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Essential Oil Compositions and Antimicrobial Activities of *Thymbra spicata* L. var. *spicata* L., *Lavandula X Intermedia* Emeric ex Loisel., *Satureja macrantha* C. A. MEYER and *Rosmarinus officinalis* L.

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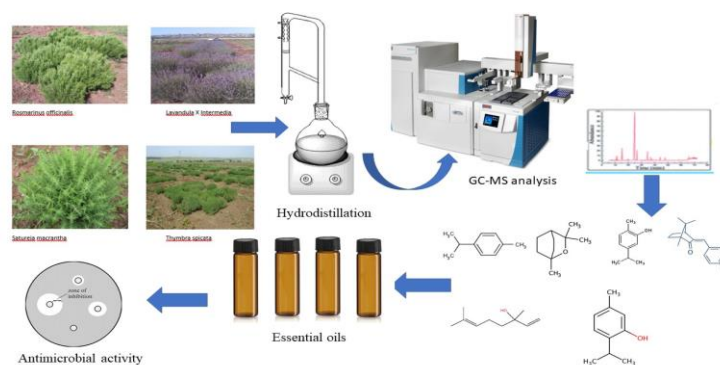
HIGHLIGHTS

- Essential oil contents of *T. spicata*, *L. X Intermedia*, *S. macrantha* and *R. officinalis* was determined.
- The main components of these plants were monoterpenoid
- *T. spicata* and *S. macrantha* showed strong effects against three microorganisms.

Abstract: Medicinal and aromatic plants have been widely using in folk medicine as antimicrobial, anti-inflammatory and antinociceptive agents. The aim of this study was to determine essential oil composition and antimicrobial activity of *T. spicata*, *L. X Intermedia*, *S. macrantha* and *R. officinalis*. Essential oil components of these plants were obtained by water vapor distillation method using Neo-Clevenger apparatus. Essential oil components were determined by gas chromatography-mass spectroscopy (GC-MS). The main components of these plants are carvacrol (74.26 %) and γ -terpinene (10.28%) in *T. spicata*, 1,8-cineol (32.48%), linalool (24.38%) and camphor (14.73%) in *L. X Intermedia*, p-cymene (56.70%), carvacrol (10.96 %) in *S. macrantha* and camphor (18.26 %), α -pinene (15.51%), 1,8-cineole (11.86%) and borneol (10.39%) in *R. officinalis* were determined. *T. spicata* and *S. macrantha* showed strong effects against three microorganisms. *L. X Intermedia* and *R. officinalis* showed strong activity against *Candida albicans*, while they had moderate effects against *Staphylococcus aureus*, *Escherichia coli*.

Keywords: medicinal and aromatic plants; essential oils; major compounds; GC-MS.

GRAPHICAL ABSTRACT



INTRODUCTION

Antibiotics have contributed to saving lives of millions of people by treating the microbial infections and achieving significant gains in life expectancy. However, clinical efficacy of many existing antibiotics is threatened by the emergence of multiple drug resistant (MDR) pathogens [1], and unwanted side effects of some antibiotics [2]. Antibiotic resistance is rising to dangerously high levels in all around the world. The overuse and misuse of these drugs are also important factors in development of antibiotic resistance. The emergence of antibiotic resistance increases the burden on the health system and economy because of prolonged hospital stays and higher medical costs [3,4]. Antibiotics are not taken only by using for their therapeutic purposes, but also with consumption as food by humans of livestock given antibiotic [5].

One of the MDR pathogens that has become a major problem all over the world, *Staphylococcus aureus* (Gram positive bacterium) is among the most common bacterial pathogens that a significant cause of soft tissue infections, arthritis, pneumonia, sepsis, osteomyelitis and skin infections [6], *Escherichia coli*, a Gram-negative bacterium, is a pathogen that cause food-borne outbreaks [7] and *Candida albicans* is a pathogen thrush [8].

Plants synthesize a wide range of molecules formed as a result of their metabolic activities. These compounds are known as secondary metabolites including; phenolics, flavonoids, alkaloids and terpenoids, and normally produced to protect themselves against viruses, fungi, bacteria and environment stresses [9], at the same time plant secondary metabolites are a main source of many raw materials used in cosmetics, flavouring and in making drugs or direct use as herbal medicine to treat sickness and ailments [10]. Almost 25% of medicines used in treatment are originated from medicinal plants [11]. Medicinal and aromatic plants are the main source of many essential oils [12,13]. Carvacrol, thymol, menthol, and 1,8-cineol are the most common essential oils in medicinal and aromatic plants including oregano, mint and thyme [14].

