

Antibacterial activity and phytochemical screening of *Citrus sinensis* (orange peel) extract against bacteria associated with wound infections from patients attending Ahmad Yariman Bakura Specialist Hospital, Gusau

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ABSTRACT: This study investigated the antibacterial activity and phytochemical profile of *Citrus sinensis* (orange) peel extracts against bacteria associated with wound infections from patients attending Ahmad Sani Yariman Bakura Specialist Hospital, Gusau. Phytochemical screening of both ethanolic and aqueous extracts revealed the presence of flavonoids, phenolic compounds, saponins, tannins, and terpenoids. Alkaloids, triterpenes, steroids, and resins were absent in both extracts, while glycosides were uniquely present in the aqueous extract. The identification of the isolates was based on colonial morphology and standard biochemical tests. The antibacterial activity was assessed using the agar well diffusion method. The ethanolic extract exhibited the highest zones of inhibition of 23 ± 00 mm, and the aqueous extract also demonstrated activity with zones of inhibition of 10 ± 00 mm, for *Staphylococcus aureus*. The ethanolic and aqueous extract exhibited the lowest zones of inhibition of 10 ± 00 mm, for *Pseudomonas aeruginosa*, respectively. The minimum inhibitory concentration for the ethanolic extract ranges from 12.5 to 25 mg/ml, the minimum bactericidal concentrations of 50 mg/ml. The aqueous extract demonstrated a minimum inhibitory concentration range from 25 mg/ml to 50 mg/ml with a corresponding minimum bactericidal concentration of 100mg/ml across all isolates. The findings of this study demonstrated the antibacterial properties of *Citrus sinensis* peel extract, which is attributable to its diverse phytochemical composition, indicating its potential as a natural therapeutic agent for managing wound infections.

Keywords: Antibacterial, ciprofloxacin, *Citrus sinensis*, glycoside.

INTRODUCTION

Wound infections continue to cause a significant public health concern, especially in developing nations where inadequate hygiene, scarce healthcare facilities, and the growing prevalence of antibiotic-resistant microbes hinder effective treatment. Open wounds provide a suitable environment for microbial growth, which can result in prolonged healing, tissue injury, systemic spread of infection, and, in severe instances, life-threatening outcomes (Maheswary *et al.*, 2021). Common bacterial pathogens implicated in wound infections include *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. The increasing

prevalence of multidrug-resistant strains of these organisms has further intensified the search for alternative therapies that are affordable, effective, and accessible.

Medicinal plants and natural products have long played a vital role in traditional medicine, offering bioactive compounds with antimicrobial, antioxidant, and anti-inflammatory activities (Dar *et al.*, 2023). Among these, *Citrus sinensis* (orange) is widely consumed as a fruit but also possesses medicinal value. Its peel, often considered waste, is rich in phytochemical components such as flavonoids, alkaloids, tannins, phenols, saponins, and essential oils, many of which have demonstrated antimicrobial

properties. Utilising orange peel extract for antibacterial purposes not only provides a natural therapeutic option but also adds value to agricultural by-products, contributing to waste reduction and sustainable healthcare solutions (Mohsin *et al.*, 2022). Investigating the antibacterial activity of *Citrus sinensis* peel against wound-associated pathogens is particularly relevant in the face of escalating antibiotic resistance. By evaluating both its phytochemical composition and antimicrobial efficacy, this study seeks to provide scientific evidence supporting the traditional and potential clinical use of orange peel as a natural antibacterial agent.

Therefore, the present research focuses on the antibacterial activity and phytochemical screening of *Citrus sinensis* peel extract against bacteria isolated from wound infections among patients attending Ahmad Sani Yariman Bakura Specialist Hospital, Gusau.

MATERIALS AND METHODS

Collection of orange peel samples

Oranges were purchased from Tudun Wada Market, Gusau, Zamfara State. The peels were obtained and authenticated at the Herbarium of the Department of Biological Sciences, Federal University Gusau. The voucher number FUG/BIO/HEB/162 was obtained. The peels were rinsed with clean water and air-dried in shade for seven days. The peels were ground into powder and kept in an air-tight container for further use.

Consents and ethics

Ethical clearance was obtained from Ahmad Sani Yariman Bakura Specialist Hospital, Ethics Committee (ASYBSH/SUB/205/VOL.1).

