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Contributed Paper

Comparative GC-MS Analysis, *In-vitro* Antioxidant and Antimicrobial Activities of the Essential Oils Isolated from the Peel of Omani Lime

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ABSTRACT

The aim of this study was, to analyze and compare the composition of volatile constituents in the essential oils from the peel of *Citrus aurantifolia* L. grown in three different regions of Oman and to investigate their antioxidant and antibacterial potential. The essential oil from the fresh peels was isolated by hydro-distillation method and chemical composition analysis of oils was carried out by Gas chromatography-Mass spectrometry (GC-MS). The antioxidant activity was investigated by a non enzymatic free radical scavenging method while antibacterial activity was tested against gram positive and gram negative bacteria by disc diffusion method. Molecular docking studies were also carried out to probe the mechanism of antioxidant activity. GC-MS analysis indicated variation in content and composition of chemical constituents in the peel oils. Limonene was found to be the major volatile constituent thus establishing lime peel oil as limonene chemotype. The peel essential oils of *C. aurantifolia* showed moderate antioxidant and antibacterial activitities. Molecular docking studies indicated α-farnesene to have better binding affinity for *Xanthine oxidoreductase* in comparison to limonene and pinene. Further studies are needed to develop the lime peel oil as an antimicrobial preservative and an alternative source of natural antioxidant(s).

Keywords: *Citrus aurantifolia* L, lime peel, antioxidant, antibacterial activity, limonene

1. INTRODUCTION

Essential oils are volatile oily liquids chiefly responsible for the characteristic aroma of the plant materials such as fruits, flowers, leaves, bark etc [1]. Chemically they are mixtures of terpenic hydrocarbons $[(C_5H_8)_n]$ or their oxygenated derivatives such as aldehydes, alcohols, ketones, esters etc. These oils are chemically different from edible oils as they are not esters of glycerides. Plant essential oils and extracts have been used by mankind for many centuries in food preservation, pharmaceuticals, alternative

medicine, natural therapies, cosmetic and perfumes [2-4]. These essential oils have been reported to possess anticancer, antidiabetic, antifungal, antibacterial, anti-inflammatory and antioxidant activities [5]. Essential oils of clove and rosemary had shown antibacterial and antifungal activity while basil is reported to have anti-inflammatory activity. Cinnamon oil exhibits antidiabetic activity, peppermint and orange oils have shown promise as anticancer agents [6]. Essential oils, therefore, are considered as important source of bioactive compounds.

The genus Citrus of the family Rutaceae includes several important fruits such as oranges, mandarins, limes, lemons, grapefruits etc., and is famous for the source of essential oils [7]. Citrus fruit essential oils and their major components have gained acceptance in the food industry since they have been generally recognized as safe, and many foods tolerate their presence. Currently, there is a global interest in finding new and safe antioxidants and antibacterial agents from natural sources, to prevent oxidative deterioration of foods, to minimize oxidative damage to the living cells and to combat the spread of multi drug resistant microorganisms.

Omani lime (locally known as Lomi) or *Citrus aurantifolia* (L). Swingle belongs to the family Rutaceae. It is one of the main citrus crops in terms of cultivated area and production all over the country, with production being concentrated in Al Batinah region, Oman [8]. It is a very popular citrus species in gulf region due to its high acid content and distinct flavor. Omani lime is used as a traditional medicine for many ailments viz. cataract, cold, fever, chest pain, earache, stomachache etc [9]. Lime peel oil has shown antimicrobial, radical scavenging, anti-cholinesterase, anthelmintic, and anticancer activities [10]. Limonene and β-myrcene were reported as the major components of the peel oil [11]. However, chemical profiling and composition can vary from species to species depending upon geographical and seasonal variations i.e. Omani lime may have the same volatile constituents but in different proportion as compared to same species grown in other countries. It is also possible that it might contain some new terpenes or terpenoids that could influence the biological activity of volatile oil. Also previous studies done elsewhere have shown significant differences in the qualitative and quantitative composition of the oils obtained from peel and leaves [12,13].

Although the chemical compositions of the volatile oils of *C. auranifolia* are well studied [2,14,15] to our best knowledge no comparative research has so far been conducted on the peel oil of Omani species and their *in- vitro* antioxidant and antibacterial effects. The aim of this study was, therefore, to analyze and compare the composition of volatile constituents in the essential oils from the peel of *C. aurantifolia* L. grown in three different regions of Oman and to investigate their antioxidant and antibacterial potential.

2. MATERIALS AND METHODS

2.1 Chemicals and Test Microorganisms

All the drugs, chemicals and solvents used in the present study were of analytical grade and procured locally. Clevenger apparatus used for the isolation of essential oils was from Borosil®, India. Two bacterial strains viz. *Escherichia coli* (gram negative) and *Staphylococcus aureus* (Gram-positive) were obtained from the Department of Natural Sciences, Oman Medical College, Sultanate of Oman.

