IN VITRO STUDY OF ANTIMICROBIAL ACTIVITY OF COMMERCIAL ESSENTIAL OILS OF THE LAMIACEAE FAMILY AGAINST STAPHYLOCOCCUS AUREUS ATCC 29213, ESCHERICHIA COLI ATCC 25922 AND CANDIDA ALBICANS ATCC 10231

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Abstract: The aim of this study is to evaluate the potential antimicrobial activity of four commercial essential oils: *Thymus vulgaris* and *Origanum vulgare* (two for external use and two for internal one). The antimicrobial activity of the tested essential oils was determined by using the serial two-fold dilutions method. The following bacterial and yeast strains were purchased and used in the study: *E. coli, S. aureus, and C. albicans*. The minimal inhibitory concentration (MIC) of Thyme oil (external use) against *E. coli,* and *C. albicans* was 2 µg/ml and 4 µg/ml, respectively. The observed minimum bactericidal concentration (MBC) for Thyme oil (external use) against *S. aureus* was 8 µg/ml. The Thyme oil for internal use demonstrated higher antimicrobial activity than the topical form. The evaluated MBC against *S. aureus* was 4 µg/ml, and for *E.coli,* and *C. albicans,* it was even lower than 1 µg/ml. While determining the MBC of Oregano oil (external) against *S. aureus, E. coli,* and *C. albicans*, antimicrobial activity is reported as : 16 µg/ml against *S. aureus*, 4 µg/ml for *E.coli* and 32 µg/ml against *C. albicans.* Also, the observed antimicrobial activity of Oregano oil for internal use is higher than external form – 2 µg/ml against *S. aureus* and lower than 1 µg/ml for the other pathogens. The results of MIC and MBC determination of Thyme and Oregano oils showed that *E. coli, S. aureus* and *C. albicans* demonstrate high sensitivity and can be used in therapeutic practice, and some of them probably in combination with other antimicrobial agents.

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Introduction

Antibiotic resistance is a global health problem. In 2020, Europe's 18% decline in antibiotic consumption signals a positive development. Covid 19 mitigation strategies including wearing masks, hand hygiene, and social distancing are primary responsible for the decline. However, Bulgaria is the only country in the European Union reporting an increase in antibiotic consumption, approximately 30% in its community sector. Overconsumption has been observed in macrolide and fluoroquinolone antibiotic classes. Also, Bulgaria reports over 100% increase in the use of azithromycin in the community sector (Bozukova, 2021). The pathogens causing resistant infections thrive in the community and hospital sector, putting all patients at risk, regardless of the severity of their medical condition. Widespread and overuse of antibiotics result in the emergence and spread of resistant pathogens, involving the transfer of bacteria and genes between humans, animals, and the environment (Kraemer et al., 2019), (Prestinaci et al., 2015), (Avershina et al., 2021). Therefore, new antimicrobial agents to overcome this resistance need to be discovered. Over the past few decades, scientists have focused on designing, developing, and producing therapeutic agents that do not cause resistance and can be used to treat infections caused by resistant pathogens. Medicinal plants are the sources for discovering new drugs. By synthesizing specific substances, plants are easily protected from various enemies such as insects and microorganisms. In addition, they maintain their normal growth and development by producing secondary antimicrobial metabolites (Al-Mariri & Safi, 2013).

The *Lamiaceae family* is one of the most diverse and common plant families in terms of traditional medicine in Bulgaria. Its medicinal value is primarily based on the composition of volatile oils. Essential oils are volatile fractions derived from medicinal and aromatic plants, and they have been tested over time as alternative remedies for many infections and as natural food preservatives (Moumni et al., 2020).

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In 2019, six pathogens were responsible for more than 250 000 deaths associated with AMR – *E. coli, S. aureus, K. pneumoniae, S. pneumoniae, A. baumannii*, and *P. aeruginosa* (Murray et al., 2022). The essential oil plants of *Lamiaceae 's family,* like oregano and thyme, etc., possessed significant antibacterial and antifungal activities against resistant microbial isolates and referent strains such as G (-) – *A. baumannii, K. pneumoniae, E. coli, P. aeuruginosa*; G(+) – *S. aureus* and fungus *Tr. Rubium* and *C. albicans* (Chouhan et al., 2017), (Fournomiti et al., 2015). In addition, thyme and oregano essential oils exhibit strong antioxidant properties (Kulisic et al., 2005), (Amiri, 2012), (Rodriguez-Garcia et al., 2016).

