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# Antibacterial Efficacy of Citrullus lanatus (Watermelon) Seeds Extracts against Wound Infecting Staphyloccocus aureus and Pseudomonas Aeruginosa

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***Abstract***

**Watermelon seeds, originally from vine plants, are rich in nutrients and bioactive compounds. This study aimed to assess the antibacterial efficacy of *Citrullus lanatus* (Watermelon) seed extracts against wound-infecting *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Preliminary phytochemical screening of the ethanol and aqueous extracts of the watermelon seed was carried out using standard analytical methods. The two extracts were screened for antibacterial activity against *S. aureus* and *P. aeruginosa*, isolated from wound swabs, using both agar well diffusion and broth dilution assays. The result of the phytochemical screening revealed the presence of saponins, steroids, tannins, phenols, quinones, and terpenoids in the ethanol extract, while saponins, steroids, quinones, phenols, terpenoids, and tannins were present in the aqueous extract. The inhibitory zone of the ethanol extract against *S. aureus* ranged between 12.25±3.18 mm to 15.50±0.71 mm, while that of *P. aeruginosa* ranged between 12.80±0.00 mm to 15.25±1.06 mm at 50 and 100 (mg/mL), respectively. The inhibitory zone of the aqueous extract against the clinical isolate of *S. aureus* ranged between 11.50±0.71 mm to 11.75±2.87 mm at 50 and 100 (mg/mL), respectively, while *P. aeruginosa* exhibited inhibitory activity of 14.40±0.00 mm at a concentration of 100 mg/mL only. The Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC), on average, were 50 mg/mL and 100 mg/mL, respectively. The observed inhibitory activity of the ethanol and aqueous extracts of the seed against the clinical isolates could be due to the presence of phytochemical components within the seed.**

**Keywords: Phytochemical, Antibacterial, *Citrullus lanatus* seeds, *Staphylococcus aureus*, and *Pseudomonas aeruginosa***

## INTRODUCTION

The increasing prevalence of antibiotic-resistant bacterial infections poses a significant challenge to public health globally (Dewu *et al.*, 2023). Infectious diseases were estimated to be among the causes of more than half of all fatalities globally. This is largely related to inadequate medical facilities in impoverished nations, and more significantly, microorganisms that are resistant to previously effective antibiotics. These have sparked a fresh interest in finding chemicals with unique structures that may help provide healthcare more effectively and reduce the threat of resistance (Dewu et *et al.*, 2023). Plant-derived chemicals are therefore promising antimicrobials for the treatment of infectious disorders, according to numerous studies (Bello *et al.*, 2016).

*Citrullus lanatus* (water melon) is the fruit of a plant, originally from a vine of Southern Africa. It produces about 92% water; hence named "water" melon (Baker *et al.*, 2012). *Citrullus lanatus* (watermelon) is a member of the *Cucurbitaceae* family and widely cultivated for its large sweet fruit. *Citrullus lanatus* is a prostrate annual plant with several herbaceous, firm, and stout stems measuring up to three (3) metres in length (Hlaing *et al.*, 2020). Watermelon may help prevent several types of cancer due to its antioxidant qualities, including beta-carotene and vitamin C, which are abundant in one substantial slice of watermelon (approximately one-sixteenth of a melon).

Additionally, watermelon contains a significant amount of potassium, which helps regulate blood pressure and maintain cardiac function (Benmeziane *et al.*, 2023). It is also a good source of fiber, which helps to prevent colon and kidney cancer and preserve bowel regularity. The plant is a natural source of lycopene and contains a high level of beta-carotene. Citruline, a potent precursor of L-arginine, is also abundant in it. Both the plant's edible and inedible portions contain phenolic compounds (Nwankwo *et al.*, 2024).

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The flesh of the watermelon is often consumed for its refreshing taste and hydrating properties (Athar *et al.*, 2020). However, after eating the fruit, the seed is frequently discarded, making it one of the planet's neglected resources. An emulsion made from watermelon seed water extract is used to treat fever, catarrhal infections, and bowel and urinary tract issues (Nwankwo *et al.*, 2024).

