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ORIGINAL ARTICLE

The composition of the essential oil and aqueous distillate of *Origanum vulgare* L. growing in Saudi Arabia and evaluation of their antibacterial activity

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Abstract The essential oil and aqueous distillate composition of *Origanum vulgare* L. were analyzed by GC/MS. Sixty-seven different components were detected in both oils. Sixty-four components were characterized for the oil derived from the aerial parts, whereas thirty-three components in the volatile oil from the aqueous distillates of *O. vulgare* L., representing 99.8% and 98.5% of the oils, respectively. The main components of the volatile oil from the aerial parts of *O. vulgare* L. were carvacrol (70.2 ± 1.37%), γ-terpinene (5.6 ± 0.11%), *p*-cymene (4.5 ± 0.42 %), *trans*-sabinene hydrate (3.8 ± 0.07%), and thymol (2.2 ± 0.12%). In comparison, the main compounds of the volatile oil of the *O. vulgare* L. aqueous distillates were carvacrol (92.5 ± 0.97 %), thymol (2.5 ± 0.09%), and terpinen-4-ol (1.0 ± 0.03%). The antibacterial activity of both oils, along with that of the purified major component, carvacrol, against Gram-positive and Gram-negative strains was assessed. The results revealed that all three samples showed significant antibacterial activity against all tested strains. The IC_{50} values of the oils derived from the aerial parts and aqueous distillates of *O. vulgare* L. against the tested strains was in the range of 107–383 $\mu g \cdot mL^{-1}$, whereas, the IC_{50} value of carvacrol was in the range of 53–151 $\mu g \cdot mL^{-1}$. The data suggest that carvacrol, a major component of both oils, possesses the highest antibacterial activity of all the

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constituents and is the main component responsible for the antibacterial activity of Saudi *O. vulgare* L. oils.

1. Introduction

Medicinal and aromatic plants (MAPs) have been utilized in traditional medicine for centuries. About four-hundred compounds derived from plants are currently being used in drug formulations (Musthaba et al., 2010; Sewell and Rafieian-Kopaei, 2014). The active compounds of MAPs are generally employed directly in medicines, food flavoring and preservation, cosmetics, and pharmaceuticals. MAPs contain biologically active chemical substances in the form of secondary metabolites. Secondary metabolites are generally produced by plant secondary metabolism and belong to various chemical classes such as alkaloids, flavonoids, saponins, steroids, and terpenoids. These secondary metabolites are considered to be the main constituents that impart medicinal properties to the plants. Moreover, the volatile oils obtained from aromatic and medicinal plants are composed of a complex mixture of secondary metabolites and can be used in various applications such as aromatherapy, perfumery, pharmaceuticals, foods, detergents, and cosmetics.

The genus *Origanum* is a perennial herbaceous aromatic plant that belongs to the family Lamiaceae. These plants are indigenous to the Mediterranean, Euro-Siberian, North Africa, and many other Asian countries having moderate temperatures, where the plants grow in mountainous or open terrains. Some species are also dispersed in areas of North America and other places (Aligiannis et al., 2001). The plants possess intensely fragrant leaves and ample cylindrical flowers with colorful bracts. The genus comprises an important section of culinary herbs, which includes marjoram and oregano (Kindersley, 2008), and is well known for its volatile oils and other chemical substances.

Origanum vulgare L. (oregano) is a medium-sized perennial aromatic herb of the genus *Origanum*. This species is considered to be one of the most extensively used aromatic plants within the Lamiaceae family. Its volatile oils contain mono- and sesquiterpenoids as major chemical classes of secondary metabolites. In the majority of *O. vulgare* L. essential oils, phenolic monoterpenoids constitute up to 70% of the total oil; these monoterpenoids mainly comprise polar phenolic compounds such as thymol and carvacrol. Moreover, γ-terpinene and *p*-cymene have been detected in appreciable amounts (Bozin et al., 2006; Sarikurkcu et al., 2015). The volatile oils of *O. vulgare* L. reportedly display anti-inflammatory, antispasmodic, antibacterial, diaphoretic, antioxidant, antifungal, analgesic, and carminative activity (Faleiro et al., 2005; Souza et al., 2007; Tommasi et al., 2009).

The chemical components of volatile oils in plants vary considerably based on the geographical origin and the developmental stage of the plants. Therefore, this widens the area of research for the same plant species grown in diverse topographical locations as the chemical composition of the volatile oils may vary (Gupta et al., 2002; Holm et al., 1998; Verma et al., 2011). Although the volatile oils of *O. vulgare* L. grown under diverse geographical conditions of the world have been studied (Afsharypour et al., 1997; Al-Kalaldeh et al., 2010; Camiletti et al., 2016; Chorianopoulos et al., 2004; Gong et al., 2014; Kula et al., 2007; Sarikurkcu et al., 2015; Suzuki et al., 2015), to the best of our knowledge, the chemical composition of the volatile oil of *O. vulgare* L. grown in Saudi Arabia has not been reported to date. Moreover, water distillates produced during hydro/steam distillation of the plants for extraction of the essential oils may contain various valuable aromatic constituents. These slightly hydrophilic aroma-compounds in the aqueous distillates are called hydrosol and may have a very pleasant odor, and thus can be used as high-quality flavoring agents in the food, cosmetics, soap, and perfume industries. Because hydrosols are the by-products obtained during the hydro/steam distillation of aromatic plants and contain various valuable aroma chemicals, recently, a few methods of recovering these valuable aroma-compounds from water distillates have been developed (Bohra et al., 1994; Fleisher 1990; Verma et al., 2016). However, to date, studies on the chemical constituents of hydrosols are limited to only a few plants (Eikani et al., 2005; Nakagawa et al., 2016; Rao et al., 2005; Verma et al., 2016). Therefore, in the present work, we investigate the chemical composition of the volatile oils extracted from the aerial parts (SOVAD) and aqueous distillates (SOVADH) of *O. vulgare* L. grown in Saudi Arabia. Chemical profiling of the SOVAD and SOVADH obtained through hydro-distillation is performed by gas chromatography with flame ionization and mass spectral detection (GC-FID and GC-MS) techniques using two different stationary phase (polar and non-polar) columns, as well as by employing nuclear magnetic resonance (NMR) spectroscopy. The antimicrobial properties of the SOVAD and SOVADH and their purified compounds against Gram-negative and Gram-positive bacterial strains were also determined.

2. Materials and methods

2.1. Plant material

The aerial parts of *O. vulgare* L. grown in Al-Kharj, central province of Saudi Arabia, were collected before flowering stage in the month of March 2013. Authentication of the plant material was assured by a plant taxonomist (Dr. Jacob Thomas Pandalayil, Herbarium Division, King Saud University, Riyadh, Saudi Arabia). A token sample of the plant materials with voucher specimen number (OVHZK-303) is retained in our laboratory.

