

Antioxidant activities of the constituents of *Picris echoides*

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Some flavonoids with antioxidant properties from the aerial parts of the plant species *Picris echoides* (family Asteraceae) were identified. Upon chromatography, the ethyl acetate extract afforded flavonoids, such as: flavone apigenin (**1**) and its glucoside, cosmosiin (**2**), as well as common plant constituents from this family, flavonol galetin (**3**, 3,4',5,6,7-pentahydroxyflavone) and 4,4',6,7-tetrahydroxyaurone (**4**). The structure of the aurone **4** has not been described so far in the literature and presented a very rare type of aurone skeleton. The structures of the isolated compounds were determined by interpretation of their physical and spectral data. The antioxidant activities of different extracts from *Picris echoides* were measured by the Schaal oven test at 60 °C and by the Rancimat method at 100 °C. Water/ethanol extracts (2:8, v/v), in concentrations of 0.02 and 0.05 %, showed lower activity than commercial tocopherol (Tch). On the contrary, the purified ethyl acetate extracts showed a strong concentration-dependent antioxidant effect. The investigation demonstrated that galetin was the main flavonol from this origin. According to the results of the two methods, galetin (**3**) showed a two-fold better activity than did Tch and a lower activity than did butylated hydroxyanisole (BHA). The aurone **4** exhibited significantly lower antioxidant activity than did galetin at the same concentration level. Thus, the plant species *P. echoides* is a new and favorable source of natural lipid antioxidants.

Keywords: *Picris echoides*, Lactuceae, antioxidant activity, flavonoids.

INTRODUCTION

Autoxidation is a phenomenon with significant effects *in vivo* and *in vitro*. Peroxidation of unsaturated lipids is a catalytic process involving a free-radical chain reaction mechanism, with the formation of hydroperoxides, and further reactions of oxidative breakdown and polymerization.^{1,2} Lipid peroxidation can also result in rancidity in finished products and can make them unacceptable to consumers.

Lipid oxidation *in vivo* of proteins and nucleic acids, caused by free radicals, may be related to aging and diseases.^{3,4} Highly reactive free radicals are capable of oxidiz-

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ing biomolecules, resulting in cell death and tissue damage. Almost all organisms are well protected against free-radical damage by either enzymes, or compounds, such as ascorbic acid, α -tocopherol and glutathione. When the mechanism of antioxidant protection becomes unbalanced by the deterioration of different factors, physiological functions can occur which result in diseases or accelerated aging. Consequently, it is important to find compounds that prevent oxidation. Antioxidants have important preventive roles, not only on undesirable changes in the flavor and nutritional quality of foods, but also on tissue damage in various human diseases.

The safety of synthetic antioxidants, however, has been a cause of concern and has stimulated the evaluation of the effectiveness of natural compounds, or extracts with potent antioxidative activities.⁵ Tocopherols are widely used as natural antioxidants, although their protective ability is not always sufficient. Naturally occurring phenolic compounds, such as flavonoids and tannins, have been shown to scavenge active oxygen species and to effectively prevent oxidative cell damage.⁶ In addition to essential oils, flavonoids are characteristic constituents of the great number of species of the family Asteraceae.

Picris echoides (family Asteraceae), an aromatic herb, is a native of central Europe and is the main floral element of the Mediterranean regions, where it creates a major weed problem. The small genus *Helminthia*, from the tribe Lactuceae, family Asteraceae comprises 6 species. In Serbia only one grows: *Helminthia echoides*, which has the extensive name syn. *Picris echoides*. Alcoholic extracts of this origin have been used in traditional medicine for the treatment of indigestion, intestinal nematodes and other parasites.⁷ As the result of numerous biological activities, secondary metabolites of the members of this genus have received considerable attention. When its biological activity was observed, among the most interesting isolated compounds were sesquiterpene lactones (germacranolides and guaianolides), mainly in the aerial parts,^{8,9} some flavonoids¹⁰ and terpenoid compounds.^{11,12}

In the present study, under conditions of *in vitro* oxidation, the flavonoid content of the plant origin *Picris echoides* was examined as a potential source for active antioxidants.

