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(Research Article)

Antimicrobial and Phytochemical Screening of Water Melon (*Citrillus lanatus)* Seeds on Selected Bacterial species

Susana Ekere Ukpong [1, *], Magnus Chinedu Onwuzuruigbe [2] and Mini Prestige Ugwugwueli [3]

[1] *Department of Microbiology, Faculty of Science, Madonna University, Elele, Rivers State.*
[2] *Department of Pharmacology and Therapeutics, Faculty of Clinical Sciences, College of Medicine, Madonna University, Elele, Rivers State.*
[3] *Department of Microbiology, Federal University of Environment and Technology, Sakpenwa / Koroma, Ogoni, Rivers State.*

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Abstract

Watermelon seeds are rich in protein, B vitamins, minerals (such as magnesium. potassium, phosphorous, sodium, iron, zinc, manganese and copper) and fat among others, the antimicrobial compounds found in plants are of great interest because antibiotic-resistance is becoming a worldwide public health concern especially in terms of food-borne illness and hospital-acquired infections. This study investigates the antimicrobial and phytochemical attributes of watermelon *(Citrillus lanatus)* seed extracts against selected bacterial isolates. Ethanol and ethyl acetate were used as solvents to extract bioactive compounds from the seeds. The extract gotten were subjected for phytochemical screening and antimicrobial activity against the following microorganisms; Escherichia coli, Staphylococcus aureus, Salmonella typhi, Klebsiella pneumonia, and Pseudomonas aeruginosa. The result of phytochemical screening revealed the presence of flavonoids, tannins, phenols, alkaloids, glycosides, and saponins. Furthermore, the results of antibacterial activity showed that the ethanol extract exhibited significantly higher antimicrobial activity compared to the ethyl acetate extract, as shown by larger zones of inhibition and lower MIC and MBC values against the test organisms where Escherichia coli was the most susceptible organisms with a minimal inhibitory concentration at 50mg/ml zone of inhibition of 16.0 mm respectively. Similarly, ethyl acetate showed activity against the test isolates where Staphylococcus aureus emerged the most susceptible organisms with a minimal inhibitory concentration at 50mg/ml and zone of inhibition of 15.0 mm respectively. Klebsiella pneumonia, showed resistance to both extracts even at minimal inhibitory concentration above >250mg/ml. using the agar well diffusion method, to determine their minimum inhibitory concentrations (MICs) and minimum bactericidal concentration (MBCs). However, ethyl acetate extracts contained fewer phytochemicals. These investigations support the practical application of watermelon seed extracts, especially ethanol-based, as a natural source of antimicrobial agents. It is also a prove to the growing body of research supporting the advancement of plant-based alternatives to synthetic antibiotics.

Keywords: Antimicrobial; Phytochemical; Screening; Water melon seeds and Selected test microorganisms

1. Introduction

Watermelon *(Citrillus lanatus)* seeds are a source of protein, B-vitamins, minerals (such as magnesium, potassium, phosphorous, sodium, iron, zinc, manganese and copper) and fat among others (Adebo *et al.,* 2025). The antimicrobial compounds found in plants are of interest because antibiotic resistance is becoming a worldwide public health concern especially in terms of food-borne illness and nosocomial infections (Lee, 2025; WHO, 2025). Naturally occurring antimicrobials are being sought as replacements for synthetic preservatives such as parabens (ethyl, methyl, butyl and propyl parabens), butylated hydro-xytoluene (BHT) and butylated hydroxanisole (BHA) that are under scrutiny because

* Corresponding author: Susana Ekere Ukpong

it's been suspected as cancer causing agents (Bergfeld *et al*., 2005; Byford *et al.,* 2002; Sun *et al.*, 2003 Wangensteen *et al*., 2004). The plant kingdom has proven to be the most useful in the treatment of diseases and they provide an important source of all the world's pharmaceuticals (Latif *et al.*, 2025).

