GLOBAL JOURNAL OF PURE AND APPLIED SCIENCES VOL. 6 NO. 1 JANUARY 2000: 83-87
 ISSN 1118-0579

STUDIES ON CERTAIN CHARACTERISTICS OF EXTRACTS OF BARK OF PAUSINYSTALIA JOHIMBE AND PAUSINYSTALIA MACROCERAS (K.SCHUM.) PIERRE EX BEILLE

OLUWOLE A. SODIPO, JOHN A. AKINNIYI and JOSEPHINE V. OGUNBAMERU

(Received 30 March 1999; Revision accepted 29 June 1999)

ABSTRACT

Phytochemical screening revealed the probable presence of alkaloids, saponins, phlobatannins, anthraquinones, coumarins and cardiac glycosides in the bark of two species of *Pausinystalia (P. macroceras* and *P. johimbe)*. Besides, the results also showed that while tannins and combined anthraquinones were probably present in *P. johimbe*, the same extract of *P. macroceras* was probably devoid of them. Extracts, phosphate buffered saline (PBS) and methanol/phosphate buffered saline (M/PBS) of the same sample were also screened for potential haemolytic activity. The results showed that the two extracts were haemolytic with human red blood cells. However, generally the haemolytic activities of the M/PBS extracts of the two species of plant were higher than those of PBS extracts, using the various types of blood groups (O,A,B,AB). The foamability of the extracts was also investigated. The results showed that PBS extracts of *P. macroceras* and *P. johimbe* produced long-lasting foams of 67.39 ± 9.03 hr and 392.55 ± 4.44 hr respectively. Moreover, while the M/PBS extract of *P. johimbe* produced a long-lasting foam of 283.43 ± 8.49 hr, that of *P. macroceras* was only 0.11 ± 0.01 hr. The results have been discussed with respect to the probable presence of saponins in the samples. The potentials of this class of secondary metabolites of plant origin in medicine pharmaceutical industry and technology have also been highlighted.

Key words: Saponins, *Pausinystalia johimbe*, *P. macroceras*, bark extracts.

INTRODUCTION

Pausinystalia johimbe and *P. macroceras* are trees which extend from South-Western Nigeria to Gabon and Democratic Republic of Congo (Keay, 1989), *P. johimbe* is commonly called 'idagbon' (Yoruba), while *P. macroceras* is known as 'abo-idagbon' (Yoruba), 'nikiba' or 'likiba' (Edo), 'jombe-wà' or 'djombe-wa-njombe' (Duala, Cameroons) and 'nloue (S. Cameroons) (Dalziel, 1937; Keay, 1989). *P. johimbe* is a tall forest tree of about 30.5 m with distinctive umbel-like clusters of white flowers, sometimes yellowish and arranged in panicles among the leaves at the end of the shoots (Dalziel, 1937; Keay et al., 1964). The fruits are spindle-shaped, about 1.91 cm long, with winged seeds which are narrowly elongated, about 0.64 cm long. The bark is grey, flaking off in patches and brown beneath; slash pinkish to brown and sometimes yellow beneath. The twigs and branches are rather knobbly. The tree of *P. macroceras* seldom exceeds 18.0 m in height and 1.2 m in girth. The flowers and fruits are similar to those of *P. johimbe*, but the leaves are smaller, elliptic or vaguely oblanceolate, cuneate at the base and fairly long stalked. The bark is also grey, rapidly darkening to brownish or yellowish and fibrous (Keay et al., 1964).

The bark of *P. johimbe* is reputed in traditional medicine for being an aphrodisiac. It yields two alkaloids yohimbine and yohimbinine.

Yohimbine is poisonous, exerting a local anaesthetic action similar to cocaine, but without dilating the pupil and without a harmful effect on the cornea. It is an aphrodisiac mainly in veterinary medicine. In contrast, yohimbinine has no physiological action. The closely related specie, *P. macroceras*, sometimes called 'false yohimbine' is richer in yohimbinine than the active yohimbine (Dalziel, 1937).

Commercially, the bark appears in the form of thin, flat or curved pieces of grey-brown and red tinged colour, longitudinally and transversely fissured, and corky externally with a soft velvet texture. The bark of *P. johimbe* which is used for medicinal purposes can be distinguished from P. macroceras by the bushy hair on its broken surface which produce an itching or tingling effect on the skin. Traditionally, the bark is stripped into 0.9m from the felled tree, dried and then sold in the market. The high demand

OLUWOLE A. SODIPO, Department of Biochemistry , University of Port Harcourt, P.M.B. 5323, Port Harcourt, Nigeria
JOHN A. AKINNIYI, Department of Biochemistry , University of Port Harcourt, P.M.B. 5323, Port Harcourt, Nigeria
JOSEPHINE V. OGUNBAMERU, Department of Biochemistry , University of Port Harcourt, P.M.B. 5323, Port Harcourt, Nigeria

Table 1: Phytochemical Analysis of Bark of Species of *Pausinystalia*.