2.2. Isolation of essential oils by hydro-distillation

The freshly collected aerial parts of *O. vulgare* L. were sliced into tiny sections and air-dried in the shade at 20 °C. The resultant dried aerial parts of *O. vulgare* L. (326.4 g) were subjected

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to hydro-distillation in a Clevenger apparatus for 3 h to give a light-yellow oil. The yield of the oil was 1.7% (*w*/*w*) on a dry weight basis. The organic constituents recovered from the aqueous distillate were dried using anhydrous Na_2SO_4 as the dehydrating agent and stored at 4 °C until further use. The aqueous distillate (300 mL) obtained during hydro-distillation of the dried aerial parts of *O. vulgare* L. was exhaustively extracted (three times) with ethyl acetate (50 mL) in a separatory funnel. The combined ethyl acetate extracts (150 mL) were then dried using anhydrous Na_2SO_4, filtered, and the solvent was removed by rotary evaporation under reduced pressure to acquire the volatile oil.

2.3. Gas chromatography (GC) and gas chromatography–mass spectrometry (GC-MS) analysis of the essential oil and organic content in the aqueous distillate

The volatile oils were analyzed by using GC–MS and GC–FID techniques with two different stationary phase columns. For instance, the DB-Wax column was used for polar column analysis and the HP-5MS column was used for apolar column analysis. GC–MS analysis was executed on an Agilent single-quadrupole mass spectrometer fitted with an inert mass selective detector (MSD-5975C detector, Agilent Technologies, USA) attached directly to an Agilent 7890A gas chromatograph that was equipped with an auto-sampler (Agilent model 7693), a quickswap assembly, a split–splitless injector, and a HP-5MS column (5% phenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm i.d., film thickness: 0.25 μm, Agilent Technologies, USA). Additional analyses were performed on the same instrument using the polar DB-Wax column (polyethylene glycol, 30 m × 0.25 mm i.d., film thickness: 0.25 μm, Agilent Technologies, USA). The non-polar column was utilized at an injector temperature of 250 °C with the following oven temperature programming: initially, the oven temperature was kept isothermal for 4 min at 50 °C and was then raised to 220 °C at a rate of 4 °C·min^{-1}, followed by another isothermal hold for 2 min; in the second ramp, the temperature was raised to 280 °C at a rate of 20 °C·min^{-1}, and finally, the temperature was kept isothermal for 15 min. In contrast, the polar column was utilized at an injector temperature of 250 °C with the following oven temperature programming: initially, the oven temperature was maintained isothermal for 4 min at 40 °C, followed by a temperature increment of 4 °C·min^{-1} to 220 °C, and then finally kept isothermal for 10 min.

Approximately 0.2 μL of the respective *O. vulgare* L. oils dissolved in acetone (5% *O. vulgare* L. oil solution in acetone) was injected by utilizing the split injection approach; the split flow ratio was 10:1. Helium was used as the carrier gas at a rate of 1 mL·min^{-1}. The mass spectra and gas chromatography-total ion chromatogram (GC–TIC) profiles were acquired with the help of ChemStation data analysis software (version E-02.00.493, Agilent). All mass spectra were obtained in the electron ionization (EI) mode by using an ionization energy of 70 eV and an *m*/*z* scan range of 45–600. The temperature of the electronic-impact ion source was maintained at 230 °C, whereas the MS quadrupole temperature was kept at 150 °C. The MSD transfer line temperature was held at 280 °C for both the polar and nonpolar analysis. GC analysis was performed on an Agilent GC-7890A dual-channel gas chromatograph (Agilent Technologies, USA) connected to a flame ionization detector (FID) using both polar and nonpolar columns by applying the aforementioned parameters. The temperature of the FID was kept at 300 °C for all analyses. The relative percentage of the volatile oil constituents was computed on the basis of the areas of the peaks in the GC–FID profile, acquired by using the non-polar column without applying a correction factor. The results obtained for the *O. vulgare* oils are recorded in Table 1 based on the order of elution of each component on the non-polar column.

2.4. Identification of oil constituents

The GC–FID chromatograms of the volatile oils obtained from the dried aerial parts of *O. vulgare* L. and its aqueous distillates and the identified peaks of the oil constituents separated on the non-polar (HP-5MS) column are displayed in Figs. 1 and 2, respectively. The volatile oil components were characterized by matching their mass spectra with the library entries in the mass spectra databases (NIST-08 MS Library, version 2.0 f, WILEY 9th edition, Flavor and Adams libraries) and by comparing their mass spectra and LRIs with the data reported using both the DB-Wax and HP-5MS columns (Adams, 2007; Babushok et al., 2011; NIST 2017), and by co-injection of genuine standards accessible in our laboratory.

2.5. Isolation of major constituents from volatile oils

Column chromatography (CC) and thin layer chromatography (TLC) were used to purify carvacrol, a major component of the volatile oil from the aerial parts of *O. vulgare* L. A 1.0 g aliquot of the oil was subjected to CC using a silica gel column (60–120 mesh, 27 gm) and gradient elution was applied using hexane and chloroform mixtures in various ratios (100:0, 75:25, 65:35, 50:50, and 0:100), with increasing polarity, as the mobile phase. In total, forty-five fractions (15 mL each) were collected, of which fractions 29 to 33 corresponded to carvacrol based on the thin layer chromatography (TLC) profiles. These fractions were combined and the solvent was removed under reduced pressure using a rotary evaporator to produce pure carvacrol (**1**).

2.6. Carvacrol (*1*)

Dark-orange oil. ^{1}H NMR (400 MHz, $CHCl_3$-*d*): δ (ppm) = 1.38 (6H, m, H-8, H-9), 2.42 (3H, s, H-10), 2.99 (1H, m, H-7), 5.89 (1H, br s, OH), 6.83 (1H, s, H-6), 6.94 (1H, d, J = 7 .3 Hz, H-4), 7.24 (1H, d, J = 7.3 Hz, H-3); ^{13}C NMR (100 MHz, $CHCl_3$-*d*): δ 15.50 q (C-10), 24.02 q (C-8, C-9), 33.75 d (C-7),113.36 d (C-6), 119.05 d (C-4), 121.42 s (C-2), 131.04 d (C-3), 148.49 s (C-5), 153.48 s (C-1); EI-MS *m*/*z*: 150 ([M]$^+$).

2.7. Chemicals

Analytical-grade 2-propanone (purchased from Sigma-Aldrich, Germany) was employed for dilution of the volatile oil samples. The constituents of the pure essential oil, such as *β*-pinene, *γ*-terpinene, *α*-pinene, and thymol, in addition to

Table 1 Chemical constituents of volatile oils extracted from aerial parts and aqueous distillates of *O. vulgare* L. grown in Saudi Arabia.

