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Chemical Components and Antibacterial Activities of Essential Oils Obtained from Iranian Local Lavandula officinalis and Thymus vulgaris against Pathogenic Bacteria Isolated from Human

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ABSTRACT: Essential oils are commonly used to treat minor health problems. In this study the chemical compositions of Lavandula officinalis and Thymus vulgaris were determined by gas chromatography and mass spectrometry. GC/MS analysis of T. vulgaris resulted in thymol as the major oil component where as methyl sulfony exhibited as the most abundant constituent of L. officinalis. The antibacterial activities of these essential oils (Eos) against Five gram negative bacteria, namely *Pseudomonas aeruginosa*, *Salmonella paratyphi* (*D*), *Citrobacter*, *Enterobacter*, *Escherichia coli* and gram positive bacteria *Staphylococcus aureus coagulase* were investigated. Among the tested plants, Thymus vulgaris showed higher activity against different bacteria, while *S.paratyphi* and *S. aureus* were the most resistance bacteria. All the tested plant extracts possessed antimicrobial growth activities with MIC values ranging from 100 to 150µL/mL. The results suggested that due to the potential antimicrobial activities of these essential oils they might be employed in food and pharmaceutical products.

Keywords: *Antimicrobial Activity, Essential Oil, Lavandula officinalis, Minimum Inhibitory Concentration, Thymus vulgaris.*

Introduction

Antimicrobial drugs are subjected to the microbial resistance and this has become a growing problem in recent years. Therefore, sufficient research to discover potent natural antibiotics is desirable and compulsory. Since many essential oils have been reported to possess strong antimicrobial effects (Orhan *et al*., 2012), two different essential oils obtained from the various plants of Iran namely Lavandula officinalis and Thymus vulgaris locally known as lavand and avishan were examined regarding this matter.

Essential oils are widely used in food preservation, pharmaceuticals, alternative medicine and natural therapies (Imelouane *et al*., 2009). Moreover, the chemical composition of several kinds of lavender essential oils have been extensively investigated by gas chromatography–mass spectrometry (GC–MS) with varying results (Yang *et al*., 2010). Lavender essential oil is produced by steam distillation, from both the flower heads and foliage, but the chemical composition differs greatly with the sweeter and most aromatic oil being derived from the flowers (Abad *et al*., 2012). On the other hand, Thymus oil is widely used as an

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antiseptic agent in many pharmaceutical preparations and as a flavoring agent for many kinds of food products (Karaman *et al*., 2001).

Some bacteria like *Pseudomonas aeruginosa*, *Salmonella paratyphi* (*D*), *Citrobacter*, *Enterobacter*, *Escherichia coli* and *Staphylococcus aureus coagulase* are implicated to cause severe infections in human, as they are found in multiple environmental habitats.

This paper reports the results of GC/MS analyses and the antibacterial perspectives of the essential oils from aerial parts of Lavandula officinalis and Thymus vulgaris.

Materials and Methods

The aerial parts of Lavandula officinalis and Thymus vulgar were collected from Kermanshah (west of Iran) in June 2014. For the isolation of the essential oil, air-dried plant material was submitted to water distillation for 3 hours using a Clevenger-type apparatus (Pina-Vaz *et al*., 2004). The essential oil was collected and stored at 4°C until used. The oils were analyzed by gas chromatography/mass spectroscopy (GC/MS) (Pina-Vaz *et al*., 2004). The GC/MS analyses were carried out on HP-6890 fitted with 30 m×0.25 mm × 0.32 µm capillary column. Helium was used as the carrier gas at 1 ml/min and 1 µl of the sample was injected for analysis.

The antimicrobial activities of the essential oils were examined on 6 different microorganisms. Five gram-negative bacteria, namely *Pseudomonas aeruginosa*, *Salmonella paratyphi* (*D*), *Citrobacter*, *Enterobacter* , *Escherichia coli* and a gram positive bacteria *Staphylococcus aureus coagulase*. All of the microorganisms were kindly donated by local hospitals in Kermanshah.

The cultures of bacteria were maintained on their appropriate agar slants at 4 °C throughout and were used as stock cultures. The culture media Mueller-Hinton (MHA--Oxoid, Ltd.) broth and agar were used for bacteria.