2.2 Collection of Lime Fruits

Mature fresh lime fruits of uniform size, free of physical damage from insects or

fungal infection grown in North Al-Batinah, Muscat and Al-Sharqia (Eastern) regions of Sultanate of Oman were collected from the respective local farms in July, 2015. The fruits were identified by the subject expert of Oman Medical College and voucher specimens (PHAR425/2015/VSL 1-3) were kept in the herbarium of Department of Pharmacy, Oman Medical College, Oman for future reference.

2.3 Isolation of Essential Oils by Hydro-distillation Method

The fresh peels of *C. aurantifolia* L. (250 g) were washed out with water and cut into small pieces. The material was transferred to 1L round bottom flask and covered with sufficient quantity of water. The hydro-distillation by Clevenger apparatus for 6 hours produced the yellow-greenish volatile oil, which was separated from aqueous layer and collected in plastic sample tubes. The isolated oils were then dried by the addition of anhydrous sodium sulfate and stored at 4 °C in dark place until further use. The % yield of each isolated essential oil was calculated based on the dry weight of peel used for extraction and quantity of oil obtained.

2.4 Gas Chromatography-mass Spectrometry Analysis

Small amount of essential oil of *C. aurantifolia* L. peels were diluted in diethyl ether and analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) which was equipped with auto sampler. GC-MS analysis was performed on Perkin Elmer Clarus 600 GC System, fitted with Rtx-5MS capillary column (30m × 0.25mmi.d. × 0.25 µm film thickness; maximum temperature, 350 °C), coupled to a Perkin Elmer Clarus 600C MS at SQU. Ultra- high purity helium (99.9999%) was used as a carrier gas at a constant flow of 1.0 mL/min. The injection, transfer line and ion source temperatures were 280, 260 and 260 °C, respectively. An electron ionization (EI) system, with ionization energy of 70 eV was used for GC-MS analysis. Electron multiplier (EM) voltage was obtained from auto-tune. All data were obtained by collecting the full-scan mass spectra within the scan range 40-550 amu. The injected sample volume was 1 µL with a split ratio of 100:1. The oven temperature program was 60 °C at rate of 3 °C/min-280 °C hold for 2 minutes.

2.5 Identification of Volatile Constituents of Essential Oils

Volatile constituents present in the oil were identified based on their retention time relative to *n*-alkanes (C_6-C_{24}), with corresponding literature data, as well as by comparing the spectra obtained with mass spectrum libraries (NIST 2011 v.2.3 and Wiley , 9th edition) [16]. The relative peak areas for individual volatile constituents were averaged. Quantification was computed as the percentage contribution of each compound to the total amount present. The percentage composition of the oil was calculated by the normalization method from the GC peak areas.

2.6 *In vitro* Free Radical-scavenging Activity

In vitro antioxidant activity of the lime peel essential oils was determined by using 1,1 diphenyl picryl hydrazyl (DPPH) free radical as per the method of Owaisi *et al.*, with slight modification [17]. Briefly, 50 µL of various concentrations (5-50 µg/mL) of essential oils in methanol were measured by using a micropipette and added to 2.95 mL of methanolic solution of DPPH (0.01mM) in a test tube. After keeping it for 30 min

in the dark at room temperature, the absorbance (A_1) of the reaction mixture was measured at 517 nm on a UV-Vis spectrophotometer. Absorption of a control sample containing the same amount of methanol and DPPH solution was used as the negative control (A_0). The % inhibition or antioxidant activity was calculated by using formula $[(A_0 - A_1/A_0) \times 100]$. The IC_{50} value (concentration of essential oil or standard which is able to scavenger 50% of DPPH free radical) was also calculated from the plot of percentage inhibition versus concentration of sample.

2.7 Evaluation of Antibacterial Activity

The antibacterial activity of the lime peel essential oils were evaluated against gram positive (*S. aureus*) and gram negative pathogenic bacteria, *E.coli*. Antibacterial activity was determined by disc diffusion method using standard Mueller Hinton agar (MHA) media [18]. Sterile filter paper discs (6 mm diameter) were impregnated with 5 & 10 µL of extracted essential oils. Then the impregnated discs of filter paper were placed on the inoculated petri plates. The plates were then incubated at 37 °C for 24 h following which the diameter of zone of inhibition (clear zone) around the disc was measured in mm. Antimicrobial activity of the test samples was compared with the positive control, Amoxicillin (10 µg/disc). All experiments were performed in triplicate.

