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CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF *OCIMUM BASILICUM L., SALVIA OFFICINALIS L., ROSMARINUS OFFICINALIS L.,* AND *ALLIUM SATIVUM L*. ESSENTIAL OILS

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ABSTRACT

The study aimed to determine the chemical composition and antimicrobial activity of basil (Ocimum basilicum L. var. Genovese), sage (Salvia officinalis L.), rosemary (Rosmarinus officinalis L.), and garlic (Allium sativum L) essential oils (EOs). The extraction was made by hydrodistillation (Clevenger apparatus). Compound identification was performed using gas chromatography–mass spectrometry (GC–MS). The antimicrobial activity was tested on Gram-positive bacteria (Staphylococcus aureus, Listeria monocytogenes, Bacillus cereus, Bacillus subtilis) and Gram-negative bacteria (Escherichia coli, Enterococcus faecalis, Salmonella typhimurium). The antimicrobial effect varied significantly, with sage and rosemary EOs showing a strong effect on bacteria, especially on E. faecalis, while garlic oil proved to have a much-reduced effect on all tested species.

INTRODUCTION

Aromatic plants, through their metabolism, have the ability to accumulate in their leaves, stems, seeds, or underground parts (bulbs, rhizomes) different low molecular weight compounds that can be extracted through hydrodistillation or other methods (Chan et al., 2009). Essential oils (EOs) from aromatic and medicinal plants are known to have antibacterial, antifungal, and antioxidant activities (Carović-Stanko et al., 2010; Macwan et al., 2013; Nazzaro et al., 2013; 2017). It has been demonstrated that most EOs act on the cell membrane of microorganisms (Goñi et al., 2009). On the other hand, Gram-negative bacteria are more resistant than Gram-positive bacteria to the action of EOs. This phenomenon can be easily explained by the chemical composition and different structure of the cell walls of Gram-positive and Gram-negative bacteria. Gram-positive bacteria allow hydrophobic molecules to penetrate the cell, acting on the cell wall or cytoplasm. These bacteria are very sensitive to the action of phenolic compounds present in EOs. High concentrations of these compounds lead to protein denaturation, while at low concentrations, they interfere with enzymes involved in energy production. The cell wall of Gram-negative bacteria has an outer membrane that allows hydrophilic molecules to enter the cell but is almost impermeable to hydrophobic molecules. However, the susceptibility of a species of microorganisms to the action of an EO cannot

be discussed, but rather the action of the compounds found in the oil on a strain belonging to a species of microorganisms. The compounds found in EOs differ depending on the species, the anatomical part of the plant, the time of harvest, and the extraction method. Numerous studies have been conducted to determine which compounds present in EOs have more pronounced antimicrobial activity, but this research must continue. The use of EOs extracted from aromatic plants in the food industry is limited because they can produce changes in aroma. The Food and Drug Administration (FDA) recognizes as safe the crude oils of cloves, oregano, thyme, nutmeg, mustard, and cinnamon (Kayode et al., 2018). Basil is an annual plant originating from tropical regions, found in Asia, Africa, Central and South America, and Europe (da Silva et al., 2022). Taxonomically, it belongs to the *Ocimum* genus, *Lamiaceae* family, *Lamiales* order, *Magnoliopsida* class, *Magnoliophyta* division, *Plantae* kingdom. Breeding work has resulted in varieties with mint, cinnamon, lemon, and rose aromas. Basil EO has been shown to have antimicrobial activity against Gram-positive bacteria such as *Bacillus cereus*, *Staphylococcus aureus*, and Gram-negative bacteria from the *Enterobacteriaceae* family, such as *Escherichia coli* and *Salmonella* spp. (Nazzaro et al., 2013; Burt et al., 2004; De Martinoet al., 2010; Ezeorba et al., 2024). Sage is a perennial plant native to the Mediterranean region. It belongs to the genus *Salvia*, *Lamiaceae* family, *Lamiales* order, *Magnoliopsida* class, *Magnoliophyta* division, *Plantae* kingdom. Sage EO has been widely studied for its antimicrobial properties. Studies have shown that sage EO exhibits significant antibacterial activity against various pathogenic bacteria, including *S. aureus*, *E. coli*, and *Salmonella* spp. (Al Bayati et al., 2024). Rosemary is a perennial herb native to the Mediterranean region. It belongs to the *Rosmarinus* genus, *Lamiaceae* family, *Lamiales* order, *Magnoliopsida* class, *Magnoliophyta* division, *Plantae* kingdom. Rosemary EO is known for its strong antibacterial, antifungal, and antioxidant properties. It has been shown to be effective against a wide range of bacteria, including *E. coli*, *S. aureus*, and *B. subtilis* (Benissa et al., 2024). Garlic is a species native to central Asia and northeastern Iran. It belongs to the *Allium* genus, *Amaryllidaceae* family, *Asparagales* order, *Monocotyledonatae* class, *Angiospermae* division, *Plantae* kingdom. Garlic oil's primary component is allicin, which has been shown to be highly effective against Gram-positive bacteria due to its ability to disrupt the bacterial cell membrane and inhibit enzyme activity. Studies have demonstrated that garlic oil exhibits antimicrobial activity against a variety of pathogenic bacteria, including *E. coli*, *S. aureus*, and *Salmonella typhimurium* (Dziri et al., 2013).

