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Original Article

Phytochemical Profiling and Antibacterial Activity of Orange Peel Extract

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ABSTRACT

Background: Orange peel (Citrus sinensis), an abundant agro-industrial by-product, is a rich source of phytochemicals with potential antibacterial properties. Increasing antimicrobial resistance has stimulated interest in plant-derived antimicrobial agents as sustainable alternatives to conventional antibiotics. Aim of the study: To characterize the phytochemical profile of aqueous and methanolic orange peel extracts and evaluate their in vitro antibacterial activity against selected pathogenic bacteria. **Methods & Materials**: This experimental study was conducted in the Department of Pharmacology in collaboration with the Department of Microbiology, Mymensingh Medical College, Bangladesh, from January to December 2025. Fresh orange peels were extracted separately using distilled water and methanol by maceration. Qualitative and quantitative phytochemical analyses were performed to determine the presence of major bioactive compounds and estimate total phenolic, flavonoid, and tannin contents. Antibacterial activity against Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa was assessed using the Kirby–Bauer disc diffusion method. Minimum inhibitory concentration (MIC) was determined by broth dilution method. **Result**: The methanolic orange peel extract (MOPE) produced a higher extraction yield than the aqueous extract (14.36% vs. 9.7%) and contained higher concentrations of total phenolics (89.6 ± 3.2 vs. 52.8 ± 2.4 mg GAE/g), flavonoids (68.4 ± 2.7 vs. 31.5 ± 1.8 mg QE/g), and tannins (33.7 ± 2.1 vs. 18.2 ± 1.3 mg TAE/g). MOPE exhibited stronger concentration-dependent antibacterial activity, producing larger inhibition zones and lower MIC value against S. aureus and E. coli than the aqueous extract. Both extracts demonstrated comparatively weaker activity against P. aeruginosa. **Conclusion**: Methanolic orange peel extract possesses superior phytochemical content and antibacterial activity compared with the aqueous extract, suggesting that orange peel is a promising natural source of antimicrobial compounds. This study highlights the potential of citrus peel waste for developing value-added antimicrobial products and supports further investigation into the isolation and characterization of its active compounds.

Keywords: *Citrus sinensis, orange peel, phytochemicals, antibacterial activity, methanolic extract, phenolic compounds*

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INTRODUCTION

Orange peel (*Citrus sinensis*), a major by-product of the citrus industry, is a rich source of bioactive phytochemicals, including flavonoids, phenolic compounds, tannins, alkaloids, and essential oils, which possess diverse biological activities, particularly antimicrobial properties [1]. Phytochemical profiling and antibacterial activity of orange peel extract involve the identification of these phytochemical constituents and the evaluation of their inhibitory effects against pathogenic bacteria. Increasing antimicrobial resistance has stimulated growing interest in plant-derived antibacterial agents, highlighting orange peel as a promising, sustainable, and cost-effective natural resource for pharmaceutical and biomedical applications [2]. Globally, approximately 13-29% of the total weight of processed oranges is converted into peel waste, generating an estimated 55 million tons of orange peel annually, much of which remains underutilized despite its rich phytochemical composition [3]. In Bangladesh, citrus fruits-including oranges, mandarins, lemons, and indigenous citrus species-are widely cultivated and consumed; however, no national estimate of orange peel waste generation has been reported. Nevertheless, substantial quantities of citrus peel are produced as agro-industrial and household by-products, representing a largely untapped source of bioactive compounds with potential antibacterial applications [4]. Orange peel contains abundant secondary metabolites, particularly flavonoids such as hesperidin and naringin, phenolic compounds, limonoids, carotenoids, and volatile constituents dominated by D-limonene [5]. These compounds are commonly extracted using aqueous or organic solvents, including ethanol and methanol, followed by phytochemical characterization through qualitative or quantitative analytical techniques. Antibacterial efficacy is subsequently assessed against selected Gram-positive and Gram-negative bacterial strains using standardized microbiological methods such as agar diffusion assays and determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values [6]. The antimicrobial action of these phytochemicals involves disruption of bacterial cell membrane integrity, alteration of membrane permeability, inhibition of essential metabolic enzymes, induction of oxidative stress, and suppression of nucleic acid and protein synthesis, collectively leading to bacterial growth inhibition or cell death [7]. The antibacterial potential of orange peel extract has important implications for human health, food preservation, and pharmaceutical development. Natural antimicrobial agents may reduce dependence on synthetic antibiotics, help address antimicrobial resistance, and promote sustainable utilization of agricultural waste [8]. Furthermore, converting citrus peel waste into valuable bioactive products supports environmental sustainability and contributes to the circular bioeconomy by transforming low-value agro-industrial residues into high-value bioactive products [9]. Despite these advantages, variations in citrus varieties, extraction solvents, extraction conditions, and analytical methods often yield inconsistent phytochemical profiles and antibacterial results, making comparisons among studies difficult [10]. In addition, other studies focus only on preliminary phytochemical screening or antibacterial evaluation without establishing a clear

