Analysis of the phytochemical and *invivo* antimalaria properties of P*hyllanthus fraternus* webster extract

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Abstract
The Phytochemical screening and *in vivo* antimalaria and acute toxicity properties of the crude (water) plant extract of *Phyllanthus fraternus* Webster was investigated. The phytochemical screening of the plant extract revealed the presence of alkaloids, flavonoids, tannins, glycosides, saponin, carbohydrates, resins and phenols. Acute toxicity (LD_{50}) test of the crude extract on malaria parasites *P. berghei* gave a value of 692.8mg/kg ±2.8. The *in vivo* antimalaria activity of the extract against *Plasmodium berghei berghei* was assessed within 4 days of suppressive test. *P. fraternus* extract showed antimalaria property for both suppressive and curative tests. Chemo suppression of 77.23%, 85.15% and 86.39% was recorded on mice at 50mg/kg, 100mg/kg and 200mg/kg body weight dosages respectively as compared to 97.03% curative rate obtained from chloroquine (reference drug). The finding highlights the importance of plant products for the treatment of diseases such as malaria and other major tropical disease in Africa. [New York Science Journal. 2009;2(5):12-19]. (ISSN: 1554-0200).

Key words: *phytochemical*, *invivo*, *antimalaria properties*, P*hyllanthus fraternus*, *extract*

Introduction

Ethno-medicinal study is today recognized as the most viable method of identifying new medicinal plants or refocusing on those earlier reported for bioactive constituents (Adjanahoun *et al*., 1991; Farnsworth, 1966).

Medicinal plant, according to Sofowora (1982), is one which (one or more of) its organs contain substances that can be used for therapeutic purposes. It may be in form of vegetable drug, which may either be organized (material which possess a cellular structure e.g. leaf, bark, petal, root etc) or unorganized (non cellular structural medicinal agents such as gum, latex etc). It may be a decoction, which may be in cold water or prepared by bringing it to boil and allowing it to cool, or tisane, which is tea made by either decoction, or infusion (Peters, 1965). Today, traditional medicine has brought to focus a wider coverage of primary healthcare delivery, not only in the African region but also, to various countries of the world. It is the first choice of healthcare treatment for at least 80% of Africans suffering from high fever and other common ailments (Elujoba *et al.*, 2005).

Traditional medicine, a major African socio-cultural heritage, obviously in existence for several decades, was once believed to be primitive and wrongly challenged with animosity by foreign religions and conventional or orthodox medical practitioners (Elujoba *et al*., 2005). In recent years, natural products are of interest because drug resistance by diseases is on the increase (White and Nosten, 1993) and herbal remedies are being sought by a cross section of scientist for various ailments (Odetola and Bassir, 1980). The use of herbs for disease management in Africa and Nigeria in particular could be traced to early man who probably acquired the skill of healing through deliberate or accidental selection of plants and their parts (Sofowora, 1982).

The plant *Phyllanthus fraternus* Webster (**Family:** *Euphorbiaceae)* commonly called; gulf leaf-flower, Chanca piedra, quebra pedra, stone braker, arranca-pedras, carry-me-seed, hurricane weed, para-parai mi, quinine weed Mache da goyo (Hausa), Gbogbon owun lese (Yoruba) is a small, erect, annual herb

(Dicotyledonous) that grows 30–40 cm in height (Wunderlin and Hansen, 2002). It is indigenous to the rainforests of the Amazon and other tropical areas throughout the world, including the Bahamas, southern India, and China. *P. fraternus* is quite prevalent in the Amazon and other wet rainforests, growing and spreading freely (much like a weed). 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).

The plant is employed for numerous uses by the indigenous peoples. These include treatment of blennorrhagia, colic, diabetes, dysentery, fever, flu, tumors, jaundice, vaginitis, and dyspepsia. Based on its long documented history of use in the region, the plant is considered analgesic and as an aperitif, carminative, digestive, laxative, stomachic, tonic, and vermifuge (Leslie, 2003).

The wide usage of this plant in the treatment of disease suggest it trial test against the causative agent of malaria (*P. berghei).*

MATERIALS AND METHODS

Study area

This research was carried out at the National Institute for Pharmaceutical Research and Development (NIPRD), Idu – Abuja, Nigeria. The plant *Phyllanthus fraternus* Webster was collected from Minna in Niger State and identified at the herbarium of NIPRD, Idu – Abuja.

