JNCS Journal of Nepal Chemical Society July 2024, Vol. 44, No. 2, 70-77
ISSN: 2091-0304 (print)
DOI: https://doi.org/10.3126/jncs.v44i2.68307
RESEARCH ARTICLE

Bio-efficacy of Essential Oil Extracted from Locally Available Orange (*Citrus sinensis*) Peels from Nepal

Sujan Poudel[1], Bishan Datt Bhatt[1*]

[1]*Department of Chemistry, Tri-Chandra Multiple Campus, Tribhuvan University, Ghantaghar, Kathmandu, Nepal*

[*]*Corresponding E-mail: bdbhatta@gmail.com*

(Received: June.8, 2024; revised: July 7, 2024; accepted: July 15, 2024)

Abstract

Essential oils from various plants are valued for their therapeutic, aromatic, antimicrobial, and antioxidant properties. The majority of them are employed in cleaning procedures, aromatherapy, stress reduction, food flavouring and preservation, and beauty products. The present investigation focuses on elucidating the chemical composition along with the antimicrobial and antioxidant properties of essential oil extracted from locally available orange (*Citrus sinensis*) peels, mainly exploring its bio-efficacy. The antibacterial property was studied against the gram-negative bacterium *Escherichia coli* and the gram-positive bacterium *Bacillus subtilis*. The analysis results revealed significant antimicrobial activity with zones of inhibition of 4 mm and 5.5 mm, respectively. The antifungal property was studied against the opportunistic pathogenic yeast *Candida albicans*, which showed positive activity, producing 4.5 mm of zone of inhibition. The antioxidant activity of the oil was determined using DPPH free radical scavenging assay, which showed an IC_{50} value of 1.3532 µL/mL. Qualitative phytochemical screening of the essential oil as determined using some standard colour-developing methods showed the presence of alkaloids, flavonoids, terpenoids, phenols, cardiac glycosides, and saponins. The above results were validated by FT-IR and GC/MS analysis. The FT-IR analysis revealed the presence of nitrogen-containing and oxygen-containing functional groups. The GC/MS analysis showed the presence of limonene, d-limonene, 1S-α-Pinene, 1R-α-Pinene, β-myrcene, linalool, α-humulene, α-terpineol, etc. These findings demonstrated that orange peels from Nepal that are readily available locally could be a source of compounds with antibacterial, antifungal, and antioxidant properties.

Keywords: Orange peel; Essential oil, Antibacterial; Antifungal, Antioxidant

Introduction

The use of essential oils is a mounting directive due to their wide range of applications, such as biological applications [1], soaps, detergents, cosmetics products [2], pharmaceutical products, perfumes, aromatherapy [3], confectionery food products, soft drinks and hard drinks, distilled alcoholic beverages, and insecticides [4]. Due to its perfumatory value, its production and consumption are increasing day by day in an exciting way [5]. Essential oil production technologies are being refined day by day to improve their overall yield and quality. The various well-known methods of extraction are infusion, maceration, Soxhlet extraction, solid-liquid extraction, and liquid-liquid extraction [6]. Some methods of extraction consume high heat energy and also take a long time for extraction [7]. Some methods also require toxic solvents,

which make them difficult to handle. The high temperature used in some extraction processes may damage thermolabile compounds present in the sample [8]. The use of waste orange peels for the production of an essential oil is one of the best ways to manage solid waste materials. The residue left after the extraction of essential oil can also be used for various purposes [9]. Orange peels are common solid waste materials sucked out of orange juice factories and juice shops. The consumption of oranges is in increasing demand with the increasing population due to their health benefits. The greater the use of orange juice, the more solid waste materials are produced. Hence, managing the produced orange peels in a novel way is a very important step in solid waste management. The orange essential oil has a pronounced prominence due to its antibacterial [10], antifungal [11], antioxidant [12], antiulcer [13], and anticancer [14] properties. Even a low concentration of essential oil in cell culture is found to kill bacteria without harming the cultured cell. The growth of fungi causing spoilage of the food is also prevented [11].

