Imamović, B., Komlen, V., Gavrić, T., Sunulahpašić, A., Lalević, B., Hamidović, S. (2021): Antimicrobial activity of ginger (Zingiber officinale) and rosemary (Rosmarinus officinalis) essential oils. Agriculture and Forestry, 67 (1): 231-238.

DOI: 10.17707/AgricultForest.67.1.19

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ANTIMICROBIAL ACTIVITY OF GINGER (*Zingiber officinale*) AND ROSEMARY (*Rosmarinus officinalis*) ESSENTIAL OILS

SUMMARY

New advances in the food industry are directed towards exploiting natural resources. Nowadays, essential oils and their antimicrobial activities are the subject of many researches. Their possible use as natural food additives is particularly prominent. This study analyzed the influence of ginger and rosemary oil on the growth of pure bacterial culture using the disk diffusion method. *Escherichia coli, Staphylococcus aureus* and *Salmonella spp*. were used as test organisms for antimicrobial susceptibility testing.

The results showed that both types of oil inhibit bacterial growth, although inhibition rate varies between different bacterial species. It certainly depends on the type of plant used for oil extraction. Study has shown that ginger and rosemary oil can potentially be used in treating diseases caused by these bacteria.

Keywords: antimicrobial activity; ginger (*Zingiber officinale* Roscoe), human pathogens, rosemary (*Rosmarinus officinalis* L.).

INTRODUCTION

In the last decades, the interest in essential oils has been increased (Chouhan *et al*., 2017). They have been used for centuries in natural medicine (Boesl and Saarinen, 2016), mainly due to the large number of biologically active phytochemicals in their composition. Many essential oils are claimed to have antimicrobial activity and are used for the prevention and treatment of many infectious diseases as alternative medicines (Stea *et al*., 2014). The food industry

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Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

Recieved:21/01/2021 *Accepted:22/03/2021*

offers a wide range of products that have antimicrobial activity. Preventing microbial proliferation and protecting food from oxidation is a burning issue. Therefore researches have been directed towards essential oils and plant extracts, as potential natural additives in food (Bellou *et al*., 2016). Fresh and dry roots of ginger (*Zingiber officinale* Roscoe) contain essential oil (ginger oil) and oleoresin (ginger extract). The benefits of ginger oil mainly come from mono- and sespe-terpenoids such as neral, geranium, 1,8-cineol, α-zingiberen, β-bisabolene and β-sesquiphellandrene (Shirooye *et al*., 2016). It also contains a-pinen, B-pinen, stone, linalool, borneol, y-terpineol, nerol, geranol and geranyl acetate (Ekundayo *et al*., 1988). Ginger oil consists of 90% sesquiterpenes, which are responsible for anti-inflammatory, antibacterial and other medicinal properties. Research has shown that rosemary (*Rosmarinus officinalis* L.) is rich in volatile oils, flavonoids and phenolic acids, which are characterized by antiseptic and antiinflammatory effect (Haida *et al*., 2015). The leaf extract showed an extremely high antioxidant activity (Monino *et al*., 2008).

Many higher plant species have been used for spice and medical application; however, recent researches have addressed the aplication of essential oils and spice extracts in control of diseases caused by pathogens (Cui *et al*., 2018; Teles *et al*., 2019) and improvement of food safety. Antunes *et al*. (2012) found that essential oils inhibit synthesis of macromolecules in pathogenic bacteria. Taking into account the expansed amount of antibiotic-resistant microbes, it is necessairy to find novel antipathogenic agents and assess their ability to protect food products from spoilage (Wang *et al*., 2020).

Although the impact of ginger and rosemary oils in the suppression of human pathogen`s growth is well documented, only a few reports in Bosnia and Herzegovina have addressed the influence of essentials oils on human pathogens (Dzaferovic *et al*., 2019; Gavric *et al*., 2018). Thus, the main focus of this paper was to determine the impact of ginger and rosemary essential oils on three human pathogens.

MATERIAL AND METHODS

The study analyzed the effect of ginger and rosemary oil on the growth of bacteria from the *Enterobacteriaceae* (*Escherichia coli* and *Salmonella spp*.). and the *Staphylococcaceae* family (*Staphylococcus aureus*). Oil extraction from rosemary and ginger leaves and root was carried out in the Microbiology Laboratory at the Faculty of Agriculture and Food Sciences in Sarajevo. The oils were obtained by distillation technique using Clevenger apparatus. Antimicrobial susceptibility testing of essential oils on the growth of *S. aureus*, *E. coli*, and *Salmonella spp*., was done using the disk diffusion method. This method is based on the diffusion of oil through a filter paper applied on the surface of the sown substrate. Mueller-Hinton's (MH) agar was used as the experimental medium. 18-hour cultures of *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922) and *Salmonella spp.* were used for the experiment, which were seeded in a nutrient medium and incubated at 37°C. Petri dishes with MH medium were inoculated with 0.1 ml of bacterial suspension. Filter paper sterile disks with a 6 mm diameter were placed on the surface of prepared Petri dishes using aseptic technique. Each disc was then immersed with 10 µl of essential oil. The experiment was performed in six replications. Petri dishes were incubated for 24 h at 37 ºC. The results were examined after 24 hours by measuring the diameter of inhibition zones. Afterward, the effect of the essential oils was determined. Statistical analysis was done using ANOVA and Bivariate Pearson Correlation test in SPSS v24 programme.

