Chemical Composition and Antimicrobial Activity of Extracts from Thyme and Rosemary Against *Staphylococcus aureus* and *Candida albicans*

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Different concentrations of ethanolic extracts of thyme (*Zataria multiflora*) and rosemary (*Rosmarinus officinalis*) were evaluated to determine their antimicrobial activity using the agar-well diffusion method. The values of inhibition zone diameter (IZD) for *Candida albicans* fungus and *Staphylococcus aureus* Gram-positive bacteria were determined. The bioactivities of two various extracts were studied, and the chemical composition of the extracts were identified using gas chromatography-mass spectrometry (GC-MS) technique. The results of the test showed that at concentrations of 10% and 40% thyme extract, the values of IZD were 12.5 mm and 23.3 mm, respectively, against the growth of *S. aureus*, which were higher than *C. albicans* (7.0 mm and 22.5 mm, respectively). The rosemary extract at concentrations of 20% and 60% showed lower antibacterial activity against *S. aureus* (4.7 mm and 8.7 mm IZD, respectively) and lower antifungal activity against *C. albicans* (12.2 mm and 1.7 mm IZD, respectively). At a concentration of 40% thyme extract, the highest antibacterial (23.3 mm IZD) and antifungal (22.5 mm IZD) activities were observed. The GC/MS analysis showed that carvacrol (52.3%), linalool L (16%), and thymol (9.6%) were the main components of thyme extract, while in the rosemary extract β-amyrone (18.0%), verbenone (8.0%), and 1,8-cineole (7.26%) were the major constituents.

Keywords: Thyme and rosemary extracts; Antimicrobial activities; GC-MS; Carvacrol; β-amyrone

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INTRODUCTION

In recent years, due to worldwide antibiotic resistance, the tendency of using herbal medicine has increased and application of extracts instead of chemical preservatives in controlling microorganisms has received significant interest and attention (Bidaki *et al*. 2015; Raeisi *et al*. 2019).

The rosemary (*Rosmarinus officinalis*, family Lamiaceae) is a small evergreen shrub, growing approximately to 1 to 2 m tall and exists all over the world with slender, long, sharp, and slightly rough leaves along with purple-white flowers (Mirheydar 2001; Atti-Santos *et al*. 2005). Various parts of this plant, including the stems, branches, flowers, and leaves, have been used extensively for pharmaceutical products and traditional medicinal purposes (Mirheydar 2001; Tavassoli *et al*. 2011; Azizkhani and Tooryan 2015). Furthermore, rosemary is extensively used as a food additive and preservative (Frankel *et al*. 1996a).

Zataria multiflora Boiss. (thyme) is a perennial plant of the Lamiaceae family, which is mainly grown in Iran, Pakistan, and Afghanistan (Ali *et al*. 2000). It is a medicinal plant used in traditional folk remedies due to positive effects for human health, such as its carminative, antiseptic, and analgesic properties (Shafiee and Javidnia 1997). Recent studies have revealed that thyme has antibacterial, antifungal, and antioxidant properties (Ramezani *et al*. 2004; Shokri *et al*. 2006; Babaie *et al*. 2007).

The traditional uses of thyme are as an antiseptic, vermifuge, anesthetic, antispasmodic, anthelmintic, antidiarrheal, and analgesic, but its current uses of pharmaceutical forms include treatment of irritable bowel syndrome, stomachache, flatulence, cough, bronchitis, laryngitis, candidiasis, and trichomoniasis (Sajed *et al*. 2013).

For example, the dry parts of the plant are used as flavor additive and preservative in a variety of foodstuffs in Iran (Gandomi *et al*. 2009). It has inhibitory effects against radial fungal growth and aflatoxin production by *Aspergillus flavus* in cheese (Gandomi *et al*. 2009). The extracts and essential oil of thyme prevent the growth of some bacteria associated with gastrointestinal infections from *Staphylococcus aureus* (Fazlara *et al*. 2008), enterohemorrhagic *Escherichia coli* (Goudarzi *et al*. 2006), *Salmonella* Typhi and Paratyphi (Fazeli *et al*. 2007), and *Shigella flexneri* and *Bacillus cereus* (Fazeli *et al*. 2007; Misaghi and Akhondzadeh Basti 2007).

