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Preliminary *in vivo* evaluation of anti-inflammatory activities of aqueous and ethanolic whole plant extracts of *Phyllanthus fraternus* on Carrageenan-induced Paw Oedema in Sprague-Dawley Rats

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ABSTRACT

Medicinal plants represent potential sources for the discovery of new anti-inflammatory agents. Aqueous and ethanolic whole plant extracts of *Phyllanthus fraternus* were evaluated *in vivo* for their anti-inflammatory activities on carrageenan-induced paw oedema in Sprague-Dawley rats. The extracts were also screened for phytochemicals. Some of the phytochemicals found in the extracts have previously been implicated as anti-inflammatory agents. The LD_{50} of both extracts was investigated and found to be greater than 5000mg/kg. The extracts at doses 100mg/kg and 200mg/kg showed modest anti-inflammatory activity in a dose dependent manner. The aqueous extract demonstrated better inhibition of paw oedema compared with the ethanolic extract at 200mg/kg after 4hrs. The activity of the standard drug, indomethacin at 25.0 mg/kg was significantly higher ($p< 0.05$) than those of the extracts. The results suggest that whole plant extract of *P. fraternus* possess anti-inflammatory activity and will be useful in the search for novel anti-inflammatory agents.

INTRODUCTION

Inflammation is protective and defense mechanism of the body and thus, during inflammation various pathological changes take place. The production of active inflammatory mediators is triggered by microbial products or by host proteins, such as proteins of the complement, kinins, and coagulation systems that are activated by damaged tissues. In preclinical studies, these changes can be induced by administration of the agents causing inflammation. There are several reports suggesting an increased incidence of inflammatory condition in lifestyle diseases like diabetes, as inflammation is one of the most important natural defense mechanisms. Among the most common prescribed drugs for treatment of inflammation are non-steroidal anti-inflammatory drugs (NSAID). This is due to their consistent effectiveness in the treatment of pain, fever, inflammation and rheumatic disorders. Their use however, is associated with several adverse effects The most common are dyspeptic symptoms, gastrointestinal. erosions, peptic ulcers, over bleeding and perforation at the level of the digestive tract (Corrado *et al.*, 2009).Thus the development of new anti-inflammatory drugs is still necessary as this may overcome the toxicity associated with NSAID. Medicinal plants have consistently been screened for various pharmacological activities. This is because of the fact that medicinal plants contain biologically active compounds with less undesirable effects (Halliwell *et al*., 1992). They offer an alternative source for primary health care particularly in rural communities. Several plants have shown potential for anti-inflammatory activities. Thus medicinal plants contain a wide variety of chemicals that can present a source for the discovery of novel anti-inflammatory agents. The plant *Phyllanthusfraternus* (Family: *Euphorbiaceae)* commonly called; gulf leaf- flower, Chancapiedra, quebrapedra, stone braker, arranca-pedras, carry-me-seed, hurricane weed, para-parai mi, quinine weed, Mache da goyo (Hausa), Gbogbonowun lese (Yoruba) OfobiOkpabi (Ga) is a small, erect, annual herb (Dicotyledonous) that grows 30–40 cm in height (Wunderlin and Hansen, 2003). This plant is indigenous to tropical rain forest areas throughout the world. *P. fraternus*is quite prevalent in the wet rainforests, growing and spreading freely (much like a weed).

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Closely related species are *P. amarus, P. sellowianus* and *P. niruri.* The *Phyllanthus* genus contains over 600 species of shrubs, trees, and annual or biennial herbs distributed throughout the tropical and subtropical regions of both hemispheres (Leslie, 2003). *P. fraternus* has been reported for its numerous ethnomedicinal uses by the indigenous people. These include treatment of blennorrhagia, colic, diabetes, dysentery, fever, flu, tumors, jaundice, vaginitis, and dyspepsia(Matur *et al*., 2009). Following its long documented history of use in the West African region, the plant is considered analgesic and as an aperitif, carminative, digestive, laxative, stomachic, tonic, and vermifuge (Leslie, 2003).