Medicinal plants are preferred in the treatment of diseases due to their possibility of use in various forms [15, 16], effective, economical, and easily accessible [17] and not toxic. Plant essential oils are odorous and volatile compounds accumulated in special cells of many medicinal and aromatic plants including glandular hair, glands, resin and oil ducts. These components are obtained with various extraction methods by hydro distillation, pressing and steam distillation from different parts of plants such as the parts of leaves, flowers and seeds. Essential oils are used in the treatment of several disorders in animals, humans, foods and plants, safer than antibiotics and chemical drugs [18, 19]. Because synthetic drugs and antibiotics have the serious adverse effects and the development of resistance mechanisms in pathogenic microorganisms [20].

The Lamiaceae family includes a large number of plants that are well known for their antioxidant properties [21]. *T. spicata*, *L. X Intermedia*, *S. macrantha* and *R. officinalis* belonging to the Lamiaceae family are essential oil plants and are used as medicinal herbs. The part of *T. spicata* used as food is fresh shoots collected in spring and dried leaves and flower cases. The essential oil 'thyme oil' obtained by steam distillation from the dried flowers and leaves of the plant is used as a folk medicine [22]. There are 39 *Lavandula* species, mostly of Mediterranean origin [23]. Essential oils obtained from *Lavandula* are especially used in the production of perfumes, cosmetics and medicines [24]. In addition, *Lavandula* oil is used as a nervous system treatment, dermatological treatment, antiseptic and antibiotic effects [25].



Figure 1. *T. spicata*



Figure 2. *L. X Intermedia*

The genus *Satureja* is representing by 16 species and 17 taxa [26]. The essential oil obtained from the leaves of this genus is used in the treatment of some diseases such as diarrhea, wounds, gastroenteritis and upper respiratory and urinary tract infections [27]. *R. officinalis* is an economically important plant that grows naturally mainly in the Mediterranean region. Essential oil derived from rosemary is widely used in cosmetics, sweetening and preserving food products. It also has anti-inflammatory, chemo preventive, anti-cancer, anti-proliferative, antimicrobial activities [28].



Figure 3. *S. macrantha*



Figure 4. *R. officinalis*

The aim of this study was to investigate the essential oil contents of *T. spicata*, *L. X Intermediata*, *S. macrantha* and *R. officinalis* and their antimicrobial efficacy against *C. albicans*, *S. aureus* and *E. coli*.

MATERIAL AND METHODS

Plant Materials

The leaves of *T. spicata*, *L. X Intermediata*, *S. macrantha* and *R. officinalis* harvested in the pre-flowering period and grown in the trial field of Republic of Turkey Ministry of Agriculture and Forestry GAP International Agricultural Research and Training Center (GAPUTAEM) were used as material.

The plants were identified by Dr. Mehmet FİDAN from Siirt University, Department of Biology. Voucher specimens (*T. spicata*: SUFAF 1558, *L. X Intermedia*: SUFAF 1559, *S. macrantha*: SUFAF 1560, *R. officinalis*: SUFAF 1561) were deposited at herbarium of Siirt University Flora and Fauna Center (SUFAF) Siirt University, Siirt, Turkey.

Extraction of the essential oil

The leaves of each plant were dried at room temperature and powdered in a laboratory mill. The essential oil extraction (mL g^{-1}) was carried out by hydro distillation using Neo-Clevenger. Therefore, 20 grams of dry

herb were weighed, and 200 mL of water was added in 1 L round bottom flask. The balloon was boiled for 2 h using an Electromantle™ (EM2000 CE, Electrothermal Engineering Ltd., UK, 500 W). Oils obtained were dried with anhydrous sodium sulphate and stored tightly closed vials at 4 °C prior to analyses. The qualitative and quantitative analyses of the essential oils were performed by Gas chromatography–mass spectrometry (GC-MS).

