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Antimicrobial Activity of Methanolic Leaf Extract of *Terminalia brownii* on *Streptococcus pneumoniae* Isolates

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Abstract

Pneumococcal infections are a major cause of morbidity and mortality worldwide, particularly in vulnerable populations such as young children, the elderly, and immunocompromised individuals. Despite the availability of antibiotics, the emergence of antibiotic-resistant strains of pneumococcus poses a significant threat to public health. Therefore, there is a need for alternative sources of antimicrobial agents to scale down pneumococcal infections. Ethnobotanical studies have shown that specific plant species, including Terminalia brownii, possess anti-streptococcal activity. However, there is a dearth of scientific literature on the antimicrobial properties of *Terminalia brownii* against pneumococci. In this study, we explored the antimicrobial activity of Terminalia brownii on pneumococci. In vitro antimicrobial susceptibility testing was carried out using the disk diffusion method. Green leaves of Terminalia brownii were obtained from the botanical garden at Kabarak University. The percentage yield of extract obtained after preparation was 7%. Phytochemical analysis was performed to determine the major classes of compounds in the extract. Four concentrations of leaf extract were used: 7, 3.5, 1.75, 0.875mg/ml. Notably, a decrease in concentration was associated with decrease in antistreptococcal activity. The study demonstrated that *Terminalia brownii* possesses antistreptococcal activity against pneumococcus. However, the activity of the extract was less potent than that of amoxicillin. Further studies are necessary to determine the potential of *Terminalia brownii* as an alternative source of antimicrobial agents.

KEYWORDS:
Terminalia brownii, pneumococcus, tannins, flavonoids, Terpenoids.

I. Introduction

Antimicrobial resistance is a global public health concern that poses a threat to the effective treatment of infectious diseases. *Streptococcus pneumoniae* is an important pathogen responsible for causing a wide range of diseases, including pneumonia, meningitis, sepsis, and otitis media (Weiser et al., 2018). The emergence of antibiotic-resistant strains of S. pneumoniae has made it difficult to treat infections caused by this bacterium, leading to an increase in morbidity and mortality rates globally. According to the World Health Organization (WHO), S. pneumoniae is responsible for approximately 1.6 million deaths annually, with most of the deaths occurring in children under the age of five years in developing countries (Rankine-Mullings and Owusu-Ofori, 2021).

Pneumococcal infections pose a significant global burden, and vaccination is recognized as an effective preventive strategy. However, the implementation of pneumococcal vaccination programs is impeded by the high cost of vaccines, especially in low-income countries, where most of the pneumococcal disease burden occurs (Tricarico et al., 2017). For instance, it is estimated that the cost of a single dose of the pneumococcal conjugate vaccine (PCV) can be as high as US$ 69 in some countries, which is a significant financial burden for many families and healthcare systems. As a result, only a small proportion of eligible individuals have access to the vaccine, particularly in developing countries. For instance, in Africa, only 25% of children who need PCV receive it, and coverage rates are even lower in some countries (Porchia et al., 2017).

Furthermore, pneumococcal vaccines have limited effectiveness against all pneumococcal strains, as there are over 100 known serotypes of S. pneumoniae (Balsells et al., 2017). The current pneumococcal vaccines only target a limited number of serotypes that are responsible for a significant proportion of pneumococcal disease cases. However, the vaccines do not protect against all pneumococcal serotypes, and new strains emerge over time. For example, in the United States, the prevalence of pneumococcal serotypes not included in the vaccine increased from 35% in 2009 to 51% in 2015 (Suaya et al., 2020). This trend suggests that the effectiveness of the current pneumococcal vaccines may decline over time, highlighting the need for alternative strategies to prevent and treat pneumococcal infections.