Collection of wound swab sample

Wound samples were obtained with the use of swab sticks from 55 patients in Ahmad Sani Yariman Bakura Specialist Hospital, Gusau. The wounds were cleaned with a cotton swab inserted in normal saline. The swab was aseptically collected by gently twirling the cotton swab over the wound bed tissue with adequate pressure to draw out fluid from the wound tissue of each patient without contaminating the patient's wound. The specimens were transported immediately to the microbiology laboratory of the Federal University of Gusau, and the samples were analysed.

Isolation and identification of bacteria isolates

Each wound sample collected with the sterile swab stick was streaked on Nutrient agar plates and incubated for 24 hours at 37°C. The colonies were further subcultured onto

Mannitol salt agar, Cetrimide agar, and Eosin methylene blue for isolation of the bacteria present in the wound (Bobai *et al.*, 2022). Suggestive colonies were further inoculated on biochemical test reagents for further identification. The bacteria isolates were identified based on their standard cultural, morphological and biochemical characteristics.

Preservation of bacteria isolates

Colonies obtained after the incubation period were further sub-cultured on Nutrient agar and incubated for 24 hours at 37°C. Pure culture of the bacteria isolate was sub-cultured and preserved on agar slants and refrigerated at a temperature of 4°C (Alagoa *et al.*, 2025)

Gram staining

A drop of water was added to a slide, and a small amount of the colony was transferred aseptically from the Petri dish using a sterile wire loop. A drop of water was placed on a clean microscope slide, and a small amount of the colony was aseptically transferred from the Petri dish using a sterile wire loop and mixed gently. With the use of an inoculating loop to make a thin smear. The smear was air-dried, heat fixed and allowed to cool. Crystal violet was added over the fixed smear. It was allowed to stand for 60 seconds; the stain was drained off, and the excess stain was rinsed with water from a faucet. The basic objective of this step is to wash off the stain. Iodine solution was added to the smear. It was allowed to stand for 10 to 60 seconds. The iodine solution was poured off, and the slide was rinsed under running water. Excess water was shaken off from the surface. A few drops of alcohol were added. It was rinsed with water after 5 seconds. It was counterstained with safranin. The slides were allowed to dry. The slide was examined under the microscope using an oil immersion objective. Gram-positive organisms appear purple to blue, while Gram-negative organisms appear pink or red (Dimri *et al.*, 2020).

Biochemical tests

Citrate utilisation test

The Simmons citrate agar was prepared according to the specifications of the manufacturer in a sterilised petri dish. The test organism was inoculated into it and incubated at 37°C in the Incubator for 3 days (Adeoye *et al.*, 2021). A change in colour from green to blue and the presence of growth indicated a positive result.

Coagulase test

This test is essential in differentiating *Staphylococcus aureus* from other *Staphylococcal* species. The test was

performed by preparing a suspension of bacterial cells mixed into a drop of rabbit plasma on a microscope slide (Fernandes *et al.*, 2021). Visible clumping of bacterial cells indicated a positive result.

Catalase test

The colonies of the isolates were collected with the use of a sterile wire loop and emulsified in a drop of hydrogen peroxide (Okafor *et al.*, 2024). The presence of bubbles indicated that the test was a positive result.

Urease test

Christensen's urea agar was prepared, and 2 drops of urea solution were added and left to solidify. It was then inoculated with the test organism and incubated at 37°C for 48 hours (Sivaramakrishnan and Razia, 2021). A change in colour from yellow to pink indicated a positive result.

Methyl red

The test isolates were inoculated into the methyl red test tubes and were incubated at 35-37°C for 48 hours. After incubation, a few drops of methyl red indicator were added to the culture, and a resultant red colouration indicated a positive result (Mahmud *et al.*, 2023).

Voges-Proskauer test

2mls of sterile Methyl red-Voges Proskauer broth was inoculated with test isolates and incubated at 37°C for 24 hours. 10% α -naphthalol was added and then mixed, about 3 mls of KOH was added and shaken. The setup was then left for an hour at room temperature. A change from pink to red colour indicated a positive result (Muslem *et al.*, 2022).

Indole test

The test tubes containing the isolates were incubated at 37°C for 48 hours. After the Incubation period, 3-4 drops (0.5ml) of indole reagent, known as Kovac's reagent, were added and shaken gently (Hasan *et al.*, 2022). A positive result yields a red-coloured ring at the top layer of the medium.

