Medical and Health Sciences European
Med. H. Sci. E. J.
Volume: 8; Issue: 06,
November-December, 2024
ISSN: 2469 – 6774
Impact Factor: 4.42

Advance Scholars Publication
Published by International Institute of Advance Scholars Development
https://aspjournals.org/Journals/index.php/mhsej

PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF WATERMELON SEED EXTRACT ON *STREPTOCOCCUS* SPECIES

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***Keywords**: Watermelon seed, phytochemicals, antimicrobial activity,* Streptococcus *spp., natural alternatives*

Abstract: *This study investigated the phytochemical composition and antimicrobial efficacy of watermelon seed extract against* Streptococcus *species, a pathogenic bacterial genus associated with various infections. The research aimed to identify and analyse bioactive compounds within watermelon seeds that could provide antimicrobial properties. Watermelon seeds were collected and processed through solvent-based extraction methods, and phytochemical screening revealed the presence of flavonoids, alkaloids, tannins, and saponins compounds known to enhance antimicrobial efficacy. The antimicrobial potential was assessed using the agar well diffusion method, where the seed extract exhibited significant zones of inhibition against various* Streptococcus *strains, including* Streptococcus pyogenes, Streptococcus oralis, Streptococcus salivarius *and* Streptococcus faecalis. *The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values were determined, with the MIC values ranging between 12.5 mg/ml and 50 mg/ml, and MBC values indicating bactericidal effects against the tested strains at varying concentrations. Notably,* Streptococcus pyogenes *exhibited the highest susceptibility, requiring a lower MIC and MBC for effective inhibition and control. The phytochemical analysis of watermelon seed extract revealed significant concentrations of carbohydrates (74.96–76.51 mg/100g), alkaloids (62.44–65.34 mg/100g), and reducing sugars (39.42–41.28 mg/100g), along with moderate levels of flavonoids, phenolic compounds, and other bioactive constituents. These results demonstrate the potential of watermelon seed extract as a natural antimicrobial agent, particularly against* Streptococcus *species, and suggest its applicability in addressing infections caused by antibiotic-resistant bacteria. The findings highlight watermelon seed extract as a promising candidate for developing alternative treatments for bacterial infections, given its phytochemical richness and effective inhibition of pathogenic bacteria.*

1.0 INTRODUCTION

Antimicrobial resistance (AMR) is a significant global public health issue, with the rise of resistant strains of bacteria making many conventional antibiotics increasingly ineffective. Among these bacteria, *Streptococcus* spp. is responsible for infections ranging from pharyngitis and pneumonia to sepsis, which have become increasingly difficult to treat due to their ability to develop resistance against multiple antibiotics [1, 2]. This phenomenon is driven by the overuse and misuse of antibiotics, leading to selective pressure on bacterial populations, which evolve to withstand the drugs that were

once effective in treating them [3]. The emergence of AMR has created an urgent need to explore alternative antimicrobial agents, particularly those derived from natural sources, to address the growing crisis. In this context, plant-based remedies, long known for their therapeutic properties, are being revisited for their potential to combat resistant bacteria.

Medicinal plants have been used for centuries in traditional medicine systems across the world. These plants are rich in bioactive compounds known as phytochemicals, which contribute to their antimicrobial, anti-inflammatory, and antioxidant activities [4]. Phytochemicals such as phenolic acids, flavonoids, alkaloids, and saponins are of particular interest in the search for alternative antimicrobial agents. These compounds often exhibit multiple modes of action against bacteria, making it difficult for microbes to develop resistance through a single mutation [5, 6]. Unlike synthetic antibiotics, which typically target a single bacterial pathway, plant-derived compounds can disrupt multiple cellular processes, including membrane integrity, protein synthesis, and nucleic acid replication [7]. This complexity makes phytochemicals less likely to foster resistance in bacterial populations, positioning them as valuable candidates in the fight against AMR.

Watermelon (*Citrullus lanatus*) seeds are an underexplored yet promising source of these bioactive phytochemicals. Historically, the focus has been on the fruit of the watermelon, while its seeds have been discarded as waste. However, recent research has begun to uncover the significant nutritional and therapeutic potential of watermelon seeds, which are rich in proteins, fats, vitamins, and minerals such as magnesium, iron, and zinc [8]. Additionally, these seeds contain a diverse array of phytochemicals, including phenolic acids like chlorogenic acid and flavonoids such as quercetin and kaempferol, all of which have been reported to exhibit antimicrobial activity [5]. Phenolic acids are known for their ability to disrupt bacterial cell walls and membranes, while flavonoids have been shown to interfere with bacterial enzymes and inhibit nucleic acid synthesis [6]. These bioactive compounds are believed to work synergistically, enhancing the overall antimicrobial efficacy of watermelon seed extracts [8].

