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Chemical composition and antimicrobial potency of locally grown lemon essential oil against selected bacterial strains

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Abstract The hydro distillation method was used to isolate and identify essential oils from locally available lemon samples and determine their antimicrobial potency against selected gram positive and negative bacterial strains using the disk diffusion method. Two locally available and highly consumed lemon fruit samples were used for the isolation of essential oils by hydro distillation using Clevenger type apparatus. The obtained essential oils were analyzed and identified by gas chromatography–mass spectrometry (GC–MS). The essential oils at different concentrations were used to determine antimicrobial potency using one gram (+) *Staphylococcus aureus* (*S. aureus*) and three gram (−) bacteria, *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Proteus vulgaris* (*P. vulgaris*), through the disk diffusion method. Both the essential oils contain twenty-two active compounds with a variation of percentage identified based on GC retention. The main bioactive compounds with high content in Omani sweet lemon essential oil were limonene (84.73%), α-pinene (1.06%), α-terpineol (2.80%), β-myrcene (2.16%), β-pinene (3.36%), terpinen-4-ol (1.16%) and α-terpinolene (2.33%) and several other minor compounds. Similarly, Omani sour lemon essential oil was composed of limonene (53.57%), α-terpineol (14.69%), β-pinene (8.23%), α-pinene (1.84%), β-myrcene (1.51%), α-terpinolene (4.33%), terpinen-4-ol (3.38%), cymene (1.80%), β-bisabolene (1.43%), β-linalool (0.85%) and E-citral (1.08%) with several other minor chemical compounds. Antimicrobial results showed that most concentrations of both essential oils did not give any potential antimicrobial against employing bacterial strains. Therefore, the obtained results show that both essential oils could be needed for further extensive biological study and their mechanism of action.

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1. Introduction

Lemon is a flowering medicinal plant belongs to the family Rutaceae. It is a small evergreen tree native to Asia (Ahmad et al., 2006). Several varieties of lemon are available

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with ellipsoidal yellow fruits (Ahmad et al., 2006; AL-Jabri and Hossain, 2014). The lemon is considered as a major citrus fruit after oranges and mandarins (Frederick and Xulan, 1990). The "word" lemon originates from old French known as "limon". It has also a different name in Italy "limone", Arabic "laymūn" or "līmūn" and Persian "līmūn". The generic term for citrus fruit is related to the Sanskrit nimbū, "lime" (Freitas et al., 2003; Bhuiyan et al., 2009; Andrews, 1961).

The lemon first originates from Southeast Asia. Then it spread worldwide, especially to Northeast India, Burma and China. Since ancient times, the citrus fruit, especially lemon has been cultivated all over the world (Kirbaslar et al., 2006; AL-Jabri and Hossain, 2014). Now, it is commercially cultivated worldwide due to its medicinal importance. The tree is small with alternate leaves, which are shiny and leathery and dotted with oil glands. Stems are winged and jointed. The flowers have five petals and a very strong sweet smell. Normally, the shape of the fruit is spherical or egg-shaped. The fruits have 8–14 juicy sections containing white or greenish seeds (Kirbaslar et al., 2006). Traditionally, the lemon is used to cure soothe sore throats and as an additive for flavoring to our foods (Kirbaslar et al., 2006; AL-Jabri and Hossain, 2014). In Oman, it is used traditionally to reduce high blood pressure, mental health, respiratory problems, arthritis and rheumatism (Bhuiyan et al., 2009; Silalahi, 2002; Faleiro et al., 2003; AL-Jabri and Hossain, 2014). Recently, it has been a very good herbal medicine for the prevention of kidney stones (Faleiro et al., 2003; Kamal et al., 2011). In addition, lemon fruit and leaves are used traditionally as wash for oral health to freshen your breath and to treat flaky dandruff, headaches and reduce asthma symptoms (Burt, 2002; Reichling et al., 2009). Traditionally, in India, the lemon fruits are used for the treatment of dysentery and asthma (Rajbir, 2015). The essential oil of lemon showed fungitoxicity against some fungi. *Citrus medica* is relevant to treatment of diabetes and Alzheimer's disease reported by Emmanuel et al., 2008. The crude extracts from different parts of the lemon showed anticancer and antibacterial potency (Bhuiyan et al., 2009; Rainer, 1975). In Oman, the lemon is a famous crop and is completely different from other varieties of lemon available in the world (Burt, 2002; Reichling et al., 2009). The majority Omani lemons is cultivated in Batinah region, Sohar and Saham area (Miyazawa and Hisama, 2013). It contains a large amount of citrus acid and juice (Mondello et al., 2006; AL-Jabri and Hossain, 2014). The majority of chemical compounds in lemon fruits are alkaloids (Ehigbai et al., 2016). Similarly, Omani sour lemon essential oil was composed of limonene (53.57%), α-terpineol (14.69%), β-pinene (8.23%), α-pinene (1.84%), β-myrcene (1.51%), α-terpinolene (4.33%), terpinen-4-ol (3.38%), cymene (1.80%), β-bisabolene (1.43%), β-linalool (0.85%) and E-citral (1.08%) (AL-Jabri and Hossain, 2014; Tadtong et al., 2015). Three important chemicals in lemon juice are water, citric acid and carboxylic acid (Burt, 2002; Reichling et al., 2009; Mondello et al., 2006). The literature search reveals that no work has been done on essential oil composition and antimicrobial activity of locally available lemon samples. Therefore, the aim of this work is to isolate and identify the chemical compounds of essential oils from locally available lemon fruits by GC–MS and determine antimicrobial potency against the selected bacterial strains.

