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Full Length Research Article

Screening for Antibacterial Properties of Some Traditional Medicinal Plants in Kebbi State, Nigeria

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ABSTRACT

Infections caused by bacteria continue to be a major threat to public health. The impact of this is especially alarming in developing countries due to the relative shortages of medicines and the emergence of widespread drug resistance. This has led to the search for new antimicrobial agents mainly among plant extracts. As part of an ongoing research to isolate and purify antibacterial compounds from the extract of traditionally used medicinal plants from Kebbi State, Nigeria, some selected plants were screened for their antibacterial potentials. The preliminary phytochemical screening of the extracts was carried out using standard methods. The bioactivity (antibacterial) test was done using agar well diffusion method, while MIC and MBC were tested using broth dilution method. The results for the phytochemical screening showed the presence of flavonoids, tannins, saponins, glycosides, alkaloids and terpenoids in the selected plants extracts. The results for the antibacterial activity of the crude methanolic extracts of the selected plants showed varying degree of antibacterial activities against selected bacterial isolates. However, the stem bark extract of *Acacia nilotica* showed relatively high zone of inhibition (mm), minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The extract was found to inhibit the growth of most of the tested bacterial isolates comprising of both Gram-positive and Gram-negative organisms. These findings support previous reports on the antimicrobial activity of this plant. The result of the present study signifies the potential of *Acacia nilotica* stem bark as a source of therapeutic agents, which may provide leads in the ongoing search for antibacterial agents from plants.

KEYWORDS: Plant extracts, Methanol, Phytochemicals, Anti-bacterial activity, Stem bark.

INTRODUCTION

The problem of bacterial resistance to most of the available antibiotic agents and the high costs of treatments consequent upon resistance, has necessitated the search for new, safe, efficient and cost-effective ways for the management of infectious diseases. It was warned that unless concerted efforts are made to acquire new agents, very soon the population of bacteria developing resistance will overwhelm the arsenal to fight (Akinpelu and Onakaya, 2006; Breman, 2021). Extracts of higher plants have served as good sources of antibiotics against various bacterial and fungal pathogens (Falodun *et al*., 2006). Plant-based antimicrobial compounds have great therapeutic potential as they can serve the purpose without any side effects often associated with synthetic drugs and also little chance of development of resistance. The common view in the society and the medical community is that plant-based products are healthier, safer, and more reliable than synthetic products (Benli *et al*., 2008), even though safety and efficacy data are available for only a few numbers of plant materials.

For decades, medicinal plants and herbal practices have been used to treat infectious and other non-infectious diseases through the traditional practice of herbal medicine practitioners (HMPs) in Nigeria. In fact, several studies have reported the ethnomedicinal application of plants in treating diseases such as cancer, malaria, bacterial infections, etc. (Newman and Cragg, 2020; Breman, 2021). Consequently, the area of ethnopharmacology of medicinal plants has attracted increasing attention in new drugs research and development (Geyid *et al*., 2005; Kilani-Jaziri *et al*., 2011; Breman, 2021). It is estimated that two-thirds of the world population depend on traditional medications due to the limited availability, the high prices of most pharmaceutical products and the various side effects that they cause (Tagboto and Townson, 2001). This further justifies the search for alternative products from plants used in traditional medicine.

As part of our ongoing research to purify, isolate and characterized antibacterial compounds from the extracts of some traditionally used medicinal plants in Kebbi State, Nigeria, the extracts of twelve medicinal plants were screened for their antibacterial activity. The ethnobotanical properties of the selected plants are presented in Table 1. The plants have varied ethnomedicinal applications in Kebbi traditional medicinal practice including the treatment of dysentery, syphilis, toothache, typhoid fever, malaria, jaundice, body pains, stomach ache, diarrhea, Acquired Immune Deficiency Syndrome (AIDS), hepatitis, yellow fever, skin infections, etc. (Ekeanyanwu, 2011; Ekor, 2014; Keta, 2016; Abdulhamid *et al*., 2019; Aisha *et al*., 2022; Gudu *et al*., 2022).