2.8 Molecular Docking Studies

The X-ray crystal structure of *Xanthine oxidoreductase* enzyme [PDB ID:4YRW] was downloaded from Protein data Bank and was docked with the five major chemical constituents of lime essential oils. D-limonene, β- pinene, E-citral, γ-terpinene and α-farnesene were selected as ligands for binding with molecular target. Docking server (https://www.dockingserver.com), a free web based interface was used for docking calculations.

3. RESULTS AND DISCUSSION

Essential oils are the concentrated essences of plant materials which have been shown to possess useful medicinal properties [1]. Plant essential oils being of natural origin are considered as safe and have found their application in flavor and food industries as antioxidant, preservative or flavoring agent. They are also used as starting materials in the synthesis of new chemicals and fragrances for the cosmetic industry. Many volatile oils and/or their chief constituents are used in pharmaceutical industry to mask the odor, bitter and unpleasant taste of drugs [19].

Omani lime or *C. aurantifolia* (L) is a citrus fruit which looks very similar to lemons, but limes are somewhat smaller in size, less sour and ripe fruits are green in color in comparison to yellow ripe lemons [8]. It is considered as one of the most consumable fruits in the gulf region because of its numerous health benefits. Its juice or essential oil is equipopular among traditional medicine practitioners for the treatment of several acute and chronic diseases. However, the yield, composition and characteristic of any essential oil depend upon geographical distribution, environmental conditions, irrigation, harvest time etc., which in turn can affect its medicinal activity [16].

3.1 Percentage (%) Yield and Chemical Composition of Lime Peel Essential Oils

The % yield of the collected yellow-greenish essential oils varied considerably and was found to be 0.20, 0.35 and 0.45% v/w on fresh weight basis for lime peels collected from Muscat, Al-Sharqia and

Al-Batinah region, respectively. The difference in % yield of essential oils from three varieties might be due to fruit stage, extraction process, different environmental and geographical conditions. Our results are comparable with the findings of Kamal et al., who also obtained the similar yield (0.20-0.30%) for essential oils extracted from peels of three citrus fruits [20]. However, Saleem et al., (2008) obtained slightly higher yield (0.47%) of essential oil from the peels of *C. aurantifolia* [15].

The volatile constituents in the peel essential oils were identified with the help of GC-MS analysis (figure 1-3). A total of 25, 38 and 16 chemical compounds were identified in the essential oils from the peel of *C. aurantifolia* L grown in North Al-Batinah, Al-Sharqia and Muscat, representing 100%, 100% and 82.18% of the total oil, respectively. The list of identified compounds along with their % composition is populated in tables 1-3. The major aromatic constituents detected in the essential oil of North Al-Batinah peel were found to be D-limonene (54.44%), *E*-citral (7.28%), *Z*-citral (5.81%), γ-terpinene (5.60%) and *E, E*-α-farnesene (5.27%). Remaining twenty compounds were obtained in the low yield (0.20-2.81%) and are presented in table-1. Al- Sharqia lime peel essential oil showed presence of 38 compounds in varying composition (0.05-34.46%). It showed presence of additional 13 compounds which were not detected in the Al-Batinah variety. The major constituents identified include; D-limonene (34.46%), β-pinene (14.48%), *E,E*-α-farnesene (13.33%), (*E*)-citral (4.73%) and *Z*-citral (3.18%) (Table 2). While in essential oil from Muscat cultivar, only 16 compounds could be identified by GC-MS while unidentified compounds accounted for 17.82% (Table 3). The five chief volatile chemical components in Muscat lime peels are D-limonene (36.47%), β-pinene (16.75%), γ.-terpinene (5.22%), *E,E*-α-farnesene (4.78%) and α.-terpineol (3.91%). Though, limonene was the predominant monoterpenic hydrocarbon (34.46-54.44%) present in all the three lime cultivars classifying them as limonene chemotype but its concentration was highest in the Al-Batinah variety while the other two varieties had lower content. It has been reported that limonene contributes significantly to the sharp distinct aroma as well as antibacterial activity of the oil [21]. It was surprising to note that β-pinene was present in appreciable quantity in Al-Sharqia (14.48%) and Muscat variety (16. 75%) while it was absent in Al- Batinah variety. Citral, γ-terpinene and α-farnesene were the other major constituents besides limonene present in all the three varieties. Saleem et al., (2008) also analyzed the essential oil isolated from peels of *C. aurantifolia* grown in Pakistan and were able to identify only 14 components out of 21. They found limonene to be the major constituent but its concentration was quite high (82.84%) in contrast to our results (34.46-54.44%) [15]. On the other hand, the Irani lime peel is reported to contain 53.53% of limonene and 6.6% of γ-terpinene in addition to other chemical constituents [21]. Lawal et al., studied the composition of Nigerian lime grown in two different localities in Lagos. In essential oil of *C. aurantifolia* from Ijanikin, caryophyllene oxide (32.2%), caryophylla-3(15),7(14)-dien-6-ol (30.0%), α-pinene (7.9%) were observed to be the major components while Ikotun variety was rich in limonene (44.7%) and geraniol (38.2%) [22]. In all the peel essential oils, monoterpene hydrocarbons constituted the major chemical class followed by oxygenated monoterpenes and sesquiterpene hydrocarbons.