Analysis of essential oils

Samples were diluted with *n*-hexane in a 1:100 ratio for analysis. GC-MS analysis of the essential oil was performed on a GC-MS. GC (Agilent 7890A) was equipped with HP Innowax capillary column (60.0 mm x 0.25 mm x 0.25 µm). Helium was used as a carrier gas at a flow rate of 0.8 mL min⁻¹. The oven temperature was kept at 60 °C for 10 min, heated at 4 °C min⁻¹ to 220 °C at 10 min held, finally raised 240 at a rate of 1 °C min⁻¹. Injector and transfer line temperature were 250 °C and 260 °C, respectively [29] and 1 µL of essential oil was injected, with split ratio of 40:1, to the instrument, for analysis. The total run time was 60 min. The scanning range (m z⁻¹) 35-450 atomic mass units and electron bombardment ionization 70 eV were used for the mass detector (Agilent 5975C). Flame ionization detector (FID) temperature was 250 °C. To calculate the retention indices, standard alkane series (C₈-C₄₀) were used. The percentage amounts of components were measured electronically from a FID peak areas, without using correction factors, and the identification of the components of the essential oils was performed by matching data from Wiley GC-MS Library and NIST Chemistry WebBook, SRD 69 [30].

Antimicrobial activity

The antimicrobial activities of the plant essential oils were determined according to the Bauer and coauthors method [31]. Briefly, the disc diffusion test was carried out in sterile petri dishes of 90 mm diameter containing a suitable sterile solid media. It was used the test microorganisms (*S. aureus* ATCC 25923, *E. coli* ATCC 25922 and *C. albicans* ATCC 14053) providing from the culture collections of the Medical Microbiology Laboratory, Research Hospital of Dicle University in Diyarbakir, Turkey.

The isolates were separately sub-cultured in liquid growth media and incubated at 37 °C for overnight in an orbital shaker. The cultures were harvested using 5 mL of sterile 0.9% salt solution and absorbances were adjusted to a 0.5 McFarland turbidity standard (5 × 10⁵ CFU mL⁻¹) via spectrophotometer (Biochrom).

The bacteria of *S. aureus* and *E. coli* were inoculated on Mueller-Hinton agar (Oxoid, UK) and *C. albicans* was inoculated on Sabouraud dextrose agar (Oxoid, UK). 200 µL of each microbial suspension was inoculated to the solid culture medium then allowed the surface of the media to dry for 3–5 min. Sterilized filter paper (Whatman No. 1) discs (6 mm in diameter) containing various concentrations (15, 7.5, 3 and 1.5 µL) of essential oils were placed in the centre of the medium surface. After impregnation with different doses (1.5, 3, 7 and 15 µL) of the essential oil to the sterile paper discs, they were placed on the surface of agar. The plates were incubated at room temperature for 1 h to ensure the diffusion of essential oils and then incubated at 37 °C for 24-48 h. The negative control was a disc containing 15 µL of *n*-hexane. Reference antibiotics of gentamicin (10 µg/disc), ciprofloxacin (10 µg/disc), clindamycin (2 µg/disc), ampicillin (10 µg/disc) and fluconazole (25 µg/disc) discs (Oxoid, UK) were used as the positive control. The antimicrobial activities of the oils were measured by a inhibition zone (mm) around the paper disc (6 mm). All experiments were performed in triplicate, and standard deviations were calculated using Microsoft Excel as the square root of the sum of the squares of the differences from the arithmetic mean of the obtained data divided by the number of elements of the data minus one. All the data were expressed as Means ± SD of triplicate determinations.

RESULTS AND DISCUSSIONS

Essential Oil Composition of the Plants

In the present study, it was identified the essential oils of four plant species using GC/MS. Essential oil contents in the aerial parts of selected medicinal and aromatic plant varieties are given in Table 1. According to the results of GC/MS analyzes, a total of 45 compounds were identified between 97.53-98.63% (Table 1). The major part of the components was monoterpenes, in all tested plant samples. Oxygenated monoterpenes (28.38-80.47%) had the highest fraction followed by monoterpene hydrocarbons (11.56-65.85%). The quantities of sesquiterpenes was quite low, according to the monoterpenes. The sesquiterpene hydrocarbons and oxygenated sesquiterpenes were between 0.91-4.09% and 1.12-4.40%, respectively (Table 1).

