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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF STEM BARK OF BAOBAB TREE (*Adansonia digitata*) ON SOME PATHOGENIC BACTERIA

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ABSTRACT

Powdered stem bark of *Adansonia digitata* was extracted with chloroform and methanol using percolation method of extraction. The chloroform and methanolic extracts were screened for the presence of secondary metabolite using a standard technique. The result of the phytochemical screening indicated the presence of alkaloid, Flavanoid, tannin, reducing sugar and steroid in one or both the extracts. The extracts were further tested on confirmed clinical isolates of *Escherichia coli*, *staphylococcus aureus and proteus mirabilis* using disc diffusion method and micro-broth dilution technique. Stem bark extracts of adansonia digitata was found to have antimicrobial activity against the clinical isolates used in the study.

Keywords: Medicinal plants, Phytochemicals, Antimicrobials

Introduction

Many plants are known to have medicinal constituents which impacts medicinal value on the plant which can be derived from whole or part of the plant such as stem, flowers, seeds or roots. Apart from the importance placed by man on some plants as source of food, their greatest use has been in area of medication. The medicinal use of plants and their products date back to antiquity (*Ogunyemi*; 1979). From earliest time, man has use parts of plants to concord healing portions, to eliminate pain, contion suffering and counteract disease (*Iwu*, 1982).

The use of traditional medicine to cure infection has been practiced since the origin of mankind (Sofowora, 1987) and in the past it was the only method of available. Currently due to absence of sufficient health care system particularly in the rural areas, people prefer to visit traditional healers and herbal medicine (Andrew, 1982, Abebe, 1996).

Development of resistance by microorganisms reduces the effectiveness of modern drugs (WHO, 2000). This type of resistance to antibacterial agents is a problem in many areas of the world especially in the developing countries (Shears, 2000, Assefa and Yohannes, 1997). The integrations of traditional and modern medicine are gaining recognition globally (Abebe, 1996, WHO, 2000).

Baobab tree (*Adansonia digitata*) is a deciduous massive, majestic tree up to 25m high which may live for hundreds of years. It has thick angular, wide spreading branches which attains 10-14cm or more in girth and often become deeply eluded. The form of the trunk varies. In young trees, it is conical in mature trees, it may be cylindrical, bottle shaped, or tapering with branching near the base. Villagers often plant baobabs within their own courtyards and nurture them until they are 2-3m tall, before transplanting them along the edges of cultivated fields. It is used as boundary markers to make the dividing lines between plots (*Rocheleau et al.,* 1998).

The fruits pulp probably is the most important food stuff. It is dried and used in cool and hot drinks. Pulp can be dissolved in water or milk and the liquid is used as a drink, as food, as fermentation agent in local brewing or as a substitute for cream or tartar in baking. The energy value of pulp is similar to that of baobab leaves. The leaves of baobab tree are a stable food source for rural population. In many part of Africa, especially the central regions of the continent.They are eaten both fresh and as a dry powder. In the market, the powder is the most common form (*Sidebe et al.,* 1998).The leaves of *Adansonia digitata* are importance source of protein completing the amino acid profile aid in thereby improving the protein quality of the diet. Dried green leaves are used throughout the year; mostly in food served with the stable food dish of millet (*Delisle et al., 1997*). Flowers can be eaten raw or used as flavor in drinks.

The seeds are characterized as a potential source of protein and roasted seeds are used as coffee substitute in some areas (Dirar, 1993). The seeds are mostly used as thickener for soups, but may also be fermented into a seasoning, roasted for direct consumption or pounded to extract vegetable oil.

Pathogenic bacteria are bacteria that cause diseases in humans, in other animals and also in plant. Some can only make one particular host ill. Other causes sickness in a number of hosts. Depending on the host specificity of the bacteria, the diseases causes by bacteria are diverse. Some cause disease in different parts of the body. For instance Staphylococcus aureus caused urinary tract infection and as well as skin infection. Pathogenic bacteria have some structures that aid them to evade and attached itself to the host cell thereby causing disease to the host. Some bacteria have flagella and somatic antigens while some have k antigen which prevent phagocyte from engulfing them.