The agar disc diffusion method was employed to determine the antimicrobial activities of the essential oils. Suspension of the tested microorganisms (adjusted with MacFarland 0.5) was spread on the solid media plates. Filter paper discs (6 mm in diameter) were soaked with 10 µl of the oils and placed on the inoculated plates. After keeping at 2 ºC for 2 h, they were incubated at 37 ºC for 24h. The diameters of the inhibition zones were measured in millimeters (Azaza *et al*., 2003).

Determination of minimum inhibitory concentration (MIC) and the antibacterial assays were carried out by microdilution method in order to determine the antibacterial activity of the oils against different gram negative and gram positive bacteria (Sokovic *et al*., 2006). The serial dilutions of the essential oils were prepared in an LB broth medium in 96-well microtiter plates, using a range of concentrations for each essential oil from 25, 50, 100, 120 and 150µl/ml. The plates were then spot inoculated with 3 µl of freshly grown bacteria standardized by approximately 10^8 CFU/ml (McFarland No: 0.5) of each isolate. Positive control was carried out under the same conditions without essential oils; and negative control was also carried out under the same conditions without adding the bacteria. The plate was incubated for 24 h at 37°C. The lowest concentration of the essential oils that completely inhibited the visual growth was recorded and interpreted as the minimum inhibitory concentration (Al-Mariri *et al*., 2013, Sokmen *et al*., 2003).

Results and Discussion

High prevalence of antibiotic resistance among bacteria has lead to the recovery of interest in essential oils. The overall quality and quantity of the essential oil of particular species vary according to season, geographical location and the location of plants. In some species, the essence is well made in warm and sunny season. Climate and soil conditions can affect the composition of the oil (Arnold *et al*., 1997). The oils were analyzed by GC/MS and their components are presented in Tables 1 and 2 showing major constituents of both essential oils examined.

The genus Thymus has numerous species and varieties and their essential oils composition have been studied earlier (Guillen & Manzanos, 1998, Jordán *et al*., 2003). Our result differs from Vaz et al, 2004 who found that the main components carvacrol (70.3%) and p -cymene (11.7%). In contrast, our findings were similar to Rota et al (2007) who showed thymol as the major constituent. For the Spanish thyme essential oil, the major components were 1, 8-cineole, followed by terpenyl acetate, borneol, linalool, beta - pinene, alphaterpineol and camphor (Jordán *et al*., 2006).

In lavandula the most constituents consisted of 2-methyl-5-(4-methylphenyl) sulfony (25.63%) and 9, 12-octadecadienoic acid (11.22%) and borneol (9.55%). On contrary the presence of α-pinene (18.12%), camphor (10.12%) and 1, 8-cineole (15.32%) has been reported in L. officinalis in Iran (Rostami *et al*., 2012), whereas we didn't find α-pinene in this essential oil.

The antibacterial activities exhibited by the essential oils have been reported by several researchers. Deans and Ritchie (1987) and Deans *et al*. (1995) showed the susceptibility of gram-positive and gram negative bacteria to plant volatile oils had a little influence on their growth inhibition. Though, some oils appeared more active with respect to gram reaction, exerting a greater inhibitory activity against gram-positive bacteria. It was often reported that gram-negative bacteria were more resistant to the essential oils present in the plants (Smith-Palmer *et al*., 1998).

The cell wall structure of gram-negative bacteria is constituted essentially with Lipopolysaccharides (LPS). This constituent avoids the accumulation of the oils on the cell membrane (Bezić *et al*., 2003).

Table 1. Chemical composition (%) of the essential oil from *L. officinalis*

Components	**L.ooficinalis%**	**RT**
1,8Cineole	3.33	1228
L-Linanlol	2.63	1356
Camphor	2.46	1483
Silane	2.52	1491
Borneol L	9.55	1524
Terpinene-4-ol	1.29	1539
2-Cyclohexen-1-one	0.97	1562
Bornyl formate	0.69	1642
β -myrcene	1.02	1718
2-Methyl-5-(4-methylphenyl)sulfony	25.63	2012
2-Methyl-5-(4´-methylphenyl)sulfony	6.64	2048
9,12-Octadecadienoic acid	11.22	2130
9,12-Octadecadienoic acid	7.91	2147
(-)-Caryophyllene oxide	1.79	2275
1-(2-trimethylsiloxy-1)	10.1	2306
Trimethylsilyl ester of 4-methyl	2.16	2313
Epi-bicyclosesquiphellandrene	3.36	2347
β-tumerone	0.69	2360
(-)-Isopulegol	6.06	2452

