

INHIBITORY EFFECTS OF POD EXTRACT OF *ACACIA NILOTICA* AGAINST SOME PATHOGENIC BACTERIA

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ABSTRACT

This study assessed the inhibitory effects of the pod extract of *Acacia nilotica* against some pathogenic bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pyogenes*). Fresh pods of *Acacia nilotica* were air-dried and then ground into fine powder. Thereafter, pod powder was extracted using ethanol and distilled water in the ratio 3 to 2 (v/v). The crude extract was concentrated *in vacuo* and lyophilized. Afterwards, screened for phytochemicals and tested for antibacterial activity against the bacterial isolates. Antibacterial potential, minimum inhibitory concentrations (MIC), and minimum bactericidal concentrations (MBC) of the extract were determined using standard microbiological method. Zone of inhibition shown by the crude extract at 50 mg/mL on the isolates ranged between 10 mm and 18 mm while MIC ranged between 1.56 mg/mL and 25 mg/mL. Minimum bactericidal concentrations ranged between 3.13 mg/mL and 50.00 mg/mL. The phytochemical screening of the extract revealed the presence of tannins, alkaloids, flavonoids, saponins and cardiac glycosides. This study, therefore, showed that pod extract of *Acacia nilotica* exhibited appreciable inhibitory effects on the test isolates. Hence, there is need for *in vivo* studies to complement the present findings.

Keywords: Inhibitory effect, *Acacia nilotica*, Pathogenic bacteria, Pods, Antibacterial activity, Phytochemicals

INTRODUCTION

Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years and in many parts of the world (Pavithra *et al.*, 2010). The medicinal value of these plants lies in some bioactive compounds that produce a definite physiological action on the human body and these bioactive compounds are called phytochemicals (Joshi *et al.*, 2009). The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins, phenolic compounds, steroids, resins, fatty acids and gums which are capable of producing definite physiological action on body (Joshi *et al.*, 2009).

The bioactive compounds present in plants have been known to confer pharmacological potentials on them, thereby, enabling them combat many disease-causing pathogens (Hussain *et al.*, 2011). Different plant parts, including herbs, spices, fruits, vegetables and tropical plants have been showed to contain these natural antimicrobials which are of intense medicinal benefits (Ciocan and Bara, 2007). Among the plants that contains these natural antimicrobial is *Acacia nilotica*.

Acacia nilotica (Family Fabaceae) is a pioneer species, relatively high in bioactive compound and are important for a variety of functions (Abdulhamid *et al.*, 2019). It is used as medicinal plants in parts of Northern Nigeria, West Africa, North Africa and other parts of the world (Gurib-Fakim *et al.*,

2010). The plant is used to treat infections such as diarrhea, dysentery, oxidative stress, intestinal pains, ulcer, cold, hemorrhages, tuberculosis, congestion, coughs and fever in Nigeria (Aliyu, 2006; Saini *et al.*, 2008). It is economically used as a source of tannins, gums, timber, fuel and fodder. Several investigators have demonstrated the antimicrobial, antioxidant and health benefits of this plant (Banso, 2009; Kalaivani and Matthew *et al.*, 2010). To date, there has been a paucity of information on the antibacterial activities of pod extract of *Acacia nilotica* against the pathogenic bacteria commonly implicated in some human infection. Hence, this present study was therefore designed, to investigate the inhibitory effects of pod extract of *Acacia nilotica* on some pathogenic bacteria.

MATERIALS AND METHODS

Collection of test isolates

Microbial isolates used in this study include typed strains as well as locally isolated pathogens (LIPs). The LIPs which comprise clinical isolates were isolated from stool and sputum samples. Locally isolated pathogens were collected from culture collections of Department of Microbiology, Federal University, Dutsin-Ma, Katsina State, Nigeria while the typed strains were obtained from National Collection of Industrial Bacteria (NCIB), Aberdeen, Scotland, United Kingdom. The bacterial isolates include *Escherichia coli* (NCIB 86),

Klebsiella pneumoniae (NCIB 418), *Proteus mirabilis* (LIP), *Pseudomonas aeruginosa* (NCIB 950), *Staphylococcus aureus* (NCIB 8588), *Streptococcus pyogenes* (LIP). The isolates identities were confirmed based on cultural, morphological and biochemical laboratory tests. Afterwards, the isolates were maintained on a nutrient agar slants and stored at 4°C until further use.

