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Phytochemical Screening and Antibacterial Activity of Stem Bark of Baobab Tree (*Adonsonia digitata*) on some clinical isolate

Ummu Rabi'u RA[1]*, Haruna Sa'idu[2], Hajara Sani Labaran[1], Salau Abiola Olanrewaju[3], Musa Muntari[1], Sani Aliyu Ibrahim[1]

[1]Department of Microbiology, Faculty of Sciences, Gombe State University, P.M.B 127, Tudun Wada, Gombe, Gombe State, Nigeria.
[2]Department of Biology, Faculty of Sciences, Gombe State University, P.M.B 127, Tudun Wada, Gombe, Gombe State, Nigeria.
[3]Department of Natural Sciences, Gombe State College of Education,Billiri, Gombe State, Nigeria.

*Corresponding author:
Ummu Rabi'u RA
Department of Biology,
Faculty of Sciences,
Gombe State University, P.M.B 127,
Tudun Wada, Gombe,
Gombe State,
Nigeria.
Email: ummurabiura@gmail.com

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ABSTRACT

The use of plant and their secondary metabolite for the treatment of several illnesses is gaining attention across the globe. For this reason, baobab tree was procured in order to test its effect on some bacterial isolate. Powdered stem bark of *Adansonia digitata* was extracted with chloroform and methanol using percolation method of extraction. The extracts were dark brown and reddish brown in color respectively with a gummy texture. The chloroform and methanolic extracts were screened from the presence of secondary metabolites using standard technique. The result of photochemical screening indicated the presence of alkaloid, flavonoid, tannin, reducing sugar and steroid in one or both the extracts. The extracts were further tested, on confirmed clinical isolates of *Salmonella typhi* and *Staphylococcus aureus* using disc diffusion method and micro – broth dilution technique. The result of sensitivity test indicated that the tested clinical isolates were more sensitive to chloroform extract than the methanolic extract because larger zones of inhibitions were obtained with chloroform extract. In both methods used, the organisms were susceptible to all extracts even at the lowest concentration of 15 µg with zones of inhibitions ranging from (8 – 10 mm).

INTRODUCTION

Medicinal plant has for long been known to contain several secondary metabolites giving then an ability to have medicinal value. These metabolites can be extracted from different part of plant such as stem, flowers, seeds and roots. History has stated that the use of plants and their products can be dated back to earliest time when man uses parts of plants as healing portions, to eliminate pain, continuous suffering and counteract disease [1,2]. Although the use of traditional medicine to cure infection has been in practiced since the origin of mankind as reported by [3], this means that during this period the use of traditional medicine is the sole method of treatment. Despite the emergence of synthetic drugs nowadays, there is still absence of sufficient health care system especially to the rural areas having no or less access health facilities. This is regarded as major problem to the inhabitant of that area. This is why people of that area prefer visiting traditional healers and herbal medicine [4].

The major problem with synthetic drug is that several microorganisms are gradually developing strong mechanism that will make them capable of resisting those drugs thereby reducing their effectiveness for the treatment of particular ailment [5,6]. This type of resistance to antibacterial agents is a problem in many areas of the world especially in the developing countries [7]. In addition, synthetic drugs used for treatment of diseases have devastating side effect. This is why acceptance of the use of traditional of modern medicine are gaining increase recognition globally [5].

Baobab tree (*Adansonia digitata*) can be describe as deciduous and large tree that is about 25 m high, with an ability

to live for hundreds of years. The physiognomy of the tree revealed that it has thick angular, wide spreading branches which attains about 10-14 cm or more and often become deeply eluded. The have different kind of trunk, for young trees, the truck have characteristic of cylindrical, bottle shaped, or tapering with 10 – 14 cm or more in girth and often become deeply eluded [8] whereas conical in mature trees.

Among the several uses of that plant (Baobabs), local inhabitant often uses it within their own courtyards and nurture them until they are 2–3 m tall thereby providing boundary to their homes and land [9]. While [10] reported that stem bark of *Adansonia digitata* when prepared into decoction cane be used for the treatment of certain diseases such typhoid fever, malaria and urinary tract infections. Moreover, information on the microbiological and phytochemical properties of Baobab tree is scarce in literature [6]. Thus, the aim of the research is to evaluate the activity of stem bark extract of *Adansonia digitata* on some clinical isolates and phytochemical constituents and anti-bacterial.

MATERIALS AND METHODS

Collection and identification of plant materials

Stem bark of *Adansonia digitata* was scraped using a sterile knife at Malam inna Gombe State. The tree was identified using guide as described by [11]. The scraping was air dried and ground into fine powder using motor and pestle.