Unfortunately, the resistance of the bacteria to antibiotics has increased in recent decades. Previous studies reported that the dry leaves of thyme of Shiraz are used in food products as a retentive and to add flavour (Gandomi *et al*. 2009), which inhibits the progress of some microorganisms like bacteria and fungi (Shafiee and Javidnia 1997). The thyme of Shiraz is a potential alternative to antibiotics against bacteria. It has antioxidant and antimicrobial essences having monoterpenes combinations (Saei-Dehkordi *et al*. 2010). Previous research reported that thyme has phenolic components such as thymol, carvacrol, linalool, and parasmyn. Thymol is one of the important compounds of the oxidized monoterpene and is antibacterial, antifungal, and avoids mycotoxins production, which is found in the essence of thyme and many other herbs (Tiwari *et al*. 2009). Interest in natural flavours in the food packing industry has increased in the last two decades due to their significant advantages, such as being a sustainable and environmentally friendly raw material, and being non-toxic for humans as compared to the chemical antimicrobial and synthetic substances (Yanishlieva *et al*. 1999). Using natural flavours as antibacterial combinations is a good solution to control pathogenic bacteria, increase the durability of processed foods, and reduce economical losses resulting from food-deteriorating microorganisms (Ali *et al*. 2000). In general, the increase in the phenolic contents positively affects the antibacterial resistance to the nutritional pathogens.

Candida albicans belongs to the *Candida* species and is one of common pathogens in patients with organ transplantation, acquired immune deficiency syndrome (AIDS), neoplastic disease, immunocompromised patients, users of immunosuppressive drugs, and broad-spectrum antibiotics (Bineshian *et al*. 2018).

One of the widespread pathogens that can cause a wide spectrum of infections, from superficial skin infections to severe and potentially fatal invasive disease, is *Staphylococcus aureus* (Lowy 1998). The *S. aureus* can rapidly grow in foods and is known as the most common foodborne microorganism (Kadariya *et al*. 2014).

Currently, there have been no reports on the chemical composition and antimicrobial properties comparison of the aerial parts of the thyme (Shirazi thyme) and rosemary extracts. Therefore, the present study aimed to evaluate and compare the

chemical composition, antibacterial, and antifungal properties of extracts of *Z. multiflora* and *R. officinalis*.

EXPERIMENTAL

Plant Materials

The leaves and branches of *Z. multiflora* and *R. officinalis* plants were obtained from the Taleghan province, Qazvin, Iran and Nazarabad province, Karaj, Iran, respectively. Identification of the plants was carried out in the Department of Horticulture, Faculty of Agriculture and Natural Resources at Islamic Azad University, Karaj Branch, Iran.

Removal of Extracts

The plant parts of rosemary and thyme, including leaves and branches, were separately cut into small pieces, and chopped to obtain lignocellulosic flour. The particle size was between 30- and 40-mesh. In the first step, a little cotton was compressed and placed at the bottom of the 250 mL separatory funnel at the beginning of the outlet valve. In the second step, approximately 50 g powder of each tested plant material was poured into a separatory funnel, followed by the addition of 150 mL of ethanol (96%). The mixture macerated in the closed separatory funnel for 48 h. The outlet of the separatory funnel was opened, and the liquid allowed drip slowly as specified in modified method by Rathi *et al*. (2006). The liquid was clarified by filtration and finally concentrated to dryness, in a rotary evaporator at a low temperature (35 to 40 °C) to avoid chemical alteration in the bioactive compounds with loss of their properties. The extracts were accumulated and dried over anhydrous sodium sulfate and then stored at 4 ºC (Hashemi *et al*. 2013).

Culture Media and Inoculum

The strains of *S. aureus* and *C. albicans* were sourced from the Iran's fungus and bacteria center and then transferred on tryptic soy broth (TSB). The applied TSB contained 17 g/L tryptone (pancreatic digest of casein), 3 g/L peptone (soybean digest), 2.5 g/L glucose, 5 g/L NaCl, and 2.5 g/L K_2HPO_4. To regenerate the *S. aureus*, the culture media was placed in an oven at 37 °C for 48 h and to regenerate the *C. albicans*, the culture media was placed in an incubator at 25 °C for 24 h.

According to the culture media datasheet, 65 g powder of Sabouraud dextrose agar (SDA) for the fungus cultivation media and 34 g powder of Müller-Hinton agar (MHA) for bacteria cultivation media was added to 1000 mL of distilled water, separately, and placed on a heater with stirrer until a uniform solution was obtained. Next, the solution was sterilized in an autoclave at 121 °C and 1.2 atm for 20 min. Approximately 25 mL of the prepared culture media was poured into each sterile Petri plates.