The genus Phyllanthus has also been reported to possess good anti-inflammatory potential (Jay *et al*., 2011).Several scientific reports, (Kiemer *et al*., 2003; Atul & Fahim, 2012; Catapan *et al*., 2000), havealso suggested that leaf extracts of *P. fraternus* contain anti-inflammatory agents. However, to the best of our knowledge, there are limited documented reports on the anti-inflammatory activity of the whole plant extract of *P. fraternus* grown in Ghana. Phytochemical composition of plants varies according to geographical location, origin and prevailing weather condition (Oseni and Kadiri, 2012). The current investigation was therefore aimed at evaluating the *in vivo* anti-inflammatory potentials of aqueous and ethanolic whole plant extracts of Ghanaian *P. fraternus*. The plant was screened for phytochemicals and the degree of inhibition of paw-oedema in carrageenan-induced inflammation in Sprague-Dawley rats was investigated.

MATERIALS AND METHODS

Drugs and chemicals

All drugs and chemicals used were ofanalytical grade unless otherwise stated. Sodium Chloride is obtained from British Drug House (BDH) , Indomethacin (Chemiron Ltd) , Saline (BDH) and Carrageenan (Sigma Aldrich).

Plant material

Adequate quantities of whole *Phyllanthus fraternus*plant were collected from the Plant Production Department of Centre For Scientific Research Into Plant Medicine (CSRPM) at Akuapem-Mampong , Ghana in the month of February and authenticated by Dr. Yaw Ameyaw, a Botanist of the Production Department. Immediately after collection, the plant samples were cut into small pieces and spread thinly on a flat, clean tray to prevent spoilage by moisture condensation and allowed to dry at room temperature for 3 days.

Animals

Sprague Dawley males Rats (156 to 201g average weight) were obtained from the Animal Unit of the CSRPM. The animals were fed on powdered feed obtained from Ghana Agro Food Company (GAFCO), Tema – Ghana. They were allowed free access to sterile distilled water.

Preparation of extracts

To about 300g of the plant sample was added 4liters of distilledwater. The mixture was boiled for 45 minutes, sieved through a wire mesh and allowed to cool. The extract was freeze dried and stored in a cool dry place. This was reconstituted in sterilized distilled water before use. To another 300g of the plant sample was added 4liters of 70% ethanol. This was thencovered for 72hrs and sieved through a wire mesh. The extract was subjected to rotary evaporator to evaporate the ethanol before freeze driedand store in a desiccator.

Acute toxicity test

The acute oral toxicity study (Carl, 1963) was carried out as per the guideline set by the Organization for Co-operation and Development (OECD guidelines 425) received from the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Anti-inflammatory activity

The anti-inflammatory activity of the extract was evaluated in Sprague- Dawley males Rats in six (6) groups of six (6) animals per group for each dose according to the carrageenan-induced paw oedema described by Winter *et al.*, (1963). The aqueous extract at doses of 100 and 200mg/kg body weight were given to groups (3 & 4) respectively and the ethanolic extract at doses of 100 and 200mg/kg body weight were given to groups (5 &6) respectively and were administered orally an hour before the subcutaneous injection of 0.1ml of sterile normal saline solution of carrageenan 1% (w/v) into the sub planter region of the right hind paw. The control group, (2) received distilled water whiles the reference drug indomethacin 25.0mg/kg was also given to group (1) prior to induction of oedema (baseline). Paw volumes were measured using plethysmometer 30 minutes before administration of carrageenan and thereafter, readings were taken hourly until the 4^{th} hour past plant extracts administration. The anti-inflammatory activities were calculated as the degree of paw edema (*e*) and the degree of paw oedema inhibition (*i*) using the formulae:

$$e = \frac{E_0 - E_T}{E_0} \times 100\% \qquad i = \frac{e_0 - e_T}{e_0} \times 100\%$$

E_0= The pawvolume at the baseline

E_t = The paw volume at a particular reading time of the right hind paw.

e_o = the degree of paw edema of untreated

e_t = The degree of paw edema of test group.

