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Phytochemical screening and antimicrobial activity of stem bark extracts of *Antidesma Venosum*

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ABSTRACT

Chemical studies on stem bark of Antidesma venosum used in phytotherapy were done in vitro. The stem bark was extracted by Soxhlet in absolute methanol, and partitioned using ethyl acetate, n-pentanol and distilled water to give fractions coded EAA, NPE and AQE, respectively. Phytochemical screening of crude methanol extract (CME) and EAA showed presence of alkaloids, saponins, tannins and flavonoids; Saponins and tannins in NPE and AQE. These fractions together with CME were screened for antimicrobial activity against Staphylococcus aureus, Salmonella typhi and Escherichia coli. The highest antimicrobial activity was observed with EAA and least with AQE. All fractions and extract however, exhibited some activity on tested microorganisms.

Key Words: *Antidesma venosum*, stem bark, methanol extract, phyntochemical screening, antimicrobial activity.

INTRODUCTION

Higher plants can be regarded as biochemical factories that produce their basic needs using air, water, minerals and energy from sunlight. Many species of higher plants biosynthesise and accumulate extractable organic substances in their various organs which have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years. Such extensive dependence of human beings on plants has invoked tremendous interest in the scientific world, which ultimately led to the isolation of a vast number of chemical agents with potentials for multipurpose uses [1].

The renewed interest in medicinal plants is evidenced by the recommendations given by the World Health Organization (WHO) in 1970 [2]. There has been recent moves towards professionalization of African Traditional Medicine where proven traditional remedies are suggested to be incorporated within national drug policies [3].

Antidesma venosum tul. (Etulo: *Tsetse-ogbe*; Idoma: *Okoto*; Tiv *Baverkpua*; Hausa: *Kirni; Kisni*) [4] is a savanna specie with small leaves and of Euphorbiaceae family. A tree up to 9m high and 60cm in girth. Bole straight, grey; slash pinkish- brown and fibrous. Twigs are thickly hairy.

Leaves 3.5-11cm long by 2-5cm broad, sometimes slightly elongated, rounded or very shortly acuminate at the apex, cuneate or rounded at the base. Flowers pale yellow; male flower rather distantly arranged along the central stalk;

female flower very small, with very short stalks. Fruits eventually black, edible; almost spherical, about 6mm across, very shortly stalked [5].

The powdered bark of this tree is used in wound dressing and macerate of root and bark used to wash syphilitic and gonorrheal eruptions; leafy sap is taken with other medicinal plant for diarrhea and amoebic dysentery [6].

The common practice of taking crude extracts is often associated with hazards as a result of other undesirable constituents that might be present in them. It is therefore, important to investigate each medicinal plant to ascertain its full potentials [3].

Also, with the emergence and prevalence of diseases such as HIV/AIDS and cancer, and resistance of pathogenic organisms to known agents, it is of great importance to explore the rich world of plants with a view to finding new cures to these diseases that are threatening mankind. It is a growing truism that traditional medicine has a key role to play in health care worldwide. The search for scientific information about efficacy and safety of plants used in alternative medicine is a continuous process.

MATERIALS AND METHODS

Sample collection, Preparation and Extraction

The stem bark of Antidesma venosum was collected in August, 2010, from the campus of the University of Agriculture, Makurdi, Benue State and was identified in the Department of Forestry and Wildlife, University of Agriculture, Makurdi where a specimen sample has been deposited.

The fresh stem bark was air dried for two, then pulverized with aid of a mortar and pestle. The fine powdered sample was stored in a cellophane bag until needed for analysis.

Two hundred grams of the powdered plant material was defatted with 1000cm^3 pet. Ether (6O - 8O ^{o}C) using batch method of extraction in a conical flask for 24 hours, with intermittent shaking. The mixture was filtered and filtrate allowed to evaporate to dryness at room temperature to give a brownish extract 1.3g (0.65% w/w) and coded “PEE”. One hundred grams of the resulting marc was exhaustively extracted by Soxhlet for about six hours in absolute MeOH to give a reddish-brown gummy mass 30.1g (30.1% w/w) coded “CME” after evaporation over steam bath.
About 19g of “CME” was suspended in 150ml distilled water and then filtered. The resulting filtrate was sequentially partitioned using ethyl acetate and n-pentanol. The ethyl acetate and n-pentanol soluble portions were evaporated over a steam bath to give a yellowish-brown mass, 1.6g (8.42% w/w) and a reddish-brown mass, 4.Og (21.05% w/w) coded “EAA” and “NPE” respectively. The residual aqueous layer was evaporated over steam bath to give a dark-brown mass, 8.2g (43.16% w/w) coded “AQE”.