Antifungal and Antibacterial Activity Assessment

Agar-well diffusion is one of several bioassessment methods that is well known and commonly used (Balouiri *et al*. 2016). Therefore, agar-well diffusion method was applied to determine the zone diameter of inhibition. The MHA (34 g/L) for the cultivation of *S. aureus* Gram-positive bacteria and SDA (65 g/L) for the cultivation of *C. albicans* fungus were used. Approximately 25 mL of molten media cooled to 45 °C was added to pre-sterilized Petri plates. After that, 48-h-old cultures of *S. aureus*, 24-h-old cultures of *C.*

albicans were spread using a sterile cotton swab and loop, and each microbe was evenly spread over the entire surface of the agar dish to obtain a uniform dish surface growth. Next, the Petri plate (s) contents were cooled and dried. Three wells with 6-mm diameter and 5-mm depth were punched into the agar using a sterile glass Pasteur pipette for placing the extracted samples and dimethyl sulfoxide (DMSO) as the control sample. The extract solution was passed by syringe from a 0.45-μm Microsolve filter and poured into a glass vial.

Approximately 0.05 mL (one drop) of the diluted extracts of each plant was dispensed into respective wells by syringe at various concentrations (20%, 30%, 40%, 50%, and 60% for rosemary and 10%, 20%, 30%, and 40% for thyme extracts on two wells and three Petri plates as replicates). The Petri plates were then left at room temperature for 30 min and then incubated at 30 ºC for 7 days. After incubation, the diameter of inhibition zones was measured using a ruler and the results were reported in millimeters (mm). All the tests were run in triplicate, and the average result was calculated (Sadeghi-Nejad *et al*. 2010; Bachheti *et al*. 2011).

Analysis of Extracts

The gas chromatography-mass spectrometry (GC/MS) analysis of the *Z. multiflora* and *R. officinalis* extracts was performed. Next, 1 μL of each extract was dissolved with 100 μL of ethanol, separately and run on a GC Agilent 7890A and MS Agilent 5975C mass spectrometer detector (Agilent Technologies, Palo Alto, CA, USA) equipped with a HP-5MS cross-linked capillary column (30 m long and 0.25 mm internal diameter, 0.25 μm film thickness). Helium was used as the carrier gas with a flow rate of 1 mL/min.

The GC/MS operation conditions were: injector temperature 260 °C; transfer line 270 °C; oven temperature program 60 °C for 4 min, 3 °C/min to 100 °C for 2 min, then 4 °C/min to 250 °C for 5 min; carrier gas was He at 1 mL/min. The intrinsic energy that hits the sample in the MS system was 70 eV. The split ratio of the sample was 50:1 with a split flow of 1 mL/min. Individual components were identified using mass spectra with data from literature, two mass spectrometric libraries (Wiley 275 L, 1998 and NIST-05), mass database matching, and by comparing the retention times and mass spectra of constituents with published data (Joulain and König 1988; Adams 1995, 2001). Retention indices (R_I) were determined with reference to a homologous series of normal alkanes (C_{10} to C_{32}) using Eq. (1) (Kováts 1958),

$$R_I = 100 [(n + (N\text{-}n) \times \log t_{1R} (x) - \log t_{1R} (C_n)/\log t_{1R} (C_N) - \log t_{1R} (C_n)] \quad (1)$$

where R_I is the retention index of the compound of interest, t_{1R} (min) is the net retention time (t_R-t_0), t_0 (min) is the retention time of the solvent (dead time), t_R (min) is the retention time of the compound of interest, C_n and C_N are the number of carbons in the n-alkanes eluting immediately before and after the compound of interest, respectively, and N and n are the numbers of carbon atoms in the n-alkanes eluting immediately before and after the compound of interest, respectively.

Statistical Analysis

The results are given in mean values with their standard deviations. Statistical analysis was carried out using the SPSS program, version 22 (International Business Machines (IBM) Corp., Armonk, NY, USA). One-way analysis of variance (ANOVA) was conducted to determine the significance of differences between analytical results at $p < 0.05$ significance level.

RESULTS AND DISCUSSION

Antibacterial Activity

Inhibition zone diameter (IZD) determination

Statistically, there were significant differences among the microbes (*S. aureus* and *C. albicans*), extracts (thyme and rosemary), and their concentrations (Tables 1, 2, and 3). The effect of *Z. multiflora* ethanolic extract against *S. aureus* and *C. albicans* is provided in Fig. 1. The *Z. multiflora* ethanolic extract showed higher inhibition effect against *S. aureus* than *C. albicans*.

