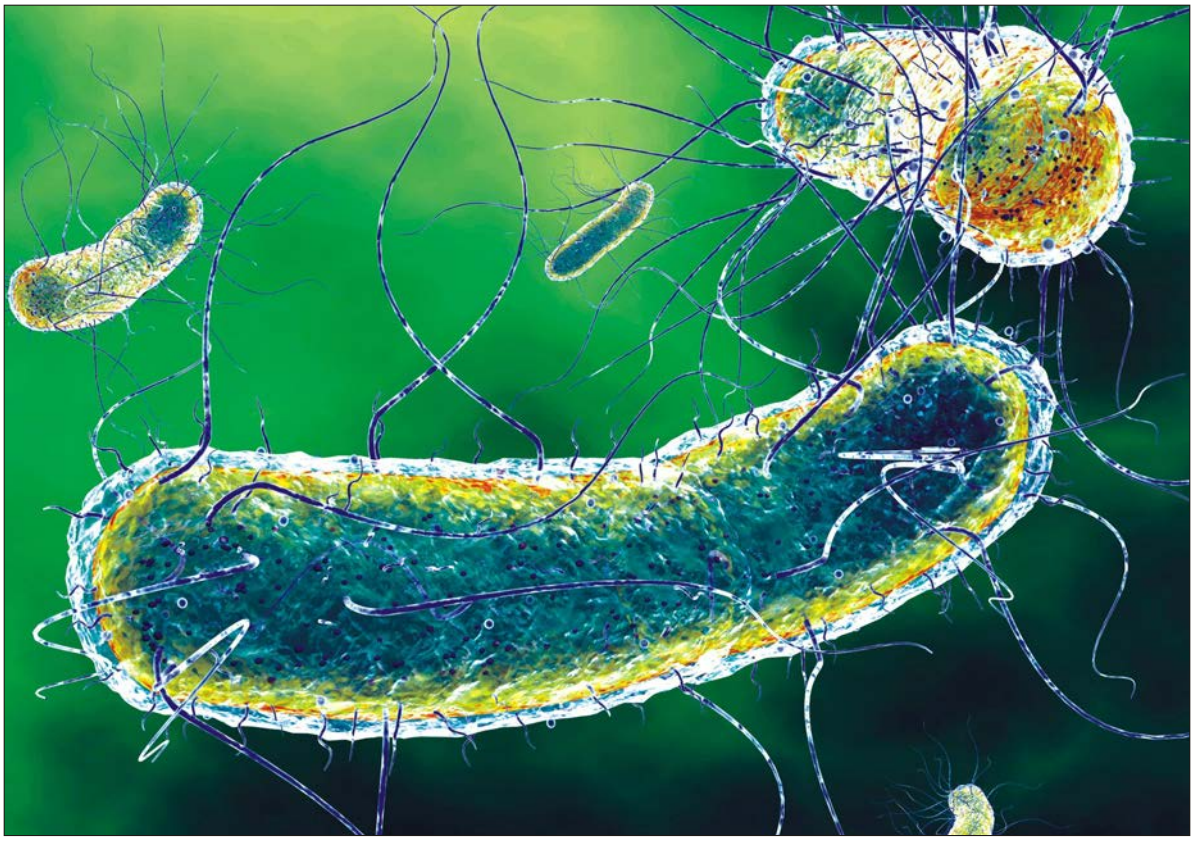

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The Study of Compositions and Antimicrobial Properties of Essential Oil of *Origanum vulgare* and *Rosmarinus officinalis* on Human Pathogens

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ABSTRACT

Aromatic and medicinal plants are widespread throughout world. The experiment include to evaluation of effect essential oil of *Origanum vulgare* and *Rosmarinus officinalis* on human pathogen also study their composition. The essential oils of *Origanum vulgare* and *Rosmarinus officinalis* collected in Iran were obtained by hydrodistillation of the aerial parts and analysed by gas chromatography equipped with flame ionisation detector (GC-FID) and gas chromatography coupled to a mass spectrometry system (GC/MS) for their chemical composition. The major compounds were carvacrol, followed by thymol, terpinolene, limonene, α-pinene and germacrene-D. From the obtained results it can be noticed that streptomycin and Nystatin possessed lower antibacterial and antifungal activity than *Origanum vulgare* and *Rosmarinus officinalis*. Carvone showed higher antifungal activities than previous components and Nystatin while, Thymol, a-Pinene and Carvacrol showed higher antibacterial activity than streptomycin. The results of this experiment indicated inhibitory effect of essential oil on human pathogen.