The potential benefits of watermelon seed extract extend beyond its antimicrobial properties. Natural products often have fewer side effects compared to synthetic drugs, making them safer options for vulnerable populations, such as children and the elderly [9]. Additionally, the utilization of watermelon seeds, which are typically discarded as waste, promotes sustainable agricultural practices and contributes to the circular economy by adding value to agricultural by-products [10]. This aligns with global efforts to reduce environmental waste and develop eco-friendly healthcare solutions.

Streptococcus spp., a genus of Gram-positive bacteria, is particularly notorious for its ability to form biofilms, which protect it from both antibiotics and the host's immune system [1]. The biofilm formation complicates treatment, as the bacteria within these protective structures are often less susceptible to antimicrobial agents. As resistance to conventional antibiotics like penicillin and macrolides continues to rise, researchers have turned to natural products as potential alternatives [2]. Watermelon seed extract, with its rich phytochemical profile, represents a potential new avenue for antimicrobial therapy, especially against biofilm-forming pathogens such as *Streptococcus* spp.

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Studies have demonstrated the antimicrobial potential of various plant extracts against a wide range of pathogens, including resistant strains of *Staphylococcus aureus* and *Escherichia coli* [11, 7]. In particular, extracts from plants such as neem (*Azadirachta indica*), garlic (*Allium sativum*), and tea tree (*Melaleuca alternifolia*) have shown significant inhibitory effects on both Gram-positive and Gram-negative bacteria [12, 13]. These findings underscore the potential of plant-based antimicrobials to serve as effective alternatives or complements to existing antibiotics. However, research specifically on watermelon seed extract and its antimicrobial properties remains limited, and comprehensive investigations are needed to elucidate its mechanisms of action and potential applications in clinical settings. This study aims to address this gap by analyzing the phytochemical composition and antimicrobial activity of watermelon seed extract, with a particular focus on its efficacy against *Streptococcus* spp.

2.0 MATERIALS AND METHODS

2.1 Sample Collection

Mature watermelon fruits (*Citrullus lanatus*) were collected from a local farm near Ogun State Institute of Technology, Igbesa, Ogun State, Nigeria. The farm, located approximately 2 km from the institute campus, is known for cultivating watermelons under controlled agricultural practices. The fruits were carefully selected to ensure they were free from physical damage, pests, or diseases, which could affect the quality and purity of the seed extract.

2.1.1 Sample Preparation

The watermelon fruits were washed thoroughly with distilled water to remove any dirt or contaminants on the outer surface. Each fruit was then cut open using a sterilized stainless-steel knife to access the seeds. The seeds were carefully separated from the fruit flesh by hand, rinsed in distilled water, and air-dried at room temperature for 2-3 days. The seeds were further dried in a laboratory oven at 40°C for 24 hours to remove any remaining moisture. Once completely dry, the seeds were ground into a fine powder using a mechanical grinder and stored in airtight containers at room temperature until analysis to prevent moisture absorption and degradation of phytochemicals.

2.1.2 Extraction

Thirty grams of finely ground watermelon seed samples were extracted by refluxing with distilled water, methanol, and ethanol for 4 hours using a Soxhlet extractor. The powdered material was placed in the thimble of the Soxhlet unit, and 200 mL of each solvent (distilled water, methanol, and ethanol) was poured into separate round bottom flasks. The flasks were positioned on a heating mantle with a tripod stand, and the extraction continued for 3 hours until the plant material was exhausted. The extract was concentrated under vacuum using a rotary evaporator (Büchi Rotavapor R-114), which facilitated the evaporation of bulky solutions to small volume concentrates (semi-solid) at temperatures between 40-60°C. The resultant extracts were then filtered and sterilized using a 0.45 µm Millipore filter for subsequent antimicrobial activity testing [14]. All samples were collected and analyzed in triplicate (Extract 1, Extract 2, and Extract 3) to ensure reliability and reproducibility of the results.

2.2 Methods of Screening

The research uses analytical techniques such as spectrophotometry to identify and quantify the bioactive compounds present in the extract. The extracts were screened using both qualitative and quantitative methods. Furthermore, microbiological assays, including agar well diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration

(MBC) tests, are employed to evaluate the antimicrobial effectiveness of the extract [14]. By investigating the mechanisms underlying the antimicrobial activity, this study seeks to provide valuable insights into how watermelon seed extract can be utilized as a natural alternative or complementary therapy to conventional antibiotics in the treatment of infections caused by antibiotic-resistant bacteria.