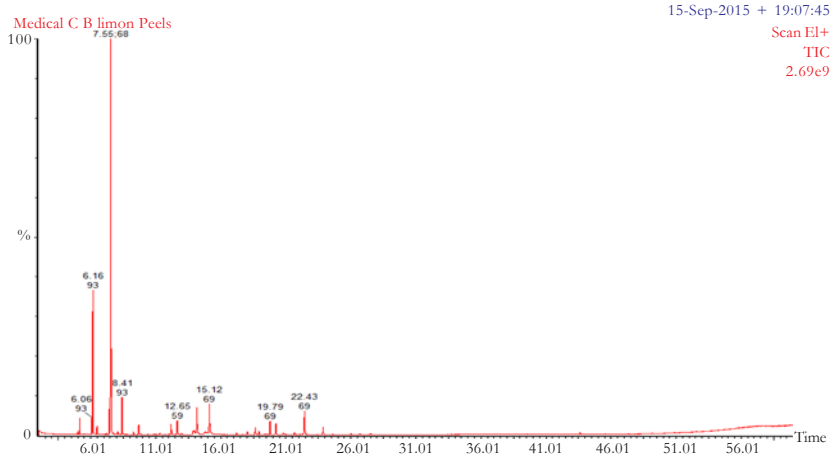

Figure 1. Chromatogram of lime peel essential oil from Al-Batinah region.

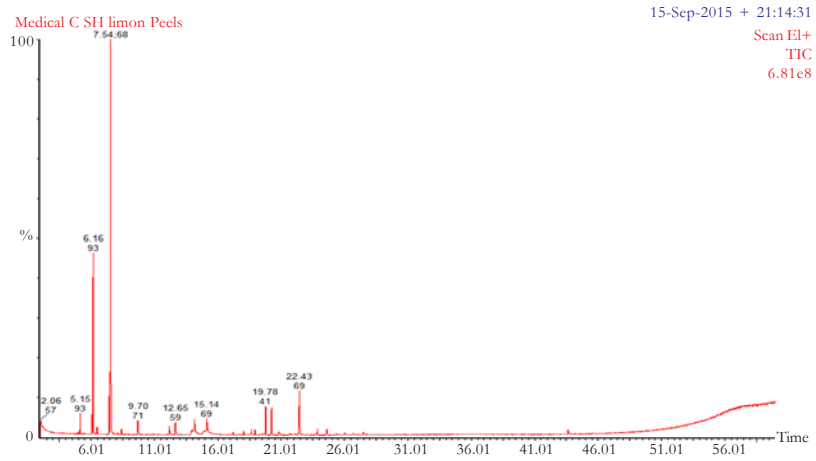

Figure 2. Chromatogram of lime peel essential oil from Al-Sharqia region.

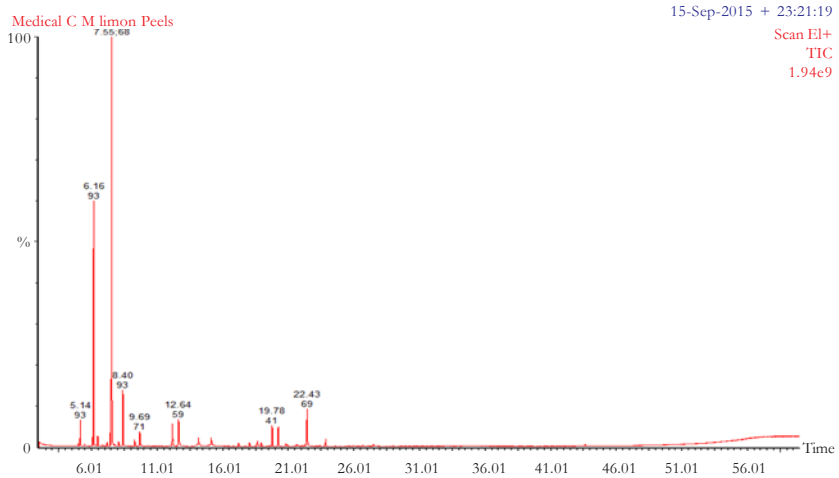

Figure 3. Chromatogram of lime peel essential oil from Muscat region.

