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Phytochemical and antimicrobial screening of three Nigerian medicinal plants used to treat infectious diseases traditionally

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Abstract

Three medicinal plants: *Carpolobia lutea, Curculigo pilosa* and *Strophanthus hispidus* used in the treatment of infectious diseases and other ailments traditionally were screened for secondary metabolites and antimicrobial activity. All the three plants contained saponins; *C. lutea* and *S. hispidus* contained cardenolides, while only *C. lutea* contained alkaloid in detectable quantity. Using agar cup diffusion method the crude extracts of the plants inhibited most of the test organisms: *Staphylococcus aureus* NCTC 6571, *Bacillus subtilis, Escherichia coli* NCTC 9001, *Pseudomonas aeruginosa* NCTC 6570, *Aspergillus niger* and *Candida albicans* at concentrations 10–100mg/ml. *S. hispidus* extracts were the most active. The study has demonstrated the antimicrobial potential of the plants and the basis for their inclusion in herbal preparations used to treat infectious diseases.

Keywords: Phytochemical screening; *Carpolobia lutea; Curculigo pilosa; Strophanthus hispidus.*

Introduction

Medicinal plants have continued to attract attention in the global search for effective antimicrobial agents that can combat resistant pathogens that have been rendering many conventional drugs obsolete in the treatment of infections (Cox, 1990). There are many medicinal plants used in Nigeria for the treatment of infectious diseases traditionally whose antimicrobial potentials and secondary metabolites have not been established.

Carpolobia lutea G. Don (Polygalaceae), *Curculigo pilosa* Engl. (Hypoxidaceae) and *Strophanthus hispidus* DC (Apocynaceae) have been employed traditionally to treat gonorrhoea, dysentery, cough, ulcers and some other ailments in Southwestern Nigeria and elsewhere (Dalziel, 1937; Irvine 1961; Abdurahaman and Omede 1990; Burkill 1985, 1994 and 1997). *Capolobia lutea* (called *Òsúnsún* in Yoruba language) is a shrub or small tree known for its edible sweet fruits; stems as chewing and walking sticks and barks for medicinal purposes (Dalziel, 1937; Irvine, 1961; Iwu, 1993). *Curculigo pilosa* (known as *èpà ikún* that is, the food of the ground squirrel) is a stemless herb with short tuberous rhizome used to treat abdominal troubles in Yoruba land and as a

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purgative in Northern Nigeria (Dalziel, 1937; Dahlgren *et al.*, 1985; A.C.C.T., 1989; Burkill, 1994). *Strophanthus hispidus* (called *isàgere* in Yoruba land) is a climbing shrub with a milky sap, the roots of which are used to treat sexually transmitted diseases and other ailments (Dalziel, 1937; Burkill, 1985).

The three medicinal plants, despite their common use in the local treatment of ailments, are yet to be established phytochemically and antimicrobially. Reports of their pharmacological activities, if any, are very scanty. In this study, the plants were screened phytochemically to determine the secondary metabolites present and investigate their possible antimicrobial activities.

Experimental

Collection and preparation of plant materials: C. lutea (root bark), *S. hispidus* (roots) and *C. pilosa* (underground rhizomes) were collected in Ibadan and authenticated at the Forestry Research Institute of Nigeria (FRIN), Ibadan, where herbarium specimens have been deposited. Thirty grams of each plant parts, previously dried in the oven at 50^0C were extracted with successive quantities of chloroform and methanol in a Soxhlet extractor. The extracts were concentrated under reduced pressure in a rotary evaporator and the yields were recorded (Table 1).

Phytochemical Screening: Powdered samples of the plants were screened for the presence of saponins, alkaloids, cardenolides, tannins and anthraquinones using standard methods (Sofowora, 1982; Harbone, 1984). The results are shown in Table 2.

Antimicrobial Screening: Agar cup diffusion method was used to determine the activity of the crude extracts on the following Gram-negative and Gram-positive bacteria, and fungi: *Staphylococcus aureus* NCTC 6571; *Bacillus subtilis* (Lab. stock); *Escherichia coli* NCTC 9001; *Pseudomonas aeruginosa* NCTC 6750; *Aspergillus niger* and *Candida albicans* (Lab. stock).

A 0.1ml of a 10^{-2} dilution of an overnight broth culture of each organism (containing an inoculum size $10^6 - 10^7$ cells/ml) seeded into molten but cooled 20ml nutrient agar (pH 7.4 Oxoid) was used for bacteria and Sabouraud dextrose agar (pH 5.4 Oxoid) was used to prepare fungal plates by surface spread method (Reeves *et al*, 1978). The extracts prepared (using methanol) as 10 and 20mg/ml of *S. hispidus* and 20 and 100mg/ml of *C. lutea* and *C. pilosa* were dropped into the 7mm diameter wells bored in the agar. Ampicillin (Beecham, England) 25µg/ml and 1%w/v tioconazole (Pfizer, NY) 0.5mg/ml were used as positive control, and 50% methanol as negative control.. The plates were incubated at 37^0C for 24hr for bacteria and at 25^0C for 48-72hr for fungi. The diameter of the zone of inhibition was measured to determine antimicrobial activity. The test were performed in duplicates and average values were recorded (Table 3.)