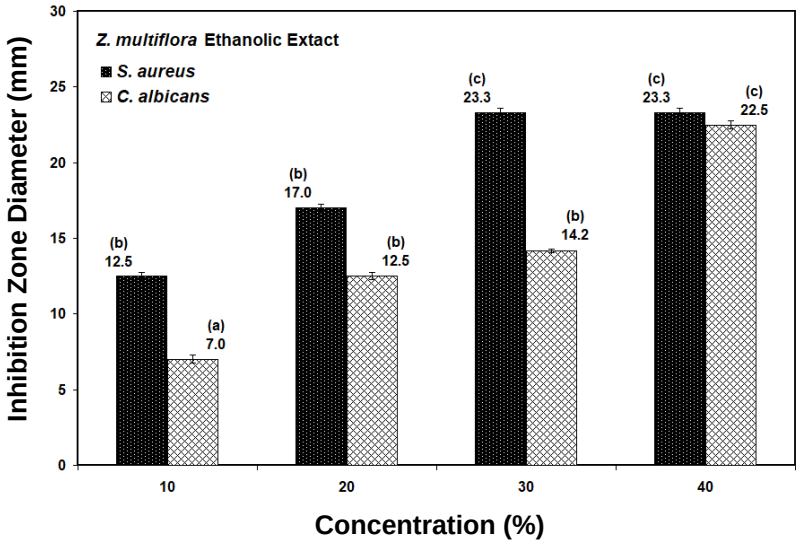

Fig. 1. Mean values ± standard deviation of the IZD of the effect of *Z. multiflora* ethanolic extract against *S. aureus* and *C. albicans* at different concentration levels. Different letters in each column indicate a statistical difference ($p < 0.05$) among the treatment groups.

There was a significant difference between *Z. multiflora* ethanolic extract against *S. aureus* and *C. albicans* at concentrations of 10% and 30% and at $p<0.05$ significance level (Fig. 1 and Table 1).

Table 1. Statistical Analysis of the Effect of Thyme Ethanolic Extract and Its Concentrations (Four Concentrations) on the IZD of *S. aureus* and *C. albicans*

Source	Sum of Squares	df	Mean Square	F	Sig.
Between groups	7.720	7	1.103	17.131	0.000
Within groups	1.030	16	0.064	-	-
Total	8.750	23	-	-	-

In general, with the increasing of extract concentration from 10 to 40%, the inhibition effect of extract against two microbial pathogens increased. The highest IZD values obtained from the thyme extract were 23.3 mm and 22.5 mm for *S. aureus* and *C. albicans*, respectively. The lowest IZD values obtained from the thyme extract were 12.5 mm and 7 mm for *S. aureus* and *C. albicans*, respectively (Fig. 1).

Comparing the effect of rosemary extract on bacteria at the statistical level of 5%, the change in the IZD up to 50% concentration range was not significant but at a concentration of 60% was significant, which was determined as the optimum concentration (Table 2 and Fig. 2).

Table 2. Statistical Analysis of the Effect of Rosemary Ethanolic Extract and Its Concentrations (Five Concentrations) on the IZD of *S. aureus* and *C. albicans*

Source	Sum of Squares	df	Mean Square	F	Sig.
Between groups	2.867	9	0.319	17.949	0.000
Within groups	0.355	20	0.018	-	-
Total	3.222	29	-	-	-

