

## ANTIBACTERIAL ACTIVITY OF AROMATIC PLANTS ESSENTIAL OILS FROM SERBIA AGAINST THE *LISTERIA MONOCYTOGENES*

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**Abstract:** The purpose of this study was to examine the effectiveness of selected essential oils for the control of the growth and survival of pathogenic bacteria *Listeria monocytogenes* ATCC 19112 and *Listeria monocytogenes* ATCC 19115, which are of significant importance in food hygiene. Essential oils extracted from *Salvia officinalis* L., *Rosmarinus officinalis* L., *Majorana hortensis* Moench., *Thymus vulgaris* L., *Carum carvi* L., *Pimpinella anisum* L. and *Coriandrum sativum* L. were evaluated. Antibacterial activity was done by the disk diffusion method in the presence of pure essential oils and four suspensions in alcohol. The best results obtained with *Thymus vulgaris* and *Majorana hortensis* essential oils, which were acting microbicidally on both observed strains of *Listeria monocytogenes*, even in the small concentration. Because some of the essential oils were highly inhibitory even in small quantities to selected pathogenic bacteria, they may provide alternatives to conventional antimicrobial additives in foods.

**Key words:** *Listeria monocytogenes*, essential oils, antibacterial activity.

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## Introduction

*Listeria monocytogenes* is a Gram-positive, rod-shaped bacteria, found in soil and water. Vegetables can become contaminated from the soil or from manure used as fertilizer. Animals can carry the bacteria without appearing ill and can contaminate foods of animal origin such as meats and dairy products. The bacteria can be found in a variety of raw foods, such as uncooked meats and vegetables, as well as in processed foods that become contaminated after processing, such as soft cheeses and cold cuts at the deli counter. Potential sources of listeriosis are ready-to-eat processed foods such as meat products and unpasteurized dairy products that are stored for long periods at refrigerator temperature (4°C) (Madigan et al., 2000.). Both unpasteurized (raw) and pasteurized milk may contain the bacteria, too (X). *Listeria monocytogenes* is the agent of listeriosis, a serious infection caused by eating food contaminated with the bacteria. Listeriosis is a serious disease for humans; the overt form of the disease has a mortality greater than 25 percent. The two main clinical manifestations are sepsis and meningitis. Meningitis is often complicated by encephalitis, a pathology that is unusual for bacterial infections.

Medicinal, aromatic and herb spices from family *Apiaceae* like Caraway (*Carum carvi* L.), Anise (*Pimpinella anisum* L.), Coriander (*Coriandrum sativum* L.) and from family *Lamiaceae* like Sage (*Salvia officinalis* L.), Rosemary (*Rosmarinus officinalis* L.), Marjoram (*Majorana hortensis* Moench.) and Thymi (*Thymus vulgaris* L.) are widely distributed in Serbia growing as wild or cultivated species (Sarić, 1989.). These plants are used like stomachic, spasmolytic, carminative and expectorant in traditional folk medicine and also in official medicine. Essential oils extracted from *Apiaceae* and *Lamiaceae* family plants can contribute the quality of food with better odor and flavor what is considered as very important quality parameter in food manufacturing (Kovačević, 2001). Other benefits could be application of essential oils in therapeutics purposes due to their antimicrobial effects on some pathogen microorganisms (Stefanini et al., 2001., Klaus et al, 2007.).

Essential oils of *Carvi aetheroleum*, *Anisi aetheroleum* and *Coriandri aetheroleum* are usually obtained from the ground, mature and dry fruits. The fruit of *Carvi fructus* contains 2-7% of essential oils. The major ingredients of essential oils are monoterpenes, ketone-carvone (50-80%) and

carbohydrates d-limonen (15-35%), which are well known to contribute to the carminative effect (Micklefield et al., 2000.).

The fruit of Anise (*Anisi fructus*) contains 1.5-5% of essential oils. The major ingredient being anethole (80-90%). With characteristic odour and taste it is often used in the food processing industry to enhance the odour and the taste of food commodities (Kubeczka et al., 1986.). It is also used in medicine as expectorant and carminative.