Table 1. Chemical composition of lime peel essential oil from North Al-Batinah region of Oman.

S. No.	Compound name	RT (min)	KI[a]	% composition
1.	α-Pinene	5.152	934.59	1.826
2.	Sabinene	6.063	974.37	1.872
3.	D-Limonene	7.547	1031.01	54.444
4.	Cis-Ocimene	8.078	1049.58	0.444
5.	γ-Terpinene	8.414	1000.61	5.603
6.	Linalool	9.703	1100.06	1.887
7.	Terpinene-4-ol	12.174	1100.83	1.721
8.	α-Terpineol	12.65	1100.98	2.814
9.	Z-Citral	14.167	1200.46	5.810
10.	E-Citral	15.121	1200.75	7.276
11.	Δ-Elemene	17.223	1341.76	0.297
12.	Neryl Acetate	18.653	1305.36	1.253
13.	(-)-β-Elemene	18.946	1396.98	0.522
14.	Trans-Caryophyllene	19.791	1400.03	2.479
15.	Trans-α-Bergamotene	20.246	1400.40	2.099
16.	α-Caryophyllene	20.831	1459.31	0.490
17.	D -Germacrene	21.655	1400.86	0.583
18.	E,E-α-Farnesene	22.435	1500.17	5.272
19.	γ-Elemene	23.865	1500.63	1.407
20.	Lyratyl Acetate	26.01	1639.92	0.308
21.	E-Limonene-1,2-Epoxide	26.682	1664.54	0.220
22.	α-Bisabolol	27.506	1694.72	0.424
23.	Hexanedioic Acid	43.629	2400.03	0.540
24.	Tricosane	45.525	2501.71	0.196
25.	Heptacosane	47.378	3916.07	0.213

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

Table 2. Chemical composition of lime peel essential oil from Al-Sharqia region of Oman.

S. No.	Compound name	RT (min)	KI[a]	% composition
1.	α-Pinene	5.152	934.59	1.473
2.	Camphene	5.488	949.25	0.156
3.	Sabinene	6.073	900.75	1.467
4.	β-Pinene	6.16	900.82	14.483
5.	β-Myrcene	6.485	900.92	0.676
6.	β-Cymene	7.428	1000.26	3.052
7.	D-Limonene	7.536	1002.97	34.464
8.	Cis-Ocimene	8.078	1049.58	0.199
9.	γ-Terpinene	8.414	1000.61	0.530
10.	Linalool	9.703	1100.05	1.866
11.	Cis-Sabinene Hydrate	10.386	1127.01	0.118
12.	Artemiseole	10.841	1141.35	0.102
13.	Terpinen-4-ol	12.174	1100.83	0.971
14.	L-α-Terpineol	12.65	1100.98	1.940

Table 2. Continued.

S. No.	Compound name	RT (min)	KI[a]	% composition
15.	Geranyl Butyrate	14.016	1200.91	1.836
16.	Z-Citral	14.178	1200.46	3.181
17.	Geranyl Butyrate	14.958	1200.14	1.736
18.	(E)-Citral	15.143	1200.76	4.734
19.	Isoterpinolene	17.223	1341.76	0.275
20.	2,6-Dimethyl-2,6-Octadiene	17.678	1356.34	0.131
21.	Geranyl Acetate,	18.046	1368.14	0.548
22.	Neryl Acetate	18.653	1305.36	0.779
23.	β-Elemene	18.946	1396.98	0.564
24.	Cis-9,10-Epoxyoctadecan-1-ol	19.444	1413.37	0.158
25.	Cis-Caryophyllene	19.78	1400.25	3.155
26.	Trans-α-Bergamotene	20.246	1400.40	3.079
27.	Laevo-β-Pinene	20.82	1458.94	0.662
28.	β-Santalene	21.004	1400.97	0.152
29.	β-Bisabolene	21.47	1480.46	0.045
30.	E,E-α-Farnesene	22.435	1500.17	13.332
31.	β-Elemene	23.865	1563.11	0.648
32.	Caryophyllene oxide	24.623	1502.86	1.041
33.	1-Silacyclo-2,4-hexadiene	25.371	1616.52	0.1742
34.	(Z)-2,6-Dimethyl-2,7-Octadiene-1,6-diol	25.534	1622.49	0.118
35.	Geranyl-α-Terpinene	26.01	1639.92	0.349
36.	2-Methyl-3-(3-methyl-but-2-enyl)-2-(4-methyl-pent-3-enyl)-Oxetane	26.682	1664.54	0.390
37.	α-Bisabolol	27.495	1694.32	0.624
38.	Hexanedioic Acid	43.64	2403.64	0.794

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

Table 3. Chemical composition of lime peel essential oil from Muscat region of Oman.

