PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol x(x): xx–xx
https://doi.org/10.14719/pst.7217

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RESEARCH ARTICLE

Phytochemical analysis and assessment of the *in vitro* antibacterial and antioxidant potential of *Citrus* × *aurantiifolia* (Christm.) Swingle fruit peel

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OPEN ACCESS

ARTICLE HISTORY
Received: 14 January 2025
Accepted: 28 February 2025
Available online
Version 1.0 : 22 April 2025

Check for updates

Additional information
Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

CITE THIS ARTICLE
Sana SS, Supriya MK, Manish SH. Phytochemical analysis and assessment of the *in vitro* antibacterial and antioxidant potential of *Citrus* × *aurantiifolia* (Christm.) Swingle fruit peel. Plant Science Today (Early Access). https:/doi.org/10.14719/pst.7217

Abstract

This study investigates the bioactive compounds in *Citrus* × *aurantiifolia* (Christm.) Swingle (CA) fruit peel using qualitative phytochemical tests, UV-visible spectroscopy, FT-IR and GC-MS techniques. The hydroalcoholic extract was obtained through preparation using aqueous ethanol (Distilled water: Ethanol, 3:1) through maceration, yielding secondary metabolites amounting to approximately 32% of the initial dry weight of the plant material. Phytochemical screening confirmed the occurrence of alkaloids, flavonoids, carbohydrates, glycosides, phenolic compounds, proteins, steroids, terpenoids and tannins. The total phenolic content (TPC) was determined to be 81.72 ± 0.126 mg/g, while the total flavonoid content (TFC) was recorded at 4.71 ± 0.052 mg/g, highlighting their phytochemical richness. The extract demonstrated moderate antibacterial activity against uropathogens, including *Acinetobacter* sp., *Enterobacter* sp., *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis*. The antimicrobial activity is attributed to the presence of phenolic compounds, flavonoids and alkaloids, which are known to disrupt microbial cell walls, interfere with enzyme activity and inhibit nucleic acid synthesis. Terpenoids and tannins further enhance this effect by disrupting the microbial membranes' integrity, leading to cell lysis. Additionally, the methanolic extract demonstrated significant antioxidant potential. Elemental analysis through ICP-MS identified essential minerals, reinforcing their medicinal potential. This study highlights the potential of CA fruit peel, typically considered agricultural waste, in sustainable resource utilization by transforming waste into valuable bioactive products, thereby reducing environmental impact and supporting waste valorization. The integration of antibacterial and antioxidant properties and its rich phytochemical and mineral profile suggests its potential use in herbal medicine and the discovery of novel antibiotics and drugs. Utilizing such by-products aligns with sustainable practices, supporting pharmaceutical advancements while reducing environmental impact.

Keywords

agricultural waste utilization; antioxidant properties; bacterial inhibition; phytochemical constituents; uropathogens

Introduction

Citrus × *aurantiifolia* (Christm.) Swingle, known as Key lime or Mexican lime, is a small evergreen tree belonging to the Rutaceae family. It is widely cultivated in tropical and subtropical regions due to its economic and medicinal significance. The plant is valued for its refreshing, tangy fruits and rich phytochemical profile, including flavonoids, alkaloids, coumarins and essential oils. Various parts of *C.* ×

aurantiifolia, including the fruit, peel, leaves and seeds, have been extensively utilized in traditional medicine for their antimicrobial, anti-inflammatory, antioxidant and gastroprotective properties. Ethnopharmacologically, Key lime has been used in diverse cultures to treat ailments such as sore throat, fever, digestive disorders and infections. The fruit peel is known for its bioactive potential, making it an attractive candidate for pharmaceutical and nutraceutical applications. (1-4).

Urinary tract infections (UTIs) are common bacterial infections that impact millions of people globally, with a higher incidence in females due to anatomical factors. These infections occur commonly across all ages but are especially frequent in sexually active women, expectant mothers, aged individuals and those with certain medical conditions. (5-7). Common UTI pathogens include *Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis* and *Staphylococcus saprophyticus*, with *Escherichia coli* being the predominant cause (8). Untreated UTIs may result in serious complications, including pyelonephritis and urosepsis. The treatment of UTIs typically includes antibiotics; however, their extensive use raises concerns about antimicrobial resistance, which complicates treatment and increases illness severity, healthcare costs and mortality rates. Given the global impact of UTIs, promoting the prudent use of antibiotics, researching new treatments and exploring alternative therapies is crucial (9-11).

Herbs like Buchu (*Barosma betulina*), Cranberry (*Vaccinium macrocarpon*), Dandelion (*Taraxacum officinale*), Goldenseal (*Hydrastis canadensis*) and Uva Ursi (*Arctostaphylos uva-ursi*) have traditionally been utilized to support UTI treatment. Antioxidants derived from natural sources are crucial in safeguarding cells from damage induced by free radicals and oxidative stress linked to numerous chronic diseases. This study aims to identify phytochemicals in *Citrus aurantiifolia* fruit peel extract, determine its total phenolic and flavonoid contents, evaluate its antioxidant activity and assess its antibacterial effectiveness against clinically isolated uropathogens (12).

