INHIBITORY ACTIVITY OF ESSENTIAL OILS ON *STAPHYLOCOCCUS AUREUS*

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Abstract. *Staphylococcus aureus is one of the most resistant Gram-positive bacteria, capable of producing serious infections difficult to treat. The purpose of this paper is to show the inhibitory property of some essential oils against this species. The antibacterial action of herbal essential oils has been extensively studied but their effectiveness differs depending on the origin of the essential oil, the extraction method, and the plant parts from which the extraction was made. The essential oils used in this study are widely marketed oils from different producers. Overall, 19 essential oils were tested: oregano, garden thyme, wild thyme, lavender, peppermint, basil, rosemary, fennel, cumin, black cumin, dill, garden sage, grapefruit, bay laurel, patchouli, and tea tree. The bacteria tested for sensitivity to these essential oils was Staphylococcus aureus, obtained from the "Horia Cernescu" Research Laboratory Complex of the "King Michael I of Romania" University of Agricultural Sciences and Veterinary Medicine of Banat in Timisoara, Romania. The method used was the Kirby-Bauer diffusion method, commonly used to test the antibiotic sensitivity of various bacteria. The culture medium used to grow bacteria was supplemented with blood. For the interpretation of the results, a classification of essential oils was carried out according to the inhibition zone diameter (IZD). For oils with an IZD larger than 20 mm, Staphylococcus aureus has been characterized as highly sensitive; for a diameter between 15 and 19 mm, it has been characterized as very sensitive; in the case of diameters of 9-14 mm, it has been called sensitive; it was considered non-sensitive for diameters smaller than 8 mm. Thus, oregano, garden thyme, wild thyme, clove, and vervain oils have been classified in the extremely sensitive category. Bay laurel, patchouli, peppermint, and tea tree oils have been classified as highly sensitive, and lavender, basil, dill, rosemary, garden sage, rose geranium, and cumin oils have been classified as sensitive.*

Keywords: *Staphylococcus aureus, essential oils, diffusion method, inhibition zone.*

INTRODUCTION

Over time, scientists have been looking for new ways to emerge victorious in the "fight" against bacteria. Thus, in 1928, Alexander Fleming discovered penicillin, bringing science back to the fore. The same Alexander Fleming, a year later, was among the first to mention that many bacteria were already resistant to the drug he had discovered. In 1945, during an interview with the New York Times, Fleming warned of the side effects that inappropriate use of penicillin may have. At that time, 14% of *Staphylococcus* bacteria were resistant to penicillin, five years later the percentage would rise to 50% and, in 1955, it would reach 95% (BUHNER, 2014).

The genus *Staphylococcus* comprises about 50 species, of which *S. aureus*, also known as the "queen of resistant bacteria" (BUHNER, 2014); it is one of the most important pathogens, which constantly evolves through the mutation and absorption of movable genetic elements, which give it increasing strength and virulence (BAGNOLI *ET AL.*, 2017).

Depending on the strain, *S. aureus* produces a wide variety of toxins capable of directly harm the host cell. These are toxins that affect the membrane (haemolysis and leukocidins), toxins that interfere with the function of the receptor, but do not affect the membrane; at the same time, *S. aureus* secretes enzymes that are capable of degrading the host molecules or affecting the defence mechanisms of the host cell (FETSCH, 2018).

The purpose of this paper is to test the inhibitory property of 19 herbal essential oils against the *S. aureus* bacterium. The essential oils used are widely marketed oils from different producers. In total, 19 essential oils were tested: oregano, garden thyme, wild thyme, lavender, peppermint, basil, rosemary, fennel, cumin, black cumin, dill, garden sage, grapefruit, bay laurel, patchouli, and tea tree.

As regards the current knowledge of the antibacterial action of these oils, there are several studies in which their efficacy has been tested, but the results differ depending on the origin of the essential oil, the extraction method, and the parts of the plant from which the extraction was carried out. In some cases, essential oils may come from several species of the same botanical genus, difficult to differentiate when plants are harvested from spontaneous flora (IMBREA *ET AL.*, 2009, 2010).

