



Phytochemical analysis and inhibitory effect of *Pyrenacantha staudtii* leaf extract on isolated rat uterus

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Received 15th March 2005; Revised, accepted 15th August 2005

Abstract

Pyrenacantha staudtii leaf (Icacinaceae) is used ethno - medicinally in Nigeria and some African countries for the prevention of miscarriage (threatened abortion) in pregnant women. The study was therefore aimed at validating the claim by the traditional herbal practitioners. The crude methanolic extract was subjected to isolated rat uterine preparation in an organ bath with oxytocin and salbutamol as controls. Both the oxytocin and the spontaneous contractions of the uterus were recorded with an Ugo basil recorder. The phytochemical constituents of the crude methanolic extract of *Pyrenacantha staudtii* leaves revealed the presence of alkaloids (benzylisoquinoline type) and saponins. The extract was found to exert significant ($P < 0.05$) inhibitory effect on oxytocin induced contractions of isolated rat uterus at a dose dependent level, comparable to that of salbutamol.

Keywords: *Pyrenacantha staudtii*, extract, rat uterus

Introduction

The use of herbal medicines is attracting attention worldwide particularly in the developing countries where some people are dying as a result of hunger and diseases due to poverty and the rising cost of orthodox medicines. The dependence on local medicinal plants and traditional medicine for primary health care in the developing countries is on the increase (WHO, 1995, Sofowora, 1982). It has also been observed that the incidence of threatened abortion is rising as a result of stress and other pathological conditions. In Nigeria, especially in the rural areas, the people rely on the use of herbal medicines such as the leaves of *Pyrenacantha staudtii* for the

treatment of dysmenorrhoeal and threatened abortion (Agbakwuru *et al.*, 1988).

Pyrenacantha staudtii Hutch and Dalz belongs to the family Icacinaceae and it is a shrub found in the light tropical forest and bush farmlands. It is a woody climber, and the leaves are intensively bitter. Traditional herbalists in many parts of Nigeria have achieved success with the use of the aqueous extract of the leaves of the plant in the treatment of threatened abortion or miscarriage in gravid uterus (Aguwa and Mittal, 1978). The aqueous infusion was also reported to have anti-ulcer and gastrointestinal activity (Aguwa and Mittal, 1978, Akubue *et al.*, 1983, Aguwa and Okunji, 1986).

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The main objective of this research work is to validate the claims by the herbalists on the use of the *Pyrenacantha staudtii* in preventing threatened abortion using isolated rat uterus.

Experimental

Preparation of the plant infusion/ extract. The matured leaves of *Pyrenacantha staudtii* were collected from the Ikpoba river area around January in Ugbowo campus of the University of Benin, Benin City, Nigeria. The leaves used for the project were all plucked in one day from the leaf stalk of the plant and sun dried for a period of one week. After showing brittle properties, when crushed with hand, they were reduced to very fine powder using a mechanical grinder. Three hundred grammes (300g) of the leaf powder were extracted by maceration using fractionated methanol. The solvent was removed *in vacuo* using a rotary evaporator and the percentage yield calculated. The concentrated extract was stored ant -4°C until when required. The crude methanolic extract was subjected to phytochemical screening testing for the presence of secondary metabolites using the method of Trease and Evans (1989) and Harbone (1973).

Animal preparation. Female Wistar rats weighing about 150-160g were pretreated with 1mg/kg of stilbesterol 48 hours prior to the actual experiment. A single blow on the head killed the rats. 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's solution with 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, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.08 g/L. The tissue was aerated with 95% oxygen 5% carbon (IV) oxide at 37°C . The spontaneous contraction of the uterus was recorded with FT 03 transducer connected to an Ugo Basil recorder. The transducer was previously calibrated to establish a relationship between

the force applied to the transducer and the gauge deflection (500mg). The tissue was allowed to equilibrate for 30 minutes before the commencement of the experiment. The effect of methanolic extract and oxytocin were determined. The effect of the extract on oxytocin-induced contraction was also compared with the effect of salbutamol. The effect of varying doses of *P. staudtii* leaf extract on oxytocin contraction was also studied.

Statistical Analysis. The In stat statistical package was used for the analysis of data making use of the Student t-test. The standard deviation and standard error of mean were calculated and the dose response curves obtained.

Drugs. Oxytocin, Atropine sulphate, Salbutamol (ventolin^R) from GlaxoSmithKline, England

Results

The results of the phytochemical screening revealed the presence of saponins, phenolic constituents and alkaloids. Further investigation of the type of alkaloids revealed the presence of the benzyloisoquinoline (table 1). The crude extract of *P. staudtii* had no contractile action on the rat uterus; it caused relaxation of the rat uterus. The extract reduced oxytocin induced contractions. 4mg/ml extract depressed the dose response curve but the change is not significantly different from oxytocin curve.

8mg/ml of extract however shifted the dose response curve to the right and also depressed the maximum response. This change was significantly different from the curve obtained using oxytocin alone. The effect of the extract on oxytocin contraction was compared to that of salbutamol. 8mg/ml of the extract produced 50% of the relaxation caused by $30\mu\text{g/ml}$ salbutamol (Fig. 1).

Discussion

The results of phytochemical investigation of the methanol extract of *Pyrenacantha staudtii* leaves indicated that the extract contains alkaloids of the benzyloisoquinoline group, saponins, tannins and glycosides of the anthraquinone group. It was also shown that the yield of these benzyloisoquinoline alkaloids was high compared with the other secondary metabolites. This was deduced from the broadness of the spot during the thin layer chromatography.

