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Antibacterial activity of *Adansonia digitata* stem bark extracts on some clinical bacterial isolates

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ABSTRACT: Powdered stem bark of *Adansonia digitata (L.)* was extracted with ethanol and chloroform using percolation method and Sohxlet extractor. The extracts were tested for antimicrobial activity against clinical bacterial isolates of *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis* and *Staphylococcus specie* using disc diffusion and microbroth dilution technique. The extracts were further screened for the presence of secondary metabolites using standard techniques. Results of sensitivity test using both procedures showed that ethanol extracts of the plant were more active than chloroform extract on the isolates tested irrespective of the method of extraction employed. The results of phytochemical screening indicated the presence of alkaloids, flavonoids, reducing sugars, steroids and tannins in either or both extracts. This indicates that *Adansonia digitata* root bark has the potential for the production of drugs against some clinical bacterial isolates.

Keywords: *Antibacterial activity, Adansonia digitata, Extracts, Clinical isolates*

Introduction

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 and a short, stout trunk which attains 10 – 14m or more in girth and often becomes deeply fluted. 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 a boundary marker to make the dividing line between plots (Rocheleau *et al*., 1988).

The fruit pulp is probably the most important food stuff. It is dry and mealy and it is used in cool and hot drinks. Pulp can be dissolved in water or milk and the liquid is used as a drink, as a sauce for food, as a fermenting agent in local brewing or as a substitute for cream of tartar in baking. The energy value of pulp is similar to that of baobab leaves (Becker, 1983).

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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" (http://en.wikipedia.org/wiki/baobab). During the rainy season the baobab leaves are tender and people harvest the leaves fresh. During the last month of the rainy season, leaves are harvested in great abundance and are dried for domestic use and for marketing during the dry season. 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 (Sidebe *et al*., 1998b). The leaves of *Adansonia digitata* are important protein sources in complementing the amino acid profile and thereby improving the protein quality of the diet (Nordeide *et al*., 1996). Young leaves are commonly used as a vegetable in soups or cooked and eaten as spinach (Venter and Venter, 1996). Dried green leaves are used throughout the year, mostly in soups served with the staple dish of millet (Delisle *et al.,* 1997). Flowers can be eaten raw or used as flavour 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 a thickener for soups, but may be also be fermented into a seasoning, roasted for direct consumption, or pounded to extract vegetable oil. (http://en.wikipedia.org/wiki/baobab).

The bark produces strong fibres 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 320 kgm^{-3} and is used mainly for fuel (Venter and Venter, 1996). Hollow trees provide reservoirs of fresh water which are used by nomads, particularly in the western part of Sudan (Tothill, 1954). Water storage capacities range from 1000 to 9000 litres per tree (Craig, 1991).
The use of trees as cattle feed is extremely important in the savanna areas especially in the arid zones, where animals obtain much of their feed in the form of pods and leaves. The pulp and seeds have a high nutritional value and are recommended for feeding the herd late in the dry season when grazing is poor (Venter and Venter, 1996).

In the folk medicine baobab pulp is used in the treatment of fevers, diarrhoea, malaria, haemoptysis and scorbutic complaints (vitamin C deficiency) and dysentery. Pulp extract is applied as eye-drops in cases of measles (FAO, 1988). In many medicinal uses, stem bark is used. When prepared it is made into a decoction for internal use and functions due to its soluble and insoluble tannin, and gummy and albuminious constituents beta-sitosterol has been studied and this occurs in the bark and also the seed oil (Asolkar *et al*., 1992). Root bark is a also used in traditional medicine. This contains Beta-sitosterol and two glycosides (Ramesh *et al*., 1992). The leaves form a component or herbal remedies and a mash prepared from the dried powdered roots is given to malarial patients as a tonic. A semi-fluid gum obtained from baobab bark is used to treat sores (FAO, 1988).

Materials and Methods

(a) Collection and Identification of plant materials

Stem bark of *Adansonia digitata* was scraped using sterile knife at Kirikasamma Local Government Area of Jigawa state. The tree was identified using guide (Aliyu, 2006). The scrapings were air-dried and ground into fine powder using mortar and pestle in the laboratory as described by Mukhtar and Tukur (1999).

(b) Extraction

Fifteen grams of the powdered plant material was dispensed in 150ml of ethanol and chloroform in separate conical flasks, kept for two weeks with shaking at regular intervals after which the content was filtered and the filtrate was evaporated at 30^{o}C. They were labeled EPE and CPE for ethanol and chloroform percolation extracts respectively. Two batches of fifteen grams were extracted with the same solvents using soxhlet extractor for one hour and labeled ESE and CSE for ethanol and chloroform soxhlet extracts respectively. All the extracts were allowed to evaporate at room temperature (Fatope *et al.*, 1993).