Key words: Essential oil, human pathogen, *Origanum vulgare*, *Rosmarinus officinalis*

INTRODUCTION

Aromatic and medicinal plants can be used to cure human and other animal diseases (Biswas *et al*., 2002). Medicinal plants have pharmaceutical and antibacterial properties (Cragg *et al*., 1997; Padma, 2005; Ishtiaq *et al*., 2007; Bitiren *et al*., 2010; Manek *et al*., 2011; Ahmida, 2011; Zahmatkash and Vafaeenasab, 2011). Essential oils aromatic and medicinal plants obtaining of different parts of aromatic and medicinal plants such as leave and seed, Essential oils have antimicrobial, antifungal and antioxidative properties (Palevitch, 1994; Sawamura, 2000; Ahmad *et al*., 2005; Sacchetti *et al*., 2005; Ganjewala and Luthra, 2007a, b; Kumar and Ganjewala, 2007; Reza and Abbas, 2007; Swamy and Rao, 2008; Soltan *et al*., 2009; Fortes *et al*., 2011; Ismail *et al*., 2011; Louis *et al*., 2011; Patra, 2011; Upadhyay and Patra, 2011). Their most important characteristics are their anti-infection, antimicrobial, antifungal and antioxidative effects (Skerget *et al*., 2005; Derwich *et al*., 2010a, b; Momtaz and Abdollahi, 2010; Onocha *et al*., 2011). *R. officinalis* is a medicinal plants, It is used for flavoring food and in traditional medicine for hepatoprotective and antitumorigenic activity (Slamenova *et al*., 2002; Jamshidi *et al*., 2009). Takaki *et al*. (2008), Wang *et al*. (2008) and

Moghtader and Afzali (2009) reported that essential oil of *R. officinalis* has antibacterial, Antinociceptive and antifungal properties. The leaves of *O. vulgare* as well as its essential oil are used medicinally (Hammer *et al.*, 1999; Force *et al.*, 2000; Dailami *et al.*, 2010). Stiycharz and Shetty (2002), Baydar *et al.* (2004) and Cleff *et al.* (2010) reported that the essential oil of *O. vulgare* has antifungal, antibacterial, antioxidant and cytotoxic activity properties. Moreover, screening of such plant extracts for antimicrobial and antifungal activities has always been of great interest to scientists looking for new sources for drugs for the treatment of various diseases (Oka *et al.*, 2000). In this study, antimicrobial and antifungal of *R. officinalis* and *O. vulgare* oils were examined using different bacterial and fungi species. In addition, chemical composition of volatile constituents, were also determined.