EXPERIMENTAL

Plant material. *Picris echoides* L. Gaertner (family Asteraceae, tribe Lactuceae).^{7,13} The aerial parts of the plant material (1.8 kg) were collected in July 1997, at the time of full blossom in the vicinity of Mt. Rudnik, near Belgrade, Serbia. A herbarium specimen was deposited at the Botany Department, Faculty of Science, University of Belgrade.

Extractions and isolations of the chemically pure compounds. The air-dried and ground plant material was extracted three times with 10 liters of solvent mixture ethanol/water (8:2, v/v) for 48 h at 25 °C. After removal of ethanol under reduced pressure in a rotary evaporator, the remaining aqueous solution of the extracted plant material was extracted with ethyl acetate. The green oily crude extract (3.5 g) obtained after evaporation of ethyl acetate *in vacuo* was treated with lead acetate (II) in the usual manner,¹⁴ and the purified ethyl acetate extract (2.8 g) was collected. The brown oil remaining (1.6 g) as a flavonoid mixture after purification on a Sephadex LH-20 column with methanol as the eluant, was chromatographed on a polyamide column. The elution was commenced with an Eggers solvent mixture (water/methanol/methyl ethyl ketone/acetone, 13:3:3:1, v/v/v/v) and the polarity of the solvent was gradually increased by the addition of water. The first crystalline compound eluted

with the Eggers solvent mixture was identified as aurone **4** after purification by silica gel column chromatography (CC), elution with ethyl acetate (100 %), and recrystallization from methanol (16 mg). The aurone was obtained as bright yellow crystals, melting at 333–335 °C. IR (ν_{\max} cm^{-1}) (KBr): 3400, 3300, 1738, 1720, 1680, 1600, 1100, 1020. UV (nm, log ϵ): MeOH: 332 (4.09), 249 (4.39). +NaOMe: 398 (4.19), 247 (4.47). +AlCl₃: 422 (4.13), 271 (4.13). +AlCl₃/HCl: 380 (3.95), 274 (4.05). +NaOAc: 389 (4.04), 375 (4.03) and 244 (4.47). +NaOAc/H₃BO₃: 363 (4.08), 244 (4.47). ¹H NMR (CD₃OD, 600 MHz, TMS) δ : 7.77 (2H, *d*, *J* = 8.5 Hz, H-2',6'), 6.84 (2H, *d*, *J* = 8 Hz, H-3',5'), 6.66 (1H, *s*, benzylic proton) and 6.49 (1H, *s*, H-5). EIMS produced peaks *m/z* (rel. int.): 286 (M⁺, C₁₅H₁₀O₆)⁺ (95 %), 270 (C₁₅H₁₀O₅)⁺ (100 %), 258, (C₁₄H₁₀O₅)⁺ (22 %), 242 (C₁₄H₁₀O₄)⁺ (22 %), 222 (C₁₅H₁₀O₂)⁺ (70 %) and 152 (C₇H₄O₄)⁺ (44 %). Aurone **4** was converted into the corresponding tetraacetate **4a**, with acetic anhydride in pyridine and on a repeated crystallization from acetone-methanol (1:1, v/v) gave white crystals, which melted at 165–167 °C. ¹H NMR (CDCl₃, 200 MHz, TMS) δ : 2.01–2.35 (12H, *m*, four acetyl groups), 7.80 (2H, *d*, H-2',6'), 6.95 (2H, *d*, H-3',5'), 6.80 (1H, *s*, benzylic proton) and 6.52 (1H, *s*, H-5).

