



Natural products application: Health, Cosmetic and Food

Provided by nature, adapted scientifically for industry



Book of abstracts
1st International Online Conference
4th - 5th February 2021

Title

1st Natural products application: Health, Cosmetic and Food: book of abstracts

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1° Online Congress on Natural products application: Health, Cosmetic and Food

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1° Online Congress on Natural products application: Health, Cosmetic and Food

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The Mountain Research Center is one of the 5 research centers within the Polytechnic Institute of Bragança and is an RD unit of excellence. CIMO conducts research on the Mediterranean mountain systems following an interdisciplinary strategy that goes from Nature to Products.

In all these years, we have had the commitment of disseminating science around the world, creating solid and robust bonds and partnerships with both, academia and industry, and we are always looking for more challenging collaborations.

In this sense, the mountain research center gathers different ways to keep evolving in our main mission of science dissemination, especially now during this difficult pandemic situation, in which science dissemination has been extremely affected.

Therefore, one of our responses was the creation of the first edition of the Natural Product Applications Online Congress, which consists in the dissemination of research using natural products applied in 3 different areas: cosmetic, food, and health.

Thanks to all of you in less than a month the congress reached more than 483 registration from universities and important companies from different parts of the world, such as Algeria, Argentina, Brazil, Colombia, France, Greece, Italy, Mexico, Netherlands, Poland, Russia, Serbia, Slovenia, Spain, Ukraine, and USA.

The NPA congress received and processed more than 211 communications, from which the scientific committee has selected the most appropriate for each type of communication, considering the limited time we have for this conference.

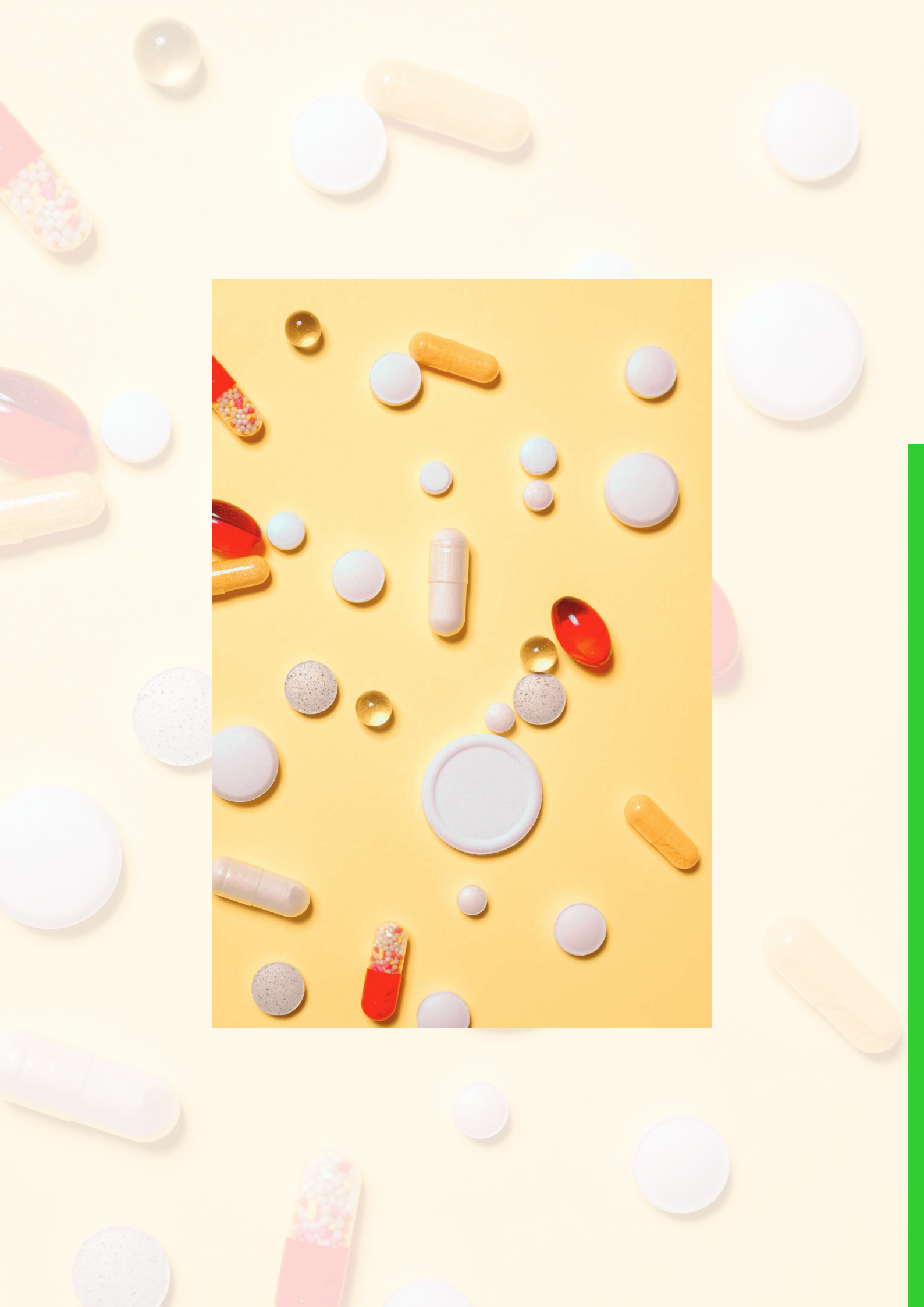
All the submitted works were divided into three main categories, Oral, Pitch, and Poster communications, which will join 9 Keynote lectures and one invited oral communication, to which, we would also like to thank for their availability and for accepting this invitation.

We could not thank you more for your participation, and we hope to see you next year on the second edition of the Natural Product Applications Congress.

NPA Team.

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Keynotes Information



Health Area Keynotes



Maria Graça Campos, PhD

Professor of Pharmacognosy at the Faculty of Pharmacy, University of Coimbra and she is the Coordinator of Observatory of Drug-Herb Interactions. The main topics of research includes Drug Discovery, especially with Bee products and Pharmacovigilance in drug-herb interactions mainly with Oncologic patients.

Pedro Cantista, MD, PhD

Medical Doctor, specialized in Physical and Rehabilitation Medicine; competences in Medical Hydrology and Pain Medicine; Consultant at the Centro Hospitalar e Universitário do Porto; Professor at the Universidade do Porto; President of the International Society of Medical Hydrology and of the Portuguese Society of Medical Hydrology.



Ana Novo Barros, PhD

Ana Novo Barros, Assistant Professor with habilitation at the Chemistry Department of the UTAD, is Director from the Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB). The area of expertise is the valorization of by-products, taking into account the circular economy and industrial symbioses. The work has been recognized with the attribution of several prizes.

Adelar Bracht, PhD

received his Doctorate in Natural Sciences from the University of Munich, and is now senior biochemistry professor at the State University of Maringá. He has been researching liver metabolism, including oxidative stress, since 1980.





Food Area Keynotes



Manuela Pintado, PhD

has a PhD in Biotechnology in the specialty of Engineering and Food Sciences. She is currently a Professor at the Faculty of Biotechnology at UCP (ESB-UCP), in which she is also Director of the Biotechnology school and of the biotechnology and fine chemistry centre. In the field of research, she is Coordinator of the group Biological and Biomedical Products group and the Leader of the thematic research laboratory for Bioactive and Bioproducts. Professor Manuel Pintado is also a Member of the IBET advisory board (since 2016), Member of the Strategic Group for AgroFood, Forest and Biodiversity and Member of the Strategic Group for

Circular Economy FCT, vice-president of the Scientific Council for Natural and Environmental Sciences (CCCNA) of FCT, Coordinator of the Evaluation Committee for Final Reports of FCT projects, Vice President of the European Chitin Society, President of the SIG (Group of Bioactive Compounds of the ISEKI Food Association (since 2014). From her research work, Professor Manuela has published hundreds of research manuscripts that led to a recognition from the Ciência Viva Program as one of the female faces of the Portuguese science community in its book dedicated to Portuguese scientist women.

Jesus Simal, PhD

Full Prof. in Food Sci. at the Univ. of Vigo (Spain) since 1999.

-Leader of a research group of excellence at Galicia (NW Spain) since 2006 till now.

-Leader of CIA3 (Environmental, Agricultural and Food Research Centre) formed by 10 research groups from different fields (botany, plant physiology, soil science and agricultural chemistry, biochemistry and molecular biology, nutrition and food science, biotechnology, food technology, food rheology, chemical engineering, and colloidal chemistry) at Univ. of Vigo since 2008 till 2018.

-Head of the Department of Analytical Chemistry and Food Science at the Univ. of Vigo between 2013 and 2018.

-Vice-Chancellor for Internationalisation in 2018.



Luis Angel Fernandez, PhD

Luis is biochemist with a post-degree in food science from the University of Buenos Aires. His doctoral thesis relates to allulose as a rare sugar very low in calories to replace saccharose in jams. Study of the technological aspects, sensory and biological response in vivo.

Fernandez has more than 35 years of professional experience in the food ingredient industry (Tate & Lyle, Cargill, Danisco, Rhodia, DuPont, Sanofi). He lived and worked in Argentina, France, Belgium and the United States.

Luis was elected fellow of the IFT (Institute of Food Technologists) in 2009 and also fellow of the IAFoST (International Academy of Food Science and Technology) in 2010.

Currently, Luis Angel Fernandez is Vice President of Strategic R&D for MANE from 2017. He is based in its headquarters in Le-Bar-sur-Loup, France and is responsible of the group innovation for sweet and savory categories and strategic research partnership (open innovation).



Food Area Keynotes



Ana Palmeira de Oliveira, PhD

Graduated (2003) and Master (2009) in Pharmaceutical Sciences, University of Porto and PhD in Pharmaceutical Sciences (2012), University of Beira Interior. Ana is invited professor at University of Beira Interior, Researcher at Health Sciences Research Center (CICS-UBI) and CEO at Health Products Research and Development Lda (Labfit®/Pharmapoli®). Ana completed the Master in Business Administration (MBA) at UBI in 2018 and is author of more than 60 papers, 1 book chapter and 3 patents. Her work has been recognized with 8 prizes/distinctions. Her research field is in the health sciences specially dedicated to the study of skin and mucosa disorders, mainly in the study of bioactive compounds as cosmetics ingredients and pharmaceutical actives against pathogens; the host-pathogens interaction and the safety assessment using in vitro tests.

Paola Perugini, PhD

did her PhD in Pharmaceutical Technology in 1998 and she is a professor in the Pharmaceutical and Cosmetic Technology fields at the Department of Drug Sciences at the University of Pavia since 2001.

She is Director of the Second Level Master Course in Cosmetological Sciences at the University of Pavia.

She is Founder and Scientific Director of Etichub s.r.l., Academic spin-off of the University of Pavia.

Her research fields concerning pharmaceutical and cosmetic technology led to several patents and more than 150 international publications and congress presentations. In cosmetic field Dr. Paola Perugini received the Maison de Navarre award for a research regarding studies on photostability of sunscreens.



Isabel Monteiro, PhD

In 2009, Isabel Monteiro starts her professional path in the cosmetics and cleaning products formulation area. Since 2013, she works at Colep Portugal, S.A., first as a New Product Implementation and then as a Blending Specialist at CPD Technical Department. In 2018, she joined the R&D team as a Product Developer and she continued her academic studies taking her Master's in Pharmaceutical Technology in the Faculty of Pharmacy, University of Porto. At this moment, Isabel is attending the first year of the PhD in Sustainable Chemistry at the Faculty of Science,

University of Porto, where she graduated in Chemistry.

Congress Program

Congress Program

Morning - 4 February

Health

9:00
-9:30

Welcome
Congress chair: Lillian Barros

Opening Session
Isabel Ferreira
Secretary of State of Inland
Improvement (Portugal)

9:30
-10:00

Maria Graça Campos
Globalization of beehive
products in food & health

Keynote 1
University of
Coimbra

10:00
-10:30

Pedro Cantista
Thermal products: mineral
waters, peloids and gases

Keynote 2
University of
Porto

10:30
-10:40

Ana Novo Barros
Invited Oral Communication

Universidade de
Trás-os-Montes
e Alto Douro

10:40
-11:40

Selected Abstracts
6 x 7 min + 3 min Q&A

**Oral
Sessions**

11:40
-12:00

Selected Abstracts
6 x 3 min

**Pitch
Sessions**

12:00
-12:30

Adelar Bracht
The adjuvant-induced arthritis model
and its utility for investigating
antiinflammatory & antioxidant effects

Keynote 3
State
University
of Maringa

12:30
-14:30







Lunch Break









Morning - 4 February

Health

Oral Presentations (10:40 - 11:40)

- 
Ana Teresa Serra
 Unveiling the protective role of natural bioactive compounds towards colorectal cancer
- 
Carlos A. Martins Gomes
 Anti-aging and neuroprotective activity of *Thymus carnosus* aqueous and hydroethanolic extracts
- 
Carlos Seiti Hurtado Shiraishi
 Anti-inflammatory evaluation of a lib. of natural compounds identified in mushrooms using *in silico* studies against cox enzymes
- 
Clara Grosso
 Silverbrain: neuroprotective potential of seaweeds subcritical water extracts
- 
Katja Kramberger
Helichrysum italicum infusion stimulates energy expenditure and fat oxidation after acute ingestion in humans: a pilot study
- 
Teresa Brás
 Chitosan based films loaded with Cynaropicrin enriched extract from *Cynara cardunculus*: anti-inflammatory potential

Pitch Presentations (11:40 - 12:00)

- 
Ana Sofia Pereira de Freitas
 Antibacterial and antioxidant synergisms between propolis and gentamicin or honey
- 
André Folgado
 Hairy roots cultures of *Cynara cardunculus* l. as a valuable source of hydroxycinnamic acid compounds
- 
Bruna Mara Machado Ribeiro
 Anti-inflammatory effect of N-3 polyunsaturated fatty acids on virus-infected neurons
- 
Catarina Faria-Silva
 Extraction of alpha-tomatine from green tomatoes to treat skin diseases
- 
Hala Haddad
 Optimization of anti-inflammatory methodologies to evaluate natural products activity as inflammation inhibitors
- 
Naiara Fernández
 Industrial waste streams valorization at IBET: health related bioactives from fisheries and wineries

Afternoon - 4 February

Food

14:30
-15:00

Manuela Pintado
Antimicrobial solutions from
natural resources for food
applications

Keynote 4
Catholic
University of
Portugal

15:00
-15:30

Jesús Simal
Metabolomics: The basics &
applications in food & function

Keynote 5
University of
Vigo

15:30
-16:30

Selected Abstracts
6 x 7 min + 3 min Q&A

**Oral
Sessions**

16:30
-16:40

Break & Poster Session

16:40
-17:00

Selected Abstracts
6 x 3 min

**Pitch
Sessions**

17:00
-17:30

Luís Angel Fernández
Protein Transition:
Plant protein off-notes: myth
or reality?

Keynote 6
MANE
France

17:30
-18:30

Poster Session

Afternoon - 4 February

Food

Oral Presentations (15:30 - 16:30)



Antón Soria López

Effect of pressurized liquid extraction (PLE) on the lipid composition of *Fucus vesiculosus*



Filipa Fernandes

Tools to develop dairy ingredients: bioactive and preservative purposes



Filipa Mandim

Phenolic composition and bioactive properties of cardoon bracts: influence of the growth cycle



Ingrida Kraujutienė

A new generation of foods for the prevention of diabetes



Jonata Massao Ueda

Natural food preservatives: application of rosemary, basil, and sage in yogurts, using sustainable, low cost, and efficient processes



Ricardo Miguel Moura Ferreira

The effect of high-pressure processing and thermal pasteurization on the microbial and physical-chemical properties of 3 varieties of *OFI*

Pitch Presentations (16:40 - 17:00)



Andreia Quaresma

ITS2 metabarcoding: a promising approach for identification of botanical origin of bee-collected pollen



Bianca Rodrigues de Albuquerque

Flavonoid composition and in vitro anti-proliferative activity of the hydroethanolic extract of *Garcinia mangostana* L. pericarp.



Francesco Di Gioia

Tailoring food crops to address specific dietary needs: the case of microgreens



Isabel A.C. Ribeiro

Exploring cranberry extract chitosan films towards the improvement of antimicrobial food packaging



José Virgílio Santulhão Pinela

Table tomato leaves are a sustainable source of rutin and display antioxidant, antihemolytic and antimicrobial activity



Seymanur Ertosun

Nutritional, technological and sensory attributes of bread enriched with bee pollen and bee bread

Morning - 5 February

Cosmetics

9:30
-10:00

Ana Oliveira
Natural cosmetics: a market trend sustained by science

Keynote 7
University of Beira Interior

10:00
-10:30

Paola Perugini
Upcycling: transforming byproducts from waste and agriculture into active ingredients for cosmetic use

Keynote 8
University of Pavia

10:30
-11:30

Selected Abstracts
6 x 7 min + 3 min Q&A

Oral Sessions

11:30
-11:40

Break & Poster Session

11:40
-12:00

Selected Abstracts
6 x 3 min

Pitch Sessions

12:00
-12:30

Isabel Maria Monteiro
Natural cosmetic products: more than a market trend

Keynote 9
Colep Portugal

12:30
-13:00

Closing Session



Morning - 5 February

Cosmetic

Oral Presentations (10:30 - 11:30)

Anna Szemik-Hojniak

Biomimetic short peptides in medicine and cosmetology

Celso Afonso Ferraz

Contributions towards the ecotoxicological evaluation of plant extracts and essential oils

Javier Echave Álvarez

Fatty acid comp., antioxidant and antibacterial act. of ethanolic PLE extracts of 4 macroalgae species from Galicia

Paula Plasencia

Bioactive properties of different extracts obtained from the aerial parts of blueberry and raspberry raw materials

Sara Gonçalves

Evaluation of cosmetic properties of natural ingredients in the Trás-os-montes area: a PhD project

Silena Silva Delgado Alves

Humulus lupulus L.: cosmetic application of extracts obtained from cones and vegetative parts

Pitch Presentations (11:40 - 12:00)

Ana Costa

From garbage to glamour: assessing the organoleptic prop. of formulations containing lycopene-enriched extracts from tomato waste

Ana Rita Silva

Optimization of a tannin-rich extract using response surface methodology

Bruno Melgar Castañeda

Time dependency on bioactive compounds UAE extractions

Diana Andreia Tavares Pinto

Castanea sativa shells: from an undervalued agro-residue to a valuable raw material for cosmetic industry

Maria Aurora Soares da Silva

Bioactive properties of six macro-alga from the iberic peninsula sea

Patrycja Brudzyńska

Application of plant-derived colorants in cosmetic products



Keynote presentation

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KLH & IOCH
Keynote
Lectures
& Invited
Communication
in Health area



KLH-01

GLOBALIZATION OF BEEHIVE PRODUCTS IN FOOD & HEALTH

Maria G. Campos,^{1,2*}, El-Mehdi Iouraouine³ and Ofélia Anjos,^{4,5,6}

¹Coimbra Chemistry Centre (CQC, FCT Unit 313) (FCTUC), University of Coimbra, Rua Larga, 3000-548 Coimbra, Portugal;

²Observatory of Drug-herb Interactions, University of Coimbra, Health Sciences Campus, Portugal

³Clinical Neuroscience Laboratory, Faculty of Medicine and Pharmacy, Sidi Mohamed Ben Abdellah University Fez, Morocco;

⁴Centro de Biotecnologia de Plantas da Beira Interior, Castelo Branco, Portugal;

⁵Instituto Politécnico de Castelo Branco, Castelo Branco, Portugal;

⁶Centro de Estudos Florestais, Instituto Superior de Agronomia, Universidade Lisboa, Lisboa, Portugal.

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The development of new and more efficient technological methods and the global quality control standards opens a new perspective in the use of various bee products. The food, pharmaceutical and cosmetics industries have an enormous source of possibilities that can be explored. In general, most of them can be used in all these three aspects. Thus, for example Honey, it can be used as food and in foods process, in cosmetics and even as a medicinal product, for instance in colds, surgical dressings or for burn wounds. In the case of bee pollen, it has been used in traditional medicine, among other purposes, to alleviate or cure colds, flu, ulcers, premature aging, anemia, colitis, allergies, enteritis. The biological properties of bee pollen are very well known and justify their application in foods, cosmetics and future medicinal products. The Bee Wax can also integrate products in a transversal way and the same occurs for Propolis, Royal jelly and Bee venom. For instance, Apitoxin shows the potential to inhibit the proliferation of melanoma K1735M2 cells *in vitro*, B16 melanoma, a transplantable solid melanoma in C57BL/6 mice, *in vivo*. Bee wax, due to its good stability and consistency is commonly used as release retardants that provides unique, but predictable, *in-vitro* and *in-vivo* release profiles, in different pharmaceutical applications (as for example, widely used as hydrophobic matrices for sustained drug delivery), such as tablets, suspensions, implants and microspheres [1,2]. Bee bread is an emerging product with high potential due to its nutritional value [3]. Royal Jelly restrains both gram positive and gram negative bacteria and these antibacterial activities are due to the presence of 10-HAD and the peptides jellenie I, II, III and IV [4]. Those are only some examples among the many studies developed in Drug Discovery that increasingly validate the bioactivities of these various Bee Products which could drive them to further new medicines, in a near future. Nevertheless, the standardization of them, as well as the correct definition and identification of their floral origin, is a key point to their quality and use [2,4,5]. Although much research is yet to take place in the near future, there is already a good scientific basis for the use of these bee products more and more safely and, above all, bringing added value to them, which allow a better implementation in the market.

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KLH-02

THERMAL PRODUCTS: MINERAL WATER, PELOIDS AND GASES

Pedro Cantista, MD, PhD^{1*} Margarida Cantista, MD ²

¹ Instituto de Ciências Biomédicas Abel Salazar / Universidade do Porto, Porto, Portugal

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Thermal Products include mineral waters, peloids and natural gases. Its therapeutic effects are studied by Medical Hydrology and Climatology (or Balneology). The clinical use of these substances is called Balneotherapy in many countries but other equivalent terms exist like Crenotherapy or Thermal Medicine. Together with Thalassotherapy and Climatotherapy, Crenotherapy is included in what the World Health Organization (WHO) considers Health Resort Medicine. It may be defined by all medical activities originated and derived in health resorts based on scientific evidence aiming at health promotion, prevention, therapy and rehabilitation. Health Resort Medicine includes core elements, modalities (methods) and agents (substances, factors). Substances used for balneotherapy may be administrated by several methods like bathing, drinking or inhalation. The use of plain water (tap water) for therapy is called hydrotherapy and the use of climatic factors for therapy is called climatotherapy. Reflecting the effects of health resort medicine, it is important to take other environmental factors into account. These can be classified within the framework of the WHO's International Classification of Functioning, Disability and Health (ICF). Natural mineral waters may be classified using several criteria such as physical, chemical and biological.

Peloids classification was established in 1949 in Dax and accepted by the International Society of Medical Hydrology. This classification was recently updated based on a full revision and systematization published by Gomes et al.

Natural gases used in thermal medicine are the Radon and Carbon dioxide. The use of natural products start by an empiric practice but progressively they are more and more sustained by evidence based medicine.

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IOCH-1

FIGHTING DIABETIC FOOT ULCERS RESORTING TO WINERY BY PRODUCTS: GRAPE STEMS' CASE

Irene Pereira Gouvinhas,¹ Carla Leal,¹ Eduardo Rosa,¹ Maria José Saavedra¹, Ana Novo Barros^{*1}

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University of Trás-os-Montes and Alto Douro (UTAD), 5000-801, Vila Real, Portugal.
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Phenolic compounds are secondary metabolites produced in plant responses against stresses (biotic and abiotic) and are increasingly important in the scientific community because of their beneficial properties in fighting degenerative diseases. Grapes are a rich source of these phytochemicals, and their by-products generated by the wine industry also contain these bioactive compounds. Since these by-products cause environmental problems and compromise the sustainability of industries, it is necessary to create alternatives for their use. In the case of grape stem, its phenolic composition is mostly constituted by flavanols, flavonols, phenolic acids, and stilbenes, which can be used in the production of innovative products.

Grape stems confirm to be a potential source of phenolic compounds being able to be used in the projection of new products in the cosmetic, pharmaceutical and food industries.

Antibiotic resistance is a public health problem worldwide, having impact in morbidity and/or high mortality rates, especially in developing countries [1]. Specific microorganisms such as *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus* are present frequently in processed food, and consequently transmit diseases, constituting a health risk [2]. In addition, one of the main causes of hospitalization worldwide are diabetic foot ulcer infections, mostly caused by *Staphylococcus aureus*, and due to frequent use of antibiotics, *S. aureus* strains have developed resistance to treatment [3]. To combat bacterial resistance, alternative antibiotics should be searched and developed to overcome the effectiveness of those currently available and, in these sense, data show that phenolic compounds may be an alternative due to their antibacterial properties [1]. Phenolic compounds, acting as antimicrobials, weaken the phospholipid bilayer of the bacterial cell membrane, causing the release of vital cytoplasmatic constituents, and damaging bacterial enzyme systems [4].

Thus, in order to replace synthetic drugs/antibiotics with more effective natural compounds, there is a growing demand to find natural compounds that can be used in the development of new products. In this way, the aim of this work was to evaluate some biological activities of phenolic compounds present in grape stems, namely, antioxidant capacity by ABTS^{•+}, DPPH[•], and FRAP methods; and antimicrobial activity against diabetic foot wound bacteria by disc diffusion and MIC assays.

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KLH-03

THE ADJUVANT-INDUCED ARTHRITIS MODEL AND ITS UTILITY FOR INVESTIGATING ANTI-INFLAMMATORY AND ANTIOXIDANT EFFECTS

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Anti-inflammatory and antioxidant actions are frequently the first bioactivities tested when new extracts or purified natural products are described. Many *in vitro* systems allow a preliminary evaluation of these properties. But, these characterizations are relatively uncertain, as the conditions are artificial. Especially the required doses are uncertain. These are the main reasons why *in vivo* models are needed. The adjuvant-induced arthritic rat presents several favourable features for being used as an *in vivo* model. Several inflammatory, oxidative and metabolic indicators are modified, as illustrated for the heart in **Figure 1** [1]. Modifications are essential because it is extremely difficult to improve conditions that are already close to normal. Adjuvant-induced arthritis in rats is very similar to rheumatoid arthritis in humans. Additionally, in patients, limited interventions are possible (*e.g.*, blood samples), but in the rat all organs can be analysed. Taking into account these advantages a fair number of studies about the effects of natural products on

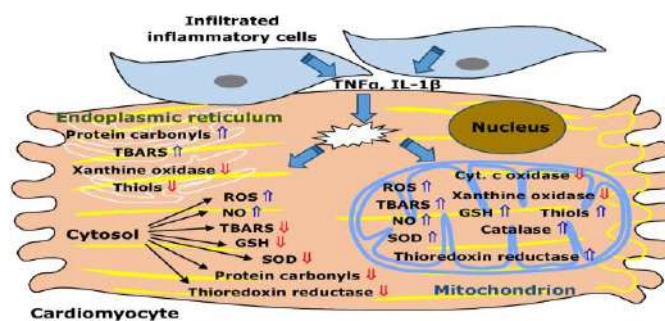


Figure 1: Main oxidative stress markers that are modified in the heart of adjuvant-induced arthritic rats [1].

inflammatory, oxidative and even metabolic markers is now available. Our group, for example, has investigated the actions of green tea, Merlot grape pomace, yerba-mate, rosemary, *Agaricus brasiliensis*, copaíba oil, methyl jasmonate and resveratrol. All these studies were based on an extensive previous characterization of the influence of arthritis on blood, liver, heart and brain. In general, anti-inflammatory and antioxidant effects were confirmed, but with a pronounced diversification concerning the various tissues and cell compartments. The effectiveness of each natural product in modifying the diverse markers in the various tissues or cell compartments can only exceptionally be predicted based on *in vitro* or *in vivo* parallel observations. Furthermore, the effects can have complex dose dependencies, some of them revealing a concomitant prooxidant action to the point that multiple dosing experiments are highly recommendable [2].

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KLF
Keynote
Lectures
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KLF-04

ANTIMICROBIAL SOLUTIONS FROM NATURAL RESOURCES FOR FOOD APPLICATIONSManuela Pintado,¹

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Consumers are demanding more from their food than ever before, reading food labels carefully to guarantee the freshness, quality, safety and nutritional value. Extension of shelf life usually involves packaging solutions, which frequently increase the unsustainable packaging in the food chain, or product formulation incorporating preservatives. An increase of shelf life also prevents up household food waste. However, most of the current solutions are synthetic preservatives and recent awareness of consumers for these additives have led to an increasing demand for clean label, this means less additives, and more natural. Therefore, to consider extending shelf life through preservatives (antimicrobials and antioxidants), they must be efficient at low levels and natural rather than artificial. Therefore, during this presentation, the production of natural preservatives inspired in natural sources and byproducts (plants extracts, bacteria, peptides, polysaccharides) will be explored in terms of biological efficiency and applications to extend shelf life of products with a short life, and demonstrate the impact in quality and safety. This will also encourage the valorisation of unexplored natural resources and food losses and wastes to assure a more sustainable food supply chain.

KLF-05

METABOLOMICS: THE BASICS AND APPLICATIONS IN FOOD AND FUNCTION

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Metabolomics is a young field of knowledge that arises linked to other omics such as genomics, transcriptomics, and proteomics. This discipline seeks to understand the performance of metabolites, identifying, quantifying them, and thus understanding its mechanism of action. This new branch of omics science shows high potential, due to its noninvasive character and its close relation with phenotype. Several techniques have been developed to study the metabolome of biological samples, fundamentally nuclear magnetic resonance (NMR), and mass spectrometry (MS), or a combination of several techniques. These techniques are focused to separate, detect, characterize, and quantify metabolites, as well as elucidate their structures and their function on the metabolic pathways they are involved. However, due to the complexity of the metabolome, in most cases it is necessary to apply several of these techniques to understand completely the whole scenery. This contribution is aimed to offer a summary of the current knowledge of these analytical techniques for metabolomics and their application to different fields as food or health sciences. Each technique shows different advantages and drawbacks depending on their technical characteristics and limitations, some factors, such as the aim of the study or the nature of the biological sample will condition the choice. Regarding their applications, NMR has been employed specially to identify new compounds and elucidate structures. The use of MS has gained popularity because of its versatility, easiness to be coupled to separation techniques and its high sensitivity. Metabolomics applications in different science fields are growing each year, due to advances in analytical techniques and combination with other omics that allow to increase the comprehension of metabolic processes. Further development of analytical tools is necessary to continue exploiting all the possibilities of metabolomics [1].

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KLF-06

PLANT PROTEIN OFF-NOTES: MYTH OR REALITY?

Luis Angel Fernández,¹

¹MANE Industry

Is it true to say that we tend to prefer the taste of animal protein over the taste of plant-based protein? Does it necessarily have to be a toss-up between chicken and tofu? Seitan and beef?

In a growing world population expected to reach 10 billion in 2050, we are trying to cut back on our animal protein consumption for various reasons such as sustainability, health, availability, cost, animal welfare or just for new food experiences. One of the most pressing questions when it comes to achieving this is whether plant-based proteins can be consumed as alternatives to our traditional animal-derived products. Meat has been the central protein part of our meals, which is one aspect that is currently changing. However, these plant-based alternatives can also be consumed in other ways such as snacks, desserts or beverages.

In order to be an acceptable stand-in for our favourite food items, plant-based products need to deliver a taste experience that is on-par with meat or dairy while keeping a simple and clear labelling. As such, there are some issues that are holding them back from reaching that position, which we will look into this conference.



KLC
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KLC-07

COSMETIC PRODUCTS: MARKET TENDENCY BASED IN SCIENCEAna Palmeira de Oliveira¹

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The cosmetic market is growing impressively for several years, anticipating and bringing solutions to consumers expectations and demands. Natural, organic, vegan cosmetic products are just some examples of trends that have been gaining place in the cosmetic market. In this talk we will present some examples of how science related to natural products supports and contributes to the innovation process in this field. We will give a special attention to the research made at Portugal and some examples of science translation from academia to companies and vice-versa, will be presented.

KLH-08

UPCYCLING: TRANSFORMING BYPRODUCTS FROM WASTE AND AGRICULTURE INTO ACTIVE INGREDIENTS FOR COSMETIC USE”Paola Perugini,PhD,^{1*}¹Department of Drug Sciences, University of Pavia, Italy.

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Upcycling is the process of conversion of waste materials to something useful or valuable; in this way the waste can be re-adapted and/or re-purposed in several fields of application.

A new trend in the cosmetics industry is Zero Waste. One aspect of this trend is using existing material coming from agriculture waste to obtain cosmetic ingredients of high quality and efficacy.

In the present work some examples of these ingredients will be presented.

Extracted oils from shell and cluster of nuts, like pistachio or pecan, are examples of very interesting compounds to value the industrial application of the nut waste.

In fact pecan nut, for example, is a dried fruit, appreciated worldwide because of its particularly pleasant flavor and its wealth of healthy bioactive components. The nut oil is rich in monounsaturated fatty acids and vitamin E, currently recognized as antioxidant agents. The oil extracted from the pecan nut cake, has demonstrated antioxidant activity and good feeling for hair application.

Furthermore, the grape pomace, a winemaking industry byproduct, can represent an other chance to obtain ingredients to be used in skin care and make up. In fact the grape pomace contains several substances in addition to the well known antioxidant resveratrol, such as polyphenols, that can act like a natural colorant for cosmetic application. The pomace as well, suitably treated, can be used as functional powder with excellent antioxidant properties; the small natural exfoliating grains obtained from the waste can also used to improve skin texture.

KLC-09

NATURAL COSMETIC PRODUCTS: MORE THAN A MARKET TRENDIsabel Monteiro,^{1*}

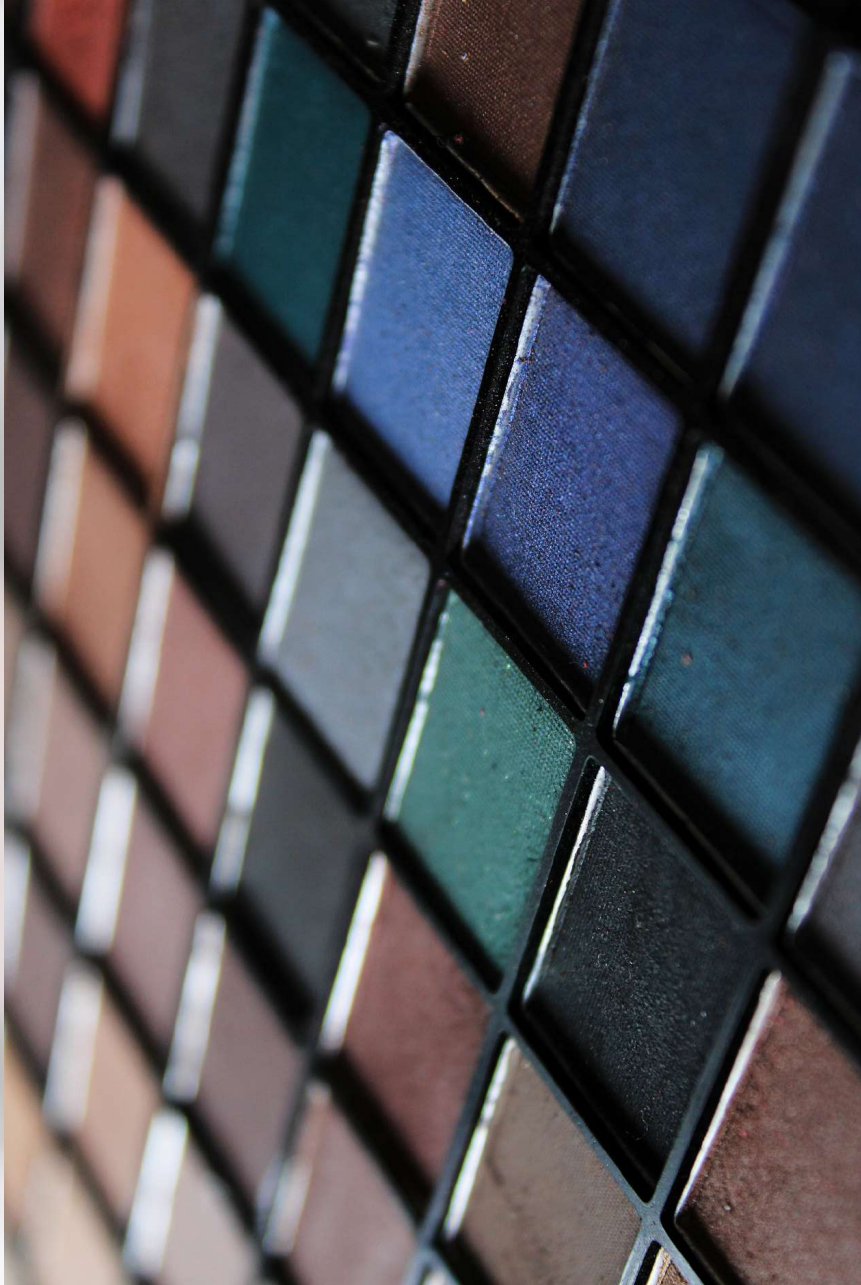
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New cosmetic products are constantly appearing on the market, driven by consumer needs. The demand for natural alternatives to traditional cosmetics is growing. This is more than a market trend, this reflects an informed choice of the final consumer, as well as of the entire value chain, taking into account the challenges of our times.

But, after all, what does "natural" mean? Although there is no official cosmetic definition for this word, it brings different perspectives from companies and consumers expectations. More than an isolated concept, "natural" is just the tip of the iceberg, meaning that the concern with economic, social and environmental sustainability issues is increasing. This translates into the life cycle ending up being more important than the cosmetic product itself.

With all of these changes happening so fast, what challenges and opportunities does the industry face in terms of safety, quality and functionality? Are the cosmetic regulations and certifications adapted to the new reality? Are consumers well informed? These are the main questions that need to be answered in order to develop natural cosmetic products.



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OCH
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OCH-01

UNVEILING THE PROTECTIVE ROLE OF NATURAL BIOACTIVE COMPOUNDS TOWARDS COLORECTAL CANCER

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Colorectal cancer (CRC) is among the leading causes of mortality and morbidity through the world [1]. Extensive research has been suggesting that nutrition may carry a protective role in CRC development [2]. Within this context, the benefits of Mediterranean diet on CRC protection has been reported [3]. In particular, the intake of cruciferous vegetables of the Brassicaceae family (e.g., broccoli and watercress) has been associated to a reduced risk of CRC, due to their high content of glucosinolates that were further converted by plant myrosinase and/or gut microbiota into more bioactive products namely isothiocyanates [4]. Citrus fruits and virgin olive oil are also recognized to be rich sources of phenolic compounds where polymethoxylated flavones and hydroxytyrosol are compounds of interest due to their anticancer activity reported *in vitro* in several types of tumors [5,6]. However, the molecular mechanisms underlying the protective action of these food bioactives compounds and their effect on cancer stem cell subpopulation are still unclear. In this presentation, three case studies focused on the identification of key bioactive compounds, derived from different food matrices, and their evaluation in 3D cell models of colorectal cancer, will be presented:

Case study 1: Brassicaceae extracts enriched in isothiocyanates target colorectal cancer cell proliferation, stemness and metastatic potential [7];

Case study 2: Polymethoxylated flavones from orange peels improve the antiproliferative effect of a chemotherapeutic drug [8];

Case study 3: Olive oil phenolics and colonic metabolites modulate cancer stemness. The knowledge generated from these studies provides important insights regarding the molecular mechanisms by which food bioactive compounds could prevent CRC. This will hopefully strengthen the design of nutritional intervention studies for cancer treatment.

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OCH-02

ANTI-AGING AND NEUROPROTECTIVE ACTIVITY OF THYMUS CARNOSUS AQUEOUS AND HYDROETHANOLIC EXTRACTS

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Medicinal and aromatic plants (MAPs) have been extensively used through human history for the treatment of the most various illnesses, contributing to traditional medicine development and increasing ethnobotanical knowledge, which now serves as basis to further explore the potential of the wide variety of species with potential to be used in the pharmaceutical and cosmetic industries. Nowadays, the correlation between a species phytochemical composition and its bioactivities is well established, which also unveils the major limitation for their use in the industry: many species are still uncharacterized regarding the phytochemicals that play a role in their bioactivities. By filling this lack of knowledge in the phytochemical composition of a given species, it would be provided scientific evidence supporting traditional medicine uses and increase the interest in its use. Various MAPs within the *Thymus* genus have been studied for industry application, as for example, *Thymus pulegioides*, a species with extracts rich in rosmarinic acid, that has been described to have, neuroprotective, anti-diabetic, anti-aging, and anti-proliferative properties [1]. However, not all *Thymus* species have been described concerning the above-mentioned bioactivities. For example, *Thymus carnosus*, an endemic and endangered species from the Iberian Peninsula, was recently reported for its extracts rich in rosmarinic acid and salvianolic acids A and K, and studied to evaluate and characterize the potential bioactivities and uses. In addition to a high content in phenolic acids, chromatographic analysis revealed that *T. carnosus* hydroethanolic extract was also very rich in the pentacyclic triterpenoids ursolic and oleanolic acids, which makes it stand from other species in the same genus [2].

The neuroprotective and anti-aging potential was evaluated for *T. carnosus* aqueous decoction and hydroethanolic extracts, as well as for some of their major phytochemicals (rosmarinic, ursolic and oleanolic acids) in order to correlate the phytochemical composition to the observed bioactivities. Both extracts greatly inhibited acetylcholinesterase activity, proving to be a valuable source of phytochemicals with neuroprotective activity. A phytochemical composition-dependent inhibition of elastase and tyrosinase was observed, as aqueous decoction only inhibited tyrosinase and hydroethanolic extract only inhibited elastase, supported by the inhibition observed for individual compounds. These findings highlight the potential application of *Thymus carnosus* in the pharmaceutical and cosmetic industries.

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OCH-03

ANTI-INFLAMMATORY EVALUATION OF A LIBRARY OF NATURAL COMPOUNDS IDENTIFIED IN MUSHROOMS USING *IN SILICO* STUDIES AGAINST COX ENZYMES

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According to the European Medicines Agency, the use of non-steroidal anti-inflammatory drugs (NSAIDs) creates risks to the individual such as gastrointestinal, renal and cardiovascular, so alternatives to compounds with similar properties are sought after [1]. NSAIDs act mostly by inhibition of cyclooxygenase (COX) enzymes, specifically COX-1 and COX-2 isoenzymes. The research of natural occurring metabolites as potential anti-inflammatory drugs, through inhibition of COX enzymes, has thus been proposed.

In the present study, a library of 115 compounds [2], naturally occurring in mushrooms with medicinal properties, was expanded to 190 compounds, thus developing an enhanced LMW (Low Molecular Weight) 3.0 library. *In silico* studies were then carried out using Virtual Screening tools (Pharmacokinetics, Docking and Molecular Dynamics) to identify compounds from the LMW 3.0 library with the best predicted anti-inflammatory potential through inhibition of COX-1 and COX-2.

From the docking studies performed using AutoDock Vina software, Aurisin A, present in *Neonothopanus nambi* mushroom [3], produced the lowest predicted Ki value against COX-1 (152,4 nM). For COX-2, Clavilactone C (**Figure 1a**), present in *Clitocybe clavipes* mushroom [4], produced the lowest predicted Ki value (52,3 nM). When both COX enzymes were considered, Officimalonic Acid B, present in the mushroom *Fomitopsis officinalis* [5], provided the best profile for dual COX inhibition, with predicted Ki values of 974,5 nM (COX-1) and 231,2 nM (COX-2). Clavilactone C was further analyzed, and the stability of the obtained docking conformation was confirmed through molecular dynamics (**Figure 1b**). The 3 highlighted compounds may have anti-inflammatory potential, having as potential mechanisms of action the inhibition of COX-1 and/or COX-2, however experimental verification must be performed.

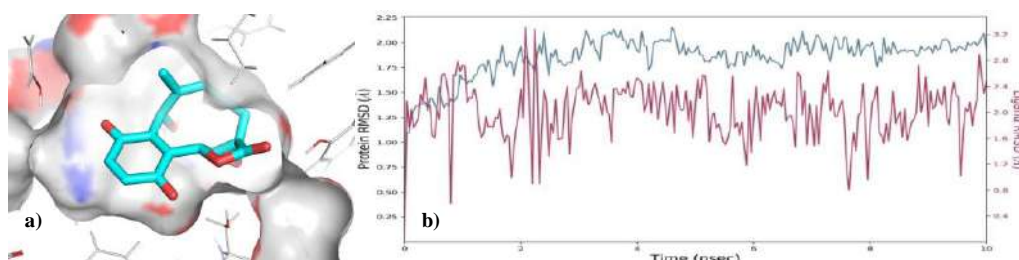


Figure 1: Clavilactone C: a) docking conformation against COX-2 and b) molecular dynamics constant stability

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OCH-04

**SILVERBRAIN: NEUROPROTECTIVE POTENTIAL OF SEAWEEDS
SUBCRITICAL WATER EXTRACTS**

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Oceans cover 70% of the earth's surface and host a vast diversity of life, including approximately 1500 brown, 900 green, and 4000 red seaweeds worldwide. This still unexplored biodiversity offers unlimited sources of novel metabolites with possible applications in food, health, and cosmetic industries [1]. Being mental health and active aging one of the biggest concerns in the XXI century, extracts prepared from *Codium tomentosum* Stackhouse (green seaweed) and *Fucus vesiculosus* L. (brown seaweed) were evaluated for their neuroprotective effect. Extracts were prepared by subcritical water extraction (sCWE), in order to respect the principles of green chemistry, using a gradient of temperatures: room temperature to 90°C (step 1), 90-140°C (step 2), 140-190°C (step 3) and 190-250°C (step 4). Being the anthropogenic contamination of the oceans a common problem, 82 pharmaceuticals and 33 pesticides were screened in the obtained extracts by UHPLC-MS/MS. All extracts were free of these contaminants. However, arsenic and a high iodine content were found in the first two extraction steps (steps 1 and 2). Therefore, the health application of both seaweeds was only assessed for fractions obtained in the steps 3 and 4 from the extraction process. These fractions were tested against five brain enzymes - acetylcholinesterase, butyrylcholinesterase, tyrosinase, monoamine oxidase A, and monoamine oxidase B implicated in Alzheimer's and in Parkinson's diseases and major depressive disorder etiology – and reactive oxygen and nitrogen species, revealing strong activities for step 4 for both seaweed species. Regarding the variation of the chemical composition during the extraction process, it was observed that fractions obtained in step 1 are richer in phenolic compounds than the subsequent fractions. Moreover, with the increase of temperature, reactions such as Maillard, caramelization, and thermo-oxidation reactions occur, and the resultant products positively affect the antioxidant capacity and neuroprotective effects. These results are in accordance with those previously obtained by other authors using sCWE [2].

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OCH-05

HELICHRYSUM ITALICUM INFUSION STIMULATES ENERGY EXPENDITURE AND FAT OXIDATION AFTER ACUTE INGESTION IN HUMANS: A PILOT STUDY

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Helichrysum italicum is an iconic Mediterranean plant with yellow fade-resistant inflorescences that are a treasury of versatile bioactive secondary metabolites with antioxidant and anti-inflammatory properties. The use of whole plant extracts with a full spectrum of bioactive compounds may be well suited for multifactorial diseases such as metabolic syndrome [1]. Our aim was to investigate the acute effects of *H. italicum* infusion on resting metabolic rate and possible differences in substrate oxidation in healthy male subjects.

For this purpose, dried ground plant material of *H. italicum* grown in Slovenia was infused with hot water and subjected to phytochemical screening with HPLC-DAD-ESI-QTOF-MS. Based on the experimental [2] and literature data, a crossover clinical pilot study was performed in eleven healthy volunteers to observe the effects of *H. italicum* infusion compared to hot water (**Figure 1**). Metabolic rate and substrate oxidation were measured for 15 minutes via indirect calorimetry at baseline (pre-ingestion) and at 30 minutes and 120 minutes post-ingestion.

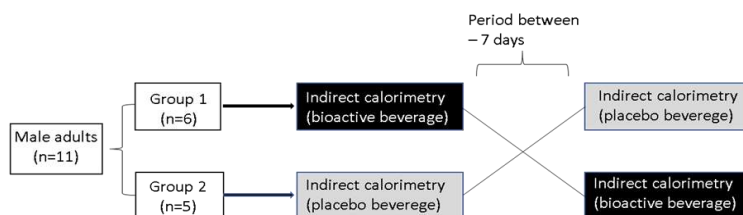


Figure 1: Study protocol for the acute pilot trial; randomized, placebo-controlled, crossover study.

Various phenolic classes were identified in the infusion, among which caffeoylquinic acids, followed by pyrones and flavonols, were the most abundant. A single ingestion of *H. italicum* infusion showed a slight but significant increase in energy expenditure in healthy adults compared to a placebo beverage as well as a significant decrease in respiratory quotient after 30 and still after 120 minutes. A significantly higher percentage of fat oxidation was observed when the bioactive beverage was ingested compared to placebo, which was also confirmed *in vitro* by the upregulation of fatty acid oxidation genes in HepG2 cells.

In the present study, infusion of *H. italicum* was found to possess bioactive compounds with potential application in the prevention of obesity. However, a longer clinical study needs to be conducted to investigate the chronic *in vivo* effects of *H. italicum* infusion under pathophysiological conditions (i.e. metabolic syndrome).

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OCH-06

**CHITOSAN BASED FILMS LOADED WITH CYNAROPICRIN ENRICHED EXTRACT FROM
CYNARA CARDUNCULUS: ANTI-INFLAMMATORY POTENTIAL**T. Brás*,^{1,2} D. Rosa,^{1,3,4} L.A. Neves², J. Crespo², M.F. Duarte^{1,4}¹Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo/ Instituto Politécnico de Beja, Beja, Portugal²LAQV/REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Campus de Caparica, 2829-516 Caparica, Portugal³Allelopathy Group, Department of Organic Chemistry, Institute of Biomolecules, Campus de Excelencia Internacional Agroalimentario (ceiA3), University of Cádiz, Puerto Real, Cádiz, Spain⁴Mediterranean Institute for Agriculture, Environment and Development, Universidade de Évora, Évora, Portugal Portugal

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Natural compounds derived from plants are presently an alternative for the discovery of new effective drugs, from which new products may be developed, potentially overcoming the current limitations of synthetic compounds for use in pharma and biomedical applications. The development of advanced biomaterials for transdermal drug delivery is a growing area of biomedicine wherein intensive research is carried out, especially for chronic wound healing and/or efficient delivery of active pharmaceutical ingredients across skin.

Cynaropicrin, a guaianolide SL, has raised high scientific interest, due to biological activities such as regulation of major adhesion molecules CD29 and CD98 [1] and anti-inflammatory response [2]. In this work chitosan-based films were prepared with different concentrations of cynaropicrin enriched extracts from *Cynara cardunculus* leaves (EtPUAE).

Incorporation of the cynaropicrin enriched extract in chitosan films, was performed by the solvent evaporation method, with different concentrations of the extract. With no cytotoxic effect on B_j5-ta cell line observed, for films with an extract concentration lower to 5%, a positive effect on skin inflammation was achieved. An 86% reduction on IL-6 cytokine levels production after exposure to chitosan with 5% extract, by indirect contact, was obtained being this effect positively correlated to the cynaropicrin content in the extract loaded into the chitosan films.

This work shows that cynaropicrin enriched extracts from *C. cardunculus* leaves presents potential to be use as chronic skin wound dressing, allied to chitosan, being a promising approach overcoming the application of synthetic drugs, and/or potentiate their application spectrum on what regards to anti-inflammatory action.

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OCF
Oral
Communications
in Food area

OCF-01

Effect of pressurized liquid extraction (PLE) on the lipid composition of *Fucus vesiculosus*

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Algae are rich sources of health beneficial compounds useful for food industry applications [1]. To exploit algae potential, it is necessary to develop new extraction methodologies without the use of high amounts of toxic organic solvents [2]. In this work, we investigated the lipid composition of the algae *Fucus vesiculosus* (*F. vesiculosus*) from the Northwest of Spain by gas chromatography- mass spectrometry (GC-MS). Results showed that this specie contain high quality fatty acids (FAs) like eicosapentaenoic acid (EPA) and arachidonic acid (ARA) in quantities of 0.4 mg/g and 1.3 mg/g, respectively. After that, we optimized the pressurized liquid extraction (PLE) technique to obtain extracts rich in these highquality FAs. Algae was extracted using five solvents of different polarities (hexane, ethyl acetate, acetone, ethanol and ethanol 50%) at three temperatures (80°C, 120°C and 160°C) and it was shown this technique using ethyl acetate is a selective method to enrich long chain FAs such as oleic acid, ARA and EPA. Therefore, PLE technology is an eco-friendly method useful for the extraction of high-quality lipid from *F. vesiculosus* algae.

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OCF-02

TOOLS TO DEVELOP DAIRY INGREDIENTS: BIOACTIVE AND PRESERVATIVE PURPOSES

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Phytosterols can be ingested and consequently absorbed by the human body, leading to a direct absorption competition with cholesterol, thus reducing its absorption [1]. Mushrooms are described as having several bioactive components, such as mycosterols (ergosterol), which, similarly to phytosterols, exhibit a strong hypocholesterolemic potential [2]. Thus, the objective of this work was to obtain mycosterol enriched extracts from *Agaricus bisporus* L. bio-waste for further incorporation in sheep cottage cheese, in order to develop a functional food with hypocholesterolemic effects. Sheep cottage cheeses with pure ergosterol, and a control cheese with no incorporation were prepared. The extracts were obtained by ultrasound assisted extraction (UAE) and the identification and quantification of their compounds was achieved through HPLC-UV. The toxicity and hypocholesterolemic activity were sought through a CaCo2 cell line. In terms of analysis, the nutritional value was analyzed according to AOAC procedures, the physical parameters included texture, external colour and water activity, and the microbial load (total aerobic mesophiles, enterobacteria, Psychrotrophic bacteria, yeasts, molds and *Staphylococcus aureus*) was also analyzed along a shelf life of 9 days according to the International Organization for Standardization (ISO) 6887-1:2003 [3,4]. No significant differences were verified for the nutritional parameters. The color of the cheeses incorporated with the extract hinted towards a browner tone when compared to the other two samples (cottage cheese with ergosterol and control cottage cheese). A dominance of palmitic acid followed by oleic and capric acids was detected, being the saturated fatty acids the predominant ones. Lactose and glucose were the two detected soluble sugars; being glucose only observed in cheese incorporated with *A. bisporus*, probably present in the extract. Also, the incorporations did not cause any significant alterations to normal microbial growth. Despite an increase of enterobacteria in the cheeses with the incorporation of the two agents, this increase was also verified for the control cottage cheese. CaCo2 cells absorbed 43.89% of cholesterol from the control cheese, while the cheese with pure ergosterol reduced cholesterol absorption by approximately 21.1%, and cheese with *A. bisporus* by approximately 30.24%. These results highlight the capacity of ergosterol to reduce the absorption of cholesterol, being an interesting candidate for the development of functional foods.

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OCF-03

PHENOLIC COMPOSITION AND BIOACTIVE PROPERTIES OF CARDOON BRACTS: INFLUENCE OF THE GROWTH CYCLE

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Cynara cardunculus L. (cardoon) is a typical Mediterranean species that comprises the ancestor wild cardoon (var. *sylvestris*), the cultivated leafy cardoon (var. *altilis*) and globe artichoke (var. *scolymus*). It can be found worldwide due to its high adaptability to diverse climate conditions (e.g., resistance to extreme temperatures, water stress, and soils with variable pH) [1,2]. It is also considered a multipurpose crop due to its nutritional, pharmacological and industrial applications [2]. Due to the increase of its commercial and economic value, this study aimed to evaluate the polyphenolic composition and bioactive potential of cardoon bracts in relation to plant growth cycle. Cardoon bracts were collected in central Greece at eight different maturation stages (samples C1 – lower maturity to C8 – higher maturity). The phenolic profile was determined by HPLC-DAD-ESI/MS. The antioxidant activity was measured through two cell-based assays: TBARS (thiobarbituric acid reactive substances formation inhibition) and OxHLIA (oxidative hemolysis inhibition). Cytotoxic effects were screened against four human tumor cell lines and hepatotoxicity against a non-tumor cell line (PLP2) by the sulforhodamine B assay. The anti-inflammatory potential was tested through the inhibition of NO production by a murine macrophage cell line (RAW 264.7). Finally, the antibacterial and antifungal activities were evaluated by the broth microdilution method. Twelve phenolic compounds were tentatively identified in the cardoon bract extracts and quantified in higher amounts in immature samples. Immature bracts (C1) also revealed the highest cytotoxic (GI₅₀ of 30 – 79 µg/mL) and anti-inflammatory (IC₅₀ = 72 µg/mL) activities, while they presented the highest capacity to efficiently inhibit the formation of TBARS (IC₅₀ = 26.8 µg/mL). In contrast, the extract with the higher maturity grade (C7) revealed superior efficacy against oxidative hemolysis (IC₅₀ of 38 and 75 µg/mL at Δt=60 min and 120 min, respectively). The highest antibacterial and antifungal activities were attributed to samples C1 and C6 and samples C2 and C4, respectively. The results obtained in this work could be helpful to choose the best harvesting time of cardoon bracts, allowing obtaining a greater variety of phenolic compounds, and consequently, a higher bioactive potential, and ultimately the most appropriate use of their constituents. Nevertheless, further studies are needed to better understand the compounds responsible for the observed activities, as well as to reveal the mechanisms involved in these activities.

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OCF-04

A NEW GENERATION OF FOODS FOR THE PREVENTION OF DIABETES

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The new generation of extruded foods is very popular in the world. Onwulata et al. [1] claim that the extrusion process opens up unlimited possibilities for the creation of new high-fibre foods. According to Asgary et al. [2] nutritional fibres can reduce blood glucose level by slowing down digestion and absorption of carbohydrates. The results of a study conducted by Phanindra et al. [3] show that a reduction in blood glucose levels has been observed with prolonged use of extruded products. Meynier et al. [4] found that reducing glycaemic response is crucial for the prevention of metabolic diseases. A new product by using the extruded grain was produced in the Department of Food Technologies Kaunas University of Applied Sciences. The aim of research: to ascertain the influence of the dragées candies, made from extruded products, on the amount of glucose in the capillary blood.

Methodology. A study of glucose levels in capillary blood involved 57 volunteers (23 nondiabetic, 34 diabetic (I type diabetes N 5; II type N 29) aged 18 to 84 years. Participants of the research were asked arrive in the morning on an empty stomach. Glucose concentration in capillary blood was measured using Bayer's ContourPlus[®] systems. After determination of glucose concentration in capillary blood, the participant was given 40 g of the product (No.1 – dragées candies coated with chocolate of 4 % sugar, No. 2 – dragées candies coated with chocolate of 30 % sugar). Subsequently, glucose concentration in capillary blood was measured 5 more times (after 15 min, 30 min, 60 min, 90 min and 120 min) program.

Consumption of dragées (Figure 1 and 2) made from extruded corn and dark chocolate does not cause jumps in blood glucose levels in either healthy or diabetic people. Therefore, we can say that this new product is suitable for people with high blood glucose.

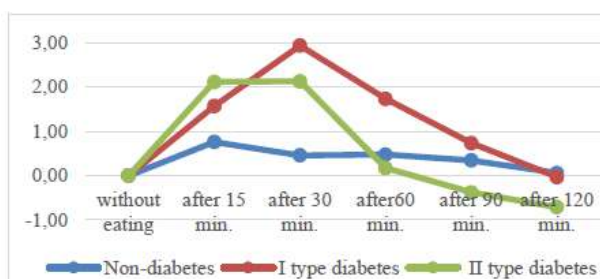


Figure 1: Changes in blood glucose levels after consumption dragée No 1

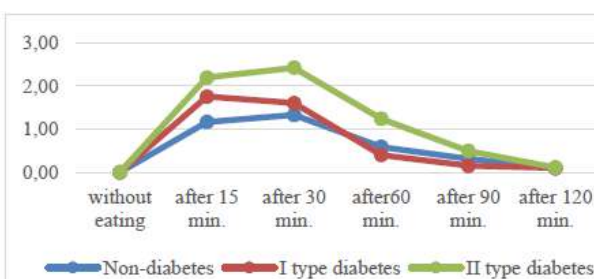


Figure 1: Changes in blood glucose levels after consumption dragées No 2

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OPF-05

NATURAL FOOD PRESERVATIVES: APPLICATION OF ROSEMARY, BASIL AND SAGE IN YOGURTS AS AVIABLE ALTERNATIVE TO ARTIFICIAL ONES, USING SUSTAINABLE, LOW COST AND EFFICIENT PROCESSES

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Foods are susceptible to various forms of degradation, either by oxygen present in the atmosphere, microbiological factors, enzymatic degradation and others, being essential to study new ways of preserving food. Due to the need of creating “stock” and preserve food in the pre-industrial era, consumers were essentially concerned on obtaining food as quickly as possible, opting for “industrialized” food. Nowadays, the demand for non-processed foods or with a minimum of artificial additives is increasing due to an increased awareness of consumers towards healthier diets, studies related to health risks and public opinions generated in the media [1,2]. Plants are known to be rich sources of bioactive compounds, since these are considered a defense mechanism against external agents such as microorganisms, oxidizing agents and ultraviolet radiation, and for these reasons their extracts are investigated as promising sources of natural additives for the food industry [3]. Thus, the main objective of this work was the exploitation of rosemary (*Rosmarinus officinalis* L.), basil (*Ocimum basilicum* L.) and sage (*Salvia officinalis* L.) as sources of preservative molecules, for incorporation in yogurts. This included: i) the optimization of the extraction of bioactive molecules through ultrasound assisted extraction (UAE), a sustainable and lowcost extraction technology, using green solvents (water and ethanol); ii) chemical characterization of the extracts obtained by HPLC-DAD-ESI/MS techniques; iii) evaluation of the bioactivities of the extracts (antioxidant, antimicrobial and cytotoxic activities); iv) incorporation of the most promising extracts into the yogurts, and v) the evaluation of the stability of the ingredients over the shelf-life through physical parameters (texture, color and pH); microbiological analysis [4]; nutritional value [5] and monitoring the molecules with preservative capacity. All the formulations were compared with control samples (without additives and with potassium sorbate, a common artificial additive used in yogurts). According to the obtained results, for the extraction optimization, it was found that the percentage of solvent is the most relevant factor for obtaining an extract rich in rosmarinic acid, followed by the extraction time and ultrasonic power. For the antioxidant and antimicrobial activity, rosemary extract showed the best result, followed by sage and basil. Moreover, none of the three plant extracts showed hepatotoxicity, at the maximum concentration tested. Finally, the plant extracts did not show changes in the physico-chemical and nutritional characteristics of the yogurts and the incorporation of the preservative extracts did not affect the normal lactic bacteria growth, which are fundamental for the yogurt manufacturing process. Moreover, the extracts were obtained through sustainable, green and low-cost process, providing the industry with safer and economically viable alternatives.