MATERIALS AND METHODS

The leaves of *O. vulgare* and *R. officinalis* have been collected during March-April 2010 in Iran (Ilam: Elevation 1339 m, Latitude East 33.638, Longitude North 46.431) then the plants were isolated from the other specimen and conserved for extraction. The essential oils were extracted by hydrodistillation using an apparatus of Clevenger. For this, mixing 250 g of plants was used in 1600 mL of distilled water the extraction took 3 h. After filtration the solvent is eliminated by reduced pressure distillation in rotary evaporator and pure oil was stored at 4°C in obscurity till the beginning of analysis. GC analysis was performed, using a Shimadzu GC-9A gas chromatograph equipped with a DB-5 fused silica column (30 m×0.25 mm i.d., film thickness 0.25 μm). The oven temperature was held at 50°C for 5 min and then programmed to 250°C at a rate of 3°C min^{-1}. Injector and detector (FID) temperatures were 290°C; helium was used as carrier gas with a linear velocity of 32 cm sec^{-1}. The percentages were calculated by electronic integration of FID peak areas without the use of response factors correction. Linear retention indices for all components were determined by co injection of the samples with a solution containing homologous series of C8-C22 n-alkanes. GC-MS analyses were carried out on a Varian 3400 GC-MS system equipped with a DB-5 fused silica column (30 m×0.25 mm i.d.); oven temperature was 40-240°C at a rate of 4°C. Transfer line temperature was 260°C. Carrier gas was helium with a linear velocity of 31.5 cm sec^{-1}, split ratio 1/60. In addition, ionization energy was 70 eV, scan time 1 sec and mass range 40-300 amu. Identification of components in the oil was based on retention indices relatives to n-alkanes and computer matching with the WILLEY 275. L library, as well as by comparison of the fragmentation patterns of mass spectra with those reported in the literature (Adams, 2001). The chromatographic conditions were identical to those used for GC analysis.

Tests for antibacterial activity: The microorganisms used in the present study were six Gram positive (*S. intermedius, B. sphaericus, S. aureus, M. luteus, S. lutea* and *E. faecalis*) and nine Gram negative (*S. typhi, K. pneumonia, S. shiga, S. sonnei, S. boydii, S. marcescens, K. oxytoca, P. aeruginosa* and *E. coli*) human pathogenic bacteria as well as eight pathogenic fungi (*A. fumigatus, A. niger, A. flavus, V. factum, Mucor* sp., *C. albicans, F. oxysporum* and *C. falcatum*). The antibacterial assays were carried out by the disc-diffusion (Verpoorte *et al.*, 1983) and microdilution method (Daouk *et al.*, 1995; Hanel and Raether, 1988; Espinel-Ingroff, 2001) in order to determine the antibacterial activity of oils and their components against the human pathogenic bacteria. The bacterial suspensions were adjusted with sterile saline to a concentration of 1.0×10^5 CFU mL^{-1}. The inocula were prepared daily and stored at +4°C until use. Dilutions of the inocula were cultured on solid medium to verify the absence of contamination and to check the validity of the inoculum.

Disc-diffusion test: Compounds were investigated by the disc diffusion using 4 mm filter discs. Bacteria were cultured overnight at 28°C in LB medium and then adjusted with sterile saline to a concentration of 1.0×10^5 CFU mL^{-1}. The suspension was added to the top of agar (6 mL) and dissolved in Petri dishes (2 mL/agar plate) with solid peptone agar. Filter discs with essential oils and main components (1.0 μg mL^{-1}) were placed on agar plates (1 disc per agar plate). The fungi were grown in Potato Dextrose Agar (PDA) and/or Nutrient agar media. After 24 h of incubation at 28°C for bacteria or at 25°C for fungi the diameter of the growth inhibition zones was measured. Streptomycin was used as a positive control and 1 μL was applied to the discs from stock solution (1 mg mL^{-1}), whereas antifungal drug is Nystatin at 50 μg $disc^{-1}$. All tests were done in duplicate; Three replications were done for each oil and for each component (Sokovic *et al*., 2007).

Microdilution test: The minimum inhibitory and bactericidal and fungicidal concentrations (MICs and MBCs) were determined using microtitre plates. The bacterial suspension was adjusted with sterile saline to a concentration of 1.0×10^5 CFU mL^{-1}. Compounds to be investigated were dissolved in broth LB medium (100 μL) with bacterial inoculum (1.0×10^4 CFU per well) to achieve the wanted concentrations (0.02-15.0 μg mL^{-1}). The microplates were incubated for 24 h at 28°C. The lowest concentrations without visible growth (at the binocular microscope) were defined as concentrations that completely inhibited bacterial growth (MICs). The MBCs were determined by serial sub-cultivation of 2 μL into microtitre plates containing 100 μL of broth per well and further incubation for 72 h. The lowest concentration with no visible growth was defined as the MBC, indicating 99.5% killing of the original inoculum. The optical density of each well was measured at a wavelength of 655 nm by microplate manager 4.0 (Bio-Rad Laboratories) and compared with a blank and the positive control. Streptomycin was used as a positive control using the same concentrations as in the disc diffusion test, whereas antifungal drug is Nystatin at 50 μg $disc^{-1}$. Three replications were done for each oil and each component (Sokovic *et al*., 2007).

