Pakistan Journal of Pharmaceutical Sciences
Vol. 18, No.4, October 2005, pp.31-35

# PHYTOCHEMICAL AND ACTIVE COLUMN FRACTIONS OF *PYRENACANTHA STAUDTII* LEAF EXTRACTS ON ISOLATED RAT UTERUS

**A. FALODUN[+], C.O. USIFOH AND Z.A.M. NWORGU***
***Department of Pharmaceutical Chemistry and *Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Edo State, Nigeria***

Four methanolic leaf fraction extracts of *Pyrenacantha staudtii* obtained from accelerated gradient chromatography (AGC) were tested on the isolated rat uterus. Various fractions – M1, M2, M3 and M4 through bioassay guided isolation were obtained. Fractions $M_2$ and $M_4$, containing saponins and alkaloids respectively, significantly ($P<0.05$) exerted high smooth muscle relaxant activity on the uterus. Fractions $M_1$ and $M_3$ containing fatty acids and tannins respectively did not exhibit significant effect on the isolated rat uterus.
The results indicate the presence of active principles in the leaf extracts of *P. staudtii* which may be responsible for some of the applications in traditional medicines as remedy against threatened abortion and dysmenorrheal.

**Keywords**: *Pyrenacantha staudtii*, rat uterus, leaf extract.

## INTRODUCTION

The use of medicinal herbs is as old as mankind and throughout the ages, traditional medicinal plants have long contributed tremendously to alleviate the suffering of the human race. In the developing countries there are about one billion people living in extreme poverty and vast numbers suffering and dying for the want of safe water and basic medicines (WHO, 1995). In these peculiar environments, the population has no other alternative rather than to rely on traditional medical practitioners and local medicinal plants for primary health care (WHO, 1995). Today, there is no doubt that complementary medicines have gained wide acceptability and it is increasingly attracting attention from scientific community world wide (Newal *et al*, 1986).

*Pyrenacantha staudtii* is a shrub found in light tropical forest and farmland bushes. It is a woody climber, older ones of which bears greenish almost, inconspicuous flower (Sofowora, 1982).The leaves are intensively bitter and the aqueous extract is used by traditional healers for the treatment of stomach colic, dysmenorrheal and threatened abortion (Hutchison and Dalziel, 1958). Recent studies showed that the aqueous extract of the leaves of *Pyrenacantha staudtii* was significantly effective for the treatment of malaria (Mesia *et al*, 2005) and has also been reported to reduce gastric ulcer in experimental animals (Aguwa and Mittal, 1978). Further research also showed that the crude methanolic extract showed inhibitory activity on the isolated rat uterus.

[+]Correspondence address: faloabi25@yahoo.com

This study is therefore aimed at fractionating the crude methanolic extract, subjecting it to accelerated column chromatography (AGC) with different solvent systems and testing the various fractions collected on the isolated rat uterus and identifying the fraction possessing the chemical principle(s) responsible for the pharmacological action of the leaves, using a bioassay guided isolation.

## EXPERIMENTALS

***Plant materials***

The fresh leaves of *Pyrencantha staudtii* were collected from the forest at Ikpoba River axis near the Ugbowo campus of the University of Benin, Benin City, Nigeria on February 21, 2004. An authenticated voucher specimen in the Department of Pharmacognosy, Faculty of Pharmacy, University Nigeria and identified by herbarium specialist Mr. Alasa Abubakar and a higher technical staff of the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City.

***Extraction and chromatography***

The air dried plant material (300g) was ground into powder and extracted exhaustively by marceration at room temperature with 100% MeOH. After filtration, the extract was concentrated under vacuum to yield 35g of a greenish residue. Part of the active extract (27g) was adsorbed into silica gel column 230 – 400 mesh on accelerated gradient chromatography, AGC, using a solvent system of a gradient of n- hexane 100%,[$CHCl_3$ – MeOH ( 9:1), $CHCl_3$ – MeOH ( 8:2), $CHCl_3$ – MeOH ( 7:3), $CHCl_3$ – MeOH (6:4), $CHCl_3$ – MeOH (5:5), $CHCl_3$ – MeOH (4:6), $CHCl_3$ – MeOH (3:7), $CHCl_3$ – MeOH (2:8), $CHCl_3$ – MeOH (1:9)] to yield four secondary fractions $M_1$ to $M_4$, which were subjected to phytochemical tests in accordance with the method of Stahl (1973). Pharmacological testing indicated two active fractions $M_2$ and $M_4$. TLC analyses were performed on silica gel 60 $F_{254}$ plates (Merck), and visualization of plates was carried out using Ninhydrin and Dragendorf's reagents.