2. Materials and methods

2.1. Materials

Chemicals like dimethyl sulphoxide (DMSO) and dichloromethane, used in this experiment were obtained from Fisher Scientific Company, UK. Sodium sulfate and filter paper were purchased from Scharlau, European Union and Whatmann (GE Healthcare Company, China) respectively.

2.2. Bacterial strains

One gram (+) bacteria *Staphylococcus aureus* (*S. aureus*) and three gram (−) bacteria, *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Proteus vulgaris* (*P. vulgaris*), used in this experiment were collected from Nizwa Hospital, Nizwa, Sultanate of Oman during the month of March, 2014.

2.3. Fruit samples

Omani lemon fruit samples (sweet and sour) were collected from a local farmer in the Al Batinah region, Barkah, Sultanate of Oman on February 19, 2014 at 9.00 am. The morphological identification of the lemon fruits was carried out by a botanist using a voucher specimen (Omani Sweet lemon No. 201 and Sour no 202) deposited in the Biological laboratory, SQU, Sultanate of Oman. After collection, the lemon fruit samples were packed instantly in a two separate polyethylene bag. The samples were carried to the Natural Products Laboratory, Nizwa University, Nizwa for preparation of samples.

2.4. Preparation of samples

The collected lemon fruit samples such as sweet and sour were washed carefully with water to remove unwanted materials. After washing, healthy lemon fruit samples were separated from affected fruits.

2.5. Extraction of essential oil by Clevenger apparatus

The clean, sweet and sour lemon fruit samples were cut into small pieces with the knife. Then the small pieces were put into a round bottom flask (3 L) and water (1.5 L) was added. The Clevenger apparatus was connected to the round bottom flask and the other part was connected to the condenser (Hussain et al., 2010). Then it was heated at 100 °C for 5 h. The essential oil was isolated from the sweet and sour lemon fruit samples and collected separately. The collected essential oils were kept in amber color polyethylene vials and stored at 4 °C until analysis. The water molecules were removed by adding anhydrous sodium sulfate (5 g).

2.6. GC–MS analysis

The essential oils were analyzed by using a Perkin Elmer Clarus 600 GC System, fitted with a Rtx-5MS capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness). It was coupled to a Perkin Elmer Clarus 600C MS. High purity helium was used as the carrier gas at a constant flow of

1.0 ml/min. The temperatures of the injector, transfer line and ion source were 280, 270 and 270 °C, respectively. The ionizing energy of MS was 70 eV. All data were obtained by collecting the full-scan mass spectra within the scan range 40–550 amu. The injected sample volume was 0.5 µl with a split ratio of 200:1. The oven temperature program was 50 °C at a rate of 3 °C/min – 280 °C held for 5 min. The unknown compounds were identified by comparing the spectra obtained with mass spectrum libraries (NIST 2011 v.2.3 and Wiley, 9th edition) (Hossain et al., 2014).