Limonene is one of the most abundant components of an essential oil commonly found in orange (*Citrus sinensis*) peels. It has been found to possess anti-stress, anti-inflammatory, and antioxidant properties. Moreover, d-limonene is one of the most common terpenes and is an abundantly available constituent of an orange essential oil, which is a chemopreventive and chemotherapeutic agent to inhibit manifold kinds of tumors [15, 16]. Some workers have reported that citrus essential oil has anticancer activity [12, 17].

The present work focuses on the extraction of essential oil from locally available orange peels from Nepal, and studying its antibacterial, antifungal, and antioxidant efficacies. Additionally, FT-IR, GC-MS, and phytochemical analyses of the oil have been performed to validate the results thus obtained. Research results are expected to contribute to fields such as pharmaceuticals, fragrances, and solid-waste management.

Materials and Methods

Materials

The orange peel waste was collected from a local juice shop located in Thamel, Kathmandu. The collected orange peels were washed several times. Then it was cut into small pieces. After making small pieces, the sample was crushed in mortar in order to make powder. This powder sample was collected in an airtight bottle for further use.

Preparation of microbial culture media

The Mueller-Hinton Agar (MHA) plates were prepared by dissolving 28 g of nutrient agar powder (Sisco Research Laboratories Pvt. Ltd., India) in 1 L of water. The mixture was autoclaved at 15 psi pressure at 121 °C for 15 minutes. The sterilized media were cooled down to 40–50 °C, followed by transfer into Petri dishes (25 mL each). The prepared media plates were stored in the refrigerator for further use.

Extraction of orange peel essential oil

Essential oils are commonly extracted using hydro distillation, water and steam distillation, steam distillation, cohobation, maceration, and enfleurage. In the present work, orange peel essential oil was extracted by the hydro distillation method using the Clevenger apparatus. The 150 g of powdered orange peel sample was poured into a round-bottom flask of Clevenger apparatus and rinsed with 250 mL of pure distilled water. The Clevenger apparatus was well fitted, and a regular supply of water was made for the condensation of steam produced from the sample mixture by a heat supply of 45 °C. After 2 hours, separation of

steam and essential oil was observed in the Florentine vase. This was collected in a conical flask, and the oil was separated using a separating funnel.

Characterization of the essential oil by GC-MS and FT-IR

The extracted essential oil was characterized by both FT-IR and GC/MS techniques. FT-IR analysis of the essential oil was carried out by the KBr pellet method, and the presence of various vibrational modes was investigated. The FT-IR analysis was used to identify functional groups present in the essential oil. The GC was equipped with Agilent 190915-433 (30 m × 250 µm × 0.25 µm). The mass spectrum was obtained by electron ionization (EI) at 70 eV with a thermal aux 2 heater at 230 °C. The injection volume was 2 µL with an inlet heater at 230 °C and a pressure of 6.6018 psi, maintaining the rate flow to be 1 mL per minute. The oven temperature was 320 °C, and the split ratio was 75:7. The sample was programmed at 32 °C for 5 minutes up to 230 °C with 5 °C per minute, and then the hold time was 15 minutes, resulting in a total time of 59.6 minutes. This GC-MS technique was used to identify the compounds and obtain information about the unit cell molecules.

Phytochemical Analysis

The qualitative phytochemical analysis of the essential oil was carried out using colour reactions for the identification of medicinally active secondary metabolites present in the essential oil. The screening of various secondary metabolites was performed as described in the literature [18].

Antimicrobial Analysis

The antimicrobial analysis was carried out by the disc diffusion method against the microbial strains *Escherichia coli*, *Bacillus subtilis*, and *Candida albicans*, as described in the literature [19].

Antioxidant Analysis

Antioxidants are substances that protect cells against free radicals, which may cause heart disease, cancer, and other diseases. DPPH free radical scavenging is a well-accepted mechanism for screening the antioxidant activity of plant extracts and essential oil extracts. The radical scavenging activity of the plant sample was evaluated using the 96-well microplate method as described in the literature [20], which is a modified colorimetric method.