For example, from a 2014 study from Romania, which balances the antimicrobial activity of garden thyme essential oil on some bacteria and fungi, it appears that garden thyme oil inhibits the growth of *Escherichia coli*, *S. aureus*, *Salmonella typhium*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Klebsiella pneumoniae* (BORUGĂ *ET AL.*, 2014). Vervain oil has also been shown to be effective in inhibiting the development of Gram-positive bacteria (*Bacillus cereus*, *E. faecalis* and *S. aureus*) (DE MARTINO *ET AL.*, 2009). Another relevant study showed that rosemary essential oil, along with other oils, contributes to the weakening of the cell walls of antibiotic-resistant bacteria to facilitate the penetration of antibiotics (YAP *ET AL.*, 2014).

MATERIAL AND METHODS

The bacteria tested for sensitivity to these essential oils is *Staphylococcus aureus* Rosenbach 1884, obtained from the "Horia Cernescu" Research Laboratory Complex at the Banat University of Agricultural Sciences and Veterinary Medicine "King Mihai I of Romania" in Timisoara.

Essential oils are oils purchased commercially from various manufacturing companies. In total, 19 essential oils were used, as shown in Table 1.

Table 1

Herbal essential oils used to test the sensitivity of *S. aureus*

Essential oil		Plant	
Romanian name	**English name**	**Botanical name**	**Botanical family**
Oregano	Oregano	*Origanum vulgare* L.	*Lamiaceae*
Cimbru	Garden thyme	*Thymus vulgaris* L.	*Lamiaceae*
Cimbrișor	Wild thyme	*Thymus serpyllum* L.	*Lamiaceae*
Lavandă	Lavender	*Lavandula angustifolia* Mill.	*Lamiaceae*
Mentă	Peppermint	*Mentha x piperita* L.	*Lamiaceae*
Busuioc	Basil	*Ocimum basilicum* L.	*Lamiaceae*
Rozmarin	Rosemary	*Rosmarinus officinalis* L.	*Lamiaceae*
Salvie	Garden sage	*Salvia officinalis* L.	*Lamiaceae*
Patchouli	Patchouli	*Pogostemon cablin* (Blanco) Benth.	*Lamiaceae*

Fenicul	Fennel	*Foeniculum vulgare* Mill.	*Apiaceae*
Chimion	Cumin	*Cuminum cyminum* L.	*Apiaceae*
Mărar	Dill	*Anethum graveolens* L.	*Apiaceae*
Chimen negru	Black cumin	*Nigella sativa* L.	*Ranunculaceae*
Dafin	Bay laurel	*Laurus nobilis* L.	*Lauraceae*
Grapefruit	Grapefruit	*Citrus x paradisi* Macfad.	*Rutaceae*
Verbină	Vervain	*Verbena officinalis* L.	*Verbenaceae*
Geranium	Rose geranium	*Pelargonium graveolens* L'Hér.	*Geraniaceae*
Cuișoare	Clove	*Syzygium aromaticum* (L.) Merrill & Perry	*Myrtaceae*
Melaleuca	Tea tree	*Melaleuca alternifolia* (Maiden & Betche) Cheel	*Myrtaceae*

The method used was the Kirby-Bauer test diffusion method. This is a method often used in microbiology laboratories to test the antibiotic sensitivity of various bacteria. The culture medium used to grow bacteria was agar supplemented with blood.

In the first work phase, the sown of the bacterial strain was carried out on the solid medium, using a single-use handle with the technique of sowing by exhaustion. After inoculation, Petri plates were left at room temperature for 5 minutes, which helped to absorb the inoculum into the environment. Filter paper discs, 6 mm in diameter, were placed at a distance of at least 25 mm and at least 15 mm from the edge of the plate. Once the discs were placed, the essential oil, 10 µl, was added to each disc on the plate. In our case, the maximum number of discs added to a plate was four. The plates prepared in this way were incubated for 24 hours at 37ºC.

All tests and interpretation of the results were carried out in the laboratories of the "Horia Cernescu" Research Laboratory Complex. For the interpretation of the results, after incubation, the reading of the IZDs was carried out by measuring with a ruler. The diameter of the disc is also included in the final value.

