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Research Article

EVALUATION OF THE ANTIBACTERIAL ACTIVITY AND PRELIMINARY PHYTOCHEMICAL ANALYSIS OF THE STEM BARK AND LEAVES EXTRACTS OF *HUNTERIA UMBELLATA K. SCHUM*, APOCYNACEAE

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ABSTRACT
Hunteria umbellata K. Schum, family Apocynaceae is used in traditional medicine in Southern Nigeria to treat various ailments using its various parts. Amongst its ethnomedicinal uses is its use in the treatment of microbial infections. This study focused on the evaluation of the antibacterial activity of the methanol extracts of its stem bark and leaves and preliminary phytochemical screening of the constituents of its stem bark and leaves using standard methods. In-vitro microbiological technique was employed using the minimum inhibitory concentration (MIC) assay. The antibacterial activity of the extracts was evaluated using nutrient agar final concentrations of 15, 20, 25, 50, 75, 100 and 150mg/ml. Ciprofloxacin was used as the reference drug for comparison using final nutrient agar concentrations of 0.3, 0.4, 0.5, 1.0 and 1.5µg/ml. The stem bark extract inhibited the growth of *Staphylococcus aureus* and *Escherichia coli* at the concentrations of 50, 75, 100 and 150 mg/ml, with 50mg/ml as the minimum inhibitory concentration. While the leaves extract had a minimum inhibitory concentration of 150mg/ml against *Staphylococcus aureus* and *Escherichia coli.* Ciprofloxacin inhibited the growth of both bacteria at concentrations of 0.5, 1.0 and 1.5µg/ml, with a minimum inhibitory concentration of 0.5µg/ml. In conclusion, the results indicate that the methanol extracts of the stem bark and leaves of *Hunteria umbellata* possess antibacterial activity against gram – positive and gram – negative bacterial isolates. This activity may be attributed to the reported phytochemical constituents in the plant with known antibacterial actions. This authenticates its ethnomedicine use in the treatment of bacterial infections.

Keywords: *Hunteria Umbellata*, stem bark, leaves, pathogenic bacteria isolates, antibacterial activity

INTRODUCTION

Hunteria umbellata K. Schum, family Apocynaceae, is a tree, about 15 – 22m in height that grows in most parts of Southern Nigeria, Ghana and the rainforest region of Cameroon. In Nigeria, it is known as Osu (Edo), erin (Yoruba, Southwest) and nkpokiri (Igbo, Southeast). In Southern Nigeria, the various parts of the plant are used in traditional medicine to treat various ailments[1,2,3]. The powdered seeds are used in the treatment of fever, pain, abdominal discomforts, piles, yaws, dysmenorrhoea, infertility and diabetes[3]. Its stem bark and leaves are used in ethnomedicine to treat pathogenic microbial infections. The juice from the leaves is used to arrest bleeding in wounds and facilitate wound healing. The leaf decoction is used to treat dysentery while the mixture of the leaf and stem bark is used to treat cholera and other bacterial infections. The decoction of the stem bark and roots is used to treat helminths infestation, although, there are some available scientific reports on the seeds and fruit pulp[4-9] to support the folkloric uses of the seeds and fruit pulp, however, similar scientific reports on the stem bark and the leaves of *Hunteria umbellata* are scanty. This study focused on the preliminary phytochemical analysis and evaluation of the antibacterial activity of the methanol extracts of the stem bark and leaves of *Hunteria umbellata* to provide scientific basis for the ethnomedicinal use of these parts in microbial infections.

MATERIALS AND METHODS

Drugs and chemicals

The chemicals and solvent were of analytical grade. These include ciprofloxacin, nutrient broth and nutrient agar (Sigma Aldrich laborchemikallein, GmBH, Germany) and methanol (Scharlau chemie S.A, Spain).

Plant materials

The fresh stems and leaves of the plant were collected in October, 2010 from Irrua, Esan-Central Local Government of Edo State, Nigeria. The preliminary identification of the plant was done by Dr B.A. Ayinde of the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Nigeria. Identification and botanical authentication was at the Forestry Research Institute of Nigeria (FRIN), Ibadan where voucher specimen FHI 107678 was deposited for future reference.

