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ORIGINAL RESEARCH ARTICLE

Antibacterial activity of *Moringa oleifera* (lam) seed against *Streptococcus pneumoniae* and *Staphylococcus aureus*

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ABSTRACT

Medicinal plants represent a rich source from which antimicrobial agents may be obtained and are used medicinally in different countries as source of many potent and powerful drugs. *Moringa oleifera* Lam. Is found to be very useful tree in tropical countries. All parts of the tree are used in different healing procedures for different diseases. The antibacterial activity of *Moringa oleifera* (lam) seed against *Streptococcus pneumoniae* and *Staphylococcus aureus* was investigated in this study. The maceration method was used to extract the plant seed material. Phytochemical analyses of the seeds in ethanol solvents were also performed. The antibacterial effect of the extracts on the test organisms was evaluated using the agar-well diffusion method. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the seed crude extract were evaluated to determine the static and cidal effect of the seed extract against the test bacteria. The results showed that the extract contained steroids, terpenoids, phenol, alkaloids, and tannins but no saponins, flavonoids, and anthraquinones. The antibacterial activity was exceptional against both bacteria with zones of inhibition of 17.33±0.58mm at 200 mg/ml, 16.33±0.34mm at 100 mg/ml, and 15.33±0.11mm at 50 mg/ml for *Staphylococcus aureus*, and 13.67±0.21mm at 200mg/ml 12.67±0.22mm at 100mg/ml, 11.67±0.05mm at 50mg/ml for *Streptococcus pneumoniae*. Because *Moringa oleifera* seeds were found to have significant antibacterial activity against the microorganisms tested, the findings of this study support *Moringa oleifera's* traditional use and can be recommended for use as an antimicrobial agent.

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INTRODUCTION

Antimicrobial agents are abundant in medicinal plants (Kubmarawa *et al.*, 2007). Moringa species are well-documented plant herbs due to their exceptional nutritional and medicinal properties. The most widely cultivated Moringaceae species are *Moringa oleifera Lam.* and *Moringa stenopetala Baker f.* (Fahey, 2005). They have long been recognized in folk medicine as beneficial in the treatment of a variety of ailments. They are anti-helminthic, antibiotic, detoxifiers, and immune boosters used in the treatment of malaria(Thilza *et al.*, 2010).

Moringa oleifera is also known as "Hausa Zogale," "Yoruba Ewe-igbale," and "Okwe Oyibo" (Igbo). *Moringa oleifera* has long been used in traditional medicine to treat a wide range of ailments, including digestion, skin diseases, and diarrhea, as well as a stimulant in paralytic illnesses, epilepsy, and hysteria (Farooq *et al.*, 2012; Mishra *et al.*, 2011) They are anti-helminthic, antibiotic, detoxifiers, and immune boosters used in the treatment of malaria (Thilza *et al.*, 2010).*Streptococcus pneumoniae* (the pneumococcus) is the classic example of a highly invasive, Gram-positive, extracellular bacterial pathogen. It is a major cause of morbidity and mortality globally causing more deaths than any other infectious disease (CDC, 2008). *Streptococcus pneumoniae* (the pneumococcus) colonizes the human nasopharynx and can cause diseases such as otitis media, pneumonia, bacteremia, and meningitis (Jonsson *et al.*, 1985). *Streptococcus pneumoniae*, discovered independently in 1881 by Louis Pasteur and George Sternberg, is a Gram-positive bacterial pathogen that can cause infections such as conjunctivitis, otitis media, lower respiratory tract infections, bacteremia, and meningitis (Henriques-normark and Tuomanen, 2013). *Staphylococcus aureus* is a commensal gram-positive bacterium that colonizes 30 percent of healthy people from various body parts (Oliveira *et al.*, 2018).

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It is responsible for many infections in both community and hospital settings, ranging from minor to fatal (Bitrus *et al*., 2018; Oliveira et al., 2018; Taylor and Unakal, 2018). *S. aureus* can adapt to different environments and colonize human skin, nails, nares, and mucus membranes, spreading through physical contact and aerosols to recipient host populations (Lowy, 1998). *S. aureus* colonization is a significant risk factor for subsequent *S. aureus* infection (Von Eiff *et al*., 2001; Wertheim *et al., 2004)*. *S. aureus* causes a wide range of infections, including skin, wound, and deep tissue infections, as well as potentially fatal conditions such as pneumonia, endocarditis, septic arthritis, and septicemia. This bacterium is also one of the most prevalent in nosocomial infections (Winn *et al*., 2006). Pathogenic bacteria and fungi infections pose a serious threat to human health (Eswarappa, 2009). Despite widespread use of antibiotics and vaccination campaigns, infectious diseases continue to be a leading cause of morbidity and mortality globally (Bloom *et al*., 2000). The leading cause of inflammation has been identified as microbial infections (Chien *et al*., 2009). It is unknown whether different concentrations of M. oleifera seed extract have antibacterial activity against the aforementioned microorganisms. As a result, the antibacterial activity of Moringa seed extracts on pathogenic bacteria must be determined. The purpose of this study was to see if Moringa oleifera (lam) seed had antibacterial activity against *Streptococcus pneumoniae* and *Staphylococcus aureus*.