The extracts were stored aseptically in a desiccator at room temperature until needed.

Phytochemical screening

The crude methanol extract and its partitioned fractions were screened phytochemically for the presence of its constituents utilizing standard methods of analyses [7,8]

Antimicrobial Tests

The microorganisms used in this study were *Escherichia coli*, *Salmonella typhi* and *Staphylocous aureus*. They were clinical isolates obtained from Tosema diagnostic specialist laboratory, Makurdi.

The paper disc method was used, the culture for the medium was prepared by weighing 7g of nutrient agar dissolved in 250 m1 of distilled water, heated and dispersed into bijoux bottles, and sterilized by autoclaving at 121^{0}C for l5mins at l5psi.

The microbes under test were grown in this nutrient broth at 37°C for 24hours in an incubator. Sterilized Petri dishes containing the nutrient agar were inoculated with the microbes and allowed to stand for 3O mins.
Whatman filter paper cut into small discs were then soaked in the extracts and placed in the plates and incubated at 37°C for 24hours. The experiment was carried out in triplicates. Plates with pure solvents used in preparing the extracts served as control.

After the incubation period, the antimicrobial activities of the extracts were determined by measuring their inhibition zone diameter (mm). Zone of observable inhibition of growth of each microorganism served as criteria for declaring a plant extract bioactive and is indicated by a clear zone around the filter paper disc [9].

RESULTS AND DISCUSSION

The result of phytochemical screening of the crude methanol extract (CME) and its fractions (EAA, NPE and AQE), has revealed the presence of some classes of secondary metabolites (Table 1).These classes of compounds are known to show curative activity against several pathogens responsible for a number of ailments in man, [10] and therefore could explain its use traditionally for the treatment of some bacterial related illnesses.

Table 1: Phytochemical Analysis of Stem Bark of A. venosum

Plant extract/ fractions	Alkaloids	flavonoids	saponins	tannins	phenols	Glycosides
CME	+	+	+	+	-	-
EAA	+	+	+	+	-	-
NPE	+	-	+	+	-	-
AQE	+	-	+	-	-	-

The antimicrobial screening result indicated zones of inhibition of microbial growth. CME, EAA, and to a lesser extent, NPE exhibited higher levels of inhibition against tested organisms with highest inhibition zone on *Escherichia coli* (Table 2).

The crude stem bark methanol extract of *Antidesma venosum* and its fractions exhibited activity against both gram positive and gram negative bacteria tested.

Table 2: Antimicrobial activity of extract/fractions of *A. venosun*

	Mean zone of Inhibition (mm) of the Extract/Fractions				
Microorganism	Concentration (g/ml)	CME	EAA	NPE	AQE
Salmonella typhi	10^{-1}	4.2	5.3	2.0	1.1
	10^{-2}	2.3	4.0	0.9	0.0
	10^{-3}	1.1	1.1	0.0	0.0
	Control	0.0	0.0	0.0	0.0
Escherichia coli	10^{-1}	4.4	6.3	4.3	1.2
	10^{-2}	2.1	5.0	3.2	0.0
	10^{-3}	0.8	3.4	1.1	0.0
	Control	0.0	0.9	0.0	0.0
Staphylococcus aureus	10^{-1}	2.1	2.2	1.3	1.1
	10^{-2}	1.3	1.4	0.7	0.3
	10^{-3}	0.0	0.6	0.0	0.0
	Control	0.0	0.0	0.0	0.0

The antibacterial activity exhibited by CME and EAA could not be unrelated with the presence of flavonoids. The higher activity of CME and EAA could possibly be due to additive and synergistic effects of other phytoconstituents such as saponins, tannins and alkaloids in these extracts [11,12]. Tannins had been used as an application to sprains, bruises and superficial wounds [13]. Saponins are effective in the treatment of syphilis, rheumatism and certain skin disease, and for the treatment of abscesses and other swelling, ulcer and septic wounds. The analgesic properties of alkaloids, as well as the diuretic and antibacterial properties of flavonoids- containing plants, have also been reported [9].

The inability of AQE to significantly inhibit the growth of some gram negative organism could probably be due to little of secondary metabolites left after the exhaustive partitioning. The quantity left in AQE was too small to elicit any significant inhibition.

The fact that the extracts produced inhibitory activities against all the tested microorganism provides some scientific basis for its use in traditional medicine against some bacterial pathogens.

CONCLUSION

The sensitivity of all extracts of *A. venosum* to tested organisms had justified the ethnomedicinal usage of this plant in treating bacterial related infections such as diarrhea and amoebic dysentery.

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