Extraction of plant materials

The stem bark was carefully separated from the woody part, cut into small bits, shade- dried and pulverized using a grinder (Lab. Mill, serial NO. 4745, Christy and Norris Ltd, England) and stored in dry, airtight glass jars. The leaves were separately, similarly shade – dried and pulverized and were stored in dry, airtight glass jars and labelled accordingly. Each of the powdered plant materials (500 g) were macerated separately in 98% methanol (2 L) in glass jars for 72 hours and were shaken intermittently throughout the period. The extracts were filtered separately. Each filtrate was evaporated to dryness at reduced pressure using a rotary evaporator to obtain a dark brownish residue (stem bark extract) and greenish - dark blue residue (leaf extract) until a constant weight was obtained and the yield with reference to the powdered plant material in each case was noted. The extracts obtained were stored separately in closed containers in the refrigerator until when required for use in experiments reported in our study.

Phytochemical screening

The powdered stem bark and leaves were subjected separately to various tests in order to determine the classes of the various chemical constituents present in them, by using standard methods[10].

Preparation of concentrations of extracts and ciprofloxacin hydrochloride

From a stock solution of 500mg/ml of either the stem bark extract or leaves extract, final concentrations of 15, 20, 25, 50, 75, 100 and 150 mg/ml respectively were used for antibacterial assay in 10ml of nutrient agar. From a stock solution of 100µg/ml of ciprofloxacin, final concentrations of 0.3, 0.4, 0.5, 1.0and 1.5µg/ml respectively were used for antibacterial assay in 20ml of nutrient agar.

Bacterial isolates

Two clinical bacterial isolates, gram positive (*Staphylococcus aureus - Staph. aureus*) and gram negative (*Escherichia coli, - E. coli*) were used in the study. The pure isolates were obtained from the Department of Pharmaceutical Microbiology stock culture unit.

ANTIBACTERIAL ASSAY

Minimum inhibitory concentration (MIC) of ciprofloxacin

The agar dilution method described by George and Robert was used[11].

The minimum inhibitory concentration (MIC) assay was evaluated using five (5) final concentrations of 0.3, 0.4, 0.5, 1.0 and 1.5µg/ml respectively in 20ml of nutrient agar. In the first set of assays *Staphylococcus aureus* was used and each concentration was in triplicate plates of 20ml of nutrient agar each. Each plate was inoculated with 0.02ml of over- night nutrient broth culture of *Staphylococcus aureus*. In the second set of assays *Escherichia coli* was used and each concentration was in triplicate plates of 20ml of nutrient agar each. Each plate was inoculated with 0.02ml of over-night nutrient broth culture of *Escherichia coli*. The inoculated plates were incubated at 37 ^{o}C for 24 hours and were examined for growth.

Minimum inhibitory concentrations (MIC) of methanol stem bark extract and leaves extract of *Hunteria umbellata*

The agar dilution method described by George and Robert was used[11].

The minimum inhibitory concentration (MIC) assay was evaluated using seven (7) final concentrations namely 15, 20, 25, 50, 75, 100 and 150 mg/ml respectively in 10ml of nutrient agar. In the first set of assays *Staphylococcus aureus* was used and each concentration of the stem bark extract was in triplicate plates of 10ml of nutrient agar each. Each plate was inoculated with 0.02ml of over- night nutrient broth culture of *Staphylococcus aureus*.

In the second set of assays *Escherichia coli was* used and each concentration of the stem bark extract was in triplicate plates of 10ml of nutrient agar each. Each plate was inoculated with 0.02ml of over- night nutrient broth culture of *Escherichia coli*.

In the third set of assays *Staphylococcus aureus* was used and each concentration of the leaves extract was in triplicate plates of 10ml of nutrient agar each. Each plate was inoculated with 0.02ml of over-night nutrient broth culture of *Staphylococcus aureus.*

In the fourth set of assays *Escherichia coli* was used and each concentration of the leaves extract was in triplicate plates of 10ml of nutrient agar each. Each plate was inoculated with 0.02ml of over-night nutrient broth culture of *Escherichia coli*. The inoculated plates were incubated at 37 ^{o}C for 24 hours and were examined for growth.