MATERIALS AND METHODS

Plant Sample Collection and Identification

The selected plant samples were collected separately from different locations around Kebbi State, Nigeria. The plants samples were selected based on the outcome of ethnobotanical survey of plants used in the treatment of infectious diseases in Kebbi State, Nigeria. The samples were identified and authenticated by a Botanist at the Department of Plant Science and Biotechnology, Kebbi State University of Science and Technology, Aliero. The identified names and voucher numbers are presented in Table 1. The samples were shed-dried, ground and kept in air-tight containers till further use.

Plant Extracts Preparation

The methanol crude extracts of the plant samples were prepared by soaking 100 g of each plant powdered sample in 90% methanol (300 mL) for 72 h. At the end of the extraction, the extract was filtered using Whatman filter paper. The filtrate was concentrated in vacuum at 30^{o}C and stored in sterile sample containers at 4 ^{o}C until further use.

Phytochemical Screening

The extract was screened for the presence of major phytochemicals using standard qualitative methods as described previously (Sofowora, 2008; Trease and Evans, 2009). The selected plant extracts were screened for the presence of alkaloids, flavonoids, glycosides, saponins, tannins and terpenoids.

The Test Organisms

The bacteria used for the test include *E. coli, K. Pneumoniae, S. aureus,* and *S. typhi.* They were obtained from the Microbiology Laboratory, Federal Medical Centre, Birnin Kebbi, Kebbi State, Nigeria. All the isolates were checked and maintained in a slant of Mueller Hinton Agar.

Antibiotic Sensitivity Test

This was done by pouring nutrient agar media into petri-dishes and allowed to solidify. Bacterial inoculum was prepared by diluting the agar culture to match the 0.5 Mcfarland turbidity standard. A sterilized swap was used to collect the culture, excess culture was removed by gently pressing the swap against the surface of the tube. The swap was then streaked across the nutrient agar plates to form a bacterial lawn. The antibiotic discs were then gently pressed into the plates. The plates were then incubated at 35 ^{o}C overnight. The antibacterial activity was interpreted by a clear zone around a disc which was measured in mm with a ruler (Abioye *et al*., 2013).

Antibacterial Activity Screening of the Selected Crude Extracts

The antibacterial activity of the crude methanol extract selected plants was determined using agar well diffusion method (Njoku *et al*., 2010). The standardized inocula of the isolates were uniformly streaked unto freshly prepared Mueller Hinton agar plates with the aid of a sterile swab stick. Three appropriately labeled wells were bored into each agar plate using a sterile cork borer (6 mm in diameter). A 0.2 mL of the appropriate extract concentrate was placed in each well and then incubated at 37 ^{o}C for 24 h. Zone of inhibition (mm) formed was determined as an indication of antibacterial activity.

Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentrations of the selected plant extracts were carried out on the bacteria that were sensitive to the extract and were done using broth dilution method (Abdulhamid *et al*., 2018). Different concentrations of the extract that exhibited antimicrobial activity against the test organisms were prepared in the test tube containing Mueller Hinton Broth (MHB). The organisms were inoculated into each tube containing the diluted extracts. The plates were incubated at 37 ^{o}C for 24 h. The lowest concentrations of the extract which shows no turbidity was recorded as the minimum inhibitory concentrations.

Determination of Minimum Bactericidal Concentration (MBC)

Minimum bactericidal concentrations of the selected plant extracts were determined to check whether the test microbes were killed or only their growth was inhibited. Mueller Hilton agar was prepared according to the manufacturer's instruction, boiled to dissolve and was sterilized at 121^{o}C for 15 min, the media were cooled to 45^{o}C and the medium (20 mL) was poured in to sterile petri dishes, the plates were covered and allowed to cool and solidify. The content of the MIC in the serial dilution was inoculated on to the media, the plates were incubated at 37^{o}C for 24 h, after which the plate were observed for colonies growth. The MBC was the plate with lowest concentrations of the extract without colony growth (Abdulhamid *et al*., 2018).