Natural products have been identified as a promising source of novel antimicrobial agents that could complement or replace conventional antibiotics (Challinor and Bode, 2015). Plant extracts and essential oils have been shown to have antibacterial activity against a range of pathogenic bacteria, including S. pneumoniae. The use of natural products is particularly attractive in low-income countries, where many communities rely on traditional medicine for their healthcare needs. Several studies have reported on the antibacterial activity of plant extracts against pneumococcal strains (Matura et al., 2022; Moghtaderi et al., 2021). For instance, extracts from the leaves and bark of *Terminalia brownii* have been shown to inhibit the growth of S. pneumoniae in vitro. Such findings suggest that plant extracts could serve as a potential source of novel antimicrobial agents to combat pneumococcal infections.

In this context, the present study investigated the antibacterial activity of methanolic leaf extract of *Terminalia brownii* against *S. pneumoniae* isolates. The study aimed to evaluate the potential of *Terminalia brownii* as a source of novel antimicrobial agents to combat pneumococcal infections. The findings of the study have implications for the development of new strategies to prevent and treat pneumococcal infections, particularly in low-income countries where access to conventional antibiotics and vaccines is limited.

II. Methodology

Plant Material Collection and Drying

Fresh green mature leaves of *Terminalia brownii* were collected from the Kabarak University School of Pharmacy Botanical Garden, located in Nakuru County, Kenya. The plant was identified and authenticated by a Botanist, and an herbarium voucher specimen (No. KABUPHARM/2023/TB/01) was prepared and deposited at the University's Pharmacognosy laboratory for future reference. The leaves were carefully selected based on the plant maturity and freedom from insect infestations or any visible damage, and transported to the laboratory for further processing. The leaves were dried at normal room temperature in a well-ventilated laboratory for 8 days to ensure complete drying, and a constant weight was achieved. The dried leaves were then pulverized to a coarse powder using a grinding machine, and the resulting powder was transferred into a sterile zip lock bag for storage until further use. Careful precautions were taken during the drying and powdering process to prevent contamination and ensure the quality of the plant material.

Materials Reagents and Apparatus

The reagents used were hexane, methanol, Dimethylsulphoxide (DMSO), lead acetate, α-naphthol, sulphuric acid, 10% ammonia solution, Mayer's reagent, Wagner's reagent, Hager's reagent, Dragengroff's reagent, Ferric chloride solution, distilled water, NaCl, NaOH, 10% Gelatin solution, HCl, chloroform, GAA, 90% alcohol, Acetic anhydride, Millon's reagent, Benedicts's reagent, Muller Hinton agar.

The apparatus used were mortar and pestle, reagent bottles, rotary evaporator, mechanical shaker, filter paper, petri dish, weighing scale, aluminum foil, universal bottle, cotton wool, filter paper disc, Whatmann paper no 1.

Preparation of Plant Extracts

The plant extract was prepared using the maceration method with methanol as the solvent (Adam et al., 2019). A total of 100g of coarsely grounded powder was placed in a flask and extracted using methanol for 48 hours under continuous agitation using a mechanical shaker. The process was repeated three times to ensure complete and exhaustive extraction. Gravitational filtration was carried out at every stage using Whatman No. 1 filter paper to separate the menstrum from the marc.

The menstrum obtained was then concentrated using a rotary vacuum evaporator at a temperature of 45 degrees Celsius. The concentrate was transferred to a sterile labeled beaker and dried completely at 30 degrees Celsius in an oven to obtain a final extract in grams. The percentage yield of the extract was calculated in terms of air-dried weight of the crude powder used. Finally, the dried extract was transferred for storage into a refrigerator at 4 degrees Celsius awaiting further analysis. The use of methanol as a solvent in the maceration process ensures the extraction of a wide range of bioactive compounds from the plant material. The efficient concentration of the extract further enhances the concentration of these bioactive compounds, making it suitable for further analysis.

Phytochemical Analysis

Phytochemical analysis of the methanolic extract of *Terminalia brownii* leaves was performed to determine the presence of various active metabolites. The extract was subjected to qualitative analysis for the detection of alkaloids, saponins, coumarins, terpenoids, flavonoids, cardiac glycosides, and tannins.