RESULTS

The maceration of the powdered plant materials (500g) in methanol yield 3.6 % and 2.4% stem bark and leaves extracts respectively.

The preliminary phytochemical screening revealed the presence of alkaloids, saponins, flavonoids, cardiac glycosides and reducing sugars in the stem bark (Table 1) while similar screening revealed the presence of tannins, alkaloids, saponins, cardiac glycosides, volatile oils and reducing sugars in the leaves (Table1).

The reference drug, ciprofloxacin in a minimum inhibitory assay, inhibited the growth of the gram positive isolate, *Staphylococcus aureus* and gram negative isolate, *Escherichia coli* at the concentrations of 0.5, 1.0 and 1.5 µg/ml in nutrient agar, with 0.5µg/ml as the minimum inhibitory concentration (Table 2).

The stem bark extract in a minimum concentration inhibitory assay, inhibited the growth of the gram positive isolate, *Staphylococcus aureus* and gram negative isolate, *Escherichia coli* at the concentrations of 50, 75, 100 and 150 mg/ml in nutrient agar, with 50mg/ml as the minimum inhibitory concentration (Table 3).

The results in Table 3 indicate that the leaves extract inhibited the growth of *Staphylococcus aureus* and *Escherichia coli* at the minimum concentration of 150 mg/ml in nutrient agar.

DISCUSSION

The emergence of antibiotic resistance against *Staphylococcus aureus, Escherichia coli* and other clinical isolates document the need for search for newer sources of antibiotics to ensure appropriate chemotherapy and therapeutic success. *Staphylococcus* species have traditionally been one of the most common gram positive pathogens in major bacterial infections[12]. The successful use of medicinal plants in the management of most microbial infections in most of the developing countries tends to suggest that these plants could offer future promise in this regard. The leaf decoction and a mixture of the leaf and stem bark of *Hunteria umbellata* are used to treat bacterial infections in Southern Nigeria. Our findings in this study indicate that the methanol extract of stem bark and leaves of *Hunteria umbellata* possess antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* which were sensitive to the reference drug, ciprofloxacin at minute concentrations *in vitro*. When compared to ciprofloxacin, the antibacterial activity of the extracts were relatively lower, however, the isolation and concentration of the active ingredients of the extracts will ameliorate their activity. The stem bark extract exhibited a more potent antibacterial action against the isolates when compared to the leaves extract as indicated by the lower minimum inhibitory concentration of the stem bark compared to that of the leaves extract. The presence of the reported phytochemical constituents in the stem bark and leaves extracts may have contributed to the observed antibacterial activity. The alkaloids, cardiac glycosides and flavonoids are known to elicit antimicrobial actions[13] and this may have contributed to the antibacterial actions observed in this study.

Although the present study could not establish the exact mechanism(s) of the antibacterial activity of the extracts, it provides evidence for the use of the plant in the management or control of bacterial infections. The findings show that the methanol extracts of the stem bark and leaves possess antibacterial actions against gram – positive and gram-negative bacteria.

In conclusion, the stem bark and leaves of *Hunteria umbellata* possess antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* and thus lend pharmacological credence to the traditionally claimed and ethno medical use of the plant parts in the treatment of infections caused by these organisms in rural communities in Nigeria and other West African countries.

