SHORT COMMUNICATIONS

Antibacterial Activity of *Euphorbia poissoni* Pax Extracts

U. AJALI*, G. B. OKIDE, AND B. K. C. CHUKWURAH
Department of Pharmaceutical Chemistry,
University of Nigeria, Nsukka, Nigeria.

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The dried pulverised stem bark of *Euphorbia poissoni*, family Euphorbiaceae, was exhaustively and successively extracted with diethylether, chloroform and methanol using Soxhlet extractor. The methanol extract was fractionated into two: alkaloid extract and alkaloid free methanol extract. The extracts were screened for antimicrobial activity. The methanol and alkaloid free methanol extracts both showed antibacterial activity. The alkaloid free methanol extract was fractionated using preparative TLC (coated with silica gel 245 HF), developed with benzene:ethylacetate (9:1) to obtain 8 fractions. The fractions were screened for antibacterial activity and fractions, F1, F3, F4 and F5 showed activity. The chemical classes of the fractions and extracts were identified. Major constituents of the plant are alkaloids, tannins, saponins, glycosides, flavonoids and carbohydrates. Steroidal glycosides are the antibacterial agent of the plant.

Plants are able to synthesize a very wide range of chemical substances which are not necessarily involved in the essential metabolism of the cells. The therapeutic properties of herbs owe their activities to these chemical substances produced in the plant. As there is need to add more drugs into therapeutic use since some diseases are developing resistance to most of the existing drugs and some others do not have appropriate therapeutic agent, research on plants intensifies as this may unlock more active therapeutic agents in the plants[1].

Euphorbia poissoni is an erect shrub and is six feet high. The branches are silvery grey and stout. Leaves are pale green and deciduous. Flowers are greenish with red stamens. Rudimentary spine are some times present only on young plants[2]. The fleshy obovate leaves of *Euphorbia*, which is deeply notched at the apex, resemble those of *Euphorbia desmondi* but *E. des*mondi is different because of its angled branches or pair of spines[3]. *E. poissoni* is commonly found in the savanna.

*For correspondence

A piece of the stem is commonly mixed with other ingredients in preparing arrow poison[4]. The latex is a powerful irritant to mucous membranes and in direct contact with the tissues, but has no systemic action on either heart or nervous system[1]. The juice is added to food or drinking water as poison. The antidote is cow's milk. In Sokoto province of Nigeria, the plant is used as a fish poison. The latex is added to tabacco snuff to increase it pungency. It is used to relieve toothache. It is poisonous preparations for catching guinea fowl. Aqueous solution of the stem bark of the plant is used for bath in treatment of skin infections[5]. From other plants in the genus; anthraquinone, glycosides, alkaloids, fixed oil[6], flavoncids and vitamin B[7], have been isolated but not from *Euphorbia poissoni.*

The objectives of this work are to detect the classes of compounds present in the plant and to examine the antimicrobial properties of the plant. The work will go further to detect the class of the compounds that has the antimicrobial activity using bio-guided extraction techniques.

The reagents are obtained commercially and used as supplied. Methanol, chloroform, diethylether are product of

May and Baker, England, while toluene, diethylamine, benzene, ethylacetate and silica gel 245HF are products of Merch, Darmstadst. The reference drugs used are nystatin an antifungal from Squibb and chloromycetin an antibacterial from Parke Davis. The standard microorganisms used were *Bacillues subtilis* (NCTC 8326), *Staphylococcus aureus* (NCTC 3761), *Escherichia coli* (NCTC 9001), *Pseudomonas aeruginosa* (NCTC 6750), *Salmonella typhi, Candida albicans* and *Aspergillus niger* (laboratory strain). These were standard cultures ontained from the Pharmaceutical Microbiology Department of the University.

Stem bark of Euphorbia poissoni PAX (Euphorbiaceae) was harvested from Nsukka in Nigeria and was authenticated in the Botany Department of the University. A voucher specimen has been deposited in the Herbarium of Department of Botany of the same University. The stem bark was air dried and pulverised.