Statistical analysis

All data from oedema paw volumes were means (n =6). Values for oedema inhibition were expressed as percentages and compared to control using one way-ANOVA. Data were statistically analyzed using students t-test and $P < 0.05$ was considered significant.

Phytochemical screening of *Phyllanthus fraternus*whole plant extracts

The aqueous and ethanolic whole plant extracts of *P. fraternus* were screened for the presence of groups of phytochemicals according to standard methods(Trease and Evans, 2002; Sofowora, 1993).

RESULTS AND DISCUSSION

Preliminary phytochemical screening of the extracts showed that saponin, phenolics, reducing sugars, triterpenes and phytosterols were present in both extracts while cynogenic glycoside, alkaloids and anthraquinones were absent in both extracts.Flavonoids and polyuronides were however present only in the aqueous extract.

The result slightly varies from those reported by Olonisakin *et al.* (2004), Okokon *et al.* (2005) and Matur *et al.*(2009). The variation may be attributed to differences in plant location, mode of extraction as well as season of plant sample collection. Reports have shown that anti-inflammatory activities of many agents have been through membrane stabilization and inhibition of protein denaturation. Several reports have implicatedsterols, Sparzak *et al*., (2009); flavonoids, saponins and tannins, Padmanabhan and Jangle (2012) and triterpenes, Toshihiro *et al.*, (2010) as anti-inflammatory agents.

The anti-inflammatory activity demonstrated by both extracts may be attributed to the presence of some these phytochemicals. The extracts showed modest anti-inflammatory activity in a dose dependent manner as manifested in the results (Table 3). Our result seems to be in agreement with similar investigations. The activity of the standard drug however, was significantly higher than those of the extracts.

From the graph, the degree of paw inhibition by the standard drug (indomethacin, 25.0 mg/kg) increased significantly with time as compared to the test groups. Among the test groups (plant extracts), however, the aqueous treatment inhibited paw oedema with time better than the ethanolic extract treatment in a dose dependent pattern.

The presence of flavonoids in the aqueous extract could be the basis of the better activity shown by the aqueous extract. Flavonoids have been considered to possess significant anti-inflammatory properties, both *in vitro* and *in vivo* (Vasudevan *et al.*, 2007; Ihantola-Vormisto *et al.*, 1997; Raja *et al.*, 2012; Khatoon *et al.*, 2006).

There are several reports that suggest that flavonoids act through a variety of mechanisms to prevent and attenuate inflammatory responses and serve as possible cardioprotective, neuroprotective and chemoprotective agents (Gomes *et al.*, 2008). Generally, the crude nature of the extracts could be responsible for their low anti-inflammatory activities compared with the standard control. Further separation of the various phytochemicals found in the crude could yield better results.

Table. 1: Phytochemical constituents of aqueous and ethanolic extracts of whole *Phyllanthus fraternus* plant.

Phytochemical	Extract	
	Aqueous	Ethanolic
Saponins	Present	Present
Phenolics	Present	Present
Phytosterols	Present	Present
Reducing sugar	Present	Present
Cynogenic glycosides	Absent	Absent
Alkaloids	Absent	Absent
Anthraquinones	Absent	Absent
Flavonoids	Present	Absent
Triterpenes	Present	Present
Polyuronides	Present	Absent

Table. 2: Acute toxicity test for *P. fraternus* whole plant aqueous and ethanolic extracts.

	Phyllantus fraternus whole plant	
	Aqueous extract	Ethanolic extract
Species and strain	Sprague-Dawley rats	Sprague-Dawley rats
No. of animals	12	12
Sex	Females	Females
No. of groups	3 (N=4)	3 (N=4)
Route of administration	Oral	Oral
Formulation	Freeze dried	Freeze dried
Dose administered (mg/kg)	1250, 2500, 5000	1250, 2500, 5000
Period of observation	48 hours	48 hours
No. of deaths	0	0
Approximate LD_{50}	>5000 mg/kg	>5000 mg/kg
Signs of toxicity	Nil	Nil

The LD_{50} is greater than 5000 mg/kg and may be classified as practically non-toxic and within the acceptable margin of safety (Hodge and Sterner scale) at the recommended dose. Thus $1/50^{th}$ and $1/25^{th}$ (i.e. 100mg/kg and 200mg/kg) were selected for the study.

