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Antianaemic and Antimicrobial Activity of *Eremomastax speciosa*

[1]J.E. Okokon, [2]B.S. Antia, [1]A.E. Udoh and [1]M.M. Akpan
[1]Department of Pharmacology and Toxicology,
Faculty of Pharmacy,
[2]Department of Chemistry, University of Uyo, Uyo, Nigeria

Abstract: The ethanolic leaf extract of *Eremomastax speciosa* was evaluated for antimicrobial and antianaemic activities. The crude ethanolic extract, as well as n-hexane and acqueous fractions were tested against pure clinical cultures of *Staphylococcus aureus*, *E. coli*, *Candida albicans* and *Aspergillus niger*. The acqueous fraction showed a higher activity against all tested organisms except *A. niger*. This was followed by n-hexane fraction which showed a broad activity against all tested organisms. The crude extract was only active against *E. coli* and *Staph. aureus*. The ethanolic crude extract (500-2000 mg kg^{-1}) also demonstrated antianaemic property by significantly ($p<0.01$) elevating Red blood cell counts, packed cell volume, Haemoglobin concentration and white blood cell counts of rats treated with it. These findings justify the ethnomedical use of this plant.

Key words: Antianaemic antimicrobial, *Eremomastax speciosa*, ethanolic leaf extract

INTRODUCTION

Eremomastax speciosa (Hochst) Acanthaceae is a tropical stout erect multi-branched herb, which grows as a weed in the forests (Heine, 1966). Due to its numerous medicinal values the plants is cultivated by many Nigerians and Camerounians. The plant is use to treat dysentery, diarrhea, anaemia (Oben *et al*., 2006) as well as irregular menstruation and Spurious labour pains. The leaves are reported to be use in the treatment of fracture, hemorrhoids and urinary tract infection (Adjanohoun *et al*., 1996). Herbalists in Southern Nigeria make use of the leaves in the treatment of internal heat, infertility, burns, haemorrhage in women after child birth and skin escoriation due to fungal infection in babies. Oben *et al*. (2006) reported that the leaves contain alkaloids, flavonoids, saponin and tannins. Studies have also report on the antidiarrhoea (Oben *et al*., 2006) and antiulcer (Tan *et al*., 1996, 1999) activities of the plant. There is no previous *in vitro* antimicrobial and antianaemic activity of *Eremomastax speciosa* in literature to the best of our knowledge. Therefore, we report the antianaemic and antimicrobial activities of this plants on few clinical isolates.

MATERIALS AND METHODS

Preparation of Plant Extract

Eremomastax speciosa leaves were collected in July, 2005 at Ikot Okoro Village, Abak, Akwa Ibom State Nigeria and authenticated by Dr. Magaret Bassey a taxonomist in the Department of Botany, University of Uyo, Uyo, Nigeria Hebarium specimen was deposited at University of Uyo herbarium with voucher No. UUH 125. The fresh leaves were shade dried and reduced to powder. The

Corresponding Author: J.E. Okokon, Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria Tel: +234-802-345-3678

powder (500 g) was macerated in 95% ethanol for 75 h in a conical flask. The flask was shaken gently at intervals during the period of extraction, after which the ethanolic filtrate was concentrated at reduced pressure *in vacuo* at 40°C. The yield was 4.13% W/W. 20.09 g of the crude extract was dissolved in ethanol: H_2O mixture (1:1) and partitioned with n-hexane to yield a n-hexane soluble fraction (2.5 g) and water soluble fraction (12.7 g). The fractions were individually concentrated in vacuo at 40°C. The extract and fractions were stored in a refrigerator at 4°C until used for experiments reported in this study.

Test Organisms

Bacteria and fungi used were pure isolates obtained from stock cultures in Pharmaceutical Microbiology Laboratory, Faculty of Pharmacy, University of Uyo, Uyo. They included *Staphylococcus aureus*, *Candida albicans*, *Escherichia coli* and *Aspergillus niger*.

Animals

Albino wistar rats (120-176 g) and albino swiss mice (20-31 g) of either sex were obtained from the University of Uyo animal house. They were maintained on standard animal pellets and water *ad libitum*. Permission and approval for animal study was obtained from College of Health Sciences Animal Ethics Committee, University of Uyo.

Determination of LD_{50}

The LD_{50} of the extract was estimated using Swiss albino mice by intraperitoneal route using the method of Lorke (1983).

Antimicrobial Tests

The antimicrobial activity of the extract and fractions were determined using agar well diffusion technique (Adeniyi *et al*., 1996). Nutrient agar plates were each seeded with 0.1 mL of an overnight culture of each bacterial isolates, while the sabourand dextrose agar plates were each similarly seeded with each fungal strain. The seeded plates were allowed to set and then dried in the incubator at 37°C for 30 min. A standard cork borer of 5 mm diameter was used to cut uniform wells on the surface of the agar, into which was added 50 µL solution of each extract or fraction at varying concentrations of 25, 50, 100 and 200 mg mL^{-1}. Nutrient agar plates with bacterial isolates were incubated at 37°C for 24 h and sabourand dextrose agar plates seeded with fungal strains were incubated at 25°C for 72 h after which the diameters of zones of inhibition were measured. Standard drugs, Gentimicin and Nystatin were used for bacteria and fungi, respectively. All assays were carried out in triplicates.

