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BIOACTIVE EVALUATION AND PHYTOCHEMICAL SCREENING OF THE ROOTS OF *Aristolochia albida* AGAINST SOME BACTERIAL PATHOGENS

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ABSTRACT
Biological evaluation and phytochemical investigation of the efficacy of the ethanol extract of the roots of *Aristolochia albida*, locally used for the treatment of diarrhea and in the form of decoction for the treatment of malaria fever in kaltungo LGA of Gombe State and Gwoza LGA of Borno State, both in North Eastern Nigeria. The pyhtochemical screening confirms the presence of bioactive secondary metabolites: Terpenoids, tannins, glycosides, resins, phlobatannins, alkaloids, flavonoids and saponnins, while sterols and phenols were absent. Antimicrobial activity of the extracts indicated significant activity on *Escherichia coli, Shigella dysentry, Pseudomonas aeruginosa, Staphylococcus aureus* and inactivity in *Salmonella typhii* and *Streptococcus pyogene*. This correlates ethnomedical claims and thus provides for the first time, the pharmacological basis for the folkloric usage of these roots as diarrhea and malaria cure in both States.

KEYWORDS: phytochemical, Estracts, *Aristolochia albida*, *Shigella dysentery,* Bacterial pathogens, antimicrobial investigation

INTRODUCTION

Thomas Jefferson wrote that "The greatest service which can be rendered any country is to add a useful plant to its culture." Plants have forever been a catalyst for our healing. In order to halt the trend of increased emerging and resistant infectious diseases, it will require a multi-pronged approach that includes the development of new drugs. Using plants as the inspiration for new drugs provides an infusion of novel compounds or substances for healing disease (Iwu *et al*., 1999). Evaluating plants from the traditional African system for medicine, provides us with clues as to how these plants can be used in the treatment of diseases and finding healing in them is an old idea. Plant based drugs are gaining popularity because of several advantages such as fewer side effects, better tolerance, relatively less expensive and acceptance due to a long history of use(Iniaghe *et al*., 2009).

Infectious diseases account for approximately one-half of all deaths in tropical countries. In industrialized nations, despite the progress made in the understanding of microbiology and their control, incidents of epidemics due to drug resistant microorganisms and the emergence of hitherto unknown disease-causing microbes, pose enormous public health concerns. Historically, plants have provided a good source of anti-infective agents; emetine, quinine, and berberine remain highly effective instruments in the fight against microbial infections. Phytomedicines derived from plants have shown great promise in the treatment of intractable infectious diseases including opportunistic HIV infections (Turano *et al*., 1989). Plants containing alkaloids, picralima-type indole alkaloids and garcinia biflavonones used in traditional African system of medicine, have been found to be active against a wide variety of micro-organisms (Deeni and Hussain, 1991).

Regarding history, they have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well-being. Their role is two fold in the development of new drugs: (1) they may become the base for the development of a medicine, a natural blueprint for the development of new drugs, or; (2) a phytomedicine to be used for the treatment of diseases. There are numerous illustrations of plant derived drugs such as: isoquinoline alkaloid, emetine, obtained from the underground part of *Cephaelis ipecacuanha*, and related species, has been used for many years as and amoebicidal drug as well as for the treatment of abscesses due to the spread of *Escherichia histolytica* infections. Quinine from the Cinchoma tree for the treatment of malaria fever is also of long

history, it can also be used to relieve nocturnal leg cramps. Currently, the widely prescribed drugs are analogs of quinine such as chloroquine, maldox, lonart, amalar plus etc. Some strains of malarial parasites have become resistant to the quinines, therefore antimalarial drugs with novel mode of action are required.eg most recently, three new atropisomeric naphthylisoquinoline alkaloid dimers, michellamines A, B, and C were isolated from a newly described species tropical liana, *Ancistrocladus korupensis* from the rainforest of Cameroon. The three compounds showed potential anti-HIV with michellamine B being the most potent and abundant member of the series. These compounds were capable of complete inhibition of the cytopathic effects of HIV-1 and HIV-2 on human lymphoblastoid target cell in vitro (Boyd *et al*., 1994).

The histochemical and phytochemical study of plants confirms the presence of various secondary metabolites in their tissues leading to increasing evidence for their being used as drugs. In recent years; there has been a gradual revival of interest in the use of medicinal plants in developing countries. It is an established fact that 80% of the world`s population depends primarily on traditional medicine for the treatments of their ailments (Cunningham, 1993). Plants remain the source of most natural and synthetic drugs (Clark, 1996).