RESULTS AND DISCUSSION

Chemical composition of the essential oil: The chemical composition of *O. vulgare* essential oil is listed in Table 1, in which the percentages of components are given. In total, 33 volatile constituents, representing 98.73% of the total composition, were identified in the leaves oils (Table 1). The most abundant components found in the leaf oil were thymol (18.36%), followed by carvacrol (18%), terpinolene (17%), carvone (10%), ledene (2.03%), linalool (3.23%), limonene (5%), j-cymene (3.02%), caryophyllene (2.14%), germacrene-D (2.14%), y-terpineol (1.13%), camphor (1.7%), cymene (2%), caryophyllene oxide (1%), pulegone (1.05%), sabinyl acetate (1%) and α-pinene (1.11%). The essential oils yield of *O. vulgare* collected from Iran was 1.7%, it is relatively higher than other plants industrially exploited as a source of essential oils: *O. vulgare* (1.15%), thymus (1%) and lavender (0.8%) (Imelouane *et al*., 2009; Derwich *et al*., 2010a, b) and this yield is relatively lower than other plants: *L. nobilis* (1.86%) and *J. phoenicea* (1.62%) (Derwich *et al*., 2009a, b; 2010a, b). The phytochemistry revealed that this leaves had compositions similar to those of other *O. vulgare* essential oils analyzed by Derwich *et al*. (2010a, b) which the major compounds was carvacrol, thymol, y-terpinene and p-cymene representing 76.62% of the total oil. Vokou *et al*. (1993) reported carvacrol, thymol, y-terpinene and p-cymene as main constituents of *O. vulgare* essential oil. Previously, it was reported that *O. vulgare* growing in Brazil contained 4-terpineol (47.95%), carvacrol (9.42%), thymol (8.42%) and α-terpineol (7.57%) as major components (Cleff *et al*., 2010). The essential oil from the same plant from Turkey contained only caryophyllene

Table 1: Chemical composition of essential oils investigated

Components	*Origanum vulgare* (%)	*Rosmarinus officinalis* (%)
Sabinyl acetate	1.18	0.00
Pulegone	1.05	0.00
Caryophyllene oxide	1.00	0.00
Terpinen-4-ol	0.82	1.01
Sabinene	0.69	1.00
α-Copaene	0.40	0.00
Verbenol	0.20	4.12
Terpinolene	17.00	0.00
Cymen-8-ol	0.23	0.00
Ledene	2.03	0.00
Limonene	5.00	8.41
Carvacrol	18.00	0.00
1.8-Cineole	0.16	2.40
Linalool	3.23	3.02
Thymol	18.36	0.00
y-Terpinene	1.20	0.00
a-Pinene	1.11	52.01
Camphene	0.20	3.17
j-cymene	3.02	0.00
Caryophyllene	2.14	0.00
Cymene	2.00	0.00
Camphor	1.70	3.11
Isosativene	0.22	0.00
Solanone	0.17	0.00
Calarene	0.20	0.00
y-Terpineol	2.00	0.00
Germacrene-D	2.14	1.08
Cadinene	0.99	0.00
α-Cubebene	0.99	0.00
Carvone	10.00	0.00
y-cardinene	0.50	0.00
Bornyl acetate	0.70	1.00
b-pinene	0.00	2.01
Sabina ketone	0.10	0.00
Myrcene	0.00	2.11
α-phellandrene	0.00	0.50
g-terpinene	0.00	0.00
Para-cymene	0.00	0.84
α-terpinolene	0.00	1.00
Chrysanthenone	0.00	0.42
β-caryophyllene	0.00	2.16
Borneol	0.00	3.00
Geraniol	0.00	3.00
Total	98.73	95.37

