Original Research Article

PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL EVALUATION OF *CITRUS SINENSIS* PEEL EXTRACT AGAINST *STAPHYLOCOCCUS AUREUS* AND *ESCHERICHIA COLI*

ABSTRACT

Background: *Citrus sinensis* is an important commercial fruit crop widely cultivated worldwide, including Nigeria, and belongs to the family Rutaceae, with nutritional, medicinal, and economic value.

Aims: This study evaluated the phytochemical composition and antibacterial activity of the methanolic peel extract of *Citrus sinensis* against *Staphylococcus aureus* and *Escherichia coli.*

Methods: The test organisms were obtained from the Specialist Hospital Gombe; the plant part was extracted by maceration using methanol as the solvent. The phytochemical screening was carried out using standard protocol, while the inoculation of the test organisms was performed using standard microbiological procedures, and the antibacterial evaluation of the crude extract was conducted using the agar well diffusion method.

Results: Phytochemical screening revealed the presence of some bioactive compounds such as tannins, steroids, flavonoids, alkaloids, saponins, and phenolic compounds. The crude extract exhibited concentration-dependent inhibition, with zones of 22, 17, 13, 9, and 6 mm for *Escherichia coli,* and 16, 14, 10, 7, and 6 mm for *Staphylococcus aureus.* Ciprofloxacin and clindamycin served as positive controls, producing inhibition zones of 30 mm and 23 mm, respectively. MIC and MBC values were 25 mg/mL and 50 mg/mL for *Escherichia coli,* and 50 mg/mL and 100 mg/mL for *Staphylococcus aureus.*

Conclusion: These findings indicate that *Citrus sinensis* peel possesses significant antibacterial potential and may serve as a natural alternative for combating bacterial infections.

Keyword

Citrus sinensis, Methanolic extract, Phytochemical screening, Antibacterial activity, *Staphylococcus aureus* and *Escherichia coli*

1.0 INTRODUCTION

Medicinal plants have long played a vital role in managing infections and promoting health due to their rich phytochemical profiles (Ahameethunisa & Hopper, 2010; Bibi et al., 2011; Umer et al., 2013; Awouafack et al., 2013) . Species such as *Azadirachta indica* (neem), *Allium sativum* (garlic), *Zingiber officinale* (ginger), *ca1purnia aurea* (Ahameethunisa & Hopper, 2010; Umer et al., 2013; Pendota et al., 2017), *Arisaema flavum* (Bibi et al., 2011), *syzygium cumini* (Jahan et al., 2011a) and *Citrus sinensis* (sweet orange) (Atwaa et al., 2022) possess strong antibacterial properties that inhibit the growth of various pathogenic microorganisms (Thielmann et al., 2019). The peels of *Citrus sinensis* (Atwaa et al., 2022), in particular, are rich in flavonoids (Ahameethunisa & Hopper, 2010), alkaloids (Djeussi et al., 2013), essential oils (Thielmann et al., 2019), and phenolic compounds that have been reported to exhibit potent antibacterial activity (Atwaa et al., 2022). These natural agents provide significant therapeutic value and serve as important alternatives or supplements to modern antibiotic treatments.

Citrus sinensis (sweet orange) (Atwaa et al., 2022) is a widely cultivated *citrus species* known for its nutritional, aromatic, and medicinal value. Botanically belonging to the family Rutaceae, *Citrus sinensis* contains numerous bioactive secondary metabolites distributed across its peel, pulp, leaves, and seeds. Several phytochemical studies have demonstrated that extracts of *Citrus sinensis* are rich in flavonoids, alkaloids, quinones, tannins, saponins, terpenoids, essential oils, phenolic acids (Atwaa et al., 2022), and other antioxidant compounds (Pendota et al., 2017). These phytochemicals are commonly identified through solvent extraction methods using ethanol (Jahan et al., 2011; Pirtarighat et al., 2019), methanol (Bibi et al., 2011; Umer et al., 2013), and aqueous ethanol (Atwaa et al., 2022), which have consistently shown the ability to extract significant quantities of hesperidin, naringin, limonene, linalool, polymethoxylated flavones, and other antimicrobial constituents. Because these compounds possess broad-spectrum antibacterial and antioxidant properties (Pendota et al., 2017), *Citrus sinensis* has gained research attention for inhibiting several pathogenic microorganisms.

Previous studies have reported that extracts especially methanolic and ethanolic peel extracts of *Citrus sinensis* exhibit measurable antibacterial activity against both Gram-positive and Gram-negative bacteria (Djeussi et al., 2013). In particular, research has shown strong inhibitory potential against *Escherichia coli* and *Staphylococcus aureus*, two medically significant pathogens responsible for common gastrointestinal, respiratory, skin, and systemic infections (Okigbo & Mmeka, 2008; Blanche et al., 1996; Snowden et al., 2014; Thielmann et al., 2019; Elisha et al., 2017) These findings support the increasing interest in using *citrus*-based phytochemicals as alternative or complementary antimicrobial agents, especially as global antibiotic resistance continues to rise.
The exploration of medicinal plants for therapeutic drug development is not new.