Statistical Analysis

The results were expressed as mean ± S.D (n = 3). The data was statistically analyzed using IBM-SPSS (version 20) statistical tool (IBM Corp., Armonk, NY, USA). One-way ANOVA was used followed by Duncan's multiple comparison test. P value <0.05 was considered statistically significant.

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Table 1: Ethnobotanical properties of selected plants used in Kebbi State

SCIENTIFIC NAME	FAMILY NAME	LOCAL NAME	COMMON NAME	VOUCHER NUMBER	PART USED	MODE OF PREPARATION	ROUTE
***Parinari curatellifolia* Planch. Ex Benth.**	Chrysobalanaceae	Gawasa	Hissing tree	114A	Stem bark	Concoction mixes with potash	Orally
***Adansonia digitata* L.**	Bombacaceae	Kuka	Baobab	266	Stem bark	Decoction	Orally
***Ficus platyphylla* Dell.**	Moraceae	Gamji	Guttapercha	122A	Leaves	Boil with tea	Orally
***Allium sativum* L.**	Amaryllidaceae	Tafarnuwa	Garlic	136	Whole plant	Concoction mixed with potash	Orally
***Cassia occidentalis* L.**	Fabaceae	Sangasanga	Coffee senna	223A	Leaf	Decoction	Orally
***Mitragyna inermis* (Willd.) Kuntze**	Rubiaceae	Giiyayaa	False abura	139A	Leaf	Concoction	Orally
***Cassia tora* L.**	Fabaceae	Tafasa	Sickle senna	212	Leaf	Decoction	Orally
***Citrullus lanatus* Thunb.**	Cucurbitaceae	Kankana	Water melon	285A	Seed	Decoction	Orally
***Diospyros mespiliformis* Hochst.**	Ebenaceae	Kanya	Jackalberry	182	Stem bark	Concoction mixed with shear butter	Orally
***Guiera senegalensis* J.F. Gmel.**	Combretaceae	Sabara	Moshi medicine	67	Leaf	Powdered	Orally
***Acacia nilotica* L.**	Cucurbitaceae	Garahuni	Balsam pear	276	Stem bark	Concoction/ the powder is boiled with tea	Orally
***Prosopis Africana* Guill. & Perr.**	Fabaceae	Kirya	Iron tree	112	Leaf	Decoction	Orally

RESULTS

Phytochemical Screening

The result of the phytochemical screening of the twelve (12) plant extracts is presented in Table 2. The result reveals the presence of flavonoids, tannins, saponins, glycosides, alkaloids and terpenoids in most of the extracts studied. However, all the tested phytochemical constituents were detected in the extract of *Cassia occidentalis* and *Acacia nilotica*.

Antibiotic Sensitivity Pattern of the Test Bacterial

The antibiotic sensitivity profile of the test bacteria to the common antibiotics is presented in Table 3. The clinical isolates showed different sensitivity pattern towards the antibiotics used. The isolates are resistant to most of the antibiotics. However, all the test isolates were found to be susceptible to Augmentin.

Antibacterial Activity of the Selected Plant Extracts

The antibacterial activities of the selected plant extracts against test isolates show different degrees of activity (Table 4). However, four plant extracts; *Ficus platyphylla, Cassia occidentalis, Diospyros mespiliformis* and *Acacia nilotica* showed better activity (higher zone of inhibition) compared to other plant extracts. *Acacia nilotica* stem bark extract shows a zone of inhibition ranging from 18.0±0.20 mm against *E. coli* to 25.4±1.53 mm against *S. aureus*. The zone of inhibition exhibited by *Acacia nilotica* stem bark extract against *S. aureus, S.typhi* and *E. coli* is presented in Figure 1 (a – c).

