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Original scientific paper

ANTIMICROBIAL EFFECT OF SELECTED ESSENTIAL OILS ON PATHOGENIC BACTERIA OF THE UROGENITAL TRACT

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ABSTRACT. Urinary tract infections (UTI) represent a significant cause of morbidity affecting individuals of all ages, including the pediatric population. In about 80% of cases, the cause of UTI in humans is *Escherichia coli*. In this paper, we have investigated the antibacterial effect of essential oils of ten species of plants on clinical isolates of pathogenic bacteria isolated from the pediatric population. The existing differences in the antimicrobial effect of essential oils were examined by the one-way ANOVA method, while Tukey's test was used to compare pairs. The results have shown that the essential oil of *Origanum vulgare* L. had the strongest antimicrobial activity (> 25 mm). The weakest effect was shown by the oil of *Chamomilla recutita* (L.) Raushert, which showed the antimicrobial effect only towards *E. coli*. Based on the results, we can conclude that essential oils can be a natural alternative to the use of antibiotics.

Keywords: essential oils, pathogenic bacteria, urogenital tract.

INTRODUCTION

Urinary tract infections (UTI) represent a significant cause of morbidity affecting people of all ages, as well as the pediatric population (TORO *et al.*, 2005; NABTI *et al*., 2019a). In about 80% of cases, the cause of UTI in humans is *Escherichia coli* (NABTI *et al.*, 2019b), while in the remaining percentage is caused by the bacteria *Proteus mirabilis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterococcus* spp., *Enterobacter* spp., Group B *Streptococcus* and *Staphylococcus saprophyticus* (FOXMAN, 2002). Uropathogenic bacteria contain virulence factors that enable them to resist various defense mechanisms of the host. Biofilm is responsible for the long-term persistence of bacteria in the urogenital tract. A significant number of studies have shown that biofilm-forming bacteria are more resistant to antimicrobial agents than planktonic bacteria (TABIBIAN *et al.*, 2008; LAGHA *et al*., 2019).

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Leading mechanisms of antibiotic resistance of the biofilm include exopolysaccharide (EPS) diffusion barrier formation, horizontal gene transfer, physiological latency of persistent cells, pH change, and quorum sensing (SHARMA *et al.*, 2019; ABEBE, 2020). It has been found that *E. coli* has a tendency to form microcolonies in the bladder membrane, favouring in this way the microorganism's resistance to the host's immune response and leading to the resistance to antibacterial drugs. It has been proved that *E. coli* has the ability to horizontally transfer resistance genes through biofilm formation (LEVIN-REISMAN *et al.*, 2017). After *E. coli* and *K. pneumoniae, P. mirabilis* is the most common cause of complicated urinary tract infections and one of the most common causes of catheter-related bacterial infections (HASAN *et al*., 2020). The most important virulence factor is swarming, which directly affects the spread of infection in parts of the urinary tract (KUAN *et al*., 2014). It has been shown that the more *P. mirabilis* swarms, the more virulence expression increases (KOTIAN *et al*., 2020). One of the bacteria that shows increasing multidrug resistance, thus becoming resistant to the latest lines of antibiotics, such as carbapenems and colistin, is *Enterobacter* spp. (LE-HA *et al*., 2019). Pathogenicity of *Enterobacter* spp. is dependent on the presence of multiple virulence genes such as operative adhesins through fimbriae, O antigens, K capsular antigens, serum resistance, production of hemolysis and others (DOUGNON *et al*. 2020). Due to their ability to transfer or acquire resistance genes through chromosomal exchanges, *Enterococcus* spp. can cause an increase in dangerous hospital infections with limited therapeutic options. Their virulence depends on surface factors that promote colonization in host cells, as well as on proteins secreted by cells, which damage tissues (CHAJĘCKA-WIERZCHOWSKA *et al*., 2017).

During the recent decades, an increasing number of studies have focused on searching for new compounds with potential antimicrobial activity. A review of recent literature indicates the importance of use of essential oils, known for their pharmacological properties, including antibacterial and antifungal activity. Currently, about 3000 essential oils are known, of which 300 are commercially available in the food and pharmaceutical industry (BAKKALI *et al.,* 2008). The bactericidal activity of essential oils, as well as their ability to interfere with bacterial replication, have encouraged their use in the pharmaceutical, agricultural and food industries (PANDEY *et al.,* 2022).

Essential oils are mixtures of volatile compounds of low molecular weight and are products of secondary metabolism of aromatic plants. They show numerous biological properties, such as anti-inflammatory, antimicrobial, antiviral or antioxidant activities (CAMERO *et al.,* 2019). Recent studies have confirmed the antibacterial effect of essential oils on *Staphylococcus aureus* (XIAO *et al.,* 2020), *Enterobacter aerogenes, Klebsiella oxytoca, P. mirabilis* and *E. coli* (PEREIRA *et al.,* 2004).

