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[Research Article]

ANTIMALARIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF ETHANOLIC LEAF EXTRACT OF *PHYLLANTHUS NIRURI AND MIMOSA PUDICA*

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ABSTRACT

The ethanolic extract of *Phyllanthus niruri and Mimosa pudica* leaves was investigated for antimalarial activity against *Plasmodium berghei* infections in mice. The median lethal dose was determined to ascertain the safety of the extract in mice. The antimalarial activities during early and established infections were evaluated. Phytochemical screening was also investigated to elucidate the possible mechanism of the antimalarial properties. The extract of *P.niruri* and *M.pudica* leaf demonstrated significant antiplasmodial activity in all the three models of the antimalarial evaluations. Phytochemical screening revealed the presence of some vital antiplasmodial constituents such as terpenoids ,flavonoids and alkaloids. The leaf extract of *P.niruri* and *M.pudica* thus possesses antimalarial activity, which explains the rational usage of this plant in traditional medicine.

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Key Words
P.niruri, M.pudica, ethanolic extract; antimalarial activity.

INTRODUCTION

Malaria is one of the most important health problems in sub-tropical and tropical countries. The World Health Organization estimates that 2,300 million people, or 41% of the total world population, live in areas with malaria risk. More than 300 to 500 million clinical cases are reported annually resulting in at least 1.5 to 2.7 million deaths. Approximately 1 million deaths among children under 5 years old are attributed to malaria alone or in combination with other diseases [WHO, 1997, 1998]. As such, there is need for continuous search for newer drugs that can retard or reverse this resistance. This has also been the reason for an increase in the search for antimalarial plants from the Indian flora. Since many modern drugs such as quinine and artemisinin originate from plants, it is essential that other medicinal plants which have folklore reputation for antimalarial properties are investigated, in order to establish their safety and efficacy, and to determine their potential as sources of new antimalarial drugs [Gessler, et al., 1994]. Currently, multi-drug resistance has become one of the most important problems impeding malaria control efforts [Htut *et al*., 2009, Sendagire *et al*., 2005.) This has led to attempts to discover other antimalarial agents, mainly from plant sources. Medicinal plants may provide antimalarial drugs directly, as in the case of quinine from cinchona bark, or they may supply template molecules on which to base further new structures by organic synthesis—artemisinin from *Artemisia annua*. *Phyllanthus niruri*, locally named kellanelli, is one of the medicinal plants traditionally used to treat malaria in India. *Phyllanthus niruri* has been used traditionally to treat various illnesses, including renal stones, gastrointestinal disturbances, cough, hepatitis, gonorrhea, fever and malaria. This plant was reported to possess hypoglycemic activity [Hukuri *et al.,* 1988], angiotensin-converting enzyme inhibition [Ueno, *et al.,* 1988], lipid lowering activity [Khanna *et al.,* 2002], anticancer activity [Giridharan *et al.,* 2002] and anti-HIV activity [Qian-Cutrone *et al.,* 1996]. Mimosa pudica is another plant called locally as touch me not. It is used to treat various diseases like Anthelminthic; antibacterial; antibiotic; antihyperglycemic; anti-implantation; anti-inflammatory antimicrobial; anti-pyretic, anti-spasmodic, antiviral; bactericide. However, very little scientific information is available about its activity against *P. falciparum* although this plant is extensively used to treat malaria. Hence in the present study an attempt has been taken to investigate it antimalarial activity.

Objective of the study:

The objective of the present study is to evaluate the antimalarial property of *P.niruri* and *M.pudica* plant extracts.

MATERIALS AND METHODS:

Plant extracts

The plant *P. niruri and M.pudica* was collected in its natural habitat in and around Bharathiar University campus, Tamil Nadu, India and the herb was air-dried and ground to provide a fine powder. Extracts were then prepared by soxhalation of the powder with methanol solvent. Two hundred grams of the powder was soxhalated with 1,000 ml of methanol for 24 hours. Upon evaporation under reduced pressure, methanolic extracts were obtained.

Preliminary Phytochemical analysis:

Phytochemical screening of the *P.niruri and M.pudica* extracts were carried out using standard procedures to test the presence of alkaloids, saponin glycosides, cardenolides, flavonoids, tannins, polyphenolic compounds, anthraquinones.

Parasites and Innoculum

P. berghei were used to assess the in-vivo intrinsic antimalarial activity. The test protocol was based on the 4-day suppressive test described by Peters et al. [Peters *et al.,* 1975]. Parasite strain was maintained by serial passage of blood from mouse to mouse. A standard inoculum of 1×10^7 of parasitized erythrocytes from a donor mouse in volumes of 0.1ml was used to infect the experimental animals intra-peritoneally.

Animals

Male albino mice weighing between 27–30 g were used for this study. The animals were fed Standard mouse cubes and clean drinking water *ad libitum.* Animals were caged in groups of five. The animals were housed in the Animal House in Kovai Medical Centre and Hospital, College of Pharmacy, Coimbatore.