Triple sugar iron test

The Triple Sugar Iron (TSI) is a biochemical test used to differentiate among enteric bacteria based on their ability

to ferment sugars and produce gas and hydrogen sulphide (H_2S). It is particularly useful for identifying members of the Enterobacteriaceae family, including *Salmonella* and *Escherichia coli*. A discrete bacterial colony was picked using a sterile wire loop and inoculated into TSI agar slant by stabbing the bottom (butt) and streaking on the surface. It was then incubated at 37°C for 18-24 hours. After incubation, the following reactions were observed, which indicated a positive result: presence of yellow colour in the slant or bottom indicated acid production from sugar fermentation, presence of cracks or lifting of the agar indicated gas production, while a black precipitate (blackening of the agar) in the butt indicated hydrogen sulphide production (Hafezi and Khamar, 2024).

Preparation of the orange peel extract

With the use of the cold maceration method, two different solvents, ethanol and water, were used for extraction. 100 grams each of the ground orange peel was separately soaked in a maceration apparatus containing 500 mL of ethanol and cold water, respectively. It was agitated for 6 hours and kept for 18 hours, then filtered. It was allowed to settle and evaporated to dryness in a water bath at 45°C. The extracts were stored in sterile capped bottles and refrigerated at 4°C until needed for further analysis.

Preparation of different concentrations of the extracts

A stock solution was prepared by dissolving 0.4 g of the extract in 2ml dimethylsulfoxide (DMSO) for the ethanolic extract only. Then, 3 ml of sterile distilled water (1.5 ml for the aqueous extract) to obtain a 200 mg/ml concentration. Serial dilution was then carried out in three test tubes containing 1.5 ml of distilled water each to obtain the concentrations 100 mg/ml, 50 mg/ml and 25 mg/ml, respectively, by transferring 1.5 ml of the stock solution into 1.5 ml of sterile distilled water and 1.5ml from there to the next test tube until the final concentration is reached. The procedure was carried out for both the ethanolic and aqueous extracts.

Phytochemical analysis of the orange peel crude extracts

Test for alkaloids

A few drops of Mayer's reagent were added to the extract. Rose red precipitate indicates the presence of an alkaloid (Balamurugan *et al.*, 2019).

Test for flavonoids

About 0.5 g of the extract was dissolved in 1-2 ml of methanol in a heat. Metallic magnesium and four or five

drops of concentrated HCl were added. A red or orange colour indicates the presence of flavonoid aglycones (Nagori *et al.*, 2025). A few drops of sodium hydroxide were added, and a small yellow colouration showed the presence of a flavonoid.

Test for glycoside

The extract was dissolved in glacial acetic acid. The test tube was held at an angle of 45°C, 1 ml of concentrated H₂SO₄ was added down the side. Purple ring at the interface indicates cardiac glycosides (Lawal *et al.*, 2019).

Test for phenol compounds

A few drops of neutral ferric chloride (FeCl₃) were added to the solution of the extract. Formation of a deep blue, green, purple, or black colouration indicates the presence of phenolic compounds (Ahmed *et al.*, 2022).

Test for resins

The extract was dissolved in acetone, and an equal volume of water was added. A turbid or cloudy precipitate indicates the presence of resins (Amini *et al.*, 2022).

Test for saponins

A small quantity of the extract was dissolved in 10 ml of distilled water. This was then shaken vigorously for 30 seconds and was allowed to stand for 30 minutes (Shaikh *et al.*, 2022). A honeycomb formed for more than 30 minutes indicates saponins.

Test for steroids and triterpenes (Lieberman Burchard's test)

An equal volume of acetic anhydride was added to the extract. 1 ml of concentrated sulphuric acid was added down the side of the tube. The colour change was observed immediately, and later red-pink or purple colour indicates the presence of triterpenes, while blue or green colour indicates steroids.

Test for tannins

Three drops of lead sub-acetate solution were added to a solution of the extract. A coloured precipitate indicates tannins (Ibrahim *et al.*, 2023). 0.5 ml of the extract was dissolved in 10 ml of distilled water, then filtered. A few drops of ferric chloride solution were added to the filtrate. Formation of blue-black precipitate indicates tannins, and green precipitate indicates the presence of condensed tannin.

Test for terpenoids

2 ml of chloroform was added to 5 mL of the extract, carefully, add 3 mL of concentrated sulfuric acid (H₂SO₄) along the sides of the test tube to form a layer (Balamurugan *et al.*, 2019). A reddish-brown colouration at the interface indicates the presence of terpenoids.