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OCF-06

THE EFFECT OF HIGH-PRESSURE PROCESSING AND THERMAL PASTEURIZATION ON THE MICROBIAL AND PHYSICAL- CHEMICAL PROPERTIES OF 3 VARIETIES OF *OPUNTIA FICUS-INDICA*

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The genus *Opuntia*, native to Mexico, is cultivated in arid and semiarid regions of the world representing one of the most agro-economically important cactus crop species. It possesses so some well-known health-promoting and nutritional benefits and for that, crops of cactus pear are increasingly gaining momentum [1,2]. *Opuntia* fruits are claimed for their richness in nutritional and bioactive compounds, which include fibers, polyphenols, betalains, carotenoids, phytosterols and some key minerals such as magnesium, calcium, iron, potassium, sodium, and phosphorus [3]. Prickly pear fruits are perishable and must be preserved by e.g. freezing or drying, or processed further to products like juice/nectars and jam. Prickly pear beverages are one of the most attractive opportunities amongst the fruit manufactured products since they are perceived as healthy, tasty and refreshing [4]. However, without any further processing, prickly pear juices and nectars have a short shelf-life, hampering its commercial use [5]. Thus, in this work thermal (TP) and high-pressure (HPP) pasteurization methods were used to ensure the microbial safety of the beverage. Furthermore, the variation of the physical-chemical properties, such as pH, titratable acidity, cloudiness, browning and total soluble solids, as well as the reducing sugar content were analyzed.

In general, the applied treatments, 71.1 °C for 30 s for TP and 500 MPa for 10 min for HPP, allowed the *Opuntia* nectars to expand their shelf life in approximately 25 days, when treated with temperature, and about 40 days, when treated using high pressure (**Figure 1**). Moreover, depending on the *Opuntia* variety, the nectars were able to maintain their physical-chemical properties up to 5-8 days for the non-pasteurized samples, up to 30-45 days for the TP samples and up to 45-60 day for the ones treated using HPP.

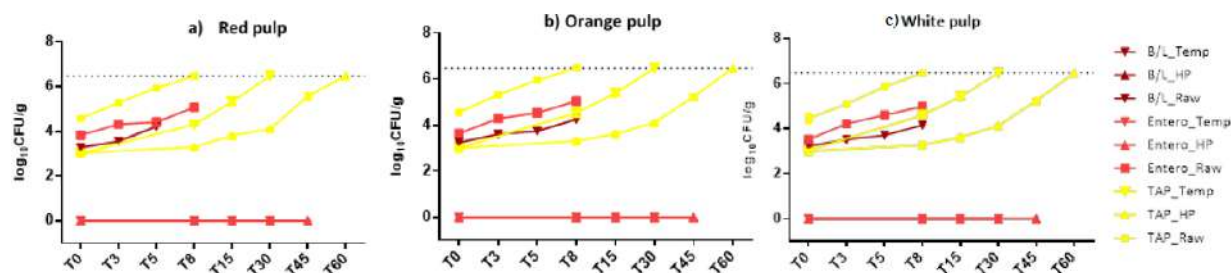


Figure 1: a) Microbial load of the red pulp of prickly pear after the three processing methods. b) Microbial load of the orange pulp of prickly pear after the three processing methods. c) Microbial load of the white pulp of prickly pear after the three processing methods

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oCC
Oral
Communications
in Cosmetic area



OCC-01

BIOMIMETIC SHORT PEPTIDES IN MEDICINE AND COSMETOLOGY

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Peptides are shorter or longer chains of amino acids and many of them occur naturally (Somatostatin, Bombesin, Ghrelin, Insulin...). In the human body they play important role in various regulatory and physiological processes as for example growth, reproduction, stress, homeostasis, immunity and many others. On the other hand a great number of biomimetic peptides is synthesized by means of biotechnological methods. These compounds have an identical amino acid fragment with respect to the natural oligopeptides and are able to mimic some of physiological actions. Many of them are used for skin dysfunction problems in Cosmetic Industry. For example, the action of growth factors due to interactions with receptors may result in positive clinical effects, e.g. in slowing of aging, synthesis of collagen and elastin, increasing fibroblast proliferation and others. The biomimetic peptides may be functionalized by heterocyclic compound of recognized biological activity forming a new hybrid class of short peptides. Recently, we synthesized dipeptides [1] as analogs of 3-(quinoxalin-6-yl) alanine, including diphenylquinoxaline (Pe-DPhQ) and dipyrrolyl-iso-quinoxaline (Pe-DPyQ).



Fig.1. Studied peptides (L:Pe-DPhQ; R:Pe-DPyQ) and their fluorescence spectra (L:RT; R:temp:298-363K) in BuCN.

In this talk their photophysical properties studied by a combined solution-state study using steady state (absorption, emission) and time-resolved spectroscopy (emission) as well as quantum-mechanical (*ab-initio*, DFT, DFT B3LYP/6-31G (d,p)) calculations will be presented. The second project involving the short peptides is concerned with analogs of the RGD tripeptide used in mycobacterial infections. Tuberculosis is evoked by *Mycobacterium tuberculosis*. It lives and proliferates in leucocyte cells that normally kill the bacteria after the phagocytic act. It has to form a complex between the bacterial Ag85 antigen, located on the bacterial cell wall, and the serum protein – fibronectin. Only in this form bacteria can interact with leucocyte cell receptors of the integrin type and penetrate the host cell. On this basis the hypothesis was put forward that the phagocytosis of *Mycobacterium* can be inhibited by blocking the interaction of fibronectin with the integrin receptor. The area responsible for that interaction is sequence Gly-Arg-Gly-Asp-Ser-Pro. Our studies of biological activity shown [2] that the pentapeptide fragment (RGDVY) of the HLA-DQ protein and the GRGD fragment of fibronectin selectively inhibit the *Mycobacteria* phagocytosis by 50-60%. We also examined 3 further series of peptides [3]. They are the fragments of the plant signaling protein, antimicrobial peptide cecropine A, produced by the silk moth. Its active fragment GRGDVVNGRG of the BRCT protein, is a regulator of transcriptional process. Further, we found that KRVDY is a weaker inhibitor of phagocytosis than its analog RKVDY. On the other hand, the introduction of the RDG sequence in place RGD (present in cecropine A) decreases the antiphagocytic activity in comparison with the RGDVY peptide. More details will be given during presentation.

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OCC-02

CONTRIBUTIONS TOWARDS THE ECOTOXICOLOGICAL EVALUATION OF PLANT EXTRACTS AND ESSENTIAL OILS

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Due to the increasing interest from customers for natural and safer products, the demand for natural-based ingredients has increased during the last years throughout many economic sectors. This demand is leading to research on new alternatives to the traditionally used plants as a source of bioactive compounds that can be used in the cosmetic, food and health industries [1]. The InovEP Project aims to provide data on new possible applications of essential oils and extracts from several plant species, present in the Portuguese territory, identified as promising sources of bioactive compounds, such as the gum rockrose (*Cistus ladanifer*), the curry plant (*Helichrysum italicum*), the Mediterranean thyme (*Thymbra capitata*) and basil (*Ocimum basilicum*). Considering the possible applications of new products derived from these plants, and following the precautionary principle laid down by European regulations, particularly REACH [2], it becomes necessary to evaluate the possible effects that the introduction of these products may have in the ecosystem. To achieve this goal, the acute toxicity of essential oils and extracts from these plants was evaluated using the model organism *Daphnia magna*. The EC₅₀ (concentration estimated to immobilize 50 per cent of the *Daphnia*) values obtained were 11.2±0.4095 mg.L⁻¹ for the *T. capitata* essential oil and 199.5±9410x10⁻⁵ mg.L⁻¹ for *C. ladanifer* essential oil. For the *H. italicum* essential oil, all the *H. lupulus* extracts (aqueous, ethanolic and methanolic), *O. basilicum* hydrolate and *T. capitata* floral water no immobilization was observed up to the highest concentrations tested. These results show that these essential oils and extracts present low to negligible acute effects towards *D. magna*, except for the *T. capitata* essential oil that showed moderate toxicity to this organism.

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OCC-03

FATTY ACID COMPOSITION AND ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF ETHANOLIC PRESSURIZED LIQUID EXTRACTION (PLE) EXTRACTS OF 4 MACROALGAE SPECIES FROM GALICIA (NW SPAIN)

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Marine seaweeds are recognized as a potential source of a wide variety of bioactive compounds, such as polyphenols, polysaccharides and high quality fatty acids. Thus, they constitute useful leads in the development of new high added-value products for the nutraceutical and cosmetic industry [1]. In this work, we studied the fatty acid (FA) profile of four macroalgae species from Galicia (NW Spain): *Ulva intestinalis*, *Ulva lactuca*, *Cystoseira baccata* and *Himanthalia elongata*. Lipids were determined by gas chromatography coupled with mass spectrometry (GC-MS) after a solid-liquid extraction using the Folch method (chloroform:methanol, 2:1). Additionally, ethanolic extracts obtained by pressurized liquid extraction (PLE) (ethanol 100% / 120°C) were used to determine the antioxidant and antibacterial activities. Green extraction methods like PLE may prove an efficient alternative technique for obtaining lipids from these biological matrixes, while avoiding the use of hazardous solvents [2]. *C. baccata* ethanolic PLE extract shows considerably higher capacity of inhibiting 50% of DPPH (1,1-diphenyl-2-picryl hydrazyl) in comparison with the other species studied with an IC₅₀ of 28.49 µg/mL. Moreover, the potential antimicrobial activity tested on *Escherichia coli* and *Staphylococcus aureus* shows that *C. baccata* extract exerted the highest inhibition: 37.9% and 58.8%, respectively. Results suggest that these algae contain valuable bioactive fatty acids and PLE extraction could be a feasible method to apply on macroalgae.

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OCC-04

BIOACTIVE PROPERTIES OF DIFFERENT EXTRACTS OBTAINED FROM THE AERIAL PARTS OF BLUEBERRY AND RASPBERRY RAW MATERIALS

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The waste biomass derived from Berry crops is a new focus of study since producers are becoming increasingly interested in its valorisation, namely to obtain high added-value products. In this context, the leaves, and other aerial parts of this raw material, are examples of waste biomass that can be exploited for several applications, namely in cosmetic formulations due to their unique chemical composition and associated bioactive properties [1,2]. In this context, this work was focused on upgrading the commercial potential of blueberry and raspberry plant by-products, which are typically discarded, presenting no economic value.

Different extracts of *Vaccinium myrtillus* L. (blueberry) and *Rubus idaeus* L. (raspberry) aerial parts were obtained using green methodologies after being air-dried, grounded at 20 mesh, and protected from light. Different extraction techniques were applied, such as infusion, decoction, maceration, and ultrasound-assisted extraction (UAE), being the latter two extractions performed with EtOH:H₂O (80:20 v/v) as the extraction medium.

The obtained extracts were studied regarding their antioxidant and antimicrobial properties through the oxidative haemolysis inhibition assay (OxHLIA) and the microdilution method against pathogenic bacteria, respectively.

According to the obtained antioxidant results, both raspberry and blueberry samples exhibited a similar activity, with decoction extracts revealing the most promising results, namely by displaying the lowest IC₅₀ values after 60 min ($21.9 \pm 0.3 \mu\text{g/mL}$ and $20 \pm 1 \mu\text{g/mL}$, respectively). It should be highlighted that these extracts presented similar activity, but higher than the one of Trolox (control), with an IC₅₀ value of $21.8 \pm 0.2 \mu\text{g/mL}$. For the antimicrobial activity, all the extracts presented inhibition capacity against most of the tested bacterial strains, being the UAE extracts the most active ones. Raspberry samples showed the highest activity, inhibiting the Gram-negative bacteria *Escherichia coli*, *Klebsiella pneumoniae*, *Morganella morganii*, and *Proteus mirabilis* with a MIC (minimum inhibitory concentration) of 5 mg/mL. In contrast, to inhibit these bacteria a concentration of 10 mg/mL of blueberry extract was needed. Regarding the Gram-positive bacteria, raspberry extract presented a MIC of 2.5 mg/mL against *Enterococcus faecalis* and the methicillin-resistant *Staphylococcus aureus*. For blueberry extract, a MIC of 10 mg/mL was determined.

Overall, these results highlight these two samples' bioactive potential and the importance of exploiting these bioresidues as novel candidates for industrial application, taking advantage of their biological properties.

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OPC-05

EVALUATION OF COSMETIC PROPERTIES OF NATURAL INGREDIENTS IN THE TRÁS-OS-MONTES AREA: A PHD PROJECTSara Gonçalves,^{1*} Isabel Gaivão,¹

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The term cosmetics refers to a product applied to the body for the purpose of beautifying, cleansing or improving appearance and enhancing attractive features [1]. The natural cosmetics market has grown since the consumer took consciousness of the concept of natural-based ingredients [2–4]. A great number of cosmetics have noxious and chemically-potent substances and have an ecological impact on the environment [5]. A study performed by the Danish Council THINK Chemicals [6] found that in total 65 chemicals of concern were found in 39 products. This means consumers are exposed to these chemicals, perhaps in a daily basis. They also found that three products contained illegal ingredients in the European Union. Thus, the use of natural and organic cosmetics becomes increasingly important. This requires a strong investigation into the benefits that fruits can bring to health.

The PhD project will focus on four natural ingredients common in the Trás-os-Montes area: almond (*Prunus dulcis*), elderberry (*Sambucus nigra*), olive (*Olea europaea*) and grapes (*Vitis vinifera*). The general purpose of this PhD project is to evaluate the cosmetic properties of the natural ingredients towards the DNA integrity promotion. Additionally, it is intended to evaluate genoprotection, longevity and prolificacy of the natural ingredients in *Drosophila melanogaster*. The short life cycle, the distinct developmental stages, the availability of various tools and reagents, known genome sequence and the physiological similarity of *Drosophila* with humans make them an excellent in vivo model organism to rapidly test toxicity in whole organism and elucidate the molecular mechanisms underlying the toxicity [7]. The natural product with the best result will be used to evaluate genoprotection in human lymphocytes. These are used as a surrogate tissue, as they are easily obtained, in large numbers, do not require cell culture, are diploids and are almost all in the same phase of the cell cycle [8]. This project is in an initial phase and lacks results, which will be available along this year.

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***HUMULUS LUPULUS* L.: COSMETIC APPLICATION OF EXTRACTS OBTAINED FROM CONES AND VEGETATIVE PARTS**

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Nowadays there is an increasing interest in the search for bioactive compounds obtained from aromatic and medicinal plants. Hop (*Humulus lupulus* L.) is known worldwide as an essential raw material for the beer industry [1], however, recent studies have revealed health benefits [2]. In the beer production process, only the cones of hop are used, while the vegetative part of the plant is discarded and not valorized albeit its richness in interesting compounds. In this work, we have studied the phytochemical profile of polar extracts of hop and of the essential oil of *T. zygis* subsp. *zygis*, and further evaluated their antibacterial and antifungal capacities. Moreover, gel formulations incorporating distinct levels of hop extracts (1.25%, 2.5% and 5%) from cones and vegetative parts of the varieties Nugget and Polaris of hop and spontaneous hop, combined with essential oil of *Thymus zygis* subsp. *zygis*, were developed and the stability of the new cosmetic products was evaluated.

UHPLC-DAD-ESI-MS² analysis was used for phytochemical study of hydroalcoholic hop extracts [3]. The antimicrobial properties of these extracts and of the essential oil of *T. zygis* subsp. *zygis* were determined by microdilution broth method and the diffusion in agar test. Additionally, the stability of the gel formulations was evaluated through a series of physical-chemical tests such as the pH, density, texture evaluation, mechanical vibration, centrifugation, relative humidity, light test, consecutive extreme temperature variation and evaluation of organoleptic characteristics.

The stability tests did not reveal phase separation or liquefaction of the prepared gel samples. There were no changes recorded in vibration test, in the organoleptic characteristics of the formulations, the relative humidity test, as well as in the extreme temperature variation test. The formulations showed an acid pH, between 5.93±0.047 and 6.16±0.047. The apparent density of the formulations were between 1.08±0.00 Kg/m⁻³ and 1.12±0.00 Kg/m⁻³. Although hop extracts had no antimicrobial effect, the essential oil of *T. zygis* subsp. *zygis* showed antibacterial and antifungal activities against all strains of bacteria and fungi tested, with higher potency against *S. aureus* (MIC = 0.031%) than for *P. aeruginosa* (MIC = 1.25%). The study of the phenolic profile of cones and vegetative parts of the polar extracts of hop, allowed the identification of thirty compounds, of which alpha and beta acids, phenolic acids and flavonoids. In conclusion, the study explored the vegetative part of hop that remain poorly studied allowing propose them as ingredient in cosmetic area.

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TCH-01

ANTIBACTERIAL AND ANTIOXIDANT SYNERGISMS BETWEEN PROPOLIS AND GENTAMICIN OR HONEY

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Antimicrobial resistance has become an emergent problem with the increase of resistant strains and the lack of new antibiotics [1]. Antimicrobial agents from natural sources, such as propolis, have become more attractive due to their potential for the development of alternative drugs, both to overcome and to avoid resistance. Propolis is a natural substance of sticky consistency produced by honeybees from numerous parts of plants and mixed with products of their metabolism, being used to protect the beehive by filling any crack or opening [2].

Previously we showed a synergistic antibacterial effect between Portuguese propolis and gentamicin, a well-known aminoglycoside antibiotic, against several bacteria, with particular interest against MRSA, the methicillin-resistant strain of *Staphylococcus aureus* [3]. In the present work, we studied antibacterial and antioxidant synergisms between the same Portuguese propolis and honey, both collected from the same apiary (Gerês). A synergistic effect between an ethanolic extract (EE) of propolis from Gerês collected in 2018 (G18) and honey was also observed and against all the tested bacteria, once again with particular interest against MRSA. Still, mixtures of sub-MIC concentrations of G18.EE and honey display stronger antibacterial effect than the activity of each individual sample, except for *Bacillus cereus*, against which no synergistic effect was found. On the contrary, no synergism was found between G18.EE and honey for antioxidant capacity. The higher the percentage of honey in the mixture, the lower the antioxidant capacity.

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TCH-02

HAIRY ROOTS CULTURES OF *CYNARA CARDUNCULUS* L. AS A VALUABLE SOURCE OF HYDROXYCINNAMIC ACID COMPOUNDS

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Plants are important sources of chemical compounds with various applications. These compounds usually accumulate in low amounts, sometimes leading to the overexploitation of the source plant. In this context, the development of alternative platforms for the production of these compounds represents a more sustainable solution for the production of these valuable metabolites. Among such platforms, hairy roots cultures are often used for their ability to produce the same secondary metabolites as the original plant associated with a high genetic stability that allows predictable production of the compound of interest [1]. *Cynara cardunculus* L. or cardoon has been used in folk medicine to treat various diseases. Distinct parts of the plant have been studied and found to produce secondary metabolites with health promoting properties [2]. In this work, cardoon roots as well as hairy roots cultures were characterized regarding their secondary metabolites production and potential biological activities (Figure 1). Methanolic extracts were analyzed by HPLC and LC-MS. The detected compounds were putatively identified by comparing with available literature and standards. These were found to belong, almost exclusively, to the hydroxycinnamic acid group. The highest phenolic content and antioxidant activity (46 mg GAE/g DW and 98 μ mol TE/g DW) was obtained for the extract derived from hairy roots of day 35 of the growth curve. This extract also showed antiproliferative activity on HT29, a human colorectal cancer cell line (EC₅₀ 1.16 \pm 0.07 mg/mL). This work demonstrates the potential of cardoon hairy roots cultures as a valuable alternative source for bioactive hydroxycinnamic acid compounds.

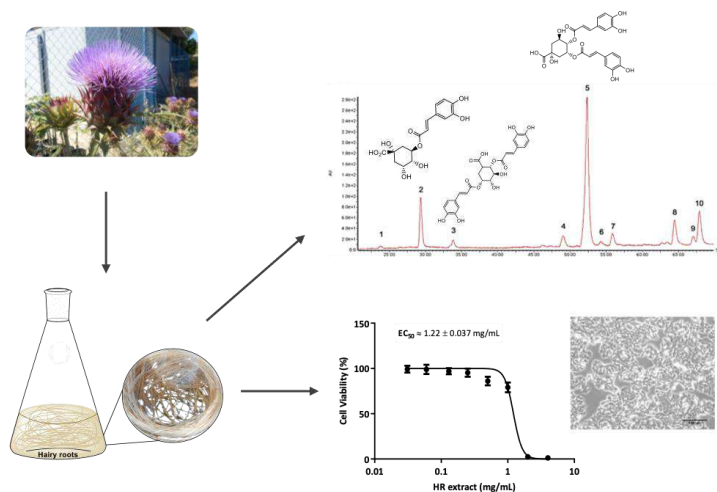


Figure 1: Graphical abstract

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ANTI-INFLAMMATORY EFFECT OF N-3 POLYUNSATURATED FATTY ACIDS ON VIRUS-INFECTED NEURONS

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The Viral Mimetic Polyinosinic:Polycytidylic Acid (Poly I:C) is an important tool to study the consequences of viral infection to the development of neuropsychiatric disorders. Here, based on the premise of Omega-3 Polyunsaturated Fatty Acids (N-3 PUFAs) presents an anti-inflammatory effect, we investigated the involvement of NFκB pathway in the effects of N-3 PUFAs in hippocampal poly I:C-challenged neurons. Primary hippocampal neuronal cultures were exposed to N-3 PUFAs (DHA 4.35 μM/EPA 7.10 μM, DHA 8.7 μM/EPA 14 μM or DHA 17 μM/EPA 27 μM) in the presence or absence of poly I:C. MTT assay revealed that poly I:C-induced reduction in cell viability was prevented by N-3 PUFAs. N-3 PUFAs (DHA 8.7 μM/EPA 14.21 μM) significantly reduced poly I:C-induced increase in iNOS, NFκB p50/p65 (**Figure 1**), IL-6 and nitrite when compared to non-treated cells. Only N-3 PUFAs prevented poly I:C-induced deficits in BDNF. On the other hand, poly I:C caused a marked reduction in DCX immunoexpression. Thus, N-3 PUFAs exert *in vitro* neuroprotective effects against Poly I:C immune challenge in hippocampal neurons, by mechanisms possibly involving the inhibition of canonical NFκB pathway. The present study adds further evidences to the mechanisms underlying N-3 PUFAs neuroprotective and anti-inflammatory effects against viral immune challenges. Since N-3 PUFAs is a safe strategy for use during pregnancy, our results also add further evidence for the use of this supplement in order to prevent alterations induced by viral hits during this developmental period.

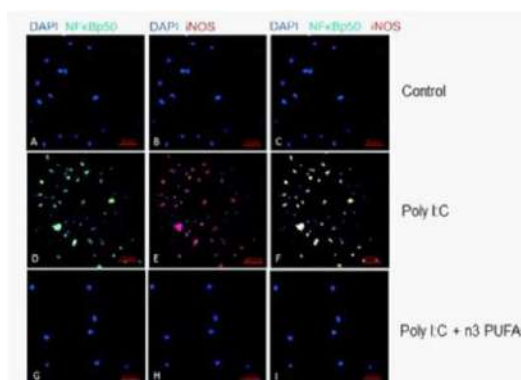


Figure 1: Poly I:C immune challenge increases p65 NFκB subunit expression in hippocampal neurons: prevention by N-3 PUFAs.

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TCH-04

EXTRACTION OF ALPHA-TOMATINE FROM GREEN TOMATOES TO TREAT SKIN DISEASES

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Green tomatoes, an industrial waste, are rich in glycoalkaloids, namely tomatine [1]. It is known that they possess anticarcinogenic, anti-inflammatory and fungicide activities, among others [2,3,4], making them a valuable new source of bioactive molecules. This work seeks to establish a circular economy, using the green tomatoes biomass to extract alpha-tomatine by subcritical water, an environmentally friendly method. The final aim is to incorporate the extracted alpha-tomatine in a topical formulation to treat skin inflammatory diseases.

In the literature, quantification and extraction methods for alpha-tomatine are not undisputed. For that reason, a quantification method by High Performance Liquid Chromatography (HPLC) was developed, using a Purospher STAR RP-18 endcapped 5 µm column, with a flow rate of 0.7 mL/min, a mobile phase consisting of acetonitrile: KH₂PO₄ 20 mM pH 3 (30:70), at a wavelength of 205 nm. Stability of the calibration curve was performed, detection and quantification limits were also calculated. A tomatine extraction process by subcritical water was developed, and extraction with conventional organic solvents, based on the method of Choi et al. [5], was also conducted for comparison purposes. Tomatine was extracted in both methods, namely 200 mg alpha-tomatine/100 g fresh tomatoes by the subcritical water extraction method, or 4 mg of alpha-tomatine/100 g fresh tomatoes, when using conventional organic solvents. Both these methods can still be improved. In particular, a purification step is required, in order to better characterize the extracts. As future work, a complete characterization of the composition of the extract must be done, to fully exploit the *in vitro* and *in vivo* biological potential provided by the discarded green tomatoes.

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TCH-05

OPTIMIZATION OF ANTI-INFLAMMATORY METHODOLOGIES TO EVALUATE NATURAL PRODUCTS ACTIVITY AS INFLAMMATION INHIBITORS

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Inflammation is a defense mechanism that is designed to eradicate microbes or irritants to protect living tissues from infection, injuries, and to potentiate tissue repair [1]. Non-steroidal and glucocorticoids are used as synthetic anti-inflammatory drugs, but unfortunately, they have been associated with an increased risk of serious upper gastrointestinal complications and many others side effects. Therefore, the discovering of novel, efficient and safer anti-inflammatory agents is crucial to prevent and treat inflammation processes. Natural matrices such as tubers have been exploited and described as possessing a huge diversity of bioactive molecules with promising anti-inflammatory capacity, with less or no undesirable effects. Thus, in the present work, the hydroalcoholic Besides the anti-inflammatory assays, the cell cycle analysis and apoptosis will be performed by flow cytometry. Moreover, these extracts will also be characterized in terms of phenolic compounds through HPLC-DAD-MS in order to establish a structure-activity relationship.

According to the obtained results until the moment, the two tubers are promising anti-inflammatory agents, by exhibiting the capacity to inhibit the NO production at very low IC₅₀ values. The remaining assays and cell cycle and apoptosis assessment are being performed. These findings are very favourable and highlight the anti-inflammatory potential of these tubers.

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TCH-06

INDUSTRIAL WASTE STREAMS VALORIZATION AT IBET: HEALTH RELATED BIOACTIVES FROM FISHERIES AND WINERIES

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iBET is a private not for profit research centre in the area of biotechnology and life sciences based in Portugal. Food & Health Division has strong competences studying food products, considering aspects related to their quality, safety, authenticity, sensory and functionality. One of the core areas of the division is the efficient extraction of high added-value products from agro- and food industry related waste.

By-products from fisheries wastes (e. g. blue shark, crab, shrimp, codfish, sardine, algae) and agro-industries (e. g. brassica, citrus, apple, olive, grape) among others have been explored as sources of health-related molecules. Focused on the bioactivity of the target extract or purified compounds, clean and sustainable extraction processes are designed. Supercritical CO₂, pressurized liquids, and alternative solvents, such as deep eutectic solvents, are used for the recovery of bioactive compounds.

Chemical, enzymatic and cell-based assays are used to evaluate the potential of the obtained compounds for further use in food, nutraceutical and cosmetic applications. Antioxidant, anti-inflammatory, anti-cancer, antimicrobial, anti-obesity, anti-hypertension, wound healing and anti-aging effect, among others are some of the bioactivity tests available at iBET.

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TCF-01

ITS2 METABARCODING: A PROMISING APPROACH FOR IDENTIFICATION OF BOTANICAL ORIGIN OF BEE-COLLECTED POLLEN

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Bee products have long been used in human's diet and their consumption has increasingly been recognized as beneficial for human's health. One such product is pollen, which is a particularly interesting food as it contains bioactive compounds and all the essential amino-acids needed by humans. However, the composition of bee-collected pollen depends on the environment where the visited plants grow (e.g.: climatic conditions, soil type) and, above all, on the plant species [1]. Therefore, identification of the botanical origin of bee-collected pollen is important for a fuller characterization of this food product. Until recently, pollen identification has been carried out using light microscopy, a costly approach that often provides low taxonomic resolution. However, with high-throughput sequencing (HTS) becoming increasingly affordable, DNA metabarcoding is emerging as a promising alternative to light microscopy. In addition to be time- and cost-effective for large sample sizes, metabarcoding has the potential to allow identification of pollen mixtures at the species level. However, before it can be widely employed in pollen analysis, the reliability of this molecular tool must be appraised. Herein, we compared the relative abundances obtained by the two approaches on 108 bee-collected pollen samples from 10 European countries. To that end, the 108 samples were first homogenized and split into two identical sub-sets. One sub-set was analysed by palynology experts from the "Institut für Bienenkunde", Germany, and the other one was subjected to HTS, using ITS2 as the barcode, in the labs of CIMO and CIBIO. Pairwise comparisons of the relative abundances at the family level of the 108 samples show no significant differences ($P \geq 0.1057$, Wilcoxon signed-rank test) and high correlation values ($0.2736 \leq r \leq 0.9842$, Pearson's correlation) between the two approaches. The highest correlation values were observed for Italian samples ($0.7245 \leq r \leq 0.9842$; global $r = 0.8958$) and the lowest for Greek samples ($0.0266 \leq r \leq 0.9703$; global $r = 0.4929$). Despite the few outliers, which can be improved by further optimization of the protocols, these results suggest that ITS2 metabarcoding promises to be a reliable alternative to light microscopy. This molecular approach is now being employed in the European project INSIGNIA (<https://www.insignia-bee.eu/>), which is developing a standard protocol for using the honey bee as a tool for environmental monitoring.

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TCF-02

FLAVONOID COMPOSITION AND *IN VITRO* ANTI-PROLIFERATIVE ACTIVITY OF THE HYDROETHANOLIC EXTRACT OF *GARCINIA MANGOSTANA* L. PERICARP.

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Garcinia mangostana L., known as mangosteen, is a tropical fruit belonging to the Clusiaceae family, native from South Asia but can also be found in other tropical territories, such as South America [1,2,3]. The fruit comprises an inedible dark purple epicarp (> 60%) that encases an edible succulent pulp [2]. Nowadays, mangosteen pulp and pericarp have been used in beverages as food supplement by virtue of the traditional knowledge about its health benefits. However, correlation studies between the folk medicine usage and its chemical composition are scarce in the literature [2,3]. Aiming to elucidate part of the chemical composition, the present study carried out the determination of the main flavonoids, including anthocyanin compounds, present in mangosteen pericarp by High Performance Liquid Chromatography coupled to a diode array detector and mass spectrometry by electrospray ionization (HPLC-DAD-ESI/MSn). Furthermore, the cytotoxicity effects of its hydroethanolic extracts were evaluated on four human tumor cell lines (NCI-H460 - lung carcinoma, MCF-7 - breast carcinoma, HepG2 - hepatocellular carcinoma, and HeLa - cervical carcinoma) by the Sulforhodamine B (SRB) assay.

Mangosteen pericarp presented nine non-anthocyanin flavonoid compounds, most of which belonging to the procyanidin class (seven compounds), one taxifolin derivative (taxifolin-*O*-rhamnoside, found in low concentrations), and one quercetin derivative (quercetin-3-*O*-rutinose, found in trace amounts). Regarding the anthocyanin flavonoids compounds group, two were found and tentatively identified as cyanidin-*O*-dihexoside and delphinidin-*O*-dihexoside. Regarding the total amount of flavonoids, the extracts presented 53 ± 1 mg of non-anthocyanin flavonoids/g of extract, 3.66 ± 0.02 mg of anthocyanins/g of extract. Concerning the cytotoxic activity, the hydroethanolic extracts presented activity against all tumor cell lines studied ($GI_{50} < 75$ μ g/mL).

The results obtained from the present study showed that mangosteen pericarp could be an interesting natural source of high added value and bioactive compounds, with the potential to be applied in several industrial fields including pharmaceutical, nutraceutical, among others.

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TCF-03

TAILORING FOOD CROPS TO ADDRESS SPECIFIC DIETARY NEEDS: THE CASE OF MICROGREENS

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Microgreens are an emerging category of greens, primarily used as garnish greens for their diversity of colors, shapes, and flavors, and are progressively recognized for their rich nutrient profile [1, 2]. The wide range of vegetables, grains, herbs, and even wild edible species used to produce microgreens are expression of our rich agrobiodiversity and offers the opportunity to select species with unique nutrient profiles [2]. Examining the mineral profile of species belonging to different botanical families it is possible to identify species that are a good source or have very low content of certain minerals. Moreover, in recent studies we have demonstrated how agronomic biofortification techniques may be applied to microgreens to modulate and tailor their mineral profile to address specific dietary needs [3]. Using Brassica microgreens, we present the opportunity to i) modify the micronutrient profile by increasing the content of micronutrients such as iron and zinc that when deficient are the cause of severe malnutrition disorders (**Figure 1**); ii) reduce the content of potassium to address the needs of patients affected by hyperkalemia, a disorder often associated with chronic kidney disease and other comorbidities. On-going research is also examining the role that radiation intensity and quality may play in modulating microgreens mineral uptake and composition.

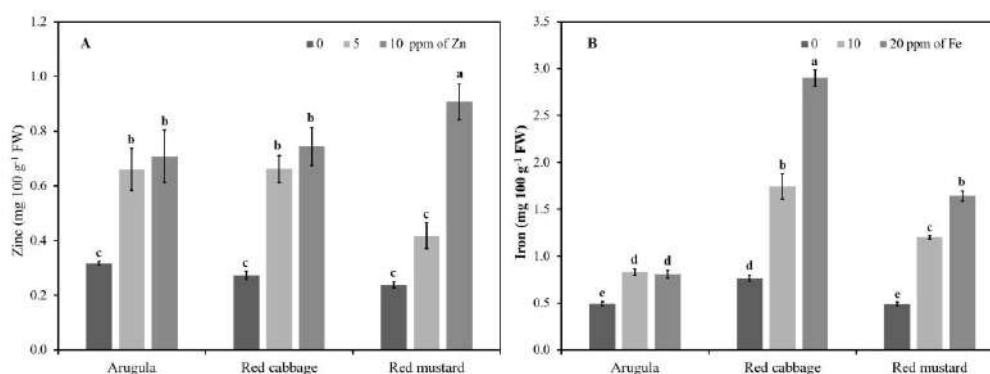


Figure 1: Example of (A) enrichment with zinc (Zn) and (B) iron (Fe) content in three Brassica microgreens species.

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TCF-04

EXPLORING CRANBERRY EXTRACT CHITOSAN FILMS TOWARDS THE IMPROVEMENT OF ANTIMICROBIAL FOOD PACKAGING

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Numerous outbreaks of foodborne pathogens have been found to be associated with biofilms, which poses a concern to the food industry since foodborne pathogens and their biofilm can adhere to a wide range of food contact materials [1]. Thus, there is a need for the development of novel strategies that can control biofilm formation, especially regarding smart and intelligent packaging systems able to incorporate antimicrobial agents into the packaging material [2].

The reported work highlights the development and characterization studies of chitosan films enhanced for their antioxidant and antimicrobial activity by cranberry extracts. Firstly, the extracts alone were characterized and subsequently they were incorporated into two types of chitosan-films differing in the plasticizers content (PEG alone or PEG and glycerol).

Results showed that all cranberry extracts presented antimicrobial properties against *Escherichia coli* and *Staphylococcus aureus* and antioxidant properties (i.e. DPPH scavenging ability). Also, by HPLC-MS/MS, it was possible to identify the presence of different phenolic compounds (i.e. isorhamnetin 3-glucoside, phloridzin, p-hydroxybenzoic acid, epicatechin and chlorogenic acid) and also the antibiofilm compound i.e., A-type procyanidin. All the produced chitosan-films added with cranberry extracts showed adequate food preservation characteristics (light transmission, water and oxygen permeability) and mechanical properties. Also, the incorporation of the cranberry extracts into the films did not affect their antioxidant activity. Based on the antimicrobial study, chitosan films were able to inhibit *E. coli* biofilm formation (a reduction of 5 and 4 log units was obtained with films added of PEG or PEG/glycerol, respectively). Moreover, both films were able to completely prevent *S. aureus* biofilm development.

Overall, the investigation revealed the potential of cranberry extract chitosan-films for the improvement of food packaging material antioxidant and antimicrobial properties.

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TCF-05

TABLE TOMATO LEAVES ARE A SUSTAINABLE SOURCE OF RUTIN AND DISPLAY ANTIOXIDANT, ANTIHEMOLYTIC AND ANTIMICROBIAL ACTIVITY

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Table tomato (*Solanum lycopersicum* L.) is highly consumed worldwide and its annual production generates a considerable amount of plant by-products [1]. Although tomatoes are well-known for their composition in nutrients and bioactive compounds [2,3], little information is available in literature regarding tomato leaves, probably because these by-products have no commercial value. Therefore, considering the enormous production of this biomass and the current trends of circularity and sustainable development [3], this study was carried out to evaluate the polyphenolic composition and bioactive properties of tomato leaves resulting from the regeneration of table tomato landraces conserved *ex-situ* in the Portuguese Genebank (BPGV), in Braga, Portugal. The tomato plant material resulting from pruning performed after the flowering season was lyophilized and ground to a fine powder, used to prepare hydroethanolic extracts [4]. These extracts were characterized for their composition in polyphenols (by HPLC-DAD-ESI/MSⁿ) and used to evaluate the antioxidant (by DPPH[•] scavenging capacity and reduction power), antihemolytic (using sheep erythrocytes and AAPH as a free radical generator), and antimicrobial (tested against food-borne bacterial and fungal strains by the serial microdilution and *p*-iodonitrotetrazolium violet colorimetric methods) activities [4]. The chromatographic analysis allowed identifying phenolic acids and flavonoids, with prevalence of quercetin-3-*O*-rutinoside (rutin). The leaf extracts showed antioxidant activity, with EC₅₀ values lower than those previously reported for the respective tomato fruits, thus translating a higher activity [2]. The extracts were also effective in some extent in protecting the erythrocytes from the oxidative hemolysis caused the thermal decomposition of the free-radical initiator AAPH. Despite their low activity against the tested microfungi, some extracts had ability to inhibit and kill some bacteria (including *Salmonella typhimurium*, *Listeria monocytogenes*, *Bacillus cereus*, and *Enterobacter cloacae*) more effectively than the antibiotic ampicillin. It was concluded that table tomato crop by-products can be used to produce rutin-rich extracts with antioxidant and antibacterial activities for possible use in the agri-food sector as natural preservatives.

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TCF-06

NUTRITIONAL, TECHNOLOGICAL AND SENSORY ATTRIBUTES OF BREAD ENRICHED WITH BEE POLLEN AND BEE BREAD

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Over the last decades, people became aware of the relation between continuous diet and health, opening roads to different dietary habits, where healthier, enriched or innovative foods are gaining their space. Thus, there is a considerable interest in developing healthy food products. Bee pollen and bee bread are apicultural products composed mainly of flower pollen, and used in both traditional medicine and human nutrition for thousands of years due to its nutritional, physiological properties and recognized therapeutic and disease-preventing functions [1]. Both bee pollen and bee bread are natural sources of carbohydrates, crude fibres, proteins and lipids but also for minor components such as phenolic compounds [2]. Given these nutritional characteristics, its inclusion as functional ingredient on bread has potential to enhance the healthy attributes of this dairy product besides diversifying the applications of both bee pollen and bee bread [2].

This study, focused on the development of enriched bread samples with bee pollen and bee bread and the evaluation of the final product in terms of nutritional parameters, such as protein, fat and sugars, on its physical and sensorial characteristic. According to the results, enriched breads presented an enhancement of the nutritional parameters compared to the standard breads. The addition of these bee products also impact bread properties such as odour, colour, specific volume, texture and overall appearance. The sensorial evaluation revealed a widely acceptancy by the consumer panel. Consequently, this study shows that bee pollen and bee bread products have potential usage as ingredients for functional foods, with no negative impact in the basic properties of the enriched product.

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TCC
Pitch
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TCC-01

FROM GARBAGE TO GLAMOUR: ASSESSING THE ORGANOLEPTIC PROPERTIES OF FORMULATIONS CONTAINING LYCOPENE-ENRICHED EXTRACTS FROM TOMATO WASTE

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Tomato is produced worldwide and its industrial processing produces a large amount of tomato waste, rich in compounds with benefic properties for health. Lycopene is one of the most studied bioactive agents from tomato, which presents a great cosmetic interest due to its antioxidant and anti-aging properties [1,2]. In this work, lycopene-enriched extracts (LEE) were obtained from tomato waste using supercritical fluid extraction methods and incorporated in topical formulations. Through the double blind assay in volunteers, the organoleptic properties (color and odor) and the acceptability of unloaded macroemulsions (Unl-Macro) and LEE-loaded macroemulsions (LEE-Macro) were evaluated. The majority of the volunteers indicated that the odor of both macroemulsions was slightly perceptible or perceptible. When the volunteers were informed that the LEE-Macro contains a tomato extract with antioxidant properties (LEE Macro (AI)), the opinions did not change. The odor perception for macroemulsions was mainly classified as undefined. Concerning the color, Unl-Macro displayed a white color, while LEE-Macro presented a yellowish color. The majority of the volunteers classified the color of both formulations as indifferent or pleasant. The acceptability of each formulation was evaluated by analyzing the probability of buying different care products with the odor and color of the respective formulations. The probability of buying a face cream, a body cream or a shampoo with the odor of LEE-Macro was higher than Unl-Macro, but the probability was even higher for LEE-Macro (AI). On the other hand, the probability of buying the same care products with the color of the respective macroemulsions was higher for Unl-Macro than LEE-Macro, but increased in the case of LEE-Macro (AI). Concerning the toothpaste more than 75% of the volunteers answered “unlikely” or even “would never buy” toothpastes with the odor of both macroemulsions, and with the color of LEE-Macro, indicating that the odor of tomato extract and the yellow color presented a negative impact in the commercialization of toothpastes. As a conclusion, the presence of LEE did not influence the odor and the color perception, but the acceptability of LEE-Macro is strongly dependent on the type of care product. Information about the composition of the final product and the beneficial skin effect can be useful for the product acceptability by the consumers and its commercialization.

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TCC-02

OPTIMISATION OF A TANNIN-RICH EXTRACT USING RESPONSE SURFACE METHODOLOGY

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Tannins are a class of phenolic compounds commonly found in plants and studied for their *in vitro* bioactive and enzyme inhibitory properties [1,2]. *Cytinus hypocistis* (L.) L. is a wild edible parasitic plant on various Cistaceae family members, which biological properties (antioxidant, anti-tyrosinase, anti-inflammatory, and antimicrobial) have been correlated with its high tannin content. Thus, studying the extraction optimisation of tannins from *C. hypocistis* will give comprehensive clues for the enhanced recovery of these high added-value bioactive compounds and their potential cosmeceutical applications [2].

The present work used Response Surface Methodology (RSM) to optimise tannins extraction using both a conventional (Heat-Assisted Extraction – HAE) and a sustainable extraction method (Ultrasound-Assisted Extraction – UAE). Two three-factor Rotatable Central Compound Designs (RCCDs) were applied to evaluate the linear, quadratic, and interactive effects of the independent variables X_1 [t : time (min)], X_2 [T : temperature (°C) or P : ultrasonic power (W)], and X_3 [S : solvent ratio (% of ethanol/water, v/v)]. The software Design-Expert v11 was used to generate the 20 experimental runs by entering the factor ranges in alphas ($\alpha = 1.68$). Each variable to be optimised was coded at five levels: -1.68, -1, 0, +1, and +1.68.

The constructed polynomial models were statistically validated based on high R^2 values and non-significant lack-of-fit and used to predict the optimal extraction conditions. The obtained results from both extraction systems revealed higher ethanol percentages as the critical factor for increasing the final response (mg total tannins/g extract). The optimum global processing conditions predicted by the models were 95 min, 46 °C, and 74% ethanol for HAE, and 18 min, 327 W, and 69% ethanol for UAE. Following these conditions, 234 mg and 175 mg of total tannins per g of extract were recovered using HAE and UAE, correspondingly.

Even though the HAE technique presented a higher final response, the UAE offers sustainable and economic advantages in time and energy consumption. These results confirm the scale-up potential of *C. hypocistis* as a valuable source of a tannin-rich extract.

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TCC-03

TIME DEPENDENCY ON BIOACTIVE COMPOUNDS UAE EXTRACTIONS

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Natural products have been used in the health, cosmetics, and food industries (HCFI), as well as in other disruptive fields. Within the HCFI, bioactive compounds are promising scientific responses to new polarized market trends, where consumers have increased their awareness of their body, mind, and environment. The same awareness has been around the scientific and industrial sectors, which acknowledge that trying to characterize natural products still have a long way upfront, however, alongside has been an increasing interest in reducing wastes, maximizing energy usage, and other current trends alike. Therefore, the HCFI characterization processes have been adapting to the usage of previously ignored matrixes or part of those matrixes which are a potential source of bioactive compounds, and the need for better extraction yields has prompt hand-by-hand.

In order to explore those better extractions yields, optimization process has been used as a recurring technique implemented to many scientific experiments in order to reduce experimental runs while maximizing outputs. For this reason, Response Surface Methodology (RSM) usually coupled to Central Composite Design (CCD) or Box-Behnken designs has been extensively adopted within bioactive compounds extraction techniques in natural products.

Further steps are also key in the overall maximization of processes, and this has also lead to the usage of modern extraction techniques like the Ultrasound-Assisted technique (UAE) which has been also integrated into the coupled RSM-CCD mention before in a plethora of natural matrixes [1]. Unfortunately, novel methodologies and technology are not a synonym of accurate results and consequently, better yields of bioactive compounds, recurrent usage of extended times have been spotted in many of the works available in the scientific literature. However, in our own experience, identification of non-significant changes on times employed, indicated that very short times are enough to extract a wide percentage of compound [2]. Therefore, a broader discussion on the topic would be advisable, before even starting the Design of Experiment (DoE), For instance, prior screening analysis with wider ranges could help enormously to identify magnitudes and significance of the whole essay, which then would lead to better DoE, and finally to efficient usage of technologies, methodologies, and maximization of target concentration of those valuable bioactive compounds.

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TCC-04

CASTANEA SATIVA SHELLS: FROM AN UNDERVALUED AGRO-RESIDUE TO A VALUABLE RAW MATERIAL FOR COSMETIC INDUSTRY

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Castanea sativa shells (CSS), an abundant and underexploited by-product, are excellent sources of bioactive compounds mostly reused as fuel for energy production or as sources of tannins with benefits in wine aging process [1]. Nevertheless, the shells produced during chestnut processing are not entirely depleted by these applications and large amounts of this undervalued agro-residue without profitable uses are still available. Therefore, ongoing studies have focused on finding novel interesting applications for CSS. The richness in bioactive compounds (polyphenols, lignin and vitamin E) reinforced the potential of shells' valorization as natural antioxidants, highlighting its potentiality for designing high-added value products with attractive applications in cosmetic field [1-3]. The valorization of agro-wastes, such as CSS, embraces a huge challenge for industries entailing the implementation of efficient and eco-friendly extraction processes [2]. The aim of this study was to employ the ultrasound-assisted extraction (UAE) to the recovery of polyphenols from CSS at 70°C for 40 min using water as eco-friendly solvent. The total phenolic content (TPC), antioxidant and antiradical activity were screened. The CSS extract was further characterized concerning *in vitro* radical scavenging ability, phenolic composition and skin cells effects. The *ex vivo* skin permeation of the identified polyphenols in CSS was assessed on Franz diffusion cells using human skin as barrier.

The CSS-UAE extract contained high TPC (393.1 mg of gallic acid equivalents (GAE)/g on dry weight (dw)) and strong antioxidant and antiradical properties (DPPH: IC₅₀=44.1 µg/mL; ABTS: IC₅₀=65.4 µg/mL; FRAP: IC₅₀=32.0 µg/mL). Ellagic acid (40.42 µg/mg dw), caffeic acid derivative (15.4 µg/mg dw) and epigallocatechin (15.3 µg/mg dw) were identified as the major phenolic compounds. The CSS extract was also an effective scavenger of NO• (IC₅₀=0.1 µg/mL) and HOCl (IC₅₀=0.7 µg/mL). In addition, the results proved that the HaCaT and HFF-1 viability did not decrease up to 100 µg/mL and the permeation of ellagic acid (732.1 µg/L) and epicatechin (71.8 µg/L) through human skin tissue. Overall, these findings emphasize the efficacy and safety of polyphenols-enriched CSS extract obtained by UAE, pointing out a promising application as an active ingredient for cosmetic purposes.

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TCC-05

BIOACTIVE PROPERTIES OF SIX MACROALGA FROM THE IBERIC PENINSULA SEA

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Algae are one of the most important richness provided by oceans. This bioresource has numerous applications, being traditionally used as fertilizer, food source or in traditional medicine, among others. Recently, macroalgae extracts have been successfully associated with powerful biological activities such as antioxidant, anti-inflammatory, antimicrobial, antifungal, antibiofilm or antifouling [1,2].

In this work, the antimicrobial capacity of *Undaria pinnatifida* (Harvey) Suringar, *Sargassum muticum* (Yendo) Fensholt, *Himantalia elongata* (L.) S.F. Gray, *Ascophyllum nodosum* (L.) Le Jolis, *Fucus spiralis* L. and *Bifurcaria bifurcata* R. Ross crude extracts were tested against six microbial strains: *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella Enteritidis* and *Staphylococcus epidermidis*.

The extracts were obtained by microwave-assisted extraction (MAE), using an ethanol/water mixture as solvent. The extraction conditions were previously optimized using response surface methodology (RSM). The capacity of the extracts to inhibit the growth of the target microorganisms was evaluated by plate diffusion method. In this methodology, the antimicrobial agent diffuses into the agar and inhibits germination and growth of the tested microorganism. The response is the diameter of inhibition growth zones. In addition, the neuroprotective activity of the same extracts was also evaluated, to assess their capacity to inhibit enzymes involved in pathologies such as Alzheimer's disease. In fact, it was observed that *B. bifurcata* extracts showed to be an active and promising butyrylcholinesterase inhibitor [3].

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TCC-06

APPLICATION OF PLANT DERIVED COLORANTS IN COSMETIC PRODUCTS

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Currently, a rebirth of the application of natural materials is observed in a wide range of areas like food, health, medicine and cosmetics. A growing interest in phytotherapy and usage of plant derived ingredients in different products is noticed but also there is a need among consumers and companies to create cosmetics based on natural raw materials. The cosmetic industry is converting into less harmful for the environment, more healthy and ecological. In natural cosmetic products a wide spectrum of plant derived ingredients are applied, usually vegetable oils, herbal extracts, or natural essential oils. But there is also a group of materials, which are dyes, that are essential particularly in color cosmetics. Nowadays mainly synthetic or mineral ones are used. Natural dyes obtained from different plants in the past were almost the only ones that were applied in different areas of life to give the desired colour, but for some time they got replaced by cheaper, easier to obtain, but not beneficial for the environment and our health synthetic colorants. In nature there is still a large selection of available colorants, despite the fact that many biomolecules are colourless. Among natural chemical compounds anthocyanins, carotenoids, betalains or quinones are distinguished that can be a source of colour and are obtained from a large number of fruits or vegetables. Natural colorants have enormous potential to be applied in food, textiles and cosmetics. Additionally natural, plant colorants thanks to the content of polyphenols can have others, besides the dyeing, desired properties in cosmetics like antioxidant or antimicrobial. Furthermore, they are characterized by biodegradability and less toxicity but their main disadvantage in an application is that their stability under the influence of light, high temperature or pH can be not satisfied - usually they are unstable during time and different conditions. Dyes are soluble in medium and can be used to dye cosmetic formulations, but to change the skin colour by cosmetics pigments are needed. To obtain plant derived insoluble colorants and simultaneously to improve their stability different methods are possible for example obtaining polymeric dyes or laking natural dyes onto an inorganic substrate. These compounds can be inorganic oxides or salts with adsorbing properties like calcium carbonate or titanium dioxide. In this study it was examined how different plant extracts containing anthocyanins like strawberry extracts, chokeberry extracts, blackcurrants extracts, black lilac extracts, carotenoids like watermelon extracts or cocoa and turmeric have the ability to be adsorbed onto inorganic substrates like talc and hydroxyapatite. Hydroxyapatite belongs to ceramic biomaterials and is applied in medicine and cosmetology as a biocompatible skin filler eliminating skin defects and as a cosmetic ingredient in different formulations. Additionally, hydroxyapatite has properties to protect skin against radiation and optically smoothes wrinkles, which can be an advantage in cosmetic products. Obtaining compounds were examined by a skin colorimeter to check whether they are able to colour the skin and to what extent. To check their chemical structure measurements using ATR-FTIR (Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy) were made. To verify their stability under the influence of light samples have been treated by UV radiation and to verify their stability under the influence of temperature samples were placed in an incubator. For the obtained compounds intensity and stability of the colour were varied, what may be caused by different content of colouring substances in tested plant extracts and different amount of adsorbed colorants onto substrates, but some of tested plant extracts can have the potential to be applied in cosmetics what requires further research.



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PCH
Poster
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PCH-01

ANTIMICROBIAL ACTIVITY OF *Diaporthe terebinthifolii* AGAINST *Staphylococcus aureus* AND METHICILLIN-RESISTANT *S. aureus* (MRSA)

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Medicinal plants represent a great source of endophytic fungi, potential producers of novel bioactive compounds that may be useful to pharmaceutical industries [1]. *Diaporthe terebinthifolii* is a fungus described in 2013 [2] and isolated from *Schinus terebinthifolius*, which is used in popular medicine to combat many health problems [3]. In this study, the strain LGMF907 was tested in different culture conditions and its biological activity was investigated. The crude extracts were obtained from malt extract broth, pH 5.8, 28°C, 120 rpm and 10 days of fermentation, demonstrating high antibacterial activity against *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) (**Table 1**). The compounds diaporthin and orthosporin were identified, and showed low activity against both *S. aureus* and MRSA (**Table 1**). *Diaporthe terebinthifolii* can be a great source of bioactive compounds and further studies should be carried out in order to isolate and examine their bioactivity.

Table 1: Evaluation of the inhibition zone (mm) of LGMF907 crude extracts and compounds tested against *S. aureus* and methicillin-resistant *S. aureus* (MRSA) at 100 µg/disk

Microorganism	methanol [#]	ampicillin	oxacillin	oxacillin	diaporthin	orthosporin	crude extract 1*	crude extract 2*
		100µg/mL	100µg/mL	600µg/ml				
<i>S. aureus</i>	0Ag	15.67Ae	26.67Ab	31.00Aa	9.0Af	9.33Af	16.00Ad	19.67Ac
MRSA	0Ad	0Bd	15.67Bb	20.00Ba	9.5Ac	9.00Ac	16.33Ab	18.00Aab

Tukey results significant with p-value < 0.001. The capital letters compare the microorganisms between the lines whereas the lowercase letters compare the treatments among the columns. [#] Solvent.

*Crude extract 1 was obtained from fermentation method (a) and crude extract 2 from fermentation method (b)

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PCH-02

SCREENING OF THE ANTIOXIDANT AND ENZYME-INHIBITING POTENTIAL OF ESSENTIAL OILS OF SOME COMMERCIAL LAMIACEAE SPECIES

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Lamiaceae species, especially those belonging to the subfamily Nepetoideae, are extensively used for centuries in traditional medicine, cookery, perfumery and cosmetics due to their prominent flavoring and curative properties. There is a hot topic on oxidative stress as one of the main triggers of diabetes mellitus and neurodegenerative diseases, hence this research was aimed to examine the antioxidant effects as well as α -amylase, acetylcholinesterase and tyrosinase inhibiting potential of essential oils of 11 Lamiaceae species commercially grown on the fields of the Institute for Medicinal Plant Research „Dr Josif Pančić“ in Pančevo, Serbia (**Table 1**). The essential oils were isolated by hydrodistillation and tested at concentrations of 0.5, 1, and 2 mg/mL. Antioxidant effects were assessed using DPPH and β -carotene bleaching assays and the results indicated that *Oh*, *Tv*, and *Ts* oils inhibit above 50% free radicals in both tests, which is in accordance with our previous findings [1]. The tested oils did not inhibit efficiently α amylase (values were ranged from 0.11-5.62%), while results of our previous study showed a promising α -glucosidase inhibitory activity, especially for *Ts*, *Tv*, and *Oh* oils [1]. The strongest inhibition of acetylcholinesterase was performed by *Oh* (even 78.90%), followed by *So* and *Ro* essential oils. The essential oil of *La* showed the strongest inhibition of tyrosinase (68.77%), followed by *Mp* and *Ho* (**Table 1**). Comparing the obtained results with positive controls and literature data, it could be concluded that essential oils of the examined Lamiaceae species, particularly those of *Ts*, *Tv*, and *Oh*, have significant potential for further research as an antioxidant and antineurodegenerative agents.

Table 1: Antioxidant and enzyme inhibition activity of 11 Lamiaceae species essential oils tested at concentration of 2 mg/mL.

Plant species	Antioxidant assays ^a		Enzyme inhibition assays ^b		
	DPPH	β -carotene bleaching	α -amylase	acetylcholinesterase	tyrosinase
<i>Hyssopus officinalis</i> (<i>Ho</i>)	13.28±0.78	4.64±0.44	3.97±0.36	12.69±0.33	61.40±1.40
<i>Lavandula angustifolia</i> (<i>La</i>)	12.07±0.28	na	0.11±0.04	24.99±0.33	68.77±0.35
<i>Mentha x piperita</i> (<i>Mp</i>)	14.08±0.56	na	1.27±0.22	48.83±0.39	63.86±0.35
<i>Ocimum basilicum</i> (<i>Ob</i>)	13.04±0.44	na	2.48±0.13	40.45±0.32	54.04±0.70
<i>Origanum heracleoticum</i> (<i>Oh</i>)	55.27±0.91	63.57±0.77	na	78.90±0.69	27.78±1.11
<i>Origanum vulgare</i> (<i>Ov</i>)	10.42±0.48	na	1.63±0.30	53.26±0.67	57.41±0.37
<i>Rosmarinus officinalis</i> (<i>Ro</i>)	10.85±0.06	na	na	68.79±0.37	54.81±0.37
<i>Salvia officinalis</i> (<i>So</i>)	14.93±0.34	8.67±0.45	5.62±0.22	72.53±0.31	42.22±1.28
<i>Satureja montana</i> (<i>Sm</i>)	22.30±0.66	31.72±0.54	na	21.68±0.56	52.27±0.76
<i>Thymus serpyllum</i> (<i>Ts</i>)	51.07±0.70	52.40±0.64	na	42.46±0.15	35.23±1.31
<i>Thymus vulgaris</i> (<i>Tv</i>)	51.25±0.69	64.08±0.96	na	57.49±0.64	6.44±0.38

Results are presented as a percent of inhibition of free radicals^a and enzymes activity^b (mean values±standard error); na - no activity

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PCH-03

EVALUATION OF AZOLE-RESISTANT MYCOBIOTA AND MYCOTOXINS PROFILE IN TEA

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Tea is one of the most common beverages worldwide, prepared by infusion of the *Camellia sinensis* leaves with boiling water, the main ones being black tea and green tea (1). Fungal contamination of tea raised the question of mycotoxins at any stage of the tea circuit, being influenced by pre- and post-harvest factors (2). Mycotoxins are secondary fungal metabolites produced by specific strains of filamentous fungi, such as *Aspergillus* and *Penicillium* (2) that confer potential risks to human health and can cause serious diseases.

Aspergillus spp. cause diseases in humans and animals, namely aspergillosis, with azole antifungals being the first-line therapy (3). While some species (*A. fumigatus*, *A. lentulus* and *A. pseudofischeri*) commonly found in soil, are sensitive to azole fungicides, its continued exposure to fungicides in conventional agricultural practices, can result in the emergence of acquired azole resistance.

Azole resistances is a threat to public health due to the potential loss of therapeutic effectiveness, and hampered treatment of fungal infections.

Considering the growing risks to public health, this study aims to evaluate the mycotoxigenic and azolic resistance profile of mycobiota present in tea samples commercially available in Portugal. Firstly, different tea samples will be obtained from Portuguese supermarkets and herbalist shops. Tea infusions will be then prepared and inoculated in culture media selective for fungi and later incubated. Colony forming units (CFU) will be counted and morphological identified through microscopy. Specific *Aspergillus* sections will be detected through Real-Time PCR (RT-PCR), namely: sections *Circumdati*, *Flavi*, *Fumigati* and *Versicolores*. The fungal resistance profile to azole drugs will be determined by inoculating the tea samples in azole-supplemented media with fixed concentrations (itraconazole, voriconazole and posaconazole). Finally, the analysis of mycotoxins will be performed by HPLC, with MS MS detection, and the results will be statistical analysed by descriptive and correlational analysis. The results of the project will be disseminated in scientific events and scientific papers, and monitoring and control measures to avoid the fungal contamination of tea will be shared with stakeholders.

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PCH-04

IN VITRO ASSAY OF BIOACTIVE POTENTIAL OF ZIZIPHUS JUJUBA MILL. EXTRACTS

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Jujube (*Zizifus jujuba* Mill., Rhamnaceae) is a warm climate plant. The fruit has high energy and nutritional value, contains a lot of sugar, dietary fiber, vitamin C, minerals, phenolic compounds. Therefore, this plant is a valuable source of phytochemicals, bioactives, vitamins etc. which makes it to be effective in curing or prevention from various diseases. The present work investigate quantification of total phenols and flavonoids, antioxidant and antimicrobial potential of different extracts *Z. jujuba*, originating from different localities of Montenegro (Podgorica, Ulcinj, and Bar). Four different dry extracts were extracted from dried fruits, by ultrasound assisted extraction using various solvents (methanol, water, dichloromethane and n-butanol). Quantification of total phenols and flavonoids was done by spectrophotometric methods. The results showed that these extracts are rich in phenolic compounds, but do not contain a high percentage of flavonoids. The antioxidant activity of the extracts was performed by DPPH and ABTS method. The best antioxidant potential in the DPPH test was shown for n-butanol extracts, while in the ABTS test methanol, dichloromethane and aqueous extracts were shown to be significantly better antioxidants. The antimicrobial effect of *Z. jujuba* extracts was tested on seven bacterial species, belonging to human infectious agents and food contaminants and four clinical isolates of dermatomycetes, by microdilution method. Based on the results, all extracts possessed antibacterial/antifungal activity against tested bacteria and fungi. The extracts showed very high antimicrobial potential, with the aqueous extract being slightly weaker compared to the other three ones. In addition to small differences in the effect of the extracts, resistant *Escherichia coli* (R) proved to be the most resistant bacteria, with MIC of 0.19 mg/mL and a MBC of 0.38 mg/mL. Tested extracts possessed similar activities against dermatomycetes, but n-butanol extracts had the best potential (MIC and MFC values was in the ranges of 0.045-0.38 and 0.09-0.75 mg/mL, respectively). Also, there is no difference in antifungal potential according to the origin of the extracts. The extracts from all three localities show approximately the same antifungal effect. *Microsporium canis* proved to be the most sensitive fungus, whose growth was inhibited at very low concentrations (MIC 0.09-0.19 mg/mL and MFC 0.19-0.38 mg/mL).