Two more pure solid fractions were isolated by elution with a solvent mixture of water/methanol/methyl ethyl ketone (13:3:3, v/v/v). The first one to be eluted was a yellow powdery product, which, after recrystallization from ethanol (100 %), was identified as the flavonol galetin with a melting point of 306–307 °C (**3**, 42 mg). Elemental analysis of **3** showed that this compound contained 59.96 % of carbon and 3.48 % of hydrogen. These percentages are almost identical to the values calculated from the molecular formula of this compound C₁₅H₁₀O₇, 59.81 % of carbon and 3.37 % of hydrogen. ¹H NMR (CD₃OD, 200 MHz, TMS) δ : 6.86 (1H, *s*, H-8), 7.05 (2H, *d*, *J* = 8 Hz, H-3', 5') and 7.95 (2H, *d*, *J* = 8.5 Hz, H-2', 6'). IR (ν_{\max} cm^{-1}) (KBr): 3450, 1695, 1600, 1290, 1170, 1100, 1030. UV (nm, log ϵ): MeOH: 373 (4.30), 253 (4.27). + NaOMe: 329 (4.33), 240 (4.11). + AlCl₃: 451 (4.34), 244 (4.47). + AlCl₃/HCl: 247 (4.22), 244 (4.47). + NaOAc: 393 (4.15), 271 (4.20) and + NaOAc/H₃BO₃: 378 (4.16), 258 (4.25). Acetylation of **3** afforded the penta-acetate, as **3a**. The precipitate was recrystallized from ethanol, giving white crystals, which melted at 200–202 °C. ¹H NMR of **3a** (CDCl₃, 200 MHz, TMS) δ : 2.30 (12H, *m*, 4 acetyl groups) 2.45 (3H, *s*, 1 acetyl group), 8.86 (1H, *d*, *J* = 2 Hz, H-8), 7.30 (2H, *d*, *J* = 9 Hz, H-3', 5') and 7.70 (2H, *d*, *J* = 8.5 Hz, H-2', 6'). The second crystalline product, isolated with the same solvent system, was identified as the flavone apigenin (**1**, 24 mg) which was recrystallized from ethanol (100 %). Further elution with a mixture of the Eggers solvent system did not produce any additional flavonoid compounds. The final eluent of this separation was water/methanol (1:1, v/v) which afforded the cosmosiin (**2**, 35 mg), which was purified by silica-gel CC by elution with ethyl acetate (9:1, v/v). A total of twenty-nine fractions resulted from this elution pattern, but only four fractions crystallized.

All elutions were controlled by thin-layer chromatography (TLC). Silica gel plates (20 × 20 cm, 0.25 mm thick; Merck, Darmstadt, Germany) were used for TLC. The solvents used to develop the TLC were *n*-butanol/acetic acid/water (BAW, 4:1:5, v/v/v) for all extracts, while a methanol/ethyl acetate (1:9, v/v) solvent mixture was used for the chemically pure compounds. Volumes of 50 μl of the concentrated extracts were streaked onto each precoated TLC silica gel plate, which had previously been activated for 30 min at 105 °C. After development, the chromatograms were dried and examined by spraying with aqueous sulfuric acid (1:1, v/v), with subsequent charring at 100 °C and spraying with, α , α' -dipyridyl reagent (1 % ethanol solution).¹⁵ All spots were visualized after spraying under ultraviolet (UV) (360 nm) light, after the TLC plates had dried.

Spectroscopic procedures. Melting points (uncorrected) were taken on a Micro-Heiztish-Boettius apparatus. The infrared (IR) spectra of the antioxidant compounds were recorded on a Perkin-Elmer-457 spectrophotometer (in KBr discs). ¹H nuclear magnetic resonance (NMR) spectra were taken on a Perkin-Elmer instrument A-200 (200 MHz) and on a Bruker-Rainer AM-600 (600 MHz) spectrometer in DMSO-*d*₆, CD₃OD and CDCl₃ solutions, using tetramethylsilane (TMS), as a reference marker. The chemical shifts are given in ppm as δ values, and coupling constants in Hz. The symbols *s*, *d*, *t* and *m* stand for singlet, doublet, triplet and multiplet, respectively. The UV spectra