Standardization of the test isolates

Overnight culture of pure and discrete colonies of each isolate was transferred into each sterile test tube containing 5 ml sterile nutrient broth and standardized to 0.5 McFarlands (10^6 cfu/ml) (Ikram-ul-Haq and Mukhtar, 2016).

Collection and preparation of plant sample

Fresh pods sample of *Acacia nilotica* used were collected during the month of July, 2019 at Dutsin-Ma, Katsina, Katsina State, Nigeria. The sample was identified and authenticated by a Botanist at the Department of Biological Sciences, Federal University, Dutsin-Ma, Katsina State, Nigeria. The plant samples were air-dried until a constant weight of the pods were obtained. The pods were later powdered using an electric blender and stored in an air tight container for further use.

Extraction of the plant sample

Exactly 100g of the powdered pods of *Acacia nilotica* was soaked in the mixture of ethanol and sterile distilled water in ratio 3:2 (v/v) and left on the laboratory bench for four days with regular agitation and then filtered using number 1 Whatman filter paper. The filtrate was concentrated *in vacuo* using a rotary evaporator to eliminate the ethanol leaving aqueous portion. The aqueous part was then lyophilized to collect the crude extract which was gummy and dark brown in colour. The yield collected was 28.6g.

Phytochemical screening of the crude extract

The crude extract of the plant was subjected to phytochemical screening using method described by Trease and Evans. (2002) and Harborne (2006).

Sensitivity testing of the crude extract against bacterial isolates

The sensitivity testing of the crude extract was determined using agar-well diffusion method as described by Hugo and Russell. (2015) with some modifications. The bacterial isolates were first grown in nutrient broth for 18 hours before use. Exactly 0.2 ml of the standardized test isolates (10^6 cfu/ml of 0.5 McFarland standards) was then subcultured on to sterile Mueller-Hinton agar (Oxoid, UK). The medium was allowed to set and wells were then bored into the agar medium using a sterile 6 mm cork borer. The wells were then filled up with prepared solution of the extract (50 mg/mL) and care was taken not to allow solution to spill on the surface of the medium. Streptomycin and Ampicillin were used as positive control at concentrations of 1 mg/mL. The plates were allowed to stand on

the laboratory bench for about 1-2 hours to allow proper inflow of the solution into the medium before incubating at 37°C for 24 hours. The plates were later observed for the zones of inhibition. The diameter of zones of inhibition was measured by a transparent ruler to the nearest millimeter.

Determination of minimum inhibitory concentrations (MIC) of the crude extract against bacterial isolates

The MIC of the extract was determined using the method described by Akinpelu *et al.* (2015). Two-fold serial dilution of the extract was prepared using 50 mg/mL of the crude extract. Exactly 2 ml of different concentrations of the solution was added to 18 ml of pre-sterilized molten nutrient agar to give final concentrations regimes of 0.78 mg/mL to 50.00 mg/mL. The medium was then poured into sterile petri dishes and allowed to set. The surfaces of the medium were allowed to dry before streaking with 18 hours old standardized bacterial cultures grown in sterile nutrient broth. The plates were incubated at 37°C for up to 72 hours after which they were examined for the presence or absence of growth. The MIC was taken as the lowest concentration that prevented the growth of the bacteria.

Determination of minimum bactericidal concentrations (MBC) of crude extract against bacterial isolates

The MBC of the crude extract was determined by the method of Spence and Spencer. (2004) with some modifications. Samples were taken from plates with no visible growth in the MIC assay and sub-cultured onto freshly prepared nutrient agar plates and later incubated at 37°C for 48 hours. The lowest concentration of the extract that did not show any growth on a new set of plates was taken as the minimum bactericidal concentration of the extract.