Results and Discussion

The extracted essential oil was characterized by the FT-IR technique to identify functional groups present in the compounds of the oil. The FT-IR spectrum has been displayed in **Fig. 1**.This illustrates the presence of various kinds of biomolecules in the essential oil. The adsorption band located at 3000 cm^{-1} corresponds to stretching vibrations of the alkenyl (C=C-H) group. The band at 3460.30 cm^{-1} represents the amine N-H stretch. The peak at 2372.44 cm^{-1} represents the presence of C-O stretch. The absorption bands at 1645.28 cm^{-1}, 1440.83 cm^{-1} and 1375.25 cm^{-1} represent amide, nitrosamine, and isopropyl groups, respectively. The absorptions at 887.26 cm^{-1} and 794.67 cm^{-1} represent the presence of aromatic C-H bonding and octadiene, respectively.

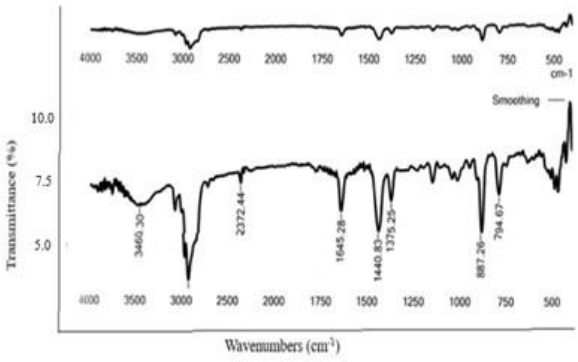

Figure 1: *FTIR spectra of the orange peel essential oil*

GC-MS Analysis

To obtain information about the chemical composition of the extracted essential oil, GC-MS techniques were utilized. The components of the essential oil as recorded by GC-MS have been recorded in **Table 1**

Table 1. Chemical composition of the orange peel essential oil

Peak	RT	Compounds	Area %
1	2.454	Cyclohexane	8.77
2	2.497	Hexane, 2-methyl	0.57
3	2.616	Hexane, 3-methyl	0.30
4	11.020	Bicyclo hex-2-ene, 2-methyl-5- (1-methylethyl) Bicycle hexane, 4-methyl-1- (1-methylethyl), didehydro derive.	0.25
5	11.203	1S α Pinene, 1R α Pinene Bicycle hept-2-ene, 2,6,6-trimethyl	1.09
6	12.725	β Phellandrene Bicyclo hexane, 4-methylene1- (1-methylethyl)	1.32
7	13.469	β Myrcene Bicyclo hex-2-ene, 4-methy-1- (1-methylethyl)	1.78
8	13.900	Octanal	0.21
9	14.257	Bicycle hept-2-ene,3,7,7-trimethyl- Cyclohexene, 4-methyl-3-(1-methylethylidene) 1,3-cyclohexadiene, 1-methyl-4-(1-methylethyl)	0.23
10	14.893	d-Limonene, Limonene	75.68
11	15.745	1,4 cyclohexadiene, 1-methyl-4-(1-methylethyl)	6.22
12	16.652	Cyclohexene, 1-methyl-4-(1-methylethylidene)	0.35
13	17.137	1,6-octadien-3-ol, 3,7-dimethyl, 2-aminobenzoate	1.52
14	19.446	3-cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)	0.45
15	19.867	3-cyclohexane-1-methanol, $\propto,\propto$, 4-trimethyl	0.31
16	20.298	Decanal	0.29
17	21.129	Benzene, 2-methoxy-4-methyl-1-(1-methylethyl) Phenol, 2-(1,1-dimethylethyl)-5-methyl	0.20
18	22.823	Thymol, Phenol, 2-methyl-5-(1-methylethyl)	0.43

RT: retention indices determined on Agilent 190915-433 column (using He gas 1 mL per minute).

The total ion chromatogram of orange essential oil has been shown in the **Fig. 2**. The major compounds were found to be 1S-α-Pinene (a terpene), 1R-α-Pinene (a terpene), β-myrcene (a monoterpene), d-limonene (a cyclic monoterpene), linalool (a terpene alcohol), α-humulene (a terpene), and α-terpineol (an alcohol). These compounds have several health benefits, like anti-inflammatory, anti-cancer, antioxidant, and antimicrobial activities [14-16]. The results from the GC-MS analysis reveal the potential of the essential oil from orange peel as an important source of bioactive compounds.