Drying / micronization of plant parts

The plant material (leaves and stems), was spread thinly on a flat, clean tray (to prevent spoilage by moisture condensation) and allowed to dry at room temperature for seven days (Sofowora, 1982). The dried plant material was pounded using a clean mortar and pestle and then blended into fine powder with electric blender (Binatone model BLG – 400). Mercerization was done to enhance the penetration of the extracting solvent (water) into the cells, thus facilitating the release of active ingredients (Sofowora, 1982).

Extraction

Sixty two grams (62g) of the powdered plant was macerated in 200ml of water for 48hours. It was filtered and evaporated with a rotary evaporator to concentrate the filtrate. The semi-solid extract was transferred into a sterile container and stored in the refrigerator.

Column Chromatographic analysis of extract

2.20g of water extract of *Phyllanthus fraternus* Webster was dissolved in a mixed solvent of hexane, ethyl acetate and methanol (table 1). 30g of silica gel was used as stationary phase.

Hexane:	Ethyl Acetate:	Methanol:	Volume (ml)
100	0	0	100
70	30	0	100
50	50	0	100
0	100	0	100
0	70	30	100
0	50	50	100
0	30	70	100
0	0	100	100

Eight fractions eluted from the column and thin layer chromatography (TLC) of the fractions was carried out using the solvent system hexane: ethyl acetate: methanol in the ratio 3:2:1. The retention factor (Rf) was calculated thus:

Rf = Distance moved by solute/Distance moved by solvent

Phytochemical screening of extract

The phytochemical screening of *Phyllanthus fraternus* extract was carried out to determine the following compounds; alkaloid, flavonoids, tannins, anthraquinones, saponins, glycosides, resins, terpenes and phenols (Sofowora 1993).

In-Vivo antimalaria test
Swiss albino mice (18-25g) obtained from the National Veterinary Research Institute, Vom, Nigeria were acclimatized for a period of 10 days at National Institute for Pharmaceutical Research Development, Idu, Abuja. The mice were infected with 0.2ml of infected blood containing about 1 x 107 dose of *P. berghei berghei* (about 64.0%) from a donor mouse (obtained from IPRD Idu) using a hemocytometer. Each mouse was inoculated on day one, intraperitoneally (Odetola & Bassir, 1980).

Drug administration: The drug (chloroquine) was positive control, distilled water (placebo) was negative control and the extracts of *P. fraternus* used in the study was administered intraperitoneally (treatment drug).

Acute toxicity (LD_{50}) test: Lorke's (1983) method of determining LD_{50} was used to determine the toxicity level of the extracts in mice. Three groups (A,B,C) containing four mice each were subjected to treatment intraperitoneally with the extract at 500mg/kg, 1000mg/kg and 1500mg/kg body weight respectively for phase I test. They were kept in check for ten days and mortality recorded from each group.
In view of the result obtained from phase I treatment, phase II treatment was carried out using a dosage of 600mg/kg, 800mg/kg and 1000mg/kg on another three groups of four mice respectively. Route of administration was also intraperitoneal and mortality was recorded.
Toxicity was calculated using the formula:
LD_{50} = √maximum dose for all survival x minimum dose for all death

SUPPRESSIVE TEST (Evaluation of schizontocidal activity on early infection**):** Peters' 4 –day suppressive test against *Plasmodium berghei berghei* infection in mice was employed (Peters, 1970).
The mice were divided into five groups of five mice each. The first three groups were administered 50,100 and 200 mg/kg/day doses of the extract for four consecutive days, while the fourth group was administered chloroquine 5mg/kg/day and the fifth group was administered an equivalent volume (5ml) of normal saline (control group) for four consecutive days (D_1-D_4).
On the fifth day (D_5), thin blood films were prepared from blood collected from the tail. The films were air-dried, fixed in methanol for 30seconds, and stained with 10% giemsa for 20minutes. The slide was rinsed carefully and thoroughly under running tap water and left to stand in an upright position to dry (Inger *et al*., 2004).
Prepared slides were viewed under the X100 objective (oil immersion) light microscope with special ocular and the condenser sufficiently close to give a good contrast. Parasites were search in -an area of a giemsa-stained thin blood film and without moving the slide; the numbers of infected erythrocytes in the whole area (i.e. the big and small squares) were counted. The slide was moved to randomly adjacent fields and counting continued as above. More fields were counted until the sum of 100 erythrocytes in the small fields was reached. The average percentage suppression of parasitaemia was calculated in comparison to controls as shown below:
Average % suppression = average % Parasitaemia in control groups – average % parasitaemia in treated groups X 100/Average % parasitaemia in control group