Table 2: Phytochemical composition of the selected plant extracts

	RESULT					
PLANT EXTRACT	**ALKALOIDS**	**FLAVONOIDS**	**GLYCOSIDES**	**SAPONINS**	**TANNINS**	**TERPENOIDS**
Parinari curatellifolia	ND	+	+	+	N.D	+
Adansonia digitata	+	+	+	+	+	+
Ficus platyphylla	+	N.D	N.D	+	+	+
Allium sativum	+	+	+	+	N.D	+
Cassia occidentalis	+	+	+	+	+	+
Mitragyna inermis	+	+	+	+	+	N.D
Cassia tora	+	+	+	+	N.D	+
Citrullus lanatus	N.D	N.D	N.D	N.D	+	N.D
Diospyros mespiliformis	+	+	N.D	+	N.D	+
Guierase negalensis	+	N.D	+	+	+	+
Acacia nilotica	+	+	+	+	+	+
Prosopis africana	+	+	+	+	+	N.D

Key: + = present; N.D = Not detected.

Table 3: Antibiotic sensitivity pattern of the test bacteria

BACTERIUM	**SENSITIVITY**									
	PG	**AM**	**CPX**	**E**	**APX**	**AMP**	**CH**	**AZM**	**AT**	**SXT**
E. coli	-	-	-	+	-	-	-	-	+	-
S. aureus	-	+	+	-	-	-	-	-	+	-
S. typhi	+	-	+	-	-	-	-	+	+	+
K. pneumoniae	-	+	-	+	+	-	-	-	+	+

PG = Defloxacine; AM = Amoxicillin; CPX = Ciprofloxacin; E = Erythromycin; PX = Ampiclox; AMP = Ampicillin; CH = Chloramphenicol; AZM = Azithromycin; AT = Augmentin; SXT = Septrin; + = Susceptible; - = resistant; All the test isolates were found to be susceptible to Augmentin

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the four (4) plants with the highest antibacterial activity is presented in Table 5. The MIC exhibited by *Ficus platyphylla, Cassia occidentalis, Diospyros mespiliformis* and *Acacia nilotica* were in the range of 25.0 – 50.0, 12.5 – 25.0, 12.5 – 50.0 and 6.25 – 12.5 mg mL^{-1}, respectively. The MIC results showed that the test organisms were responsive to the plant extracts.

Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentrations (MBC) exhibited by the crude extract, ethyl acetate and aqueous fractions against the susceptible test isolates was shown in Table 6. The MBC exhibited by *Ficus platyphylla, Cassia occidentalis, Diospyros mespiliformis* and *Acacia nilotica* extracts against the test isolates ranged between 25 and 100 mg mL^{-1}.

DISCUSSION

In search for a solution to the problem of resistance to commonly used antibiotics among bacterial strains that has become a global threat, the antibacterial activities of some commonly used medicinal plants were investigated on four clinically isolated resistant bacterial strains isolated from patients attending Federal Medical Center (F.M.C) BirninKebbi. The ethno medicinal importance of the selected plants has been reported in various indigenous system of folk medicine and scientific documents. The plants have been widely used in the traditional management of diseases such as dysentery, syphilis, toothache, typhoid fever, malaria, jaundice, stomach ache, diarrhea, Acquired Immune Deficiency Syndrome (AIDS), hepatitis, yellow fever, skin infections, etc. (Hussain and Deeni, 1991; Keta, 2016; Aisha *et al*., 2022; Gudu *et al*., 2022).

The plant materials used in this study were initially extracted with methanol; the choice of methanol as a solvent of extraction was based on the previous research works (Lourens *et al*., 2004; Parekh *et al.,* 2006; Nebedum *et al*., 2009) They are of the opinion that an organic solvent, especially methanol, was a better solvent for consistent extraction of antimicrobial compounds from medicinal plants in comparison to other solvents such as water, hexane and ethanol.