Materials and Methods

Procurement of plant material

The fruits belonging to *Citrus* × *aurantiifolia* (Christm.) Swingle were collected from Kalusta village, Chiplun taluka, district Ratnagiri, Maharashtra, India. The Blatter Herbarium at St. Xaviers' College, Mumbai, Maharashtra, India, verified the plant material. This sample has been confirmed as a cultivar of *Citrus* × *aurantiifolia* (Christm.) Swingle is part of the Rutaceae family. It closely matches the Blatter Herbarium specimen JP-4380, identified by J. Pallithanam. The fruit peel, an agricultural waste, was collected, cleaned and shade air dried. The dried peels were pulverized using an electrical grinder into a fine powder and passed through an 80-mesh sieve. The powder was transferred into airtight containers labelled appropriately for future use.

Chemicals

All the chemicals utilized in this study are analytical-grade substances. They were used directly without any additional purification. When required, cell culture-grade Milli-Q water was employed. Acetone, Chloroform, Methanol, Ethyl acetate, Hexane, PET Ether, 1,1-Diphenyl-2-picrylhydrazyl (DPPH), Quercetin, Ascorbic acid and Gallic acid were acquired from Sigma Aldrich Pvt. Ltd., India. Ampicillin nutrient agar was obtained from Hi Media Laboratories Pvt. Ltd., India.

Determination of percentage yield of phytoconstituents in various polar and nonpolar solvents

20 g of the peel powder was extracted in a sequential manner using Acetone, Aqueous ethanol (Distilled water: Ethanol, 3:1), Chloroform, Ethanol, Ethyl acetate, Hexane and PET Ether using the maceration method. The extraction was carried out at RT for 72 hrs with mild shaking. The filtrates from the extraction process were concentrated and filtered. The weight of each resulting residue was documented and the percentage yield was computed using the following Equation 1 formula:

$$\text{Percentage Yield (\%)} = \frac{\text{Weight of residue}}{\text{Initial weight of plant material}} \text{ X } 100$$

Eqn. 1

Phytochemical screening

Extraction Process: The crude plant extract was formulated using the Soxhlet extraction method. 5 g of fruit peel powder was carefully packed into a thimble and subjected to extraction using 250 mL of Aqueous ethanol (Distilled water: Ethanol, 3:1). The extraction persists for 18 hrs or until the solvent in the siphon chamber of the extractor becomes colourless. Following this, the solvent was removed using rotavapor. The dried extract was stored in the refrigerator at 4°C for subsequent use in phytochemical analysis.

Preliminary phytochemicals analysis: The hydroalcoholic extract prepared using the Soxhlet extraction method was subsequently employed for initial qualitative phytochemical screening to identify various active metabolites present using standard procedures (13).

Characterization of Methanolic extract of Citrus Aurantifolia fruit peel

UV-Visible spectrum: The CA fruit peel extract was analyzed using UV-visible spectrophotometry on a Shimadzu UV 1800 spectrophotometer. The analysis was performed at room temperature with a 2nm slit width in a 10-mm cell. The extract was examined across a 200-800 nm wavelength range under visible and UV light. Before analysis, the extract was centrifuged for 10 minutes at 3000 rpm and filtered through Whatman No. 1 filter paper. The sample was subsequently diluted with the same solvent at a 1:10 ratio.

FT-IR spectrum: A Perkin-Elmer Spectrum two FT-IR spectrometer with a universal ATR (single reflection diamond) L1600107 was employed to perform FT-IR analysis. The scanning range covered 400 to 4000 cm^{-1}. The dried powder of the methanolic extract from the fruit peel was used for the study.

GC-MS analysis: A Shimadzu GC-MS QP 2010 ultra system was employed to obtain the GC–MS chromatogram of the methanolic fruit peel extract. The system incorporated a fused silica column containing a Rxi-5SilMS capillary column (30 m length, 0.25 mm diameter, 0.25 µm thickness). High-purity helium (99.99%) was utilized as the carrier gas at a steady flow rate of 1 mL/min. The GC–MS spectral detection employed electron ionization with 70 eV ionization energy, analyzing fragments from 50 to 500 m/z. A 1 µL injection volume was used in split mode, with the injector maintained at 260°C. The column oven temperature program began at 50°C for 2 minutes, then increased by 10°C per minute to 260° C, where it remained for 15 minutes with a 100.1 split ratio. The ion source and interface temperatures were set at 200°C and 260°C, respectively. Phytochemical constituents in the test samples were identified by comparing their retention time (in minutes), peak area and mass spectral patterns with those in the National Institute of Standards and Technology (NIST) librarys' spectral databases of authentic compounds.

Elemental analysis using ICP-MS

ICP-MS Analysis Sample Preparation via Microwave Digestion: A quantity of 123.5 mg of plant material, previously dried and pulverized, was placed in a clean, dry Teflon vessel designed for microwave digestion. The vessel then received 8.0 mL of concentrated HNO_3. The sample underwent digestion in a Microwave Reaction System (Anton Paar model: Multiwave PRO) at 200° C for 10 min, utilizing 1200 W of microwave power, followed by a 20-min holding period at 200°C. The systems' temperature and pressure were capped at 200°C and 60 bar (870.23 psi). Once cooled, the digested sample was transferred to a 40 mL volumetric flask and brought to volume with deionized water. An identical procedure was employed to prepare a blank digest for comparison.

