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Original Article

Assessment of antibacterial activities of methanolic orange peel extracts against pathogenic bacteria

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ABSTRACT

This study investigates the antibacterial efficacy of methanolic extracts obtained from Iraqi orange peels and provides a comprehensive phytochemical screening of the extracts. Orange peel samples were carefully collected, washed, dried, and ground before undergoing cold maceration in methanol. The extracts were subsequently concentrated using a rotary evaporator under reduced pressure, yielding a dark green residue that was subjected to bioactivity assays. Preliminary phytochemical screening confirmed the presence of several classes of bioactive compounds within the orange peel extract, including terpenoids, tannins, flavonoids, saponins, and phenols. These phytochemicals are recognized for their broad pharmacological activities and are believed to contribute synergistically to the extract's antibacterial properties. Standard protocols were used for testing, and specific color reactions indicated these compounds in the crude extracts. To evaluate antibacterial activity, the agar well diffusion technique was applied .Mueller-Hinton agar plates were seeded with bacterial stock cultures, with extract doses applied in wells created on the agar surface. After 24 hours of incubation at 37°C, zones of bacterial growth inhibition were measured to quantify the extracts' effects. Results highlighted that the methanolic extracts exhibited notable antibacterial activity against Gram-positive and Gram-negative bacteria, supporting the traditional application of orange peel in treating bacterial infections. Both Staphylococcus aureus (Gram-positive) and Escherichia coli (Gram-negative) were found to be susceptible to the orange peel methanolic extract, contradicting some earlier reports of resistance and affirming the potential therapeutic value of these phytochemicals. The research suggests that orange peel extracts have promising applications in managing bacterial diseases.

Keywords: Orange peel, Methanolic extract, Antibacterial activity, Phytochemical screening, Citrus sinensis, Gram-positive bacteria

Introduction

Juice factories rely heavily on citrus fruit, however, much of the fruit is thrown away along with the peels. Since only around 40% of citrus fruit can be turned into juice. Waste products from oranges, such as peels, accumulate annually in enormous quantities [1]. Increased focus is continually being placed on bringing valuable goods from waste resources, and citrus wastes are no exception. The orange peel and pulp may be turned into useful items if the right processes are use [2, 3]. Pollution in the environment is also something that may be mitigated. Orange and lemon peels are packed with healthy elements and phytochemicals, making them useful as both dietary supplements and pharmaceuticals. More and more bacteria are becoming resistant to antibiotics, thus scientists are always looking for safe alternatives [4-7]. Vitamin C, found abundantly in orange juices, is often regarded as the most important water-soluble antioxidant. The primary function of vitamin C is to prevent scurvy, a condition characterized by the appearance of skin patches, gum swelling, and mucous membrane hemorrhage. Because of its instability, vitamin C may be broken down by either aerobic or anaerobic processes. Some goods, including citrus juice concentrates, may be particularly sensitive to vitamin C loss [8, 9]. The early phytochemical analysis of citrus fruit peels

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and pulp confirmed the presence of several active compounds. The tabular presentation of the findings follows. One group of phytochemicals was revealed by one solvent, whereas another was shown by another. Tannins, saponins, etc., were detected; however, anthraquinones were nowhere to be found in the citrus peel or pulp. It is difficult to attribute the antibacterial activity to a particular phytochemical, although these components may be to blame. Citrus sinensis, sometimes called sweet orange to distinguish it from the related species. When referring to citrus fruits, the term "orange" most often refers to Citrus aurantium or bitter orange. The sweet orange may reproduce asexually (through apomixis via nucellar embryogenesis), and its variants emerge by the process of natural selection [10-15]. The orange is the result of a hybridization between the pomelo (Citrus maxima) and the mandarin (Citrus reticulata) [16, 17]. Since it is the chloroplast genome in the pomelo that represents the maternal line, it is fitting that this fruit be the maternal ancestor. The genome of the sweet orange has been sequenced in its entirety [18]. The sweet orange tree is the most frequently planted fruit tree in the world, with its origins in current-day Southen China, Northeast India, and Myanmar [19-22]. The juicy fruit of the orange tree is commonly cultivated in tropical and subtropical regions. Oranges are versatile trees that may be used for their fresh fruit, juice, and aromatic peel [23]. In 2012, sweet oranges made up around 70% of the world's citrus harvest [24-27]. Numerous useful phytochemicals were found in orange peels, the most numerous of which were terpenoids, tannins, saponins, flavonoids, and phenols. There is evidence that the phytochemicals found in orange peels have anticancer, anti-diabetic, antihypertensive, and proliferative properties, and these benefits have prompted much research [28-31]. the study Aim To examine the terpenoids, flavonoids, saponins, tannins, and phenols present in C. Sinensis by phytochemical screening and assessing the antibacterial effects of Iraqi orange peel in Petri Dish.