Standardization of bacterial inoculum

1% v/v solution of sulphuric acid was prepared by adding 1 ml of concentrated H₂SO₄ to 99 ml of water and mixing. 1% w/v of BaCl₂ was also prepared by dissolving 0.5 g of dehydrated BaCl₂ in 50 ml of distilled water. 0.6 ml of BaCl₂ solution was added to 99.4 ml solution of sulphuric acid and mixed (Saâ *et al.*, 2022). A loopful of each of the recovered isolates was transferred into bottles containing normal saline and compared with a 0.5 MacFarland standard.

Screening of the orange peel extract for antibacterial activity

Agar well diffusion method

Muller-Hinton agar was prepared for testing the antibacterial activity of the extract against the bacterial isolates. A sterile cotton swab was used to inoculate the freshly prepared standardised inoculum of the test organisms on the Muller-Hinton agar plates. The plates were allowed to stay for a few minutes. A sterile cork borer (6mm in diameter) was used to bore four (4) wells on the inoculated plates labelled 200 mg/ml, 100 mg/ml, 50 mg/ml and 25 mg/ml. About 0.1 ml of different concentrations of the extracts was transferred into the wells, commercially prepared antibiotic disc was added at the centre of the plate to serve as a control. The same procedure was carried out for each bacterial isolate and for both the ethanolic and aqueous extracts. The plates were kept for 20 minutes so as to allow the extract to diffuse into the agar. The plates were incubated at 37°C for 24 hours, after which the antibacterial activity was determined by measurement of the diameter of the zone of inhibition (mm) against the bacterial isolates (Oghenejobo *et al.*, 2022).

Determination of minimum inhibitory concentration and minimum bactericidal concentration (MIC and MBC)

The initial concentration of the orange peel extracts (200 mg/mL) was diluted using double-fold serial dilution by transferring 5 mL of the sterile peel extract (stock solution) into 5 mL of sterile nutrient broth to obtain 100 mg/mL concentration. That was repeated to subsequently obtain

Table1. Morphological and biochemical characterization of the isolates.

S/N	Morp. Shape	Gram	Ind.	Cit.	Urea	MR	VP	Coa.	Cat.	TSI						Prob. Org.
										Lac.	Glu.	Suc.	Gas.	H2S	Mot.	
1.	Cocci	+ve	-	-	+	+	+	+	+	+	+	+	-	-	-	<i>S. aureus</i>
2.	Rod	-ve	+	-	-	+	-	-	+	+	+	-	+	-	+	<i>E. coli</i>
3.	Rod	-ve	-	+	-	-	-	-	+	-	+	-	+	-	+	<i>P. aeruginosa</i>

KEY: TSI = Triple Sugar Iron, Lac. = Lactose, Glu. = Glucose, Suc. = Sucrose, H2S. = Hydrogen sulfide, Mot. = Motility, Ind. = Indole, Cit. = Citrate, MR = Methylene Red, VR = Voges-proskauer, Coa. = Coagulase, Cat. = Catalase, Prob. Org = Probable organisms, Morp. Shape = Morphological shape, *E. coli* = *Escherichia coli*, *S. aureus* = *Staphylococcus aureus*, *P. aeruginosa* = *Pseudomonas aeruginosa*, Positive = +ve, Negative = -ve.

dilutions of 50 mg/mL, 25 mg/mL, 12.5 mg/mL and 6.25 mg/mL, respectively. Each concentration was inoculated with 0.1 mL of the standardised bacterial cell suspension and incubated at 37°C for 24 hours. The turbidity or cloudiness of the broth indicated the growth of the bacterial inoculum in the broth medium. The lowest concentration of extract that inhibited bacterial isolates' growth was considered the Minimum Inhibitory Concentration (Chikezie, 2017).

RESULTS

Table 1 shows the microbiological and biochemical characteristics of the isolates, in which *Staphylococcus aureus* appeared as Gram-positive cocci and reacted positively to urease, methyl-red, Voges-proskauer, coagulase, catalase, Lactose, glucose, sucrose and negatively to indole, citrate, gas, hydrogen sulfide and motility. *Escherichia coli* appeared Gram-negative Rod and reacted positively to indole, Methyl red, catalase, lactose, glucose, gas, motility and negatively to citrate, urease, Voges-Proskauer, coagulase, sucrose and hydrogen sulfide. *Pseudomonas aeruginosa* appeared as a Gram-negative rod and reacted positively to citrate, catalase, glucose, motility and negatively to indole, urease, Methyl

red, Voges-proskauer, coagulase, lactose, sucrose, gas, and hydrogen sulfide.