The active components found in the plants of *Lamiaceae family* are carvacrol and thymol. They are cyclic organic compounds containing a methyl group, a hydroxyl group, and an isopropyl group. These two compounds are structurally different from each other according to the position of the hydroxyl group. Carvacrol contains a hydroxyl group in its ortho position of its benzene ring, while, thymol contains a hydroxyl group in the meta position of the benzene ring (Li et al., 2018).

Aim

The aim of this study is to evaluate the potential antimicrobial activity of four commercial essential oils of *Thymus vulgaris* and *Origanum vulgare* (two for external use and two for internal one).

Materials and methods

The antimicrobial activity of Thyme and Oregano (two for external use and two for internal one) essential oils was determined using a serial two-fold dilutions method. The following bacterial and yeast strains were purchased and used in the study: *Escherichia coli, Staphylococcus aureus, and Candida albicans*. The study started with a concentration of 1024 µg/ml of essential oils dissolved in DMSO, and the lowest testing concentration was 1 µg/ml. For MIC determination, the inoculum was prepared using 4-6 h of a broth culture of each microbial strain, adjusted to a turbidity equivalent to a 0.5 McFarland standard, and diluted in Brain heart infusion broth to give the concentration of ≈106 cfu/mL for each strain. Two-fold serial dilutions of oregano were prepared in BHI broth in eleven tubes starting from 1024 mg/ml. Microbial inoculums in volume of 0.1 ml were added to each tube. The samples with *E. coli.* and *S. aureus* were incubated at 37°C for 24 h with *C. albicans* – 35°C/48 hours. The lowest concentration that prevented visible growth was taken as the MIC.

After determining the MICs of the tested solutions from all tubes, in which no visual turbidity was reported, bacterial seeds were made on Blood agar, and the samples were incubated under the same conditions as MICs determination. The lowest concentration at which bacterial growth is inhibited to 99.9% is reported as the minimum bactericidal concentration (MBC).

Statistical Analysis

The data were presented as means ± standard deviation. The mean separations were analyzed using Student's t-test and one-way analysis of variance (One-Way ANOVA) using Tukey's test; Differences were considered statistically significant at $P < 0.05$.

Results and Discussion

The MICs of Thyme oil (external use) against *S. aureus, E. coli,* and *C. albicans* was 8, 2, and 4 µg/ml, respectively. The Thyme oil for internal use demonstrated higher antimicrobial activity than the external form. It showed activity against *S.aureus, E. coli,* and *C. albicans* strains with the best results, with MICs ranging from 2 to 1 µg/ml ($P<0.05$). IN addition, the Thyme oil (internal use) inhibited the growth in the three strains analyzed *S.aureus, E. coli,* and *C. albicans* with MICs 2, 1, and 1 µg/ml. The findings for Thyme oils (internal and external use) are summarized in Table 1.

Meanwhile, the Oregano oil (external use) was active against *S.aureus, E. coli,* and *C. albicans* with showing a MICs of 1, 2 and 4 µg/ml, respectively (Table 2).

After MICs were determined, a volume equal to one bacterial loop was taken from each test tubes and transferred to Mueller-Hinton agar for MBC determination. The observed MBC for Thyme oil (external use) against *S. aureus* was 8 µg/ml. The evaluated MBC against *E. coli*, and *C. albicans* was 2 and 8 µg/ml, respectively. The obtained data for MBC of Thyme oil (internal use) are as follows: 4 µg/ml for *S. aureus* and lower than 1 µg/ml for *E.coli* and *C. albicans* ($P<0.05$). When determining the MBC of Oregano oil (external) against *S. aureus, E. coli, and C. albicans,* antimicrobial activity is reported as follows: 16 µg/ml against *S. aureus,* 4 µg/ml for *E.coli* and 32 µg/ml against *C. albicans*. Also, the observed antimicrobial activity of Oregano oil for internal use is higher than external form – 2 μg/ml against *S. aureus* and lower than 1 μg/ml for the other pathogens (Table 2). The evaluated data for MBC of Oregano oils (internal and external use) are statistically significant against *S. aureus* and *C. albicans* (P<0.01).