Over the years watermelon, like many fruits and vegetables, has been used as a form, now known as Traditional Health Care, for many medical complications. Furthermore, it plays a significant role in Ayurveda and Indian medicine. Watermelon can be described as a fruit which releases vital nutrients such as minerals and bioactive compounds which have plant-based medicinal benefits (Gupta *et al.,* 2023). The antibacterial activity of watermelon is one of its plant-based medicinal benefits which has recently come into focus (Sultan 2017). Watermelon along with other high value food items bring great prospects to human diets. Antimicrobial resistance ("AMR") is a global health concern, the World Health Organization marked for action (WHO, 2018). What occurs when microorganisms evolve the ability to resist our attempts to eliminate them, is what we know as AMR which makes infection immensely more difficult to treat (Ventola, 2015). Furthermore, over and misuse of what we put out to fight micro-organisms does greatly contribute to the causes of AMR (Masoko *et al.,* 2022), also to that we add poor infection control practices, lack of sanitation and lack of access to clean water which also play a role in the spread of AMR (World Health Organization, 2024). The results of AMR are very serious as it results in increased morbidity, mortality and health care costs (Smith *et al*., 2013). To go about AMR, we need what we put out to fight infection and also other approaches to treatment (Coates *et al.,* 2013) Phytochemicals which come from plants have been shown to have anti-microbial properties and may present a very good solution to the problem of AMR (Musa, 2025). Watermelon by products with great nutritional value has caught our eye. We are seeing great interest in the use of watermelon rind and watermelon seeds has a cost-effective raw material which is also abundant and which we think has valuable components that can be used in many industries (Zia *et al.,* 2021). Watermelon is composed of about 92% water, making it an excellent hydrating agent. Yet the remaining 8% contains an impressive array of phytochemicals naturally occurring plant compounds that provide biological benefits. These include: Lycopene: A powerful antioxidant responsible for watermelon's red color, shown to reduce the risk of cardiovascular diseases and certain cancers. Citrulline: An amino acid that promotes blood vessel health and may improve exercise performance. Vitamins: Including vitamin A, B6, and C, which support immunity, skin health, and energy metabolism. Phenolic compounds, flavonoids, tannins, saponins, and alkaloids especially concentrated in the seeds and rind known for their antimicrobial, anti-inflammatory, and antioxidant properties. Thus, watermelon is no longer viewed as just a refreshing summer fruit; it is increasingly studied for its role in disease prevention, immune modulation, and even natural antimicrobial therapy (Hashem *et al.*, 2023).

Watermelon Seeds, in traditional medicine, especially across Africa and Asia, different parts of the watermelon plant are used to treat various ailments. The seeds are used to relieve urinary tract infections, the rind for wound healing, and the juice for fever reduction and hydration during illness. In Nigeria and other parts of West Africa, watermelon seeds are roasted or crushed into powders used in ethno-pharmacological remedies for bacterial and parasitic infections (Yusuf *et al*. 2022). Modern science is now catching up, validating these traditional practices with laboratory studies have shown that extracts from watermelon seeds and rind possess significant antibacterial and antifungal properties, especially against common pathogens like Staphylococcus aureus, Escherichia coli, and Candida albicans. This suggests an exciting future where watermelon-based compounds may be incorporated into Phyto therapeutics plant derived alternatives to synthetic antibiotics (Nissar *et al.*, 2025). As the world grapples with rising cases of antimicrobial resistance (AMR) – a global health crisis where common infections become untreatable, there is a growing call to explore plant-based solutions. Watermelon, with its non-toxic, accessible, and biologically active phytochemicals, presents a promising candidate in this fight. Not only does it offer nutritional support, but its bioactive compounds have the potential to inhibit microbial growth, disrupt bacterial cell walls, and boost immune responses all without the harsh side effects associated with conventional antibiotics (Sola, *et al.*,2019).