Table 2. Chemical composition (%) of the essential oil from *T. vulgaris*

Components	T.vulgaris%	RT
Butanoic acid	0.45	606
Alpha-Thujene	2.3	981
α-pinene	1.65	1004
Camphene	0.96	1045
1-Octene-3-ol	1.18	1089
β-pinene	0.61	1110
Myrcene	2.35	1118
1-Phellandrene	0.47	1164
α-Terpinene	3.47	1191
Benzene	14.17	1212
Limonene	0.77	1220
1,8Cineole	2.02	1231
gamma.Terpinene	12.8	1258
trans-Sabinene hydrate	0.61	1306
Terpinolene	0.42	1347
Linalool L	2.98	1358
Borneol L	3.22	1525
1-4-Terpineoll	2.11	1540
1-Isopropyl-2-methoxy-4-methylbenze	2.01	1630
Carvacrol methyl ether	2.4	1650
Thymol	29.98	1743
phenol,5-methyl-2-(1-methylethyl)	0.76	1753
phenol,2-methyl-5-(1-methylethyl)	4.5	17.62
trans-Caryophyllene	3.56	2004
Cyclotetrasiloxane	0.41	2053
gamma-Cadiene	0.25	2148
Cis-α-Bisabolene	0.77	2167
Caryophyllene oxide	1.29	2276
Bicyclo dec-1-en	1.53	2347

Both essential oils showed antibacterial activities at different degrees (Table 3). The results proved that thyme essential oil had significant activity against *Citrobacter* and *E. coli* with diameters of inhibition zones being 2.7 mm and 1.7 mm, respectively. In addition, Lavandula also showed higher activity against *Citrobacter*. In contrast, *S. aureus* and *S.paratyphi* showed resistance to both essential oils. This result is in agreement with Imelouane *et al* (2009) who found that gram-negative bacteria were more sensitive to the essential oil of thyme. On contrary, Kon and Rai (2012) finding is not in agreement with our results that found *S. aureus* the most sensitive toward thyme.Most of the antimicrobial activities of the essential oils from Thymus genus might be associated to the presence of phenolic compounds like thymol and carvacrol (Consentino *et al*., 1999; Davidson & Naidu, 2000). In our study, carvacrol concentration was very low (2.40%), however it exhibited in antimicrobial activity. The antimicrobial activities of the oils might be due to the borneol (Tabanca *et al*., 2001; Vardar *et al*., 2003) and Pinene-type monoterpene hydrocarbons that are well-known chemicals having antimicrobial potentials. The essential oils containing terpenes are also reported to possess antimicrobial activities (Dorman and Deans, 2000).

Unlike *Staphylococcus aureus coagulase, Salmonella paratyphi* (D) and *Escherichia coli*, MIC for both essential oils is similar (100 µl/mL). Furthermore, Thymus vulgaris oil showed the lowest MIC (120µl/mL) against other strains (*Pseudomonas aeruginosa, Citrobacter*, *Enterobacter*) while the figure for lavandula officinalis was

Table 3. Antibacterial activities of essential oils against pathogens by disc diffusion method

	Zone of inhibition (mm)	
Microorganism	***L. officinalis***	***T. vulgaris***
S.paratayphi (D)	-	-
P.aerogenosa	-	0.8
Citrobacter	1.4	1.7
Enterobacter	0.8	1.2
E.coli	0.9	2.7
S.aureus coagolase (+)	-	-

Inhibition zone diameter (mm)

higher (150µl/mL). In contrast, Tarek et al., (2014) showed that Lavender oil exhibited antimicrobial activities against *S. aureus* with MIC (≤1 µl/ml) except *P. aeruginosa* and *S. Typhi* that were resistant to lavender oil at the highest used concentration (16µl/ml).

Conclusion

Our results confirm that many essential oils possess antimicrobial activities against pathogens. Moreover, the demand for the natural extracts to be employed in the manufacturers of foods, cosmetics and pharmaceuticals is increasing. Therefore, studies concerned with the essential oils lie not only in the chemical characterization but also in the possibility of linking the chemical contents with particular functional properties.

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