Research indicates that these seeds are a good source of protein, essential unsaturated fats, minerals, and other nutrients (Athar *et al.*, 2020). Watermelon seeds are known to contain a variety of phytochemicals, including saponins, flavonoids, tannins, and alkaloids (Athanasiadis *et al.*, 2023). These compounds have been associated with numerous health benefits, including antioxidant, anti-inflammatory, and antimicrobial activities (Dewu *et al.*, 2023). Tettey *et al.* (2021) carried out a study on the peels, seeds, rind and pulp of *Citrullus lanatus* to investigate the antibacterial activity of the fruit on a range of bacteria, including *Staphylococcus aureus* (NCIMB 6571) and *Escherichia coli* (ATCC 29722) where moderate activity was found by the extracts of the fruits against the bacteria used. Since the seeds and seed coats contain a variety of chemical compounds, it is expected that the seeds may be just as effective as the other parts of the plant, even though the majority of studies on the medicinal use of this plant used the leaves, fruits, roots, or flowers (Athanasiadis *et al.*, 2023). Although these chemicals are believed to be for the plant's protection, they can also play a role as antimicrobial agents. Preliminary studies have demonstrated that extracts from these seeds possess antibacterial activity against various pathogens (Athanasiadis *et al.*, 2023; Gupta *et al.*, 2018).

However, comprehensive research is lacking on the specific mechanisms and efficacy of these extracts against various clinical isolates from wound infections (Gupta *et al.*, 2018). Developing effective, natural antibacterial agents from *Citrulluslanatus* seeds could significantly impact public health by providing new treatment options for infections that currently difficult to manage due to antibiotic resistance, thereby reducing wastage of the seeds (Gupta *et al.*, 2018).

This research aimed to determine the antibacterial efficacy of *Citrullus lanatus* (Watermelon) seed extracts against wound-infecting *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

## MATERIALS AND METHODS

### Study Site

The research work was conducted at the Microbiology Laboratory of the Department of Microbiology, Kaduna State University (10.5167°N, 7.4505°E).

### Collection of Seed Materials

Watermelons (*Citrullus lanatus) were obtained from the Station Market, Kachia R*oad, Kaduna State, Nigeria. To extract the seeds, the fruits were cleaned and sliced open. The resulting seeds were cleaned, allowed to air dry for two days, and then ground into a powder using a mortar and pestle in an aseptic setting. The powdered seed material was then weighed and kept in airtight containers until further use (Raji-Idowu, 2023).

### Preparation of Extracts

The extraction was performed using the cold maceration method, as described by Tiwari et al. (2011) and Benmeziane et al. (2023), with sterile distilled water and 70% ethanol as solvents. In the aqueous extraction, 150 g of the seed powder was weighed and soaked in 750 mL of distilled water in a vessel. Similarly, for the ethanol extract, 150g of the powdered was weighed and soaked in 750 mL of 70% ethanol in a vessel. The mixtures were kept in the sealed vessels at room temperature for 72 hours with intermittent agitation to ensure proper extraction.

Whatman No. 1 filter paper and muslin cloths were then used to filter the mixtures into a sterile beaker. To eliminate the solvents, the filtrates were evaporated in a water bath set at 40°C. Following full evaporation, the leftovers in the beaker were gathered, weighed, and then placed in airtight containers for additional examination (Raji-Idowu, 2023). After the extracts were weighed, some were used for the susceptibility test, and the other part was used for phytochemical screening. The following formula was used to determine the percentage yield of the watermelon seed extracts in ethanol and aqueous form.

$$\text{Yield } (\%) = [(W_2 - W_1)/ W_o] \times 100$$

Where $W_1$ = Weight of the container in grams

$W_2$ = Weight of container + extract

$W_o$ = Weight of powdered seed

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**Phytochemical Screening**

Qualitative phytochemical screening of the various extracts was carried out according to standard procedure (Ibrahim *et al.*, 2017) to ascertain the qualitative composition of the seed. Phytochemicals screened include alkaloids, saponins, steroids, flavonoids, and cardiac glycosides.

**Test for Saponins**

0.5 g of the extract was dissolved in 10 mL of distilled water in a test tube, and the tube was capped. The mixture was shake vigorously for 30 seconds and allowed to stand for 45 minutes. The appearance of frothing indicates the presence of saponins.

**Test for Phytosteroids**

Two (2) mL of acetic anhydride was added to 0.5g of the extracts. 2 mL $H_2SO_4$ was also be added. Steroids were present when the color shifts from violet to blue.

**Test for Phenols**

Two (2) mL of distilled water, followed by a few drops of 10% Ferric chloride, was added to 1 mL of the extract. The formation of a blue or green color will indicate the presence of phenols.

**Test for Terpenoids**

Two (2) mL of chloroform was mixed with 0.5g of plant extract. 3 mL of concentrated $H_2SO_4$ was added to form a layer. A reddish brown coloration at the interface indicated a positive result.

