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Antimicrobial Activity of three Essential Oils against Several Human Pathogens

Anesa Dzaferovic[1], Teofil Gavric[1], Simin Hagh Nazari[2], Monika Stojanova[3], Muamer Bezdrob[1], Blazo Lalevic[4*], Saud Hamidovic[1]

[1] The University of Sarajevo, Faculty of agricultural and food sciences, Sarajevo, Bosnia, and Herzegovina
[2] The University of Zanjan, Faculty of agriculture, Zanjan, Iran
[3] University Ss. Cyril and Methodius, Faculty of agricultural sciences and food, Skopje, North Macedonia
[4] The University of Belgrade, Faculty of agriculture, Belgrade - Zemun, Serbia

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Abstract

Essential oils are products derived from various parts of plants. These products have therapeutic, pharmacological and antimicrobial properties against human pathogens. In this paper, the impact of clove, spruce and pine essential oils against *Escherichia coli*, *Salmonella* spp. and *Staphylococcus aureus* was determined using disc diffusion method. Liquid bacterial inoculum was streaked onto Muller-Hinton agar using the sterile swab. Antimicrobial activity was estimated by measurement of inhibition zone around the discs previously impregnated by essential oil and placed onto agar. Results showed that the antimicrobial activity of essential oils depended on the type of oil and bacterial species. The significantly highest diameter of inhibition zone against tested bacteria was recorded using clove essential oil, whilst the lowest diameter was noticed using pine essential oil. Clove oil was most effective against *Staphylococcus aureus*, whilst spruce and pine oil against *Salmonella* spp. compared to other bacteria. Strong positive correlation between the effect of the spruce oil against *Escherichia coli* and *Staphylococcus aureus*, spruce and with clove oil against *Staphylococcus aureus* was recorded. The results of this research indicate the possible application of essential oil of clove against human pathogens.

Keywords: antimicrobial activity, essential oil, human pathogens

1 Introduction

Essential (volatile) oils are natural extracts obtained from plants and have been commonly used since the 16th century [1]. These plants have medicinal interests and have received great attention due to low toxicity and pharmacological properties [2]. Besides, ancient reports showed that essential oils may be used in control of microorganisms, including Gram-positive and Gram-negative bacteria [3], as well as for treatment of several diseases, such as diabetes, cancer, cardiovascular diseases, etc. [4].

The antimicrobial activity of essential oils has been well documented [5]. Unfortunately, some synthetic drugs may not be used in control of human pathogens [6]. Furthermore, multidrug resistance of main human pathogens, such as *Pseudomonas aeruginosa*, *Salmonella* spp., *Staphylococcus aureus*, *Enterococcus* sp. and *Escherichia coli* is well described [7].

In few last decades, the resistance of antimicrobial drugs led to the development of techniques for the production of novel compounds, which may inhibit the growth of human pathogenic bacteria [8]. One of most efficient methods is the use of plant extracts and essential oils containing novel antibacterial properties with potential use as food preservatives and alternatives to treat infections [9, 10].

Several types of research have addressed the antimicrobial activity of essential oils produced by conifers, such as *Picea* [11, 12] and *Pinus* [13]. Odugbemi [14] found similar properties using clove (*Syzygium aromaticum*) essential oil.

Although the impact of essential oil on human pathogens have been studied worldwide [15], in Bosnia and Herzegovina only a few reports have addressed the influence of essential oils on pathogenic bacteria. This paper aimed to determinate the antimicrobial activity of spruce (*Picea* sp.), pine (*Pinus* sp.) and clove (*Syzygium aromaticum*) essential oils.

2 Material and methods

Flower buds of clove were obtained from local distributer and used for extraction. The essential oil of clove

Corresponding author: Blazo Lalevic, University of Belgrade, Faculty of agriculture, Department of environmental microbiology, Nemanjina 6, 11080 Belgrade-Zemun, Serbia, Email: blazol@agrif.bg.ac.rs.

was produced using distillation technique by Clevenger apparatus. *Picea* sp. and *Pinus* sp. essential oils were obtained from a local distributor. In this research, two bacteria from family *Enterobacteriaceae* (*Escherichia coli*, and *Salmonella* spp.) and one member of family *Staphylococcaceae* (*Staphylococcus aureus*) were used. Pure cultures of bacteria originated from the collection of Department of microbiology at Faculty of agriculture and food sciences (Sarajevo, Bosnia, and Herzegovina).

