



Research Article

Effect of Aqueous and Methanolic Extracts of *Acacia nolitica* Seed on Selected Gram Positive Bacteria Isolated from Patients Attending University of Maiduguri Teaching Hospital, Borno State, Nigeria

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ARTICLE INFO:

ABSTRACT

Keyword:

Phytochemical,
Antibacterial Activity,
Acacia nolitica,
Nasal Swab,
In-Patient

Acacia nolitica (Linn.) has an inspiring range of medical uses. This plant contains several compounds which have medicinal values. This study aimed to examine the antimicrobial activity of the seed extract of *Acacia nolitica* against gram-positive bacteria isolated from a nasal swab of patients attending the University of Maiduguri Teaching Hospital using different solvents (Methanol, acetone, and distilled water) of different concentrations (50µl, 75µl, and 100µl). The results revealed that the phytochemical analysis of acetone extract of *Acacia nolitica* showed the presence of Saponins, Alkaloid, Volatile oil, and Terpenoid, while distilled water extract showed the presence of Alkaloid, Volatile oil and Terpenoid. Similarly, methanol extract revealed the presence of Saponins, flavonoids, alkaloids, terpenoids and cardiac glycosides. *Staphylococcus aureus* and *Streptococcus species* were isolated from the nasal swab samples of the patients attending the University of Maiduguri Teaching Hospital. The sensitivity of the extract against the two isolates revealed that the extract exhibited activity against *Staphylococcus aureus* while resistant to *Streptococcus species*. Therefore, *Acacia nolitica* may represent a new source of antimicrobial with a stable biological active component that can establish a scientific base for the use in modern medicine for the treatment of diseases caused by *Staphylococcus aureus*.

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INTRODUCTION

Medicinal plants include parts of various types of plants used in traditional herbal medical practice for the management and cure of diseases. Many of these plants are very useful in the pharmaceutical industries in the production of drugs used today (Bekele *et al.*, 1993). Among many plants studied for possible biologically interesting and useful natural product; *Mormodica balsamina* (Geidam *et al.*, 2007), *Guiera senegalensis* (Oshoba and Geidam, 2014), *Carissa edulis Vahl* (Ngulde *et al.*, 2021) and *Adenopus breviflorus* (Oyedeki *et al.*, 2021). These medicinal plants are considered to be rich sources of ingredients that can be used in drug development and synthesis. Besides, these plants play a critical role in the development of human cultures around the world. Moreover, some plants consider an important source of nutrition, and are recommended for their therapeutic values (Bein, 1996). Medicinal plants have been playing an essential role in the development of human culture. Medicinal plants have always been at the forefront of virtually all cultures of civilizations. It is regarded as sources of traditional medicines, and from these plants, many modern medicines are produced. For thousands of years, medicinal plants have been used to treat health disorders, add flavour, and conserve food and prevent disease epidemics. The secondary metabolites produced by the plants are usually responsible for the biological characteristics of plant species used throughout the world. Microbial growth in diverse situations is controlled by plant-derived products.

Acacia nilotica Leaves, bark, gum and seeds are used medicinally. The paste made of tender leaves with water and sugar is useful as a demulcent in cough. Water extract from leaves is used as astringent. It is useful in cases of gonorrhoea, dropsy, and leucorrhoea. It also used in a gargle for relaxing sore throat and spongyls, which is used for washing bleeding wounds and ulcers. A decoction of the bark is used in diarrhoea, dysentery and diabetic patients. It is

used as an astringent for ulcer, cancerous and syphilitic affections. Juice of bark and its extract is used as a styptic, especially to stop bleeding from wounds. The bark is also useful in snakebite, and the bitten part is dusted with pulverized pods. Good tooth powder is made with a mixture of burned bark well pulverized with burnt almond shells and salt. Juice mixed with burned milk is used in eye conjunctivitis (Dastur, 1966). Therefore, the objective of this study is to determine the phytochemical and antibacterial activity of *Acacia nilotica* seed against bacterial isolated from a nasal swab of patients attending the University of Maiduguri Teaching Hospital.

METHODOLOGY

Collection of Plant Sample

The *Acacia nilotica* seeds were collected from the University of Maiduguri Staff Quarters, Borno State, Nigeria. The plant was identified and authenticated by plant taxonomists at the herbarium unit in the Department of Biological Sciences, University of Maiduguri. The samples were washed and allowed it to dry before pulverization into powder form using sterile mortar and pestle.