**Test for Tannins**

The ferric chloride test was used, where 0.5 g of seed extracts was dissolved in 10 mL of distilled water and then filtered. Two drops of a 5% Ferric chloride solution were added to the filtrate. Green precipitate indicated the presence or absence of condensed tannins while blue black color indicates hydrolysable or non-hydrolysable tannins.

**Test for Cardiac Glycosides**

0.5 g of the extract was dissolved in 2 mL of glacial acetic acid in a test tube. One drop of ferric chloride solution was added, and 1 mL of concentrated H2SO4 was added to the solution. A brown ring at the interface indicated the presence of cardiac glycosides.

**Sources and Characterization of the Isolates Used**

Wound sample was collected using a sterile swab stick. The swab stick was then streaked onto the surface of prepared Mannitol Salt Agar and Cetrimide Agar for *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Patra et al., 2020), and incubated at 37 °C for 24 hours. The test isolates were sub-cultured by picking a loopful using sterilized wire loop on Nutrient Agar and incubated at 37°C for 24 hours (Athanasiadis *et al.*, 2023). The test isolates obtained from the plates were examined through morphological characterization (Patra et al., 2020), Gram staining, microscopic examination, and biochemical tests to confirm the *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolates (Ibrahim *et al.*, 2017).

**Biochemical Tests**

**Catalase Test**

A drop of 3% Hydrogen peroxide was placed on a clean, grease-free microscope glass slide using a sterile wire loop; a small portion of the colonies from the plates was emulsified with the Hydrogen peroxide. A positive result is indicated by the rapid formation of bubbles, while a negative result is indicated by the absence of bubbles (Ibrahim *et al.*, 2017).

**Coagulase Test**

The *Staphylococcus aureus* and *Pseudomonas aerugionosa* colonies were selected and tested in agar culture before being gently emulsified with two drops of sterile saline. A drop of undiluted bacterial suspension plasma was added and mixed with a wooden applicator stick. A second drop of saline was placed on the other half of the slide as a control and rocked back and forth for 10 to 15 seconds to observe the clumping of the bacterial suspension, which indicated a positive result (Patra *et al.*, 2020).

**Indole Test**

A loopful of the test organisms was inoculated into two test tubes containing 5 mL of Tryptone broth, with one test tube remaining uninoculated to serve as a control. The tubes were then incubated for 48 hours at 37°C. Following this, 0.5 mL of Kovac's reagent was added and gently shaken, and the mixture was left to stand for 20 minutes to allow the reagent to settle. A red color indicates a positive result,

while a negative result is indicated by a yellow color (Roy *et al.*, 2023).

**Oxidase test**

A filter paper was soaked with few drops of oxidase reagent. A sterile inoculating loop was used to pick a colony of the test organisms, which were then smeared on filter paper and allowed to stand for a few seconds. Presence or absence of a dark purple color indicated a positive or negative result (Roy *et al.*, 2023).

**Simmon's Citrate Test**

After being sterilized, roughly 10 mL of Simmon's citrate medium was transferred into two to three test tubes and let to cool slantedly. Following solidification, the test organism was streaked once across the agar surface to inoculate the tubes. The presence of blue indicates a positive outcome, whereas a poor outcome is indicated by the presence of green (Roy *et al.*, 2023).

**Standardization of Clinical Isolates**

The method of Oyeleke and Manga (2008) was used to standardize the organisms. The isolates being tested were grown on Mueller-Hinton Agar (MHA) that was prepared according to the manufacturer's instructions at 37°C overnight. Colonies from the overnight growth on the MHA were inoculated into a tube containing Mueller-Hinton broth until the turbidity was equivalent to that of 0.5 McFarland standards.

**Preparation of Extract Concentration**

One (1)g of the crude extract was weighed and dissolved in 10 mL of 2% Dimethyl Sulfoxide (DMSO) in a test tube to get 100 mg/mL as the stock concentration. Three test tubes were arranged and 5 mL of 2% DMSO was dispensed. From the stock solution, 1 mL was dispensed into a tube containing 5 mL of 2% DMSO, resulting in a concentration of 50 mg/mL. From the second test tube, 1 mL was dispensed into the third test tube containing 5 mL of 2% DMSO to give concentration of 25 mg/mL. From the third test tube, 1 mL was dispensed into the fourth test tube to achieve a concentration of 12.5 mg/mL (Hassan *et al.*, 2011).

