

**ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF STEM BARK EXTRACTS OF *Adansonia digitata* ON SOME CLINICAL ISOLATES**^aMagashi, A. M. and ^bAbdulmalik, U.^aMicrobiology Department, Faculty of Sciences, Bayero University, P. M. B. 3011, Kano, Nigeria, ^b Microbiology Department Federal University, Dutsinma P. M. B 5001 Dutsinma, Katsina State, Nigeria.*Correspondence author: ammagashi93@gmail.com,**Abstract**

Adansonia digitata stem bark extract is been used in the treatment of stomach upset, diarrhoea, dysentery, antioxidant, antimalaria, antiinflammation. This work was aimed at determine the phytochemical constituents and the antibacterial activities of petroleum ether, ethanol and aqueous crude extracts of the stem bark from *Adansonia digitata* on some clinical isolates. The Dried stem barks powdered of *Adansonia digitata* were extracted using three solvents. The crude extracts were subjected to Phytochemical screening using stand ard procedureThe crude extracts were further tested for their antibacterial activity using disc d iffusion method against *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Klebsiellapneumoniae*, *Escherichia coli* and *Proteusmirabilis*. MICs were determined using micro broth dilution technique. Acute toxicity study was carried out using albino Wister rats. The ethanol extract as the highest percentage yields of 5.14%. The result of phytochemical screening shows the presence of tannins, saponins, alkaloids, flavonoids, triterpenoids, glycosides, steroids and reducing sugar. The results of antibacterial activity show that all the isolates showed significant susceptibility except *Escherichia coli* that was resistance to all the test concentration of the three extracts. The MIC and MBC of the different extract ranged from 500µg/ml to 2000 µg/ml and 1000µg/ml to 4000µg/ml respectively. The result of this study showed that the stem bark extract of *Adansonia digitata* contain bioactive constituents which are responsible for the antibacterial activity and may have the potential for the production of drugs against some clinical isolates.

Keywords: *Adansonia digitata*, Extracts, Phytochemical screening, Antibacterial activity, Minimum Inhibitory Concentration and Minimum Bactericidal Concentration.

INTRODUCTION

Plant parts have been used in the treatment of human disease for ages, and herbal medicine was the major form of medicine because they contain large varieties of chemical substances that posses important preventive and curative therapies (Nascimento *et al.*, 2000). According to World Health Organization (WHO, 2001), as many as 80% of world population depend on traditional medicine for their primary health care needs and that 25% of the drugs are based on plants and their derivatives (Azu and Onyeagba, 2007, Yahaya *et al.*, 2011). The detection of the antimicrobial properties of a plant indicates that, such plant could be a good source for the development of antimicrobial agent which is based on their Phytochemical constitutes (Maria *et al.*, 2007).

Medicinal plants have bioactive compounds which help to treat various ailments caused by microorganisms. These compounds may have

evolved in plants as self defence mechanism against pests and pathogens to help plants to establish themselves in their environment (Sukumaran *et al.*, 2011). Our concern is shifting towards traditional medicinal plants to tap their unexplored bioactive potential, as nature has been a source of medicinal agents for thousand of years and an impressive number of modern drugs have been isolated from natural sources, notably from plant origin, many based on their use in traditional medicine (Cowan, 1999). Herbal therapy medicine uses plant extracts for their therapeutic value. It is the oldest form of healthcare known to mankind (Manzoor and Maksuda, 2000) and remains an important element of human and livestock healthcare systems in many developing countries (Lambert, *et al.*, 2005).