Table. 3: Anti-inflammatory activity of aqueous and ethanolic whole plant extracts of *Phyllanthus fraternus.* Mean degree of paw oedema inhibition(%)

Time (hr)	Control drug	Aqueous extract of *P. fraternus*		Ethanolic extract of *P. fraternus*	
		Gp 3 (100mg/kg)	Gp 4 (200mg/kg)	Gp 5 (100mg/kg)	Gp 6 (200mg/kg)
1	57.55	18.66*	25.39*	10.49*	12.74*
2	59.13	23.78*	33.11*	10.56*	19.38*
3	63.10	30.67*	40.39*	11.83*	26.86*
4	65.32	35.23*	49.07*	13.49*	28.00*

Degree of paw oedema inhibition after treatment with standard/extracts.

* Values significantly different from standard drug indomethacin ($p<0.05$)

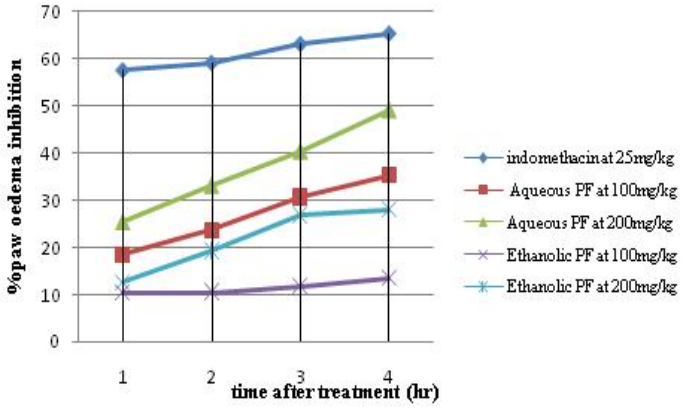

Fig. 1: Graph showing % hind paw-oedema inhibition of the various extracts of *Phyllanthus fraternus* (PF) after treatment.

CONCLUSION

Aqueous and ethanolic whole plants extracts of *P. fraternus* have shown modest anti-inflammatory activity on carrageenan-induced paw oedema in a dose dependent fashion. This investigation suggests that *P. fraternus* is a potential candidate for the discovery of new anti-inflammatory agents.

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REFERENCES

Atul R.C. and Fahim J.S. Evaluation of membrane stabilizing and inhibition of protein denaturation activity of *Phyllanthus fraternus* Webster. Asian Pacific Journal of Tropical Biomedicine. 2012; 1-4

Catapan E., Otuki M.F., Viana A.M., Yunes R.A., Bresciani L.F.V., Ferreira J., Santos A.R.S., Calixto J.B. & Cechinel-Filho V. Pharmacological activity and chemical composition of callus culture extracts from selected species of Phyllanthus. Pharmazie. 2000; 55: 945-946

Carl M.P. Experimental joint disease observations and adjuvant-induced arthritis. Journal of Chronic Disease. 1963; 16: 863-874

Corrado B., Marco T., Colucci R., Fornai M., Antonioli L., Ghisu N. and Tacca M.D. Role of coxibs in the strategies for gastrointestinal protection in patients requiring chronic non-steroidal anti-inflammatory therapy. Pharm. Res. 2009; 59: 90-100

Gomes A., Fernandes E., Lima J.L. and Corvo M.L. Molecular mechanisms of anti-inflammatory activity mediated by flavonoids. Curr. Med Chem. 2008; 15: 1586-1605

Halliwell B., Cross C.E. and Gutteridge J.M.C. Free radicals, antioxidants and human disease: where are we now? Journal of Lab Clin Med. 1992; 119: 598-620

Ihantola-Vormisto A., Summanen J., Kankaanranta H., Vuorela H., Asmawi Z.M., Moilanen E. Anti-inflammatory activity of extracts from leaves of *Phyllanthus emblica*. Planta Medica. 1997; 63: 518-524