In order to form as clear a conclusion as possible, a classification of essential oils according to the IZD was made (Figure 1). Thus, for oils with an IZD greater than 20 mm, *S. aureus* was characterized as extremely sensitive; a diameter between 15 and 19 mm was characterized as very sensitive; and diameters 9-14 mm were called sensitive; were considered non-sensitive diameters less than 8 mm (BABU *ET AL.*, 2011).

Extremely sensitive: IZD > 20 mm	Very sensitive: IZD 15-19 mm	Sensitive: IZD 9-14 mm	Non-sensitive: IZD < 8 mm

Figure 1. Classification scale by IZD

RESULTS AND DISCUSSION

Essential oils have proven to be a good source of bioactive compounds with antibacterial, antifungal, antiviral, and antioxidant properties (BASER & BUCHBAUER, 2010). Essential oils are concentrated natural extracts obtained from plants, used since ancient times and, sometimes, used inappropriately or in excess, especially in recent years. With the advent of more and more publications, volatile or essential oils have begun to be used in the production of perfumes, food, OR beverages (ANDREI *ET AL.*, 2018; MAN *ET AL.*, 2019).

Given the growing popularity of commercially available essential oils as well as of the curative claims attributed to them, in this study was tested the effectiveness of 19 essential oils against the *S. aureus* bacterium.

In the first stage, we carried out the tests on a Petri plate (Table 2), for the oils of garden thyme, wild thyme, vervain, bay laurel, basil, dill, rosemary, garden sage, rose geranium, fennel, black cumin, and grapefruit. Subsequently, for a higher measurement accuracy, we used two plates for oregano, garden thyme, cloves, tea tree, peppermint, lavender, and patchouli (Table 3).

Table 2

Results of tests carried out on a single Petri plate

Essential oil	**Inhibition zone diameter - *S. aureus* (mm)**
Garden thyme	47
Wild thyme	30
Vervain	21
Bay laurel	19
Basil	11
Dill	10
Rosemary	10
Garden sage	10
Rose geranium	10
Cumin	10
Fennel	8
Black cumin	8
Grapefruit	8

Thus, according to our classification, *S. aureus* was characterized as highly sensitive to garden thyme (47 mm), wild thyme (30 mm), and vervain (21 mm) oils, as highly sensitive to bay laurel (19 mm) oil, as sensitive to basil (11 mm), dill (10 mm), rosemary (10 mm), garden sage (10 mm), rose geranium (10 mm), and cumin (10 mm) oils, and non-sensitive to fennel (8 mm), black cumin (8 mm), and grapefruit (8 mm) oils (Figure 2).

Extremely sensitive: IZD > 20 mm	Very sensitive: IZD 15-19 mm	Sensitive: IZD 9-14 mm	Non-sensitive: IZD < 8 mm
Garden thyme	Bay laurel	Basil	Fennel
Wild thyme		Dill	Black cumin
Vervain		Rosemary	Grapefruit
		Garden sage	
		Rose geranium	
		Cumin	

Figure 2. Classification by results obtained on a Petri plate

The effect of some essential oils and their inhibition zones are shown in Figures 3-5.

Figure 3. IZD for garden thyme (47 mm)

Figure 4. IZD for wild thyme (300 mm)

Figure 5. IZD for vervain (21 mm)

Oregano ranks first from the results on two plates with an average of 27.5 mm IZDs, followed by garden thyme with 29 mm, and by clove with 22.5 mm, classified in the highly sensitive category. There was also good inhibitory activity in tea tree (18 mm), patchouli (17 mm), and peppermint (15.5 mm) oils, which fall into the highly sensitive category. Lavender oil (12.5 mm), classified as sensitive, has significantly reduced inhibitory activity compared to garden thyme, oregano, and clove oil (Table 3, Figure 6).

Table 3

Results from testing on two plates

Essential oil	**IZD *S. aureus* I (mm)**	**IZD *S. aureus* II (mm)**	**Average IZD (mm)**
Garden thyme	23	35	29
Oregano	25	30	27.5
Clove	20	25	22.5
Tea tree	11	25	18
Patchouli	16	18	17
Peppermint	18	13	15.5
Lavender	10	15	12.5

Extremely sensitive: IZD > 20 mm	Very sensitive: IZD 15-19 mm	Sensitive: IZD 9-14 mm	Non-sensitive: IZD < 8 mm
Garden thyme	Tea tree	Lavender	
Oregano	Patchouli		
Clove	Peppermint		

Figure 6. Classification of oils by results obtained on two plates

The effect of some essential oils and their IZDs are shown in Figures 7-9.

