Nig. J. Pharm Res. Vol. 2 April - Sept. 2003

SUSCEPTIBILITY PATTERNS OF COMMON BACTERIA AND FUNGAL SPECIES ISOLATED FROM SEXUALLY TRANSMITTED DISEASED PATIENTS TO *XYLOPIA AETHIOPICA* (*ANNONACEAE*)

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Abstract

The hexane and methanolic extracts of the leaves, stem, stem bark, and root bark of *Xylopia aethiopica* were investigated for their anti-microbial activities against some common bacteria and fungal strains isolated from sexually transmitted diseased patients and some standard organisms. The hexane and methanol crude extracts showed varying degrees of anti-microbial activities at varying concentrations while the stem bark exhibited significant broad-spectrum anti-microbial activity. The methanolic extract of the stem bark showed better activity than hexane, was fractionated and the four fractions collected were tested for their antimicrobial activity against the organisms implicated in STD at a concentration of 10mg/ml. The results suggest that the active fractions A and B contain constituents which can be isolated, characterized and identified for effective management of both bacterial and fungal STD's. Preliminary Phytochemical screening revealed the presence of alkaloids, sugars, cardiac glycoside and saponins and the absence of anthraquinones and tannins.

Keywords:
Xylopia aethiopica, Antimicrobial activity, Sexually transmitted diseased patients .

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INTRODUCTION

Xylopia aethiopica, a tree up to 20 metre high or more is mostly found in the lowland rain forests and in the evergreen or deciduous lowland forests of many African countries including Nigeria where it is commonly used as a spice and flavouring for food and medicine.

Several ethnopharmaceutical claims made in respect of *Xylopia aethiopica* include the use of the leaf decoction as an emetic and for rheumatism and broncho-pneumonia, the fruit as a cough medicine, carminative and purgative. It is given as a woman remedy to encourage fertility and for ease in childbirth and to induce menstrual flow. The root decoction is used as a mouth wash and for toothaces while the root and bark powder is used for ulcers, sores and on gums for pyorrhoea. The fruit and bark in decoction are used as a treatment for asthma, bronchitis, dysenteric conditions, biliousness and febrile pains.

Information on *in-vitro* antimicrobial activity of both the extracts and essential oils of this species showed antimicrobial activity against a wide range of Gram-positive and Gram-negative bacteria and fungi (Malcom and Sofowora, 1969; Boakaiji *et al*, 1977; Kande *et al*, 1994; Masotti *et al*, 1997 and Harrigan *et al*, 1994). However, information on its in-vitro anti-microbial activity on specific bacteria and fungi implicated in sexually transmitted infections are not available in current literature hence this study is designed to identify its antimicrobial potential in this respect and further justify scientifically its encouraging fertility anti-microbial relevance in traditional medicine.

MATERIALSAND METHODS

Plant materials:

The plant materials (leaves, stem, stem-bark and root bark) were collected in March 2001 from Etam village in Ogun-State, Nigeria. Identification and authentication of the plant species was done at the Forestry Research Institute of Nigeria, Ibadan, Nigeria. The F.H.I No is 10627. The plant parts were dried under mild sunshine for several days and pulverized into coarse powder.

Extraction and Isolation:

Known weights of the powered plant materials of leaf, stem, stem bark and root bark were soxhlet extracted at a temperature of 70-80°C with hexane and methanol in succession for 24 hrs each. The different extracts were each concentrated until dried under vacuum and stored at 40°C until used.

Phytochemical screening:

The quantitative chemical analysis of the stem bark powder was carried out for the presence of secondary metabolites using conventional method adopted in similar surveys (Hugo and Russel, 1983).

Chromatographic fractionation:

Dried stem bark methanolic extract was subjected to chromatographic fractionation.

Thin Layer Chromatography of Extracts:

Silical gel pre-coated aluminium plates were used with the following mobile phases:

a). Dichloro-methane:methanol - 3:1
b). Dichloro-methane:methanol - 7:3
c) Dicholoro-methane: methanol - 8:2
d) Chloroform: methanol - 8:2
e) Chloroform: methanol - 7: 3.

The plates were developed in iodine tank after drying.

Vacuum Liquid chromatography (VLC):

Five grammes (5g) of the dried extract was reconsti tuted with small quantity of methanol and uniformly mixed together with about 5g of silica gel. This was chromatographed on silica gel column (grade 3 pore diameter). Gradient elution with dichloromethane/ methanol solvent polarity being increased slowly and gradually resulted in several 50ml fractions which were combined on the basis of TLC analysis to give four major fractions namely; A (fraction 1-5, 6.4 %) B (fractions 6-8, 6.4 %), C (fractions 9-12, 13.6%), and D (fractions 13-15, 46%).

Organisms:

The micro-organisms employed in the study were clinical isolates collected from the Sexually Transmitted Infection (STI) Clinic, University College Hospital (UCH), and the Department of Pharmaceutical Microbiology Laboratory, University of Ibadan (UI), Ibadan, Nigeria and some standard typed cultures (Table 1).