2.3 Qualitative Phytochemical Screening

The extracts were screened for various phytochemicals as follows:

2.3.1 Tannins

Two grammes (2 g) of each extract were boiled with 5 mL of distilled water for 5 minutes in a water bath, filtered while hot, and allowed to cool. Ten percent (10%) Ferric chloride (1 mL) was added to 1 mL of each filtrate. The presence of tannins was indicated by a color change from red to green [14].

2.3.2 Flavonoids

One gramne of each dried and washed seed was boiled with 5 mL of distilled water for 10 minutes and filtered while hot. After cooling, one drop of 10% ferric chloride solution was added to each filtrate. Additionally, 1 mL of dilute NaOH was added to each filtrate. A greenish-brown color indicated the presence of flavonoids [14].

2.3.3 Saponins (Froth Test)

Two grammes of each extract were boiled in 10 mL of distilled water in a water bath for 5 minutes. The solution was filtered while hot, cooled, and then treated with 3 mL of dilute tetraoxosulphate (VI) solution (H_2SO_4) and boiled for 15 minutes. Following filtration, 3 mL of 20% NaOH and 2.6 mL of Benedict's quantitative solution were added for observation [14].

2.3.4 Alkaloids (Wagner's Test)

One gramme of each extract was dissolved in dilute hydrochloric acid and filtered. The filtrates were then treated with Wagner's reagent, a solution of iodine in potassium iodide. The appearance of a brown or reddish precipitate confirmed the presence of alkaloids [15].

2.3.5 Glycosides (Modified Borntrager's Test)

One gramme of extract was hydrolyzed with dilute hydrochloric acid (HCl) and tested for glycosides. The extract was treated with ferric chloride solution and placed in boiling water for 5 minutes. After cooling, the solution was extracted with an equal volume of benzene. The benzene layer was separated and treated with ammonia solution. A rose-pink color in the ammoniacal layer indicated the presence of anthranol glycosides [16].

2.3.6 Detection of Phenols (Ferric Chloride Test)

One gramme of extract was treated with four drops of ferric chloride solution. The appearance of a bluish-black color indicated the presence of phenolic compounds [15].

2.3.7 Steroids (Salkowski's Test)

One milligramme of the crude extract was dissolved in 10 mL of chloroform, and concentrated sulphuric acid was added carefully along the sides of the test tube. A red upper layer and a yellow lower layer with green fluorescence indicated the presence of steroids [15].

2.3.8 Terpenoids (Salkowski's Test)

Two millilitres of chloroform was added to 5 mL of the plant extract and mixed with 3 mL of concentrated sulphuric acid. The appearance of a reddish-brown colour indicated the presence of terpenoids [17].

2.3.9 Triterpenoids

Five milligrammes of dried crude extract were dissolved in 2 mL of chloroform, and 1 mL of acetic anhydride was added, followed by 1 mL of concentrated sulphuric acid. The formation of a

reddish-violet colour indicated the presence of triterpenoids [18].

2.3.10 Anthraquinones

One gramme of each plant extract was shaken with 2 mL of 10% ammonium hydroxide solution, then filtered. Ten mL of concentrated H_2SO_4 was added carefully, and color changes were observed. A pink-red or violet color in the lower ammoniacal phase indicated the presence of anthraquinones [14].

2.3.11 Carbohydrates (Benedict's Test)

One gramme of each extract was dissolved in 5 mL of distilled water, filtered, and subjected to Benedict's test by adding Benedict's reagent and gently heating the solution. The formation of an orange-red precipitate indicated the presence of reducing sugars [19].

2.3.12 Extraction of Saponin

In a separate 500 mL beakers, 62.5 g of each of macerated ripe and unripe fruit and leaves were introduced. Fifty-two mL of 95% ethanol was added, and the mixture was left to stand for 48 hours before being filtered through muslin cloth. The filtrates were dried in an oven at 50°C. Seventy-five mL of ethanol was added to each beaker to dissolve the contents. The beakers were left to stand for an additional 48 hours, and the contents were filtered again. The combined filtrates were shaken with a bronze ether mixture (1:1 v/v) and discarded. The alcoholic extracts were concentrated under reduced pressure. The resulting crystals were removed by filtration, dissolved in 95% ethanol, decolorized with activated charcoal, passed through a short column of alumina, and eluted with 95% ethanol. The elute was left at room temperature, and the crystals obtained were removed by filtration and washed with distilled water before recrystallization from 95% ethanol [18].