The antimicrobial activity of essential oils varies and depends on their chemical composition, concentration and climate where the plants grow (PERRINO *et al*., 2021). The effects of essential oils on bacteria are multiple, as some of the components affect the lipid bilayer of the cell membrane, and others affect negatively the cell cycle of bacteria and inhibit protein synthesis and DNA replication. Gram-negative bacteria are more resistant to essential oil than gram-positive bacteria (NAZZARO *et al.,* 2013). Essential oils break down relatively quickly in the mammalian body and show low toxicity, which makes them safe for use (HUMA *et al.,* 2014).

The aim of this research was to examine the potential antibacterial activity of the essential oils of medicinal and spice plants collected in the region of Southeastern Serbia, on bacteria associated with urinary tract infection in the pediatric population.

MATERIALS AND METHODS

Plant material

The aerial parts of the plants used were collected in Southeastern Serbia in 2023, during the flowering period of the plants. An overview of the sites where the plants were collected, along with data on geographic location and altitude, is given in Table 1. 500 g of fresh plant material was collected from each species.

The collected plant material was pressed, and the plant specimens were deposited in the herbarium collection of the Department of Agricultural and Food Studies in Prokuplje. Relevant, dichotomous keys were used to identify the plant material (TUTIN *et al.*, 1964-1980; JOSIFOVIĆ, 1970-1980). The nomenclature of the recorded taxa was harmonized with the Euro+Med database.

The herb was air-dried immediately after harvesting in a shady site for 15 days, packed in a paper bag and kept in a dark, dry and cool place. The dry plant material was comminuted by a mill. Hydrodistillation process was performed using a Clevenger-type apparatus. Pure essential oils were kept in dark glass ampoules at +4°C (European Pharmacopoeia, 2013.)

Table 1. An overview of plant collection sites, with data on geographic location and altitude

No	Plant name	Locality	Longitude	Latitude	Altitude (m)
1.	*Lavandula angustifolia* Mill.	Mala Plana	21° 28′ 23″	43° 15′ 6″	280
2.	*Anethum graveolens* L.	Prekopuce	21° 25′ 6″	43° 16′ 21″	345
3.	*Allium cepa* L.	Prekopuce	21° 25′ 6″	43° 16′ 21″	345
4.	*Origanum vulgare* L.	Rastovnica	21° 36′ 11″	43° 12′ 08″	516
5.	*Thymus serpyllum* L.	Rastovnica	21° 36′ 11″	43° 12′ 08″	516
6.	*Mentha spicata* L.	Niška banja	22° 00′ 29″	43° 17’ 37”	248
7.	*Chamomilla recutita* (L.) Raushert	Niška Banja	22° 00’ 29”	43° 17’ 37”	248
8.	*Salvia officinalis* L.	Gornja Trnava	21° 31′ 19″	43° 16’ 35”	383
9.	*Urtica dioica* L.	Gornja Trnava	21° 31′ 19″	43° 16’ 35”	383
10.	*Petroselinum crispum* (Mill.) Fuss	Mala Plana	21° 28′ 23″	43° 15′ 6″	280

Disk - diffusion method

The analyses in question dealt with isolating and identifying the bacterial pathogens that cause urinary infections. The research was conducted in the microbiological laboratory "Živković", based on the instructions given by a primary health care pediatrician. Only urine samples from children up to twelve years of age were considered in this paper.

When it comes to sampling urine from children, two basic methods of sampling are used: sampling the mid-stream urine during spontaneous urination and collection of urine using a sterile plastic bag. The number and type of bacteria was determined by the standard urine culture protocol. A positive result is one in which pathogenic bacteria are found in significant numbers in the sample (≥ 100,000 CFU/mL), while less than that is considered contamination (KARAH *et al.*, 2020). At least 15 µL of urine was used for analysis. In order to identify the organism causing UTI, it was necessary to inoculate the samples on the appropriate substrates, after which the material was incubated for 24 hours. Isolates were identified to the species or genus level, based on growth on selective and differential media.