The phytochemical screening reveals the presence of flavonoids, tannins, saponins, glycosides, alkaloids and terpenoids in most of the extracts studied. Previous research reported relatively similar phytochemicals from the selected plants (Davis *et al*., 2003; Jang *et al*., 2007; Halilu *et al*., 2008; Banso, 2009; Okoro *et a*l., 2014; Chinmay *et al*., 2015). These findings support previous reports on the phytochemical composition screening of these plants. Phytochemical compounds are known to be biologically active (Ververidis *et al.,* 2007) and thus may contribute to the observed antibacterial activities in these plants.

Phytochemicals exert antimicrobial activity through different modes. For instance, flavonoids possess a wide range of biological activities which include antimicrobial, anti-inflammatory, analgesic, anti-allergic effects, cytostatic and antioxidant properties (Maika *et al*., 2009). 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. Thus, these plants are traditionally used in treating diarrhoea and dysentery among communities in Northern Nigeria.Saponins are known to produce inhibitory effects on inflammatory processes. They were also reported to possess antibacterial property. Their mode of action attributed to their ability to cause leakage of proteins and certain enzymes from bacterial cells (Tamil *et al*., 2011). Alkaloids are another kind of phytochemicals detected in most of the plant extracts tested. Alkaloids have been associated with medicinal uses for centuries.

The antibacterial activities of the selected plant extracts were investigated against the bacterial isolates and the results are presented in Table 4. The extracts at a concentration of 100 mg/mL were found to inhibit the growth of most of the test bacterial isolates comprising of both Gram-positive and Gram-negative organisms. The zones of inhibition exhibited by the extracts ranged between 0.00 ± 0.00 mm and 25.4± 1.53 mm. The extracts tested displayed a varying degree of antibacterial activity against the bacterial isolates. These findings support previous reports on the antimicrobial activity of these plants (Jang *et al*., 2007; Halilu *et al*., 2008; Masola *et al*., 2008; Banso, 2009; Kubmarawa *et al*., 2009; Okoro *et al*., 2014; Chinmay *et al*., 2015).

The bacteria isolates used in this study include pathogens such as *E. coli* known to cause urinary tract infections (Dromigny *et al*., 2005); *S. typhi* known to cause typhoid fever and *K. pneumoniae* known to be the causative agent of pneumonia. All these pathogens were susceptible to specifically four of the plant extracts used in this study, thus supporting the use of these plants in folklore remedies in the treatment of diseases caused by these pathogens. However, the antibacterial activity of *Acacia nilotica* stem bark extract against the bacterial isolates is relatively higher than all the other extracts used in this study. The zone of inhibition exhibited by the *Acacia nilotica* stem bark extract ranged between 18.00 ± 2.00 for *E. coli* and 25.4 ± 1.53 for *S. aureus.*

The results of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) revealed that the four plant extracts have relatively high activity against the tested strains. The MIC for *Acacia nilotica* stem bark extract range between 6.25 mg/ml against *S. aureus* and 12.5 mg/ml against *E. coli, S. typhi* and *K. pneumoniae.* While the MBC for *Acacia nilotica* stem bark extract range between 25.0 mg/ml against *E. coli* and *S. aureus* and 50.0 mg/ml against *S. typhi* and *K. pneumoniae.* The results of the MIC and MBC indicate how active an extract is against the tested organisms. High MIC and MBC results indicate low activity, while low MIC and MBC indicate high activity (El-Mahmood and Doughari, 2008).

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Table 4: Antibacterial activity of the selected plant extracts