Furthermore, its broad cultural acceptance, environmental sustainability, and economic importance make watermelon a uniquely positioned crop in the quest for natural health solutions. Watermelon is not just a tale of sweetness and hydration - it is a narrative of resilience, healing, and biological power, from ancient tombs to modern laboratories, watermelon continues to reveal its depth and purpose. As science delves deeper into its phytochemistry and pharmacological properties, we are beginning to understand what traditional healers have long known: within this humble fruit lies a medicine chest of immense value. By embracing watermelon not only as food but as functional therapy, we not only honor its past but we unlock its potential for the future, a future where nature and science work hand in hand. In this research we aim at looking at the antimicrobial properties of phytochemical from water melon and determine their value as alternative for the treatment of bacterial infection.

2. Materials and methods

2.1. Sources of Raw Materials

Fresh seeds of water melon (*Citrullus lanatus*) were obtained from Oil-mill market, Port Harcourt, Rivers State. The seeds were thoroughly washed with distilled water remove attached pulp, after the seeds were air-dried at room temperature for 7-10 days, it was ground into fine powder using a sterilized electric blender. The powdered sample obtained was stored in a sterile airtight bottle at room temperature for further analysis.

2.2. Preparation of Aqueous Extract

The aqueous extraction was done using the maceration method as described by Sofowora, (1993); Trigo *et al.,* 2025) Approximately, 50g of each ground seeds of *Citrullus lanatus* were soaked in both 250 methanol and ethyl acetate respectively for 24 hours with intermittent shaking. This was filtered through Whatman filter paper No 1. The liquid extracts obtained was air-dried and stored for further analysis. Furthermore, three mill of the dried residues were weighed into bijou bottles and 4ml of Di methyl sulphuric oxide (DMSO) was added to make a stock solution, having different concentration of 250mg/ml, 200mg/ml, 150mgml, 100mg/ml, 50mg/ml for the different extract which are ethyl acetate and ethanol extract and kept in the refrigerator (Collins *et al.,* 1995).

2.3. Phytochemical Analysis

Qualitative phytochemical evaluation of extracts was conducted on both extracts to determine the active phytochemical composition of the extract using standard procedures described by Trease *et al.,* (2002); Sofowora (1993). The presence of alkaloid, flavonoids, phenols, saponins and tannin, glycosides and terpenoids was evaluated using different qualitative test, including Meyer's, Dragendorff's, ferric chloride, fronthing, keller-killiani and Salkowski tests, respectively.

2.4. Collection of Test Microorganisms

Test microorganisms mainly, clinical Isolates of *Salmonella typhi, Escherichia coli' Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae* were obtained from the microbiology laboratory, University of Port Harcourt Teaching Hospital, Rivers State. The isolates were identified using standard biochemical tests such as catalase test, coagulase test, citrate test, indole test, oxidase test, methyl-red, sugar fermentation test and urease test. The confirmed isolates were maintained on nutrient agar slants at 4^{0}C for further usage (CLSI,2021).

2.5. Antimicrobial Susceptibility Screening

The Antibacterial activity of the extract was determined using the agar well diffusion method as described by Bauer et al., (1966), with little adjustments. Sterile Mueller-Hinton Agar (MHA) plates were prepared and allowed to solidify, the plates were inoculated with standard bacterial suspensions using a sterile swab, wells (6mm diameter) were bored aseptically into the agar using a sterile cork borer. An aliquot of the extract of ethanolic or ethyl acetate at different concentrations (i.e.,250, 200, 150, 100, and 5Omg/mL) were introduced into the wells. DMSO (Dimethyl sulfoxide) was used as a control to validate the assay and compare effectiveness. The plates were allowed to pre-diffuse for an hour at room temperature before incubated at 37°C for 24 hours to allow bacterial growth and potential. Zones of clearance were measured in millimeters using a calibrated ruler. The larger the zone of clearance, the more effective the extract is at inhibiting the growth of the test bacteria.