Clinical isolates from the urogenital tract of children under the age of 12, *E. coli*, *Proteus* spp., *Enterobacter* spp., *Enterococcus* spp., were used to determine the antimicrobial

activity of plant essential oils. A turbidity suspension of 0.5 McFarland containing 1.5 x 10^8 CFU/mL was made from overnight cultures of tested strains of microorganisms grown on nutrient agar (NCCLS - National Committee for Clinical Laboratory Standards, 2003). On sterile Müller-Hinton agar (Torlak) substrates, 0.1 mL of all prepared microorganism suspensions were inoculated. Sterile cellulose discs with a diameter of 6 mm (HiMedia, Milano, Italy) were placed on the inoculated surface of the agar plate. The discs were impregnated with 1 μL of solutions of essential oils. A disc with gentamicin (10 μg/mL) was used as a positive control. Petri dishes were incubated at 37 °C for 24 hours. The absence of bacterial growth results in a transparent halo around the disc that represents the zone of inhibition. The zone of inhibition was read in mm. The larger the diameter, the more sensitive the strain is to a given essential oil. All growth inhibition assays were performed in triplicate.

Statistical analysis

One-way ANOVA was used for statistical analysis of the obtained results. If the existence of significant differences between groups was detected, post-hoc tests were used to determine which groups are significantly different. Tukey's test was used to compare all possible pairs of group means and to determine whether the differences between them are statistically significant.

RESULTS AND DISCUSSION

In this paper, we investigated the antibacterial effect of essential oils of ten types of plants on clinical isolates of pathogenic bacteria isolated from the pediatric population. The results of testing of the antimicrobial effect of essential oils are presented in Table 2.

The essential oil of *O. vulgare* L. showed the strongest antimicrobial activity (> 25 mm). The essential oil of *T. serpyllum* L., beside *O. vulgare* L., is the only oil with an inhibition zone greater than 20 mm. The weakest effect was shown by *C. recutita* (L.) Raushert oil, which showed an antimicrobial effect only against *E. coli*, with an inhibition zone of 7.63 mm. Also, the essential oils of *S. officinalis* L. and *U. dioica* L. did not show antimicrobial activity against *Enterobacter* spp.

Most of the tested oils showed bacteriostatic activity using the disk diffusion method. *O. vulgare* L. showed the strongest antimicrobial effect against *E. coli*, while *A. cepa* L. showed the weakest effect.

The essential oils of *O. vulgare* L. and *T. serpyllum* L. oils showed significant zones of inhibition on both *Proteus* species ($p<0.05$). The essential oil of the plant *C. recutita* (L.) Raushert showed no inhibitory effect on either *P. vulgaris* or *P. mirabilis*. *A. cepa* L. oil had the weakest effect on *P. vulgaris*, while the zone of inhibition was absent in *P. mirabilis*. The essential oil of *P. crispum* (Mill.) Fuss showed the strongest inhibitory effect with a mean zone of inhibition of 12.10 mm. The oils of *C. recutita* (L.) Raushert, *S. officinalis* L. and *U. dioica* L. did not show any inhibitory effect against *Enterobacter* spp. In the case of *Enterococcus* spp., *C. recutita* (L.) Raushert did not show an inhibition zone, while *T. serpyllum* L. had the strongest effect with an inhibition zone of 18.80 mm. *O. vulgare* L. essential oil has shown the strongest antimicrobial effect (12.10 – 25.47 mm), while the oil of *C. recutita* (L.) Raushert has shown the weakest antimicrobial effect.

Tabela 2. Antibacterial activity of essential oils of selected plant species expressed as diameter inhibition zones

Bacteria	Plant species	Inhibition zones (mm)*
E. coli	*L. angustifolia* Mill.	11.27 ± 0.87
	A. graveolens L.	13.77 ± 0.25
	A. cepa L.	7.10 ± 0.36
	O. vulgare L.	25.47 ± 1.95
	T. serpyllum L.	23.97 ± 1.04
	M. spicata L.	19.20 ± 0.79
	C. recutita (L.) Raushert	7.63 ± 0.71
	S. officinalis L.	8.60 ± 0.40
	U. dioica L.	9.07 ± 0.12
	P. crispum (Mill.) Fuss	19.67 ± 1.61
Proteus vulgaris	*L. angustifolia* Mill.	8.43 ± 0.60
	A. graveolens L.	13.03 ± 0.40
	A. cepa L.	6.67 ± 0.29
	O. vulgare L.	19.00 ± 1.25
	T. serpyllum L.	19.17 ± 0.29
	M. spicata L.	16.13 ± 0.12
	C. recutita (L.) Raushert	/
	S. officinalis L.	7.37 ± 0.12
	U. dioica L.	8.47 ± 0.06
	P. crispum (Mill.) Fuss	16.17 ± 0.29
Proteus mirabilis	*L. angustifolia* Mill.	7.87 ± 0.12
	A. graveolens L.	11.33 ± 0.76
	A. cepa L.	/
	O. vulgare L.	19.93 ± 0.51
	T. serpyllum L.	19.07 ± 1.51
	M. spicata L.	15.77 ± 0.75
	C. recutita (L.) Raushert	/
	S. officinalis L.	7.10 ± 0.36
	U. dioica L.	7.83 ± 0.29
	P. crispum (Mill.) Fuss	15.60 ± 0.53
***Enterobacter* spp.**	*L. angustifolia* Mill.	8.33 ± 0.29
	A. graveolens L.	10.13 ± 0.93
	A. cepa L.	/
	O. vulgare L.	12.10 ± 0.69
	T. serpyllum L.	11.43 ± 0.12
	M. spicata L.	10.57 ± 1.67
	C. recutita (L.) Raushert	/
	S. officinalis L.	/
	U. dioica L.	/
	P. crispum (Mill.) Fuss	12.90 ± 0.36
***Enterococcus* spp.**	*L. angustifolia* Mill.	12.50 ± 0.50
	A. graveolens L.	10.40 ± 0.53
	A. cepa L.	6.97 ± 0.47
	O. vulgare L.	16.60 ± 1.06
	T. serpyllum L.	18.80 ± 1.21
	M. spicata L.	10.60 ± 0.17
	C. recutita (L.) Raushert	/
	S. officinalis L.	6.83 ± 0.29
	U. dioica L.	8.93 ± 0.40
	P. crispum (Mill.) Fuss	15.03 ± 0.42

