African Journal of Biotechnology Vol. 5 (19), pp. 1789-1791, 2 October 2006
Available online at http://www.academicjournals.org/ajb
ISSN 1684–5315 © 2006 Academic Journals

Short Communication

Phytochemical and antimicrobial studies on the leaves and stem of *Desmodium scorpiurus* Der (sw)

Ndukwe I. G.*, Habila J. D. Bello I. A and Okoh J. I.

Department of Chemistry, Ahmadu Bello University, Zaria, Nigeria.

Accepted 25 August, 2006

Phytochemical studies on the aerial parts of *Desmodium scorpiurus* Der (sw) revealed the presence of alkaloids, saponins, glycosides, steroids and carbohydrates. The petroleum spirit, chloroform and methanol extracts were screened for antimicrobial activity using clinical isolates of *Escherichia coli, Staphylococcus aureus, Salmonella typhi, Bacillus cereus, Streptococcus pyrogenes* and *Klebsiella pneumoniae.* The results showed that the plant is very active against *Pseudomonas aeruginosa, Escherichia coli* and *Streptococcus pyrogenes* with minimum inhibitory concentration (MIC) of 2 x 10^2 mg/ml.

Key words: *Desmodium scorpiurus*, crude extracts, antimicrobial activity, MIC.

INTRODUCTION

In our continuous search for plants with pharmacological properties *Desmodium Scorpiurus* which belongs to the family-Papilionaceae was investigated. The plant is commonly found in various parts of West Tropical Africa including Nigeria. *D. scorpiurus* and other related species have been variously utilized in a number of folk medicine (Watt et al., 1962; Dalziel, 1955). It is claimed to be of use in the treatment of constipation, cough, convulsion, haemauria, general venereal infections, ringworm etc. In this paper we are reporting the result of our investigations on the phytochemical and antimicrobial studies of the plant.

MATERIALS AND METHOD

Plant material

The aerial parts of the plant were collected from Zaria, Nigeria in August, 2005. The plant was confirmed and authenticated at the Herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria, and a voucher specimen, number 344, was deposited there.

*Corresponding Author. E-mail: ndukweilogeorge@yahoo.com

Preparation of plant material and extraction

The aerial parts of the plant were air dried under a shade, powdered to fine powder using a porcelain mortar and pestle. The powdered material weighing 79 g was packed into a soxhlet extractor and extracted exhaustively and successively with petroleum spirit (60 –80°C), chloroform and methanol. The various extracts were respectively concentrated *in vacuo* at 40°C using a *rota vapor* after which 2.34 g of petroleum spirit, 0.74 g of chloroform and 9.85 g of methanol extracts were, respectively, realized.

Phytochemical screening

The crude pulverized plant sample was screened for plant metabolites using standard techniques of Brain and Turner (1975) and the results are as shown in Table 1.

Clinical isolates

Clinical isolates of *Klebsiella pneumoniae, Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi* and *Beta-haemolytic streptococcus* obtained from the Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria, were used in this study. All cultures were tested for purity.

Table 1. Phytochemical screening of *Desmodium scorpiurus.*

Constituents	Occurence
Alkaloids	+
Saponins	+
Glycosides	+
Steroids	+
Flavonoids	-
Cardinolids	+
Carbohydrates	+

Key: + = present, - = absent

Table 2. Susceptibility results of the microorganism against the plant extracts.

Test Organism	Mean zone of inhibition (mm)								
	1000 mg/ml			750 mg/ml			500 mg/ml		
	P	C	M	P	C	M	P	C	M
Klebsiella Pneumoniae	17.0	18.0	19.0	14.5	14.5	16.0	10.0	10.0	12.5
Staphylococcus aureus	17.5	17.5	16.5	14.5	14.5	14.0	11.0	11.0	11.0
Bacillus cereus	18.5	16.5	18.0	16.0	14.5	15.5	13.0	11.0	13.0
Pseudomonas aeruginosa	12.0	12.0	13.5	10.5	11.5	12.5	10.0	10.0	11.5
Escherichia coli	10.5	10.0	10.0	9.5	9.0	9.0	9.0	8.0	8.0
Salmonella typhi	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Streptococcus	10.5	8.5	0.0	9.5	8.0	0.0	9.0	8.0	0.0

P = Petroleum spirit extract, C = Chloroform extract, M = methanol extract.

Preparation of media/inoculum

Nutrient agar obtained from Fluka Biochemika was used in the preparation of the media of the microorganisms and was prepared according to the manufacturer's standard of 28 g/1000 ml. It was then sterilized in an autoclave at 121°C for 15 min, cooled to 45°C and dispensed into sterile Petri-dishes, after which they were stored in the refrigerator until the need arose. Nutrient broth was also prepared and each culture prepared contained 10^6 cell/ml.

Crude extract prepaaration

Three concentrations 1000, 750 and 500 mg/ml of the petroleum spirit, chloroform and methanol extracts were prepared in distilled water (Table 2). Where an extract was insoluble in water, 10% solution of DMSO in water was used. This 10% solution of DMSO in water was used as control and there was no inhibition to the growth of the microorganisms all through in it.

Susceptibility tests

The sensitivities of all the organisms to the various extracts were tested using the cork and bore diffusion method of Bauer et al. (1966) and Barry and Thornsberry (1985). Inoculation of the prepared plates with the organism was done using a sterilized wire-loop to transfer a strand of the organism into the plate followed by cross-streaking with the same wire-loop to achieve uniform spread on the plate. A control was set up using 10% DMSO in water for each organism. The plates were incubated at 37°C for 24 h after which they were examined for zones of inhibition of growth.