(14.4%), spathulenol (11.6%), germacrene-D (8.1%) and α-terpineol (7.5%) as the main constituent (Tepe *et al.*, 2004). The chemical composition of essential oils of *R. officinalis* are presented in Table 1. In total, 20 volatile compounds, representing 95.37% of the total composition, were

identified in the leaves oils (Table 1). Monoterpene hydrocarbons were found to be the major group of compounds, the main one being α-pinene (52.01%) followed limonene (8.41%). The most abundant components found in the leaf oil were α-pinene (52.01%), other predominant components were terpinen-4-ol (1.01%), sabinene (1%), verbenol (4.12%), limonene (8.41%), 1.8-cineole (2.4%), linalool (3.02%), camphene (3.17%), camphor (3.11%), germacrene-D (1.08%), β-pinene (2.01%), myrcene (2.11%), α-terpinolene (1%), β-caryophyllene (2.16%), borneol (3%) and cymene (3%). The essential oils yield of *R. officinalis* was 0.92%, it is relatively higher than other plants industrially exploited as a source of essential oils: *A. herba-alba* (0.59%) and *A. absinthium* (0.57%) (Derwich *et al*., 2009a, b) and this yield is relatively lower than other plants: *Thymus* (1%) (Imelouane *et al*., 2009) and *R. officinalis* (0.48-1.75%) (Angioni *et al*., 2004). Tomei *et al*. (1995) investigated the essential oil from flowers and leaves of *R. officinalis* and found the main components to be camphor (32.33%), 1,8-cineole (14.41%) and α-pinene (11.56%). Kadri *et al*. (2011) found that α-pinene, 1,8-cineole, camphor, verbenone and borneol constituted and represented about 77.32% of the total *R. officinalis* oil.

Antibacterial and antifungal activity: The essential oil extracted from the leaves of *O. vulgare* and *R. officinalis* were used in the present study to investigate their antibacterial and antifungal potential. The results obtained and screening of antibacterial and antifungal activity of essential oil of *O. vulgare* and *R. officinalis* are summarized in Table 2, 3. All the oils tested in the disc-

Table 2: Antibacterial activity of essential oils (1.0 μg mL^{-1}) in disc-diffusion method, inhibition zones in mm

Bacteria and fungi	*Origanum vulgare*	*Rosmarinus officinalis*	*Streptomycin*	Nystatin
Gram positive				
S. intermedius	38	30	22	0
B. sphaericus	33	22	19	0
S. aureus	33	30	18	0
M. luteus	32	30	21	0
S. lutea	31	24	20	0
E. faecalis	30	22	20	0
Gram negative				
S. typhi	21	18	15	0
K. pneumonia	21	15	14	0
S. shiga	22	20	15	0
S. sonnei	20	15	11	0
S. boydii	21	22	12	0
S. marcescens	22	21	12	0
K. oxytoca	20	22	10	0
P. aeruginosa	20	20	8	0
E. coli	20	20	8	0
Fungi				
A. fumigatus	20	16	0	22
A. niger	15	15	0	21
A. flavus	15	15	0	20
V. factum	20	18	0	12
Mucor sp.	14	15	0	14
C. albicans	15	16	0	15
F. oxysporum	13	16	0	20
C. falcatum	15	15	0	15

Table 3: Antibacterial activity of essential oils (MIC and MBC μg mL^{-1}), microdilution method