relationship between phytochemical composition and antimicrobial efficacy [11]. Therefore, comprehensive phytochemical profiling, combined with antibacterial assessment, is essential for identifying the bioactive compounds responsible for antimicrobial activity and providing scientific evidence for future therapeutic applications. The study aimed to characterize the phytochemical profile of orange peel extract and evaluate its in vitro antibacterial activity against selected pathogenic bacterial isolates.

METHODS & MATERIALS

This experimental study was conducted in the Department of Pharmacology in collaboration with the Department of Microbiology, Mymensingh Medical College, Bangladesh, from January to December 2025.

Plant material and preparation of extracts

Fresh oranges (*Citrus sinensis*) were collected from a local market in Mymensingh, Bangladesh. The peels were washed thoroughly with tap water, cut into small pieces, shade-dried at room temperature for two weeks, and ground into a fine powder. 500 grams of peel powder were separately extracted with distilled water and methanol (1:10, w/v) by maceration for 72 hours at room temperature with intermittent shaking. The extracts were filtered through Whatman No. 1 filter paper, and the filtrates were concentrated by evaporation at 50°C to obtain the dried aqueous orange peel extract (AOPE) and methanolic orange peel extract (MOPE). The dried extracts were weighed to determine percentage yield, dissolved in 10% dimethyl sulfoxide (DMSO) to prepare a stock solution of 1 g/mL, and stored at 4°C until further phytochemical and antibacterial analyses.

Preliminary phytochemical screening

Qualitative phytochemical screening of the AOPE and MOPE was carried out using standard phytochemical methods to detect the presence of alkaloids, flavonoids, phenolic compounds, tannins, saponins, glycosides, terpenoids, steroids, carbohydrates, and proteins.

Quantitative phytochemical analysis

Total phenolic content (TPC), total flavonoid content (TFC), and total tannin content (TTC) were determined using standard spectrophotometric methods. Total phenolic content was expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g extract), total flavonoid content as milligrams of quercetin equivalent per gram of extract (mg QE/g extract), and total tannin content as milligrams of tannic acid equivalent per gram of extract (mg TAE/g extract). All analyses were performed in triplicate.

Test microorganisms

Standard reference strains of *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853 were obtained from the Department of Microbiology, Mymensingh Medical College. Bacterial inocula were prepared from fresh cultures and adjusted to 0.5 McFarland turbidity standard (approximately 1.5×10^8 CFU/mL).

Antibacterial activity

Antibacterial activity of AOPE and MOPE was evaluated using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar. Sterile filter paper discs (6 mm) were impregnated with 10 µL of extract at concentrations of 100, 200, 400, 600, 800, and 1000 mg/mL and placed on agar plates inoculated with the test organisms. Plates were incubated at 37°C for 24 hours, after which the diameters of inhibition zones were measured in millimetres. Discs containing 10% DMSO served as the negative control.