Instrumentation for ICP-MS: The trace element analysis was performed using an Agilent Technologies Model 7900 single quadrupole Inductively Coupled Plasma Mass Spectrometer (ICP-MS). The operational parameters of the equipment employed in this study are outlined in Table 1.

ICP-MS calibration and Internal standard: The instrument was standardized using an internal standard solution containing 10 to 200 ppb of various elements in 1% nitric acid. These elements included Chromium (52 Cr), Cobalt (59 Co), Nickel (60 Ni), Copper (63 Cu), Zinc (66 Zn), Arsenic (75 As), Silver (107 Ag), Cadmium (111 Cd), Cesium (133 Cs), Platinum (195 Pt), Gold (197 Au), Lead (208 Pb) and Bismuth (209 Bi). This standard solution was also utilized to fine-tune gas flow, mass calibration, resolution and Auto Lens calibration.

Estimation of total phenol: The modified Folin-Ciocalteus' (FC) method was employed to determine the total phenol content in the sample. A standard Gallic acid (GA) solution was prepared in methanol at 1 mg/mL. For GA measurements, standard solution concentrations ranged from 43.5 to 435 µg/mL, while extract concentrations varied from 180 to 1800 µg/mL. The procedure involved adding 150 µL of FC reagent (diluted 1:1 v/v with distilled water) to these solutions and vertexing. Subsequently, 500 µL of 20 % (w/v) Na2CO3 was added and the mixture was incubated in darkness for 1 hr. The resulting greenish-blue colour absorbance was measured at 650 nm using a Shimadzu UV-visible spectrophotometer (UV-1800). A blank was prepared by combining all reagents with 500 µL of methanol, excluding the plant extract.

Table 1. Instrument operating conditions for determination of elements in *Citrus × aurantiifolia* (Christm.) Swingle fruit peel

Nebulizer gas flow	: ~ 1 L/min
Auxiliary gas flow	: ~ 1 L/min
Plasma gas flow	:~15 L/min
He gas flow in the reaction cell	:~ 0.2 mL/min
Reflected power	: ~ 45 W
Forward power	: ~ 1500 W
Analyser vacuum	: ~$6x10^{-5}$

Quantification of total flavonoids: The aluminium chloride method was employed to determine the total flavonoids in the fruit peel extract. A standard Quercetin solution was prepared in methanol at a concentration of 10 mg/mL. Quercetin measurements utilized standard solutions ranging from 8.3 to 83.3 µg/mL. For the sample analysis, 100 µL of methanolic extract was used. These solutions were combined with 100 µL of 10% aluminium chloride solution and thoroughly mixed. Subsequently, 100 µL of 1M sodium acetate was introduced and the mixture was left to incubate in darkness at ambient temperature for 45 min. The resulting golden-yellow colour absorbance was measured at 415 nm using a Shimadzu UV-visible spectrophotometer (UV-1800). A blank was prepared by combining 500 µL of methanol with all reagents, excluding the plant extract.

Antibacterial activity

Bacterial Strains: Five different uropathogenic bacterial strains of the following uropathogens, namely *Acinetobacter sp., Enterobacter sp., Escherichia coli, Klebsiella pneumonia, Proteus mirabilis sp.,* isolated and identified (from urine samples of patients clinically suspected to be suffering from UTI) were collected from Dr Purandares' diagnostic centre, Girgaon, Mumbai, Maharashtra. The uropathogens were obtained on culture plates of blood agar and nutrient agar. The microorganisms were subsequently cultured on nutrient agar slants and kept at 37°C for 24 hr. Afterwards, they were stored in a refrigerator at 5 to 10°C.

Antibacterial screening: The agar cup plate method was employed to investigate the antibacterial effectiveness of the *Citrus × aurantiifolia* (Christm.) Swingle (CA) fruit peel against all five uropathogens. The broth medium of all gram-negative bacterial strains *Acinetobacter sp., Enterobacter sp., Escherichia coli, Klebsiella pneumonia* and *Proteus mirabilis sp*. were distributed and spread across the agar plates to establish a uniform growth surface. Wells measuring 6 mm in diameter were created using a cork borer in each plate. The control group included aqueous ethanol (Distilled water: Ethanol, 3:1) to account for solvent effects and Ampicillin 0.01% as a positive control for antibacterial activity. The petri dishes were then incubated for 24 hrs at 37°C. The inhibition zones' dimensions were measured and compared to those produced by the antibiotic Meropenem to assess the antibacterial efficacy. These experiments were conducted twice in triplicate and the diameter of the inhibition zone was recorded in millimetres.