Antimicrobial activity of essential oils was determined using paper-disc diffusion method [16]. Pure bacterial cultures were grown on Muller-Hinton agar plates at 37°C for 24h. Colonies of bacteria were transferred into sterile normal saline. Bacterial inoculum was streaked onto Muller-Hinton agar using the sterile swab. Six sterile filter discs (diameter 6mm, Whatman paper No. 3) were previously impregnated by essential oil (10 µl/disc) and placed onto Muller-Hinton agar. Incubation was performed in an incubator at 37°C for 16-24h. Antimicrobial activity was estimated by measurement of the inhibition zone around the discs. All experiments were carried out in triplicate. The occurrence of an inhibition zone > 18 mm was characterized as good antimicrobial activity, 16-18 mm as an average activity, 14-16 as moderate activity, and 10-14 mm as low activity. Presented data are statistically processed with ANOVA test using SPSS 20 package program.

3 Results and discussion

Our results showed that antimicrobial activity of essential oils depended on the type of oil and bacterial species (Table 1, 2 and 3). From Table 1 it is obvious that clove essential oil has the highest zone of inhibition against *Escherichia coli* compared to other essential oils (average 26.7 mm). Antibacterial activity of spruce essential oils can be classified as average, whilst pine oil has moderate antibacterial activity. Based on the statistical analysis, can be seen that the effect of clove oil against *Escherichia coli* has a statistically significant difference (p<0.05) compared to the determined effects of spruce and pine oil. There is no statistically significant difference between the effect of spruce and pine oil.

Table 1: The diameter of inhibition zone (mm) of clove, spruce and pine essential oils against *Escherichia coli*

Essential oil	Clove	Spruce	Pine
	Diameter of inhibition zone (mm)		
Disc 1	26	21	17
Disc 2	25	15	12
Disk 3	29	14	14
Disk 4	25	18	13
Disk 5	27	15	15
Disk 6	28	13	14
Average	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$
	26.7 ± 1.63^a	16.0 ± 2.96^b	14.2 ± 1.72^b

$^{a, b}$ – Values marked with different letters have a statistically significant difference between the examined essential oils (p<0.05)

Similar results were obtained for selected oils and antimicrobial activity against *Staphylococcus aureus* (Table 2). The more pronounced activity of all tested essential oils against *S. aureus* compared to *E. coli* was noticed. As previously, the activity of clove oil against *S. aureus* can be characterized as good, which is significantly different (p<0.05) from spruce and pine oil, whilst both of them have average antibacterial activity.

Table 2: The diameter of inhibition zone (mm) of clove, spruce and pine essential oils against *Staphylococcus aureus*

Essential oil	Clove	Spruce	Pine
	Diameter of inhibition zone (mm)		
Disc 1	30	20	15
Disc 2	28	14	16
Disk 3	26	15	15
Disk 4	27	18	14
Disk 5	31	17	20
Disk 6	26	15	16
Average	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$
	28.0 ± 2.10^a	16.5 ± 2.26^b	16.0 ± 2.10^b

$^{a, b}$ – Values marked with different letters have a statistically significant difference between the examined essential oils (p<0.05)

As can be seen from Table 3, highest antimicrobial activity against *Salmonella* spp. was obtained using clove oil (diameter of inhibition zone was 27.7 mm), whilst another essential oil has a lower diameter of inhibition zone. However, the antibacterial activity of all tested oils can be classified as good. According to that, a statistically significant difference (p<0.05) between the effect of all the examined essential oils against *Salmonella* spp. was determined.

Table 3: The diameter of inhibition zone (mm) of clove, spruce and pine essential oils against *Salmonella* spp.

Essential oil	Clove	Spruce	Pine
	Diameter of inhibition zone (mm)		
Disc 1	30	20	18
Disc 2	27	28	19
Disk 3	25	26	19
Disk 4	29	20	19
Disk 5	28	19	19
Disk 6	27	20	18
Average	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$
	27.7 ± 1.75^a	22.2 ± 3.82^b	18.7 ± 0.52^c

$^{a, b, c}$ – Values marked with different letters have a statistically significant difference between the examined essential oils (p<0.05)

Obviously, there is no statistically significant difference in the effect of the clove oil against three determined pathogenic bacteria (Table 4). Nevertheless, there is a statistically significant difference (p<0.05) in the effect of spruce and pine oil against *Salmonella* spp., compared to that against *Escherichia coli* and *Staphylococcus aureus.* Results presented in Table 5 showed that there is a strong positive correlation (r= +.896*) between the effect of the spruce oil against *Escherichia coli* and *Staphylococcus aureus*. This oil has a strong positive correlation (r= +.847*) with clove oil against *Staphylococcus aureus*, too. On the other hand, the effect of spruce oil against *Staphylococcus aureus* has a strong positive correlation (r= +.860*) with the effect of clove oil against *Salmonella* spp.

Several previous researchers suggest that eugenol is a major component of clove essential oil [17]. Eugenol belongs to the phenolic compounds. These compounds have antiseptic, antimicrobial and antioxidant properties [18]. Hamad et al. [19] detected β-caryophyllene as a constituent of clove essential oil, which also posses antibacterial influence [20]. According to Hamad et al. [19], clove essential oil showed strong antimicrobial activity against *S. aureus*, which is confirmed in this research. On the other hand, inhibition of *E. coli* growth was not detected in the same research, which is in contrast with our results. Ayoola et al. [18] found that the antimicrobial activity of clove oil was most expressed against *S. aureus*.