Determination of minimum inhibitory concentration (MIC)

The MIC of AOPE, MOPE, and ciprofloxacin was determined using the broth dilution method. Serial concentrations of the extracts were prepared in nutrient broth and inoculated with standardized bacterial suspensions. Following incubation at 37°C for 24 hours, MIC was defined as the lowest concentration that completely inhibited visible bacterial growth.

RESULT

Methanolic extraction demonstrated greater extraction efficiency than aqueous extraction. Using 500 g of dried orange peel, the methanolic extract yielded 71.8 g, corresponding to a 14.36% extraction yield, whereas the aqueous extract produced 48.5 g, equivalent to a 9.7% yield (*Table I*).

Table I
Percentage yield of orange peel extracts.

Extract	Weight of dried peel (g)	Weight of extract (g)	Percentage yield (%)
Aqueous extract	500	48.5	9.7
Methanolic extract	500	71.8	14.36

The methanolic extract showed strong presence of flavonoids, phenolic compounds, and terpenoids, with moderate levels of alkaloids, tannins, and glycosides. Steroids were detected only in the methanolic extract. In contrast, the aqueous extract showed moderate levels of flavonoids, phenolic compounds, saponins, and carbohydrates, while alkaloids, tannins, glycosides, and terpenoids were present. Proteins were absent in both extracts (*Table II*).

Table II
Preliminary phytochemical screening of orange peel extracts.

Phytochemical constituent	Aqueous extract	Methanolic extract
Alkaloids	Present	Moderately present
Flavonoids	Moderately present	Strongly present
Phenolic compounds	Moderately present	Strongly present
Tannins	Present	Moderately present
Saponins	Moderately present	Present
Glycosides	Present	Moderately present
Terpenoids	Present	Strongly present
Steroids	Absent	Present
Carbohydrates	Moderately present	Present
Proteins	Absent	Absent

Total phenolic content was 89.6±3.2 mg GAE/g extract in the methanolic extract compared with 52.8±2.4 mg GAE/g extract in the aqueous extract. Similarly, total flavonoid content measured 68.4±2.7 mg QE/g extract in methanolic and 31.5±1.8 mg QE/g extract in aqueous, while total tannin content reached 33.7±2.1 and 18.2±1.3 mg TAE/g extract, respectively (*Table III*).

Table III
Quantitative phytochemical analysis of orange peel extracts.

Parameter	Aqueous extract	Methanolic extract
Total phenolic content (mg GAE/g extract)	52.8 ± 2.4	89.6 ± 3.2
Total flavonoid content (mg QE/g extract)	31.5 ± 1.8	68.4 ± 2.7
Total tannin content (mg TAE/g extract)	18.2 ± 1.3	33.7 ± 2.1

The antibacterial activity demonstrated concentration-dependent activity, with MOPE consistently producing larger inhibition zones than AOPE. At 1000 mg/ml, inhibition against *Staphylococcus aureus* measured 22 mm for MOPE and 21 mm for AOPE, while corresponding values against *Escherichia coli* were 18 mm and 16 mm. Against *Pseudomonas aeruginosa*, both extracts produced an inhibition zone of 18 mm. At 800 mg/ml, MOPE maintained greater inhibitory activity than AOPE, particularly against *S. aureus* (18 vs 11 mm) and *E. coli* (15 vs 12 mm). Lower concentrations showed minimal activity, with inhibition zones comparable to the control (6 mm) (*Table IV*).

Table IV
Comparative antibacterial activity of aqueous and methanolic extracts.