Table 1. Statistical data of MIC and MBC of Thyme oils

	Thymus vulgaris essential oil (internal use)	**Thymus vulgaris essential oil (external use)**	P-value	**Thymus vulgaris essential oil (internal use)**	**Thymus vulgaris essential oil (external use)**	P-value
	MIC (μg/ml)	**MIC (μg/ml)**		**MBC (μg/ml)**	**MBC (μg/ml)**	
S.aureus ATCC 29213	2	8	P<0.05*	4	8	P<0.05*
E. coli ATCC 25922	1	2	P>0.05	1	2	P>0.05
C. albicans ATCC 10231	1	4	P>0.05	1	4	P>0.05

Source: Authors

Table 2. Statistical data of MIC and MBC of Oregano oils

	Origanum vulgaris essential oil (internal use)	**Origanum vulgaris essential oil (external use)**	P-value	**Origanum vulgaris essential oil (internal use)**	**Origanum vulgaris essential oil (external use)**	P-value
	MIC (μg/ml)	**MIC (μg/ml)**		**MBC (μg/ml)**	**MBC (μg/ml)**	
S.aureus ATCC 29213	2	1	P>0.05	2	16	P<0.01
E. coli ATCC 25922	1	2	P>0.05	1	4	P>0.05
C. albicans ATCC 10231	1	4	P>0.05	1	32	P<0.01

Source: Authors

Table 3. Determination of MIC and MBC of Thyme and Oregano essential oils

	S. aureus* *ATCC 29213		***E. coli* *ATCC 25922***		***C. albicans* *ATCC 10231***	
	MIC **(μg/ml)**	MBC **(μg/ml)**	MIC **(μg/ml)**	MBC **(μg/ml)**	MIC **(μg/ml)**	MBC **(μg/ml)**
Thymus vulgaris essential oil (internal use)	2 (SD±0.58)	4 (SD±0.58)	1 (SD±0.58)	1 (SD±0.58)	1 (SD±0.58)	1 (SD±0.00)
Thymus vulgaris essential oil (external use)	8 (SD±1.00)	8 (SD±1.00)	2 (SD±0.58)	2 (SD±0.58)	4 (SD±0.58)	4 (SD±0.58)
Origanum vulgaris essential oil (internal use)	2 (SD±0.58)	2 (SD±0.58)	1 (SD±0.58)	1 (SD±0.58)	1 (SD±0.00)	1 (SD±0.00)
Origanum vulgaris essential oil (external use)	≤ 1 (SD±0.58)	16 (SD±0.58)	2 (SD±0.58)	4 (SD±0.58)	4 (SD±0.58)	32 (SD±0.58)

Source: Authors

The antimicrobial activity of the Thyme essential oil has been evaluated in other studies. Ivanovic et al. (Ivanovic, 2012) reported significant activity of essential oil of thyme against *E. coli*, and *Salmonella* strains, with MIC of 640 μg mL^{-1}. An important role of bacteriostatic and bactericidal activity of the essential oils of thyme and oregano against E. coli O157:H7 isolated from bovine feces has also been observed (Burt & Reinders, 2003). The essential oil of thyme has also shown activity against yeasts susceptible and resistant to antifungal drugs (Pozzatti et al., 2010), (Santurio et al., 2014).

The results of MIC and MBC determination of Thyme and Oregano oils showed that *E. coli, S. aureus* and *C. albicans* demonstrate high sensitivity and can be used in therapeutic practice, and some of them probably in combination with other antimicrobial agents. The obtained data for MICs and MBC are presented in Table 3.

Conclusion

Antimicrobial drug resistance can be prevented in several ways, and using essential oils with intrinsic antimicrobial properties has proven to be a relatively effective method. The observed data in our study showed that Thyme and Oregano oils demonstrate high sensitivity against *E. coli, S. aureus,* and *C. albicans*. A combinational approach that allows additive interaction between essential oils and conventional narrow spectrum antibiotics is a reasonably effective method in the fight against antimicrobial resistance. Future explorations of synergistic or additive therapies could lead to the delivery of multi-target activity, and reduced hospital stays, respectively, low-cost therapy.

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