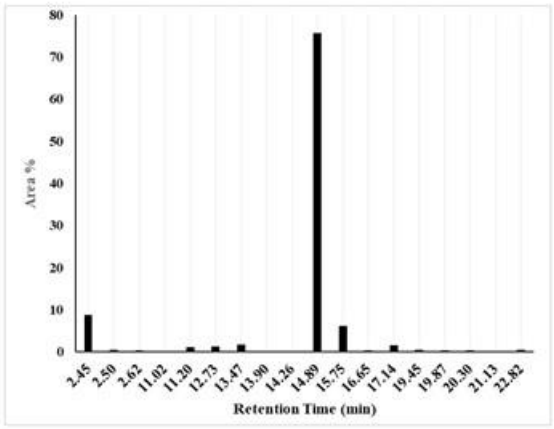

Figure 2: *GC-MS spectra of the orange peel essential oil*

Antibacterial Analysis

The antibacterial analysis of the essential oil extracted from the orange peels was performed against *Escherichia coli* (*E. coli*), a gram-negative, facultative anaerobic, rod-shaped, and coliform bacterium, and *Bacillus subtilis* (*B. subtilis*), a gram-positive bacterium, also known as *Hay bacillus* or *Grass bacillus*. The zone of inhibition was determined in both cases. The experimental results have been shown in **Fig. 3 and Fig. 4**.

Figure 3: *Antibacterial activity of the orange peel essential oil against E. coli*

The zone of inhibition in *E. coli* was found to be 4.0 mm, and that in *B. subtilis* was found to be 5.5 mm. These findings show that orange peel essential oil (extracted by hydro-distillation method) has fairly good antibacterial action against B. subtilis and E. coli, yet it appears to be less potent than that of methanolic extract in earlier research [21]. To get rid of over load of *E. coli* and *B. subtilis* beyond their normal balance in the body, the essential oil extracted from *Citrus sinensis* has been expected to be very effective.

Figure 4*. Antibacterial activity of the orange peel essential oil against B. subtilis*

Antifungal Analysis

The antifungal analysis of the essential oil extracted from the orange peels was performed against the fungus *C. albicans,* which is an opportunistic pathogenic yeast of the Saccharomycetaceae family. The zone of inhibition was determined, and the result is displayed in **Fig. 5.**

Figure 5: *Antifungal activity of the orange peel essential oil against C. albicans*

The zone of inhibition was found to be 4.5 mm demonstrating fairly good antifungal activity

against *Candida albicans*. However, it appears that this extract's effectiveness is lower than that of the wild fruit extract employed in earlier research [22]. This reveals the application of the oil as a potential source of antifungal substances.

Antioxidant Analysis

The antioxidant activity of the methanol extract was measured by DPPH radical scavenging assay. The inhibition curve was plotted by plotting extract concentration versus the corresponding % scavenging effect, which is displayed in **Fig. 6.** The IC_{50} (50% inhibitory concentration) value was determined using this curve. The IC_{50} value indicates the effective concentration of the sample, which is required to scavenge 50% of the DPPH free radicals.The antioxidant activity assay revealed the IC_{50} value of the oil to be 1.3532 µL/mL. This result indicates that the extracted essential oil exhibits excellent antioxidant efficacy. Previous researchers have demonstrated this efficacy as well [12]. Thus, the oil is capable of reducing the risk of many diseases including heart disease and certain cancers.

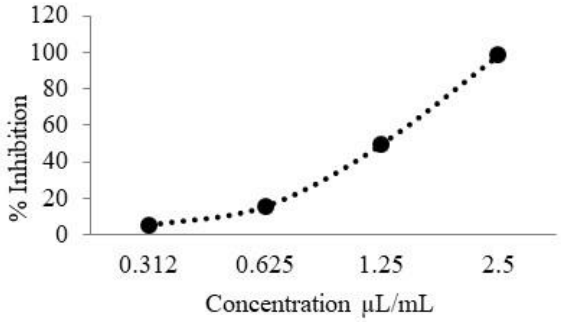

Figure 6: *A plot of percentage radical scavenging activity versus concentration*