2.7. Identification of chemical constituents

Both isolated essential oils were analyzed by GC–MS and chemical compounds were identified on the basis of their retention time. Their retention indices were calculated under temperatures programed for n-alkanes (C_6–C_{24}). The condition for the analysis of both oils and *n-alkanes* (C_6–C_{24}) were the same. The individual chemical compounds were identified by comparison of their mass spectra matched with the GC–MS exiting library (NIST 2005 v.2.0 and Wiley Access Pak v.7, 2003 of GC–MS systems) or authentic compounds (Inouye et al., 2003).

2.8. Antimicrobial assay

The essential oils from sweet and sour lemon fruit samples collected locally were evaluated for their antimicrobial activity. The disk diffusion method was used for the determination of antimicrobial activity (Alawiya and Hossain, 2016). The essential oils were subjected to serial dilution by using dimethyl sulphoxide (DMSO) to prepare concentrations of 2 mg/ml, 1 mg/ml, 0.5 mg/ml, and 0.25 mg/ml. The antibiotic amoxicillin was used as a standard for this study at a concentration of 1 mg/ml. Each prepared concentration of both isolated essential oils was tested for their antimicrobial activity against one gram (+) bacteria (*S. aureus*) and three gram (−) bacteria (*E. coli*, *P. vulgaris* and *P. aeruginosa*) on nutrient agar plates. Sterile filter paper disks (6 mm diameter) were saturated with each concentration of both essential oils isolated from sweet and sour lemon fruit samples. Then the soaked filter paper disk was placed on the inoculated agar plate. The positive control amoxicillin was used without essential oil on the inoculated agar plate. After inoculation, all inoculated plates were incubated at 37 °C for 24 h. Finally, the antibacterial activity was determined by measuring diameter zones of inhibition against the tested bacterial strains. Each method in this experiment was replicated three times.

3. Results

3.1. Essential oil composition

Both the essential oils from lemon fruit samples were used for extraction through hydro distillation method using Clevenger apparatus (Hussain et al., 2010). After extraction, the collected essential oils were yellowish in color with a strong smell. The isolated essential oils were preserved in amber color polyethylene vials which were kept in the refrigerator to avoid evaporation and the decomposition of essential oils.

3.2. Omani sweet essential oil

The sweet lemon fruits collected from a local farmer in the Al Batinah region were a source of essential oils which were analyzed the oil by GC–MS. A total of twenty-two chemical compounds were identified in the Omani sweet essential oil by GC–MS representing about 97.5%. Various chemical compounds identified in the essential oil by GC–MS are listed in Fig. 1 and Table 1. The detected aromatic aroma compounds with high percentage found in the essential oil were limonene (84.73%), β-pinene (3.36%), α-terpineol (2.80%), β-myrcene (2.16%), terpinen-4-ol (1.18%), α-pinene (1.06%) and α-terpinolene (0.98%). Other compounds were also detected at a low percentage of sweet oil as presented in Fig. 1 and Table 1.

3.3. Omani sour lemon essential oil

The collected sour essential oil was analyzed by GC–MS which identified different chemical compounds with various percentages. Similarly, a total of twenty-two different compounds were identified, representing about 98.35%. The identified chemical compounds in sour essential oil based on GC–MS retention time is presented in Fig. 2 and Table 2. The low molecular weight with high percentage aromatic chemical compounds found in the sour essential oil were limonene (53.57%), α-terpineol (15.15%), β-pinene (7.44%), α-terpinolene (4.33%), terpinen-4-ol (3.55%), cymene (2.88%) and E-citral (2.38%). Similarly, other minor chemical compounds with low percentage were identified in the sour essential oil (Fig. 2 and Table 2).

3.4. Antimicrobial activity

The essential oils at different concentrations were used to test for their antimicrobial activity against four pathogenic bacterial strains. The presence or absence of inhibition zones based on amoxicillin standard was measured quantitatively for the calculation of percentage zones of inhibition. Most of the prepared concentrations of both essential oils did not give any zone of inhibition. Omani sweet essential oil at the concentrations of 2 mg/ml and 0.5 mg/ml gave very small antimicrobial activity against *E. coli*. However, the oil gave high activity against *S. aureus* at the concentration of 2 mg/ml. Similarly, Omani sour lemon oil gave small antimicrobial activity against *E. coli* and *P. aeruginosa* at the concentration of 2 mg/ml. However, the sour oil gave very strong activity against *P. aeruginosa* at the concentration of 0.25 mg/ml (Table 3).