Plant Extraction

The seed extraction was carried out using Plant Tissue Homogenization methods. A 20g of extract powder was weighted using a digital weighing balance and dissolved into 80ml of three different solvents (Methanol, Acetone and Distilled water). The solvents were shaken vigorously for 5-10 minutes using an orbital Shaker. The samples were made homogenous and then incubated for 24 hours as described by Banu and Cathrine (2015).

Phytochemical Analysis

The plant extract i.e. methanol, Distilled water and Acetone were tested for the absence or presence of phytochemical constituents, known as secondary metabolites like alkaloids, tannins, Phlobatannins, flavonoids, carbohydrates, phenols, saponin, cardiac glycosides, proteins and terpenoids (Soni *et al.*, 2011). It is well-known that plants produced these chemicals to

protect themselves, but recent research demonstrates that they can also protect against diseases (Breslin and Andrew, 2017).

Flavonoid screening

A 5ml of the plant extract was put in a test tube and treated with sodium hydroxide (NaOH) solution and water. A formation of intense yellow-orange colour in the test tube indicates the presence of flavonoids.

Tannins screening

A 5ml of plant extract was mix with (5%) ferric chloride solution (FeCl_3). A dark-green or blue-black coloration was observed to indicate the presence of tannins (Kokate *et al.*, 2008).

Saponin screening

A 5ml of plant extract was placed in a test tube, 3ml of distilled water was be added and shake vigorously for 3-5 minutes. The formation of 2 cm layer foam indicates the presence of saponins.

Alkaloid screening

A 4ml of extract in a test tube and a 3-5 drop of Wagner's reagents were added to the same tube. The reaction mixtures formed reddish-brown precipitate and indicate the presence of alkaloids.

Cardiac glycoside screening

For cardiac glycosides detection, 2ml of ferric chloride and 2-4 drop of concentrated sulphuric acid (H_2SO_4) was added to 3 ml of plant extract. Observation of a reddish-brown coloration indicates the presence of cardiac glycosides.

Steroid screening

A 3ml of the extract was mix with few drops of concentrated sulphuric acid (H_2SO_4). The formation of reddish-brown coloration at the interface indicates a positive result.

Volatile oil screening

The 4ml of the extract was mixed with dilute hydrochloric acid (HCL). White precipitates at the bottom of the test tube indicate the presence of volatile oil.

Terpenoid screening

Four ml of plant extracts were added to 3 ml of chloroform and a few drops of concentrated

sulphuric acid (H_2SO_4) was added to the tube and formed the layer of reddish-brown coloration (Dahiru *et al.*, 2006).

Sample Collection and Preparation

A nasal swab was collected using a sterile swab stick and transferred to sterile bottles (200ml) appropriately labeled with participants' study numbers. These collected samples were immediately transported to the Microbiology laboratory and inoculated onto Mannitol Salt agar (Hi-Media Laboratories, Mumbai), Blood agar and Nutrient agar plates. These plates were incubated at 37°C for 24-48 hours. Plates were observed for growth, and a Gram smear was performed. Characteristic colonies were identified by Gram reaction, colony morphology and pigment formation. Further biochemical tests such as catalase, coagulase tests, urease test, methyl red (MR), Voges Proskauer (VP) and DNase test were carried out to identify the genus and species of the bacteria (Dauner *et al.*, 2010).

Antimicrobial Sensitivity Test

The sensitivity test was carried out using impregnated Grams positive disc as control and the plant extract for the test. The bacterial strains isolated from the clinical samples were *Staphylococcus aureus* and *Streptococcus species*. The test colonies were taken from a 24-hour culture grown on nutrient agar and were introduced evenly on a Mueller Hinton Agar in a sterile petri dish using the streak plate method. A sterile cork borer of 6mm was used to punch a hole in each of the Petri dishes. The wells were filled with different concentrations of the extracts (100ul, 50ul and 75ul) of various solvents and incubated for 48 hours. The zone of inhibition was measured around each of the wells using meter ruler.

RESULTS

The phytochemical analysis of distilled water extract showed the presence of Alkaloid, Volatile oil and Terpenoid, while Methanolic extract

revealed the presence of Saponin, flavonoids, Alkaloids, Terpenoid and Cardiac glycosides as shown in Table 1.

Table 1: Phytochemical analysis of *Acacia nilotica* seeds using different solvents

Phytochemical Components	Distilled water	Methanol
Saponins	–	+
Flavonoids	–	+
Alkaloids	+	+
Steroid	–	–
Volatile oil	+	+
Terpenoid	+	+
Tannins	–	–
Cardiac glycosides	–	+

The result of the gram staining revealed that the isolates were gram-positive cocci and suspected to be *Staphylococcus aureus* and *Streptococcus species* due to the below biochemical characteristics (Table 2).

