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CHROMATOGRAPHIC CHARACTERIZATION, PROXIMATE ANALYSIS AND IN VITRO INHIBITORY EVALUATION OF *PYRENACANTHA STAUDTII* HUTCH AND DALZ (ICACACINANCAE) ON THE GUINEA PIG ILEUM

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ABSTRACT

Pyrenacantha staudtii (Hutch and Dalz), Icacinaceae is a medicinal plant used in trado – medical practice for the treatment of dysmenorrheal and gastrointestinal disorders. The leaf extract is also used in ethno - medicine for the treatment of threatened abortion. The present study aimed at assessing the scientific evaluation of the ethnomedicinal claim on isolated guinea pig ileum suspended in an organ bath containing a physiological salt solution of Tyrode solution at a pH of 7.35, and also to investigate the phytochemical constituents and quantitative parameters like the moisture content, ash value, acid insoluble ash, water soluble ash and extractive values such as alcohol and water extractives which can be used in the identification of the leaf of *P. staudtii*. The crude extract of the plant was subjected to thin layer and vacuum liquid chromatography with different solvent systems. The phytochemical evaluation revealed the presence of alkaloids, tannins and saponins in the leaves of *P. staudtii*. The proximate analysis gave 5.40 ± 0.02 % as the moisture content, 5.25 ± 0.01 % as ash value, 2.58 ± 0.13 % as acid insoluble ash and 3.25 ± 0.08 % as water soluble ash value. The alcohol and water extractives of 7.20 ± 0.04 % and 8.10 ± 0.13 % respectively were obtained. The thin layer chromatography profile of the fractionated crude extract indicated the presence of compounds with different R_f values. The pharmacological evaluation revealed a very significant ($P<0.05$) inhibitory effect of the extract on histamine and acetylcholine induced ileum contractions. The relaxation of the guinea ileum by the extract, has justified the claims for which the plant is known and used.

Keywords: *Pyrenacantha staudtii*, extract, ileum, chromatography.

INTRODUCTION

Despite the amazing progress in the fields of genomics and genetics, and the importance of synthetic pharmaceutical Chemistry and microbial fermentation, plants remain an essential source of new lead compounds (Hosettmann and Marston, 2001). Natural products are an important source of new structures leading to drugs in all major diseases areas. Traditional medicine practice in the treatment of diseases and infections have assumed a more scientific and wider dimension as the emphasis on ethnomedicine is on the increase especially in the developing countries where the primary health care needs of the populace are not easily affordable. In the developed countries, the use of herbal medicine is attracting attention as a result of the increased resistance posed by orthodox drugs which are expensive and with numerous adverse side effects. The support for the use of medicinal plants by the World Health organization (WHO) is quite encouraging due to the numerous therapeutic benefits (WHO, 1995). For example, acetylsalicylic acid was obtained from *Flipendula umaria*, the antihypertensive and antipsychotic agent, reserpine from *Rawolfia vomitoria* is still clinically in use today. This support for traditional medicine still needs the support and cooperation of the regulatory agents in various countries to discourage the abuse and misuse of these herbal medicines. This attraction for herbal medicine led to the present scientific investigation of the local medicinal plant *Pyrenacantha staudtii* used in the treatment of spastic conditions of the gastrointestinal tract disorders. The aqueous extract is used for the treatment of dysmenorrheal and of threatened abortion. Previous phytochemical studies reported the isolation and characterization of 3-Carbomethoxylpyridine and Hexahydroxylcyclohexane (Falodun and Usifoh, 2006; 2007). The present study aimed at validating the ethnnomedicinal use as anti - spamolytic agent via an in vitro experimental model.

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MATERIALS AND METHODS

Collection and preparation of plant material

The fresh leaves of the plant were collected from Ikpoba river axis of the University of Benin, Ugbowo campus, identified by Mr. Sunny of the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City, Nigeria. Botanical authentification

was done by Dr. BA Ayinde of the same Department. [A voucher specimen was kept in the herbarium of Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Nigeria].

The plant sample was dried at room temperature and powdered with the aid of a mechanical mil.

Quantitative analysis of the crude powdered sample
Proximate composition of the sample was determined according to standard methods (African Pharmacopoeia, 1986, British Pharmacopoeia, 1988, and AOAC, 1990) with Analytical Codex Number14.062, 14.063, 14.064 and 14.065.