MATERIAL AND METHODS

2.1. Plant material

Basil (*Ocimum basilicum* L.), sage (*Salvia officinalis* L.), rosemary (*Rosmarinus officinalis* L.), and garlic (*Allium sativum* L.) plants were cultivated at the Banu Mărăcine Didactic Station of the University of Craiova (44°20'0" N 23°49'0" E) in the Oltenia region, Romania and were harvested in August 2023. The cultivation process followed all technological steps specific to each plant species, excluding disease and pest control treatments. EOs were extracted from the leaves of basil, sage, and rosemary, and matured bulbs of garlic. The plant materials were naturally dried in shaded, cool areas, and then ground for extraction.

2.2. Extraction of essential oils

The EOs were extracted through hydrodistillation using a Clevenger apparatus. 100 g of dried and ground plant material were mixed with 1000 mL of

distilled water (1/10, *w/w*) in a round-bottom flask. The mixture was heated, and the released EO was collected in a graded column after passing through a condenser. The distillation process lasted approximately three hours, yielding between 2–4 mL of EO per 100 g of dried leaves and bulbs. The collected EOs were stored at 2–4ºC in dark, tightly sealed bottles until use.

2.3. GC–MS analysis

The chemical composition of the EOs was analyzed using gas chromatography–mass spectrometry (GC–MS). The analysis was performed using a Thermo Scientific Focus GC coupled with a DSQ II mass detector. The column used was TraceGOLD TG-5SilMS (60 m × 0.25 mm × 0.25 µm). The initial oven temperature was set at 110 °C for two minutes, increased to 120 °C at 3 °C/min, held for three minutes, then increased to 140 °C at 3 °C/min, held for three minutes, and finally increased to 230 °C at 3 °C/min, held for seven minutes. The carrier gas was helium at a flow rate of 1.5 mL/min, with a split ratio of 1:100. The MS transfer line temperature was 250 °C, and the ion source temperature was 230 °C, with electron impact ionization at 70 eV. The mass range scanned was 35–350 *m/z*. All constituents' retention times (RTs) were meticulously recorded. The resulting mass spectra were then scrutinized and compared with reference spectra from the NIST Library 2020 to ensure accurate identification and quantification of each component (Adams, 2017). Each analysis was performed in triplicate.

2.4. Microorganisms

Cultures of *S. aureus* (ATCC 25923), *Listeria monocytogenes* (ATCC 13932), *B. cereus* (ATCC 11778), *B. subtilis* (ATCC 6633), *E. coli* (ATCC 25922), *Enterococcus faecalis* (ATCC 19433), *Salmonella typhimurium* (ATCC 14028), were used for antimicrobial testing.