That is: $\frac{\text{Control mean - Dose mean}}{\text{Control mean}} \times \frac{100}{1}$
The means were calculated as Mean ± Standard Error of Mean (SEM) where
SEM = $\frac{\text{Standard deviation}}{\sqrt{n}}$

CURATIVE TEST: Peter's (1970) method was used.
25 mice infected with 0.2ml of the standard inoculum were weighed, labeled and grouped into five. Doses of 50, 100 and 200mg/kg body weight/day of the plant extract and 5mg/kg/body weight per day of chloroquine (standard group) and 5ml/kg/body weight per day normal saline (control group) were administered for five days (D_1-D_5). At D_6 thin blood films collected from the tail region were prepared for parasitaemia. The blood films were examined using a light microscope and the parasitized erythrocytes on each slide counted.

Results

Of the 62 g weight of powdered *Phyllanthus fraternus* 9 g (14.52%) was extracted and used for treatment test. Phytochemical screening of the extract showed that alkaloids, flavonoids, tannins, saponin, glycosides, phenols and resins were present in the extract while anthraquinones and terpenes were absent. Based on the lethal concentration, dose treatments of up to 200 mg/kg were prepared for the suppressive test and the result are as presented in table 2 and 3 below;

Table 1: Result of Acute Toxicity (LD_{50}) Test in Mice.

CODE	WEIGHT (g)	DOSE	SURVIVAL
RA	16.5		
LL	20.2	500mg/kg	All survived beyond 24 hours
BKTL	20.0		
RLRS	23.4		
RLLA	18.6		
RLRA	15.3	600mg/kg	All survived beyond 24 hours
RALL	20.6		
HDBKTL	23.5		
TLRS	20.7		
HDLS	21.4	800mg/kg	All died within 24 hours
LARA	23.5		
RLLS	24.2		
RL	16.9		
HDRL	21.4	1000mg/kg	All died within 24 hours
RS	19.5		
HDLL	20.4		
LS	17.4		
HDTL	22.3	1500mg/kg	All died within 24 hours
LA	18.2		
HDRA	24.0		

KEY:

TL = Tail, HD = Head, BK = Back, RL = Right Leg, R = Right, LL = Left Leg, L = Left or Leg, A = Arm and S = Side. For example RA= right arm, HDLS= head left side and HDBKTL= head back tail. These are the parts of the animal that were marked with ink for identification.

The LD_{50} was calculated using Lorke's method (1983) as:

LD_{50} = √maximum dose for all survival X minimum dose for all death

LD_{50} = √600 x 800 = √480000

LD_{50} = ± 692.8mg/kg.

Table 2: Antimalaria Properties of Water Extract of *Phyllanthus fraternus* (Suppressive Test on *Plasmodium berghei*)

Code	**Wt (g)**	**Vol. of Treatment**	**Dose**	**No. of Parasites**	**Mean Parasitaemia**
RS	24	0.12		20	
RL	21.4	0.15	5mg/kg/day	26	
BKRL	24.3	0.12	Normal Saline	18	20.2±1.69
HD	20.2	0.1	(control)	16	
LS	27	0.13		21	
TLRA	21.2	0.1		10	
TLLS	24	0.12	50mg/kg/day	6	4.6 ± 1.54
BK	25.4	0.12	Extract	2	
TLLA	27	0.13		3	
LL	29.1	0.14		2	
NM	22	0.22		2	
RLLA	24	0.24	100mg/kg/day	4	3 ± 0.63
RALS	25	0.25	Extract	5	
HDBK	27	0.27		2	
LARA	29	0.29		2	
BKLL	23	0.46		2	
TLRS	23	0.46	200mg/kg/day	4	2.75 ± 0.42
HDRL	26	0.52	Extract	0	
RA	26	0.52		3	
LLRL	31	0.62		2	
BKTL	17.3	0.08		0	
HDTL	24.1	0.12	5mg/kg/day	0	0.6 ± 0.40
TL	25.2	0.12	Chloroquine	2	
LA	27	0.13	(Standard)	1	
LSRS	27.4	0.14		0	

Standard Error of Mean (SEM) = $\frac{\text{Standard Deviation}}{\sqrt{n}}$

Table 3: Summary of Suppressive Test with Water extract

TREATMENT	DOSE	MEAN PARASITAEMIA COUNTS	% INHIBITION
NORMAL SALINE (CONTROL)	5ml/kg	20.2 ± 1.69	0.00
EXTRACT	50mg/kg	4.6 ± 1.54	77.23
EXTRACT	100mg/kg	3.0 ± 0.63	85.15
EXTRACT	200mg/kg	2.75 ± 0.42	86.39
CHLOROQUINE (STANDARD)	5mg/kg	0.6 ± 0.40	97.03