Adansonia digitata belongs to the malvaceae family. It is wide spread throughout the hot drier regions of tropical Africa (*FAO,* 1988). It is mostly found in the very north of southern Africa. In eastern Africa, the trees grow also in scrubland and on the coast, in the baobab grow in woodlands, and in the coastal regions in addition to savannah.

The tree bears large heavy, white flowers. The showy flowers are pendulous with a very large number of stamens. The carrion scene and researchers have shown that they appear to be pollinated by fruit bats of the subfamily pteropodinae' The fruits are filled with pulp that dries, harden and fall to pieces which look like a chunks of powder, dry bread (*FAO,* 1998).

The baobab is a traditional food plant in Africa but little known elsewhere. The vegetables is been suggested to have the potential to improve nutritional boost food security, faster rural development and support sustainable land care. The different parts of the tree are used as herbal medicine in Africa and other part of the world and it leaves and fruits have high nutritional value.

Baobab (*A. digitata*), a tree plant belonging to the Malvaceae family, is widespread throughout the hot, drier regions of tropical Africa (*FAO*, 1988). It is a deciduous, massive and majestic tree up to 25 m high, which may live for hundreds of years (*Gebauer et al.*, 2002). The trunk is swollen and stout, up to 10 m in diameter, usually tapering or cylindrical and abruptly bottle-shaped; often buttressed. Branches are distributed irregularly and large. The bark is smooth, reddish brown to grey, soft and fibrous (*Gebauer et al.*, 2002). The tree produces an extensive lateral root system and the roots end in tuber.

Leaves are alternate and foliate. Leaves of young tree are often simple. Overall mature leaf size may reach a diameter of 20 cm. Flowers are pendulous, solitary or Paired in leaf axils, large and showy. Flower bud is globose, sometimes ovoid (*Sidibe and William.*, 2002).

The fruit of the baobab tree hangs singly on long stalks with an ovoid, woody and indehiscent shell 20 to 30cm long and up to 10 cm in diameter (*Nnam and Obiakor, 2003*). The shell contains numerous hard, brownish seeds, round or ovoid, up to 15 mm long, which are embedded in a yellowish-white, floury acidic pulp (*Nnam and Obiakor*, 2003). The ripe fruit pulp appears as naturally dehydrated, powdery, whitish colored and with a slightly acidulous taste (*Vertuani et al.*, 2002). Baobab leaves, bark, roots, pulp and seeds are used for multy- medicinal purposes. In many parts of Africa baobab tree were found to have interesting medicinal properties including antioxidant, periodic-like activity, anti-diarrhea ,anti-dysentery activity and excipient. In folk medicine baobab pulp is used in the treatment of fever, diarrhea, malaria, hemoptysis and ascorbic acid complaints (vitamin C deficiency) and dysentery. Pulp extracts is applied as eye-drop in cases of measles (FAO, 1988).In many medicinal uses, stem bark is used when prepared, it is made to its soluble and insoluble tannin, gummy, and aluminous constituents. Beta-sitesteroll has been studied and this occurs in the bark and also in the seed oil.

The widest use in tradition medicine comes from the baobab bark as a substitute for quinine in case of fever or as a prophylactic. A decoction of the bark deteriorates rapidly due to the mucilaginous substances present (Sidibe and Williams, 2002). Baobab bark is used in Europe as a febrifuge (antipyretic). In the Gold Coast (Ghana), the bark is used instead of quinine for curing fever (Shukla et al., 2001). In Indian medicine, baobab bark is used internally as a refrigerant, antipyretic and antiperiodic (Sidibe and Williams, 2002). The bark, however, is certainly used for the treatment of fever in Nigeria (Wickens, 1979). Moreover, the bark contains a white, semi-fluid gum that can be obtained from bark wounds and is used for cleansing sores (Wickens, 1979).According to

the same authors, there are no alkaloids present in the bark. In Congo Brazzaville, a bark decoction is used to bath rickety children.