Species of *Pausinystalia*	Test*											
	Alkaloids		Others						Cardiac Glycosides			
	Wagner's	Mayer's	Tannins	Saponins	Phlobatannins	Anthraquinones	Combined anthraquinones	Coumarins	Legal	Lieberman's	Salkowski's	Keller-Killiani
P. johimbe	+	+	+	+	+	+	+	+	+	+	+	+
P. macroceras	+	+	-	+	+	+	-	+	+	+	+	+

* +, positive; -, negative.

for the bark in the early 20th century led to excessive cutting down of the trees. And since the plant does not reproduce from the stump of the cut shoot but from bark itself in two years when carefully cut by special methods from the standing plant, the trees are not common any more (Dalziel, 1973). In addition to this, the farming activities of man, deforestation as well as use of the wood as a substitute for mahogany and for railway work, bridge piles, etc., also combine to make the tree a scarce commodity.

The presence of alkaloids in the bark has been documented (Dalziel, 1973). This study was carried out to further screen the bark for the presence of some other natural products.

MATERIALS AND METHODS

SAMPLE COLLECTION

Dried samples of the bark of *P. johimbe* and *P. macroceras* were supplied by Prof. J. A. Akinniyi of the Department of Chemistry, University of Maiduguri. Verification of the samples was carried out by the Botany Section of the Department of Biological Sciences and voucher specimens deposited in the Herbarium of the same University and also available from the authors.

PREPARATION OF EXTRACTS

The dried samples were each ground into a fine powder to pass through a 0.25 mm mesh (Endecott's Ltd, London), using a blender (Moulinex Blender Mill 2, Surrey, England). Each of the ground sample (1 g) was extracted with 50 ml of phosphate buffered saline (PBS), pH 7.2 for 10 min at 100°C. with occasional shaking. The mixture was filtered hot and filtrate stored at 4°C (Thermocool 250, Thermocool Eng. Co. Ltd. Nigeria) until required.

Another portion (1 g) of the ground sample was extracted with 50 ml boiling methanol for 10 min. with occasional shaking. The methanol in the filtrate was distilled off at 65°C and residue dried *in vacuo* (LTE Qualivac Vacuum oven, Bard and Tatlock, Ltd., London). The residue was then re-suspended in 50 ml PBS to produce the methanol/phosphate buffered saline (M/PBS) extract which was also stored at 4°C (Thermocool 250, Thermocool Eng. Co. Ltd., Nigeria).

PHYTOCHEMICAL ANALYSIS

Phytochemical analysis for various plant constituents including alkaloids, saponins, tannins, phlobatannins, anthraquinones, coumarins and cardiac glycosides was carried out using the methanol extract (Sofowora, 1984).

FOAM-FORMING ACTIVITY

Foam-forming activity was measured using essentially the method of O'Dell et al., (1959). A 5ml portion of each extract (PBS and M/PBS) was shaken vigorously for 1 min in a 15ml graduated stoppered centrifuge tube, using a Griffin Vortex Mixer/Shaker (Gallenkamp & Co. Ltd., England). The foam height produced after 1 min was measured and the time taken for the foam to disappear completely.

TREATMENT OF ERYTHROCYTES

Fresh blood (A, B, AB and O) was collected into clean, sterile tubes containing ethylenediamine tetraacetic acid (EDTA) and used within 24 hr. They were centrifuged at 3,000 rpm for 5 min. (Hettich, Universal II, Western Germany) and the packed cells washed (x 4) with PBS, pH 7.2 and the supernatant

Table 2: Foam Forming Activity of Extracts of Bark of *Pausinystalia*

Species of *Pausinystalia*	Extract	Foam-Forming Activity*	
		Foam Height (mm)	Foaming Time (hr)
P. johimbe	PBS	53.00 ± 1.00	392.55 ± 4.44
	M/PBS	9.30 ± 2.00	283.43 ± 8.49
P. macroceras	PBS	43.00 ± 1.60	67.39 ± 9.03
	M/PBS	10.30 ± 0.40	0.11 ± 0.01

* Values are mean of 4 determinations ± S.E.M.

Table 3: Haemolytic Activity of Extracts of Bark of *Pausinystalia*

Species of	Title*															
	PBS Extract								M/PBS Extract							
Pausinystalia	A		B		AB		O		A		B		AB		O	
	FH	PH	FH	PH	FH	PH	FH	PH	FH	PH	FH	PH	FH	PH	FH	PH
P. johimbe	2^0	2^2	Neg	2^2	Neg	2^2	Neg	2^2	2^0	2^1	2^0	2^1	2^0	2^1	2^0	2^2
P. macroceras	Neg	2^2	Neg	2^0	Neg	2^1	Neg	2^0	2^1	2^2	2^0	2^2	2^0	2^1	2^0	2^1

FH, full haemolysis; PH, partial haemolysis; Neg, no haemolysis.