## MATERIALS AND METHODS

***Determination of the $LD_{50}$ of the methanolic extract***

Toxicity test in mice were determined (Lorke, 1983) by administering a single dose of the extract to a group of animals and observing the effect over twenty four hours. The animals were fasted for 24 hours prior to the administration of the extract. The mice were grouped into four groups, each containing four mice. 10,100 and 1000mg/Kg of the extract were administered to the animals

**Table 1**

TLC profile of the fractions of *Pyrenacantha staudtii* leaf extract

| Tube number | Fractions from AGC | $R_f$ values | Dragendorf's reagent | Ninhydrin reagent | Uv 254nm and 366nm |
|---|---|---|---|---|---|
| 21 - 40 | $M_1$ | 0.82 | - ve | -ve | Fluorescence at 366nm |
| 41 - 57 | $M_2$ | 0.79, 0.22 | -ve | +ve | Fluorescence at 366nm |
| 58 - 69 | $M_3$ | - | -ve | -ve | Fluorescence at 366nm |
| 70 - 95 | $M_4$ | 0.18 | +ve | +ve | Quenched at 254nm |

-ve; indicates negative reaction; +ve; indicates positive reaction

**Table 2**

Phytochemical compositions of the fractions of *Pyrenacantha staudtii*

| Phytochemical Composition | Fraction $M_1$ | Fraction $M_2$ | Fraction $M_3$ | Fraction $M_4$ |
|---|---|---|---|---|
| Carbohydrates | - | + | + | - |
| Saponins | - | + | - | - |
| Alkaloids | - | - | - | + |
| Flavonoids | - | - | - | - |
| Tannins | - | - | + | - |
| Fats and oils | + | - | - | - |
| Steroidal saponins | - | - | - | - |
| Benzylisoquinoline alkaloid | - | - | - | + |

orally. No death was recorded after 24 hours. 200, 300 and 400mg/kg was administered for the second time and no death was recorded. The dose of the extract was increased to 500mg/Kg for the third stage and no death was recorded.

***Treatment of animals***

Female Wistar rats weighing about 150-160 g were pretreated with 1mg/kg of stilbesterol 48 hours prior to the actual experiment (Veale, 1998). The rat was killed by cervical dislocation and exanguination. The abdomen was opened and the two horns of the uterus carefully isolated, freed of mesenteric fat and a 1cm piece was mounted in a 50ml organ bath containing De Jalon solution having the following chemical composition NaCl, 9 g/l, $NaHCO_3$, 0.5 g/l, D-glucose, 0.5 g/l, KCl, 0.402 g/l, $CaCl_2.2H_2O$, 0.08 g/l was used (Staff of the Department of Pharmacology, 1970).

The tissue was aerated with 95% oxygen 5% carbon (IV) oxide at 37$^oC$, the spontaneous contraction of the uterus was recorded with FT 03 transducer connected to an Ugo basil recorder (7075). The transducer was previously calibrated to establish a relationship between the force applied to the transducer and the gauge deflection (500 mg). The tissue was allowed to equilibrate for 30 minutes before the commencement of the experiment.

The dose-response curves of oxytocin induced contractions were obtained, the effect of various fractions and that of the positive control (salbutamol) were also determined.

## STATISTICAL ANALYSIS

All results are expressed as the mean of four experiments ± SEM. The statistical package used was SAS, 1994. Users guide, Version 8.2. SAS Institute Inc., Cary, NC, USA. The statistical significance ($P<0.05$) of differences between means was assessed by an analysis of variance (ANOVA) followed by Duncan's multiple test.

## RESULTS AND DISCUSSION

The eluates from the AGC chromatography were bulked together based on similar $R_f$ values, to obtain fractions $M_1$, $M_2$, $M_3$ and $M_4$ as shown in table 1. These fractions from the plant extract contain saponins, alkaloids, tannins, and fatty acids and oils, as reflected in table 2. Fraction $M_1$ contains mainly the fatty acids while fraction $M_3$ contains some tannins. Fraction $M_2$ contains the saponins and it was necessary to identify the type(s) of saponins. This is because it gave a negative reaction to the Lieberman's test for steroidal saponins suggesting the presence of triterpenoids saponins. The thin layer chromatography (TLC) profile of the four fractions was obtained. Fraction $M_1$ revealed the presence of a large greenish spot containing chlorophyll and other non - polar materials closed to the solvent front. Fraction $M_2$ had two spots with $R_f$ 0.79 and 0.22. $M_3$ did not move from the origin suggesting the increased polarity of the sample. Fraction $M_4$ had only one spot $R_f$ 0.18 which gave positive test to ninhydrin and Dragendorf's reagent indicative of alkaloid (benzyliso-quinoline type).