Historically, several commercially important drugs originate from plant-derived compounds such as quinine, artemisinin, morphine, aspirin, and vincristine demonstrating the pharmaceutical potential of natural products. Numerous modern antibiotics, antivirals, antifungals (Sulub-Tun et al., 2020), and anticancer agents have been developed or inspired by phytochemicals, emphasizing the significance of plant-based research in discovering new, effective, and safer therapeutic agents. This has encouraged continued investigation into easily accessible medicinal plants like *Citrus sinensis* for their antimicrobial value.

Escherichia coli is a Gram-negative, rod-shaped bacterium commonly found in the human gastrointestinal tract (Djeussi et al., 2013). While many strains are harmless, pathogenic strains can cause severe diarrheal diseases (Umer et al., 2013), urinary tract infections, and foodborne illnesses (Umer et al., 2013). Its intrinsic resistance mechanisms including outer membrane impermeability often make it difficult to treat, thereby increasing the need for alternative antimicrobial agents derived from natural sources.

sStaphylococcus aureus, on the other hand, is a Gram-positive coccus associated with skin infections, wound infections, pneumonia, sepsis, and toxin-mediated diseases (Okigbo & Mmeka, 2008; Palma & Cheung, 2001). Due to the widespread emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) and other multidrug-resistant strains, this pathogen represents a major clinical challenge (Blanche et al., 1996). Natural plant extracts with potent antibacterial properties (Palma & Cheung, 2001; Snowden et al., 2014), such as those from *Citrus sinensis*, may offer promising complementary strategies for limiting the growth and pathogenicity of *S. aureus*.

Therefore, evaluating the antibacterial activity of *Citrus sinensis* against *Escherichia coli* and *Staphylococcus aureus* is essential for understanding its therapeutic potential and for expanding the scientific basis for plant-derived antimicrobial agents.

The aim of the study is to evaluate the antibacterial activity peel of *Citrus sinensis* extract against *Staphylococcus aureus* and *Escherichia coli.*

2.0 MATERIALS AND METHODS

2.1 Study Area

This study was carried out in Tudun Wada, Gombe State, Nigeria, within the premises of Gombe State University (GSU). Gombe State is located in the North-eastern region of Nigeria and experiences a tropical savannah climate characterized by distinct dry and rainy seasons, with average ambient temperatures ranging between 27–35°C. These climatic conditions are suitable for the collection and processing of plant materials such as *Citrus sinensis*. All laboratory analyses were conducted within two specialized laboratories at GSU, The Microbiology Laboratory was utilized for all bacteriological procedures, including the handling of clinical isolates, preparation of bacterial cultures, inoculum standardization, antibacterial sensitivity testing using agar well diffusion, and the determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). This laboratory is equipped with essential microbiological facilities such as incubators, autoclaves, laminar flow cabinets, analytical balances, microscopes, and sterilization equipment, ensuring aseptic conditions and reliability of experimental results. Botany Laboratory served as the center for plant-related analyses, including botanical authentication of *Citrus sinensis* and qualitative phytochemical screening of the methanolic peel extract. This section ensured accurate identification of the plant material and characterization of its bioactive constituents prior to antimicrobial evaluation. All experimental procedures were performed following standard laboratory safety and research ethics guidelines within the controlled environment of the university campus, thereby ensuring the accuracy, reproducibility, and safety of the study.

2.2 Samples Collection, Confirmation and Preparation

Clinical isolates of *Staphylococcus aureus* and *Escherichia coli* were obtained from the Microbiology Laboratory of the Specialist Hospital, Gombe. The isolates were collected as pure cultures on nutrient agar slants and transported aseptically to the laboratory for further analysis. Preliminary confirmation of the isolates was performed using Gram staining and observation of cellular morphology under the microscope. Isolates that appeared as Gram-positive cocci arranged in clusters were presumptively identified as *Staphylococcus aureus*, whereas those appearing as Gram-negative rod-shaped cells were considered presumptive *Escherichia coli.*

2.2.1 Biochemical Tests

Further confirmation of the isolates was carried out using standard biochemical tests following the methods described by Woo et al. (2008), Catalase Test is used to differentiate *Staphylococcus* species (catalase-positive) from *Streptococcus* species. *Staphylococcus aureus* produced immediate bubbling, indicating a positive reaction. Indole Test is used to detect the production of indole from tryptophan metabolism. *Escherichia coli* showed a positive reaction, confirming its identity. Citrate Utilization Test is used to determine the ability of the isolate to utilize citrate as the sole carbon source. *Escherichia coli* gave a negative result, supporting identification. Oxidase Test is used to differentiate oxidase-negative enteric bacteria such as *Escherichia coli* from oxidase-positive organisms. *Escherichia coli* tested negative. Motility Test is used to determine the presence of flagella. *Escherichia coli* was motile, whereas *Staphylococcus aureus* was non-motile. The combined results of Gram staining, colony morphology, and biochemical reactions confirmed the identities of the isolates as *Staphylococcus aureus* and *Escherichia coli*, which were subsequently used for antibacterial sensitivity testing.