The obtained results show that fruit of *Z. jujuba* and its extracts represent a potential source of new compounds with antioxidant and antimicrobial activity. It is an appropriate candidate for treatment of microbial infections and for therapeutic application.

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PCH-05

EFFECTS OF GREEN TEA IN URINARY BLADDER CANCER: DATA FROM A MOUSE MODEL

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Urinary bladder cancer is one of the most common diseases around the world, associated with several risk factors [1-2]. *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine (BBN) is a carcinogen able to induce preneoplastic and neoplastic urothelial lesions development in rodents [3]. Green tea (GT) is one of the most popular beverages whose beneficial effects on health have been demonstrated [4]. This study aimed to evaluate the effects of whole GT on urinary bladder cancer in male and female mice. The experiment followed the European (Directive 2010/63/EU) legislation. Forty-one ICR mice of five weeks of age (21 males and 20 females) were used. Animals from each gender were randomly divided into three experimental groups, as follows: *Males* - group I (BBN+GT) (n=8); group II (BBN) (n=7); group III (GT) (n=6); *Females* - group IV (BBN+GT) (n=7); group V (BBN) (n=7); group VI (GT) (n=6). BBN was administered to animals from groups I, II, IV and V by gavage, at a dose of 7.25 mg/mouse, 2 times/week, during 10 consecutive weeks. The whole GT (0.5%) was daily prepared and given *ad libitum* to groups I, III, IV and VI for 20 consecutive weeks. Animals were sacrificed and a complete necropsy was performed. A histological analysis of the urinary bladder was performed. Data was analyzed with ANOVA. Results were considered statistically significant for $p < 0.05$. Animals from groups not exposed to BBN (III and VI) did not develop any urothelial lesion. Animals from groups BBN+GT (I and IV) and BBN (II and V) developed only preneoplastic lesions. The number of inflammatory aggregates was lower in animals exposed to BBN that drank GT (I and IV), when compared with those only exposed to BBN (II and V). A statistically significant difference was observed between groups BBN (II and V) and groups GT (III and VI) ($p < 0.05$) (**Table 1**). The administration of GT infusion had no effect on urinary bladder cancer development, but reduced urothelial inflammation.

Table 1: Urothelial histopathological analysis (n; %) and inflammatory aggregates (mean±S.D).

Analysis	Groups	Male				Female	
		I (BBN+GT) n=8	II (BBN) n=7	III (GT) n=6	IV (BBN+GT) n=7	V (BBN) n=7	VI (GT) n=6
Histologica l analysis	Normal urothelium	0 (0%)	0 (0%)	6 (100%)	0 (0%)	0 (0%)	6 (100%)
	Simple hyperplasia	6 (75%)	3 (50.0%)	0 (0%)	3 (42.8%)	4 (66.7%)	0 (0%)
	Dysplasia	7 (87.5%)	5 (83.3%)	0 (0%)	4 (57.1%)	4 (66.7%)	0 (0%)
	Papilloma	2 (25%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	Squamous metaplasia	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (16.7%)	0 (0%)
	Inflammatory aggregates	3.50 ± 4.46	4.67 ± 2.99 ^a	0.42 ± 0.90	3.50 ± 2.56	3.83 ± 3.13 ^b	0.25 ± 0.45

^a $p < 0.05$ vs Group III; ^b $p < 0.05$ vs Group VI

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PCH-06

C. SCOLYMUS LEAF EXTRACT CHARACTERIZATION AND BIOLOGICAL PROPERTIES

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Cynara scolymus L. is an herbaceous and perennial crop. Originally native to the Mediterranean basin, the arid, high temperatures and elevated salinity conditions are ideal for growth and reproduction. The artichoke plant is known for its varied medicinal properties, such as hepatoprotective and antioxidant, and nutritional value since ancient times [1]. The increasing interest for these biological properties in the dietary and for healthier foods triggered the worldwide production and economic valorization of artichoke. Artichoke leaves, non-edible parts often discarded, are rich in bioactive secondary metabolites, mainly polyphenolic compounds like flavonoids (0.1-1.0%) and phenolic acids (2%), and sesquiterpenes, such as cynaropicrin (0.44-1.6%) [2,3]. Cynaropicrin has anti-inflammatory, choleric, antibacterial, and antitumoral properties and prevents skin photoaging induced by UV radiation [4]. While chlorogenic acid, a phenolic acid, has antioxidant properties and reduces blood cholesterol [1]. This knowledge enhances the use of plant bio-products for extracting pharmacologically active compounds or producing extracts for nutraceutical and cosmeceutical applications. Thus, this work aimed to obtain artichoke leaf extract by sustainable techniques and characterize the structure and the biological potential. The original extract obtained by a decoction of leaves in hot water was partitioned by liquid-liquid extraction, and aqueous (Aq) and organic (O) fractions were examined separately to assess the *in vitro* and *in vivo* antioxidant activities of their components. Firstly, the determination of the Total Phenolic Content (TPC) by colorimetric tests. Secondly, the antioxidant capacity was evaluated by the DPPH scavenging activity and reducing power *in vitro* assays and by flow cytometry *in vivo* assay, using *Saccharomyces cerevisiae* cells. Finally, *in vitro* DNA topology assay analyzed the genotoxic potential and the antigenotoxic activity. The composition of leaf extracts obtained by decoction in water was analyzed by NMR spectroscopy, and the results showed that chlorogenic acid (1) and cynaropicrin (2) are the main compounds. These compounds were efficiently separated by liquid-liquid extraction, as 1 was only found in the aqueous (Aq) fraction, and 2 moved to the organic (O) fraction. The Aq fraction showed the higher TPC value, *in vitro* antioxidant activity by scavenging activity, and reducing power, however, was not able to protect living *S. cerevisiae* cells from oxidative stress. O fraction exhibited *in vitro* antioxidant potential by scavenging activity and was able to protect *S. cerevisiae*. Additionally, the O fraction displayed antigenotoxic activity, and both fractions proved not to be genotoxic. This study valorizes artichoke consumption and recognizes the plant as a source of bioactivities. The different properties presented by the extract highlights the use of cynaropicrin as a potential drug for genome protection and the prevention of degenerative diseases like cancer and, the high antioxidant potential for nutraceutical applications.

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ANTIOXIDANT ACTIVITY OF PORTUGUESE COMMERCIAL ESSENTIAL OILS OBTAINED FROM FOREST MAINTENANCE

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Currently, there has been an emergent interest in the production and commercialization of essential oils (EOs). In an attempt, to validate the claims of EOs bioactivity, and in a context of sustainability, this work assessed the antioxidant activity of EOs obtained from biomass wastes resulting from Portuguese forest maintenance.

Eleven EO samples from *Eucalyptus globulus*, *Pinus pinaster*, *Pinus pinea* and *Cryptomeria japonica*, were obtained from local producers, from the mainland Portugal and Azores archipelago. Using gas chromatography and gas chromatography associated with mass spectrometry, the evaluated EOs were chemically characterized as detailed in [1]. Subsequently, using the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) assay [2], the antioxidant activity of these EOs was evaluated. 1,8-Cineole (eucalyptol) was the main component of the 6 *E. globulus* EO samples (49-65%). From the three samples of *P. pinaster* EOs, two were dominated by α -pinene (37-45%) and the third showed similar amounts of α -pinene and β -pinene (28% and 29%, respectively). Limonene (73%) was the main component in *P. pinea* EO, and α -pinene (26%) in *C. japonica* EO.

All EOs showed antioxidant activity: *C. japonica* EO showed the highest antioxidant activity, whereas one of the *E. globulus* EO samples showed the lowest antioxidant capacity. Among *P. pinaster*, *P. pinea* and *E. globulus* EOs the results obtained were very broad, differing from the producers, probably due to the composition of each sample.

It is possible to conclude from these preliminary results, that EO's studied have relevant antioxidant activity, which can be a key benefit justified by their promising health promoting properties.

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PCH-08

HEALTH FROM THE SEA: *BIFURCARIA BIFURCATA* AS A SOURCE OF BIOACTIVE COMPOUNDS

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The marine environment appears to be a source of compounds used in the manufacture of food, cosmetic and medicinal products. Within all the richness of this medium, algae are potential candidates for the discovery and extraction of bioactive compounds [1]. Brown algae are especially rich in a carotenoid compound called fucoxanthin. Fucoxanthin is considered to be the most abundant natural carotenoid and its consumption has been related to the improvement of various biochemical parameters and with a possible beneficial effect on the loss of fat weight [2].

This work performed an optimization of the main variables of ultrasound assisted extraction (UAE) for fucoxanthin obtaining from *Bifurcaria bifurcata*, an alga typically found in the Galician coast. The optimization procedure was built with a Response Surface Methodology (RSM) with 5 levels of three variables: the extraction time (*t*, 5 to 55 min), the solvent as a percentage of ethanol (*S*, 35 to 100%) and the power applied (*P*, 100 to 500 W). In addition, the solid-to-liquid ratios were optimized at the optimal conditions of the main variables. The obtained results suggest that the application of UAE can be considered as an ecological, fast and simple method, suitable to obtain fucoxanthin in short times from *Bifurcaria bifurcata*, allowing the valorization of the algae.

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PCH-09

IMPROVING THE ANTI-INFLAMMATORY ACTIVITY OF CLOVE OIL-ISOLATED β -CARYOPHYLLENE USING A SELF-EMULSIFYING SYSTEM: AN *IN VIVO* STUDY

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(-)- β -Caryophyllene (β -Cary) is a sesquiterpene reported to present anti-inflammatory activity [1] that can be obtained from asymmetric synthesis or, alternatively, from essential oils [2]. Clove buds are rich in eugenol (70-90%), which is widely used in perfumes and flavorings, but also has a considerable amount of β -Cary [3]. Eugenol is extracted from clove oil by fractionated distillation, which also permits to separate a fraction exceptionally rich in β -Cary [3]. The lipophilic character of β -Cary makes it difficult to interact with the gastrointestinal aqueous environment [1], but nano-emulsifying carriers have been shown to be very effective as systems for oral administration of active compounds with low water solubility. Therefore, they should improve the anti-inflammatory activity of the orally administered β -Cary. To test this hypothesis, a lipid-based self-emulsifying drug delivery system (SEDSS) containing β -Cary was prepared and its acute anti-inflammatory effect when orally administered was evaluated by using the carrageenan-induced paw edema in rats. β -Cary from clove bud oil was purchased from Quinarí Fragrâncias Ltda (Ponta Grossa, PR, Brazil) and its composition determined by GC-MS. The SEDSS system, consisting of β -Cary, surfactant and oily phase, was characterized in relation to morphology and rheology. Data from rheology were used to obtain the consistency index (k) and flow behavior index (n). For *in vivo* study, Wistar rats were distributed into three groups (n=5): Controls, which received saline; CAR-50, which received unformulated 50 mg/kg β -Cary; and F-CAR, which received formulated 50 mg/kg β -Cary. All animals were treated 1 h before the carrageenan injection in the left hind paw. The edema was monitored by plethysmography at 0, 1, 2 and 4 h after the carrageenan injection. After 4 h, the animals were euthanized and the plantar tissue collected to measure the myeloperoxidase (MPO) activity, which is an indicative of polymorphonuclear cells (PMN) infiltration. The animal protocol was approved by the Ethics Committee for Animal Experimentation of UEM. The clove oil-isolated β -Cary contained β -Cary (91%), α -humulene (8.2%) and copaene (0.7%). SEDSS containing β -Cary were spherical droplets, whose viscosity tended to decrease following a Newtonian behavior as the temperature increased. The paw edema was similar for the 3 groups at 0, 1 and 2 h, but at 4 h it was lower in F-CAR (-40%) and CAR-50 (-20%) when compared to the control group. The MPO activity was equally decreased by 35% in CAR-50 and F-CAR. The results show that SEDSS containing β -Cary was more effective in decreasing the carrageenan-paw edema than unformulated β -Cary, but the mechanism does not seem to involve the prevention of PMN migration.

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PCH-10

PHYTOCHEMICAL CHARACTERIZATION AND EVALUATION OF THE BIOACTIVE PROPERTIES OF TISANES PREPARED FROM AROMATIC AND MEDICINAL PLANTS

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The antioxidant, antidiabetic and anti-inflammatory properties of different species of Medicinal and Aromatic plants (MAP) have been extensively described, being its most common form of consumption the tisane, or popularly known as herbal teas or infusions [1-3]. The present study aimed to characterize the phenolic compounds by HPLC-DAD/ESI-MS of five tisanes prepared from mixtures of MAP, as also study the bioactive properties, namely antioxidant, antimicrobial, anti-inflammatory, cytotoxic, anti-tyrosinase, and anti-diabetic activities. It was also proposed to characterize its composition in organic acids and tocopherols by HPLC coupled to a DAD and fluorescence detector, respectively. Thus, the tisane composed by lemon thyme, Shrubby St. John's Wort, cloves and cinnamon, was the most promising mixture, presenting the lowest values for the lipid peroxidation inhibition, anti-inflammatory, cytotoxicity against breast adenocarcinoma tumor cell line and anti-diabetic activity. In addition, it showed the highest concentration of tocopherols (γ -isoform), phenolic acids (caffeoylquinic acids derivatives) and flavan-3-ols (catechin derivatives). For the anti-hemolytic, antimicrobial (Gram-negative bacteria), and cytotoxic (cell lines HepG2, NCI H460 and HeLa) activities, the tisanes containing also lemon thyme as the major component, were highlighted as the best mixtures. Rosmarinic acid and its derivatives were the most abundant phenolic compounds in all tisanes, followed by glycosylated flavanols/flavones. These results suggest that tisanes are promising sources of bioactive compounds with high added value that can be applied in several industrial fields, such as the food industry.

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PCH-11

ANTIMICROBIAL POTENTIAL OF *CYMBOPOGON MARTINII* ESSENTIAL OIL AND DENTAL IRRIGANT BASED ON IT AGAINST ROOT CANAL BIOFILMS

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The interest in natural products applications as antimicrobial agents is rapidly growing, mainly due to increasing microbial resistance to conventional therapeutics. Essential oils (EOs) seem to be especially attractive, since many of them possess high antimicrobial potential and none revealed resistance issue. EOs have already found applications in endodontic practice, mainly as sealers, but their antimicrobial potential has not been explored in this field yet. Taking this into account, the objective of this study was to investigate the antibacterial and antibiofilm efficacy of *Cymbopogon martinii* EO against bacterial strains relevant for endodontic therapy. Chemical composition of EO was determined by GC-MS/FID analysis. Antibacterial potential was screened in microdilution assay, detecting minimal inhibitory and bactericidal concentrations (MICs and MBCs) and performed on *Enterococcus faecalis*, *Streptococcus mitis* and *Streptococcus sanguinis*. Eradicating potential against biofilms preformed of each bacterial species as well as of all three bacteria, was determined *in vitro* in crystal violet (CV) assay. For screening of *in situ* effect against biofilm preformed in root canals of extracted teeth, the EO-based irrigant was formulated. Its antibiofilm potential was evaluated by plate counting (PC) assay, performed with single *E. faecalis* biofilm and multispecies biofilm composed of all three strains. Single *E. faecalis* biofilm was selected since it is the most relevant intracanal pathogen, while multispecies biofilm was screened to better mimic clinical conditions.

Chemical analysis revealed that geraniol was the most abundant constituent (83.5%), followed by geranyl acetate (8.4%). Determined MIC and MBC values were the lowest for *E. faecalis* (0.12 and 0.25 mg mL⁻¹, respectively), and the highest for *S. sanguinis* (2.95 and 5.90 mg mL⁻¹, respectively). CV assay pointed out that EO successfully eradicated *in vitro* preformed biofilms of *E. faecalis* and *S. mitis* (approximately 30% reduction), but had no effect on *S. sanguinis* and multispecies biofilms. EO-based irrigant used for determination of *in situ* antibiofilm potential was pre-screened for its physicochemical characteristics: a mean particle diameter and polydispersity index was 101 ± 3 nm and 0.36 ± 0.06, respectively. PC assay showed that eradicating potential of applied irrigant was weak against both *E. faecalis* and multispecies intracanal biofilm (reduction of logCFU was 0.76 and 0.52, respectively). However, successive irrigation with conventional dental irrigants (sodium hypochlorite and sterile saline), followed by *C. martini* EO-based irrigant, notably increased the biofilm reduction (1.89 and 1.69, respectively).

In conclusion, *C. martini* EO possesses notable antibacterial potential that could be further investigated for different dental applications.

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ANETHOLE IMPROVES THE OXIDATIVE STATE OF RATS WITH ADJUVANT-INDUCED ARTHRITIS

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Rheumatoid arthritis (RA) is a chronic multisystem disease, which the main feature is a persistent inflammatory synovitis [1]. Anethole, a substance present in the essential oil of star anise (*Illicium verum*), can reduce inflammation and joint damage in adjuvant-induced rheumatoid arthritis model (AIA) [2]. However, studies investigating the effects of anethole on oxidative stress induced by arthritis are lacking. Thus, the aim of the present study was to evaluate the effects of anethole treatment on oxidative stress in arthritic bearing rats. Male Holtzman rats (180-220 g) were used. All experiments were approved by the ethics committee of State University of Maringá. Arthritis was induced by injection of Freund's complete adjuvant on the plantar surface of animals' posterior paw. Animals were treated daily with oral anethole, at doses of 62.5 and 250 mg/kg. Treatment started on the day of AIA induction and continued for 21 days. Plasma, liver and kidneys were used to determine oxidative stress[3]. Data were analyzed by ANOVA followed by Tukey post-test ($p < 0.05$). Anethole treatment (62.5 and 250 mg/kg) decreased plasma protein carbonylation in 20%, when compared to arthritic control animals. The increased myeloperoxidase (MPO) activity, observed in arthritic animals, was prevented by anethole treatment at a dose of 250 mg/kg. However, anethole treatment was not able to prevent the decreased levels of thiols or the reduced total antioxidant capacity caused by arthritis. Indicators of oxidative damage in the liver and kidneys, namely, levels of carbonylated proteins, total ROS and lipid peroxidation (TBARS), were significantly increased in control arthritic animals. Treatment of arthritic animals with anethole at a dose of 62.5 mg/kg did not improve any parameters of oxidative damage in the liver or kidneys. Treatment with AN at a dose of 250 mg/kg decreased the levels of carbonylated protein and TBARS in the liver, although it was not able to prevent the increase in ROS levels in both organs, nor the increase in protein carbonylation in the kidneys. AIA also caused a marked reduction in the activity of the antioxidant enzymes catalase and superoxide dismutase (SOD) in both organs. Anethole treatment (62.5 and 250 mg/kg) was not able to prevent the decreased catalase activity in the liver and kidneys. In contrast, there was an increase in SOD activity in the liver. Treatment with anethole in both doses was able to increase the levels of reduced glutathione (GSH) and decrease oxidized glutathione, improving the GSH/GSSG ratio in the liver. Therefore, our results demonstrate that anethole, specially at the dose of 250 mg/Kg, is able to improve the antioxidant defense and to reduce oxidative damage in arthritic animals.

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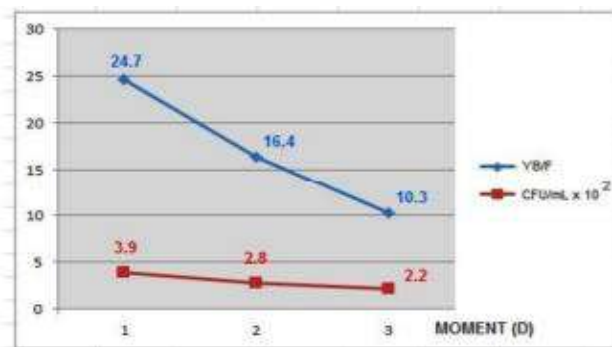
***IN VIVO* EFFECT OF AQUEOUS 20% PROPOLIS EXTRACT ON THE CONCENTRATION OF *Malassezia* SPP. IN DOGS WITH BILATERAL CHRONIC EXTERNAL OTITIS**

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Chronic external otitis (CEO) is a common syndrome in adult dogs, causing financial and psychological damage to the owner, in addition to affecting animal health [1]. In these conditions, it is common to see otopathic conditions with a marked presence of yeasts of the genus *Malassezia*, worsening the clinical presentation, making therapy difficult and preventing the restoration of the patient's health [1]. It is necessary to control this fungal population, either through traditional antimycotic drugs or other substances with similar properties, such as herbal medicines and animal derived products. Propolis is a natural resin collected by bees (*Apis mellifera*), having proven antifungal activity *in vitro*, but its effectiveness *in vivo* is still the subject of discussion in the scientific community [1]. The objective of this research was to evaluate the concentration of *Malassezia* spp. present in the ear canal of dogs with CEO and subjected to topical exposure to aqueous propolis extract (APE) 20%. Fifteen adult dogs (varied weight, size and breed) were used, who had a history, clinical signs and ear exams compatible with CEO. Three moments were selected to collect auditory exudate (D0 = pre-treatment; D5 = five days of treatment; and D10 = ten days of treatment) for yeast counting in culture medium (CFU/mLx10²) and conventional optical microscopy (YB/F) [2]. The experiment started with the instillation of six drops / animal of the APE in each ear every eight hours, for ten consecutive days. No other medication or therapeutic maneuver was used in these patients. The analysis of the data shows that there was a significant regression (p<0.05%) in the yeast population, both in the counting in culture medium and in conventional optical microscopy (**Graphic 1**). It is concluded that the isolated use of 20% aqueous propolis extract in dogs with chronic external otitis leads to a reduction in the yeast population of *Malassezia* spp. *in vivo*, suggesting that this practice may be associated with conventional canine otitis externa therapy.

Graphic 1: Concentration of *Malassezia* spp. in the ear canal of dogs with chronic otitis externa before and after exposure to therapy with 20% aqueous propolis extract.



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PCH-14

RELEVANCE OF HORSE CHESTNUT (*AESCULUS HIPPOCASTANUM* L.) SAPONINS IN HEALTH AND COSMETICS

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Saponins are steroidal glycosides or polycyclic terpenes resulting from the secondary plant metabolism and have many beneficial characteristics and applications. They can be used to fight and cure many diseases and health issues, as well as in cosmetics and as natural alternatives for food preservation and packaging [1,2]. Due to the finite resources our planet can provide and the increasing potential for saponins in various sectors, it is necessary to find alternative sources of these pharmacologically active high-added values compounds. This study aims to provide an overview of the bioactive properties of saponins that can be found in *Aesculus hippocastanum* L. The saponins found in this plant have a trisaccharide chain at C3 of the aglycone and a β -glucopyranosyl unit attached at C4 of the glucuronic acid. Aescin (Figure 1) is the main active component and responsible for most of its medicinal properties [2,3]. These saponins have already been described as more antioxidants than vitamin E and capable of protecting cells from free radicals and reactive species [2,4]. These compounds shown positive effects on venous insufficiency and associated conditions, reduce capillary fragility, and help prevent the leakage of fluids into surrounding tissues, thus presenting beneficial effects on human skin [2]. Due to these properties, *A. hippocastanum* saponins can be used in cosmetic formulations with anti-aging capacity [2]. The bark of this plant has also been used to treat dermatitis, while the leaves and seeds are used due to their anti-inflammatory effects [3]. β -Aescin (the main active compound of aescin) is a natural inhibitor of leukemic cell proliferation and may be useful for new anti-leukemic drugs [5]. β -Aescin has also been reported to be effective for the treatment of colon cancer [4]. Therefore, given the high added-value of aescin, it would be interesting to develop an extraction method that maximizes the recovery of these compounds from this plant in a sustainable way to promote resource use efficiency.

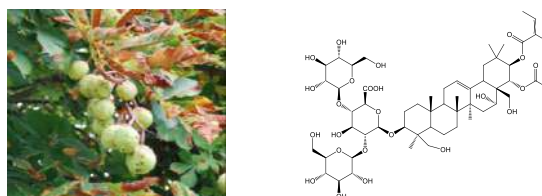


Figure 1: *A. hippocastanum* fruits (<https://flora-on.pt/>) and chemical structure of the main component of aescin.

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PCH-15

LEE INDEX VARIATION AND GLUCOSE CLEARANCE RATE IN AN ANIMAL MODEL OF OBESITY WITH ANIMALS SUPPLEMENTED WITH *GANODERMA LUCIDUM*

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The consumption of diets high in sugars and saturated fat are associated with obesity and other comorbidities, as well as glucose intolerance¹. Obesity can be influenced by the regular consumption of natural bioactive compounds like those found in *Ganoderma lucidum* mushroom (GL)². The main objective of this work is to determine body mass variations (Lee Index) and serum glucose levels in animals fed with diets supplemented with three concentrations of an hydroethanolic extract of GL. The fruiting bodies of GL were provided by Bioreishi- Agricultura Biologica, Lda. Forty-eight male mice (C57BL/6) were acquired and divided into 5 groups: G1-Western Diet 0.2% Cholesterol (WD); G2-Western Control (WC); G3-WD+0.7%g/kg of *G. lucidum*; G4-WD+1.4%g/kg of *G. lucidum*; G5- WD+2.8%g/kg of *G. lucidum*. At 7th and 13th weeks of study, the animals were measured (nasoanal distance in cm) in order to calculate the Lee Index (cubicle root of the weight (g)/the nasoanal length (cm) x 1000). Obesity was defined by a Lee index >310. Glucose intolerance test was performed on 4 animals in each group. The animals were fasted overnight and in the early morning, a 50% glucose solution (2g/kg) was injected intraperitoneally. Blood samples were collected one hour before glucose (time zero) administration and 30, 60 and 120 minutes later. Serum glucose was determined with the OGCare equipment. Glucose concentration values at time zero were considered control values. All ethical issues were followed (approval n° 8776). The chemical composition of the extract was profiled by HPLC-DAD-ESI/MS. All data were analyzed using the GraphPad Prism[®] for Windows software (version 7.0). Ganoderic acid H and *p*-hydroxybenzoic acid were the main triterpenic and phenolic acids found in the extract, respectively. In respect to the Lee index, the values show that all animals in the experimental groups became obese. In both weeks, G5 showed the highest values (353.45 ± 12.43 and 351.77 ± 12.24 respectively). At week 7, G1 and G2 differed statistically (p<0,05) from G5. Although G1 and G2 have the lowest Lee values in both weeks, they are the only ones that increase from week 7 to week 13, in contrast to the groups supplemented with GL. Regarding the glucose intolerance test, recorded glucose values at 30 minutes after the injection were increased in all groups, returning to normal values two hours later. At 13th week, the glucose values were increased in all groups in the 30 and 60 minutes after the intraperitoneal injection. At the end of two hours, the values decreased, but did not return to normal values in all the groups. Although animals in all experimental groups remained obese, animals supplemented with GL decreased their Lee Index. At 7th week the animals showed a normal glucose clearance, but in the last week of the study glucose values did not return to the values initially recorded after two hours, showing that the animals were not able to maintain the glucose clearance rate.

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PCH-16

DETERMINATION OF ANTIARTHRITIC ACTIVITY OF NIGELLA SATIVA L. SEEDS EXTRACTS *IN VITRO*.

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Rheumatoid arthritis is the most common chronic inflammatory rheumatism (between 0.4 and 0.8% of the general population). It is also the most serious of rheumatism in particular by the risk of developing irreversible joint destruction, joint deformities and a sometimes significant handicap [1]. *Nigella sativa* L. is an annual herb of the *Ranunculaceae* family, with the seeds called black cumin seed or black seed. It's one of the most widely used plants in medicine traditional because of the various properties of its different parts including its seed. It is used as treatment for a variety of health conditions pertaining to the digestive tract and immune system support [2]. For this, the objective of this study was the evaluation of the antiarthritic activity [3, 4] as well as the phytochemical screening of *Nigella sativa* L. seeds extracts *in vitro*. The dosage of flavonoids have shown that the content found in the hydroalcoholic extract (134.7 ± 0.289 mg QE / g) was significantly higher compared to the aqueous extract (48.495 ± 0.035 mg QE / g)

(**Table 1**). The phytochemicals analysis of the extracts revealed the presence of tannins, saponins and reducing compounds and the absence of alkaloids. The results of antiarthritic activity showed an important effect of the aqueous and hydroalcoholic extracts on the inhibition of inflammation (**Table 1**). The results of this study show that the seed extracts exhibited an important anti-inflammatory effect.

Table 1: Results of flavonoid content and inhibition of protein and albumin denaturation.

Extracts	Flavonoids content (mg QE/g of extract)	Inhibition of protein denaturation (IC50 mg/ml)	Inhibition of Albumin denaturation (IC50 mg/ml)
Hydroalcoholic extract	$134.7 \pm 0.28a$	$52.74 \pm 1.86b$	$46.75 \pm 1.74b$
Aqueous extract	$48.495 \pm 0.03b$	$37.79 \pm 0.67a$	$34.09 \pm 1.26a$
Diclofinac sodium	/	$48.55 \pm 1.09b$	$48.55 \pm 1.09b$

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PCH-17

**CHEMICAL CHARACTERIZATION OF ESSENTIAL OILS FROM
CULTIVATED POPULATIONS IN W. GREECE OF *THYMUS VULGARIS* L.,
SATUREJA MONTANA L. AND *ORIGANUM VULGARE* L.
A SURVEY OF CARVACROL AND THYMOL AS ANTIMICROBIAL AGENTS**

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Throughout European flora, Greece is characterized by high plant diversity, remarkable endemism, and polymorphism. A great example of this unique identity is attributed to the aromatic and medicinal plants, which are of high economic interest. The essential oils (EOs) produced by such plants are valuable products with many applications in pharmaceutical, cosmetic, food and flavouring industries. Lamiaceae is one of the most important families in the production of EOs rich in monoterpene phenols, especially thymol and carvacrol. This class of secondary metabolites are one of the most potent components of the EOs possessing significant biological properties including antioxidant, antispasmodic, antitussive, expectorant, antiinflammatory, analgesic, antibacterial, and antimicrobial [1]. Due to the alarming increase in antibiotic resistance, the interest in natural products containing plant antimicrobial compounds as alternative or adjunct antimicrobial agents has been revived [2]. Thus, we investigated the EOs [1] of *Thymus vulgaris* L., *Satureja montana* L. and *Origanum vulgare* L. from cultivated populations in W. Greece. The results herein are supported by a literature survey on the major constituents i.e. phenolic monoterpenes. The antimicrobial compounds thymol and carvacrol represented by these EOs might be useful as potential therapeutic agents alternatively or synergistically with synthetic chemical agents to enhance their antimicrobial activity.

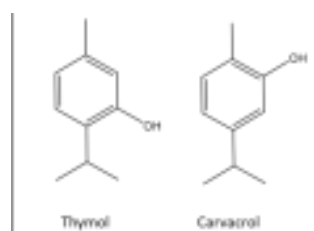


Figure 1: Structure of major EOs constituents.

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PCH-18

DEVELOPMENT OF A QSAR MODEL TO EVALUATE THE ANTIOXIDANT ACTIVITY OF DI (HETERO) ARYL COMPOUNDS

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QSAR (Quantitative Structure - Activity Relationship) relationships are useful to understand and explain the mechanism of action of compounds at a molecular level and allow the development of new compounds with desirable biological properties. A QSAR quantitative model is represented by a mathematical equation that relates calculated properties of the studied compounds (descriptors), with their biological activities. QSAR models can have multiple applications, including in natural compounds. For example, they may help in the prediction of different natural products bioactivities, as long as the QSAR model in question is developed using similar compounds [1,2].

The QSAR study presented was carried out to model the antioxidant activity, specifically the ability to eliminate free radicals, from 70 di(hetero)aryl compounds. This model is an enhancement of a model previously developed in our group, although using only 26 compounds [3].

The QSAR model was developed using PyQSAR, a package present in PYTHON programming language and Jupyter Notebook software. The OCHEM molecular descriptor calculation tool was used to calculate a total of 8.235 molecular descriptors from which PyQSAR selected 4 descriptors: B06 [C-O], Eig04_AEA (dm), JGI2 and J_Dz (p), in order to build the QSAR model. With this model, an equation was obtained capable of making a prediction of the antioxidant activity (pIC50) of new compounds (**Figure 1**). This model presented quite satisfactory statistical data, such as the correlation coefficient ($R^2=0.8905$), the square root value of the average error (RMSE=0.2328) and the mean score value of the multiple linear regression method ($Q^2CV=0,8676$).

Finally, this model proved that it can be a useful tool for the prediction of free radical scavenging capacity of compounds with similar di(hetero)aryl structures and may guide the synthesis of new compounds with better antioxidant capabilities or the analysis of natural compounds with similar structures.

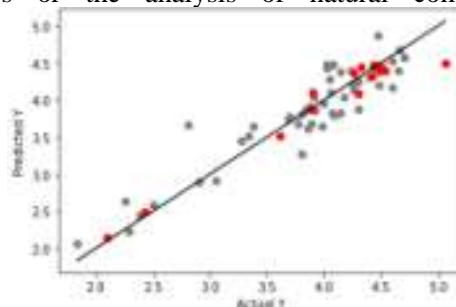


Figure 1: Correlation between experimental and predicted antioxidant activity for the 70 di(hetero)aryl compounds.

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PCH-19

BIOHERBICIDE CYNARA: WEED CONTROL THROUGH NATURAL RESOURCES FOR SUSTAINABLE AGRICULTURE

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Weeds represent a negative impact on agriculture, decreasing production yields by 34%. [1] After decades of extensive chemical control, herbicide-resistance increased significantly and bioaccumulation of synthetic compounds in the environment has caused great concerns not only on human health but also to soil and ecosystems health. [1] As the world population continues to grow, much more effort and innovation will be needed in order to sustainably increase agricultural production. [2] As an alternative to synthetic compounds, allelochemicals – secondary metabolites from plants – has gained attention for their influence on the growth and development of agricultural and biological systems, demonstrating to be a sustainable tool for weed management. [3] A vast list of allelochemicals has already been described due to their phytotoxic activity, namely Sesquiterpene Lactones (SL). SL, abundantly present in *Cynara cardunculus* leaves (Ccl) (95 mg/ g DW), [4] have recently been described for its allelopathic effect on common target species showing strong evidence of their phytotoxicity and potential use as bioherbicides. [5, 6] The main objective of this PhD proposal is to develop a green bioherbicide based on Ccl extract, rich in SL to be used in olive orchards, grapevines and cereals production. Ccl extract prepared by ultrasound assisted extraction methodology [7], followed by membrane nanofiltration technology to obtain Ccl-derived SL rich fractions [8], will be chemically characterized using HPLC-MS/MS/MS. Phytotoxic activity will be assessed using standard wheat coleoptile bioassay and a panel of weed species commonly found on Mediterranean agriculture crops. Formulations will be developed considering type, SL stability in solution and ecological excipients. The formulations produced will be first tested in vitro against weeds, at different stages of the plant development in order to observe physiological effects, and then tested on crop fields to verify phytotoxicity potential.

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PCH-20

BIOLOGICAL ACTIVITY OF CHICORY EXTRACTS AND COMPOUNDS

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Cichorium intybus L. (Asteraceae) or chicory is a leafy biennial or perennial plant, with a strong fleshy taproot, that can grow up to 75 cm in length. Chicory has a long history being used as medicinal plant. The major sesquiterpene lactones (STLs) of chicory belong to the class of guaianolide sesquiterpene lactones and are commonly derived from a single sesquiterpene, germacrene A. The main STLs in chicory are lactucin, lactucopicrin, 8-deoxylactucin and their oxalate and glucoside derivatives.

The aim of this study was to screen the different types of chicory extracts and terpene compounds for their bioactivities and to understand better how the valuable currently unused part of chicory could be taken to use.

Antimicrobial activity of terpene samples was tested via analysing the type of growth inhibition (biostatic or biocidal) using modified microdilution method. For the determination of the antimicrobial activity of terpene samples, a modified bioautographic method was used as well. Impact of extracts and compounds on biofilm formation were determined on *Candida albicans* 475/15 and *Pseudomonas aeruginosa* (AMP_R). Cristal violet assay was used for the determination of antiproliferative effect, on human gingival fibroblasts cells HGF-1 (ATCC® CRL-2014™).

It was observed that EtOAc extract of chicory root showed good antimicrobial potential against all tested bacteria (MIC 0.75-3 mg/mL) except for *P. aeruginosa* (AMP_R) strain, while water root extract did not display antimicrobial effect with tested concentrations. Costunolide, lactucopicrin, 11 β ,13-dihydrolactucin, compounds found in chicory roots, showed the most prominent effect against resistant strain of *P. aeruginosa*. All tested standard compounds demonstrated very good antifungal activity against *Candida* species (MIC 0.03-1.00 mg/mL). By using bioautographic method we have shown that none of the extract fractions were active against *Escherichia coli*, while two fractions of EtOAc extract were active against *P. aeruginosa*. EtOAc extract of chicory showed the most promising activity in inhibiting *C. albicans* biofilm formation, while slightly lower activity was observed for compound parthenolide. Water extract did not exhibit any cytotoxicity up to 1 mg/mL. On the other hand, EtOAc extract was cytotoxic to HGF-1 cell at GI₅₀(%) concentration of 0.750 mg/mL. As for the tested compounds, 11 β -13-dihydrolactucin was the less toxic to HGF-1 cells. All other compounds were toxic at the lowest concentration used.

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PCH-21

ANTI-INFLAMMATORY AND IMMUNOMODULATORY ACTIVITY OF *LENTINULA EDODES* EXTRACTS

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The use of mushrooms for their medicinal and nutritional properties has been reported throughout history. Nowadays, the market for edible mushrooms is dominated by *Agaricus bisporus*, followed by *Lentinula edodes* and *Pleurotus* spp.. *Lentinula edodes*, commonly known as shiitake, is the second most cultivated mushroom in the world and classified as a medicinal species. Mushrooms have also been studied for their functional and medicinal properties and their application for pharmaceutical use. Their composition stands out due to the presence of medicinal/bioactive compounds, such as polysaccharides, terpenoids, lipids, and sterols, which have presented prominent results for the treatment of various tumors and infections (e.g. [1]). In this study, lyophilized *Lentinula edodes* mushroom was extracted using an exhaustive hydroethanolic extraction, followed by dialysis and subsequent purification of some fractions using ion exchange chromatography, as described by Lemieszek *et al.* (2018) [1]. In order to obtain extracts free from bacterial contamination, a protocol for endotoxins removal was implemented to prevent further undesired interaction with the macrophages cellular model RAW 264.7. The extracts' effect on RAW 264.7 cells proliferation was evaluated using different concentrations, with cell viability being assessed by the Alamar Blue method [2]. The effect of extracts on anti-inflammatory activity was also evaluated using the same cell line, by quantifying the nitric oxide (NO) released by the Griess method, as described in [3]. It was found that the extracts did not present cytotoxicity in RAW 264.7 cells at any of the tested concentrations. However, significant differences were found in the anti-inflammatory activity as well as immunomodulatory effect of *Lentinula edodes* extracts.

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PCH-22

PHYTOCHEMICAL SCREENING AND CYTOTOXIC POTENTIAL OF SIDEROL ISOLATED FROM CULTIVATED *SIDERITIS EUBOEA* HELDR.

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Diterpenes are characteristic compounds from the genus *Sideritis* L. (Lamiaceae) [1]. One of the major diterpenes of the Mediterranean *Sideritis* species is siderol. The aim of the present study was the phytochemical screening of the non-polar extract from the aerial parts of the cultivated *S. euboea* Heldr. by the means of NMR spectroscopy. Rapid characterization through 1D ¹H NMR spectrum of the total crude plant extract enabled us to track the characteristic proton signals of siderol, as well as to spot the main chemical groups of the included specialized products. Overall, eleven compounds, one fatty acid ester, 2-(p-hydroxyphenyl)ethylstearate; three phytosterols, β -sitosterol, stigmasterol, and campesterol; one triterpenoid, ursolic acid; four diterpenoids, siderol, eubol, eubotriol, 7-epicandicandiol and two methylated flavonoids, xanthomicrol and penduletin were isolated from the specific extract, using several analytical techniques. Furthermore, the cytotoxic activity of siderol was evaluated by the MTT assay on three cancer cell lines DLD1, HeLa, and A549. The IC₅₀ values were 26.4 ± 3.7, 44.7 ± 7.2, and 46.0 ± 4.9 μ M, respectively. As a result, siderol demonstrated the strongest activity in the human colon cancer cell line DLD1. To explore the potential similarity of the isolated chemotype of siderol with other compounds with known bioactivity, we conducted a 3D-based virtual screening in the ChEMBL database [2], unveiling its chemical similarity to chemotypes with a broad range of biological activities. Of great interest was its similarity with another anticancer compound of colon cancer, confirming its potent cytotoxic effect in the tested DLD1 cell line. The current study revealed the beneficial potential of siderol as a remarkable cytotoxic agent and the powerful contribution of NMR spectroscopy in the isolation and identification of natural products. As far as we know, this is the first study to report the phytochemical content of the dichloromethane extract of this cultivated species, as well the cytotoxic potential of siderol [3].

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PCH-23

PINE NUT SHELLS AS A SOURCE OF BIOACTIVE COMPOUNDS: HEALTH-PROMOTING POTENTIAL

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The agro-industrial sector generates a high quantity of by-products and waste with low economic value, making it urgent to seek viable solutions for their valorisation and better use of natural resources. *Pinus pinea* L. is a forest specie widely present in the Iberian Peninsula and is well known for the economic importance of its edible pine nuts. Pine nut shells, the main by-product obtained from this industry, represent over 77% of the weight of the whole pine nut and are not presently valorised [1]. Although the studies concerning the characterization of *Pinus pinea* pine nut shell are very limited, literature data suggests promising results concerning the potential use of this by-product as a cheap source of bioactive compounds. *P. pinea* shells are reported to have a total phenolic content similar to that found in walnut shells [2]. Among the phenolic compounds identified in the shells of *Pinus pinea* pine nuts, vallinic acid, syringic acid and isoferulic acid are the most commonly cited. Also, the antioxidant capacity of a pine nut shell polar extract was found to be superior to that present in walnut and almond shells. In addition, other compounds such as triterpenoids/triterpenes (isopimaric acid, lupeol, betulinic acid) and sterols (campesterol, stigmaterol, B sitosterol) have been already identified in lipophilic extracts of pine nut shell [2]. The preceding compounds have been investigated for pharmacological prospects, regarding their antihypercholesterolemic, anticarcinogenic, hypoglycaemic, antimutagenic, antioxidant, anti-inflammatory and antimicrobial potential.

Thus, this work aims to comprehend the health-promoting potential of pine nut shell compounds, aiming their prospective integrated valorisation as added-value compounds.

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PCH-24

**EFFECT OF HIDROETHANOLIC EXTRACT OF *LAVANDULA PEDUNCULATA* (MILL.) CAV.
ON MORPHOMETRIC PARAMETERS IN HPV-16 TRANSGENIC MICE**

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Lavandula pedunculata (Mill.) Cav., common English name French lavender, belongs to the Lamiaceae family and has been used as a medicinal plant in infusions for respiratory and digestive systems and as a therapeutic agent with antiseptic action for cleaning wounds [1,2]. The K14HPV16 mice is a skin squamous carcinoma model that can be used to test the antitumoral properties of several chemical and natural products [3]. The aim of this work was to evaluate the effect of the hydroethanolic French lavender extract (FLE) on body weight, relative organs weights, food and water consumption in an HPV-16-transgenic mice model. The extract was obtained from a maceration with ethanol/water (80:20, v/v), and the phenolic composition was determined through HPLC-DAD-ESI/MS. Twenty-eight male mice were randomly divided into four groups (n=7/group) according to their genotype: group I (HPV16- control); II (HPV16- FLE); III (HPV16+ control) and IV (HPV16+ FLE). The FLE was administrated orally in drinking water at 6.8 mg/10mL/animal to animals from groups II and IV and changed every 4 days. The animals were kept under controlled conditions such as temperature, light and humidity. Food and water were kept *ad libitum* regime. Animals' body weight, food and water consumption were measured weekly as well as animal welfare. After twenty-nine days, all animals were sacrificed by anaesthetic overdose and blood was obtained from cardiac puncture. The organs were collected and immediately weighted. Data was analysed using SPSS 25. The differences were considered statistically significant at $p < 0.05$. A total of thirteen compounds were identified in the hydroethanolic extract, being salvianolic acid B and rosmarinic acid the main molecules present. Moreover, the compounds revealed to be stable in the drinking water during the 5 tested days. HPV animals exposed to FLE (group III) showed higher values of body weight variation than HPV animals not exposed to lavender in week 1, 2 and 3 ($p < 0.05$), suggesting that the FLE was highly palatable. However, the values of food consumption were identical between groups and water intake was higher in transgenic animals as expected. The relative organ weight of heart, lung, kidneys, adrenals and liver did not demonstrate differences between groups ($p < 0.05$). According to our results the consumption of French lavender demonstrated a favourable and safe toxicological profile using these experimental conditions.

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PCH-25

CASSAVA STARCH SPOUTED BED FOR MANUFACTURING INCLUSION COMPLEXES OF *Equisetum hyemale* EXTRACT

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Equisetum hyemale Linnaeus. (Equisetaceae) is a species rich in phenolic compounds, flavonoids, and alkaloids [1,2,3], being used in traditional medicine to treat hypertension, stroke and inflammatory diseases [2]. This study verified the best condition for the coating of granules of cassava starch, with polymeric solution containing *E. hyemale* liquid extract, with the purpose of protecting and slowly releasing the chemical markers of the plant. A Box-Behnken factorial design (3^3) [4], varying the granule load (80g; 120g; 160g), flow of the coating solution (1mL / min; 2mL / min; 3mL / min) and percentage polymeric solution (1%; 2%; 3%) was employed to find the best condition of granulation coating regarding the contents of phenols and flavonoids [5,6]. The determination test of the levels of phenols and flavonoids for each experiment allowed to observe a correlation between the variables of load, flow of the coating solution and HPMC content applied. The ANOVA showed that linear and quadratic effects exerted effect on the total phenols (TP). The fitted equation in terms of coded factors presented $R_{adj}^2 = 0.85$. Thus, the results presented here was suitable to predict the best condition to apply *E. hyemale* liquid extract in cassava starch. The knowledge gained from this study should be useful for further exploitation and application of the resource.

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PCH-26

NEUROPROTECTIVE EFFECTS OF MULTICOMPONENT CHINESE HERBAL TEAS

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The Traditional Chinese medicine is one of the oldest medical systems and was developed in China about 3000 years ago. One of its branches, the Chinese herbal medicine, has a long history of use for prevention and treatment of several diseases, including mental health, with several preparations being prescribed against major depressive disorder (MDD) and dementia [1,2].

In this study, four multicomponent Chinese herbal teas were evaluated, for the first time, against two enzymes related with Alzheimer's disease (acetylcholinesterase – AChE - and butyrylcholinesterase - BuChE) and MDD (monoamine oxidase A – MAO-A). Regarding cholinesterases inhibition, the most promising ones were the Shangxi Yiyang tea (IC_{50} [AChE] = 619 μ g/mL and IC_{50} [BuChE] = 2327 μ g/mL), the Wei Er Le tea (IC_{50} [AChE] = 3889 μ g/mL and IC_{50} [BuChE] = 3645 μ g/mL), and the Honeysuckle tea (IC_{50} [AChE] = 3544 μ g/mL and IC_{50} [BuChE] = 3826 μ g/mL). On the other hand, the order of potency against MAO-A was: Shangxi Yiyang tea > Shangxi Fuyi tea > Wei Er Le tea > Honeysuckle tea. These bioactivities can be correlated with the total phenolic content (TPC) of the samples, since the Shangxi Yiyang tea is the most potent one and showed the highest TPC value (210 μ g GAE/mg dried extract).

The results obtained showed the potentialities of these phenolic-rich multicomponent herbal formulations to affect three different targets implicated in the etiology of brain disorders.

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PCH-27

EFFECTS OF *MYRCIARIA JABOTICABA* PEEL EXTRACT ON THE OXIDATIVE STATE OF THE BRAIN, LIVER AND PLASMA OF RATS WITH ADJUVANT INDUCED ARTHRITIS

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Adjuvant-induced arthritis (AIA) is an experimental immunopathology in rats that shares many characteristics of rheumatoid arthritis in humans and is often used as a model for this disease [1]. Jaboticaba, *Myrciaria jaboticaba*, is a fruit native to Brazil and has been widely studied due to its high nutritional content and for being a good source of phenolic compounds. These compounds have antioxidant and anti-inflammatory activity [2]. The objective of the study was to investigate the actions of the *M. jaboticaba* peel extract (250 and 500 mg/Kg) on the oxidative state of the brain, liver and plasma of arthritic rats. For the induction of arthritis Freund's complete adjuvant was used. Controls and arthritic animals were treated once a day by oral administration (gavage) of the extract during 23 days. Healthy control rats received water for the same period. Oxidative damage markers (TBARS and protein carbonyl groups) and the activity of antioxidant enzymes were determined by spectrophotometry. All procedures were previously approved by the Ethics Committee for Animal Experimentation. Catalase (CAT) and superoxide dismutase (SOD) activities were reduced (24% e 34% respectively) in the brain of arthritic rats when compared to the control animals. *M. jaboticaba* extract 250 and 500 mg/Kg normalized the brain CAT activity in arthritic rats. Brain SOD activity was maintained close to control levels by treatment with the lower dose of the extract. However, the higher dose of the extract (500 mg/Kg) increased the SOD activity in the brain above to the control by 66%. Levels of TBARS were 21% higher in the liver of nontreated arthritic rats (compared to the controls). Treatment of arthritic rats with 500 mg/Kg of the extract maintained lipoperoxidation levels close to the control ones. SOD activity was 23% lower in the liver of arthritic rats. Treatment with 500 mg/Kg of the extract increased SOD activity in the liver to values close to the control. Levels of protein carbonyl groups were 110% higher in the plasma of arthritic rats (compared to the controls). Treatment of arthritic rats with 250 and 500 mg/Kg extract reduced this parameter by 23% and 21% respectively. The results showed that *M. jaboticaba* peel extract was able to reduce the levels of oxidative damage in lipids and proteins mainly in the plasma and improve the enzymatic antioxidant defense system, protecting tissues from oxidative stress induced by arthritis.

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PCH-28

***PTEROSPARTUM TRIDENTATUM*: PHYTOCHEMICAL PROFILE AND ANTIOXIDANT ACTIVITY OF METHANOLIC EXTRACTS**

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Pterospartum tridentatum is a fabaceae plant native of the North-West Iberian Peninsula and Morocco [1]. This plant is used in folk medicine for the management of several pathologies as diabetes [2, 3, 4, 5], hypertension [3, 5, 6], respiratory [3, 4, 5, 6, 7] and digestive disorders [3, 6, 7, 8]. The aim of this work was i- to produce methanolic extracts (7:3, v/v) of leaves (PtL) and flowers (PtF) of *Pterospartum tridentatum* collected in the littoral of Portugal (Esposende), ii- to determine the antioxidant activity of the extracts, and iii- to correlate it with total phenolic content by Folin-Ciocalteu spectrophotometric method and, iv- identified the phenolic compounds present in the extracts by HPLC DAD. Both extracts exhibited strong antioxidant activities, as evidenced by the low EC50 values in both 2,2-diphenyl-1-picryl hydrazyl (DPPH) (PtL: 101 ± 23 µg/ml; PtF: 116 ± 19 µg/ml), nitric oxide (PtL: 154 ± 72 µg/ml; PtF: 185 ± 72 µg/ml) and superoxide (PtL: 42 ± 8 µg/ml; PtF: 47 ± 8 µg/ml) scavenging methods and Iron Chelating Activity (ICA) (PtL: 199 ± 40 µg/ml; PtF: 161 ± 41 µg/ml). Additionally, antioxidant activity and the total phenolic content demonstrated a strong positive correlation. Phytochemical profile of the extracts, characterized by HPLC-DAD, demonstrated the presence of several phenolic acids and flavonoids; these compounds could contribute to the antioxidant activity. Our work highlights the potential of *Pterospartum tridentatum* methanolic extracts as a source of compounds for the development of new therapies targeting oxidative stress-related diseases.

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PCH-29

BIOACTIVE COMPOUNDS OF OLIVE SEEDS FROM THREE CULTIVARS WITH VALUABLE BIOLOGICAL PROPERTIES

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The present work aimed to evaluate the phytochemical composition of seed extracts from different olive cultivars (Cobrançosa, Galega and Picual), as well as their antioxidant capacity. The content of total phenols ranged between 11.90 ± 1.56 and 14.71 ± 1.37 mg GA g⁻¹ (Table 1). Regarding *ortho*-diphenols and flavonoids content, Galega seed extracts revealed to present significantly higher concentrations than Cobrançosa and Picual samples with 7.05 ± 0.44 mg GA g⁻¹ and 2.40 ± 0.18 mg CAT g⁻¹, respectively. The antiradical scavenging capacity of phenolic olive seed extracts have been performed by ABTS and DPPH methods. As expected, Galega cultivar samples presented the highest activity compared to the other cultivars studied with 0.065 ± 0.004 and 0.016 ± 0.002 mmol Trolox g⁻¹ for ABTS and DPPH, respectively (Table 1)

Table 1. Total phenols (mg GA g⁻¹DW), *ortho*-diphenols (mg GA g⁻¹DW), and flavonoids (mg CAT g⁻¹DW) content and antioxidant capacity (mmol Trolox g⁻¹DW) of olive seeds from different cultivars.

	Phenolic content			Antioxidant Capacity	
	Total phenols	<i>Ortho</i> -diphenols	Flavonoids	ABTS	DPPH
Cobrançosa	11.90 ± 1.56^a	4.69 ± 0.16^a	1.50 ± 0.20^a	0.054 ± 0.005^a	0.004 ± 0.000^a
Galega Vulgar	14.71 ± 1.37^a	7.05 ± 0.44^b	2.40 ± 0.18^b	0.065 ± 0.004^b	0.016 ± 0.002^b
Picual	13.03 ± 0.10^a	4.00 ± 0.28^a	1.40 ± 0.05^a	0.057 ± 0.002^{ab}	0.006 ± 0.001^a
<i>P</i> -value	N.S.	***	**	*	***

^zData presented as Mean (n=3) \pm SD values for the same parameter evaluated followed by different superscript lowercase letters are significantly different at $p < 0.001$, according to Tukey's test.

^y Level of significance: N.S.: not significant ($p > 0.05$); *significant at $p < 0.05$; **significant at $p < 0.01$; ***significant at $p < 0.001$.

The HPLC–DAD–ESI/MSn screening of the three cultivars of olive seeds studied revealed similar chromatograms. Six phenolic compounds were identified and recorded at 280 nm: one phenyl alcohol, two flavonoids, three secoiridoids. The most abundant compounds are nüzhenide (32.45 mg g⁻¹, on average), ligstroside (4.52 mg g⁻¹, on average), and rutin (3.08 mg g⁻¹, on average). Another three phenolic compounds were found in Cobrançosa, Galega, and Picual cultivars, namely tyrosol, luteolin-7-glucoside, and oleuropein, values ranging between 2.84 ± 0.02 and 2.90 ± 0.04 mg g⁻¹, between 2.10 ± 0.00 and 2.21 ± 0.00 mg g⁻¹, and between 2.35 ± 0.01 and 2.57 ± 0.02 mg g⁻¹, respectively.

Some studies allowed to identify some phenolic compounds in olive seeds, however, very few investigations have quantified these compounds in this matrix. Overall, our results indicate that olive seeds extracts may provide a new and alternative source of agents for medical and industrial applications.

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EFEITOS TERAPÊUTICOS DO GENGIBRE (*Zingiber officinale*): REVISÃO DE LITERATURA

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Ao longo dos anos e devido ao avanço da medicina, cada vez mais há um interesse pela fitoterapia, que consiste num conjunto de técnicas de utilização dos produtos de origem vegetal com finalidade terapêutica, ou seja, para prevenir ou curar doenças. De entre as diversas plantas estudadas na fitoterapia podemos destacar o gengibre pelas suas propriedades farmacológicas, sendo dotado de uma rica composição nutricional, conforme descrito na Tabela 1, funcionando como fonte de fibra e potássio, o que faz com que seja bastante comercializado e utilizado na medicina. Este trabalho foi realizado através de pesquisas realizadas em diversos sites online. Utilizaram-se as palavras-chaves: Gengibre, *Zingiber officinale*, propriedades terapêuticas e farmacológicas. Foram utilizados artigos em língua portuguesa, sendo selecionados os que apresentavam informações relevantes para a elaboração do trabalho e foram excluídos artigos de pouca credibilidade. O objetivo deste trabalho foi apresentar e discutir os efeitos terapêuticos do gengibre, incluindo o uso em geral desta planta para fins medicinais. Os trabalhos encontrados na literatura apontam o gengibre como uma planta originária do sul da Ásia, de onde se difundiu para outras regiões do mundo, tendo reconhecimento na Europa na época dos descobrimentos tendo sido trazido da Índia juntamente com outras especiarias. Em Portugal existe registro da sua presença a partir do século XVI mas antes disso já era utilizada há mais de 2000 anos no Oriente. Nos últimos anos a fitoterapia tornou-se mais popular e enquanto que a utilização no passado era feita na maioria das vezes de forma empírica, podemos hoje em dia identificar os componentes ativos de cada planta e com a nova compreensão da fisiologia humana, explicar o seu mecanismo de ação. Quanto ao gengibre, temos descrito na Literatura Científica as principais propriedades e efeito farmacológicos sendo dotado de ação antimicrobiana, anti-inflamatória, diurética, antipirética, hepatoprotetora e antioxidante.

Valores nutricionais por 100g de Gengibre		
Valores por:		% Dose de referência*
Energia (Kcal)	81	4
Lípidos Totais (g)	1	1
Ácidos Gordos Saturados (g)	0	0
Hidratos de Carbono (g)	16	12
dos quais açúcares (g)	2	2
Fibra (g)	2	7
Proteínas (g)	2	4
Sal (g)	0,33	6
Vitamina E (mg)	1,1	9
Vitamina B6 (mg)	0,2	14
Potássio (mg)	415	21
Magnésio (mg)	43	11

* Doses de referência de acordo com Regulamento da União Europeia nº1169/2011 de 25 de outubro de 2011.

Tabela 1: Valores Nutricionais do Gengibre (*Zingiber officinale*).

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PCH-31

CATHARANTHUS ROSEUS FLOWERS AQUEOUS EXTRACT INEFFECTIVENESS LARVICIDAL ACTIVITY AGAINST AEDES AEGYPTI L. IN VITRO.

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The mosquito species *Aedes aegypti* L. are important vectors of several diseases, and control of these insects is imperative for human health. The use of insecticides for prolonged periods to control vectors without adequate monitoring of the population in relation to its consequences may promote the mosquito vectors resistant populations selection [1]. With the growing concern for the mosquito borne diseases, urge for different botanicals as mosquitocidal or larvicidal agents has increased.

Catharanthus roseus L. belongs to the *Apocynaceae* family, is native to Madagascar, and has compounds that can stimulate the microtubules depolarization, to β -tubulins binding for polymerization preventing vinblastine and vincristine [2]. Vincristine has anti-cancer activity and toxicity different from vinblastine, even though it has similar chemical characteristics [3]. Because of all this fact, the *Catharanthus roseus* L. flowers aqueous extract was tested, in of 10, 50 and 100 $\mu\text{g/ml}$ concentrations, on the *Aedes aegypti* L. L4 larvae, for a period of 48 hours (**Figure 1**). Its flowers aqueous extract proved to be harmless to the larvae of the mosquito *Aedes aegypti* L., under the conditions tested here.

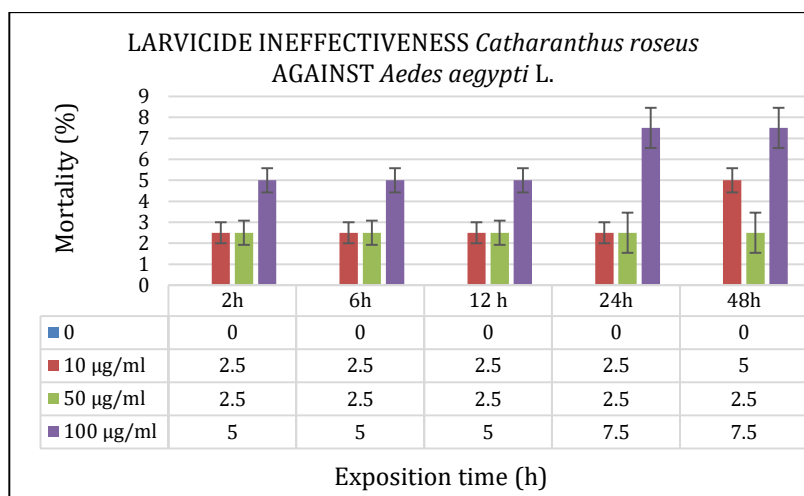


Figure 1. Larvicide ineffectiveness *Catharanthus roseus* flowers aqueous extract against *Aedes aegypti* L.

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PCH-32

TOPICAL ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITY OF COENZYME Q10

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Coenzyme Q10 (CoQ10), or ubiquinone, is a lipid-soluble benzoquinone, with a long isoprenoid side chain, which has antioxidant and photoprotective properties [1]. It is commercialized as a food supplement and used in cosmetic formulations. Although some studies have already shown that CoQ10 has *in vitro* [2] and *in vivo* [1] anti-inflammatory activity, no studies have demonstrated the effects of topical application of CoQ10 on a model of skin inflammation. Thus, the aim of this work was to investigate the effects of CoQ10 administered topically on ear edema induced by croton oil (OC) in mice. Swiss mice weighing between 30-40 g were used. The protocol was approved by the Animal Experimentation Ethics Committee of the State University of Maringá (protocol n. 9987080420). Ear edema was induced by the application of croton oil to the inner ear of mice. Animals received CoQ10 (0.03 to 0.5 mg/ear) or dexamethasone (positive control, 0.1 mg/ear) topically, dissolved in acetone. After 6 hours, animals were euthanized, ears were sectioned in circular discs of 6.0 mm in diameter and weighed. Myeloperoxidase [3] (MPO) and N acetylglucosaminidase (NAG) [4] activities were evaluated in the homogenate supernatant of the ear sections (controls and treated). Parameters indicating oxidative stress (reduced glutathione – GSH - content, protein carbonyls, reactive oxygen – ROS - species and antioxidant enzymes) were measured in ear tissue homogenate [3]. Antioxidant capacity of CoQ10 was also evaluated *in vitro* in chemical assays and on the real time ROS generation using mice liver isolated mitochondria [5]. The results obtained in the present study demonstrated that the topical application of CoQ10 was able to significantly reduce the ear edema induced by croton oil and the migration of polymorphonuclear and mononuclear leukocytes (inferred as the reduced activities of MPO and NAG). Additionally, topically applied CoQ10 reduced ROS content in the tissue and normalized the reduced GSH content, although it did not improve catalase activity or protein carbonylation. The *in vitro* experiments demonstrated a low direct antioxidant activity of CoQ10 in chemical tests (FRAP and ABTS). However, CoQ10 was able to significantly reduce real time generation of ROS in mice liver isolated mitochondria. Therefore, our results clearly demonstrate that topically applied CoQ10 is able to reduce the skin inflammatory process and improve some oxidative stress parameters in the croton oil ear edema model in mice. Additionally, *in vivo* antioxidant properties could be partially related to the ability of CoQ10 to reduce mitochondrial ROS generation. At least to our knowledge, this is the first report of a direct topical anti-inflammatory effect of CoQ10 in a *in vivo* model of skin inflammation.

