

## Full Length Research Paper

# Phytochemical screening and *in vivo* antimalarial activities of crude extracts of *Lantana trifolia* root and *Premna oligotricha* leaves in *Plasmodium berghei* infected mice

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Resistance of malaria parasites to several antimalarial drugs mandates the need for new compounds from affordable sources. Thus the current study was conducted to justify the traditional usage of *Lantana trifolia* roots and *Premna oligotricha* leaves to combat malaria. The powdered roots of *Lantana trifolia* and leaves of *Premna oligotricha* were macerated using 70% ethanol. Secondary metabolites present in the extracts were determined using the standard chemical method. The *in vivo* antiplasmodial effect of the crude extracts were evaluated using the four-day suppressive test in chloroquine (CQ) sensitive strain of *Plasmodium berghei* infected mice. Parameters such as parasitaemia, packed cell volume, body weight change and survival time of mice were then determined as using standard tests. The ethanol extracts showed a dose-dependent chemosuppression when compared to the negative control in this study. The chemosuppressive effect produced by all doses was very significant ( $P < 0.001$ ). Concentrations at 350, 500 mg/kg of leaf extract and 200 mg/kg from root extract have demonstrated prevention effect on weight loss of mice ( $P < 0.001$ ). All the test extracts employed in this study have no significant prevention effect on PCV loss of mice at all dose levels. The present study has demonstrated the efficacy of the extracts of *Premna oligotricha* leaf and *Lantana trifolia* root traditionally used in chemotherapy of malaria infection. These observations provide the basis for the traditional use of these plants in treatments of malaria and potential for development of novel therapeutics in the conventional medicine.

**Key words:** Antimalarial, *in vivo*, *Lantana trifolia*, parasitaemia, *Plasmodium berghei*, *Premna oligotricha*.

## INTRODUCTION

Malaria is a life-threatening infectious disease caused by plasmodium parasite. It affects more than 200 million

people worldwide with an estimated death of nearly 1 million people, mostly children. The disease is also

responsible for causing significant economic loss, especially in developing countries (WHO, 2015). In Ethiopia, for example, malaria adversely affects the health of the public and the country's economy as a result of significant expansion to previously non-affected areas with endemicity of about 75% (WHO, 2004; Adhanom et al., 2006; Gebreyohannes et al., 2017). Subsequently, it attracts significant attention from various stakeholders in designing a strategy to prevent and control the disease.

Treatment of malaria infection with conventionally used anti-malarial drugs are puzzled by the emergence of resistant parasites, which reduces the importance of these drugs in controlling malaria infection (Kilama, 2005; Birru et al., 2017). Various strategies are designed and undertaken to enhance the treatment outcomes of anti-malarial drugs, including combination of drugs and the discovery of new compounds from plants (Tchoumboungang et al., 2004; Okafor et al., 2013). Most of the currently used antimalarial drugs including chloroquine, primaquine and artemisinin derivatives are derived from plants which have been traditionally used against malaria.

In addition, the society relies on traditional medicine practice; and plants having antimalarial properties is commonly used as an alternative therapy to combat malaria (Waako et al., 2007; Builders et al., 2011). These necessitates the importance of further studies on medicinal plants which are reported to have antimalarial activity. In Ethiopia, despite the presence of a huge number of plants which are claimed to have anti-malarial activity, only few studies have been conducted to evaluate the anti-malarial activity and safety margins of these plants (Tadesse and Wubneh, 2017).

The roots of *Lanata trifolia* are traditionally used for the treatment of various human ailments such as malaria, fever, dermatitis, wound, bleeding, asthma, cough, colds, diarrhea, and bronchitis (Atkin and Kadercit, 2004). The plant is also used in East Africa to treat fever, epilepsy and cerebral malaria (Mukungu et al., 2016; Nalubega et al., 2013). Consumption of *L. trifolia* fruit has also been reported in Konso, Ethiopia (Ocho, 2012). Previous study demonstrated that the plant has shown anti-inflammatory and anti-nociceptive activity (Silva et al., 2005; Johnson et al., 2017).

Based on ethnobotanical survey conducted in South Omo, the leaves of *Premna oligotricha* is used for the treatment of malaria (Waako, 2007). *P. oligotricha*, is distributed in Ethiopia, Kenya and Somalia. The plant is locally known as 'yeweba medhanit' or 'drug of malaria' by the society in South Omo, Southern Ethiopia. As a result of pleasant smelling, the plant is also used to fumigate and cleanse gourds in Marsabit districts of Ethiopia and Kenyas (Ketemma et al., 2013). Studies

also revealed the presence of two antibacterial diterpenes, a clerodane and labdane (WHO, 2005; Habtemariam et al., 1990).

Although *L. trifolia* and *P. oligotricha* are traditionally used for the treatment of malaria in Ethiopia, there is no study which evaluates the anti-malarial activities and safety of these plants. Therefore, this study is designed to evaluate the in vivo antimalarial activities of crude extract of *L. trifolia* and *P. oligotricha* leave in Plasmodium berghei infected mice. The study also examined the safety profiles and phytochemical constituents of both plants.