No.	Compound*	LRI_{Lit}	LRI^{a}_{Exp}	LRI^{p}_{Exp}	SOVAD (%)[b]	SOVADH (%)[b]	Identification[c]
1	*trans*-2-Hexenal	846	852	1217	t	t	1,2
2	*cis*-3-Hexen-1-ol	850	854	1389	–	t	1,2,3
3	2-Heptanol	–	898	–	t	–	1,2
4	**α-Thujene**	**924**	**927**	**1024**	**1.1 ± 0.05**	**–**	**1,2**
5	α-Pinene	932	934	1017	0.6	–	1,2,3
6	Camphene	946	949	1059	0.1	–	1,2,3
7	Benzaldehyde	952	961	1523	t	0.1	1,2
8	Sabinene	969	974	1117	0.3	–	1,2
9	β-Pinene	974	977	1104	0.2	–	1,2,3
10	1-Octen-3-ol	974	979	1455	0.3	0.1	1,2,3
11	3-Octanone	979	988	1254	t	–	1,2,3
12	**β-Myrcene**	**988**	**991**	**1164**	**1.7 ± 0.11**	t	**1,2,3**
13	3-Octanol	988	995	1398	0.4	t	1,2,3
14	α-Phellandrene	1002	1005		0.2	–	1,2,3
15	δ-3-Carene	1008	1011	1146	0.1	–	1,2
16	**α-Terpinene**	**1014**	**1017**	**1177**	**1.7 ± 0.09**	t	**1,2,3**
17	***p*-Cymene**	**1020**	**1025**	**1269**	**4.5 ± 0.42**	0.1	**1,2,3**
18	β-Phellandrene	1025	1030	1205	0.5	–	1,2
19	1,8-Cineole	1026	1033	1212	t	–	1,2,3
20	Benzyl alcohol	1026	1035		–	0.1	1,2
21	*cis*-β-Ocimene	1032	1039	1236	t	–	1,2
22	*trans*-β-Ocimene	1044	1049	1251	t	–	1,2
23	**γ-Terpinene**	**1054**	**1060**	**1245**	**5.6 ± 0.11**	0.1	**1,2**
24	*cis*-Sabinene hydrate	1065	1068	1470	0.8	0.2	1,2
25	α-Terpinolene	1086	1089	1282	0.3	t	1,2
26	***trans*-Sabinene hydrate**	**1098**	**1099**	**1555**	**3.8 ± 0.07**	0.9	**1,2,3**
27	1-Octen-3-yl acetate	1110	1113	1380	t	–	1,2
28	*cis*-*p*-Menth-2-en-1-ol	1118	1123	–	0.1	0.1	1,2
29	α-Campholenal	1122	1128	1491	t	–	1,2
30	*trans*-*p*-Mentha-2-en-1-ol	1135	1141	1591	0.1	0.1	1,2
31	Camphor	1141	1148	1518	t	–	1,2
32	Borneol	1165	1168	1708	0.2	0.1	1,2
33	**Terpinen-4-ol**	**1174**	**1180**	**1608**	**1.9 ± 0.10**	**1.0 ± 0.03**	**1,2,3**
34	*p*-Cymene-8-ol	1179	1188	1854	0.1	0.1	1,2
35	α-Terpineol	1186	1193	1703	0.3	0.2	1,2,3
36	*cis*-Dihydrocarvone	1191	1199	1611	0.2	0.1	1,2
37	*n*-Decanal	1201	1208	1495	t	–	1,2
38	Verbenone	1204	1210	–	0.1	–	1,2
39	*trans*-Carveol	1215	1213	1842	t	–	1,2
40	*cis*-Carveol	1226	1228	–	t	–	1,2
41	Methyl carvacrol	1241	1246	–	t	t	1,2
42	Linalool acetate	1254	1258	–	t	–	1,2
43	Bornyl acetate	1284	1287	1584	t	–	1,2
44	**Thymol**	**1289**	**1293**	**2190**	**2.2 ± 0.12**	**2.5 ± 0.09**	**1,2,3**
45	**Carvacrol**	**1298**	**1309**	**2224**	**70.2 ± 1.37**	**92.5 ± 0.97**	**1,2,3,4**
46	δ-Elemene	1335	1342	–	t	–	1,2
47	Eugenol	1356	1361	2172	t	0.1	1,2
48	Carvacrol acetate	1370	1375	1876	0.2	0.1	1,2
49	**β-Caryophyllene**	**1417**	**1427**	**1600**	**1.2 ± 0.08**	t	**1,2,3**
50	*trans*-α-Bergamotene	1432	1440	1588	t	–	1,2
51	α-Guaiene	1437	1446	1595	0.1	0.1	1,2
52	Seychellene	1444	1450	1644	t	–	1,2
53	α-Humulene	1452	1461	1672	0.1	–	1,2
54	Germacrene-D	1484	1490	1712	t	–	1,2
55	α-Selinene	1498	1502	1728	0.1	–	1,2
56	α-Bulnesene	1509	1513	1718	–	t	1,2
57	γ-Cadinene	1513	1521	1763	t	–	1,2
58	*trans*-Calamenene	1521	1529	1835	t	–	1,2
59	Spathulenol	1577	1586	2131	t	t	1,2
60	Caryophyllene oxide	1582	1592	1989	0.1	0.1	1,2,3
61	Viridiflorol	1592	1600	–	t	0.1	1,2
62	1,10-Di-*epi*-cubenol	1618	1619	2065	t	–	1,2

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Table 1 (*continued*)

No.	Compound*	LRI_{Lit}	LRI^{a}_{Exp}	LRI^{p}_{Exp}	SOVAD (%)[b]	SOVADH (%)[b]	Identification[c]
63	τ-Cadinol	1638	1648	2180	t	t	1,2
64	Pentadecanoic acid	–	1871	–	0.1	t	1,2
65	2-Heptadecanone	–	1909	–	0.1	–	1,2
66	*n*-Hexadecyl acetate	2003	2005	2305	t	–	1,2
67	Phytol	1942	2108	2621	t	–	1,2
Chemical class Composition							
Monoterpene hydrocarbons					16.88	0.23	
Oxygenated monoterpenes					80.28	97.66	
Sesquiterpene hydrocarbons					1.66	0.12	
Oxygenated sesquiterpenes					0.18	0.23	
Oxygenated aliphatic hydrocarbons					0.88	0.22	
Aromatics					0.0	0.07	
Diterpenes					0.021	0.0	
Total identified					99.90	98.53	
Oil yield (%, w/w-dry weight basis)					1.70	0.13	