2.4 Quantitative Estimation of Phytochemical Components

2.4.1 Determination of Total Steroids

The total steroid content of watermelon seed extracts was determined colorimetrically based on the reference saponin content. Saponin crystals were dissolved in a 50 mL mixture of formaldehyde and concentrated sulphuric acid (H_2SO_4). The absorbance (Abs) of this solution was measured at 470 nm using a UV-Vis spectrophotometer [15, 16]. The total steroid content was calculated using the formula:

$$\text{Total Steroid} = \frac{\text{Absorbance of Sample} \times \text{Saponin Content x 100}}{\text{Absorbance of standard x Sample Weight}}$$

2.4.2 Determination of Phenols

Two grammes of fine ground sample were weighed into a 100 mL conical flask and extracted with 20 mL of chloroform at 25°C for 40 minutes. Two mL of distilled water was added to the sample with ten drops of H_2SO_4 solution. Then, 200 mL of 85% ethanol was added, followed by 5 drops of a 10% sodium nitrate solution diluted in H_2SO_4. After 30 minutes, 20 mL of alcoholic NHPG ammonium hydroxide was added. The absorbance of the mixture was measured at 525 nm using a UV/VIS spectrophotometer [20]. The phenolic content was calculated as follows:

$$\text{Phenols Content (\%)} = \frac{\text{Absorbance of Sample} \times \text{Standard Concentration}}{\text{Absorbance of Standard} \times \text{Sample Weight}}$$

where:

Absorbance of Sample is the measured absorbance for the sample solution,

Standard Concentration is the concentration of the phenol standard used for calibration,

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https://aspjournals.org/Journals/index.php/mhsej

Absorbance of Standard is the absorbance of the phenol standard,
Sample Weight is the weight of the sample in grams.

2.4.3 Determination of Alkaloids

Two grammes of the ground sample were weighed and extracted with 5.0 mL of distilled water. The sample was filtered, and the filtrate was used for the determination of alkaloids. Five millilitres (5.0 mL) of the sample extract were measured in a test tube, to which 2.0 mL of 0.2 M sodium nitrate solution and 1.0% sulphuric acid solution were added. The mixtures were thoroughly mixed, and then 2.0 mL of 20% ammonia solution was added. The resulting solution was gently shaken and allowed to stand at room temperature. A red precipitate formed, which was dissolved by adding Nessler's reagent. The resulting extract was read at 560 nm using a UV/visible spectrophotometer. A known standard alkaloid (Sigma product) (5 mg/mL) was treated as described above [21]. The alkaloid content in the sample was calculated using the formula:

$$\text{Alkaloids} = \frac{\text{Absorbance of Sample} \times \text{Concentration of Standard x100}}{\text{Absorbance of Standard} \times \text{Sample Weight}}$$

2.4.4 Determination of Terpenoids

One hundred milligrammes of the sample was weighed into 50 mL of ethanol for reflux, then filtered using Whatman No. 4 filter paper. To 10 mL of the extract, 30 mL of chloroform was added, and the mixture was vortexed and left for 3 minutes. Two hundred mL of concentrated sulfuric acid (H_2SO_4) was added, and reddish-brown precipitation formed. The supernatant was discarded without disturbing the precipitate. Three mL of 95% (v/v) methanol was added and mixed thoroughly until all precipitates dissolved. The absorbance was read at 538 nm using a UV/visible spectrophotometer [17]. The total terpenoid content was calculated as follows using 0.1 mL of Linalool as standard:

$$\text{Terpenoids} = \frac{\text{Absorbance of Sample}}{\text{Weight of sample}} \times \frac{\text{Concentration of Standard}}{\text{Absorbance of Standard}} \text{ x } 100$$

2.4.5 Determination of Glycosides

Five miligrammes of strychnine sulphate was weighed and transferred into a 100 mL volumetric flask. The solution was diluted with distilled water. Ten mL of the diluted sample was mixed with 0.07 M phosphate buffer to a volume of 100 mL. Five mL of the sample extract was adjusted to pH 7 and heated at 100°C for 30 minutes. The total glycoside content was determined colorimetrically at 525 nm [19]. The glycoside content was calculated using:

$$\text{Glycosides} = \frac{\text{Standard Concentration} \times \text{Sample Absorbance x100}}{\text{Standard Absorbance} \times \text{Sample Weight}}$$

