Chemical composition and in-vitro antioxidant and antimicrobial activity of the essential oil of Citrus aurantifolia L. leaves grown in Eastern Oman

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Abstract

Objectives: This study investigated the chemical composition and the antioxidant and antimicrobial activities of the essential oil isolated from the aerial parts of Citrus aurantifolia L.

Methods: Fresh Citrus aurantifolia L. leaves were collected from farms in Sur city, located in the Al-Sharqia (Eastern) region of the Sultanate of Oman, during June—July of 2015. The essential oil was isolated using hydrodistillation. Gas chromatography—mass spectrometry (GC–MS) was used to identify and quantify the chemical constituents of the oil. An in-vitro 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method was used to determine the antioxidant activity of the isolated oil from the lime leaves while a disc diffusion method was used to evaluate the antibacterial activity against Gram-negative and Gram-positive bacteria.

Results: Thirty-three chemical compounds were identified, with D-limonene (63.35%) forming the major constituent. Other prominent constituents include 3,7-dimethyl-2,6-octadien-1-ol (7.07%), geraniol (6.23%), E-citral (4.35%), Z-citral (3.29%), and β-ocimene (2.25%). The essential oil of Citrus aurantifolia L. leaves showed excellent antibacterial activity against Staphylococcus aureus and moderate activity against pathogenic Escherichia coli strains. The oil exhibited promising in-vitro antioxidant activity (IC50 value = 21.57 µg/mL) but showed moderate antibacterial activities.

Conclusions: The essential oil from Omani lime leaves is characterized by a high D-limonene content, making it a promising source of natural antioxidants and antimicrobials.

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Introduction

*Citrus aurantifolia* (L.) Swingle or Omani lime (Family: Rutaceae), is a major citrus crop in the Sultanate of Oman in terms of cultivation, production, and consumption. This edible and medicinal plant is native to Southeast Asia and was widely cultivated in the tropics and subtropics before its introduction to the African and European continents through Oman. It is a perennial, flowering, evergreen tree, which is 3–5 m in height. Its stem is unusually slender and branched, with sharp thorns and spines. Leaves are alternately arranged and elliptical to oval in shape with rounded teeth on their perimeters; they usually measure 4–6 cm in length by 2.5–4.5 cm in width. The tree’s flowers are white in color and have a strong aroma. The lime fruits are round in shape (3–5 cm in diameter), and green to yellow in color with a thin skin; they are juicy, aromatic, and acidic in nature. The fruit is known as lime in English and lomi or limah in Arabic. The Omani lime variety resembles the Indian, Mexican, or Floridian key lime.

*Citrus aurantifolia* L. is a very popular and valued citrus species in the Gulf region due to its nutritional qualities, distinct flavor, and health benefits. Various parts of the plant are used in traditional medicine to treat cataract, cold, sore throat, fever, chest pain, earache, headache, stomach ailments, and edema, and it is considered an antiseptic, anthelmintic, mosquito repellent, anti-scurvy, astringent, digestive, and appetite stimulant, among others. Lime juice and its essential oil are also commonly used in the food, drug, and cosmetic industries because of their medicinal properties and fragrance. The traditional and pharmacological uses of *Citrus aurantifolia* L. plants are attributed to the presence of secondary plant metabolites including flavonoids, coumarins, and terpenoids.

Biologists have recently become increasingly interested in the useful biological activities of essential oils, especially their broad antimicrobial abilities against a wide range of pathogenic microbes. This antimicrobial activity is primarily due to their complex chemical composition, including substances belonging to a broad range of chemical classes including terpenes, aldehydes, alcohols, esters, phenols, ethers, and ketones. Thus, understanding the chemical constitution of volatile natural essential oils could prove a viable approach to identify and develop novel antimicrobial agents to overcome the problem of antimicrobial drug resistance.

An extensive literature review revealed that very little is known about the chemical composition and antimicrobial activity of the essential oil of *C. aurantifolia* L. leaves grown in Eastern Oman. Hence, the goals of this study were to (1) analyze the composition of the essential oil of *C. aurantifolia* leaves by GC–MS and (2) investigate the antioxidant and antibacterial activity of the isolated oil.