were recorded on a Beckman DU-50 spectrometer. When necessary, the UV spectra (λ max in nm) were measured after the addition of different reagents and the ϵ coefficient was also included. The mass spectra were measured on a Varian-MAT CH-5. Column chromatography was performed on a silica gel 60 (Merck, 0.063-0.200 mm), Sephadex LH-20 (Merck), polyamide CC-6 (Marchery-Nagel, 0.05-0.16 mm). Silica gel cards (SIF E. Marck) were used for TLC.

Antioxidant activity analysis. Ten grams of air-dried and ground plant material were extracted in a Soxhlet-type apparatus with petroleum ether for 24 h. After concentration at reduced pressure, the residue was extracted with the solvent mixture ethanol/water (8:2, v/v) for 48 h at 25 °C. After removal of ethanol, the remaining aqueous solution of the plant material residue was extracted separately with chloroform and ethyl acetate. Finally, the residue was extracted with ether, as shown in Fig. 1. All the extracts were concentrated on a rotary evaporator at 40 °C.

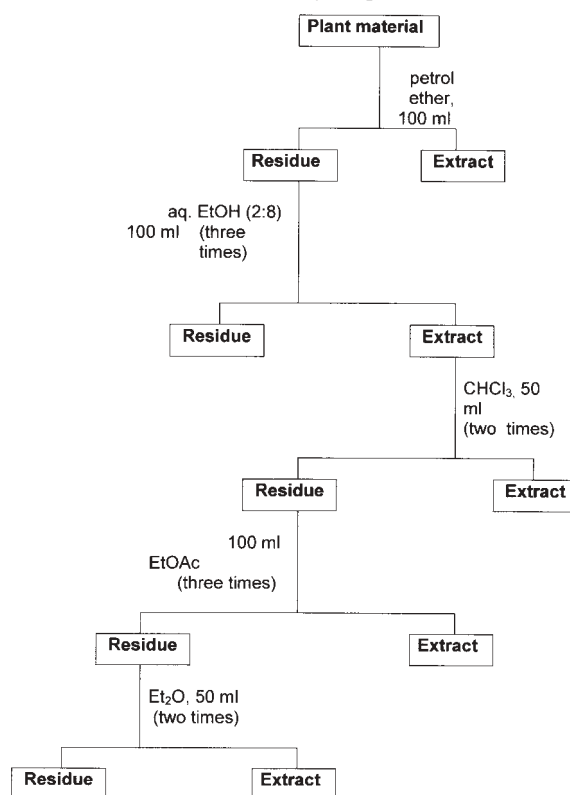


Fig. 1. Scheme for the separation of extracts from *Picris echioides*; EtOH – ethanol; CHCl₃ – chloroform; EtOAc – ethyl acetate; Et₂O – ether.

The antioxidant activity of each sample tested was based on its ability to prevent the formation of peroxides and secondary products of oxidation in prime steam lard, as a substrate with no antioxidants. The antioxidant activities of the different extracts of *P. echioides* and of the flavonoids galetin and some aurone **4** were determined by the Schaal oven test at 60 °C¹⁶ and by the Rancimat method at 100 °C.¹⁷ For the Schaal oven test, glass tubes (100 ml, 15 cm i.d.) with a flat bottom each containing 50.0 ± 0.01 g of prime steam lard and 0.02 or 0.05 % of one of the samples were placed into an incubator and kept at 60 ± 1 °C in the dark. A tocopherol mixture (Tch) and butylated hydroxyanisole (BHA) were used as commercial antioxidants for comparing the antioxidant activities of the tested samples.