MATERIALS AND METHODS

Study Area

This research work was carried out in Aliero Local Government Area of Kebbi State. Aliero is a town located along Jega-Sokoto road of Kebbi State. Located in the southeast of Kebbi State between latitude 12°16'42"N and longitude4°27'6"E. The name Aliero originated from two prominent Fulani scholars Ali and Yero. It has an area of 350 km² and a population of 65,973 at the 2006 census. In Aliero the wet season is hot, oppressive, and mostly cloudy and the dry season is sweltering and partly cloudy.

Sample Collection

Fresh *Moringa oleifera* plant seed was collected in a clean polythene bag from the Zuru Local Government Area of Kebbi State and transported to the herbarium of the Plant Science and Biotechnology Department, Faculty of Life Sciences, Kebbi State University of Science and Technology, Aliero, Kebbi State, Nigeria. A voucher number was assigned to the plant after it was identified (*Moringa oleifera* lam 121).The freshly collected seeds were thoroughly washed under running tap water and air-dried in the shade at room temperature; they were ground into powder using a wooden mortar and pestle. The plant was identified and a voucher number was obtained (*Moringa oleifera* lam 121). The freshly collected seeds were washed thoroughly under running tap water and air-dried under shade at ambient temperature; it was grinded into powder using a wooden mortar and pestle.

Extraction of Plant Material

In 250ml Ethanol, 50g powdered *Moringa oleifera* seed was soaked and allowed to percolate for 72 hours while shaking frequently. After being filtered through a clean, sterile muslin cloth, it was evaporated in a 45^{o}C water bath. The extract was weighed, labelled, and stored in an airtight bottle at room temperature.

Qualitative Phytochemical Screening

By dissolving three (3g) of powdered crude extract in 15ml of ethanol and 15ml of ethyl acetate to serve as a stock solution for the phytochemical screening, the crude extract of *Moringa oleifera* (seeds) extract was subjected to qualitative phytochemical screening. Following the methods of, this was used to detect the presence of bioactive constituents such as phenol, saponins, tannins, alkaloids, flavonoids, steroids, and anthraquinones (Awoyinka *et al*., 2007; Sofowora, 1993).

Test for Phenol

Two (2ml) of the seed extract were added to 1ml of distilled water and 1-2 drops of Iron III Chloride ($feCl_3$). A blue, green, red, or purple coloration indicated the presence of phenol.

Test for Alkaloids

On a steam bath, three (3) mls of aqueous extract were stirred with three (3) mls of 1 percent HCl. The mixture was then treated with Mayer's and Wagner's reagents. The presence of alkaloids was indicated by the turbidity of the resulting precipitate.

Test for Tannins

Two (2.0) mls of aqueous extract were mixed with two (2.0) mls of distilled water before adding a few drops of $FeCl_3$ solution. The presence of tannins was indicated by the formation of a green precipitate.

Test for Saponins

In a test tube, five (5.0) ml of aqueous extract was vigorously shaken with five (5.0) ml of distilled water and warmed. The formation of stable foam indicated the presence of saponins.

Test for Flavonoids

One (1.0) mls of aqueous extract was added to one (1) ml of 10% lead acetate solution. The formation of a yellow precipitate was an indication of the presence of Flavonoids.

Test for Terpenoids

A layer was formed by carefully adding two (2ml) of chloroform and three (3) ml of concentrated H_2SO_4. Terpenoids were detected at the interface by a reddish-brown colour.

Test for Steroids

In a test tube, two (2ml) of the seed extract and two (2ml) of chloroform were added. 2ml of sulphoric acid was carefully added via the test tube's side. The presence of steroids was indicated by the reddish-brown color at the interface.

Test for Anthraquinones

Two (2ml) of the seed extract, 5ml of benzene, and 2ml of a 10% ammonia solution were shacked into a test tube. The presence of anthraquinones was indicated by

the presence of pink, red, or violet color in the ammonia (lower) phase.

Preparation of Varying Concentration

Positive controls for *Staphylococcus aureus* were (ciprofloxacin 500mg) and *Streptococcus pneumoniae* (erythromycin 500mg). In 1ml of sterile distilled water, 0.2, 0.1, and 0.05 grams of the extract were dissolved to obtain the following concentrations: 200mg/ml, 100mg/ml, and 50mg/ml following the procedure outlined by (Banso & Ayodele, 2001).

