ANTIFUNGAL AND ANTIBACTERIAL ACTIVITIES OF THE CRUDE EXTRACT OF ANTHOCLEISTA DJALONENSIS CHEV.

Ogbeche, K.A.,[1] Alabe, O.K.[2] and Olagbende-dada, S.O.[2]

Dept Of Pharmaceutical Chemistry and Pharmacognosy, School Of Pharmacy, College Of Medicine, Idi- Araba, University Of Lagos. Nigeria

Correspondence: Ogbeche, K.a.abs

SUMMARY

Studies on antimicrobial and antifungal activities of the roots of Anthocleista djalonensis chev., Family legoanaceae, a medicinal plant used in West African coast most especially in Nigeria for the treatment of various ailments and infections was carried out using agar diffusion technique.

The result revealed that the crude methanolic and water extracts exhibited antibacterial activities against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Candida albicans while the petroleum ether extract fraction exhibited little or no antibacterial or antifungal action when compared with a standard ampicillin concentration of 10mg/ml. The minimum inhibitory concentration (MIC) for the methanolic root extract ranged between (0.25-4.68mg/ml) while that of the water extract ranged between (0.67-12.62mg/ml).

INTRODUCTION

Anthocleista djalonensis chev. Family, Loganiaceae is a plant that is widely distributed in the savanah forest and West African coast , most especially Cameroun, Nigeria, Ghana, Ivory Coast and Guinea from low lands to 500M altitude Some other species of the plant such as Anthocleista vogelli has been found in West African coast with some anticonvulsant activities [2].

Different parts of this plant have been claimed to serve various purposes in different parts of the world especially the West African coast where it is used mainly for the treatment of infections and skin rashes [3]. The stems are sometimes hollowed out in Northern Nigeria for use as quivers, hence the Hausa name Kwari [3].

The root decoction is used in Ivory Coast as a poison-antidote for leprosy and also for the treatment of oedema and elephantiasis of the scrotum [4]. While in Sierra Leone the root decoction is used in the treatment of Gonorrhea [5]. Previous studies of the methanolic extract of the root of Anthocleista vogelli showed some antimicrobial activities against Bacilus subtilis [6]. Alcoholic extract of the dried leaves also exhibited intestinal motility of the guinea pig and relaxed the smooth muscle of rabbit [7]. There is however no report on the antibacterial properties of the roots and stem of this plant.

This report therefore presents studies on the antimicrobial properties of the methanolic, petroleum ether and water extract of the roots using standard and local strains of bacteria.

MATERIAL AND METHODS

Plant collection : The roots of Anthocleista djalonensis were collected based on ethnopharmacological information and with the assistance of a traditional healer Olatunde Aliyu of Number 10 Ladipo Street, Mushin, Lagos. The roots were collected along Lagos/Badagry express way in Lagos state of Nigeria in the months of May through June 2001. The botanical identity of the plant and its roots was authenticated at the Department of Pharmacognosy, College of Medicine of the University of Lagos, Idi-Araba and the Forestry Research Institute of Nigeria, Ibadan by comparison with a herbarium samples. Immediately after collection, the roots were cut into small pieces and oven dried at 40 C for 5 days. The dried roots were pulverized to a smooth powder using impact mill, weighed and kept for further analysis in a clean polythene bag for easy identification.

Extract preparation: The technique described by Irobi and Daramola 1994. was used with slight modification. 300g of the powdered material were separately mixed with 700ml distilled water, methanol (95%) and petroleum ether for 2 days. Each mixture was stirred every 24hr using a sterile glass rod and at the end the extracts were passed through Whatman filter paper No.1. The filtrates were concentrated in vacuum at 60 °C and stored in universal bottles and refrigerated at 4 °C prior to use.

Micro-organisms : The standard strains of Escherica coli ATCC 25922, Staphylococcus aureus ATCC 25923, Pseudomonas aeruginosa ATCC 27853 and the yeast Candida albicans were all supplied by the Medical Microbiology Department of the College of Medicine, University of Lagos.

Preparation of Medium: Mueller-Hinton Agar was supplied by the Department of Medical Microbiology and Parasitology of College of Medicine, Idi-Araba, Lagos for preparing the medium . The pH of the medium was adjusted with 0.1N HCL to pH 7.2-7.4 before autoclaving. This was refrigerated for further use.

Preliminary screening of the extracts: 2g quantity of the extracts were reconstituted in 50ml of the extracting solvents and thereafter tested by agar diffusion technique for antibacterial activity [11].
The extracts were tested at 15, 10 and 5mg/ml concentrations which were delivered into four wells (6mm in diameter) bored unto the surface of Mueller Hinton agar plates previously inoculated with 10 micro-organisms/ml.
After incubation at 37 °C for 24hrs the presence of zones of inhibition around the wells was interpreted as a preliminary indication of antibacterial activity.