The fruit of Coriander (*Coriandri fructus*) contains up to 1% of essential oils. Monoterpenes are the major ingredients of this oil with limonen (40-60%) as predominate constituent. The essential oils of Coriander has stomachic, spasmolytic and carminative effect. It also has bactericidal and fungicidal effect (Baratta et al., 1998.). It is used in the food processing industry as a spice and aroma enhancer.

The folium of Sage (*Salvia officinalis* L.) contains 1-2,8% of essential oil. Monoterpenes are the major ingredients of this oil with thujon (30-60%) as predominate constituent, cineol (15%) and camphor. The essential oils of Sage has spasmolytic, adstringent and carminative effects (Baratta et al., 1998.).

The folium of Rosemary (*Rosmarinus officinalis* L.) contains up to 2% of essential oil. The major ingredients of essential oil of *Rosmarini aetheroleum* are cineol (35%), pinenes, camphor and borneol. It is used in the food processing industry as a spice and aroma enhancer (Angioni et al., 2004.).

The herba of Thyme (*Thymus vulgaris* L.) contains 1-2% of essential oil. Monoterpenes are the major ingredients of this oil with thymol (40%) as predominate constituent and caracrol, which are well known like antimicrobial substances. The essential oils of Thyme has spasmolytic and expectorant effects (Marino et al., 1999.).

The herba of Marjoram (*Majorana hortensis* Moench.) contains 1-2% of essential oil (terpinen-4-ol, borneol). It is also used in folk medicine as stomachic and as a spice and aroma enhancer in food processing industry (Biondi et al., 1993.).

The aim of this work was to investigate antibacterial activity of these essential oils on bacteria *Listeria monocytogenes* ATCC 19112 and *Listeria monocytogenes* ATCC 19115.

## Materials and Methods

Antibacterial effects of essential oils extracted from *Apiaceae* and *Lamiaceae* family plants on Gram-positive bacterial strains *Listeria monocytogenes* ATCC-19115 and *Listeria monocytogenes* ATCC-19112 were investigated. ATCC cultures were taken from American Type Culture Collection, Rockville, Maryland.

Essential oils were obtained from Sage (*Salvia officinalis* L.), Rosemary (*Rosmarinus officinalis* L.), Marjoram (*Majorana hortensis* Moench.), Thymi (*Thymus vulgaris* L.), Caraway (*Carum carvi* L.), Anise (*Pimpinella anisum* L.) and Coriander (*Coriandrum sativum* L.). The fruits and herba of Sage, Rosemary, Marjoram, Thymi, Caraway, Anise and Coriander represent a domestic raw material from Serbia. The foliums of Sage (variety Primorska), Rosemary (domestic variety), Thymi (variety N-19) Marjoram (variety Holand olfactory) and fruits of Caraway (early maturing domestic variety), Coriander (variety Nikola), Anise (variety N.210) were used in this experiment. Essential oils were extracted from dry foliums and herbs of Sage, Rosemary, Thymi and Marjoram and from ground mature and dry fruits of Caraway, Anise and Coriander by destillation and vapouration with Clavenger according to the methods of Pharmacopea Jugoslavia V (Ph. Jug. V, 2000).

Screening of essential oils for antibacterial activity was done by the disk diffusion method with pure oil and four different concentrations (2:1, 1:1, 1:2, 1:3). Essential oils were dissolved in 96% C<sub>2</sub>H<sub>5</sub>OH in ratio 2:1 (2 ml essential oil:1ml 96% C<sub>2</sub>H<sub>5</sub>OH), 1:1 (1ml essential oil:1 ml 96% C<sub>2</sub>H<sub>5</sub>OH), 1:2 (1 ml essential oil:2 ml 96% C<sub>2</sub>H<sub>5</sub>OH) and in ratio 1:3 (1 ml essential oil:3 ml 96% C<sub>2</sub>H<sub>5</sub>OH) (Klaus et al., 2008.).