Our study showed that the highest antimicrobial activity of spruce oil was detected against *Salmonella* spp. These results are in accordance with observations of Radulescu et al. [21]. Several studies have addressed the presence of antibacterial compounds in spruce essential oil, such as p-cymene [22], limonene [23], camphene [24], etc. Nevertheless, the efficiency of spruce essential oils against human pathogens was not reported in several kinds of research. Canilac and Mourey [25] found that coliform bacteria were more resistant in the presence of essential oil of *Picea excelsa*. On the other hand, Tanase et al. [26] reported that spruce extract may be effective against *E. coli*. Monoterpenes compounds in *Pinus* sp. essential oil are known to have antimicrobial properties [27]. Antimicrobial activity of various pine essential oils is well described [13].

Table 4: Statistical analysis of average values from the effects of the examined essential oils against three different pathogens

Essential oil	nn	Clove	Spruce	Pine
		$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD
Escherichia coli	66	26.7 ± 1.63^{a}	16.0 ± 2.96^{a}	14.2 ± 1.72^{a}
Staphylococcus aureus	66	28.0 ± 2.10^{a}	16.5 ± 2.26^{a}	16.0 ± 2.10^{a}
Salmonella spp.	66	27.7 ± 1.75^{a}	22.2 ± 3.82^{b}	18.7 ± 0.52^{b}

$^{a, b}$ – Values for the essential oils marked with different letters have a statistically significant difference between the examined pathogens (p<0.05)

Table 5: Correlation between the effects of examined essential oils against three different pathogens

		Clove *E. coli*	Spruce *E. coli*	Pine *E. coli*	Clove *S. aureus*	Spruce *S. aureus*	Pine *S. aureus*	Clove *Salmonella* spp.	Spruce *Salmonella* spp.	Prine *Salmonella* spp.
Clove *E.coli*	Pearson Correlation	1	(.578)	.237	(.350)	(.325)	.175	(.676)	.011	(.158)
	Sig. (2-tailed)		.230	.651	.496	.529	.740	.140	.984	.765
	N		6	6	6	6	6	6	6	6
Spruce *E.coli*	Pearson Correlation		1	.548	.482	.896*	(.354)	.847*	(.336)	(.261)
	Sig. (2-tailed)			.260	.333	.016	.492	.033	.515	.617
	N			6	6	6	6	6	6	6
Pine *E. coli*	Pearson Correlation			1	.554	.746	.166	.486	(.583)	(.600)
	Sig. (2-tailed)				.254	.089	.753	.328	.224	.208
	N				6	6	6	6	6	6
Clove *S. aureus*	Pearson Correlation				1	.549	.636	.599	(.375)	.000
	Sig. (2-tailed)					.259	.174	.209	.464	1.000
	N					6	6	6	6	6
Spruce *S. aureus*	Pearson Correlation					1	(.127)	.860*	(.685)	(.343)
	Sig. (2-tailed)						811	.028	.134	.506
	N						6	6	6	6
Pine *S. aureus*	Pearson Correlation						1	(.054)	(.250)	.185
	Sig. (2-tailed)							.918	.633	.726
	N							6	6	6
Clove *Salmonel la* spp.	Pearson Correlation							1	(.648)	(.369)
	Sig. (2-tailed)								.164	.472
	N								6	6
Spruce *Salmonel la* spp.	Pearson Correlation								1	.440
	Sig. (2-tailed)									.383
	N									6
Prine *Salmonel la* spp.	Pearson Correlation									1
	Sig. (2-tailed)									
	N									

* Correlation is significant at the 0.05 level (2-tailed).

In our research, the various antibacterial activity of pine oil was noticed, which corroborate with previous findings [28,29]. Ait Mimoune et al. [30] reported that *S. aureus* was one of the most sensitive bacteria against pine essential oil, which is confirmed in this study.

4 Conclusion

The results of the present research have shown that essential oils of clove, spruce and pine inhibit the growth of *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* spp. Clove oil was most effective against *Staphylococcus aureus*, whilst spruce and pine oil against *Salmonella* spp. compared to other bacteria. In all treatments, clove oil showed the highest inhibition of bacterial growth compared to other oils. Thus, the results of current research indicate the possible application of essential oil of clove in medicinal protocol against human pathogens.

Ethical issue

Authors are aware of, and comply with, best practice in publication ethics specifically with regard to authorship (avoidance of guest authorship), dual submission, manipulation of figures, competing interests and compliance with policies on research ethics. Authors adhere to publication requirements that submitted work is original and has not been published elsewhere in any language.

Competing interests

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

Authors' contribution

All authors of this study have a complete contribution for data collection, data analyses and manuscript writing.

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