**Antibacterial Activities of *Citrullus lanatus* Seed Extracts against *Staphylococcus aureus* and *Pseudomonas aeruginosa***

Covington *et al.* (2021) used the Agar well diffusion method to test the antibacterial properties of the ethanol and aqueous seed extracts. Mueller Hinton agar was prepared as directed by the manufacturer and sterilized at 121°C for 15 minutes. Using sterile cotton swabs, 0.1 mL of the standardized inoculum of the test bacteria was added to the solidified sterile medium in petri plates. Additionally, wells were drilled in each agar plate using a sterile cork borer with a diameter of 6.0 mm. Then, 0.5 mL of the extract concentrations were poured into each well.The plates were then left at room temperature for one hour to allow for proper diffusion of the extracts into the agar. The plates were incubated at 37°C for 24 hours. Ciprofloxacin (5 µg) was used as positive control and DMSO was used as negative control for all the isolates. At the end of the incubation period, diameters of inhibition was measured using a well calibrated meter ruler in mm.

**Determination of Minimum Inhibitory Concentration (MIC)**

The extracts underwent additional testing to determine their minimum inhibitory concentration (MIC). Mueller Hinton broth was used in the broth dilution procedure (Covington *et al.*, 2021). The test clinical isolates from the susceptibility tests were serially diluted twice. In test tubes, 2 mL of Mueller-Hinton broth was mixed with 2 milliliters (2 mL) of the test concentration of each extract that inhibited growth. The aforementioned mixes were inoculated with 1 mL of the standardized inoculum and then incubated for 24 hours at 37°C. The lowest concentration of the extracts that prevented the organisms from growing visibly after the incubation time was known as the minimum inhibitory concentration, or MIC. Negative controls were set up containing Mueller-Hinton broth only and Mueller broth with extracts only, while positive controls were set up containing Mueller-Hinton broth and the bacteria.

**Determination of Minimum Bactericidal Concentration (MBC)**

The minimum bactericidal concentration (MBC) was determined by pipetting 0.1 mL of broth from the MIC tubes that showed no visible growth using a sterile pipette and dispensing it onto the surface of Mueller-Hinton agar plates. A sterile rod was used to spread the inoculum, and the plates were then incubated at 37°C for 24 hours. Sterile Mueller-Hinton agar was used as the control.The MBC was determined to be the lowest concentration of the extracts that allowed any bacterial growth on the surface of a

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Mueller-Hinton agar plate (Adebayo *et al.*, 2017).

**Statistical Analysis of Data**

The significant differences were analyzed using SPSS V 23.0 (2018). Mean ± Standard deviation of the zones of inhibition were calculated.

## RESULTS

The results of the seed extracts' yield are presented in Table 1. 150g of each coarse seed was used for extraction. Yields obtained were 35.70% and 26.67% for the aqueous and ethanol extracts respectively. The crude aqueous and ethanol extracts of the seeds showed characteristics sticky texture brownish color. However, the aqueous extract had the highest yield (35.70%).

The phytochemical screening of ethanolic extracts and aqueous extracts of *Citrullus lanatus* seed extracts is presented in Table 2. The phytochemical screening of aqueous and ethanol extracts of *Citrullus lanatus* seed detected the presence of saponins, phytosteroids, tannins, phenol, and terpenoids in both extracts, while glycosides were not detected.

**Table 1: Percentage Yield and Physical Characteristics of *CitrullusLanantus* Seed Extracts.**

| Plant Extract | Initial Weight of Sample (g) | Weight of Extract (g) | Yield of Extract (%) | Extract Appearance |
|---|---|---|---|---|
| Aqueous | 150.00 | 53.57 | 35.70 | Dark brown and gummy |
| Ethanol | 150.00 | 40.00 | 26.67 | Dark brown and gummy |

**Table 2: Phytochemical Screening of Aqueous and Ethanol Extracts of *Citrullus lanatus* Seeds.**

| PhytochemicalComponents | Aqueous Extract | Ethanol Extract |
|---|---|---|
| **Saponin** | + | + |
| **Steroids** | + | + |
| **Phenol** | + | + |
| **Terpenoids** | + | + |
| **Tannins** | + | + |
| **Glycosides** | - | - |

**Key:** + = Detected, - = Not Detected

**Table 3: Cultural and Biochemical Characteristics of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.**

| Gram Reaction | Cell/Colony Morphology | Biochemical Test | | | | | Probable Organism |
|---|---|---|---|---|---|---|---|
| | | Catalase | Coagulase | Oxidase | Indole | Citrate | |
| Positive | Cocci/Golden yellow, circular, smooth, and mucoid | + | + | - | - | + | ***Staphylococcus aureus*** |
| Negative | Rod/Smooth, round colonies with greenish coloration | + | - | + | - | + | ***Pseudomonas aeruginosa*** |