4. Discussion

The isolated essential oils from locally grown lemon samples were analyzed through GC–MS. The chemical compounds in both essential oils were identified based on their retention time. So far we know, this is the first report of the analysis of Omani lemon oils. The variation of chemical compounds and their percentage in the essential oil from natural sources depends on geographical distribution as well as the environmental conditions such as temperature, rainfall, altitude, and hours of sunshine (Hussain et al., 2010; Rahma et al., 2013; AL-Jabri

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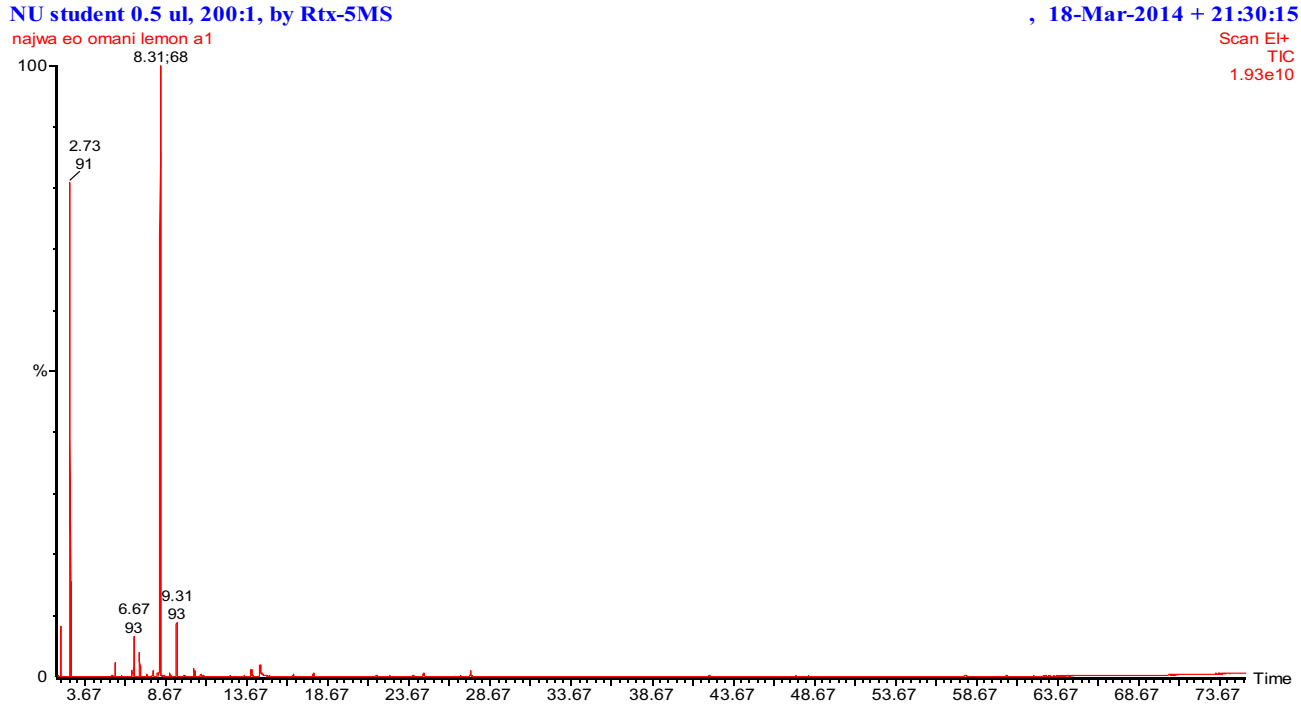

Figure 1 A typical chromatogram of essential oil isolated from Omani sweet lemon fruits sample.

Table 1 Chemical composition of essential oil from Oman sweet lemon fruits samples.