Statistical analysis

Data were expressed as mean \pm SD (standard deviation) of three replicates and were statistically analyzed using one way analysis of variance (ANOVA). Values were considered significant at $p < 0.05$.

RESULTS

The crude extract exhibited a considerable level of antibacterial activities against all the bacterial isolates used for this study (Table 1). The crude extract exhibited antibacterial activity at a concentration of 50 mg/mL. The zones of inhibition exhibited by the extract against the bacterial isolates ranged between 10 mm and 18 mm. The highest zone of inhibition (18 mm) was exhibited against *Escherichia coli* while the lowest (10 mm) zone was exhibited against *Streptococcus pyogenes*. On the other hand, the standard antibiotics, streptomycin and ampicillin, each at a concentration of 1 mg/mL inhibited the growth of all the bacterial isolates. The zones of inhibition

exhibited by streptomycin and ampicillin used as a positive control ranged between 14 mm and 28 mm.

Table 1: Sensitivity patterns exhibited by the crude extract and the standard antibiotics against bacterial isolates

Bacterial Isolates	Zones of inhibition (mm)**		
	Crude extract (50 mg/mL)	Streptomycin (1 mg/mL)	Ampicillin (1 mg/mL)
<i>Staphylococcus aureus</i> (NCIB 8588)	16 ±2.00	24 ±1.00	20 ±3.60
<i>Escherichia coli</i> (NCIB 86)	18 ±1.00	20 ±0.00	16 ±1.00
<i>Pseudomonas aeruginosa</i> (NCIB 950)	12 ±1.00	26 ±2.00	22 ±3.46
<i>Streptococcus pyogenes</i> (LIP)	10 ±0.00	14 ±0.00	16 ±2.65
<i>Klebsiella pneumoniae</i> (NCIB418)	14 ±1.00	28 ±1.00	16 ±1.00
<i>Proteus mirabilis</i> (LIP)	16 ±2.65	20 ±4.36	22 ±2.00

Keys: LIP: Locally Isolated Pathogen, NCIB: National Collection of Industrial Bacteria, (mm) **= Mean of three replicates

The crude extract of *Acacia nilotica* exhibited a varying degree of minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) against the bacteria isolates as shown in Table 2. The MIC exhibited by the crude extract against the bacterial isolates ranged between 1.56 mg/mL to 25 mg/mL while the MBC ranged between 3.13 mg/mL to 50 mg/mL.

Table 2: The minimum inhibitory and bactericidal concentrations exhibited by the crude extract against bacterial isolates

Bacterial Isolates	MIC (mg/mL)	MBC (mg/mL)
<i>Staphylococcus aureus</i> (NCIB 8588)	1.56	3.13
<i>Escherichia coli</i> (NCIB 86)	3.13	6.25
<i>Pseudomonas aeruginosa</i> (NCIB 950)	12.50	25.00
<i>Streptococcus pyogenes</i> (LIP)	25.00	50.00
<i>Klebsiella pneumoniae</i> (NCIB 418)	3.13	6.25
<i>Proteus mirabilis</i> (LIP)d	1.56	3.13

Key: LIO: Locally Isolated Pathogen, NCIB: National Collection of Industrial Bacteria

Table 3 shows the results of phytochemical screening of the pod extract of *Acacia nilotica*. The phytochemical screening of the pod extract of *Acacia nilotica* revealed the presence of tannins, alkaloids, flavonoids, saponins and cardiac glycosides.

Table 3: Phytochemical screening of *Acacia nilotica*

Phytochemical test	Results
Tannins	+
Alkaloids	+
Flavonoids	+
Steroids	-
Saponins	+
Cardiac glycosides	+

Key: +=present, - = absent

DISCUSSION

The inhibitory effects of the crude extract of *Acacia nilotica* were investigated against some pathogenic bacteria. The crude extract was found to possess antibacterial activities against the bacterial isolates used for this study. The crude extract at a concentration of 50 mg/mL inhibited the growth of all the test isolates (Table 1). The zones of inhibition exhibited by the crude extract against the test isolates ranged between 10 mm and 18 mm. The results support previous reports on the antibacterial activity of this plant (Banso, 2009; Okoro *et al.*, 2014; Abdulhamid *et al.*, 2019). Thus, the pod extract of *Acacia nilotica* appears to be a potential source of antibacterial compounds that could be relevant in the treatment of infections caused by these organisms.