The use of plant extracts or chemicals derived from plants to treat diseases has stood the test of time (Chowdhury *et al*., 2002, Khan *et al*., 2006). Plant based antimicrobials represent a vast untapped source for medicines. Continued and further exploration of plant antimicrobials needs to occur. Plants based antimicrobials have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. They are effective, yet gentle. Many plants have tropisms to specific organs or systems in the body. Phytomedicines usually have multiple effects on the body. Their actions often act beyond the symptomatic treatment of disease. An example of this is *Hydrastis canadensis*. *Hydrastis* not only has antimicrobial activity, but also increases blood supply to the spleen promoting optimal activity of the spleen to release mediating compounds (Murray, 1995).

It is therefore, necessary for natural product and organic chemists to find and analyze the phytochemical components of plants origin because it is likely that these phytochemicals will find their ways in to composition of antimicrobial drugs and eventually the human system.

Reports of antibiotic resistance of human pathogens, to the present synthetic /modern drugs is of concern world wide (Mitsuyama *et al*., 1987; Gutmann *et al*., 1988; Mathias *et al*., 2000; Ganguly *et al*., 2001; Martino *et al*., 2002). Biomolecules of plant origin appear to serve as alternatives for the control of these human pathogens, more so, with a scientific back-up.

Aristolochia albida is a Herb/shrub, rarely lianas, sub shrubs root, stems and leaves with oil cells . Leaves alternate; stipules absent; petiole present and well defined; leaf blade simple, usually pinnately. Inflorescences terminal or axillary, racemes , cymes, corymbs or flowers solitary. Flowers bisexual veined, sometimes palmately 3-5-veined, margin usually entire, rarely 3-5-lobed zygomorphic or actinomorphic. Perianth usually with 1 petaloid whorl (in *Saruma* with 2 whorls: outer one sepaloid , inner one petaloid), mostly connate into distinct tube , cylindric to campanulate or subglobose; limb rotate, urceolate , cylindric, or ligulate , 1-3-lobed; lobes valvate . Stamens 6-12 (in China), in 1 or 2 series; filaments adnate to ovary (in *Asarum*) or style column (in *Thottea*) with anthers free , or filaments and anthers fully adnate to style column to form gynostemium (in *Aristolochia*) ; anthers 2-loculed, dehiscence longitudinal . Ovary inferior to superior, 6-loculed (in *Thottea* 4-loculed) carpels connate only at base or fully fused; ovules numerous , anatropous , usually in 1 or 2 series; placentation parietal . Styles free or connate, column 3- or 6-lobed (in *Thottea* 5-20-lobed). Fruit a fleshy or dry capsule, rarely siliquiform or follicular. Seeds many; testa somewhat hard or crustaceous; endosperm copious, fleshy; embryo minute (Turrill, 1952; Keay, 1972)

It is used for the benefit of mankind. The flowers are used in Social: religion, superstitions, magic; sayings, and aphorisms, the leaves for the treatment of skin and mucosae diseases and the roots an antidote

(venomous stings, bites etc), medicine for cutaneous and subcutaneous parasitic infections, vermifuges, and general healing and stomach troubles. The crude methanol extract of the root has antifeedant activities and contain a good yield of aristolochic acid at 0.1% concentrations (Labunmi, 1993).

Aristolochia albida [*Aristolochiaceae*] *is a* twining climber of the Sahel zone of the Region. An infusion of the dried leaves, sometimes with dried root added, is used in Nigeria by Hausa and Fulanis as an anthelmintic (Iwu, 1993). The leaf is applied in Nigeria to certain (unspecified) painful skin-diseases, and crushed and mixed with castor-oil and applied topically on pimples (Johnston, 1997). To get rid of guinea-worm, the leaf may be applied, or a poultice composed of powdered root with seeds of *Lepidium sativum* Linn. (Cruciferae), garlic and native natron, and an infusion of the same mixture is drunk (Johnston, 1997). The root is bitter. It is sold in markets in light-coloured pieces 8–10 cm long for taking as a stomachic and tonic for which an infusion is made by pouring water repeatedly on to it through a strainer. The root mixed with lime-juice is given in cases of snake-bite, scorpion-stings, etc., against which the flowers are sometimes worn as a juju or charm (Iwu, 1993).