Anti-microbial agents:

The following chemotherapeutic agents were included in the study as positive controls; Gentamycin sulphate at 100µg/ml (Taylek Nig. Ltd.) and Tioconazole 1% w/v (Pfizer Inc.,) Nigeria.

Media:

Nutrient agar pH 7.4 (Oxoid), Nutrient broth pH 7.4 (Oxoid), Sabourand dextrose agar pH 5.4 (Oxoid) and Starch agar were used in this study.

Determination of anti-microbial activity:

An agar diffusion method (Hugo and Russel, 1983) was used to determine the antimicrobial properties of the extract. Nutrient agar plates were each seeded (starch agar was used for Neisseria strains) with 0.1ml of a 10^{-2} dilution of an overnight culture of each bacterial isolate, while sabourand dextrose agar plates were similarly seeded with the fungal strains. The seeded plates were allowed to dry in the incubator at 37°C for 20mins. A standard cork borer of 6mm diameter was used to cut uniform equidistant wells on the surface of the agar into which was added 80µl solution of each extract/fraction reconstituted in methanol/sterile distil water at concentration of 300mg/ml and 100mg/ml. Nutrient agar and starch agar plates seeded with the bacterial isolates were incubated at 37°C for 24 hrs while sabourand dextrose agar plate seeded with fungal strains were incubated at 25°C for 48 hrs, after which diameters of zones of inhibition were measured. The reconstituting solvent was included in each plate as a sol

vent control. The stem bark hexane and methanol extracts were further tested on the test organism s at lower concentrations of 50mg/ml and 25mg/ml.

Determination of Minimum Inhibitory Concentrations (MIC):

The MIC of the stem bark extracts were determined using the agar diffusion method. Desired concentrations of the extracts were prepared and introduced into already made wells that have been cut into the surface of the seeded plates. The lowest concentration that inhibited growth was taken as the MIC.

RESULTS

The percentage yield of the hexane and methanolic extract are 2.9 and 13.8 for the leaves, 6.9 and 12.2 for the stem bark, 6.0 and 12.3 for the root bark and 1.3 and 9.8 for the stem. Phytochemical screening of the stem bark reveals the presence of alkaloids, sugars, cardiac glycosides and saponins while anthraquinones and tannins were absent. Results of the antimicrobial activity are shown in Tables 2 and 3. Extracts were found to possess varying degrees of antimicrobial activities. Zones of inhibition obtained for the stem bark methanol and hexane extracts were found to be higher than for the other plant parts for the bacteria isolates (Table 4). The methanolic extract showed greater antimicrobial activity than hexane extract for the stem bark. It is interesting to note the broad-spectrum antimicrobial activity displayed by the stem bark methanol extract against the test microorganisms. This interesting trend led to the fractionation of this extract. The four major fractions collected from the fraction ation procedure showed varying degrees of antimicrobial potential with fraction B being the most active (Table 5) with an interesting broad spectrum pattern of antimicrobial activities. Furthermore, zones of inhibition obtained in respect of the three active fractions compared favourably with those obtained from the drug controls (Gentamycin and Tioconazole).

DISCUSSION

The stem bark extract distinctively showed a broader spectrum of anti-microbial activity against the test microorganisms than the leaf, stem, and root bark extracts. Most of the microorganisms used in the study were hospital isolates collected from sexually transmitted patients and the were mostly commonly implicated in sexually transmitted infections with the exception of *P.aeruginosa, Klebsiella species,* and *E. coli*. These have also been said to be among the most frequent microorganisms in the vagina which even though may not cause any disease manifestations, they can be associated with infections under certain circumstances and determined concentrations (Provenzana, 1999). Their inclusion in this study was therefore on this basis. The strong anti-microbial activities demonstrated by crude extracts of this plant parts may therefore be a justification of its use in some part of Nigeria and Africa as an ingredient of a medicine to aid conception, encourage fertility and for inducing menstrual flow (Dalziel, 1937).

Other studies have shown the crude extracts of antimicrobial activities against both Gram - positive and Gram – negative bacteria but in active against *E.coli* (Malcom and Sofowora, 1969; Boakaiji *et al*, 1977). The essential oils of the fruits have been reported to have anti-microbial activities against a number of bacteria including *E.coli* (Chalchat *et al*, 1977).