The tocopherol mixture consisting of 12 % α -tocopherol, 1 % β -tocopherol, 61 % γ -tocopherol and 26 % of δ -tocopherol (Coviox T-70, Chemie Fabrik Grunau GmbH, Illertissen, Germany) was used as a positive control. Changes in peroxide values (PV) were determined by the AOAC method.¹⁸ A sample size of 5.00 ± 0.01 g was used in each PV analysis every 24 h. All Rancimat analyses were performed with a 617 Rancimat (METROHM AG, Ch-9100, Metrohm, Herisau, Switzerland) at 100 °C, with an airflow of 18–20 mL/min on 2.5 g samples.

All experiments were repeated in triplicate and typical results are shown from one of the three independent experiments.

RESULTS AND DISCUSSION

Identifications of the flavonoids. Extracts from aerial part of the plant species, *P. echioides*, showed significant antioxidant activity measured on the stability of prime steam lard. The objective of this study was to determine the flavonoids content of this specimen. Thus, to isolate and identify the active compounds, the water extract was purified by a simplified Geissman procedure for extraction with ethyl acetate,¹⁴ the extract being further fractionated by Sephadex LH-20 CC, with the flavonoid mixture obtained. In the next step, the flavonoid mixture was further chromatographed on polyamide, affording four pure flavonoid compounds whose structures are presented in Fig. 2.

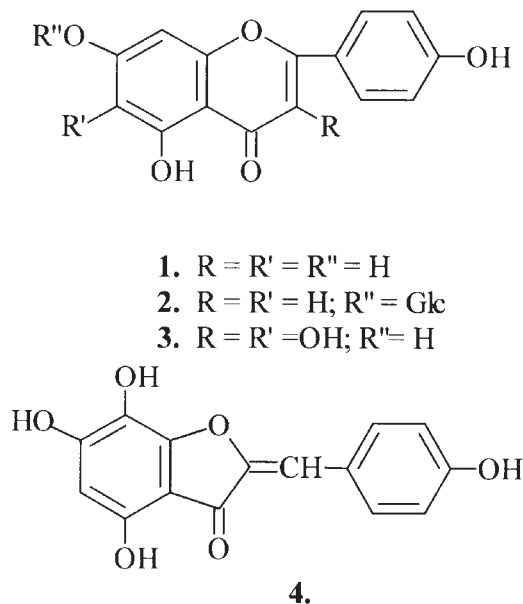


Fig. 2. Chemical structure of the flavonoid compounds isolated from the plant species *Picris echioides*.

Aurone **4** (4,4',6,7-tetrahydroxyaurone), was isolated first as bright yellow crystals and identified by spectral values. No data related to the chemical determination of the aurone **4** could be located in the literature. In the IR spectrum of **4**, the bands at 3400 and 3300 cm^{-1} show the presence of hydroxy groups; the band at 1680 cm^{-1} belongs to the conjugated keto group; the bands at 1738 and 1720 cm^{-1} correspond to keto groups, and the other bands are due to aryl ethers. The mass spectra showed the molecular ion M^+ at m/z 286