Extraction and Partitioning of extract
The powdered sample (400g) was extracted exhaustively by maceration at room temperature with ethanol for 72hrs. Removal of the solvent under pressure at 40˚C yielded a greenish residue (7.86g). The concentrated extract was stored in air tight containers, labeled and refrigerated at - 4˚C prior to use. The crude ethanolic extract was partitioned with petroleum ether 40-60^{O}C, chloroform and then ethyl acetate.

Phytochmemical screening of crude powdered and biologically active extract
The crude powdered and the solvent free petroleum ether and ethanolic extracts of the plant was subjected to phytochemical examination using chemical and chromatographic methods. The extracts were separately screened for following constituents, carbohydrates alkaloids and / or nitrogenous bases, tannins, saponins and flavonoids, using standard procedures (Trease and Evans, 1989, Sofowora 1980 and Harborne, 1984).

Fractionation of crude extracts using open column chromatography with Sephadex
The bioactive extract of *P. staudtii* was subjected to chromatographic investigation using thin layer, column and vacuum liquid chromatography. The ethylacetate, ethanolic and petroleum ether fractions were subjected to thin layer chromatographic analysis with different solvent systems of chloroform, methanol and petroleum ether ratio. The R_f values recorded.

Ethylacetate fraction (305mg) was applied on a column of sephadex, elution was done with 100 % toluene followed by a mixture of toluene/methanol of increasing polarity (1:9; 2:8; 3:7; 4:6; 5:5; 6:4; 7:3; 8:2; and 9:1 v/v) and then 100 % methanol followed by a mixture of MeOH/H_20 (75:25 and 50:50 v/v) each 50ml. The spots were visualized with sulphuric acid and heating at 105^{o}C for 10 minutes. The spots were also characterized with UV lamp at 254nm and 366nm.

Pharmacological evaluation
Drugs and Chemicals
The following drugs were used: Acetylcholine (Roche), Histamine (Roche), DMSO (Sigma Aldrich). Stock solutions of the various drugs, ethanolic and petroleum ether of *Pyrenacantha staudtii* extracts were prepared fresh for each experiment.

Animals
Guinea pigs of either sex weighing 250 -300g were obtained from the Animal house of the College of Medicine, Ambrose Alli University, Ekpoma, Edo State, Nigeria. Animals were acclimatized for two weeks before being subjected to the experimental protocol. The animals were maintained on a standard diet (Ladokun feeds, Ibadan, Oyo State, Nigeria) and had free access to food and water *ad libitum.* Animals were housed in a cage with a twelve hour light-dark cycle. [Approval for use of animals in this work was obtained from the Ethical Committee on the use of Animals for Experiments of the Faculty of Pharmacy, University of Benin, Benin City].

Animal preparation
The guinea pigs were killed by cervical dislocation and exanguinations. The abdomen was opened and the ileum carefully isolated, freed of mesenteric fat and about 3 - 4cm piece in length was mounted in a 50 ml organ bath containing Tyrode physiological salt solution having the following chemical composition: NaCL, 8 g/l, $NaHCO_3$, 1.0 g/l, D-glucose, 1.0 g/l NaH_2PO_4, 0.05g/l, $MgCl_2$, 0.1g/l, KCL, 0.2 g/l, $CaCL_2.2H_20$, 0.26 g/l. Each intestinal strip was suspended vertically with the lower end fastened to the tissue holder and the upper end attached to an isometric or isotonic transducer connected to a chart recorder.

The tissue was aerated with 95 % O_2 and 5 % CO_2 and temperature maintained at 37^{o}C with a pH of 7.4. The contractions of the ileum which occurs both circularly and longitudinally were recorded with FT 03 transducer connected to an Ugo Basile recorder (7075). The transducer was previously calibrated to establish a relationship between the force applied to the transducer and the gauge deflection (800mg). The tissue was allowed to equilibrate for 30 minutes before the commencement of the experiment.