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PCH-33

NEW INSIGHTS INTO ANTI-STREPTOCOCCAL POTENTIAL OF SELECTED ESSENTIAL OILS RELEVANT TO DENTISTRY

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Essential oils (EOs) are potent antibacterial agents that have numerous applications in different fields, such as traditional medicine, pharmacy and cosmetics, food and beverage industry. However, their use in dentistry is limited and not adequately explored. In order to investigate the potential to expand their applications in dentistry, we screened for antibacterial and antibiofilm activity of selected EOs toward *Streptococcus salivarius* dental isolates. Selected EOs were originated from *Pelargonium roseum*, *Pelargonium graveolens*, *Leptospermum petersonii* and *Eucalyptus citriodora*. In order to take insight into the variation in sensitivity among bacterial strains of different origin, both isolates from symptomatic and asymptomatic root canal infections, as well as the reference *S. salivarius* ATCC 9222 strain, were involved in this study. EOs were chemically characterized by GC/MS-FID analysis. Microdilution assay, was used to determine minimal inhibitory concentrations (MICs). Crystal violet (CV) assay was provided to explore the potential to disturb biofilm formation. Statistical analysis performed by One Way ANOVA was used to compare sensitivity between tested strains.

Chemical characterization revealed that all EOs were abundant in oxygenated monoterpenes (76.8 – 92.4% composition). Furthermore, the content of sesquiterpene hydrocarbons was relatively high in *P. roseum* and *P. graveolens* EOs (12.3% and 16.7%, respectively). The major constituents of *Pelargonium* oils were citronellol, geraniol, citronellyl formate and geranyl formate, contributing together with 71.3% and 57.1% of total *P. roseum* and *P. graveolens* EOs, respectively. EO of *L. petersonii* was abundant with following dominant constituents: geraniol, neral, citronellal, citronellol and isopulegol (accounting for 81.2% of the total EO). Some of these constituents, namely citronellal, citronellol and isopulegol, were also dominant in *E. citriodora* oil, constituting 84.8% of its composition. MIC assay demonstrated that referent strain was the most sensitive, while the strains isolated from symptomatic infections were highly resistant. Considering all tested strains, the comparison of EOs antibacterial activity pointed out the following order, calculated in mg mL⁻¹: *E. citriodora* (MIC values 0.08±0.03 – 5.42±1.71) > *L. petersonii* (MIC values 0.53±0.00 – 4.74±0.64) > *P. graveolens* (MIC values 0.36±0.15 – 5.54±0.00) > *P. roseum* (MIC values 1.50±0.68 – 13.77±0.69). The inhibition of biofilm formation by EOs was also strain specific; clinical isolates were less sensitive than the reference strain (the ranges of biofilm inhibition were 24%-75% and 84%-89%, respectively). Similarly as in the microdilution assay, the *E. citriodora* EO was the most potent biofilm inhibitor.

Taking into account all obtained results, *Eucalyptus citriodora* EO proved promising antibacterial and antibiofilm activity and could be recommended for further investigation as alternative antimicrobial in dental practice.

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PCH-34

CHEMICAL CHARACTERISATION OF CHOKEBERRY SEED OILJelena Živković^{1*}, Mihailo Ristić¹, Angelika Malarski², Volker Böhm²¹Institute for Medicinal Plants Research “Dr. Josif Pančić”, Tadeuša Košćuška 1, 11070 Belgrade, Serbia;²Institute of Nutrition, Friedrich Schiller University Jena, Dornburger Straße 25-29, 07743 Jena, Germany.

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Numerous reports state that introduction of vegetable oils in the diet has beneficial effects in prevention of cardiovascular diseases, preserving healthy cholesterol levels, improving brain function and fighting with free radical species. The interest in finding newer sources of oils for edible and non-edible applications such as unconventional plant seeds increased recently. Although there is a broad range of vegetable oil sources, world consumption is prevailed by palm, soybean, rapeseed and sunflower oils [1]. Black chokeberry (*Aronia melanocarpa*) fruits are particularly rich in polyphenol compounds and are among the fruits with the highest antioxidant activity [2]. In the last decade, there is an increasing research interest in black chokeberries, still only small number of studies has been dedicated to investigation of chemical and pharmacological potential of oils obtained from its seeds. The aim of the present study was to explore the composition (fatty acids, carotenoids and tocopherols) of fatty oil obtained from chokeberry seeds.

Oil was extracted from ground seeds using Soxhlet extraction with *n*-hexane. Before GC/MS analysis, fatty acids were converted to fatty acid methyl esters. Tocopherols and tocotrienols were analysed using a normal-phase HPLC equipped with a fluorescence detector. Carotenoids were analysed using reversed-phase HPLC with diode array detector.

Accurate composition of fatty acids is important in order to determine possible adulterations and overall stability of the product. According to our results, unsaturated fatty acids comprised around 90% of all of the fatty acids. The predominant fatty acids in chokeberry seed oil were linoleic acid (80%) and oleic acid. Tocopherols and carotenoids have strong antioxidant activity and their main role is to protect unsaturated fatty acids against oxidation [18]. In the seed oil chokeberry two tocopherols were detected. The dominant one was α -tocopherol (133 mg/100 g oil) followed by β -tocopherol (14 mg/100 g oil). Concerning carotenoids, (*E*)- β -carotin followed by lutein were predominant in the oil. (13*Z*)- β -Carotin, zeaxanthine, (9*Z*)- β -carotin and α -carotin were also present. The total amount of carotenoids was 31.88 μ mol/100 g of oil.

In summary, the results obtained in our study demonstrated that from a nutritional viewpoint seeds of *A. melanocarpa* may have potential as non-traditional sources of oils due to high content of antioxidant compounds.

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PCH-35

EVALUATION OF ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES OF A *Luehea divaricata* MARTIUS EXTRACT

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The search for herbal medicines has grown in recent years, what confers a strong importance to studies about active plant of compounds. *Luehea divaricata* Martius is a tree native to Brazil that is among those widely used in folk medicine as a herbal medicine against various diseases [1]. This work aims to evaluate the antioxidant and antimicrobial activities of the hydroethanolic extract of the bark of the *L. divaricata* stem. The extract was prepared by adding 20 mL of 70% ethanol to 1 g of the *L. divaricata* bark. The mixture was filtered, frozen and lyophilized. The antioxidant activity of the extract was evaluated by two methods: reduction of the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) and reduction of 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) cation (ABTS⁺). Both tests were performed as described previously [2]. The results were expressed as IC₅₀ values (sample concentration that causes 50% inhibition of radical reduction). The antimicrobial and antifungal activity of the extract was evaluated by the microdilution method, in which Gram-negative (*Escherichia coli*, *Salmonella typhimurium*, *Enterobacter cloacae*) and Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus cereus* and *Listeria monocytogenes*) were used. The antifungal activity was evaluated against *Aspergillus fumigatus*, *Aspergillus ochraceus*, *Aspergillus niger*, *Penicillium funiculosum*, *Penicillium ochrochloron* and *Penicillium verrucosum*. The results were expressed as the minimum extract concentrations that completely inhibited bacterial growth (MICs), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC). The bark extract of *L. divaricata* showed high antioxidant capacity. An IC₅₀ of 13.31 µg/mL was found for DPPH bleaching and 7.30 µg/mL in the ABTS assay. Regarding the antimicrobial activity, the values referring to the minimum inhibitory, bactericidal and fungicidal concentrations of the extract are presented in **Table 1**. When analyzing the results, it appears that the *L. divaricata* bark extract is more active against fungi than against bacteria. The inhibitory activity presented by the extract indicates the presence of an extensive spectrum of phytochemical constituents with antibiotic potential. Based on the results it can be concluded that the hydroethanolic extract of the stem bark of *L. divaricata*, in addition to having a high antioxidant capacity, also presents antimicrobial activity.

Table 1: Antibacterial and antifungal activities of *L. divaricata* stem bark extract (mg/mL)

		<i>S. aureus</i>	<i>B. cereus</i>	<i>L. monocytogenes</i>	<i>E. coli</i>	<i>S. typhimurium</i>	<i>E. cloacae</i>
Antibacterial activity	MIC	3	1.5	1.5	1.5	3	3
	MIB	6	3	3	3	6	6
		<i>A. fumigatus</i>	<i>A. niger</i>	<i>A. ochraceus</i>	<i>P. funiculosum</i>	<i>P. verrucosum</i>	<i>P. ochrochloron</i>
Antifungal activity	MIC	0.75	0.75	0.75	0.75	0.75	1.5
	MFC	1.5	1.5	1.5	1.5	1.5	3

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PCH-36

ROOT OF *RUMEX ACETOSA* AS A POSSIBLE ANTIBIOTIC ADJUVANT

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The discovery of antibiotics was one of the most relevant breakthroughs of modern medicine. Antibiotics allow humans to live longer and with more quality of life because they can control bacterial infections by killing or inhibiting bacterial growth. However, due to acquisitions of genes, genetic changes and nonheritable phenomena, bacteria are able to survive and grow in the presence of antibiotics [1]. For this reason, the use of natural products has increased and, consequently, several microbiological studies have been conducted with plants. Common plants of our community could be an excellent way to fight this issue [2]. This study was designed to explore the antibacterial properties of the roots of *Rumex acetosa*. The extraction of the root's phenolic compounds was performed with two different solvents: a water/ethanol (20:80) mixture and an absolute solution of methanol. The extracts were redissolved in dimethyl-sulfoxide (DMSO) to five different concentrations (100, 75, 50, 25, 10 mg/mL). The antimicrobial potential was screened against nine pathogenic bacteria (*L. monocytogenes*, *E. faecium*, *E. faecalis*, *B. cereus*, *S. aureus*, *S. epidermidis*, *P. aeruginosa*, *S. enteritidis* and *E. coli*) using the Kirby-Bauer disk diffusion method. Both of the extraction methodologies showed results against *L. monocytogenes*, *E. faecium*, *S. aureus*, *E. faecalis* and *B. cereus*. The methanol extraction also showed results against *S. epidermidis* with the highest inhibition zone (15 mm). The water/ethanol extraction had a minimum inhibitory concentration (MIC) at 25 mg/mL while the methanol extraction had a MIC at 10 mg/mL. Our work confirms the efficacy of the roots of *R. acetosa* as a natural antibacterial agent and a possible antibiotic adjuvant for the treatment of infectious diseases caused by drug-resistant bacteria.

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PCH-37

TRAMETES VERSICOLOR (L.) LLOYD EXTRACTS: ASSESSMENT OF THEIR ANTIOXIDANT AND NEUROPROTECTIVE EFFECTS

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Mushrooms have been used for medicinal and food purposes for centuries due to their nutritional value and bioactive compounds; however, their pharmacological potential is still underestimated [1]. The reported medicinal properties are vast, including immunity-stimulating, antitumor, antimicrobial, antioxidant, anti-diabetic, anti-hypercholesterolemic, hepatoprotective, and anti-inflammatory effects, among others, and they are usually correlated to different classes of metabolites found in mushrooms (e.g. polysaccharides, phenolic compounds, terpenoids, ergosterols, and volatile organic compounds) [2]. This study aimed to explore the chemical composition, antioxidant and neuroprotective activities of two different extracts (aqueous and methanolic) of *Trametes versicolor*. The aqueous extract showed higher total phenolic and ortho-diphenols contents than the methanolic one which was reflected in stronger scavenging of ABTS radical. Extracts were also evaluated against monoamine oxidase A (an enzyme implicated in neuropsychiatric disorders) and B (involved in Parkinson's disease). The same pattern was observed for enzyme inhibition, the aqueous extract being also slightly more active against both isoforms of monoamine oxidase. However, none of the extracts displayed anticholinesterase activity. These results highlight the potential of phenolic-rich extracts as potential inhibitors of monoamine oxidases.

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PCH-38

ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF *LENTINULA EDODES* DONKO AND KOSHIN VARIETIES EXTRACTS AGAINST METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS*

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Methicillin-resistant *Staphylococcus aureus* (MRSA), a Gram-positive bacterium, has become the most recurrent global cause of skin and soft tissue infections, belonging to the WHO high priority pathogens list. Effective therapy remains challenging and entails the assessment of novel and successful antibiotics. In this context, mushrooms are considered a valuable source of single and natural compounds to be applied for fighting multidrug resistant bacteria. Consequently, the present study aimed to evaluate, by diffusion disk method and concentration min, *Lentinula edodes* Donko and Koshin methanolic and aqueous extracts against to MRSA isolates from clinical wound infections. Moreover, the phytochemical constituents, as well as the antioxidant activity of these extracts were also assessed. The results showed that there is a strong correlation between the total content of phenolic compounds, antioxidant activity, and antimicrobial activity suggesting that the presence of phenolic compounds may explain the biological effects of the extracts. Highest total phenolic compounds, as well as antioxidant activity was shown by Koshin variety aqueous extract, which presented the more effective antimicrobial activity against MRSA. Although more detailed phytochemical compounds are required to identify the specific antimicrobial compounds in each extract, our results suggest that Koshin variety could be a significant source of compounds for the treatment of global multidrug-resistant pathogens.

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PCH-39

 β -CARYOPHYLLENE ISOLATED FROM CLOVE ESSENTIAL OIL IMPROVES LIPOPOLYSACCHARIDE (LPS)-INDUCED ACUTE LUNG INJURY IN MICE

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Acute lung injury (ALI) is a respiratory failure syndrome characterized by diffuse pulmonary infiltration, edema and severe hypoxemia. Risk factors for ALI include pneumonia, trauma, sepsis, and, more recently, COVID-19 [1], with nearly 30% of patients with COVID-19 developing ALI. The pathophysiology of ALI involves an intense pulmonary inflammatory response associated with edema, macrophages activation and neutrophils recruitment. Neutrophils and activated macrophages secrete reactive species and proteases, which damage the alveolar epithelium. The lipopolysaccharide (LPS)-induced acute lung injury (ALI) is a model of lung inflammation in mice that shares many features of human ALI. (-)- β -caryophyllene (β -Cary) is a sesquiterpene found in essential oils and has been reported to present anti-inflammatory activity [2]. This compound is the second most abundant (6-17%) of the essential oil extracted from clove buds, after eugenol that comprises 70-90% [3]. To date, no study has been carried out to verify whether β -Cary improves the inflammation in ALI. Thus, this work aimed at evaluating the effects of β -Cary isolated from clove oil on LPS-induced ALI in mice, when orally administered. β -Cary from clove bud oil was purchased from Quinari Fragrâncias e Cosméticos Ltda (Ponta Grossa, PR, Brazil) and chemical characterized by gas chromatography with mass spectrometry (GC-MS), revealing a purity of 91%. BALB-c mice were orally treated with vehicle or β -Cary at doses of 50-400 mg/kg. After 1 h, anesthetized animals received 5 mg/kg LPS or saline (control) intranasally to induce ALI. After 24 h, the animals were anesthetized and bronchial alveolar lavage (BAL) and lung tissue was collected. Total and polymorphonuclear cells (PMN) were counted in the BAL and myeloperoxidase (MPO) activity, carbonylated proteins and reduced glutathione (GSH) were measured in the lung tissue. The animal protocol was approved by the Ethics Committee for Animal Experimentation of UEM. Mice of the LPS group showed a significant increase of the number of leukocytes in the BAL, which was related to the migration of PMN. Treatment with 200 and 400 mg/kg β -Cary reduced the leucocytes migration by 30% as well as the number of PMN. The accumulation of neutrophils in the lung was assessed by the MPO activity, which increased after LPS administration. Treatment with 200 and 400 mg/kg β -Cary reduced MPO activity by 50%. Regarding oxidative stress, carbonylated proteins and GSH were, respectively, 60% higher and 35% lower in the lung of LPS group. The treatment with 200 and 400 mg/kg β -Cary practically reestablished the control values for these parameters. Overall, this study suggests that β -Cary improves ALI in mice by means of attenuating the neutrophils migration to the lungs, a phenomenon that also decreases the oxidative stress.

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PCH-40

LARVICIDAL ACTIVITY OF *PARAHANCORNIA AMAPA* (HUBER) DUCKE LATEX AGAINST *AEDES AEGYPTI* L LARVAE.

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The mosquito species *Aedes aegypti* is one of the main vectors of arboviruses, including yellow fever, dengue, Zika, chikungunya, oropouche and mayaro. A vector control remains an important strategy because the absence of vaccines to prevent these diseases and insecticides resistance acquired by the mosquito, contribute to a serious public health problem. The use of insecticides based on natural plant products represents an alternative to chemical insecticides, as they are less harmful to the environment and health, not to mention less toxic to other insects [1].

Tests were carried out with the *Parahancornia amapa* (Huber) Ducke latex, at 0, 10, 50 and 100 µg/ml concentrations, on the *Aedes aegypti* L. L4 larvae, for a period of 48 hours. The results obtained here demonstrate that *P. amapa* latex showed effective larvicidal activity in the 100 µg/ml concentration, for an exposure period of 48 hours, LC50 <50 µg/ml, with a result of 36.84 µg/ml, being considered as a potent larvicide or highly active, under the experimental conditions tested here (Figure 1).

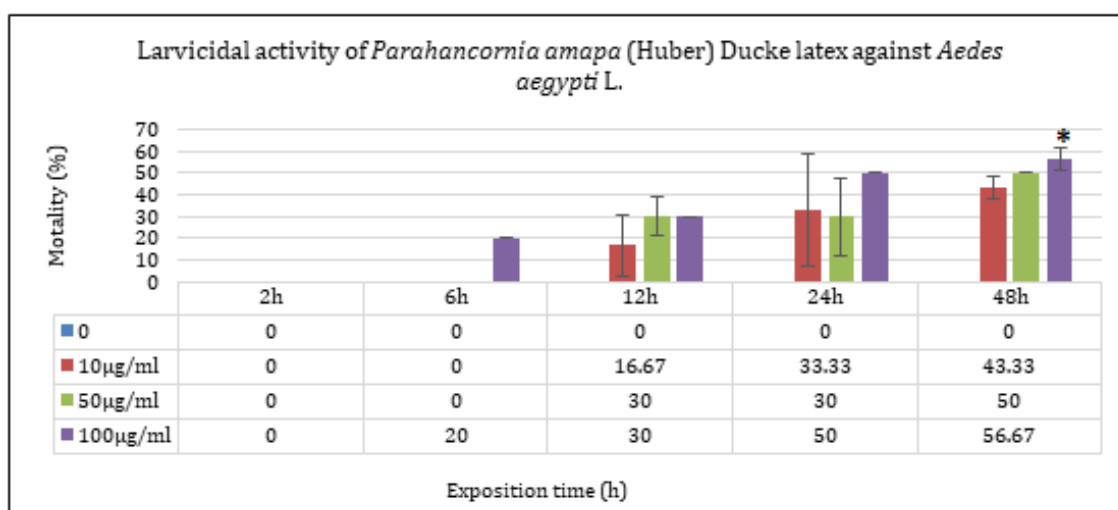


Figure 1. *Parahancornia amapa* (Huber) Ducke latex larvicide action, against *Aedes aegypti* L. larvae.

Parahancornia amapa altered the development of the fly *Chrysomya megacephala* (Diptera: Calliphoridae) larvae, in high concentrations, and influenced its pupation, in lower concentrations [2].

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PCH-41

THE ROLE OF COSMETOLOGIST IN THE REDUCTION OF ACNE VULGARIS

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Abstract: Acne vulgaris is a disease that occurs in up to more than 80 % of people. Acne may be present as comedones (whiteheads, blackheads), pustules, nodules, cysts and papules [1]. Factors that are causing the acne vulgaris are: the abnormal keratinization of the pilosebaceous canal, increased production of sebum, skin inflammatory, bacterial colonization (*Cutibacterium acnes*), hormonal problems and genotopic factors [2-5]. The study was examining the effectiveness of hydrogen purification cosmetological new treatment (containing alkaline water) on improving the skin condition of the women with A. vulgaris. The group of 30 women, had four treatment carried out at 7 day intervals. The Hellgren Vincent Scale was used to diagnose severity of the disease (**Table 1**), and sebum level was taken before, and after the sessions (between the eyebrows, nose area, bottom of lip). The level of sebum decreased after the four session of hydrogen purification.

Table 1. Characteristics of acne vulgaris severity in terms of the Hellgren–Vincent Scale (HVS) among participants of the acne vulgaris (AV) group ($n = 30$).

HVS	Baseline		Days 14 After Finishing Treatment	
	<i>n</i>	%	<i>n</i>	%
I	0	0.00	16	53.33
II	23	76.67	14	46.67
III	7	23.33	0	0.00

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PCH-42

ANTIMICROBIAL ACTIVITY OF CHOSEN ESSENTIAL OILS FROM *LAMIACEAE* FAMILY

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Essential oils are complex, natural volatile compounds of plants origin, produced as their secondary metabolites. These natural substances are mixtures of many chemical compounds, in various concentration ranges, therefore they exhibit broad biological activity as for instance antioxidant or antimicrobial effect. One of the plant group well-known for essential oils content is *Lamiaceae* family. *Lamiaceae* or *Labiatae* family is a group of flowering plants known from ancient times which are cultivated worldwide but are very common in Mediterranean Europe. The family includes over 7 thousand species. Most species are trees or shrubs, sometimes vines. A great number of *Lamiaceae* members contain essential oils, which are used e.g. as a flavors, fragrances and also pharmaceuticals thanks to their properties.

In the presented study antimicrobial activity of five essential oils from *Lamiaceae* family herbs: thyme (*Thymus vulgaris*), sage (*Salvia sclarea*), spearmint (*Mentha piperita*), oregano (*Origanum vulgare*) and rosemary (*Rosmarinus officinalis*) were investigated. Indicator microorganisms chosen for the research included *Pseudomonas putida* ATCC 11774, *Candida albicans* ATCC 10231 and *Micrococcus luteus* ATCC 4698. Serial dilution method, with 96-well microplates, was used to determine the minimal inhibitory concentration (MIC) of *Lamiaceae* family essential oils. Chosen essential oils were tested in concentration range 5.00-0.04 mg/ml. Spectrophotometric measurements were made using the BioTek Instruments EPOCH 2 microplate reader.

Conducted laboratory trials indicate on differentiated antimicrobial activity of tested essential oils. The degree of growth inhibition by the essential oils depended on the strain of the microorganism used in the research. The greatest susceptibility to tested essential oils showed *P. putida*. The MIC value for mint and oregano essential oils was 0.31 mg/ml, and for others MIC was 0.62 mg/ml. *M. luteus* was also very sensitive to the action of tested essential oils. Three essential oils inhibited the growth of this bacteria at a low concentration (MIC < 1 mg/ml). *C. albicans* was also susceptible towards tested volatile compounds as MIC for four out of five tested essential oils was below 1 mg/ml. The lowest MIC values for all tested microorganisms were obtained for thyme and sage essential oils (0.62 mg/ml). This study shows that all tested essential oils presented inhibitory effect on the growth of indicator microorganisms.

PCH-43

DIET, MICROBIOTA AND EPIGENETICS: A DYNAMIC AND ADJUSTABLE TRIADE TO EXPLORE TREATMENT APPROACHESKaterina Reva¹¹Faculty of Pharmacy, University of Coimbra, Azinhaga de Santa Comba, 3000-548, Coimbra, Portugal.

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It is known since early days that diet has a strong influence on human physiology, being an important adjustable variable in determining health and disease. More recently, diet-driven gut microbiota changes emerged as a pathway in influencing the host's physiology, establishing microbiotic metabolites as a key mechanistic link underlying microbial actions. In connection with these notions, epigenetics has brought new insights to our understanding of how environmental factors influence cellular genetic expression, pinpointing gut microbiota metabolites as one of the relevant factors. Intense efforts have been dedicated to mechanistically establish the links between aberrant epigenetic modifications and disease, and how variations in specific metabolites influence epigenetic agent's activity. Overall, this has brought new insights on how specific metabolites shape the epigenetic landscape of cells and organs, highlighting new therapeutic strategies for the use of epigenetic drugs.

Herein, we discuss the effect of diet in shaping microbiotic intestinal populations and how microbiotic metabolites interact with the host. These notions will be instrumental to address the modulatory role of environmental factors, including microbiotic metabolites, on epigenetic changes in health and disease development. Finally, we propose a dynamic diet-microbiota-epigenetics triade as concept of relevance in terms of health and disease, suggesting that a dietary planning according to specific epigenetic targets constitutes a potential innovating strategy for treatment and disease prevention.

PCH-44

ENZYME INHIBITORY ACTIVITY OF *LASERPITIUM SILER* L. (APIACEAE) EXTRACTSMileski Ksenija,^{1*} Krivošej Zoran,² Marin D. Petar¹, Džamić Ana¹¹Department of Morphology and Systematics of Plants, University of Belgrade - Faculty of Biology, Studentski trg 16, 11000 Belgrade, Serbia;²Department of Biology, University of Priština with temporary seat in Kosovska Mitrovica, Faculty of Natural Science, Lole Ribara 29, 38220 Kosovska Mitrovica, Serbia.

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Laserwort (*Laserpitium siler* L., Apiaceae) is one of 14 European *Laserpitium* species wide distributed at mountains of Southern and Central Europe. This plant is one of five laserworts that can be found in Serbia [1, 2]. This species has traditional value as food and folk remedy in the Alpine region and Russian tradition. The study on *L. siler* from Serbia presents the enzyme inhibitory activity of methanol, ethanol, methylene chloride and ethyl acetate extracts from fruits and aerial parts of the plant. Performed bioassays included determination of *in vitro* inhibition of acetylcholinesterase, tyrosinase, α -amylase and α -glucosidase enzymes by various extracts. Obtained results were expressed as IC₅₀ values (mg/mL) (Table 1). Enzyme inhibitory effects of tested samples showed that aerial parts of the plant possessed higher potential than the fruits. Still, all extracts showed lower activity in comparison to used standards. The highest inhibitory effects showed methanol and especially ethyl acetate aerial parts extract, which exhibited slightly lower activity on diabetes-associated enzymes α -amylase and α -glucosidase than acarbose (Table 1). Previously, it was proven that methanol extracts of some Apiaceae species also inhibited selected enzymes in different ranges [3]. Obtained results provide initial information on anti hyperglycemic and neuroprotective effects of *L. siler* extracts.

Table 1: Inhibitory activity of *L. siler* extracts on selected enzymes expressed as IC₅₀ in mg/mL (means \pm SD).

<i>L. siler</i> extracts (C=0.25-5 mg/mL)/ Standards (C= 0.00625-1 mg/mL)		Acetylcholinesterase	Tyrosinase	α -amylase	α -glucosidase
Fruits	MeOH	5.954 \pm 0.024 ^c	5.522 \pm 0.516 ^b	6.252 \pm 0.050 ^b	4.250 \pm 0.181 ^d
	EtOH	3.671 \pm 0.014 ^b	7.154 \pm 0.365 ^c	9.239 \pm 0.285 ^c	1.145 \pm 0.091 ^b
	MeCl ₂	2.605 \pm 0.108 ^b	7.168 \pm 0.145 ^c	4.956 \pm 0.080 ^b	1.491 \pm 0.069 ^b
	EtOAc	2.510 \pm 0.002 ^b	6.424 \pm 0.036 ^b	9.174 \pm 0.168 ^c	2.737 \pm 0.000 ^c
Aerial parts	MeOH	1.760 \pm 0.344 ^a	4.973 \pm 0.100 ^b	4.189 \pm 0.011 ^b	3.454 \pm 0.063 ^c
	EtOH	2.223 \pm 0.036 ^b	4.891 \pm 0.089 ^b	5.831 \pm 0.076 ^b	1.985 \pm 0.154 ^b
	MeCl ₂	4.939 \pm 0.001 ^c	9.909 \pm 0.201 ^c	4.514 \pm 0.045 ^b	5.655 \pm 0.159 ^d
Standards	EtOAc	1.837 \pm 0.038 ^a	5.687 \pm 0.147	2.919 \pm 0.006 ^a	0.479 \pm 0.016 ^a
	Galantamine/ Kojic acid	0.130 \pm 0.005 ^a	0.180 \pm 0.001 ^a		
	Acarbose			0.832 \pm 0.057 ^a	0.296 \pm 0.013 ^a

Indicated letters mean significant difference in the same column (p < 0.05).

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PCH-45

COMMELINA ERECTA AS A SOURCE OF NATURAL BIOACTIVE COMPOUNDS

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Plants are an important source for the discovery of new products of medicinal value and unique sources for food additives, flavors, and other compounds with industrial value *Commelina erecta* L. (Commelinaceae) is an herbaceous flowering plant, popularly known as “trapoeraba” and “erva-de-santa-luzia”. In folk medicine, *C. erecta* has been used as antiviral, for the treatment of haemorrhages, skin rashes and sores, and for the treatment of infections [1]. Moreover, it is also used as a wild edible plant in salads, preserves or decorating the dishes [1]. The present study aims at evaluating the bioactive potential, namely, antioxidant, cytotoxicity to tumor and non-tumor cell lines, and anti-inflammatory activities of the stem and flower of *C. erecta*. Antioxidant activity was evaluated in the hydroethanolic extracts by two *in vitro* assays measuring inhibition of lipid peroxidation using thiobarbituric acid reactive substances (TBARS) assay and antihaemolytic activity (OxHLIA). The cytotoxicity was tested using four human tumor cell lines: AGS (gastric adenocarcinoma), CaCo (colorectal adenocarcinoma), NCI-H460 (non-small cell lung cancer), MCF-7 (breast carcinoma), as well a non-tumor culture from African green monkey (Vero). Anti-inflammatory activity was determined based on the nitric oxide (NO) production by a murine macrophage (RAW 264.7) cell line.

Significant differences between the evaluated extracts were observed, the hydroethanolic extract showed the best activity in the TBARS assay for the stem sample, with EC₅₀ value of 0.63±0.01 mg/mL. On the other hand, in the OxHLIA assay, flower hydroethanolic extract present an IC₅₀ of 5.1±0.2 µg/mL, value required to protect half of the erythrocyte population from the hemolytic action caused by the used oxidative agent at Δt = 60 min. Stem sample presented effective results in the inhibition of the tested tumor cell lines, namely, AGS, CaCo and MCF-7, while flower is more effective against NCI-H460. Both extracts exhibited toxicity against non-tumoral cell lines, denoting that these extracts have cytotoxicity. The *in vitro* anti-inflammatory potential of extracts was evaluated by measuring NO inhibition and the most effective extract was the flower with an EC₅₀ values of 41±1 µg/mL. These results indicate that stems and flowers of *C. erecta* might be a potential source of natural biomolecules for pharmaceutical and food applications.

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PCH-46

COPAIBA OIL PREVENTS OBESITY AND IMPROVES ANTIOXIDANT DEFENSES IN ADIPOSE TISSUE

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Obesity, a worldwide public health problem, is a chronic disease characterized by an excessive or abnormal body fat accumulation that may lead to adipose tissue dysfunction [1, 2]. Active compounds were described in copaiba oil (CO), a product of a typical tree at the north of Brazil, which present anti-inflammatory, antimicrobial and antioxidant properties [3, 4]. However, there is no data regard the effects of CO in adipose tissue. Thus, the aim of this study was to evaluate the effect of CO on the obesity development and adipose tissue dysfunction in high-sucrose feed rats. Male *Wistar* rats were randomly assigned for receiving standard diet (C, n=8), standard diet and 30% sucrose in the drinking water (S, n=8), and standard diet and sucrose in the water + copaiba oil (S+CO, n=8). Copaiba oil was given at dose of 200 mg/kg/day by gavage for eighth weeks. C and S animals received vehicle, at equivalent volume, by gavage. At the end of experiment, animals were killed by decapitation, and blood samples and epididymal adipose tissue were collected for biochemists and oxidative stress determinations. Data were expressed as mean and standard deviation. The comparison between groups was performed using one-way ANOVA complemented *post-hoc* Tuke's test, with significance level adopted at P<0.05. High-sucrose diet model was efficient to promote obesity, increased fasting glycemia, dyslipidemia and insulin resistance, also increased oxidative stress in the adipose tissue from S animals. CO supplementation prevented weight gain, adiposity and insulin resistance, in addition, partially inhibited the increase of fasting glucose levels and dyslipidemia, and improved the antioxidant defense system in adipose tissue. Therefore, it is possible to conclude that CO displays benefic effects, suggesting its use as a good strategy for preventing of obesity and its complications.

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PCH-47

SAMBUCUS NIGRA L. EXTRACT-LOADED POLYMERIC NANOPARTICLES AS A POTENTIAL ANTI-INFLAMMATORY TOPICAL FORMULATION

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Natural products are a great source of potential bioactive compounds. *Sambucus nigra* L. is one of medicinal plants that has been used due to its promising biological activities. In our previous studies, several extracts were obtained from fresh flowers and fruits (fresh and dried) of *S. nigra* and the most interesting results in terms of anti-inflammatory activity (AIA) were achieved with methanolic extract prepared from fresh flowers [1,2]. The aim of the present work was to evaluate if the encapsulation process using polymeric nanoparticles (NPs) would improve the AIA of the methanolic extract [2]. Two different types of NPs were developed: 1) extract-loaded PLGA NPs prepared by emulsification/solvent diffusion method; 2) extract-loaded PCL NPs prepared by solvent-displacement method [2]. Both extract-loaded NPs were stable over time (0, 3, 6 and 12 months) [2]. DLS, SEM, TEM and AFM techniques demonstrated that the developed NPs presented a small mean size (< 480 nm), a spherical shape and a smooth surface. The encapsulation efficiency was determined in terms of rutin concentration (the extract's major compound) and the value was very similar for both NPs. The cell viability assays using HaCaT and HFF cells did not reveal cytotoxicity for both NPs after 24 h incubation and using a concentration of 30 µg/mL. The AIA of the methanolic extract, using a carrageenan paw oedema model, was evaluated following the topical administration of developed formulations as depicted in **Table 1**. Histological analysis and pro anti-inflammatory/anti-inflammatory factors analysis (TNF α , IL-6 and IL-10) corroborate the previous results.

Table 1: Characterization of the tested parameters (mean \pm S.E.M, n \geq 3, * p < 0.05 and *** p < 0.001, when compared to negative control, ^a mean \pm S.D.) [2].

Samples	Dose (mg/kg)	AIA <i>in vivo</i> (%)	TNF- α (pg/mL) ^a	IL-6 (pg/mL) ^a	IL-10 (pg/mL) ^a
Extract	1.0	41.2 \pm 15.3	133.3 \pm 26.5	106.9 \pm 7.7	10.7 \pm 1.2
Extract-loaded PLGA NPs	1.0	60.7 \pm 9.0 *	117.6 \pm 15.1	96.3 \pm 7.6	27.3 \pm 10.9
Extract-loaded PCL NPs	1.0	21.7 \pm 12.7	123.4 \pm 17.3	114.6 \pm 16.1	18.8 \pm 2.9
Negative Control (Carbopol 940 [®] gel)	---	0.0 \pm 0.0	168.5 \pm 42.2	98.6 \pm 12.1	14.6 \pm 5.8
Positive Control (Diclofenac)	1.0	133.5 \pm 12.6 ***	95.0 \pm 9.6	89.5 \pm 11.9	28.8 \pm 6.7

Thus, the current study provides a scientific evidence of AIA properties for the methanolic extract obtained from *S. nigra* L. flowers. This effect was even more noticeable after encapsulation in PLGA NPs. Comparing the two encapsulate polymers, PLGA NPs displayed a higher *in vivo* AIA effect following topical administration, leading to a 61% oedema inhibition. Similar values of IL-6 and IL-10 were observed for extract-loaded PLGA NPs and for the positive control, a commercial formulation of diclofenac. More detailed studies should be performed to identify the exact mechanism associated to this biological activity, as well as, to verify if the developed system in this work can be applied to a chronic model of inflammation.

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PCH-48

Development and validation of an HPTLC densitometry method for psoralen and bergapten quantification in *Brosimum gaudichaudii* Trécul.

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The species *Brosimum gaudichaudii* Trécul is popularly known as “mama cadela”, “arbóreo de cadela” or “algodãozinho do campo”. Traditionally, its roots are used as a therapeutic resource for the treatment of vitiligo, which is a specific form of depigmenting leukoderma. The word vitiligo is of Latin origin and comes from “vitium”, which means defect, and “vitelium”, which means white spots^[1,2]. The methodology for the analysis of bergapten and psoralen compounds present in the lyophilized extract was developed and validated by thin layer chromatography (TLC)/densitometry. The best resolution, separation and identification of the markers occurred in RP 18 F254 (Merck) silica chromatoplate and on the mobile phase composed of acetonitrile: water (60:40). The retention factors (RF) for psoralen and bergapten were 0.41 and 0.35, respectively (Figure 1A e Figure 1B). The developed method of analysis was linear, precise and accurate. Thus, TLC/densitometry or HPTLC proved to be an alternative technique for the identification and separation of these furanocoumarins, being important for the development of new products containing *B. gaudichaudii* extracts for the treatment of vitiligo.

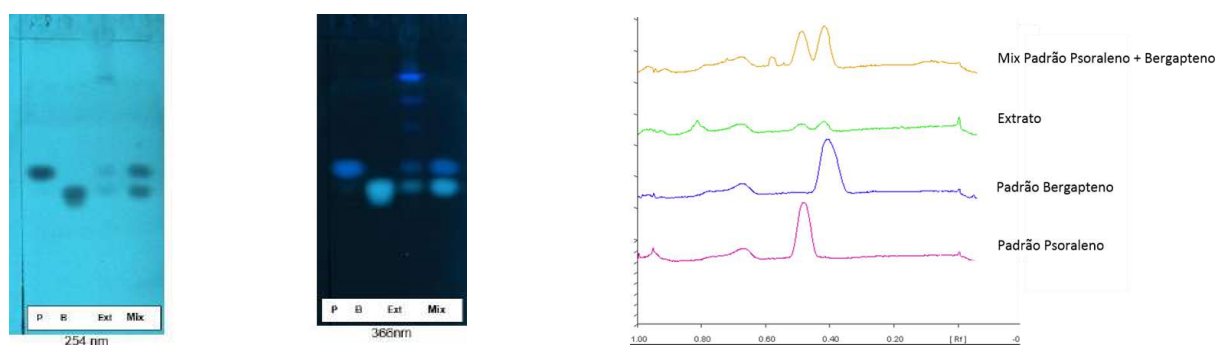


Figure 1A- Photos of the RP18 reverse phase silica chromatoplates (10 cm x 10 cm), mobile phase acetonitrile: water 60:40 (v / v), a condition that allowed the best separation of psoralen and bergapten in the extract of *B. gaudichaudii*. **Figure 1 B-**Densitograms of the standards and lyophilized extract of *B. gaudichaudii*, confirming the presence of the markers and with good resolution.

Subtitles: P- Psoraleno; B- Bergapteno; Ext- Extrato liofilizado de *B. gaudichaudii*; Mix- Padrão de psoraleno + bergapteno (1:1).

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PCH-49

OREGANO AND THYME HYDROLATS AS POTENTIAL NUTRACEUTICALS

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Medicinal plants contain pharmacologically active secondary metabolites that exhibit beneficial health effects and their extracts, essential oils, and hydrolats are often used as therapeutic agents. Species from genera *Origanum* L. (oregano) and *Thymus* L. (thyme) belong to the Lamiaceae family and are commonly recognized as spicy, fragrant, and ornamental plants, while a number of them are often used in traditional medicine for treating a plethora of conditions, such as sore throat, bronchitis, cold and gastrointestinal disorders [1]. The aim of this research was to examine the chemical composition, as well as antioxidant, antidiabetic and antineurodegenerative activities of hydrolats of two *Origanum* and two *Thymus* species, commercially grown on the fields of the Institute for Medicinal Plant Research „Dr. Josif Pančić“ in Pančevo, Serbia. The hydrolats were obtained as by-products of hydro-distillation and were tested without further dilution. The GC-FID/GC-MS analysis showed that the *Oh* hydrolat consisted mostly of carvacrol (99.00%), while *Ov* hydrolat was the most abundant in linalool (47.92%), α -terpineol (23.87%) and 1,8-cineole (8.70%). On the other hand, *Ts* and *Tv* hydrolats consisted mostly of thymol (98.04 % and 94.70%, respectively). Since plant products establish their health-boosting actions through various mechanisms, for each of the tested biological activity we employed multiple assays based on different principles (**Table 1**). Overall, *Tv* showed the highest antioxidant potential, while *Ts* slightly exceeded *Tv*'s capacity as an antidiabetic agent. Nevertheless, *Oh* exhibited higher antineurodegenerative potential compared to the other tested hydrolats. Altogether, *Tv*, *Ts*, and *Oh* exerted excellent biological potential, while carvacrol and thymol seem to be responsible for them being considered auspicious nutraceuticals.

Table 1: Biological activities of *Origanum* and *Thymus* hydrolats.

Biological activity	Assay	Plant species			
		<i>O. heracleoticum</i> (<i>Oh</i>) ^{a,b}	<i>O. vulgare</i> (<i>Ov</i>)	<i>Th. serpyllum</i> (<i>Ts</i>)	<i>Th. vulgaris</i> (<i>Tv</i>)
Antioxidant	DPPH	54.12±0.55	14.45±1.94	56.78±1.46	59.00±0.34
	Total reducing power	57.12±1.45	7.24±0.72	67.10±0.62	67.17±0.87
	β -carotene bleaching	44.04±0.12	nd	nd	40.83±3.75
Antidiabetic	α -amylase	0.96±0.12	0.07±0.26	0.97±0.32	nd
	α -glucosidase	60.49±1.03	nd	87.39±0.21	83.44±0.74
Antineurodegenerative	Acetylcholinesterase	78.51±0.55	43.74±2.20	64.96±1.16	67.42±0.37
	Tyrosinase	nd	7.09±1.20	11.81±0.91	nd

^a The results are expressed as percentages of inhibition for all the assays, except for the total reducing power (μ g AAE/g), nd – not detected.

^b The results are presented as a mean of three measurements \pm standard error.

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PCH-50

ROSMARINIC ACID –PLANT POLYPHENOL WITH ANTIMICROBIAL AND ANTIBIOFILM POTENTIAL

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Increasing antimicrobial resistance accompanied by range of side effects linked to antibiotics overuse are urging the demand for the novel antimicrobials with the ones obtained from natural sources considered as an appealing alternative. Rosmarinic acid is naturally occurring plant polyphenol. This study aimed to enlighten its potential role as inhibitor of planktonic and biofilm microorganism growth, along with its antifungal mechanisms. In this study, rosmarinic acid has shown promising anticandidal (MIC 0.1-0.2 mg/mL) (**Table 1**) and antibacterial (MIC 0.05->0.8 mg/mL) activity. To some extent rosmarinic acid was able to reduce attachment of fungal cells to abiotic surface, first stage in biofilm formation process, and eradicate preformed biofilms. Rosmarinic acid antifungal mode of action involves interference with membrane integrity, but not binding to membrane lipid, ergosterol, or reduction in protease production; while its antibiofilm activity is moderately attributed to decrease in exopolysaccharide production. Considering its wide antimicrobial and antibiofilm capacity rosmarinic acid could be further examined as antimicrobial agent along with range of medicinal plants with rosmarinic acid as the dominant compound.

Table 1: Minimal inhibitory (MIC) and minimal fungicidal (MFC) concentrations of rosmarinic acid, mg/mL.

Yeasts	Rosmarinic acid		Ketoconazole	
	MIC	MFC	MIC	MFC
<i>C. albicans</i> 475/15	0.1	0.2	0.003	0.006
<i>C. albicans</i> 13/15	0.1	0.2	0.0016	0.05
<i>C. albicans</i> 17/15	0.1	0.2	0.0016	0.05
<i>C. albicans</i> 527/14	0.15	0.3	0.0031	0.0062
<i>C. albicans</i> 10/15	0.15	0.3	0.0031	0.05
<i>C. albicans</i> 532/15	0.1	0.2	0.0031	0.0062
<i>C. albicans</i> ATCC 10231	0.2	0.4	0.0016	0.006
<i>C. krusei</i> H1/16	0.2	0.4	0.0016	0.003
<i>C. glabrata</i> 4/6/15	0.1	0.2	0.0016	0.006
<i>C. tropicalis</i> ATCC 750	0.2	0.4	0.0016	0.006
<i>C. parapsilosis</i> ATCC 22019	0.1	0.2	0.003	0.006

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PCH-51

SEASONAL CHANGES IN THE BIOCHEMICAL PROFILE AND ANTIOXIDANT PROPERTIES OF *GELIDIUM CORNEUM* FROM SÃO MARTINHO DO PORTO, PORTUGAL

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The marine environment holds a few of the richest ecosystems on the planet and astounding genetic diversity in the species that inhabit it [1]. The existing species of marine algae remain a mystery to scientist even today, but their biotechnological potential has become appealing to various industries [2,3]. Besides the natural production of bioactive compounds, abiotic factors such as nutrient starvation, UV radiation or temperature may trigger metabolic reorientations [1]. Nowadays the algae resources from São Martinho do Porto, Portugal, namely the red algae *G. corneum* is mostly used for agar extraction and soil fertilization [4]. In this study, the seasonal variations of antioxidant capacity, carbohydrate content and lipidic content were evaluated to further deepen the biotechnological potential of these macroalgae.

The antioxidant potential was evaluated through the DPPH, ABTS and FRAP assays and total phenolic content (TPC) was determined [5,6,7,8]. Antioxidant activities for DPPH, ABTS, FRAP and TPC assays show average values (5-15% inhibition, 10-14% inhibition, 0.009-0.02 AAE/g_{extract} and 4-7 GAE/g_{extract}, respectively), in line with the expected. The lipidic content, evaluated through the Folch method [9], showed the lowest and highest values in springtime and summertime, respectively. The carbohydrate content was evaluated through the Dubois method [10]. Samples from springtime showed the highest values (61.1 ± 2.0% DW).

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PCH-52

**MEDICAL AND PHARMACOLOGICAL POTENTIAL OF LOW-MOLECULAR COMPOUNDS
DERIVED FROM THE LATEX OF A MEDICINAL PLANT *CHELIDONIUM MAJUS* L.**

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Chelidonium majus L., also known as Greater Celandine, is a perennial, herbaceous plant widely used in traditional medicine. The plant produces yellow milky sap (**Figure 1**) which is rich in biologically active substances, like alkaloids, flavonoids, phenolic acids and defence-related proteins with antipathogenic activities [1]. The milky sap is used in folk medicine to treat warts, papillae and condylomas, which are caused by human papillomavirus (HPV) infections [2]. The plant's extracts, containing mostly hydrophilic molecules such as alkaloids, have various medicinal properties as antimicrobial, anti-inflammatory, antiviral, antifungal and antitumor activities [1]. *C. majus* alkaloids, such as chelidonine, sanguinarine or protoberberine compounds, are intensively researched for potentially medical properties. For instance, the antibacterial and antiviral activity of *C. majus* alkaloids was confirmed [3]. Moreover, the alkaloids' properties such as cytotoxicity and ability to overcome the multidrug resistance mechanisms in tumor cells extend the potential use of the plant extracts in tumor therapies [4]. In the presentation, the current state of knowledge about medical and pharmacological potential of *C. majus* low-molecular compounds, with special emphasis on its alkaloids, will be discussed. In the future study, the antitumor and antiviral properties of two compounds of *C. majus* milky sap, defense proteins and alkaloids, will be examined. This approach will allow investigating the potential synergistic action of milky sap compounds.

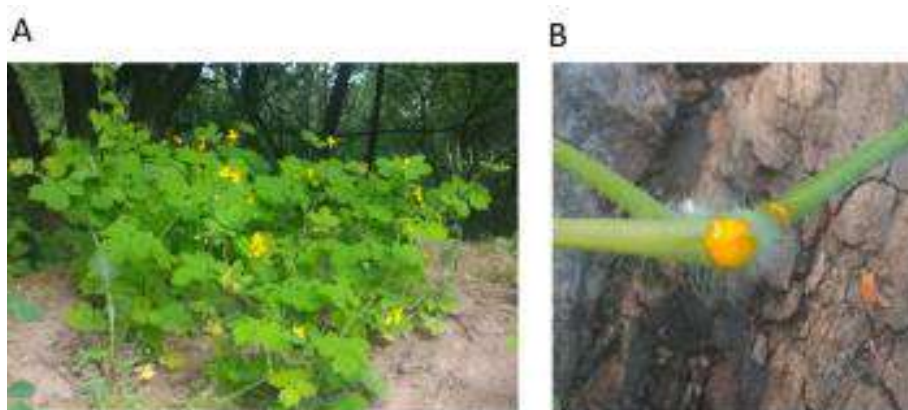


Figure 1: [A]*Chelidonium majus* L. plant.[B] *C.majus* milky sap.

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PCH-53

HEALTH PROMOTING PROPERTIES OF BEE ROYAL JELLY: FOOD OF THE QUEENS

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Over the last few years, the interest of consumers and food industry in healthy natural products has gradually increased [1]. Royal Jelly (RJ) is one of the most attractive functional foods due to its excellent biological properties [2]. RJ is an acidic colloid formed by different substances, mainly carbohydrates, proteins and lipids and in lower proportion vitamins, minerals and phenolic or volatile compounds [3,4]. The Mayor Royal Jelly Proteins (MRJP) together with 10-hydroxy-2-decenoic acid (10-HDA), a unique lipid of the RJ, are mainly responsible for their different biological properties [5]. In this way, the use of RJ, which has been historically produced in China and used in traditional medicine, has been expanding throughout the world [6]. Currently, RJ is one of the most attractive functional foods, being used as a dietary supplement and in various industries such as pharmaceutical, food and cosmetics [2,7]. Several studies have reported many pharmacological activities and thus, RJ or its specific components can be considered as a potential compounds or ingredients to prevent or combat numerous diseases [2]. Within these biological properties, anti-lipidemic, antioxidant, antiproliferative, antimicrobial, neuroprotective, anti-inflammatory, immunomodulatory, antiaging and estrogenic are the most outstanding activities [6]. Furthermore, RJ is considered an attractive but expensive functional food and thus it can be submitted to adulteration processes. However, further studies are still needed to understand its mechanism of action. RJ components, its biological activities and related health applications have been revised.

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PCH-54

Effects of a *Myrciaria jaboticaba* Peel Extract and role of cyanidin-3-*O*-Glucoside on α -amylase and lipase in mice.

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This study consists of a parallel and comparative investigation of the effects of a *Myrciaria jaboticaba* peel extract and one of its most prominent constituents, cyanidin-3-*O*-glucoside [1], on the pancreatic α -amylase and lipase activities. All procedures in animals were previously approved by the Ethics Committee for Animal Experimentation. The peel extract inhibited both α -amylase and lipase but acted 13.6 times more strongly on the latter. Cyanidin-3-*O*-glucoside contributed minimally to these inhibitions. The *Myrciaria jaboticaba* extract, but not cyanidin-3-*O*-glucoside, inhibited starch absorption in mice at doses compatible with its inhibitory action in α -amylase. Both the extract and cyanidin-3-*O*-glucoside (**Figure 1**) inhibited triglyceride absorption, but at doses that were considerably smaller than those predicted by their strength in inhibiting the pancreatic lipase *in vitro*. It was concluded that inhibition of triglyceride absorption by extract can result from another action mechanism that is concomitant or even independent of the inhibition of lipase. Due to the low active doses, *Myrciaria jaboticaba* peel extract presents many favourable perspectives as inhibitors of fat absorption, and cyanidin- 3-*O*-glucoside seems to play a decisive role.

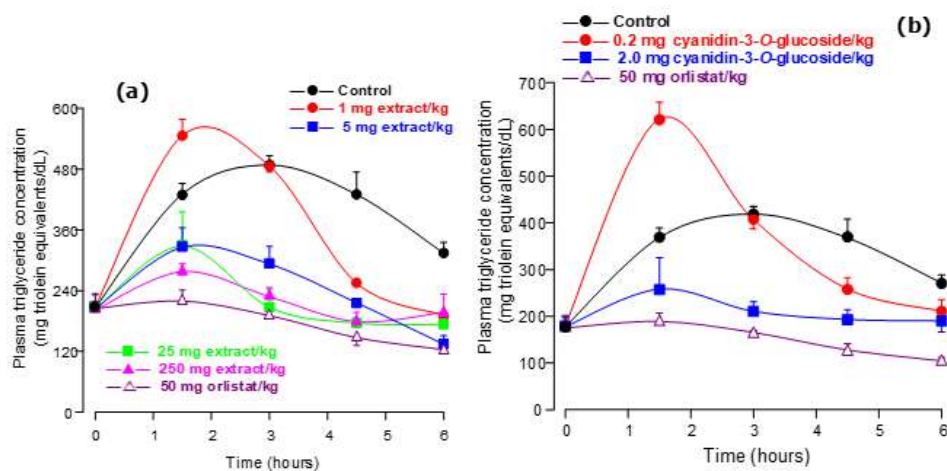


Figure 1: Effect of the different concentrations of *M. jaboticaba* peel extract (a) and of the cyanidin-3-*O*-glucoside (b) on the plasma triglyceride concentration profiles after intragastric olive oil loads in mice.

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Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES)

PCH-55

Developmental effects of natural fungicides (*Equisetum arvense*, *Mimosa tenuiflora*, and Thymol) on zebrafish (*Danio rerio*)Raquel Vieira,^{1*} Carlos Vênancio,^{1,2} Luís Félix,^{1,3}¹Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal;²Department of Animal Science, School of Agrarian and Veterinary Sciences (ECAV), University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal;³Institute for Research and Innovation in Health (i3s), Laboratory Animal Science (LAS), Institute of Molecular and Cell Biology (IBMC), University of Porto (UP), Porto, Portugal.

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The excessive use of synthetic fungicides is a problem for the environment. The use of natural products with fungicidal properties has been claimed as one of the most viable alternatives. Yet, there is a lack of ecotoxicological information on these products. Therefore, the objective of this study was to evaluate the effects of three natural extracts (*Equisetum arvense*, *Mimosa tenuiflora*, Thymol) with antifungal properties, by evaluating the teratogenic, oxidative stress and behavioural effects on the early development of zebrafish. Collected embryos with ~2 h post-fertilization (hpf) were exposed to serial dilutions of *E. arvense* and *M. tenuiflora* commercial formulations and to Thymol for a period of 96 h and the LC₅₀ was determined. After that, embryos were exposed for 96 h to sublethal concentrations (1/300–1/200 to 1/3–1/2 below the LC₅₀) with lethal and sublethal parameters being assessed during the exposure period. After the exposure, larvae were collected for biochemical determination of different biomarkers associated with oxidative stress, energetic metabolism, and neurotransmission or allowed to develop until 120 hpf, at which time the locomotor activity of the zebrafish larvae was assessed using different behavioural paradigms. Thymol increased lethality, pericardial oedema, yolk and eye deformations, and decreased body length. The higher concentration of Thymol induced an increase in the reduced and oxidized glutathione (GSH:GSSG) ratio, as well as increased the glutathione-s-transferase (GST) activity. In addition, the disruption of behavioural states (fear- and anxiety-like disorders) were noted in Thymol-exposed animals. The results evidenced the teratogenic effects of Thymol, which might have consequences for non-target species, while showed the safety profile of *E. arvense* and *M. tenuiflora* at sublethal concentrations.

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PCH-56

THE EFFECT OF A TANNIN ENRICHED TOPICAL FORMULATION ON MICE EARS FUNGAL BIODIVERSITY

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Cytinus hypocistis (L.) L. is a holoparasitic plant on various *Cistaceae* family members occurring in the Mediterranean region. This specie has long been used in European popular medicine due to their astringent and haemostatic properties. Current studies attributed its antioxidant, anti-inflammatory, and antimicrobial properties to their hydrolysable tannin content. Therefore, the extracts of these plants might be a natural alternative treatment for many skin conditions.

The main objective of the present work was to study the effect of *C. hypocistis* enriched topical formulation (EF) in mice ears fungal isolates using routine mycological techniques [1]. Three EF were prepared with different concentrations of *C. hypocistis* extracts: a) 3.1 mg extract (E)/g of cream; b) 6.2 mg E/g of cream; and c) 12.4 mg E/g of cream. The formulations were carefully mixed to guarantee sample homogeneity. A negative control (base cream) and the three EF were stored at 4 °C during the experiments. Thirty samples were collected in the ears of 30 female mice. The mice were divided in six groups of 5 mice each, to evaluate the correct dosage and toxicity of the extract for the mice, although these were accessed in another essay. Two of the groups were of wild type, one was treated with the base cream (G6) and the other with the cream with highest concentration of extract (G4), and the four others of Papillomavirus models, a positive control on which was applied the base cream (G5) and rest were treated with three creams with rising concentrations of the extract (G1, G2 and G3, respectively). The experiment procedure was approved by a Animal Ethics Committee-DGAV number 0421/000/000/2014, 24/09/2014 (020172).

Before the application, the surface of the ear was rubbed for 1 minute, using a swab and saline solution. The samples were transferred to Petri dishes with the culture medium Potato Dextrose Agar and the microscopic identification was carried out using the Lactophenol with Cotton Blue technique for staining the filamentous fungi and identification of the genera. In the entirety of samples collected on the first collection, 4 filamentous fungal genera were isolated: *Penicillium* (16.7%) in G1, G4 and G5, *Mucor* (16.7%) G1, G2, G4 and in G5, *Cladosporium* (3.33%) in G1, *Aspergillus* (3.33%) in G6. In total, fungi were isolated in 14 (46.7%) animals. Fungal genera were not identified in three mice from G1 and G5. The remaining samples exhibiting either other types of microorganisms or no growth at all. After the application of *C. hypocistis* cream for 17 days in the ears, only one fungal colony was isolated in a mouse from G4. The genus was not identified. The results obtained suggest that *C. hypocistis* reduces the presence of fungal in the ears of mice.

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PCH-57

ANTIOXIDANT ACTIVITY OF AGRO-INDUSTRIAL WASTE FROM GUAVA (*PSIDIUM GUAJAVA*)

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Psidium guajava L. is a plant originated in Mexico, belonging to Myrtaceae family. The guava fruit is growth in economic value for the production of derivatives in the Brazilian agribusiness. However the use of guava pulp generates waste discarded in the environment, without the correct use, ie an environmental liability. The guava residue consists of pulp, peel, but mainly seeds. The guava residues used in the work were supplied by the company Predilecta Alimentos®, located in the city of Aparecida de Goiânia. The material was dehydrated in an oven with forced circulation and air renewal at 40 ° C for seven days. After dehydration, the dry material was ground un a knife mill and stored under light and moisture, under refrigeration (-20°C). The characterization of the guava residue was carried out according to pharmacopoeial parameters of: loss on desiccation, determination of the swelling index, total ash, total tannins and flavonoids. The 96% ethanolic extract was then produced using the maceration method, followed by percolation. The antioxidant activity was carried out by the methods of capturing the free radical 2,2-diphenyl-1-picryl-hydrazil (DPPH) following that described by [1], as well as by capturing ferrous sulfate (FRAP) described by [2]. The drying loss content found in the powder of *P.guajava* residues was 8.39% ± 0.06%, remaining within all limits and favorable to the conservation of the product. The swelling index was found to be 0.00 ± 1.8mL. The total ash content was 1.91% ± 0.01%. The content of total tannins found in residues from the processing of guava was 4.80% ± 0.01% and flavonoids 1.85% ± 0.03. Regarding the antioxidant potential, with an EC 50 of 31.24 mg/mL for the capture of DPPH and a content of 3.67 10⁻⁶ µM of ferrous sulfate capture per gram of residue. A study with several guava species, carried out in Colombia, demonstrated a correlation between the content of phenolic compounds and antioxidante activity by the FRAP and DPPH methodologies [3]. The literature considers it important to study the physical and chemical composition of plant materials, since methods used to determine antioxidant activity, when applied alone, may not provide safe results. The activity against the DPPH radical and against ferrous sulfate demonstrated the potencial of guava residue in preventing oxidative, which can be useful for the development of new pharmaceutical formulations.

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PCH-58

OPTIMIZATION AND RECOVERY OF VITAMIN D₂ FROM SURPLUS PRODUCTION OF *AGARICUS BISPORUS* PORTOBELLO

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During mushroom production a percentage as high as 20% of surplus can be generated. These unused mushrooms have high nutritional value and valuable chemical compounds. In this sense, finding innovative alternatives to valorizing this surplus mushroom production needs to be explored [1,2]. Irradiation of mushrooms surplus to obtain vitamin D₂ is a sustainable strategy to increment vitamin D availability.

Under this perspective, the objective of this study was setting the UV-C irradiation and extraction conditions that maximize vitamin D₂ contents in surplus mushrooms production (*Agaricus bisporus* Portobello). The bioactive effects and potential toxicity of vitamin D₂-enriched extracts were also evaluated. The surplus production from *A. bisporus* Portobello were supplied by the Ponto Agrícola, Baião, North of Portugal. The irradiation was performed using an ultraviolet (UV-C at 200 mJ/cm², 800 mJ/cm² and 3200 mJ/cm²) radiation chamber with different exposure times: 0, 2, 6 and 10 min. Sensitivity and linearity of the high performance liquid chromatography (HPLC) coupled to ultraviolet detector (UV) were determined and the method was validated by the instrumental precision, repeatability and accuracy, and the extracts rich in vitamin D₂ were also quantified by HPLC-UV. The cytotoxicity of the vitamin D₂ extract were evaluated using three tumoral cell lines (MCF-7 - breast adenocarcinoma, NCI-H460 - non-small cell lung cancer and AGS - gastric cancer) and one non-tumoral cell line of bone origin (h-FOB 1.19 - human osteoblasts).

The chromatographic method revealed a great reproducibility and accuracy, thus being the method validated. Independently of the UV-C irradiation dose, the effect in the conversion of vitamin D₂ concentration was very high, allowing it to increase from ~3 µg/g dw to more than 100 µg/g dw in *A. bisporus* portobello. The extract enriched in vitamin D₂ presented an effective activity in AGS (82 µg/mL) tumoral cell line and a moderate activity in NCI-H460 (293 µg/mL) and CaCo (377 µg/mL) tumoral cell lines. Furthermore, the extract did not show cytotoxicity against the non-tumor bone cell h-FOB 1.19 (GI₅₀ > 400 µg/mL). Accordingly, development food applications of mushroom extracts enriched in vitamin D₂, from surplus mushroom production, can be considered and valorized, supporting and adding value to the agriculture sector or pharmaceutical industries.