RI[a]	Compound Name	Retention time	Area	%
927	α-Thujene	5.35	1300022.75	0.10
933	α-Pinene	5.54	13732783	1.06
973	Camphene	5.93	679135.875	0.05
986	Fenchene	5.88	255010	0.02
988	β-Pinene	6.70	43476908	3.36
990	β-Myrcene	7.05	27942058	2.16
994	α-Phellandrene	7.51	2382033	0.18
998	Cymene	8.15	4573739.5	0.35
1001	dl-limonene	8.32	1096144896	84.73
1021	α-Terpinolene	10.41	12622165	0.98
1044	β-linalool	10.82	3459146.5	0.27
1078	Terpinen-4-ol	13.93	15208953	1.18
1092	L- α-Terpineol	14.50	36217392	2.80
1134	Decanal	15.06	1146553.63	0.09
1187	Z-Citral	16.55	3167777.5	0.24
1206	E-citral	17.79	5705800.5	0.44
1265	Neryl acetate	21.70	2611245.25	0.20
1289	Geranyl acetate	22.49	1253888.5	0.10
1297	Isocaryophllene	23.93	2113133.5	0.16
1327	α-Bergamotene	24.59	6463821	0.50
1355	Valencene	26.89	842178.25	0.07
1369	β-bisabolene	27.48	10952934	0.85
Total			1293630128	97.5

[a] Retention index relative to n-alkanes on Rtx-5MS capillary column.

and Hossain, 2014). In addition, the biological and chemical activities of the essential oils depend on the chemical compounds present in the isolated essential oils (Hossain et al., 2014; Rahma et al., 2013; Rajbir, 2015; Alawiya and Hossain, 2016).

Almost all identified chemical compounds in both essential oils have low molecular weights compounds (Kirbaslar et al., 2006; Rahma et al., 2013). The low molecular weight compounds can be easily evaporated at room temperature during the processing of oil samples due to their volatility compounds