(c) Phytochemical screening

(i) Test for alkaloids

To 0.1ml of the extract and fractions in a test tube, 2 – 3 drops of Dragendoff's reagent was added. Orange red precipitate with turbidity denoted the presence of alkaloids (Ciulci, 1994).

(ii) Test for flavonoids

To 4mg/ml of the extracts and fractions a piece of magnesium ribbon was added followed by drop-wise addition of concentrated HCl. A colour change from orange to red indicated the presence of flavones; red to crimson indicated the presence of flavonoids (Sofowora, 1993).

(iii) Test for reducing sugars

To 1ml of extract and fractions in separate test tubes, 2.0mls of distilled water were added followed by addition of Fehling's solution (A + B) and the mixtures were warmed at 40^{o}C. Appearance of brick red precipitate at the bottom of the test tube indicated the presence of reducing sugar (Brain and Turner, 1975).

(iv) Test for steroids

Two milliliters of the extracts were evaporated to dryness in separate test tubes and the residues dissolved in acetic anhydride followed by addition of chloroform. Concentrated sulphuric acid was added by means of a pipette via the side of the test tubes. Formation of brown ring at the interface of the two liquids and violet colour in the supernatant layer denoted the presence of steroids (Ciulci, 1994).

(v) Test for tannins

Two milliliters of the extract/fraction was diluted with distilled water in separate test tubes, 2 – 3 drop of 5% ferric chloride ($FeCl_3$) solution was added. A green – black or blue colouration indicated tannin (Ciulci, 1994).

(d) Bioassay studies

(i) Disc preparation

Sensitivity discs were punched from Whatman No. 1 filter paper, sterilized in Bijou bottles by autoclaving at 121^{o}C for 15mins. Sensitivity discs were prepared by weighing the appropriate amount of the extract or fraction and serial doubling dilution in Dimethyl-sulfoxide (DMSO) followed by placing the improvised paper discs in the solution such that each disc absorbed 0.01ml to make the disc potency of 15µg, 30µg and 60µg (Akinyemi *et al.*, 2005; Vallekobia *et al.*, 2001).

(ii) Test isolates

Identified clinical tract isolates were collected from the microbiology laboratory of Aminu Kano Teaching Hospital (AKTH) and maintained on nutrient agar slants in the refrigerator (4^{o}C) prior to use.

(iii) Inoculum standardization

A loopful of the test isolate was picked using a sterile wire loop and emulsified in 3 – 4mls of sterile physiological saline. The turbidity of the suspension was matched with that of 0.5 McFarland Standard (Cheesbrough, 2004).

(iv) Sensitivity testing

Using sterile swab stick, standardized inocula of each isolate was swabbed onto the surface of Mueller Hinton Agar in separate Petri dishes. Discs of the extracts and standard Tetracycline (TET 30µg) were placed onto the surface of the inoculated media. The plates were inverted and allowed to stand for 30mins for the extract to diffuse into the agar after which the plates were incubated aerobically at 35^0C for 18 hours. This was followed by measurement of zone of inhibition formed by the test organisms around each of the extract and standard antibiotic discs (NCCLS, 1999).

(e) Micro-broth dilution technique

(i) Minimum inhibitory concentration (MIC)

Minimum inhibitory concentrations of the extract and fractions were prepared by serial doubling dilution using distilled water to obtain concentrations of 2000µg/ml, 1000µg/ml and 500µg/ml. Equal volume (2mls) of extract and Mueller – Hinton broth were mixed. Specifically 0.1ml of standardized inocula (3.3 x 10^6 CFU/ml) was added to each of the test tubes above. The tubes were incubated aerobically at 35^0C for 24 hours. Tubes containing broth and leaf 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 to determine minimum inhibitory concentration. That is the lowest concentration that showed no evidence of growth (Akinyemi *et al.*, 2005; Vallekobia *et al.*, 2001).

(ii) Minimum Bactericidal Concentration (MBC)

Sterile Mueller-Hinton agar plates were separately inoculated with sample from each of the test tubes that showed no evidence of growth. The plates were further incubated at 35^oC for 24 hours and observed. The highest dilution that yielded no bacterial growth was regarded as MBC (Akinyemi *et al.*, 2005; Vallekobia *et al.*, 2001).

Results and Discussion

High yield of extract was obtained at the end of Sohxlet extraction using chloroform, with extract having yellow colour and gummy texture. The high yield of this extract may be due to the high solubility of the compounds present in low polar solvent coupled with the effect of temperature provided by the Sohxlet extraction system. There exist differences in the colour of the extracts based on the extraction solvent irrespective of the method with ethanol extracts being reddish brown and chloroform extracts being yellow but except for ethanol percolation extract that has powdery texture, all the extracts had gummy texture.