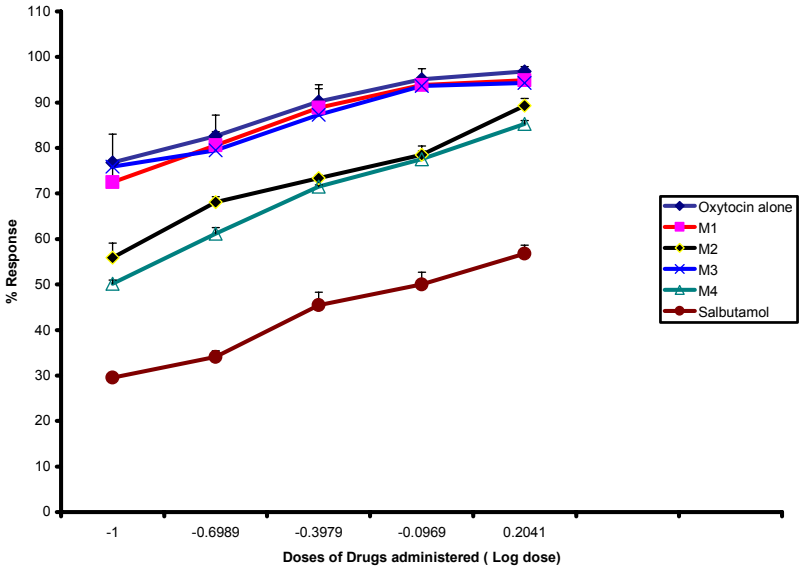


Fig. 1: Inhibitory activity of fractions of *Pyrenacantha staudtii* leaf on isolated rat uterus.

**Table 3**
Percentage response produced by the fractions in oxytocin induced contraction of isolated rat uterus

| Dose volumes of oxytocin (ml) 1 IU | Oxytocin alone (%) | Fraction $M_1$ (%) | Fraction $M_2$ (%) | Fraction $M_3$ (%) | Fraction $M_4$ (%) | Salbutamol (%) |
|---|---|---|---|---|---|---|
| 0.1ml | 76.81±6.28 | 72.50±4.50 | 55.93±3.15 | 75.90±0.92 | 50.15±0.84 | 29.55±0.63 |
| 0.2ml | 82.60±4.59 | 80.60±2.98 | 68.10±1.19 | 79.50±0.88 | 61.20±1.33 | 34.09±1.34 |
| 0.4ml | 90.21±3.68 | 88.90±4.12 | 73.40±0.64 | 87.32±0.55 | 71.55±0.38 | 45.45±2.85 |
| 0.8ml | 95.15±2.29 | 93.85±1.51 | 80.49±1.95 | 93.62±0.93 | 77.55±1.80 | 50.00±2.70 |
| 1.6ml | 96.80±1.10 | 95.90±1.07 | 89.30±1.58 | 94.26±1.14 | 85.30±0.73 | 56.82±1.86 |

Results are expressed as Mean ± S.E.M. (n = 5)

The four fractions $M_1$, $M_2$, $M_3$, and $M_4$ were subjected to pharmacological testing on the isolated rat uterus. Fractions $M_2$ and $M_4$ gave an inhibitory effect on the rat uterus while fractions $M_1$ and $M_3$ showed no positive inhibitory activity (fig. 1). The absence of activity in $M_1$ could also be adduced to the presence of chlorophyll and some fatty acids. Fraction $M_2$ showed an inhibitory activity on the rat uterus at 80mg/ml with $P<0.05$ with a reduction in the spontaneous contraction of the uterus (fig. 1). The inhibitory effect of fraction $M_2$ could be due to the presence of triterpenoid saponins which are known to exert smooth relaxant and antispasmodic activity (Akubue *et al*., 1983).

Fractions $M_2$ and $M_4$ induced a significant ($P<0.005$ concentration – dependent inhibition of the spontaneous concentrations of the isolated rat uterus (table 3). This probably due to the presence of benzyl isoquinoline alkaloids in $M_4$ which are known to have smooth muscle relaxant activity. The activity of the two active fractions $M_2$ and $M_4$ at a dose of 40mg/ml were also compared to the relaxation produced by salbutamol 30 µg/ml clinically used in the treatment of threatened abortion. Salbutamol which is a positive control significantly ($P<0.001$) relaxed the uterus (fig. 1).

In conclusion, fractions of *Pyrenacantha staudtii* leaves possess inhibitory activity on the uterine smooth muscles, which is consistent with the popular use of this plant for the treatment of threatened abortion and dysmenorrheal. From the chemotaxonomic point of view this is the first report of benzylisoquinoline alkaloids in this plant. Further work is in progress to determine the mechanism of action, at the receptor site of the fractions, and also to elucidate and characterize the chemical structure of the triterpenoid saponins and the benzylisoquinoline alkaloid(s) in fractions $M_2$ and $M_4$ respectively. It is envisaged that the active ingredients (compounds) will have a real potential for being added to the present list of tocolytic agents used clinically.