Concentration (mg/ml)	Zone of Inhibition (ZOI)					
	Staphylococcus aureus (mm)		Escherichia coli (mm)		Pseudomonas aeruginosa (mm)	
	AOPE	MOPE	AOPE	MOPE	AOPE	MOPE
1000	21	22	16	18	18	18
800	11	18	12	15	6	6
600	6	11	6	6	6	6
400	6	6	6	6	6	6
200	6	6	6	6	6	6
100	6	6	6	6	6	6
Control	6	6	6	6	6	6

The methanolic orange peel extract exhibited lower MIC values than the aqueous extract against *Staphylococcus aureus* (600 and 750 mg/ml) and *Escherichia coli* (700 and 800 mg/ml), indicating greater inhibitory activity. Both extracts showed the same MIC (800 mg/ml) against *Pseudomonas aeruginosa*. The reference antibiotic ciprofloxacin demonstrated substantially lower MIC values of 0.75, 1, and 0.5 µg/ml against *S. aureus*, *E. coli*, and *P. aeruginosa*, respectively (*Table V*).

Table V
Minimum inhibitory concentration (MIC).

Test organism	AOPE (mg/ml)	MOPE (mg/ml)	Ciprofloxacin (µg/ml)
S. aureus	750	600	0.75
E. coli	800	700	1
P. aeruginosa	800	800	0.5

DISCUSSION

The increasing demand for natural antimicrobial agents has renewed interest in citrus peel, owing to its abundance of biologically active phytochemicals with diverse pharmacological properties [9]. The higher extraction yield obtained with methanol (14.36%) than with water (9.7%) indicates that methanol is a more efficient solvent for extracting bioactive constituents from orange peel because of its greater ability to dissolve phenolic compounds and flavonoids. Similar observations were reported by Saleem and Saeed (2020), who found that methanolic citrus peel extracts produced significantly higher extraction yields than aqueous extracts owing to improved recovery of polyphenolic compounds [12]. Likewise, organic or binary solvent systems enhance the extraction of phytochemicals from *Citrus sinensis* peel by improving penetration of the pectin-rich flavedo layer. However, extraction efficiency varies with solvent polarity, extraction conditions, and the target phytochemicals [13]. Conversely, a study by Ashraf et al observed a higher raw yield in aqueous extractions under prolonged hydrothermal conditions [14]. Preliminary phytochemical screening showed that both extracts contained alkaloids, flavonoids, phenolics, tannins, saponins, glycosides, terpenoids, and carbohydrates, while proteins were absent. However, flavonoids, phenolics, and terpenoids were more abundant in the methanolic extract, and steroids were detected only in MOPE. These findings are consistent with those of Mahato et al., who reported that citrus peels are naturally rich in flavonoids, phenolic acids, and terpenoids, while methanol and other organic solvents generally provide higher extraction efficiency than water [15]. Similarly, Ghasemi et al. observed that orange peel contains abundant phenolic and flavonoid compounds responsible for its antioxidant and antimicrobial properties [16]. Another research validated that while water successfully dissolves polar elements like proteins and certain carbohydrates, it fails to recover structural alkaloids and free-state terpenoids efficiently, leaving them bound to the discarded peel cake [17]. Quantitative phytochemical analysis further confirmed that MOPE contained substantially higher levels of total phenolic, flavonoid, and tannin content than AOPE. Similar findings have been reported by Bocco et al., who demonstrated that methanolic citrus peel extracts possess significantly greater total phenolic concentrations than aqueous extracts [1]. Furthermore, Eddour et al reported comparable ranges of total