PLANT EXTRACTS (100 mg/ml)	ZONE OF INHIBITION (mm)*			
	E. coli	*S. aureus*	*S. typhi*	*K. pneumoniae*
Parinari curatellifolia	4.33±0.58^{b}	16.7±1.52^{c}	NA	6.7±2.52^{a}
Adansonia digitata	14.3±1.52^{cd}	N.D	6.3±1.54^{a}	NA
Ficus platyphylla	17.0±1.00^{d}	14.0±2.65^{b}	15.3±1.52^{d}	4.0±1.00^{a}
Allium sativum	NA	17.0±1.00^{c}	NA	NA
Cassia occidentalis	17.0± 1.00^{d}	21.3±1.53^{d}	15.0±4.58^{d}	15.7±1.15^{c}
Mitragyna inermis	NA	18.0±1.00^{c}	5.67±2.31^{a}	12.3±2.08^{b}
Cassia tora	12.3±0.46^{cd}	NA	4.67±2.08^{a}	14.3±1.53^{c}
Citrullus lanatus	9.4±0.55^{c}	NA	7.67±3.06^{b}	12.7±0.58^{b}
Diospyros mespiliformis	19.0±0.10^{de}	19.3±0.58^{cd}	6.00±1.73^{a}	14.3±1.53^{c}
Guiera senegalensis	2.00±1.00^{a}	NA	5.33±1.52^{a}	NA
Acacia nilotica	18.0±0.20^{d}	25.4±1.53	22.7±2.01^{e}	24.3±1.53^{e}
Prosopis africana	NA	10.0±0.31^{a}	9.68±0.38^{c}	14.0±2.00^{c}
Augmentin (10mg/ml)	23.7±1.15^{f}	22.8±0.23^{d}	21.3±2.08^{e}	20.7±2.31^{d}
Methanol (15%)	NA	NA	NA	NA

Data presented as mean ± S.D (n=3), values having different superscript along the column are significantly different at $p<0.05$; NA: No Activity

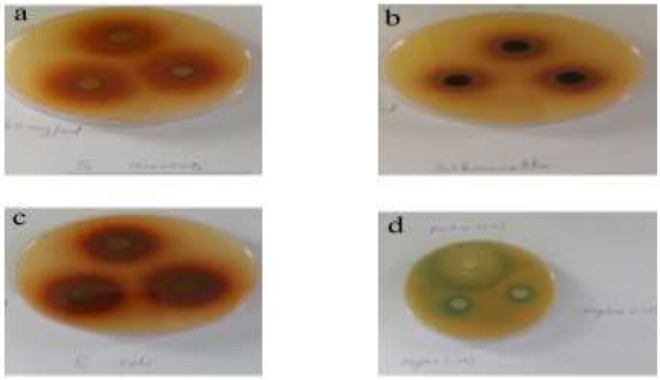

Figure 1: Zone of inhibition of *Acacia nilotica* stem bark extract against (a) *S. aureus,* (b) *S.typhi,* (c) *E. coli* and (d) positive and negative controls

Table 5: The minimum inhibitory concentrations (mg/ml) of the selected plants

BACTERIAL ISOLATES	*E. coli*	*S. aureus*	*S. typhi*	*K. pneumoniae*
Ficus platyphylla	25.0	25.0	50.0	50.0
Cassia occidentalis	12.5	25.0	25.0	25.0
Diospyros	50.0	12.5	25.0	12.5
mespiliformis	12.5	6.25	12.5	12.5
Acacia nilotica				

Table 6: The minimum bactericidal concentration (mg/ml) of the selected plants

BACTERIAL ISOLATES	*E. coli*	*S. aureus*	*S. typhi*	*K. pneumoniae*
Ficus platyphylla	50.0	N.D	50.0	N.D
Cassia occidentalis	100	50.0	N.D	50.0
Diospyros mespiliformis	N.D	25.0	100	25.0
Acacia nilotica	25.0	25.0	50.0	50.0

In this study, the lowest MIC and MBC values exhibited by *Acacia nilotica* extract indicates the highest activity of the extract against the tested bacteria.

CONCLUSION

The result of the present study signifies the potential of the selected plant extracts as source of therapeutic agents, which may provide leads in the ongoing search for antibacterial agents from plants. It further scientifically justified the ethnobotanical use of the selected plants in the management of infectious diseases in Kebbi State, Nigeria. Further, the activity exhibited by the plant extracts against tested bacteria species that are associated with various infectious diseases may provide scientific justification for the ethnomedicinal uses of these plants.

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