

6. Jeney E. and Zsolnai T., Zentr, *Bakteriol, Parasitenk. Abt. I. Orig.*, 1956, 167, 55.; *Chem. Abstr.*, 1957, 51, 7579.
7. Bito T., *Bull. Nagoya Inst. Technol.*, 1952, 4, 218; *Chem. Abstr.*, 1954, 48, 2991.
8. Vogel A. I, A text book of Practical Organic Chemistry 1971, 909.
9. Gilfillan F.A. and Merritt John R., *J. Am. Pharm. Assoc.*, 1936, 25, 860; *Chem. Abstr.*, 1937, 31, 214.
10. D. Sh. Rozina and Snytkovskaya R.G., *Metody Polucheniya Pokhim*, 1962, 4-5, 109. Organic Synthesis collective Volume I, Henry Gilman, Editor in Chief IInd Edition John Wiley and Sons, Inc. London, 1941, 511.

In Vivo Antisnake Venom Activity of A Furanoid Diterpene from *Aristolochia albida* Duch (Aristolochiaceae)

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The antisnake venom activity of a furanoid diterpene lactone isolated from the rhizome of *Aristolochia albida* (Family : Aristolochiaceae) was evaluated. The whole animal (*in vivo*) studies were conducted using the mortality of male Swiss albino mice after Intra-peritoneal (i.p.) injection of lethal doses (LD₁₀₀), 8.75 mg/kg and 4.20 mg/kg of venoms of *Naja nigricollis* (spitting cobra) and *Bitis arietans* (puff-adder) respectively. The diterpene was found to significantly reduce the toxic symptoms and protect the mice against the lethal doses of these two snake species commonly found in Northern Nigeria. However, the compound is more effective against the venom of *N.nigricollis* (ED₅₀=45 mg/kg) than that of *B.arietans* (ED₅₀=74 mg/kg).

ARISTOLOCHIA ALBIDA is a climbing shrub commonly found in the tropical West Africa¹ and is used in several gastro-intestinal disorders². There are reports that the rhizomes are used in skin diseases and against snake bites^{3,4}. The isolation of fargesin (neolignan) was reported earlier⁵. Recently, the isolation of phytosterols and glucose has been reported⁶. No pharmacological work on this medicinal plant has been reported so far to validate the claims of the folk-loric uses except for the molluscidal activity of the plant.⁷ Ethnopharmacological reports from northern Nigeria about the use of the rhizomes of this plant against snake bites⁸ have

prompted us to investigate the plant phytochemically and pharmacologically. This communication reports the antisnake venom activity of a diterpene isolated from *A.albida*.

The plant was collected in mid-July and authenticated by the Ahmadu Bello University herbarium, Zaria. The air dried, powder of the rhizomes was defatted with petroleum ether (b.p.60- 80°) and then extracted with methanol in a Soxhelt apparatus. The column chromatography (silica gel) of the methanolic extract furnished the diterpene which upon crystallisation from chloroform/methanol gave fine colorless needlelike colourless needles, m.p. 182°.

*For Correspondence

Table

Group number	Dose of diterpene (mg/Kg) administered	Envenomation with LD ₁₀₀ of N. nigricollis		Envenomation with LD ₁₀₀ of B. arietans	
		Number of survival out of 15 (n=15)	% of survival	Number of survival out of 15 (n=15)	% of survival
1	5.0	0	0.0	0	0.0
2	10.0	1	6.7	2	13.3
3	20.0	3	20.0	3	20.0
4	40.0	7	46.7	6	40.0
5	80.0	11	73.3	9	60.0
6	Saline	0	0.0	0	0.0

The venoms of **N.nigricollis** and **B.arietans** were collected by holding the snakes over small beakers covered with thin sheets of polythene. The snakes were made to strike the polythene and penetrate it with their fangs just to release the venom into the containers (the milking process). The venoms were lyophilised immediately. The LD₅₀ and LD₁₀₀ of these venoms were determined by dissolving the venoms separately in physiological saline solution to make a stock solution of 1 mg/ml each. The solutions were further diluted to give (0.1 ml) doses between 1 mg/kg to mg/kg body weight. These different doses were administered (i.p) into six groups of mice consisting of fifteen in each group and the mortality was recorded up to 24 hours as described by Theakston and Reid⁹. The values of the LD₅₀ and LD₁₀₀ were found to be 5.25 mg/kg and 8.75 mg/kg respectively for **N.nigricollis** and 2.58 mg/kg and 4.20 mg/kg respectively for **B.arietans**.