Evaluation of antioxidant potential

DPPH Test : The antioxidant properties of CA fruit peel extract were assessed using a modified DPPH (2,2′-diphenyl-1-picrylhydrazyl) technique. A 0.004% DPPH solution was prepared by dissolving 4 mg of DPPH in 100 mL of methanol and a 1 µg/mL standard Ascorbic acid (ASC) solution was made in water. ASC standard solutions ranged from 50 to 250 µg/mL, while extract concentrations varied from 5 to 25 µg/mL. To each sample, 200 µL of 0.004% DPPH solution was introduced. After homogenization, the mixtures were kept in darkness for 2 hr before measuring optical density at 517 nm, with methanol as the blank. Each test was performed three times. The antioxidant activity percentage was calculated using the Equation 2:

Radical scavenging activity (RSA)% =

$$\frac{Abs_{(control)} - Abs_{(sample)}}{Abs_{(control)}} \times 100 \quad \text{(Eqn 2)}$$

In this formula, the control consisted of 600 µL methanol + 200 µL DPPH solution, while the sample contained CA fruit peel extract brought to 600 µL with methanol + 200 µL DPPH solution. Antioxidant activity was expressed as the half maximal inhibitory concentration (IC50), defined as the antioxidant amount required to decrease the controls' absorbance by 50%. These values were determined by graphing the obtained % RSA against the various solution concentrations used in the test. To express each samples' antioxidant capacity relative to ascorbic acid, the "ascorbic acid equivalent antioxidant capacity" (AAEAC) was also determined as follows in Equation 3:

$$AAEAC = \frac{IC_{50} \text{ of ASC mg/mL}}{IC_{50} \text{ of sample mg / mL}} \quad \text{(Eqn 3)}$$

Statistical analysis: The findings were presented as Mean ± SD. Statistical analysis was conducted using a one-way analysis of variance (95% confidence interval) followed by Duncans' multiple range tests to determine significant differences ($p < 0.05$) among means. These statistical methods were implemented using SPSS software (Version 29.0.2.0).

Results

The powder was extracted from the fruit peel using a range of solvents, including Acetone, Aqueous ethanol (Distilled water: Ethanol, 3:1), Chloroform, Methanol, Ethyl acetate, Hexane and PET Ether and the obtained % yield value was expressed as mean ± standard deviation (SD) based on triplicate extractions: 7.0 ± 0.2%, 31.0 ± 1.0%, 9.0 ± 1.0%, 20.0 ± 1.0%, 4.0 ± 1.0%, 1.0 ± 0.2% and 0.6 ± 0.1% respectively. In this study, hydroalcoholic (Distilled water: Ethanol, 3:1) fruit peel extract of CA possesses high solubility properties and a high % yield value; hence, it is utilized for initial phytochemical screening and evaluating the antibacterial and antioxidant activity. The phytoconstituents detected in the hydroalcoholic extracts of *Citrus* × *aurantiifolia* (Christm.) Swingle fruit peels are alkaloids, carbohydrates, flavonoids, glycosides, phenols, proteins, steroids, tannins and terpenoids, as listed in Table 2. To identify compounds with σ- bonds, π-bonds, lone electron pairs, chromophores and aromatic rings, a UV-visible spectrum was analyzed (Fig. 1). The analysis revealed three distinct peaks at 255, 420 and 660 nm, with corresponding absorbance values of 0.492, 2.652 and 0.274. Earlier research has shown that absorption peaks between 234-676 nm indicate alkaloids, flavonoids, phenols and glycosidic compounds. Since all observed peaks fall within this range, it suggests the presence of these secondary metabolites in the fruit peel (12). FTIR spectrum of the fruit peel as shown in Fig. 2, depicts strong absorption peak at 924 cm^{-1} due to C-H vibrations in aromatic compounds, weak absorption peaks in the range from 1092 cm^{-1} to 3038 cm^{-1} for C-O, C-N, C-H, C=C and C=O vibrations in aliphatic and aromatic hydrocarbons, alcohol, phenols, aldehydes, ketones, amines and esters and medium absorption peak at 3333 cm^{-1} due to N-H vibration in primary and secondary amines (14-16). Thus, the FTIR data suggested the existence of the above compounds, as outlined in Table 3. The GC-MS chromatogram (Fig. 3) indicates that the fruit peel contains an array of phytochemicals in the methanolic extract, with 38 compounds identified and categorized into alcohol, aldehyde, alkanes, benzoxazine derivatives, coumarin derivatives, disaccharide, fatty acids and their esters, glycoside and sterols (see Table 4) reflecting the complexity in its chemical composition. Benzoxazinone derivative, glycoside and fatty acids were the most abundant identified compounds (17). The findings from the Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) analysis indicated the detection of 13 heavy metals, as outlined in Table 5. Notably, according to the WHO guidelines, all potentially harmful metals were found to fall within the permissible limits. Gold and Zinc were the most abundant elements, with the highest mean values of 11.816 ± 0.71 mg/kg and 7.613 ± 0.84 mg/kg, respectively. Platinum and Cadmium had the lowest mean values of 0.003 ± 25.72 mg/kg and 0.007 ± 22.44 mg/kg, respectively (17-20). In the methanolic CA fruit peel extract, the TPC and TFC expressed in Gallic acid (GAE) and Quercetin (QE) equivalent were quantified to be 81.72 ± 0.126 mg GAE /g FP and 4.71 ± 0.052 mg QE /g FP respectively. Phenolic compounds and flavonoids are acknowledged for their antioxidant activities and potential health benefits. Elevated levels of TPC and TFC indicate a greater antioxidant capacity in the peel, which could be significant for its potential health-promoting effects.