Table 2 shows the phytochemical components of orange peel extract. In which there is an absence of alkaloids, resins, steroids, and triterpenes in both the aqueous and ethanol extracts. While presence of flavonoid, phenol compounds, saponins, tannins, and triterpenoids in both the aqueous and ethanol extracts. And glycosides were present in the aqueous extract, while absent in the ethanol extract.

Table 3 shows the antibacterial activity of the ethanolic extracts of the orange peel on the test isolates. Which *Staphylococcus aureus* presented a high zone of inhibition of 23 mm at 200 mg/ml, and a low zone of inhibition of 13 mm at 25 mg/ml, with ciprofloxacin 30 mm zone of inhibition. *Escherichia coli* presented a high zone of inhibition of 20mm at 200mg/ml and a low zone of inhibition of 12mm at 25 mg/ml, with a ciprofloxacin 27mm zone of inhibition. *Pseudomonas aeruginosa* presented a high zone of inhibition of 19mm at 200mg/ml and a low zone of inhibition of 10mm at 25mg/ml, with ciprofloxacin 26 mm zone of inhibition. That is, all the isolates were more susceptible to the ethanolic extract at the highest concentration.

Table 4 shows the antibacterial activity of the aqueous extracts of the orange peel on the test

isolates. Which *Staphylococcus aureus* presented a high zone of inhibition of 21 mm at 200 mg/ml, and a low zone of inhibition of 12 mm at 25 mg/ml, with a ciprofloxacin 30 mm zone of inhibition. *Escherichia coli* presented a high zone of inhibition of 19 mm at 200 mg/ml, and a low zone of inhibition of 11mm at 25 mg/ml, with ciprofloxacin 27 mm zone of inhibition. *Pseudomonas aeruginosa* presented a high zone of inhibition of 18mm at 200 mg/ml, and a low zone of inhibition of 10 mm at 25 mg/ml, with ciprofloxacin 26 mm zone of inhibition. That is, all the isolates were also more susceptible to the aqueous extract at the highest concentration.

Table 5 shows the minimum inhibitory and minimum bactericidal concentrations of the ethanolic extract. *Staphylococcus aureus* presents an MIC of 12.5 mg/ml, *Escherichia coli* and *Pseudomonas aeruginosa* present an MIC of 25 mg/ml. *Staphylococcus aureus* and *Escherichia coli* present an MBC of 50 mg/ml, while *Pseudomonas aeruginosa* presents an MBC of 150 mg/ml.

Table 6 shows the minimum inhibitory and minimum bactericidal concentrations of aqueous extract. *Staphylococcus aureus* and *Escherichia coli* present an MIC of 25 mg/ml, and *Pseudomonas aeruginosa* present an MIC of 50 mg/ml. All isolates present an MBC of 100 mg/ml.

Table 2. Phytochemical components of orange peel.

Phytochemical components	Extracts	
	Ethanol	Aqueous
Alkaloids	-	-
Flavonoids	+	+
Glycosides	-	+
Phenol compounds	+	+
Resins	-	-
Saponins	+	+
Steroids	-	-
Tanins	+	+
Terpenoids	+	+
Triterpenes	-	-

Key: + = phytochemical present, - = phytochemical absent.

Table 3. Antibacterial activity of ethanolic extracts of orange peel on test isolates.

Bacterial Isolates	Zone of inhibition (mm)			Ciprofloxacin disc	
	200 (mg/ml)	100 (mg/ml)	50 (mg/ml)	25 (mg/ml)	Potency
<i>S. aureus</i>	23	18	17	13	30
<i>E. coli</i>	20	16	14	12	27
<i>P. aeruginosa</i>	19	16	13	10	26

Key: mg/ml = Milligram per mil, mm = Millimetre.

Table 4. Antibacterial activity of aqueous extract of orange peel on test isolates.