Antimicrobial susceptibility testing: The susceptibility assay was carried out with 10mg/ml concentration of each of the extracts with bacterial suspensions of 10 organisms/ml . Ampicillin 10mg/ml (Beecham) and 10mg/ml Niridazole (Janssen-Cilag) served as positive control for the antibacterial and antifungal activities. The plates were incubated at 37 °C for 24hrs while the plates containing the Fungi were incubated at 25 °C for 48hrs. After incubation zone of inhibition around the wells and the disc were measured with ruler and recorded.

Minimum Inhibitory Concentration: The agar diffusion method described by Ver-poorte was used [12]. The extracts were incorporated into Mueller Hinton broth at concentrations ranging from 0.01- 10mg/ml. A control tube containing the growth medium and each of the bacteria was also set up. The mixtures were incubated at appropriate temperatures of 37 °C for 24hrs and 25 °C for 48hrs for the bacteria and fungi respectively. The minimum inhibitory concentration (MIC) of the extracts was regarded as the lowest concentration of the extract that did not permit and turbidity or growth of the test organism

RESULTS.

Table 1. showed that the methanolic extract of the roots of Anthocleista djalonensis possess greater antibacterial and antifungal properties against S. aureus, E. coli, P. aeruginosa and C. albicans as compared to the water extract respectively . The petroleum ether extract had little or no effect on the organisms tested hence no further action was carried out with this fraction.
The methanolic extract Table 2. produced greater inhibitory action against S.aureus , E. coli, P. aeruginosa and C. albicans as against the water extract when compared with the standard ampicillin 50mg/ml and Niridazole 50mg/ml that was used. The minimum inhibitory concentration (MIC) of the methanolic extract (0.25-4.68mg/ml) were lower than those of the water extract of (0.67- 12.62mg/ml) as can be seen in Table 3.

The higher MIC value of 4.68mg/ml obtained with the methanolic extract and the 12.62mg/ml for the water extract against P. aeruginosa showed that the extracts are not very active against this particular organism.

Table 1
Preliminary screening of the root extracts of Anthocleista djalonensis chev. For antimicrobial activities.

Micro-organisms	Methanolic ext. (mg)				Hot water ext. (mg)				Pet. Ether ext. (mg)			
	Neat	15	10	5	Neat	15	10	5	Neat	15	10	
Staph. Aureus	3+	3+	3+	2+	3+	3+	3+	2+	+	+	-	-
E. coli	3+	3+	3+	+	2+	2+	+	-	-	-	-	-
P. aeruginosa	3+	3+	3	+ 2+	+	+	+	-	+	-	-	-
C. albicans	3+	3+	2+	+	3+	3+	3+	2+	+	+	-	-

Key: 3+, 2+, and + presence of anti-microbial activity, - absence of activity

Table 2.
Antibacterial susceptibility pattern of root extract of Anthocleista djalonensis chev.

Micro-organisms	Mean S.E.M. Zone of Inhibition (mm)[a] Methanolic ext. 10mg/ml	Water ext. 10mg/ml	Ampicillin 10mg/ml
Staphylococcus Aureus	18.02 0.7	16.32 0.8	22.14 1.3
Escherichia Coli	21.62 1.8	19.52 1.2	21.85 0.6
Pseudomonas Aeruginosa	14.98 0.5	10.68 0.4	22.49 0.1
Candida Albicans	13.121.6	11.48 0.2	Niridazole 10mg/ml 17.341.3

a = Mean of four assays.

Table 3.
MIC values showing the bacterial properties of root extracts of Anthocleista djalonensis.

Micro-organism	Methanolic ext. 10mg/ml	Water ext. 10mg/ml	Ampicillin 10mg/ml
Staphylococcus Aureus	0.25	0.67	0.06
Escherichia Coli	1.87	-	0.07
Pseudomonas Aeruginosa	4.68	12.62	0.88
Candida Albicans	2.98	4.52	Niridazole 10mg/ml 0.12

DISCUSSION

The fact that the crude extract of the roots of Anthocleista djalonensis produced zones of inhibition against gram - negative organisms such as P. aeruginosa and E. coli and gram-positive organisms, S. aureus and yeast fungus, C. albicans indicate the presence of potent antibacterial activity which confirms its use as anti-infectives . However the petroleum ether extract did not show any appreciable effect on the test organisms. This could be due to the chemical constituents of which were found to be mainly fatty acids and glycerides . Although both the methanolic and water extracts of Anthocleista djalonensis root

produced inhibitory actions against the bacteria and the fungus in test, the latter had more inhibitory action as shown by the larger zone of inhibition and lower inhibitory concentration.
This also suggest that methanol would be a better solvent of choice for extraction to isolate the antibacterial or antifungal principles. However the most susceptible of all the micro-organisms tested was Staphylococcus aureus. This organism and Candida albicans have been implicated in pathology of skin diseases
From the investigation carried out, it shows that at low doses of 0.25 12.62mg/ml the crude extract of the methanolic or probably the water extract would inhibit the effect of the aetiologic agents causing these infections (rashes). This gives credence to its ethnopharmacological use as a remedy for skin infections.

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