Sensitivity of the test isolates to *A. digitata* root bark extracts using disc diffusion method was indicated by observation and measurement of inhibition zones formed around discs prepared from various concentrations of the extracts. Absence of turbidity in tube cultures indicates the activity of the extract using micro-broth dilution technique, the least concentration amongst the tubes without evidence of turbidity was considered the minimum inhibitory concentration (MIC). Microbroth dilution technique employed in this research is important in determining whether the extract and fractions are capable of inhibiting the growth or completely killing the test isolates.

The results of sensitivity tests using both procedures indicated that ethanol extracts of the plant were more active than chloroform extract on the isolates tested irrespective of the method of extraction employed. The activity exhibited by the extracts may be related to the presence of tannins in addition to flavonoids that are reported to be responsible for antimicrobial properties of some ethno-medicinal plants (Singh and Bhat, 2003).

The results of phytochemical screening of ethanol and chloroform extracts of *A. digitata* using percolation and Sohxlet extraction method revealed the presence of flavonoids and Steroids in all extracts irrespective of either the solvent used or extraction method employed. Alkaloids were present in Sohxlet extracts while tannins were present in percolation extracts hence their presence is dependent upon method

of extraction employed. Reducing sugars is present in all extract with the exception of ethanol sohxlet extracts (Table 2). 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 ethnomedicinal plants (Singh and Bhat, 2003).

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

Physical parameters	EPE	ESE	CPE	CSE
Weight extracted	15	15	15	15
Weight of extract	1.3	1.2	1.3	2.0
Percentage yield	8.67	8.67	8.67	13.33
Colour	Reddish brown	Reddish brown	Yellow	Yellow
Texture	Powdery	Gummy	Gummy	Gummy

Key: EPE – Ethanol Percolation Extracts, **ESE** – Ethanol Soxhlet Extract, **CPE –** Chloroform Percolation Extract, **CSE** - Chloroform Soxhlet Extract

Table 2: Phytochemical properties of *Adansonia digitata* extracts.

	Phytochemical Tests				
Extracts	**Alkaloids**	**Flavonoids**	**Reducing sugars**	**Steroids**	**Tannins**
EP	**-**	**+**	**+**	**+**	**+**
ES	**+**	**+**	**-**	**+**	**-**
CP	**-**	**+**	**+**	**+**	**+**
CS	**+**	**+**	**+**	**+**	**-**

Key: EP – Ethanol Percolation, **ES** – Ethanol Soxhlet, **CP –** Chloroform Percolation,
CS - Chloroform Soxhlet, **+ -** Present, **- -** Absent

Table 3: Sensitivity of clinical isolates (mm) to *Adansonia digitata* extracts using Disc Diffusion Method.

	EP			ES			CP			CS			TET(S)
Isolates	**15**	**30**	**60**	**15**	**30**	**60**	**15**	**30**	**60**	**15**	**30**	**60**	**30**
E. coli	8	9	9	6	6	6	6	6	6	6	6	6	6
Klebsiella pneumoniae	6	8	8	6	6	6	6	6	6	6	6	6	6
Proteus mirabilis	6	6	6	6	6	6	6	6	6	6	6	10	9
Staphylococcus specie	6	6	6	6	6	9	6	6	6	6	6	6	(33)

Key: EP – Ethanol Percolation, **ES** – Ethanol Soxhlet, **CP – C**hloroform Percolation,
CS - Chloroform Soxhlet, **TET** – Tetracycline, **(S)** - Streptomycin
TET - Tetracycline**, S –** Streptomycin

Table 4: Sensitivity of clinical isolates to *Adansonia digitata* extracts using Micro-broth Dilution Technique.

Isolates	EPE (µg/ml) MIC	MBC	ESE (µg /ml) MIC	MBC
Escherichia coli	1000	**	2000	**
Klebsiella pneumoniae	**	**	**	**
Proteus mirabilis	**	**	**	**
Staphylococcus specie	**	**	**	**

Key: EPE – Ethanol Percolation Extracts, **ESE** – Ethanol Soxhlet Extract,
CPE – Chloroform Percolation Extract, **CSE** - Chloroform Soxhlet Extract

Conclusion

From the results of this work, it can be concluded that *Adansonia digitata* has the potential for the production of drug for the treatment of bacterial infections.

References

Asolkar L. V., Kakkar, K. K. and Chakre, O. S. (1992): Second Supplement to Glossary of Indian Medicinal Plants with Active Principles Parts I, CSIR, New Delhi. Pp65-83.

