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Phytochemical Analysis, Antioxidant Activity, and Antimicrobial Evaluation of Kinnow Fruit Peel Extract

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Abstract: This study investigates the bioactive constituents, antioxidant efficacy, and antimicrobial activities of kinnow fruit peel extract. Qualitative phytochemical analysis confirmed the presence of flavonoids, phenolics, tannins, and alkaloids. The extract's antioxidant capacity, assessed using DPPH assay, exhibited notable radical scavenging activity. Antimicrobial properties were tested against E. coli and S. aureus using agar well diffusion technique, showing significant zones of inhibition. These results indicate the potential of kinnow peel as a natural therapeutic agent and sustainable by-product for the development of nutraceuticals and natural antimicrobial products.

Keywords: Phytochemicals Analysis, Antioxidant Activity, Antimicrobial Evaluation, Kinnow Fruit Peel Extract, Citrus Bioactives, Natural Therapeutics.

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I. INTRODUCTION

Plants have long been recognized for their medicinal properties and are often considered safer than synthetic alternatives due to fewer side effects. The rising global interest in natural remedies has brought attention to citrus fruits, especially kinnow (Citrus reticulata × Citrus sinensis), known for its high vitamin C content and rich phytochemical profile. Despite its nutritional benefits, kinnow peel, often discarded as waste, contains numerous bioactive compounds. This study explores the potential of kinnow fruit peel extract for antioxidant and antimicrobial applications, aligning with global trends towards sustainable and natural therapeutics.

II. MATERIALS AND METHODS

The study employed standardized protocols for sample preparation and phytochemical screening. Kinnow fruit peels were collected, washed, shade-dried, and powdered. Solvent extraction was performed using ethanol, methanol, acetone, and distilled water. Soxhlet extraction and maceration techniques were used. Extracts were filtered and stored under refrigeration until use. Phytochemical screening involved qualitative tests for alkaloids (Mayer's and Wagner's),

flavonoids (NaOH test), phenolics (Ferric Chloride), tannins, saponins, and carbohydrates (Benedict's and Fehling's).

Antioxidant activity was determined using the DPPH assay. A 1 mM DPPH solution in methanol was prepared, and various concentrations (20–100 $\mu g/mL$) of the extract were mixed with the solution. Absorbance was measured at 515 nm after 15 minutes of incubation in the dark. IC50 values were calculated. Antimicrobial activity was assessed using the agar well diffusion method. Bacterial strains Escherichia coli and Staphylococcus aureus were tested against the extract. Wells were loaded with 100, 50, and 25 mg/mL concentrations of extract. Zones of inhibition were measured after 24-hour incubation at 37°C.

III. RESULTS

Phytochemical screening confirmed the presence of flavonoids, tannins, alkaloids, phenolics, and saponins in the kinnow peel extract, indicating its rich phytochemical profile. The antioxidant activity revealed a dose-dependent increase in radical scavenging, with IC50 value of 64.47 $\mu g/mL$ compared to 58.66 $\mu g/mL$ for standard vitamin C, demonstrating comparable efficacy.

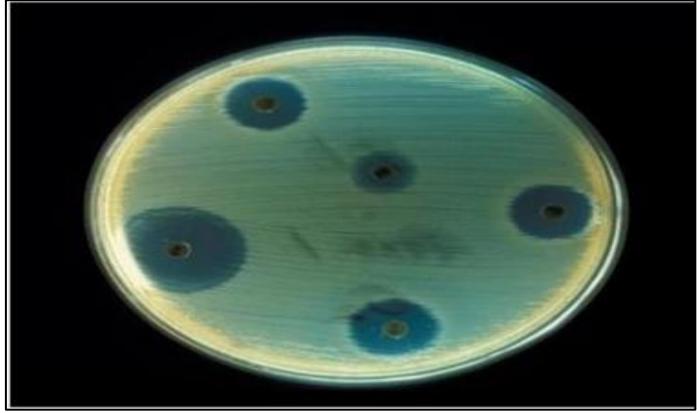


Fig 1 Zone of Inhibition – E. coli

This figure illustrates the antibacterial effect of kinnow peel extract on Escherichia coli using the agar well diffusion method. A clear zone around the well indicates microbial

growth inhibition. The highest activity was observed at $100 \,\mu\text{g/mL}$ with a zone diameter of 9 mm, demonstrating the extract's potential efficacy against Gram- negative bacteria.

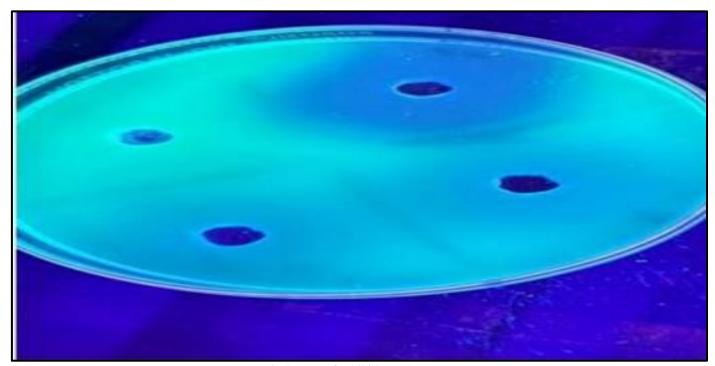


Fig 2 Zone of Inhibition – S. aureus

This figure shows the inhibition zone produced by the extract against Staphylococcus aureus. A zone diameter of 8 mm was recorded at $100~\mu g/mL$ concentration, confirming

moderate antibacterial activity against Gram-positive bacteria. The results support the broad-spectrum antimicrobial potential of the kinnow peel extract.