RESULTS AND DISCUSSION

Research was done in order to analyze the antimicrobial activity of ginger and rosemary oil on three human pathogens using the disk diffusion method. The results have been established using the standard values for the inhibition zones of gentamicin and chloramphenicol antibiotics for *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella spp.* (Table 1).

Table 1: Standard values for the inhibion zones of gentamicin and chloramphenicol antibiotics for *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* spp.

Human pathogens	Antibiotics	Inhibition zone (mm)		
		S	I	R
Escherichia coli	Gentamicin	≥15	13-14	≤12
	Chloramphenicol	≥18	13-17	≤12
Staphylococcus aureus	Gentamicin	≥15	13-14	≤12
	Chloramphenicol	≥18	13-17	≤12
Salmonella spp.	Gentamicin	≥17	13-16	≤14
	Chloramphenicol	≥17	/	≤17

Legend: S – sensitive; I – moderately sensitive; R – resistant

For *E. coli*, ginger oil has an average inhibition zone of 23.67 mm and therefore belongs to S category (sensitive) compared to both types of antibiotics. Correspondingly with the results, ginger oil shows a strong antimicrobial and therapeutic effect. Rosemary also shows an inhibitory effect on *E. coli* whose inhibition zones diameter has an average value of 17.33 mm (Table 2), and therefore belongs to S category (sensitive) in comparison with gentamicin antibiotic. *E. coli* is sensitive to gentamicin and correspondingly the rosemary oil has a very therapeutic effect. In comparison with the chloramphenicol, this oil belongs to the I category (moderately sensitive) which leads to the conclusion that *E. coli* is sensitive to rosemary oil and has the same effect as the aforementioned antibiotic in an increased dosage. Ginger essential oil has a strong inhibitory effect on *S. aureus* with an average value of the inhibitory zone of 23.17 mm, which is included in category S (sensitive) for both types of antibiotics.

This oil has a strong potential to inhibit the growth of *S. aureus* and act therapeutically.

Table 2. Impact of ginger and rosemary essential oil on growth of *Escherichia coli, Staphylococcus aureus and Salmonella spp.*

Human pathogen	Essential oil	Inhibition zone (mm)						
		Discs						AVG ±SD
		1	2	3	4	5	6	
E. coli	Ginger	20	22	25	28	22	25	23.67±2.87
	Rosemary	17	23	12	23	12	17	17.33±4.93
S. aureus	Ginger	20	18	24	26	26	25	23.17±3.37
	Rosemary	20	20	21	23	15	10	18.17±4.79
Salmonella spp.	Ginger	20	19	25	28	23	24	23.17±3.31
	Rosemary	20	25	20	19	20	26	21.67±3.01

Legend: AVG – average; SD - standard deviation

Table 3. Comparison of differences in the antimicrobial activity of ginger and rosemary essential oils between three human pathogens

ANOVA		Sum of Squares	df	Mean Square	F	Sig.
E. coli	between groups	120.333	1	120.333	7.398	.022
	within groups	162.667	10	16.267		
	total	283.000	11			
S. aureus	between groups	75.000	1	75.000	4.369	.063
	within groups	171.667	10	17.167		
	total	246.667	11			
Salmonella spp.	between groups	6.750	1	6.750	.674	.431
	within groups	100.167	10	10.017		
	total	106.917	11			

Rosemary oil has an average inhibition zone of 18.17 mm (Table 2) and therefore belongs to category S (sensitive) when it comes to both types of antibiotics. This indicates the sensitivity of *S. aureus* to this essential oil, and we can assume that it can be used in therapy.