Materials and Methods

Plant material

Orange Peel leaves were collected from Tikrit Salah Aldin in November 2021. Peel from oranges was cleaned, dried in the shade, and then processed into a powder in a mechanical grinder.

Extraction method (cold method)

The 250 g of plant powder was steeped in 1500 ml of methanol at room temperature with intermittent shaking. When the three days were over, the methanol-soluble components were separated through filtration. A rotary evaporator was used to remove all of the moisture from the filtrate while operating at very low pressure. It produced a residue that looked dark green. All that was left after the liquid evaporated was taken to the lab and tested for antibacterial activity.

Preliminary phytochemical examination of crude extracts

Standard protocols for phytochemical analysis were applied to crude extracts, fractions, and powder specimens from the medicinal plants under investigation with the purpose of screening and identifying bioactive chemical ingredients.

Saponins test

Each plant extract and fraction was dissolved in 0.5 grams of boiling water in a test tube, allowed to cool, and then agitated ferociously to produce froth.

Using a boiling water bath, 2.0 g of the plant powder was cooked in a test tube containing distilled water before being filtered. By rapidly shaking together 10 ml of the filtrate and 5 ml Of distilled water, A stable long-lasting froth was produced. Because emulsion formation is a hallmark of saponins, the foam was combined with three drops of olive oil and aggressively agitated to ensure proper incorporation.

Tannins test

Filtered distilled water was used to dissolve 0.25 g of plant extract and fraction in 10 ml. The filtered liquid was mixed with a 1% ferric chloride (FeCl3) solution in water. When testing samples, a strong green, blue, or black hue indicates the presence of tannins.

Terpenoids test

The (Salkowski) Initially, 5 ml of each plant extract and fraction were combined in 2 ml of chloroform, and then 3 ml of concentrated (H2SO4). The presence of terpenoids was confirmed by the formation of a layer of the characteristic reddish-brown hue at the contact.

Flavonoids test

To remove the oils, 0.5 g of each plant extract and fraction was shaken in petroleum ether (to extract the lipid layer). After filtering, 20 ml of 80% ethanol was added to the defatted residue. Experiments were conducted using the filtrate:

(a) The hue was seen after combining 3 ml of the filtrate with 4 ml of aluminum chloride in methanol at a concentration of 1%. Flavonoids were present because of their ability to have a yellowing effect.

(b) In a test tube, 3 ml of the filtrate was combined with 4 ml of 1% potassium hydroxide, and the resulting color was examined. Flavonoids were present when the liquid became a deep yellow.

Activity of antibacterial

Extracts of orange peel were tested for their antibacterial effects on both Gram-negative and Gram-positive bacteria in an agar well diffusion experiment [32-35]. Aseptically, around 20 millilitres of Muller-Hinton (MH) agar was put onto sterile Petri plates. Using a sterile wire loop, we were able to extract the

bacteria from their respective stock cultures [36]. After the organisms were cultured, wells were drilled with a sterile point into the agar plates using a sterile pipette tip at a diameter of 6 mm. The extracted Indigofera suffruticosa was injected at varying doses into the drilled wells. The extract and test organisms were placed on culture plates, and after incubation at 37°C for day, the average diameter of the zones of inhibition was measured and recorded [37, 38].

Statistical analysis

Data analysis was done statistically using GraphPad Prism [39]. The results of three independent tests are shown as the mean and standard deviation, indicating a statistically significant differences p 0.05 [40-43].

Results and Discussion

Extraction methods

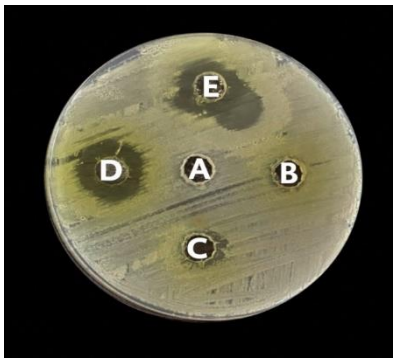
Separating the final products from the basic components begins with extraction. To successfully isolate a material, the method of extraction must be tailored to that substance. Two separate extraction procedures were performed, each at a different temperature, to get a sample of the plants contents, the optimal solvent for extraction was chosen after taking into account the percentage yield from each method and the TLC examination of the crude extract for its constituents. According to the rule of similarity and impermissibility, when selecting a solvent for solvent extraction, selectivity, solubility, cost, and safety should all be taken into consideration (like dissolves). In general, the performance of a solvent improves when its polarity value is closer to that of the solute, and vice versa.