The presence of this benzyl isoquinoline alkaloid probably is responsible for the pharmacological actions observed with the plant leaf (i.e. smooth muscle relaxant effects) from its ethno medicinal uses in the prevention of premature labour and also in the prevention of abortion during pregnancy. This hypothesis is based on the pharmacology effects, which seem to characterize other

chemical compounds which share a close resemblance to the benzyloisoquinoline nucleus or the pharmacological effects of other benzyloisoquinoline alkaloids (e.g. Morphine and Papaverine). The smooth muscle relaxant activity of the uterus could also be due to the saponin constituents especially the triterpenoid type. The crude extract has dose-dependent relaxant effect on the uterus and was comparable to that of salbutamol. The spontaneous activity of the uterus was also drastically reduced with increasing doses of the crude extract.

Further work is currently on to establish the mechanism of action and to also carry out a bioactivity guided isolation using different fractions of the crude extract and to finally characterize and elucidate the active chemical constituents.

Dose response curves of Oxytocin and extracts of *P. staudtii*

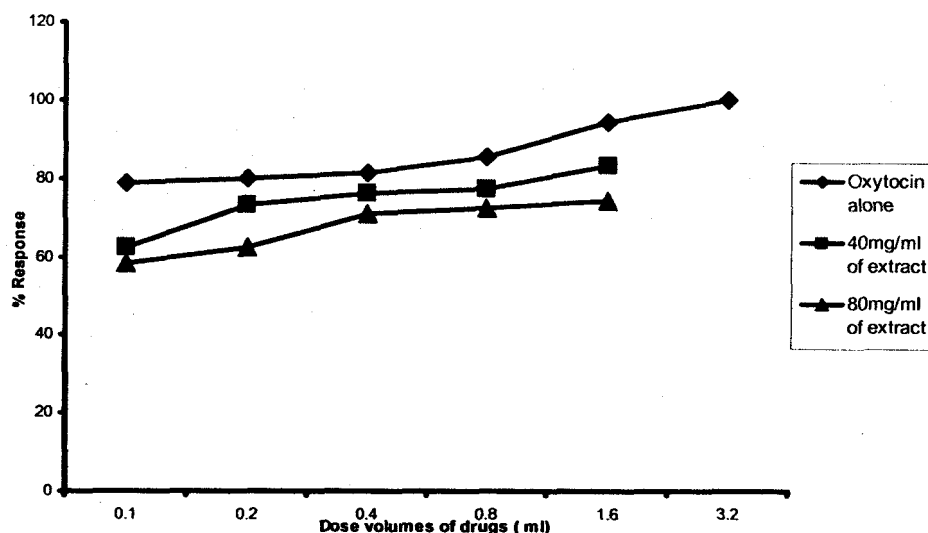


Fig. 1: Dose-Response curves of oxytocin and extracts of *Pyrenacantha staudtii*

Table 1: Phytochemical composition of the extract of *Pyrenacantha staudtii*

Compounds Class	Occurrence
Alkaloids	+
Benzylisoquinoline	+
Saponins	+
Phenolic compounds	+
Anthraquinone glycosides	-

+ = present; - = absent

Conclusion

The methanolic extract of *Pyrenacantha staudtii* leaves contains saponins and alkaloids. The extract has an inhibitory effect on the rat uterus and could therefore be very effective in the prevention of threatened abortion, thus justifying the traditional use of the medicinal plant in the management of threatened abortion and dysmenorrheal in traditional medicine.

Acknowledgement

We acknowledged the effort of Professor E.O.P Agbakwuru, who apart from introducing the plant to us, also gave his technical assistance during the research work.

References

- Agbakwuru E.O.P., Rucker P.G., Mayer R. (1988). Chemical constituents of *Pyrenacantha staudtii* Hutch and Dalz. Nigerian Journal of Pharm. 19, 24 – 26.
- Aguwa C.N and Mittal G.C. (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 C.N and Okunji C.O. (1986). Gastrointestinal studies of *Pyrenacantha staudtii* leaf extracts. Journal of Ethnopharmacology 15, 45 – 55.
- Akubue P. I., Mittal G.C. and Aguwa C.N. (1983). Preliminary Pharmacological study of some Nigeria Medicinal Plants. Journal of Ethnopharmacology 8, 53 - 63.
- Ejimadu I. M. Falodun. A. (2002). Chemical and Biological Studies of Seed Extracts of *Hunteria umbellata* (K.Schum). International Jour. Chem. 12, (3) 241 – 248.
- Falodun A, Agbakwuru E.O.P and Ukoh G.C. (2003) Antibacterial Activity of *Euphorbia heterophylla* Linn (Family Euphorbiaceae). Pak. J. Sci. Res. 46 (6) 471 – 472.
- Falodun A, Usifoh C.O and Nworgu Z.A.M (2005) Phytochemical and Active column fractions of *Pyrenacantha staudtii* leaf extracts on isolated rat uterus. Pakistan Journal of Pharmaceutical sciences 18 (4), p 31 – 35.
- Harbone, J.B (1973). Phytochemical methods. Chapman and Hall Limited, London. p 27.
- Hutchinson J. and Dalziel J.M. (1958). Flora of West Tropical Africa. Crown Agents for Overseas Government and Administration, London.
- Sofowora E.A. (1982). Medicinal Plants and Traditional medicine In Africa. 1st Ed., Published by John Wiley and Sons, England pp 6 – 16.
- Trease G.E., Evans W.C. (1989). Pharmacognosy, 13th Ed., Bailleire Tindall, London pp 199.
- World Health Organization, WHO (1995). The World Health Report. Bridging the gap. Geneva 1, p.118.