The cultures were adjusted to approximately 10<sup>5</sup>CFU/ml with sterile saline solution. Petri dishes were inoculated with 0.2ml suspension of microorganisms and overlaid with 20ml of the medium. For cultivating bacteria *Listeria monocytogenes* TSA-YE were used. Three filter disks (Sigma-Aldrich's Whatman ® Schleicher & Schuell, 6mm in diameter) were placed on every agar and diffusion method was performed by adding 10µl of appropriate suspension on every disk. Blind probe contained only 96% C<sub>2</sub>H<sub>5</sub>OH, without any ethereal oils. Bacteria were incubated at 37°C for 24 h and after the incubation period the zone of inhibition was measured. Small sectors from the zone of inhibition were taken and inoculated in TSB-YE for cultivating *Listeria monocytogenes*. Sectors in TSB-YE were incubated

at 37°C for 24 h to see if the effect of essential oils were microbicidal or microbistatic (Klaus et al., 2007.). Studies were performed in triplicate, and mean value was calculated.

## Results and Discussion

After 24 h incubation at 37°C the zone of inhibition around the filter disks were measured and results are presented in Table 1. If TSB-YE with sectors taken from the zone of inhibition showed turbidity after incubation, influence of applied essential oils were regarded as microbistatic. In the cases when TSB-YE with sectors taken from the zone of inhibition stayed clear after incubation, influence of applied essential oil were microbicide. Blind probe showed that 96% C<sub>2</sub>H<sub>5</sub>OH had no antimicrobial influence on investigated microorganisms.

Tab. 1. - The zone of inhibition procured by presence of pure essential oils and in concentrations 2:1 (2 ml essential oil: 1 ml 96% C<sub>2</sub>H<sub>5</sub>OH) and 1:1 (1 ml essential oil : 1 ml 96% C<sub>2</sub>H<sub>5</sub>OH)

micro organism	Average size of the zone of inhibition in diameter (mm)																				
	<i>Carum carvi</i>			<i>Pimpinella anisum</i>			<i>Coriandrum sativum</i>			<i>Rosmarinus officinalis</i>			<i>Thymus vulgaris</i>			<i>Majorana hortensis</i>			<i>Salvia officinalis</i>		
	pure oil	2:1	1:1	pure oil	2:1	1:1	pure oil	2:1	1:1	pure oil	2:1	1:1	pure oil	2:1	1:1	pure oil	2:1	1:1	pure oil	2:1	1:1
<i>Listeria monocytogenes</i> ATCC 19115	13	8	6	2	3	4	13	17	7	8.67	4.67	5.67	20.67	16	-	38	57	51.67	6.67	4.67	4.67
<i>Listeria monocytogenes</i> ATCC 19112	6	3	4	4	3	4	6	11	8	9	4	4	18.67	22.67	-	31	32	36.33	9.67	5.67	5.67

- with no influence on the growth

In the case of the bacteria *Listeria monocytogenes* ATCC 19115 all concentrations and pure essential oil obtained from *Majorana hortensis* showed microbicide effect (inhibitory zone 57 mm with suspension 2:1, Fig. 1; 51.67 mm with suspension 1:1 and 38 mm in the presence of pure oil). In the presence of *Thymus vulgaris* essential oil microbicide effect appeared (inhibitory zone 20.67 mm with pure oil and 16 mm with suspension 2:1, while suspension 1:1 had no effect on the growth of bacteria). Pure oils from *Carum carvi* and *Coriandrum sativum* showed microbistatic effect on *Listeria monocytogenes* ATCC 19115 with inhibitory zone of 13 mm. Suspension 2:1 of essential oil extracted from *Coriandrum sativum* made inhibitory zone of 17 mm. In the presence of *Carum carvi* essential oil microbistatic effect appeared with inhibitory zone of 8 mm. When essential

oil from *Rosmarinus officinalis* were applied microbistatic effect appeared (inhibitory zone 8.67 mm with pure oil, inhibitory zone 4.67 mm with suspension 2:1, 5.67 mm with suspension 1:1). Essential oil from *Salvia officinalis* showed microbistatic effect (inhibitory zone 6.67mm with pure oil, inhibitory zone 4.67 mm with suspension 2:1 and suspension 1:1; Tab.1.).