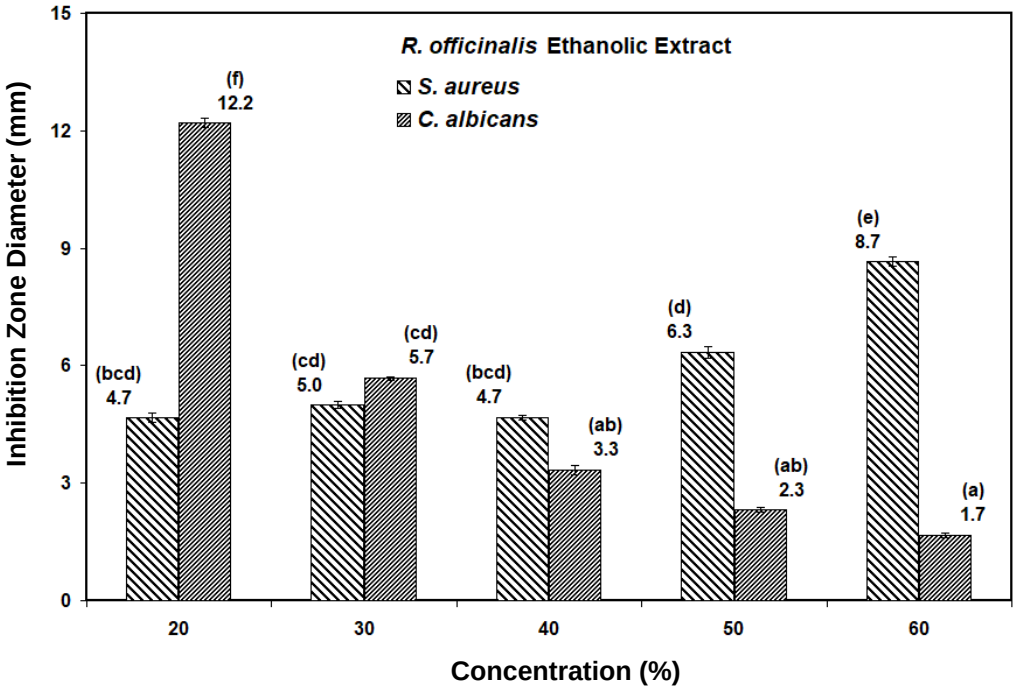

Fig. 2. Mean values ± standard deviation of the IZD of the effect of *R. officinalis* ethanolic extract against *S. aureus* and *C. albicans* at different concentration levels. Different letters in each column indicate a statistical difference ($p < 0.05$) among the treatment groups.

With the increasing of rosemary extract concentration levels from 20 to 60%, the inhibition effect showed a significant reduction against *C. albicans* (Swari *et al*. 2020), while with the increasing of rosemary extract concentration levels from 20 to 60%, the inhibition effect showed an increasing trend against *S. aureus* (Fig. 2). The highest IZD values obtained from the rosemary extract were 12.2 mm and 8.7 mm for *S. aureus* and *C. albicans*, respectively. The lowest IZD values obtained from the rosemary extract were 1.7 mm and 4.7 mm for *S. aureus* and *C. albicans*, respectively (Fig. 2). According to the studies, the authors found that the type and content of some compounds can affect the antifungal and antibacterial activities of extracts at different concentrations (Matsuzaki *et al*. 2013; Abdulaziz *et al*. 2015). Based on the comparison of the IZD mean values, a significant difference was observed between the effects of rosemary and thyme extracts on the bacteria. The antibacterial effect of thyme extract was optimized more than the rosemary extract. Statistically, there was a significant difference between the extracts and their concentrations on the value of growth inhibition of microbes, bacteria, and fungus (Table 3 and Fig. 3).

Table 3. Univariate Test Results of the Effect of Thyme and Rosemary Ethanolic Extracts and Their Concentrations (Three Concentrations, 20%, 30%, and 40%) on the IZD of *S. aureus* and *C. albicans*

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected model	20.024	11	1.820	42.280	0.000
Intercept	55.502	1	55.502	1289.090	0.000
Microbes	0.122	1	0.122	2.845	0.105
Extracts	14.694	1	14.694	341.290	0.000
Concentrations (Conc.)	0.200	2	0.100	2.327	0.119
Microbes × Extracts × Conc.	1.210	1	1.210	28.103	0.000
Microbes × Conc.	0.555	2	0.278	6.450	0.006
Extracts × Conc.	2.521	2	1.260	29.276	0.000
Microbes × Extracts × Conc.	0.720	2	0.360	8.366	0.002
Error	1.033	24	0.043	-	-
Total	76.560	36	-	-	-
Corrected Total	21.058	35	-	-	-