* = Components are recorded as per their order of elution from a nonpolar column; [b] = Mean percentage calculated from FID data and compounds higher than 1.0% are highlighted in boldface and their ± SD (n = 2) are mentioned; LRI_{Lit} = Linear retention index from the literature (Adams, 2007); LRI^{a}_{Exp} = Computed LRI with reference to *n*-alkanes mixture (C8-C31) on nonpolar column; LRI^{p}_{Exp} = Computed LRI with reference to *n*-alkanes mixture (C8-C31) on polar column; SOVAD = Oil from dried aerial parts of *O. vulgare* L.; SOVADH = Oil from hydrosol of dried aerial parts of *O. vulgare* L.; [c] = Identification by; 1 = Linear retention index (LRI) identical to literatures (*cf.* exp. part); 2 = Comparison of mass spectra (MS) with the library entries of mass spectra databases (*cf.* exp. part); 3 = co-injection/comparison with the LRI and mass spectra of standards; 4 = ^{1}H and ^{13}C NMR; t = trace (< 0.05%).

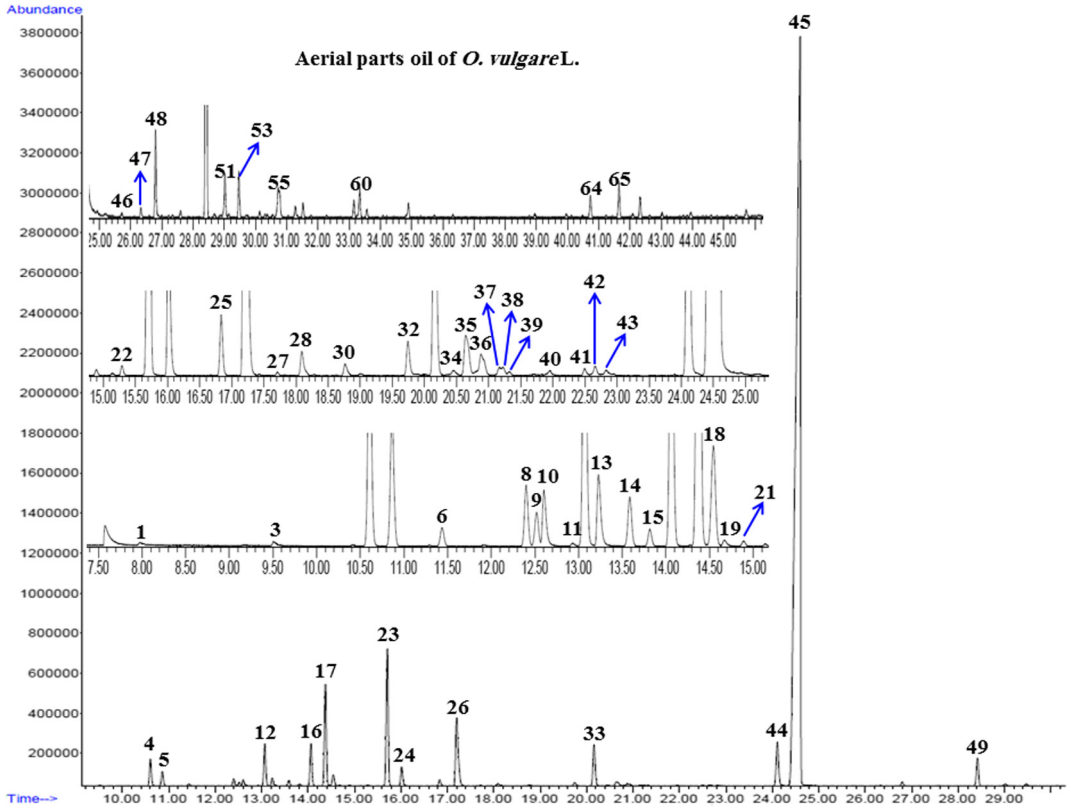

Fig. 1 GC–FID chromatogram of volatile oil from aerial parts of *O. vulgare* L. obtained using HP-5MS column. The characterized peaks are numbered according to their serial number in Table 1.

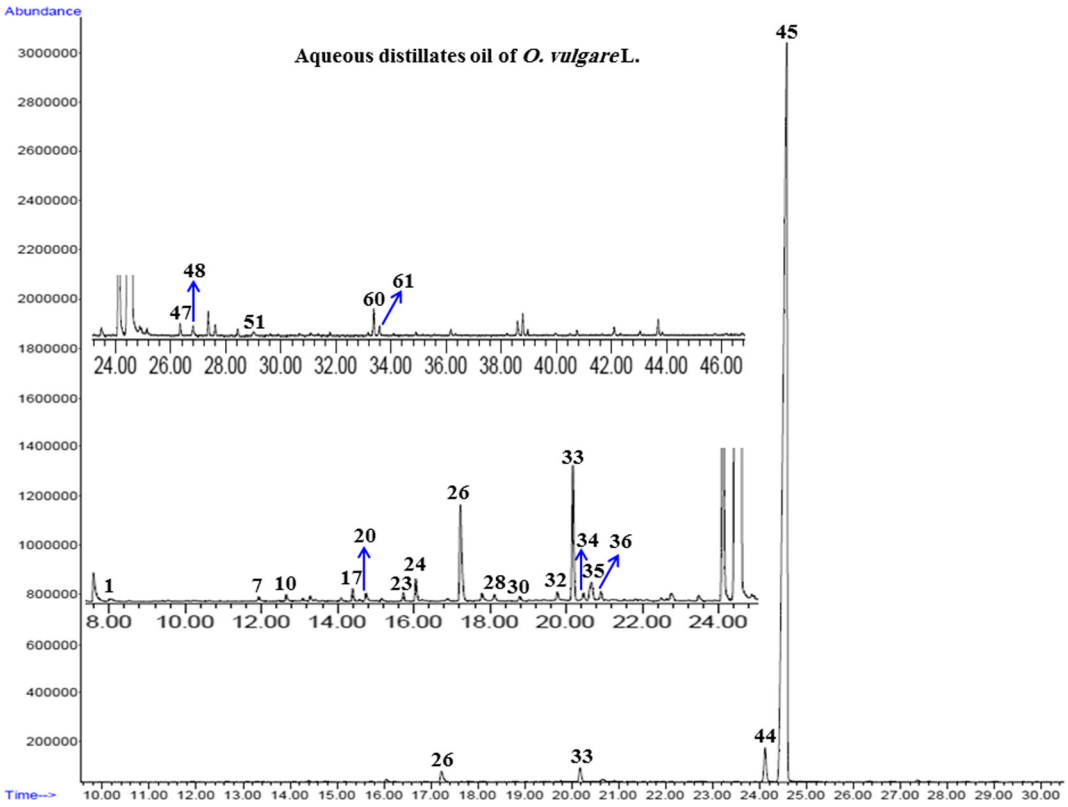

Fig. 2 GC–FID chromatogram of essential oil from aqueous distillate of *O. vulgare* L. using HP-5MS column. The characterized peaks are numbered according to their serial number in Table 1.

some volatile oil fractions mainly comprising compounds such as camphene, *β*-caryophyllene, *p*-cymene, 1-octen-3-ol, *α*-terpinene, terpinen-4-ol, *cis*-3-hexen-1-ol, 3-octanone, and caryophyllene oxide, were available and were used for co-injection/comparative analysis.