Bacteria and fungi	*Origanum vulgare*	*Rosmarinus officinalis*	*Streptomycin*	Nystatin
Gram positive				
S. intermedius	0.5	0.5	1.0	0.0
	1.0	1.0	1.0	0.0
B. sphaericus	0.5	0.5	1.0	0.0
S. aureus	1.0	0.5	1.0	0.0
	1.5	1.5	0.5	0.0
M. luteus	1.5	1.0	1.0	0.0
	0.5	1.0	1.0	0.0
S. lutea	0.5	1.0	1.0	0.0
E. faecalis	1.5	1.5	0.5	0.0
	1.5	1.5	1.0	0.0
Gram negative				
S. typhi	2.0	3.0	2.0	0.0
	3.0	3.0	3.0	0.0
K. pneumonia	2.0	3.0	1.0	0.0
	3.0	2.0	1.0	0.0
S. shiga	3.0	3.0	2.0	0.0
	3.0	1.5	3.0	0.0
	3.0	5.0	5.0	0.0
S. sonnei	2.0	4.0	1.0	0.0
	2.5	3.0	5.0	0.0
S. boydii	2.5	2.0	2.0	0.0
	3.0	2.0	5.0	0.0
S. marcescens	1.5	2.0	1.0	0.0
	2.0	5.0	5.0	0.0
K. oxytoca	2.0	3.0	4.0	0.0
	2.0	3.0	3.0	0.0
P. aeruginosa	2.0	4.0	1.5	0.0
Fungi				
A. fumigatus	1.5	3.0	0.0	1.5
	2.0	1.5	0.0	1.0
A. niger	1.5	1.5	0.0	0.5
	2.5	3.0	0.0	1.0
A. flavus	1.5	2.5	0.0	1.0
	2.0	2.0	0.0	2.5
V. factum	2.5	2.5	0.0	1.5
	1.5	1.5	0.0	1.5

diffusion method showed bacteriostatic activity in concentration of 1 μg $disc^{-1}$. The essential oil of *O. vulgare* and *R. officinalis* tested by disc-diffusion method, showed very strong antibacterial and antifungal activity. The essential oils which showed the best antibacterial and antifungal activity in disc-diffusion method were *O. vulgare* (13.0-38.0 mm) and *R. officinalis* (15.0-30.0 mm). Streptomycin at 1 μg $disc^{-1}$ showed inhibition zones in the range of 8-22.0 mm (Table 2). Nystatin at 50 μg $disc^{-1}$ showed inhibition zones in the range of 12-22.0 mm (Table 2). It can be seen that essential oils from *O. vulgare* and *R. officinalis* possess a higher antibacterial and antifungal effect than streptomycin and Nystatin. The essential oils from *O. vulgare* and *R. officinalis* inhibited all the bacteria and fungi in very small concentrations. Streptomycin showed MIC at 1.0-3.0 μg mL^{-1} and MBC at 1.0-3.0 μg mL^{-1}. Nystatin showed MIC at 1.5-1.5 μg mL^{-1} and MBC at 1.0-2.5 μg mL^{-1}.

Table 4: Antibacterial activity of essential oils components (1.0 μg mL^{-1}) in disc-diffusion method, inhibition zones in mm

Bacteria and fungi	Terpinolene	Limonene	Carvacrol	Carvone	α-Pinene	Thymol	Streptomycin	Nystatin
Gram positive								
S. intermedius	20	18	30	32	28	35	25	0
B. sphaericus	18	18	30	32	25	28	15	0
S. aureus	15	15	25	32	24	28	17	0
M. luteus	15	14	21	30	25	24	20	0
S. lutea	16	17	22	30	22	25	18	0
E. faecalis	20	17	25	28	21	25	18	0
Gram negative								
S. typhi	14	11	18	20	15	20	14	0
K. pneumonia	12	12	18	20	15	20	13	0
S. shiga	11	14	20	22	16	20	15	0
S. sonnei	14	13	20	21	16	22	12	0
S. boydii	13	15	20	25	18	21	14	0
S. marcescens	10	15	20	25	15	20	15	0
K. oxytoca	10	13	18	23	16	20	12	0
P. aeruginosa	12	14	18	21	20	20	13	0
E. coli	11	13	20	20	15	20	10	0
Fungi								
A. fumigatus	10	11	15	24	16	20	0	20
A. niger	10	10	18	24	15	20	0	25
A. flavus	10	13	15	25	15	20	0	15
V. factum	13	11	15	21	15	20	0	20
Mucor sp.	12	11	15	20	15	20	0	20
C. albicans	12	12	16	27	16	21	0	18
F. oxysporum	11	10	20	23	15	20	0	15
C. falcatum	10	10	20	21	15	22	0	10