Extraction method (cold methods)

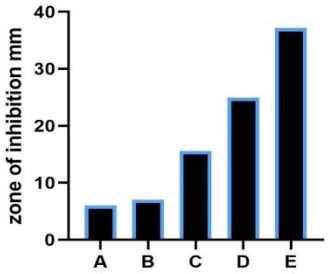
This is a relatively basic extraction technique; however, it has the drawbacks of being time-consuming and inefficient. Thermolabile substances might be extracted using this method. aprocess that takes two days and includes macerating leaves. In the event of pharmacological action, this approach was opted for.

Antibacterial activity

Extracts and physiologically active chemicals derived from common plant species have received considerable study and media interest in recent years. Medicinal plants have an important role in meeting the fundamental health requirements of people in underdeveloped nations, and they may provide a new supply of medicines with substantial action against bacterial, fungal, and viral infections. The findings of our maceration-derived methanolic extract of orange peel show that it has potent antibacterial activity against both Gram-positive and Gram-negative bacteria. Both Gram-negative bacterium E. coli **(Figure 1)** and Gram-positive bacterium S. aureus **(Figure 2)** are susceptible to the antibacterial effects of orange peel methanolic extracts. The findings of this research provide credence to the traditional usage of this plant in the treatment of infectious disorders, particularly those caused by bacteria. To further understand the potential antibacterial and antifungal actions, the extracts are now undergoing purification of the bioactive component(s).

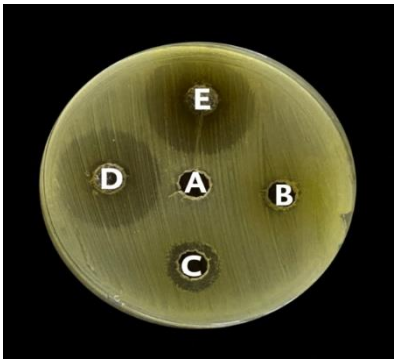


a)



b)

Figure 1. Antibacterial activity of *C. Sinensis* against *E. coli.* (A) Control, (B) 62.5 microgram/ml, (C) 125 microgram/ml, (D) 250 microgram/ml, (E) 500 microgram/ml.



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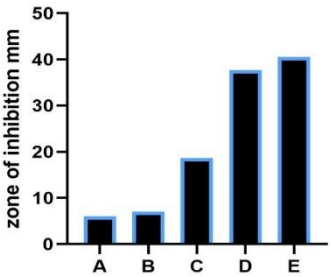

b)

Figure 2. Antibacterial activity of *C. Sinensis* against *S. aureus.* (A) Control, (B) 62.5 microgram/ml, (C) 125 microgram/ml, (D) 250 microgram/ml, (E) 500 microgram/ml.

Conclusion

The results show that methanolic extracts of Iraqi *Citrus sinensis* peels possess significant antibacterial activity against both Gram-positive and Gram-negative pathogens. Phytochemical screening confirmed the presence of diverse bioactive compounds—including terpenoids, flavonoids, tannins, saponins, and phenols—which likely act synergistically to inhibit bacterial growth. The clear zones of inhibition observed against *Staphylococcus aureus* and *Escherichia coli* validate the traditional use of orange peel in managing infectious diseases and highlight its potential as a natural source of antimicrobial agents. Further purification and characterization of the active constituents are recommended to better understand their mechanisms of action and to support the development of novel therapeutic alternatives derived from citrus waste.