**Key :**+ = Present, - = Absent

Cultural and Biochemical Characteristics of *Staphyloccocus aureus* and *Pseudomonas aeruginosa* is presented in Table 3. The results show that *Staphylococcus aureus* was Gram-positive, with chrome-yellow-colored colonies and the presence of catalase, coagulase, and citrate, while Pseudomonas aeruginosa was Gram-negative, with light-greenish colonies, and was positive for catalase, citrate, and oxidase, with coagulase and indole being negative.

Antibacterial activity of ethanol and aqueous extracts of *Citrullus lanatus* seeds against Staphylococcus *aureus* and *Pseudomonas aeruginosa* is presented in Table 4. The inhibitory zone of the aqueous extract against

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the clinical isolate of *S. aureus* ranged between 11.50±0.71 mm to 11.75±2.87 mm at 50 and 100 (mg/mL), respectively, while *P. aeruginosa* had inhibitory activity of 14.40±0.00 mm at 100 mg/mL only. The inhibitory zone of the ethanol extract against *S. aureus* ranged between 12.25±3.18 mm to 15.50±0.71 mm, while that of *P. aeruginosa* ranged between 12.80±0.00 mm to 15.25±1.06 mm at 50 and 100 (mg/mL), respectively.

**Table 4: Antibacterial activity of *Citrullus lanantus* Seed Extracts against the Test Isolates.**

| Organisms | Solvents | Zone of Inhibition (mm) | | | | Control Ciprofloxacin |
|---|---|---|---|---|---|---|
| | | 100 mg/mL | 50 mg/mL | 25 mg/mL | 12.5 mg/mL | |
| *S. aureus* | Aqueous | 11.75 ±2.87[a] | 11.50±0.71[b] | - | - | 30.50±0.71 |
| *P. aeruginosa* | | 14.40±0.00[a] | - | - | - | |
| *S. aureus* | Ethanol | 15.50±0.71[a] | 12.25±3.1[b] | - | - | 26.00±0.00 |
| *P. aeruginosa* | | 15.25±1.06[a] | 12.80±0.00[b] | - | - | |

**Key:**- = No Zone Detected

Values are presented as mean ± SD. Values with similar superscript within the same column indicate no statistical significant difference at $p \leq 0.05$.

**Table 5: Minimum Inhibitory Concentration (MIC and Minimum Bactericidal Concentration (MBC) of Aqueous and Ethanol Extracts of *Citrullus lanatus* Seed against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.**

| Test Organism | Solvent | MIC(mg/mL) | MBC (mg/mL) |
|---|---|---|---|
| *Staphylococcus aureus* | Ethanol | 50 | 100 |
| | Aqueous | 50 | 100 |
| *Pseudomonas aeruginosa* | Ethanol | 50 | 100 |
| | Aqueous | 50 | 100 |

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of aqueous and ethanol extracts of *Citrullus lanatus* seeds against *Staphylococcus aureus* and *Pseudomonas aeruginosa* are presented in Table 5. The Minimum Inhibitory Concentration (MIC) of *Citrullus lanatus* seed extracts on *Staphylococcus aureus* and *Pseudomonas aeruginosa* indicated that aqueous and ethanol tubes containing 50 mg/mL showed no growth, which means that it is the lowest concentration of the extract that inhibits growth. The Minimum Bacterial Concentration (MBC) of *Citrullus lanatus* seed extracts against *Staphylococcus aureus* and Pseudomonas aeruginosa indicated that aqueous and ethanol tubes containing 100 mg/mL killed the test isolates, meaning that it was the lowest concentration of the extract that showed no visible growth on the agar plate.

## DISCUSSION

Reports of medicinal plants' antibacterial and antifungal qualities are growing from all over the world. According to Mogana *et al.* (2020), the World Health Organization promotes the use of plant extracts or their active ingredients as sources of antimicrobials in traditional therapies and folk medicine. Some of these observations have contributed to the development of medications for therapeutic use in humans, as well as the identification of the active chemicals responsible for these effects. Thus, the purpose of this work is to assess the antibacterial properties of ethanol and aqueous extracts made from *Citrullus lanatus* seeds against *Pseudomonas aeruginosa* and *Staphyloccocus aureus* isolated from wound infections.