From the obtained results it can be noticed that streptomycin and Nystatin possessed lower antibacterial and antifungal activity than *O. vulgare* and *R. officinalis* (Table 3). The results of antibacterial and antifungal activity of essential oil components are presented in Table 4 and 5. linalool inhibited bacterial growth of all bacteria and fungi and inhibition zones were 10.0-18.0 mm, Terpinolene reacted slightly better (inhibition zones 10.0-20.0 mm) while streptomycin and Nystatin showed inhibition with zones of 10.0-25.0 mm. Strong antibacterial and antifungal activity was noticed for a-Pinene (15.0-28.0 mm) and especially for carvacrol (15.0-35.0 mm), thymol (20.0-35.0 mm) and carvone (20.0-32.0 mm). It can be seen that thymol, α-pinene and carvacrol showed higher antibacterial activity than streptomycin while, carvone showed higher antifungal activities than previous components and nystatin. Terpinolene showed MIC of 4.0-6.0 μg mL^{-1} and MBC at 5.0-10.0 μg mL^{-1}. Similarity, limonene showed MIC of 4.0-6.0 μg mL^{-1} and MBC at 5.0-10.0 μg mL^{-1}. α-Pinene showed MIC of 1.5-2.5 μg mL^{-1} and MBC at 1.0-2.5 μg mL^{-1}. Limonene showed MIC of 1.5-1.5 μg mL^{-1} and MBC at 1.0-1.5 μg mL^{-1}. Carvacrol showed MIC of 1.0-1.0 μg mL^{-1} and MBC at 1.0-1.0 μg mL^{-1}. Similarity, thymol showed MIC of 1.0-1.0 μg mL^{-1} and MBC at 10-1.0 μg mL^{-1}. Only carvone, carvacrol and thymol showed higher antibacterial and antifungal activity than streptomycin and Nystatin (MIC 3.0-5.0 μg mL^{-1} and MBC 5.0-5.0 μg mL^{-1}) (Table 5). These results are similar to those found by (Baydar *et al*., 2004; Chaudhry *et al*., 2007; Derwich *et al*., 2009a, b; Derwich *et al*., 2010a, b). Carvone, carvacrol and thymol are the main components of the essential oil of *O. vulgare* and *R. officinalis* which are

Table 5: Antibacterial activity of essential oils components (MIC and MBC μg mL^{-1}), microdilution method