2.5. MIC assay

Minimum inhibitory concentration (MIC) values were determined using the microdilution method in 96-well plates (Preuss et al., 2005). Microbial suspensions were adjusted to a turbidity of 0.5 McFarland units. Each well received 100 µL of sterile nutrient broth supplemented with 0.5% Tween 80 and 100 µL of undiluted essential oil. Serial dilutions were performed, and 10 µL of microbial suspension was added to each well. The plates were incubated at 37°C for bacteria and 25°C for yeast and molds, with incubation times extended for *L. monocytogenes*. After incubation, 20 µL of resazurin solution (0.2 mg/mL) was added to each well, and the plates were incubated for an additional two hours. Wells showing blue color indicated inhibition of microbial growth (MIC). Each microorganism was tested in triplicate.

2.6. Statistical analysis

The data were statistically analyzed using Statgraphics Centurion XVIII software. One-way analysis of variance (ANOVA) was used to determine significant differences between the means of the EOs and the seven pathogens tested, with $p<0.05$ indicating statistical significance. A least significant difference (LSD) test was performed to establish the statistical differences at the 0.05 significance level.

RESULTS AND DISCUSSIONS

3.1. Oil yield of the study plants

The oil yield of the plants taken in the study ranged from 1.4% for basil, to 2.4 % for rosemary. It should be noted that in the case of basil, sage and rosemary,

dried leaves were used in the shade, while crushed and dried bulbils were used for garlic. Table 1 shows the oil yield for the four plants taken in the study.

Table 1.

Yield in oil (ml/100 g dried herb) for basil, sage, rosemary and garlic

Plant	Basil	Sage	Rosemary	Garlic
Percentage of volatile oil extracted	1,4	2,2	2,4	2,2

3.2. GC–MS analysis

The GC–MS analysis (Figures 1–4) revealed the chemical composition of the EOs extracted from *O. basilicum*, *S. officinalis*, *R. officinalis*, and *A. sativum*. The identified compounds and their relative percentages are presented in Table 2 and Figure 1-4.

Table 2.

Chemical composition of basil, sage, rosemary and garlic EOs analyzed by GC–MS

Compound	RT (min)	Basil (%)	Sage (%)	Rosemary (%)	Garlic (%)
Diallyl sulfide	10.21	ND	ND	ND	0.529
β-Thujene	11.95	ND	0.544	ND	ND
Tricyclene	12.08	ND	ND	0.383	ND
α-Pinene	12.47	ND	1.804	21.703	ND
Allyl methyl disulfide	12.95	ND	ND	ND	4.414
Camphene	13.38	ND	3.057	7.523	ND
1,2,3,4,5,8-Hexahydronaphthalene	13.66	ND	ND	0.913	ND
Sabinene	14.32	ND	0.450	ND	ND
α-Myrcene	14.48	ND	1.538	2.528	ND
β-Pinene	14.54	0.271	5.685	0.427	ND
3-Hexyl hydroperoxide	14.60	1.621	ND	ND	ND
2-Hexyl hydroperoxide	14.98	1.414	ND	ND	ND
1-Octen-3-ol	15.54	ND	0.428	0.247	ND
Dimethyl trisulfide	15.76	ND	ND	ND	0.700
1,2-Dithiole	15.88	ND	ND	ND	0.495
3-Octanone	15.89	ND	ND	3.196	ND
3-Octanol	16.16	ND	ND	0.189	ND
α-Ocimene	16.59	ND	0.170	ND	ND
D-Limonene	16.81	ND	1.831	5.514	ND
o-Cymene	17.09	ND	0.315	2.537	ND
Eucalyptol	17.61	4.642	17.623	11.124	ND
γ-Terpinene	18.33	ND	0.825	ND	ND
Diallyl disulphide	21.47	ND	ND	ND	38.241
Linalool	22.24	35.719	0.225	1.646	ND
Allyl methyl trisulfide	25.26	ND	ND	ND	17.235
Terpinolene	26.30	ND	0.882	0.286	ND
Camphor	26.84	ND	24.911	9.904	ND
Terpinen-4-ol	27.22	0.790	1.486	1.088	ND
endo-Borneol	27.72	ND	0.871	7.426	ND
Estragole	28.03	8.615	ND	ND	ND