2.8. Retention indices

A mixture of an uninterrupted sequence of straight-chain hydrocarbons starting from C8 to C31 (C8–C20, 04070, Sigma-Aldrich, USA and C20–C31, S23747, AccuStandard, USA) was injected into both the DB-Wax (a polar column) and HP-5MS (a nonpolar column) columns using the conditions described above for the *O. vulgare* L. oil samples in order to calculate the linear retention indices (LRIs) of the *O. vulgare* L. oil components (Table 1). The LRIs of the oil components of *O. vulgare* L. were computed using van den Dool and Kratz's equation.

2.9. Nuclear magnetic resonance (NMR) analysis

The ^{1}H and ^{13}C NMR spectra of the *O. vulgare* L. oil samples and their purified compounds were obtained by using a JEOL ECP-400 spectrophotometer. The NMR samples were prepared in deuterated chloroform ($CDCl_3$) and tetramethylsilane (TMS) was used as an internal standard. The chemical shifts and coupling constants (*J*) are expressed in *δ* (ppm) and Hz, respectively. Thin layer chromatography (TLC) was carried out on pre-coated silica gel 60 F_{254} (0.2 mm, Merck) plates and the compounds were detected under UV light.

2.10. Determination of antimicrobial activity

Inhibition of the growth of *E. coli* ATCC 25922, *P. aeruginosa* ATCC 75853, *M. luteus* ATCC 10240, and *S. aureus* ATCC 25,923 in the presence of the *O. vulgare* L. volatile oils and their purified compounds was evaluated by calculating the change in the optical density of cells grown with or without the test compounds. *P. aeruginosa*, *E. coli*, *S. aureus*, and *M. luteus* were grown in sterile nutrient broth, Luria broth, and Müeller-Hinton broth, respectively, at their optimal growth temperatures. A 10 µL aliquot of the cultures grown to the log phase was added to 90 µL of sterile broth. The test compounds were diluted and were added to the wells at final concentrations of 50, 100, 200, 300, and 500 µg mL^{-1}, where the test compounds were diluted in 5% DMSO. Finally, the plates were incubated for 12 h at the optimal growth temperatures (Haque et al., 2017). The absorbance was determined at 600 nm using a Multiskan microtiter plate reader (Multiskan Ex, Thermo Scientific, Finland).

The OD_{600} at 0 h was subtracted from the OD_{600} at a given time interval to calculate the change in the optical density. The values presented are the mean ± standard error of three independent experiments. Moreover, the unpaired *t*-test implemented in GraphPad software was used to determine the statistical significance (*p*-values). The IC_{50} values were calculated from the average OD_{600} values for each treatment.

3. Results and discussion

In continuation of our research on Saudi plants (Alkhathlan et al., 2015; Al-Saleem et al., 2016; Khan et al., 2016), the present study focuses on chemical characterization and the antimicrobial activities of the volatile oils and their major components isolated from the aerial parts and aqueous distillates of *O. vulgare* L. grown in Saudi Arabia. Hydro-distillation of the aerial parts of *O. vulgare* L. in a traditional Clevenger apparatus furnished a light-yellow essential oil with a significant yield of 1.7%, *w*/*w*, on a dry weight basis. The yield of volatile oil from the aqueous distillates was found to be 0.13%. Comparing the yield of the volatile oil derived from the aerial parts of *O. vulgare* L. grown in Saudi Arabia with those recorded earlier for *O. vulgare* L. plants grown in various regions of the world (Gong et al., 2014; Lukas et al., 2008; Sarikurkcu et al., 2015) revealed that the Saudi *O. vulgare* L. plant contained an appreciable amount of essential oil, the content being much higher than those reported previously for *O. vulgare* L. grown in several parts of the world (Bozin et al., 2006; Gong et al., 2014). The highest percentage of volatile oil reported earlier was for *O. vulgare* L. plants grown in Greece (Kokkini and Vokou, 1989), where the yield of essential oil was as high as 8.0%. The lowest oil yield was reported for *O. vulgare* L. plants grown in China and Corsica, where yield of essential oil was as low as 0.1% (Gong et al., 2014; Lukas et al., 2008).

The phytochemical constituents of the volatile oils derived from the aerial parts and aqueous distillates of *O. vulgare* L. grown in Saudi Arabia were analyzed by NMR as well as by GC–FID and GC–MS by utilizing two different columns (polar and nonpolar). These analyses led to the identification of a total of sixty-seven different compounds from both oils. Identification of the most representative components in both oils was further confirmed by ^{1}H and ^{13}C NMR (Figs. S1a and S1b). Sixty-four constituents were identified in the oil derived from the aerial parts of *O. vulgare* L., whereas thirty-three constituents were identified in the oils attained from the hydrosol of *O. vulgare* L.; these components respectively represent 99.8% and 98.5% of the overall oil compositions. The identified volatile constituents and their relative percentages are shown in Table 1 in the order of elution of each oil component on the HP-5MS column.

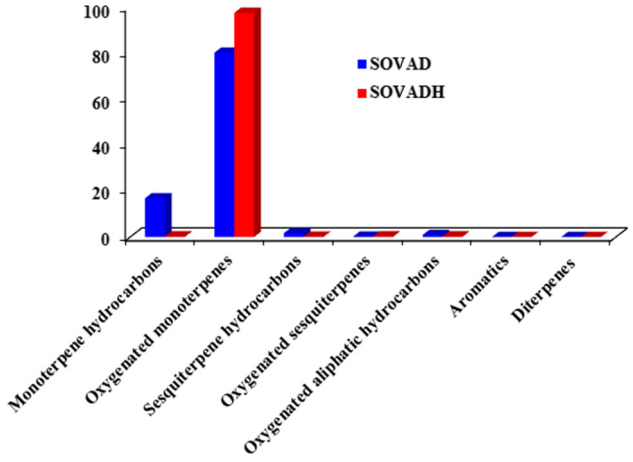

Fig. 3 Chemical classes of the identified essential oils from aerial parts and aqueous distillates of *O. vulgare* L.