Extraction of powdered plant

About 30 g of the powdered plant of stem bark of *Adansonia digitata* was dispensed in 300 mL of ethanol in a conical flask and another batch of the 30 g of the powdered materials was dispensed in 300 mL of chloroform then kept for two days with shaking at regular intervals after which the content were filtered and the filtrates were evaporated at 40 °C. These were labeled as chloroform percolation extracts (CPE) and methanol percolation extract (MPE). All extracts were allowed to evaporate at room temperature 12].

Phytochemical screening of stem back of *Adansonia digitata*

Test for alkaloid

About 0.1mL of the plant extract were place in a test tube, it was then followed by 2–3 drops of reagent. The formation of orange red precipitates plus turbidity indicated the presence of alkaloid .

Test for flavonoid

1 mL of 10% of sodium hydroxide (Naoh) was added to 3mL of the stem bark extract. Appearance of yellow color indicated the presence of flavonoid [13].

Test for reducing sugar

About 1mL of extract and fractions were place in test tubes and 2 mL of distilled water were added. It was then followed by addition of Fehling's solution (a/b) under 40 °C temperature. The formation of brick red precipitate at the base of the test tube confirms the presence of reducing sugar [12].

Test for steroid

To the portion of stem bark of the extract, a few drops of concentrated hydrochloric acid were added was gradually swirled to ensure uniformity. The appearance of reddish-brown color indicated a positive result [3].

Test for tannin

About 2 mL of the plant extract and fraction were diluted with distilled water in separate test tube. 2-3 drop of 5% ferric chloride ($FeCL_3$) was added. The presence of green black or blue coloration confirms the presence of tannin [12].

Bioassay study

Preparation of sensitivity disc

Sensitivity discs of about 6mm in diameter were pouched from Whatman's no. I filter paper using a file punch and put in bijou bottle. The sensitivity discs were then sterilized in autoclave at 121 °C for 15 min and were allowed to cool. Sensitivity discs were prepared by weighing the appropriate amount of the extract or fraction and serial dilution in dimethyl– sulphoxide (DMSO) followed by placing the improvised paper discs in the solution such that each disc absorb 0.01mL to make potency of 15 µg, 30 µg and 60 µg [14].

Test isolates

Clinical isolates *Staphylococcus aureus and Salmonella typhi* were collected from Gombe State specialist and Doma hospital and maintained in agar slants in refrigerator (40 °C) prior to use. Appropriate confirmatory biochemical tests were carried out on each of the isolates.

Inoculum standardization

A loopful of each of the test's isolates using sterile were picked using wire loop and emulsified in 3.4mL of sterile physiological saline. The turbidity of the suspension was then matched with that of 0.5 McFarlands standard [15].

Sensitivity testing

Sterile swab stick was used to swab inoculum of isolate onto the surface of Mueller Hinton agar petri dishes; it was then followed with addition of disc of the extracts and standard tetracycline (tet 30 µg) to the surface of inoculated media. In order for the extract to diffuse into the agar, the plates were place upside down for about 30 min and then incubated aerobically at 35 °C for 18 h. Next, the zone of inhibition formed around each of the extract and standard antibiotic discs were measured [12].

Minimum inhibitory concentration (mic)

Minimum inhibitory concentration (MIC) of the extracts was measured by serial dilution using distilled water to obtain concentration of 3000 µg/mL, 200 µg/mL, 100 µg/mL. An equal quantity of extract and nutrient broth were both mixed in 2mLs tubes. 0.1 mL of standardized inoculum was appended to the different test tube and was incubated aerobically at 35 °C for 24 h. Control experiment was prepared by having tubes containing broth [14].

Minimum bacterial concentration (MBC)
Sterile Mueller–Hinton agar plates were separately inoculated with sample from each of the test tubes that indicated no evidence of bacterial growth. The plates were further incubated at 35 ºC for 24 h and the highest dilution that yielded no bacterial growth was regarded as MBC [14].

RESULTS

The result for phytochemical screening and bacterial activity of stem bark of *Adansonia digitata* on some clinical isolates were presented in the **Table 1** below:

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

Physical Parameters	CPE	MPE
weight extracted (g)	30±2	30±2
weight of extracts (g)	1.24±0.15	2.56±0.5
percentage yield (%)	4.1±0.8	8.5±0.3
color	dark brown	reddish
texture	gummy	gummy

Key: Cpe Chloroform Percolation Extract, Mpe: Methanol Percolation Extract

Table 2. Result of phytochemical screening of *Adansonia digitata* stem bark extracts.

extracts	result of phytochemical screening				
	alkaloid	flavonoid	reducing sugar	steroid	tannin
CPE	+	+	-	-	+
MPE	+	-	+	+	-

Key: + = presence of metabolites, = absence of metabolites

Table 3. Sensitivity of clinical isolates (mm) to chloroform and methanolic extracts of *Adansonia digitata.*

Isolates	CPE µg			MPE µg			TET µg
	15	30	60	15	30	60	30
Staphylococcus aureus	8±1.2,	9±1	10±1.5	10±1.5	,10±1.5,	0±0	11±1.5
Salmonella typhi	11±1.5	10±1.5	10±1.5	0±0	0±0	9±0	10±1.5

Table 4. Sensitivity of clinical isolates to chloroform percolation extract of stem bark of *Adansonia digitata*.