* Mean value ± standard deviation (three replicates); / - not actvity.

The results have shown that there are statistically significant differences between the essential oils in relation to their effect on each of the microorganisms. So, in the case of *E. coli, O. vulgare L.* and *T. serpyllum* L. oils have the strongest effect with no significant difference. What is particularly interesting is that the oils of *M. spicata* L. and *P. crispum* (Mill.) Fuss act without significant difference on *E. coli, P. vulgaris* and *P. mirabilis*. *T. serpyllum* L. had the strongest effect on *Enterococcus* spp. while the oils of *P. crispum* (Mill.) Fuss and *O. vulgare* L. act without a significant difference. Although *Enterobacter* spp. shows the highest resistance to essential oils, the oils that had effect on it (*M. spicata* L., *T. serpyllum* L., *O. vulgare* L. and *P. crispum* (Mill). Fuss acted without significant difference.

SOKOVIĆ *et al.* (2007) showed that the essential oils with the best antibacterial activity in the disc-diffusion method were the oils of *Thymus vulgaris* L. (16.0-30.0 mm) and *O. vulgare* L. (20.0-35.0 mm). Also, in the work of SOKOVIĆ *et al.* (2007) the oil of *M. spicata* L. showed an effect on uropathogenic bacteria, which is in accordance with our results, while the antimicrobial effect of the *L. angustifolia* Mill. oil proved to be weak. In a study by JIANU *et al.* (2013), the antimicrobial activity of the oil of *L. angustifolia* Mill. on *E. coli* proved to be satisfactory. CHELARU *et al.* (2023) confirmed the strongest antimicrobial effect of *Mentha piperita* L., *O. vulgare* L. and *T. vulgaris* L. oils on uropathogenic strains of *E. coli*. The positive antibacterial effect of *Mentha pulegium* L. oil on *P. mirabilis* was confirmed by ZANJANI *et al.* (2015). The results of testing the effect of *A. cepa* L. oil on *E. coli* and *Enterococcus* spp. are in accordance with the research results of several authors (ZIARLARIMI *et al.*, 2011; YE *et al*., 2013; SADEGHIAN *et al.,* 2020). Our work showed good results in relation to the effect of *P. crispum* (Mill.) Fuss oil on *E. coli* and *Enterococcus* spp., but that is in contradiction with other authors, which can be explained by the fact that the plants were harvested in different areas and have different concentrations of antimicrobial components (NAWEL *et al.,* 2014). In relation to that, the work of DIMKIĆ *et al.* (2021) proved a good antimicrobial effect of parsley against *Enterococcus* species, both alone and in synergy with other plants. *A. graveolens* L. oil has been used in aromatherapy since ancient times, and recent studies confirm its antibacterial effect (BADAR *et al.,* 2008; RASHEED *et al.,* 2010; JIANU *et al.,* 2012). The essential oil of *U. dioica* L. showed better activity against gram-positive bacteria in comparison to gram-negative bacteria (MODARRESI-CHAHARDEHI *et al.,* 2012). As for our work, it proved to have best effect against *E. coli*, which is in contrast to the research of RAMTIN *et al.* (2014) in which it showed best effect against *E. faecalis*.

CONCLUSION

This research was conducted in order to conclude which essential oils of the certain types of plants from the area of southwestern Serbia show the best antimicrobial effect. The tested plant species showed different antimicrobial effects on selected bacteria. Further research on the effectiveness of essential oils in terms of bactericidal effect can be a significant help in the synthesis of natural antimicrobial drugs.

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