Standardization of Inoculum

One gram (1g) of anhydrous barium chloride ($BaCL_2$) was mixed with 100ml of distilled water to make a 1% (1%) solution of barium chloride ($BaCl_2$). In addition, 1ml of concentrated H_2SO_4 solution was combined with 1ml of water to create a 1 percent sulfuric acid solution. The resulting mixture was kept in a screw-capped tube covered with aluminum foil to prevent evaporation. The McFarland standard density solution was made and allowed to clump over time, resulting in a concentration of 0.5 (0.5108 cells) (Cheesbrough, 2002). Each test bacteria was subcultured on a nutrient agar plate and incubated for 18-24 hours at 37oC to obtain pure colonies. Following an overnight incubation period, the test bacterial colonies were transferred to a glass tube containing sterilized physiological saline and thoroughly mixed by shaking until the suspension's turbidity matched that of the 0.5 McFarland standard, which contained approximately 1.5 108 CFU/ml (Cheesbrough, 2002).

Sensitivity Test

The test bacteria were inoculated onto a sterile Mueller-Hinton agar plate and given fifteen minutes to adapt to the medium. The plant extract concentrations were dispensed into each well using a sterile cork-borer and a 6mm diameter well. After allowing the extract to diffuse into the medium for 30 minutes, it was incubated at 37°C for 24 hours, and the zone of inhibition was measured and recorded in millimeters. Positive controls included standard antibiotics (ciprofloxacin and erythromycin), while negative controls included sterile distilled water (Rios *et al.,* 1988)

Minimum Inhibitory Concentration

The MIC was determined by serial doubling dilution of 1ml of crude extract and 1ml of each standardized inoculum into each test tube and incubating at 37^{0}C for 24 hours, with the least concentration without turbidity being the MIC (Eloff, 1998).

Minimum Bactericidal Concentration

The Minimum Bactericidal Concentration was calculated by inoculating a sample from the MIC tubes that showed no bacterial growth on Nutrient agar plates and incubating the plates for 24 hours at 37°C.The MBC was determined to be the lowest extract concentration that demonstrated no bacterial growth (Wikler, 2006).

Analysis

All experiments were performed in triplicate, and inhibition zone diameter data were expressed as mean standard deviation. Analysis of variance (ANOVA) was used in the statistical analysis, and the statistical package for social science students (SPSS) version 20.0 was used to express the significant difference between mean and standard deviation.

RESULTS

Table I shows the results of a qualitative phytochemical screening of *Moringa oleifera* ethanolic seed extract. Some active phytochemical components found in the seed extract included alkaloids, tannins, terpenoids, steroids, and phenol, while saponins, anthraquinones, and flavonoids were found to be absent.

Table II showed the zone of inhibition of *Moringa oleifera* ethanolic seed extract against *Staphylococcus aureus.* The result showed that the extract has potent activity against *S. aureus* at concentrations of 50mg/ml, 100mg/ml, and 200mg/ml with zones of inhibition of 17.33±0.05mm, 16.33±0.34mm and 15±0.11mm respectively. Furthermore, the results showed that at concentrations of 50mg/ml, 100mg/ml, and 200mg/ml, *Streptococcus pneumonia* was susceptible with zones of inhibition of 13.67±0.21mm, 12.67±0.33mm and 11.67±0.05mm respectively. The result is presented in table III.

Comparison between the antibacterial potential of the seed extracts and Control antibiotic (ciprofloxacin 500mg) for *Staphylococcus aureus* revealed that extracts at 50mg/ml(17.33) and 100(16.33mm) are above the control while at 200mg/ml(15mm), the zone of inhibition is same as the control (15mm). Also, for *Streptococcus pneumonia* the zones of inhibitions for all the concentration are below the control (15mm).

Table IV and V displayed the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) results for *S. pneumonia* and *Staphylococcus aureus. Staphylococcus aureus* was found to be sensitive against extracts of *Moringa oleifera* at the concentration of 12.5mg/ml while *S. pnemoniae* was found to be sensitive at the concentration of 25 and 12.5mg/ml. Based on the MIC results the minimum bactericidal concentration (MBC) was performed against *Staphylococcus aureus and S. pneumonia*, no visible growth of any of these microorganisms were found.

Table I: Qualitative Phytochemical screening of *Moringa oleifera* (lam) seed extract.

Phytochemical Compound	Inference
Steroids	+
Terpenoids	+
Tannins	+
Alkaloids	+
Phenol	+
Saponins	-
Flavonoids	-
Anthraquinones	-

Key: (+) present, (-) absent

Table II: Antibacterial activity of the seed extract of *Moringa oleifera* against *Staphylococcus aureus*.