Materials and Methods

Chemicals and test microorganisms

Chemicals and reagents were obtained from a local supplier. A medium-size glass Clevenger apparatus made by Borosil® India, was used to isolate the essential oil. To evaluate the antibacterial activity, two pathogenic bacterial strains (*Escherichia coli* ATCC 8739, Gram-negative and *Staphylococcus aureus* ATCC 29213, Gram-positive) were obtained from the Department of Natural Sciences, Oman Medical College, Sultanate of Oman.

Collection of lime leaves

Fresh *C. aurantifolia* L. leaves grown in Sur city, which is located in the Al-Sharqia region of the Sultanate of Oman, were collected from farms in June–July of 2015. The leaves were identified by a subject expert from Oman Medical College, and a voucher specimen (PHAR425/2015/4) was deposited in the Department of Pharmacy, Oman Medical College, Oman.

Isolation of essential oil by hydro-distillation method

*C. aurantifolia* leaves (150 g) were washed under running water to remove dust and insects and then cut into small pieces to increase their surface area. The material was transferred to a 1-L round bottom flask and covered with a sufficient quantity of water (approximately 700 mL). Hydro-distillation in a Clevenger apparatus for 6 h yielded a strongly aromatic light green volatile oil. The oil was separated from the aqueous layer, collected in plastic sample tubes, dried over anhydrous sodium sulfate, and stored in the dark at 4 °C until further use. The yield of the isolated essential oil was calculated based on the weight of the fresh leaves.

Gas chromatography–mass spectrometry analysis

A small portion of the essential oil was diluted in diethyl ether to determine the chemical composition using a gas chromatography–mass spectrometry (GC–MS) instrument equipped with an auto sampler, including 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; maximum temperature = 350 °C) which was 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. The ionizing energy was 70 eV. The electron multiplier (EM) voltage was obtained using auto-tune. All data were obtained by collecting a full-scan mass spectrum within the range of 40–550 amu. The injected sample volume was 1 µL with a split ratio of 100:1. The oven temperature was programmed to begin at 60 °C and heat at a ramp rate of 3 °C/min to a final temperature of 280 °C, which was held for

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2 min. Unknown compounds were identified by comparing the experimental spectra with those in mass spectrum libraries (NIST 2011 v.2.2 and Wiley, 9th edition).

Identification of volatile constituents of the essential oil

Volatile constituents were identified based on their retention time relative to n-alkanes (C₅–C₂₄), with corresponding literature data in conjunction with that available in mass spectrometry libraries (NIST 2011 v.2.3 and Wiley, 9th edition).

In vitro anti-oxidant activity

The in vitro free radical scavenging activity of the essential oil of lime leaves was determined with DPPH using a slightly modified adaptation of a previously reported method. Briefly, 50 μL solutions of the essential oil in ethyl acetate at various concentrations (5–50 μg/mL) were measured using a micropipette and added to 2.95 mL of a DPPH/ethyl acetate solution (0.01 mM) in a test tube. After 30 min in the dark at room temperature 23–28°C, the absorbance (Aₜ) of the reaction mixture was measured at 517 nm on a UV–Vis spectrophotometer (UV Analyst-CT 8200). Ethyl acetate was used as a blank while DPPH solution was used as the control (A₀). The % inhibition of DPPH radical was calculated using the formula \( \frac{(A₀ - Aₜ/A₀) \times 100}{A₀} \). The IC₅₀ value was also calculated using the plot of percentage inhibition versus sample concentration.

Evaluation of antibacterial activity

The antibacterial activity of the lime leaves essential oil was evaluated against Gram-positive and negative pathogenic bacteria, S. aureus and E. coli, respectively. The antibacterial activity was determined using the disc diffusion method with standard Mueller Hinton agar (MHA) media. Sterile filter paper discs (6 mm in diameter) were impregnated with 5 and 10 μL of pure extracted essential oil and then placed on inoculated petri plates. The plates were then incubated at 37 °C for 24 h before measuring the diameter of the zone of inhibition (clear zone) around the disc. The zone of inhibition (clear zone) around the disc. The results confirm that the Omani lime leaves belong to the limonene chemotype. In a similar study of various lime species, Lota et al., detected a total of 59 chemical constituents with γ-limonene, pinene, and sabinene as the major components, followed by citronellal, geranial, linalool, and neral. Lawal et al., analyzed the essential oil of C. aurantifolia grown in the Lagos state of Nigeria and determined that γ-limonene (45%) and geranial (38%) form the chief constituents in the oil.