Bacterial Isolates	Zone of inhibition (mm)			Ciprofloxacin disc	
	200 (mg/ml)	100 (mg/ml)	50 (mg/ml)	25 (mg/ml)	Potency
<i>S. aureus</i>	21	17	14	12	30
<i>E. coli</i>	19	16	13	11	27
<i>P. aeruginosa</i>	18	15	12	10	26

Key: mg/ml = Milligram per mil, mm = Millimetre.

Table 5. Minimum Inhibitory and Minimum Bactericidal concentration of Ethanol extract.

Bacterial isolates	MIC (mg/ml)	MBC (mg/ml)
<i>S. aureus</i>	12.5	50
<i>E. coli</i>	25	50
<i>P. aeruginosa</i>	25	150

Key: MIC = Minimum inhibitory concentration, MBC = Minimum bactericidal concentration, mg/ml = milligram per mil.

Table 6. Minimum inhibitory and minimum bactericidal concentration of aqueous extract.

Bacterial isolates	MIC (mg/ml)	MBC (mg/ml)
<i>S. aureus</i>	25	100
<i>E. coli</i>	25	100
<i>P. aeruginosa</i>	50	100

Key: MIC = Minimum inhibitory concentration, MBC = Minimum bactericidal concentration, mg/ml = milligram per mil.

DISCUSSION

This study evaluates the potential of *Citrus sinensis* (orange) peel extracts as an antibacterial agent against common bacterial isolates from wounds of patients attending Ahmad Sani Yariman Bakura Specialist Hospital, Gusau. The study focused on identifying the active phytochemical constituents and determining the antibacterial efficacy, including minimum inhibitory and bactericidal concentrations. The phytochemical screening revealed the presence of several bioactive compounds in both ethanolic and aqueous extracts of *Citrus sinensis* peel, namely: flavonoids, phenolic compounds, saponins, tannins, and terpenoids. Glucoside was exclusively found in the aqueous extract. Notably, alkaloids, triterpenes, steroids, and resins were absent in both extracts. The presence of these phytochemicals is significant, as they are widely recognised for their antimicrobial, antioxidant, and anti-inflammatory properties. This report is in agreement with the findings of Shin *et al.* (2020), who similarly observed the presence and effectiveness of both ethanol and aqueous extracts in their study. Their results support this current finding, suggesting that different solvent types can extract a wide range of bioactive compounds with potential antimicrobial properties. Rodríguez *et al.* (2023) also reported that flavonoids and phenolic compounds are known to exert antibacterial effects by disrupting bacterial cell membranes, inhibiting enzyme activity, or interfering with DNA synthesis. Saponins can cause leakage of cell membranes, while tannins are known to precipitate proteins, which can inhibit microbial growth (Kholif, 2023). Terpenoids also possess documented antimicrobial activities.

A study by Abdelazem *et al.* (2021) also reported the presence of flavonoids, tannins, saponins, and terpenoids in *Citrus sinensis* peel extracts. However, Abdelazem *et al.* (2021) have reported the presence of alkaloids and some other compounds in *Citrus sinensis* peel that were absent in this current study. This could be attributed to variations in extraction methods, geographical location, plant variety, or analytical sensitivity. The unique presence of glucoside in the aqueous extract highlights potential differences in the polarity of active compounds and their solubility in different solvents (Kaczorová *et al.*, 2021).

The antibacterial assay demonstrated that both ethanolic and aqueous extracts of *Citrus sinensis* peel possess significant inhibitory activity against the tested wound pathogens. The activity was observed to be dose-dependent, with higher concentrations yielding larger zones of inhibition, which is a typical characteristic of antimicrobial agents.

The ethanolic extract showed strong activity, with zones of inhibition reaching 23±00 mm for *Staphylococcus aureus*, 20±00 mm for *Escherichia coli*, and 19±00 mm for *Pseudomonas aeruginosa* at 200 mg/ml. The aqueous extract also exhibited considerable activity, with zones of 21±00 mm for *Staphylococcus aureus*, 19±00 mm for

Escherichia coli, and 18±00 mm for *Pseudomonas aeruginosa* at 200 mg/ml, respectively. Comparing the two extracts, the ethanolic extract generally showed slightly higher activity, suggesting that some of the active compounds with better antibacterial properties are more soluble in ethanol. However, both extracts demonstrated promising results.