Concentration mg/ml	Mean ± SD zone of inhibition(mm)
200	17.33±0.58
100	16.33±0.34
50	15.00±0.11
Control (ciprofloxacin 500mg)	15.00±0.24

Table III: Antibacterial activity of the seed extract of *Moringa oleifera* against *Streptococcus pneumoniae*.

Concentration mg/ml	Mean ± SD Zone of inhibition (mm)
200	13.67±0.21
100	12.67±0.33
50	11.67±0.05
Control (erythromycin 500mg)	15.00±0.12

Table IV: Minimum Inhibitory Concentration of *Moringa oleifera* ethanol seed extract against *S. aureus* and *S. pneumoniae*

Organism	Concentration in mg/ml				
	200	100	50	25	12.5
S. aureus	-	-	-	-	+
S.pneumoniae	-	-	-	+	+

Table V: Minimum Bactericidal Concentration of *Moringa oleifera* ethanol seed extract against *S. aureus* and *S. pneumoniae*.

Organism	Concentration in mg/ml				
	200	100	50	25	12.5
S. aureus	-	-	-	-	-
S.pneumonia	-	-	-	-	-

DISCUSSION

The purpose of this study was to determine the antibacterial activity of *Moringa oleifera* seeds against *Staphylococcus aureus* and *Streptococcus pneumonia*. Tannins, steroids, terpenoids, phenol, and alkaloids were found in the seed extract, which agrees with previous findings (Bukar, *et. al.,* 2010; Saadabi and Abu Zaid, 2011; Paulista & Brasil, 2021). The identified phytochemical compounds may be the bioactive constituents responsible for *Moringa oleifera* seeds' efficacy, because some of these constituents have antimicrobial activity (Kubmarawa *et al*., 2007). The absence of saponins, anthraquinones and flavonoids corresponds to the findings of (Anwar & Rashid, 2007; Dodiya *et al*., 2015) .This could be because other compounds in the seed extract were impeding or interfering with the pure compound's activity.

Also the results revealed that the seed extract possessed antibacterial activity against *Staphylococcus aureus* (17.33mm at 200 mg/ml, 16.33mm at 100 mg/ml, and 15.00mm at 50 mg/ml). This result is in concordance to that reported by Bukar et al., (2010) and Fowoyo & Oladoja, (2015) in which *Staphylococcus aureus* was also found to be susceptible with inhibitory zone of 15.0mm at 200mg/ml and 10mm at 200mg/ml respectively. The result is also in agreement with a study where *Staphylococcus aureus* was susceptible to seed extract with 14.9mm zone of inhibition (Alzohairy, 2017). In addition, the seed extract exhibited antibacterial activity against *Streptococcus pneumonia* (13.67mm at 200mg/ml, 12.67mm at 100mg/ml, and 11.67mm at 50mg/ml). These results are in close agreement with other findings obtained by other workers (Anwar & Rashid, 2007; Jamil *et al*., 2007; Paulista & Brasil, 2021). The antimicrobial nature of *Moringa oleifera* seeds is attributed to the oil contained in it, which on consumption forms a thin film over the intestinal wall, thus reducing or preventing pathogens from penetrating the intestinal walls (Nwosu & Okafor, 1995). Some studies have also shown that the antibacterial activity of *Moringa oleifera* seeds is linked with a gum produced in the seed (Harristoy *et al*., 2005). Other studies have shown that the antibacterial activity is linked with the presence of various phytochemical constituents such as saponins, tannins, flavonoids, alkaloids, cardiac glycosides and terpenoids in the seed (Nepolean *et al*., 2009).

Furthermore, the antibacterial effects of seed extract tested for *Staphylococcus aureus* revealed that extracts at 100(16.33mm) and 200(17.33mm) concentration are greater than the effects of Control antibiotic (ciprofloxacin 500mg). Therefore, at this concentrations the seed extract is more potent than the antibiotics. Also, for *Streptococcus pneumonia* the zones of inhibitions for all the concentration are below the control (erythromycin 500mg) (15mm). As a result, the control antibiotic showed more potency than seed extracts. The effectiveness may be improved, for instance, by testing different extractions methods to increase the concentration of the active chemical components.

CONCLUSIONS

This study showed that Moringa oleifera seeds extracts have antibacterial activity on *Staphylococcus aureus* and *Streptococcus pneumonia* indicating a potent source of new antibiotic alternative. This inhibitory action of the extracts could be attributed to the presence of phytochemical constituents in the extracts. The findings of this study support Moringa oleifera's traditional use and can be recommended for use as an antimicrobial agent. More work should be carried out to determine the antibacterial constituents that can be used for drug formulation.

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