Detection of Alkaloids

To confirm the presence of alkaloids in the methanolic extract, three different chemical tests were employed, namely Mayer's test, Wagner's test, and Dragendorff test. According to Ahmed and Sulaiman (2018), Mayer's test involved dissolving a small amount of the extract in 2 ml of distilled water and adding a few drops of Mayer's reagent (potassium mercuric iodide solution). The resulting mixture was then observed for the formation of a cream-colored precipitate which was indicative of the presence of alkaloids.

In Wagner's test, a small quantity of extract was also dissolved in 2 ml of distilled water, and a few drops of Wagner's reagent (iodine solution in potassium iodide) were added. A reddish-brown precipitate formation would confirm the presence of alkaloids in the extract. Similarly, for Dragendorff test, a small amount of extract was dissolved in 2 ml of distilled water and a few drops of Dragendorff reagent (solution of bismuth potassium iodide) were added. The mixture was then observed for the formation of an orange or red precipitate that would indicate the presence of alkaloids. All the three tests were conducted in triplicates and the results were recorded. The concentration of the extract was adjusted to standard concentrations to ensure accuracy and reproducibility of the tests. The presence of alkaloids in the extract was further confirmed through the use of thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) (Doshi and Une, 2016).

Test for Saponins

To test for the presence of saponins, a frothing test was performed. A sample of 0.5g of the extract was weighed and transferred into a clean test tube. Five millilitres of distilled water were added to the extract, and the mixture was shaken vigorously for about a minute. The mixture was then left to stand for 15 minutes. The observation was made for the formation of froth, which persisted for more than 15 minutes. The formation of stable froth indicates the presence of saponins in the extract. This method of testing for saponins has been previously described by Khandelwal and Vrunda (2015).

Detection of Flavonoids

Two methods were used to detect the presence of flavonoids in the extract. The first method was the Shinoda test, which involved the extraction of the extract using 70% alcohol in a water bath. A sample of 0.5g of the extract was weighed and transferred into a clean test tube. The extract was then extracted by 70% alcohol in a water bath, and 0.1g of magnesium ribbon was added. A few drops of concentrated hydrochloric acid were then added to the mixture, and the development of orange, pink or red to pink color to be observed, indicating the presence of flavonols, dehydro-derivatives and xanthene's in the extract (Khandelwal and Vrunda (2015).

The second method used was the ammonia test. A sample of 0.5g of the extract was weighed and transferred into a clean test tube. The extract was then boiled with 5ml of 70% alcohol in a water bath for 3 minutes. The solution was then cooled, and a strip of Whatmann filter paper No. 1 was inserted to soak it in the extract. The paper strip was impregnated in a tube containing ammonia fumes for a few minutes, and the strip was observed for a yellow color or yellow brown spot. The development of a yellow color indicated the presence of flavonoids in the extract (Harborne, 1998). Both the Shinoda and ammonia tests are reliable methods

for the detection of flavonoids in plant extracts. The Shinoda test is based on the reaction between flavonoids and magnesium in the presence of hydrochloric acid, which results in the formation of a yellow color. The ammonia test, on the other hand, is based on the reaction between flavonoids and ammonia, which results in the formation of a yellow color on the filter paper strip (Sofowora, 1993; Harborne, 1998).

In alkaline test, addition of increasing amount of sodium hydroxide to the extract solution shows fluorescent yellow color, which disappears on addition of acid. (Khandelwal and Vrunda (2015).

Test for Terpenoids

To test for the presence of terpenoids in the extract, the Salkowski test was performed. A sample of 0.5g of the extract was weighed and dissolved in 5ml of chloroform. The solution was then filtered, and a sample of 2ml was transferred into a clean test tube. A few drops of concentrated sulfuric acid were gradually added to the solution and allowed to stand for 5 minutes. The mixture was then shaken, and the lower layer was observed for any color change. The development of a red color in the lower layer indicated the presence of terpenoids in the extract (Brahmachari, 2013). The Salkowski test is based on the reaction between terpenoids and concentrated sulfuric acid, which results in the formation of a red color in the lower layer. Terpenoids are a class of compounds found in plants that are known to exhibit various biological activities, including antioxidant, antimicrobial, and anticancer properties (Brahmachari, 2013).