The broad spectrum antimicrobial activity of fraction B on this sexually transmitted infections-implicated bacteria and fungi isolates and the selective antimicrobial activity of fractions A and C indicate that they are very promising in further improving the management of both bacteria and fungi sexually transmitted infections world wide. The strong presence of alkaloids in fractions A and B indicate that the strong antimicrobial activity may not be unrelated to its presence as reported by Kande *et al* (1994) and Harrigan *et al* (1994). Since no part of this plant used in different African communities including Nigeria has spice and for various ailments, has any reported adverse or toxic effect, phytochemical studies are in progress to isolate, characterized and identify the specific active compounds in this plant responsible for the anti-microbial activity especially in the stem bark

Table 1
The types of microorganisms used and their sources

Microorganisms	Code	Source
Escherichia coli	STI/EC_1	STI Clinic, UCH
Escherichia. coli NCTC 9001	EC_2	Dept of Pharm.Micro Lab, U.I.
Klebsiella spp.$_1$	STI/K_1	STI Clinic, UCH
Klebsiella spp.$_2$ *NCTC 10896*	K_2	Dept. of Pharm. Micro Lab, UI
Gardnerella spp.	STI/G	STI Clinic, UCH
Neisseria gonorrhoae	STI/Ng_1	STI Clinic, UCH
Neisseria gonorrhoae ATCC 19424	STI/Ng_2	Dept of Pharm. Micro. Lab, UI
Staphylococcus aureus NCTC 6571	S.a	Dept of Pharm. Micro Lab, UI
Pseudomonas aeruginosa NCTC 6750	P.a	Dept of Pharm Micro Lab, UI
Candida albicans$_1$	STI/C_1	STI Clinic, UCH
Candida albicans$_2$	STI/C_2	STI Clinic, UCH

Table 2
Antimicrobial activity of X.. *aethiopica* crude extracts on all the tested organisms

Organisms	Leaves		Root bark		Stem		Stem bark				Meth/Tween80
	Methano		Methanol		Methanol		Methanol		Hexane		
	300	100	300	100	300	100	300	100	300	100	
EC_2	-	-	-	-	-	-	-	-	-	-	-
STI/EC_1	-	-	-	-	-	-	-	-	-	-	-
STI/K_1	-	-	-	-	-	-	26	22	16	14	-
K_2	-	-	-	-	-	-	20	14	14	11	-
STI/G_1	-	-	-	-	14	13	22	16	15	12	-
STI/G_2	-	-	-	-	-	-	22	15	13	12	-
$N.g_2$	-	-	14	-	-	-	20	15	-	-	-
$STI/N.g_2$	13	11	-	-	-	-	18	12	-	-	-
Pr	-	-	-	-	15	12	18	12	12	-	-
S.a	20	17	-	-	-	-	22	15	18	12	-
P.a	-	-	-	-	-	-	20	15	20	14	-
$STI/C.a_1$	-	12	10	-	20	15	15	12	-	-	-
$STI/C.a_2$	13	10	-	-	19	15	12	10	-	-	-

Meth = Methanol; - = No zone of inhibition

Table 3

Antimicrobial activity of *X. aethiopica stem* bark extracts on selected organisms

Organisms	Hexane Extracts				Methanol Extracts				Gent.	Tio	Meth/Tween80
										100µg/ml	4µg/ml
	200	100	50	25	200	100	50	25			
STI/K_1	15	14	14	12	25	22	20	18	14	NT	-
STI/G_2	15	13	12	12	20	17	15	13	20	NT	-
STI/N.g_1	-	-	-	-	18	15	13	12	14	NT	-
S.a	18	12	-	-	20	15	12	-	24	NT	-
P.a	16	14	12	10	16	14	12	10	12	NT	-
STI/C.a_2	-	-	-	-	15	12	-	-		NT	23

Meth = Methanol,; Gent = Gentamycin; Tio = Tioconazole; - = No zone of inhibition;
NT = Not tested

Table 4

Determination of MIC of X. *aethiopica* stem bark extracts against the test organisms

Organisms	*X. aethiopica* stem bark										
	Hexane Extracts				Methanol Extracts				Gent.	Tio	Meth/tween80
	50	25	12.5	6.25	50	25	12.5	6.25	100µg/ml	4µg/ml	
Kleb $_1$	14	12	10	-	19	18	16	-	14	NT	-
Gard $_2$	13	12	10	-	14	12	10	-	20	NT	-
Neiss $_1$	-	-	-	-	13	12	11	-	14	NT	-
S. aureus	14	12	10	-	16	15	12	-	24	NT	-
Pseud $_{NCTC}$	12	10	-	-	12	10	-	-12	NT	-	
Cand $_2$	-	-	-	-	-	-	-	-	NT	23	-

Meth = Methanol,; Gent = Gentamycin; Tio = Tioconazole; - = No zone of inhibition,; NT = Not tested

Table 5

Zones of inhibition (mm) of fractions obtained from the methanol extract of stem bark at concentration 10mg/ml

Organisms	A	B	C	D
STI/N.g_2	13	18	-	-
N.g_2	-	14	-	-
STI/G_1	-	15	-	-
STI/G_2	-	15	-	-
STI/K_1	-	15	-	-
.P.a	20	27	-	-
S.a	18	25	25	-
STI/C.a_2	23	30	15	-

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