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ANTIMICROBIAL EFFECT OF LEAF EXTRACTS OF BLOOD TREE *(HARUNGANA MADAGASCARIENSIS* LAM EX. PIOR) ON SOME HUMAN PATHOGENS

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ABSTRACT.

In developing countries like Nigeria, it is important that serious attention be given to most human pathogens. Five pathogens were used for the study of the antimicrobial effect of *Harungana madagascariensis* leaf extract on some human pathogens. *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Kleibsiella pneumonia,* and *Pseudomonas aeruginosa* were the bacteria isolates used. Solvents used for the extraction of active ingredients of the medicinal plant leaf were, ethanol, chloroform, methanol and petroleum ether (organic solvents). The antibacterial effect of the plant extract showed that the organic solvents extracts were inhibitory to *S. typhi* and *S. aureus,* while *E. coli, K. pneumonia* and *P. aeruginosa* showed resistance to all the extracts. The minimum inhibitory concentration value of the extracts on the organisms ranged between 0.0625-0.125mg/ml for *S. typhi* and 0.125-0.25mg/ml for *S. aureus*. Static effects varied with solvent type of extracts, concentrations of the leaf extracts and the organisms.

KEYWORDS: *Antimicrobial, human pathogen, medicinal plant, Nigeria*

INTODUCTION

Harungana madagascariensis lam.ex poir is a shrub ubiquitous to the tropical rainforest margins and stream bank. Its English name include "blood tree", "orange – milk tree", " dragon's blood tree", "*Harongana*" (Dalziel, 1959). Among the Igbo, Yoruba, and Bini , the plant is known as "uturu", "elopo" and "itue", respectively. (Gill, 1992) The plant grows to a height of about 4-7m, sometimes reaching 10 -25m with cylindrical trunk and golden –green and spreading crown. Its leaves are opposite, simple, ovate or tapering apex (Dalziel, 1959).

In Africa traditional medicine, different parts of the plant *H. madagascariensis* are highly valued for the treatment of diverse human diseases. For example, In Sierra Leone, the red juice is employed to arrest postpartum bleeding. While in Liberia; puerperal infection is treated by eating the unopened bud sheaths beaten up with the *Pentaclethra Macrophylla which* is equally employed in treating leprosy while the red sap washed out of the stem bark is drunk as a remedy for tapeworm infection (Adjanohoun, 1981). The leaf extract is also drunk for the cure of Craw craw or as dressing materials for wounds among Ghanaians (Irvine, 1961). Decoction of the plant root and stem bark is also used as remedy for dysentery, bleeding piles, *trypanosomosis*, *fever*, cold and cough (Gill, 1992). The plant exudates are used by the Ondo people (South –West Nigeria) to cure acute enteritis, scabies and jaundice (Gill, 1992). The boiled decoction of plant leaves is equally reputed for the treatment of malaria (Agbor, *et al.*, 2007). Among the Yoruba herbalists (South West Nigeria), the aqueous root decoction of the plant is employed in the treatment of suspected

liver or kidney disease (Adeneye, *et al.*, 2008). Leaf decoction of *H. Madagascariensis* is used in Rwanda for malaria treatment (Gill, 1992).

Many human pathogens require serious attention in developing countries like Nigeria. *S. typhi* is associated with malaria. *S. aureus* constitutes a nuisance in post-infection and post operative wound infection, causing formation and production of wound diseases in both cases (Daneji, 1981). S. aureus and E. coli (enteropathogenic) have been frequently incrimated in food poisoining incidences associated with gastroenteritis (Etani, et al 1998). K. pneumonia causes pneumonia in man and cow (Lin, et al., 1999). The prevalence of microbial resistance to existing antimicrobial drugs underscores the need for the continuous search for new antimicrobials (Olorundare, et al.,1998). One of the avenues for such a search is to screen local medicinal plants for likely antimicrobial activities. Information on the biological activities of *H. madagascariensis* is scanty and its antimicrobial effects have not been documented in this part of the world. This work therefore examines the antimicrobial activities of *H. madagascariensis* on some human pathogens.