These findings are in agreement with the study by Chaturvedi *et al.* (2024) which also reported antibacterial activity of *Citrus sinensis* peel extracts against *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* isolated from wound infections, with zones of inhibition of 7±0.0 mm at 50 mg/ml and 16±2.0 mm at 200 mg/ml against *Escherichia coli*; *Pseudomonas aeruginosa* at 7±0.0 mm, 50 mg/ml and 15±1.0 mm at 200 mg/ml *Klebsiella pneumoniae* 6±0.0 mm at 50 mg/ml and 15±1.0 mm at 200 mg/ml for the aqueous extract, Ethyl acetate produced a zone of inhibition of 9±0.0 mm and 19±1.0mm with *Klebsiella pneumoniae* at 50mg/ml and 200 mg/ml; *Escherichia coli* at 8±0.0 mm and 14±1.0 mm at 50 mg/ml and 200 mg/ml respectively; *Pseudomonas aeruginosa* was susceptible to ethanol extract, giving zones of inhibition of 6±0.0 mm and 14±0.0 mm at the concentrations of 50mg/ml and 200 mg/ml, respectively.

The Ciprofloxacin control consistently produced larger zones of inhibition (26±00, 27±00 and 30±00 mm, respectively), indicating its superior potency. This is expected, as Ciprofloxacin is a purified, potent synthetic antibiotic. However, the demonstrated activity of the orange peel extracts suggests their potential as complementary or alternative natural agents, especially in an era of increasing antibiotic resistance. The minimum inhibitory concentration and minimum bactericidal concentration values further quantify the antibacterial potency of the extracts. Ethanolic extract showed the minimum inhibitory concentration of 12.5 mg/mL and minimum bactericidal concentration of 50 mg/ml for *Staphylococcus aureus*. *Escherichia coli* and *Pseudomonas aeruginosa* showed a minimum inhibitory concentration of 25 mg/ml and a minimum bactericidal concentration of 50 mg/ml. Aqueous extract showed a minimum inhibitory concentration of 25 mg/ml and a minimum bactericidal concentration of 100mg/ml for *Staphylococcus aureus* and *Escherichia coli*. *Pseudomonas aeruginosa* minimum inhibitory concentration of 50 mg/ml and a minimum bactericidal concentration of 100 mg/ml. The lower minimum inhibitory concentration values of the ethanolic extract, particularly for *Staphylococcus aureus*, suggest its higher potency compared to the aqueous extract.

The minimum bactericidal concentration values being higher than minimum inhibitory concentration values are expected, as a higher concentration is usually required to kill bacteria than merely inhibit their growth. For *S. aureus* and *E. coli*, the minimum inhibitory concentration of 12.5-25 mg/ml are within the range reported by Tayyab *et al.* (2021) with the minimum inhibitory concentration values

for *Citrus sinensis* peel ethanol extract against *S. aureus* as low as 0.625 mg/ml and 10 mg/ml for the minimum bactericidal concentration, The higher minimum inhibitory concentration and minimum bactericidal concentration for *Pseudomonas aeruginosa* (50 mg/ml and 100 mg/ml for aqueous extract) indicate that this pathogen is comparatively more resistant to the orange peel extracts than *Staphylococcus aureus* and *Escherichia coli*, which aligns with its known intrinsic resistance mechanisms.

Conclusion

This study demonstrated that both ethanolic and aqueous extracts of *Citrus sinensis* (orange) peel possess significant antibacterial activity against bacterial pathogens commonly associated with wound infections, namely *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The phytochemical analysis confirmed the presence of various bioactive compounds, including flavonoids, phenolic compounds, saponins, tannins, and terpenoids, which are likely responsible for the observed antimicrobial effects. While the synthetic antibiotic Ciprofloxacin exhibited stronger activity, the promising inhibitory and bactericidal concentrations were achieved. These findings underscore the traditional knowledge of medicinal plants and suggest that *Citrus sinensis* peel, often considered a waste product, could be a valuable source for developing novel antimicrobial interventions.

Recommendations

Based on the findings of this study, the following are recommended;

1. The isolation and purification of the specific phytochemical compounds responsible for the most potent antibacterial activity from the orange peel extracts should be prioritised so as to allow for a more targeted approach to drug development.
2. Comprehensive toxicological studies should be performed to ensure the safety of the extracts for therapeutic use, including acute, sub-chronic, and chronic toxicity assessments.
3. Studies that demonstrate promising results and safety should proceed to clinical trials to assess the efficacy and safety of the formulated products in human patients with wound infections.

CONFLICT OF INTEREST

The authors declared no conflict of interest exists.

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