2.2.2 Preparation of *Citrus sinensis*

Fresh fruits of *Citrus sinensis* (sweet orange) were obtained from Gombe Tudun Wada main market and authenticated by a qualified botanist at the Department of Biological Sciences, Gombe State University to ensure correct taxonomic identification of the plant material. The fruits were thoroughly washed under running water to remove soil particles, debris, and surface contaminants. The peels were carefully separated from the pulp using sterile knives to prevent contamination and subsequently rinsed with distilled water to eliminate residual sugars and microorganisms. The cleaned peels were air-dried under shade for 14 days at ambient room temperature ranging between 35°C. Shade drying was employed to prevent photodegradation and thermal destruction of heat-sensitive phytochemicals that may occur under direct sunlight. Drying was continued until the peels became brittle and moisture-free, ensuring effective grinding and long-term stability of the bioactive compounds. The dried peels were initially crushed using a mortar and pestle to reduce particle size and then milled into a fine powder using a mechanical grinder. This step increased the surface area of the plant material, thereby enhancing solvent penetration and extraction efficiency during the maceration process. A measured quantity of 30 g of the powdered peel was weighed using an analytical balance and stored in an airtight container in a cool, dry environment prior to extraction. Proper storage was necessary to protect the sample from moisture absorption, oxidation, and microbial contamination, thereby preserving the integrity of the phytochemicals until further analysis.

2.2.3 Extraction of Peel Material

Fresh peels of *Citrus sinensis* were thoroughly washed with clean water to remove adhering dirt and contaminants, air-dried at room temperature, and then ground into a fine powder using a sterile mechanical grinder. The powdered material was stored in an airtight container until extraction. Maceration extraction was carried out by immersing 30 g of the powdered peel in 100 mL of analytical-grade methanol in a sterile conical flask (Jmii et al., 2020). Methanol was selected as the extraction solvent due to its high polarity and efficiency in dissolving a wide range of phytochemicals, including flavonoids, phenolic acids, and other bioactive compounds known for antimicrobial activity. The flask was tightly sealed with aluminum foil to prevent solvent evaporation and contamination and then kept at room temperature for 48 hours with intermittent shaking. The shaking process enhanced solvent penetration into the plant matrix and facilitated the release of intracellular bioactive constituents into the solvent phase. After the maceration period, the extract was first filtered through sterilized cotton cloth to remove coarse plant debris, followed by filtration through Whatman No. 1 filter paper to obtain a clear filtrate. The filtrates were then concentrated using a rotary vacuum evaporator at 40°C and 110 rpm, which allowed removal of methanol under reduced pressure, thereby preserving heat-labile compounds. The semi-solid concentrate was further dried on a water bath and in a fume cupboard to eliminate any residual solvent, yielding a dark-colored crude extract. The dried extract was weighed, labeled as "Methanolic Peel Extract of *Citrus sinensis,*" and stored in a sterile container at 4°C until further use (Pendota et al., 2017). This extraction method ensured the recovery of a broad spectrum of phytochemicals responsible for the antibacterial activity observed in subsequent sensitivity tests.

2.2.4 Phytochemical Screening of the Plant Extract

Qualitative phytochemical screening was conducted on the crude extract to detect major secondary metabolite classes. One gram of crude extract was dissolved in 10 mL deionized water and used for the following standard tests. Alkaloids were detected using Wagner's reagent with the appearance of a reddish-brown precipitate indicating a positive result (Kaya et al., 2017). Tannins were identified by adding ferric chloride and observing dark green or blue coloration. Flavonoids were detected by adding a few drops of 2 % sodium hydroxide and noting a pale-yellow coloration. Phenolic compounds were screened using gelatin solution where a white precipitate indicated presence. Saponins were tested with the frothing (foam) test where persistent foam indicated saponins. Steroids were screened by adding chloroform and concentrated sulfuric acid and observing a red ring at the interface. Glycosides were assessed by hydrolyzing with dilute acid, neutralizing and adding Fehling's solution; a brick-red precipitate indicated reducing glycosides. All phytochemical tests were performed in duplicate and observations recorded.

2.3 Preparation of Extract Concentrations

A stock solution of *Citrus sinensis* extract was prepared at a concentration of 100 mg/mL by dissolving 1.0 g of the dried extract in 10 mL of dimethyl sulfoxide (DMSO). DMSO was selected as the solvent because of its ability to dissolve both polar and non-polar phytochemicals and its minimal antibacterial activity at low concentrations. From the stock solution, a series of two-fold serial dilutions were prepared to obtain working concentrations of 50 mg/mL, 25 mg/mL, and 12.5 mg/mL. These concentrations were used in the antibacterial sensitivity assay to evaluate the dose-dependent effect of the extract against the test organisms.

