PHYTOCHEMICAL AND ANTIMICROBIAL STUDIES OF LEAVES OF ACALYPHA RACEMOSA

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ABSTRACT
Phytochemical studies on the powdered leaves of *Acalypha racemosa* revealed the presence of alkaloids, tannins, flavonoids and terpenes. Antimicrobial activities of cold water, hot water and methanolic extracts were studied against standard organisms *(E. coli* NCTC 10418, *Staphylococcus aureus* NCTC *6571)* and a clinical isolate *(Candida albicans).* The cold water extract showed better antibacterial activity than the hot water and methanolic extracts. Similarly, S. *aureus* was more susceptible than *E. coli,* but *Candida albicans* was completely resistant to the extracts. The cold water extract showed activity with MIC range from 3.0mg/ml (against S. *aureus)* to 4.0mg/ml (against *E. coli).* The methanolic extract had MIC of 6.0mg/ml for the two isolates respectively, while the MBC values range from 5.0mg/ml *(S. aureus)* to 6.0mg/ml *(E. coli)* for cold water and 7.0mg/ml for the two isolates (methanolic extract). The MBC of the cold water extract (6.0mg/ml) was able to cause 2 log cycle reduction of cell population in 90 minutes.

INTRODUCTION

Acalypha racemosa Dawoudu, (Euphorbiaceae) is a herb widely distributed in tropical Africa and Asia. In Nigeria, it is found both in forest and Savannah regions [1]. The plant leaves are used in traditional medicine for treatment of sores and skin diseases among children popularly known among the Yorubas as "Ela"[2].

Medicinal plants are cheap and renewable source of pharmacologically active substances. Some of the well known Nigerian plants such as *Mitracarpus scaber* and *Acalypha ornate* have been known to exert some inhibitory effects on some bacteria and fungi [3, 4, 5].

In this paper, we report the results of a phytochemical screening of the leaves of *Acalypha racemosa* and the antimicrobial activities of the leaf extracts.

RESULTS AND DISCUSSION

A. Phytochemical studies

The results of phytochemical screening are shown in Table 1 It showed that the plant leaves contains alkaloids, flavonoids, tannins, terpenes, mucilages and absence of saponins.

B. Microbiologica stidies

Initial assessment of the antimicrobial activity of crude extract of *A. racemosa* revealed that the extracts possessed antibacterial activities, but was not inhibitory to *Candida albicans* at the maximum concentration of 10.0mg/ml used in these studies. 2.0 mg/ml of the cold water extract was able to cause marked inhibition of *Staphylococcus aureus,* while 5.0mg/ml of the same extract inhibited *E. coli* (Table 2). The results of the MIC and MBC determinations are presented in Table 2. Generally, the cold water extract was more active than the hot water and methanolic extracts. Similarly, *Staphylococcus aureus* appeared more susceptible to the extracts than *E. coli* (Tables 2, 3, Figure 1). Also 6.0mg/ml of the cold water extract was able to reduce the cell population from 10^6cfu/ml to $1\ 0^4$cfu/ml in 90minutes (S. *aureus)* and in 120 minutes *(E. coli)* respectively (Figure1)

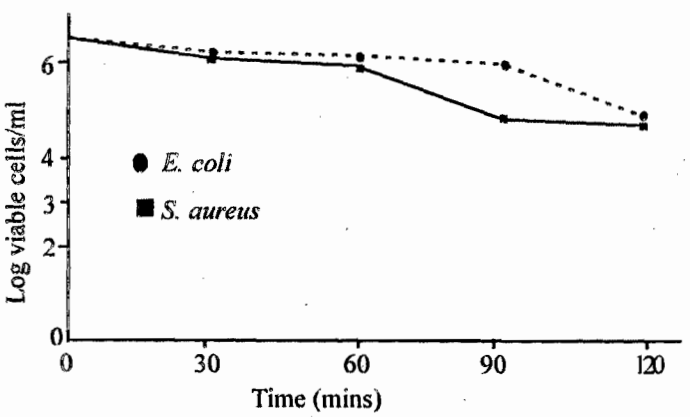

Figure 1: Survival of *S. aureus* and *E. coli* in a 6% (w/v) of cold water extract of *Acalypha racemosa*

The observed antibacterial actions of extracts of *A.. racemosa* in this study is significant and therefore justifies the use of the plant in Nigerian ethnomedicine. There is a need to conduct toxicity studies on the plant extracts so as to ascertain their safety in human systems.

Table 1: Phytochemical screening *of Acalypha racemosa.*

Constituents	Occurrence
Alkaloids	+
Flavonoids	+
Tannins	+
Mucilages	+
Terpenes	+
Saponins	-

KEY: + = present
- = absent.