Test for Coumarone Glycosides

To detect the presence of coumarin glycosides in the extract, a total of 0.5g of the extract was weighed and transferred into a clean test tube. Then, 5ml of 70% alcohol was added, and the mixture was boiled for 3 minutes. After boiling, the mixture was filtered while hot and left to cool. A sample of 2ml of the extract was measured and transferred into a new test tube. Then, one drop of aqueous ferric chloride was added to the extract, and the mixture was observed for any colour change. The formation of a dark green colour indicated the presence of coumarin glycosides in the extract (Gadgoli & Mishra, 1999). The test is based on the reaction between coumarin glycosides and ferric chloride, which results in the formation of a green colour. Coumarin glycosides are a class of compounds found in plants that have various biological activities, including anti-inflammatory, antioxidant, and anticoagulant properties (Gadgoli & Mishra, 1999).

Test for Tannins

To detect the presence of tannins in the plant extract, a total of 0.5g of the extract was weighed and boiled with 5ml of distilled water. After boiling, the extract was filtered, and 2ml of the filtrate was transferred to two different test tubes.

To the first test tube, three drops of ferric chloride solution were added, and the mixture was observed for any colour change. The development of a brown-green precipitate indicated the presence of tannins in the extract. To the second test tube, 1ml of lead sub-acetate was added, and the mixture was observed for any precipitate formation. The development of a creamy-brown precipitate indicated the presence of tannins in the extract (Uma et al., 2014). Tannins are a group of polyphenolic compounds that are widely distributed in the plant kingdom. They have astringent properties and are known to have various biological activities, including antioxidant, anti-inflammatory, and antimicrobial properties (Salah et al., 2020).

Detection of Protein and Amino Acid

Xanthopoetic Test

The extracts were treated with a few drops of Conc. Nitric acid. Formation of yellow colour indicates the presence of proteins. (Uma et al., 2014)

B. Ninhydrin test: to the extract, 0.25% w/v Ninhydrin reagent was added and boiled for a few minutes. Formation of blue colour indicates the presence of amino acid.

Detection of Carbohydrates

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test the presence of carbohydrates. (Uma et al., 2014)

Molisch's Test

Filtrates were treated with 2 drops of alcoholic α-Naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of carbohydrates.

Fehling's Test

Filtrates were hydrolyzed with dilute HCl, neutralized with alkali and heated with Fehling's A and B solutions. Formation of a red precipitate indicates the presence of reducing sugars.

Detection of Phenol

Ferric chloride test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenol. (Uma et al., 2014)

10. Detection of glycosides: Extracts were hydrolysed with dilute HCl, and then subjected to test for glycosides. (Uma et al., 2014)

Modified Borntrager Test

Extracts were treated with ferric chloride solution and immersed in boiling water for about 5 min. The mixture was cooled and extracted with equal volumes of benzene layer was separated and treated with ammonia solution. Formation of rose pink colour in the ammoniacal layer indicates the presence of anthranol glycosides.

Test for Cardiac Glycosides

About 5 ml of each extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1ml of concentrated sulphuric acid. A brown ring at the interface indicated the deoxy sugar characteristics of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may be formed.

Test for Combined Anthraquinones

A total of 0.5 g of extract sample was boiled with 5 ml of 10% hydrochloride acid for 5 mins. The mixture was filtered while hot and the filtrate was allowed to cool. The cooled filtrate was portioned against the equal volume of chloroform and the chloroform layer was transferred into a clean dry test tube using a clean pipette. Equal volume of 10 % ammonia solution was added into the chloroform layer, shaken and allowed to separate. The separated aqueous layer was observed for any change, delicate rose pink colour showed the presence of an anthraquinone. (Uma et al., 2014)

Preparation of Bacterial Strains of *Streptococcus pneumoniae*

To prepare bacterial strains of *Streptococcus pneumoniae*, frozen samples stored at the Kabarak University Microbiology Laboratory were retrieved and thawed. The viability of the bacteria was ensured by sub-culturing them on both blood agar and Mueller Hinton agar. To perform sub-culturing, a sterile swab was used to streak the S. pneumoniae isolate onto a blood agar plate. The plate was then incubated overnight at 37 degrees Celsius with 5% carbon dioxide to allow the bacteria to grow. The sub-culturing procedure was repeated for a total of five plates.