The study's high extract yield may be attributed to the solvent's polarity and the amount of plant material used. Other researchers extracted active components from some medicinal plants with similar yields (Bereksi *et al.*, 2018). The greater solubility of the active ingredients in watermay be the reason why the aqueous extract produced a higher yield than the ethanol extract.

The presence of these phytocomponents has been linked to the antimicrobial properties of the seeds. According to Braide *et al.* (2011), *plants with higher concentrations of phytochemicals are believed to exhibit better antibacterial activity*. Particularly in Gram-positive bacteria, saponins have been linked to the production of cell membrane pores, complex formation with cholesterol, and morphological changes (Guerrero-Alveraz and Giraldo-Rivera, 2023). In a prior study, the presence of saponins was linked to the susceptibility of *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Adunola *et al.*, 2015). Our findings also support the idea that other phytochemicals, besides saponins, are active against Gram-negative bacteria. Cell lysis may result from tannins' inactivation of microbial adhesions, enzymes, cell membranes, and the transport system (Mogana *et al.*, 2020). Glycosides were not found in the aqueous and ethanol extracts of watermelon seeds in this study, which contradicts the reports of studies conducted by Bello et al. (2016) and Nwankwo et al. (2024), where glycosides were detected in both the aqueous and ethanol extracts of watermelon. The discrepancy may be due to geographical location, as it can influence the active constituents of plants, which can be affected by various factors such as climate, soil, and propagation technique.

The antibacterial activity of the seed extracts revealed that the organisms tested showed higher susceptibility to the ethanol extract against both Staphylococcus aureus and Pseudomonas aeruginosa compared to the aqueous extract. This could be because organic compounds like terpenoids, flavonoids and phenols are more soluble in ethanol. It may also be due to the ability of ethanol to penetrate the cell walls of the seeds more efficiently than water, resulting in more potent extracts and a reduction in the enzymatic degradation of active compounds that could occur in an aqueous environment. Braide *et al.* (2012) observed that water extracts exhibit a better response to antibacterial activities than ethanol, while Nwankwo *et al.* (2024) reported the opposite.

The variation in the susceptibility of clinical test isolates to different concentrations of extract could be due to differences in the cell wall of the bacteria. Gram-positive *Staphylococcus aureus* bacteria lack lipopolysaccharides, which tend to allow diffusion of the active components, while Gram-negative *P. aeruginosa* bacteria have lipopolysaccharides, which may have prevented penetration of extracts' active components. This finding is consistent with the work of Guerrero-Alveraz and Giraldo-Rivera (2023), who observed a larger zone of inhibition against Gram-positive bacteria than against Gram-negative bacteria. Although it contradicted the findings of Sharma and Kaur (2016), who found that Gram-negative bacteria were more susceptible than Gram-positive bacteria to plant extracts. However, all activities of the extracts were dose-dependent, which was also observed in the research reported by Guerrero-Alvarez and Giraldo-Rivera (2023), where at higher concentrations, more antibacterial activity was detected.

A high Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) indicate that more extracts are needed to inhibit the bacteria, which could require a higher dose and lead to an increased toxicity risk and the promotion of resistance. The efficiency of the seed extracts, the region where the plants were collected, and the extraction technique may all have contributed to the plant's Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) against the isolates. This study's MIC and MBC values were comparable to those reported by Saquib *et al.* (2019). This runs counter to the findings of Alemu *et al.* (2017), who reported MIC and MBC of 6.25 mg/mL and 12.5 mg/mL against *S. aureus*, respectively.

## CONCLUSION

Steroids, phenol, terpenoids, tannins, and saponins were the phytochemicals detected in the extracts. At concentrations of 100 mg/mL and 50 mg/mL, respectively, the study's findings demonstrated that both aqueous and ethanol extracts of *Citrullus lanatus* seeds exhibited antibacterial activity against Staphylococ*cus aureus* and *Pseudomonas aeruginosa*. However, the ethanol extracts exhibited a greater inhibition than the aqueous extract. The MIC and MBC of both extracts against the two test isolates were 50 mg/mL and 100 mg/mL, respectively. The antibacterial properties and phytochemical analysis results of *Citrullus lanatus* seed extract validate its use in ethnomedicine and make it a valuable source for pharmaceutical manufacturing. The observed antibacterial activity is a clear indication of the therapeutic properties possessed by the seeds, similar to those of other plant parts. Therefore, the seeds of watermelon could be a good source of antibacterial agents and thus, should be harnessed. Further research into the toxicity profile, as well as the fractionation of the

extracts to identify the active compounds, is recommended.

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