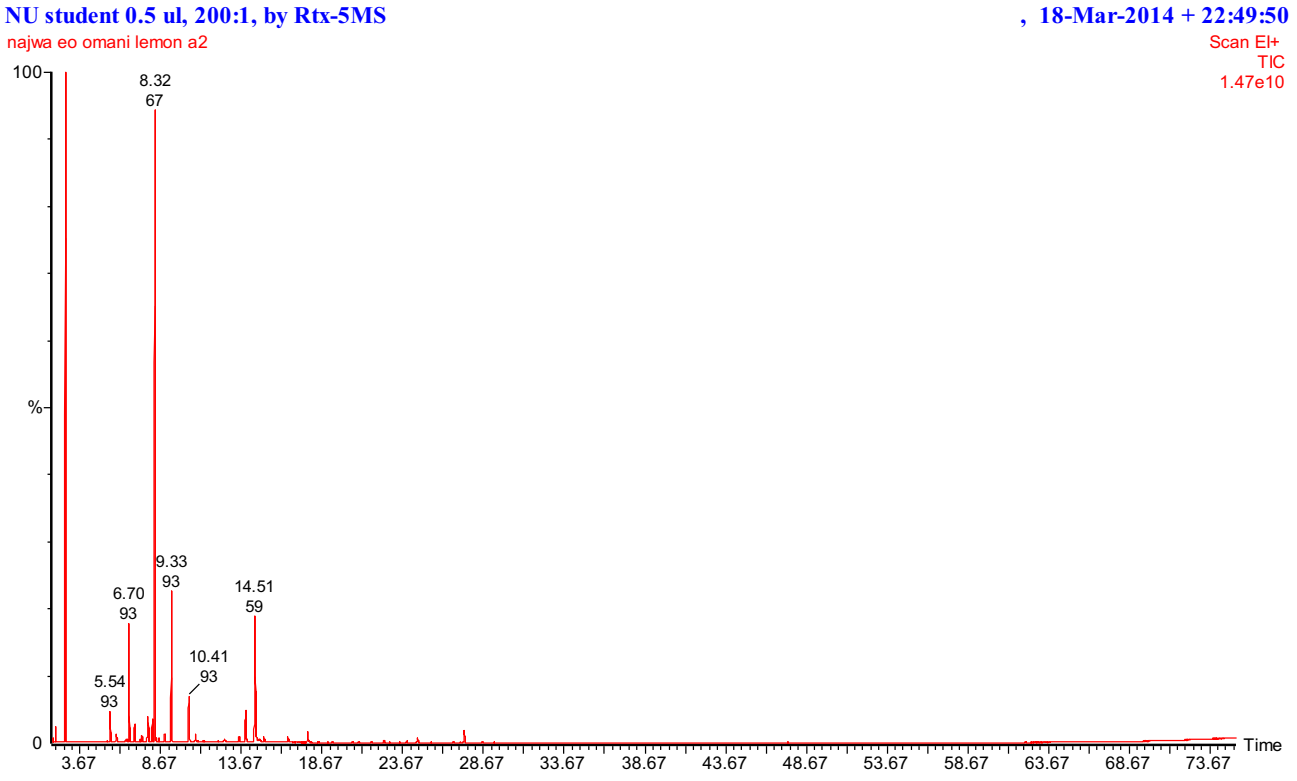

Figure 2 A typical chromatogram of essential oil isolated from Omani sour lemon sample.

Table 2 Chemical composition of essential oil from Oman sour lemon fruits samples.

RI[a]	Compound Name	Retention time	Area	%
927	α-Thujene	5.35	1360245	0.12
933	α-Pinene	5.54	21341906	1.84
973	Camphene	5.93	5923825	0.51
986	Fenchene	5.88	2106882	0.18
988	β-Pinene	6.70	95359504	8.23
990	β-Myrcene	7.05	17540130	1.51
994	α-Phellandrene	7.51	5906753	0.51
998	Cymene	8.15	20816108	1.80
1001	dl-Limonene	8.32	6.55E + 08	56.51
1021	α-Terpinolene	10.41	50198388	4.33
1044	β-Linalool	10.82	9858320	0.85
1078	Terpinen-4-ol	13.93	39183932	3.38
1092	L-α-Terpineol	14.50	1.7E + 08	14.69
1134	Decanal	15.06	5365937	0.46
1187	Z-Citral	16.55	7019990	0.61
1206	E-citral	17.79	12452409	1.07
1265	Neryl acetate	21.70	1516738	0.13
1289	Geranyl acetate	22.49	3379084	0.29
1297	Isocaryophllene	23.93	2803388	0.24
1327	α-Bergamotene	24.59	6838028	0.59
1355	Valencene	26.89	708031.1	0.06
1369	β-Bisabolene	27.48	16487993	1.42
	Total		1.16E + 09	98.35

[a] Retention index relative to n-alkanes on Rtx-5MS capillary column.

(Kirbaslar et al., 2006; Bhuiyan et al., 2009). Some bioactive compounds from both lemons samples are evaporated during the processing and isolation of the lemon samples. Therefore, in our present experiment, we did not find all chemical compounds in the lemon essential oils which were reported in the literature.

Table 3 Antimicrobial activity of essential oils from Omani sweet and sour lemon fruits samples.

Essential oil	Conc	*E. coli* (mm)	*P. aeruginosa* (mm)	*S. aureus* (mm)	*P. vulgaris* (mm)
Omani sweet	2 mg/ml	5 ± 0.12	nd	30 ± 0.51	nd
	1 mg/ml	nd	nd	nd	nd
	0.5 mg/ml	5 ± 0.17	nd	nd	nd
	0.25 mg/ml	nd	27 ± 0.77	nd	nd
	Control	15 ± 0.42	nd	nd	nd
Omani sour	2 mg/ml	4 ± 0.18	6±0.10	nd	nd
	1 mg/ml	nd	nd	nd	nd
	0.5 mg/ml	nd	nd	15 ± 0.09	nd
	0.25 mg/ml	nd	29 ± 0.47	nd	nd
	Control	35 ± 0.15	6 ± 0.32	20 ± 0.21	28 ± 0.14

nd = not detectable.