Preparation of Impregnated Discs

To prepare impregnated discs, a stock solution of the *Terminalia brownii* extract was first prepared by dissolving 7mg of the extract in 1ml of dimethyl sulfoxide. Different concentrations of the extract (7mg/ml, 3.5mg/ml, 1.75mg/ml, and 0.4375mg/ml) were then used to impregnate sterilized blank discs. Negative controls were prepared by impregnating discs with dimethyl sulfoxide only, while treatment discs were soaked in the methanolic extract. A positive control was prepared using amoxicillin 500mg.

All the impregnated discs were then dried in an incubator at 45°C and stored aseptically for 24 hours prior to bacterial application. The dried discs were placed equidistant from each other on a blood agar plate, and incubated at 37 degrees Celsius with 5% carbon dioxide overnight. The use of different concentrations of the extract in the impregnated discs allows for a dose-dependent effect to be observed on bacterial growth inhibition. The use of dimethyl sulfoxide in the negative control discs ensures that any observed effects are due to the presence of the extract and not the solvent. The inclusion of a positive control (amoxicillin) allows for the comparison of the effectiveness of the extract to a known antibiotic.

Application of Impregnated Discs

The prepared bacterial strains were used to test the effectiveness of the impregnated discs. To ensure consistency, each test plate contained three discs; one positive control (ceftriaxone 30mcg), one negative control (100% DMSO), and one treated disc loaded with different concentrations of the *Terminalia brownii* extract. The treated discs were placed equidistant to each other on the Mueller Hinton agar surface using sterile forceps. The plates were then inverted and incubated at 37°C for 24 hours under 5% carbon IV oxide conditions. After incubation, the plates were observed for the presence of susceptibility around the impregnated discs. The zones of inhibition were measured using vernier calipers, and the results were recorded. Each test was repeated three times to ensure reproducibility and accuracy of results.

In addition, the diameter of the inhibition zones was measured and compared with the positive control (ceftriaxone 30mcg) to evaluate the effectiveness of the extract. The results were tabulated and statistically analyzed to determine the significant differences between the treated and control groups. The statistical analysis included ANOVA and Student's t-test, and p-values less than 0.05 were considered statistically significant. The data obtained were then interpreted, and conclusions were drawn. It is worth noting that the experiment was conducted in a sterile environment to minimize contamination and ensure accurate results. The use of appropriate controls and replicates improved the reliability of the results. Also, the use of different concentrations of the extract on the impregnated discs enabled the determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the extract on the tested bacteria.

Results

Calculation of the Percentage Yield of the Extract

In order to fully utilize the potential of this extract for medicinal purposes, it was important to accurately determine the percentage yield of the extract obtained from the leaves. The percentage yield is a measure of the efficiency of the extraction process and gives an idea of the quantity of the extract that can be obtained from a given amount of plant material.

$$Percentage\ Yield = \left(\frac{Actual\ Yield}{Theoretical\ Yield}\right) \times 100$$

$$Percentage\ Yield = \left(\frac{7g}{100g}\right) \times 100$$

Percentage yield = 7%

Antimicrobial Susceptibility and Activity

Table 1 shows results for antimicrobial susceptibility testing. Four concentrations of methanolic leaf extract were used. At 7mg/ml, the furthest zone of inhibition was recorded at 9.12mm. Comparatively, this was slightly lower than amoxicillin's zone of inhibition (10.26mm) notably, as concentration of leaf extract was decreased, the average zone of inhibition also reduced.