Acute Toxicity Tests

The oral acute toxicity of the ethanol extract was estimated in albino mice (27 - 30g) by medium lethal dose (LD50) described by Lorke's method [Lorke, 1983]. 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 hrs [CDER, 1996.]. The mice were observed for gross behavioral changes such as feeding, hair erection, lacrimation, mortality and other signs of toxicity manifestation [Pillai, 1984.]. The mice have free access to food and clean water during the experiment.

Test on early malaria infection (4-day suppressive test)

This test was a modified *Makinde et al.* [1989] and Peters and Robinson [1992] methods. Twenty five mice were divided into five groups of five mice each were inoculated with the parasite at the commencement of the experiment (day 1). Group's 1-3 mice received 400, 600 and 800mg extract/kg body weight i.p. respectively. While the 4th group which served as the positive control received 5mg chloroquine/ kg body weight, mice in 5th group received 1ml distilled water and served as the negative control. On the fifth day (i.e., day 5) two drops of blood samples from the animals' caudal vein were taken and transferred on slides, thus, making thin film from each mouse and staining with Giemsa stain, Then, each stained slide was examined under the microscope with an oil immersion objective of 100x magnification power to evaluate the percent suppression of each extract with respect to the control groups so that the average percentage (%) parasitaemia could be evaluated as

$$\text{Average \% suppression} = \frac{\text{Average Parasitaemia in negative control} - \text{Average Parasitaemia in drug treated group}}{\text{Average Parasitaemia in negative control}} \times 100$$

Test on established infection (curative or Rane test)

The method was a modified on Ryley and Peters, [1970]. Twenty five mice were divided into five groups of five mice each were inoculated with the parasite on the first day of the experiment (day 1). The mice were not treated until the parasitaemia was established. On day 4 i.e., 72h after the animals were infected. Group's 1-3 mice received 400, 600and 8000mg extract/kg body weight per day for 4 days i.p. While the 4th group which served as the positive control received 5mg chloroquine/kg bodyweight i.p for, mice in 5th group received 1ml distilled water and served as negative control for the same period. On the fifth day (i.e., day 5) two drops of blood samples from the animals' caudal vein were taken and transferred on slides, thus, making thin film from each mouse and staining with Giemsa stain so that the average percentage (%) parasitaemia could be evaluated for each of the doses using the formula above. After the sixth day, the animals were fed *ad libitum* and observed for 28 days. Any death that occurred during this period was noted and used to determine the mean survival time.

Statistical analysis

Data obtained by this study were analyzed using SPSS (version 16, 2004). The Student's t-test and ANOVA (one- or two-way) were used to test the differences between groups. Differences between means at 5% level ($P \leq 0.05$) were considered significant.

RESULTS:

The mortality rate and the acute toxicity symptoms of orally administered *P.niruri* and *M.pudica* leaf extract increased as the dose increased from 500 to 1500 mg/kg (Table 1). The main observed behavioural signs of toxicity were asthenia, piloerection, ataxia, anorexia, urination, diarrhea, lethargy and coma. There were no signs of toxicity as above said. According to Horn [1956] and Rhiouani *et al*. [2008], plants or plant products with LD50 values higher than 2,000–3,000 mg/kg are considered free of any toxicity. This supports the logical usage of this plant in folk medicine practices.

All the treated mice were carefully observed for 24 hours for any signs of toxicity (behavioural 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 chemosuppressive activity test for the ethanol leaf extract of *P.niruri* produced a dose dependent chemosuppression activity and was shown in the table 2. The highest suppression of parasitaemia was observed at the dose of 800mg/kg body weight of mice. Percentage suppression was observed to increase as extract concentration increased. After four days treatment with the different extract doses, the mean parasitaemia of the test groups ranged from 16.0±1.0% to 37.6±0.9% while the corresponding value of the negative control group being 49.4±0.7%. The mice treated with CQ were completely free from the parasites on day four. The antimalarial activity produced by the extract was statistically significant ($P < 0.05$) when related to control.

The result of the *in vivo* evaluation of the *P.niruri* extract on established infection showed a slight increase in chemo-suppressive activity. The extract was marginally active at 400 mg/kg per day (40%) and active at 600 and 800mg/kg per day doses (60% for both), respectively (Table 3).The mice that received 5mg of chloroquine/kg per day however showed 100% chemo-suppression. The antimalarial activity produced by the extract was statistically significant ($P < 0.05$) when related to control.

Early malaria infection or Peters four days chemosuppressive activity test or for the methanol leaf extract of *M.pudica* produced a dose dependent chemosuppression activity and was shown in the table 4. The highest suppression of parasitaemia was observed at the dose of 800mg/kg body weight of mice. Percentage suppression was observed to increase as extract concentration increased. After four days treatment with the different extract doses, the mean parasitaemia of the test groups ranged from 15.8±1.0% to 28.0±0.9% while the corresponding value of the negative control group being 34.0±1.0%. The mice treated with CQ were completely free from the parasites on day four.

The result of the *in vivo* evaluation of the *M.pudica* extract on established infection showed a slight increase in chemo-suppressive activity. The extract was marginally active at 12.5mg/kg per day (40%) and active at 25 and 50mg/kg per day doses (60% for both), respectively (Table 5).The mice that received 5mg of chloroquine/kg per day however showed 100% chemo-suppression.