Extraction

50g of the powder was extracted with 250ml petroleum ether (60° - 80°C) by maceration with constant shaking for 6 hours using electric shaker. The extract was filtered and the marc dried. The dried marc was extracted with 250ml ethanol using the same method and left to stand for 18 hours, and then filtered. The dried marc was then soaked in 250ml of distilled water applying similar method. All the extracts concentrated at 50°C and used for phytochemical screening.

The cold water, hot water and methanolic extracts were prepared by macerating 50g of the powdered leaves in 50ml of the solvent in a conical flask with constant shaking for about 18 hours [7]. T he extracts were evaporated to dryness on a water bath in case of the methanol extract, and by freeze-drying for cold and hot water extracts. These were finally used in the antimicrobial studies.

Table 2: Zones of cell inhibition (mm) by extracts of *Acalypha racemosa* using the cup plate method

Test Organisms	Cold water extract					Hot water extract					Methanolic extract				
	Concentrations of extracts (mg/ml) and zones of inlubition (mm)														
	10	7.5	5	2	1	10	7.5	5	2	1	10	7.5	5	2	1
E. coli	19	14.5	12	0	0	18.5	15	12.5	0	0	19	14	11	0	0
S. aureus	23	21	15	10	0	21.5	17	12.5	0	0	24.5	18	15	0	0
C. albicans	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*

Table 3: The MIC and MBC of *Acalypha racemosa* extracts against the test organisms.

	Antibacterial activities (mg/ml)			
	Cold water extract		Methanolic extract	
Organisms	MIC	MBC	MIC	MBC
E. coli	4.0	6.0	6.0	7
S. aureus	3.0	5.0	6.0	7.0

EXPERIMENTAL

Plant collection and identification

The plant samples of *Acalypha racemosa* were collected at Samaru-Zaria in March, 1999. The plant was identified by the authors and further authenticated by Mallam Musa at the Herbarium unit of Department of Biological Sciences, Ahmadu Bello University, Zaria. (No. 2805)

The leaves were air-dried and complete dryness was achieved in an oven at a temperature not exceeding 60°C. The dried leaves were ground to coarse powder using mortar and pestle and sieved with 20 mesh sieve (British Standard). The powdered leaf was kept for the studies.

Phytochemical studies

The methods used were that published in Brain and Turner [7], Ciulei [8] and Trease and Evans [9].

Microbiological studies

Three microbial isolates were utilised in the initial assessment of antimicrobial activities of various extracts of the plant. These are *Escherichia coli* (NCTC 10418), *Staphylococcus aureus* (NCTC 675) and a clinical isolate of *Candida albicans.* The test organisms were purified and standardised to 106 cfu/ml using the standard spectrophotometric method (SP6 200 spectrophotometer; Pye Unicam ltd, England). The initial susceptibilities of the test organisms to the plant extracts were assessed using the agar cup plate method. One millilitre of the standardised culture was mixed with 20ml of sterile melted agar, poured into sterilised Petri dishes and allowed to set. The set agar was hardened at 4°C for *5* minutes, and cups bored into them with No 4 cork borer. The cups were filled with the desired concentrations of the plant extracts, allowed to stand for hours and then incubated at 37°C overnight.

The minimum inhibitory concentrations (MIC) of the plant extracts were determined using the agar dilution method. The desired concentrations of the extracts were prepared in 20ml sterile, melted agar, poured into sterile Petri dishes and allowed to set. Sterile paper discs (5mm diameter) were then placed firmly on the plates in triplicates/organism. The 20µl sample of the

standardised culture was then placed on such discs aseptically and incubated overnight. This method was adopted as it allowed a direct assessment of both the MIC and MBC for several organisms in the same experimental set-up). The lowest concentration that inhibited growth was taken as the MIC.

Paper discs in the MIC determination above that showed no visible growth around its edge was sub-cultured into recovery broths (supplemented with 3% Tween 80 and 0.5% egg lecithin) and incubated at 37°C for 48 hours. Concentrations at which there was no growth in the subculture were taken as the minimum bactericidal concentration (MBC) of the extract [10].

Determination of the rate of cell kill by the plant extract was based on the methods of Olonitola *et al* [3]. 10ml volume of the desired concentration of the plant extract (usually the MBC as obtained above) was prepared aseptically. This was inoculated with 10^6cfu/ml of the bacteria under test.

At different time intervals, 1 ml of the reaction mixture was withdrawn and ten-fold serial dilutions made in inactivating diluent (sterile normal saline with 3% Tween 80). lml of each dilution was mixed with 20m1 of 0.5% yeast extract - enriched melted nutrient agar (45°C) and plated out in duplicates. These plates were incubated at 37°C for 72hours and viable cells counted. The rates of cell death were characterised by means of the log cycle reduction in cell population.

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