Sufferers of malaria in Africa, India, Sri Lanka and west Indies are said to consume a mash containing dried baobab bark as a febrifuge in order to treat the fever associated with this illness(Wickens and Lowe,2008: Brandy, 2011).Fruit. Pulp and seeds are widely used for anti-pyretic properties (Ramadan et al, 1993: Wickens and Lowe, 2008).

The antimicrobial activity of the stem bark of the baobab tree is as a result of some metabolites present in it which are tannin, Flovanoid, alkaloid, and steroids. The activity exhibited by extracts may be related to the presence of tannins in addition to Flovanoid that reported to be responsible for antimicrobial properties of some ethno-medicinal plants(Singh and Bhat,2003).these metabolites have been reported to posses antimicrobial activity. In particular the Flovanoid were reported to be responsible for antimicrobial activity associated with some ethno medicinal plant (Cowan, 1999).

Methodology

Clinical isolates of *Staphylococcus aureus*, *Escherichia* coli and *Proteus mirabilis* were collected from Gombe specialist hospital and appropriates confirmatory biochemical test were carried out on the bacteria isolates.

Catalase

This test was used to differentiate those bacteria that produce the enzyme catalase, such as staphylococci, from non-catalase producing bacteria such as streptococci. Approximately 2–3 ml of the hydrogen peroxide solution was poured into a test tube. Using a sterile wooden stick or a glass rod several colonies of the test organism was removed and immerse in the hydrogen peroxide solution. Bubbling was observed for positive result.

Citrate Utilization

Slopes of Simon Citrate medium was prepared in bijou bottles as recommended by the manufacturer.

Using a sterile straight wire, the slope was streaked with a saline suspension of the test organism and then the butt stabbed. Bright Blue coloration indicates a positive result.

Coagulase Test

This test was used to identify *S. aureus* which produces the enzyme coagulase. Approximately 0.2 ml of plasma mixed with a loopful of the test organism and incubated at 35^0C. Clotting was observed for a positive test.

Indole Test

This was used for identification of E.coli. the test organism was inoculated in a bijou bottle containing 3 ml of sterile tryptone water. Incubated at 35–37 _C for up to 48 h. Indole was tested by adding 0.5 ml of Kovac's reagent. Red colouration in the surface layer was observed within 10 minutes for a positive result.

Collection and identification of plant materials

Stem bark of *Adansonia digitata* was scraped using a sterile knife at Pantami Quarter's of

Gombe state. The tree was identified using guide (Aliyu, 2006). The scrapings were air-dried and ground into fine powder using mortar and pestle in as described by *Muktar and Tukur* (1999).

Extraction

30g of the powdered plant materials was dispensed in 300ml of methanol in a conical flask and another batch of the 30g of the powdered plant materials was dispensed in 300ml of chloroform and kept for two days with shaking at regular intervals after which the content were filtered and the filtrates were evaporated at 40^0C.These were labeled as chloroform percolation extracts (CPE) and methanol percolation extract (MPE). All extracts were allowed to evaporate at room temperature (*Fatope et al.,* 1993).

Phytochemical screening

Tests for the presence of alkanoid, Flavonoid, steroids, Tannins and Reducing sugars was done based on the methods described by (*Cuilci*, 1994), (Sofowora, 1975), (*Sofowora*, 1978) and (*Brain and Turner*, 1975).