Jay R.P., Priyanka T., Vikas S., Nagendra S.C., Vinod K.D. *Phyllanthus amarus*: Ethnomedicinal uses, phytochemistry and pharmacology: A review. Journal of Ethnopharmacology. 2011; 138: 286-313

Kiemer A.K., Hartung T., Huber C. and Vollmar A.M. Phyllanthus amarus has anti-inflammatory potential by inhibition of iNOS, cox-2 and cytokinine via the NF-KB pathway. J. Hepatol. 2003; 38: 289-297

Khatoon S., Rai V., Rawat A.K.S., Mehrotra S. Comparative pharmacognostic studies of three Phyllanthus species. Journal of Ethnopharmacology, 2006; 104: 79-86

Leslie T. Herbal Secrets of the Rainforest. 2nd Edition. Sage Press Incorporated. WHO (2003). Traditional medicine. WHO (ed). Genevapp1-334.

Matur B.M, Mathew T. and Ifeanyi C.I.C. Analysis of phytochemical and invivo antimalarial properties of *P. fraternus* Webster extract. New York Science Journal. 2009; 2 (5): 12-19

Okokon J.E., Ofodum K.C., Ajibesin K.K., Danladi B. and Gamaniel K.S. Pharmacological screening and evaluation of antiplasmodial activity of Croton zambesicus against Plasmodium berghei berghei infection in mice. Indian Journal of pharmacology. 2005; 37 (4): 243-246

Olonisokan A., Aremu M.O. and Omonigbehin E.A. Phytochemical and antimicrobial investigations of extractive from Phyllanthus amarus. Biosciences, Biotechnology Research Asia. 2004; 2 (1)

Oseni L. A. and Kadiri O. A comparative evaluation of in vitro growth inhibitory activities of different solvent extracts of some medicinal plants in Northern Ghana against selected human pathogens. IOSR Journal of Pharmacy. 2012; 2(2): 199-206

Padmanabhan P. and Jangle S.N. Evaluation of in-vitro anti-inflammatory activity of herbal preparation, a combination of four medicinal plants. International Journal of Basic and Applied Medical Sciences. 2012; 2(1) : 109-116

Raja C., Biplab D., Nayakanti D., Saikat S. Anti-inflammatory, antinociceptive and antioxidant activities of *Phyllanthus acidus* L. extracts. Asian Pacific Journal of Tropical Biomedicine. 2012; S953- S961

Sofowora A. Medicinal Plants and Traditional Medicine in Africa. 2nd Ed. Sunshine House, Spectrum Books Ltd, Ibadan (1993) 134-156

Sparzak B., Miroslawa K-B. and Loretta P-O. High performance Thin-layer Chromatography Densitometric determination of β-Sitosterol in Phyllanthus species. Journal of AOAC International. 2009; 92 (5): 1343-1348

Toshihiro A., Nobuo K., Takashi K., Ken Y., Harukuni T., Eliot T. M., Aranya M., and Jiradej M. Anti-inflammatory and Chemopreventive Effects of Triterpene Cinnamates and Acetates from Shea fat. Journal of Oleo Science. 2010; 59 (6) : 273-280

Trease G.E. and Evans W.C. Pharmacognosy. 15th Ed. Saunders Publishers, London (2002) 42-44. 221-229, 246-249, 304-306, 331-332, 391-393

Vasudevan M., Gunman K.K. and Parle M. Antinociceptive and anti-inflammatory effects of Thespesia populnea bark extract. Journal of Ethnopharmacology. 2007; 109: 264-270

Winter C.A., Risley E.A. and Nuss G.W. Carrageenin-induced oedema in hind-paw of the rat as an assay for anti-inflammatory drugs. Journal of Pharmacology and Experimental Therapeutics. 1963; 141: 369-373

Wunderlin, R.P., and Hansen, B.F. Guide to the Vascular Plants of Florida. 2nd ed. University Press of Florida. Gainsville, Florida (2003) 787.

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