which is in agreement with the molecular formula $C_{15}H_{10}O_6$. The characteristic features of the aurone skeleton are ion peaks at m/z 222 ($C_{15}H_{10}O_2$) and at m/z 270 ($C_{15}H_{10}O_5$), as the base peaks which correspond to the known fragmentation pattern of the aurone skeleton. The fragment ion peak at m/z 152 suggests the presence of a hydroxy group in the B-ring, and the other ion peak at m/z 258 gives information about hydroxy function in the A-ring.¹⁹ The 1H NMR spectrum indicates that the aurone structure is present, because the benzylic proton at 6.66 ppm appears as a singlet in the range of 6.5–6.7 ppm. The singlet at δ 6.49 originates from the C-5 proton, when the compound contains the common pattern 4,6-dihydroxy-substitution. The proton order for the B-ring, indicates that the position C-4 is substituted (two doublets at δ 6.84 and 7.77, for the H-3',5' and H-2',6', respectively). This assignment was also based on a comparison of the 1H NMR data of its tetraacetate **4a**, mostly differing in chemical shifts at δ 2.01–2.35, as a multiplet for four acetyl groups. The results of the UV spectra for the determination of the structure of aurone, **4**, also are in good agreement with the proposed structure.²⁰ The addition of sodium methoxide to a methanol solution of **4**, produced a bathochromic shift of the Band 1 of 66 nm, indicating the presence of free 6 and 4'-hydroxyl groups. This substitution was also determined in the sodium acetate spectrum, as a shift of Band 1 of 43–57 nm, compared to the methanol spectrum. Moreover, the absence of *ortho*-dihydroxy groups in the B-ring was also proved by the addition of boric acid, which did not change the sodium acetate spectrum already obtained. The presence of a 4-hydroxy group was identified in the aluminium chloride spectrum, as a shift of Band 1 of 90 nm towards higher wavelengths, compared with the methanol spectrum. Finally, the addition of hydrochloric acid produced a shift of Band 1 of 42 nm, compared to the aluminium chloride spectrum, which is typical for the positions of 6- and 7-OH groups in the aurone skeleton.¹⁹

The second yellow powder, **3**, namely galetin (3,4',5,6,7-pentahydroxyflavone) has not hitherto been isolated as a natural product. According to the literature data, heteroside galetin rhamnoside was isolated and identified only from the plant species *Galega officinalis* L.²¹ The flavonol galetin was isolated as the main flavonoid (the overall yield in this separation being 42 mg) and identified on the bases of the spectral data. The IR spectrum showed strong absorption of hydroxyl groups at 3450 cm^{-1} , the band at 1695 cm^{-1} belongs to the conjugated carbonyl group and the band at 1600 cm^{-1} corresponds to C=C. The other absorptions at 1290, 1170, 1100 cm^{-1} are due to aryl ethers. In the 1H NMR spectrum the proton order in the A-ring indicates that the positions 5, 6 and 7 are substituted, with a singlet for H-8 at δ 6.86. A typical four-peak pattern of two doublets at δ 7.05 and 7.95 corresponds to the protons at the 3', 5' and 2', 6' positions in the B-ring, respectively.²⁰ The results of elemental and UV spectral analyses for the determination of the proposed structure of **3** are included. The nature of hydroxyl groups was confirmed by acetylation to the corresponding penta-acetate, as **3a**. The acetate **3a** gave changes in its 1H NMR spectrum as twelve protons at δ 2.3 for four acetyl groups and three protons at δ 2.45 for one acetyl group. These two compounds **3** and **3a** were also easily deduced from the similarities of their UV data, with those already reported in the literature.²¹

In order of elution, two further yellow crystalline compounds, flavone apigenin, **1** and its glucoside cosmosiin **2**, whose structures had been previously described,²² were

also isolated and identified. From the chemosystematic point of view, the presence of the certain apigenin and apigenin-7-*O*-glucoside is important as a means for positive detection.²³

Antioxidant activities: The results of this research showed that the water/ethanol and ethyl acetate extracts of domestic origin *Picris echooides* possess significant antioxidant ability. The antioxidant activities of these extracts, aurone **4** and galetin were measured by the Schaal oven test at 60 °C and the Rancimat method at 100 °C and compared with BHA and a tocopherol mixture. Table I summarizes the values of the induction time for prime steam lard with and without antioxidants. As the different accelerated stability methods were conducted under different conditions with different mechanisms, a comparison of the absolute values of these induction times is not meaningful. However, the relative antioxidant activity of the examined samples can be concluded, based on the values of the induction times assessed by the different methods. The results of both methods show the same trends. Table I shows that all the investigated samples (at a concentration of 0.02 %) inhibited lipid oxidation (regardless of applied method) in the following order: BHA > galetin > ethyl acetate extract > aurone **4** > water/ethanol extract. The petroleum ether and ether extracts were not further investigated as they showed negative results in antioxidant tests with ferric chloride, sulphuric acid and α , α' -dipyridyl.