Compound	RT (min)	Basil (%)	Sage (%)	Rosemary (%)	Garlic (%)
α-Terpinyl propionate	28.34	0.457	0.335	1.932	ND
(-)-Myrtenol	29.26	ND	ND	0.210	ND
Verbenone	31.64	ND	ND	9.994	ND
Dihydrocarveol	32.06	ND	ND	1.229	ND
Bornyl acetate	32.81	ND	ND	3.850	ND
Allyl trisulfide	34.06	ND	ND	ND	7.835
Trisulfide	35.28	ND	ND	ND	3.280
β-Elemene	37.04	0.963	ND	ND	ND
m-Eugenol	37.80	1.998	ND	ND	ND
Piperitenone	37.90	ND	ND	0.476	ND
trans-α-Bergamotene	38.53	1.591	ND	ND	ND
Caryophyllene	38.99	ND	1.110	0.558	ND
α-Guaiene	39.02	0.805	ND	ND	ND
δ-Guaijene	42.31	0.699	ND	ND	ND
γ-Muurolene	42.77	1.956	ND	ND	ND
Disulfide	44.47	ND	ND	ND	25.003
Nerolidol	45.26	0.507	ND	ND	ND
(-)-Spathulenol	47.64	0.408	ND	ND	ND
Viridiflorol	48.18	ND	5.076	ND	ND
Thujone	48.67	ND	27.587	ND	ND
Cadinol	49.63	4.302	ND	ND	ND
Pinocamphone	55.58	ND	ND	2.278	ND
Cinnamic acid	73.48	31.139	ND	ND	ND
Total		97.898	96.753	97.159	97.733

EO: Essential oils; GC–MS: Gas chromatography–mass spectrometry; RT: Retention time.