Conclusions

In the present investigation, essential oil was extracted from locally available orange peels from Nepal. The extracted oil was subjected to bio-efficacy analysis. The oil was found to have good biomedical properties. Antimicrobial analysis was carried out against gram-negative bacteria *Escherichia coli (E. coli)*, gram-positive bacteria *Bacillus subtilis (B. subtilis),* and the fungus *Candida albicans (C. albicans).* Excellent antimicrobial activity was noted, with zones of inhibition of 4 mm, 5.5 mm, and 4.5 mm, respectively. DPPH free radical scavenging assay of the oil revealed excellent antioxidant activity, with an IC_{50} value of 1.3532 µL/mL. The phytochemical screening of the oil revealed the presence of alkaloids, flavonoids, terpenoids, phenols, cardiac glycosides, and saponins. These secondary metabolites are responsible for their antimicrobial, and antioxidant activities. GC-MS analysis was performed to explore the presence of possible biomedically important compounds. The compounds found in abundance were limonene, d-limonene, 1S-α-Pinene, 1R-α-Pinene, β-myrcene, linalool, α-humulene, and α-terpineol. These compounds have various marvelous and unique properties that can be used in various fields of life sciences astoundingly. These results vouch for the applicability of the locally available orange peels from Nepal for the extraction of various biomedical substances. Moreover, this investigation also explores a way for solid waste management.

Acknowledgements

Author's Contribution Statement

Sujan Poudel: Conceptualization, Methodology, Formal analysis, Data curation, Writing-original draft preparation, Writing-review and editing, **Bishan Datt Bhatt:** Conceptualization, Methodology, Formal analysis, Data curation, Writing-review and editing, Supervision

Conflict of Interest

The authors do not have any conflict of interest throughout this research work.

Data Availability Statement

The data supporting this study's findings are available from the corresponding authors upon

reasonable request.

References

1 N.S. Dosoky and W. N. Setzer, Biological activities and safety of *Citrus* spp. essential oils, *Int J Mol Sci,* 2018, 19, 1966. (DOI: 10.3390/ijms19071966)

2. E. Palazzolo, V.A. Laudicina and M. A. Germanà, Current and potential use of *Citrus* essential oils, *Curr Org Chem,* 2013, 17, 3042–3049. (DOI: 10.2174/13852728113179990122)

3. B. Ali, N. A. Al-Wabel, S. Shams, A. Ahamad, S A. Khan and F. Anwar, Essential oils used in aromatherapy: A systemic revie, *Asian Pacific Journal of Tropical Biomedicine*, 2015, 5(8*),* 601–611. (DOI: https://doi.org/10.1016/j.apjtb.2015.05.007)

4. H. Bora, M. Kamle, D. K. Mahato, P. Tiwari and P. Kumar, *Citrus* Essential Oils (CEOs) and Their Applications in Food: An Overview, *Plants (Basel).* 2020, 9(3), 357. (DOI: 10.3390/plants9030357)

5. J. Lawless, The Complete Illustrated Guide to Aromatherapy: A Practical Approach to the Use of Essential Oils for Health and Well-Being, *Harper Collins, London, UK.* 2002.

6. N. Mahato, M. Sinha, K. Sharma, R. Koteswararao and M. H. Cho, Modern Extraction and Purification Techniques for Obtaining High Purity Food-Grade Bioactive Compounds and Value-Added Co-Products from *Citrus* Wastes, *Foods*, 2019, 8, 523. (DOI: https://doi.org/10.3390/foods8110523)

7. M. Elyemni, B. Louaste, I. Nechad, T. Elkamli, A. Bouia, M. Taleb, M. Chaouch and N. Eloutassi, Extraction of Essential Oils of *Rosmarinus officinalis L.* by Two Different Methods: Hydrodistillation and Microwave Assisted Hydrodistillation, *The Scientific World Journal*, 2019, Article ID 3659432. (DOI: https://doi.org/10.1155/2019/3659432)

8. S. R. Shirsath, S. H. Sonawane and P. R. Gogate, Intensification of extraction of natural products using ultrasonic irradiations-A review of current status, *Chem. Eng. Process*, 2012, 53, 10–23. (DOI: https://doi.org/10.1016/j.cep.2012.01.003)

9. P. S. Calabrò, E. Paone and D. Komilis, Strategies for the sustainable management of orange peel waste through anaerobic digestion, *Journal of Environmental Management*, 2018, 212, 462–468. (DOI: 10.1016/j.jenvman.2018.02.039)

10. M. E. Abalaka and A. O. Bello, Antibacterial activity of *Citrus sinensis* (Orange) peel on bacterial isolates from wound, *Journal of Microbiology Research,* 2016, **1,** 161-168.