Antioxidant activity

A number of studies have recommended the use of essential oils in the food and drug industries as natural antioxidants because of the combination of their promising antioxidant activities and relatively safe toxicological profiles. In 2000, Choi et al., tested the antioxidant activity of 31 essential oils from citrus fruits and found them to be similar or better antioxidants than Trolox. Prompted by these and other studies, a simple and reliable in vitro assay method using DPPH free radicals was used to investigate the antioxidant potential of the essential oil isolated from lime leaves from the Al-Sharqia region. The results of the antioxidant assay, which are presented in Table 2, indicate that in concentrations

| S. No. | Compound name | RT (min) | KI | % composition |
|-------|---------------|----------|----|---------------|
| 1.    | α-Pinene      | 5.152    | 924.24 | 1.7485        |
| 2.    | Sabineine     | 6.063    | 974.36 | 0.2596        |
| 3.    | β-Pinene      | 6.16     | 911.26 | 0.3195        |
| 4.    | γ-Limonene    | 7.558    | 1026.03 | 63.3539     |
| 5.    | Trans-β-Ocimene | 7.774 | 1039.02 | 0.4397       |
| 6.    | β-Ocimene     | 8.078    | 1028.74 | 2.2450       |
| 7.    | β-Thujene     | 8.403    | 1060.86 | 0.0625       |
| 8.    | Isoterpinolene | 9.302  | 1092.08 | 0.0503       |
| 9.    | Linalool      | 9.692    | 1090.2  | 1.6491        |
| 10.   | UI            | 10.386   | 1127.01 | 0.0476       |
| 11.   | Limonene oxide| 10.7     | 1136.90 | 0.0563       |
| 12.   | Trans-Limonene oxide | 10.841 | 1141.35 | 0.0749 |
| 13.   | Citronellal   | 11.307   | 105.06  | 0.8985        |
| 14.   | Terpinen-4-ol | 12.174  | 1183.4  | 0.0562       |
| 15.   | Cit-Verbenol  | 12.271   | 1186.46 | 0.0885       |
| 16.   | l-α-Terpineol | 12.64    | 1222.44 | 0.2821       |
| 17.   | 3,7-dimethyl-2-6-Octadien-1-ol | 13.821 | 1234.81 | 7.07054     |
| 18.   | Z-Citral      | 14.157   | 1236.01 | 3.2800       |
| 19.   | Geraniol      | 14.666   | 1261.05 | 6.2331       |
| 20.   | E-Citral      | 15.11    | 1263.75 | 4.3481       |
| 21.   | UI            | 17.83    | 1361.21 | 0.0439       |
| 22.   | Neryl acetate | 18.046   | 1368.14 | 0.0839       |
| 23.   | Geranyl acetate | 18.653 | 1387.59 | 0.1838       |
| 24.   | α-Elemene     | 18.935   | 1396.63 | 0.1800       |
| 25.   | Trans-Caryophyllene | 19.78 | 1407.75 | 1.6035 |
| 26.   | Humulene      | 20.82    | 1458.94 | 0.2702       |
| 27.   | (–)-Germacrene D | 21.644 | 1486.22 | 0.0965       |
| 28.   | UI            | 22.424   | 1512.72 | 0.1776       |
| 29.   | α-Springene   | 23.41    | 1547.20 | 0.0958       |
| 30.   | γ-Elemene     | 23.865   | 1563.11 | 0.2437       |
| 31.   | Caryophyllene oxide | 24.613 | 1565.53 | 0.4910 |
| 32.   | Spathulenol   | 26.01    | 1639.92 | 0.2303       |
| 33.   | α-Bisabolol   | 27.506   | 1694.72 | 0.0777       |
of 5–50 µg/mL, the oil has a comparable antioxidant activity (27.9–87.54%) to that of a reference compound, ascorbic acid (42.18–93.83%). The free radical scavenging activities of the test and reference compounds increased as concentrations increased; however, ascorbic acid (IC50 = 13.68) was found to be approximately 1.5 times as potent as the essential oil based on the IC50 value.