S. No.	Compound name	RT (min)	KI[a]	% composition
1.	α-Pinene	5.152	934.59	0.604
2.	β-Pinene	6.16	900.82	16.749
3.	β-Myrcene	6.474	900.92	0.779
4.	β-Phellandrene	6.864	1000.08	0.107
5	α-Terpinolene	7.189	1000.19	0.348
6.	D-Limonene	7.547	1031.15	36.471
7.	Trans-β-Ocimene	7.764	1000.39	0.155
8.	γ-Terpinene	8.414	1000.61	5.220
9.	L-Linalool	9.692	1100.05	1.754
10.	Terpinene-4-ol	12.174	1100.83	2.705
11.	α-Terpineol	12.65	1100.99	3.909
12.	Z-Citral	14.167	1200.46	2.013
13.	E-Citral	15.121	1200.75	2.099
14	Cis-Caryophyllene	19.78	1400.25	2.224
15.	Bergamotene	20.246	1400.39	2.264
16.	E,E-α-Farnesene	22.435	1500.17	4.781

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

3.2 Antioxidant Activity

In last decade or so, a number of studies have been conducted worldwide in search of potent and safer antioxidants as well as antimicrobial agents from natural sources which can be used for food preservation, prevention of several chronic diseases and to treat infectious diseases [1]. Citrus volatile oils have shown great potential as antioxidant agents in experimental studies which are attributed to their complex chemical composition. The results of an earlier study carried out using 31 citrus essential oils against DPPH have indicated the essentials oils to have the similar or better antioxidant spectrum in comparison of Trolox [11]. An *in vitro* free radical scavenging (colorimetric) assay method was used to probe the antioxidant activity of lime peel essential oils [17,23]. Essential oils from the lime peel of North Al-Batinah, Al-Sharqia and Muscat region showed good antioxidant activity (12.72-58.22, 13.45-50.89 and 12.14-59.87%, respectively) as compared to ascorbic acid (31.22-96.12%) at a concentration of (5-50 µg/mL). Results of antioxidant assay presented in table 4 indicated that free radical scavenging activity of test and reference compounds increased with an increase in concentration. Peel oil from Muscat region showed the highest antioxidant activity (59.87%) followed by Al-Batinah (58.22%) and Al-Sharqia (50.89%) while at the same concentration, ascorbic acid, the positive control, exhibited 96.12±2.9 % inhibition. It was also observed that ascorbic acid (IC_{50} =14.01) is approximately thrice as potent as the essential oil based on IC_{50} value. The IC_{50} value of the essential oils were found in the following order 41.02>41.62>46.84 for Muscat >Al-Batinah>Al-Sharqia variety. Kamal and co-workers in 2013 also evaluated the antioxidant activity of citrus peel essential oils by DPPH method but surprisingly they found the essential oils to possess very weak antioxidant activity (18-47-24.08 % inhibition) in comparison to BHT (87.3%) [20]. Previous studies have indicated that limonene possess antioxidant activity and is more powerful than α-pinene [24]. All the three lime varieties have limonene as the major chemical constituent, but their antioxidant activities cannot be attributed alone to the limonene level. The % composition of limonene in essential oils form Muscat region (36.47%) is observed to be lower than in Al-Batinah region (54.44%), however, it exhibited better antioxidant activity than the other two oils. Therefore, it is evident that the difference in antioxidant activity of these oils could be due to the synergistic action of their different chemical composition. Choi in 2010 have reported that the antioxidant activity of citrus oils is primarily due to the presence of β-pinene, sabinene and γ-terpinene rather than limonene and myrcene [25].

Table 4. Percentage inhibition of free radical scavenging (DPPH) activity of essential oils and positive control.

Concentration (ug/mL)	Ascorbic acid	Al-Batinah E. oil	Al- Sharqia E. oil	Muscat E. oil
5	31.22±2.2	12.72±1.8	13.45±1.1	12.14±1.2
10	48.88±2.5	19.65±2.1	21.61±1.8	20.34±1.2
25	79.98±3.5	33.45±1.6	35.87±2.1	31.54±2.8
50	96.12±2.9	58.22±2.3	50.89±1.7	59.87±3.6
IC_{50}	14.01	41.62	46.84	41.02