11. M. Viuda-Martos, Y. Ruiz-Navajas, J. Fernández-López and J. Pérez-Álvarez, Antifungal activity of lemon (*Citrus lemon L.*), mandarin (*Citrus reticulata L.*), grapefruit (*Citrus paradisi L.*) and orange (*Citrus sinensis L.*) essential oils, *Food Control*, 2008 19 (12). 1130-1138. (DOI: https://doi.org/10.1016/j.foodcont.2007.12.003)

12. C. Yang, H. Chen, H. Chen, B. Zhong, X. Luo and J. Chun, Antioxidant and Anticancer Activities of Essential Oil from Gannan Navel Orange Peel, *Molecules,* 2017, 22(8), 1391. (DOI: 10.3390/molecules22081391)

13. F. D. A. Oliveira, L. N. Andrade, E. B. DeSousa and D. P. DeSousa, Anti-Ulcer activity of essential oil constituents, *Molecules,* 2014, 19, 5717–5747. (DOI: 10.3390/molecules19055717)

14. Y. Bhalla, V. K. Gupta and V. Jaitak, Anticancer activity of essential oils: A review, *J Sci Food Agric,* 2013, 93, 3643–3653. (DOI: https://doi.org/10.1002/jsfa.6267)

15. P. L. Crowell and M. N. Gould, Chemoprevention and therapy of cancer by d-limonene, *Crit Rev Oncog*, 1994, 5, 1–22. (DOI: 10.1615/critrevoncog.v5.i1.10)

16. D. M. Vigushin, G, K, Poon, A, Boddy, J. English, G. W. Halbert, C. Pagonis, M. Jarman and R. C. Coombes, Cancer Research Campaign Phase I/II Clinical Trials Committee. Phase I and pharmacokinetic study of d-limonene in patients with advanced cancer, *Cancer Chemother Pharmacol,* 1998 42, 111–117. (DOI:

10.1007/s002800050793)

17. J. A. Miller, P. A. Thompson, I. A. Hakim, A. M. Lopez, C. A. Thomson, W. Chew, C. H. Hsu and H. H, Chow, Safety and Feasibility of Topical Application of Limonene as a Massage Oil to the Breast, *Journal of cancer therapy*, 2012, 3(5A). (DOI: 10.4236/jct.2012.325094
18. R. Gul, S. U. Jan, S. Faridullah, S. Sherani and N. Jahan, Preliminary Phytochemical Screening, Quantitative Analysis of Alkaloids, and Antioxidant Activity of Crude Plant Extracts from *Ephedra* intermedia Indigenous to Balochistan, *The Scientific World Journal*, 2017, ID5873648. (DOI: https://doi.org/10.1155/2017/5873648)
19. B. D. Bhatt and C. Chhetry, Comparative study of antioxidant and antibacterial activities in the methanol and ethyl acetate extract of leaf and stem bark of *Semecarpus anacardium Linn, Journal of Nepal Chemical Society*, 2018, 38, 58–65. (DOI: https://doi.org/10.3126/jncs.v38i0.27789)
20. J. Tu, D. Shi, L. Wen, Y. Jiang, Y. Zhao, J. Yang, H. Liua, G. Liu and B. Yang, Identification of moracin N in mulberry leaf and evaluation of antioxidant activity, *Food and Chemical Toxicology*, 2019, 132, 110730. (DOI: 10.1016/j.fct.2019.110730)
21. D. Dubey, K. Balamurugan, R.C. Agrawal, R. Verma, R Jain. Evalution of Antibacterial and Antioxidant Activity of Methanolic and Hydromethanolic Extract of Sweet Orange Peels, Recent Research in Science and Technology, 2011, 3(11), 22-25.
22. Q. Phan-Nguyen, T. Tran-Le, H. Nguyen, V. Le. Investigation of biological activities of essential oil obtained from peel of wild orange in Quang Ngai by steam distillation, *Earth and Environmental Science*, 2024, 1340, 012024. (DOI: 10.1088/1755-1315/1340/1/012024).