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PCH-59

REPRODUCTIVE TOXICITY OF PLANT EXTRACTS PRODUCED IN PORTUGAL

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Plant extracts are increasingly being used in healthcare and cosmetics, as well as pharmaceutical formulations. The reasons behind this interest are related with the bioactivity of plant extracts, specifically their intrinsic antimicrobial activity, antioxidant, anti-proliferative and anti-inflammatory, among many other beneficial effects. Nonetheless, not much is known about the overall safety of these plant extracts for the use in humans. In this study, we aim to determine the reproductive toxicity of extracts of plants produced/endogenous in Portugal: *Cistus ladanifer*, *Thymra capitata*, *Helychrisium italicum* and *Cupressus lusitanica*. Hydrolates for each plant were obtained by hydrodistillation of aerial parts. The chemical composition of the hydrolates were determined by GC-MS (gas chromatography mass spectrometry). Reproductive toxicity was assessed by exposing freshly harvested bovine oocytes to five different concentrations of each hydrolate (0,2%-0,002%). After incubation, the oocytes were visually inspected to assess maturation by optical microscopy. In parallel, oocyte viability was determined for each concentration by staining with trypan blue. We found that for all concentrations tested, the viabilities of the bovine oocytes for each plant were superior to 70% (*C. ladanifer*: 79,02±7,25; *T. capitata*: 76,56±4,26; *H. italicum*: 87,28±4,87; *C. lusitanica*: 86,72±7,04). The EC50 was determined by estimating the concentration of the hydrolate able to inhibit maturation of 50% of bovine oocytes in vitro (*T. capitata*: 1,26%; *H. italicum*: 1,27%; *C. ladanifer*: 2,07%; *C. lusitanica*: 2,46%;). The results show that the hydrolates in test do not compromise oocyte viability. We observed some inhibition of oocyte maturation, at concentrations ranging 1-2,5%. In our study, *C. ladanifer* and *C. lusitanica* were less toxic to oocytes than *T. capitata* and *H. italicum*.

Our results suggest that plant extracts should be tested prior to their inclusion in formulations to be used in humans, to assess the safe concentration to be used in the final product. This information should be complemented with dermal permeation experiments to properly assess the reproductive toxicity of the ingredient by considering its body distribution.

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PCH-60

POTENTIAL OF SWEET CHERRY BY-PRODUCTS: PHENOLIC PROFILE AND ANTIMICROBIAL ACTIVITY OF STEMS AND KERNELS

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During sweet cherry processing, large amounts of by-products, are generated [1]. There is no substantial use of this waste, which increase environmental and managements costs each year to deal with the excess of such residues. Although sweet cherry stems are widely recognized by traditional medicine [2, 3], detailed and feasible information about their bioactive composition or biological value is still scarce, as well as the characterization of kernels (**Figure 1**). These by-products only recently received attention and this new interest is focused in finding ways to achieve their valorization [4]. Thus, we conducted a study in which saponins, phenolic profile composition of stems and kernels of four sweet cherry cultivars (*Early Bigi* (grown under net cover (C) and without net cover (NC)), *Burlat*, *Lapins*, and *Van*) and antibacterial activities against important Gram negative and Gram positive bacterial human isolates were examined. Extracts from stems of cv. *Lapins* and kernels of *Early Bigi* NC presented high levels of saponins. Apart from cv. *Early Bigi* NC, major phenolic compounds identified in stems and kernels were sakuranetin and catechin, respectively. In cv. *Early Bigi* NC the most abundant compounds were ellagic acid for stems and protocatechuic acid for kernels. Antimicrobial activity assays showed that only stem's extracts were capable of inhibiting the growth of Gram positive isolates, although extracts from kernels resulted in only a small synergistic effect in three situations. This new data is intended to provide new possibilities of valorization of these by-products and their valuable properties.

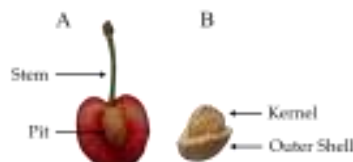


Figure 1: Unvalued sweet cherry by-products: view of sweet cherry stem and pit (A) and view of sweet cherry pit (B).

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PCH-61

EDIBLE FLOWERS AS FUNCTIONAL FOODS WITH THERAPEUTIC POTENTIAL

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Plants have been used in traditional medicine throughout the centuries, being flowers some of the most important organs not only for ornamental purposes but also due to bioactive compounds and applications. For example, borage flowers have been used as antihypertensives or diuretics and some types of pansies have been used to treat Central Nervous System diseases. The many properties attributed to those flowers are given by their rich phytochemical composition.

Edible flowers are currently widely used in gastronomy as healthy ingredients, but little it is known about their bioactive properties as functional foods. Therefore, we hypothesized if some species of edible flowers could be considered as functional food due to their antidiabetic and antioxidant properties. To develop that, polyphenolic extracts obtained from flowers such as borage (*Borago officinalis*) and pansies (*Viola x wittrockiana*) were studied in terms of their antioxidant and antidiabetic properties through *in vitro* biochemical procedures.

The antioxidant properties were quantified by *in vitro* elimination of superoxide radicals ($O_2^{\cdot-}$) generated through the xanthine/xanthine oxidase reaction. The antidiabetic potential of the extracts was quantified by inhibition of the enzyme α -glucosidase [1] and the capacity of these extracts to inhibit advanced glycation end products (AGEs) formation [2].

Viola extract showed a lower IC_{50} value than acarbose, an α -glucosidase reference inhibitor. Both extracts (*Viola x wittrockiana* and *Borago officinalis*) showed great antioxidant ability against the superoxide radicals created through the xanthine/xanthine oxidase reaction, being *Viola* the best antioxidant. Both flower extracts displayed important activity inhibiting protein glycation.

In conclusion, pansies and borage flowers can be considered as source of bioactive compounds with interesting properties in the field of nutrition as functional foods or food supplements in the prevention and improvement of metabolic of chronic diseases such as diabetes.

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PCH-62

ANTIBIOFILM ACTIVITY OF EMODIN ON *STAPHYLOCOCCUS AUREUS* AND ITS EFFECT ON AEROBIC RESPIRATION

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Bacterial resistance to conventional antibiotics is a global problem nowadays and it is necessary to find an appropriate solution in the form of new therapeutics, and strategies in the fight against resistance [1]. Bearing in mind that plants are rich in secondary metabolites and often used in traditional medicine, they represent an interesting source of new and alternative chemotherapeutics [2]. Emodin, (derivative of anthraquinones) is an abundant constituent of many plants and possesses a wide range of biological activities [3]. Guided by the fact that *Staphylococcus aureus* is main causative of numerous infections and resistant to many antibiotics, the aim of the study was to investigate antibiofilm potential of emodin and its influence on bacterial respiration of *S. aureus* ATCC 25923 (MSSA), ATCC 43300 (MRSA) and clinical isolates from nasal carriage – Gp19 (MSSA) and Gp7 (MRSA). For the effect of emodin on biofilm disruption, crystal violet staining of biomass was performed. O₂ consumption and CO₂ production in treated and untreated biofilms was monitored using Micro-Oxymax respirometer. RT-PCR was used to investigate the effect of emodin on the expression of *icaA* and *icaD* (responsible for biofilm formation), and *srrA* and *srrB* (staphylococcal respiratory regulatory complex). Results obtained in this study revealed that emodin disrupted biofilm of both MRSA strains at all tested concentrations (up to 33%) while biofilm of MSSA strains was not affected by emodin. Aerobic respiration of ATCC 43300 strain was decreased in treated biofilm while in other strains that effect was less pronounced. In addition, emodin reduced expression of *icaA* and *icaD* genes in all tested strains except ATCC 43300 where emodin upregulated this genes. On the other hand, expression of *srrA* and *srrB* was slightly increased in all MSSA strains and decreased in both MRSA strains. The obtained results demonstrated that emodin possesses significant antibiofilm activity which is related to specific strain. Furthermore, the results indicate multiple pathways through which emodin acts and affects on the biofilm and metabolism of *S. aureus*. Overall, emodin could be recommended for further investigation in order to be used in medicine treatment of infections.

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PCH-63

**ASSESSMENT OF *FRANGULA ALNUS* ETHYL-ACETATE EXTRACT ON BIOFILM
DISRUPTION AND BACTERIAL RESPIRATION OF *STAPHYLOCOCCUS AUREUS* STRAINS**

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Staphylococcus aureus is one of the most serious pathogens, known to the world health community. It is able to form biofilm, which can cause numerous acute and chronic infections [1]. These infections can be difficult to treat because of its wide range of resistance on different antibiotics in biofilm form. Therefore, scientific community has been looking for new approach in order to overcome this problem. It is proven that many secondary metabolites produced by plants have antibacterial activities [2]. *Frangula alnus* has been used in traditional medicine for years and previous studies have shown that plants from genus *Frangula* express various biological activities [3]. During lack of knowledge about antibiofilm activity of *Frangula alnus* (FA) extract, the aim of this study was to examine influence of previously chemically characterized ethyl-acetate extract on *Staphylococcus aureus* ATCC strains (MSSA-25923 and MRSA-43300) and clinical isolates (Gp19 and Gp7 both from nasal carriage). Antibiofilm activity was studied using crystal violet staining of biomass, while Scanning electron microscopy (SEM) attested effect of FA extract on biofilm structure and morphology. Respiration, O₂ consumption and CO₂ production was monitored by Micro-Oxymax respirometer. Furthermore, the effect of FA extract on expression of *icaA*, *icaD*, *srrA*, *srrB* and RNA III was investigated by RT-PCR. Gathered results, showed that disruption of biofilm biomass was significant decreased in ATCC 43300 biofilm (up to 28%), but increased in ATCC 25923. Scanning electron micrographics showed changes in matrix structure for Gp19 isolate, slight disruption of biofilm biomass for ATCC 43300 and also slight changes in structure and morphology of biofilms for Gp7. Respiration in treated biofilm of both clinical isolates, has been decreased, while respiration of tested ATCC strains in treated biofilms have not been changed. Gene expressions of MSSA strain are slightly decreased, except *icaD* gene, but in MRSA *icaA*, *srrB* and RNA III are increased and *icaD* and *srrA* decreased. In clinical isolates, Gp19 all gene expressions are slightly increased, except for *icaA* gene. In Gp7 *icaA* and *icaD* genes were upregulated, but all other were decreased. Taking into account the obtained results, FA extract has demonstrated great potential for further studies, which could provide better knowledge about its mechanism of action and provide its potential use as therapeutic agent.

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PCH-64

FROM OXIDATIVE STRESS PROTECTION TO CELL CYCLE ARREST: *CUCURBITA FICIFOLIA* BOUCHÉ EFFECTS

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Cucurbita ficifolia Bouché (*chila* pumpkin) is a type of squash native from México, being used for human nutrition especially in crystallized pastry and deserts and also as medicinal purposes, such as for the treatment of diabetes type 2, in wound-healing and for fever. There are a few ethnopharmacological studies reporting its usage as well as some studies performed in which a hypoglycaemic effect was demonstrated in animal models fed with this pumpkin, but there is a lack of information concerning its phytochemical profile as well as of other bioactivities.

Thus, in this study we have performed hydroethanolic ((80:20) % v/v) extracts, from pulp and from peel, that were further fractionated into methanolic (H.E-Fr.MeOH) and aqueous fraction (H.E-Fr.Aq). The phenolic profile was determined and the extracts were screened for biological activities. The extracts and fractions shown to be rich in polyphenolic compounds and in sugars. Extracts exerted a moderate anti-proliferative activity against Caco-2 cells (dose- and time-dependent). Using flow cytometry and DCFDA probe (2',7'-Dichlorofluorescein diacetate) we evaluate the Caco-2 cell's content in reactive oxygen species (ROS) after being exposed to different concentrations of the extracts. We observed that the pre-exposure of Caco-2 cells to aqueous fraction of pulp extract protected the cells against, but when cells were pre-exposed to the methanolic fractions this protection was not observed. We also have observed that methanolic extracts dose-dependently increased intracellular ROS and modulated the cell cycle in respect to control cells.

In conclusion, consumption of *Cucurbita ficifolia* pulp has a positive effect concerning to ROS protection and has a moderate role as anti-cancer agent against colorectal carcinoma cells (Caco-2 cells).

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PCH-65

The anti-tumour effect of spearmint (*Mentha spicata*) in a HPV-16-transgenic mouse model

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Infection by Human Papillomavirus (HPV) is the main cause of cervical cancer, highlighting the importance of studying compounds that may reduce viral activity and its lesions/symptoms. The aromatic herb spearmint (*Mentha spicata*) (MS) has proven anti-tumour properties¹. Thus, this study aimed to evaluate the effects of an hydroethanolic extract obtained from spearmint in HPV16-transgenic (HPV+/-) mice. The extract was obtained through maceration with ethanol/water (80:20, v/v), and the phenolic composition was determined through HPLC-DAD-ESI/MS. Thirty-three female mice (16 HPV-/- and 17 HPV+/-) were randomly divided into six groups: Group (G) I – HPV-/- w/o Mentha (n=5); G II – HPV-/- w/ Mentha (0.5 mg/ml; n=6); G III – HPV-/- w/ Mentha (0.55 mg/ml; n=5); G IV – HPV+/- w/o Mentha (n=6); G V – HPV+/- w/ Mentha (0.5 mg/ml; n=6); G VI – HPV+/- w/ Mentha (0.55 mg/ml; n=5). The spearmint extract was administered in the animal's drinking water for 28 days. During the study, water and food intake as well the animals' weights were recorded weekly. Afterwards, the animals were sacrificed, and their organs were collected for oxidative stress and genetic damage analysis. A total of thirteen compounds were identified in the hydroethanolic extract, being salvianolic acid B, rosmarinic acid and luteolin-7-*O*-glucuronide, the main compounds found. Moreover, the compounds revealed to be stable in the drinking water during the 5 tested days. Results show that HPV+/- (GIV, V and VI) have lower weight but higher water and food intake, as expected. Analysis of HPV+/- mice GIV showed a significant increase of superoxide dismutase activity when compared with GV (p=0.0029) and VI (p=0.0011). This suggests that group IV was subjected to a higher oxidative stress, as expected¹. These results could also mean that MS is responsible for a decrease in the oxidative stress known to be induced by HPV, which in turn decreases SOD, further the role of MS as an antioxidant. Regarding genetic damage, no statistically significant changes between groups were found in the comet and micronucleus assays, implying that spearmint has no influence on genotoxicity at the concentrations employed. There were no significant differences concerning basal DNA damage between WT and transgenic individuals as seen in other studies². Further studies are required to clarify the antioxidant and antigenotoxic effects of spearmint.

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PCH-66

**EPIGALLOCATECHIN-3-GALLATE ANTIMYCOTIC AND AZOLE RESISTANT MODULATOR
POTENTIAL AGAINST TRIAZOLE-RESISTANT *ASPERGILLUS FUMIGATUS***

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Systemic–opportunistic fungal infections caused by azole-resistant fungi, including *Aspergillus fumigatus*, are emerging as an important cause of human disease with high associated morbimortality rates worldwide [1]. The resistance to the limited arsenal of antifungal drugs is a serious concern, making it imperative to assess new compounds with antimicrobial properties and no cytotoxic effects, to be utilized as adjuvants in fungal therapy. Epigallocatechin-3-gallate (EGCG), the largest constituent of green tea, has demonstrated anti-infective properties in several *in vitro* studies [2] however, information regarding EGCG antimycotic activity and synergistic interactions between EGCG and triazoles against *A. fumigatus* is scarce. This work aimed to assess the antimycotic potential of EGCG against azole-resistant isolates of *A. fumigatus*, and the potential synergistic effects between EGCG and commonly used triazole antifungal drugs, namely itraconazole (ITR), voriconazole (VOR) and posaconazole (POS).

Azole-resistant *A. fumigatus* isolates (N=4) collected during exposure assessment campaigns performed in the last ten years in Portugal and abroad, and previously characterized through conventional culture-based (agar-plate screening) and molecular (sequencing the calmodulin or β -tubulin genes for *Aspergillus* spp.) methods, were grown in MEA medium. The obtained pure colonies were suspended in 1mL solutions with EGCG at final concentrations of 250 $\mu\text{g/ml}$, 100 $\mu\text{g/ml}$, 50 $\mu\text{g/ml}$, 25 $\mu\text{g/ml}$ and with no EGCG at all. Each suspension was then spread onto Sabouraud dextrose agar media supplemented with 4 mg.L^{-1} ITR, 2 mg.L^{-1}

VOR or 0.5 mg.L^{-1} POS and with no supplementation, for screening of antifungal resistance (adapted from the EUCAST 2020 guidelines). The inoculated plates were incubated at 25°C for 7 days. After the incubation period, all resistant isolates grew on Sabouraud dextrose agar media with no azole supplementation. The addition of 25 $\mu\text{g/ml}$ of EGCG to the suspensions canceled the growth observed in Sabouraud dextrose agar media of the 4 isolates, building evidence towards antimycotic potential of EGCG against azole-resistant isolates of *A. fumigatus*. One isolate grew on Sabouraud dextrose agar media supplemented with 2 mg.L^{-1} VOR, but showed no growth when 25 $\mu\text{g/ml}$ of EGCG was added to the suspension, indicating sensitization of the azole-resistant isolate when EGCG and VOR were used together. This study suggests that EGCG is a potential therapeutic adjuvant for therapies against azole-resistant fungi. We recommend increasing the sample size in further studies and evaluating EGCG potential against other fungal pathogens of clinical interest, such as dermatophytes.

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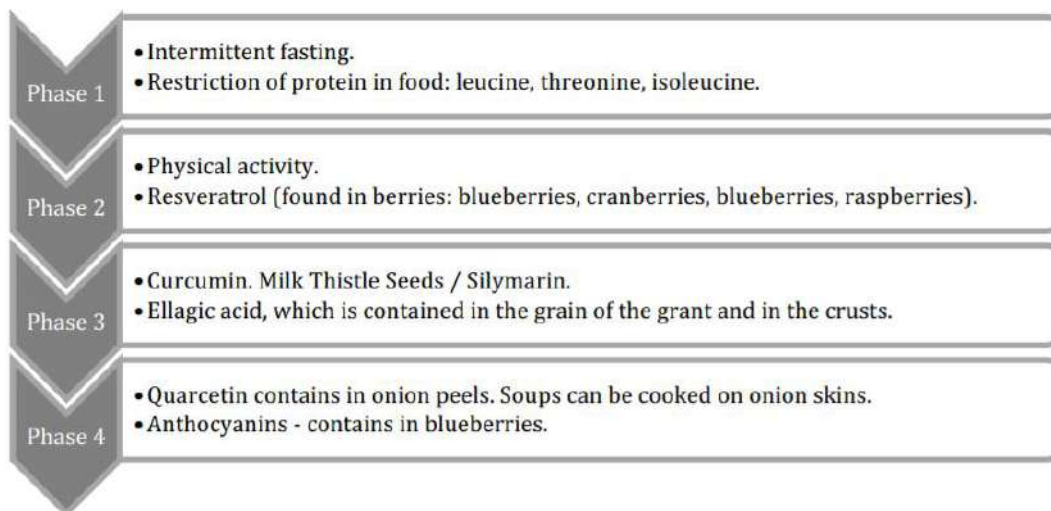
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PCH-67

ROLE OF mTOR HYPERACTIVATION IN DISEASEVladyslava Harkusha ^{1*}¹International Business Department, Simon Kuznets Kharkiv National University of Economics, Nauki Avenue, 9A, Kharkiv, Ukraine.

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Chronic diseases of civilization are largely associated with hyperactivation of mTOR (a key regulator of cell growth and metabolism): acne, cancer, type 2 diabetes [1]. After 25 years, a person's growth ends, and excessive mTOR activity makes not only muscles grow, but also increases the growth of malignant tumors, moles, and acne. Excessive activation of mTOR work leads to uncontrolled cell division - that is, to their transformation into cancerous ones. Normally, the work of mTOR primarily regulates cell growth and reproduction. These processes are very subtle: the slightest bias in one direction can lead to aging or death of the cell, in the other - to its uncontrolled multiplication and the development of a cancerous tumor. Therefore, too weak, and, especially, too "zealous" work of mTOR is very dangerous. In particular, mTOR is hyperactive in almost all cases of prostate cancer (which is the most common cancer in men) and in several other cancers. A group of American researchers led by Davide Ruggiero and Christian Rommel investigated the relationship between mTOR activity and cancer development [2]. What activates it the most? Dairy and meat products stimulate mTOR as strongly as possible. Cow's milk is an extremely potent evolutionary rapid growth program that overstimulates mTOR in people who consume this product. Increase in calf weight during the first year of feeding on 0.8 kg per day. This is nearly 40 times faster than that of a human infant [3]. Also, the essential amino acid leucine, which is found in large quantities in meat, has the ability to directly stimulate mTOR. mTOR reduction factors (**Figure 1**):



Take as an example the traditional diet of Okinawa island inhabitants, where there are only 10% of protein and no dairy products. They eat mostly plant foods. Hence the secret of centenarians who do not have acne, diabetes, and women have menopause after 60. Less than 1% of their diet consists of fish and meat (one serving of meat a month). Their life expectancy is the highest among any population groups [3].

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PCH-68

ANALYSIS OF NATURAL PRODUCT BIO-MARKERS USING ANTIBODY ENGINEERING AND IMMUNOASSAYS

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Natural product bio-markers such as active compounds, toxic components, pesticide residues and heavy metal contamination have been considered as important elements to evaluate and control the quality of natural products [1]. To date, numerous natural products still have not been estimated, and most of instrumental analytical methods are time-consuming and require expensive and professional operations.

Under such conjunction, there is huge need in the development of sensitive, quick and simple analytic methods. Rapid detection method is a major technique could be applied in biology, it divides into three main category such as microbiological detection, chemical and physical testing and immunoassay. Immunoassay,

a quick analytical technique is applying immunology theory to design and determine the antigens, antibodies, immune cells and cytokines.

Different approaches can be used to classify/characterize immunoassays [2]:

Enzyme-Linked Immunosorbent Assay (ELISA) [3] is the most widely used immunoassay, including indirect ELISA, Dual-Colorimetric Enzyme-Linked Immunosorbent Assay (DC-ELISA);

Chemiluminescent immunoassay (CLIA) [4]: Chemiluminescence enzyme immunoassay (CLEIA),

Multiplex CLIA, Chemiluminescence Resonance, Energy Transfer Competitive Immunoassay;

Fluorescence polarization immunoassay (FPIA);

Enzyme-multiplied immunoassay technique (EMIT);

Lateral flow immunoassay: Multiplex lateral flow immunoassay;

Phage display-mediated immuno-polymerase chain reaction (PD-IPCR);

Novel detection method based on aggregation of immunomagnetic beads.

There is a huge demand for the detection of natural products bio-markers in the reagent on the market and it gradually becomes the intrinsic driving force of the development of industry. Diversified antibody generation platforms/methods promote the screening and assessment of high qualified antibodies. Various immunoassays used to simplify and empower the immune-detection applications for different biomedical purposes.

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PCH-69

SOLID DISPERSIONS OF LEAVES EXTRACT of *Eugenia florida*Nóbrega AB,^{1,2*} Bizarri CHB,¹ Conceição EC,²¹Laboratório de Química da Biodiversidade, Plataforma Agroecológica de Fitomedicamentos, Centro de Inovação em Biodiversidade e Saúde, Farmanguinhos/Fiocruz, Rio de Janeiro, Brasil;²Laboratório de Pesquisa, Desenvolvimento e Inovação de Bioprodutos, Faculdade de Farmácia, Universidade Federal de Goiás, Goiânia, Brasil.

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Eugenia florida DC belongs to the family Myrtaceae [1]. Previous phytochemical studies with extracts of *E. florida* leaves showed the occurrence of triterpenes and, among its constituents, the betulinic acid (BA) [2], which presents a vast literature with different biological activities, such as anti-inflammatory [3,4], antimalarial [4], antiviral [4,5] and anticancer [4,6,7]. However, BA low aqueous solubility limits its bioavailability [8] (Figure 1A). The use of solid dispersions is highlighted in order to increase bioavailability, where lipophilic drugs are dispersed in hydrophilic carriers, improving solubility and dissolution profile. In this work, solid dispersions containing extracts of *E. florida* were prepared by fusion technique using the carrier Gelucire® 50/13 as a polymeric agent and lactose monohydrate as a hydrophilic support (Figure 1B). The solid dispersion technique increased in eleven times-the solubility of betulinic acid in *Eugenia florida* leaves extract, suggesting a higher bioavailability of the chemical marker. These results justify the continuity of studies to develop a phyto pharmaceutical drug.

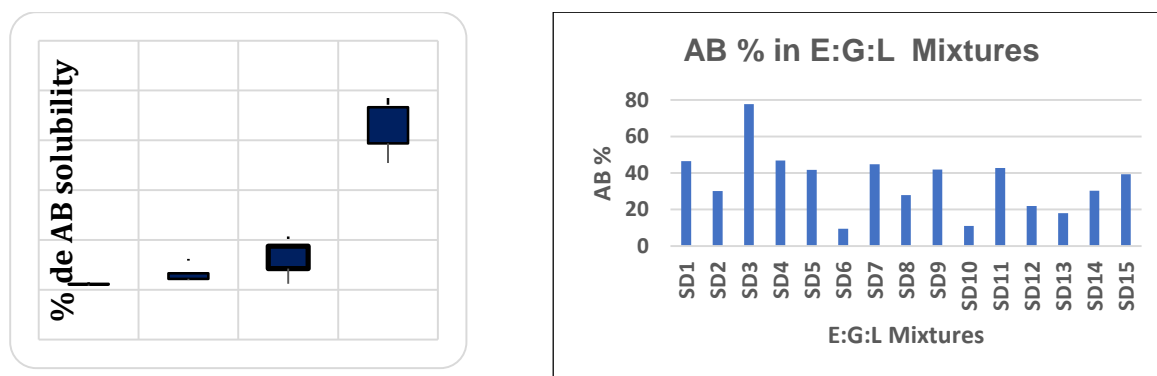


Figure 1. A. Betulinic acidsolubility present in crude extract at different pHs. **B.** Solubility response in pH 7 buffer solution of the betulinic acid present in the *Eugenia florida* leaves extract in solid dispersions. The different solid dispersions are described in Table 1. Solid dispersions of extract, gelucire and lactose in the following proportions: SD1 (50:500:100); SD2 (500:500:100); SD3 (50:950:100); SD4 (500:950:100); SD5 (50:500:300); SD6 (500:500:300); SD7 (50:950:300); SD8 (50: 950:300); SD9 (50:725:200); SD10 (500:725:200); SD11 (275:50:200); SD12 (275:95:200); SD13 (275:725:100); SD14 (275:725: 300); SD15 (275:725:200).

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MICROCRYSTAL SCOPE DETERMINATION OF CALCIUM SALTS IN TOOTHPASTES

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In the course of inorganic chemistry students of St. Petersburg State Technical University (TU) perform reactions in the laboratory for the qualitative determination of certain elements in different compounds. Calcium salts are the objects of study, their analysis allows to get acquainted with the chemical properties of this element. The well-known microscopic method is based on the reaction of characteristic crystals of gypsum $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ formation [1, 2]. This method can be recommended in the research work of schoolchildren and applicants due to its simplicity and clarity. It can be used to confirm the presence of calcium salts in some natural objects, such as limestone, eggshells, as well as in calcium toothpastes.

In limestone, eggshells and some pastes, calcium is present in the form of carbonate CaCO_3 , in other pastes - in the form of hydroxyapatite $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, glycerophosphate $\text{Ca}(\text{CH}_2\text{OH})_2\text{CHOPO}_3$, lactate $\text{Ca}(\text{C}_3\text{H}_5\text{O}_3)_2$ and other weak acids' salts [3,4].

Experiment. The analyzed sample was dissolved in hydrochloric acid, centrifuged, a drop of filtrate was evaporated on a glass slide, sulfuric acid was added, and the crystals formed were observed using an optical microscope. The formation of characteristic gypsum needle-shaped crystals indicated the presence of calcium salts. The table shows the composition of the initial calcium salts in the analyzed substances.

Table. Composition of calcium salts in the analyzed objects

Studied object / toothpaste	Calcium salt	Manufacturer
New Pearl Calcium	Calcium carbonate Calcium lactate	RF (St. Petersburg)
Splat Professional Biocalcium	Calcis (eggshell calcium carbonate) Calcium lactate, Hydroxyapatite	RF
R.O.C.S. Active calcium	Calcium glycerophosphate	RF (Moscow)
Karex	Hydroxyapatite	Germany
SPLAT Professional Whitening plus	Dicalcium phosphate dihydrate Calcium pyrophosphate	RF
Colgate Sensitive	Calcium carbonate	Poland
Eggshell	Calcium carbonate	
Limestone	Calcium carbonate	
Sensodyne	---	England
Elmex children	---	Switzerland

Conclusions. The microscopic analysis of the investigated substances confirmed the expected presence or absence of calcium salts. Natural substances, such as limestone, eggshells, as well as calcium toothpastes can be offered as interesting objects for research in the course of inorganic chemistry.

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in Food area



PCF-01

***POLYPODIUM VULGARE* LINN (*POLYPODIACEAE*), AS A SOURCE OF BIOACTIVE COMPOUNDS**

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The *pteridophytes*, popularly known as ferns, due to their independent evolution with respect to the rest of the species, constitute a source of new phytoconstituents [1,2]. In the last decades, studies published on the aqueous extract of the fronds of the fern *Polypodium leucotomos* Linn (*Polypodiaceae*) have highlighted the potential of new applications of this fern as a nutraceutical and cosmeceutical agent due to photoprotective properties [3,4].

The European Medicines Agency (EMA) has also published a monograph on the therapeutic use of the *Polypodium vulgare* Linn (*Polypodiaceae*) rhizome for its beneficial expectorant properties in cough and cold as well as for short-term use in cases of occasional constipation. However, other uses and potential applications are here explored.

In the present study we focused on the phytocharacterization and *in vitro* bioactivity of the methanol extract from the fronds of *Polypodium vulgare* (PVM) in the cytotoxicity and phototoxicity assays and the evaluation of the cytoprotective and neutralization capacity of ROS against H₂O₂ as an oxidative stress agent. The polyphenolic profile obtained by HPLC-DAD has revealed a high content of polyphenolic compounds (77823.8 mg/kg) with a predominance of 3-O-caffeoylquinic acid (58778.3 mg/kg) over the rest of the phytoconstituents analysed. The extract did not induced cytotoxicity against tumoral cell lines, HaCaT being the cell line that has experienced a greater decrease in cell viability (LC_{50, HaCaT} = 0.390 mg/mL). The 3T3 cell line has experienced better results than HaCat in terms of cytoprotection using different models of toxic agents.

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PCF-02

BIOACTIVE FOOD COLORANTS OBTAINED FROM *LONICERA CAERULEA* L., *MORUS NIGRA* L., AND *RUBUS FRUTICOSUS* L. FRUITS

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Lonicera caerulea L., *Morus nigra* L., and *Rubus fruticosus* L. fruits are widely known for their nutritional and bioactive properties. Their richness in anthocyanins, which are the main responsible compounds for the reported beneficial properties, justify their exploitation not only as functional foods but also as sources of natural colorants, in alternative to some artificial compounds with reported adverse effects to human health [1,2]. In this context, the fruits were characterized in terms of anthocyanin and non-anthocyanin compounds, by HPLC-DAD/ESI-MS, and two solid colouring formulations were prepared through the spray-drying technique with maltodextrin and mixtures of Arabic gum and maltodextrin in different proportions, according to the characteristics of each fruit juice and the efficiency of the process. The stability of the prepared colorants was assessed over three months of storage at room and refrigerated temperature. For that purpose, the microbial load, the cytotoxicity, and the bioactive properties (antioxidant and antimicrobial) were evaluated, along with their anthocyanin concentration and colouring capacity.

Different phenolic compounds were detected in the three fruits, among which, some anthocyanins as cyanidin-3-*O*-glucoside and cyanidin-*O*-hexose, as the most abundant ones. All the formulations revealed great colouring, antioxidant, and antimicrobial properties, with a slight variation of anthocyanin concentration along the three months of storage at room and refrigerated temperature, which validate their application for colouring purposes. None of these formulations revealed cytotoxic properties, being, then, considered safe for food application. Through the present study, it was possible to obtain stable anthocyanin based colorants with potential application in several industrial fields.

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PCF-03

**PROSPECTION FOR THE BEHAVIOR OF ENZYMIC ACTIVITY FOR
POLYPHENOLOXIDASE (PPO), PEROXIDASE (PO),
PECTINAMETILESTERASE (PME) IN THE ASSAYS OF THE FERMENTATIONS OF
CACAU (*THEOBROMA CACAO L.*)**

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The work presents the behavior of the enzymes polyphenoloxidase (PPO), peroxidase (PO) and pectinamethylesterase (PME) extracted from cocoa beans during the total process of 168 hours of conventional fermentation (*f1*) and a prospection of this activity for the fermentation test with inoculum start (*f2*). They were monitored beyond the time (7 days), temperature (Digital Thermometer MINIPA MT - 450), pH expressed through direct reading in digital pH-meter (QUIMIX) and Total Soluble Solids (° Brix) in bench refractometer Abbe [1]. The cocoa fruits were obtained in the open market, ver-o-peso, Belém/PA. The results of Figures 1 to 3 show that in the parameters of maximum temperature, at pH 4.5 and SST the prospecting for enzymes present better and constant performance than the enzymatic activities in conventional fermentation. With this, it is concluded that the fermentation start will present greater efficiency in the enzymatic activity.

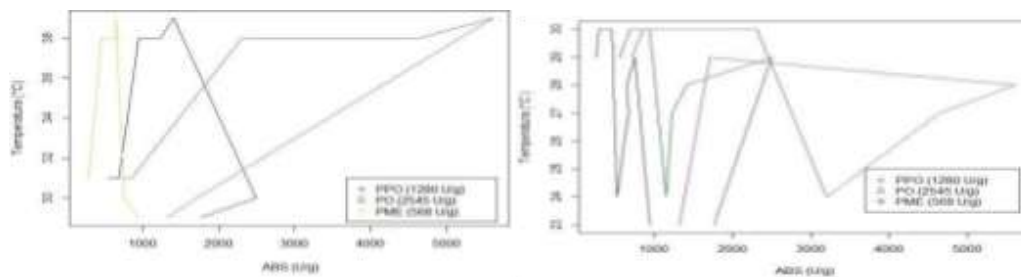


Figure 1 - Temperature and ABS (absorbance) of the enzymatic activity in the fermentation monitored assay (*f1*) and (*f2*).

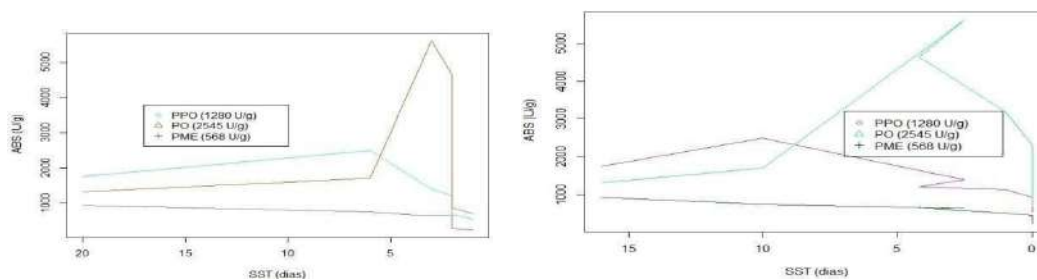


Figure 2 - Relationship of TSS and ABS of enzymatic activity in the fermentation assay (*f1*) and (*f2*).

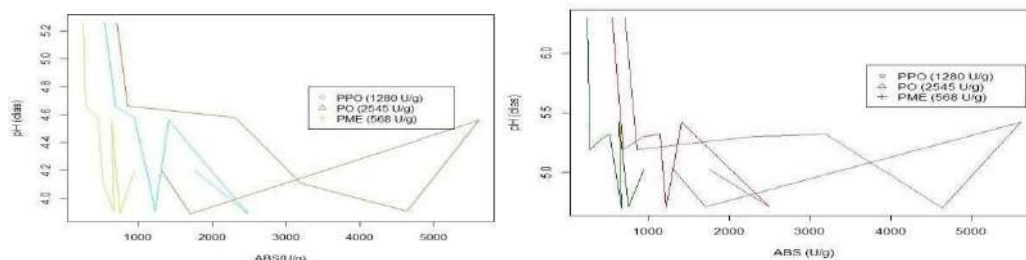


Figure 3 - Relationship of pH and ABS of enzymatic activities in the assay with fermentation (*f1*) and (*f2*).

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PCF-04

BY-PRODUCTS OF AGRI-FOOD INDUSTRY AS TANNIN-RICH SOURCES: A REVIEW OF TANNINS' BIOLOGICAL ACTIVITIES AND THEIR POTENTIAL FOR VALORIZATION

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In recent decades, consumers have been continuously moving towards the substitution of synthetic ingredients used in industries by natural products, extracted from animal, vegetal or microbial sources. Additionally, circular economy has been proposed as the most efficient production system since it allows reducing and reutilizing different wastes that are commonly discharged in landfills causing economic and environmental damage [1]. Currently, the agri-food industry has been engendering tons of liquid and solid wastes during manufacturing, along with agriculture high production rate of organic agricultural waste (e.g. discarded fruits and vegetables, peels, leaves, seeds or forestall residues). These wastes are not considered environmentally friendly, and usually end up underutilized and accumulated. During their degradation, the soil is left inhabitable due to the high toxicity of the released greenhouse gases. Furthermore, these agri-food by-products are potential sources of valuable bioactive molecules such as tannins. Tannins are phenolic compounds, naturally found in vegetables [2]. Their presence in nature has prompted their historical use in different sectors. They are secondary metabolites of plants, widespread distributed in terrestrial and aquatic natural environments. As they can be found in plenty of plants, herbs and even algae, they have been traditionally used for medicinal and other purposes, such as the leather industry. This reality is clarified by the fact that they exert several distinctive biological activities, and in this way, they involve an interesting potential to be used within the food, nutraceutical, pharmaceutical and cosmetic industry [1]. Consequently, this article is tended to towards the description of the biological activities exerted by tannins as they may be further extracted from by-products of the agri-food industry to create new high-added-value products.

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PCF-05

**BLUEBERRY JUICE AS A NUTRITIOUS AND BIOACTIVE BEVERAGE TO BE INCLUDED
IN NOVEL FOOD PRODUCTS**

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Blueberry (*Vaccinium myrtillus* L.) is a very popular fruit, native from the northern hemisphere and consumed worldwide. It has been widely studied for being a rich source of bioactive compounds with recognized beneficial properties for Human health [1]. Therefore, several industrialized products, such as juices and derivatives, have been developed from blueberry fruit, aiming at most practical forms of consumption. In this sense, the present work aimed to analyse the blueberry juice in terms of nutritional value, following AOAC procedures, and chemical compositions, namely in free sugars, by HPLC-RI, organic acids, by UFLC-PDA, and phenolic compounds, by HPLC-DAD/ESI-MS. Moreover, the antioxidant capacity, by TBARS and OxHLIA assays, was also assessed to validate its bioactive properties.

Regarding the nutritional value, carbohydrates were the major macronutrients found in blueberry juice, followed by ash and protein. In terms of free sugars, fructose and glucose were detected, with a higher concentration of fructose. Four organic acids were also identified in the juice, namely oxalic, quinic, malic, and shikimic acids, being quinic acid the most abundant one. In what concerns the phenolic composition, four phenolic acids (quinic acid, caffeic acid hexoside, cis 5-*O*-caffeoylquinic acid, and trans 5-*O*-caffeoylquinic acid) and six anthocyanins (cyanidin-3-*O*-glucoside, cyanidin-3-*O*-pentoside, peonidin-3-*O* glucoside, peonidin-3-*O*-pentoside, malvidin-3-*O*-glucoside, and malvidin-3-*O*-pentoside) were detected. As expected, considering its chemical composition, the juice also presented a strong antioxidant capacity, being able of inhibiting the lipid peroxidation and the oxidative hemolysis. The results obtained in the present study validate the nutritional and bioactive quality of the juice obtained from *Vaccinium myrtillus* L., justifying its application in the development of novel foodstuff.

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PCF-06

SUBCRITICAL WATER EXTRACTION OF *ACTINIDIA ARGUTA* LEAVES: EVALUATION OF TEMPERATURE EFFECTS ON BIOACTIVITY AND RADICAL SCAVENGING ACTIVITY

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Actinidia arguta fruit (kiwiberry) is considered an excellent source of bioactive compounds, such as antioxidants, contributing to human's health [1,2]. However, the by-products of this vine, such as leaves, were also associated to different health benefits due to its varied chemical composition [3,4]. The aim of this study was to evaluate the bioactivity and antioxidant radical scavenging activity of *A. arguta* leaves extracts, employing a sustainable extraction technique: Subcritical Water Extraction (SWE). In this way, the antioxidant activity (DPPH assay), the total phenolic content (TPC) and the total flavonoid content (TFC) were evaluated as well as the scavenging capacity against the superoxide $\text{O}_2^{\cdot-}$ and the hypochlorous acid (HOCl) species. The obtained results (Table 1) revealed that the sample extracted at 123 °C achieved the best results in all assays, with the superoxide assay. The TPC, TFC and DPPH assay results were higher than the ones reported by other authors for this matrix using other extraction techniques [4,5,6]. In what concerns to the radical scavenging activity, the best $\text{O}_2^{\cdot-}$ result was $228.87 \pm 1.30 \mu\text{g/mL}$ (110 °C), while for the HOCl assay was $18.22 \pm 1.29 \mu\text{g/mL}$. Considering all findings, the best temperature was 123 °C. These results demonstrate that *A. arguta* leaves extracted by SWE has a large bioactive potential for application in several industries.

Table 1: Extraction yield, bioactivity, and radical scavenging activity of *A. arguta* leaves extracts by SWE.

Samples	Extraction yield (%)	TPC (mg GAE/g dw)	TFC (mg CE/g dw)	DPPH ($\mu\text{g/mL}$)	$\text{O}_2^{\cdot-}$ ($\mu\text{g/mL}$)	HOCl ($\mu\text{g/mL}$)
110°C	29.67	106.48 \pm 4.71	46.07 \pm 4.11	648.57 \pm 28.03	228.87 \pm 1.30	20.48 \pm 1.21
123°C	25.76	109.72 \pm 4.98	53.11 \pm 4.52	559.31 \pm 54.33	343.33 \pm 2.06	18.22 \pm 1.29
135°C	25.44	68.78 \pm 2.72	33.68 \pm 3.38	615.78 \pm 45.46	433.34 \pm 2.80	22.29 \pm 0.32
148°C	26.43	72.92 \pm 1.18	32.69 \pm 1.60	670.36 \pm 62.09	471.26 \pm 1.80	21.45 \pm 3.45
160°C	24.71	77.37 \pm 3.01	32.72 \pm 1.27	649.14 \pm 18.17	551.15 \pm 13.84	27.59 \pm 2.30

*dw: dry weight; GAE: gallic acid equivalents; CE: Catechin equivalents

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NUTRITIONAL AND FUNCTIONAL PROPERTIES UNCONVENTIONAL FOOD PLANTS (UFP): ORA-PRO-NOBIS (*Pereskia aculeata* Miller) AND YACON (*Smallanthus sonchifolius*)

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Unconventional Food Plants (UFP) can function as alternative natural sources to diversify human food [1]. However, several of these food sources have fallen out of use or are little known, due to the recent patterns of globalized food. Can find a wealth of edible plants in nature, with an estimated of about 30,000 species with food potential [2]. The ora-pro-nóbis (*Pereskia aculeata* Miller) is a *Cactaceae* plant with leaves rich in protein, which is why it is also known as poor meat [3]. It is referred to in food because it has a high protein content and is used as a nutritional supplement, in addition, it is considered a functional food (Table 1). Its leaves are edible and succulent and can be used in various preparations, such as stews, flour, pasta, pies, etc. [4]. Yacon (*Smallanthus sonchifolius*) is a plant of the *asteraceae* species that has tuberous roots, preferably consumed in natura, as it contains a sweet and refreshing taste, but its form of ingestion may vary according to its use. Obtaining flour by dehydrating Yacon proved to be interesting, as it increases its useful life and facilitates its use in the formulation of cookies, juices, sweets, cakes, etc. [5].

Table 1: Nutritional and functional benefits of ora-pro-nobis e Yacon.

Benefits	Ora-pro-nóbis	Yacon
Nutricional	Features of about 20% of protein of higher content of amino acids lysine and tryptophan, rich in fibers soluble, minerals calcium, iron and phosphorus, and further shows the vitamins A, B and C.	Rich in antioxidants, phenolic compounds and fructooligosaccharides.
Functional	Helps in treatment of the anemia iron deficiency, osteoporosis and constipation bowel, arrest of womb, varicose veins and hemorrhoids and other problems bowel	It aids in the control of diabetes, regulating intestinal decreasing the levels of cholesterol, increasing the immunity and absorption of magnesium, iron and calcium

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PCF-08

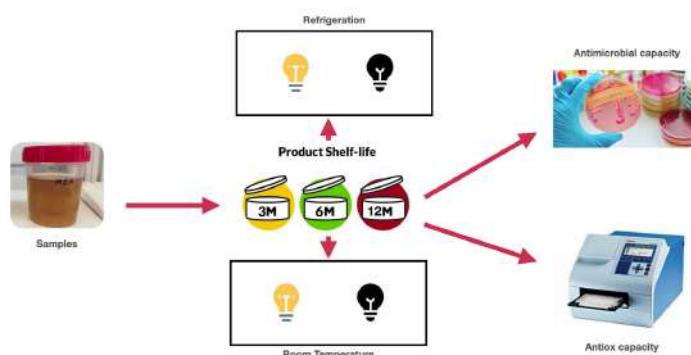
ANTIOXIDANT ACTIVITY OF A NEWLY DEVELOPED BIOPOLYMER-BASED COATING

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Plastic has become one of the key environmental issues of our time, over 8 million tons of plastic are thrown into the ocean every year [1]. The vast majority of it being plastic from food packaging. Plastic waste is considered a global problem; therefore, solutions are needed to reduce the pollution resulting from the overuse of this material [2]. In this context, this work explores the possibility of manufacturing of a solution from a natural biopolymer to use as a spray to coat and protect food as an alternative barrier. The contact between the spray and food is harmless for human health. The Spray solution is composed of a primary solution of a natural biopolymer and a mixture of different natural antioxidants extracted from specific herbs tested, the extractions were made using 80% thanol and 100% water, lyophilized and then mixed on an aseptic environment. The current work focuses on monitoring antioxidant and antimicrobial activity using a shelf-life span of 1 year, divided in 7 different times (0, 7, 15 days and 1, 2, 6, 12 month), in 4 different conditions 2 in refrigeration ($\pm 3^{\circ}\text{C}$) and 2 at room temperature, each temperature with a pool of light protected and another one without light protection. In this study, reports within our research group have indicated that the solution could help protecting certain type of foods [3]. The main objective of this study is to explore the antioxidant and antimicrobial activity in order to screen the solution shelf-life span.



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PCF-09

INTESTINAL MORPHOLOGICAL CHANGES PRODUCED BY MALNUTRITION : EVALUATION OF A SUPPLEMENT BASED ON BURITI FLOUR AND MILK DERIVATIVES

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Malnutrition induces a series of changes that affect several body parameters, including the integrity of the intestinal mucosa [1]. Thus, we evaluated the effects of a supplement based on buriti flour and whey, on the recovery of the intestinal mucosa of malnourished Swiss mice, focusing on histological parameters. Two dietary supplements were used: one already commercialized and another, test supplement. There was a phase of induction of malnutrition and one of renutrition: control group, two isocaloric groups to control (commercial supplement and test), one malnourished group and two isolaloric groups to the malnourished. It was observed, after renutrition, that the intestinal villi of the groups in the test supplement were thicker and higher (Figure 1). The nutritional quality of the ingredients of the test supplement, whey and buriti flour may have contributed to these results. The buriti fruit (*Mauritia flexuosa*), is rich in vitamins, antioxidants, unsaturated oils and dietary fibers. The fiber content of buriti flour stimulates intestinal fermentation and the production of short-chain fatty acids, with beneficial effects on this organ [2]. This fact corroborates and reinforces our findings related to the recovery of the intestinal structure. The high bioavailability of whey proteins and the higher leucine content stimulate protein synthesis and renewal [3]. Our work presents a new tested and effective supplement composition in the treatment of malnutrition.

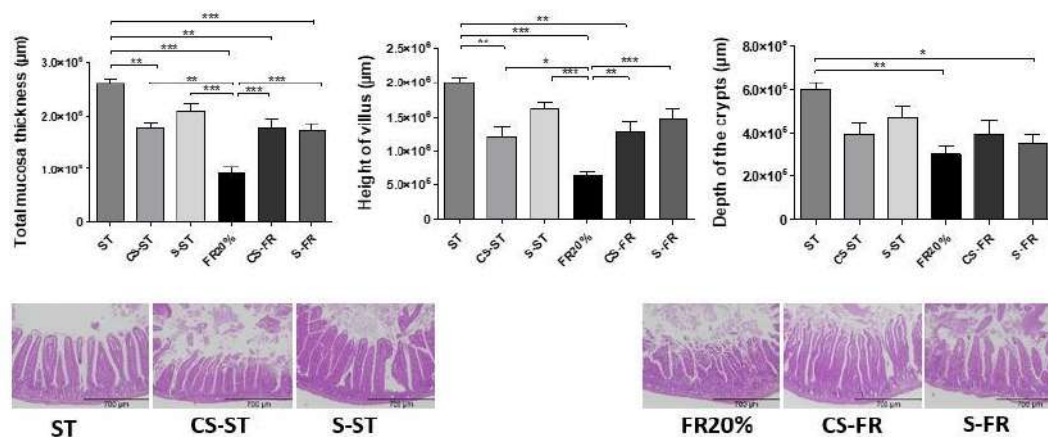


Fig. 1 Intestinal villi of mice submitted to different diets: ST (Standard Chow), CS-ST (Standard Chow + Commercial Supplement), S-ST (Standard Chow + Test Supplement), FR20% (Standard Chow - 20% food restriction), CS-FR (Standard Chow + Commercial Supplement) and S-FR (Standard Chow + Test Supplement). Data presented as mean ± SEM; * p < 0.05, ** p < 0.01; *** p < 0.001 versus indicated group.

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PCF-10

***Bixa orellana* L. pods and seeds: nutritional and chemical characterization, bioactivity studies, and development of a carotenoid-based food colorant**

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Bixa orellana L. is worldwide known as a source of bixin, a carotenoid compound with high colorant capacity [1]. The growing tendency in the food industry to use new and safer colorant compounds with higher stability coupled to the high demand of consumers concern, became of the utmost importance. Moreover, sustainable extraction technologies coupled with greener solvents are increasingly required to maximize the recovery of high added value compounds [2]. The present work intended to deepen the study of the nutritional and chemical profile and bioactive potential of *B. orellana* seeds. Furthermore, to maximize the extraction of bixin from seeds ultrasound-assisted technologies combined with RSM were used. As an externality of this work, the pods of this plant (bio-residues resulting from the processing of seeds) were also studied, in relation to its bioactive properties and profile in phenolic compounds. The most abundant macronutrients found in seeds were carbohydrates, followed by fat, proteins, and ash. Sucrose and trehalose were the only sugars found; and malic acid and α -tocopherol were the main organic acid and tocopherols present, respectively. Monounsaturated fatty acids (eicosenoic acid) were found in higher amounts. Pods hydroethanolic extract presented lower IC₅₀ values for antioxidant activity; while seeds sample revealed lower IC₅₀ values for $\Delta t = 60$ min and $\Delta t = 120$ min for OxHLIA assay, and lower GI₅₀ values for cytotoxic and hepatotoxic assays. Both samples presented lower MIC, MBC, and MFC in comparison to the two positive controls, against all bacterial and fungal strains. Pods presented the highest amounts of phenolic compounds (due to protocatechuic acid). The model developed for the extraction process was validated, with optimal processing conditions (sonication - 348 W, 6 min, 79 % (v/v) ethanol), being possible to obtain 27.1 mg of bixin per g of extract.

Overall, this study allowed to present innovative results in relation to the nutritional, chemical and bioactive properties of the seeds, and as an externality the great potential of the pods regarding its biological activity. Considering the good results obtained in the optimization procedure, it is worth mentioning the sustainable way in which this extract can be obtained.

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PCF-11

PRODUCTION OF NATIVE TREE PLANTS FOR FOOD, RESTORATION AND CONSERVATION PURPOSES

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The Upper Parana Atlantic Forest, a hotspot of highly threatened biodiversity, extends to northwestern in Argentina in the province of Misiones is known as the Selva Misionera or Selva Paranaense. National and provincial laws establish and promote the restoration of degraded forest areas, mainly linked to protective forests of natural water sources. This is a crucial process necessary to the establishment of biodiversity islands, which requires plant propagation material of native species to ensure their viability and diversity. As part of our previous work on restoration of degraded lands with native tree species that can be useful for productive purposes [1], and considering that the local diversity contains more than 100 species of edible fruits [2], species of the Myrtaceae family are considered, as: *Eugenia involucrata*, *Eugenia uniflora*, *Eugenia myrcianthes*, *Eugenia pyriformis*, *Plinia peruviana*, *Plinia rivularis*, *Myrcianthes pungens*. Trees are registered in every landscape where they are naturally present, whether they are part of forests, riverine strips or growing in fields. The harvest should be done when the fruits are ripe on the tree or in the ground at the time of dispersal. The immediate separation of the seed from the pulp must be carried out and can be used for the production of juices, liqueurs, vinegars and frozen pulp. While the seeds that due to their recalcitrance must be sown directly in the nurseries for the production of plants. If is necessary to be stored, the humidity should be generally maintained above 35%, and they should be preserved in moist sand under cold temperatures (4 to 8°C), conditions to which the seeds can survive for more than one year [3]. The sowing is done directly in containers with composted pine bark substrate and slow release fertilizer and after 12 to 24 months (depending on the species) the plants are ready to be planted in the field, preferably in humid places and medium shade. The economic valuation of the goods and services produced by the native forest includes fruits and seeds of the species of interest for their propagation in nurseries. Being an incipient market to develop it is suggested, to apply the methodology of the cost-price ratio, as a way to cover the costs incurred and reasonable for the market which includes the cost of conservation. Conserving biodiversity can also be a profitable renewable resource through the non-timer use of local multiple use species for economic purposes.

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PCF-12

PHENOLIC COMPOUNDS FROM AMARANTHACEAE SPECIES: EXTRACTION, CHARACTERIZATION AND BIOLOGICAL PROPERTIES

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Species of the Amaranthaceae family have become a potential group of plants for their latent beneficial properties. Moreover, in most cases, they are plants with a low price and easy to collect as they could be cultivated and they grow wildly in cosmopolitan areas. Their use in traditional medicine and potential biological properties can be considered as the basis to guide further investigations about their characteristics and beneficial uses. In this work, three species of the Amaranthaceae family traditional from China (*Alternanthera sessilis*, *Dicliptera chinensis* and *Dysphania ambrosioides*) were proposed as an alternative source of bioactive compounds, namely phenolic compounds. Some of the species of this family have already been reported for their antioxidant and antimicrobial activities, so they open the possibility to find other bioactive ingredients of interest [1].

The study aimed to extract and characterize the phenolic compounds of the three species and investigate possible antitumor, antimicrobial, antioxidant and anti-inflammatory activities. For this purpose, the plant extracts were prepared by solid-liquid extraction with an hydroethanolic mixture, and the phenolic profiles were characterized by HPLC-DAD-ESI/MSⁿ. The antioxidant activity was assessed by two *in vitro* assays: TBARS (thiobarbituric acid reactive substances formation inhibition) and OxHLIA (oxidative hemolysis inhibition). The antimicrobial activity was tested against Gram (-) and Gram (+) pathogenic bacteria and various fungi species. The antitumor properties were assessed *in vitro* by the ability to inhibit the growth of tumor (MCF-7, NCI-H460, HeLa and HepG2) and non-tumor (PLP2) cell lines. The anti-inflammatory activity was evaluated by the extracts capacity to inhibit the NO production by the lipopolysaccharide (LPS)-stimulated macrophage-like RAW 246.7 cell line. Overall, this study showed that the studied species of the Amaranthaceae family could be an alternative source of bioactive compounds to formulate new innovative products and incorporate them into the food, cosmetic and pharmaceutical industry.

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PCF-13

WINTER SAVORY (*Satureja montana*) ESSENTIAL OIL AS A NATURAL ANTIMICROBIAL FOR MEAT PRESERVATION

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Microbial contamination is considered one of the most important causes of food spoilage [1]. In order to control pathogens, numerous preservatives and procedures are used. However, due to possible side effects of the artificial food additives, the natural ones, including essential oils (EOs), are enthusiastically accepted [2]. In addition, marinating process that is commonly used in food preparation could also contribute to better conservation [3]. Assessing different foods, fresh meat could be considered as an extremely subjected to microbial contamination. Among its contaminants, *Listeria monocytogenes*, a well-known foodborne pathogen that causes listeriosis, is listed as one of dominant [4]. Taking into account all abovementioned, the aim of this study was to chemically characterized and examine antibacterial effect of winter savory (*Satureja montana*) EO, both *in vitro* and *in situ* in wine-marinated beef meat. *In vitro* antibacterial effect was screened in microdilution and time kill assays, both performed on *L. monocytogenes* strains (reference ATCC 19111 and three isolates originated from meat industry: LMB, LMS and LMT). *In situ* analysis involved monitoring of antilisterial effect, as well as activity against meat spoilage bacteria: aerobic heterotrophic mesophyll bacteria (AHMB), Enterobacteriaceae (ENT) and lactic acid bacteria (LAB).

GC-MS analysis revealed carvacrol (30.7%) and thymol (18.0%) as the most abundant among identified constituents. In microdilution assay EO induced antilisterial effect against all tested strains, with MIC values determined at 0.5% for referent strain and 1% for isolates, and MBC values determined at 1% for all the strains. *In vitro* time kill assay was performed on selected listerial strains (ATCC 19111 and LMB) and pointed out the dynamics of growth inhibition. Additionally, it enabled us to estimate curve MIC values, which indicated a higher bacterial sensitivity under aerobic than hypoxic conditions. Considering *in situ* analysis, red wine-marination of a beef reduced the growth of previously inoculated listeria (ATCC 19111 and LMB isolate), as well as pre-existing meat spoiling contaminants AHMB, ENT and LAB. Furthermore, addition of winter savory EO notably enhanced antimicrobial effect of marinade.

In conclusion, obtained results suggest possible application of winter savory EO in the form of a natural preservative in beef and recommend further research directed to other foodstuffs.

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PCF-14

***ANANAS COMOSUS* L. BIO-WASTE AS A SOURCE OF BIOACTIVE COMPOUNDS WITH HEALTH BENEFITS**

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Pineapple (*Ananas comosus* L.) is a fruit appreciated and consumed worldwide not only because it is recognized for nutritional properties, but also for the beneficial characteristics that help in the development of the organism [1]. Although only the pulp is consumed, several studies have been exploring different parts of the fruit, as they have high amounts of bioactive compounds of interest. Thus, and since the food industry annually produces tons of waste that are not properly used [2], this work aimed at the characterization of the pineapple peel and crown in order to enhance this bio-waste and a circular bioeconomy.

Heat-assisted hydroethanolic extraction was used to recover compounds subsequently identified and quantified by High-Performance Liquid Chromatography coupled with a diode array detector and electrospray ionization mass spectrometry (HPLC-DAD-ESI/MS). Twenty phenolic compounds were identified in both peel and crown extracts, among them, phenolic acids and flavonoids. The main detected compounds were caffeic acid derivatives, namely caffeine putrescine and flavones such as apigenin 6,8-*C*-diglucoside. The antioxidant activity of the extracts was tested and proved through two *in vitro* tests: the lipid peroxidation inhibition test (TBARS) and the oxidative hemolysis inhibition test (OxHLIA). The antiproliferative activity of both extracts was evaluated in tumor and non-tumor cell lines using the sulforhodamine B method, and the anti-inflammatory activity in lipopolysaccharide-activated RAW 264.7 macrophages by the ability to inhibit NO production.

The results showed that both extracts had an excellent performance in the cell-based tests of antioxidant activity, highlighting the lower EC₅₀ values and consequently greater activity for the bark extract. The same trend was seen in the tests of anti-tumor activity, with none of the extracts showing toxicity up to the maximum concentration tested (GI₅₀ > 400 µg/mL).

This study confirms the potential application of pineapple bio-residues, especially the peel, in the food industry as a source of compounds with bioactive properties, contributing to the valorization of this bio-waste.

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PCF-15

ANTIOXIDANT COMPOUNDS FROM *UNDARIA PINNATIFIDA*: MICROWAVE-ASSISTED EXTRACTION AND OPTIMIZATION USING RESPONSE SURFACE METHODOLOGY

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Macroalgae are a rich source of diverse secondary metabolites such as carotenes and phenolic compounds, with several health benefits. These compounds have attracted special attention in both, scientific and industrial fields, due to their bioactivities [1]. Therefore, the aim of this study was to simultaneously optimize the total phenolics content (TPC), total flavonoids content (TFC), extraction yield (EY), radical scavenging activity (DPPH) and trolox equivalent antioxidant capacity (TEAC) from *Undaria pinnatifida* using microwave assisted extraction (MAE). To perform this study, a response surface methodology (RSM) was applied with a five-level central composite design combining the independent variables: the solvent as a percentage of ethanol (*S*, 0 to 100%), time (*t*, 3-25 min) and pressure (*P*, 2-20 bar) in 28 experiments, maintaining a constant value of power during the process. To maximize all responses at the same time, each response was individually optimized and compared with a global optimization that allowed obtaining the optimum extraction conditions. Analysis of variance of each response variable model showed that they were all significant ($p < 0.05$), suggesting that these models could be used to describe the effects of the selected independent variables on TPC, TFC, EY, DPPH, TEAC and BC of *Undaria pinnatifida* extracts. The optimum conditions for extracting bioactive compounds with antioxidant activity from the algae in study were: 10.25 ± 0.34 min, 20.00 ± 4.00 bar and 0% of ethanol, which means that water was more receptive polar solvent for microwave energy absorption than ethanol. Additionally, a validation study was performed and a close agreement between experimental and predicted values was observed, indicating the suitability of the model and the success of RSM in modelling responses to characterize their dependence with extraction conditions under evaluation. Concluding that an aqueous MAE (20 bar, 10 min) appears to be the optimum processing approach for the extraction of phenolic compounds with antioxidant activity from *Undaria pinnatifida* without using organic solvents.

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PCF-16

DEVELOPMENT OF ENERGY BARS WITH BEE POLLEN AND BEE BREAD

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Bee pollen and bee bread are two beehive products that have a high nutritional value, result of the presence of proteins, amino acids, fatty acids, carbohydrates, vitamins, minerals and phenolic compounds [1]. Therefore, these products can be considered as excellent ingredients for energy bars.