Values are mean ± SD, n=3,

3.3 Antibacterial Activity of Essential Oils

Citrus essential oils have been used as a flavoring agent in food and drug industry but there are several reports which have highlighted the beneficial role of these volatile oils as possible antimicrobial preservative [26]. Few studies have also demonstrated the antibacterial and antifungal activity of citrus peel essential oils [21,27,28]. The antimicrobial activity of essential oils is partly due to their lipophilic character that leads to interaction of its constituents with the lipid layer eventually disrupting the cell membrane integrity of microorganisms and causing death of microorganisms [29]. The antibacterial activity of the lime peel essential oils evaluated at two different concentrations (5 and 10 µL) against two bacteria, one gram positive and one gram negative pathogenic strain, is reported as diameter of zone of inhibition (mm). The results of antibacterial activity presented in table 5 showed that all three essential oils inhibited the growth of *S. aureus* and *E. coli* at a higher concentration of 10 µL, however; essential oils from Al-Sharqia and North Al-Batinah variety were resistant to *E. coli* at 5 µL. In general, antibacterial actions of essential oils were weaker than standard amoxicillin drug, though both test and standard samples exhibited better activity against gram positive bacteria in comparison to gram negative bacteria. Peel oil from Muscat region exhibited the highest antimicrobial activity against both the pathogenic bacteria at each tested concentration while Al-Sharqia peel oil was found to be the least active. The variation in antibacterial activity could be because of the difference in chemical composition of these oils.

Table 5. Antibacterial activities of lime peel essential oils against two pathogenic strains of Gram positive and Gram negative bacteria.

Lime peel Essential oil/Standard	Conc/disc	Inhibition zone (mm)[a] against microbes	
		S. aureus	*E. coli*
Al- Sharqia region	5 µL	6.3±2.2	NZ
	10 µL	8.1±1.6	2.2±0.5
Al- Batinah region	5 µL	5.5±0.9	NZ
	10 µL	9.2±1.1	3.1±0.8
Muscat region	5 µL	7.5±1.4	5.8±1.5
	10 µL	10.2±2.1	8.2±1.7
Amoxicillin	10 µg	19.5±2.6	18.8±2.1

[a]Values are mean ± SD; n=3; NZ=No zone of inhibition.

The review of literature confirmed that α-pinene, limonene, linalool and other terpenes are known to exhibit antibacterial activity [30]. Limonene was found to be the chief constituent of isolated peel oils while α-pinene along with β-pinene were also present in lesser amount. The difference in antibacterial actions could be due to some other minor oxygenated and non oxygenated terpenes that might be helpful in altering the metabolic functions of microbial cells and therefore augment the actions of limonene and pinene [31].

3.4 Molecular Docking Studies

In order to identify the principal constituent(s) of essential oils responsible for exhibiting antioxidant activity, molecular

docking studies were performed with the well established antioxidant target [32]. Five major chemical constituents of lime essential oils viz. D-limonene, β-pinene, *E*-citral, γ-terpinene and α-farnesene (figure 4) were docked with *Xanthine oxidoreductase* enzyme by docking server. The best conformation for docking observed for each test ligands is shown is figures 5-9. The binding energy for the best pose for each test ligands illustrated in figure 10 was found in the range of -4.45- -6.18 kcal/mol. A ligand with the least binding energy indicates strong affinity and interaction with the receptor while high binding energy accounts for weak interaction. α-farnesene displayed the least binding energy while citral showed the highest binding energy. All the docked ligands form hydrophobic interaction with the target protein but α-farnesene interact and bind strongly to the enzyme closely followed by pinene and limonene. Hence, based on the results of docking studies, it can be concluded that the antioxidant activity of lime peel oil is not primarily because of the higher content of limonene but other oxygenated and non oxygenated hydrocarbons seem to play an important role.

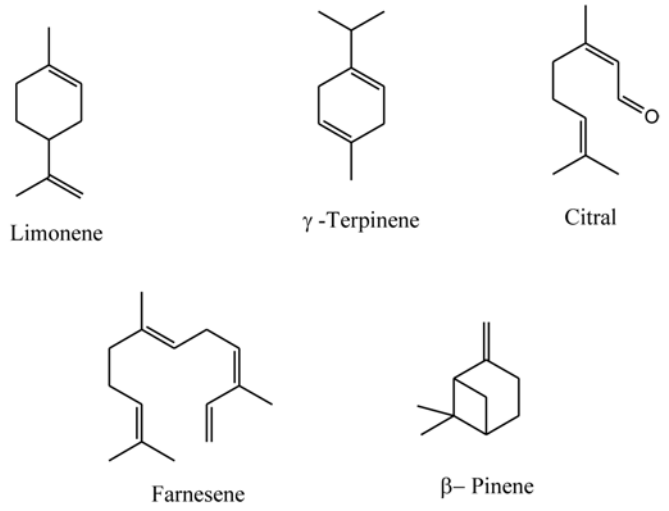

Figure 4. Chemical structures of some major chemical constituents identified in the lime peel essential oil and used as ligands for docking.