Adansonia digitata Linn Commonly known as “Baobab” is a deciduous tree and belongs to the plant family called Bombacaceae. The leaves of the baobab tree are a staple food source for rural populations in many parts of Africa, especially the central region of the continent (Yazzie *et al.*, 1994). They are eaten both fresh and as a dry powder. In Nigeria, the leaves are locally known as “kuka” and are used to make “kuka soup”. The leaves are typically sun-dried and either stored as whole leaves or pounded and sieved into a fine powder. In markets the powder is the most common form (Sidibe and William, 2002). The seeds are characterized as a potential source of protein and roasted seeds are used as coffee substitute in Sudan and North Africa (Dirar, 1993). The seeds are mostly used as a thickener for soups, but may be also be fermented into a seasoning, roasted for direct consumption, or pounded to extract vegetable oil. (Gebauer *et al.*, 2002).

Adansonia digitata stem bark extract is been used in the treatment of stomach upset, diarrhoea, dysentery, antioxidant, antimalaria, antiinflammation. A semi-fluid gum obtained from baobab bark is used to treat sore throat (FAO, 1988). The bark produces strong fibers used in making ropes, mats, bags and hats. The smooth fibers of the inner side of the bark are more important than the outer bark for weaving (Igboeldi *et al.*, 1997). The wood is whitish, spongy and light air dried and is used mainly for fuel (Venter and Venter, 1996). Water storage capacities range from 1000 to 9000 litres per tree (Craig, 1991). Baobab contains a number of substances usually employed for the treatment of numerous diseases in the African traditional medicine and for that reason it is also named “the small pharmacy” (Obizoba and Anyika, 1994). In many medicinal uses the stem bark is used when prepared is made into a decoction for internal use and functions due to its soluble and insoluble tannin (Yusha’u *et al.* 2010). This work was aimed at determine the antibacterial activities of the crude extract using some clinical isolate so as to reveal the most sensitive organism.

MATERIALS AND METHODS

Collection, Identification and authentication of the plant

The plant material (stem bark) of *Adansonia digitata* was collected by scraping the tree bark using sterile knife from Bayero University, campus (Old Site) Kano, Nigeria. in the month of February, 2015. Identification and authentication was confirmed by a

Taxonomist at the Department of Plant Biology, Bayero University, Kano and a voucher specimen number was provided as Accession Number (NO.BUKHAN 0036), from their herbarium.

Preparation of *A. digitata* stem bark extracts

The plant stem bark was air-dried at room temperature in the laboratory for two weeks. After drying the stem bark was pounded into a powder form using clean mortar and pestle and stored till use in according to Mukhtar and Okafor (2002).

Extraction of *A. digitata* (stem bark)

One hundred grams (100g) of the powdered plant was weighed into 3 different 1L capacity bottles and percolated with 500ml each of petroleum ether, ethanol (for two weeks) and water (for one week) with shaking at regular intervals. The mixture was then filtered through a clean muslin cloth followed by filtration with whatman No.1 filter paper and the filtrate was allowed to evaporate at ambient temperature. The crude extract was kept under refrigerated condition at 4°C until required for further use (Betoni *et al.*, 2006).

Phytochemical Screening

Test for Flavonoids, tannins, Triterpenoids, glycosides, alkaloids, saponins, steroids and reducing sugar were carried out as described by Poongothai *et al.*, (2011).

Test Organisms

The clinical test isolates which include *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pneumoniae* were obtained from Microbiology department of Aminu Kano Teaching Hospital (AKTH), Kano and maintained on nutrient agar slants in the refrigerator (4°C) prior to use (Cheesbrough, 2000).

Bioassay procedure

Preparation of Sensitivity Discs

Discs of 6.0mm diameter were punched from Whatman No.1 filter paper with the aid of paper puncher and placed into screw capped Bijou bottles in batches of 100 discs per bottle. The discs were then sterilized by autoclaving at 121°C for 15 mins and then allowed to cool. Sensitivity discs were prepared by weighing the appropriate amount of the extract by serial doubling dilution in Dimethyl-Sulfoxide (DMSO). This was followed by the addition of 100 discs into each of the solution bottle containing 1ml such that each disc absorbed 0.01ml of the solution to arrive at concentration of disc potencies of 4,000µg/disc, 6,000µg/disc, 8,000µg/disc and 10,000µg/disc respectively (Akinyemi *et al.*, 2005; Vallekobia *et al.*, 2001).