The data in Fig. 3 clearly demonstrate that the volatile oil from the aerial parts of *O. vulgare* contained oxygenated monoterpenes as the dominant constituents (80.3%), followed by monoterpene (16.9%) and sesquiterpene (1.7%) hydrocarbons. Other classes of compounds such as oxygenated aliphatic hydrocarbons (0.9%) and oxygenated sesquiterpenes (0.2%) were present in minute concentrations. On the other hand, as expected, the principal chemical class in the oil derived from the hydrosol of *O. vulgare* was oxygenated monoterpenes, accounting for 97.7% of the total oil composition. Other chemical classes of compounds were present in trace amounts.

The major components of the volatile oil from the aerial parts of *O. vulgare* L. were carvacrol (70.2 ± 1.37%), γ-terpinene (5.6 ± 0.11%), *p*-cimene (4.5 ± 0.42%), *trans*-sabinene hydrate (3.8 ± 0.07%), and thymol (2.2 ± 0.12%). In contrast, as anticipated, the main compounds in the volatile oil of the *O. vulgare* hydrosol were carvacrol (92.5 ± 0.97%), thymol (2.5 ± 0.09%), and terpinen-4-ol (1.0 ± 0.03%). Comparison of the chemical compositions of the volatile oils derived from the aerial parts and the hydrosol of *O. vulgare* L. shows that the volatile oil derived from the aqueous distillates contained higher proportions of oxygenated compounds such as carvacrol, thymol, and terpinen-4-ol than that of the oil derived from the aerial parts of *O. vulgare* L. These variations could be expected because oxygenated/phenolic compounds have a greater tendency to form hydrogen bonds with water than terpene hydrocarbons; thus, the oxygenated/phenolic compounds are more soluble in the hydrosol. Further, the chemical structure of the dominant compound, carvacrol was identified from the mass fragmentation pattern and the LRI values obtained from the two different stationary phase columns (polar and non-polar); the compound was also purified and its identification was further confirmed from the ^{1}H NMR and ^{13}C NMR data (Figs. S2a and S2b). The chemical structure and electron ionization mass spectrum (EIMS) fragmentation pattern of carvacrol are presented in Fig. 4.

Previous reports on the composition of the volatile oil of *O. vulgare* L. from diverse regions of the world revealed that this plant species contains numerous polymorphs of phytomolecules and occurs as various chemo-types. Thus far, at least 12 chemo-types of *O. vulgare* L. volatile oil from various regions of the world have been described in the literature (Table 2). However, the most common and economically important chemo-type for *O. vulgare* L. oil is the 'cymyl' type, with either carvacrol or thymol as the main compound. Based on the present findings, Saudi *O. vulgare* L. oil contains a high percentage of phenolic constituents, with carvacrol as the main component, and hence, Saudi *O. vulgare* L. oil can be characterized as a carvacrol chemo-type, similar to those reported in some earlier studies (Bozin et al., 2006; Chorianopoulos et al., 2004). The other main components of Saudi *O. vulgare* L. oil are γ-terpinene and *p*-cymene.

Notably, *O. vulgare* L. plants containing a large amount of essential oil with a high concentration of phenolic monoterpenoids originating from the 'cymyl'-pathway (with main components such as thymol and/or carvacrol) are known to produce a sharp and strong oregano aroma. This type of *O. vulgare* L. plant is considered an essential source of oregano and is recognized among the most-traded and commercially important plants (Lukas et al., 2015). The *O. vulgare* L. grown

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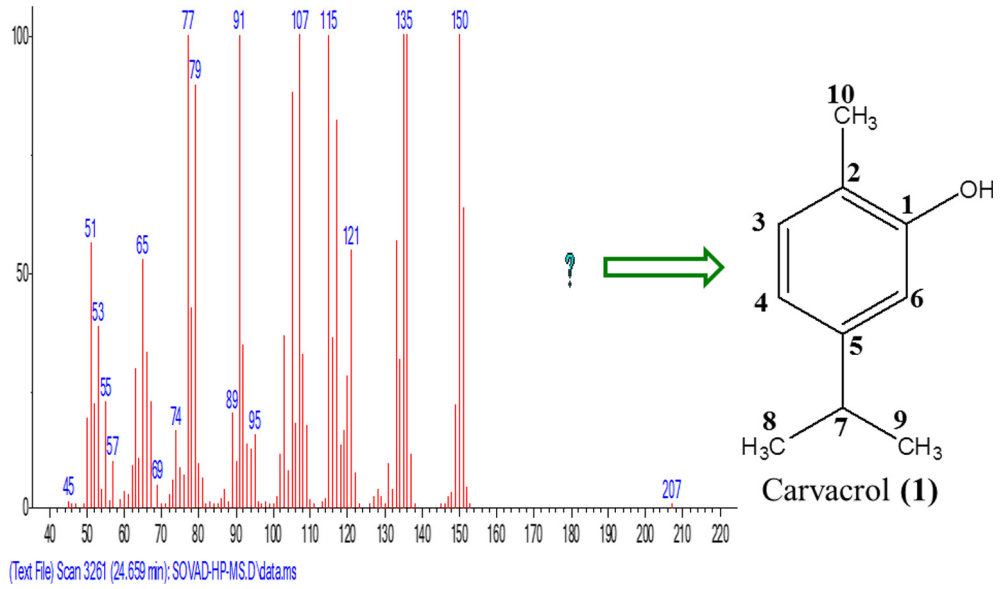

Fig. 4 EIMS fragmentation pattern of the most dominant peak of carvacrol (**1**) and its chemical structure.

Table 2 Chemotypes in essential oil of *O. vulgare* L. grown in various parts of the world.