2.3.1 Standardization of Inoculum

Fresh bacterial isolates less than 24 hours old were used to ensure active growth. Colonies were suspended in sterile normal saline and mixed thoroughly. The turbidity of each suspension was visually adjusted to match the 0.5 McFarland standard, which corresponds to approximately 1.5×10^8 CFU/mL. This standardization step is critical because variations in inoculum density can significantly influence antibacterial susceptibility results. By adjusting all suspensions to the same turbidity, reproducibility and reliability of the sensitivity testing were ensured, allowing meaningful comparison of the antibacterial activity of *Citrus sinensis* extract against both test organisms.

2.4 Antibacterial Sensitivity Testing

The antibacterial activity of *Citrus sinensis* extract was evaluated using the Agar Well Diffusion Method as described by Pendota et al. (2017). Mueller–Hinton agar (MHA) was selected because of its reproducibility, uniform diffusion characteristics, and acceptance by the Clinical and Laboratory Standards Institute (CLSI) for antimicrobial susceptibility testing. Standardized bacterial suspensions of *Staphylococcus aureus* and *Escherichia coli* were prepared to match the turbidity of 0.5 McFarland standard (approximately 1.5×10^8 CFU/ml). Each Mueller–Hinton agar (MHA) plate was uniformly inoculated using a sterile cotton swab to produce a confluent lawn of bacterial growth, ensuring even exposure of the organisms to the test extract. Wells of 6 mm diameter were aseptically bored into the agar using a sterile cork-borer as described by Pirtarighat et al. (2019). Each well was carefully filled with different concentrations of *C. sinensis* extract ranging from 12.5 to 100 mg/ml. Care was taken to avoid overflow and ensure proper diffusion of the extract into the agar matrix. Ciprofloxacin (for *E. coli*) and Clindamycin (for *S. aureus*) were used as positive controls according to CLSI guidelines (Jahan et al., 2011) to validate the susceptibility of the test organisms and confirm the reliability of the assay. Sterile distilled water or solvent served as the negative control. The plates were incubated at 37°C for 24 hours. After incubation, antibacterial activity was assessed by observing the presence of zones of inhibition clear areas around the wells where bacterial growth was suppressed. The diameters of these zones were measured in millimeters using a transparent ruler. Larger zones of inhibition indicated greater sensitivity of the organism to the extract, whereas smaller or absent zones suggested resistance or low susceptibility.

2.4.1 Determination and Interpretation of MIC and MBC

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Citrus sinensis* extract were determined using the broth dilution method as described by Elisha et al. (2017). A standardized inoculum (10 µL) of each test organism was added to tubes containing serial dilutions of the extract. Following incubation at 37 °C for 24 h, the tubes were examined for turbidity. The lowest concentration that showed no visible growth was recorded as the MIC. For MBC determination, aliquots from tubes that showed no turbidity during MIC testing were streaked onto fresh nutrient agar plates and incubated at 37 °C for 18–24 h (Elisha et al., 2017). The lowest concentration at which no bacterial colonies were observed on the agar plates was recorded as the MBC.

Organism *Escherichia coli* MIC =25 mg/mL, MBC =50 mg/mL

Organism *Staphylococcus aureus* MIC =50 mg/mL, MBC =100 mg/mL

The results indicate that *Escherichia coli* was inhibited at a lower concentration (MIC = 25 mg/mL) compared with *Staphylococcus aureus* (MIC = 50 mg/mL). This suggests that *E. coli* is more sensitive to the antibacterial constituents of *Citrus sinensis* extract. The lower MIC value for *E. coli* implies that a smaller amount of the extract is sufficient to suppress its visible growth. Similarly, the MBC values show that *E. coli* was completely killed at 50 mg/mL, whereas *S. aureus* required a higher concentration of 100 mg/mL for bactericidal activity. The difference between MIC and MBC for both organisms indicates that the extract exerts a bacteriostatic effect at lower concentrations and a bactericidal effect at higher concentrations. The ratio of MBC to MIC provides insight into the killing efficiency of the extract. For both organisms, the MBC values are approximately twice the MIC values (MBC/MIC ≈ 2). This suggests that *C. sinensis* extract possesses strong bactericidal properties rather than being merely inhibitory. The higher MIC and MBC values observed for *S. aureus*

may be attributed to its thicker peptidoglycan layer, which can act as a barrier to the penetration of plant-derived antimicrobial compounds. In contrast, *E. coli* appears more susceptible to the phytochemicals present in *C. sinensis,* such as flavonoids, citric acid, and essential oils, which may disrupt membrane integrity and interfere with essential cellular processes.

These findings confirm that *Citrus sinensis* extract demonstrates significant antibacterial activity, with greater efficacy against *Escherichia coli* than *Staphylococcus aureus,* and exhibits both inhibitory and bactericidal effects depending on concentration.

3. 0 RESULTS AND DISCUSSION

3. 1 Physical Properties of *Citrus sinensis* Extract

The result for physical characteristics of *Citrus sinensis* extract were recorded in table 1. The

properties include the color, odor, texture, consistency and the weight of both the sample and the extract including the total percentage of the extract yield.