Ginger oil has a strong inhibitory effect on *Salmonella* spp., with its average value of 23.17 mm (Table 2), which defines it as S (sensitive) compared to both types of antibiotics. This oil has a strong potential to inhibit the growth of *Salmonella* spp. and act therapeutically. Rosemary oil with an average 21.67 mm of inhibition zone belongs to the S (sensitive) category when it comes to both types of antibiotics, meaning *Salmonella* spp. is sensitive to this oil and will act in therapy. Using the ANOVA test, we compared the antimicrobial activity of ginger and rosemary oil among three bacterial species tested (Table 3). According to the obtained results ginger and rosemary oil have a statistically different effect only on *E.coli* (p=0.022<0.05). For *S.aureus* and *Salmonella spp*., ginger and rosemary oil did not show any significant difference in antimicrobial activity (*S.aureus* p=0.063>0.05, *Salmonella spp.* p=0.431>0.05).

Table 4. Results of the correlation test

Correlations							
		Ginger	Rosemary	Ginger	Rosemary	Ginger	Rosemary
		E. coli		*S. aureus*		*Salmonella*	
Ginger *E.coli*	Pearson Correlation	1	.249	.646	.135	.910*	-.108
	Sig. (2-tailed)		.634	.165	.798	.012	.839
	N	6	6	6	6	6	6
Rosemary *E.coli*	Pearson Correlation	.249	1	-.389	.353	-.053	.279
	Sig. (2-tailed)	.634		.445	.493	.920	.593
	N	6	6	6	6	6	6
Ginger *S.aureus*	Pearson Correlation	.646	-.389	1	-.287	.857*	-.348
	Sig. (2-tailed)	.165	.445		.582	.029	.499
	N	6	6	6	6	6	6
Rosemary *S.aureus*	Pearson Correlation	.135	.353	-.287	1	.111	-.619
	Sig. (2-tailed)	.798	.493	.582		.834	.190
	N	6	6	6	6	6	6
Ginger *Salmo-nella*	Pearson Correlation	.910*	-.053	.857*	.111	1	-.415
	Sig. (2-tailed)	.012	.920	.029	.834		.414
	N	6	6	6	6	6	6
Rosemary *Salmo-nella*	Pearson Correlation	-.108	.279	-.348	-.619	-.415	1
	Sig. (2-tailed)	.839	.593	.499	.190	.414	
	N	6	6	6	6	6	6

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

Correlation can take on any value in the range [-1, 1]. The sign of the correlation coefficient indicates the direction of the relationship, while the magnitude of the correlation (how close it is to -1 or +1) indicates the strength of the relationship. According to the correlation results (Table 4), there is a strong positive correlation (r= +.910*) between the effect of the ginger oil against

Escherichia coli and *Salmonella spp.* Also, the effect of ginger oil on *S. aureus* and *Salmonella spp*. have a strong positive correlation as well (r=+.857*).

Several researches have addressed the mechanisms of action of essential oils and their compounds. Gutierrez *et al*. (2008) found that monoterpenes and phenols exist in rosemary essential oil are responsible for antimicrobial activity. Rosemary essential oil is more effective against *E. coli* and *S. aureus* compared to *Pseudomonas* spp. (De Martino *et al*., 2009), which is in accordance with our results. Furthermore, rosemary essential oil showed more expressed antibacterial activity against *Salmonella* spp. compared with *E. coli*. Similar observation was noticed by Ouwehand *et al*. (2010). Genena *et al*. (2008) found that Gram-positive *S. aureus* was more sensitive to rosemary essential oil, which is in accordance with our results. Ojeda-Sana *et al*. (2013) reported that the cell wall structure of Gram-negative bacteria blocks the absorption of components of oils, which explain the resistance of these bacteria.

In previous studies, ginger essential oil also showed high efficiency in the suppression of the growth of pathogenic bacteria. Hanan *et al*. (2016) found that an increase of concentration of ginger essential oil has an inhibitory effect on the growth of *Salmonella typhimurium*, *E. coli*, *S. aureus*, and *Bacillus cereus*. As shown previously, the antimicrobial activity of ginger essential oil may be due to gingerols and phenolic components (Mascolo *et al*., 1989). In contrast, Indue *et al*. (2006) reported the absence of antimicrobial activity of ginger extracts against *E.coli* and *Salmonella spp*.

CONCLUSIONS

The disk diffusion method confirmed that ginger and rosemary oil has antimicrobial activity and that in certain conditions they prevent the growth of some human pathogens. Ginger oil showed to be more effective on the growth of *E. coli* compared to rosemary oil. Results for antimicrobial activity of ginger oil did not show any major differences between different bacterial species tested. This confirms that ginger oil has a very strong antimicrobial and therapeutic effect. Based on the results, rosemary oil has pronounced antimicrobial activity and strong therapeutic effect, especially when it comes to *Salmonella spp* and *S. aureus* while for *E. coli* it has to be used in a higher dosage than standard to demonstrate its therapeutic effect.

ACKNOWLEDGEMENTS

This study was supported by the Ministry of Education, Science and Technological development of the Republic of Serbia (grant number 451-03-9/2021-14/200116).

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