Table 1:

Antimicrobial Susceptibility Testing of Methanolic Leaf Extract at Various Concentrations

Plates	Concentration	Average Zone of Inhibition
1	7mg/ml	9.12mm
2	3.5mg/ml	8.03mm
3	1.75mg/ml	7.32mm
4	0.875mg/ml	6.52mm
	Negative control	6.00mm
	Positive control	10.26mm

Phytochemical Analysis

Table 2 shows the phytochemical compounds that were detected from the leaf extract of *Terminalia brownii*. Flavonoids, coumarins, tannins and Terpenoids were present in high concentration while alkaloids and cardiac glycosides were undetectable. Saponins were moderately present in the extract. See table 2.

Table 2:

Phytochemical Analysis of Leaf Extract of Terminalia brownii

Chemical Test	Observation	Results
Alkaloids test		
Mayer's	Absence of white-brown buffy precipitate	absent
Wagner's	Absence of red-brown color	absent
Dragendroff's	Absence of orange-red color	absent
Hager's test	Absence of yellow precipitate	absent
Flavonoids test		
Shinoda test	Presence of yellow color	++
Sulphuric acid test	Presence of deep yellow color	+++
Alkaline test	Presence of yellow color, no reaction on addition of acid.	+++
Ammonia test (fumes)	Presence of yellow color/spot on a filter paper strip	+++
Lead acetate test	Presence of yellow precipitate	+++
Tannins test		
Ferric chloride test	Deep dark green/dark brown colour-	+++
Lead acetate	White-yellow precipitate	+++
Steroids		
Liebermann's test	Reddish brown ring at the interface of two layers was observed, upper layer bluish green and lower layer clear	+++
Terpenoids		
Salkowski test	Red-purple color	++
Saponins test		
Foam test	Forms froth, the froth disappears after 10-15 mins.	++
Haemolytic test	Formation of fragments in a blood sample, RBCs ruptured	++
Cardiac glycosides		
Keller killiani test	Absence of bluish-green color in the upper acetic acid layer	Absence of deoxy sugars
Kedde reaction	Absence of violet/purple color for lactones	absence of lactones
Liebermann's test	Positive formation of reddish-brown ring at the junction	+++ presence of steroids
Coumarin test		
Fluorescence test	Formation of a yellow color/fluorescence	+++ high presence
Ferric chloride test	Dark-brown color/precipitate	+++high presence
Test for combined Anthraquinone		
Borntrager test	Absence of rose-pink color in the ammoniacal layer-trace of brown	Traces amount
Modified Borntrager	Absence of pink-red color at the junction	absence
Detection of phenol		
Ferric chloride test	Presence of dark green color	+++presence of phenolic compounds
Detection of carbohydrates		
Molisch's test	Presence of red-purple ring at the junction	++ presence of carbohydrates
Fehling's test	Presence of a brick red precipitate	+++ presence of carbohydrates

Discussion

The phytochemical screening tests conducted on the *Terminalia brownii* extract revealed the absence of alkaloids as indicated by the lack of color change in both Mayer's and Wagner's tests. Alkaloids are nitrogen-containing compounds that are often associated with biological activities such as antimicrobial, anti-inflammatory, and analgesic effects (Mandal and Mandal, 2011). The absence of alkaloids in *Terminalia brownii* extract suggests that other phytochemicals may be responsible for its biological activities.

On the other hand, the presence of flavonoids in the extract was confirmed by the intense color change observed in Shinoda's and ammonia tests. Flavonoids are well-known for their antioxidant, anti-inflammatory, and antimicrobial properties, which makes them useful in the treatment of various diseases (Ghasemzadeh et al., 2015). The presence of flavonoids in *Terminalia brownii* extract suggests that it may have potential as a source of natural antimicrobial agents against *Streptococcus pneumoniae*. Similarly, the moderate presence of coumarins in the extract as confirmed by the ferric chloride test suggests that *Terminalia brownii* may possess anti-inflammatory and anti-thrombotic properties (Kumar and Prakash, 2013). Coumarins have been reported to have antibacterial and antifungal activities against various pathogens (Boeira et al., 2018). Therefore, the presence of coumarins in *Terminalia brownii* extract may contribute to its antimicrobial activity against *Streptococcus pneumoniae*.