Table 3: Antimalaria Properties of Water Extract of *Phyllanthus fraternus* (Curative Test on *Plasmodium berghei*)

Code	**Wt (g)**	**Vol. of Treatment**	**Dose**	**No. of Parasites D4**	**D7**
NM	19	0.08		21	30
HDRL	19	0.08	5ml/kg/day	24	35
BKLS	22	0.11	Normal Saline	28	40
RS	22	0.11	(Control)	16	38
RA	33	0.16		22	28
RL	20	0.1		20	7
LL	22	0.11	50mg/kg/day	19	4
BK	24	0.12	(Extract)	17	10
HD	27	0.14		24	10
HDBK	29	0.15		15	2
HDLL	21	0.21		35	8
TLRS	22	0.22	100mg/kg/day	20	5
BKRA	25	0.25	(Extract)	26	2
BKLA	27	0.27		18	1
LS	29	0.29		22	7
TLRA	21	0.42		27	8
LSRS	22	0.44	200mg/kg/day	30	2
LLRL	25	0.50	(Extract)	28	2
RLLS	26	0.52		18	5
TLLS	30	0.60		34	0
BKTL	27.8	0.14	5mg/kg	30	1
TL	22	0.11	Chloroquine	22	0
HDTL	24	0.12	(Standard)	18	2
LA	27	0.13		21	2
TLLA	28	0.14		19	0

Table 4: Summary of Curative Test for Water Extract.

TREATMENT	DOSE	PRETREATMENT	POSTTREATMENT
NORMAL SALINE (CONTROL)	5ml/kg/day	22.2 ± 1.96	34.2 ± 2.29
EXTRACT	50mg/kg/day	19 ± 1.52	6.6 ± 1.60
EXTRACT	100mg/kg/day	24.2 ± 3.01	4.6 ± 1.36
EXTRACT	200mg/kg/day	27.4 ± 2.64	3.4 ± 1.40
CHLOROQUINE (STANDARD)	5mg/kg/day	2 ± 2.12	1 ± 0.45

Table 5: Mean survival time of mice receiving various doses of water extract of *P. fraternus* during an established *P. berghei berghei* infection in mice.

Drug/Extract	**Dose (mg/kg/day)**	**Mean Survival Time (Day)**
P. fraternus Extract	50	11.5±3.51
	100	20.5 ±0.63
	200	25.5± 0.54
Chloroquine (Standard)	5ml	27.5± 0.73
Normal saline (Control)	5ml	7.5 ±0.76
One-way ANOVA	F	3.176
	P	<0.05

Discussion

Phytochemical screening of the *Phyllanthus fraternus* plant extract revealed the presence of alkaloids, tannins, saponin, flavonoids, glycosides, resins, phenols and carbohydrates. This is similar to research findings on *Phyllanthus amarus* plant by Olonisakin et al (2004) and Okokon et al (2005). Of all of these metabolites, carbohydrate was the most frequent followed by alkaloids, flavonoids, saponin and glycosides. Resins and phenols were the least frequent.

The suppressive activity of the extract is shown on table 2(a) with the summary on table 3(b). The extract at 50mg/kg, 100mg/kg and 200mg/kg weight of mice yielded 77.23%, 85.15% and 86.39% inhibition respectively as against 97.03% for chloroquine. Results after 4 days treatment showed mean Parasitaemia of 4.6±1.54, 3.00±0.63, 2.75±0.42, 0.6±0.40 and 20.2±1.69 for 50mg/kg, 100mg/kg, and 200mg/kg of extract, chloroquine and normal saline respectively. Percentage chemo suppression was also observed to increase as extract concentration increased. The curative activity showed decrease in Parasitaemia with increase in dose similar to but lower than the chloroquine standard group as shown on table 3(b).

The plant Phyllanthus fraternus was observed to show some intrinsic antimalaria activity by its percentage chemo suppression and even curative ability compared to that of chloroquine which is the standard drug. The activity might be attributed to the presence of alkaloids or flavonoids which has been identified present in this work; or even a combined action of more than one metabolite. However, the active compound(s) known to give this observed activity need to be identified.

This study has however, established the rationale for traditional use of this plant in Nigeria as remedy for malaria infection.

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