2.6. Minimum Bactericidal Concentration (MBC) of Watermelon Seed Extracts

The MBC of the seed of Citrullus lanatus extracts was determined using the standard broth dilution method. This procedure was conducted with the Minimum Inhibitory Concentration (MIC) determination to establish the lowest concentration of extracts that resulted in the death and inhibition of bacteria. After determining the MIC, organisms from the cleared zones were sub-cultured by streaking onto freshly prepared nutrient agar plates using a sterile wire loop. The plates were incubated at 37°C for 24 hours. The MBC was recorded as the lowest concentration of extract that produced no visible growth on the agar surface, indicating complete bactericidal activity. (CLSI, 2021).

2.7. Statistical Analyses

Mean and standard deviations were calculated to analyze sample attributes; Statistical analysis was conducted using appropriate software, where the difference means were evaluated using one-way analysis of variance (ANOVA), with significance set at < 0.05.

3. Results

Table 1 shows the result of the phytochemical analysis of the various bioactive compounds in the seeds of *Citrillus lanatus* extracts. Ethanolic extract revealed the presence of saponins, tannins, flavonoids, alkaloids while in ethyl acetate of the seeds of *Citrullus lanatus;* tannins, flavonoids, alkaloids were present, while saponins, phenols were absent. It showed that ethanol was able to extract more phytochemicals from the seeds of *Citrullus lanatus* more than the ethyl acetate.

Table 2, shows the result of the biochemical test conducted on the selected test organisms to confirm the ideal organism.

3.1. Antimicrobial Activity of *Citrillus lanatus* Extracts on Selected Test Organisms

Tables 3 and 4 shows the antibacterial activity of the watermelon seeds extracts against five organisms: *Salmonella* (stool), *Escherichia coli* (urine), *Pseudomonas* (wound), *Staphylococcus aureus* (urine), *Klebsiella pneumoniae* (urine). The inhibition zones, recorded in millimeters (mm) at various extracts concentration (250 mg/ml, 200 mg/ml, 150 mg/ml, 100 mg/ml, 50 mg/ml), demonstrate variable susceptibility. The ethanolic extracts were moderately effective against *staphylococcus* and while highly effective against *Escherichia coli,* suggesting the presence of strong antibacterial gram-negative bacteria. For the *Salmonella spp.*, it showed moderate antibacterial activity while observed to be ineffective against Klebsiella. It showed moderate sensitivity to *Pseudomonas*, the ethyl acetate extract showed more efficacy against *staphylococcus*. it was consistently active against *Escherichia coli* and moderately against *Salmonella*.

3.2. Minimum Bactericidal Concentration (MBC) of Watermelon Seeds

Table 5 and 6 shows the Minimum Bactericidal Concentration (MBC) of the two watermelon seeds (Extracts land 2) which were evaluated against five organisms: *Salmonella* (stool), *Escherichia coli* (urine), *Pseudomonas* (wound), *Staphylococcus aureus* (urine), *Klebsiella* (urine). The bactericidal effects of each extract were tested at concentration of 250 mg/ml, 200 mg/ml, 150 mg/ml, 100 mg/ml, 50 mg/ml. The presence of growth ('+") or no growth (-) was recorded to determine the minimum concentration required for bactericidal activity.

4. Discussion

This study investigated the phytochemical components, quantification of bioactive compounds and antibacterial efficacy of two watermelon seed extracts, phytochemical screening and antibiotic susceptibility testing to assess potential health benefits and therapeutic applications. phytochemicals are widely documented for their diverse health benefits and antimicrobial activities through different mechanisms such as membrane destruction, enzymatic inhibition and interruption with microbial metabolism (Neamah *et al.,* 2021; Wijerathna *et al.,* 2025). The elementary phytochemical screening revealed the presence of several bioactive compounds in the watermelon seed extracts including tannins, saponins, flavonoids, phenols, glycosides, alkaloids compounds.