In the case of the bacteria *Listeria monocytogenes* ATCC 19112 the best results appeared in the presence of suspensions and pure oil from *Majorana hortensis*. Suspensions 1:1 (with inhibitory zone of 36.33 mm), 2:1 (with inhibitory zone of 32 mm) and pure oil (with inhibitory zone of 31 mm) showed microbicide effect. In the presence of *Thymus vulgaris* essential oil microbicide effect appeared (inhibitory zone 18.67 mm with pure oil and 22.67 mm with suspension 2:1, while suspension 1:1 had no effect on the growth of bacteria). Essential oil extracted from *Coriandrum sativum* showed microbistatic effect on the *Listeria monocytogenes* ATCC 19112 (inhibitory zone of 11 mm with suspension 2:1, inhibitory zone of 8 mm with suspension 1:1 and inhibitory zone of 6 mm with pure oil). In the presence of *Salvia officinalis* essential oil microbistatic effect appeared (inhibitory zone 9.67 mm with pure oil, inhibitory zone 5.67 mm with suspension 2:1 and suspension 1:1). Pure oils extracted from *Rosmarinus officinalis* (inhibitory zone 9 mm), *Carum carvi* and *Coriandrum sativum* (inhibitory zone 6 mm) and *Pimpinella anisum* (inhibitory zone 4 mm) showed microbistatic effect (Tab. 1.).

Essential oils obtained from *Majorana hortensis* and *Thymus vulgaris* were additionally dissolved in 96% C<sub>2</sub>H<sub>5</sub>OH in ratio 1:2 (1ml essential oil:2ml 96% C<sub>2</sub>H<sub>5</sub>OH) and 1:3 (1ml essential oil:3ml 96% C<sub>2</sub>H<sub>5</sub>OH), because previous investigations with dissolving of essential oils in 96% C<sub>2</sub>H<sub>5</sub>OH in ratio 2:1 (2ml essential oil:1ml 96% C<sub>2</sub>H<sub>5</sub>OH) and in ratio 1:1 (1ml essential oil:1ml 96% C<sub>2</sub>H<sub>5</sub>OH) showed very good results. Results are presented in Table 2.

Suspensions of *Thymus vulgaris* essential oil in 96% C<sub>2</sub>H<sub>5</sub>OH in ratio 1:2 and 1:3 showed microbicide effect in contact with *Listeria monocytogenes* ATCC 19112 (inhibitory zone 38 mm in suspension 1:2 and 34 mm in suspension 1:3, Fig. 2) and *Listeria monocytogenes* ATCC 19115 (inhibitory zone 31mm in suspension 1:2 and 37 mm in suspension 1:3).

Tab. 2. - The zone of inhibition procured by presence of essential oils in concentrations 1:2 (1 ml essential oil : 2 ml 96% C<sub>2</sub>H<sub>5</sub>OH) and 1:3 (1 ml essential oil : 3 ml 96% C<sub>2</sub>H<sub>5</sub>OH)

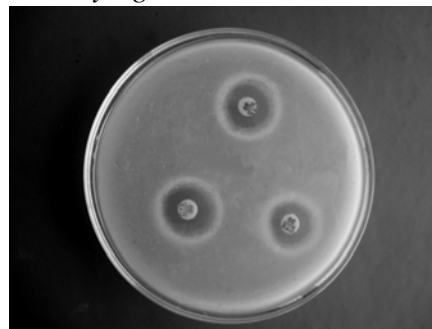
microorganism	Average size of the zone of inhibition in diameter (mm)			
	<i>Thymus vulgaris</i>		<i>Majorana hortensis</i>	
	suspension			
	1:2	1:3	1:2	1:3
<i>Listeria monocytogenes</i> 19115	31	37	36.33	36.33
<i>Listeria monocytogenes</i> 19112	38	34	34	42.33