The dried coarse powder (100 g) of *Euphorbia posissioni* was extracted exhaustively and successively with 500 ml each of diethylether, chloroform and methanol using a Soxhlet extractor and the extracts were named De, Ch, Me extracts, respectively. The methanolic extract was extracted with 200 ml of 0.1 N HCl in water and the residue was collected. The filtrate was treated with 200 ml of 0.1 N NaOH in water and a precipitate was formed and was collected and called alkaloid extract (Me3). The liquor was concentrated to dryness and mixed with the insoluble part form O.1 N HCl in water (the extract is called alkaloid free methanolic extract Me2).

Preparative thin layer chromatography (PTLC) plates (20 cm by 20 cm) were coated to a thickness of 0.25 cm with silica gel 254HF using a standard method with Kenso CJK 520 spreader[8]. The coated plates were dried at room temperature and activated in the oven for 1 h at 110°. It was cooled to room temperature.

The activated plates were spotted (band) with methanol solution of the Me2 extract at a distance of 1 cm from the origin. The spotted plates were allowed to dry at room

TABLE 1: ANTIMICROBIAL ACTIVITY OF *EUPHORBIA POISSONI* FRACTIONS[a].

Samples	*S. aereus*	*B. subtilis*	*S. typhi*	*P. aeruginosa*	*E. coli*	*A. niger*	*C. albicans*
F1	300	380	250	-	-	-	-
F2	-	-	-	-	-	-	-
F3	100	120	80	-	-	-	-
F4	200	150	120	-	-	-	-
F5	180	150	120	-	-	-	-
F6	-	-	-	-	-	-	-
F7	-	-	-	-	-	-	-
F8	-	-	-	-	-	-	-
Me	1150	900	560	-	-	-	-
Me2	800	690	420	-	-	-	-
Me3	-	-	-	-	-	-	-
Ce	-	-	-	-	-	-	-
De	-	-	-	-	-	-	-
Chloromycetin	6.5	6.5	6.5	6.5	10.0	-	-
Nystatin	-	-	-	-	-	20.0	14.0

F1 to F9 are alkaloid free methanol extract (Me2) fractions, Me, Ce and De are methanol, chloroform and diethylether extracts, respectively. Me2 and Me3 are the alkaloid free extract and alkaloid extract, respectively. -means not determined since inactive in the agar diffusion assay. [a]The values are minimal inhibitory concentration (MIC) in μg/ml.s.

temperature and were developed in the solvent system (benzene:ethylacetate, 9:1). The solvent system was allowed to travel a predetermined distance of 15 cm from the origin, the plates were removed from the chamber and dried. The developed plates were viewed under UV light and some were sprayed with standard reagents to locate the bands[9]. The located bands were scrapped out and each eluted with enough quantity of methanol. The solvent of the filtrates was distilled off. The R_f values and weights of the fractions were determined.

The antibacterial and antifungal activity of the fractions was studied using agar diffusion assay method[10]. Dimethylsulphoxide (DMSO) was used to dissolve the fractions. The minimal inhibitory concentration (MIC) was determined by the agar dilution method[11]. Nystatin and chloromycetin were used as standard drugs.

An isolated colony of each organism was transferred into the appropriate medium and propagated consecutively for 5 d. One hundred-fold serial dilution of the 24 h broth culture was made. The inoculum (0.2 ml of each of the bacterial culture) was added into 20 ml of molten agar and properly mixed. This was poured into sterile petri dishes and was allowed to set for 10 min. Then using a 8 mm cork borer, wells were made on the agar plates and into the holes were added the solutions of each fractions. The solutions were allowed 30 min to diffuse in the agar medium. The inoculated agar petri dishes were incubated at 37° for bacteria and at 25° for fungi for 24 h. The zones of inhibitions were measured and recorded.