The study focused on the formulation of different energy bars with compositional variation of pollen and bee bread. Additionally, the composition included chestnuts, almonds, walnuts, hazelnuts, common in Trás-os-Montes region, and ingredients such as white quinoa, sesame and oat flake. Here we report the first stage of the study, namely the nutritional characteristics of all the raw materials and the sensory analysis of the energy bars. The results of the pollen analysis for bee pollen and bee bread, identified as the predominant origin the *Fabaceae* family (21%) and *Fagaceae* family (39%), respectively. In nutritional terms, bee pollen showed a higher content of moisture, proteins, fat, and energy when compared to bee bread. In opposition, the bee bread had a higher content of ash, fiber, and carbohydrates. The sugar profile for bee bread highlighted the high fructose content when compared to pollen. For the dry fruits, walnuts showed a high fat content, while almonds have presented high levels of protein and carbohydrates. The hazelnut, on the other hand, was characterized by its rich fiber and sucrose contents. Regarding seeds, the sesame seed exhibited the highest contents of ash, fat, protein, energy, and fiber. For the sugar profile, the seeds only contained sucrose, with the highest value found in white quinoa. According to a sensory analysis (**Table 1**), the panel acceptability was good for all the energy bars, with the two highest scores registered for the formulations containing pollen, particularly those with white quinoa/hazelnut and sesame seed/almond combinations.

Table 1: Sensorial analysis for the different energetic bars formulations, measure as general acceptancy.

Formulations of energy bars	Minimum	Maximum	Average	Formulations of energy bars	Minimum	Maximum	Average
Oat flake, pollen, walnut	3	8	4.9	Oat flake, bee bread, walnut	3	7	5.5
Oat flake, pollen, hazelnut	4	9	5.8	Oat flake, bee bread, hazelnut	4	8	6.7
Oat flake, pollen, almond	5	8	6.3	Oat flake, bee bread, almond	5	8	5.9
Sesame seed, pollen, walnut	3	8	6.0	Sesame seed, bee bread, walnut	4	9	6.4
Sesame seed, pollen, hazelnut	4	9	6.2	Sesame seed, bee bread, hazelnut	4	9	6.6
Sesame seed, pollen, almond	4	9	7.1	Sesame seed, bee bread, almond	4	9	6.4
White quinoa, pollen, walnut	3	9	6.3	White quinoa, bee bread, walnut	4	8	5.9
White quinoa, pollen, hazelnut	5	8	7.0	White quinoa, bee bread, hazelnut	4	8	6.7
White quinoa, pollen, almond	4	8	6.5	White quinoa, bee bread, almond	4	7	5.5

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PCF-17

CHEMICAL COMPOSITION AND BIOACTIVE PROPERTIES OF PUMPKIN SEEDS AND SEED CAKES

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Pumpkin, (*Cucurbita pepo* L.) is one of the most important vegetables of the Cucurbitaceae family which is widely used for its edible fleshy fruit, especially fruit pulp. However, other fruit parts such as seeds are a good source of phytochemical and usually consumed in raw or processed form. In the present work, the chemical composition and bioactive properties of pumpkin seeds and seed cakes were evaluated. Plants from local landrace “Nychaki” were cultivated at the experimental farm of the University of Thessaly during the summer-autumn growing period of 2020 and fruit were collected at marketable maturity. Seeds were removed from 15 randomly selected after cutting each fruit at the equatorial axis. Then seeds were air-dried at room temperature and pressed with a cold-press to obtain the seed oils and seed cakes, while whole air-dried seeds were ground to fine powder. The ground seeds were rich in fat and proteins (42.7 g/100 g dw and 37.7 g/100 g dw, respectively) while carbohydrates and ash content were 16.1 g/100 dw and 3.5 g/100 g dw. On the other hand, seed cakes contained a high amount of protein (58.6 g/100 g dw) and carbohydrates (28.4 g/100 g dw). Ground seeds and seed cakes contained all the four vitamin E isoforms (α -, β -, γ - and δ -tocopherols) with γ -tocopherol being the most abundant isomer in both samples (6.59 mg/100 g dw and 1.07 mg/100 g dw, respectively). The main detected free sugar in ground seeds and seed cakes were sucrose, followed by trehalose, fructose and glucose, while seed cakes contained a higher amount of sucrose and total free sugars than ground seeds. Regarding the organic acids content, oxalic acid content was the only detected compound in seed cakes, whereas no organic acids were detected in ground seeds. The main detected fatty acids were linoleic acid (43.9% and 41.5% in ground seeds and seed cakes, respectively) and oleic acid (37.0% and 36.3% in ground seeds and seed cakes, respectively), followed by stearic acid (4.83% and 5.46% in ground seeds and seed cakes, respectively). Polyunsaturated and unsaturated fatty acids were the main fatty acids class and accounted for 81.9% and 79.0% of total fatty acids in ground seeds and seed cakes, respectively. Finally, both materials showed no toxic effects against non-tumor PLP2 cell lines indicating that they are safe for human consumption. In conclusion, the presented results highlighted the nutritional value of the tested materials which could be considered a rich source of protein. Moreover, the high content in polyunsaturated fatty acids and tocopherols could be further valorized for pharmaceutical and nutraceutical purposes and increase the added value of pumpkin crop.

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PCF-18

EFFECT OF PRE- AND POST-HARVEST TREATMENTS ON QUALITY, ORGANOLEPTIC AND NUTRACEUTICAL PROPERTIES OF WILD EDIBLE PLANTS

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The Mediterranean basin is a biodiversity hotspot of wild edible plant species, and their therapeutic and culinary uses have long been documented [1,2]. Owing to the growing demand for wild edible species, there are increasing concerns about the safety, standardization, quality, and availability of fresh or stored products derived from these species collected in the wild. Moreover, the maintenance of the nutraceutical profile of wild edible species in the same domesticated species is another request by consumers. After a literature screening of the most interesting Mediterranean wild edible species [3], *S. minor* samples were analysed as fresh and then exposed to different preservation processes (oven-drying at 60 °C until constant weight or freeze-drying until constant weight), and studied for their content in phenolic compounds, antioxidant, antimicrobial, cytotoxic and anti-inflammatory properties. In most of the cases, the oven-dried samples showed higher bioactive properties and higher content in phenolic compounds than the freeze-dried samples. The most abundant phenolic compounds in both samples were kaempferol-3-*O*-glucoside and caffeoyl ester, with some differences between wild and domesticated samples. This study provides important information to choose the most adequate methodology to maintain secondary metabolites and bioactive properties of *S. minor*. Further researches are occurring to introduce *S. minor* extract in fresh pasta, trying, in this way, to enhance the nutraceutical profile of a common food.

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PCF-19

**CHITIN ISOLATION AND CHITOSAN PRODUCTION FROM AN INVASIVE
CRAYFISH PRESENT IN PORTUGUESE FRESHWATERS**

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Chitin, considered to be the most abundant biopolymer after cellulose and usually extracted from crustaceans exoskeletons, can be used as source to produce chitosan, a biopolymer with attractive characteristics including non-toxicity, antimicrobial activity, biocompatibility and biodegradability [1], showing potential to be used in diverse areas, namely biomedicine, tissue engineering or agricultural applications. Obtaining chitin from natural sources has been positioned as a challenging strategy in the development of sustainable processes. Among other approaches, the gradual elimination of invasive species from specific ecosystems to produce green materials such as chitin, has become a key topic the last decades.

In this context, *Procambarus clarkii* is a species of freshwater crayfish, native to northern Mexico, and southern/southeastern of United States, but also introduced elsewhere, where it is often an invasive pest. The ability of crayfish to adapt to different environments is huge, causing great damage to local fauna and flora, and significantly affect the biodiversity of the ecosystems [2]. In the present study, we explored the abundance of *Procambarus clarkii* in local rivers and used their shells for the extraction of chitin and production of pure chitosan, with the application of classical methodology which includes demineralization, deproteinization, decolorization and sodium hydroxide treatment. The chitosan content in the shells, analyzed by FTIR, was recorded as 15%. The FTIR spectra of the extracted material enable the identification of all major peaks associated to chitosan, corroborating the extraction of chitin and chitosan production by this approach.

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PCF-20

EFFECT OF LEMON JUICE AND PACKAGING ON SHELF-LIFE OF FRESH SALMON (*Salmo salar*)

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Fish has a relatively short shelf-life due to several mechanisms involved in the process of fish deterioration, such as physical and chemical changes and microbiological activity. Fish microbiota is quite influenced by capture location and fish handling during distribution and storage.

The aim of this work was to estimate the onset of spoilage of fresh Atlantic salmon (*Salmo salar*) fillets stored at 3 and 8°C, under different atmospheres.

Salmon specimens were obtained in the local market (Portugal) and were cut in 3x4x1 cm³ samples, and individually packed, in duplicate, in three different conditions: air packaging (AP), and modified atmosphere packaging (MAP) without and with lemon juice addition (MAPL). After packaging, the samples were stored at 3 and 8°C and analyzed at intervals of 24, 72, 144, 216 and 312h for the two temperatures. The effect of lemon juice in combination with MAP, 60%CO₂/40%CO₂/10%N₂, on microbial, physical-chemical and sensory properties was studied.

The relation between the studied parameters and spoilage of fresh Salmon fillets was calculated using principal component analysis (PCA) and discriminant analysis (DA). Comparison between TVB-N values and the limits of sensory rejection is also presented.

For lactic acid bacteria, yeasts and moulds, Enterobacteriaceae, total viable counts and H₂S producers an effect of lemon juice (MAPL) was observed, with less growth rate, particularly evident in the final stage of storage.

It was established that the viscosity, off-odours and overall assessment of freshness were the best sensory attributes for evaluation of freshness. Through the OAF the point of sensory rejection was defined on the following storage time (hours): 144 (AP, 3 °C); 72 (AP, 8 °C); 216 (MAP, 3 °C); 216 (MAP, 8 °C); 312 (MAPL, 3 °C); 216 (MAPL, 8 °C).

The results showed that modified packaging with lemon juice addition applied to salmon fillets had a pronounced effect on shelf-life, retarding the growth of microorganisms thereby increasing the shelf-life of the salmon.

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PCF-21

AMARANTHUS CAUDATUS L. FLOWERS: RECOVERING OF ADDED VALUE COLOURING MOLECULES, CHEMICAL AND BIOACTIVE CHARACTERIZATION

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The vibrant colors of many plants are due to secondary metabolites, such as nitrogen-containing compounds, where betacyanins are included. *Amaranthus caudatus* L., a plant that has aroused enormous interest due to the nutritional profile of its seeds [1,2], presents a very strong pink colour on its flowers, disclosing the interest for the exploitation of these flowers in terms of natural colourants. In this perspective, and due to an overproduction of this crop that results in the accumulation of large amounts of bio-residues, including flowers without any economic value or subsequent destination, these flowers can be exploited as sources of betacyanins for industrial application. In addition to an excellent colouring power, these compounds (betacyanins), also present strong bioactivities [3]. The present work aimed at exploiting the flowers of *A. caudatus* L. as a source of bioactive (tocopherols, organic acids), but specially of colouring compounds (betacyanins). The ultrasound assisted extraction (UAE) was applied and optimized through the Response surface methodology (RSM), a statistical tool widely used in the optimization of extraction processes, that allows the evaluation of several factors that affect the extractability and stability of the target molecules. Moreover, the bioactive potential of these extracts was also evaluated for the antioxidant activity through the OxHLIA assay, the antimicrobial potential by the microdilution method, as also the hepatotoxicity for normal cells using the sulphorhodamine B assay. From the obtained results, three isoforms of tocopherols were detected, being β -tocopherol (0.884 ± 0.003 mg/100 g dw) the most abundant one. Regarding the organic acids, oxalic (2.48 ± 0.05 mg/100 g dw), shikimic (0.170 ± 0.003 mg/100 g dw) and traces of fumaric acid were found in the extract. Concerning the colouring agents, four betacyanins were identified and quantified, namely: amaranthine (171 ± 1 mg/g extract), isomaranthine (38 ± 1 mg/g), betanin (1.6 ± 0.1 mg/g), and isobetanin (1.3 ± 0.1 mg/g). The obtained extract also presented antioxidant activity with IC_{50} values of 29.0 ± 0.4 μ g/mL and 114 ± 4 μ g/mL for Δt of 60 min and 120 min, respectively in the OxHLIA assay. An interesting antibacterial activity was also verified, with minimum inhibitory concentrations ranging from 5-20 mg/mL against pathogenic bacteria; and no toxicity for normal cells was observed at the maximum tested concentration of 400 μ g/mL. After such promising results, this plant can be a viable alternative to obtain natural colorant ingredients.

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PCF-22

THERMAL AND SPECTROSCOPIC CHARACTERIZATION OF POLYMERIC NANOPARTICLES WITH SILYMARIN

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Introduction: Silymarin is the active principle extracted from the plant *S. marianum*, its composition consists of a mixture of flavonolignans, which have antioxidant activity [1]. Nanoencapsulation is an alternative to preserve and protect bioactive compounds from unfavourable environmental conditions, in addition to increasing their bioavailability and stability [2]. The present study aimed to obtain and characterize silymarin-loaded nanoparticles by the solid dispersion.

Methodology: Kolliphor® Poloxamer 407 polymer (Sigma-Aldrich) was used as encapsulant, ethanol was used as solvent, Tween 80 PS as surfactant and silymarin was acquired from Sigma-Aldrich. The polymer and Tween 80 were solubilized in ethanol with the aid of magnetic stirring and the silymarin was added. The obtained dispersion was taken to evaporation in an oven at 150 ° C for 6 hours. The nanoencapsulated compound was characterized by Differential Scanning Calorimetry (DSC) heated from 0 to 400 ° C at 10 ° C.min⁻¹ and nitrogen flow (50 mL.min⁻¹) and Infrared Spectroscopy Fourier Transform (FTIR) with about 2 mg of compound were weighed to produce potassium bromide tablets conducted with a resolution of 1 cm⁻¹ from 4000 to 400 cm⁻¹.

Results: The DSC thermogram demonstrated that the melting peak of silymarin was not detected in the physical mixture or in the nanoparticles (Figure 1-A).

The FTIR spectrum of silymarin showed a peak at approximately 1600 cm⁻¹, a characteristic peak of silymarin at approximately 3000 cm⁻¹, and between 1460-1500 cm⁻¹ (Figure 1-B)

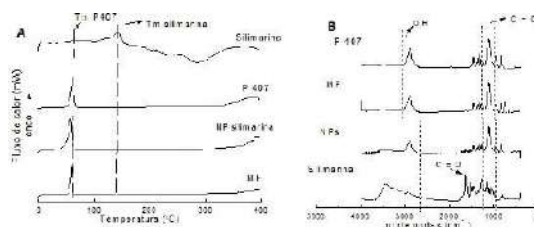


Figure 1: A- DSC thermograms for silymarin, P 407, NP, MF; B- FTIR spectrum.

Conclusion: Characterization analyzes showed that silymarin was efficiently encapsulated in the polymeric matrix, going from a crystalline state to a solid amorphous solution, forming the nanoparticles. Further works in the group will focus on the bioactivity of the nanoencapsulated silymarin in *in silico*, *in vitro*, *in vivo* and *ex vivo* tests.

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PCF-23

**CHEMICAL AND BIOACTIVE CHARACTERIZATION OF *IMPATIENS BALSAMINA* L. PINK FLOWERS
AND THEIR APPLICATION IN A PORTUGUESE PASTRY PRODUCT**

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Edible flowers have been described as presenting several health benefits, associated with powerful bioactive compounds present in their composition [1]. These matrices have also been explored and applied in several fields, such as in cosmetic, pharmaceutical, but specially in gastronomy, due to the with increasing demand of the common consumer for safer and healthier food products [2]. The plants from the genus *Impatiens* are popularly known for the attractive colours of the petals, and preliminary studies have demonstrated a high potential bioactivity of these plants [3]. Thus, the present investigation aimed to characterize the nutritional value (ash, protein, fat, and carbohydrate content, and energy value, by AOAC methodology) and the phenolic compounds profile (by High-Performance Liquid Chromatography coupled with a diode array detector and mass spectrometry by electrospray ionization - HPLC-DAD-ESI/MS) of *Impatiens balsamina* L. pink petals. Moreover, the antioxidant, antimicrobial, cytotoxic, and anti-inflammatory evaluation (by the oxidative hemolysis inhibition assay - OxHLIA, microdilution method with ATCC strains, the sulforhodamine B method in four human tumour cell lines, and analysis in macrophage cells of rats (RAW 264.7), to inhibit the production of NO, respectively) in the hydroethanolic extracts was also accessed. Finally, the enriched-coloured extract was applied as a colorant in a cake filling called "bombocas", and its colorant capacity was compared with an artificial additive (E163). In the pink petals, proteins stood out as the main macronutrient, and only fructose and glucose were found in sugars profile. As for the phenolic composition, eighteen compounds were tentatively identified, five non-anthocyanin compounds (caffeic and coumaric acids, and eryodictiol-*O*-hexoside) and ten anthocyanin compounds (mainly acylated *O*-glycosylated malvidin, pelargonidin, and peonidin derivatives). In addition, the hydroethanolic extracts demonstrated anti-inflammatory and cytotoxicity for all cell lines studied, presenting also a remarkable antifungal activity. Finally, the coloured extract applied in the formulations conferred a more natural colour to the "bombocas, as also functional properties such as antioxidant activity. Further studies will be performed in order to optimize the extractions conditions of colorant compounds, requiring also stabilization strategies for further evaluation of the colorant extract efficacy.

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PCF-24

ANTIMICROBIAL SENSITIVITY OF *Escherichia coli* TO COFFEE EXTRACTS (*Coffea arabica* AND *Coffea canephora*)

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Plant extracts as coffee is recognized by its bioactive potential. In this research, the antimicrobial activity of extracts from *Coffea arabica* roasted coffee (AR), *Coffea canephora* roasted coffee (CR) and *Coffea canephora* green coffee (CG) were evaluated against the growth of *Escherichia coli* ATCC 43888 (reactivated in BHI broth, 35 ± 1 °C/12 h). Strain suspension were standardized at 10^8 CFU mL⁻¹ for sensitivity testing by agar diffusion [1] [2]. Coffee extract solutions were prepared in sterilized distilled water (0.5, 1.5, 2.0, 2.5 and 5.0% (m v⁻¹)), which was impregnated in sterile disks (20 µL). Sterile commercial antibiotics disks (amoxicillin clavulanic acid, trimethoprim and ampicillin) were tested as a positive control. The strain suspension was inoculated (100 µL) on the surface of Mueller-Hinton agar in Petri dishes and spread. The sterile disks impregnated with coffee extracts and sterile antibiotics disks were deposited in the inoculated agar; the inverted plates were incubated at 35 ± 1 °C for 16 to 20 h. Sequentially, the plates were stored under refrigeration from zero to twenty days. The presence of a translucent inhibition halo around the deposited disks suggests growth inhibition (Table 1). *E. coli* was sensitive against all coffee extract from 0.5% to 5.0%, but in concentrations greater than 1.5%, larger halos were obtained for all coffee extracts, and no differences were observed in the concentration range from 1.5% to 5.0%. Comparing the studied coffee extracts, the CR had a greater inhibition evidenced by greater inhibition halos below 5.0% coffee extract. Moreover, *Escherichia coli* were sensitivity to the positive control (halos were greater than the halos with coffee extracts). The results observed for CR is probably associated with the effect of melanoidins, produced during roasting. The antimicrobial action of coffee extracts against *E. coli* opens new possibilities for the use of these extracts as a compound to microbial growth control in foods.

Table 1- Results of halo diameter (in mm) for *Escherichia coli* against the coffee extracts.

Coffee extract	Concentration (%; w v ⁻¹)				
	0.5%	1.5%	2.0%	2.5%	5.0%
AR	9.1 ± 0.1 ^{bb}	11.5 ± 0.6 ^{aA}	11.6 ± 0.4 ^{aA}	10.8 ± 1.0 ^{bAB}	11.2 ± 0.8 ^{aA}
CR	11.1 ± 0.4 ^{ab}	11.8 ± 0.8 ^{aAB}	12.3 ± 0.7 ^{aAB}	12.7 ± 0.5 ^{aA}	12.5 ± 0.2 ^{aAB}
CG	7.8 ± 0.2 ^{cb}	11.5 ± 0.8 ^{aA}	10.3 ± 0.6 ^{ba}	10.3 ± 0.3 ^{ba}	11.4 ± 0.8 ^{aA}

AR: *Coffea arabica* roasted; CR: *Coffea canephora* roasted; CG: *Coffea canephora* green; results expressed by mean ± standard deviation (n = 3); different lowercase superscript letters (same column) indicate difference among the coffee extracts in the same concentration (Tukey test, p ≤ 0.05); different capitalized superscript letters (same line) indicate difference among concentrations of the same extract (Tukey test (p ≤ 0.05).

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PCF-25

APPLICATION OF GUABIJÚ POWDER (*MYRCIANTHES PUNGENS*) IN ICE CREAM DEVELOPMENT: PHYSICO CHEMICAL APPROACH

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The use of natural ingredients in the development of food products is of interest to industries to meet the consumers demand for healthier foods. Fruits have essential health elements, such as bioactive compounds and may improve the sensory characteristics of products developed. Some native species are little known, such as the guabijú berry fruit (*Myrcianthes pungens*). Native from Brazil, guabijú is characterized by its pleasant sweet taste, yellowish and juicy pulp. The ripe fruit has velvety bark with dark purple color [1]. The berry is also reported as a source of bioactive compounds, such as anthocyanins, which are associated with antioxidant activity [1]. Additionally, ice cream is a popularly consumed milk product, which has a suitable matrix for adding healthier ingredients, making it more nutritious. The objective of this study was developed ice cream formulations with lyophilized guabijú powder (LGP), and determine the proximal composition and bioactive compounds of the formulations. Three ice cream formulations were developed with 4%, 8% and 12% of LGP and characterized as the proximal composition [2], antioxidant activity by DPPH (EC₅₀) [3], total anthocyanin [4] and phenolic compounds [5]. The data were evaluated by ANOVA and Tukey test ($p < 0.05$). According to Table 01, the formulation with 12% LGP had the lowest content of moisture, ash and lipids and the highest content of carbohydrates. The proteins were not significantly different among the formulations. Moreover, the formulation with 12% LGP had a higher bioactive content and consequently greater antioxidant activity compared with other formulations. The addition of 4%, 8% and 12% of LGP allowed obtaining ice creams with natural antioxidants, emerging as a possibility of use LGP in food preparations, especially in the production of ice creams.

Table 1: Physical chemical and bioactive characterization of ice cream with LGP

Parameters	4%	8%	12%
Moisture (g 100 g ⁻¹)	63.47 ± 2.12 ^a	62.08 ± 2.17 ^{ab}	59.24 ± 1.87 ^b
Ash (g 100 g ⁻¹)	1.05 ± 0.06 ^a	1.05 ± 0.05 ^a	0.85 ± 0.06 ^a
Total lipids (g 100 g ⁻¹)	9.47 ± 0.10 ^a	7.40 ± 0.17 ^b	6.92 ± 0.15 ^b
Crude protein (g 100 g ⁻¹)	3.93 ± 0.04 ^a	3.98 ± 0.11 ^a	4.00 ± 0.20 ^a
Carbohydrates (g 100 g ⁻¹)	13.78 ± 0.29 ^c	25.48 ± 0.41 ^b	27.02 ± 0.15 ^a
Anthocyanin (mg 100 g ⁻¹)	7.41 ± 1.18 ^c	10.43 ± 0.19 ^b	11.41 ± 0.66 ^a
EC ₅₀ (g g ⁻¹ DPPH)	1829.72 ± 48.27 ^a	528.82 ± 0.48 ^b	472.05 ± 3.54 ^c
Phenolic compounds (mg EGA 100 g ⁻¹)	215.36 ± 0.00 ^c	280.12 ± 5.90 ^b	317.81 ± 10.22 ^a

EGA: Equal gallic acid; Means ± standard deviations (n = 3); ^{a,b,c}: different superscript letters in the lines indicate a significant difference by the Tukey ($p < 0.05$). Source: the authors (2021).

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PCF-26

LACCASE PRODUCTION BY TRAMETES VERSICOLOR USING FOOD AND NON-FOOD WASTES AS SUBSTRATES

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Laccases (EC 1.10.3.2) are multi-copper oxidases capable of catalyzing the oxidation of a wide variety of organic and inorganic substrates with concomitant reduction of molecular oxygen to water. Fungal laccases display a high redox potential, which amplifies the range of oxidizable substrates, and are useful in several biotechnological processes [1]. Laccases have been successfully applied in the bakery, dairy, and beverage industries where they are used to improve several technological and sensorial properties of various products

[2]. The present work aimed at evaluating the suitability of various food and non-food wastes as sources for the production of laccase by *Trametes versicolor* on solid-state fermentation (SSF), taking into account that these materials are cheap and renewable. *T. versicolor* was previously grown and maintained on potato dextrose agar at 4 °C. Three agar plugs (10 mm diameter) were used as inoculum for 5 g of each substrate. The initial moisture content was adjusted at 80% with a mineral solution. The SSF was run for 5 days at 28±2 °C. The laccase activity was measured with ABTS (2,2'-azinodi-[3-ethyl-benzo-thiazolin-sulfonate]) as a substrate. One activity unit of laccase (U) is the amount of enzyme that catalyzes the transformation of 1 µmol of substrate per minute. All the investigated substrates promoted fungal growth and laccase production, but with varying efficiencies (Fig. 1). PW was the most effective one, having allowed the production of a laccase activity of 18.27±0.17 U/g, which was somewhat higher than those ones found with PP, WB, PFP, and OB. The laccase productions found with the latter group of substrates were similar and much higher than those found with ES, CC, SB, and SP. It is known that slight changes in the substrate composition, e.g., phenolic compounds content, can affect the capacity of inducing laccase production. The use of agro-wastes as substrates can be regarded as an eco-friendly approach that is expected to allow the production of high amounts of enzymes at a low cost for industrial purposes. Investigations at finding the most appropriate wastes, thus, are highly desirable and promising undertakings.

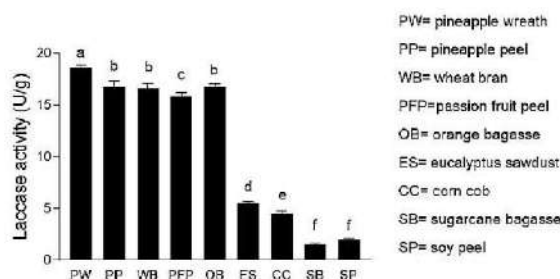


Figure 1: Influence of various substrates on laccase production. Columns with the same letter are not significantly different at the 0.05% level (Tukey's test).

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PCF-27

FERMENTED MILK DRINK WITH TECHNOLOGICAL APPLICATION OF FRUIT BREAD FLOUR: A CONCEPT TEST

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Abstract

Obtaining breadfruit flour (*Artocarpus altilis*) provides broad forms of technological use, from its application in bakery and beverages to the development of edible films. The present study aimed to evaluate the concept of a fermented milk drink containing breadfruit flour in its composition. The study was carried out with 212 participants, who received a questionnaire describing the concept of the product to be formulated and composed of 10 objective questions, following the standards of a concept test. The questionnaires were made available through various social networks, including Facebook, whatsapp, instagram and discord, in order to identify the consumer's profile and frequency of consumption of dairy drinks as well as the verification of purchase intention of this proposed new product, among other issues. The results indicated that the majority of respondents were people between 19 and 39 years old, with complete or incomplete higher education and who consume milk drink at least once a week, with the preferred flavor being strawberry, in addition to consuming some functional food regularly. The majority knew and tried the bread fruit and liked it and claimed to be interested in the proposed fermented milk drink due to the benefits presented by it and therefore would be interested in buying such a product at an average price of 4.50 R\$. It is concluded that the applied concept test suggests that the fermented milk drink added with breadfruit flour can proceed to a stage of product development and later to its physical-chemical, microbiological and sensory evaluation.

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PCF-28

Berry pomace: Chemical composition and applicationsFilipa Costa¹, [Ana Ferreira](#)¹, Élia Fogueiro¹, Cláudia Neves¹, Dulcineia F. Wessel^{1,2,3*}¹ Polytechnic Institute of Viseu, School of Agriculture, Quinta da Alagoa – Estrada de Nelas, Ranhados, 3500-606 Viseu, Portugal² CITAB, University of Trás-os-Montes e Alto Douro, 5001-801 Vila Real, Portugal³ LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, 3810-193 Aveiro

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Food wastes are rich sources of different classes of compounds with recognizes biological activities that may be recovered, applying existent and emerging technologies to produce value-added products [1]. The processing of berries into juice generally results in approximately 20-30% of by-product. During juice processing, berries are crushed and pressed, and after extracting the juice, a solid residue, commonly denoted as pomace is obtained. Berry pomace comprises the seeds, skins, stems of the fruit and pulp rests [2]. Each constituent is rich in distinct chemical compounds: seeds are rich in lipophilic compounds such as polyunsaturated fatty acids, tocopherols, tocotrienols, sterols and carotenoids, while the other parts are rich in cell wall dietary fiber, anthocyanins and other polyphenols. These compounds present antioxidant, anti-inflammatory and antimicrobial properties, and therefore can be used as food additives and as valuable ingredients for functional foods, nutraceuticals, medicines, and other products [3]. Therefore, valorization of berry pomace provides an opportunity for circular economy and can efficiently reduce environmental stress and system sustainability recovery [4]. This work intends to summarize the last developments on berry pomace valorization with focus laid on the extraction of bioactive compounds and their potential applications.

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PCF-29

NUTRITIONAL VALUE AND ANTIOXIDANT ACTIVITY OF BEE POLLEN SUBMITTED TO DIFFERENT PRESERVATION TECHNIQUES

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The moisture content in bee pollen is one parameter of greatest interest for the preservation and quality of this product, since bee pollen is subject to the proliferation of microbiological contaminations that can make its consumption and commercialization unfeasible [1]. Various studies have stated that the choice of preservation technique can have an impact on the chemical composition and antioxidant activity of pollen [2,3,4,5]. Thus, this work intends to evaluate the immediate impact, and also over a storage period of 9 months, of the application of several preservation techniques in the chemical composition and antioxidant activity of the pollen. The bee pollen was collected in Bragança region and frozen at -18°C, being subsequently preserved through lyophilization or drying in an oven at 35°C, 40°C or 45°C. The chemical composition (moisture content, ash, fat, protein, fructose, glucose and total phenolic compounds) and antioxidant activity (DPPH and reducing power) were analysed immediately after the application of the preservation technique and after 1, 3, 6, and 9 months of storage. In addition to the expected reduction in moisture content, other chemical parameters were significantly affected during the storage period. In general, considering the maintenance of the nutritional value of the pollen, lyophilization was shown to be the most appropriate preservation technique. However, the use of lyophilization appears to have a more evident negative impact on the content of total phenolic compounds and on the antioxidant activity of the pollen, when compared with the oven drying technique, performed at a temperature of 45°C.

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PCF-30

KIWI BY-PRODUCTS AS POTENTIAL NEW FOOD ADDITIVES

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The food processing industry generates a large amount of organic waste from both crops and the industrialization of the product itself. The processing of fruits and vegetables constitutes the second generator of waste to the environment, only surpassed by wastewater [1]. These wastes, typically accumulated in landfills, are used for composting, or they are burned, causing environmental problems. However, numerous scientific studies suggest that these residues are rich in bioactive compounds that contain many reusable substances with great economic potential [1]. The production of kiwi, the main fruit of the *Actinidia genus* (Actinidiaceae family), generates skin, pulp, seeds and pruning residues, which have been shown to have important bioactive compounds such as phenolic compounds, vitamins and pigments. These molecules possess biological properties like antioxidant, antimicrobial, proteolytic and prebiotic, among others [2]. Therefore, these matrices could be revalued since they are novel, natural and economic sources of flavorings, colorants, proteins, coagulants, dietary fiber, antimicrobials and antioxidant compounds, which can be used in the food industry as natural food additives [3]. Also, the substitution of synthetic additive for natural ones can cause a positive effect on consumer's health and the environment, contributing to the satisfaction of the current consumers' demand of more natural foods. This article reviews the potential of the residues derived from the industrial processing and agricultural maintenance of kiwi, as promising matrices for the development of new food additives, obtaining, at the same time, economic returns and a reduction of the environmental impact of this industry.

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PCF-31

CASSAVA PRODUCTIVE CHAIN (*Manihot esculenta* Crantz), AS AN INSTRUMENT FOR RURAL DEVELOPMENT: ANALYSIS OF THE MUNICIPALITY OF FORMIGA, MG

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Cassava of the species *Manihot esculenta* Crantz (1766), serves as an energy food in underdeveloped and developing countries; it is a source of carbohydrates for human and animal food; it also serves as a generator of employment and income, in mainly rural areas. The present research project aims to establish a research on cassava culture, as an instrument of rural development, in the Brazilian context, being developed in the municipality of Formiga - MG. It is intended to evaluate how the cassava culture contributes to the rural development of the municipality, considering the structure, dynamics and coordination of the production systems used, as well as the characteristics related to the plant, climate and soil. For data collection, a semi structured interview will be applied to the group of associated producers, consumers, technical assistance technicians and, a diagnostic analysis of agrarian systems will be applied. It is expected that the reach of public policies for the development of productive activities in the association is known; the association's social productive system is known; an action plan is proposed to assist in the development of the members' economy.

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PCF-32

ACCEPTABILITY MUESLI FORMULATIONS FROM HOVENIA DULCIS PSEUDOFRUITS

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Hovenia dulcis Thunb., known as Japanese grape, is a tree belonging to the Rhamnaceae family, originally from China, Japan and Korea. When dehydrated, pseudofruits can be stored for months and are energy sources and can be used in the form of raisins. It has functional potential because it contains significant values of polyunsaturated fatty acids, soluble fibers and phenolic compounds [1,2]. Muesli is a very common breakfast cereal in countries like Switzerland and Germany, as well as in other countries in Europe. In addition to other ingredients, muesli is composed of oats, some cereal rich in dietary fiber, which represents the major ingredient in the preparation. The nutritional composition of the muesli may vary according to the ingredients [3]. As *Hovenia dulcis* is considered a source of dietary fiber, the present study proposed two formulations of muesli made from sugar and raisins obtained from pseudofruits from *Hovenia dulcis* Thunb. (**Table 1**).

Table 1: Tested muesli formulations and energy values.

Ingredients	FI (%)	FII (%)	FIII (%)
Whole grain oats	23.8	23.8	23.8
Pumpkin seed	7.1	7.1	7.1
Flaxseed	7.1	7.1	7.1
Traditional raisin	7.1	-	7.1
Raisin <i>Hovenia dulcis</i> Thunb.	-	9.52	-
Sugar <i>Hovenia dulcis</i> Thunb.	-	-	2.3
Milk	47.6	45.5	45.5
Honey	7.1	4.7	4.7
Energy Value (kcal/100g)	194	196	195

Muesli is a food that has several beneficial health effects and could be incorporated into the diet and it is a product composed of a mix of dried fruits, dried oil fruits, whole grains such as wheat, oats and rye [4]. It's daily consumption, combined with healthy habits, can bring benefits such as lowering cholesterol levels, reducing the risk of cardiovascular disease, among others. An consumer acceptance study (n=97), FI, FII and FIII formulations were tested by women (69.1%) and men (30.9%), aged 18 to 63 years, using the ordination test (preference). The FI formulation (control) was preferred by the evaluators (44.7%), with the proposal of formulation FII (raisins) being the second preferred form (34.0%). The pseudofruits of *Hovenia dulcis* Thunb. have a sweet taste and an excellent source of dietary fiber, but it still does not have a good acceptability, when it is incorporated in a muesli formulation. Thus, its consumption in other preparations or the incentive *in natura* must be proposed.

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PCF-33

INDUSTRIAL PRODUCTION OF FRESH DOUBLE CREAM CHEESE SUPPLEMENTED WITH CLOVE ESSENTIAL OIL

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Clove essential oil was identified by GC-MS. Eugenol (57.66%) and caryophyllene (14.57%), acetyl eugenol (5.96%), α -caryophyllene (4.57 %) were identified as the major constituents in CEO. These constituents were identified in previous studies with comparable amounts [1, 2].

Antimicrobial activities against *E.coli*, *Salmonella enteritidis* and *Staphylococcus aureus* (Table 1) and the antioxidant activity were evaluated.

Clove Essential Oil (CEO) was incorporated in industrial fresh double cream cheese in order to lengthen its conservation and to improve its antioxidant stability [3, 4].

An optimum content of CEO (0.37 %) was determined by mixing plan carried out by Expert design software. The responses used for this mixing plan were: minimum inhibitory concentration (MIC), antioxidant activity (IC₅₀), taste and odor intensity and global appreciation (Table 1). The results have shown that the antioxidant activity of the cheese incorporated with CEO was relatively important. In fact, the incorporation of CEO in cheese may strongly improve its oxidative stability [5]. Cheese antioxidant activity was not affected during the storage period for 13 days. The fresh double cream cheese supplemented with CEO could be stored at refrigerated temperature (4°C) with good taste and odor during the period of 13 days that suggest lengthening its shelf life.

Table 1: Optimization of the suitable CEO concentration by Expert design software

Solutions Number	CEO %	Cheese %	Taste	Smell	Global appreciation	Antioxidant activity (IC ₅₀)
1	0.37	99.63	1.96017	3.00024	1.5	0.146335

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PCF-34

VALORIZATION OF BREAD WASTE INTO A HIGH-VALUE PRODUCT: STARCH

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Bread is one of the most wasted products of all food in many countries around the world due its short shelf life. In Tunisia, about 900,000 units of bread are thrown daily, at a cost estimated at \$50 million each year [1]. Thereby, bread residues present inexpensive, abundant and underutilized renewable substrate which is highly available for valorization into value-added products. In the present study, the feasibility of starch extraction from white bread waste is investigated. Chemical composition of dried bread waste used as substrate was performed. Initial moisture content, ash content, lipid content, protein content, the carbohydrate content and starch content were assessed. Extraction of starch using several procedures: artisanal, chemical, and enzymatic method with protease and cellulase were investigated. These methods were compared according to their extraction yields and the results provided by spectroscopic analysis: Infrared (IR) and Nuclear Magnetic Resonance (NMR) data. The main results showed that the enzymatic method using cellulase from *Aspergillus niger* (500 units per 10 g of grinded bread) could be an effective alternative for bread waste valorization ensuring an extraction yield of 85%.

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PCF-35

ANTIOXIDANT ACTIVITY OF MATE HERB IN ITS DIFFERENT PREPARATIONS

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Yerba mate, a plant native to South America, is appreciated as a hot drink, in the preparation of chimarrão, cold, through tereré, and in a unified way it is used in the preparation of mate tea. The regional production of its different presentations, and reports encouraging its benefits in view of its wide variety of compounds, aroused interest in his research. For this purpose, the extraction of phenolic compounds was carried out by means of the infusion technique, under extract: water of 10 mL / g for 5 minutes at 80 °C for mate and mate, and 10°C for tereré, respectively. The determination of phenolic compounds was carried out using the Folin- Ciocalteu colorimetric method [1]; determination of flavonoids [2]; determination of antioxidant activity by DPPH free radical scavenging activity. A large amount of phenolic compounds was observed in yerba mate (7 - 10%) proving its antioxidant action. The chimarrão showed a higher concentration of phenolic compounds and flavonodes (Table 1) caused by the high temperature of preparation. The antioxidant activity was higher in chimarrão and tereré (Table 2) due to the processing temperature. In addition, the granulometry and the production process of the herb can be factors that affect the antioxidant activity of the product.

Table 1. Concentrations of chimarrão, tereré and mate extracts and their average absorbances and amounts of phenolic and flavonoid compounds

Extracts	Concentration of extract (µg/ml)	Absorbance média	µg ec/mg of extract
phenolic compounds			
Chimarrão	25	0,318	205,81
Tereré	25	0,300	194,16
Mate	25	0,297	192,87
Flavonoid compounds			
Chimarrão	25	0,216	285,21
Tereré	25	0,167	220,51
Mate	25	0,242	159,77

Table 2. Average absorbance obtained for chimarrão, tereré and mate extracts in different concentrations and their percentages of antioxidant activity

Concentration of extract (µg/ml)	Chimarrão		Tereré		Mate torrado	
	Abs. mean	% AA	Abs. mean	% AA	Abs. mean	% AA
400	0,253	70,95	0,229	73,71	0,354	58,02
300	0,355	59,24	0,364	58,21	0,476	43,51
200	0,534	38,69	0,544	37,54	0,619	26,58
100	0,705	19,05	0,683	21,58	0,703	13,43
50	0,805	7,58	0,785	9,87	0,792	6,06
25	0,850	2,41	0,840	4,09	0,809	4,27
Controle	0,871		0,871		0,843	

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PHYSICAL PROPERTIES OF 29 COLORED POTATO VARIETIES

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The use of different varieties of potatoes (*Solanum tuberosum* L.) with different colors may be appealing to consumers, namely in terms of their visual appearance, allowing their use as “chips” and other ready-to-eat snacks. This work studied the physical properties of 29 varieties of colored potatoes, analyzing their external color and the different dimensions of texture. The external color was measured with a portable colorimeter, analyzing the CIELab color space according to the L* (lightness), a* (red-green) and b* (blue-yellow) coordinates. The texture was determined using a “texture profile analysis” assay allowing the classification of their hardness, adhesiveness, springiness, cohesiveness and resilience. The samples were classified through an analysis of variance using Tukey’s test as a post-hoc, and finally were subjected to a linear discriminant analysis for further discrimination based on the studied parameters. According to the obtained results, the color of the potatoes showed significant statistical differences, although many of the samples were related to each other. The sample with the darkest color was “Blaue veltlin” variety, with an L* of 27.8, whereas the sample with the lightest colour was “Blaue ajanhuiri” variety. In terms of the values of parameter a* the cultivar with the greenest tone was also the “Blaue ajanhuiri”, while the one closer to the red quadrant was “Purple from Peru”. Finally, the analysis of parameter b* showed that “Blaue Neuseeländer” sample was the one with the highest blue tone and “Purple fiesta” the one with the highest intensity of yellow. Regarding the different texture dimensions, the samples showed homogenous values. All samples revealed no statistical difference for springiness, whereas regarding adhesiveness, the sample “Purple from Congo” was the most adhesive at -144 g/sec and “Linzer blaue” the less adhesive at -15 g/sec. Concerning the hardness parameter, “Blaue Anneliese” and “Blaue neuseeländer” were the samples with the hardest and softest texture, respectively. In addition, the most cohesive sample was “Pink from Bolivia”, whereas the least cohesive one was “Linzer blaue”. Chewiness had three different levels of significant difference with “Blaue Anneliese” being the least chewy and “Black princess” the most, while for resilience, the least resilient were “Pink from Bolivia” and “Hermanns blaue” and the most resilient was “Linzer blaue”. In terms of the linear discriminant analysis, the first two of five functions described 84.8% of variability (function 1 – 72.0%, function 2 – 12.8%), while the varieties that presented the highest variability were “Lilly rose”, “Red salad potato”, “Purple from Peru”, “Königspurpur” and “Highland burgundy”. In conclusion, the presented results showed a great variability among the tested potato varieties indicating that these could dictate the different uses of the final food product.

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PCF-37

***CALOCYBE GAMBOSA* (FR.) DONK WILD GROWING IN SERBIA AS FUNCTIONAL INGREDIENT IN OATMEAL**

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Edible mushrooms have been appreciated globally for their organoleptic, nutritional and chemical properties. In the present study, fruiting body of *Calocybe gambosa*, wild growing in Serbia has been chemically characterized (content of macronutrients, soluble sugars, tocopherols, fatty and organic acids) and its bioactive properties (antimicrobial and antioxidant) evaluated. Obtained results suggest that this mushroom is a source of carbohydrates and proteins, with low fat content. Sugar analysis revealed presence of trehalose and mannitol. Tocopherol composition revealed presence of α -tocopherol, while fatty acid analysis revealed presence of 24 fatty acids with prevalence of polyunsaturated fatty acids. Amongst organic acids, oxalic, quinic, malic, citric and fumaric acid were detected. Comprehensive antioxidant analysis (reducing power, DPPH scavenging activity, β -caroten/linoleic acid and TBARS assay) indicate that mushroom is a perspective antioxidant, whereas its antimicrobial potential turned out to be moderate. Nevertheless, at sub-inhibitory level methanolic extract disrupted cell-to-cell communication using *Pseudomonas aeruginosa* PAO1 as a model system. Finally, enrichment of oatmeal cookies with *C. gambosa* flakes not only improved nutritional value of cookies, but was praised among participants in sensory evaluation test (**Table 1**), indicating that along with results of chemical composition and biological activity, this mushroom has potential to be regarded as functional food.

Table 1. Oatmeal cookies enriched with *C. gambosa* flakes.

Amount per serving (1 cookie ~ 25 g)	Cookie with <i>C. gambosa</i>	Cookie without <i>C. gambosa</i>
Fat (g/100 g dw)	3.40	3.37
Proteins (g/100 g dw)	1.82	1.47
Carbohydrates (g/100 g dw)	14.95	13.47
Energy (kcal/100 g dw)	98.57	90.98
Appearance	4.66	4.25
Smell	4.25	4.25
Taste	4.66	4.25
Consistency	4.83	4.54
Overall acceptability by panelists	4.68	4.24

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PCF-38

**RED SEAWEEDS *GRATELOUPIA TURUTURU* AND *PORPHYRA UMBILICALIS* AS
NUTRACEUTICALS AND FUNCTIONAL FOOD: NUTRITIONAL/CHEMICAL
COMPOSITION AND IMMUNOSTIMULATORY ACTIVITY**

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The number of nutraceuticals and functional foods from marine origin is increasing in the worldwide market. Specifically, the red seaweeds *Grateloupia turuturu* and *Porphyra umbilicalis* have shown their potential considering relevant bioactive compounds and nutritional value [1,2]. Nevertheless, it is pertinent to explore knowledge gaps regarding their nutritional/chemical composition and bioactivities. Hence, firstly, *G. turuturu* and *P. umbilicalis* proximate composition and lipid classes were determined; secondly, after preparing hydroethanolic and aqueous (infusion and decoction) seaweed extracts, mycosporine-like amino acids (MAAs) and carbohydrates were quantified, and the immunomodulatory potential was assessed using RAW 264.7 cell line. Results show that dietary fibre was the most abundant nutritional parameter for both seaweed species, followed by relevant ash and protein contents. Free fatty acids were the main lipid class in both seaweed species, whereas tocopherols exhibited residual contents by RP-HPLC-FLD. For hydroethanolic and aqueous extracts, several MAAs were quantified by RP-HPLC-DAD and LC-DAD

ESI-MS. *P. umbilicalis* extracts demonstrated greater MAAs content than *G. turuturu*, and porphyra-334 was the main MAA. The aqueous extracts of both seaweeds showed higher carbohydrate contents than the hydroethanolic, with *P. umbilicalis* decoction exhibiting the highest level. Concerning the immunomodulatory activity, for 0.02 mg/mL, the exposure to *P. umbilicalis* aqueous extracts resulted in the most accentuated NO production by RAW 264.7 cells, showing immunostimulatory potential. MAAs and carbohydrates are believed to be responsible for the immunostimulatory activity [3,4]. *G. turuturu* and *P. umbilicalis* potential as functional food and nutraceuticals, relying on their relevance as sources of bioactive compounds to the food, pharmaceutical and cosmetic industries, is thus reinforced.

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PCF-39

**CONTROLLED FERMENTATION OF CURLY KALE JUICE WITH THE USE OF AUTOCHTHONOUS
*LACTOBACILLUS PLANTARUM***

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Recently, the interest of the functional properties of fermented fruits and vegetable products is gaining popularity among researchers, food technologists and consumers worldwide for health-related and economic reasons. As the market for probiotic fermented non-dairy products grows, new plant materials rich in biologically active compounds are being sought. For that reason, the aim of the studies was to evaluate the impact of autochthonous *L. plantarum* JS052, isolated previously during spontaneous fermentation [1], on the functional properties of curly kale juice. The research included the viability of *L. plantarum* using the standard plate method, pH monitoring, the antimicrobial properties towards Gram-positive (*S. aureus* ATCC 33862 and *L. monocytogenes* ATCC 1911) and Gram-negative (*E. coli* ATCC 35218 and *S. enterica* ser. Enteritidis ATCC 13076) pathogens performed by broth microdilution method at juice concentration ranged from 50% to 6.25% [2]. Besides, the focus was set on the evaluation of total phenolic content (TPC) [3] as well as the assessment of antioxidant activity determined by TEAC assay [4]. Firstly, the viability of *L. plantarum* increased from 8.28 log CFU/mL to 10.44 log CFU/mL after 24 hours of incubation and the pH dropped from 5.92 to 3.91. The fermentation process contributed to the improved antimicrobial properties in comparison to the fresh juice among all tested pathogens, however it was dependent on the juice concentration. The 50% and 25% of juice concentration completely inhibits the growth of all indicator microorganisms. In the case of *L. monocytogenes*, the lower juice concentrations (12.5% and 6.25%) also contributed to a significant inhibition of growth. In relation to other pathogens, the lowest concentration even stimulated their growth. Considering the effect of lactic acid fermentation on TPC it was noticed that the 24-hour process provided a slight increase in TPC, from 119 mg GAE/100 mL for fresh juice to 132 mg GAE/100 mL of fermented juice. In turn, antioxidant activities remained unchanged at the level app. 5.5 mM/100 mL. Overall, this study indicated that fermented curly kale juice with the use of autochthonous starter may be a new innovative non-dairy product characterized by potentially probiotic and functional features.

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PCF-40

CHARACTERIZATION OF NON-CONVENTIONAL FOOD PLANTS SEEDS *GUIZOTIA ABYSSINICA* (L.F.) CASS., *PANICUM MILIACEUM* L., AND *PHALARIS CANARIENSIS* L. FOR APPLICATION IN THE BAKERY INDUSTRY

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The use of unconventional food plants (PANC) has been an asset for the food industry, not only due to its abundance, but also because it does not compete with other vegetable matrices used for human consumption, and for its nutritional properties, chemical, and bioactive potentiality [1-2]. The study of new food sources and its application for the development of new food products is urgent. The present work aimed to study the granulometry and water absorption index of three flour seeds of PANC, *Guizotia abyssinica* (Lf) Cass. (niger), *Panicum miliaceum* L. (millet), and *Phalaris canariensis* L. (birdseed), followed by its proximate composition (AOAC), and composition in fatty acids (GC-FID), free sugars, organic acids, tocopherols, and phenolic compounds (HPLC-RI, DAD, fluorescence, and DAD/ESI-MS, respectively). The bioactive capacity of the hydroethanolic extracts was also assessed. Finally, bakery products were developed with partial replacement of the wheat flour (20% of the PANC's flour) and an experimental statistical design using the centroid simplex method was applied to understand the effect of applying PANC flours on the final physical-chemical characteristics of the breads. Birdseed and niger flours showed high granulometry with high water absorption index, indicating that their use in bakery should be supplemented with other flours. Niger seed revealed higher contents in total fat, PUFA (linoleic acid), sugars, tocopherols (α tocopherol), and phenolic compounds (derivatives of caffeic acid). All seeds presented relatively low IC₅₀ and MIC values for TBARS assay and antimicrobial activity, respectively, and did not show hepatotoxicity. The hydroethanolic extracts of niger and millet presented lower MIC values for antifungal activity when compared with the positive controls used (E211 and E224). Breads with 20% of millet and birdseed flour presented highest similarity to the control bread (100% wheat flour), in texture, specific volume and color. Considering their composition in bioactive compounds, the use of these seeds is highly advisable in the context of a fortified diet, being sources of compounds of high nutritional value and with beneficial effects for the health of the consumer.

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PCF-41

PHYSICO-CHEMICAL PARAMETERS OF ALGERIAN HONEYS: ROSEMARY, TAMARISK, THISTLE AND MULTIFLORAL

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Honey is a continuous subject of study in many countries due to its importance for consumers. Its quality depends on the floral and the geographic origin which is linked to the climatic conditions. The production of honey in Algeria range between 4000 and 6000 Tons per year [1]. Even though honey is commonly consumed in Algeria, there is lack of information about physico-chemical characteristics of its honeys. Algeria presents a diversity of honey types which is the result of the different phytogeographical and climatic conditions found. In the Mediterranean areas of the north, honey origin is mainly from citrus, eucalyptus, rosemary and thyme, while in south regions, spurge, ziziphus, thistle honeys are dominant [2].

The aim of this study was to characterize 10 honey samples (rosemary, tamarisk thistle and multifloral honey) from two different regions of Algeria (Sidi Belabbes and El Bayedh) based on their physico-chemical properties such as: moisture content, color, electrical conductivity, pH and acidity, hydroxymethylfurfural (HMF), diastase activity, fructose to glucose ratio and proline content. The results show that the color ranged from extra white to light amber, while the moisture ranges from 13 to 16 %, and the conductivity varied from 0.09 to 0.34 mS.cm⁻¹. The free acidity (calculated at pH=7) varied from 7 to 31.2 meq.kg⁻¹, with pH value ranged from 4.0 to 4.7. The amount of HMF range from 11.2 to 79.8 mg.kg⁻¹ while the diastase values varied from 2.1 to 14.7 DN. The proline content ranged from 0.4 to 2.8 mg/g. With the exception of HMF, which in some samples was above the maximum of 40 mg.kg⁻¹ permitted on Codex Alimentarius, the physicochemical parameters studied were within the quality standards established for honey.

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PCF-42

PRODUCTION AND CHARACTERIZATION OF BIOPOLYMER FILMS FOR THE FOOD PACKAGING MARKET

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Agro-industrial waste has been increasingly used as sources for the production of biodegradable and sustainable packaging, this market generates a potential increase in the research and development sector of biopolymeric sciences in university and industrial centres^[3]. In this sense, this project have as the main objective of the manufacture and chemical characterization, such as FTIR (Fourier transform infrared), Vis-UV (visible ultraviolet) and TGA (thermogravimetric analysis) of sustainable polymeric thin films, destined to the active packaging category. The biopolymeric film (BF) comes from an array of Soy Protein Isolate (SPI) being reinforced by kraft lignin (KL) and tannins (of *Stryphnodendron* origin). The choice of BF's for use in the packaging business can be highlighted by the fact that this material does not compromise the organoleptic characteristics of the product where it will be used, such as odor, colour and flavour, besides presenting favourable performance in the sustainable environmental and economic sector^[3]. The methodology used consisted of a matrix of SPI 35g/L, kraft lignin (applied in different proportions (0.5%, 1.0%, 2.0%), tannins (0.5% concentration) and using ethylene glycol/ethanol/glycerol (1:2:2) as plasticizing additives for the formation of bioplastics^[2]. Performing later, the tests can be evaluated that the increase in the amount of lignin increases the concentration of free hydroxyls in the medium, favouring the formation of bands 1900-1550 cm⁻¹ indicating the existence of O-H bonds, which allows great affinity of lignin with the polymermatrix and, consequently, decreases film transparency (Vis-UV)^[1]. Furthermore, with the TGA analysis it was possible to infer that the amount of lignin added is inversely proportional to its sensitivity to heat^[1]. Finally, there's no doubt that it is necessary investment and development of sustainable biopolymeric films, which present great utility and practicality to the food packaging market^[1].

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PCF-43

EXTRACTION OF CHLOROPHYLLS FROM NATURAL SOURCES

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The growing consumers' concern for possible long-term adverse effects of artificial molecules commonly used in food industry has led to an increased interest in natural products. At the same time, there is a demand for a more eco-sustainable use of natural matrices, which justifies the search for byproducts that have no other application to be explored in the development of novel food products [1,2]. In this context, the present study was designed to exploit natural pigments, more specifically chlorophylls, from bioresidues (aerial parts of carrot and tomato) for the development of food colorants. These are the most abundant pigments in plants and present, beyond their great coloring capacity, several bioactive properties, which corroborates the importance of their application in foodstuff. In this work, different extraction methodologies and techniques (maceration, ME, and ultrasound-assisted, USE) were applied to the lyophilized aerial parts of carrot and tomato to maximize the chlorophyll extraction yield. For the extraction, green solvents were prioritized, namely water, ethanol (90%), and hexane. The parameters affecting the pigments recovery were varied for each technique, namely the time, power, and solvent for USE, and the time and solvent for ME. The extractions were performed protecting the samples from light and the results were monitored through the implementation of a new chromatographic method, HPLC coupled to a diode array detector (DAD) and mass spectrometry (MS), to determine the concentration of chlorophylls and the best procedure to be performed. Both aerial parts presented chlorophylls and derivatives in significant concentrations and extraction yields up to 88% for the ethanolic extracts. The applied chromatographic method revealed to be appropriate for the analysis of this class of pigments, allowing a good peak resolution and separation, but also characteristic TIC spectrum for the tentative identification of the compounds. Therefore, the results of the present study can be explored for the development of chlorophyll-based colorants from these bioresidues, but also from similar byproducts.

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PCF-44

BIOLOGICAL CONTROL OF APPLE POST-HARVEST DISEASES BY PORTUGUESE PROPOLIS

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Diseases that arise during fruit conservation are one of the main constraints to their marketing and consumption, with negative economic repercussions. This problem leads to up to 50% of losses [1], which in turn translates into an increase of food waste. Apple culture is globally one of the most important and its main post-harvest diseases are fungal rots, like the one caused by *Penicillium expansum*, responsible for blue mold and accounting for about 5 to 20% of losses worldwide [2]. Post-harvest disease control is currently managed by using synthetic fungicides that are highly harmful to the environment and to consumers. Thus, it is necessary to find equally effective, but safer and more friendly alternatives.

Propolis, a natural product of the hive known for its various bioactivities, namely antioxidant and antimicrobial [3], becomes therefore an excellent candidate for applications other than medicine, where it has been widely used and acclaimed for centuries. In the present work, the antifungal potential of a Portuguese propolis hydroalcoholic extract was studied *in vitro* and *in vivo* against *P. expansum*. *In vitro*, a reduction of 48% in mycelium size was observed 4 days after inoculation with the highest concentration of the propolis hydroalcoholic extract tested. The same extract at concentrations of 2.5 and 5% proved to be effective at reducing the size of the lesion caused by the fungus in “Golden” apples by 58.65 and 65.38%, after 10 days, supporting the potential of this natural product as a biocontrol agent in apple and possibly other crops.

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PCF-45

PRODUCTION AND FERTILIZATION SYSTEM AFFECTS THE NUTRITIONAL, CHEMICAL, AND BIOACTIVE PROPERTIES OF SMALL RED FRUITS

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Small red fruits, such as blueberries (*Vaccinium corymbosum* L.) and currants (*Ribes rubrum* L.), are considered as emerging crops in Portugal, with a high growth potential. Although the consumption of these fruits in Portugal is still not very significant, there has been a reasonable increase, compared to the last century, with the growing interest of consumers in functional foods. In this sense, there is an increasing concern for production in more sustainable ways, such as organic and integrated production, replacing the conventional agriculture. This kind of production can enhance the quality of the fruits, rich in added-value antioxidant compounds, allowing to meet the most demanding consumers' expectations [1]. This study

aimed to analyze and compare the nutritional value (AOAC), the chemical composition (fatty acids (GC FID), free sugars (HPLC-RI), organic acids (UFLC-PDA), tocopherols (HPLC-fluorescence), and phenolic compounds (HPLC-DAD/ESI-MS)), and the antioxidant properties (TBARS and OxHLIA assays) of blueberries and currants subjected to different types of production (conventional and applying a biological fertilizer, *Ecoser*), providing information that allows a more conscious choice.

The method of production was found to influence not only the nutritional parameters, but also the composition of the fruits in free sugars, fatty acids, tocopherols, organic acids, and phenolic compounds. Thus, blueberries grown in a conventional manner revealed higher levels of carbohydrates and energy, fructose and glucose, saturated fatty acids, quinic acid, and phenolic compounds. In turn, blueberries fertilized with *Ecoser* revealed higher concentrations of γ - and δ -tocopherol, monounsaturated and polyunsaturated fatty acids, oxalic acid, quinic acid, and malic acid. Regarding currants, higher levels of carbohydrates and energy, sucrose, polyunsaturated fatty acids, and anthocyanins were found in fruits grown in conventional agriculture. On the other hand, the currants cultivated in biological mode showed higher concentrations of lipids, fructose, glucose, ascorbic acid, saturated and monounsaturated fatty acids, phenolic acids, and flavonoids. These variations were also reflected in the bioactivity of the fruits in terms of inhibition of lipid peroxidation, enhanced in the currants produced in biological way and in the blueberries produced in conventional way, and in the inhibition of oxidative hemolysis, improved in the currants and blueberries cultivated in biological way. The results obtained in the present study may serve as a basis for the definition of production parameters that best fit the culture of each fruit.

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PCF-46

STABILITY ANALYSIS OF PHENOLIC COMPOSITION AND ANTIOXIDANT ACTIVITY OF POMEGRANATE (*PUNICA GRANUTUM* L.) LEAF INFUSION OVER LONG TIME STORAGE

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Infusion made from medicinal plants is one of the most commonly consumed beverages both in ancient and modern societies, due to its eco-friendliness, economy, low toxicity, and health benefits [1, 2]. Effectively, medicinal plants have been found rich in polyphenolic antioxidants, contributing to its potent health promoting capacities in the light of recent scientific developments. These phenolics play an important role in the reduction of biomolecule (lipid, protein, DNA) oxidation in brain, liver, kidney, lung, cardiovascular system etc., mainly attributed to their favourable number and position of hydroxyl groups [3].

The current focus is toward to excavating natural antioxidant phenols and potential therapeutical effects of plants. Interestingly, different parts (juice, peel, seed, flower, leaf, root) of *punica granatum* (pomegranate) have been shown to possess distinctly higher phenolic level and antioxidant power than many fruits (apple), vegetables (broccoli), food products (green tea, wine) and other herbal plants (peppermint), resulting in many biological capabilities including anti-bacteria, anti-diabetes, anti-hypertension, anti-obesity, anti proliferation, hepato- and nephro-protection [4]. In folk medicine, pomegranate leaves are widely used as analgesics and diuretics to treat renal colic and urinary tract problems. Among other parts recognized as sources of antioxidants, pomegranate leaf has attracted increasing interest recently, which can be exploited as functional food alternatives or active pharmaceutical ingredients, in terms of growing studies *in vitro* and *in vivo* [5, 6].

For the purpose of exploring hopeful health-promoting values of pomegranate leaf, the present work aimed to evaluate the phenolic composition (total phenols, *ortho*-diphenols, flavonoids, and tannins) of pomegranate leaf infusion on time 0, 2h, 4h, 6h, 8h and 24h, as well as its antioxidant property including radical scavenging ability (ABTS, DPPH) and ferric reducing power (FRAP). Furthermore, the Reverse Phase - High Performance Liquid Chromatography - Diode Array Detector (RP-HPLC-DAD) was conducted to characterize the polyphenolic profile of infusion samples, so as to reveal the relationship between the phenolics and antioxidant activity.