Suspensions of *Majorana hortensis* essential oil in 96% C<sub>2</sub>H<sub>5</sub>OH in ratio 1:2 and 1:3 showed microbicide effect in contact with *Listeria monocytogenes* ATCC 19115 (inhibitory zone 36.33 mm in suspensions 1:2 and 1:3), and *Listeria monocytogenes* ATCC 19112 (inhibitory zone 42.33 mm in suspension 1:3 and 34 mm in suspension 1:2).

Fig. 1. - Inhibitory effect of *Majorana hortensis* essential oil (ratio 2:1) on the growth of *Listeria Monocytogenes* ATCC 19115



Fig. 2. - Inhibitory effect of *Thymus vulgaris* essential oil (ratio 1:3) on the growth of *Listeria monocytogenes* ATCC 19112



## Conclusion

*Listeria monocytogenes*, the cause of listeriosis, is emerging as an important foodborne pathogen. This is acid-tolerant, psychrotolerant (cold-tolerant) and salt-tolerant bacterium, which can make difficult the preservation of food. It can contaminate the food at any stage during food growth or processing and methods such as refrigeration, which ordinarily slow microbial growth, are ineffective in limiting growth of the organism.

This investigation showed possibility of some essential oils, especially those extracted from *Thymus vulgaris* and *Majorana hortensis*, to act microbicidally on *Listeria monocytogenes*, even in the small concentration. Suspensions of *Thymus vulgaris* and *Majorana hortensis* essential oils in 96% C<sub>2</sub>H<sub>5</sub>OH in small concentrations, 1:2 (1ml essential oil:2ml 96% C<sub>2</sub>H<sub>5</sub>OH) and 1:3 (1ml essential oil:3ml 96% C<sub>2</sub>H<sub>5</sub>OH) were acting microbicidally in contact with both investigated strains of *Listeria monocytogenes*. This could be very important regarding the fact that all microorganisms become resistant on numerous antibiotics. By adding some herbs or their essential oils in food in precisely defined concentration, by taking care of harmonizing with flavor, taste and odor of the particular groceries, it is possible to upgrade the quality of food and also, to protect the groceries from unwanted *Listeria monocytogenes*.

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ANTIBAKTERIJSKA AKTIVNOST ETARSKIH ULJA IZ  
AROMATIČNIH BILJAKA IZ SRBIJE NA *LISTERIA*  
*MONOCYTOGENES*

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Re z i m e

Svrha ovog istraživanja bila je ispitivanje efikasnosti izabranih etarskih ulja za kontrolu rasta i preživljavanja patogenih bakterija *Listeria*

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*monocytogenes* ATCC 19112 i *Listeria monocytogenes* ATCC 19115, koje su izuzetno značajne za higijenu hrane. Ispitivana su etarska ulja ekstrahovana iz žalfije (*Salvia officinalis* L.), ruzmarina (*Rosmarinus officinalis* L.), majorana (*Majorana hortensis* Moench.), timijana (*Thymus vulgaris* L.), kima (*Carum carvi* L.), anisa (*Pimpinella anisum* L.) i korijandera (*Coriandrum sativum* L.). Antibakterijska aktivnost ispitivana je difuzionim metodom pomoću filter diskova u prisustvu čistih etarskih ulja i četiri suspenzije u alkoholu. Najbolji rezultati dobijeni su sa etarskim uljima timijana i origana, koja su delovala mikrobicidno na oba ispitivana soja *Listeria monocytogenes*, čak i u vrlo malim koncentracijama. S obzirom da su neka etarska ulja visoko inhibitorna za izabrane patogene bakterije čak i u malim količinama, mogu da obezbede zamenu za konvencionalne antimikrobne aditive u hrani.

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