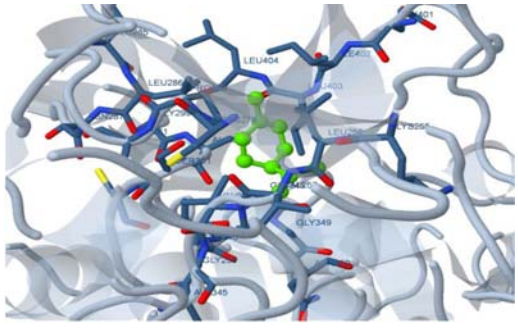

Figure 5. Docking of D-limonene with xanthine oxidoreductase.

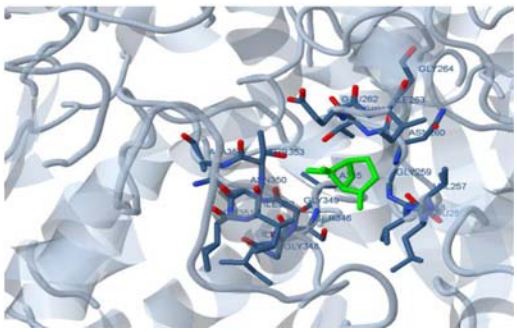

Figure 6. Docking of β-pinene with Xanthine oxidoreductase.

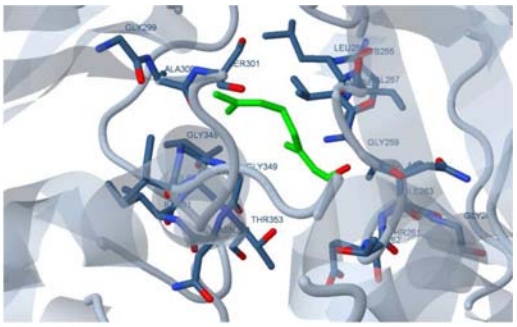

Figure 7. Docking of E-citral with xanthine oxidoreductase.

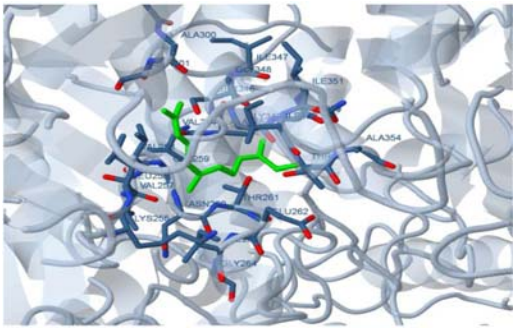

Figure 8. Docking of α-farnesene with xanthine oxidoreductase.

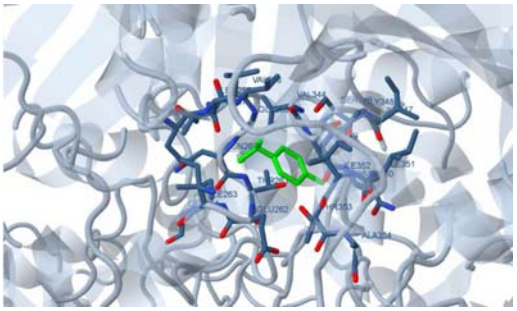

Figure 9. Docking of γ-terpinene with xanthine oxidoreductase.

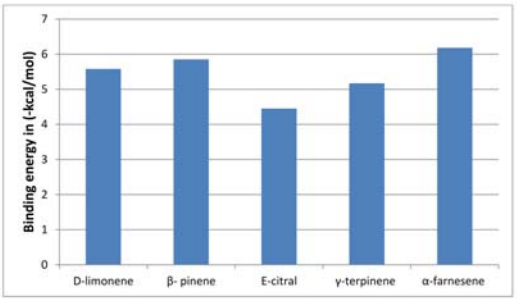

Figure 10. Binding energy values in kcal/mol for tested ligands.

4. CONCLUSIONS

The results of the current study indicate that % yield and chemical composition of lime essential oils vary from one region to other and thus are affected by environmental and geographical conditions. GC-MS analysis revealed the presence of (16 -38) chemical compounds in the essential oils. Limonene was identified as the major chemical constituent of essential oils and monoterpene hydrocarbons constituted the bulk of oil. Peel oils exhibited moderate antioxidant activity by in vitro DPPH assay method and were also observed to inhibit growth of gram positive and gram negative bacteria at 5 µL concentration. Peel oil from Muscat region yielded lower amount of oil on hydrodistillation but found to be superior to other two varieties in exhibiting antioxidant and antibacterial activities that could be attributed to difference in chemical composition of oils. It could be proposed that antioxidant and antibacterial activity of lime peel oil does not depend on limonene content alone but could be attributed to the combined effect of its complex chemical composition. The outcome of this study might be of interest to the researchers from a functional point of view and for the valorization of *C. aurantifolia* L. in Oman and in the whole gulf region.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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