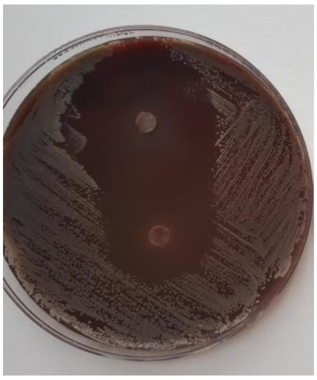

Figure 7. IZDs for garden thyme (top) and clover (bottom)

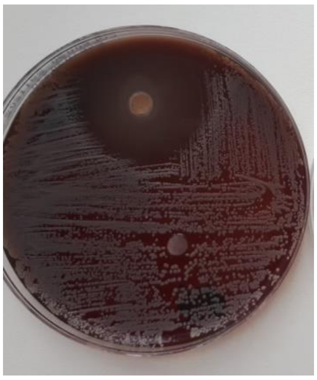

Figure 8. IZD for oregano

Figure 9. IZDs for peppermint, patchouli, tea tree (from top to bottom)

Worldwide, infections caused by antibiotic-resistant bacteria, including some strains of *S. aureus*, have become endemic, with outbreaks acquired by medical facilities frequently reported, posing an increasing threat, with significant clinical consequences for treatment options. The World Health Organisation has decided, since 2001, to give high priority to measures aimed at slowing down the occurrence of antibiotic-resistant bacteria (MUNTEAN *ET AL.*, 2019).

The results obtained by us indicate good inhibitory effects in the case of essential oils of garden thyme, wild thyme, oregano, clove, and vervain, classified in the category Extremely Sensitive. Compared to the oils of bay laurel, tea tree, patchouli, and peppermint, the bacteria turned out to be very sensitive, according to the classification presented above. For oils of basil, dill, rosemary, garden sage, rose geranium, cumin, and lavender, IZDs between 9 and 14 mm were measured, which ranked the oils in the Sensitive category. No effect or with small IZDs were fennel, black cumin, and grapefruit; for these, it cannot be said that the species have no antibacterial effect at all, but only that the oils had no significant inhibitory effect.

Compared to another study, the results obtained with oregano and garden thyme coincide with the results obtained by MAN *ET AL.* (2019). In their article, the antimicrobial activity of several essential oils on several human pathogens, including *S. aureus*, was studied, with oregano having the best inhibitory response against all bacteria studied (MAN *ET AL.*, 2019).

On the other hand, the inhibition zone of peppermint and lavender essential oils is quite close, in value, to the results reported by ROHRAFF & RODERICK (2014). Thus, they reported 19.5 mm in the case of peppermint oil (15.5 mm was the average in the study presented here) and 13 mm for lavender (12.5 mm, in the study presented here) (ROHRAFF & RODERICK, 2014).

Also, XU *ET AL.* (2016) showed that clove oil had high antibacterial activity against *S. aureus*, which was also confirmed in the study presented here.

CONCLUSIONS

Tests carried out on the bacterium *S. aureus* using commercially available herbal essential oils were carried out according to the Kirby-Bauer diffusion method.

The results obtained point to good inhibitory effects in the case of essential oils of garden thyme, wild thyme, oregano, clove, and vervain, classified in the category Extremely Sensitive. Compared to the oils of bay laurel, tea tree, patchouli, and peppermint, the bacteria turned out to be Very Sensitive, according to the classification presented here. For oils of basil, dill, rosemary, garden sage, rose geranium, cumin, and lavender, IZDs between 9 and 14 mm were measured and ranged in the Sensitive category.

No effect or with small IZDs were fennel, black cumin, and grapefruit; for these, it cannot be said that the species have no antibacterial effect at all, but only that the oils used had no significant inhibitory effect.

Therefore, herbal essential oils can be used in different therapeutic variants to treat infections with *S. aureus*, in both human medicine and veterinary medicine, to reduce the amount of antibiotics used excessively for farm animals.

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