Minimum inhibitory concentration (MIC)

The medium was nutrient agar solution which was prepared according to the manufacturers' standard of 28 g/1000 ml. In this case double strength was prepared by dissolving 28 g in 500 ml of distilled water which was swirled and mixed thoroughly by heating to allow uniform dissolution after which 5 ml of it was dispensed into 72 sets of universal bottles and sterilized in an autoclave at 121°C for 15 min. The agar was allowed to cool to 45°C and each graded solution was then mixed gently with molten double strength nutrient agar in a Petri-dish and allowed to solidify for one hour. Extracts' concentrations of 200, 100, 50, 25, 12.5 and 6.25 mg/ml were prepared by serial dilution (Table 3). Each plate was divided into six equal sections and labeled accordingly to correspond to six test organisms. Two 5 mm diameter paper discs (Whatman No.1) were placed aseptically into each labeled section of the plate using sterilized forceps. With an automatic micropipette, 0.1 ml of each bacterial suspension was taken and transferred aseptically and carefully into each appropriate pre-labeled paper disc on the agar plates. The plates were incubated for 24 h at 37°C after which they were observed for growths or death of the test organisms. The lowest concentration inhibiting growth was taken as the minimum inhibitory concentration (MIC).

Table 3. Minimum inhibitory concentration (MIC) results.

Test Organism	Concentration of extracts in mg/ml																	
	2 x 10^2			1 x 10^2			0.5 x 10^2			0.25x 10^2			0.125 x 10^2			0.0625 x 10^2		
	P	C	M	P	C	M	P	C	M	P	C	M	P	C	M	P	C	M
Klebsiella Pneumoniae	-	-	-	-	-	-	-	-	*	#	#	#	+	+	+	++	++	++
Staphylococcus aureus	-	-	-	-	-	-	-	-	*	#	#	#	+	+	+	++	++	++
Bacillus cereus	-	-	-	-	-	-	-	-	*	#	#	#	+	+	+	++	++	++
Pseudomonas aeruginosa	-	-	*	#	#	#	+	+	+	+	+	+	+	+	+	++	++	++

* = Clear solution (MIC), - = No growth, # = Cloudy solution (slight growth),
+ = Turbid solution (strong growth), ++ = Highly turbid solution (dense growth).

Table 4. Minimum bactericidal concentration (MBC).

Test Organism	Concentration of extracts in mg/ml								
	2 x 10^2			1 x 10^2			0.5 x 10^2		
	P	M	C	P	M	C	P	M	C
Staphylococcus aureus	+	+	+	++	++	++	+++	+++	+++
Bacillus cereus	-	-	*	++	++	++	+++	+++	+++
Pseudomonas aeruginosa	+	+	+	++	++	++	+++	+++	+++
Escherichia coli	-	-	*	++	++	++	+++	+++	+++
Streptococcus	+	+	+	++	++	++	+++	+++	+++

* = MBC, - = no growth, + = little growth, ++ = strong growth, +++ = dense growth.

Minimum bactericidal concentration (MBC)

This was carried out to know if the organisms could be killed completely or their growths could only be inhibited (Table 4). Another 72 sets of plates of nutrient agar were prepared according to the manufacturers' standard and sterilized in an autoclave as earlier described. The paper discs in all the plates from the MIC tests were re-activated. Emphasis was mostly paid to the MIC plates and the preceding plates. The re-activation was done in a mixture of 0.5% egg lecithin and 3% Tween 80 solution in a test tube. The reactivated organisms were sub-cultured into appropriately labeled quadrants of the sterilized nutrient agar plates using wire loop into each test tube and streaking uniformly on the labeled quadrants. This was then incubated for 24 h at 37°C after which they were observed for growths. The MBC was the quadrant with the lowest concentration of the extract without growth.

RESULTS AND DISCUSSION

The aerial parts of *D. scorpiurus* contain alkaloids, saponins, glycosides, steroids and flavonoids among others. These metabolites have varying pharmacological effects on animals. Scientists have shown that these metabolites play defensive roles in the plants producing them. For example, Haralampidis et al. (2001) reported that secondary metabolites have been implicated as chemical defenses against attack by soil fungi. In the same paper they further reported that many plants synthesize secondary metabolites as part of their normal programme of growth and development, often sequestering them in tissues where they may protect against microbial attack. From this work *D. scorpiurus* is seen to possess a wide range of antimicrobial activity against microorganisms of which *P. aeruginosa, S. aureus, E. coli, B. cereus, K. pneumoniae* and *Streptococcus* are examples. At a concentration of 2 x 10^2 mg/ml, all the extracts were able to completely destroy *Staphylococcus* and *Pseudomonas*. These bacteria are known to cause infections and other ailments. Therefore, the result justifies the use of the leaves and stem of *D. scorpiurus* in ethnomedicine.

REFERENCES

Barry AL, Thornsberry C. (1985). Susceptibility tests, Diffussion test procedure. J..Chem. Pathol. 19: 492-500.

Bauer AW, Kirby WMM, Sherris JC, Truck M (1966). Am. J. Clin. Pathol, 45(2): 493.

Brain KR, Turner TD (1975). The practical evaluation of phytopharmaceuticals, Wright-science technical.Bristol Britain, pp. 56-64.

Dalziel JM (1955). The useful plants of West Tropical Africa, Crown Agents for Colonies, London, pp. 238-239.

Haralampidis K, Bryan G, Qi X, Papadopoulou K, Bakht S, Melton R, Osbourn A (2001). Proceedings of the National Academy of Science of the United States of America. 98(23): 13431-13436.

Watt JM, Breyer-brandwijk GM (1962).The medicinal and Poisonous Plants of Southern and Eastern Africa, 2nd Edition, E &S. Livingstone Ltd. Edinburgh and London. p. 594.