As shown in Table 1., it was determined thirteen essential oil components and among these, carvacrol (74.26%), γ -terpinene (10.28%) and p-cymene (5.21%) were the main components of *T. spicata*. Gedikoğlu and coauthors [32] reported that carvacrol (68.20%), γ -terpinene (13.25%), p-cymene (5.37%), β -caryophyllene (2.59%), and thymol (1.19%) were major components of *T. spicata*. Barakat and coauthors [33], Hancı and coauthors [34], Baydar and coauthors [35] and Markovic and coauthors [36] reported that carvacrol, γ -terpinene and p-cymene were the major components of the essential oils of *T. Spicata* and their amounts varied according to the different growth seasons.

It was determined twenty-three essential oil components in *L. X Intermedia* and 1,8-cineol (32.48%), linalool (24.38%), camphor (14.73%), β -caryophyllene (4.09%) and borneol (3.92 %) were the main components (Table 1). Nogueira and Romano [37] found that the highest essential oil components of *L. viridis* were 1,8-cineole (18.2-25.1 %). Yilmaz [38] reported that linalool, linalyl acetate, eucalyptol, camphor and α -terpineol were the main components of *L. Intermedia*.

As seen in Table 1., it was determined twenty-two components and among these component, p-cymene (56.70%), carvacrol (10.96%), thymol (7.76%), γ -terpinene (3.68%) and borneol (3.50%) were major components in *S. macrantha*. Sefidkon and Jamzad [39] reported that p-cymene (25.8%), limonene (16.3%) and thymol (8.1%) were major components of *S. macrantha* grown in Iran. Aghbash and coauthors [40], also reported that p-cymene, γ -terpinene and carvacrol were the major compounds at all phenological stages.

Camphor (18.26%), α -pinene (16.81%), 1,8-cineole (14.83%), borneol (10.39%), verbenone (7.88%), camphene (6.79%), bornyl acetate (4.58%), linalool (4.26 %) and limonene (3.81%) were major components of *R. officinalis* (Table 1). Hussain and coauthors [41] found that 1,8-cineol (38.5%), camphor (17.1%), α -pinene (12.3%), limonene (6.23%), camphene (6.00%), and linalool (5.70%) were the major constituents of *R. officinalis*. Diraz Yıldırım [42], Sienkiewicz and coauthors [43] and Jiang and coauthors [44] stated that the major compounds of *R. officinalis* were found as α -pinene, 1,8-cineole, α -terpineol, α -pinene, camphor, isoborneol.

Table 1. Chemical composition of essential oils of *T. spicata*, *L. X Intermedia*, *S. macrantha* and *R. officinalis* (%)

No	RI ^a	RI ^b	Components	<i>R.</i> <i>officinalis</i>	<i>S.</i> <i>macrantha</i>	<i>L.X</i> <i>Intermedia</i>	<i>T.</i> <i>spicata</i>
1	1030	1032	α -Pinene	15.51	1.22	2.03	0.71
2	1033	1035	α -Thujene	-	0.86	-	-
3	1080	1076	Camphene	6.79	-	-	-
4	1121	1118	β -Pinene	-	0.34	2.72	-
5	1127	1131	Sabinene	-	-	1.04	-
6	1135	1136	Verbenene	0.61	-	-	-
7	1162	1159	\square -3-Carene	1.63	-	-	-
8	1172	1174	Myrcene	1.27	1.24	1.41	0.84
9	1193	1188	α -Terpinene	-	0.45	-	-
10	1203	1203	Limonene	3.81	0.47	2.55	-
11	1214	1213	1,8-Cineole	11.86	-	32.48	-
12	1241	1246	(<i>Z</i>)- β -Ocimene	-	0.89	-	-
13	1255	1255	γ -Terpinene	-	3.68	-	10.28
14	1286	1280	p-Cymene	1.88	56.70	1.81	5.21
15	1287	1290	α -Terpinolene	0.44	-	-	-
16	1451	1452	1-Octen-3-ol	-	-	0.63	-
17	1508	1503	Isomenthone	-	0.58	-	-
18	1533	1532	Camphor	18.26	-	14.73	-
19	1537	1535	Pinocamphone	2.43	-	-	-
20	1549	1553	Linalool	4.26	1.09	24.38	0.34
21	1585	1586	Pinocarvone	0.45	-	-	-
22	1591	1590	Bornyl acetate	4.58	-	0.27	-
23	1606	1604	Isobornyl acetate	0.95	-	-	-
24	1614	1611	Terpinen-4-ol	0.86	0.59	1.14	-
25	1608	1612	β -Caryophyllene	0.91	-	4.09	1.29
26	1643	1648	Myrtenal	-	-	0.31	-
27	1667	1662	Pulegone	-	1.98	-	-
28	1681	1682	\square -Terpineol	-	-	0.65	-

