



COMPARATIVE ANTIMALARIAL ACTIVITY OF *PHYLLANTHUS AMARUS* AND *ENICOSTEMMA AXILLARE* PLANTS EXTRACTS

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ABSTRACT

Malaria is a prevalent disease in India. The results show that the LD₅₀ of the ethanolic extract of *Phyllanthus amarus* and *Enicostemma axillare* was 500 to 1500 mg/Kg. The *in vivo* antimalarial activity of the extract against *Plasmodium berghei* was assessed within 4 days of suppressive test of *P. amarus* and *Enicostemma axillare*. The present study involves comparative screening of leaves of *phyllanthus* and root of *Enicostemma* for anti-malarial activity on *plasmodium berghei* induced Swiss albino mice using chloroquine as standard. Ethanolic and aqueous extracts (50, 100 and 200 mg/Kg b.w.) of leaves of *phyllanthus* and root of *Enicostemma axillare* were screened administered, produced significant ($p < 0.05$), dose-dependent activity against the parasite in the suppressive, the present study therefore validates the local use of the extracts of *Phyllanthus amarus* Schumach and Thonn as an antimalarial agent. Further studies are however recommended to identify and possibly characterize the potential antiplasmodial agents in the aqueous extract of the plant.

Key words: Antimalarial, *Phyllanthus amarus*, *Enicostemma axillare*, Chloroquine.

INTRODUCTION

Phyllanthus amarus Schum (Family Euphorbiaceae) is a widely distributed small erect, tropical annual Herbal shrub, whose stem has green capsule, and grows up to 10-50 cm high and blooms with flowers with 5 white sepals and apical acute anther. The fruit has green capsules, and smooth and fruiting pedicels. The seeds are longitudinally rugose. It is locally called Iyin-olobe¹. Increased agricultural production is attributed to excessive use of synthetic chemicals during the last century. This has raised a number of ecological and human health problems as most of them are associated with several harmful effects, e.g., prolonged existence of these chemicals in the environment leads to resurgence of resistance among various fungi, and contaminates environment and food chain, which cause serious

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ecological imbalance². The success of the natural products quinine and artemisinin, most potent antimalarial drugs provided impetus the study of plants as antimalarial agents. Ethno pharmacological and then phytopharmacological approach for the search of new antimalarial agents from plant sources has proved to be more predictive. Based on the photochemistry and traditional knowledge *Enicostemma littorale* seems to be a viable candidate. Many plants from family gentianaceae, like *Swerita chirata*, showed good antimalarial activity³. The present study determines the possible antiplasmodial effects of the aqueous extract of the leaves and stem of the plant against *Plasmodium berghei* infection using Swiss albino mice as models. The blood schizonticidal activity of the aqueous extract in early infection and in established *Plasmodium berghei* infection was assessed and compared to the activities of chloroquine and sulfadoxine/pyrimethamine. The repository activity of the extract was also assessed and compared to the activity of pyrimethamine⁴. Malaria is the most prevalent among the insect-borne diseases. Every year it kills between one and two million people, with as many as 300–500 million people being infected. It is estimated that nearly half the world population is at risk, with fatal rates being extremely high among young children below 5 years of age⁵.

Enicostemma littorale (Gentianaceae) is a glabrous perennial herb belonging to the family Gentianaceae (20). It grows throughout India upto 1.5 feet height and more frequently near the sea. It is called as Chota-kirayat or Chota chirayata in Hindi, Mamejavo in Gujarati, Nagajivha in Bengal and Vellarugu or Vallari in Tamil⁶. Hence, the present study was aimed towards the comparative screening of both the plants extracts for anti-malarial activity of ethanolic extract against *Plasmodium berghei* infected Swiss albino mice in order to scientifically.

EXPERIMENTAL

Plant material

P. amarus leaves and *Enicostemma axillare* root were collected around the Bhopal and authenticated at department of botany of the institute. Voucher specimen of the plant has been preserved in our herbarium in Pest control and Ayurvedic drug research laboratory, Vidisha for further reference.

Plant extraction

Collected leaves were dried and the dried plant material was crushed using a clean mortar and pestle and then blended into fine powder with electric blend. Powdered material was charged into soxhlet apparatus and continuous hot extraction was carried out using solvents like petroleum ether, ethanol and water.

Animal selection

Healthy Swiss albino mice of either sex weighing 25-30 g were selected for the study. The study was carried out in accordance with the rules and regulations laid by the Institutional Animal Ethical Committee. The animals were housed with free access to food and water. The basal food intake and body weight were noted. Rats were starved 24 hrs prior to the study. Both the plants extracts were evaluated in five groups of five animals each.

Acute toxicity study

The oral acute toxicity of the ethanol extract was estimated in albino mice (25 – 30 g) by medium lethal dose (LD50) described by Lorke's method⁷. A total of fifteen albino mice of both sexes were employed, acclimatization period of 24 h was allowed. The extract was weighed and dissolved in distilled water. The test was carried out. In the first, the extract was administered orally at doses of 500, 1000 and 1500 mg/Kg to three groups of 5 animals each received, respectively. The animals were monitored for 24 h and number of deaths per group recorded. Then, the mice were observed continuously for one hr after the treatment; intermittently for four hrs, and thereafter over a period of 24 hrs⁸. The mice were observed for gross behavioral changes such as feeding, hair erection, mortality and other signs of toxicity manifestation⁹. The mice have free access to food and clean water during the experiment.