REFERENCES

1. Sofowora A. Medicinal plants and traditionalmedicine in Africa, 1st.ed. , John Willey and Sons Ltd, Chichester, 1982; 168.
2. Keay RWJ, Onochie CFA, Stanfield DP. Trees of Nigeria (formerly Nigerian trees, 1960, 1964), Oxford University, Oxford, 1989; 234.
3. Gill LS. Ethnomedicinal uses of plants in Nigeria. University of Benin press, Benin, Nigeria, 1992; 276.
4. Igbe I, Ching FP and Aigbe E. Antiinflammatory activity of aqueous fruit pulp extract of *Hunteria umbellata* K.Schum in acute and chronic inflammation. Acta Pharmaceutica – Drug Research, 2010; 6(1): 81 – 85.
5. Igbe I, Ozolua RI, Okpo SO and Obasuyi O. Antipyretic and analgesic effects of the aqueous extract of the fruit pulp of Hunteria umbellata K. Schum (Apocynaceae), Trop. J. Pharm. Research, 2009; 8(4): 331 – 336.
6. Falodun A, Nworgu ZAM and Ikpomwonsa MO. Phytochemical components of *Hunteria umbellata* (K. Schum) and its effects on isolated non-pregnant rat uterus in oestrus. Pakistan Journal of Pharmaceutical Sciences, 2006; 19(3)**:** 256 – 258.
7. Ejimadu IM and Falodun A. Biological and chemical studies of *Hunteria umbellata* (K. Schum) seed . Intern. Journ. Chem. 2003;1 2(3): 241 – 248.
8. Vaele D, Nigeria JH, Furman KI and Oliver DW. South African traditional medicines used during pregnancy and childbirth. J. of Ethnopharmacology, 1992; 36: 185 – 191.
9. Onuaguluchi G. Anti-ascarid activity of certain extracts from the bark of Polydoa umbellata, West African Medical Journal, 1964; 13(4): 162 – 165.
10. Trease GE and Evans MC. Textbook of Pharmacognosy, 13rd ed., Bailliere Tindall, London, 1989; 520.

11. George ME and Robert CM. Antimicrobial combinations In: Antibiotics in medicine , 4th ed. Victor , L. Editor, Williams and Wilkens, London, 1996; 334.
12. Yah SC, Enabulele IO and Eghafona NO. Bacteriological studies on infected kerosene burn wounds in Benin City, Nigerian Journal of Biomedical Investigation (JBI), 2004; 2(1): 4 – 9.
13. Ebana RUB, Madunagu BE, Ekpe ED and Otung IN. Microbiological exploitation of cardiac glycosides and alkaloids from *Garcinia kola, Borreria ocymoides, Kola nitida* and *Citrus aurantifolia*. J. of Applied Bacteriology, 1991; 71: 398 – 401 .

TABLE 1: PHYTOCHEMICAL CONSTITUENTS OF THE EXTRACTS OF STEM BARK AND LEAVES OF *Hunteria umbellata*

Constituents	Stem Bark Extract	Leaves Extract
Tannins	-	+
Alkaloids	++	+
Saponins	++	+
Flavonoids	+	-
Reducing sugars	+	++
Cardiac glycosides	+	+
Volatile oils	-	+

+, constituents is present, + +, constituent present in copious amount, -, constituent absent.

TABLE 2 : MINIMUM INHIBITORY CONCENTRATION OF THE REFERENCE DRUG, CIPROFLOXACIN AGAINST *Staphylococcus aureus* AND *Escherichia coli*

Stock conc. of Ciprofloxacin (µg/ml)	Vol. used (ml)	Actual amt. (µg)	ciprofloxacin conc in nutrient agar (µg/ml)	*Staphylococcus aureus*	*Escherichia coli*
100	0.06	6	0.3	+	+
100	0.08	8	0.4	+	+
100	0.10	50	0.5	-	-
100	0.20	100	1.0	-	-
100	0.30	150	1.5	-	-

+ indicated that there was growth while – indicated that no growth of the organism was observed.

TABLE 3: MINIMUM INHIBITORY CONCENTRATION OF THE METHANOL EXTRACTS OF STEM BARK AND LEAVES OF *Hunteria umbellata* AGAINST *Staphylococcus aureus* and *Escherichia coli*

Stock conc of extracts (mg/ml)	Vol. used(ml)	Actual amt (mg)	Final conc in nutrient agar(mg/ml)	Stem bark extract		Leaves extract	
				Staph. aureus	*E. coli*	*Staph. aureus*	*E. coli*
500	0.3	150	15	++	+++	++	+++
500	0.4	200	20	+	++	++	+++
500	0.5	250	25	+	+	++	++
500	1.0	500	50	-	-	+	++
500	1.5	750	75	-	-	+	++
500	2.0	1000	100	-	-	+	+
500	3.0	1500	150	-	-	-	-

-, indicated No growth of colonies; +, scanty growth observed; + +, dense growth observed; + + +, very dense growth of colonies.

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