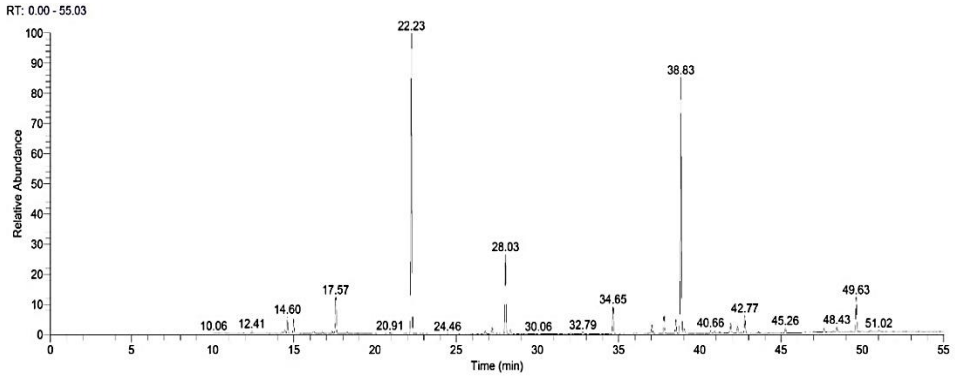


Figure 1. Gas chromatogram of *O. basilicum* EO

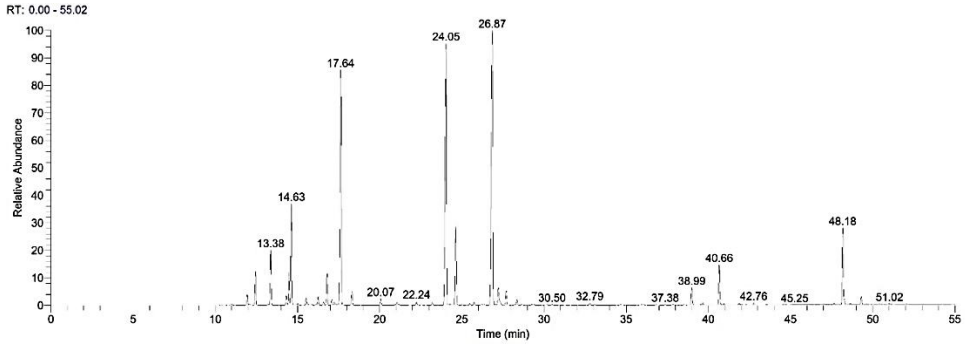


Figure 2. Gas chromatogram of *S. officinalis* EO

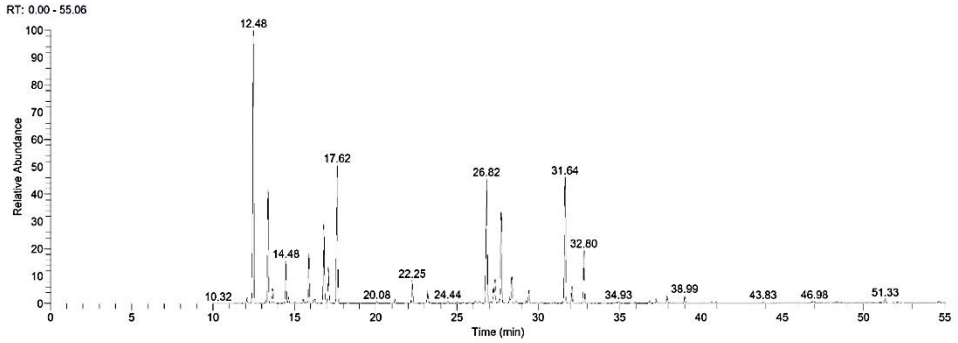


Figure 3. Gas chromatogram of *R. officinalis* EO

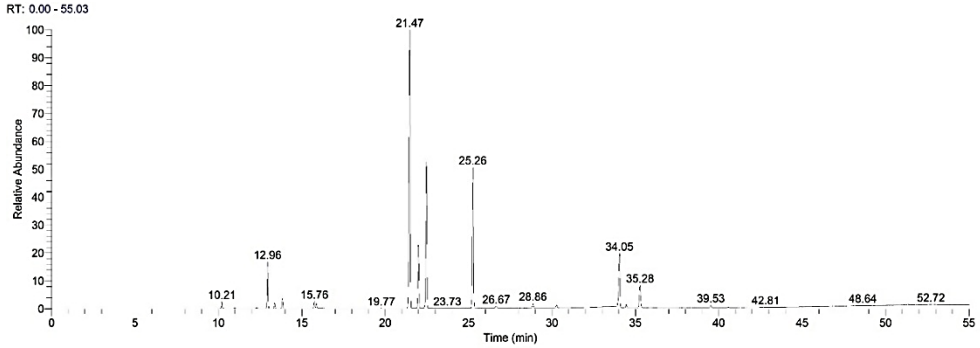


Figure 4. Gas chromatogram of *A. sativus* EO

In *O. basilicum* EO, the predominant compounds were linalool (35.719 %), estragole (8.615 %), and eucalyptol (4.642 %). For *S. officinalis* EO, the main components were eucalyptol (17.623 %), camphor (24.911 %), and thujone (27.587 %). The major constituents of *R. officinalis* EO included α-pinene (21.703%), eucalyptol (11.124 %), camphor (9.904 %), and verbenone (9.994 %). The EO of *A. sativum* was primarily composed of diallyl disulfide (38.241 %), allyl methyl trisulfide (17.235 %), and disulfide (25.003 %). The diversity in chemical composition across the different EOs reflects the unique properties and potential antimicrobial activities of each plant. The presence of significant amounts of eucalyptol in all three herb EOs

suggests a common compound contributing to their antimicrobial properties. Additionally, the high content of sulfur compounds in garlic oil, such as diallyl disulfide and allyl methyl trisulfide, is indicative of its distinctive antimicrobial efficacy. These findings are consistent with previously reported data on the chemical composition of these EOs, highlighting their complex mixture of terpenes, esters, and sulfur compounds, which are known for their antimicrobial activities. The detailed chemical profile provided by GC–MS analysis is crucial for understanding the potential mechanisms underlying the antimicrobial effects observed in these EOs.