Bioassay

Sensitivity discs of about 6mm in diameter were pouched from Whatman's no. 1 filter paper using a file punch and put in Bijou bottle. The sensitivity discs were then sterilized in autoclave at 121^0C for 15minutes and were allowed to cool. Sensitivity discs were prepared by weighing the appropriate amount of the extract or fraction and serial doubling dilution in Dimethyl-sulphoxide(DMSO) followed by placing the improvised paper discs in the solution such that each disc absorb 0.01ml to make the potency of 150ug, 30µg and 6oµg (*Akinyemi et al*., 2005; *Valekubia et al,* 2001). A loopful of test isolates were picked using sterile wire loop and emulsified in 3.4ml of sterile physiological saline. The turbidity of the suspension was then matched with that of 0.5 Mc farlands standard (*Chessbrough,* 2006). Using sterile swab stick, standardized inocular of each isolate was swabbed onto the surface of Mueller Hinton agar in separate Petri dishes. Disc of the extracts and standard tetracycline (TET 30uµg) were placed onto the surface of the inoculated media. The plates were inverted and allowed to stand for 30 minutes for extract to diffuse into the agar after which the plates were incubated aerobically at 35^oC for 18hours then followed by measurement of zone of inhibition formed around each of the extract and standard antibiotic discs (*NCCLS,* 1999).

Minimum inhibitory concentration

Minimum inhibitory concentration (MIC) of the extract were prepared by serial doubling using distilled water to obtain concentration of 3000µg/ml, 2000µg/ml, 1000ug/ml and 500µg/ml. Equal volume of extract and nutrient broth were mixed specifically 0.1ml of standardized inocular was added to each of the test tubes above. The test tubes were incubated aerobically at 35^0C for 24hours. Tubes containing broth and leaf extracts without inocular served as positive control while tubes containing broth and inocular served as negative control. The tubes were observed after 24 hours

of inoculation to determine minimum inhibitory concentration that is the lowest concentration that showed no evidence of growth (*Akinyemi et al.,* 2005; *Vallekobia et al*; 2001).

Minimum bacterial concentration (MBC)

Sterile Mueller-Hinton agar plates were separately inoculated with sample from each of the test tubes that shows no evidence of growth. The plates were further incubated at 35c for 24 hours and the highest dilution that yielded no bacterial growth was regarded as MBC (Akinyemi et al; al2005 Vallekobia et al; 2001).

Results

The result for phytochemical screening and antimicrobial activity of stem bark of *Adansonia digitata* tested on some clinical isolates were presented in the tables below.

Table 1: Physical properties of *Adansonia digitata* extracts

Physical parameters	CPE	MPE
Weight (g)	30	30
Weight of extract (g)	1.24	2.56
Percentage yield (%)	4.1	8.5
Color	Dark brown	Reddish brown
Texture	Gummy	Gummy

Keys: **CPE**- Chloroform percolation extract, **MPE**- Methanol percolation extract

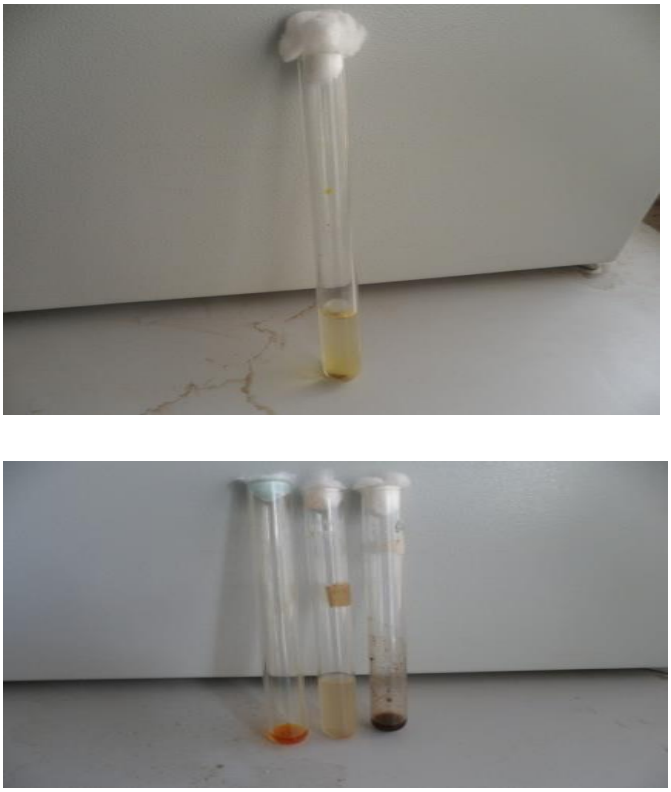

Figure 1: Phytochemical screening showing positive result for alkaloid, Flavanoid, steroid and tannin