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Conflict of interest: None

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Ethics statement: None

References

1. Manthey A, Grohmann K. Phenols in citrus peel byproducts: concentrations of hydroxycinnamates and polyethoxylated flavones in citrus peel molasses. J Agric Food Chem. 2001;49(7):3268-73.
2. Nand K. Recent advances in the treatment of liquid and solid wastes of food processing industries for biogas production and pollution abatement. Proc 4th Int Food Conv, Mysore. 1998;35.
3. Bolay Ş, Öztürk E, Tuncel B, Ertan A. Studying fracture strength of root-treated and reconstructed teeth with two types of post and core. Ann J Dent Med Assist. 2024;4(2):1-6. doi:10.51847/i57dzmzc2A
4. Wilson CL, Droby GG. Microbial food contamination. Boca Raton, FL: CRC Press; 2000. p. 1–304.
5. Friedman M, Henika RP, Mandrell ER. Bactericidal activities of plant essential oils and some of their isolated constituents against Campylobacter jejuni, Escherichia coli, Listeria monocytogenes, and Salmonella enterica. J Food Prot. 2002;65(10):1545–60.
6. Ashok K, Narayani M, Subanthini A, Jayakumar M. Antimicrobial activity and phytochemical analysis of citrus fruit peels - utilization of fruit waste. Int J Eng Sci Technol. 2011;3(6):5414–21.
7. Bulusu A, Cleary SD. Comparison of dental caries in autistic children with healthy children. Ann J Dent Med Assist. 2023;3(2):14–9. doi:10.51847/wa2pZXE4RJ
8. Micucci P, Alonso MR, Turner S, Davicino R, Anesini C. Antioxidant and antimicrobial activities of Larrea divaricata Cav. aqueous extract on vitamin C from natural orange juice. Food Nutr Sci. 2011;2(1):35–46.
9. Malcangi G, Patano A, Trilli I, Piras F, Ciocia AM, Inchingolo AD, et al. A systematic review of the role of soft tissue lasers in enhancing esthetic dental procedures. Int J Dent Res Allied Sci. 2023;3(2):1–8. doi:10.51847/DWXltUS9Lp
10. Xu Q, Chen LL, Ruan X, Chen D, Zhu A, Chen C, et al. The draft genome of sweet orange (Citrus sinensis). Nat Genet. 2013;45(1):59–66.
11. Orange fruit information. 2017 Jun 9 [cited 2018 Sep 20].
12. Orange fruit nutrition facts and health benefits. [cited 2018 Sep 20].
13. Oranges: health benefits, risks & nutrition facts. Live Science. [cited 2018 Sep 20].
14. AlHussain BS, AlFayez AA, AlDuhaymi AA, AlMulhim EA, Assiri MY, Ansari SH. Impact of different antibacterial substances in dental composite materials: a comprehensive review. Int J Dent Res Allied Sci. 2022;2(1):1–7. doi:10.51847/jg2xu2PbJK
15. Maneea ASB, Alqahtani AD, Alhazzaa AK, Albalawi AO, Alotaibi AK, Alanazi TF. Systematic review of the microbiological impact of sodium hypochlorite concentrations in endodontic treatment. Int J Dent Res Allied Sci. 2024;4(2):9–15. doi:10.51847/PH80PpWOX7
16. García Lor A. Organización de la diversidad genética de los cítricos [Thesis]. 2013. p.79.
17. Shaheen RS, Alsaffan AD, Al-Dusari RS, Helmi RN, Baseer MA. Self-reported oral hygiene and gum health among dental and medical students, dentists, and physicians in Saudi Arabia. Turk J Public Health Dent. 2023;3(1):9–16. doi:10.51847/SZCGti8lFn
18. Velasco R, Licciardello C. A genealogy of the citrus family. Nat Biotechnol. 2014;32(7):640–2.
19. Morton JF. Fruits of warm climates. 1987;134–42.
20. Talon M, Caruso M, Gmitter FG Jr. The genus Citrus. Woodhead Publishing; 2020. p.17. ISBN: 9780128122174.
21. Morton J. Orange, Citrus sinensis. In: Fruits of warm climates. NewCROP, Purdue University; 1987. p.134–42.
22. Fernandes AL, Malik JB, Ansari SR, Murali S, Thirupathii J. Saudi dentists' knowledge and approaches to managing tooth wear: a cross-sectional survey-based analysis. Turk J Public Health Dent. 2022;2(2):1–12. doi:10.51847/p7ulFD4XZm
23. Citrussinensis. Germplasm Resources Information Network (GRIN), USDA. [cited 2017 Dec 10].
24. Selli S, Canbas A, Varlet V, Kelebek H, Prost C, Serot T. Characterization of the most odor-active volatiles of orange wine made from a Turkish cv. Kozan (Citrus sinensis L. Osbeck). J Agric Food Chem. 2008;56(1):227–34.