phenolics (202.59±15.93 mg GAE/100 g DM), total flavonoids (430.70±2.78 mg QE/100 g) when examining dehydrated citrus by-products [18]. The antibacterial assay revealed concentration-dependent inhibitory activity against all tested organisms, with the largest zones of inhibition observed at 1000 mg/mL. MOPE showed slightly greater activity than AOPE against *Staphylococcus aureus* and *Escherichia coli*, whereas both extracts exhibited similar inhibition against *Pseudomonas aeruginosa*. A similar study reported that citrus peel extracts exhibit greater antibacterial activity against Gram-positive bacteria than Gram-negative bacteria, largely because the lipopolysaccharide-rich outer membrane of Gram-negative bacteria restricts the penetration of phytochemicals [9]. This is identical to findings by Raheem et al, where *S. aureus* strains from food matrices showed immediate susceptibility to citrus flavedo extracts, while *Pseudomonas* strains remained highly resistant until exposed to extreme extract volumes [19]. The MIC results demonstrated the stronger antibacterial efficacy of MOPE, which required lower concentrations to inhibit *S. aureus* and *E. coli* than AOPE. *P. aeruginosa* presented the highest resistance, requiring a high concentration () from both extract types. These are consistent with previous reports indicating that crude *Citrus sinensis* extracts generally require substantially higher concentrations than purified antibiotics because their bioactive compounds are present as complex mixtures at relatively low individual concentrations [20]. Compared with the reference antibiotic ciprofloxacin, which typically exhibits antibacterial activity at MICs of ≤0.5-1.0 µg/mL against many susceptible bacteria, the crude extracts required substantially higher concentrations (mg/mL) to achieve comparable inhibitory effects, indicating lower intrinsic potency [21].

LIMITATIONS

- The investigation was limited to crude aqueous and methanolic extracts without isolation or characterization of individual bioactive compounds responsible for antibacterial activity.
- The study evaluated orange peel collected from a single geographical source and one harvesting period, which may not reflect variations in phytochemical composition associated with cultivar, season, or environmental conditions.
- Antioxidant activity and advanced phytochemical characterization using chromatographic techniques such as HPLC or GC–MS were not performed, limiting comprehensive identification of the active constituents.

CONCLUSION

Orange peel (*Citrus sinensis*) is a valuable natural source of bioactive phytochemicals with promising antibacterial properties. The methanolic extract demonstrated higher extraction efficiency, greater concentrations of phenolics, flavonoids, and tannins, and superior antibacterial activity than the aqueous extract. Both extracts exhibited concentration-dependent inhibitory effects against the tested bacterial pathogens, with the strongest activity against *Staphylococcus aureus* and comparatively weaker activity against *Pseudomonas aeruginosa*. The higher phytochemical content, particularly phenolics, flavonoids, and tannins, observed in the methanolic extract may contribute to its greater antibacterial activity. These findings support the potential utilization of citrus peel waste as an economical and sustainable source of natural antimicrobial agents and provide a foundation for future studies involving isolation of active constituents, elucidation of their mechanisms of action, safety assessment, and in vivo evaluation.

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CONFLICT OF INTEREST

None declared

ETHICAL APPROVAL

The study was approved by the Institutional Ethics Committee.