MATERIALS AND METHODS

Collection of Plant Material

In January 2008, leaves of blood tree (*H. madagascariensis lam. Ex poir*.) were collected from plants growing wild in the rainforest zone of Arochukwu town, Abia State, Nigeria*. H. madagascariensis* was authenticated at the Forestry Research Institute of Nigeria (FRIN) Umuahia and deposited in the herbarium of the Institute.

Test for Potency of Bacteria

Microorganisms employed in this work were derived from stock culture at the Medical Centre, Michael Okpara University of Agriculture, Umudike, Nigeria. Viability tests of each isolate were carried out by resuscitating the organisms in buffered Tryptone Soy Broth (Biotic, U.K) and thereafter sub-culturing onto appropriate solid media followed by overnight incubation at 37^0C. Enteropathogenic *E. coli* and *S. typhi* were sub-cultured onto MacConkey Agar (BL9 6AU-Lab-2, England); *S. aureus* onto Nutrient Agar (BL9-6AU Lab-8, England); *K. pneumonia* and *P.aeruginosa* onto Blood Agar (Biotec, U.K) and sabouraud Dextrose Agar (Biotec, U.K) and incubated for 24h at 37^0C, followed by refrigerator storage at 4^0C until required for use.

Organic Extract Preparation

The organic solvents used were ethanol (BDH Chemicals Ltd. England), chloroform (M&B Ltd., England), petroleum x63sether (James Burougy Ltd.,England), and methanol (H.E.Chemicals Ltd., U. K)

Ethanol Extract

The leaf of *H. madagascariensis* following complete dryness were powdered and sieved. Five hundred grams of pulverized *H. madagascariensis* were weighed using electronic scale (Gibertini, Italy) and percolated with 10litres of ethanol for five days. The extract was filtered using sterile filter paper. The filtrate was concentrated on a rotary evaporator at 35^0 .The solvent was recovered in the recovery flask while the extract remained in the sample holder, which was collected and stored in the refrigerator at 4C.

A process called partitioning was employed to obtain the chloroform, petroleum and methanol extract

Chloroform Extract

Ethanol extract of weight 26g were partitioned between Chloroform and water in the ratio of 1:1 (150ml of Chloroform: 150ml of water). The mixture was shaken for 1 hr and allowed to stand inside a separating funnel for 24hrs. After which Chloroform fraction was collected. Dried extract was obtained from this fraction on the exposure to the atmosphere.

Petroleum and methanol extract

Petroleum and methanol extracts were obtained from *H. madagascariensis* by employing a second partitioning between petroleum ether and methanol as solvents using a certain portion of the chloroform extract. The procedure is same as was described for the chloroform extract.

Antimicrobial Susceptibility

Antimicrobial activity of the extracts was evaluated using the following organisms, *E. coli, S. typhi*, *K –pneumoniae, P. aeruginosa, S. aureus.* The ability of the various extracts to inhibit growth of the clinically significant bacteria isolate was determined using the 8mm diameter hole in agar - diffusion technique. Sterile glass pippets of 8mm diameter were used to make holes on prepared agar medium. Aliquots of 0.2ml of the extracts were introduced into the holes made on pre-seeded appropriate gelled media containing each isolate of organism, at different concentrations of the extract, for a specific isolate. A control hole where the solvent used for the extraction was added to one of the plates. Plates were incubated at 37^oC for 24hrs in the incubator. Following the incubation, the diameter of zone of inhibition was recorded.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the extracts was determined by incorporating constant volumes (0.2ml) of each dilution of the extracts and antibiotics solutions into sensitivity dose prepared with white man filter paper No.1. This dilution was gotten by dissolving 0.2gml of the extract as well as the antibiotics in 100ml of sterile distilled water to obtain 2.0mg/ml. This 2.0mg/ml concentration was then doubly diluted in sterile distilled water to obtain concentrations of ½, ¼, 1/8, 1/16, and 1/32mg/ml. (100mg, 50mg, 25mg, 12.5mg, and 6.25mg). 0.2ml of diluted inoculums was introduced into the Petri dishes with agar, swab before introducing the sensitivity discs. The plates were incubated at 37^{o}C and examined after 24hrs and 48hrs growth. Zones of growth of inhibition were measured in millimeters using a plastic ruler. The positive control discs contain 2mg/ml of Ciprofloxacin sulphate produced by Pfizer (5mm diameter) tested concentration. Ciprofloxacin sulphate inhibited all the test organisms of the tested concentration. The zone of inhibition is referred as the clearly visible zones of inhibition across a diameter disregarding the diameter of the disc.