Table 1. Physical Properties of The Extract

Criteria	Properties
Weight of the material prepared	30 g
Weight of the extract recovered	2.2 g
Percentage of the extract yield	7.3 %
Color of the extract	deep orange brown red pale yellow
Consistency of the extract	viscous and thick oily
Odor of the extract	citrusy aroma milder
Texture of the extract	smooth and slightly tacky
Compositional test	malic acid, citric acid and sugars
Nutritional value	mineral pigment, dietary fibers,
monoterpenes	esters and aldehyde

3.2 Phytochemical Components of *Citrus sinensis* extract

The phytochemical analysis of *Citrus sinensis* extract include testing for the present of tannin, flavonoid, saponin, phenol, steroid, alkaloid and glycoside, in which the result were recorded in table 2.

Table 2. Phytochemical Screening of the Extract

Phytochemical	Reagent	Reference
Tannings	2 % $FeCl_3$	+
Steroids	Chloroform + H_2SO_4	+
Flavonoids	2 % NaOH	+
Alkaloids	2 % NaOH	+
Saponins	Foam test	+
Phenols	Folin Ciocalteu	+

Key: + = Present, - = Absent

3.3 Cultural, Morphological and Biochemical Characteristics of *Escherichia coli* and *Staphylococcus aureus*

This involved the phenotypic properties of *Escherichia coli* and *Staphylococcus aureus* such as color of the colony on agar medium and staining for shape and staining properties, either Gram -ve or Gram +ve bacteria on microscopy. The phenotypic result was recorded in table 3. Biochemical characteristic of *Escherichia coli* and *Staphylococcus aureus*, the result of biochemical properties of *Escherichia coli* and *Staphylococcus aureus* were recorded in table 3. Which include indole, catalase, oxidase, motility and citrate test.

Table 3. Cultural, morphological and Biochemical Characteristics of the Test Isolates.

Colony Appearance	Gram Staining Presumptive Microscopy	Organism	Indole	Catalase	Oxidase	Motility	Citrate
Small circular concave pink Colony on MacConkey.	negative short Rods	*Escherichia Coli*	+	+	-	+	-
Small circular smooth yellow Colony on mannitol salt.	positive cocci in cluster	*Staphylococcus aureus*	-	+	-	-	-

Key: + = Present, - = Absent

3.4 Antibacterial sensitivity of the *Citrus sinensis* Crude extract against *Escherichia coli* and *Staphylococcus aureus*

The result of the antibacterial properties of the *citrus sinensis* crude extract against *Escherichia coli and Staphylococcus aureus* indicated that the extract can only inhibit the growth of the organism at a concentration greater than 12.5 mg/mL, with different zones of inhibition based on the concentration of the extract. The result was recorded in Table 4 and Fig. 1. Antibacterial activity of the plant extract against *Escherichia coli* and *Staphylococcus aureus,* expressed as zones of inhibition (mm) at different extract concentrations (100–6.25 mg/mL). The extract showed a concentration-dependent inhibitory effect against both test organisms, with higher concentrations producing larger zones of inhibition. Ciprofloxacin (10 µg) and clindamycin (10 µg) served as positive controls for *E. coli* and *S. aureus*, respectively.

Table 4. Zone of inhibition (mm) of *Citrus sinensis* crude extract against Test Organisms.

Test Organism		**Zone of inhibition (mm)**				**Control**	
Concentrations (Mg/mL)	100	50	25	12.5	6.25	CIP10 µg	DA10 µg
Escherichia coli	22	17	13	9	6	30	–
Staphylococcus aureus	16	14	10	7	6	–	23

Key: +ve = positive control, (mm) = millimeter, CIP = ciprofloxacin, (10µg/disc) DA = clindamycin, (10µg/disc) -ve = Negative control, (mm), (mg/mL) = milligram per milliliter, Note: 6.00 mm = 0 or Nill, zone of the well.