## ACKNOWLEDGEMENTS

The authors are grateful to Professors E.O.P. Agbakwuru (University of Benin, Benin City.) and T. Olugbade of the Faculty of Pharmacy, Obafemi Awolowo University Ile- Ife for their contributions.

## REFERENCES

Agbakwuru EOP, Rucker G and Mayer R (1988). Chemical constituents of *Pyrenacantha staudtii* (Hutch and Dalz). *Nigerian Journal of Pharm.,* **19**: 24-26.

Aguwa CN and Mittal GC (1978). Study of Anti-ulcer Activity of Aqueous extract of the leaves of *Pyrenacantha staudtii* (Icacinaceae) using various models of experimental gastric Ulcer in rats. *European Journal of Pharmacology*, **74**: 215-219.

Aguwa CN and Okunji CO (1986). Gastrointestinal studies of *Pyrenacantha staudtii* leaf extracts. *Journal of Ethnopharmacology*, **15**: 45-55.

Akbue PI, Mittal GC and Aguwa CN (1983). Preliminary Pharmacological study of some Nigeria Medicinal Plants. *Journal.of Ethnopharmacology*, **8**: 53-63.

Barttle I (2001). Conference note: bioactive molecules, plant products for the pharmaceutical and health care industries. *Phytotherapy Research,* **15**: 88.

De Alencar Cunhua KM, Paira LA, Santos FA, Gramosa NV, Silveriar FR and RaO VS (2003). Smooth muscle relaxant effect of Kaurennoic acid, a diterpene from *Copaifera Langsdorffi* on rat uterus *in vitro. Phytotherapy Research*, **17**(4): 320-324.

Falodun A and Agbakwuru EOP (2004) Phytochemical analysis and Laxative studies of the leaves of *Euphorbia heterophylla* L. *Pak. Jour. Ind. and Sci. Res*., **47**(5): 325-405.

Falodun A, Ukoh GC and Agbakwuru EOP (2003). Anti-bacterial activity of the leaves of *Euphorbia heterophylla* L. *Pak. Jour. Ind. and Sci. Res*., **46**(6): 395-477.

Furman KJ (1989). Preliminary isolated organ studies using an aqueous extract of *Clivia miniata* leaves. *Journal of Ethnopharmacology*, **37**: 341-346.

Hutchinson J and Dalziel JM (1958). Flora of West Tropical Africa crown Agents for Overseas Government and Administration, London. p.119.

Lorke D (1983). A new approach to practical acute toxicity testing: arch. *Toxicol*., **54**: 275-287.

Mesia G K, Tona G L, Penge O, Lusakibanza M, Nanga T M, Cimanga R K, Apers S, Van Miert S, Totte J, Pieters L and Vlietinck AJ (2005). Antimalarial activities and toxicities of three plants used as traditional remedies for malaria in the Democratic Republic of Congo: *Croton mubango*, *Nauclea pobeguinii* and *Pyrenacantha staudtii*. *Annals of Tropical Medicine and Parasitology*, **99**(4): 345-57.

Newal CA, Anderson LA and Phillipson JD (1986). *Herbal medicine a guide for health care professionals*. Pharmaceutical Press, pp.1-296.

Rojas A, Cruz S, Rauch V, Bye R, Linaires E and Mata R (1995). Spasmolytic potential of some plants used in Mexican traditional medicine for the treatment of gastrointestinal disorders. *Phytomedicine*, **2**: 51-5.

Sofowora A (1982). *Medicinal Plants and Traditional Medicine in Africa*. Published by John Wiley and Sons 1st ed., pp. 6-16.

Stahl E (1973). *Drug Analysis by Chromatography and Microscopy*: A Practical supplement to Pharmacopoeias. 1st ed., Ann. Arbor, Michigan, pp.219-224.

Staff of the Department of Pharmacology (1970). *Pharmacological experiments on isolated preparations*. 2nd Edition, University of Edingburgh, Churcill, Livingstone, Eding-burgh London and New York, p.2.

Trease GE, Evans WCA (1978). *Textbook of Pharmacognosy,* 11th ed., Bailierie Tindall, London, p.199.

Veale DJH, Oliver DW, Arangics NS and Furman KJ (1989). Preliminary isolated organ studies using an aqueous extract of *Clivia miniata* leaves. *Journal of Ethnopharmacology,* **37**: 341-346.

World Health Organization WHO (1995). The World Health Report. *Bridging the gap*. Geneva 1, p.118.

Zhou B, Hoch JM, Johnson RK, Mattern MR, Eng W, Ma J, Hecht SM, Newman D and Kingston DGI (2000). Use of COMPARE Analysis to Discovery New Natural Product Drugs: Isolation of camptothecin and 9- Methoxycampothecin from a new source. *Journal of Natural Products*, **63**(9): 1273-1276.