English name is dutchman's pipe, *HAUSA, dúmán duútseè* = gourd of the rocks (auctt.) fiyaka (JMD; ZOG) gaďahuka, gaďakuka, gaďaukuku from Fulani (JMD) kadacin kasa (ZOG) mádaàcín ƙ̀ásà = medicine of the earth (auctt.)

It's believed that children suffer from severe dysentery/diarrhea during teething. Scientists are still to justify this claim. But it could be inferred that this is a time when the children are exposed to lots of contaminated things as they crawl around and imbibe virtually everything. In Kaltungo and Gwoza, North Eastern Nigeria, roots of *dúmán duútseè* are used (oral decoction), for the treatment of dysentery /diarrhea and malaria both in children and adults (Per comm).

This paper reports the pyhtochemicals in this indigenous medicinal plant, its potentials against microbial activity and also viewing it as a promissory source of chemotherapy against dysentery/diarrhea and malaria, and in the long term, drug formulation.

MATERIALS AND METHODS

Collection of plant materials:

Fresh sample of the roots of *Aritolochia albida* were collected in Gwoza, Borno State and were identified in the Biological Sciences Department of Gombe State University. The FHI number is 044 and a specimen of the plant was deposited in the herbarium. The sample (1.2kg) was air dried in the laboratory before pounding to a fine powder using pestle and mortar to about 70 mesh size and then stored in a dry container.

Extraction

150g of the powdered roots was accurately weighed and percolated with 2L of distilled ethanol for 72hrs. After which there was decantation, filtration, and concentration using rotary evaporator (R110) at 40^0C to obtain ethanol soluble fractions, (F_E01), labeled, $F_{E0}R$. [23.5g]. This was divided into two portions of 12g and 13g respectively and one was used for the phytochemical screening while the other kept in the refrigerator for the biological evaluaton.

Qualitative chemical test

The method described by (Abulude *et al* 2001) and (Abulude 2007) were used to test for the presence of phytochemical in the extract.

Test for tannins.

Two drops of 5% $FeCl_3$ was added to 1ml of the extract. A dirty green precipitate indicated positive test.

Test for glycosides
Ten(10) ml of 50% H_2SO_4 was added to 1ml of extract in a test tube, this mixture was heated in boiling water for 5 minutes. 10ml Fehling's solution A and B (5 ml each) were added and boiled. Brick red precipitate indicated positive test.

Test for resins.
Two and a half (2.5) ml of Copper (II) Sulphate solution was added to 2.5 ml of the extract. The resulting solution was shaken vigorously and allowed to settle. A green colour indicated positive test

Test for saponins (Frothing test)
Two (2) ml of extract was vigorously shaken in test tube for two minutes. Frothing indicated positive test.

Test for phlobatannins.
Five (5) ml of distilled water was added to 5 ml of extract solution and boiled with 1%HCl for two minutes. A deep green colour indicated positive test.

Test flavonoids.
Two (2) ml of the extract solution was heated with 10 ml of ethyl acetate on a water bath and cooled. The layers were allowed to separate and a colour of ammonia layer (red colouration formed) indicated positive test.

Test for sterols (salkowski test)
Two (2) ml of conc. H_2SO_4 was added 2 ml of extract solution. A red precipitate indicated steroidal ring.

Test for Phenols.
Equal volumes of extract solution and $FeCl_3$ were mixed. A deep bluish green solution confirmed the presence of phenols.

Test for carbohydrate. (Fehling test)
Five (5) ml of the mixtures of equal volume Fehling solution A and B were added to 2 ml of the extract in a test tube. The resultant mixture was boiled for two minutes. A brick red precipitate of copper oxide indicated a positive test.

Test for alkaloids.
One (1) ml of conc. H_2SO_4 was added to 3 ml of the extract, then treated with few drops of Wagner reagent. Reddish brown precipitate indicated positive test.

Terpenoid (Solkowski) test
0.2g of the extract sample was mixed with 2ml of chloroform ($CHCl_3$) and conc. H_2SO_4 (3ml) was carefully added to form a layer. A reddish brown colouration of the interface was formed to indicate positive result for the presence of terpenoids.

Test Organisms
Clinical isolates of *Escherichia coli, shigella dysentriae, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogene* and *Salmonella typhii* were obtained from Yola Specialist Hospital, Adamawa State, Nigeria.