[a] *Title defined as reciprocal of greatest dilution at which haemolysis occurred.

Values are means of quadruplicate determinations.

discarded. The packed cells were resuspended to 2% (v/v) in PBS and stored at 4°C (Thermocool 250, Thermocool Eng. Co. Ltd., Nigeria).

HAEMOLYTIC ACTIVITY

Haemolytic assays were conducted by a two-fold serial dilution of extracts of the plant parts, using microtitre plates (Sever, 1962), with 2% (v/v) erythrocyte suspension. A drop of treated erythrocyte was added to each well and incubated to give the sedimentation pattern recorded. The sedimentation patterns of the erythrocyte suspension in the unperturbed plates were read after 2 hr at room temperature (34°C) to determine the titre. A positive pattern indicating full haemolysis (FH) appeared visually as a circular big spot of red solution, surrounded by a small clear zone (if any) while a negative pattern indicating no haemolysis appeared as a uniform small spot of erythrocytes at the bottom of the well, surrounded by a big concentric clear zone. Partial haemolysis (PH) was recorded in those instances where positive pattern appeared as a clumping of erythrocytes in a rather large and non-uniform spot.

RESULTS AND DISCUSSION

Some of the general characteristics of saponins, a class of glycosides which occur primarily in plants but not exclusively include formation of foams in aqueous solutions, haemolytic activity, cholesterol-binding properties and bitterness (Price et al., 1987). The foam-forming activity involving the production of a characteristic honey-comb froth has long been employed as presumptive evidence for the presence of saponins (Farnsworth, 1966). In particular, the production of long-lasting foams have been used to identify saponins (Harborne, 1984). Also, the haemolytic effect of saponins has long been employed to detect and measure saponins (Armstrong and Armstrong, 1931; Schulz-Langner, 1966; Jurzysta, 1979; Feher, 1983). Extracts of the bark of the two species of *Pansinystalia* produced foams, most of which lasted for long periods of time (Table 2). The results showed that the foam heights of the PBS extracts were generally higher than those of the M/PBS extracts (Table 2). And although the foam heights of the M/PBS extracts of *P. johimbe* and *P. macroceras* were close, yet the M/PBS extract of *P. johimbe* produced a longer-lasting foam compared with that of *P. macroceras* (Table 2). Besides, despite the closeness of the values of the foam heights of *P. johimbe* and *P. macroceras*, the foaming time differed greatly (Table 2). The foaming time of the extract of *P. johimbe* was approximately six times that of *P. macroceras*. The production of longer-lasting foams by extracts of *P. johimbe* compared with those of *P. macroceras* might be due to the distribution of some other related species of naturally occurring constituents in the samples, in addition to the saponins. For example, it has been reported that while the two alkaloids, yohimbine and yohimbinine occur in these two species of *Pausinystalia*, *P. macroceras* is

richer in the physiologically inactive yohimbinine (Dalziel, 1937). Indeed some compounds which are structurally related to saponins have been found to contribute to the foam-forming activity to saponin-containing samples (Price et al., 1987). The results of phytochemical analysis have revealed the probable presence of alkaloids and cardiac glycosides, among other constituents (Table 1).

The results of the determination of haemolytic activity showed that the extracts of the two species of *Pausinystalia* were haemolytic to varying degrees. The PBS extract of *P. johimbe* was more haemolytic than that of *P. macroceras*, while the haemolytic activities of M/PBS extracts of the two species of *Pausinystalia* were generally higher than those of PBS extracts (Table 3).

Previous studies have confirmed the occurrence of both water and methanol-soluble saponins in various species of plants (Walter et al., 1954; Kitagawa et al., 1976; Sodipo and Tizhe, 1992). The results of this study indicate the occurrence of such water – and methanol-soluble saponins in these species of *Pausinystalia* (Tables 2 and 3). Again, the production of a longer-lasting foam with a foaming time of 283.43 ± 8.49 hr and foam height of 9.30 ± 2.00 mm by the methanol extract of the bark of *P. johimbe* compared with that of the same extract of *P. macroceras* (0.11 ± 0.01 hr and 10.30 ± 0.40 mm respectively) strongly indicated the occurrence of different types of methanol-soluble saponins even in these species of the plant. The lack of correlation between haemolytic and foaming activities have been shown in previous studies (Lindahl, et al., 1957; Woodward and Alsberg, 1916). The results of this study further confirm this lack of correlation between haemolytic and foaming activities. Some of the properties of saponins have been put to use in medicine, pharmaceutical industry and technologically. The foaming ability has been put to use to produce the frothy effect in the food industry (George, 1965). In addition, some countries have also included them in the list of flavouring agents (George, 1965; Merck Index, 1983). Moreover, saponins are used in the manufacture of shampoos, insecticides, various drug preparations and synthesis of steroid hormones. Thus in view of the various uses and immense potentials of this class of natural products, research efforts should be focused at the isolation, purification and eventual structural elucidation of the saponins of the bark of *P. johimbe* and *P. macroceras*.