RESULT

Certain pathogens demonstrated susceptibility to the organic extracts solvent extracts of *H. madagascariensis*. Effects varied among the four organic solvents extracts of *H. madagascariensis*. S. typhi and *S. aureus* were the two pathogens that were susceptible to the organic sovents. In the case of extraction with ethanol, both S. typhi and S. aureus demonstrated an MIC of 0.0625mg/ml and 0.125mg/ml respectively (Table 1). It is worthy to note that *S. typhi* with an MIC of 0.0625mg/ml demonstrated the lowest MIC of all the microorganisms that were sensitive to the plant *H. madagascariensis* (Table 5). In the chloroform, methanol and petroleum extracts, the S. typhi and S.aureus have MIC 0.125mg/ml and 0.25mg/ml respectively. *S.typhi* had the highest zone of inhibition which could be seen in the MIC (Table 5). The standard drug Ciprofloxacin showed activity on all the organisms in all the organic solvents extracts except on chloroform, and petroleum ether extracts where it did not inhibit *E. coli* or *K. pneumonia* respectively. The standard drug Ciprofloxacin showed a close relationship with the zone of inhibition of the ethanol extract of *H. madagascariensis* on *S. typhi* and *S. aureus*. In general, the zone of inhibition increased with concentration of the leaf extract of *H. madagascariensis. E. coli, K. pneumonia and P. aeruginosa* were all resistant to the leaf extract of *H. madagascariensis*. Ethanol extract exhibited the highest zone of inhibition followed by the chloroform, methanol and petroleum ether extracts (Table1,2,3,4).

Table1 Effects of ethanol extract on the diameter of zones of inhibition (mm) at varying concentration (mg /ml) of *H.* m*adagascariensis* on the listed pathogens.

Con (mg/ml) Diameter of zone Of inhibition	**Drug**	**1.0**	**0.5**	**0.25**	**0.125**	**0.0625**	**Mic**

(mm)							
E. coil	16	-	-	-	-	-	R
S. typhi	15	11	10	8	6	4	0.0625
K. neumonia	14	-	-	-	-	-	R
P. eruginosa	5	-	-	-	-	-	R
S.aureus	11	7	5	3	2	-	0.125

Note:- = No zone of inhibition, R = resistance,MIC = Minimum inhibitory concentration

Table 2: Effects of chloroform extract on the diameter of zones of inhibition (mm) at varying concentration (mg /ml) of H. madagascariensis on the pathogens

Con (mg/ml) Diameter of zone Of inhibition (mm)	Drug	1.0	0.5	0.25	0.125	0.0625	Mic
E. coil	R	-	-	-	-	-	R
S. typhi	20	5	3	2	1	-	0.125
K.pneumonia	11	-	-	-	-	-	R
P.aeruginosa	-	-	-	-	-	-	R
S.aureus	17	6	4	2	-	-	0.25

Note: - = No zone of inhibition, R = resistance MIC= Minimum Inhibitory concentration

Table 3: Effects of methanol extract on the diameter of zones of inhibition (mm) at varying concentrations (mg /ml) of *H. madagascariensis* on the listed pathogens

Con (mg/ml) Diameter of zone Of inhibition (mm)	Drug	1.0	0.5	0.25	0.125	0.0625	MIC
E. coil	14	-	-	-	-	-	R
S. typhi	12	5	3	2	1	-	0.125
K.pneumonia	10	-	-	-	-	-	R
P.aeruginosa	6	-	-	-	-	-	R