TABLE I. Antioxidant activities of extracts and isolated flavonoids from *Picris echooides* measured by two different accelerated stability methods

Samples	Rancimat method IP/hours	Schaal oven test IP/days
C	2.5	2
C + E ₁	11.5	6
C + E ₂	13	7
C + E ₃	16	9
C + E ₄	18	11
BHA – 0.02 %	21	13
Tch – 0.02 %	12	6
Galetin – 0.02 %	19	10
Aurone 4 – 0.02 %	13.5	7

IP – induction time; C – lard without antioxidants; E₁ – 0.02 % water/ethanol extract; E₂ – 0.05 % water/ethanol extract; E₃ – 0.02 % ethyl acetate extract; E₄ – 0.05 % ethyl acetate extract; Tch – tocopherol mixture.

The water/ethanol and ethyl acetate extracts exhibited strong antioxidant activities and displayed increasing trends of inhibition of oxidation when their concentration was increased (0.02–0.05 %). The water/ethanol extract had almost the same effectiveness as the tocopherol mixture and was two-fold lower than BHA. The ethyl acetate extract showed a significantly stronger antioxidant effect on the elongation of the induction period than did the tocopherol mixture and a slightly lower antioxidant activity than BHA at the same concentration level. The lower antioxidant activity of the water/ethanol extract, than ethyl acetate extract is probably caused by a lower concentration of flavonoids and the existence of some minor components, which might have pro-oxidant activity.

The antioxidant activities of the chemically pure flavonoid compounds isolated from the plant species *Picris echoides* L. were also investigated. Flavonoids have been reported to scavenge free radicals in an activity-structure related manner by donating a hydrogen atom.²⁴ However, the potent antioxidant activities of compounds **3** and **4** have not been reported before. Aurone **4**, at the 0.02 % level, had an antioxidant activity similar to that of the tocopherol mixture, but significantly lower than BHA (see Table I). The results also demonstrated that galeitin **3**, in a concentration of 0.02 % was approximately two times more effective than the tocopherol mixture, but was less effective than BHA under the experimental conditions used. Thus, of the flavonoids, galeitin is mainly responsible for the antioxidant activity of the ethyl acetate extracts. The antioxidant properties of the two isolated flavonoids, apigenin and cosmosiin have been previously described.²²

The antioxidant activity of phenolic compounds with two OH groups at the *ortho* position of phenol have greater antioxidant activity than those with two OH groups in the *meta* position.⁵ The flavonol galeitin also has an OH group at the neighboring position and has greater antioxidant activity than does aurone **4** (see Fig. 2). These results suggest that the presence of OH groups at the neighboring positions is essential for strong antioxidant activity, and that the number of phenolic OH groups in the molecule is not critical. As aurone **4** and galeitin **3** have the *ortho* substitution pattern, we concluded that the presence of such substitution was necessary for these antioxidant activities.

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ИЗВОД

САСТАВ И АНТИОКСИДАТИВНА АКТИВНОСТ *Picris echoides*

МИРЈАНА МИЛОВАНОВИЋ¹, КСЕНИЈА ПИЋУРИЋ-ЈОВАНОВИЋ¹, ВЕРИЦА ЂЕРМАНОВИЋ² И МИЛУТИН СТЕФАНОВИЋ³

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Испитан је хемијски састав водено/етанолног екстракта домаће биљке *Picris echoides* (фамилија Asteraceae). Изолована су и идентификована четири флавоноида: апигенин (**1**), његов 7-*O*-глукозид (**2**), галетин (**3**, 3,4',5,6,7-пентахидрокси-флаворон) и 4,4',6,7-тетрахидрокси-аурон (**4**). Скелет типа аурона **4** врло ретко је присутан у биљкама и његова структура до сада није описана у литератури. Одређена је антиоксидативна активност различитих екстраката биљке, као и чистих флавоноидних супстанци у условима Schaal oven теста на 60 °C и Rancimat метода на 100 °C. Водено/етанолни екстракти (2:8) у концентрацијама 0,02 и 0,05 % показали су слабу активност у поређењу са токоферолом, док су етилацетатни екстракти, у истим концентрацијама, деловали као ефикасни антиоксиданти. Доказано је да галетин (**3**) показује дупло већу активност од токоферола.