ACKNOWLEDGEMENTS

The technical assistance of Mr. Nathaniel Ufaruna is gratefully acknowledged. Thanks and appreciation are also extended to the University of Maiduguri for providing the laboratory facilities. We are also grateful to the typist, Mr. Ephraim J. Akwaju who typed the script.

REFERENCES

Armstrong, E. F. and Armstrong, B. A. 1934. The Glycosides. Longmans Green and Co., New York, p. 59.

Dalziel, J. M. 1937. The Useful Plants of West Tropical Africa, being an Appendix to the Flora of West Tropical Africa by Hutchinson, J. and Dalziel, J. M. Published by the Crown Agents for the Colonies, London, pp. 406-407.

Farnsworth, N. R. 1966. Biological and Phytochemical Screening of Plants. J. Pharm. Sci. 55, 225-276.

Feher, F. 1983. Approximate determination of total saponin content in alfalfa by the haemolytic method. Novenytermeles, 32: 509.

George, A. G. 1965. Legal status and toxicity of saponins. J. Food Cosmet. Toxicol. 3: 85-91.

Harborne, J. B. 1984. Phytochemical Methods-A Guide to Modern Techniques of Plant Analysis, 2nd edn., Chapman and Hall, New York, p. 126.

Jurzysta, M. 1979. Haemolytic micromethod for rapid estimation of toxic alfalfa saponin. Acta Agrobot. 32: 5.

Keay, R. W. J., 1989. Trees of Nigeria. A revised version of Nigerian Trees (1960, 1964) by Keay, R. W. J., Onochie, C. F. A. and Stanfield, D. P. Clarendon Press, Oxford, Great Britain, pp. 424-425.

Keay, R. W. J., Onochie, C. F. A. and Standfield, D. P., 1964. Nigerian Trees. Nigerian National Press Ltd., Apapa and Published by the Department of Forest Research, Ibadan, Nigeria, pp. 406-407.

Kitagawa, I., Yoshikama, M. and Yoshioka, I., 1976. Saponin and sapognenol. XIII. Structures of three soybean saponins: Soyasaponin I, Soyasaponin II and Soyasaponin III. Chem. Pharm. Bull. 24: 121-129.

Lindahl, I. L., Davis, R. E., Tertell, R. T. et. al., 1957. Alfalfa saponins. Studies on their chemical, pharmacological and physiological properties in relation to ruminant bloat. U.S. Dept. Agri. Tech. Bull. No. 1161, Washington, D.C.

Merck Index, 1983. An Encyclopaedia of Chemicals, Drugs and Biologicals. Published by Merck and Co. Inc. Rahway, N.J. USA, pp. 1 pp. 1203-1204.

O'Dell, B. L., Reagan W. O. and B[illegible]

Toxic principle in red clover. Missouri Univ. Agr. Expt. Star. Res. Bull. 702: 12.

Price, K. R., Johnson, I. T. and Fenwick, G. R. 1987. The Chemistry and Biological Significance of Saponins in Foods and Feedingstuffs. CRC Critical Reviews in Food Science and Nutrition, 26: 27-135.

Schultz-Langner, E. 1966. Quantitative determination of very small amounts of saponin by observation of the duration of haemolysis. Planta Med., 14: 49.

Sever, J. L., 1962. Application of a microtechnique to viral serological investigations. J. Immunol. 88: 320-329.

Sodipo, O. A. and Tizhe, F. S. 1992. A preliminary study of the saponin content of neem tree (*Azadirachta indica* A Juss). Annals of Borno 8/9: 142-149.

Sofowora, A. 1984. Medicinal Plants and Traditional Medicine in Africa. Published in Association with Spectrum Books Ltd., Ibadan by John Wiley and Sons, New York, pp. 142-146.

Walter, E. D., Van Atta, G. R., Thompson, C. R. and Maclay, W. D. 1954. Alfalfa saponins. J. Am. Chem. Soc. 76: 2271-2273.

Woodward, H. E. and Alsberg, C. L. 1916. The relation of the surface tension of saponin solutions to their haemolytic activity. J. Pharm. Exp. Ther. 8: 109-110.