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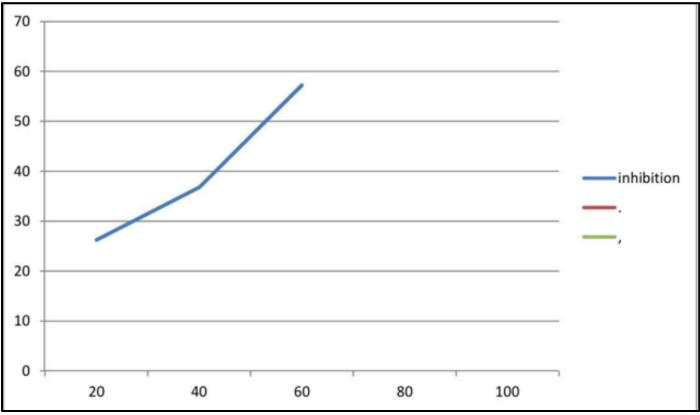


Fig 3 Free Radical Scavenging Activity (Vitamin C vs Extract)

Table 1 Comparison of Free Radical Scavenging activity between Kinnow Peel Extract and Vitamin C.

Concentration (µg/mL)	Extract % Inhibition	Vitamin C % Inhibition
20	26.25	~30
40	36.79	~42
60	57.25	~55

This figure illustrates the free radical scavenging activity of kinnow peel extract compared with standard Vitamin C (ascorbic acid), as measured by the DPPH assay. Both substances show a dose-dependent increase in antioxidant activity. The extract reached a maximum inhibition of 57.25% at 60 $\mu g/mL$, while Vitamin C achieved slightly higher inhibition (approximately 60–65%) at the same

concentration. The IC50 value of the kinnow extract (64.47 μ g/mL) was close to that of Vitamin C (58.66 μ g/mL), indicating strong antioxidant potential. These findings suggest that kinnow peel extract can serve as a natural and effective antioxidant agent, supporting its use in functional foods and herbal formulations.

Table 2 Phytochemical Screening

S. No	Phytochemical Test	Reagent Used	Result
1	Alkaloids	Mayer's reagent	Positive
2	Phenol	Ferric chloride	Positive
3	Terpenoids	Chloroform	Positive
4	Reducing Sugars	Fehling's A & B	Positive

This table presents the qualitative results of the phytochemical analysis conducted on kinnow peel extract. Standard tests confirmed the presence of key secondary metabolites such as alkaloids, phenols, terpenoids, and

reducing sugars. These compounds are known to contribute to the antioxidant and antimicrobial properties of plant-based extracts, suggesting that kinnow peel holds substantial therapeutic potential.

Table 3 IC50 values for Antioxidant activity of Kinnow Peel Extract and Standard Vitamin C

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Sample	IC50 Value (mg/mL)
Vitamin C (Standard)	58.66
Kinnow Extract	64.47

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This table compares the IC50 values of kinnow peel extract and standard vitamin C, indicating the concentration required to scavenge 50% of DPPH free radicals. The extract showed an IC50 value of $64.47 \mu g/mL$, slightly higher than

vitamin C at $58.66~\mu g/mL$. While less potent than the standard, the extract demonstrates significant antioxidant potential and could serve as a natural alternative to synthetic antioxidants.

Table 4 Antibacterial Inhibition Zones

Bacteria	Extract Conc. (µg/mL)	Zone of Inhibition (mm)
E. coli	100	9
S. aureus	100	8
E. coli	50	Moderate
S. aureus	50	Moderate

This table outlines the inhibitory effects of kinnow peel extract against two pathogenic bacteria—Escherichia coli and Staphylococcus aureus—at varying concentrations. A clear dose-dependent antibacterial response was observed, with the highest zone of inhibition recorded at 100 μ g/mL (9 mm for E. coli and 8 mm for S. aureus). These findings validate the antimicrobial potential of kinnow peel and support its future application in natural antibacterial formulations.

IV. DISCUSSION

The antioxidant and antimicrobial efficacy of kinnow peel extract can be attributed to its rich phytochemical composition, particularly the presence of phenolic compounds and flavonoids. These compounds exhibit strong redox properties, allowing them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. Their roles in disease prevention, particularly in oxidative stress-related conditions such as cancer, cardiovascular diseases, and neurodegenerative disorders, have been well-documented in previous studies.

In the context of antimicrobial activity, the observed zones of inhibition reflect the efficacy of the extract against both Gram-positive and Gram-negative bacteria. This suggests that the extract disrupts microbial cell walls or interferes with metabolic pathways critical for bacterial survival. These findings are consistent with existing literature highlighting the antimicrobial potential of citrus-derived bioactive. Notably, the use of kinnow peel extract aligns with sustainable development goals by repurposing agricultural waste into value-added products.

While the in vitro results are promising, further studies are necessary to isolate specific active constituents and to validate these findings through in vivo models. Clinical studies will be critical in determining safety, bioavailability, and efficacy in therapeutic contexts.

V. CONCLUSION

Kinnow fruit peel, a typically discarded by-product, has shown significant pharmacological potential through its rich phytochemical content and biological activities. The study highlights its antioxidant and antimicrobial efficacy, reinforcing its potential in pharmaceutical, nutraceutical, and food industries. Further research should focus on isolation and in vivo testing of active constituents to validate therapeutic applications.

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