3.3. Antibacterial assay

The antibacterial activity of the essential oils from *O. basilicum*, *S. officinalis*, *R. officinalis*, and *A. sativum* was evaluated against a selection of Gram-positive and Gram-negative bacteria, and the MICs were determined using the microdilution method (Table 3).

Table 3.

MICs of basil, sage, rosemary and garlic EOs against various pathogens

Pathogen	EO (µl/ml)			
	Basil	Sage	Rosemary	Garlic
Staphylococcus aureus (ATCC 25923)	1.01^{aA}	0.78^{bcA}	1.04^{abcA}	1.30^{aAB}
Listeria monocytogenes (ATCC 13932)	3.12^{bC}	0.39^{abA}	0.325^{abA}	1.30^{aB}
Bacillus cereus (ATCC 11778)	4.16^{bcB}	0.78^{bcA}	1.30^{bcA}	2.60^{abB}
Bacillus subtilis (ATCC 6633)	5.20^{cdB}	1.3^{cA}	2.08^{cA}	2.60
Escherichia coli (ATCC 25922)	0.194^{aA}	0.91^{bcA}	0.325^{abA}	6.25
Enterococcus faecalis (ATCC 19433)	6.25^{dC}	0.129^{aA}	0.129^{aA}	4.16^{bB}
Salmonella typhimurium (ATCC 14028)	0.162^{aA}	0.162^{aA}	1.82^{cB}	7.29^{cC}

*The data for the same essential oil, followed by different lowercase letters, are significantly different based on the least significant difference (LSD) test ($p < 0.05$). The data for the same species, followed by different uppercase letters, are significantly different based on the LSD test ($p < 0.05$).

Basil (*O. basilicum*) EO exhibited notable antimicrobial activity, particularly against *S. typhimurium*, with an MIC of 0.162 µl/ml. It was also effective against *E. coli* and *S. aureus*, with MIC values of 0.194 µl/ml and 1.01 µl/ml, respectively. However, *E. faecalis* demonstrated the highest resistance to basil oil, with an MIC of 6.25 µl/ml.

Sage (*S. officinalis*) EO displayed significant antimicrobial activity across all tested bacteria. It was particularly potent against *E. faecalis*, with an MIC of 0.129 µl/ml, and maintained strong activity against other bacteria. The highest MIC value for sage oil was 1.3 %, observed against *B. subtilis*.

Rosemary (*R. officinalis*) EO showed a broad spectrum of antibacterial activity. It was highly effective against *E. faecalis* (MIC 0.129 µl/ml) and *E. coli* (MIC 0.325 µl/ml). The highest MIC value for rosemary oil was 2.08 µl/ml, noted for *B. subtilis*.

Garlic (*A. sativum*) EO demonstrated variable antimicrobial activity. It showed the lowest MIC values against *L. monocytogenes* and *S. typhimurium*, both at 1.3 µl/ml. However, *S. aureus* and *B. subtilis* exhibited higher resistance to garlic oil, with MICs of 6.25 µl/ml and 7.29 µl/ml, respectively.

Overall, the results indicated that sage and rosemary EOs had the most potent antibacterial effects, particularly against *E. faecalis*. Basil oil was notably effective against *S. typhimurium*, while garlic oil had a broad but generally weaker antimicrobial activity compared to the other EOs.

These findings highlight the potential of these EOs as natural antimicrobial agents. The variability in MIC values among the oils and bacteria emphasizes the importance of selecting specific EOs based on the target microorganisms. Compounds such as eucalyptol, present in high concentrations in sage and rosemary EOs, may significantly contribute to their antimicrobial efficacy.