2.4.6 Determination of Anthocyanins

Anthocyanins were determined gravimetrically by the method described by Harborne [15]. Each 5 g test sample was hydrolyzed by boiling in 100 mL of 2 M hydrochloric acid (HCl) for 30 minutes. The hydrolysate was filtered using Whatman No. 42 filter paper, and the filtrate was transferred to a separation funnel where it was extracted with ethyl acetate. The ethyl acetate extract was concentrated to dryness over a steam bath, treated with concentrated amyl alcohol, and filtered. The amyl alcohol extract was transferred to a weighed evaporating dish, evaporated to dryness, and dried in an oven at

30°C. The dry weight of the extract was recorded as the anthocyanin content.

2.5 Antimicrobial Activity

The antimicrobial activity of the extracts was evaluated using the disk diffusion method [22]. Each bacterial strain was grown overnight in Mueller Hinton broth at 37°C. The inoculum was prepared by diluting the overnight cultures to match the turbidity of a 0.5 McFarland standard, approximately 1 x 10^8 CFU/mL. Mueller Hinton agar plates were prepared and allowed to solidify. Each plate was inoculated with 100 µL of the standardized bacterial suspension using a sterile spreader. Sterile filter paper discs (6 mm) were soaked in different concentrations of the extracts and placed on the inoculated agar plates. The plates were incubated at 37°C for 24 hours. After incubation, the plates were observed for zones of inhibition around the discs. The diameters of the inhibition zones were measured in millimeters.

2.6 Minimum Inhibitory Concentration (MIC) Determination

The Minimum Inhibitory Concentration (MIC) of watermelon seed extracts against *Streptococcus* spp. was determined following standardized agar well diffusion and broth dilution methods [23, 24]. The MIC test was performed to establish the lowest concentration of extract that inhibited visible bacterial growth after 24 hours of incubation at 35 ± 2°C.

2.6.1 Agar Well Diffusion Method

Muller-Hinton Agar was prepared by dissolving 38 g of the medium in 1000 mL of distilled water, sterilized at 121°C for 15 minutes, and allowed to cool to 46°C before pouring into sterile Petri dishes [25]. Fresh cultures of *Streptococcus pyogenes*, *Streptococcus oralis*, *Streptococcus faecalis*, and *Streptococcus salivarius*, adjusted to 10^6 CFU/mL, were spread evenly across the agar surface. Wells of 5 mm diameter were created on the inoculated plates, each filled with 100 µL of watermelon seed extract at concentrations of 200 mg/mL, 100 mg/mL, 50 mg/mL, and 25 mg/mL (Bauer et al., 1966). Tetracycline (30 mg) and flagyl (30 mg) served as positive controls, while 100 µL of dimethyl sulphoxide (DMSO) served as the negative control. Plates were incubated at 37°C for 24 hours, and the diameter of zones of inhibition was measured in millimeters [23].

2.6.2 Broth Dilution Method

A serial dilution of watermelon seed extract was prepared in nutrient broth at concentrations of 200 mg/mL, 100 mg/mL, 50 mg/mL, and 25 mg/mL in 12 labeled test tubes [26]. Each tube was inoculated with a fresh suspension of the test bacteria to achieve a final concentration of approximately 10^6 CFU/mL. Tubes were incubated at 35 ± 2°C for 24 hours. After incubation, each tube was observed for visible growth (turbidity). The MIC was defined as the lowest concentration with no observable bacterial growth [27].

2.7 Minimum Bactericidal Concentration (MBC) Determination

The Minimum Bactericidal Concentration (MBC) test was conducted on tubes from the MIC test that showed no growth, following guidelines established by the Clinical and Laboratory Standards Institute [23]. Fresh Mueller-Hinton Agar plates were prepared, and an inoculum from each MIC tube with no visible growth was streaked across the agar surface at concentrations of 200 mg/mL, 100 mg/mL, 50 mg/mL, and 25 mg/mL for each bacterial strain [24]. Plates were incubated at 35 ± 2°C for 24 hours. The MBC was determined as the lowest concentration at which no bacterial colonies were observed, indicating bactericidal efficacy [28].

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Impact Factor: 4.42

Advance Scholars Publication
Published by International Institute of Advance Scholars Development
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3.0 RESULTS

Table 1 shows the phytochemical analysis revealing the presence or absence of various bioactive compounds within the watermelon seed extracts.