Table 2: Morphological and biochemical characteristic of bacteria isolates

S/No.	Organisms	Gram reaction	Catalase	Coagulase	MR	Urease	DNase	VP
1.	<i>S. aureus</i>	+	+	+	+	+	+	+
2.	<i>Streptococcus species</i>	+	–	+	+	–	+	–

Key: + indicate positive result, while – indicate negative result. MR=Methyl Red, VP= Voges Proskauer

The result of the antimicrobial activity of methanol and distilled water extract of *Acacia nilotica* showed inhibitory activity against *Staphylococcus aureus*, while *Streptococcus species* exhibit resistance to extract from distilled water. The methanol extract has antibacterial activity with the zone of

inhibition of 16mm and 20mm using the concentration of 75ul and 100ul respectively. The distilled water extract showed an inhibition zone of 19mm, 17mm and 35mm using the concentration of 50ul, 75ul and 100ul respectively against *Staphylococcus aureus*, while the *Streptococcus species* were resistant to

all. Ciprofloxacin, Septrin, Erythromycin, and Pefloxacin were used as a positive control (Table 3).

Table 3: Antimicrobial activity of Methanolic and Aqueous extracts of *Acacia nilotica* against pathogenic bacteria

S/No.	Extract of different conc. and solvent with control	Methanolic extract (Zone of inhibition in millimeter (mm))		Aqueous extract (Zone of inhibition in millimeter (mm))	
		<i>S. aureus</i>	<i>Streptococcus spp.</i>	<i>S. aureus</i>	<i>Streptococcus spp.</i>
1	50µl	R	R	19	R
2	75 µl	16	R	17	R
3	100 µl	20	R	25	R
4	Rifampicin (10mcg)	19	R	12	R
5	Ciprofloxacin (10mcg)	26	20	18	R
6	Streptomycin (10mcg)	R	R	16	R
7	Septrin (25 mcg)	R	22	20	R
8	Erythromycin (10mcg)	R	17	17	R
9	Pefloxacin (10mcg)	10	R	16	R
10	Gentamicin (10mcg)	R	R	R	R
11	Ampiclox (10mcg)	R	R	R	R

R = resistance

DISCUSSION

The results of this study revealed the presence of secondary metabolites such as Saponin, alkaloids, volatile, terpenoid flavonoids and cardiac glycosides while tannins were not found in all the extracts. The result also showed the activity of *Acacia nilotica* seed extract on *Staphylococcus aureus* but resistance to *Streptococcus species*. The results of this study agrees with the findings of Mona (2017), who reported that the concentration of the extract and species of the bacteria may account for the difference in the susceptibility pattern of the test organism. Banso (2009) and El-Shanaway (1996) also reported that *Acacia nilotica* contained compounds such as tannins, saponins, essential oil, alkaloid, terpenoid and flavonoids, which have an antimicrobial effect. It has been suggested that the mechanism of the

antimicrobial effect involves inhibition of different cellular processes, like a rise in plasma permeability and ultimately ions seepage from the cells. Alkaloids contribute to a plant species' fitness for survival. They often have pharmacological effects and are used as medication and recreational drugs (Rodger, 1998). Phenolic compounds are reported to exert a wide spectrum of biological effects, such as antioxidant and free radical scavenging activity and antimicrobial activity (Bammidi *et al.*, 2011). Similarly, tannins are well known for their antioxidant and antimicrobial properties as well as skin regeneration, anti-inflammatory and diuretic properties (Cowan, 1999). Flavonoids are widely recognized for exerting antioxidant, antimicrobial, anti-carcinogenic and antitumor properties. Many

pharmacological activities such as antibiotic, antifungal, antiviral, hepatoprotective, and anti-inflammatory and anti-ulcer activities have been reported for Saponin. Steroids have been reported to exert analgesic properties while cardiac glycosides are reported to have antibacterial and antifungal activity.

CONCLUSION

Based on the result obtained from this study, the antibacterial activity of *Acacia nilotica* in methanol and distilled water showed inhibitory activities against *Staphylococcus aureus in vitro*, while *Streptococcus species* were resistant. Conclusively, *Acacia nilotica* may represent a new source of an antimicrobial with a stable biological active component that can establish a scientific base for the use in modern medicine for the treatment of diseases caused by *Staphylococcus aureus*.

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