In the determination of the MIC, the agar petri dishes were divided into four sectors. A hole was made in each sector as before. Different concentrations of the extracts that were sensitive to the organisms were made using two-fold dilutions method. These concentrations were made, transferred into the holes and were allowed 30 min to diffuse in the agar medium. The inoculated agar petri dishes were incubated at 37° for 24 h. And the zones of inhibitions (IZD) were measured as the difference of the diameter of the circle of inhibition and the diameter of the cork borer. The MIC was obtained from the intercepts of log concentration axis of a graph of IZD^2 against log of concentration.

The classes of constituents present in various extracts were detected using standard methods and standard reagents. The classes of compounds detected are alkaloids, saponins, tannins, glycosides, anthracene glycosides, cardiac glycosides, steroidal glycosides, flavonoids, carbohydrate and reducing sugar.

The De, Ch and Me extract gave 4.0, 8.0 and 6.4% (w/w) yield of the dried plant, respectively while the Me2 and Me3 gave 63 and 34.1% of the Me extract. From the phytochemical test, the presence of saponins, alkaloids, tannins, steroidal glycosides, saponins, carbohydrates and reducing sugars were detected in Me extract. Steroidal glycosides, saponins, carbohydrates and reducing sugars are found in Ce extract while De extract contained cyanogenetic glycosides, cardiac glycosides, steroidal glycosides, saponins, flavonoids, carbohydrates and reducing sugars. The Me2 contained tannins, steroidal glycosides, saponins, carbohydrates and reducing sugars while Me3 had only alkaloids.

Antimicrobial screening showed that the Me extract and

TABLE 2: SOME PHYSICAL AND PHYTOCHEMICAL PROPERTIES OF THE ALKALOID FREE METHANOL EXTRACT (ME2).

Fractions	R_f values	% yield[a] (w/w)	Chemical class
F1	0.00	40.0	tannins, saponins, glycosides
F2	0.18	7.3	saponins
F3	0.25	8.4	sterodial glcosides
F4	0.45	10.2	sterodial glcosides
F5	0.54	6.5	sterodial glcosides.
F6	0.65	5.4	saponins
F7	0.78	5.0	tannins
F8	0.88	5.0	saponins

[a]Mens percentage yield calculated on the basis of dried quantity of alkaloid free extract.

the Me2 extract possessed antibacterial activity. The extracts showed no antifungal activity against the microorganisms tested and the results are shown in Table 1. From these results it can be concluded that the alkaloids, flavonoids, cardiac glycosides, cyanogenetic glycosides of the extracts have no antimicrobial activity. This equally proves that the antibacterial agents of the plant are polar.

On further fractionation of the alkaloid-free methanol extract, eight bands were obtained. The Rf values, chemical classes and percentage yield of these bands are reported in Table 2. Out of the eight bands only three showed antibacterial activity and they belong to steroid glycosides. Fractions belonging to the same class of compounds in a plant are assumed to have the same biosynthetic origin hence the same basic structural unit responsible for antibacterial and antifungal activities in a plant[12]. These fractions may have the same basic structural unit. As some of the extracts show activity against some bacteria, the antibacterial potential has to be further investigated.

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Visible Spectrophotometric Methods for Estimation of Clarithromycin from Tablet Formulation

I. SINGHVI
Department of Pharmaceutical Sciences,
M. L. Sukhadia University, Udaipur-313 001.

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Three simple visible spectropbotometric methods have been developed for the estimation of clarithromycin from tablet formulation. The first developed method is based on formation of orange-red coloured complex of drug with concentrated hydrochloric acid and acetone. The coloured complex shows absorbance maxima at 485 nm and obeys Beer's law in concentration range of 50-500 μg/ml. Second and third developed methods are based on formation of chloroform extractable complex of drug with bromocresol green and bromopbenol blue, respectively, both of which show abaorixince maxima at 414 nm and linearity in concentration range of 0-60 μg/ml of drug. Results of analysis for all the methods were validated statistically and by recoveiy studies.

Clarithromycin, chemically 6-O-methyl erythromycin is a semi-synthetic macrolide antibiotic used in the treatment of respiratory tract infection and in skin and soft tissue infections[1]. The drug is not yet official in any of the pharmaco-