Table 2. Phytochemical screening of hydroalcoholic extract of *Citrus* x *aurantiifolia* (Christm.) Swingle peel

Sr. No.	Phytoconstituents	Hydroalcoholic extract
1.	Alkaloids	+ve
2.	Carbohydrates	+ve
3.	Flavonoids	+ve
4.	Glycosides	+ve
5.	Saponin	+ve
6.	Cholesterol	-ve
7.	Steroid	+ve
8.	Terpenoid	+ve
9.	Phenols and Tannins	+ve
10.	Proteins	+ve

Notes: (+ve) = Present, (-ve) = Absent

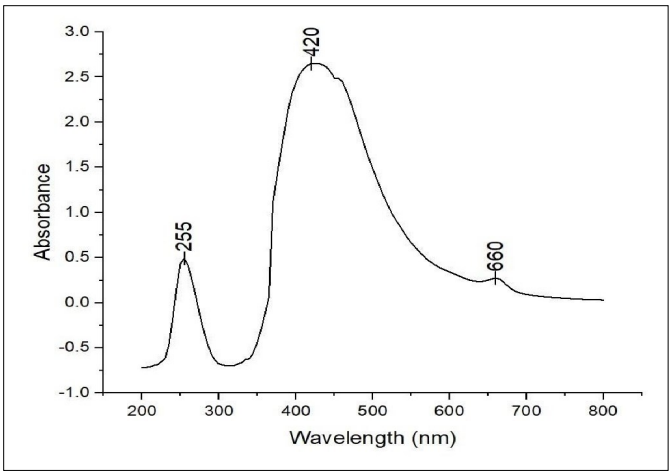


Fig. 1. UV-Visible spectrum of methanolic extract of *Citrus × aurantiifolia* (Christm.) Swingle fruit peel.

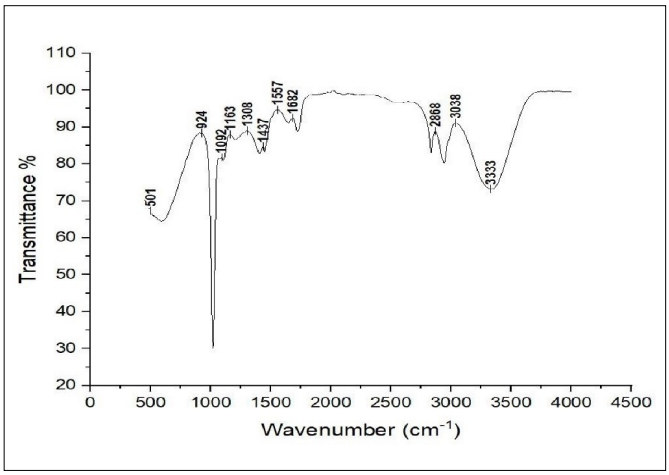


Fig. 2. FT-IR spectrum of methanolic extract of *Citrus × aurantiifolia* (Christm.) Swingle fruit peel.

Table 3. FT-IR peak values of methanolic extract of *Citrus × aurantiifolia* (Christm.) Swingle fruit peel

Sr. No.	Peak Values (Wavenumber cm^{-1})	Vibrations	Possible type of compound
1.	924	C-H bending vibrations	Aromatic
2.	1092	C-O stretching vibrations	Alcohol, phenol, ether & esters
3.	1163	C-N stretching vibrations	Amines & Amides
4.	1308	C-H bending vibrations	Alkynes
5.	1437	C-H unsymmetrical bending vibrations	Alkyl
6.	1557	C=C stretching vibrations	Aromatic
7.	1682	C=O stretching vibrations	Aldehydes & Ketones
8.	2868	C-H symmetrical stretching vibrations	Alkanes
9.	3038	C-H stretching vibrations	Aromatic
10.	3333	N-H stretching vibrations	Amines & Amides