Basil EO is rich in eucalyptol, linalool, estragole, methyl-cinnamate, eugenol, *trans*-α-bergamotene, caryophyllene, germacrene D, γ-cadinene, and τ-cadinol (Ionica et al., 2022). It has demonstrated antimicrobial activity against several pathogenic bacteria. The most sensitive bacteria to the compounds present in basil EO, in order, were *S. typhimurium*, *E. coli*, and *S. aureus*, while *E. faecalis* showed the highest resistance. These results are consistent with those recorded by Beatović et al. (2015), who demonstrated the high resistance of *E. faecalis* to basil EO. Gram-positive bacteria from the *Bacillus* genus also showed high resistance to the compounds present in basil EO.

Species of the *Bacillus* genus exhibited different resistance levels to basil EO, with *B. subtilis* (MIC 5.20 µl/ml) being more resistant than *B. cereus* (MIC 4.16 µl/ml). *E. coli* demonstrated sensitivity to basil EO, with an MIC value of 0.194 µl/ml similar to the value (MIC 0.25 µl/ml) recorded by Smith-Palmer et al. (Smith-Palmer et al., 1998). Other studies have noted that *E. coli* strains isolated from poultry exhibited moderate sensitivity to basil EO (Ebani et al., 2018), while Carović-Stanko et al. (Carović-Stanko et al., 2010) found that *E. coli* O157was inhibited by basil EO at a concentration of 400 ppm. Semeniuc et al. (Semeniuc et al., 2017) observed that tested *E. coli* strains showed moderate sensitivity to basil EO (10.80 µL/mL). Studies by Noumedem et al. (Noumedem et al., 2013) demonstrated that different *E. coli* strains present varied resistance to basil EO depending on the tested variety, with the highest sensitivity observed for strains tested with the *Genovese* variety (MIC 0.364 mg/mL). The anti-*E. coli* activity of basil EO has been attributed mainly to linalool (Ionica et al., 2022; Beatović et al., 2015).

Among the tested bacteria, *E. faecalis* exhibited the highest resistance, with an MIC of 6.25 µl/ml. These results are in line with those recorded by Beatović et al. (2015), who demonstrated the resistance of this bacterium to basil EO compared to other pathogenic bacteria, and with results recorded by Sakkas et al. (2018), who noted that tested *E. faecalis* strains had MIC values greater than 4%. Carović-Stanko et al. (2010) noted an MIC of 500 ppm for *O. americanum* EO on *E. faecalis* strains. Similar studies have shown that the sensitivity of *E. faecalis* bacterium also depends on the basil variety from which the EO was extracted, with MICs ranging from 200–500 ppm for *O. americanum* and *O. citriodorum* (Rattanachaikunsopon and Phumkhachorn, 2010). The resistance of enterococci to physicochemical factors is well known and approaches that of spore-forming bacteria.

L. monocytogenes exhibited a MIC value of 3.12 µl/ml. Comparing the obtained results, it is evident that *L. monocytogenes* is more resistant than *E. coli*, a fact also noted by Rattanachaikunsopon and Phumkhachorn (2010). For basil EO, Beatović et al. (2015) recorded the same MIC value of 5.39 µg/mL for *E. coli*, *S. aureus*, *B. cereus*, *S. typhimurium*, and *L. monocytogenes*.

S. typhimurium showed greater sensitivity to basil EO, with a MIC value of 0.162 µl/ml, like that noted by Smith-Palmer et al. (1998). The sensitivity of *S. typhimurium* to basil EO has also be en reported in other studies. Rattanachaikunsopon & Phumkhachorn (2010) observed that adding basil EO at concentrations of 50–150 ppm to food inoculated with *S. typhimurium* significantly

reduced the bacterial concentration from 5 *log* colony-forming unit (CFU)/g to 3–1 *log* CFU/g, demonstrating its inhibitory effect.

S aureus was also sensitive to basil EO, with a MIC value of 1.01 µl/ml, lower than that recorded for *E faecalis* but higher than that for *E. coli* and *S. typhimurium*. Sakkas et al. (2018) noted that selected *S. aureus* strains had MIC values for basil EO ranging from 0.25–4 %, consistent with the results of this study. The higher sensitivity of *S. aureus* strains to basil EO (MIC 200 ppm) compared to *E. faecalis* (MIC 500 ppm) was also noted by Carović-Stanko et al. (2010).