A dose response for acetylcholine obtained using 0.004ug to 4ug in geometric doses with a dose cycle of 120seconds (30sec contact period and 90sec washout period) was maintained. The dose response then repeated in the presence of 400ug of the crude extract after a contact time of 120sec. Similarly, a dose response for histamine was obtained using 0.032ug to 4ug in geometric doses with a dose cycle of 120seconds (30sec contact period and 90sec washout period) was maintained. The dose response then repeated in the presence of 400ug of

the crude extract and its fractions after a contact time of 120sec.

Statistical analysis

All results are expressed as the mean of 5 experiments ± SEM (standard error of mean) and continuous line graph. The data were analyzed statistically by student's t-test using Graphpad instant version 2.05a. The level of significance was $P<0.05$.

RESULTS AND DISCUSSION

Determination of the proximate composition is used to further establish and differentiate crude drug identity particularly where similar macroscopic and microscopic features are observed. The results of the quantitative parameters are shown in (Table 1). The moisture content shows the susceptibility of crude drug samples to microbial attack especially fungi, and also to degradation due to hydrolysis of the crude powdered drug. A moisture content of 5.55 ± 0.02 % obtained from this study is indicative of the storage quality for some time without microbial degradation or hydrolytic break down of the chemical constituents. The maximum range is between 6-8 % in African Pharmacopoeia (1986).

Table 1. Percentage (%) values of quantitative parameters examined on the leaf of *Pyrenacantha staudtii*

Parameter	Values ± SEM (%)
Moisture content	5.40 ± 0.02
Total ash	5.25 ± 0.01
Acid insoluble ash	2.58 ± 0.13
Water soluble ash	3.25 ± 0.08
Alcohol extractive	7.20 ± 0.20
Water extractive	8.10 ± 0.18

The total ash is a measure of the non-volatile inorganic constituents remaining after ashing. It is made up of two parts, the physiological and the non-physiological ash. The physiological ash consists of carbonates, phosphates, nitrates, sulphates, chlorides and silicates of metals which the plant took up when it was growing. The non-physiological ash represents ash from extraneous matter. The acid insoluble ash is residue obtained when total ash is boiled with 10 % HCl. It is a measure of the sandy matter in the crude drug samples. The values of 5.25 ± 0.01 and 2.58 ± 0.13 were obtained.

The results of the phytochemical test (Table 2) as well as the chromatographic profile (Table 3) of *Pyrenacantha staudtii* leaf revealed the presence of phyto constituents which include simple and complex sugars, tannins, saponins and alkaloids. The column chromatography with sephadex (Table 3) afforded one broad spot R_f 0.84 which tested positive for reducing sugar. Because of the intensively bitter taste of the leaf, locals usually add common salt (NaCl) to the aqueous decoction before ingestion. This has important implications since there is a co-transport of Na^+ and glucose across the epithelial cells of the ileum and glucose enhances sodium absorption and thus water uptake, these locals are actually rehydrating themselves – an important first step in diarrhea treatment.

The pharmacological evaluation as revealed in figure 1 and figure 2, showed that graded doses of acetylcholine at 0.064ug/ml, 0.128ug/ml, 0.256ug/ml, and 0.0512ug/ml elicited dose-dependent contractions of the ileum producing 77.63, 79.12, 78.73%, and 88.33% mean responses respectively. The crude ethanolic extract inhibits acetylcholine and histamine induced contractions of the isolated guinea pig ileum. In the presence of 400ug/ml of the crude ethanolic extract, there was

Table 2. Results of phytochemical screening of the crude extract and fractions of *Pyrenacantha staudtii* leaf.

Secondary metabolite	Powdered leaf	Crude ethanolic extract	Petroleum ether fraction	Chloroform fraction	Ethanolic fraction	Ethylacetate fraction
Carbohydrate	present	present	present	present	Present	present
Reducing sugar	present	present	present	present	Present	present
Saponins	present	present	absent	absent	present	absent
Tannins	present	present	present	present	absent	absent
Alkaloids	present	present	present	present	present	absent

Table 3. Resolutions of vacuum liquid chromatography.