Since ancient time, some of the chemical compounds e.g., limonene, α-pinene, β- pinene and β-myrcene have been used extensively for the preparation of the perfume and flavoring agents, and medicines for local antiseptic and anesthetic use (Burt, 2002; Reichling et al., 2009). The mentioned commercially used bioactive compounds were also present in both essential oils with high percentages. The high percentage bioactive compounds present in the sweet essential oil were limonene, β-pinene, α-terpineol, β-myrcene, terpinen-4-ol, α-pinene and α-terpinolene (Fig. 1 and Table 1). Similarly, the bioactive compounds with high percentage identified in sour essential oil were limonene, α-terpineol, β-pinene, α-terpinolene, terpinen-4-ol, cymene and E-citral (Fig. 2 and Table 2). The literature search reveals that nobody has worked on Omani sweet and sour lemon essential oils. Therefore, this is the first report by the authors of the analysis of Omani lemon oils by GC–MS. So, we are unable to compare our experimental results due to lack of data. The major compound limonene, was identified in the Turkish lemon essential oil at high percentage (Kirbaslar et al., 2006). The same compound limonene with high concentration was also present in Indian lemon essential oil reported by Ahmad et al., 2006. However, our present experimental results showed that limonene was present in both essential oils at high percentages. Our observations showed that the percentage of other chemical compounds present in both isolated essential oils was not constant. The variation in percentage of chemical compounds depends on the extraction process, samples processing and environmental conditions (Nicolosi et al., 2000; Rahma et al., 2013). Results in previous studies on lemon samples with regard to chemical ingredients are almost similar (Burt, 2002; Mondello et al., 2006). In addition, the identified chemical compounds in both essential oils were alkaloids, mono and sesquiterpenoids, acyclic and cyclic hydrocarbons, phenolic and polyphenolic derivatives as well as their oxygenated derivatives reported by several authors (Reichling et al., 2009; McKay and Blumberg, 2006; Rahma et al., 2013).

The essential oils obtained from local sweet and sour lemon fruit samples collected from the farmer were used for antibacterial activity against four pathogenic bacterial strains. The presence or absence of inhibition zones based on amoxicillin standard was measured quantitatively for the calculation of percentage zones of inhibition. Our observations showed that most of the prepared concentrations of both oils did not give any zone of inhibition against pathogenic bacterial strains. The sweet essential oil at the concentrations of 2 mg/ml and 0.5 mg/ml gave very small activity against *E. coli* bacteria. On the other hand, the sweet essential oil at the concentration of 2 mg/ml gave very high zone of inhibition against *S. aureus* bacterial strain. However, the sweet essential oil did not give any activity against *P. aeruginosa* and *P. vulgaris* at any of the applied concentrations. Similarly, sour lemon also gave small inhibition against *E. coli* at the concentration of 2 mg/ml. But the other applied concentrations of sour oil did not give any activity against *E. coli* bacteria strain. The same sour oil at the concentration of 0.25 mg/ml gave potential antimicrobial activity against *P. aeruginosa* bacterial strain with range 0–27 mm. *S. aureus* bacteria strain also gave very strong activity against sour oil at the concentration of 0.5 mg/ml. However, both the essential oils against *P. vulgaris* bacterial strain did not give any activity at all applied concentrations (Table 3). The major bioactive chemical compounds in both essential oils listed in Tables 1 and 2 have strong potential against microbial pathogens reported by several authors (Alawiya and Hossain, 2016; Burt, 2002; Saidan et al., 2004; Liakos et al., 2014). However, our experimental results showed that most of the bioactive compounds are present in both the isolated essential oils. But most of applied concentrations from both essential oils did not give any activity against employing bacterial strains. It could be due to too low a concentration applied or non-effective bacterial strains (Rahma et al., 2013; Sacchetti et al., 2004). Almost, similar antimicrobial results were obtained from lemon essential oils by others (Bhuiyan et al., 2009; Liakos et al., 2014; Alawiya and Hossain, 2016).

5. Conclusion

The aim of this present study is to isolate and identify the chemical compounds of essential oils from locally available lemon fruits by GC–MS and determine their antimicrobial potency against the selected bacterial strains. The experimental results showed that both essential oils gave very good chemical profile. Our antimicrobial activity results showed that both the essential oils at most of the concentrations did not give activity against the selected pathogenic bacterial strains. The disk diffusion method might not be an adequate tool for the evaluation of best antimicrobial activity. Therefore, further extensive studies are needed for investigating the possible

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mechanism of essential oil against pathogenic bacterial strains and their use in food.

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