Other studies have shown that basil EO at a concentration of 0.08 mL/L exhibited strong antimicrobial effects on *E. coli* O157 and *S. typhimurium* strains inoculated on lettuce (Karagözlü et al., 2011).

Compared to basil EO, which showed variable resistance among tested bacteria, sage EO proved more effective, with MIC values ranging from 0.129 µl/ml (*E. faecalis*) to 1.3 µl/ml (*B. subtilis*). Similar studies conducted on *S. aureus*, *B. cereus*, *Pseudomonas* spp., and *S. typhimurium* reported comparable results (MIC 0–1 %) (Shelef, 1984).

Rosemary EO exhibited strong inhibitory activity against *E. coli*, *E. faecalis*, and *L. monocytogenes*, while *S. aureus*, *Salmonella* spp., *B. cereus*, and *B. subtilis* were more resistant. MIC values ranged from 0.129 µl/ml (*E. faecalis*) to 2.08 µl/ml (*B. subtilis*). Abdullah et al. (Abdullah et al., 2015) reported MIC values for rosemary EO ranging from 0.312–5% for *Acinetobacter baumannii*, *P. aeruginosa*, *S. aureus*, and *E. faecalis* strains. Other studies noted an MIC of 5000 ppm for *Salmonella* spp. (Stojiljkovic et al., 2018). Kačániová et al. (2017) demonstrated the antimicrobial effect of rosemary EO, noting an MIC of 25 µg/mL for *E. coli*. Bozin et al. (2007) reported strong antibacterial activity of rosemary EO against *E. coli*, *Salmonella typhi*, *S. typhimurium*, and *Shigella sonei* strains.

The main component of garlic oil is allicin, which has been shown to be three times more effective against Gram-positive bacteria than Gram-negative bacteria due to the higher lipid content in *E. coli* compared to *S. aureus* (Mozaffari et al., 2014). The same authors, Mozaffari Nejad et al. (2014), noted variable MIC values for Gram-positive and Gram-negative bacteria, with values ranging from 15.6–48.3 µg/mL for Gram-positive bacteria and 22.9–37.2 µg/mL for Gram-negative bacteria.

In summary, these findings highlight the significant antimicrobial potential of EOs, with variability in effectiveness depending on the specific EO and bacterial strain. Further research is needed to optimize their use and understand the mechanisms underlying their antimicrobial properties.

CONCLUSIONS

The study revealed significant antimicrobial potential in the EOs extracted from *O. basilicum*, *S. officinalis*, *R. officinalis*, and *A. sativum*. The GC–MS analysis identified the primary active compounds in these EOs, which are responsible for their antimicrobial properties. Basil EO, rich in eucalyptol, linalool, and estragole, was particularly effective against *S. typhimurium* and *E. coli*. Sage EO demonstrated the broadest spectrum of activity, effectively inhibiting both Gram-positive and Gram-negative bacteria, highlighting its potential as a versatile antimicrobial agent.

The antibacterial assays showed that *E. faecalis* exhibited high resistance across all EOs, with basil EO being the least effective against this pathogen. Conversely, the strong antimicrobial action of sage and rosemary EOs against *E. faecalis* underscores their potential use in treating infections caused by this resilient

bacterium. Garlic oil, while effective against certain pathogens like *L. monocytogenes* and *S. typhimurium*, demonstrated a more variable antimicrobial profile, likely due to its distinct sulfur compounds.

These findings support the use of these EOs as natural alternatives to synthetic antimicrobials, particularly in light of rising antibiotic resistance. Further research should focus on understanding the synergistic effects of these EOs' compounds, optimizing their extraction and application methods, and exploring their efficacy in various formulations. The potential for incorporating these EOs into food preservation, healthcare, and pharmaceutical applications is vast, offering a promising natural solution to combat microbial infections.

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