No.	Origin	Chemotype	Major components (%)	Reference
1	Turkey	Thymol	Thymol (58.3), carvacrol (16.1), *p*-cymene (13.5) and γ-terpinene (4.5).	Sarikurkcu et al. (2015)
2	Greece	Carvacrol	Carvacrol (88.7), *p*-cymene (3.4), γ-terpinene (3.2) and β-caryophyllene (1.1).	Chorianopoulos et al. (2004)
3	Turkey	Linalool	Linalool (96.3).	Sarikurkcu et al. (2015)
4	Turkey	Caryophyllene	Caryophyllene (14.4), spathulenol (11.6), germacrene-D (8.1), α-terpineol (7.5) and caryophyllene oxide (5.8).	Sahin et al. (2004)
5	China	β-Citronellol	β-Citronellol (85.3), citronellol acetate (5.2).	Gong et al. (2014)
6	Jordan	*trans*-sabinene hydrate	*trans*-sabinene hydrate (27.2), terpineol-4 (19.4), γ-terpinene (7.8) and γ-terpineol (6.6).	Al-Kalaldeh et al. (2010)
7	Bulgaria	Spathulenol	Spathulenol (20.7), β-caryophyllene (9.9) and caryophyllene oxide (5.7).	Kula et al. (2007)
8	Brazil	γ-Terpinene	γ-Terpinene (30.5), carvacrol (15.7), terpinen- 4-ol (13.0), geraniol (7.1) and *cis*-ocimene (7.0).	Suzuki et al. (2015)
9	Iran	Linalyl acetate	Linalyl acetate (20.1), sabinene (13.4) and γ-terpinene (5.6).	Afsharypour et al. (1997)
10	China	Eucalyptol	Eucalyptol (20.8), carvophyllene (10.2) and eugenol methyl ether (9.8).	Gong et al. (2014)
11	Argentina	*o*-Cymene	*o*-Cymene (14.3), terpinen-4-ol (12.5), (*E*)-β-terpineol (10.4), thymol (10.1), γ-terpinene (9.1) and carvacrol (5.6).	Camiletti et al. (2016)
12	China	Caryophyllene oxide	Caryophyllene oxide (32.9), carvophyllene (17.8) and citronellol acetate (10.2).	Gong et al. (2014)

in Saudi Arabia had a notable essential oil yield of 1.7% with up to 72. 4% phenols derived from the 'cymyl'-pathway, where carvacrol was the dominant compound. Therefore, *O. vulgare* L. grown in Saudi Arabia can be considered a high-quality plant material with immense economical potential.

Remarkably, the volatile oil derived from the aqueous distillate of *O. vulgare* L. was found to be an excellent source of carvacrol (< 92%), which has widespread applications in the pharmaceutical, flavor and fragrance, cosmetics, and food industries. Carvacrol (2-methyl-5-(1-methylethyl)-phenol) (Fig. 4), an isomer of thymol, is a monoterpenic phenol with a pungent spicy-woody odor. It reportedly exhibits various pharmacological properties including antibacterial, antifungal, anticancer, anti-obesity, anti-inflammatory, antiplatelet, anti-nociceptive, anti-oxidant, antiplatelet, and antidepressant activities. Moreover, carvacrol shows the ability to modulate metabolic enzymes and protect/regenerate damaged organs (Suntres et al., 2015). Furthermore, because of the extraordinary pungent woody odor, pleasant spicy aroma, and various significant biological properties, carvacrol is extensively applied in low quantities as a food preservative, flavoring component, disinfectant, and fungicidal agent, as well as in different cosmetic formulations as a fragrance ingredient (Andersen, 2006).

As described above, carvacrol is widely used in the food, pharmaceutical, nutraceutical, agricultural, and perfumery

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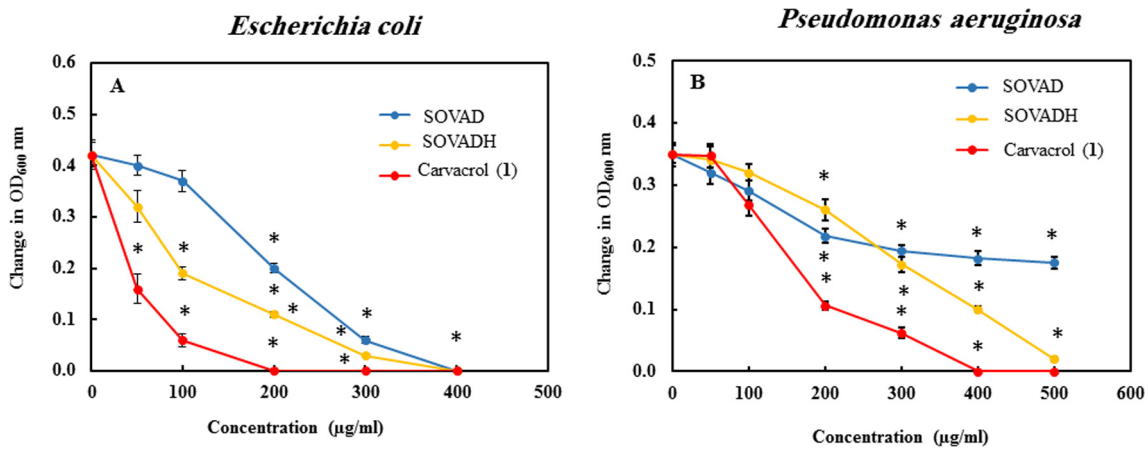

Fig. 5 Inhibition of *E. coli* (A) and *P. aeruginosa* (B) growth, as measured by the change in the OD_{600} following treatment with different test compounds. *Presents significant values that are different from the control (p value < 0.005).

Table 3 IC_{50} of test compounds in µg/mL against Gram-positive and Gram-negative bacteria.

Test organism	IC_{50} (µg/mL)				
	SOVAD	SOVADH	Carvacrol	Amp†	km†
Gram-positive					
S. aureus	270	107	53	16	10
M. luteus	263	174	66	>1	40
Gram-negative					
E. coli	214	127	42	20	10
P. aeruginosa	383	286	151	125	40

† MIC values of Ampicillin (Amp) and Kanamycin (km) against bacteria.

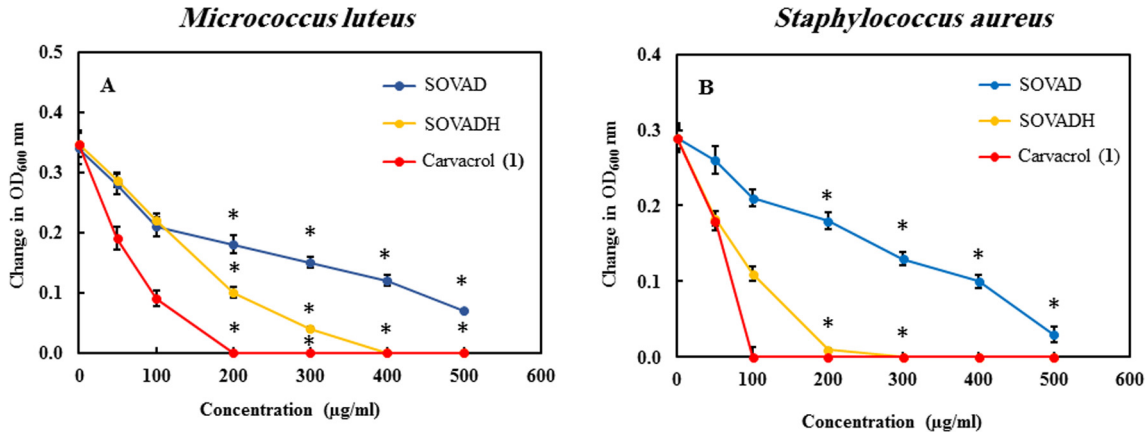

Fig. 6 Inhibition of *M. luteus* (A) and *S. aureus* (B) growth, as measured by the change in the OD_{600} following treatment with different test compounds. *Presents values that are significantly different from control (p value < 0.005).

fields. The volatile oil derived from the hydrosol of Saudi *O. vulgare* L. is demonstrated herein to be an excellent source of carvacrol. Therefore, the hydrosol (a byproduct obtained during the hydro and/or steam distillation of aromatic plants) of Saudi *O. vulgare* L. should not be considered as waste material and discarded as is usually done during the extraction of volatile oils from various aromatic plants.