Drug administration

The drug (chloroquine) was positive control, distilled water was negative control and the extracts of *P. amarus* and *Enicostemma axillare* used in the study was administered Intraperitoneal (treatment drug).

Animals and Inoculation¹⁰⁻¹²

Suppressive test (Evaluation of schizonticidal activity on early infection): Peters' 4 – day suppressive test against *Plasmodium berghei* infection in mice was employed¹³. A Total of 25 mice were used for studies on the Blood schizonticidal activity. Each mouse was subsequently given standard intra-peritoneal inoculums of 1.02×10^5 *P. berghei* parasites (chloroquine-sensitive) with the aid of a 1 mL disposable syringe 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 5 mg/Kg/day and fifth group was administered 5 mL of normal saline (control group) for four consecutive days (D1-D4). On the fifth day (D5), thin blood smears were made samples obtained from the tails of the animals. The smears were stained

with Giemsa stain and examined under the light microscope for the levels of parasitaemia. The average percentage suppression of parasitaemia was calculated in comparison to control.

Average % suppression = average % Parasitaemia in control groups – average % parasitaemia in treated groups x 100/Average % parasitaemia in control group

$$\text{That is: } \frac{\text{Control mean} - \text{Dose mean}}{\text{Control mean}} \times 100$$

The means were calculated as Mean \pm Standard Error of Mean (SEM) where

$$\text{SEM} = \frac{\text{Standard deviation}}{\sqrt{n}}$$

Table 1: Acute oral toxicity of the ethanolic leaf extracts of *Phyllanthus amarus* and aqueous root extracts of *Encostemma axillare* administered orally to mice

Dose mg/Kg	Mortality		Toxic symptoms
	D/T	Latency (h)	
0	0/5	-	None
500	0/5	-	None
1000	0/5	-	None
1500	0/5	-	None

Table 2: Effects of Ethanolic leaf extract of *Phyllanthus amarus* on early malaria infection

Treatment	Dose	Mean parasitaemia counts	% Inhibition
Extract	50 mg/Kg	8.2 \pm 0.25	53.4%
Extract	100 mg/Kg	2 \pm 0.62	88.6%
Extract	200 mg/Kg	1.8 \pm 0.59	89.7%
Chloroquine (Standard)	5 mg/Kg	0.8 \pm 0.39	95.4%
Normal saline (Control)	5 mL/Kg	017.6 \pm 1.83	0.00 %

Table 3: Effects of aqueous root extract of *Enicostemma axillary* on early malaria infection

Treatment	Dose	Mean parasitaemia counts	% Inhibition
Extract	50 mg/Kg	9.5 ± 1.3	23.38%
Extract	100 mg/Kg	3 ± 0.76	75.80%
Extract	200 mg/Kg	2.2 ± 0.66	82.25%
Chloroquine (Standard)	5 mg/Kg	2 ± 0.63	83.87%
Normal saline (Control)	5 mL/Kg	12.4 ± 1.57	0.00%

RESULTS AND DISCUSSION

The mortality rate and the acute toxicity symptoms of orally administered *P. amarus* and *Enicostemma axillare* leaf and root extract increased as the dose increased from 500 to 1500 mg/Kg (Table 1). All the treated mice were carefully observed for 24 hours for any signs of toxicity (behavioral changes and mortality). D/T: dead/treated mice; none: no toxic symptoms were recorded during the Observation period; latency: time to death (in hours) after the dose administration. Early malaria infection or Peters four days suppressive activity test for the ethanol leaf extract of *P. amarus* and aqueous root extract of *Enicostemma axillare* produced a dose dependent suppression activity and was shown in the Table 2 and Table 3. The extract of 50 mg/Kg/day, 100 mg/Kg/day and 200 mg/Kg/day weight of the mice yielded of *phyllanthus amarus* 53.4 %, 88.6% and 89.7% and *Enicostemma axillare* mice yielded 23.38%, 75.80%, 82.25% inhibition respectively as against 95.4 % and 83.87% for chloroquine. Results after 4 days treatment showed mean Parasitaemia of 8.2 ± 0.25, 2 ± 0.62, 1.8 ± 0.59, 0.8 ± 0.39 and 017.6 ± 1.83 for 50 mg/Kg, 100 mg/Kg and 200 mg/Kg of *phyllanthus amarus* extract, and 9.5 ± 1.3, 3 ± 0.76, 2.2 ± 0.66, 2 ± 0.63, 12.4 ± 1.57 for 50 mg/Kg, 100 mg/Kg and 200 mg/Kg of *Enicostemma axillare* extract, Chloroquine and normal saline respectively. The antimalarial activity produced by the extract was statistically significant ($P < 0.05$) when related to control. Percentage suppression was observed to increase as extract concentration increased. The plant *Phyllanthus amarus* and *Enicostemma axillare* was observed to show some intrinsic antimalarial activity by its percentage suppression and compared to that of chloroquine which is the standard drug.

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