Fig 2: Zones of inhibitions produced by the chloroformextract of *Adansonia digitata*

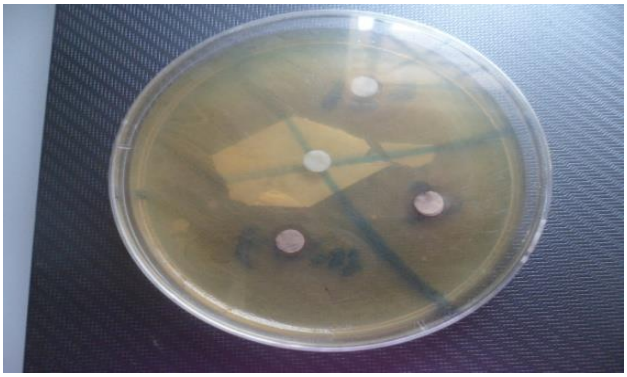

Figure 3: Zones of inhibitions produced by the different concentrations of methanolic extract of *Adansonia digitata*

Table 2: Result of Phytochemical screening of *Adansonia digitata* extracts

Extracts	Result of phytochemical screening				
	Alkaloid	Flavanoid	Reducing sugar	Steroid	Tannin
CPE	+	+	-	-	+
MPE	+	-	+	+	-

Keys: +: denotes presence of the metabolite, -: denotes absence of the metabolite

Table 3: Zones of inhibition (mm) to chloroform and methanolic extracts of *Adansonia digitata*

Isolates	CPE			MPE			TET(30ug)	
	15	30	60	15	30	60	CPE	MPE
Staphylococcus aureus	8	10	9	10	10	0	11	7
Escherichia coli	11	10	10	0	0	9	10	8
Proteus mirabilis	11	11	10	0	7	8	10	11

Table 4: Sensitivity of clinical isolates to chloroform and methanol percolation extracts of *Adansonia digitata* stem

	CPE		MPE	
	MBC	MIC	MBC	MIC
Staphylococcus aureus	-	-	+	-
Escherichia Escherichia	-	-	-	-
Proteus mirabilis	-	-	-	-

using micro-broth dilution technique.

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

Discussion

Methanol was found to be the solvent with high yield of the plant extract (8.5%) compared to choloroform (4.1%) after the extraction. In addition, the extracts also differ in terms of coloration (Dark brown versus red brown) but similar texture (Table 1).

The results of phytochemical screening of chloroform and methanolic extracts of *Adansonia digitata* stem bark using percolation method of extraction revealed the presence of alkaloid and tannin in all the extracts irrespective of the solvent used for the extraction. Flovanoid was present in chloroform percolation extract while steroid and reducing sugar were present in methanolic extract (Table 2).

The result of sensitivity test using methanolic and chloroform percolation extracts indicated that chloroform percolation extract exhibited more antimicrobial effect on the bacterial isolates than methanolic percolation extract (Table 3, Fig.2 and 3) which may be due the presence of tannin in addition to Flavanoid that were reported to be responsible for antimicrobial properties of ethno-medicinal plant which correspond with the work of *Singh and Bhat*, 2003 and M. Yusha'u et al., 2010. Minimum bacteriocidal effect was recorded only against *S.aureus* using methenolic extractsa as shown in Table 4.

Figure 4: Result of Minimum inhibitory concentration using Chloroform extract of Adansonia digitata

Figure 5: Result of Minimum inhibitory concentration using methanolic extract of Adansonia digitata

Conclusion

This study found an antibacterial activity by stem bark extracts of *Adansonia digitata* against some bacterial isolates which depends upon the type of solvents used for extraction.

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