3.1. Screening for antimicrobial activity

There are several reports on *O. vulgare* L. essential oil and its biological activity (Afsharypour et al. 1997; Al-Kalaldeh et al., 2010; Camiletti et al., 2016; Ebani et al., 2016; Faleiro et al., 2005; Souza et al., 2007; Tommasi et al., 2009). However, reports on the isolation and identification of the active

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constituents responsible for the biological activity are very rare. Therefore, to identify the main constituent of Saudi *O. vulgare* L. oil exhibiting antimicrobial activity, the bactericidal activity of the volatile oils derived from the aerial parts and hydrosol of *O. vulgare* L., along with the isolated major compound carvacrol (**1**), was screened.

The antimicrobial activity was evaluated by measuring the change in the OD_{600} for selected Gram-positive (GM+) and Gram-negative (GM−) bacteria. The antimicrobial potential of the tested oils and pure compound against two GM– bacteria, i.e., *P. aeruginosa* and *E. coli*, is shown in Fig. 5. Carvacrol was the most effective compound, and completely inhibited the growth of *E. coli* at 200 $\mu g \cdot mL^{-1}$. In comparison, compared to the control, significant inhibition ($P < 0.005$) of the bacterial growth was obtained only at concentrations of 200 $\mu g \cdot mL^{-1}$ and 100 $\mu g \cdot mL^{-1}$ for the volatile oil from the aerial parts and aqueous distillates of *O. vulgare* L., respectively (Fig. 5**A**). The IC_{50} values of the test compounds against *E. coli* are given in Table 3.

The decrease in the OD_{600} of *P. aeruginosa* following treatment with various test compounds is shown in Fig. 5**(B)**. Carvacrol (**1**) was also the most effective for retarding the growth of *P. aeruginosa*, with an IC_{50} value of 151 $\mu g \cdot mL^{-1}$. Significant inhibition ($P < 0.005$) of the growth of *P. aeruginosa* was obtained with 200 $\mu g \cdot mL^{-1}$ of all the test compounds (Fig. 5**B**). Therefore, the compounds evaluated herein could be organized in the following order based on their bactericidal activity against the GM– bacteria (*E. coli* and *P. aeruginosa*) on the basis of their IC_{50} values: carvacrol (**1**) > SOVADH > SOVAD.

The antimicrobial activity of these compounds against two GM+ bacteria was also determined. The antimicrobial activity of the test compounds against *M. luteus* is shown in Fig. 6**A**. Carvacrol (**1**) was clearly the most effective inhibitor of the growth of *M. luteus*, with significant growth inhibition at 200 $\mu g \cdot mL^{-1}$ (Fig. 6**A**). A similar trend was obtained when the antimicrobial activity of these constituents against *S. aureus*, another GM+ bacteria, was determined. In fact, *S. aureus* was more sensitive to all three test compounds than *M. luteus*. Carvacrol (**1**) most effectively inhibited the growth of *S. aureus* (Fig. 6**B**) also. Based on the results presented in Fig. 6**B** and the IC_{50} values presented in Table 3, the test compounds can be organized in the following order based on their activity against *S. aureus:* carvacrol (**1**) > SOVADH > SOVAD.

The trend in the antimicrobial activity was very similar for all four bacteria. Moreover, it is interesting that the bactericidal activity of the test compounds against the four selected organisms increased with an increase in the content of carvacrol in both essential oils, and the highest activity was observed with the purified compound carvacrol (**1**). Hence, based on the present results, it is suggested that the antimicrobial activity of Saudi *O. vulgare* L. oils is mainly due to the presence of carvacrol. Furthermore, it is notable that the IC_{50} values of carvacrol (**1**) were comparable to those of the known antibiotic drug ampicillin (Table 3), whereas, the IC_{50} value of carvacrol (**1**) was found to be 4–10 times higher than the minimum inhibitory concentration (MIC) of kanamycin, as determined in this study (Table 3). The mechanism of the antimicrobial activity of carvacrol was evaluated in our previous study by checking the cell membrane integrity through propidium iodide staining and scanning electron microscope (SEM) analysis. It was demonstrated that carvacrol damages the cell membranes of *Streptococcus mutans*, resulting in the leakage of intracellular materials, deformation of cells, leading to cell death (Khan et al., 2017). The genes involved in the apoptosis-like activity and oxidative stress were also over-expressed in the presence of carvacrol, suggesting that carvacrol induces general and oxidative stress in *S. mutans*. Therefore, carvacrol may have affected the growth of the microorganisms evaluated herein by the same mechanism of action. Because Saudi *O. vulgare* L. oils exhibited significant antimicrobial activity against all of the tested bacteria, its possible use in the control of food-borne pathogens, and the pathogens of skin and oral cavity, is suggested.

4. Conclusion

This is the first detailed study on the chemical characterization and antimicrobial activity of the volatile oils and their major constituents isolated from the aerial parts and aqueous distillates of *O. vulgare* L. grown in Saudi Arabia. The volatile oil derived from the aerial parts of *O. vulgare* L. grown in Saudi Arabia could be characterized as carvacrol chemo-type given that oxygenated monoterpenes were the main constituents, with carvacrol (73%) as the dominant compound. Moreover, the volatile oil derived from the hydrosol of *O. vulgare* L. was characterized as an excellent source of carvacrol (< 92%), which has widespread applications in the pharmaceutical, flavor and fragrance, cosmetics, and food industries. Therefore, hydrosol (a byproduct obtained during the hydro and/or steam distillation of aromatic plants) of Saudi *O. vulgare* L. has enormous potential for use in various industrial applications. Screening of the antibacterial activity of the volatile oils derived from the aerial parts and hydrosol of *O. vulgare* L., along with their purified major components, revealed that all three samples showed potent antibacterial activity against all tested strains. However, carvacrol, the dominant component of the aerial parts and hydrosol oils of *O. vulgare* L., displayed the highest activity against all tested strains. Hence, the volatile oils derived from Saudi *O. vulgare* L. are considered promising for use in various pharmaceutical and food applications, particularly as a food preservative.

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Conflict of interest

None.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.arabjc.2018.02.008.

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