The MIC and MBC exhibited by the crude extract against the test isolates were also studied. The lowest MIC exhibited by the crude extract against the test isolates was 1.56 mg/mL. On the other hand, the lowest MBC exhibited by the crude extract against the test isolates was 3.13 mg/mL (Table 2). According to Suffredini *et al.* (2006), the antibacterial activity of plant extract is considered significant if the MIC of the extract is less than or equal to 200 mg/mL. The MIC exhibited by the crude extract was below 200 mg/mL as observed in this study. This is an indication that the pod extract of *A. nilotica* exhibited significant antibacterial activity against the test isolates. In addition, plants extract with MIC index which is equal or <2mg/mL is considered as bactericidal while those above 2 mg/ml but <16mg/mL are said to be bacteriostatic (Shanmughapriya *et al.*, 2008). This result showed that *Acacia nilotica* pod extract is bactericidal in action.

The phytochemical analysis of crude extract revealed the presence of tannins, alkaloids, flavonoids, saponins and cardiac glycosides (Table 3). These compounds are known to be biologically active and contribute to the antimicrobial and antioxidant activities of medicinal plants (Trease and Evans, 2002). This is an indication that these phytochemical compounds contributed to the antibacterial potentials of this plant extract against test isolates used for this study.

Phytochemicals exert antimicrobial activity through different mechanisms. For example, tannins act by forming irreversible complexes with proline-rich proteins (Shimada, 2006) resulting in the inhibition of the cell protein synthesis. Also, tannins are known to react with proteins to provide the typical tanning effect which is important for the treatment of inflamed or ulcerative tissues (Parekh and Chanda, 2007). Herbs that have tannins as their component are astringent in nature and are used for treating intestinal disorders such as diarrhoea and dysentery (Dharmananda, 2003), thus, exhibiting antimicrobial activity. Alkaloids are another kind of phytochemical compounds revealed in the pod extract of *Acacia nilotica*. Abdulhamid *et al.*

(2019) also reported the presence of alkaloids in seedless pod extract of this plant.

Another bioactive compound detected in *Acacia nilotica* was flavonoid and have been reported to possess a wide range of biological activities which include antimicrobial, anti-inflammatory, analgesic, anti-allergic effects, cytostatic and antioxidant properties (Makai *et al.*, 2009). The antibacterial activity of flavonoids has been shown to be a result of their ability to form complexes with bacterial cell walls extracellular and soluble proteins (Scalbert, 1991). Saponins, also found in the crude extract of *Acacia nilotica* are known to possess antibacterial property. The mode of action of saponins were attributed to their ability to cause leakage of proteins from bacterial cells (Ganguly and Sainis, 2001; Tamil *et al.*, 2011). Cardiac glycosides are an important class of naturally occurring drugs whose actions help in the treatment of congestive heart failure (Ikeda *et al.*, 1995). This class of bioactive compound was detected in crude extract of *Acacia nilotica* and thus supports the usefulness of this plant for the treatment of cardiac infections (Koube *et al.*, 2016).

CONCLUSION

This study established that the pod extract of *Acacia nilotica* exhibited significant inhibitory effects on some pathogenic bacteria. This study confirmed the usefulness of this plant in folklore medicine for the treatment of infections caused by the disease causing pathogens. The plant (*Acacia nilotica*) could be recommended to pharmaceutical companies and research institute as potential source of drugs for the management of infections caused by different bacteria investigated in this study. Also, further studies should be carried out on the isolation and structural elucidation of bioactive compounds responsible for the inhibitory effect of the plant.

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