S.aureus	16	6	4	2	-	-	0.25

Note: - = No zone of inhibition, R = resistance,MIC=Minimum inhibitory Concentration

Table 4: Effects of petroleum ether extract on the diameter of zones of inhibition (mm) at different concentrations (mg/ml) of *H.madagascariensis* on the listed pathogens

Con (mg/ml) Diameter of zone Of inhibition (mm)	Drug	1.0	0.5	0.25	0.125	0.0625	MIC
E. coil	16	-	-	-	-	-	R
S. typhi	15	4	3	2	1	-	0.125
K. pneumonia	-	-	-	-	-	-	R
P. aeruginosa	5	-	-	-	-	-	R
S.aureus	11	4	2	1	-	-	0.25

Note:- = No zone of inhibition, R=Resistance MIC = Minimum inhibitory Concentration

Table 5: Minimum Inhibitory Concentration (MIC) of all the extracts on the listed Pathogens

Pathogens	**Ethanol xtract**	**Chloroform extract**	**Methanolextract**	**Pet. extract**
E .C*oli*	R	R	R	R
S. typhi	0.0625	0.125	0.125	0.125
K.pneumonia	R	R	R	R
P.aeruginosa	R	R	R	R
S. aurues	0.125	0.25	0.25	0.25

R = resistance

DISCUSSION

The results of this investigation revealed that the leaf extracts of *H.madagascariensis* possess appreciable antimicrobial activity against commonly encountered microorganisms. The result obtained from all the organic solvents extracts showed the susceptibility of the extract on *S. typhi* and *S. aureus*. This probably shows that there are bioactive ingredients that are inhibitory to the growth of these common pathogens (Etani, et al., 1998). Hence this finding indicates a possible treatment for acute malaria, typhoid, fever, and diarrhea that has been associated with these organisms. Also the high sensitivity of S. aureus to the leaf extract of *H. madagascariensis* demonstrates the use of the plant leaf extract as dressing materials for wounds (Irvin, 1961). This study also tried to show that the conservation of this plant should

be a priority of many plant scientists. The inability of the extracts to inhibit the growth of *E. coli* in this experiment indicates that this plant drug cannot be used in the treatment of gastroenteritis that has been associated with *E. coli* (Etani, et al., 1998). Also, the non inhibition of growth of *K. pneumonia* and *P. aeruginosa* which are aetiology agents of urinary tract infection (Latta, et al., 1998, Tolson, et al., 1997) and pneumonia (Lin, 1999) have shown that the active components of the plant leaf extract cannot kill or cure any disease caused by these organisms.

Salmonella typhi registered the highest diameter of zones of inhibition of all the extracts used and therefore was very sensitive to the active components of the plant leaf extract. This is in line with the local use of *H. madagascariensis* for the treatment of malaria (Agbor, et al., 2007). The ethanol leaf extract was the most competent of the extraction solvents because it produced the highest zone of inhibition on the respective organisms at different concentrations. It is also important to note that the antimicrobial activity on *S. typhi* showed a synergistic effect in combination with the standard drug Ciprofloxacin. This finding indicates a possible use of the extract of plant *H. madagscariensis* in combination with Ciprofloxacin in the treatment of *Samonellosis* and other food burn diseases. There are many factors that influence the active principles present in plant which include the age of plant, extracting solvent, method of extraction and time of harvesting plant materials (Amadioha and Obi, 1999, Qasem and Abu-Blan, 1996, Okigbo, and Emoghene, 2003, Okigbo and Emoghene, 2004, Okigbo and Nmeka, 2005).

This work has highlighted the antimicrobial effects of the leaf *H. madagascariensis* on known human pathogens. Some antibiotics have become almost obsolete because of the problem of drug resistance (Ekpendu, 1994). Though, there has been little attention to the antimicrobial effects of *H. madagascariensis* leaf on pathogens, the consequence of drug resistance implies that new drugs, both synthetic and natural, must be sought to treat diseases for which known drugs are no longer useful.

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