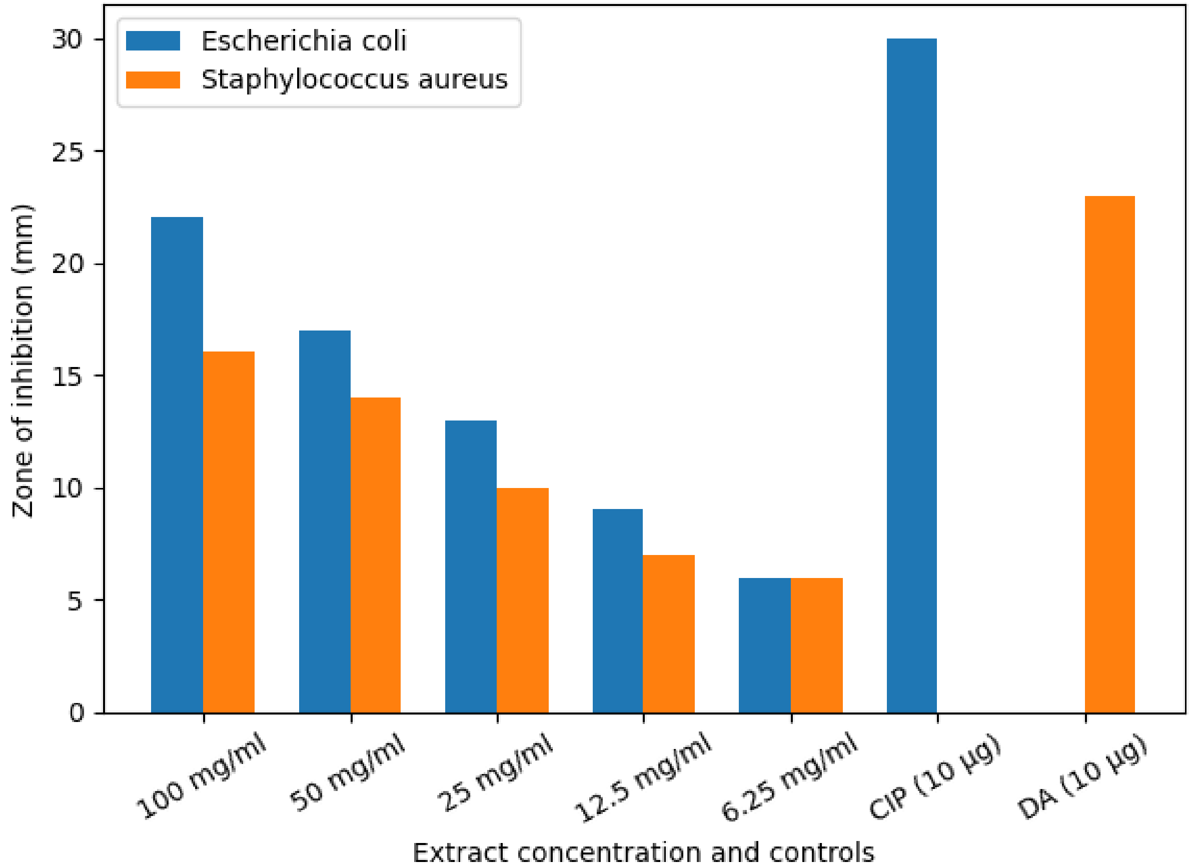

Figure 1: Zones of inhibitions of *Citrus sinensis* peel extract against *Escherichia coli* and *Staphylococcus aureus* at different concentration

3.5 MIC and MBC of methanolic extract of *Citrus sinensis* against *Escherichia coli* and *Staphylococcus aureus*

The result for MIC and MBC of the extract was presented in table 5. The result indicated that, the extract can inhibit and kill the organism at different concentration. i.e. 50 mg/mL and 100 mg/mL respectively.

Table 5. MIC/MBC of The Extract Against the Test Organism

Test of Organism	Methanolic Extract (Mg/ML)	
	MIC (mg/mL)	MBC (mg/mL)
Escherichia coli	25	50
Staphylococcus aureus	50	100

Key: MIC = Minimum Inhibitory Concentration, MBC = Maximum Bactericidal Concentration.

3.6 Discussion

The rising incidence of drugs resistance and the production of extended-spectrum beta-lactamase (ESβL) by some bacterial strains is a great global concern to infectious disease management; hence the quest for a novel plant with the desired phytopharmaceutical constituents is in the arsenal of public health. Curative potential of plants material is due particularly to the presence of pharmacologically active compounds termed phytochemicals (Kudatarkar and Nayak, 2018). In the present study, preliminary phytochemical analysis of methanol extract of *Citrus sinensis* were carried out. Qualitative analysis of methanolic extract of *Citrus sinensis* revealed the presence of bioactive compounds of alkaloids, saponins, tannins; glycoside, polyphenol, steroids and flavonoids as shown in Table 2. The presence of these phytochemicals has been reported by previous researchers (El-Desoukey et al., 2018; Musa et al., 2019 and Nwachukwu et al., 2019). The results given in Table 1 and 2 indicate that methanol extracts of *Citrus sinensis* were evaluated for their antibacterial activity against some medically significant pathogens isolated from specialist hospital (*Staphylococcus aureus*, and *Escherichia coli*) (Table 3). The extract demonstrated a clear concentration-dependent inhibitory effect against both organisms, with zones of inhibition increasing as extract concentration increased "(Fig. 1)". This observation is consistent with earlier reports that plant extracts exhibit enhanced antibacterial activity at higher concentrations due to increased availability with active constituents of bioactive phytochemicals such as alkaloids, flavonoids, tannins, and phenolic compounds responsible for antibacterial effects (Doughari et al., 2012; Zige et al., 2014; Musa et al., 2019). At the highest concentration tested presented in (Figure1), the *C. sinensis* extract produced zones of inhibition of 22 mm, 17 mm, 13 mm, 9 mm, and 6 mm against *E. coli* and 16 mm, 14 mm, 10 mm, 7 mm, and 6 mm against *S. aureus* at 100, 50, 25, 12.5, and 6.25 mg/mL, respectively. A concentration-dependent pattern was observed, with higher extract concentrations producing larger zones of inhibition, a trend commonly reported for plant-derived antibacterial agents (Akinyemi et al., 2005). At 100 mg/mL, the extract produced inhibition zones of 22 mm against *E. coli* and 16 mm against *S. aureus*. As the concentration decreased, the zones of inhibition progressively reduced, with both organisms recording a minimum zone of 6 mm at 6.25 mg/mL, corresponding to the diameter of the well (Table 4). Ciprofloxacin (10 µg) showed a strong inhibitory effect against *E. coli* with a zone of inhibition of 30 mm, while clindamycin (10 µg) produced a zone of inhibition of 23 mm against *S. aureus*.