Preparation of paper discs
Using an ordinary office two-hole puncher, paper disks with approximate diameter of 6.3mm were punched out from a What-man (Grade No. 1) filter paper; precaution taken to avoid overlapping of holes since the paper disks had a tendency to curl after punching and these were flattened by spreading them in a single layer on a clean smooth surface then pressed by rolling a bottle repeatedly. The disks were placed in vials and autoclaved for 15minutes at 121˚C. Impregnation of disks

Immersion method was used. Disks were soaked in 0.025mg / ml extract solution (extract dissolved in ethanol) and then allowed to dry for 3 hours.

Bioassay (disk diffusion test)
The sterilized petri dishes containing the set nutrient agar were inoculated with one type of microbe using separate swab sticks. The plates were labeled. The paper disks impregnated with the extract were placed on the surface of the solid medium, which was previously inoculated with the bacteria under test. The inoculated plates containing the extract (paper disks) were incubated at $37^{O}C$ for 24 hours. Observation comprising of diameter of paper disk and zone of inhibition was made for proper evaluation. Results are presented below:-

RESULTS AND DISCUSSION
Phytochemical investigation:
The phytochemical analysis of the ethanol extract from the roots of the indigenous plant *Aristolochia albida* are shown in Table 1 and the antimicrobial activities of the fractions against some human and animal pathogens are shown in Table 2.

Table 1: Phytochemical constituents of the ethanol extract of the roots of *Aristolochia albida*:

phytochemical compound(s)	Extract
Tannins	+
Saponins	+
Sterols	-
Terpenoids	+
Glycosides	+
Phlobatannins	-
Resins	+
Flavonoids	-
Phenols	-
Alkaloids	+
Carbohydrates	+

Key + = present - = absent

Table 2: Antimicrobial activity of the ethanol extract of *Aristolochia albida* roots on clinical isolates:

Microorganisms	Extract	Positive control (Ampicillin)	Negative control (Distilled water)
Diameter of zone of inhibition (mm)			
Escherichia coli	8.3	10.0	-
Shigella dysentryae	7.8	8.0	-
Pseudomonas auroginosa	11.1	12.0	-
Staphylococcus aureus	9.0	10.1	
Streptococcus pyogene	N.A	15.0	-
Salmonella typhii	N. A	13.0	-

Key N. A =Non Active
Concentration of the extract used = 0.025mg/ml
Concentration of the ampicillin =0.01mg/ml

Table 1 shows the phytochemical constituents of the extract. It reveals that tannins, saponnins terpenoids, glycosides, resins, alkaloids and Carbohydrates are present. Sterol, phlobatannins, phenols and flavovoids absent, these secondary metabolites function in a synergistic or antagonistic fashion for the treatment of diseases. Normally, the phonological age of the plant, percentage humidity of the harvested material, geographical location, climatic conditions, soil condition, time of harvest and the method of extraction are possible sources of variation for the chemical composition, toxicity and bioactivity of the extracts (Felix 1982). The presence of tannins and alkaloids confirms with literature as these secondary metabolites are used to treat cough, asthma, diarrhea and other hay fevers (Gill 1992, Burkill 1994, Rahila *et al*., 1994). The present investigation clearly reveals the antibacterial nature of this plant and portrays it as a potential source of useful drug thus suggesting that it could be exploited in the management of diseases caused by these bacteria in human and Plant systems (Raghavendra 2006).

Antimicrobial activities of the extract

The biological activity of the plant extract is shown (Table 2) and the measured diameter of zones of inhibition in (mm) of the plant extract against the microorganisms. Zones of inhibition indicate the effect of the extract on the microorganism. The result showed that the ethanol extract of the plant have antimicrobial activity on nearly all the test organisms with the exception of *Streptococcus pyogene and Salmonella typhii.* A clear indication that the plant can treat diarrhea and other diseases. This is inconsonance with the activity of plant secondary metabolites of medicinal plants ((Lin *et al.* 2001). This thus, supports the use of the plant by herbalists and Kaltungo and Gwoza local indigenes for treatment of diarrhea / dysentery and other human ailments. Ampicillin (positive control) is higher in performance, with greater zones of inhibition than the extract probably because the extract contains some impurities that are interfering with its activity. When active compounds are isolated from the extracts, they may give higher activity than ampicillin.

CONCLUSION

This strongly suggests that the indigenous plant, *dúmán duútseè* contain active agent(s) and could be a potential source for drug development. This assertion is confirmed, as their extracts indicate a relatively moderate number of phytochemicals. It is suggested that more work be conducted to further isolate, identify, characterize and elucidate the structure(s) of the bioactive compound(s) and possible mechanism(s) involved so that the use of this plant in ethno medical practice be validated.

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