Bacteria and fungi	Terpinolene	Limonene	Carvacrol	Carvone	α-Pinene	Thymol	Nystatin	Streptomycin
Gram positive								
S. intermedius	4.0	4.0	1.0	1.5	1.5	1.0	-	3.0
	5.0	5.0	1.0	1.0	1.0	1.0	-	5.0
S. aureus	6.0	7.0	1.5	2.5	1.0	1.0	-	9.0
	5.0	4.0	0.5	2.0	1.0	1.0	-	5.0
M. luteus	2.0	9.0	0.5	1.5	1.0	1.0	-	2.0
	1.0	9.0	0.5	1.5	1.0	0.5	-	3.0
S. lutea	3.0	5.0	1.0	1.5	1.0	0.5	-	6.0
	2.0	4.0	1.0	1.0	1.0	0.5	-	8.0
E. faecalis	1.0	6.0	1.5	1.5	1.0	0.5	-	1.0
	5.0	9.0	1.5	1.5	1.0	1.5	-	0.5
Gram negative								
S. typhi	4.0	4.0	2.0	3.0	2.0	2.0	-	9.0
	5.0	5.0	2.5	5.0	2.5	1.0	-	7.0
K. pneumonia	6.0	6.0	4.0	5.0	2.0	1.5	-	4.0
	5.0	2.0	2.0	5.0	2.0	3.0	-	3.0
S. shiga	5.0	3.0	3.0	4.0	2.5	3.0	-	5.0
	5.0	4.0	1.0	3.0	1.5	3.0	-	2.0
S. sonnei	1.5	0.5	3.0	6.0	1.0	1.0	-	3.0
	7.0	4.0	3.0	5.0	1.0	1.0	-	6.0
S. boydii	4.0	6.0	5.0	4.0	3.0	1.0	-	8.0
	5.0	9.0	5.0	4.0	3.0	1.5	-	4.0
S. marcescens	6.0	4.0	1.0	4.0	2.5	1.5	-	2.0
	1.0	4.0	4.0	5.0	2.0	1.5	-	3.0
K. oxytoca	5.0	7.0	3.0	8.0	3.0	1.0	-	2.0
	6.0	5.0	6.0	5.0	5.0	1.0	-	8.0
P. aeruginosa	4.0	1.0	2.5	3.0	5.0	1.5	-	1.0
	3.0	5.0	6.0	4.0	5.0	1.5	-	5.0
Fungi								
A. fumigatus	1.0	5.0	4.0	1.5	2.5	2.0	3	-
	4.0	6.0	6.0	2.5	2.0	2.0	5	-
A. niger	3.5	4.0	2.0	3.0	1.2	2.0	1	-
	5.0	4.0	14.0	3.0	2.0	2.0	5	-
A. flavus	7.0	9.0	6.0	5.0	0.5	1.5	6	-
	10.0	10.0	1.0	1.5	2.5	1.0	8	-
V. factum	1.0	4.0	4.0	5.0	2.0	1.0	4	-
	6.0	6.0	1.0	1.5	2.5	1.0	5	-

responsible of its antimicrobial and antifungal (Tian and Lai, 2006; Derwich *et al*., 2010a, b). The antimicrobial activities, in general have been mainly explained through terpenes with aromatic rings and phenolic hydroxyl groups able to form hydrogen bonds with active sites of the target enzymes, although other active terpenes, as well as alcohols, aldehydes and esters can contribute to the overall antimicrobial effect of essential oils (Belletti *et al*., 2004). Essential oils are rich in α-pinene demonstrated potential antibacterial activity (Hajji *et al*., 1993; Tantaoui-Elaraki *et al*., 1993). Essential oils rich in phenolic compounds, such as carvacrol, are widely reported to possess high levels of antimicrobial activity (Aligiannis *et al*., 2001; Baydar *et al*., 2004; Belletti *et al*., 2004). On the other hand, it has previously been shown that Carvone, Carvacrol and Thymol is capable of inhibiting bacteria and fungi (Chaudhry *et al*., 2007;

Sokovic *et al*., 2007; Derwich *et al*., 2009a, b, 2010a, b; Imelouane *et al*., 2009) observed that the susceptibility of gram-positive and gram negative bacteria to plant volatile oils had a influence on growth inhibition. However, some oils appeared more active with respect to gram reaction, exerting a greater inhibitory activity against gram-positive bacteria (Derwich *et al*., 2009a, b, 2010a, b). Gram-negative bacteria were shown to be generally more resistant than Gram-positive ones to the antagonistic effects of essential oils because of the lipopolysaccharide present in the outer membrane but this was not always true.

The study demonstrated that *O. vulgare* and *R. officinalis* represents a source of natural mixtures of antibacterial and antifungal constituents that can be as effective as modern medicine to combat pathogenic micro-organisms.

CONCLUSION

Present study was conducted to investigate the chemical composition and antibacterial and antifungal activity of essential oil extracted from *O. vulgare* and *R. officinalis*. The major compounds were carvacrol, followed by thymol, terpinolene, limonene, α-pinene and germacrene-D. However, further studies are still required to investigate its application in medicine and food industries.

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