Six groups of healthy well fed male Swiss albino mice (18-22 g) were pretreated with different doses of diterpene (i.p.) five minutes before injecting (i.p) lethal dose (LD₁₀₀) of **N.nigricollis** venom. The survival of the mice were observed within 24 hours. The same procedure was repeated using the venom of **B.arietans** and the results are shown in the table.

The median effective doses (ED₅₀) of the diterpene against the LD₁₀₀ of **N.nigricollis** and **B.arietans** venoms were calculated and found to be 45 mg/kg and 74 mg/kg, respectively, by probit analysis according to Miller and Tainter¹⁰. It was observed that the mortality of mice was reduced progressively with increasing doses of the compound and significant protection (p<0.05) was observed at 80 mg/kg dose. Death from **N.nigricollis** resulted mainly due to neurotoxicity by curare-like action of cobrotoxin on the respiratory muscles causing flaccid paralysis leading to respiratory depression¹¹. The diterpene was found to interfere with acetylcholine receptors¹² and therefore, it might mediate its actions by antagonising the actions of the neurotoxic substances in the venom at the acetylcholine receptor sites. This might be the reason for its higher activity against **N.nigricollis** venom when compared to that of **B.arietans** which (the latter)interferes primarily with haemostasis and causes cardiotoxicity. The methanolic extract of the plant was found to be nearly as potent as the diterpene. This observation suggested the possibility for the presence of other active compounds in the plant.

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REFERENCES

1. Irvine, F.R. :Woody Plants of Ghana with Special Reference to Their Uses. Ford University Press, London, 1961,p:878
2. Watt J.M. and Brayer, G.M.: Medicinal and poisonous Plants of Southern and Eastern Africa.2nd Edn and S Livingstone Ltd,1962, P:118.
3. Dalziel, J.M: The useful Plants of West Tropical Africa, volume 6, Crown Agents for the Colony, London, 1937,P:612.
4. Comley, J.C.W., *Trop.Med.Parasit*, 1990 41(1), 1.
5. Fagbule, M.O. and Olatunji, G.A.,*Cellul.Chem.Tech.*, 1984,18,293.
6. Choudhury, M.K. and Haruna, A.K., *Indian J.Pharm.Sci.*, 1994 56,230.
7. Kela, S.L., Ogususi, R.A., Ogbogu, V.C. and Nwude, N., *Rev.Elev.Med.Vet.Fays.Trop.*, 1989, 42, 195.
8. Kahina, R., Personal communication.
9. Theakston, R.D.C and Reid, H.A., *Bull.W.H.O.*, 1983,61,949.
10. Miller, L.C. and Trainter, M.L., *Proc.Soc.Expt. Biol.Med.*, 1944, 57, 261.
11. Warrel, D.A. Manson's Tropical Diseases. Balliere Tindall, 1987, P:855.
12. Haruna, A.K., Ph.D. Thesis, Ahmadu Bello University, Zaria, 1994, P:216.

Simultaneous Spectrophotometric Determination of Dipyrone and Caffeine in Pharmaceutical Formulations.

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A simple and rapid spectrophotometric method has been developed for simultaneous determination of dipyrone and caffeine without prior separation.

DIPYRONE in combination with caffeine is used as an analgesic antipyretic drug. Dipyrone or caffeine in binary combination with other analgesics has been determined by titrimetry¹⁻³ and spectrometry⁴⁻⁹. In this study, the absorbance ratio technique was applied to determination of dipyrone-caffeine mixtures.

In this method, it is necessary to choose the two wavelengths to be used in the analysis. There are wavelength at which one of the two substances exhibits maximum absorption and the isoabsorptive

point. At the isoabsorptive point, dipyrone and caffeine have the same absorbance index values. In this study, there are two isoabsorptive wavelengths (235.1 and 255.2 nm in 0.1 N HCl). Caffeine exhibits maximum absorption at 271.4 nm in 0.1 N HCl (**Fig1**).

At the chosen wavelengths, standard solutions of dipyrone and caffeine obey Beer's Law in the concentration range of 2-24 $\mu\text{g. ml}^{-1}$ and 1-18 $\mu\text{g. ml}^{-1}$ respectively.