Standardization of the Inocula

A loopful of the confirmed test isolates using biochemical test were obtained from AKTH were picked using a sterile wire loop and emulsified in to 2mls of sterile normal saline to matched with the 0.5 McFarland Standard for sensitivity test as described by Cheesbrough (2000). This turbidity is equivalent to approximately 1.5×10^8 colony forming units per ml (cfu/ml).

Sensitivity testing

Using a sterile swab stick, a standardized Inoculum of each isolate was swabbed onto the surface of Mueller Hinton Agar in separate Plates. This was followed by application of the prepared sensitivity discs of the four different concentration of the extracts (4000 μ g/ml, 6000 μ g/ml, 8000 μ g/ml and 10,000 μ g/ml) at different corners of the Petri dish while the Standard antibiotics (Ofloxacin 5 μ g) was onto the center of the surface of the inoculated media to serve as positive control, using a sterile pointed forceps. The discs prepared were kept at the distance of 2cm apart to prevent any overlapping of zones. The plates were inverted and allowed to stand for 15mins to allow pre-diffusion of the extract into the agar and subsequently incubated at 35 $^{\circ}$ C for 24 hours.

This was followed by measurement of the diameter of zones of inhibition by each of the test organisms, using venire caliper in millimeter (Cheesbrough, 2000).

Determination of Minimum Inhibitory Concentration (MIC)

The Minimum inhibitory concentration (MIC) assay was determined by serial tube dilution using Dimethyl-Sulfoxide (DMSO) to arrive at concentrations of 4000 μ g/ml, 2000 μ g/ml, 1000 μ g/ml, 500 μ g/ml, 250 μ g/ml, 125 μ g/ml and 62.5 μ g/ml. Two millitre (2mls) of extract and Mueller Hinton broth was mixed. A 0.1ml of the standardized suspension of the test organisms was added to each of the test tubes above. The tubes were incubated aerobically at 35 $^{\circ}$ C for 24

hours. Tubes containing broth and the extracts without inocula which served as positive control while tubes containing broth and inocula served as negative control. The tubes were observed after 24 hours of incubation for the presences of growth. The lowest concentration that showed no evidence of growth was regarded as minimum inhibitory concentration (MIC) (Akinoyemi *et al.*, 2005, Vallekobia *et al.*, 2001).

Minimum Bactericidal Concentration (MBC)

Sterile Mueller Hinton agar plates were separately inoculated with culture from each of the MIC test tubes that showed no evidence of turbidity. The plates were further incubated at 37 $^{\circ}$ C for 24 hours to determine the Minimum Bactericidal Concentration (MBC), as the highest dilution that yielded no single bacterial colony on the solid medium (Akinoyemi *et al.*, 2005, Vallekobia *et al.*, 2001).

Toxicological Study

All the animals uses in this research work were healthy albino Wister rats of both sexes. They were purchased from the animal house of Biological science department of Bayero University, Kano (BUK old site). They were fed with feed and water ad libitum. Acute toxicity study of *Adansonia digitata* stem bark extract was carried out and LD₅₀ determination using the method of Lorke (1983). In phase I, three groups (1, 2, and 3) of three rats each were orally administration with the extracts of doses of 10mg/kg, 100mg/kg and 1000mg/kg. The same procedure was carried out on each of the three extract (petroleum ether extract, ethanolic extract and aqueous extract). And then observed for 24hours for signs of toxicity which include salivation, stretching of the body, weakness, brushing of nose on the floor, sleep, coma and death. In phase II, three groups of one rat each were orally administered extract doses of 1600mg/kg, 2900mg/kg and 5000mg/kg and the same procedure was also repeated for each of the three extracts. They were observed for 24hours for any toxicity or mortality.

RESULTS

Table 1: Physical properties of crude extracts of *Adansonia digitata*.