29	1690	1684	Isoborneol	0.84	-	-	-
30	1705	1706	α -Terpineol	-	-	1.32	0.61
31	1719	1719	Borneol	10.39	3.53	3.92	0.52
32	1724	1725	Verbenone	7.88	-	-	-
33	1729	1733	Neryl acetate	-	-	0.35	-
34	1750	1751	Carvone	-	-	0.37	-
35	1805	1802	Cuminal	-	0.35	0.88	-
36	1798	1804	Myrtenol	0.43	-	-	-
37	1855	1857	Geraniol	-	-	-	0.93
38	1862	1864	p-Cymen-8-ol	1.49	0.62	0.29	-
39	1947	1949	Piperitenone	-	0.43	-	-
40	2011	2008	Caryophyllene oxide	-	1.76	0.67	1.73
41	2114	2113	p-Cymen-7-ol	-	0.53	-	-
42	2142	2144	Spathulenol	-	2.64	-	-
43	2203	2198	Thymol	-	7.72	-	1.69
44	2230	2232	α -Bisabolol	-	-	0.45	-
45	2241	2239	Carvacrol	-	10.96	-	74.26
Total identified (%)				97.53	98.63	98.49	98.41
Grouped components							
Monoterpene hydrocarbons				31.94	65.85	11.56	17.04
Oxygenated monoterpenes				60.10	28.38	80.47	78.35
Sesquiterpen hydrocarbons				0.91	-	4.09	1.29
Oxygenated sesquiterpenes				-	4.40	1.12	1.73
Others				4.58	-	1.25	-

RI^a: Relative retention indices on Innowax FSC calculated against *n* alkanes (C₈-C₄₀)

RI^b: Relative retention indices taken from literature (Wiley GC-MS Library and NIST Chemistry Web Book)

%: Calculated from FID peak areas

- : Compounds not detected

Antimicrobial activity

The antimicrobial activity tests were carried out with disk diffusion method. The antibacterial activities of *T. spicata*, *L. Intermedia*, *S. macrantha* and *R. officinalis* essential oils were tested against multidrug resistant *S. aureus*, *E. coli* and *C. albicans* (Table 2). The antibiotics of ampicillin, gentamicin, ciprofloxacin, clindamycin and fluconazole were tested against *S. aureus*, *E. coli* and *C. albicans* (Table 3).

The highest antimicrobial activity of essential oil of *T. spicata* was 36±1.0 mm diameter zone of inhibition against *C. Albicans*, 26±1.52 mm diameter zone of inhibition against *S. aureus* and 28±1.52 mm diameter zone of inhibition against *E. coli* (Table 2). *T. spicata* displayed the potent antimicrobial activity against *C. albicans* (36 mm) in all doses. This activity was more powerful than flukonazol (24±1.15 mm), an antibiotic using against *C. albicans*. Essential oils of *T. spicata* had highly effective against *E. coli* and *S. aureus*. The doses of 15 μ L (28±1.52 mm), 7.5 μ L (26±0.57 mm) and 3 μ L (24±1.52 mm) were more effective than ampicillin (20±1.0 mm) and gentamicin (22±0.57 mm) antibiotics against *E. coli*, but their effects were lower than ciprofloxacin (34±1.15 mm). The dose of 15 μ L essential oils of *T. spicata* showed more powerful effect than gentamicin (22±0.28 mm) and ciprofloxacin (25±0.57 mm), but its effect was lower than clindamycin (26±1.0 mm) against *S. aureus* (Table 2, 3). In studies investigating the antimicrobial activity of *T. spicata* essential oil in various bacterial and fungal strains by disk diffusion methods; It has been determined that the essential oil of the plant has an antimicrobial effect against the tested bacteria and fungi [35,45,46]. Bioactivity of essential oil might be attributed to a single major constituent or to the synergistic/ additive behaviours of minor components [47]. In this study, the essential oils were characterised by high contents of carvacrol. Similar results having been reported in other studies, Baydar and coauthors [35] stated that the essential oil of *T. spicata* (containing mainly carvacrol 75.5%) was the inhibitory against *E. coli* and *S. aureus*. It is thought that carvacrol interacts with the cytoplasmic membrane and causes passive transport of ions across the membrane [48]. Carvacrol is also capable of aligning with fatty acid chains of lipid bilayers by interacting with transmembrane proteins and forming channels through the cytoplasmic membrane leading to the increase of membrane fluidity and alteration of proton motive force and cell permeability [40]. Carvacrol is considered as one of the fast-acting essential oil compounds as it inactivates *E. coli* and *Salmonella* in about five minutes [49]