Results and Discussion

Table 1 shows that percentage yields of extracts were higher with methanol than with chloroform except for *Strophanthus hispidus*. This may mean that the majority of the constituents are in highly polar form.

Phytochemical screening showed the presence of saponins in all the plants with *Capolobia lutea* showing the highest concentration. This is in accordance with the reported phytochemistry of the *Polygalaceae* family where many saponins have been isolated; e.g. saponosides were reportedly isolated from the root bark of *C. lutea* (Delaude, 1975; Mitaine-Offer *et al.*, 2002).The presence of cardenolides in *Curculigo pilosa* needs further investigation for possible action on the cardiac system, while the presence of steroidal saponins may be important in the chemical synthesis of cortisone and other important sex homones. Although, the

authors have not seen any phytochemical reports on *C. pilosa*, allied species from Asia, *C. orchioides* and *C. recurvata* have been reported to contain cycloartane triterpenes and showed predisposition to antitumour activity (Chifundera *et al*, 1994; Venukumar *et al*, 2002). The as yet unidentified alkaloids of *Carpolobia lutea* may be responsible for many of the biological activities (along with the saponins) reported in traditional usage e.g. invigorating tonic, aphrodisiac, antirheumatism and antiinfectives. (Irvine, 1961; Iwu, 1993; Burkhill, 1994).

Table 1: Percentage yield of plants on extraction

Plant	Part used	Solvent	% Yield
Carpolobia lutea	Root bark	Chloroform	4.54
		Methanol	24.40
Curculigo pilosa	Rhizomes	Chloroform	1.64
		Methanol	14.94
Strophanthus hispidus	Roots	Chloroform	5.12
		Methanol	4.72

Table 2: Results of Phytochemical Screening of Plants

Plants	Saponins	Cardenolides	Tannins	Alkaloids	Anthraquinones
Carpolobia lutea	+ + +	-	-	+ +	
Curculigo pilosa	+ +	+ +	+ +	-	+ +
Strophanthus hispidus	+	+ + +	-	±	

Key: Present = + + + (highest), + + (higher), + = high, ± (trace), - = absent

Table 3: Antimicrobial Screening of Extracts

Plant	Extract	Conc. (mg/ml)	Diameter of zone of inhibition (mm)					
			S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*Asp. niger*	*C. albicans*
Carpolobia lutea	Chloroform	20	10.0	-	-	-	NT	14.0
		100	13.0	-	-	-	NT	20.0
	Methanol	20	12.0					
		100	15.0	-	-	-	-	-
Curculigo pilosa	Chloroform	20	10.0	10.0	9.0	-	11.0	11.5
		100	11.0	11.0	11.0	12.0	15.0	14.0
	Methanol	20	11.0	11.0	11.0	-	11.0	11.0
		100	12.0	12.0	12.5	14.0	16.0	16.0
Strophanthus hispidus	Chloroform	10	10.0	10.0	10.0	9.5	12.5	10.5
		20	11.0	13.5	13.5	10.0	15.0	12.5
	Methanol	10	11.0	11.0	10.5	10.0	13.0	13.5
		20	11.5	14.0	14.5	10.0	18.0	13.0
Ampicillin		25µg/ml	20.5	19.0	18.0	15.0	NT	19.0
Tioconazole		0.5mg/ml	NT	NT	NT	NT	NT	20.0

Key: - = not active; NT = not tested; Cup diameter = 7mm; Methanol not active.

With respect to the antimicrobial activity recorded in Table 3, *Carpolobia lutea* extracts showed a significant antibacterial activity only on *S. aureus* and a notable anti-Candida activity, which was detected only in the chloroform extract. In the work of Malcom and Sofowora 1969, *C. lutea* was mentioned among other plants which had no activity on Gram-negative bacteria and fungi. This

report is consistent with their findings except that chloroform extracts of *C. lutea* were active on *Candida albicans* at 20 and 100mg/ml.

Curculigo pilosa extracts showed only a slight antimicrobial activity at 20mg/ml but very significant broad-spectrum activity (active on all the tested organisms) at 100mg/ml.

Strophanthus hispidus showed the highest activity among the three plants being active at 10mg/ml and more active at 20mg/ml. The extracts of *S. hispidus* were active on all the tested organisms.

The phytochemical screening has shown that the plants were rich in secondary metabolites like alkaloids, saponins, cardenolides and anthraquinones which are very important constituents when searching for pharmacologically active phytochemicals. Further, the antimicrobial activities recorded have justified the traditional use of the plants in the treatment of infections diseases and encouraged further studies with the view to isolating the specific active components of the plants.

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