Saponins were also present in the extract, as indicated by the frothing test and Fehling's test. Saponins are known for their ability to form stable complexes with sterols and other lipids in the cell membrane, leading to cell lysis and death of microorganisms (Hostettmann et al., 2000). The moderate presence of saponins in *Terminalia brownii* extract may explain its potential antimicrobial activity against *Streptococcus pneumoniae*. Furthermore, the extract was found to contain terpenoids as indicated by the strong positive reaction in the Salkowski test. Terpenoids are known for their broad-spectrum antimicrobial activities against various bacteria and fungi (Burt, 2014). Therefore, the high presence of terpenoids in *Terminalia brownii* extract suggests its potential as a natural antimicrobial agent against *Streptococcus pneumoniae*. Finally, the moderate presence of tannins as confirmed by the ferric chloride and bromine water tests suggests that *Terminalia brownii* extract may possess astringent and antioxidant properties. Tannins are known to have antimicrobial activities against various pathogens by inhibiting their growth and adhesion (Gao et al., 2017). Therefore, the presence of tannins in *Terminalia brownii* extract may contribute to its antimicrobial activity against *Streptococcus pneumoniae*.

The table shows the results of the antibacterial activity of *Terminalia brownii* extract against *Streptococcus pneumoniae* at different concentrations. The extract exhibited concentration-dependent activity against the bacteria, with the highest concentration (7mg/ml) showing the greatest zone of inhibition (9.12mm). The zone of inhibition decreased as the concentration of the extract decreased, with the lowest concentration (0.875mg/ml) showing the least activity (6.52mm). The negative control, which was impregnated with 100% dimethyl sulfoxide, showed minimal activity against the bacteria, with a zone of inhibition of 6.00mm. This result indicates that the solvent used in the extraction process did not contribute significantly to the observed antibacterial activity.

The positive control, which was impregnated with amoxicillin, a standard commercial antibiotic, exhibited the highest zone of inhibition (10.26mm). The result suggests that amoxicillin was more effective than *Terminalia brownii* extract in inhibiting the growth of *Streptococcus pneumoniae*. These findings suggest that *Terminalia brownii* extract has moderate to high

antibacterial activity against *Streptococcus pneumoniae*. However, the activity is less than that of the standard commercial antibiotic, amoxicillin. Further studies are needed to determine the mechanism of action and potential use of *Terminalia brownii* extract in treating infections caused by *Streptococcus pneumoniae*.

Conclusions

We conclude as follows:

1. The results indicate that *Terminalia brownii* extract contains several phytochemicals with potential antimicrobial properties, including flavonoids, coumarins, saponins, terpenoids, and tannins.
2. The extract demonstrated significant antimicrobial activity against *Streptococcus pneumoniae*, with the highest inhibition observed at the highest concentration of 7mg/ml. This finding suggests that *Terminalia brownii* extract could be a promising alternative source of antimicrobial agents against S. pneumoniae.
3. The results also suggest that *Terminalia brownii* extract could be used in combination with conventional antibiotics to enhance their activity against S. pneumoniae. Further studies are needed to investigate the potential synergistic effects of combining *Terminalia brownii* extract with antibiotics.

Recommendations

We recommend exploration and the use of *Terminalia brownii* extract as an alternative therapy for bacterial infections, particularly against *Streptococcus pneumoniae*, as it has demonstrated significant potential as a natural antimicrobial agent. Further studies should be conducted to isolate and identify the active compounds, determine the optimal dosage and mode of administration, and evaluate potential toxicity. The use of *Terminalia brownii* extract as an alternative therapy for bacterial infections should also be explored. These findings provide a basis for further research to develop new drugs to combat antibiotic-resistant bacterial infections and promote the use of natural remedies in healthcare.

Conflict of Interest

Authors declare no conflict of interest.

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