Table 1 Qualitative Screening of Phytochemical Analyses of Seeds of *Citrillus Lanatus* Extracts

PHYTOCHHEMICALS	ETHANOL EXTRACT	ETHYL ACETATE
Tannins	+	+
Saponins	+	-
Flavonoids	+	+
Phenols	+	-
Alkanoids	+	+

Table 2 Biochemical Tests Results of selected organisms

Organism	Catalase Test	Coagulase Test	Citrate Test	Indole Test
Eschericia coli	Positive	Negative	Negative	Positive
Klebsiella spp	Positive	Negative	Positive	Negative

Staphylococcus spp	Positive	Positive	Negative	Negative
Salmonella spp	Positive	Negative	Positive	Negative
Pseudomonas	Positive	Negative	Positive	Negative

Table 3 Antibacterial Activity of Watermelon Seeds (Citrillus Lantus) Ethanolic Extracts on Selected Test Organisms

S/N	**Probable Isolates**	**Concentration(mg/ml)**						**MIC**
		250	**200**	**150**	**100**	**50**	**C**	
1.	*Staphylococcus aureus*	14.0	10.0	12.0	7.0	9.0	20.0	100.0
2	*Escherichia coli*	18.0	20.0	19.0	15.0	16.0	20.0	100.0
3	*Salmonella typhi*	12.0	9.0	9.0	11.0		10.0	50.0
4	*Klebsiella pneumonia*	0.0	0.0	0.0	0.0	0.0	0.0	>250
5	*Pseudomonas aeruginosa*	12.0	9.0	11.0	11.0	10.0	0.012.0	50.0

Table 4 Antibacterial Activity of Watermelon Seeds *(Citrillus Lanatus*) Ethylacetate Extracts on Selected Test Organisms

Probable Isolates	**250**	**200**	**150**	**100**	**50**	**C**	**MIC**
Staphylococcus aureus	10.0	8.0	20.0	10.0	15.0	20.0	200
Escherichia coli	14.0	10.0	11.0	8.0	9.0	12.0	100
Salmonella typhi	10.0	10.0	13.0	8.0	9.0	11.0	100
Klebsiella pneumonia	0.0	0.0	0.0	0.0	0.0	0.0	>250
Pseudomonas aeruginosa	9.0	18.0	10.0	9.0	7.0	10.0	50.0

Table 5 Minimum Bactericidal Concentration (MBC) of Ethanolic Extracts of seeds of *Citrillus lanatus* on Clinical Isolates

Test Organisms	**Concentration (mg/ml)** **250**
Staphylococcus aureus	-
Escherichia coli	-
Salmonella typhi	-
Klebsiella pneumonia	+
Pseudomonas aeruginosa	-

KEY: MBC-Minimum Bactericidal Concentration

Table 6 Minimum Bactericidal Concentration of Ethyl Acetate Extract of seeds of *Citrillus lanatus* on Clinical isolates

Test Organisms	**250 ml**
Staphylococcus aureus	-

Escherichia coli	-
Salmonella typhi	-
Klebsiella pneumonia	+
Pseudomonas aeruginosa	-

The discovery of the presences of these bioactive compounds in watermelon seed extracts collaborated with the earlier reports of Braide *et al.,* (2012), who discovered similar phytochemicals components in watermelon seeds and linked them to plant derived secondary metabolites that are responsible for the antimicrobial effect of the watermelon seeds. For instance, flavonoids and phenols are known for the in destruction of the cell membrane of the bacteria and at the same time stops the synthesis of nucleic acid, tannin on the other hand, exhibits antimicrobial effect by formation of complexes with protein and bacterial enzymes (Neamah, 2021). Furthermore, saponins raises the level of the permeability of the cell membrane, resulting in drastic leakage of the internal components and even sudden death of the bacterial cell.

The antimicrobial evaluation carried out indicated that the extract showed different levels of inhibition against the test bacteria isolates; *Staphylococcus aureus, Escherichia coli, Salmonella tyhi, Klebsiella pneumonia,* and *Pseudomonas aeruginosa* which is in collaboration with earlier finding by Adelani-Akande *et al.,* (2015), who reported that watermelon seed extract shows a reasonable inhibitory effect against both gram negative and positive bacteria isolates. The seeds of watermelon possess a crucial antimicrobial bioactive component due to their rich phytochemical composition. Typically, it has been earlier established that ethanol-based extracts display a more potent antibacterial activity than ethyl acetate because of their ability to extract a wider spectrum of polar and semi-polar compounds responsible microbial inhibition. Correspondingly, the indicated high level of susceptibility by gram positive bacteria isolates compared to the gram-negative isolates is similar to the fact that gram negative isolates develop more resistance due to the presence of its outer lipopolysaccharide membrane that prevents the access of antimicrobial compounds (Wijerathna *et al.,* 2025).