Isolate	MIC	CPE MBC	MIC µg/mL	MBC	MIC	MBC
	1000		2000		3000	
Staphylococcus aureus	+	+	-	-	-	-
Salmonella typhi	-	-	-	-	-	-

Keys: +: Presence of turbidity or growth, - denotes absence of turbidity or growth

DISCUSSION

High yield of the extract was obtained at the end of methanolic percolation extraction having reddish brown and gummy texture as presented in table one above. The high yield of the extract was a result of high solubility of plant material in the compound. There was a difference in the color of extracts based on the extracting solvent with methanolic extract having reddish brown color and chloroform extract being dark brown and both have gummy texture.

The results of phytochemical screening of chloroform and methanolic extracts of *Adansonia digitata* stem bark using percolation method of extraction (**Table 2**) revealed the presence of alkaloid in all the extracts irrespective of the solvent used for the extraction. Flavonoid was present in chloroform percolation extract while steroid and reducing sugar were present in methanolic extract. However, when [16] conducted his research using similar plants, he found that *A. digitata* harbors large amount of constituents such as squaline, phytol, palmitic acid and oleic acid using gas chromatography mass spectrometer (GC-MS) and the distribution of these constituent vary depending on part of the plant taken. For instance, high concentration of plant metabolite is found in leaves, followed by root then stem bark of the plant [16]. In addition, according to the research conducted by [17] on assessing the potential impact of different part of *A. digitata*, the results indicated that leave contain the largest portion of the constituent than stem bark of the plant. This is one of the justifications why the leaves of the plant are chosen for the purpose of this research.

Several researches reported that secondary metabolite found in *A. digitata* have a lot of uses. For instance, alkaloids are reported to control the development in living system and destroy the cell division. Terpenoids function as an anti-fungal and also confer prevention and ensure therapy of cancer. Flavonoids are a water-soluble antioxidant that have anti-cancer role and further prevent oxidative cells from damage [17].

Sensitivity of the confirmed clinical isolates to *Adansonia digitata* stem bark extract using disc diffusion method was determined by measurement of zones of inhibition formed around the disc impregnated with difference concentrations of the extracts (**Tables 3** and **4**). Absence of turbidity in the tube culture containing the extract, Mueller – Hinton broth and clinical isolates indicated the activity of the extract using micro – broth dilution technique. Tubes containing less concentration of the extracts without evidence of turbidity (growth) were considered as minimum inhibitory concentration.

The result of sensitivity test using methanolic and chloroform percolation extracts indicated that chloroform percolation extract was more active on the bacterial isolates than methanolic percolation extract (**Table 4**). Whereas [16], reported that chloroform and aqueous extract of *A. digitata* were less effect on bacterial isolate (*Escherichia coli* and *Salmonella typhi*) as there are no zone of inhibition at 20 and 30 g/mL concentration. In the current study, the findings revealed that chloroform is the most effect against clinical isolates (*Staphylococcus aureus* and *Salmonella typhi*). Increase in the concentration of chloroform percolation extract was found to increase the zone of inhibition from 8±1.2 to 10±1.5 mm in *Staphylococcus* unlike in the case of *S. typhi* where there is not much variation in inhibition reading.

Similar observation was seen in methanolic percolation extract (Table 3). This is as a result of the presence of tannin in addition to flavonoid that were reported to be responsible for antimicrobial properties of medicinal plant. This finding was in agreement with the research of [16] which reported that different part of *A. digitata* contain abundant number of constituents that confer antimicrobial activity against some pathogenic bacteria. Classical examples of such bacteria are *Staphylococcus aureus, Bacillus subtilis, Enterococcus faecalis, Salmonella typhi, Pseudomonas aeruginosa* and *E.coli*.

CONCLUSION

Conclusively, the analysis of stem back of *Adansonia digitata* revealed the presence of flavonoid, tannins, alkanoid, reducing sugar and steroid. These constituents are confirmed to have antimicrobial effect on *Staphylococcus* and *Salmonella typhi* giving it the potential to be used for the treatment of diseases cause by these bacteria. Further research should be carried out on *in vivo* test of the extract on laboratory animals so as to test it efficiency on laboratory animals.

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