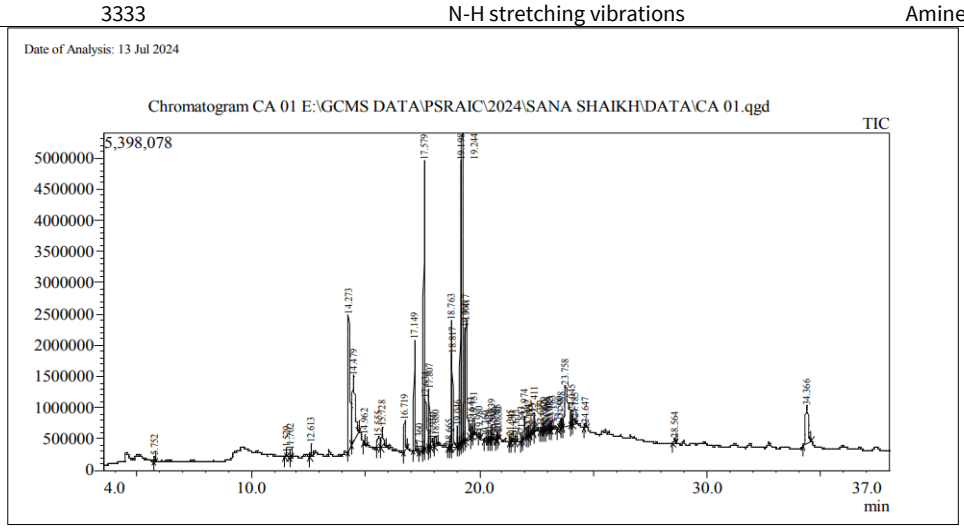
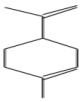
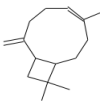
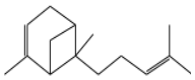
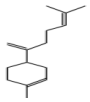
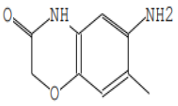
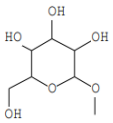
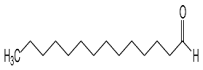
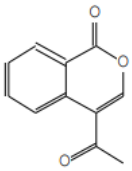
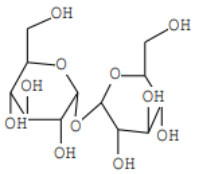
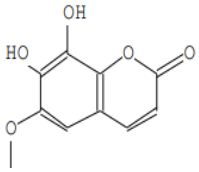
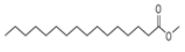
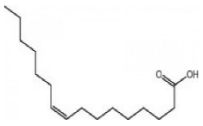
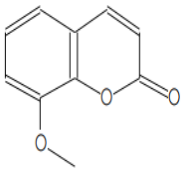
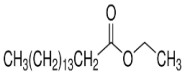
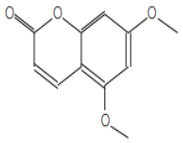
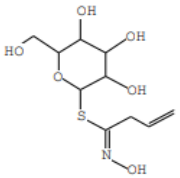
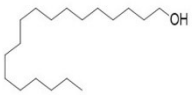
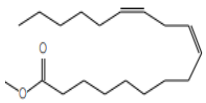
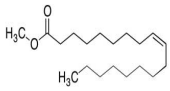
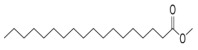
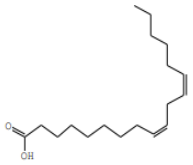
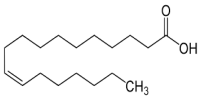
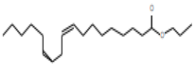
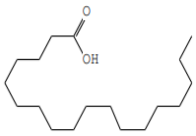
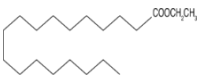
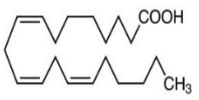
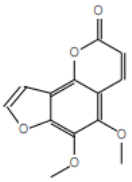


Fig. 3. GC/MS Chromatogram of *Citrus × aurantiifolia* (Christm.) Swingle fruit peel.

The effectiveness of the substance was measured by the size of inhibition zones, which included the diameter of the well and the minimum inhibitory concentrations (MICs) of the CA fruit peel extract for the examined uropathogens are presented in Table 6. Despite the time-consuming and labour-intensive nature of the agar disc diffusion method, it remains a reliable and widely utilized technique for antimicrobial screening in many laboratories. Hence, we employed this method to assess the antimicrobial activity. The agar cup plate method results indicated that *Acinetobacter sp*., *Enterobacter sp*. and *Klebsiella pneumoniae* exhibited weak inhibition zones measuring 12.7 ± 0.58 mm, 13.7 ± 0.58 mm and 11.7 ± 1.52 mm, respectively. While *Escherichia coli* showed extremely weak inhibition zones of 9.7 ± 0.58 mm and *Proteus mirabilis* was almost found to be resistant. The MIC results differed among the tested organisms. The results of MIC suggested that the CA fruit peel extract has mild potential to inhibit all the uropathogens tested. The highest MIC was for *Escherichia coli* (22.0 ± 0.115 mg/mL), while *Acinetobacter sp. and Enterobacter sp.* exhibited the lowest MIC of 10.0 ± 0.058 mg/mL and 10.0 ± 0.115 mg/mL respectively. The extracts' antibacterial potential may derive from compounds like D-Limonene, Caryophyllene, trans-α-Bergamotene, β-Bisabolene, 4-Acetylisocoumarin, Scopoletin, Palmitoleic acid, linoleic acid and Pimpinellin (18-19). The CA fruit peel extract exhibited potent antioxidant potential against DPPH radicals, as shown in Table. 4 with IC_{50} values of 4.39 ± 1.09 mg/mL (standard antioxidants) and 1.91 ± 0.005 mg/mL (test samples), as shown in Table 7. Compounds such as D-limonene, Caryophyllene, trans - α - Bergamotene, β-Bisabolene, 4-Acetylisocoumarin, Trehalose, Scopoletin, Palmitoleic acid, methyl linoleate, methyl oleate, linoleic acid, Pimpinellin and gamma-Sitosterol must have likely contributed to its efficacy (20-21).