Physical parameters	PEE	EE	AE
Weight extracted (g)	100	100	100
Weight of extract (%)	3.89	5.14	4.96
Percentage yield (%)	3.89	5.14	4.96
Colour	Yellow	Dark green	Brawn
Texture	Gummy	powdery	powdery

KEY: PEP= Petroleum Ether Extract, EE= Ethanol Extract, AE= Aqueous Extract.

Table 2: Phytochemical Components of the Extracts of Stem bark of *Adansonia digitata*.

Phytochemical Tests.	PEE	EE	AE
Alkaloids	+	+	+
Tannins	+	+	+
Saponins	-	+	+
Flavonoids	-	+	+
Glycosides	-	+	-
Steroids	-	+	-
Reducing sugar	-	+	+
Triterpenoids	+	+	-

KEY: PEE= Petroleum Ether Extract, EE= Ethanol Extract, AE= Aqueous Extract, + = Present
- = Absent.

Table 3: Antibacterial Activities of Stem Bark Extract of *Adansonia digitata* Against the Test Isolates

Test Isolates	Diameter of zones of inhibition (mm) /Extract concentration (µg/disc)														
	Solvents														
	Pet. Ether					Ethanol					Aqueous				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
<i>Staph. aureus</i>	26	0	10	15	18	26	8	18	21	24	26	0	16	19	22
<i>Strep. Pneumoniae</i>	26	0	8	10	15	26	7	15	19	22	26	0	15	17	20
<i>Pseudo. aeruginosa</i>	25	0	11	15	18	25	7	14	17	20	25	7	12	15	18
<i>Kleb. pneumoniae</i>	25	0	8	10	12	25	7	9	12	16	25	0	9	11	13
<i>Proteus mirabilis</i>	25	0	8	10	14	25	7	13	11	19	25	0	9	12	18
<i>Escherichia coli</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

KEY: 1 = 5µg (Ofloxacin as control), 2 = 4000µg, 3 = 6000µg, 4 = 8000µg, 5 = 10,000µg

Table 4: Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of different extracts against the test organisms

Values of Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) (in µg/ml)

Test Organisms	PEE(in µg/ml)		EE(in µg/ml)		AE(in µg/ml)	
	MIC	MBC	MIC	MBC	MIC	MBC
<i>Staphylococcus aureus</i>	1000	2000	500	1000	500	1000
<i>Streptococcus pneumoniae</i>	1000	4000	1000	2000	1000	2000
<i>Pseudomonas aeruginosa</i>	2000	4000	2000	4000	1000	2000
<i>Proteus mirabilis</i>	1000	2000	1000	2000	1000	4000
<i>Klebsiella pneumoniae</i>	2000	4000	2000	4000	2000	4000

KEY: PEE = Petroleum Ether Extract, EE = Ethanol Extract, AE = Aqueous Extract.

DISCUSSION

The ethanol extract as the highest percentage yields of 5.14%. Difference in the yield may be due to the difference in the accumulation of phytochemical component, solubility of the bioactive metabolites and the polarity of the solvent which tend to affect it is ability to recover compounds. The variation in the texture in may be due to the difference in the polarity of the solvent, as ethanol and aqueous appears to be powdery while petroleum ether was gummy in nature. The variation in colour in may be due to the difference in the natural colour of the bioactive component present in the plant stem bark.

The result of Phytochemical Screening of crude petroleum ether, ethanol and aqueous

extract of the stem bark of *Adansonia digitata* revealed the presence of secondary metabolites such as alkaloid, tannins, saponins, Flavonoids, glycosides, steroids, triterpenoids and reducing sugar. These metabolites have been reported to possess antimicrobial activity (Cowan, 1999). In particular the flavonoids were reported to be responsible for antimicrobial activity associated with some ethno medicinal plants (Singh and Bhat, 2003). This agreed with the work of Masola *et al.*, (2009); Lim *et al.*, (2006) who reported the presence of tannins, phlobatannins, terpenoids, cardiac glycosides and saponins in the stem bark extract of *Adansonia digitata*.