These results indicate that the extract was more effective against *E. coli* than *S. aureus*, particularly at higher concentrations. This finding contrasts with some previous studies that reported greater susceptibility of Gram-positive bacteria to *C. sinensis* extracts (Nas et al., 2018; Mohammed et al., 2018; Buah et al., 2023), but aligns with reports that susceptibility patterns may vary depending on bacterial strain, plant part used, solvent type, and extraction method not only Gram reaction alone (Shetty et al., 2016; Anibijuwon et al., 2018 Musa et al., 2019; Thielmann et al., 2019). *Citrus sinensis* extract showed good inhibitory activities “as shown in Table 2”. Although, higher abundance of saponins, tannins, and steroids in *C. sinensis* extract could have enhanced its antibacterial activities at low concentration as shown in “Table 2”. At 50 mg/mL concentration “as shown in Table 4 and 5”, *C. sinensis* extract was active against all the tested bacteria, this result is in consonance with the finding conducted in Ilorin by (Anibijuwon et al., 2018). The present study is that the methanolic extract of *Citrus sinensis* exhibited greater antibacterial activity against the Gram-negative bacterium *Escherichia coli* than against the Gram-positive *Staphylococcus aureus*, as evidenced by larger zones of inhibition “as shown in Table 4”, lower MIC (25 mg/ml), and lower MBC (50 mg/mL) values for *E. coli* “as shown in Table 5”. Although Gram-positive bacteria possess a thicker peptidoglycan layer, this structural feature does not always confer increased resistance to all antibacterial agents. In *S. aureus*, the peptidoglycan layer is highly cross-linked and associated with teichoic and lipoteichoic acids, which contribute to cell wall rigidity and reduced permeability to certain bioactive compounds (Palma and Cheung, 2001). This thick and compact structure may act as a physical barrier, limiting the diffusion of phytochemicals such as flavonoids and tannins into the cytoplasmic membrane, thereby reducing antibacterial efficacy.

In contrast, *E. coli*, despite being Gram-negative, possesses a thinner peptidoglycan layer located between the inner cytoplasmic membrane and the outer membrane. While the outer membrane of Gram-negative bacteria is often considered a permeability barrier due to the presence of lipopolysaccharides (LPS), it also contains porin proteins that facilitate the passive diffusion of low-molecular-weight hydrophilic compounds (Blanche et al., 1996; Thielmann et al., 2019). Many phytochemicals present in *C. sinensis*, particularly flavonoids, phenolics, and alkaloids, are capable of traversing these porin channels, allowing them to reach intracellular targets more effectively in *E. coli*. Furthermore, the lipid-rich outer membrane of Gram-negative bacteria may enhance susceptibility to certain plant-derived compounds with amphipathic or lipophilic properties (Nikaido, 2003; Tortora et al., 2019). *Citrus* phytochemicals, especially flavonoids and terpenoids, have been reported to disrupt membrane integrity by interacting with lipid bilayers, leading to increased permeability, leakage of intracellular contents, and eventual cell death (Woo et al., 2008; Thielmann et al., 2019). This membrane-disruptive effect may explain the higher susceptibility of *E. coli* observed in this study. Another contributing factor may be the difference in efflux pump activity between the two organisms. *Staphylococcus aureus* is known to possess efficient multidrug efflux systems that actively expel antimicrobial agents from the cell, thereby reducing intracellular accumulation of bioactive compounds (Palma and Cheung, 2001; Djeussi et al., 2013). Reduced intracellular retention of phytochemicals in *S. aureus* could account for its higher MIC and MBC values compared to *E. coli*. Furthermore, *S. aureus* may possess enzymatic mechanisms capable of detoxifying or modifying certain plant-derived compounds, further diminishing their antibacterial effectiveness (Awouafack et al., 2013; Elisha et al., 2017). These combined factors provide a plausible explanation for the higher MIC and MBC values recorded for *S. aureus* compared to *E. coli* “as shown in Table 5”.