Evaluation of Antianaemic Activity

The rats were weighed prior to treatment and randomly assigned on the basis of weight into 5 groups of 6 animals each. Animals in groups A, B and C were orally administered with 500, 1000 and 2000 mg kg^{-1} of the extract respectively, while animals in group E were orally given normal saline 2 mL kg^{-1} each day and served as control. Administration of the extract continued for 21 days between 8.00 and 9.00 am each day. Twenty-four hours after the last administration, the animals were anaesthetized with chloroform vapour and dissected. Whole blood was obtained by cardiac puncture and collected into anticoagulant treated (EDTA 0.77 m) sterile bottles. This was used for haematological study. Haemoglobin concentration was determined spectrophotometrically by cyanomethaemoglobin method (Jain, 1986), Total red blood cell and white blood cell counts were estimated according to the visual method of Dacie and Lewis (1975).

Statistical Analysis

All data obtained from the study were statistically analyzed using Student's t-test, values of $p<0.01$ were considered significant.

RESULTS

Acute Toxicity

Administration of *Eremomastax speciosa* leaf extract (1000 g-5000 mg kg^{-1}) did not produce any mortality although signs of writhing and body weakness were observed. The LD_{50} was calculated to be 5000 mg kg^{-1} according to Lorke (1983).

Antimicrobial Screening

Table 1 shows the diameters of the zones of inhibition exhibited by extracts and the fractions at various concentrations employed. The n-hexane fraction showed a broad Spectrum activity (against all the tested organism) with highest activity against *E. coli* and *A. niger*. Acqueous fraction was highly active against *Candida albicans*, *E. coli* and *Staphylocoecus aureus*. While the crude ethanolic extract was only active against *E. coli* and *Staph. aureus*. The activities of this extract and fractions were lower than that of the standard drug and were concentration dependent.

Antianaemic Activity

The administration of the ethanolic leaf extract of *E. speciosa* (500-2000 mg kg^{-1}) for 21 days resulted in a significant increase in RBC counts, Hb concentration, PCV and WBC counts (Table 2). The effect was observed to be more pronounced at the lowest and highest doses (500 and 2000 mg kg^{-1}). The levels of these haematological parameters were significantly ($p<0.05$) higher in extract treated rats than the control (Table 2).

Table 1: Antimicrobial activity of ethanolic leaf extract, n-hexane and acqueous fractions of *Eremomastax speciosa*

		Zone of inhibition (mm)				
Micro organisms	Conc. (mg mL^{-1})	Ethanolic extract	N-Hexane fraction	Acqueous fraction	Gentimicin	Nystatin
Candida albicans	200	-	5.20	17.00		20.00
	100	-	5.00	13.20		
	50	-	-	9.50		
	25	-	-	4.50		
Aspergillus niger	200	-	11.50	-		19.00
	100	-	10.20	-		
	50	-	7.50	-		
	25	-	3.00	-		
Escherichia coli	200	9.20	16.00	20.50	22.50	
	100	7.52	10.00	17.10		
	50	7.40	9.50	13.00		
	25	53.40	5.25	10.00		
Staphylococcus aureus	200	17.10	5.50	20.00	37.20	
	100	7.20	5.00	13.40		
	50	5.00	4.52	9.50		
	25	2.00	2.50	7.00		

Table 2: Effect of *Eremomastax speciosa* haematological parameters of rats

		Parameters			
Groups	Dose (mg kg^{-1})	RBC (X 10 µL)	Hb (g dL^{-1})	PCV (%)	WBC ($X10^3U\mu$)
A	500	4.6±0.15*	13.5±0.20*	42.0±0.25*	7.36±0.65*
B	1000	4.9±0.04*	14.7±0.35*	43.5±0.50*	7.64±0.4*
C	2000	4.9±0.04*	14.8±0.15*	44.0±0.05*	7.78±0.35*
D	Control	4.3±0.04*	12.0±0.30*	39.0±0.15*	11.3±0.52

*Values are expressed as mean±SEM; *$p<0.01$ vs control (n = 5)

DISCUSSION

In the study, the antimicrobial activity of the fractions and their spectrum of activity was greater than that of the crude extract. The activities of the fractions could have been enhanced through purification. The n-hexane and acqueous fractions exhibited both antibacterial and anti-fungal activities, though at varying degree with the acqueous fraction having a higher activity than n-hexane fraction except in the case of *A. niger*. The ethanolic crude extract was observed to be inactive against fungal strains tested which necessitated the purification of the extract for the same purpose. This enhanced the antifungal activity as observed in the fractions. The substances shrouding the antifungal activity of the ethanolic extract were eliminated during the course of purification. It is hoped that further purification will enhanced the activity of the fractions. The antimicrobial activities demonstrated by extract and fractions of this plant, therefore justify some of the enthnopharmacological claims about this plant in the treatment of infection related illnesses and are attributed to the chemical constituents of this plant.

The administration of the ethanolic extract to rats caused significant increases in haematological parameters; red blood cell count, packed cell volume, haemoglobin concentration and white blood cell counts. This could be attributed to the presence of alkaloids in the extract. Alkaloids such as theobromine have been reported to cause similar effects (Eteng *et al*., 2003) by inhibiting cyclic adenosine monophosphate (cAMP) phosphodiesterase leading to the accumulation of cAMP, which in turn stimulates the phosphorylation of proteins as well as proteins synethesis (Eteng *et al*., 1998). These findings justify its use in traditional medicine as blood tonic and/or antianaemic.

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