### Antibacterial activity

Essential oils have been well known to exert antimicrobial activity and are potential candidates for the development of antimicrobial agents from alternative sources.18,19 Because of their lipophilic nature, essential oils can interact with and alter the permeability of the cell membrane in microorganisms, eventually leading to the microorganism’s death.20 The antimicrobial spectrum of a volatile oil invariably depends upon its chemical composition. In the present study, d-limonene was found to be the major chemical constituent of the citrus oil, contributing to its sharp aroma and antibacterial actions.21 The results of the antibacterial activity analysis (reported as the diameter of the zone of inhibition, Table 3) indicate that the essential oil exerts dose-dependent activity with more pronounced effects against S. aureus (5.8–7.9 mm) than in E. coli (1.7–3.1 mm). However, its antibacterial activity is much weaker than that of the positive control, ampicillin.

Citrus oils are generally recognized as safe (GRAS) and therefore, lime essential oil can be used as a natural flavoring agent or food additive for its aroma as well as its antioxidant and antibacterial activities. d-limonene, the major constituent of lime oil, is an effective gastroprotective agent22; therefore, lime oil may be used in combination with anti-inflammatory agents to overcome their gastrotoxicity. Lime essential oil can also be explored as an alternative to conventional therapy for some common ailments.

### Conclusions

A GC–MS analysis of lime leaf essential oil detected 33 volatile chemical compounds, three of which remain un-identified (9.1%). d-limonene was found to be the major constituent, confirming the limonene chemotype of the Al-Sharqia lime variety. The lime leaves oil demonstrated concentration-dependent inhibition of DPPH radicals with an IC50 value of 21.87 µg/mL. Its in-vitro free radical scavenging activity was nearly comparable to that of ascorbic acid at a concentration of 50 µg/mL. On the other hand, the oil had moderate anti-bacterial activities. Further, more detailed studies are recommended to explore the potential of C. aurantifolia L. leaves essential oil as a food preservative and a source of natural antioxidants.

### Conflicts of interest statement

The authors have no conflict of interest to declare.

### Authors’ contribution

SAK designed the experiment, analyzed the results and edited the manuscript; TA performed the antioxidant activity and wrote the manuscript; MSA, NMA, and SSA performed the experiment, collected the data, and analyzed the results (all three authors contributed equally). All authors read and approved the final manuscript.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jtumed.2017.12.002.