These findings also agreed with the report of Tanko *et al.*, (2008) which also revealed the

presence of tannins, carbohydrate, terpenes, saponins, flavonoids and alkaloids in *Adansonia digitata* which may be responsible for their antibacterial activity.

The result of antibacterial activities of stem bark of the petroleum ether, ethanol and aqueous crude extracts of *Adansonia digitata* against test isolates at various concentrations revealed all the isolates to be sensitive.. Except *Escherichia coli* that were resistance all the three extracts at all the test concentrations. The highest zones of inhibition among the three extracts was observed in ethanol against *Staphylococcus aureus* at 10000µg/disc with 24mm and the least sensitive of 7mm each observed against the isolates, at 4000µg/disc in both ethanol and aqueous extracts, while the standard antibiotic have zones of inhibition of 26mm - 2mm against the isolates. This finding observed that positive control (Ofloxacin 5µg/disc) presented a wide diameter of zones of inhibition compared to the individual extracts on the tested organisms. This may be due to the fact that the positive control (Ofloxacin) is a purified form of antimicrobial agent where as the stem bark extracts are crude extract in their unpurified forms (Yusha'u, 2011). Previous studies on Stem and root barks of *Adansonia digitata* showed them to contain bioactive constituents such as tannins, phlobatannins, terpenoids, cardiac glycosides and saponins in the stem bark, as well as terpenoids in the aqueous extract of root bark, which are responsible for significant antibacterial activity of the crude extracts of this plant (Masola *et al.*, 2009) which agreed with this study. The activity exhibited by the extracts may be related to the presence of saponins, tannins in addition to flavonoids that are reported to be responsible for antimicrobial properties of some ethno-medicinal plants (Singh and Bhat, 2003).

The antibacterial activity of this finding disagrees with Yagoub (2008) that showed that the petroleum ether, ethanol and aqueous extracts of baobab showed antimicrobial activity against *Escherichia coli*. The antibacterial result showed that the aqueous extract was active against the test organisms,

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which confirms its usage traditionally. It was observed that, antibacterial activity of the extracts was enhanced by an increase in the concentration of the extracts, this correspond with the work of Mann *et al.*, (2008) i.e. the higher the concentration of the plant extract the greater the zones of inhibition. The Minimum Inhibitory Concentration (MIC) was determined in this study to be 500µg/ml - 200µg/ml and Minimum Bactericidal Concentration (MBC) was 1000µg/ml - 400µg/ml The acute toxicity study of extracts shows that no mortality was recorded in any of the experimental groups in 24hours after oral administration of the varying doses of the extracts. According to toxicity class of Hodge and Sterner (2005), any compound with oral LD₅₀ (rat) of 5000mg/kg or more should be considered as practically harmless. Hence oral administration of stem bark of *Adansonia digitata* at a dose of less than or equal to 5000mg/kg could be safe. And this also describe as practically non toxic according to the scale proposed by (Lorke, 1983). This agreed with the report that, the extracts are non-toxic to brine shrimp larvae (Musila *et al.*, 2013). This supports the results obtained in this study on the nontoxic nature of extracts on albino Wister rat. Nontoxicity of *A. digitata* explains why most of the plant parts, seeds, fruit pulps, stem and leaves are consumed by many communities (Kamatou *et al.*, 2011; Nguta *et al.*, 2011).

CONCLUSION

It can be concluded that the stem bark of *Adansonia digitata* may have the potential for the production of drug for the treatment of bacterial infections due to presence of bioactive constituents which are responsible for antimicrobial activity of the extracts.

RECOMMENDATIONS

The finding of this study shows that ethanol was the best solvent. Further studies is recommended in order to isolate, identify and purified each of the bioactive constituents present in the stem bark of *Adansonia digitata* to yield a pure compound that can be processed to synthetic drugs.

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