Table 2 shows the concentration of phytochemicals in watermelon seed extracts (labelled as Extracts 1, 2, and 3) quantified for the presence of various phytochemicals, including tannins, glycosides, saponins, terpenoids, flavonoids, alkaloids, phenolics, steroids, reducing sugars, carbohydrates, and anthocyanins. The values measured, represented in absorbance (Abs.) and their respective concentrations (mg/100g), are detailed below.

Table 3 shows the antibacterial activity of the three watermelon seed extracts against four bacterial species: *Streptococcus pyogenes*, *Streptococcus oralis*, *Enterococcus faecalis*, and *Streptococcus salivarius*. The inhibition zones, recorded in millimeters (mm) at various extract concentrations (200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml), demonstrate variable susceptibility. Watermelon Seed Extract 1 showed significant inhibition on *Streptococcus pyogenes* (25 mm at 200 mg/ml) and *Enterococcus faecalis* (25 mm at 50 mg/ml). Watermelon Seed Extract 2 demonstrated notable inhibition on *Streptococcus pyogenes* and *Streptococcus salivarius*, particularly with a minimum inhibition concentration (MIC) of 2.5 mg/ml and 50 mg/ml, respectively. Watermelon Seed Extract 3 provided strong inhibitory effects on *Streptococcus pyogenes* (24 mm at 200 mg/ml) and *Streptococcus salivarius* (25 mm at 25 mg/ml).

Table 4 shows the Minimum Bactericidal Concentrations (MBCs) of the three watermelon seed extracts (Extracts 1, 2, and 3) which were evaluated against four bacterial species: *Streptococcus pyogenes*, *Streptococcus oralis*, *Streptococcus faecalis*, and *Streptococcus salivarius*. The bactericidal effects of each extract were tested at concentrations of 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml. The presence of growth ("+") or no growth ("-") was recorded to determine the minimum concentration required for bactericidal activity.

Table 1. Qualitative Screening of Phytochemical Watermelon Seed Extract

S/N	TEST	TEST MIXTURE	OBSERVATION	INFERENCE
1.	Tannins	5.0ML sample extent +20ml of 1.0%$^{w/v}$ Fecl3 regent solution	Absence of dark green colouration was observed in all the samples	Tannis absent (-ve)
2.	Saponins	20.ml of sample in 5.0ml distilled water and the mixture was vigorous shaken + heat	Formation and Slight persistence of frothing observed	Saponnis present (+ve)

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3.	Alkaloids	20ml sample extract + 2.0ml of 2% v/v H_2SO_4 + 1.0ml iodine solution in potassium iodide	Formation of a yellow cream precipitate was observed in all the samples.	Alkaloids present (+ve)
4.	Glycosides	2.0ml sample extract + HCl, neutralized with NaOH, + few drops of 1.0% w/v $FeCl_3$ + 1.0ml conc. H_2SO_4	Formation of reddish-brown ring at junction of two liquid layers observed	Glycosides present (+ve)
5.	Flavonoids	a. 2.0ml sample extract + diluted NaOH and HCl b. Filtrates + zinc dust + conc. HCl	Formation of pink scarlet and occasional green-blue observed	Flavonoids Presence (+v)
6.	Phenolic Compounds	2.0ml sample extract + 5.0ml 95% ethanol, boiled, filtered hot + 5.0ml distilled water, evaporated ethanol, + 5 drops of 1.0% w/v $FeCl_3$ and potassium ferricyanide solution	Formation of blue or greenish coloration was observed in all samples	Phonetic compounds present (+ve)
7.	Steroids	2.0ml acetic anhydride + 2.5ml sample extract + 2.0ml conc. H_2SO_4	Formation of Reddish-brown ring at the junction of two layer was observed	Steroids present in all samples (+ve)
8.	Terpenoids	2.0ml extract + 2.0ml chloroform + 3.0ml conc. H_2SO_4	There was formation of an intense red/brown coloration in all the sample.	Terpenoids present (+ve)
9.	Triterpenoids	Sample extracts (2.0ml) was added with a few drops to conc. Sulphuric acid, them the mixture was shaken thoroughly.	There was appearance of yellow colorations in all the samples.	Triterpenoids present (+ve)
10.	Anthocyanin	2.0ml sample extract + 2.0ml 0.2M HCl, boiled, + 1.0ml ammonia	No formation of pink/red coloration was observed in the samples	Anthocyanins absent (-ve)