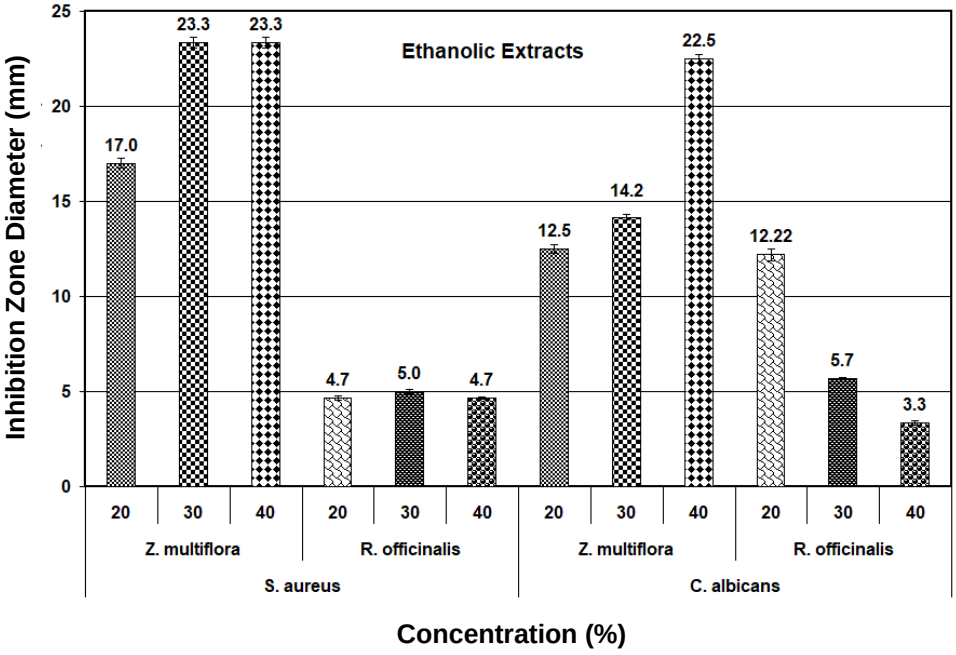

Fig. 3. Mean values ± standard deviation of the IZD of the effect of thyme and rosemary ethanolic extracts at similar concentrations against *S. aureus* and *C. albicans*

Figure 3 illustrates that at similar concentrations the highest inhibition effect on the growth of bacteria, *S. aureus* showed at 30% and 40% concentration levels by *Z. multiflora* extract, while the highest inhibition effect on the growth of fungus, *C. albicans* showed at a concentration of 40%. The antibacterial activity was classified according to Mutai *et al*. (2009): very strong response, zone diameter ≥ 30 mm; strong response, zone diameter 21 to 29 mm; moderate response, zone diameter 16 to 20 mm; weak response, zone diameter 11 to 15 mm; and little or no response, zone diameter ≤ 10 mm. According to the

classification of IZD, the thyme extract is placed in two classes of moderate (zone diameter 16 to 20 mm) to strong (zone diameter 21 to 29 mm), but the rosemary extract is placed in two classes of little or no response (zone diameter ≤ 10 mm) to weak (zone diameter 11 to 15 mm) (Mutai *et al*. 2009).

Extracts Analysis

Chemical composition of thyme extract

To further investigate the chemical constituents in these plants, in this study, a total of 24 and 32 compounds were separated, and they were identified in the thyme and rosemary extracts, respectively (Tables 4 and 5). The main constituents of the extracts of the tested two plants identified by GC/MS are presented in Tables 4 and 5 according to their percentage composition.

Table 4. Characterized Chemical Composition of Ethanolic Extract of *Z. multiflora*

No.	Compound	RT* (min)	Relative Area Percent (%)	KI**
1	Benzene, 1-methyl-4-(1-methylethyl)-	6.995	1.09	1030
2	1,8-Cineole	7.120	0.52	1037
3	.gamma-Terpinene	7.711	0.54	1065
4	Linalool oxide cis	7.996	0.52	1078
5	Linalool oxide (2)	8.328	0.55	1092
6	Linalool L	8.603	15.96	1104
7	3,7-Octadiene-2,6-diol, 2,6-dimethyl-	10.554	1.96	1198
8	Carvacrol methyl ether	11.561	0.53	1250
9	Linalyl acetate	11.753	1.16	1260
10	Thymol	12.614	9.59	1301
11	Carvacrol	12.863	52.32	1315
12	1-Methylpyrroline	13.252	3.53	1336
13	Carvacryl acetate	14.067	0.56	1379
14	*Trans*-caryophyllene	15.011	3.43	1430
15	Aromadendrene	15.364	0.40	1450
16	(+) Spathulenol	17.808	0.63	1590
17	Caryophyllene oxide	17.896	1.32	1595
18	1H-Cyclooctapyrazole, 4,5,6,7,8,9-hexahydro-	23.121	0.61	1939
19	2-Pentadecanone	24.066	0.98	2007
20	Phytol	25.539	0.34	2117
21	Di-(2-ethylhexyl)phthalate	30.707	0.63	2554
22	Squalene	33.613	0.63	2833
23	Vitamin e	37.696	0.62	3145
24	.gamma-Sitosterol	41.390	1.24	NC