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PCF-47

DEVELOPMENT OF METHODS FOR THE EXTRACTION AND DERIVATIZATION OF CARRAGEENAN FROM CHONDRUS CRISPUS AND ACTIVE COATINGS FORMULATIONS

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Plastics present one of the biggest environmental problems on a global scale, due to poor biodegradability.[1,2] Nevertheless, viable candidates exist for the replacement of conventional plastics.[3] For instance, carrageenans are a class of algal polysaccharides which have the ability to form edible films and coatings.[4] The extraction method of carrageenans (CRG) from *Chondrus crispus* using a Soxhlet extractor and hot water as extraction solvent afforded 35.15±0.64% of polysaccharide. The native carrageenan presented some antioxidant activity against DPPH and was capable of suppressing the bleaching of methyl red dye by the NaFeEDTA/acetic acid/H₂O₂ system. The treatment of native carrageenan with N-(salicyl)-L-cysteine hydrochloride in water, at ca. 90 °C, increased the antioxidant activity against DPPH and NaFeEDTA/acetic acid/H₂O₂ (Table 1). No antimicrobial activity against Gram-positive bacteria *Bacillus subtilis* was detected. Structural elucidation studies are being carried to determine nature of the interaction of N-(salicyl)-L-cysteine hydrochloride with carrageenan. Coatings prepared from native and modified carrageenans have the ability to adhere to surfaces.

Table 1: Table caption [times new roman, 8, plain, centered, line spacing 1.15].

	[D-Gal] %	Protein mg.mL ⁻¹	Perox. H ₂ O ₂ A Abs ₅₂₀ ([Abs ₅₂₀ -Abs ₅₂₀]) 15 min	DPPH %Scavenged (0.2 mg/mL)
CRG	77.35±1.77	ND*	7.05*10 ⁻³ ±5.1*10 ⁻²	3.69±1.65
CRG-CysSal	23.11±2.55	ND*	2.96*10 ⁻² ±6.2*10 ⁻³	61.43±3.43

ND* – Not detectable

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PCF-48

ANTHOCYANIN-RICH EXTRACT OBTAINED FROM *PRUNUS SPINOSA* L. BY ULTRASOUND ASSISTED EXTRACTION FOR COLORING PURPOSES

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Anthocyanins are a group of natural pigments presenting a range of colours between red, blue, and violet that are characteristic of various fruits and vegetables. A complex profile of anthocyanins, predominantly cyanidin 3-rutinoside and peonidin 3-rutinoside, was previously identified in *Prunus spinosa* L. fruit, a bitter and astringent fruit from a wild shrub that is poorly commercially exploited [1]. In this sense, the objective of this work was to develop a natural food colourant based on anthocyanins extracted from the epicarp of *P. spinosa* fruits. For that purpose, a conventional extraction method, maceration, and a rapid and low-cost ultrasound procedure were applied for the extraction of anthocyanins from this matrix. To achieve the conditions that maximize anthocyanins' extraction, a response surface methodology was applied using a circumscribed central composite design with three variables and five levels, being the variables time, temperature, and ethanol content, in the case of maceration extraction, whereas for ultrasound assisted extraction, temperature was replaced by ultrasound power. The anthocyanins were identified and quantified by HPLC-DAD-ESI/MS. The optimized extract was assessed in terms of antioxidant and antimicrobial capacity, and hepatotoxicity.

Ultrasound assisted extraction was the most efficient method, under optimum conditions of 5.00±0.15 min, 400.00±32.00 W and 47.98±2.88% ethanol, where the extraction yield was 68.60±2.06% (v/v), with a total anthocyanin content of 18.17±1.82 mg/g of dry extract and 11.76±0.82 mg/g of dry epicarp. Regarding bioactivity, the optimized extract showed antioxidant and antimicrobial activity and it did not show hepatotoxic effects in a primary culture of porcine liver cells. To validate its coloring properties, the anthocyanin-rich extract was incorporated into a typical Brazilian confectionery product "beijinho", proving its applicability as food colorant.

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PCF-49

EXPLORING THE ANTIOXIDANT AND ANTIBACTERIAL POTENTIAL OF LEMON GRASS

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There is an ongoing, extensive search for natural ingredients that either prevent or retard undesirable changes, such as oxidation or microbiological contamination, which take place in food products. Lemon grass (*Cymbopogon citratus* L.) seems to be an interesting plant that may be employed as nutritious, healthy, safe and natural ingredient. Because plant material is rather unstable, a crucial step is to obtain extracts that are rich in phenolic phytochemicals, thus, using different solvents may help to extract valuable compounds. Therefore, this study aimed to investigate the antioxidant and antibacterial activities of lemon grass and to assess the influence of the extraction solvent, that is, ethanol, water, and water:ethanol (50/50 vol/vol), on measured properties.

The research revealed the significant influence ($p < .05$) of solvent system on extracts' properties. The water:ethanol extract from lemon grass showed the highest total phenolic content, DPPH radical scavenging activity and ferric reducing antioxidant power (FRAP) value. Ethanol and water:ethanol extracts revealed high antibacterial activity. Gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*) bacteria were inhibited by lemon grass extracts at a concentration of 0.3 mg/ml, whereas Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) needed higher extract concentrations (5–20 mg/ml). Water extract inhibited only *Staphylococcus aureus* at a level not exceeding 55%.

The results of the research demonstrated potential importance of lemon grass extracts in the food industry, as their implementation in food products, food supplements, and beverages could significantly improve product safety, shelf life, and health-promoting properties.

PCF-50

EXTRACTION OF PHENOLIC COMPOUNDS FROM *ARTEMISIA ABSINTHIUM* L.: OTIMIZATION THROUGH ULTRASSOUND ASSISTED EXTRACTION USING THE RESPONSE SURFACE METHODOLOGY

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Plants throughout history have been a human resource available to be used for food and feeding and treatment of diseases, thus being considered as natural medicine agents. Plants are a huge source of secondary metabolites which have been described as strong health promoters and can be divided in primary metabolites, responsible for the processes of photosynthesis, respiration, solute transport, translocation, nutrient assimilation and differentiation; and secondary metabolites (terpenes, phenolic compounds, organic acids, among others), which are important for plant protection and pollination [1]. Recent studies have shown the relevant biological activities of phenolic compounds, consisting in their antioxidant, antimicrobial, and also cytotoxic effects activity. Among the medicinal plants it is possible to find the *Artemisia absinthium* L., an aromatic plant [2], that grows in temperate regions of Europe, Asia and North Africa. In addition to the renowned wormwood application in preparation of absinth and related beverages, *A. absinthium* has been used since ancient times for medical purposes. From the ethnopharmacological point of view, this plant has been used for its antihelmintic, stomachic, antibacterial, antifeedant, antifertility, antipyretic, cytostatic, antitumor, and antimalarial actions [3].

This work intended to optimize the extraction parameters of the ultrasound assisted extraction to obtain highly enriched extracts in phenolic compounds. This was possible using the response surface methodology (RSM) that is able to model data within certain parameters chosen by the researcher, but prior to start running multiple experiments without knowing the behaviors of certain factors involved, this technique would be only able to visualize certain magnitudes and not all the optimum point, therefore, strong complex and random screening analysis have to be performed in order to work the RSM in the optimal conditions and be able to properly model datasets. Thus, 4 different screening analyses (Fractional Factorial design, 2 factors multilevel, and 1 factorial design) were developed using a 12-run array mixture which was randomized and codify using statistical software (Design Expert). Three factors were tested in this experiment (Power [50-100%], Solvent percentage [0-50%] and Time [20-600s]) and for the interpretation of the fractional factorial design factors were aliases and estimated as Intercept= Intercept + ABC; A= A + BC; B= B + AC; and C= C + AB. Data was processed through an ANOVA analysis employing Pareto plots and the least significant difference (LSD) test. The response analysis was achieved through the phenolic compounds content, analyzed by HPLC-DAD-MS. According to the obtained results, the UAE ideal conditions for the screening analysis were 100% power, 20% ethanol and 240 s allowing a total phenolic content in the concentrations of 13.79 mg of TPC/g of extract, being chlorogenic acid derivatives, the main compounds. These results highlight the richness of *A. absinthium* in phenolic compounds and validate the UAE as an efficient extraction technology.

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PCF-51

“ECONÓMICOS” CAKES STRENGTHENED WITH CHESTNUT FLOUR: EFFECTS ON TEXTURE AND COLOR

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Cereals grains, such as wheat, rice and corn starch are one of the main sources of nutrients and energy contributors to the human diet. Furthermore, wheat baked products are historically the basis of the diet in Europe [1]. The “económicos” are traditional cakes from Portugal, made with flour, sugar, margarine, olive oils, eggs and brandy and quite appreciated by consumers, because of the good taste [2]. Different sources of flour can be used in pastry products, however, the gluten present in wheat flour, along with starch maintain the union between the ingredients, provide the structure of the baked product and also the elasticity and extensiveness of the mass. Therefore, with the use of other sources of flour, the presence of gluten becomes minimal and the cake tends to become brittle and compact [3,4], usually with an inferior quality compared to foods which contain it, namely at the level of low nutritional value, weak coloration, higher tendency to crumble and, mainly, low volume [3].

Thus, the main objectives of this work were to analyse the texture and color of “económicos” incorporated with chestnut flour, thus strengthening the económicos nutritional value and not fully reducing gluten. Color was analysed using a portable colorimeter, with analysis of the L*, a* and b* coordinates on the top, bottom and inside sections of the “económicos”. Texture was analysed using a texturometer and a texture profile analysis, rendering the dimensions of hardness, adhesiveness, resilience, cohesiveness, springiness, gomosity, chewiness and firmness. Both analysis were carried out over the course of 25 days and the samples were compared with control económicos with only wheat flour. Overall, significant interaction was sought for all analysis of color, except for red-green on the top of the “económico” and lightness of the inside, being the chestnut samples significantly darker than the control ones. Storage time showed, as expected, a higher influence on the texture profile, especially in terms of hardness, cohesiveness, springiness and chewiness, with statistical differences found from the first to the 11th day. The chestnut flour only influenced the springiness, making thus reducing this dimension. Chestnut flour can be useful to fortify these cheap snacks, although analysis on the chemical, nutritional and microbial analyses are being processed.

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PCF-52

***EISENIA FETIDA* AS A BIOLOGICAL RESEARCH MODEL FOR SOY ISOFLAVONES**

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Soy-based foods contain high concentrations of isoflavones which show some chemoprotective effects in cancer. [1]. Animal models of disease have been used; however, several ethical questions are increasing regarding the use of vertebrates. Consequently, it is essential to optimize new biological research models that do not raise ethical issues. *Eisenia fetida* is an earthworm easy to handle and inexpensive, recognized as the most used for ecotoxicology studies, being a useful bioindicator to test soil toxicity [2]. With this work, our team aims to determine the effects of isoflavones on physiological and behavioral parameters of *E. fetida* through soil exposure.

For this study, a toxicity bioassay test was prepared according the guidelines described by the Organization for Economic Co-operation and Development (OECD, 2016). Briefly, an artificial soil consisting of 10% peat, 20% kaolin, 70% sand mixture was prepared; pH (0.1M KCl, 1:2.5 ratio) was adjusted to 6.0 ± 0.5 by adding CaCO_3 and moisture content adjusted to 40% of the maximum water holding capacity. The concentrations of isoflavones selected were 140, 279, and 499 mg/Kg of soil. In addition to exposure groups, three control groups were used: (i) a group with artificial soil; (ii) a group with soil and earthworms and (iii) a group with soil and isoflavones (499 mg/Kg), without earthworms. The worms were acclimatized to the artificial soil for 24 hours, and then they were left to depurate the intestinal contents on moistened paper filter for 24 hours. For this study, adult earthworms, with a bodyweight of 0.40 ± 0.02 g were used. All procedures were performed at $20 \pm 2^\circ\text{C}$. Each group contained three reactors to which eleven earthworms were randomly allocated (except controls without earthworms). Every week, soil moisture was corrected on a weight basis through the addition of distilled water and earthworms were fed by adding of 2g ground oats. We evaluated the behavior of earthworms daily, according to their position in the soil. At the end of experimental period (56 days), the worms were weighed and euthanized.

No mortality was observed throughout the study and earthworms showed no behavioral changes during the experimental test period. The concentration of isoflavones did not reveal significant effects on the evaluated physiological parameters. However, we found that there is a tendency to increase earthworm weight with the highest concentration (499 mg / kg) and a decrease in weight with the lowest concentration (140 mg / kg) both relative to the control. In all studied treatment, earthworms were able to generate offspring.

Our results show that isoflavones did not affect physiological parameters of *Eisenia fetida*, however, the differences observed in weight among exposed groups and the controls suggest that this species is sensitive to this type of compounds. Therefore, further studies must be performed to better understand these changes.

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PCF-53

EVALUATION OF THE CHEMICAL COMPOSITION OF BIOACTIVE COMPOUNDS FROM PLANTS OF THE ASTERACEAE FAMILY

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Since ancient times, traditional plants have been used as a remedy against disease and pain. Nowadays, numerous studies have shown that these plants have different beneficial properties such as antioxidant, anti-inflammatory or antimicrobial among others and that these biological properties are linked to their content in active compounds. This fact justifies their traditional use and their potential industrial interest. Although they are still used mainly in herbal products, today their consumption is decreasing. Sometimes the mechanisms and compounds responsible for its beneficial properties are even unknown [1].

Based on this, this work is focused on five plants of the Asteraceae family: *Achillea millefolium*, *Arnica montana*, *Calendula officinalis*, *Chamaemelum nobile* and *Taraxacum officinalis*. An initial review of their status has been performed, followed by a chemical characterization of its components, as well as a heat assisted extraction (HAE) to obtain extracts rich in bioactive compounds. Phenolic compounds have been identified and quantified and the antioxidant, antimicrobial and enzymatic activities have been assessed.

Furthermore, based on the results obtained in the enzymatic tests, a molecular docking of the phenolic compounds found in the samples and the target enzymes was carried out. Also, it was observed that specific extracts showed potential inhibitory activity against acetylcholinesterase and butyrylcholinesterase, two enzymes involved in Alzheimer's-related pathologies. The obtained results suggest the possibility of obtaining new extracts that may be of interest for the development of new products for the nutraceutical, cosmetic or pharmaceutical industry.

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RED RASPBERRY WASTE AS A SOURCE OF ANTHOCYANIN-RICH FOOD COLORANTS: EXTRACTION PROCESS OPTIMIZATION AND FUNCTIONALITY ASSESSMENT

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Food colorants are increasingly used in the food industry to preserve, improve or change the food color. While the quite controversial artificial colorants are widely used in this sector, the natural counterparts have been less selected in part due to the limited availability of options and stability issues [1]. Within this class, anthocyanins are naturally occurring colorants that can be found in different plant matrices, including berries such as red raspberry (*Rubus idaeus* L.). These water-soluble pigments show attractive colors ranging from red to purple and present health-promoting effects [2,3]. Therefore, this work aimed to develop a novel anthocyanin-rich food colorant from red raspberry waste through the optimization of a sustainable extraction methodology and to characterize this ingredient for its functionality. Heat (HAE)- and ultrasound (UAE)-

assisted extraction methods were implemented to recover the anthocyanins from red raspberry. Processing time, ethanol concentration, and temperature or ultrasonic power were the independent variables analyzed in a central composite design coupled with response surface methodology for processes optimization. The extraction yield and levels of anthocyanins (cyanidin-3-*O*-sophoroside and cyanidin-3-*O*-glucoside) were monitored gravimetrically and by HPLC-DAD-ESI/MSⁿ, respectively, and used as response criteria. The constructed theoretical models were successfully fitted to the experimental data and used to determine the optimal extraction conditions. Overall, HAE originated slightly higher response values (61% extract weight and 8.7 mg anthocyanins/g extract) but needed 76 min processing at 38 °C, with 21% ethanol, while the UAE process required 16 min sonication at 466 W, using 38% ethanol, to obtain 58% extract weight and 8.3 mg anthocyanins/g extract. Then, the predictive models were experimentally validated and the purple

red extracts obtained under optimal condition showed antioxidant activity through lipid peroxidation and oxidative hemolysis inhibition, and antibacterial effects against food-related bacteria, such as *Escherichia coli* and *Enterococcus faecalis* [4]. These results highlight the potential of red raspberry extracts as natural food colorants with bioactive effects. In future studies, it will be interesting to investigate the stability of the developed anthocyanin-rich extracts when exposed to different stability factors and in real food matrices.

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PCF-55

**ANTIOXIDANT AND ANTIHEMOLYTIC ACTIVITY OF PUMPKIN BY-PRODUCTS:
A CONTRIBUTION TOWARDS RESOURCE-USE EFFICIENCY**

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The current need to obtain nutritious and healthy foods from alternative and more sustainable sources and to reduce waste in the agri-food sector has led to a great interest in the valorisation of plant parts currently treated as by-product, but which have nutritional value and food-grade potential for inclusion in the human diet. The pumpkin processing industry is a good example because it generates a large amount of biowaste in the form of seeds and peels [1], which can be recycled inside the food chain as bioactive or functional ingredients, since these matrices contain significant amounts of carotenoids, protein, fibre, and tocopherols, among other antioxidants [2]. This study aimed to measure the antioxidant activity of pumpkin by-products, in order to assess their potential for use in novel food formulations. Pumpkin seeds and peels provided by local producers in the northeast of Portugal were lyophilized, ground to a fine powder, and submitted to a solid-liquid extraction using an hydroethanolic mixture as solvent [3]. The obtained liquid extracts were lyophilized, redissolved in distilled water, and successively diluted to different concentrations to evaluate the antioxidant activity. The thiobarbituric acid reactive substances (TBARS) formation inhibition and the oxidative haemolysis inhibition assays were performed *in vitro* using porcine brain cell homogenates and sheep red blood cells as oxidizable biological substrates, respectively [3]. A Student's *t*-test was applied to assess the existence of statistical differences between both samples. Based on the results obtained with the performed cell-based assays, it was possible to conclude that both seed and peel extracts display antioxidant effects. The seed extract was more effective in inhibiting the formation of TBARS, such as the highly reactive malondialdehyde, which results from the peroxidation of polyunsaturated fatty acids that constitute the porcine brain cell membranes. In turn, the peel extract had a better performance in protecting the red blood cells from the haemolytic action of free radicals initially generated in the system by the oxidant 2,2'-azobis (2-methylpropionamide) dihydrochloride, which is thermally activated during incubation at 37 °C. Overall, these results demonstrate that the preservation and/or functionalization of food products could be achieved with the addition of bio-based antioxidant ingredients resulting from pumpkin by-products.

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PCF-56

DEVELOPMENT OF A NEW APPROACH BASED ON REAL-TIME PCR COUPLED WITH HIGH RESOLUTION MELTING (HRM) ANALYSIS TOWARDS THE ENTOMOLOGICAL AUTHENTICATION OF HONEY

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Honey is a natural product widely consumed around the globe, not only for its taste and nutritional value, but also for its health benefits. Being a product of high dietary relevance and increasing demand, it has also become a target of economically motivated adulteration. According to the 2014 European Parliament report on the food crisis, fraud in the food chain and the control thereof, honey is among the 10 food products most prone of being adulterated [1]. Up until now, honey authenticity was mainly focused on the issues of sugars addition and botanical and geographical origin. However, recently an increased attention has been paid to the entomological origin of honey. To this aim, different approaches have been proposed to differentiate honey produced by different *Apis mellifera* subspecies, including those from distinct mitochondrial (mt) DNA lineages [2]. This work aimed to develop a novel real-time PCR method coupled with HRM analysis that allows for the simultaneous differentiation of honeybee from maternal lineages A, M and C, for further application in honey authentication. In this sense, data previously obtained from the mitogenomes of a total of 112 honeybees of different lineages were considered for the development of new DNA markers. Considering the aim of further application in honey, new primer sets were designed to amplify short fragments that included different single nucleotide polymorphisms (SNPs) allowing for HRM application. Three primer sets were proposed, *amsCOI-F/amsCOI-R* targeting the Cytochrome oxidase I (COI) gene, *amsND1-F-amsND1-R* targeting the NADH-ubiquinone oxidoreductase chain 1 (ND1) gene and *amsCox3-F/amsCox3-R* targeting the Cytochrome oxidase subunit III (Cox3) gene. Each primer set was first tested using qualitative PCR using DNA extracted from honeybees of A, M and C mtDNA lineages. After optimizing the real-time PCR conditions, each primer set was tested using a series of mtDNA extracted from honeybees. While *amsCOI-F/amsCOI-R* allowed only for the separation of the honeybees in two clusters, with lineage C and M clustering together, both the *amsCox3-F/amsCox3-R* and *amsND1-F/amsND1-R* set of primers allowed to differentiate the three lineages in separate clusters, with high level of confidence. As future work, the methodology will be assayed in commercial honey samples.

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PCF-57

**RESPONSE SURFACE ANALYSIS OF ULTRASOUND AND DYNAMIC MACERATION EXTRACTIONS OF
*ARBUTUS UNEDO***

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The use of natural plant extracts in the food industry has been common practice for many decades, but, due to the pursuit of healthier diets by consumers, has gained a new momentum, in which natural food additives, namely preservatives have been extracted from plants. The most prominent example is the use of rosemary extract (E392) as a food preservative throughout the European Union, paving the way for new extracts to be used for the same purpose, possibly with better results. In this work, the extraction of leafy parts of *Arbutus unedo* L. is described both through ultrasound assisted extraction (UAE) and dynamic maceration (DM), following an optimization through Response Surface Methodology (RSM) to optimize the extraction yield. For the DM the factors analyzed were (F1) “Time” which varied between 10 and 60 minutes, (F2) “Temperature” which varied between 30 and 80 °C, and finally (F3) “Solvent” (ethanol) which varied between 0 and 100%. For the UAE, the factors were also (F3) “Solvent” and (F1) “Time”, and although the variation in solvent was the same, the time of extraction only varied between 5 and 30 minutes. Finally, the third factor was ultrasonic (F2) “Power” of the equipment that varied between 50 and 500 watts. The analyzed response for both extractions were the dry residue (Y1) which varied between 3 and 65.3 mg for UAE and 10.4 and 99.9 mg for DM. The RSM analysis rendered a quadratic model with an inverse transformation for DM, and a reduced quadratic model with no transformation for UAE. To optimize the yield of dry residue (Y1), optimization studies were performed and indicated the optimal points at which a higher yield of dry residue can be obtained, and were F1 – 57 minutes, F2 – 46 °C and F3 – 52% of ethanol for the DM extraction. For UAE, the optimal points for the same Y1 response were F1 – 17 minutes, F2 – 380 watts and F3 – 39% of ethanol. In **Figure 1**, it is clear that for DM a longer time of extraction favors the residue yield, as well as a temperature near 60 °C, while for the UAE, time did not seem such an important factor, while the power of the ultrasonic probe was quite important. Overall, due to the lower amount of ethanol needed and the lower extraction time, UAE seems to be the best extraction technique to maximize the yield of dry residue of *A. unedo*, although in terms of mass, DM yielded 99 mg while UAE only 65.3mg. Other responses are currently being studied to determine the best overall extraction technique.

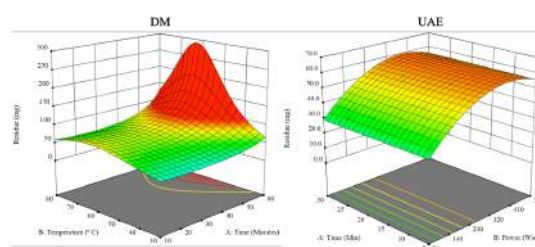


Figure1. Graphical representation of the optimal points for UAE and DM extractions of *A. unedo*

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WORLD MARKET OF SUNFLOWER OIL

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Today, the Ukrainian oil industry has modern production facilities for processing oilseeds, which are constantly growing. Sunflower oil production is one of the most important economic and food components of the agro industrial complex in Ukraine. Every year the volumes of its production and export increase, which makes the country a leader in the domestic and international markets.

The aim of the study is to analyze the current state of the oil market in the world, to consider prospects for market development and to identify ways to adapt producers to modern conditions, to monitor the Ukrainian sunflower oil market to maintain sustainable development and the title of Ukraine as world leader in sunflower oil production and export.

In the course of research the tasks concerning the analysis of the basic indicators of development of branch are solved; identification of the reasons holding back development; finding ways to increase competitiveness.

Methodology: To solve the tasks, the following statistical research methods are used to collect statistical information; analytical for forecasting development; inductions, deductions for generalization; graphic for clarity of the obtained results.

The share of sunflower in the global production of vegetable oils is about 10%. In terms of production volumes, it is the fourth indicator after palm oil (36%), soybean (28%) and rapeseed (13%). Ukraine's share in a separate market of sunflower oil can be considered confidently leading because Ukrainian suppliers account for more than 50% of global product exports. The main buyer is India, followed by China, EU countries and Turkey. Ukraine has become a world leader in the export of sunflower oil in the 2020-2021 marketing year [1]. The demand for sunflower oil is constantly growing, due to its positioning as a product for healthy eating. This is especially true of the HOSO (high oleic sunflower oil) segment.

Ukraine has the twenty largest plants, which produce more than 80% of the total production of sunflower oil in the country. Sunflower oil is the main for the Ukrainian market of vegetable oils, an important raw material for many sectors of the food industry. According to Ukroilprom, the TOP-3 producers who produced more than 40% of unrefined oil are as follows Kernel – 23,1%, Bunge – 10,7%, Mironovsky bread product – 6,3% [2]. Currently in Ukraine there is a tendency to increase production capacity for processing oilseeds, new plants are being built. Ukraine's world leadership in the foreign market of sunflower oil also has negative consequences. In particular, serious problems for the country's agricultural sector may be caused by the constant expansion of sown areas. Expansion of areas and non-compliance with cultivation technologies threatens land depletion and reduced yields.

Thus, after assessing the development trends of the sunflower oil market, the priority of further market reform is to expand foreign markets by increasing the competitiveness of sunflower seeds and domestic products and the rapid formation of appropriate customs policy depending on world market conditions.

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PCF-59

**BEE BREAD PRESERVATION METHODS: PHYSICO-CHEMICAL AND MICROBIAL STABILITY
THROUGHOUT STORAGE**

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Bee bread is an important beehive product, with growing commercial interest due to its high nutritional value and to the bioactive compounds it contains, setting it as a good supplement as a functional food. Considering human safety and the significant impact that bee bread is gaining in the field of human nutrition, this work evaluates the effect of distinct preservation techniques on the bee bread physico-chemical and microbiological parameters, throughout a storage period of 3 months.

Similar bee bread portions were submitted to four distinct conditions: i) storage at room temperature without previous treatment, ii) oven drying at 30 °C for 24 hours and storage at room temperature, iii) freeze drying and storage at room temperature, and iv) storage at -20 °C (freezing). Samples were taken from each treatment set immediately after the application of the treatments (T0), and after one (T1) and three (T3) months of storage. For each time point of analysis, the samples were analyzed for several microbiological, nutritional, and bioactive parameters [2, 3, 4].

The results showed a decrease of the concentration of the total phenolic compounds and a consequent decrease in the antioxidant activity. No variation was detected for the other physico-chemical parameters. As for the microbial analysis, the results showed a decrease in bacterial loads, but yeasts, molds and lactic acid bacteria kept a constant trend. No difference was observed throughout time between treatments. These results could be explained by the low levels of water activity ($a_w < 0.6$) and pH (< 4.1), which were maintained constant for the four conditions of preservation through time. These results suggest that, for the evaluated storage period, all the studied preservation treatments enabled to maintain water activity and pH values, which seem to contribute to effectively control the growth of microbial loads.

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PCF-60

DETERMINATION OF THE PHYTOCHEMICAL COMPOSITION, ANTIOXIDANT AND CYTOTOXICITY PROFILE OF *OPUNTIA FICUS-INDICA*

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In the past few years, synthetic colorants by natural pigments have gain popularity over synthetic dyes since natural pigments are associated with healthy living. The food industry has widely utilized colorants to make them more attractive to consumers. Nature produces numerous pigments with commercial value for coloring food, such as anthocyanins and betalains. Betalains are water-soluble nitrogenous natural colorants consisted of a backbone core, betalamic acid [1]. Betalains have been considered a current trend in food coloring, as has been pointed out to possess a large pharmacological profile. Significantly, the biological activities of betalains seem to play a major role in human health due to their robust antioxidant ability and their capacity to absorb free radicals [2]. In this frame, betalains can be used as ingredients to provide colorful foods that possess enhanced antioxidant ability. Therefore, isolation of betalains and determination of their photophysical properties as also their antioxidant capacity is of importance. The plant *Opuntia ficus-indica* (L.) Mill. (cactus or prickly pear) is one of the primary sources of betalains [3]. The prickly pear fruits are characterized by various colors. Thus, we selected two different plant varieties of *Opuntia ficus-indica*, purple and orange in order to isolate betalains and study their pharmacological potency. The purple variety is due to betacyanins, and the orange variety due to betaxanthins. This study's objective was to identify betalain patterns and content in the fruits of prickly pear cultivars/lines.

Techniques such as column chromatography and HPLC were employed for the separation of the pigments in the extracts. UV-Vis spectroscopy was used for the quantification of the betalains as also the total phenolic in the extracts. For the characterization of betalains in the isolated pigment LC/MS-MS as also Nuclear Magnetic Resonance (NMR) was also performed. Specifically, novel protocols for optimizing the ¹H-NMR spectra of complex mixture were developed to analyze the main pigments present in the extracts. In addition, the photophysical properties, as also their cell uptake capacity, were evaluated using fluorescent spectroscopy. Finally, the antioxidant effect of the extracts as also their in vitro cytotoxicity was evaluated.

To conclude, we identified numerous pigments in two *Opuntia ficus-indica* varieties. All of them demonstrated significant antioxidant capacity and proved safe for use as colorants in the food industry.

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PCF-61

NUTRITIONAL VALUE AND CHEMICAL COMPOSITION OF PURSLANE LEAVES IN RELATION TO HARVESTING STAGE

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Purslane (*Portulaca oleraceae* L.) is an omnipresent weed, while it is also considered a common ingredient of the Mediterranean diet due to its high nutritional value with particular reference to omega-3 fatty acids content. In the present study, we evaluated the nutritional value and chemical composition of purslane leaves in relation to harvesting stage. Plants were harvested at three growth stages (29, 43 and 52 days after sowing (DAS)), while leaves were separated from the aerial parts of the plant. Leaves contained high amounts of macronutrients, especially at 52 DAS. α -Tocopherol was the main isoform, which increased at 52 DAS, as well as the total tocopherols (values were in the ranges of 197–327 $\mu\text{g}/100$ g fresh weight (fw) and 302–481 $\mu\text{g}/100$ g fw, for α -tocopherol and total tocopherols, respectively). Glucose and fructose were the main detected free sugars, while total sugars content ranged between 0.160 g and 0.330 g/100 g fw). Oxalic and total organic acids content increased at late harvesting (52 DAS; 8.6 g and 30.3 g/100 g fw for oxalic acid and total organic acids, respectively). Regarding the fatty acids content, leaves were abundant in α -linolenic acid (35.4–54.92%), although a decreasing trend was observed with successive harvesting. Oleracein A and C were the major oleracein derivatives detected in leaves, regardless of the harvesting stage (values were in the ranges of 8.2–103.0 mg and 21.2–143 mg/100 g dried weight (dw) for oleraceins A and C, respectively), while the highest content was recorded at 29 DAS for both compounds. Hepatotoxicity assays showed no toxicity with GI_{50} values being higher than 400 $\mu\text{g}/\text{mL}$ for all the harvesting stages. In conclusion, early harvesting could increase the nutritional value of the final product through increasing the content of valuable compounds, such as omega-3 fatty acids, phenolic compounds and oleracein derivatives, while at the same time, the contents of anti-nutritional compounds such as oxalic acid are reduced.

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PCF-62

CHEMICAL COMPOSITION AND BIOACTIVE PROPERTIES OF VINE-CANES OBTAINED BY SUBCRITICAL WATER EXTRACTION

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Grapes are one of the major fruit crops produced throughout the world, due to their high nutritional properties, consumer appreciation and ancient domestication [1]. This production, especially concerning the wine industry, results in the generation of high amounts of by-products, with vine-canes corresponding to the largest parcel. Usually, this woody material is incorporated into the soil or incinerated; however, taking into account their phenolic content and antioxidant properties other applications can be more profitable [2]. Nowadays, one of the biggest challenges is to find an efficient environmentally friendly extraction technique, that industries could apply at a large scale.

The aim of the present work was to characterize the chemical composition and bioactive properties of vine-canes from Touriga Nacional variety. For that, the previous optimized subcritical water extraction conditions (33 min and 280 °C) were employed, and the obtained extract characterized through different spectrophotometric assays. The results obtained revealed that vine-canes presented a total phenolic content value of 229 ± 23 mg of gallic acid equivalents per g of dry extract. Regarding the antioxidant properties, the extract presented 227 ± 20 and 236 ± 10 mg ascorbic acid equivalents per g of dry extract for the ferric reducing antioxidant power and the ABTS radical scavenging activity assays, respectively. The extract also displayed strong *in vitro* neuroprotective effects, inhibiting several brain enzymes involved in neurodegeneration: acetylcholinesterase ($IC_{50} = 290.5 \mu\text{g/mL}$), butyrylcholinesterase ($IC_{50} = 244.0 \mu\text{g/mL}$), tyrosinase ($IC_{50} = 1459.0 \mu\text{g/mL}$) and monoamine oxidase B ($\sim 53.4\%$ of inhibition at $1000 \mu\text{g/mL}$).

Work is in progress in order to identify the individual phenolic compounds that can be contributing to the presented antioxidant properties, using high performance liquid chromatography with diode array detection.

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PCF-63

CAROTENOID-BASED SOLUTIONS FOR THE REPLACEMENT OF ARTIFICIAL COLORANTS IN PASTRY PRODUCTS

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Colour has a great importance in the first consumers' impression, allowing to infer about the overall quality, the taste, the smell, the texture, and even the safety of foodstuff [1]. For these reasons, there is a massive use of colorants in food products. Nevertheless, the most applied compounds are of artificial origin and some of them have been increasingly associated to health issues, with allergic reactions, children attention deficit, and cancer pointed out as the most common consequences [2]. These facts have been driving new research in this field, through the exploitation of natural sources of colouring molecules to be applied in detriment of artificial colorants. Among the numerous natural matrices potentially used for the extraction of colouring compounds, the fruits from the genus *Solanum* represent promising sources of pigments, namely carotenoids [3]. Together with the fact that large amounts of fresh tomato wastes (resulting from crop growing, packaging, processing, storage, and sale) are discarded worldwide, the recovery of valuable colorant biomolecules from these agri-food wastes represents a crucial step of the circular economy by re-introducing them into the food chain as ingredients [3]. The need to process these bio-wastes for the recovery of coloring molecules, has led to the use of more eco-sustainable extraction methodologies in detriment of more conventional techniques, such as maceration. Ultrasound-assisted extraction methodology arises as one of the most promising alternatives, with lower extraction times, use of greener solvents, and higher recovery yields, but also with the possibility to be scaled-up to respond to the high demands of the industrialized world [3]. Carotenoid compounds are lipophilic pigments responsible for the yellow, orange, and red colours of certain plant matrices, with a vast structural diversity, but prone to isomerization and oxidation [4]. However, the colouring capacity of these molecules overcomes any instability problem (that can be solved with stabilization strategies) and, therefore, carotenoid-based colorants appear as a valid solution for application in the pastry sector, that greatly relies on yellow/orange artificial colorants.

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PCF-64

STUDY OF AROMATIC AND MEDICINAL PLANTS AS POTENTIAL NATURAL INGREDIENTS FOR THE FOOD INDUSTRY

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Considering the association of plants consumption and utilization with the treatment of some illnesses and diseases, several aromatic and medicinal plants have been used in traditional and contemporary medicine. This capacity has been proven scientifically and there are numerous studies describing their therapeutic properties in the treatment of inflammatory and cardiovascular disorders, diabetes, among other conditions [1]. The antioxidant and antimicrobial activities have already been studied in a wide variety of plants, which have been shown to contain bioactive molecules effective against pathogenic microorganisms and capable of removing reactive oxygen species formed in cells [2,3].

In this sense, ten aromatic and medicinal plants (*Eucalyptus globulus* Labill., *Olea europaea* L., *Melissa officinalis* L., *Origanum vulgare* L., *Glycyrrhiza glabra* L., *Arbutus unedo* L., *Matricaria recutita* L., *Thymus vulgaris* L., *Ocimum vulgare* L., and *Salvia officinalis* L.) were selected to perform an initial screening for the exploration of natural ingredients with bioactive potential.

The plant extracts were obtained after ethanolic extraction. The antioxidant activity was evaluated through two *in vitro* cell-based assays, namely the lipid peroxidation inhibition test (TBARS) and the oxidative hemolysis inhibition test (OxHLIA), and antimicrobial activity was tested by the broth microdilution method, against a panel of bacteria and fungi, selected according to their public health importance.

These analyses presented very promising results, showing a high bioactive potential for all plant extracts.

In the TBARS assay, *E. globulus* and *O. vulgare* stood out with lower EC₅₀ values, followed by *T. vulgaris* and *S. officinalis*. In turn, in the OxHLIA assay, for Δt 60 min and Δt 120 min, the excellent antioxidant capacity of *S. officinalis* and *T. vulgaris* was also evidenced, respectively. In addition, the evaluation of the antimicrobial activity highlighted the sample of *G. glabra*, both at the bacterial and fungal levels. Overall, it was possible to prove the great bioactive potential of the *E. globulus*, *O. vulgare*, *S. officinalis*, *T. vulgaris* and *G. glabra* extracts, and to highlight them as promising options for exploitation in the food industry.

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PCF-65

EFFECTS OF BLACK-EYED-BEANS IN AN ANIMAL MODEL OF COLORECTAL CANCER

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Several studies revealed a possible protective effect on the intake of vegetables in relation to colorectal cancer (CCR) development. Black-eye-bean (*Vigna unguiculata* (L.) Walp) is ingested as a high quality plant protein source, presenting a better option to red meat [1]. Black-eye-beans have high antioxidant capacity and high flavonoids content [2]. Flavonoids are among the compounds with the most important anticancer properties of vegetables, especially isoflavones [3]. So, CCR development might be reduced by introducing black-eye-bean in the diet. This work aimed to evaluate the effects of supplementation of black eyed-beans on an animal model of chemically induced CRC, through the sequential administration of azoxymethane (AOM) and sodium dextran-sulfate (DSS).

Forty-eight female FBV/N strain mice, aged 5-6 months, were randomly divided into four groups: control group (n=9), induced group (n=13), induced group / 20% (m/m) black-eyed-bean flour (BEBF (n=12) and induced group / 50% (m/m) BEBF (n=14). AOM was administered intraperitoneally only once (7.5 mg/kg). One week later, the animals were exposed to drinking water with 1.5% DSS during seven consecutive days over three cycles, with 7-day intervals between them. The animals had *ad libitum* access to food and water. Weekly, the animals were individually weighed. After 13 weeks, all animals were humanely euthanized. Blood and organs were collected for further analysis: histopathology, immunohistochemistry, biochemical markers, and comet assay.

No differences were observed in initial and final animal weights between groups. Regarding histopathological analysis, 50% (n=6) of the animals in the induced group + 20% BEBF presented epithelial dysplasia in the colon, 16.67% (n=2) presented adenocarcinomas in the rectum, being the group with more lesions. In the three groups induced with AOM/DSS, mild to moderate inflammation was observed in the colon. Regarding the expression of the cell proliferation marker Ki-67 in the colon mucosa, there was a higher immunostaining in the + 20% BEBF induced group. Data showed no significant variations of circulating C-reactive protein, IL-6, TWEAK, MMP-9, MMP-2, and myostatin levels. The comet assay revealed no statistically significant differences between groups.

To conclude, black-eyed-beans did not show negative or positive effects on these model of CCR. New studies must be carried out using different doses of the carcinogenic compound, time of exposure to the inflammatory agent, moment of sacrifice, and/or concentration of the used black-eyed-beans.

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PCF-66

PHENOLIC AND BIOACTIVE PROFILES OF TEN COLORED POTATO PEELS

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The pharmaceutical and food industries have been exhaustively prospecting the use of natural products as sources of bioactive molecules to substitute synthetic drugs and food additives. Withal, the valorization of bio-residues abundant in bioactive phytochemicals that are commonly rejected, like potato peels, could pitch in to the development of more sustainable, both economically and socially, productive chains [1]. In this study an unprecedented in-depth characterisation of the non-anthocyanin and anthocyanin phenolic compounds of potato peels from ten colored potato varieties was performed. Beyond that, the antioxidant, cytotoxic and anti-inflammatory potentials of the samples were explored for the first time. Among non-anthocyanin phenolics, caffeic and a caffeoylquinic acid were found in the highest concentrations in all samples, which also showed the presence of *O*-glycosylated flavonol derivatives and polyamine derivatives. Acylated anthocyanins were detected in red and purple varieties, being pelargonidin, peonidin, and malvidin the most prominent aglycones. All samples displayed antioxidant and antiproliferative actions, and no toxicity towards non-tumor cell lines up to the concentration of 304 µg/mL. The Rosemary variety presented the best antioxidant and antitumor activities, and was the only sample to show anti-inflammatory effects. Such promissory findings support the exploitation of potato peels as valuable sources of bioactive compounds and as natural additives in functional food formulations.

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PCF-67

PHENOLIC COMPOUNDS FROM MONOFLORAL BEE POLLEN: A POTENTIAL SOURCE OF BIOACTIVE MOLECULES

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Bee pollen is composed of hundreds and even thousands of plant pollen that have been harvested by bees. The insects use their saliva secretions and plant nectar to agglomerate the grains and transport them into the hives. Beekeepers can collect this pollen loads at the hive entrance, with the use of appropriated pollen traps.

In the present study, the phenolic profile of seven bee pollen samples, from Nisa and Bragança, were characterized. The pollen load samples were separated by colorimetry and the botanical origin was checked by palynological analysis. After the phenolic extraction, the samples were characterized through UV-Vis analysis to determine the total phenolic and flavonoid content. The phenolic composition was assessed using high performance liquid chromatography coupled to diode array detection and electrospray mass spectrometry (HPLC-DAD-ESI/MS). The results classified all bee pollen samples as monofloral with the presence of high quantities of important flavonoid and phenylamides. P1 (*Carduus* sp), revealed important amounts of isorhamnetin glycosides, while P2 (*Ligustrum/Olea* sp.) displayed significant quercetin glycosides, specially quercetin-*O*-diglucoside (*m/z* 625). P3 (*Cistaceae* bee pollen) revealed a prominent quercetin glycoside amount, and also laricitrin glycosides. P4 and P6 belong to the same floral origin (*Rubus* sp.) displayed similarities in their phenolic profile, with different quantities, showing that each plant species owns a unique phenolic profile. These samples presented a rich composition in methyl herbacetin glycosides, mainly methyl herbacetin-*O*-dihexoside (*m/z* 639). *Echium* sp. bee pollen sample, P5, presented a rich composition in kaempferol glycosides mainly kaempferol-3-*O*-rutinoside (*m/z* 593) and kaempferol-*O*-malonyl-hexosyl-deoxyhexoside (*m/z* 679). The last sample, *Castanea* sp., displayed the highest total phenolics and flavonoids content among all the samples. Besides, a rich composition in isorhamnetin and kaempferol glycosides was found, together with a high amount of phenylamides, with seventeen compounds identified, being N¹-feruloyl-N⁵, N¹⁰-dicafeoylspermidine (*m/z* 644), the most abundant. In conclusion, some of the detected phenolic compounds can be considered as floral biomarkers such as methyl herbacetin-*O*-malonyl-hexosyl-deoxyhexoside, which was only detected in the *Rubus* samples.

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PCF-68

**ANTIOXIDATIVE PROPERTIES OF HELICHRYSUM ITALICUM AND HELICHRYSUM ARENARIUM
INFUSIONS**

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Helichrysum arenarium (HA) has a long tradition in European ethnomedicine as a medicinal plant with many known activities, it is used against digestive disorders, feeling of fullness and bloating and for free radical scavenging, and is also approved as a medicinal plant. In the Mediterranean region of Slovenia and in other regions with similar climate conditions, such as coastal Croatia, southern Italy, Spain, Portugal and Corsica, *Helichrysum italicum* (HI) is more common compared to HA, both as a wild plant and as a crop. Scientific data on HI are mostly limited to either topical applications in wound healing or to the investigation of separate compounds in *in vitro* models, despite the fact that, similar to HA, HI is widely used in traditional medicine, often as an infusion or decoction.

Therefore, we analyzed the antioxidative properties, tested *in vitro* and in cell models, of infusions prepared from HI plants in comparison to the commercially available HA.

High performance liquid chromatography-mass spectrometry analysis was first performed to identify the bioactive compounds. The amount of total identified phenolic compounds was more than twice as high in the HI infusion as in the HA. Looking at individual classes and subclasses of the identified compounds, we found hydroxycinnamic acids and total arzanol derivatives and other pyrones to be most abundant in HI, whereas HA infusion was richest in total flavonoids, especially flavonones. Infusions prepared from HI exhibited superior antioxidative properties to those from HA, which were nevertheless substantial. This was demonstrated by the *in vitro* DPPH radical scavenging activity test and confirmed with the DCFH-DA test; the later showed HI to be more successful in protecting different cell lines from induced oxidative stress. The observed difference could be due to more efficient upregulation of superoxide dismutase in HI-infusion treated cells, as was detected with RT-PCR. We also found that antioxidative potential of *Helichrysum* was higher in the green parts of the plant compared to the flowers and depended on the harvest harvest.

Altogether, our data show that in the investigated parameters, *Helichrysum italicum* is superior or of equal quality as the HA. It can thus be recommended as a good dietary source of antioxidants, even though, due to its intense fragrance, HI plantations are at present primarily dedicated to the use in cosmetic industry.

Acknowledgments

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PCF-69

PHYSICO-CHEMICAL EVALUATION OF ALGERIAN HONEYS: EUCALIPTUS, JUJUBE, SPURGE AND MULTIFLORAL

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Arid and semi-arid zones represent nearly two-thirds of Algerian area. The immensity of these territories and the absence of systematic studies of the bee flora, make honeys from these regions poorly studied and poorly understood. The aim of the present study was to evaluate the quality of semi-arid Algerian honeys and verify its compliance with the established honey standards. For that, ten samples with different botanical and geographical origin, Eucalyptus (EC), Jujube (J), Euphorbia (EF) and multifloral (MF), were analyzed regarding the following physicochemical parameters: moisture, color, pH, free acidity, electrical conductivity, hydroxymethylfurfural (HMF), diastase index and proline. Concerning the moisture content, the samples presented values below the 20 % allowed by European Community regulations, ranging from 13.6% (EF) and 18.3% (EC). Eucalyptus honeys showed a darker color when comparing to the other samples. All honey samples presented conductivity values lower than 0.8 ms.cm^{-1} , ranging between 0.27 (MF) and 0.41 ms.cm^{-1} which are in accordance with the standard results for nectar honeys. The honeys pH values varied between 4.2 (MF) and 5.1 (J) with an average value equal to 4.6. For free acidity, tested at pH 8.3, the values were between 12.2 meq.kg^{-1} (EC) and 43.9 meq.kg^{-1} (EF). The HMF levels observed for the samples had a minimum of 0.53 (J) and a maximum of 36.5 (EC) mg.kg^{-1} , while diastase values ranged between 8.8 DN and 14.3 DN, being in accordance with the required by the European legislation ($<40 \text{ mg.kg}^{-1}$ and not less than 8 DN). For proline, the values ranged between 2.2 and 4.7 mg/g indicating the maturity of the honeys and absence of adulteration. Generally, the samples were found to meet the requirements of the international honey standards and were within those found in previous studies about physicochemical properties of Algerian and Moroccan honeys [1].

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PCF-70

Anthocyanin-rich extracts from purple and red potatoes as natural colourants: bioactive properties, application in a soft drink formulation and sensory analysis

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Aqueous extracts from seven coloured potato varieties (three red-fleshed, three-purple fleshed, and one marble-fleshed genotype) were studied for their anthocyanin content, *in vitro* biological activities, colouring properties and their potential application in the food industry. Acylated glycosides or pelargonidin and petunidin aglycones were identified as the main anthocyanin forms in the red and purple varieties, respectively. The total anthocyanin content among varieties ranged from 478.3 to 886.2 mg/100 g extract. All the extracts presented *in vitro* antioxidant, antibacterial and antifungal activities, whereas no toxic effects were detected. Finally, two selected extracts were tested as colourants in a soft drink formulation and presented suitable sensory profiles as well as high colour stability during a 30-day shelf-life when compared with the commercial colourant E163. Therefore, the tested extracts could be used as natural food colourants.

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PCF-71

**CHEMICAL PROFILE AND BIOACTIVE PROPERTIES OF GREEN- AND RED-COLORED BASIL
CULTIVARS AS AFFECTED BY NITROGEN FERTILIZATION**

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Basil (*Ocimum basilicum* L.) is an aromatic herb of the Lamiaceae family which has a high commercial value for having multiple purposes such as medicinal, nutrition, ornamental, cosmetics, religious and insecticidal or insect repellent purposes. It is a species with high genetic variability and numerous cultivars that do not differ significantly in morphological terms but present differences from the chemical composition point of view. In the present work, the chemical composition and bioactive properties of green and red-coloured cultivars were evaluated in relation to nitrogen fertilizer application rate. Three red-coloured (Dark Opal, Basilico Rosso and Red Basil) and one green-coloured landrace (Mitikas) of basil were grown under four nitrogen regimes, namely Control (no fertilizer added), 200 ppm, 400 ppm and 600 ppm of nitrogen (N). Fresh yield varied depending on N input following a quadratic function in all four genotypes, and green basil performed better compared to the red cultivars. A significant interaction of genotype \times N input was recorded for most of the chemical parameters measured. Tocopherols contents of leaves were consistently higher in plants that received 200 ppm of N and lower in those receiving 600 ppm of N, especially in Dark Opal and Red Basil cultivars. Polyunsaturated fatty acids (PUFA) were the major category of fatty acids and Red basil had the lowest ratio of omega-6/omega 3 (0.29) and thus the best fatty acid profile. Polyphenols content was the highest in Red Basil and Dark Opal (25 mg/g of extract on average) and the lowest in Mitikas and decreased with increasing N input. Similarly, antioxidant activity was the highest in Dark Opal and Red basil fertigated with 200 ppm of N. In conclusion, basil chemical and bioactive profile was significantly influenced by both genotype and N input. Red-coloured basil although less productive had the best chemical profile, and moderate levels of N input may provide the best compromise between yield, nutritional value and bioactivity for the species.

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PCF-72

GENOPROTECTIVE EFFECT OF BIOLOGICALLY ACTIVE PLANT COMPOUNDS GENTIOPIROSIDE AND MANGIFERIN AGAINST FOODBORNE MUTAGENS IQ AND PHIP

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During high temperature cooking of protein rich food, particularly red meat and fish, heterocyclic aromatic amines (HAAs) are formed. They represent a class of potent mutagens that can induce serious consequences on human health [1]. Genotoxicity of many HAAs, including IQ and PhIP, can be ascribed to different mechanisms, one of them being the production of reactive oxygen species [2, 3]. To overcome the problem of their genotoxicity, investigation of biologically active genoprotective agents is of a great importance. Taking into account all the above, the main aim of this study was to investigate the antigenotoxic effect of gentiopicroside and mangiferin, known for their numerous biological activities [4, 5]. Antigenotoxicity was screened towards foodborne mutagens IQ and PhIP on human hepatocarcinoma HepG2 cells, by applying alkaline comet assay. In order to analyze the involvement of antioxidative mechanism into possible antigenotoxicity, screening of DPPH radical scavenging activity, expression of Nrf2 transcription factor and glutathione redox status of the cells was included. Nrf2 was selected in accordance with its role in up-regulation of antioxidative enzymes [6].

Inhibition of IQ/PhIP-induced genotoxicity was recorded for both gentiopicroside and mangiferin. They induced dose-dependent response, with the highest effects of gentiopicroside against IQ (up to 68% of DNA damage inhibition, $p < 0.001$) and mangiferin against PhIP (up to 67% inhibition, $p < 0.001$). Ability of test substances to scavenge DPPH radical revealed moderate antioxidative activity of gentiopicroside and remarkable of mangiferin, with IC_{50} values at $119 \mu\text{g mL}^{-1}$ and $0.9 \mu\text{g mL}^{-1}$, respectively. Further on, mangiferin, and especially gentiopicroside, up-regulated the expression of Nrf2 transcription factor. The protection of glutathione depletion in the cells was demonstrated for both test substances.

In conclusion, the results obtained showed remarkable capacity to reduce HAAs-induced DNA damage. They recommend further and more detailed investigation of gentiopicroside and mangiferin genoprotective effect in *in vitro* and *in vivo* model systems.

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PCF-73

AMONG THE TEA VARIETIES (*CAMELLIA SINENSIS*) PURPLE TEA IS THE MOST EFFECTIVE INHIBITOR OF THE PANCREATIC α -AMYLASE

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Type 2 diabetes mellitus (DM2) is the most common type of diabetes and its complications are a major global health problem [1]. Inhibition of starch absorption by ingesting α -amylase inhibitors is a common treatment, but the drugs commonly used present several negative effects [2]. For this reason there has been continuous efforts at discovering natural antidiabetic agents that could provide mild anti-hyperglycemic protection by virtue of a continuous ingestion in conjunction with the regular and daily food intake. Teas prepared with the various *Camellia sinensis* varieties are rich in phenolics and antioxidants. Green tea, for example, has been linked to several health benefits, including its ability of inhibiting α -amylase [3]. Purple tea differs from the other teas because of its color and high content in anthocyanins, which are claimed to possess functional properties [4]. Its pharmacologic potential, however, is largely unknown. In this context the objective of this study was to compare the *in vitro* inhibitory activity of purple tea on the pancreatic α amylase with that of other tea varieties (green, oolong, white and black teas). Anthocyanins were evaluated using the pH differential method and the pancreatic α -amylase was assayed by measuring the production of reducing sugars. Purple tea presented total monomeric anthocyanin levels that were much higher than those of the other teas (0.935±0.047 mg cyanidin-3-*O*-glucoside equivalents per g extract). As shown by Fig. 1, purple tea did not present stimulation at low concentrations and was much more effective than the other teas as inhibitor of the α -amylase (\approx 90% inhibition at the concentration of 4 mg/mL). Possibly, thus, regular ingestion of purple tea is potentially more likely to protect against hyperglycemia than other types of tea .

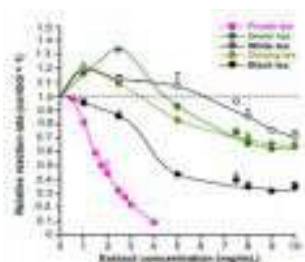


Figure 1 Dependence of the porcine pancreatic α -amylase activity on the concentration of various tea extracts.

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PCF-74

THE ANTIOXIDANT POTENTIAL OF WILD GARLIC (*ALLIUM URSINUM* L.) PLANT IN SHELF LIFE EXTENSION OF FOOD LIPIDS

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Yellow leaves of wild garlic (*Allium ursinum* L.) plant were reported to be a source of the sulphur containing compounds as well as kaempferol derivatives, flavonoid glycosides, phenolic acids and other antioxidants [1,2]. Their antioxidant potential can be utilized in two ways: as a source of bioactive compounds showing protecting properties against heart disease, cancer, oxidative damage to cells and DNA, but also as a source of natural antioxidants, showing inhibiting effect against oxidation of food lipids.

The aim of the present study was the evaluation of antioxidant activity of *Allium ursinum* L. extracts in rapeseed oil. Research material was commercially available yellow leaves of wild garlic, which were extracted with the use of 96% ethanol. Ethanolic extracts were added to the refined rapeseed oil in concentration of 0.02% and 0.1%. A synthetic antioxidant – BHT was used for comparison. Accelerated storage conditions were used (50 °C). The measure of antioxidant potential of the studied extracts were hydroperoxides determined with the use of iodometric method as peroxide value (PV) [3].

The results showed high antioxidant activity of *Allium ursinum* L. extracts in rapeseed oil. After 5 days of storage the PV of control sample (without antioxidants) reached the high level of 27.4 meq O₂/kg, whereas in samples with different *Allium ursinum* L. extracts, used in concentration of 0.02%, hydroperoxides were in the range of 2.6-8.6, and 3.9-12.2 meq O₂/kg by 0.1%. At the same time hydroperoxides in the samples with BHT reached the level of 20.3 meq O₂/kg.

Extracts of *Allium ursinum* L. can be a good alternative to synthetic antioxidants in order to retard undesirable oxidative processes and thus, to prolong shelf life of food. Concentration of 0.02% is more efficient in rapeseed oil than 0.1%. Further investigation is necessary in order to confirm consumers' acceptance of sensory attributes of the extract in particular foods but taking into consideration consumer interest in natural plant additives and in bioactive compounds, the potential of *Allium ursinum* L. extracts seems to be promising in shelf life extension of lipid containing foods.

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PCF-75

**Antioxidant properties of Subcritical Water Extracts Derived from Mushroom
*Inonotus obliquus***

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The medicinal mushroom *Inonotus obliquus* is widespread in Europe, Asia and North America. In many countries, since the 16th century, it has traditionally been used to treat gastrointestinal cancer, cardiovascular disease and diabetes [1]. This study was designed to determine the chemical composition and antioxidant activity of subcritical water extracts obtained from fruiting bodies of *I. obliquus* originating from Mongolia (IM) and from the mountain Vlasina, Serbia (IS). Chemical analysis revealed the total content of proteins, carbohydrates and phenols. High carbohydrate content was found in both extracts, and glucose was the most dominant monosaccharide. In order to identify phenolic acids, the extracts were subjected to a qualitative chemical analysis, and the presence of chlorogenic acid, catechin, p-coumaric acid and cinnamic acid has been confirmed. Chlorogenic acid was detected in the highest concentration, compared to other phenolic acids. DPPH free radical scavenging activity assay was used to measure the antioxidant properties of extracts *in vitro*. Extracts concentrations from 0.156 to 10 mg/mL were tested and a maximum of 93% of scavenging ability was reached. The results indicated that antioxidant activity in both extracts can be achieved through hydrogen atom (HAT) and single electron transfer (SET) as dominant mechanisms [2].

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PCF-76

DEVELOPMENT OF BIO-BASED INGREDIENTS FROM UNDERUSED TREES AND SHRUB SPECIES FOR INDUSTRIAL APPLICATION

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Around the world, the strategies of companies and governments are increasingly converging around the concept of using biomass in industry. Besides the benefit from moving away from fossil-based raw materials, the use of natural matrices bring health properties and functionalities to the final products and is desirable from a circular economy perspective. This is leading industries like the food, cosmetic and pharmaceutical to look for bio-based ingredients to obtain these bio-based products [1].

To not compete with the current use of biomass, one strategy to obtain these natural ingredients could be the use of underutilized species cultivated in marginal lands. This research has different purposes such as the chemical characterization of selected natural matrices from Germany, Spain and Romania (*Betula pendula*, *Calluna vulgaris*, *Carpinus betulus*, *Cistus ladanifer*, *Cupressus sempervirens*, *Cytisus scoparius*, *Euonymus europaeus*, *Hippophae rhamnoides*, *Juniperus communis*, *Populus nigra*, *Robinia pseudoacacia*, *Rosmarinus officinalis*, *Rubus fruticosus*, *Spartium junceum*, *Ulmus minor* and *Ulmus pumila*) to know and improve the contents in the target compounds (triterpenoids, phenolic compounds, including flavonoids and proanthocyanidins, hydrosoluble tannins, among others). After the chemical characterization, the extraction procedures can be optimized using experimental design tools based on response surface models to obtain high yields and highly pure essential oils and polar extracts. The obtained responses will be monitored based on extraction yield and HPLC-DAD-MS analysis. The optimized extracts in the target compounds will be refined using membrane technologies in ultrafiltration processes, semi-preparative and preparative chromatography, selective precipitation and crystallization, adsorbent bed purification to obtain purified fractions or isolated compounds. To monitor the efficiency of each process and select the most adequate methodology, HPLC-DAD will be applied. From the final extracts and/or isolated compounds, a fully characterization of the bioactive potential will be done, namely through the antioxidant, antimicrobial, cytotoxic, and anti-inflammatory properties.

From these bioactive screening, each developed extract and/or isolated compound will be analyzed according to different market requirements and regulatory documents to determine their applications as nutraceuticals, plant-based medicaments, feed additives and cosmetic ingredients.

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BIOACCESSIBILITY OF BIOACTIVE COMPOUNDS FOLLOWING GASTROINTESTINAL DIGESTION OF BEE POLLEN AND BEE BREAD

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The consumption of natural products has increased significantly due to the idea that whether improving nutrition, improves health, general well-being, and reduces the risk of developing certain diseases [1]. Bee products, in special bee pollen (BP) and bee bread BB, have demonstrated several nutritional and bioactive properties, which make them functional foods par excellence. Both BP and BB are natural products rich in phenolic compounds as well as macro and micronutrients [2]. Most of the bioactive properties are attributed to the powerful antioxidant and antiradical activity demonstrated by phenolic compounds [2]. Nevertheless, bioactive claims are made without taking into consideration the further modifications to which phenolic compounds are subjected in the gastrointestinal tract (GIT). In the present study, the bioaccessibility level of bioactive compounds in different BP and BB samples, as well as the mechanisms of action of these compounds in the GIT were investigated comparatively. In accordance with this purpose, the Folin–Ciocalteu and aluminum chloride methods were used for the quantification of total phenolic (TPC) and flavonoid content (TFC), respectively. Also, UPLC-ESI-MS analysis was performed to better understand the fate of each bioactive compound in the GIT. The findings indicated a significant reduction in phenolic content in both BP and BB samples at the end of gastrointestinal digestion compared to raw samples. Besides, the bioaccessibility level for TPC was calculated on average 31% and 38% for BP and BB, respectively, whereas the bioaccessibility level for TFC averaged 25% (BP) and 35% (BB). The results showed that both bee products are highly affected by *in vitro* digestion and BB is more accessible in bioactive compound content compared to BP. In conclusion, the current study highlighted BP and BB as good sources of bioactive compounds, with high bioaccessibility levels.

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PCF-78

ISOLATION, PURIFICATION AND *IN SILICO* MODELING OF HALOTOLERANT BACTERIAL ENDO- β -1,4-GLUCANASE

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The current demand for renewable energy sources and green components in order to improve food industry has spurred the search for alternatives that could replace the traditional applied sources in industries. One of the most promising innovations to positively impact the world energy scenario is the production of second generation bioethanol (2G-ethanol) from reducing sugars derived from enzymatic degradation of lignocellulosic material that is normally discarded in agroindustrial processes. Cellulose is the most abundant linear homopolysaccharide on the planet formed by glucose units linked by β -(1-4) glycosidic bonds. In addition to their primary structure formed by a sequence of glucose residues, cellulose microfibrils aggregate to form fibrils that are stabilized through the formation of various hydrogen bonds between the intra and inter-chain hydroxyl groups, a feature responsible for the crystalline structure. recalcitrant of this carbohydrate. The present work had as objective the structural modeling *in silico* and the purification of cellulolytic enzymes for the conversion of lignocellulosic material processed into reducing fermentable sugars. In order to predict the structure and biological function of the isolated protein, an alignment was made based on known deposited proteins was submitted to the ITASSER server^[1]. The extracellular proteins secreted from microorganisms in liquid medium were fractionated with ammonium sulfate and applied in molecular exclusion chromatography^[2]. Enzymatic purification was able to obtain an endoglucanase with an approximate molecular mass of 37 kDa while *in silico* modeling evidenced the presence of monomeric structure in the main endoglucanase involved in the process. According to the data obtained in this work, it was possible to conclude the possibility of applying this optimized bioprocess in industrial processes for bioethanol generation.