Table 4. Chemical composition of *Citrus × aurantiifolia* (Christm.) Swingle fruit peel

Sr. No.	RT (in min)	Name of the compound	Molecular formula	Structure	Molecular weight	Peak Area %	Classification of compound
1.	5.752	D-Limonene	$C_{10}H_{16}$		136	0.13	Monoterpene
2.	11.529	Caryophyllene	$C_{15}H_{24}$		204	0.10	Sesquiterpene
3.	11.702	trans-α-Bergamotene	$C_{15}H_{24}$		204	0.17	Sesquiterpene
4.	12.613	β-Bisabolene	$C_{15}H_{24}$		204	0.33	Sesquiterpene
5.	14.273	2H-1,4-Benzoxazin-3(4H)-one, 6-amino-7-methyl-	$C_9H_{10}N_2O_2$		178	7.68	Benzoxazinone derivative
6.	14.479	-D-Glucopyranoside, methyl	$C_7H_{14}O_6$		194	7.91	Glycoside
7.	14.962	Tetradecanal	$C_{14}H_{28}O$		212	0.26	Aldehyde
8.	15.555	4-Acetylisocoumarin	$C_{11}H_8O_3$		188	1.12	Isocoumarin derivative
9.	15.728	Trehalose	$C_{12}H_{22}O_{11}$		342	1.51	Disaccharide
10.	16.719	2H-1-Benzopyran-2-one, 7,8-dihydroxy-6-methoxy-	$C_{10}H_8O_5$		208	1.23	Coumarin derivative
11.	17.149	Hexadecanoic acid, methyl ester (methyl palmitate)	$C_{17}H_{34}O_2$		270	2.48	Fatty acid ester
12.	17.360	cis-9-Hexadecenoic acid (palmitoleic acid)	$C_{16}H_{30}O_2$		254	0.09	Monounsaturated fatty acid
13.	17.579	n-Hexadecanoic acid (palmitic acid)	$C_{16}H_{32}O_2$		256	8.89	Saturated fatty acid
14.	17.634	2H-1-Benzopyran-2-one, 8-methoxy- (isoscopoletin)	$C_{10}H_8O_3$		176	3.46	Coumarin derivative

15.	17.807	Hexadecanoic acid, ethyl ester (ethyl palmitate)	$C_{18}H_{36}O_2$		284	1.70	Fatty acid ester
16.	17.930	2H-1-Benzopyran-2-one, 5,7-dimethoxy- (scopoletin)	$C_{11}H_{10}O_4$		206	0.71	Coumarin derivative
17.	18.080	Desulphosinigrin (sinalbin)	$C_{10}H_{17}NO_6S$		279	0.55	Glucosinolate
18.	18.665	1-Octadecanol (stearyl alcohol)	$C_{18}H_{38}O$		270	0.10	Fatty alcohol
19.	18.763	9,12-Octadecadienoic acid (Z, Z)-, methyl ester (methyl linoleate)	$C_{19}H_{34}O_2$		294	2.91	Polyunsaturated fatty acid ester
20.	18.817	11-Octadecenoic acid, methyl ester (methyl oleate)	$C_{19}H_{36}O_2$		296	3.14	Monounsaturated fatty acid ester
21.	19.046	Methyl stearate	$C_{19}H_{38}O_2$		298	0.51	Fatty acid ester
22.	19.198	9,12-Octadecadienoic acid (Z,Z) (linoleic acid)	$C_{18}H_{32}O_2$		280	11.21	Polyunsaturated fatty acid
23.	19.244	cis-Vaccenic acid	$C_{18}H_{34}O_2$		282	13.85	Monounsaturated fatty acid
24.	19.366	n-Propyl 9,12-octadecadienoate	$C_{21}H_{38}O_2$		322	3.49	Fatty acid ester
25.	19.417	Octadecanoic acid (stearic acid)	$C_{18}H_{36}O_2$		284	5.35	Saturated fatty acid
26.	19.643	Octadecanoic acid, ethyl ester (ethyl stearate)	$C_{20}H_{40}O_2$		312	0.73	Fatty acid ester
27.	19.980	8,11,14-Eicosatrienoic acid, (Z,Z,Z)- (alpha-linolenic acid)	$C_{20}H_{34}O_2$		306	0.16	Polyunsaturated fatty acid
28.	20.260	Pimpinellin	$C_{13}H_{10}O_5$		246	0.11	Furanocoumarin derivative

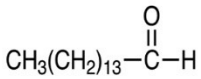
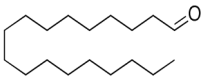
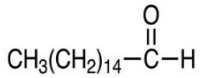
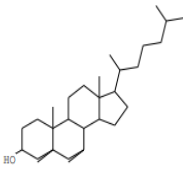
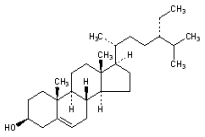
29.	20.785	Pentadecanal	$C_{15}H_{30}O$		226	0.25	Aldehyde
30.	21.345	Pentacosane	$C_{25}H_{52}$		352	0.16	Alkane
31.	21.611	Octadecanal	$C_{18}H_{36}O$		268	0.16	Aldehyde
33.	22.142	Heneicosane	$C_{21}H_{44}$		296	0.36	Alkane
34.	22.315	Glycerol 1-palmitate	$C_{19}H_{38}O_4$		330	0.17	Monoglyceride
35.	22.699	2-methylhexacosane	$C_{27}H_{56}$		380	0.11	Branched alkane
36.	24.045	Hexadecanal	$C_{16}H_{32}O$		240	0.76	Aldehyde
37.	28.564	Cholesta-4,6-dien-3-ol, (3.beta.)-	$C_{27}H_{44}O$		384	0.28	Sterol
38.	34.366	Gamma-Sitosterol	$C_{29}H_{50}O$		414	4.32	Phytosterol

Table 5. Heavy metal concentration in *Citrus* × *aurantiifolia* (Christm.) Swingle fruit peel