Phytochemical screening of the ethanolic extract of the two plants revealed that the leaf extract contains terpenoids, flavonoids, alkaloids, saponins, steroids and glycosides. (Table 6)

Table 1. Acute oral toxicity of the ethanolic leaf extracts of *Phyllanthus niruri* and *Mimosa pudica* 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 niruri* on early malaria infection

S.No	Treatment	Doses (mg/kg/day)	*Average parasitemia in percentage	% chemo-suppression	Significance
1.	Extracts	400	37.6±1.0	23.8	$P < 0.05$
2.	Extracts	600	27.4±0.6	44.5	$P < 0.05$
3.	Extracts	800	16.0±0.3	67.6	$P < 0.05$
4.	Chloroquine	5	1.0±0.0	100	-
5.	Distilled water	1ml	49.4±1.2	0	-

** = Values were presented as Mean ± SEM, n= 5*

Table 3: Effects of ethanolic leaf extract of *Phyllanthus niruri* on established malaria infection

S.No	Treatment	Doses (mg/kg/day)	*Average parasitemia in percentage	% chemo-suppression	Significance
1.	Extracts	400	39±1.0	25.8	$P < 0.05$
2.	Extracts	600	30.4±0.9	41.7	$P < 0.05$
3.	Extracts	800	20.6±0.4	60.8	$P < 0.05$
4.	Chloroquine	5	1.0±0.0	100	-
5.	Distilled water	1ml	52.2±1.5	0	-

** = Values were presented as Mean ± SEM, n= 5*

Table 4: Effects of ethanolic leaf extract of *M.pudica* on early malaria infection

S.No	Treatment	Doses (mg/kg/day)	*Average parasitemia in percentage	%chemo-suppression	Significance
1.	Extracts	400	37.6±1.1	17.2	$P < 0.05$
2.	Extracts	600	31.4±0.7	30.8	$P < 0.05$
3.	Extracts	800	28.0±0.3	38.3	$P < 0.05$
4.	Chloroquine	5	1.0±0.0	100	-
5.	Distilled water	1ml	45.4±1.5	0	-

** = Values were presented as Mean ± SEM, n= 5*

Table 5: Effects of ethanolic leaf extract of *Phyllanthus niruri* on established malaria infection

S.No	Treatment	Doses (mg/kg/day)	*Average parasitemia in percentage	%chemo-suppression	Significance
1.	Extracts	400	38.2±1.0	22.35	$P < 0.05$
2.	Extracts	600	29.8±0.6	39.40	$P < 0.05$
3.	Extracts	800	22.2±0.2	54.87	$P < 0.05$
4.	Chloroquine	5	1.0±0.0	100	-
5.	Distilled water	1ml	49.2±1.6	0	-

** = Values were presented as Mean ± SEM, n= 5*

Table 6: Phytochemical analysis of ethanolic extract of *P.niruri and M.pudica* leaves:

Phytochemical	Test	Observation	Indication
Tannins	Ferric Chloride	+ve(Blue-green colour)	Present
Anthraquinone	_	+ve(Brightpink colour)	Present
Saponins	Frothing Test	+ve	Present
Cardenolide	Keller-Killani	+ve(Bluish-green colour)	Present
Flavonoids	Ferric Chloride	+ve(Green colour)	Present
Alkaloid	Meyer	+ve(creamcolouredprecipitate.)	Present
Terpenoids	-	+ve	Present

(+ ve) Shows the Presence of phytochemical constituent

(-ve) Shows the Absence of phytochemical constituent

DISCUSSION:

Mosquitoes cause allergic responses in humans that include local skin and systemic reactions such as angioedema [Peng *et al.,* 1999]. In the present study after the treatment of plant extracts showed considerable parasites inhibitory effect. Phytochemical compounds such as terpenoids are commonly implicated in the antiprotozal and antiplasmodial activity of many plants [Philipson *et al.,* 1991, Francois *et al.,* 1996, Ghoshal et al., 1996, Asase et al., 2010.). An example of common terpenoids is artemisinin, the main active ingredient in the traditional Chinese antimalarial qinghaosu. Flavonoids are the other form of the two plants phenolic structures. Flavonoids showed significant antiparasitic activities against different strains of malaria, trypanosome and leishmania [Kim *et al.,* 2004, Monbrison *et al.,* 2006, Tasdemir *et al.,* 2006]. These chemical compounds may be acting singly or in synergy with one another to exert the observed antimalarial activity of *Phyllanthus niruri* and *Mimosa pudica*.

The plant *P.niruri and M.pudica* 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 Tamil nadu as remedy for malaria infection.

In conclusion, the present work has shown, in an animal model of malaria, the efficacy of extracts of the two plants (*Phyllanthus niruri* and *Mimosa pudica*) traditionally used in chemotherapy of *Plasmodium falciparum* infection in humans. So the traditional use of these plants to treat malaria is based on a real anti-parasitic activity. It would therefore be worthwhile to purify the active components by a bioassay-guided isolation. With the enriched fractions or the pure compounds, we would be able to assess the parasite life phase on which the plant extracts are most active.

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