References

1. Al Sadi AM, Al Moqbali HS, Al yahyai RA, Al Said FA. Citrus aurantifolia Swingle in Oman in the outbreak of witches’ broom disease of lime. *Euphytica* 2010; 188: 285–297.
2. Manner H, Buker R, Smith V, Ward D, Elevitch C. In: Elevitch C, editor. *Citrus species (Citrus). Species profiles for Pacific island agroforestry.* 2.f. Holualoa, Hawaii: Permanent Agriculture Resources; 2006.
3. Al-Yahyai R, Al-Subhi A, Al-Sabahi J, Al-Said F, Al-Wahaiib AK, Al-Sadi AM. Chemical composition of acid lime leaves infected with *Candidatus Phytoplasma aurantifolia*. *Agric Sci* 2014; 5: 66–70.
4. Apraj V, Thakur ND, Bhagwat A, Mallya R, Sawant L, Pandita N. Pharmacognostic and phytochemical evaluation of *Citrus aurantifolia* (Christm) Swingle peel. *Pharmacogenomics J* 2011; 3: 70–76.
5. Johann S, Smania A, Pizzolatti MG, Schripsema J, Branco A. Complete 1H and 13C-NMR assignments and antifungal activity of two 8-hydroxy flavonoids in mixture. *An Acad Bras Cienc* 2007; 79: 215–222.
6. Piccinelli AL, Garcia MM, Armenteros DM, Alfonso MA, Arevalo AC, Campone L, Rastrelli L. HPLC-PDA-MS and NMR characterization of C-glycosyl flavones in a hydro-alcoholic extract of *Citrus aurantifolia* leaves with antiplatelet activity. *J Agric Food Chem* 2008; 56: 1574–1581.
7. Sandoval-Montemayor NE, Garcia A, Elizondo-Treviño E, Garza-González E, Alvarez L, Camacho-Corona MR. Chemical composition of hexane extract of *Citrus aurantifolia* and anti-mycobacterium tuberculosis activity of some of its constituents. *Molecules* 2012; 17: 11173–11184.
8. Ali B, Al-Wabel NA, Shams S, Ahmad A, Khan SA, Anwar F. Essential oils used in aromatherapy: a systemic review. *Asian Pac J Trop Biomed* 2015; 5(8): 589–598.
9. Lang G, Buchbauer G. A review on recent research results (2008–2010) on essential oils as antimicrobials and antifungals. A review. *Flavour Fragrance J* 2012; 27(1): 13–39.
10. Chouhan S, Sharma K, Guleria S. Antimicrobial activity of some essential oils—present status and future perspectives. *Medicine* 2017; 4: 58.
11. Rudramurthy GR, Swamy MK, Sinniah UR, Ghasemzadeh A. Nanoparticles: alternatives against drug-resistant pathogenic microbes. *Molecules* 2016; 21(7): 836.
12. Al-Owaisi M, Al-Hadiwi N, Khan SA. GC-MS analysis, determination of total phenolics, flavonoid content and free radical scavenging activities of various crude extracts of *Moringa peregrina* (Forsk.) Fiori leaves. *Asian Pac J Trop Biomed* 2014; 4(12): 964–970.
13. Al-Abbasy DW, Pathare N, Khan SA, Al-Sabahi JN. Chemical composition and antibacterial activity of essential oil isolated from Omani basil (*Ocimum basilicum* Linn). *Asian Pac J Trop Dis* 2015; 5(8): 645–649.
14. Lota ML, de Rocca Serra D, Tomi F, Jacquesmond C, Casanova J. Volatile components of peel and leaf oils of lemon and lime species. *J Agric Food Chem* 2002; 50: 796–805.
15. Lawal OA, Ogunwande IA, Owolabi MS, Giwa-Ajeniya AO, Kasali AA, Abudu FA, Sanni AR, Opoku AR. Comparative analysis of essential oils of *Citrus aurantifolia* Swingle and *Citrus reticulata* Blanco, from two different localities of Lagos State, Nigeria. *Am J Essent Oils Nat Prod* 2014; 2(2): 8–12.
16. Amorati R, Foti MC, Valgimigli L. Antioxidant activity of essential oils. *J Agric Food Chem* 2013; 61(46): 10835–10847.
17. Choi HS, Song HS, Ukeda H, Sawamura M. Radical-scavenging activities of Citrus essential oils and their components: detection using 1, 1-Diphenyl-2-picrylhydrazyl. *J Agric Food Chem* 2010; 58: 4156–4161.
18. Mahmud S, Saleem M, Siddiqui S, Ahmad R, Khanum R, Parveen Z. Volatile components, antioxidant and antimicrobial activity of *Citrus acaida* var. sour lime peel oil. *J Saudi Chem Soc* 2009; 12: 195–198.
19. Raut JS, Karuppayil SM. A status review on the medicinal properties of essential oils. *Ind Crop Prod* 2014; 62: 250–264.
20. Costa ART, Amaral MFZJ, Martins PM, Paula JAM, Fiuza TS, Tresvenzol LMF, Paula JR, Bara MTF. *Ind Crop Prod* 2014; 45(12): 964–970.
21. Casanova J. Volatile components of peel and leaf oils of lemon and lime species. *J Agric Food Chem* 2002; 50: 796–805.
22. Jafari S, Esfahani S, Fazeli MR, Jamalifar H, Samadi N, Toosi AN, Ardekani MRS, Khanavi M. Antimicrobial activity of lime essential oil against food-borne pathogens isolated from cream-filled cakes and pastries. *Int J Biol Chem* 2011; 5: 258–265.
23. Moraes TM, Kushima H, Moleiro FC, Santos RC, Machado Rocha LR, Marques MO, Vilegas W, Hiruma-Lima CA. Effects of limonene and essential oil from *Citrus aurantium* on gastric mucosa: role of prostaglandins and gastric mucus secretion. *Chem Biol Interact* 2009; 180: 499–505.

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