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PCF-79

NANOPARTICLES CONTAINING BERBERINE: PRODUCTION AND EVALUATION OF THERMAL AND SPECTROSCOPIC PROPERTIES

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Berberine is an isoquinoline alkaloid of the protoberberine type present in plants from the Berberidaceae family. Berberine has beneficial characteristics such as antioxidant, anti-inflammatory and neuroprotective, however, it has low oral bioavailability and low water solubility, which limits its application in most foods. Nanotechnology has been used by industries in order to circumvent such limitations of bioactive compounds. The aim of this study was to encapsulate berberine and characterize the obtained nanoparticles. Berberine nanoparticles based on Poloxamer 407 polymer (P407) were obtained by the solid dispersion technique. Tween 80 was mixed with ethyl alcohol and P407 and stirred for 5 min. Subsequently, the berberine was added and stirred for another 5 min. The solution was sonicated for 3 min in an ice bath. The solvent was removed in an oven at 50 °C for 18 h [1]. The extracted and encapsulated compounds were characterized by Differential Scanning Calorimetry (DSC, 5 to 10 mg of the analyte were placed in closed aluminum sample holders and kept at 0 °C for 5 min and heated to 400 °C at 10 °C min⁻¹ under a nitrogen flow of 50 mL.min⁻¹) and Fourier Transform Infrared Spectrophotometry (FTIR, potassium bromide tablets (1%wt compound) subsequently analyzed with a resolution of 2 cm⁻¹ in the range 4750-450 cm⁻¹ and 32 cumulative scans) [2]. A mixture obtained manually from berberine and P407 (physical mixture) was also evaluated. Characterization confirmed the conversion of the physical state of the berberine particles from the crystalline to the amorphous (**Figure 1**). The solid dispersion technique for particle production proved to be efficient for the type of compound studied, thus allowing the characterization of nanoencapsulated berberine.

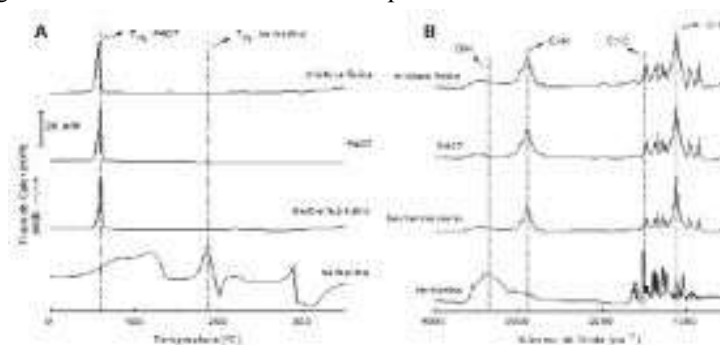


Figure 1: DSC (A) and FTIR (B).

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PCF-80

STATE OF ART OF *ATTALEA SPECIOSE* (BABASSU) MESOCARP FLOUR FOR APPLICATION IN BAKERY PRODUCT

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The term unconventional food plants (UFP) refers to plants that have one or more parts that can be used in human food, such as: roots, tubers, bulbs, rhizomes and others. The introduction of UFP on a regular diet basis could lead to diversification and improvement of food nutritional quality considering our current agricultural system, as a very small number of plants are available for commercialization and consumption [1]. *Attalea speciosa* (Mart. ex Spreng – Babassu) is a palm tree of the botanical family Arecaceae found in Brazil, considered the largest native oil resource in the world and its production chain is one of the most representative in Brazil. The potentialities of babassu range from the use of coconut, as its unfolding in all the primary fractions of the epicarp, mesocarp, endocarp and almonds. Its economic exploitation is carried out in several ways: human food, folk medicine, cleaning materials, and cosmetics [2]. The babassu mesocarp represents about 20.4% of the fruit, is a by-product of the babassu oil extraction industry, however much of its application also takes place for human nutrition. The mesocarp flour, in addition to the rich proximate composition, it has also been described as having antioxidant and antimicrobial activity due to the presence of phenolic compounds and flavonoids; and anti-inflammatory and analgesic properties [3]. The flour produced from the mesocarp of babassu coconut is also nutritious for its high carbohydrate content and the presence of fibers, which can modify the lipid profile and significantly decrease blood glucose in diets. The high content of fibers and phytochemicals with biological activity increases the quality of babassu flour for food applications, meeting the increasing demand for healthier food products [4]. With the full potential of UFP, their applicability in food products is limitless as they represent a viable and efficient alternative for replacing the food products we consume today. On the other hand, the non-use of UFP's has always been due to the lack of technology that would enable the obtaining and application of such products, making the industry incipient. The studies of the nutritional, chemical and bioactive characteristics of the babassu mesocarp flour are very scarce or even non-existing. In addition, its application in bakery products for the substitution of the widely consumed wheat flour are also scarce, making this plant by-product very appealing for the development of new foodstuffs that could be on the vanguard of the next generation of bakery products.

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PCF-81

ANTIOXIDANT ACTIVITY AND GC-MS CHARACTERIZATION OF *JUNIPERUS COMMUNIS* L. AND *CISTUS LADANIFER* L. ESSENTIAL OILS

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Juniperus communis L. and *Cistus ladanifer* L. are two abundant shrubs in the mountain areas of the Mediterranean basin, particularly in the Iberian Peninsula. Both species are known for their valuable essential oil that can be used in cosmetic, food and pharmaceutical industries for their bioactive properties [1]. Within the scope of the European Project “BeonNat”, that aims at developing innovative and bio-based products using the biomass of trees and shrubs growing on marginal and underutilised lands as feedstock for the bio-based industry, these two species were selected for evaluation. In this work, the biomass of the two species (branches milled to <20mm particle size) grown in Spain was submitted to steam distillation. The extracted essential oils (EO) were characterized regarding their chemical composition by gas chromatography coupled with mass spectrometry (GC-MS) and their antioxidant activity by the ferric reducing power assay.

An extraction yield of 0.50% and 0.08% (dry basis) was obtained for *J. communis* and *C. ladanifer* biomasses, respectively. The GC-MS analysis enabled the identification of 98.1% of compounds in *J. communis* EO, corresponding to a total of 63 identified compounds, with α -pinene being the major compound (32.3%), followed by limonene (15.8%), sabinene (7.6%), germacrene B (4.9%), cis-thujopsene (4.6%), β -myrcene (3.7%) and β -caryophyllene (3.6%). In general, the chemical composition was in good agreement with that of juniper berries essential oil defined in the European Pharmacopoeia and the ISO 8897 standard, with the exception of limonene (15.8%) that was slightly higher than the defined range (Eur. Ph from 2-12% and ISO standard from 2-8%). For *C. ladanifer* essential oil, a total of 61 compounds were identified corresponding to 92.8% of total compounds, with viridiflorol being the major compound (20.7%), followed by α -pinene (19.8%), ledol (8.1%), camphene (7.2%) and bornyl acetate (5.6%). This result is in good agreement with previous works that also report the sesquiterpene alcohol viridiflorol as the major compound in the EO obtained from *C. ladanifer* leaves and small branches [2]. Regarding the antioxidant activity, both oils showed promising results in the reducing power assay, presenting an EC₅₀ value of 1.35 \pm 0.19 mg/mL and 1.30 \pm 0.07 mg/mL for *J. communis* and *C. ladanifer*, respectively.

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BIOACTIVE POTENTIAL OF AROMATIC AND MEDICINAL PLANTS TRADITIONALLY USED AS CONDIMENTS

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Aromatic and medicinal plants are highly appreciated and used worldwide as condiments, dyes, and preservatives. Given their nutritional value and chemical composition, related to health beneficial properties, their inclusion in the Human diet has gain an increasing expression [1]. Certain mixtures of plants demonstrate greater potential when compared to isolated plants, due to synergistic effects, and these properties make them of great interest in food, pharmaceutical, and cosmetic industries. They have been consumed through direct use in prepared dishes, but also by incorporation into foodstuff, making them bioactive and functional [2]. In the present study, four mixtures of aromatic plants used for seasoning poultry, meat, fish, and salads were characterized in terms of phenolic compounds (HPLC-DAD-ESI/MS), organic acids (UFLC-PDA), tocopherols (HPLC-fluorescence), and bioactive properties (antioxidant, antimicrobial, anti-inflammatory, and antitumour).

Twenty-five phenolic compounds were identified, with apigenin-*O*-malonyl-pentoside-hexoside as the most abundant compound in all extracts. Regarding organic acids, oxalic, citric, and malic acids were detected in all of the samples. The mixtures also revealed the four isoforms of tocopherols, namely α , β , γ , and δ . In terms of bioactive properties, for antioxidant activity, the extracts of the mixtures for meat and salads revealed the best results in the TBARS assay, whereas those from mixtures for meat and poultry stood out in the OxHLIA assay. The mixtures for poultry and fish showed the highest anti-inflammatory activity and the mixture for salad showed the best antitumour properties. On the other hand, the mixtures for meat and salad revealed the highest antimicrobial activity. In conclusion, these seasoning mixtures demonstrated valuable bioactive properties, conferred by their chemical composition and cumulative and synergistic effects observed in the mixtures, which corroborates the importance of their inclusion in the Human diet.

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PCC
Poster
Communications
in Cosmetic area

PCC-01

MICROWAVE-ASSISTED EXTRACTION OF *SALICORNIA RAMOSSISIMA* BIOACTIVE COMPOUNDS: COMPARISON WITH CONVENTIONAL EXTRACTION

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The halophytes are salinity-sensitive plants that tolerate extremely high salt concentrations, being mainly found in saltmarshes and coastal areas worldwide, including Portugal [1]. Halophytes, such as *Salicornia* spp., are used by human due to commercial, ecological and gastronomic values [2]. Inside this genus, *Salicornia ramosissima* is of particular interest, being considered an alternative to salt [3]. However, few has been reported regarding the extraction of *S. ramosissima* bioactive compounds for cosmetic and nutraceutical applications. Microwave-assisted extraction (MAE) is an alternative extraction technology classified as fast and environment-friendly, that produces extracts with high extraction yields and antioxidant composition, when compared to conventional extraction techniques (CE) [4]. In the present study the total phenolic and flavonoid contents (TPC and TFC, respectively), antioxidant/antiradical activities (through FRAP and ABTS assays) and intestinal cells effects after exposure to different extracts concentration (0.1-1000 µg/mL) obtained by MAE and CE were evaluated. Considering green principles, water was the solvent employed for both methodologies. Briefly, CE was performed as a decoction preparation while MAE was executed at selected temperatures (72 – 94 °C) during 1 to 5 min with constant medium stirring. After extractions, samples were filtrated and lyophilized for further assays. The *S. ramosissima* CE extract exhibited the highest TPC (15.02 mg GAE/g) and TFC (8.44 mg CE/g) (Table 1). Regarding ABTS and FRAP assays, the MAE extract showed the highest values (60.61 µmol FSE/g dw and 16.06 % inhibition for FRAP and ABTS assays, respectively). The cell effects demonstrated that the MAE extract did not lead to a decrease of viability until de highest concentration tested, while the CE extract conducted to a viability of 91.20% after exposure to 1000 µg/mL. Further studies should be performed to analyze the bioactive profiles, in order to select the best one for further cosmetic/nutraceutical applications.

Table 1: Total phenolic and flavonoid contents (TPC and TFC, respectively), and antioxidant activities evaluated by FRAP and ABTS assays of *S. ramosissima* extracts prepared by CE and MAE. GAE, gallic acid equivalents. CE, catechin equivalents. * in the same column indicate significant differences between extracts ($p < 0.05$).

Times New Roman	TPC (mg GAE/g dw)	TFC (mg CE/g dw)	FRAP (µmol FSE/g dw)	ABTS (% inhibition)
CE extract	15.02 ± 2.01*	8.44 ± 0.45	60.61 ± 6.64	14.87 ± 0.06
MAE extract	8.34 ± 1.22	8.41 ± 0.45	65.56 ± 8.68*	16.06 ± 0.20

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PCC-02

THE ANTI-INFLAMMATORY POTENTIAL OF HYDROLATES FROM TWO THYMUS SPECIES PRODUCED IN PORTUGAL

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Background: Plants from the genus *Thymus* L. have been suggested to promote potential health benefits by their anti-inflammatory, and anti-proliferative, anti-microbial and anti-oxidant potentials [1,2]. Most studies focus on essential oils, but hydrolates, produced as co-products in the distillation process to obtain essential oils, can present several interesting applications. Therefore, we intended to explore the anti-inflammatory potential of *Thymus mastichina* and *Thymus citriodorus* hydrolates, both produced in Portugal. **Methods:** *T. mastichina* and *T. citriodorus* hydrolates were obtained from Planalto Dourado and Ervitas Catitas, respectively. Their anti-inflammatory activity was investigated on LPS-stimulated mouse macrophages (RAW 264.7), by evaluating their effect on cellular viability (MTT assay) and on the production of nitric oxide (NO) using Griess colorimetric reagent. Additionally, hydrolates were also studied for their ability to scavenge NO, using (*S*)-Nitroso-*N*-acetylpenicillamine (SNAP) as a NO donor, in a non-cellular model. **Results:** Both hydrolates affected cellular viability in a dose dependent manner, presenting cytotoxic effects only at the highest concentrations tested. Still, *T. mastichina* presented a higher biocompatibility, with a higher IC-50 value (half maximal inhibitory concentration). Similarly to cellular viability, both hydrolates were able to inhibit nitrites production in a dose-dependent manner. Interestingly, significant reductions were observed at non-cytotoxic concentrations. Using a non-cellular model, *T. citriodorus* failed to scavenge NO and *T. mastichina* presented only modest scavenging activity, thus not justifying the high reduction of nitrites by LPS-stimulated macrophages. **Conclusion:** Our preliminary results unveil an interesting anti-inflammatory potential of co-products of essential oil production, from two *Thymus* species cultivated in Portugal. This potential may be of interest to value hydrolates as active ingredients for different industries, particularly for the cosmetic industry, promoting an anti-inflammatory effect.

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PCC-03

AGROFORESTRY AS PRODUCTION SYSTEMS OF ESSENTIAL OILS

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Essential oils are complex mixtures of volatile substances derived from secondary metabolism of plants. Due to its several biological activities, these natural products are widely used in aromatherapy and healthcare, also being applied in the industry as flavourings, in pharmaceuticals, food, and cosmetics formulations [1]. Agroforestry systems are a land use system which integrate trees and agricultural crops [2]. The cultivation of medicinal plants, both shrubs [3] and woody plants [4] producing essential oils in agroforestry systems is already an alternative to make production sustainable. Thus, this study aimed to identify native Brazilian tree species which can be used in agroforestry systems proposing environmental preservation and sustainable management in order to produce essential oils in family farms.

A bibliographic database analysis was conducted based on a list of sixty-three species that have been used in the implantation of agroforestry systems projects related to environmental preservation and recovery of degraded areas developed by institutional partnerships in the South of Brazil. Three potential species to be managed on agroforestry systems in family farms to obtain essential oils were presented (**Table 1**). Further research involves the seasonal chemical analysis of essential oil of these species growing in agroforestry systems and the development of technical guidelines on crop management to obtain essential oils. This study proposes to find new sources of essential oils, understanding the productive potential of native trees, contributing to the development of local productive chains of sustainable forestry products.

Table 1: Potential species to be managed on agroforestry systems in family farms to obtain essential oils

Plant species	Plant part	Yield	Majoritary compounds
<i>Eugenia pyriformis</i> Cambess	fruits	1.23%	caryophyllene oxide (16.2%), limonene (12.4%) [5,6]
<i>Psidium cattleyanum</i> Sabine	leaves	0.26%	α -thujene (25.2%), 1,8-cineole (16.4%), β -caryophyllene (10.2%) [7]
<i>Schinus terebinthifolius</i> Raddi	seeds	2.66%	p -menth-1-en-9-ol (29%), hedicariol (11%) [8]

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PCC-04

THE POTENTIAL OF THE MUSHROOM SHIITAKE (*LENTINULA EDODES*) AQUEOUS EXTRACT AS COSMETIC ACTIVE INGREDIENT

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Skin infections occur commonly and often present therapeutic challenges to specialists due to the growing concerns regarding multidrug-resistant bacteria. Herein, mushrooms could be a promise source of new and bioactive cosmetic ingredients with several properties. In fact, the mushroom *shiitake* (*Lentinula edodes*) has been used in different cosmetic formulations with several properties such as firmness, hydration and whiteness. Medicinal products from mushrooms could be used in cosmeceuticals, applied topically, such as creams and lotions, or nutricosmetics, ingested orally.

In this context, aqueous extract of the mushroom *shiitake* was examined concerning to antimicrobial activity against to clinical wound bacterial isolates as well as to antioxidant activity. The results showed an effective antimicrobial activity against Methicillin-resistant *Staphylococcus aureus* (MRSA) [1], suggesting a potential use as antimicrobial ingredient in antiseptic cosmetic formulations.

Based on these findings an organic cream was formulated with the purpose of acting as antiseptic on wounds and skin cracks, providing healing and nourishing the skin. The organic cream containing *shiitake* aqueous extract as antiseptic agent was enriched with selected organic vegetable oils containing nourishment properties and organic essential oils as healing and skin regenerating components. Future work will be primarily focus on the prediction of the safety of the cosmetic product, namely in what concerns to skin sensitization, skin penetration, phototoxicity and cytotoxicity, in order to be ready for the market.

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PCC-05

DEVELOPMENT OF A COSMETIC ANTI-AGING GEL FORMULATION WITH EXTRACTS OF SPONTANEOUS PLANTS AND CULTIVARS OF *HUMULUS LUPULUS* L.

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Hop (*Humulus lupulus* L.) is known worldwide as an essential flavor in the beer industry. Its major compounds have been demonstrated to be associated to health benefits, due to its antimicrobial, antioxidant, anti-inflammatory and anticancer activities [1]. This study intended to develop and to evaluate the stability of an anti-aging cosmetic gel by incorporation of different percentages of hydroalcoholic extracts obtained from cones (flowers) and vegetative parts (leaves) of hop spontaneous and of the Cascade and Polaris cultivars and essential oil of *Thymus zygis*. In addition, the phytochemical profile and antimicrobial activities determination of hop extracts and of essential oil of thymus were performed.

A methylcellulose gel formulation was used as base to develop the hop cosmetic product. Subsequently, several parameters were evaluated to address its physical-chemical stability. In addition to that, the phenolic profile of hop extracts was determined by UHPLC-DAD-ESI-MS²[2] while the composition of thyme essential oil was done by GC and GC-MS. The ability to inhibit the microbial growth was performed by microdilution broth method and the diffusion in agar test. Contrary to hop extracts, thyme oil has shown to exert antibacterial and antifungal activities and thus, it was added to the formulation as preservative. Carvacrol, terpinen-4-ol and p-cimene, determined as major compounds in this oil, could be important contributors for these activities. The extracts obtained from hop cones were rich in phenolic compounds such as cohumulone, humulone and xanthohumol, which have been claimed as possessing anti-wrinkle effect [3]. Concerning stability of the formulations, no phase separation was observed in the centrifugation, mechanical vibration tests or the color tests. However, changes were observed in the UV-Visible spectra between 300nm to 350nm wavelengths, probably by presence of the phenolic compounds such as example xanthohumol, cohumulona, humulona and lupulona [3]. In texture by back extrusion, only the formulation with the extracts of the Polaris variety has high consistency and low viscosity. In the light test there was phase separation in the samples due to the occurrence of dehydration, to minimize this effect, it must be properly in a non-transparent packaging to protect from light. Overall, the incorporation of hydroalcoholic extracts of hop and essential oil of thyme in methylcellulose gel formulations, especially with Polaris variety with 2.5% hydroalcoholic extract of hop cones, allow to obtained a stable cosmetic with potential as anti aging take into account the chemical composition.

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PCC-06

LIPID OXIDATION IN COSMETIC CREAMS: THE POTENTIAL PROTECTIVE EFFECTS OF NATURAL EXTRACTS

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Skincare products are functional products, consisting of multiple ingredients with several functions. Traditionally, one of the main ingredients are lipids that can be saturated or unsaturated [1]. These unsaturated lipids and their interaction with the other components of the skincare products and external factors allow understanding and predicting the occurrence of oxidation. Lipid oxidation is a spontaneous and inevitable phenomenon and one of the most significant problems of deterioration of cosmetic products, affecting the chemical, physical, and sensorial features. Thus, it is essential to study the product's behavior during its lifetime, based on scientific and reproducible methods [2] considering the presence of flower and fruit extracts.

In this work, skincare products of different compositions (with and without natural extracts) were tested, stored at 30°C, in the absence of light, for six months. During each month, a set of samples (from A to J) was taken. Sensory analysis (color, odor, texture, and appearance changes) was made. For each skincare product, the primary oxidation products were determined by the conjugated dienes (DC) method expressed as a percentage and by the Peroxide Value (IP) method expressed in meqO₂/kg dry sample. Secondary oxidation products were also determined using the p-Anidisin Value (ipA) and the 2-Thiobarbituric Acid Reactive Substances (TBARS) test, expressed as malonaldehyde concentration (mg/g dry sample).

It was concluded that the presence of flower extracts influences the results obtained. The skincare products that have the greatest extent of lipid oxidation, based on the results obtained from Total Oxidation Value (TOTOX), is the Cream J with no natural extract in its composition followed by cream H that has fruit extracts. On the other hand, Cream D is the one with the smallest extent of lipid oxidation, having as a component *Calendula officinalis* flower extract. The presence of saturated fatty acids inhibits the process of lipid oxidation and decreases the oxidation rate. It can also be suggested that additives, such as flower extracts, can act as antioxidants preventing further degradation.

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PCC-07

THYROSINASE AS A PROTEIN TARGET FOR DERMATOLOGICAL TREATMENT

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Tyrosinase can be considered the most important enzyme for the synthesis of melanin. Alterations in the formation of melanin has been related to several dermatological disorders ranging from simple hyperpigmentation to skin cancers. Tyrosinase inhibition may be of great interest to prevent melanogenesis and, consequently, these disorders [1]. Currently, many known tyrosinase inhibitors are characterized, both natural and synthetic, but there is a great need and commercial interest in the discovery of new and better inhibitors [2].

The present study presents an *in silico* analysis, using molecular docking, in which a Tyrosinase three-dimensional structure (3NQ1), obtained from the Protein Data Bank database, was prepared and docked, using “AutoDock Vina” software, against a series of control compounds, in order to validate the structure. After validation, a library of 37 naturally derived flavonoids [3] were also docked using the same structure and the same docking software, and the predicted free energy of binding and docked conformation were analysed. Three compounds presented the lowest energy of binding: myricetin (**Figure 1**), fisetin and syringetin, with values of -8.3, -8.3 and -8.1 Kcal/mol, respectively. Although these results are promising, experimental validation will be necessary.

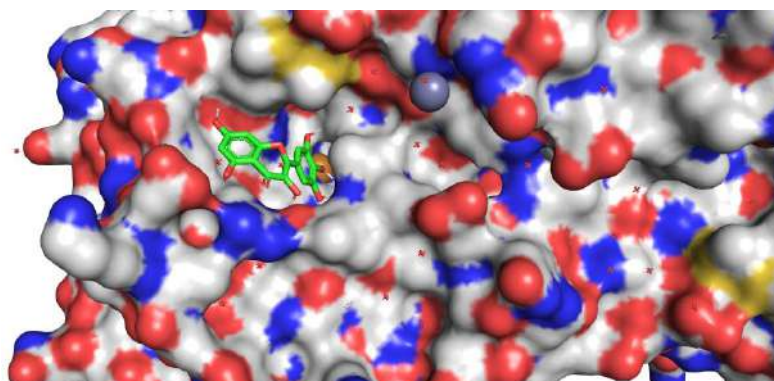


Figure 1: Docking conformation of myricetin against Tyrosinase three-dimensional structure (PDB: 3NQ1).

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PCC-08

DEVELOPMENT OF A COSMOS SOLID EMULSION

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Natural products are becoming a trend in the cosmetic market, due to a growing awareness and concern with the origin of these products. The certification of natural products, especially organic ones under the COSMOS signature preserves the integrity of the constituents, in an environmentally appropriate and safe for human health, expanding the concept of "green chemistry", guaranteeing the origin, and processing of the products, storage, manufacturing, packaging, etc. [1]. The objective of this study was to develop a moisturizing cosmetic emulsion in the solid format according to COSMOS certification, with guarantee the quality of the product in a new concept of solid cosmetic. 59 tests were carried out to optimise this emulsion, with the best possible hydration level. The different tests included variations in components and their amounts, following be evaluation of sensory aspects for each formulation. The final cosmetic emulsion formulation (Figure 1) contains components of Portuguese origin such as grape seeds oil from Douro Valley, olive oil and extract of by-products of acorn (*Quercus Ilex L.*) and essential oils of mandarin, geranium, coriander and cinnamon. The formulation has undergone accelerated stability tests with temperatures (40 °C /-12 °C). The pH, density and organoleptic characteristics were evaluate. The phenolic profile of acorn by-product (*Quercus ilex L.*) was performe by UHPLC-DAD-ESI-MS2. A questionnaire was applying to evaluate the acceptance after use of the moisturizing cosmetic emulsion. In stability tests, overall the formulation showed small color variations and less aroma intensity, maintaining hydration and solid state. The pH changes were from 4.68 ± 0.006 to 4.78 ± 0.05 and the density was maintaining at 0.73 kg/m^3 . The polar extract of acorn by-product have as major compounds trigalloyl-HHDP-glucose, valoneic acid dilactone and gallic acid, known as antioxidants compounds. [2,3]. The essential oils bring beneficial properties to the skin and the aroma. In the post use of solid emulsion survey applied shown that "aroma" was the second point most valorised (47.6%), after "hydration" (90.5%). The results shown that the majority of participants (81.0%) were "very satisfied" or "satisfied" with the solid emulsion indicating the intention to use it if it is on the market (76.2%). This study highlights the research in reformulations from liquid to solid products, and the potential of using Portuguese raw materials such as acorn.

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PCC-09

DETERMINATION OF THE TOTAL ANTIOXIDANT ACTIVITY OF THE TOASTED COFFEE BY-PRODUCT

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Introduction: *Coffea* sp. (coffee tree) is a shrub of the Rubiaceae family of great interest worldwide due to the presence of caffeine in its beans, which has a stimulating property, and for presenting a pleasant aroma and flavor.^{1,2} The habit of consuming roasted coffee ends up generating a large amount of by-products which are dispersed in the environment and, consequently, contributing to the environmental impact.³ Therefore, there is a need for in vitro studies that can prove the presence of beneficial chemical properties, such as antioxidants, in agro-industrial waste to develop new sustainable products. **Objective:** The objective of this work was to evaluate the total antioxidant activity of the by-product of roasted coffee. **Material and methods:** Samples of the roasted coffee by-product were purchased by Garoa Cafés Especiais, located in the city of Goiânia, Brazil. The plant material obtained was subjected to dehydration in a forced circulation oven and air renewal at 60 ° C. The dry material was kept away from light and moisture. The total antioxidant activity by capturing the free radical DPPH (2,2-diphenyl-1-picryl-hydrazil) was determined according to the method described by Brand-Williams, Cuvelier and Berset (1995), with adaptations.⁴ **Results:** The result found in this current study (IC₅₀ of 277 µg / mL) showed that the by-product of roasted coffee has bioactive compounds with antioxidant potential that were responsible for the reduction of absorbances, at a wavelength of 515 nm, from capture of the DPPH free radical (Figure 1). **Conclusion:** The by-product of roasted coffee contains secondary metabolites with antioxidant properties, such as phenolic compounds, which were able to capture the free radical DPPH and promote its stability. Therefore, it is suggested to continue the study from other antioxidant tests to fully characterize the compounds that neutralize or reduce the free radical and promote the application of the by-product of roasted coffee in technological innovation in the pharmaceutical, cosmetics and food industries.

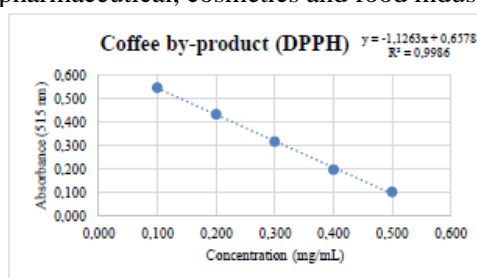


Figure 1: Antioxidant activity of the by-product of roasted coffee by the DPPH method.

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PCC-10

WATER-IN-OIL EMULSIONS BASED ON NATURAL INGREDIENTS FOR FUNCTIONAL APPLICATIONS

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The development of emulsions based on natural compounds is a topic of great interest for several industrial areas including the cosmetic industry due to their high biological value and environmental-friendly aspects [1]. Regarding the different emulsions types, water-in-oil (W/O) emulsions are biphasic delivery systems that comprise water droplets into a continuous oil phase [2]. In this context, the present work aimed to develop W/O emulsions based on sweet almond oil and green tea (*Camellia sinensis*) aqueous extract, in order to impart suitable functional antioxidant and antimicrobial properties, to produce a moisturizing agent. For this purpose, a base emulsion and emulsions formulated with the extract at concentrations of 3.75% and 5% (w/v, water base) were prepared. The used W/O ratio was 40/60 and the emulsifier system was composed of a Span 80/Tween 80 mixture (54/46 ratio, v/v). The production was made by preparing a coarse emulsion using a mechanical homogenizer (11000 rpm, 5 min). Then, the droplet size was reduced using a high-pressure homogenizer during 12 cycles. The emulsions' antioxidant activity (DPPH radical-scavenging activity method) and antimicrobial activity (agar diffusion test with the microorganisms *S. aureus* ATCC 29213 and *E. coli* ATCC 25922) were thereafter evaluated. The formulation stability was accessed by visual inspection and by optical microscopy along the storage time (3 months) at 4 °C. As result, the formulations with green tea extract showed antioxidant and antimicrobial activities, revealing the effect of the extract presence (**Figure 1**). Both emulsions with extract had a DPPH scavenging activity of approximately 83%, evidencing good antioxidant activity of the produced formulation. Regarding the antimicrobial analysis, the emulsions with green tea at 3.75% and 5% concentrations had a prolonged effect against *S. aureus* bacteria, maintaining the same inhibition zone (10 and 12 mm, respectively) after 96 hours of incubation. However, no inhibition was detected for *E. coli* bacteria. The emulsions presented macroscopic and microscopic stability with no instability phenomena for, at least, 3 months of storage. This study suggests that emulsions with green tea extract have potential to be used as antimicrobial agents in cosmetic applications.

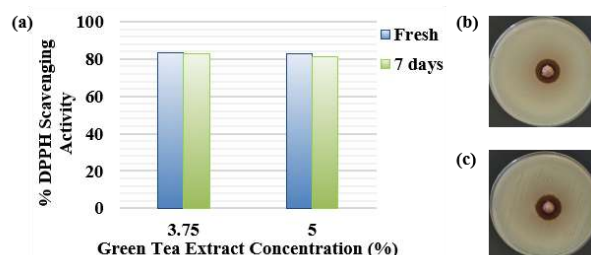


Figure 1: (a) Antioxidant results of the fresh emulsions and after 7 days of storage. Antimicrobial assay for (b) 3.75% and (c) 5% green tea emulsions against *S. aureus* after 24 hours of incubation.

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PCC-11

OLIVE OIL BY-PRODUCTS: IMPACT IN COSMETIC FORMULATIONS

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Olive oil by-products are considered a major ecological issue. However, its recovery and use are described as a key benefit as a result of their promising health promoting properties [1]. Accordingly, the aims of this work are the evaluation of three olive oil by-products extracts', obtained without using organic solvents, in vitro bioactivities and their impact and characterization in oil-in-water (O/W) creams for cosmetics. Cell viability studies in immortalized human keratinocyte cells as well as in fibroblasts were evaluated by a MTT reduction method [2], enzymatic activity against human neutrophil elastase (HNE) were performed by a fluorometric method in three olive by-products extracts. The capacity of reducing reactive oxygen species, using a hydrogen peroxide solution and UVB light to induce ROS production, were evaluated. Moreover, the incorporation of these extracts in O/W creams (Cream 1, 2 and 3) were performed and characterized regarding their organoleptic, physical-chemical and rheological properties (viscosity and oscillatory experiments) and compared to a control (cream without extract – Cream control). The results had shown that all extracts presented promising in vitro activities regarding the methods studied. Regarding the formulations, the results had shown that the creams containing the promising extracts presented a beige semi-solid appearance, while the control cream showed a white colour. All the formulations exhibited pH values suitable for topical application (4.7 and 5.5). From a rheological point of view, all formulations showed a shear-thinning behavior with yield stress, where the viscosity decreases with increasing shear rate. Additionally, the oscillatory results shown that, in all cases, the storage modulus (elastic component) overcome the loss modulus (viscous component). The loss tangent values were 0.32, 0.32, 0.28 and 0.31, for Cream 1, 2, 3 and Control cream, respectively, at a frequency of 0.1Hz. Accordingly, these creams have a strong network structure, with a suitable spreadability of the product on the skin. In conclusion, the in vitro methods demonstrated that olive by-products presented antioxidant and HNE activities. Furthermore, these olive oil by-products extracts present an impact in cosmetic formulations' appearance, pH and rheological performance being suitable for cosmetic use.

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PCC-12

SUBSTANTIATION OF HIBISCUS EXTRACT USE IN COSMETICSInga Išdonaitė-Medžiūnienė^{1*}¹University of Applied Social Sciences (SMK), Nemuno str. 2, Klaipėda, Lithuania.

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In the Indian Ayurvedic literature, different parts of the hibiscus plant are recommended for various ailments like hypertension, fever and liver disorders. The plant is traditionally used as an antiseptic, aphrodisiac, as having emollient, digestive, diuretic, brightening, soothing properties [3]. Hibiscus flowers are rich in flavonoids [3] and proanthocyanidins [6]. Chinese medicine claims that hibiscus has many phytotherapeutic possibilities. Studies prove the cosmetic skin care potential of hibiscus extract; for example, Hibiscus rosa-sinensis flower extract has been found to have a protective effect against the sun by absorbing ultraviolet radiation [6]. In addition, antioxidant and free radical reducing effects have been demonstrated [3] [1]. The properties of Hibiscus rosa-sinensis extract, which has a high antioxidant effect, are also proved by Garg *et al.* in 2012. Hibiscus plant extracts have an incredibly low degree of toxicity [1]. Therefore, hibiscus and its isolated compounds may be an important source of therapeutically useful products, given the stated nutritional and pharmacological characteristics and the relative safety of the extract [1]. As hibiscus extracts contain organic acids and minerals, they can have a direct relaxing effect on smooth muscles, and the antipyretic, antinociceptive and anti-inflammatory effects of this extract [1]. The use of hibiscus flower extract as an active ingredient in cosmetic products for skin care has been observed to have the following effects [5]: the oxidative effect of this active substance and the stimulating effect of stimulating cellular metabolic processes. Martino D., Tito A. et al. research in 2017 shows that the use of hibiscus extract increases the therapeutic potential of fibroblasts and keratinocytes [4]. Specifically, hibiscus extract significantly stimulates fibronectin and collagen synthesis by 16% and 60%, respectively, and fibroblast contraction is increased by 30%. These results were confirmed in skin care procedures where hibiscus extract markedly accelerated wound healing, epithelial formation, and fibronectin production. In addition, studies confirm that hibiscus extract increased the expression of genes involved in skin hydration and homeostasis [4]. The conducted research substantiate the positive use of hibiscus extract in cosmetics for its numerous advantages.

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PCC-13

BIOACTIVITY OF PLANT EXTRACTS: ASSESSMENT OF CYTOTOXICITY, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES

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The increase interest in natural cosmetic formulations has created the need to extensively characterize plant extracts. Plant extracts are known for their overall bio-compatibility, low toxicity and numerous health benefits, being used in traditional medicine over the ages. In this study, we aim to contribute to this huge body of knowledge by characterizing extracts of plants produced/endogenous in Portugal, with the ultimate goal of assessing the interest of inclusion of these extracts in cosmetic or pharmaceutical formulations. To achieve our aims, hydrolates of six different plants were obtained by hydrodistillation of aerial parts (*Matricaria chamomila*, *Hammamelis virginiana*, *Echinacea purpurea*, *Cistus ladanifer*, *Cupressus lusitanica*, *Thymbra capitata*). An aqueous infusion of the leaves of *Ocimum basilicum* was also studied. The antimicrobial activity against Gram-positive and Gram-negative bacteria was determined by microdilution broth assay. Antioxidant activity was assessed using the DPPH reduction assay. Cytotoxicity against skin fibroblasts (3T3) was determined using MTT assay. We found that the great majority of plant extracts had mild antibacterial activity against Gram-negative bacteria (minimum inhibitory concentration, MIC \geq 50%). The exceptions were *T. capitata* and *C. lusitanica* that were active against Gram-negatives (MIC 6,25% each). *E. purpurea*, *C. ladanifer* and *O. basilicum* hydrolates were also not very active against Gram-positives (MIC \geq 50%). The same was observed for *C. lusitanica* hydrolate. The remaining extracts showed activity against Gram-positive bacteria (*M. chamomila*, MIC 25%; *H. virginiana*, MIC 25%; *T. capitata* MIC 6,25%). Regarding the antioxidant activity, only two extracts showed a very strong ability to reduce DPPH: *T. capitata* and *H. virginiana*. *C. ladanifer* and *O. basilicum* showed a moderate antioxidant activity and the remaining extracts did not show a relevant ability to reduce DPPH. Finally, only one extract was able to reduce fibroblasts' viability by 50% at a lower concentration: *T. capitata* (EC₅₀, 18%). The remaining extracts were cytotoxic at around 30% (*M. chamomila*, *H. virginiana*, *C. lusitanica*) or 50% (*C. ladanifer*, *O. basilicum*). Our results suggest that by showing modest antibacterial activity, moderate antioxidant activity and low cytotoxicity *C. ladanifer* and *O. basilicum* extracts are excellent candidates to be included as ingredients in natural cosmetic formulations.

Acknowledgments

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IN SILICO STUDY OF A LIBRARY OF FLAVONOIDS AS POTENTIAL COLLAGENASE INHIBITORS

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Collagenase (MMP-13) is a matrix metalloproteinase involved in the degradation of collagen, being collagen an essential constituent of human tissues like cartilage, tendons and skin [1]. The inhibition of collagenase is a therapeutic target for several diseases such as osteoarthritis and skin age since these conditions can be caused by an uncontrolled degradation of collagenase. Therefore, the research for natural metabolites and products that could inhibit Collagenase is ongoing.

In the present study, a total of 37 flavonoids, prepared by Pradiba *et. al* in 2018 [2] and used against metalloproteinase-9 (MMP-9), were analyzed as potential inhibitors of Collagenase. A Molecular Docking study was performed, using AutoDock Vina software, and a three-dimensional structure of Collagenase was used as protein target.

From the docking studies with Autodock Vina and posterior virtual screening through MOLA software, 4 major compounds with theoretical good potential to inhibit Collagenase were identified. Kaempferitrin (**Figure 1**) presented the best Collagenase inhibition potential, with a predicted Ki (Constant Inhibition) value of 91,86 nM, followed by Nicotiflorin with a predicted Ki value of 108,75 nM, Rutin with a predicted Ki value of 128,75 nM and Brousoflavonol F with a predicted Ki value of 180,44 nM (**Table 1**). In conclusion, the 4 highlighted flavonoids may have good potential to inhibit Collagenase, however this predicted activity must be experimentally verified.

Table 1: Table caption [times new roman, 8, plain, centered, line spacing 1.15].

Compound	Ki (nM)
Kaempferitrin	91,86
Nicotiflorin	108,75
Rutin	128,75
Brousoflavonol F	180,44

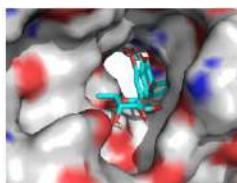


Figure 1: Predicted docking conformation of Kaempferitrin against Collagenase.

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PCC-15

FILM-FORMING PROPERTIES OF FISH COLLAGEN IN THE PRESENCE OF PLANT EXTRACTS

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Collagen is a major element of the extracellular matrices, tendons, joints and skin [1,2]. Due to its excellent biocompatibility and non-toxicity [3-6] collagen represents a natural polymer which has an application in many fields: biomedicine, cosmetics, food and pharmaceutical industry [7-9]. Nonetheless flexibility, elasticity and mechanical strength can be improved by the addition of some natural components [10].

In this work we focused on the influence of particular natural extract on film-forming properties of fish collagen. Collagen in the presence of *Melissa officinalis extract* was executed by using atomic force (AFM) and scanning electron microscopy (SEM) to assess the superficial characteristics of collagen film. Mechanical properties of the obtained film were investigated by Zwick&Roell apparatus, whereas for collagen structure analysis infrared spectroscopy (IR) was performed. It was alleged that addition of *Melissa officinalis extract* has an impact on the film-forming properties of fish collagen. Film-forming characteristics of this material are particularly significant due to their influence on adhesion to the skin and adequate application of collagen material.

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PCC-16

ELASTASE AND ITS POTENTIAL AS TARGET PROTEIN FOR BIOACTIVE COMPOUNDS

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Elastase (MMP-12) is a metalloprotein of the matrix metalloproteinase (MMP) family and plays an essential role in various biological processes and, in deregulated situations, it is associated with pathological conditions such as cancer and pulmonary emphysema. It is also associated with aging and loss of skin elasticity, so it has been studied as a potential target for dermatological and cosmetic treatments [1]. Hence the importance of compounds with an inhibitory capacity for therapeutic targets.

Due to the importance of developing inhibitors for elastase, it was discovered for example the potential of Ageladine A, from a marine sponge (*Agelas nakamurai*), which showed inhibitory capacity for MMP-12, as well as other members of the family of MMPs. In addition, Ageladine A derivatives were also synthesized to obtain compounds more effective in inhibiting elastase [2].

Knowing the generic structure of an elastase inhibitor, it is possible to investigate and potentially discover new inhibitors. For the identification of these inhibitors, we emphasize the importance of *in silico* tools. One of the most used are molecular docking tools, that have the advantage of being readily available, being fast and with the potential to replace, at least partially, experimental tests. In this work a molecular docking study was performed, using AutoDock Vina software, which made it possible to predict the inhibitory potential of a flavonoid library composed of 37 compounds of natural origin, against an elastase experimental three-dimensional structure [3]. From the flavonoid compounds studied, Ombuin presented the lowest predicted binding energy (ΔG) with a value of -11Kcal/mol. This value indicates that this compound may be a potential elastase inhibitor, and it may be a promising compound in combating pathologies associated with elastase and in possible treatments in the cosmetic area.

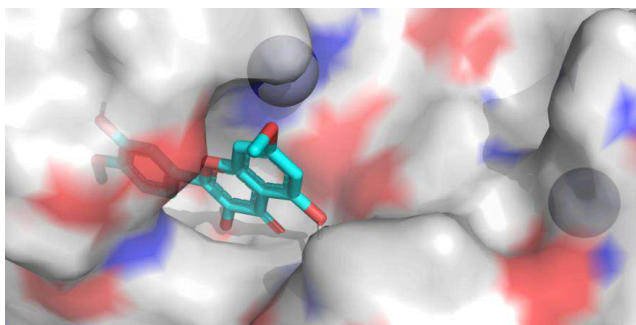


Figure 1: Predicted docked conformation of Ombuin against an Elastase protein structure (PDB:6EKN). Image prepared using Pymol.

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ASSESSMENT OF COSMECEUTICAL POTENTIAL OF *AGARICUS BRASILIENSIS* MUSHROOM: ANTIOXIDANT AND ANTI-TYROSINASE ACTIVITY

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Modern trends in the cosmetics industry prioritize ingredients or extracts from natural sources with nontoxic effects and the ability to delay the aging process. Mushrooms bioactive extracts are receiving increasing attention in the design of cosmeceutical formulations for topical application [1]. In this study polysaccharide extract of *Agaricus brasiliensis*, a cultivated edible mushroom was screened for the free radical-blocking potential which could strengthen the skin's barrier function, and inhibition of tyrosinase which could provide a skin-lightening effect. The antioxidant activity of extract was evaluated by *in vitro* models including, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) free radical scavenging activity, and inhibition of lipid peroxidation (LPx) in a linoleic model system. Carbohydrates were the most abundant components of the extract, and smaller quantities of proteins were detected (**Table 1**). The β -glucan fraction represented 71% of carbohydrate content. β -glucans, despite their considerable molecular weight, are known to enter the *stratum corneum* and epidermis, penetrating deep into the dermis. Within the dermis, they can stimulate collagen synthesis through direct interaction with fibroblasts and through indirect cytokine-mediated interaction with macrophages [2]. Collagen synthesis is one possible mechanism by which the elasticity of the skin is enhanced. Extract was found to be effective inhibitor of LPx, with an almost three-fold increased inhibition compared with ascorbic acid (EC₅₀=1.90 mg/ml), a common additive in cosmeceutical formulations used at mg levels. Likewise, extract showed moderate inhibition potential on tyrosinase (**Table 1**) compared to the anti-tyrosinase IC₅₀ value of kojic acid (0.079 mg/ml), which is currently used in topical dermatological products. Cosmeceutical potential of investigate extract confirmed that *A. brasiliensis* may represent a promising source of natural cosmeceutical ingredients.

Table 1: Chemical composition, antioxidant and anti-tyrosinase potential of *A.brasiliensis* polysaccharide extract

Properties	
Chemical composition	mg/100mg \pm SD
Carbohydrates	64.7 \pm 0.7
β -glucan	45.1 \pm 0.5
Protein	7.3 \pm 0.4
Cosmeceutical potential	
Antioxidant activity	EC ₅₀ (mg extract/ml) \pm SD
ABTS	0.09 \pm 0.001
LPx	0.65 \pm 0.04
Enzyme inhibition	IC ₅₀ (mg extract/ml) \pm SD
Anti-tyrosinase activity	1.23 \pm 0.02

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PCC-18

CONSUMERS BEHAVIOR AND PERCEPTIONS REGARDING NATURAL COSMETIC PRODUCTS

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In the last decades, the increasing concerns related with sustainability and the emergence of ethical concerns related to the production and consumption of cosmetics, as well as, the verification of the adverse effects of chemical additives present in cosmetics for human health and the environment, have driven the growth of green cosmetics. Natural substances, derived from plants, animals or other organisms are increasingly popular as ingredients in cosmetics for being considered by consumers as safety alternatives to synthetic cosmetics. This work aims to analyze the habits and perceptions of consumers regarding natural cosmetic products. A cross-sectional study was carried out based on a sample of 222 individuals' ages between 18 and 74 years old. Respondents were mainly female (75%), young urban adults, employed, with higher education. Most of the respondents use cosmetics daily, and considers them important (48%) or essential (34%), particularly the hygiene and skin care products. The median amount of monthly spend on cosmetic products are of 20 €. Super and hypermarkets (34.5%), and pharmacies and parapharmacies (31%) are the most relevant places for buying cosmetic products although the internet (8%) and catalogues sales (7%) are also significant. The importance of internet is also present as source of information about the products (40%), seconded by beauty professionals (27%). The great majority of the respondents uses natural cosmetic products, although not often organic ones. Despite the respondents' considerable familiarity with natural cosmetic products, results display some misconceptions about these products still present in consumers' minds (Figure 1).

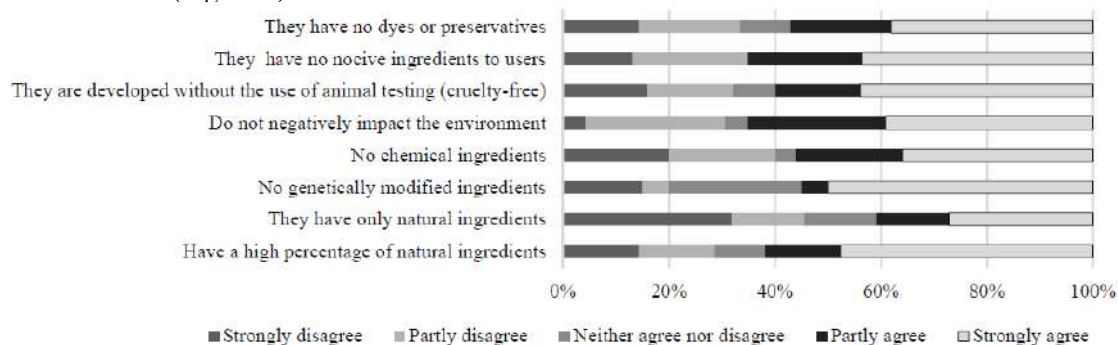


Figure 1: Perceptions of respondents about natural cosmetic products attributes

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PCC-19

PRODUCTION AND *IN VIVO* SKIN HYDRATION EVALUATION OF A COSMECEUTICAL CONTAINING *OPUNTIA FICUS-INDICA* (L.) MILL CLADODES HYDROALCOHOLIC EXTRACT

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Plants have been used for long time as a source of bioactive ingredients for cosmetic industries. Recently, several scientific data has been produced concerning bioactivities of *Opuntia-ficus indica* (L.) Mill cladodes, namely ultraviolet protection, and skin moisturizing properties[1,2]. However, cladodes use have been undervalued being often discarded in landfills [3]. A cladodes hydroalcoholic extract was prepared and used at 0.15 % as an ingredient in a cosmeceutical formulation. Skin hydration was evaluated by a non-invasive probe (Corneometer®), which measure water content of the stratum corneum. Measures were performed in the right inner forearm and upper arm of 4 volunteers. The formulation showed no allergic reactions and a significant moisturize effect in very dry skin, with a hydration increment of 42 % at 3 hours after application, and 25 % at 6 hours after reapplication. Overall, the cladode cosmeceutical showed good skin hydration potential, which can be helpful for the treatment of critical skin conditions like xerosis.

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APPLICATION OF CHITOSAN AND CHITOSAN DERIVATIVES IN COSMETICS

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The growing consumer awareness of environmental protection and ecology resulted in an increased interest in natural cosmetics. This is a stimulus for scientists who are looking for natural polymers with a wide range of properties. Chitosan and its derivatives are biopolymers made from post-production waste such as crab or shrimp shells. It is also possible to obtain chitosan from mushrooms. Chitosan is non-toxic, biocompatible, biodegradable, can bind fat, and has antibacterial, antifungal and wound healing properties. In cosmetology, it can be used as a hair smoothing, moisturizing or antibacterial substance [1].

Carboxymethyl chitosan is a derivative of chitosan in which the carboxymethyl group is attached to the amino group or to the hydroxyl group. As a result, the following can be obtained: N, N-carboxymethyl chitosan, N, O-carboxymethyl chitosan, O-carboxymethyl chitosan, N-carboxymethyl chitosan and mixtures thereof. Carboxymethyl chitosan is biocompatible and biodegradable. It has antibacterial and antioxidant properties and can chelate metals. In cosmetic products, it can be used as a rheology modifier, an antibacterial substance or as an antioxidant substance [2,3].

In the conducted preliminary studies, the conditions for carboxymethyl chitosan synthesis were selected, which allow to obtain chitosan derivatives attractive from the point of view of cosmetic products. Their characteristics such as average molecular weight, degree of deacetylation (DA), degree of substitution (DS), were compared with characteristics of chitosan.

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PCC-21

STABILITY OF NANODISPERSIONS CONTAINING *MYRCIARIA AULIFLORA* MART. EXTRACT FOR SKIN LIGHTENING PRODUCTS APPLICATION

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Myrciaria cauliflora Mart., popularly known as jaboticabeira, is extensively cultivated in Brazil [1]. Among the secondary metabolites present in *Myrciaria cauliflora*, ellagic acid can be found mainly in the peels of the fruit [2]. The antioxidant activity and capacity to complex with copper are the biological activities attributed to ellagic acid that can give the ability to inhibit the tyrosinase enzyme which has a fundamental role in the production of melanin [3]. The application of nanotechnology associated with plant extracts may increase ability to lightening skin blemishes due to the capability to promote improvements in delivery system aspects by achieving greater speed of action, increasing the synergy with the skin and permeation of active compounds resulting on greater hydration power and differentiated effectiveness [4]. The aim of the study was to develop nanodispersions from the extract of *Myrciaria cauliflora* fruit peels and evaluate their stability. The liquid extract was produced by percolation method and standardized on ellagic acid. Then, the nanodispersions were developed with low energy input [5], testing two different surfactants, and evaluated according to its particle size, polydispersity index (PDI) and zeta potential, on day one and seven. The development of nanodispersions from the standardized *Myrciaria cauliflora* extract was able to produce a stable formulation with particle size, polydispersity index and zeta potential below than 190 nm, 0,300 and -13mV, respectively, after 7 days of analysis (Table 1). Polysorbate 85 proved to be more efficient in stabilizing the formulation because it presented smaller particle size and greater homogeneity between them along the study. The application of nanotechnology on extracts can increase the stability of the secondary metabolites of interest and their efficiency when applied on the skin. The stability of the formulation it is important to achieve an increased lightening action on skin blemishes therefore the selection of surfactants composition is an important step in the development of the skin lightening active formulation.

Table 1: Particle size, polydispersity index and zeta potential of nanodispersions prepared with Polysorbate 85 and Polysorbate 80 / Sorbitan oleate 80 on the day of preparation and after 7 days.

	Day one			Day 7		
	Particle size (nm)	PDI	Zeta Potential (mV)	Particle size (nm)	PDI	Zeta Potential (mV)
Polysorbate 85	183,8 ± 2,12	0,228 ± 0,006	- 10,0 ± 0,26	186,4 ± 1,02	0,238 ± 0,006	-10,7 ± 0,25
Polysorbate 80 + Sorbitan Oleate	226,8 ± 5,92	0,410 ± 0,011	- 12,4 ± 0,06	231,9 ± 5,35	0,378 ± 0,042	-12,8 ± 0,28

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PCC-22

USERS SELF-ASSESSMENT OF A DAILY MOISTURIZING OF THE SKIN, WITH NATURAL INGREDIENTS

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The cosmetic industry keeps growing, constantly pursuing to satisfy consumers' desire for beauty. Nowadays, sustainability awareness is gaining relevance in the cosmetic sector. The increasing market trend in favour of green cosmetic products, which use ingredients from natural inputs, portrays the ecological and social weight of consumer choices when using products that contribute to nature in a sustainable way. This study aims to analyze the user self-assessment effectiveness of a cream formulation for daily moisturizing of the skin, with natural ingredients, including bee products, local plant material of the region of Trás-Os-Montes, and vegetable oils. Twenty-two individuals aged between 24 and 74 years were included in the product's effectiveness study. The pre-use self-assessment shows that most of volunteers presented mixed skin type (at least three in five individuals). For dry skin individuals (one in five individuals), skin jerking and the rapid absorption of the products are the main issues. The assessment of product efficacy was measured based on a post-use questionnaire. The results obtained for non-placebo users in the assessments are presented in **Figure 1**. The product exhibits positive performance for all the attributes under assessment. For products efficacy the highlight is for hydration and skin smoothness. For products attributes, ease application scores the maximum of 3.3 points. Different, wrinkles reduction and fragrance present less favourable results. When asked about the willingness to buy the product only one in four users respond negatively, mainly because of the strong fragrance of the preservative agent product. Users willing to buy/recommending the product to a friend mention the satisfactory results obtained, hydration performance and natural product as the determinant for that decision. The users willing to buy the product consider to do it at an average price of 10€ for a 50ml package.

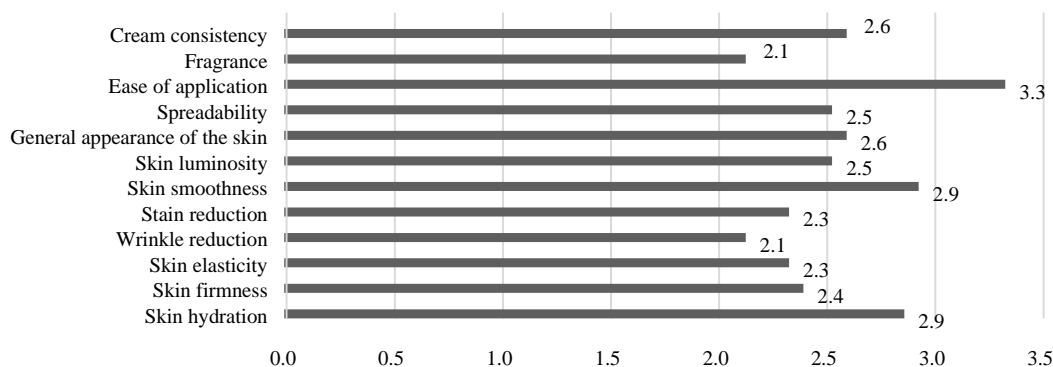


Figure 1: Results from consumers' satisfaction based on the self-assessment from the users' sample (0. Totally dissatisfied; 1. dissatisfied; 2. Indifferent; 3. Satisfied; 4. Totally satisfied)

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THE ANTIMICROBIAL EFFECTS OF THE COMMERCIAL LAVENDER AND INCENSE ESSENTIAL OILS AND EXTRACTS AS AN ECOFRIENDLY COSMETICS PRESERVATIVES

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Essential oils have been known and used by humans for centuries. They are distinguished by a multi-component, unique form and strong action, including: antibacterial, antiviral and antifungal. They have a very wide range of applications in the pharmaceutical, cosmetic, chemical and even food industries, which is undoubtedly due to the fact that more and more consumers pay attention to the composition of products and the growing demand for natural and organic cosmetics. Especially the last ones – cosmetics use the benefits of aromatic plant substances. This work indicates that essential oils and extracts can be treated as natural preservatives in ecological cosmetics in which one of the major problem is microbial purity ensuring. Contamination with microbes leads to the loss of physicochemical properties of cosmetics, and what is worse - they pose a health hazard to their users. In this context, it is worth paying special attention to the substances obtained from the aromatic plants like lavender and incense, which are known for their pro-health and physicochemical properties for a long time. Their addition, even in small amounts, can improve the safety of organic cosmetics, especially where the smell of these plants is not undesirable.

The aim of the study was to verify and compare the antimicrobial activity of commercial essential oils and extracts obtained from the lavender (*Lavandula Angustifolia*) and incense (*Boswellia Carteri*) plants against indicator microorganisms causing contaminations and infections in the cosmetics industry. The following strains were used: *Escherichia coli*, *Staphylococcus epidermidis*, *S. aureus*, *Pseudomonas aeruginosa*, *P. putida*, *Bacillus subtilis*, *Micrococcus luteus* and *Candida albicans*.. Two methods were used to determine antimicrobial activity: disc diffusion method and 96-well serial dilution method. On their basis, the width of the microorganism growth inhibition zone and the percentage values of growth inhibition at subsequent concentrations of the oils and extracts were calculated. The obtained results confirm that in the range of tested concentration (0,1-5%) the plant substances from lavender and incense are effective agents which inhibit the growth of microorganisms. Vary, nevertheless significant clearing zones were observed for each microorganisms. Some of the tested strains were more susceptible to the effects of lavender essential oils, and the others to the effect of extracts. The strongest inhibitory effect of lavender essential oils was observed against following strains: *B. subtilis* and *S. aureus* whereas of CO₂ extract against: *S. aureus* and *P. Putida*. For substances derived from incense, the strongest effect of essential oil was observed against *S. epidermidis*, *B. subtilis* and *M. luteus* while CO₂ extract was slightly less effective against tested strains. The *E. coli* strain showed the strongest resistance to all tested plant substances

The obtained results confirm that plant substances from lavender and incense are effective agents that inhibit the growth of microorganisms, and may also play an important role in microbial infection preventing especially, thanks to its aromatic qualities, in the cosmetics industry.

PCC-24

UTILIZATION OF BEE PRODUCTS AND TRÁS-OS-MONTES AROMATIC PLANTS ON THE DEVELOPMENT OF COSMETIC FORMULATIONS

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The use of natural cosmetics is nowadays becoming increasingly frequent given to the notice of consumers to the consequences of using chemically synthesized compounds [1], which may even be carcinogenic. This work aimed to develop a cream formulation for daily moisturizing of the skin, with natural ingredients, like bee products, plants of the region of Trás-Os-Montes and vegetable oils. Among bee products, beeswax was chosen for its properties, like skin repairing power and antimicrobial power [2]. In more detail, two sage plants (*Salvia officinalis* and *Salvia elegans*) and the thyme species *Thymus zygis* subsp. *zygis* were used as sources of antioxidants. The phenolic profile of the hydroalcoholic extracts of sage were elucidated by UHPLC-DAD-ESI-MS² analysis [3], while the composition of the essential oil of *T. zygis* subsp. *zygis* was determined by GC/ GC-MS. The antimicrobial activities were evaluated by microdilution broth method and by diffusion agar test. The obtained formulations were subjected to physicochemical and microbiological stability tests. The major phenolic components of *S. officinalis* extract were rosmarinic acid, apigenin-*O*-glucuronide, scutellarein-*O*-glucuronide and luteolin-7-*O*-glucuronide while *S. elegans* was mostly composed by rosmarinic acid, salvianolic acid K, luteolin-7-*O*-glucuronide and caffeic acid. The GC/ GC-MS analysis of essential oil of *T. zygis* subsp. *zygis* was mainly composed of carvacrol, cymene and trans-sabinene hydrate. The formulations had pH values slightly acid, similar to the skin pH. No phase separation or alteration of the organoleptic criteria of the formulations were observed. The stability of the products was also verified by spectrophotometer within the UV visible region between 210 and 600 nm, especially the sample containing olive oil and 5% of *S. elegans* hydroalcoholic extract. A non-Newtonian behavior, criterion sought in cosmetic creams, was also observed during the analysis by the texturometer. Additionally, the thyme volatile oil, used as a preservative in the cream, showed an inhibitory effect against *Staphylococcus aureus* (MIC and MBC < 0.031%), *Pseudomonas sp* (MIC and MBC = 1.25%), *Escherichia coli* (MIC and MBC = 0.31%) and *Candida albicans*. Above a degree of 0.31%, thyme oil showed in *C. albicans*, inhibition zones varying from 2 to 9.5 mm against *C. albicans*, in the agar diffusion test. The promising results of these work allows propose beeswax, *S. officinalis*, *S. elegans* and *T. zygis* subsp. *zygis* as active ingredients for natural cosmetic formulations.

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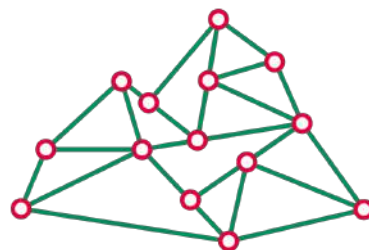
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