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11.	Anthraquinones	0.5ml sample extract + 10% v/v HCl, boiled, filtrate cooled, + 2.0ml chloroform, + 2.0ml filtrate + few drops 10% v/v ammonia	No formation of pink-red colouration observed	Anthraquinones absent (-ve)
12.	Reducing Sugars	5.0ml of mixture of Fehling's solution A and B + 5.0ml sample extract, heated	A Brick-red precipitate of cuprous oxide observed	Reducing sugars Present (+ve)
13.	Carbohydrates	0.1ml sample extract boiled with distilled water, filtered, filtrate + few drops naphthol in ethanol + conc. H_2SO_4	Formation of red/ dull violet coloration at the interfacial was observed	Presence of carbohydrates confirmed (+ve)

Table 2. Concentration of Phytochemicals in Watermelon Seed Extract.

Test Parameter	Watermelon Seed Extract 1		Watermelon Seed Extract 2		Watermelon Seed Extract 3	
	Abs. Reading	Value mg/100g	Abs. Reading	Value mg/100g	Abs. Reading	Value mg/100g
Tannins	0.0126	0.026	0.00136	0.028	0.00107	0.022
Glycosides	0.01436	12.43	0.01454	12.58	0.01421	12.29
Saponins	0.00028	2.80	0.00027	2.70	0.00026	2.60
Terpenoids	0.3506	0.56	0.3944	0.63	0.3694	0.59
Flavonoids	0.0340	5.36	0.0283	4.98	0.0294	5.18
Alkaloids	0.04208	62.44	0.04404	65.34	0.04266	63.29
Phenolic	0.7775	6.22	0.7800	6.74	0.8237	6.59
Steroids	0.0145	0.29	0.0160	0.32	0.0135	0.27
Reducing Sugar	0.1143	40.32	0.11167	39.42	0.1222	41.28
Carbohydrates	0.0938	76.51	0.2904	75.63	0.2873	74.96
Anthocyanin	0.0002	0.004	0.0001	0.002	0.0002	0.004

Table 3. Minimum Inhibitory Concentration (MIC) for Watermelon Seed Extracts on Selected Test Organisms

Test Organisms	Extent Concentration 200mglml 100mg/ml 50mg/ml 25.mg/ml	12.5mg/ml	Minimum Inhibition Concentration

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Watermelon Seeds Extract 1	(mm)				(mm)	mg/ml
Streptococcus pyogenes	25	23	22	18	14	12.5
Streptococcus oralis	18	15	09	08	11	25
Streptococcus faecalis	24	23	25	16	18	25
Streptococcus salivarius	23	20	20	17	09	12.5
Watermelon Seeds Extract 2						
Streptococcus pyogenes	18	16	22	15	20	2.5
Streptococcus oralis	23	19	16	19	15	12.5
Streptococcus faecalis	22	20	18	15	14	12.5
Streptococcus salivarius	23	20	17	20	22	50
Watermelon Seeds Extract 3						
Streptococcus pyogenes	24	22	20	21	21	12.5
Streptococcus oralis	22	17	04	09	12	25
Streptococcus feecalis	24	20	18	14	16	25

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Table 4. Minimum Bactericidal Concentrations (MBCs) of water melon extracts

Test Organisms	Extent Concentration 200mglml	100mg/ml	50mg/ml	25.mg/ml	12.5mg/ml	Minimum Bactericidal Concentration
Watermelon Seeds Extract 1	No Growth	No Growth	No Growth	No Growth	Growth	mg/ml
Streptococcus pyogenes	-	-	-	-	+	25
Streptococcus oralis	-	-	-	+	+	50
Streptococcus faecalis	-	-	-	+	+	50
Streptococcus salivarius	-	-	-	-	+	25
Watermelon Seeds Extent 2	No Growth	No Growth	No Growth	No Growth	Growth	
Streptococcus pyogenes	-	-	-	+	+	50
Streptococcus oralis	-	-	-	+	+	50
Streptococcus faecalis	-	-	-	-	+	25
Streptococcus salivarius	-	-	+	+	+	100
Watermelon Seeds Extract 3	No Growth	No Growth	No Growth	No Growth	Growth	
Streptococcus pyogenes	-	-	-	-	+	25
Streptococcus oralis	-	-	-	+	+	50
Streptococcus faecalis	-	-	-	+	+	50
Streptococcus salivarius	-	-	+	+	+	100

4.0 DISCUSSION

This study aimed to investigate the phytochemical constituents, quantification of bioactive compounds, and antibacterial efficacy of three watermelon seed extracts, leveraging phytochemical screening, spectrophotometric analysis, and antibiotic susceptibility testing to assess potential health benefits and therapeutic applications.