Basile, A., Giordano, S., Lopez-saez, J. A. and Cobianchi, R. C. (1999): Antibacterial activity of pure flavonoids isolated from mosses. *Phytochemistry*. **52**:1479-1482**.**

Becker, B. (1983); *The contribution of wild plants to human nutrition in the Ferlo* (*Northern Senegal);* Agro for System, 1: 252 – 267.

Brain, K. R. and Turner, T. D. (1975): The practical evaluation of phytochemicals. Wright Scientechina, Bristol: 57 – 58.

Ciulci, I. (1994): Methodology for the analysis of vegetable drugs. Chemical industries branch, Division of industrial operations. UNIDO, Romania: 24, 26 and 67.

Craig, G. M. (1991): The Agriculture of the Sudan*.* Oxford University Press, USA. Pp. 32-58.

Delisle, H., Bakari, S., Gevry, G., Picard, C. and Ferland, G. (1997): Provitamin A. content of traditional green leaves from Niger. *Cahiers Agricultures* **6:** 553 – 560.

Dirar, H. A. (1993): The indigenous Fermented Foods of the Sudan. *A Study in African Food and Nutrition* ; Cab International, UK. Pp. 85-97.

FAO, (1988): Traditional Food Plants. *FAO food and nutrition paper* **42:** 63 – 67.

Fatope, M. O. and Hamisu I. (1993): Screening of higher plants reputed as pesticides using Brine Shrimp lethality assay. *Int. J. of Pharmacognosy: Vol.* **31***:250 – 60.*

http://en.wikipedia.org/wiki/baobab

Igboeldi, L. C., Addy, E.O.H. and Salami, L. I. (1997): Effects of some processing techniques on the antinutrient contents of baobab seeds (Adansonia digitata). *Bioresource Technology* **59 (1):** 29 – 31.

Lin, R., Chin, Y. and Lee, M. (2005): Antimicrobial activity of Antibiotics in combination with Natural flavonoids against clinical extended spectrum betalactamase-producing *Klebsiella pneumoniae. Phytotherapy Research* **19:** 612-617.

Mukhtar, M. D. and Tukur, A. (1999): *In-vitro* screening for activity of Pistia stratiotes extracts. NISEB Journal **1(1)**:51 – 60.

National Committee for Clinical Laboratory Standards (1999): Performance standard for antimicrobial susceptibility testing. NCCL approved standard M100-59.

National Committee for Clinical Laboratory Standards (2000): Methods for dilution Anti-microbial assays for bacteria that grow aerobically. Approved standard M7- A5.

Nordeide, M. B., Hatloy, A., Folling, M., Lied, E. and Oshaug, A. (1996): Nutrient composition and nutritional importance of green leaves and wild food resources in an agricultural district, koutiala, Southern Mali. *International Journal of Food Science and Nutrition* **47:** 455 -468.

Ramesh, D., Dennis, T. J. and Shingare, M. S. (1992): Constituents of Adansonia digitata root bark. *Fitoterapia* **63:** 278 – 279.

Rocheleau, D., Weber, W. and Field Juma A. (1988): Agroforestry in Dryland. English Press, UK. Pp 88-98.

Sidibe, M., Scheuring, J. F., Tembley, D., Sidibe, M. M., Hofman, P. and Frigg, M. (1996): Baobab – homegrown vitamin C for Africa. *Agroforestry Today*. **8(2)**: 13 – 15.

Singh B. and Bhat T. K. (2003): Potential therapeutic applications of some antinutritional plant secondary metabolites. *Journ. Agric. Food Chem*. **51**:5579- 5597.

Sofowora, A. (1993): *Medicinal plants and Traditional Medicines in Africa*. Chichester John Wiley & Sons, New York Pp. 34-36.

Tothill, J. D. (1954): Agriculture of the Sudan. Oxford University Press, USA. Pp. 88-93.

Tschehe, R. (1971): Advances in the chemistry of antibiotic substances from higher plants: Pharmacognosy and phytochemistry. *In proceeding of 1st International Congress, Munich, 1970*: 274-289.

Vallekobia, A., Kostalova, D. and Sochorova, R. (2001): Isoquinolone alkaloid from *Ahonia aquifolium* stem bark is active against *Malassezia species*. *Folia Microbiology* **46**: 107-111.

Venter, F. and Venter, J. A. (1996): Making the most of Indigenous Trees: Briza Publications, Singapore. Pp. 64-72.

Yazzie, D., Vanderjagt, D. J., Pastuszyn, A., Okolo, A. and Glew, R. H. (1994): The amino acid an mineral content of baobab (Adasonia digitata L.) leaves. *Journal of Food Composition and Analysis* **7:** 189 – 193.

Yusha'u, M. and Dabo, N. T. (2004): Sensitivity of *A. fumigatus* to ketoconazole and leaf extracts of *A. godseffiana*. *BEST JOURNAL* **1**(2): 103-105.