Furthermore, the resistance indicated by Klebsiella pneumonia and the high level of susceptibility shown by *Escherichia coli* in this study aligns with the reports of Abdulhadi *et al.,* (2023) who stated that *Klebsiella pneumonia* exhibits high level of resistance due to its polysaccharide capsule and efficient defense mechanisms; enzyme production and biofilm formation, whereas in the case of *Escherichia coli* its showed high susceptibility to the plant extract because its response to extracts differs and depends on species-specific resistance mechanisms and membrane permeability.

Consequently, Among the two solvents, the ethanol extract consistently showed stronger antibacterial activity. It produced larger zones of inhibition in the agar diffusion test and lower MBC values across most of the bacterial strains tested. In particular, *E. coli* was the most sensitive organism. With an MBC of just 50 mg/ml in the ethanol was able to extract a broader and more potent range of antimicrobial compounds. from the seeds. The ethanol extracts demonstrated superior antimicrobial activity across all test organisms compared to the ethyl acetate. This observation agrees with the findings of Ezealisiji, Abubakar, and Aliyu (2014), who reported that ethanol extract of *Citrillus lanatus* seeds exhibited notable inhibition zones against E. coli and S. aureus on the other hand, the ethyl acetate extract showed less activity and required higher concentrations to achieve the same effect. Interestingly, *Klebsiella* specie was resistant to both extracts, indicating that not all bacteria are equally affected by compounds present in watermelon seeds. The MIC and MBC values further supported the efficacy of the ethanol extract, with *E. coli* showing the lowest MIC (100 mg/ml) and MBC (200mg/ml). This is in line with (D'Agostino *et al.,* 2015)

Overall, the findings suggest that ethanol is a more efficient solvent for extracting medicinal compounds from watermelon seeds. These results also support traditional uses of watermelon seed preparations in natural medicine, especially in treating infections.

5. Conclusion

This research confirms that watermelon seeds are more than just agricultural waste, they have real potential as natural antimicrobial source. The ethanol extract, in particular showed a promising antibacterial activity, especially against *E. coli, salmonella spp.* and *staphylococcus aureus,* which shows a promising potential on the use of watermelon seeds in the treatment of various illnesses. This activity is likely due to the presence of various phytochemicals such as flavonoids, tannins, phenols, and saponins. In contrast, the ethyl acetate extract was less effective, which shows how the choice of solvent can significantly affect the potency of herbal extractions. Although the extracts were not effective against all bacteria (example *Klebsiella* specie showed resistance), the overall results support the fact that watermelon seed has prospects for developing watermelon seed-based antimicrobial agents, particularly in low-income settings, where access to conventional antibiotics is limited.

Recommendations

Based on the findings, this study recommends firstly that further isolation and characterization of bioactive compound responsible for the observed antimicrobial activity using advanced techniques such as chromatography and spectroscopy for a better understanding of their mode of action This will aid in the development of novel antimicrobial agents from *Citrullus lanatus*. Secondly before any medical application, it is important to carry out toxicity studies to ensure that these extracts are safe for human use and lastly to encourage agricultural innovation, since watermelon seeds are usually discarded, there should be awareness of their medicinal value and their use in antimicrobial production which could also offer economic opportunities and reduce waste.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors have declared that no competing interests exist.

Statement of ethical approval

The ethical approval for this study was obtained from the Departmental Research and Ethics committee of the Madonna University.

Statement of informed consent

The authors declare that this research did not involve human participants or animals; therefore, consent for participation and publication was not applicable. All authors have reviewed and approved the final manuscript and consent to its publication.

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