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REFERENCES

1. A. J. St. Angelo, *Crit. Rev. Food Sci. Nutr.* **36** (1996) 175
2. E. N. Frankel, *Trends Food Sci. Technol.* **4** (1993) 220
3. K. A. Schultze-Osthoff, C. Bakker, B. Vanhaesebroeck, R. Beyeaert, W. A. Jacob, W. Fiers, *J. Biol. Chem.* **26** (1992) 5317
4. P. C. Bragt, J. I. Bansberg, I. L. Bonta, *Inflammation* **4** (1980) 289
5. Six, Pauline, *Inform* **5** (1994) 679
6. T. Okuda, Y. Kimura, T. Yoshida, T. Hatano, H. Okuda, S. Arichi, *Chem. Pharm. Bull.* **31** (1983) 1625
7. M. Gajić, M. Josifović, *Flora of the Republic of Serbia*, VII, Serbian Academy of Sciences and Arts, Belgrade, 1975, Vol. 7, p. 280
8. Marco Alberto, Sanz Juan, Carda Miguel, *Phytochemistry* **31** (1992) 2163
9. F. Bohlmann, C. Zdero, H. Robinson, *Phytochemistry* **20** (1981) 2029
10. M. Milovanović, M. Djermanović, *Fitoterapia* **4** (1994) 377
11. M. Milovanović, K. Picurić-Jovanović, V. Djermanović, M. Stefanović, *Fitoterapia* **6** (1998) 553
12. M. Milovanović, K. Picurić-Jovanović, M. Djermanović, V. Djermanović, M. Stefanović, *J. Serb. Chem. Soc.* **65** (2000) 763
13. S. Javorka, V. Csapody, *Iconographia Florae Partis Austro-Orientalis Europae Centralis*, Hung. Acad. Sci., Budapest, 1975, p. 555
14. M. Stefanović, S. Mladenović, M. Djermanović, D. Jeremić, *J. Serb. Chem. Soc.* **42** (1977) 639
15. G. M. Barton, *J. Chromatog.* **20** (1965) 189
16. E. R. Scherwin, *Synthetic Antioxidant for Fats and Oils in Flavour Chemistry of Fats and Oils*, D. B. Min and T. H. Smouse, Eds., American Oil Chemists Society, Champaign, 1985, pp. 163–169
17. M. W. Laubli, P. A. Bruttel, *J. Am. Oil Chem. Soc.* **23** (1986) 792
18. *Official Methods of Analysis of the Association of Official Analytical Chemist (AOAC)*, International 16th edition, Arlington, Virginia, 1995. Vol 2, Ch. 41, p. 9
19. K. R. Markham, *Techniques of Flavonoids Identification*, Academic Press, London, 1982, pp. 40–93
20. J. T. Marbry, R. K. Markham, B. M. Thomas, *The Systematic Identification of Flavonoids*, Springer-Verlag, New York, 1970, pp. 112–303
21. P. N. Maksyntina, V. I. Litvinenko, *Dokl. Akad. Nauk. SSSR* **154** (1964) 1123
22. M. Milovanović, K. Picurić-Jovanović, B. Vucelić-Radović, Z. Vrbaski, *J. Am. Oil Chem. Soc.* **73** (1996) 773
23. B. J. Harborne, B. L. Turner, *Plant Chemosystematics*, Academic Press, London, 1984, pp. 141–149
24. W. Bors, W. Heller, C. Michael, M. Saran, *Enzymol.* **186** (1990) 343.