The essential oil of *S. macrantha* with 38 ± 1.25 mm diameter zone of inhibition showed highest activity against *C. albicans* whereas lowest inhibition zone was observed in $1.5 \mu\text{L}$ dose of essential of *S. macrantha* against *E. coli* (8 ± 0 mm). The doses of $15 \mu\text{L}$ (32 ± 1.25 mm) and $7.5 \mu\text{L}$ (28 ± 0.57 mm) of *S. macrantha* were more effective than ampicillin (20 ± 1.0 mm) and gentamicin (22 ± 0.57 mm), but lower than ciprofloxacin (34 ± 1.15 mm) against *E. coli*. Essential oils of *S. macrantha* were very effective against *S. aureus*, in all doses. p-Cymene is hydrophobic in nature and has been reported to cause swelling of the cytoplasmic membrane and affect protein synthesis in *E. coli*. [50, 51] p-cymene and γ -terpinene are biosynthetically related to thymol and carvacrol and their presence may lead to synergistic effects in the bacterial cell [40].

Table 2. Antimicrobial activity of different doses of essential oil of *T. spicata*, *L. Intermedia*, *S. macrantha* and *R. officinalis*

Plants	Microorganisms	Essential oil doses and inhibition zones (mm)			
		15(μL)	7.5 (μL)	3(μL)	1.5(μL)
<i>T. spicata</i>	<i>E. coli</i>	28 ± 1.52	26 ± 0.57	24 ± 1.52	12 ± 1.0
	<i>S. aureus</i>	26 ± 1.52	21 ± 0.57	18 ± 0.5	14 ± 0
	<i>C. albicans</i>	36 ± 1.0	36 ± 0	36 ± 0.28	36 ± 0.46
<i>S. macrantha</i>	<i>E. coli</i>	32 ± 1.25	28 ± 0.57	16 ± 1.52	8 ± 0
	<i>S. aureus</i>	32 ± 1.0	32 ± 0.57	30 ± 1.0	22 ± 1.0
	<i>C. albicans</i>	38 ± 1.25	38 ± 0.64	38 ± 1.25	38 ± 0
<i>L. X Intermedia</i>	<i>E. coli</i>	12 ± 1.0	10 ± 1.0	8 ± 0	7 ± 1.0
	<i>S. aureus</i>	13 ± 0.57	9 ± 1.0	7 ± 1.0	6 ± 0
	<i>C. albicans</i>	30 ± 1.8	20 ± 1.15	12 ± 0.76	6 ± 1.0
<i>R. officinalis</i>	<i>E. coli</i>	12 ± 1.0	10 ± 1.0	8 ± 0	6 ± 0.57
	<i>S. aureus</i>	16 ± 1.41	10 ± 0.76	8 ± 1.15	6 ± 1.0
	<i>C. albicans</i>	30 ± 1.0	24 ± 0.76	12 ± 1.0	8 ± 0