Element	Concentration (ppm)	Element	Concentration (ppm)
Cr	1.116 ± 0.68	Cd	0.007 ± 22.44
Co	0.069 ± 5.79	Cs	0.207 ± 1.88
Ni	0.483 ± 2.29	Pt	0.003 ± 25.72
Cu	3.369 ± 1.04	Au	11.816 ± 0.71
Zn	7.613 ± 0.84	Pb	0.136 ± 1.42
As	0.024 ± 5.89	Bi	3.787 ± 0.64
Ag	0.442 ± 1.88		

Table 6. Antibacterial activities of meropenem antibiotic and *Citrus* × *aurantiifolia* (Christm.) Swingle fruit peel

Sr.No.	Uropathogens	Meropenem		*Citrus aurantiifolia* (Christm) Swingle	
		DD	MIC(µg/mL)	DD	MIC(mg/mL)
1.	*Acinetobacter sp.*	20.7 ± 1.15^{a}	1.0 ± 0.058	12.7 ± 0.58^{cd}	10.0 ± 0.058
2.	*Enterobacter sp.*	37.7 ± 0.58^{d}	1.0 ± 0.115	13.7 ± 0.58^{d}	10.0 ± 0.115
3.	*Escherichia coli*	30.3 ± 0.58^{c}	2.0 ± 0.115	9.7 ± 0.58^{b}	22.0 ± 0.115
4.	*Klebsiella pneumonia*	30.7 ± 1.15^{c}	1.2 ± 0.058	11.7 ± 1.52^{c}	20.0 ± 0.058
5.	*Proteus mirabilis*	24.7 ± 0.58^{b}	1.8 ± 0.115	7.7 ± 0.58^{a}	-

Notes: DD- diameter of the zone of inhibition (mm) including well diameter of 6 mm, MIC-minimum inhibitory concentration. Different letters within a column indicate statistically significant differences between the means ($p < 0.05$).

Table 7. Radical scavenging activities of ascorbic acid and *Citrus* × *aurantiifolia* (Christm.) Swingle fruit peel

Concentration (µg/mL)	% RSA	Concentration (µg/mL)	% RSA
Ascorbic acid		CA fruit peel	
50	48.62± 1.33^{a}	5	40.35± 0.161^{a}
100	72.82± 0.97^{b}	10	51.61± 0.097^{b}
150	84.89± 1.64^{c}	15	64.68± 0.096^{c}
200	92.36± 0.63^{d}	20	79.49± 0.060^{d}
250	94.90± 1.27^{e}	25	90.02± 0.097^{e}
IC_{50}	4.39± 1.09	IC_{50}	1.91± 0.005

Notes: Different letters within a column indicate statistically significant differences between the means ($p< 0.05$).

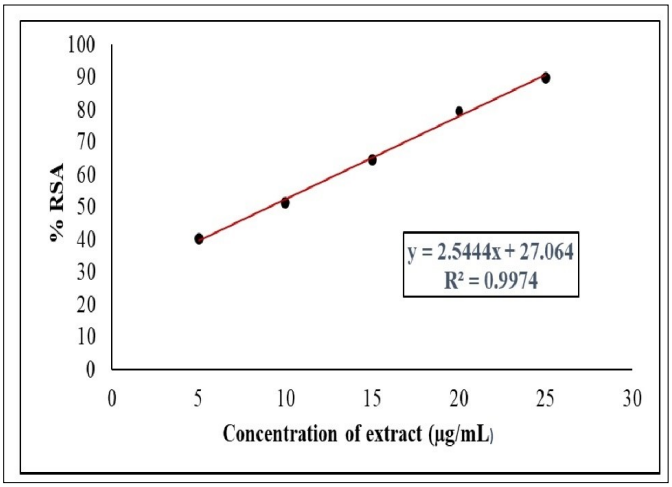


Fig. 4. % Radical scavenging activities of *Citrus* × *aurantiifolia* (Christm.) Swingle fruit peel at different concentrations.

Conclusion

Due to the adverse issues and harmful effects associated with artificial chemicals, there has been growing interest from researchers in natural substances derived from an array of plants, especially those utilized for food and medicine. *Citrus* × *aurantiifolia* (Christm.) Swingle fruit peel agricultural waste has been shown to contain important secondary metabolites. Thus, it can act as a source of some industrially important natural products. This is the initial investigation on assessing the antibacterial effectiveness of the *Citrus* × *aurantiifolia* (Christm.) Swingle fruit peel on clinically isolated uropathogens linked with urinary tract infections. Our findings indicate that the fruit peel exhibits a broad yet mild antibacterial and significant antioxidant activity. This suggests that the fruit peel of *Citrus* × *aurantiifolia* (Christm.) Swingle could serve as a novel source of therapeutic agents.

Acknowledgements

We sincerely thank Dr PS Ramanathan Advanced Instrumentation Centre for the GC-MS analysis and IIT Delhi for the ICP-MS analysis. We also thank Ramnarain Ruia Autonomous College for providing valuable resources and technical assistance throughout the project.

Authors' contributions

SS conceptualized and designed the study and conducted all experimental work related to extraction and characterization. SK assisted in performing the antibacterial assay. SS, SK and MS contributed equally to reviewing the manuscript, discussing the results and critically evaluating the revisions.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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