∗ Retention time; ∗∗ Kováts index of sample; NC means not calculated due to the lack of normal alkane atoms

Ethanolic extract of thyme was the most active against the growth by *S. aureus* and *C. albicans*, and it possessed good antibacterial and antifungal activity. The chemical components identified in the ethanolic extract from leaves and branches of *Z. multiflora* are presented in Table 4. The main constituents were carvacrol (52.32%), linalool L (15.96%), thymol (9.59%), 1-methylpyrroline (3.53%), and *trans*-caryophyllene (3.43%). Thymol was the main constituent of the fresh plant (73.21%), while carvacrol was the primary constituent in the dried plant (62.87%) (Saleem *et al*. 2004). Carvacrol and thymol

are well-known anti-fungal agents in the essential oil of *Z. multiflora* with significant amounts (Baser 2008; Shokri *et al*. 2011, 2012; Abbaszadeh *et al*. 2014). It was concluded that several factors, such as geographical variation, cultivar differences, stage of plant growth, preparation, and extraction process, may not affect only the quantitative properties of the essential oil composition but additionally influence its qualitative ones (Ali *et al*. 1999a, 1999b). The aromadendrene was found in a low content (0.40%) (Dezaki *et al*. 2016). Phytol with minor and trace concentration was found (0.34%) (Martínez-Pérez *et al*. 2007).

Chemical composition of rosemary extract

Ethanolic extract of rosemary at different concentrations did not exhibit acceptable antifungal and antibacterial effects against *C. albicans* and *S. aureus*, and it also possessed little to weak antimicrobial activity. The chemical components identified in the ethanolic extract from leaves and branches of *R. officinalis* are presented in Table 5.

The main constituents were β-amyrone (18.00%), verbenone (8.00%), 1,8-cineole (7.26%), camphor (6.09%), 3,8,9-trimethoxy-6H-dibenzo[b,d]pyran-6-one (6.63%), bornyl acetate (6.01%), *trans*-caryophyllene (4.86%), and borneol L (3.24%) (Pintore *et al*. 2001; Genena *et al*. 2008; Senanayake 2013; Karakaya *et al*. 2014; Jan *et al*. 2017).

The (23S)-ethylcholest-5-en-3.beta.-ol was found in a low content (0.53%). Pinocamphone (0.44%), also known as 3-pinanone, belongs to the class of organic compounds and as bicyclic monoterpenoids, was found with trace concentration (0.34%) (Jan *et al*. 2017; Kulak 2019).

Table 5. Characterized Chemical Composition of Ethanolic Extract of *R. officinalis*

No.	Compound	RT* (min)	Relative Area Percent (%)	KI**
1	β-Myrcene	6.274	0.85	NC
2	1,8-Cineole	7.119	7.26	1037
3	Linalool L	8.577	0.88	1103
4	Camphor	9.537	6.09	1152
5	Pinocamphone	9.875	0.44	1167
6	Borneol L	10.009	3.24	1174
7	A-Terpineol	10.534	0.66	1197
8	Verbenone	10.902	8.00	1216
9	Bornyl acetate	12.401	6.01	1291
10	*Trans*-caryophyllene	15.006	4.86	1430
11	A-Humulene	15.624	0.76	1464

∗ Retention time; ∗∗ Kováts index of sample; NC means not calculated due to the lack of normal alkane atoms

Table 5. Characterized Chemical Composition of Ethanolic Extract of *R. officinalis* (Continued)

No.	Compound	RT* (min)	Relative Area Percent (%)	KI**
12	β-elemene	19.126	0.92	1672
13	Neophytadiene	21.725	0.61	1842
14	n-Hexadecanoic acid	23.567	2.61	1971
15	Methyl heptadecyl ketone	24.066	1.23	2007
16	Phytol	25.539	1.97	2117
17	Linoleic acid	25.959	2.76	2151
18	9,12-Octadecadienoic acid (Z,Z)-	26.224	1.38	2171
19	(+)-5-Methyl-6(S)-(3-methyl-2-butenyl)tetrahydroimidazo[4,5,1-jk][1,4]benzodiazepin-2(1H)-one	27.365	1.39	2264
20	2,2'(1H,1'H)-Spirobi-s-indacene, ethanone deriv.	28.263	0.68	2338
21	Ferruginol	28.362	1.00	2347
22	1,2-Dicyano-1-(1,2-dimethyl-3-indolyl)-2-(1,2,4,5-tetramethyl-3-pyrrolyl)ethane	28.528	0.74	2361
23	Pyrido[2,3-b]indole	28.896	2.93	2392
24	Benzo[c]coumarine, 3,4,8-trimethoxy-	29.161	1.86	2415
25	4-Hydroxy-3,3',4-trimethoxystilbene	29.306	2.53	2428
26	3,8,9-Trimethoxy-6H-dibenzo[b,d]pyran-6-one	30.235	6.63	2510
27	Cyproheptadine	30.515	0.64	2536
28	Squalene	33.613	1.05	2833
29	D,α.-Tocopherol	37.701	1.16	3145
30	(23S)-Ethylcholest-5-en-3-β-ol	41.422	0.53	NC
31	β-Amyrone	41.608	18.00	NC
32	Handianol	43.455	0.66	NC