Essential oils of *L. Intermedia* showed slight effect against *E. coli* ($12-7 \pm 1.0$ mm) and *S. aureus* ($13-6 \pm 1.0$ mm) in all doses. These components showed potent effects against *C. albicans* at doses of $15 \mu\text{L}$ (30 ± 1.8 mm) and $7.5 \mu\text{L}$ (20 ± 1.15 mm), but moderate effects at doses of $3 \mu\text{L}$ (12 ± 0.76 mm) and $1.5 \mu\text{L}$ (6 ± 1.0 mm). It was reported that essential oils of *L. Intermedia* had moderate effects at the doses of 5, 10 and $15 \mu\text{L}$, while they showed powerful effect at dose of $20 \mu\text{L}$ against *E. coli* and *S. aureus* [52]. The antibacterial activity of essential oil of *Lavandula* seems closely related to amount of 1,8-cineol [53]. In other study, in vitro antimicrobial activity of essential oil of lavender against *S. aureus* and *E. coli* were investigated by Predoi and coauthors [54]. The antibacterial activity of these essential oil of lavender could be due to their ability to degrade membrane proteins and cell permeability.

Table 3. Antimicrobial activity of different antibiotics doses

Microorganisms	Antibiotics	Antibiotic doses (μg)	Inhibition zones (mm)
<i>E. coli</i>	Ampicillin	10	20 ± 1.0
	Gentamicin	10	22 ± 0.57
	Ciprofloxacin	6	34 ± 1.15
<i>S. aureus</i>	Gentamicin	10	22 ± 0.28
	Ciprofloxacin	6	25 ± 0.57
	Clindamycin	2	26 ± 1.0
<i>C. albicans</i>	Fluconazole	25	24 ± 1.15

Essential oils of *R. officinalis* showed moderate effects against *E. coli* and *S. aureus* in all doses but these components had very strong effects against *C. albicans* in at doses of $15 \mu\text{L}$ (30 ± 1.0 mm) and $7.5 \mu\text{L}$ (24 ± 0.76 mm) compared with fluconazole (24 mm). It was reported that essential oils of *R. officinalis* had weak or moderate antibacterial activity [55]. Sienkiewicz and coauthors [43], Burt [56] Sirocchi and coauthors [57] and Yildirim and coauthors [58] demonstrated the antibacterial activities of *R. officinalis* in their studies. Rosmarinic acid, rosmaridiphenol, carnosol, epirosmanol, carnosic acid, rosmanol and isorosmanol in rosemary are inhibitory in cells due to their interaction with the cell membrane, which causes loss of

membrane functionality and structure, changes in genetic material and nutrients, changes in electron transport, leakage of cellular components, and changes in fatty acid production [59]. One of the main components of rosemary is α -pinene, a monoterpene. As noted by Bajpai and coauthors [60] and Nieto and coauthors [61], the inhibitory effect may be associated with terpenes' ability to disorganize the cytoplasmic membrane and therefore promote lysis.

The results from the present study highlight the potential of the essential oils of *T. spicata*, *L. X Intermedia*, *S. macrantha* and *R. officinalis* as effective antimicrobials against *C. albicans*, *E. coli* and *S. aureus*. Comparing inhibition zones, all concentrations of *T. spicata* and *S. macrantha* and high dose of *L. X Intermedia* and *R. officinalis* were most effective on *C. albicans*. In addition, high doses of essential oils of all plants were effective *S. aureus* and *E. coli*, but the effect decreased as the concentration decreased. As a result, essential oils obtained from all plants used in the study had antimicrobial activity on test microorganisms.

CONCLUSION

The essential oils of *T. Spicata*, *L. X Intermedia*, *S. macrantha* and *R. officinalis* were constituted mainly by monoterpenoid (84.54% for *T. Spicata*, 71.59% for *L. X Intermedia*, 75.42% for *S. macrantha* and 47.95% for *R. officinalis*). The major compounds of the *T. Spicata* was carvacrol (74.26%), for *L. Intermedia* was 1,8-cineol (32.48%), for *S. macrantha* was p-cymene (56.70%) and for *R. officinalis* was camphor (18.26%). The current study demonstrated the antimicrobial activity of the essential oils of *T. spicata*, *L. X Intermedia*, *S. macrantha* and *R. officinalis*. The results indicated that *T. spicata* and *S. macrantha* had strong and the best antimicrobial activity against all test microorganisms. The high doses of essential oils of *L.X Intermedia* and *R. officinalis* had potent activity against *C. albicans* but moderate/less activity against *E. coli* and *S. aureus*.

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