Codes	Fractions	R_f Values	UV reaction (nm)
A	7	0.91, 0.82	254, 366
B	8	0.93, 0.82, 0.27	254, 366
C	10	0.93, 0.82, 0.64, 0.27	254, 366
D	11	0.94, 0.27	254, 366
E	29	0.84	254, 366

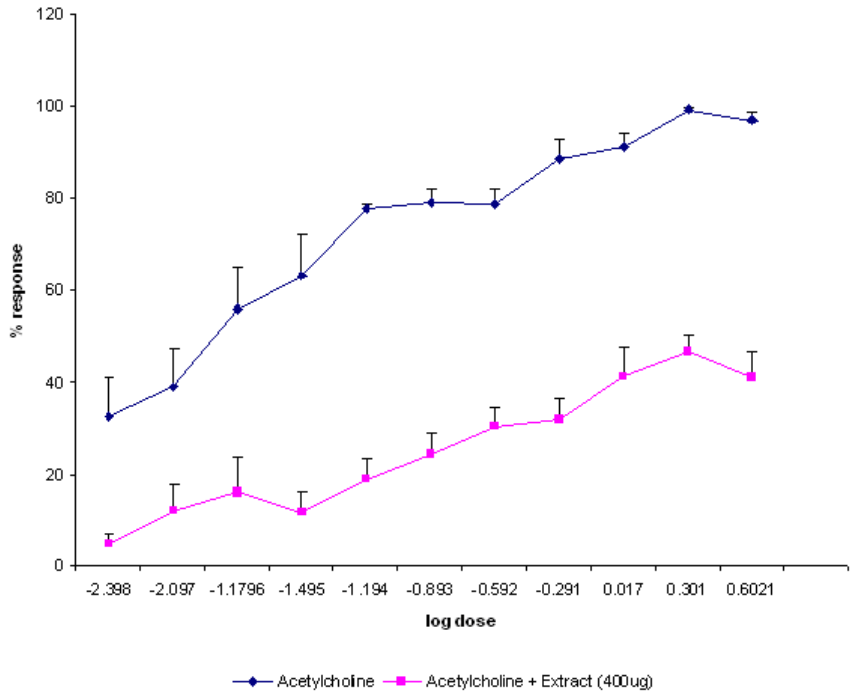

Fig. 1. Effect of acetylcholine and the crude ethanolic extract of *Pyrenacantha staudtii* on isolated guinea pig ileum.

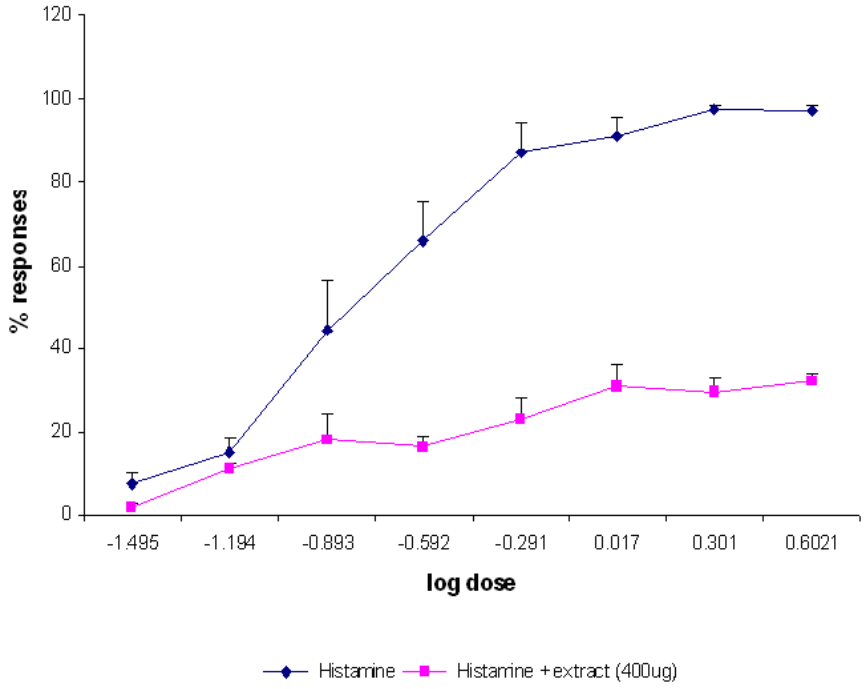

Fig. 2. Effect of histamine and the ethanolic extract of *Pyrenacantha staudtii* on isolated guinea pig ileum.