The zones of inhibition produced by the positive control antibiotics ciprofloxacin against *E. coli* (30 mm) and clindamycin against *S. aureus* (23 mm) were significantly larger than those produced by the *C. sinensis* extract at all tested concentrations. This observation is consistent with findings from numerous antibacterial studies comparing crude plant extracts with conventional antibiotics (Okigbo and Mmeka, 2008; Snowden et al., 2014; Shetty et al., 2016). Antibiotics are purified, single-compound agents with specific cellular targets ciprofloxacin inhibits DNA gyrase and topoisomerase IV, while clindamycin inhibits protein synthesis by binding to the 50S ribosomal subunit resulting in rapid and potent antibacterial activity (Blanche et al., 1996; Palma and Cheung, 2001). In contrast, plant extracts are complex mixtures of multiple compounds, many of which may not possess antibacterial activity, thereby diluting the overall potency of the extract. Another critical factor is dosage precision. Antibiotic discs contain accurately measured microgram quantities of active compounds, ensuring consistent and optimal antibacterial activity. In contrast, plant extracts are typically expressed in mg/mL concentrations, which represent crude preparations with unknown proportions of active constituents (Snowden et al., 2014; Buah et al., 2023). Standard antibiotics exhibit superior diffusion characteristics in agar media, resulting in wider zones of inhibition during agar diffusion assays. The relatively large molecular size and variable solubility of phytochemicals can limit their diffusion through agar, leading to smaller inhibition zones even when antibacterial activity is present (Okigbo and Mmeka, 2008; Jahan et al., 2011). Antibiotics such as ciprofloxacin and clindamycin have undergone extensive optimization to enhance cell penetration, target binding affinity, and metabolic stability, characteristics that crude plant extracts inherently lack (Blanche et al., 1996; Thielmann et al., 2019). the standard antibiotics used as positive controls exhibited higher zones of inhibition than the plant extract, the inhibitory activity observed in the extract confirms its antibacterial potential. This supports previous reports that medicinal plants can serve as alternative or complementary sources of antibacterial agents, especially in the face of increasing antibiotic resistance (Akinyemi et al., 2005; WHO, 2014).

These pharmacodynamic advantages translate into stronger and more consistent antibacterial effects. Despite these differences, the relatively large inhibition zones produced by the *C. sinensis* extract particularly against *E. coli* highlight its significant antibacterial potential. Several studies have emphasized that while plant extracts may not outperform conventional antibiotics, their value lies in their potential use as complementary agents, sources of novel antimicrobial compounds, or alternatives in cases of antibiotic resistance (Okigbo and

Mmeka, 2008; Djeussi et al., 2013; Buah et al., 2023).

This study evaluates that the methanolic peel extract of *Citrus sinensis* possesses meaningful antibacterial activity, with greater efficacy against Gram-negative *E. coli* than Gram-positive *S. aureus*. Although the extract was less potent than standard antibiotics, its activity coupled with its phytochemical richness suggests potential application as a complementary antibacterial agent or as a source of novel bioactive compounds for future drug development.

4.0 CONCLUSION

The increasing emergence of drug-resistant bacteria necessitates the search for alternative antimicrobial agents from plant sources. Herbal medicines are produced from different parts of particular plants, for example- seeds, roots, stems, barks, leaves, berries etc. The present study demonstrates that the methanolic peel extract of *Citrus sinensis* possesses measurable antibacterial activity against *Escherichia coli* and *Staphylococcus aureus.* The extract showed clear concentration-dependent inhibition. For *E. coli,* zones of inhibition increased from 6 mm at 6.25 mg/mL to 22 mm at 100 mg/mL, while *S. aureus* showed inhibition zones ranging from 6 mm to 16 mm across the same concentrations. The activity of the extract, although significant, was lower than that of standard antibiotics, with ciprofloxacin (positive control) producing a 30 mm inhibition zone against *E. coli* and clindamycin yielding 23 mm against *S. aureus.* Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) results further supported these findings. *E. coli* exhibited greater susceptibility, with an MIC of 25 mg/mL and MBC of 50 mg/mL, compared to *S. aureus,* which had an MIC of 50 mg/mL and MBC of 100 mg/mL. The lower MIC and MBC values for *E. coli* suggest stronger antibacterial effectiveness of the extract against the Gram-negative organism in this study. The antibacterial effects may be attributed to the presence of phytochemicals such as flavonoids, tannins, alkaloids, saponins, phenols, and terpenoids identified in the extract. While the crude extract demonstrated promising activity, its efficacy remains lower than conventional antibiotics. Therefore, further studies involving purification of active compounds, toxicity evaluation, and mechanism-of-action analysis are necessary before therapeutic application can be considered. The result of the present study revealed that the methanolic extract of the *C. sinensis* peel have antibacterial activity. It can be used as a therapeutic agent in the treatment of gastrointestinal infection. Further detailing study is necessary to observe the mechanism of impacts closely and to draw extractive conclusion.

ETHICAL APPROVAL

Bacterial isolates were obtained from clinical samples provided by Specialist Hospital, Gombe, and used strictly for research purposes under laboratory biosafety guidelines.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest regarding the publication of this study.

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