REFERENCES

1. Bocco A, Cuvelier ME, Richard H, Berset C. Antioxidant activity and phenolic composition of citrus peel and seed extracts. Journal of agricultural and food chemistry. 1998 Jun 15;46(6):2123-9.
2. Li S, Lo CY, Ho CT. Hydroxylated polymethoxyflavones and methylated flavonoids in sweet orange (Citrus sinensis) peel. Journal of agricultural and food chemistry. 2006 Jun 14;54(12):4176-85.
3. Bratovcic A. Orange Peel Waste Valorization from Bioactive Compound Recovery to Functional and Industrial Applications. Journal of Biomaterials and Nanobiotechnology. 2026;17(2):33-63.
4. Ladaniya MS. Postharvest management of citrus fruit in south Asian countries. Acta Hortic. 2015 Apr 15;1065:1669-76.
5. Mandalari G, Bennett RN, Bisignano G, Saija A, Dugo G, Lo Curto RB, Faulds CB, Waldron KW. Characterization of flavonoids and pectins from bergamot (Citrus bergamia Risso) peel, a major byproduct of essential oil extraction. Journal of agricultural and food chemistry. 2006 Jan 11;54(1):197-203.
6. Sharma K, Mahato N, Cho MH, Lee YR. Converting citrus wastes into value-added products: Economic and environmently friendly approaches. Nutrition. 2017 Feb 1;34:29-46.
7. Suja D, Bupesh G, Nivya R, Mohan V, Ramasamy P, Muthiah NS, Arul AE, Meenakumari K, Prabu K. Phytochemical screening, antioxidant, antibacterial activities of citrus limon and citrus sinensis peel extracts. International Journal of Pharmacognosy and Chinese Medicin. 2017;1(2):1-7.
8. Dassoff ES, Guo JX, Liu Y, Wang SC, Li YO. Potential development of non-synthetic food additives from orange processing by-products—a review. Food Quality and Safety. 2021 Jan 1;5:fyaa035.
9. Alsakhawy SA, Baghdadi HH, El-Shenawy MA, El-Hosseiny LS. Comparative phytochemical composition and antimicrobial activity of citrus peel essential oils and phenolic compounds. Anti-Infective Agents. 2023 Aug 1;21(4):57-68.
10. Saleem M, Durani AI, Asari A, Ahmed M, Ahmad M, Yousaf N, Muddassar M. Investigation of antioxidant and antibacterial effects of citrus fruits peels extracts using different extracting agents: Phytochemical analysis with in silico studies. Heliyon. 2023 Apr 1;9(4).
11. Zahr S, Zahr R, El Hajj R, Khalil M. Phytochemistry and biological activities of Citrus sinensis and Citrus limon: An update. Journal of Herbal Medicine. 2023 Sep 1;41:100737.
12. Saleem M, Saeed MT. Potential application of waste fruit peels (orange, yellow lemon and banana) as wide range natural antimicrobial agent. Journal of King Saud University-Science. 2020 Jan 1;32(1):805-10.
13. Liu Y, Shi J, Langrish TA. Water-based extraction of pectin from flavedo and albedo of orange peels. Chemical engineering journal. 2006 Jul 15;120(3):203-9.
14. Ashraf H, Iahtisham-Ul-Haq, Butt MS, Nayik GA, Ramniwas S, Damto T, Ali Alharbi S, Ansari MJ. Phytochemical and antioxidant profile of citrus peel extracts in relation to different extraction parameters. International Journal of Food Properties. 2024 Dec 31;27(1):286-99.
15. Mahato N, Sinha M, Sharma K, Koteswararao R, Cho MH. Modern extraction and purification techniques for obtaining high purity food-grade bioactive compounds and value-added co-products from citrus wastes. Foods. 2019 Oct 23;8(11):523.
16. Ghasemi K, Ghasemi Y, Ebrahimzadeh MA. Antioxidant activity, phenol and flavonoid contents of 13 citrus species peels and tissues. Pak J Pharm Sci. 2009 Jul 1;22(3):277-81.
17. M'hiri N, Ioannou I, Ghoul M, Boudhrioua NM. Extraction methods of citrus peel phenolic compounds. Food Reviews International. 2014 Oct 2;30(4):265-90.
18. Eddour AR, Litim M, Bouderoua K. Evaluation of the Chemical Composition and Antioxidant Capacity of Dried Orange Pulp. Vet. J.;15(4):997-1006.
19. Raheem SS, Abd Ali AH, Al-Saffar MF, Ayad ZM, Hadi OM. Antibacterial activity of orange peel extract against Staphylococcus aureus in vitro. Open Veterinary Journal. 2025 Dec 31;15(12):6224-.
20. Dongre P, Doifode C, Choudhary S, Sharma N. Botanical description, chemical

composition, traditional uses and pharmacology of Citrus sinensis: An updated review. Pharmacological Research-Modern Chinese Medicine. 2023 Sep 1;8:100272.

21. Sanders CC, Sanders Jr WE, Goering RV. Overview of preclinical studies with ciprofloxacin. The American Journal of Medicine. 1987 Apr 1;82(4A):2-11.