25. UN Food and Agriculture Organization, Corporate Statistical Database (FAOSTAT). 2020 [cited 2021 Mar 21].
26. Ravoori S, Sekhar PR, Pachava S, Pavani NPM, Shaik PS, Ramanarayana B. Perceived stress and depression among oral cancer patients: a hospital-based cross-sectional study. Turk J Public Health Dent. 2024;4(1):1–5. doi:10.51847/FoK9xAl1JW
27. Graefen B, Hasanli S, Fazal N. Behind the white coat: the prevalence of burnout among obstetrics and gynecology residents in Azerbaijan. Bull Pioneer Res Med Clin Sci. 2023;2(2):1–7. doi:10.51847/vIIhM1UG2l
28. Hanelt P. Encyclopedia of agricultural and horticultural crops. 1st English ed. Springer; 2001.
29. Abbate L, Tusa N, del Bosco SF, Strano T, Renda A, Ruberto G. Genetic improvement of citrus fruits: new somatic hybrids from Citrus sinensis (L.) Osb. and Citrus limon (L.) Burm F. Food Res Int. 2012;48(1):284–90.
30. Dhanasekar P, Rajayyan JS, Veerabadiran Y, Kumar KS, Kumar KS, Chinnadurai N. Evaluation of alum and purification process of water by coagulation method. Bull Pioneer Res Med Clin Sci. 2022;1(2):1–6. doi:10.51847/R8GyfOmMDh
31. Makhoahle P, Gaseitsiwe T. Efficacy of disinfectants on common laboratory surface microorganisms at R.S Mangaliso Hospital, NHLS Laboratory, South Africa. Bull Pioneer Res Med Clin Sci. 2022;1(1):1–12. doi:10.51847/d5bXpXAtcI
32. Ramful D, Tarnus E, Rondeau P, Robert-Da Silva C, Bahorun T, Bourdon E. Citrus fruit extracts reduce advanced glycation end products (AGEs)- and H_2O_2-induced oxidative stress in human adipocytes. J Agric Food Chem. 2010;58(20):11119–29.
33. Bahjat HH, Ismail RA, Sulaiman GM, Jabir MS. Magnetic field-assisted laser ablation of titanium dioxide nanoparticles in water for antibacterial applications. J Inorg Organomet Polym Mater. 2021;31(9):3649-56.
34. Kwatra D, Venugopal A, Anant S. Studying the efficacy of tolmetin radiosensitizing effect in radiotherapy treatment on human clonal cancer cells. Bull Pioneer Res Med Clin Sci. 2024;3(2):22–8. doi:10.51847/Uuhjk0fMC8
35. Shen F, Bao L. Studying the effects of music on the time to gain independent oral feeding in premature infants. J Integr Nurs Palliat Care. 2025;6:1–6. doi:10.51847/xBTC4CiH10
36. Khashan KS, Abdulameer FA, Jabir MS, Hadi AA, Sulaiman GM. Anticancer activity and toxicity of carbon nanoparticles produced by pulsed laser ablation of graphite in water. Adv Nat Sci Nanosci Nanotechnol. 2020;11(3):035010.
37. Khashan KS, Badr BA, Sulaiman GM, Jabir MS, Hussain SA. Antibacterial activity of zinc oxide nanostructured materials synthesis by laser ablation method. J Phys Conf Ser. 2021;1795(1):012040.
38. Jihad MA, Noori F, Jabir MS, Albukhaty S, AlMalki FA, Alyamani AA. Polyethylene glycol functionalized graphene oxide nanoparticles loaded with Nigella sativa extract: a smart antibacterial therapeutic drug delivery system. Molecules. 2021;26(11):3067.
39. Mohammed MK, Mohammad MR, Jabir MS, Ahmed DS. Functionalization, characterization, and antibacterial activity of single-wall and multi-wall carbon nanotubes. IOP Conf Ser Mater Sci Eng. 2020;757(1):012028.
40. Ali IH, Jabir MS, Al-Shmgani HS, Sulaiman GM, Sadoon AH. Pathological and immunological study on infection with Escherichia coli in albino BALB/c mice. J Phys Conf Ser. 2018;1003(1):012009.
41. Younus A, Al-Ahmer S, Jabir M. Evaluation of some immunological markers in children with bacterial meningitis caused by Streptococcus pneumonia. Res J Biotechnol. 2019;14(1):131–3.
42. Dipalma G, Inchingolo AD, Fiore A, Balestriere L, Nardelli P, Casamassima L, et al. Comparative effects of fixed and clear aligner therapy on oral microbiome dynamics. Asian J Periodontics Orthod. 2022;2:33–41. doi:10.51847/mK28wdKCIX
43. Patil RD. Structural insights into the alimentary canal of Deudorix isocrates (Fab.) larvae (Lepidoptera: Lycaenidae). Entomol Lett. 2022;2(1):28–36. doi:10.51847/PoTmk4aq6W