The preliminary phytochemical screening revealed the presence of several bioactive compounds in the watermelon seed extracts,

including saponins, alkaloids, glycosides, flavonoids, phenolic compounds, steroids, and terpenoids. Such phytochemicals are widely documented for their iverse health benefits and antimicrobial activities [29]. For instance, saponins have demonstrated immune-stimulatory and cholesterol-lowering effects, making them valuable for cardiovascular health [30]. Alkaloids are known for their analgesic and anti-inflammatory properties, hence are crucial for pharmaceutical applications [31]. The presence of glycosides and flavonoids indicates possible antioxidant activities, as flavonoids are particularly effective in scavenging free radicals and protecting cells from oxidative damage [32]. The absence of anthocyanins and anthraquinones in the extracts aligns with previous studies on non-pigmented watermelon species, suggesting that the chemical composition may vary depending on the fruit variety and extraction methods [33]. This variability underpins the importance of selecting appropriate extraction protocols to maximize the yield of target compounds [34].

The spectrophotometric analysis provided a quantitative profile of key phytochemicals in the watermelon seed extracts, revealing that the extracts were particularly rich in alkaloids and carbohydrates. Alkaloid concentrations ranged from 62.44 mg/100g to 65.34 mg/100g, aligning with findings by Chakravarthi et al [35] that support watermelon seeds as a source of bioactive alkaloids with potential health benefits. Tannins and glycosides are exhibited noteworthy concentrations, with glycoside levels reaching up to 12.58 mg/100g. Glycosides are associated with cardiotonic and diuretic properties, supporting cardiovascular health by maintaining electrolyte balance and promoting heart function [36]. The carbohydrate content, quantified between 74.96 and 76.51 mg/100g, highlights the nutritional potential of watermelon seeds as a dietary supplement. Previous research indicates that carbohydrates in seeds serve as an energy source and play a role in enhancing endurance [37].

The antibacterial potential of the extracts was assessed through antibiotic susceptibility testing and determination of Minimum Bactericidal Concentration (MBC). Results indicated varying degrees of inhibition across different bacterial strains, with *Streptococcus pyogenes* exhibiting the highest sensitivity, particularly to Extracts 1 and 3, where an MBC of 12.5 mg/ml was sufficient for bactericidal action. This efficacy is consistent with prior findings that highlight watermelon seeds' antimicrobial potential against Gram-positive bacteria [38].

Watermelon seed extracts demonstrated bactericidal effects against *Streptococcus faecalis* and *Streptococcus oralis* at an MBC of 25 mg/ml for Extracts 1 and 3. Such effectiveness could be attributed to the presence of phenolic compounds and flavonoids, known for their ability to disrupt bacterial cell walls and inhibit essential enzymatic pathways [39]. Phenolic compounds, with recorded concentrations between 6.22 mg/100g and 6.74 mg/100g, are documented to possess strong antimicrobial activity due to their hydroxyl groups, which increase hydrophobicity and enhance interaction with bacterial membranes [40].

While Extract 2 exhibited a slightly reduced efficacy, its notably inhibitory effect on *Streptococcus salivarius* at higher concentrations (50 mg/ml) demonstrates that even minor variations in extraction techniques or chemical composition can impact antibacterial properties. This observation corroborates the work of Belščak-Cvitanović et al., who emphasized that minor compositional differences among plant extracts can influence their biological activities [41].

The findings suggest that watermelon seed extracts, particularly ethanol-based ones, could serve as a natural antimicrobial agent, particularly against Gram-positive pathogens including *Streptococcus* species, which are implicated in a range of infections. The presence of multiple phytochemicals, including flavonoids and saponins, likely contributes to this activity. These compounds disrupt bacterial cell walls and inhibit enzymatic processes, thereby making the development of resistance less likely. This antimicrobial potential aligns with the increased interest in plant-based compounds as alternatives to synthetic or conventional antibiotics, especially amidst rising antibiotic resistance [42].

Conclusion

Watermelon seed extract shows great promise as a natural antimicrobial agent against *Streptococcus* spp. Given the presence of potent bioactive compounds like alkaloids, glycosides, and phenolic compounds, watermelon seeds could offer both nutritional and therapeutic benefits.

Funding
This research received no external funding.
Acknowledgments
The authors acknowledge the technical support from the postgraduate laboratory microbiology, Lagos State University, Ojo.
Conflicts of Interest
The authors declare no conflict of interest.

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