significant reduction of the mean percentage responses ($P<0.0001$). Similarly, as shown in figure 2, histamine at 0.512ug/ml, 1.04ug/ml, 2ug/ml, and 4ug/ml elicited dose-dependent contraction of the ileum producing 86.89 %, 91.19 %, 97.53 %, and 96.99 % mean responses respectively. In the presence of 400ug/ml of the crude ethanolic extract, there was significant ($P<0.0001$) reduction of the contractile responses. Except for the dose of 0.064ug/ml of histamine ($P>0.05$), there was significant reduction in the contractile responses ($P<0.05$). It therefore suggests strongly that (Fig. 1 and 2), as the concentration of the drugs (acetylcholine and histamine) are increased in the presence of the extract, there is a gradual increase in the contractile responses suggesting

that the extract act through competitive antagonism. Acetylcholine acting through muscarinic receptor (M_3-subtype) and histamine acting through histamine (H_1-subtype) both activates G-protein resulting in the cleavage of phospholipase C and formation of IP_3 (inositol triphosphate) and DAG (diacyl glycerol) both of which cause increase in intracellular calcium. The extract may work through interaction with the second messenger (Ca^{2+}) rather than by specific receptor antagonism. However, since the extract also inhibits H_2-receptor mediated ulcer in the gastrointestinal tract (Aguwa and Okonji, 1986; Aguwa and Mittal, 1983; which act through increase in cAMP (cyclic adenine monophosphate) Rang and Dale, 2003. It therefore suggests that the specific mechanism of action is unclear. More work need to be done in order to establish the precise mechanism of action of the extract. Further characterization of the extract is necessary for the structure elucidation of the bioactive chemical constituent(s) and its structure-activity relationship.

CONCLUSION

The study was carried out with the objective of investigating the ethno medical claim of the leaves of *Pyrenacantha staudtii* in folk as a remedy against diarrhoea and intestinal colic and to authenticate its use. The present work confirmed and justified its use in traditional medicine, and the active constituents when isolated and purified would be a potential anti diarrhea agent.

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REFERENCES

African Pharmacopoeia. 1986. Vol. 2. 1st ed. OAU/ STRC Publications. pp.128-144.

Aguwa, CN. and Mittal, GC. 1983. Study of the Antiulcer Activities of the Aqueous Extract of the Leaves of *Pyrenacantha Staudtii* (Icacinaceae) Using Various Models of Experimental Ulcer in Rats. European Journal Pharmacology. 74: 215-219.

Aguwa, CN. and Okunji, CO. 1986. Gastrointestinal Studies Of *Pyrenacantha Staudtii* Leaf Extracts. European Journal Pharmacology. 15: 45-55.

AOAC. 1990. Ref. No. 968.08, 946.06, Official Method of Analysis Association of Official Analytical Chemists (13th ed.) Washington, DC, USA.

British Pharmacopoeia BP. 1988. Vol.II. Her Majesty's Stationary Office, London. p.1022.

Falodun, A. and Usifoh, CO. 2006. Isolation and Characterization Of 3-Carboxymethypyridine from the Leaves of *Pyrenacantha Staudtii* (Hutch and Dalz) (Icacinaceae). Acta Poloniae Pharmaceutica-Drug Research. 63 (3): 235-237.

Falodun, A. and Usifoh, CO. 2007. Isolation and Characterization of hexahydroxylcyclohexane from the leaves of *Pyrenacantha staudtii* (Hutch and Dalz). J. Pharm. Sci. & Pharm. Pract. 8(3): 60-63.

Harborne, JB. 1984. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 2nd Ed. Chapman and Hall, USA, p.46.

Hosettmann, K. and Marston, A. 2001. The Search for New Drugs From Higher Plants; CHIMIAD. 61(6): 322-324.

Rang, HP, Dale RT. and Ritter, JM. and Moore, PK. 2003. The Gastrointestinal Tract. Pharmacology 5th Ed. 24: 367.

Sofowora, A. 1980. Screening Plants for Bioactive Agents. In: Medicinal plants and traditional Medicine in Africa. Spectrum Books Limited, 1st Ed. 128-161.

Trease, GE. and Evans, WC. 1989**.** Pharmacognosy (13th Ed.). English Language Book Society, Bailliere Tindall, Britain. 378: 386-480.

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