∗ Retention time; ∗∗ Kováts index of sample; NC means not calculated due to the lack of normal alkane atoms

Based on the previous reports, the biological activities, including antioxidant and antimicrobial activities of rosemary, are related to the non-nutrient secondary metabolites of the plant such as the phenolic diterpenes, carnosol, carnosic acid, methyl carnosate, rosmanol, epirosmanol, and phenolic acids such as ferulic, rosmarinic, and chlorogenic and caffeic acids (Chen *et al*. 1996; Cuvelier *et al*. 1996; Frankel *et al*. 1996b; Richheimer *et al*. 1996; Huang *et al*. 1997; Campo *et al*. 2000; Wellwood and Cole 2004; Peñuelas and Munné-Bosch 2005; Moreno *et al*. 2006).

The thyme oil antifungal effects against *Coniophora puteana* and *Aspergillus niger* was also confirmed by Jones *et al*. (2011). Bahmani and Schmidt (2018) after impregnation of *Fagus orientalis* and *Pinus taeda* wood with lavender, lemongrass, and thyme oils confirmed that these oils could ensure efficient protection against *A. niger*, *Penicillium commune*, *C. puteana*, *Trametes versicolor*, and *Chaetomium globosum*.

Voda *et al*. (2003) using the agar dilution method showed that the anise, basil, cumin, oregano, and thyme oils have had high antifungal effects against brown-rot fungus *C. puteana* and white-rot fungus *T. versicolor*. They also concluded that thymol, carvacrol,

trans-anethole, methyl chavicol, and cuminaldehyde were the most effective compounds in inhibiting the growth of both fungi.

Additionally, Reinprecht *et al*. (2019) in their studies on five different essential oils (basil, cinnamon, clove, oregano, and thyme), found that the highest antifungal activity against brown-rot fungus *Serpula lacrymans* and the white-rot fungus *T. versicolor* was shown for basil oil (containing mainly linalool), and the lowest was noted for clove oil (containing mainly eugenol).

The antifungal effectiveness and stability of beech wood treated with 10% solutions of ten different essential oils (birch, clove, lavender, oregano, sweet flag, savory, sage, tea tree, thyme, and a mixture of eucalypt, lavender, lemon, sage, and thyme oils) against brown-rot fungus *C. puteana* and white-rot fungus *T. versicolor* were examined by Pánek *et al*. (2014). They showed that after a complex accelerated ageing procedure the most effective against *C. puteana* were clove, oregano, sweet flag, and thyme oils that contain phenol compounds such as carvacol, eugenol, thymol, and cis-isoasarol trimethylether.

According to the previous studies and findings of researchers, the authors found that the thyme ethanolic (obtained by 96% ethanol) extract containing the mentioned compounds with a concentration of 40% and probably the rosemary ethanolic extract with low concentration can have potential effects on wood protection as a benign environment-friendly preservative.

CONCLUSIONS

In this study, the inhibitory effect of ethanolic extracts of thyme and rosemary on fungus and bacteria was tested.

1. According to the fungal inhibition results, *Zataria multiflora* extract had a higher antibacterial and antifungal effects than rosemary extract.
2. *Z. multiflora* at 30% and 40% concentrations showed the highest effect on the growth of bacteria *S. aureus* and at 40% concentration showed the highest effect on the growth of fungus *C. albicans*.
3. According to the classification of IZD, the thyme extract is placed in two classes of moderate to strong, but the rosemary extract is placed in two classes of little or no response to weak.
4. The thyme ethanolic (obtained by 96% ethanol) extract with a concentration of 40% can have potential effect on wood protection as a benign environment-friendly preservative.

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