## MATERIALS AND METHODS

### Collections and preparation of plant materials

The roots of *L. trifolia* were collected from the premises of Hawassa city, South Ethiopia. Whereas, the leaves of *P. oligotricha* were collected from the location 50 km from Bulle Hora on the highway to Yabello, Oromiya regional state, Ethiopia. After collection, the plant materials were confirmed by a taxonomist and a voucher specimen (AE-001/08) was deposited at the herbarium of Addis Ababa University (AAU), Ethiopia.

### Extraction

Prior to extraction, the plant parts were washed with distilled water and dried under shade just after specimen collection. The powdered roots and leaves were macerated using 70% ethanol. Then, the extracts of both plants were filtered using Whatmann filter paper No. 1 and the ethanol was removed using rotary evaporator at average of 40 RPM under reduced pressure at a temperature of 40 to 45°C. The crude extracts obtained were kept in a refrigerator at 4°C and fresh solutions using 3% tween 80 were prepared for each extract immediately before the test.

### Laboratory animal

Swiss albino mice of 6 to 8 weeks of age, weighing 25 to 32g, were maintained in a standard room and acclimatized to the laboratory condition for 14 days in 12 h light and dark cycle. Animals were provided with a standard pellet diet and water on *ad libitum*. Mice were handled according to the international guidelines for the care and use of animals in the experiments (European Community Guidelines, 1986). The study was also approved by the institutional review board (IRB/026/08) of Hawassa University, Ethiopia.

### The parasite strain

The anti-malarial activity of *L. trifolia* roots and *P. oligotricha* leaves were tested on mice infected with chloroquine-sensitive *P. berghei* strain ANKA which was obtained from the Department of Biomedical Sciences, Addis Ababa University. Viable strains of *P. berghei* was maintained by a weekly passage of blood from an

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infected donor mouse (with a rising parasitemia of 30%) to non-infected mice. Blood from infected mouse was collected through cardiac puncture. Taking into account the level of parasitemia of infected mouse and the erythrocyte count of non-infected mouse, the blood was diluted to get  $1 \times 10^7$  *P. berghei*-infected RBCs in 0.2 ml normal saline diluted blood. On the first day (D0), mice were injected intraperitoneally with the 0.2 ml of infected and diluted blood (Knight and Peters, 1980).

### Grouping of animals

Then, infected mice were allocated randomly into 8 groups of 5 mice each. The first 6 groups received an oral dose of 200, 350 and 500 mg/kg b. wt root and leave extracts of *L. trifolia* and *P. oligotricha*, respectively. The other two groups were served as positive and negative control groups, and received an equal volume of 3% of Tween 80 (vehicle) and chloroquine (25 mg/kg/day; orally) (Ethiopian Pharmaceutical Manufacturing, Ethiopia), respectively.

### In vivo anti-malarial test

Antiplasmodial activity of the test extracts was assessed following

$$\% \text{ Suppression} = \frac{\text{Parasitemia in the negative control group} - \text{Parasitemia in test group}}{\text{Parasitemia in the negative control group}} \times 100$$

### Body weight changes and packed cell volume (PCV)

The body weights and PCV at D0 and D4 of mice were taken to observe whether the test extracts prevented the weight loss and reduction in PCV that are commonly observed with increasing parasitaemia in *P. berghei* infected mice. The PCV was determined by the following equation:

$$\text{PCV} = \frac{\text{Total volume erythrocytes in a given volume of blood}}{\text{Total volume of blood}} \times 100$$

### Determination of the percentage survival

The curative potential of the fractions was assessed by monitoring the mortality starting from the day of treatment in infected mice, daily. The number of animals survived from the time injecting blood containing the parasite up to 10th day was determined for each group. The comparison was made based on the percentage of animals that survived on the 10th day after treatment.

### Test for acute oral toxicity studies

In order to assess the safety profile of crude extracts of *L. trifolia* and *P. oligotricha*, mice were divided randomly into two groups of six animals each (three male and three female). Mice were fasted for one night prior to administration of the extracts and provided with only water. Then, the extracts were given with an increasing doses of 2000, 3500, 5000 mg/kg using oral gavage following the standard guidelines (WHO, 2001). Animals were observed for any signs of toxicity for 14 days.

Observation was carried out for any signs of overt toxicity such as hair erection, salivation, lacrimation, diarrhea, tremor, convulsion, and mortality.

the standard four-day suppressive test protocol (Waako et al., 2005). The mice were divided randomly into five groups consisting of five mice each. 3 h after infection, 200, 350 and 500 mg/kg/day oral doses of *L. trifolia* and *P. oligotricha* crude extracts were given to the test groups. The control groups were treated with chloroquine (25 mg/kg/day, orally) and an equal volume of 3% Tween 80. Treatment of the test and control groups was conducted for four consecutive days (day 0 to day 3). On day 4, blood samples were collected from the tail of each mouse. Thin blood smears were made and stained with 10% Giemsa stain. The numbers of parasitized RBCs out of 100 RBCs in random fields were used to determine the parasitemia level. Then, average percentage parasitemia was determined using the following formula:

$$\% \text{ Parasitemia} = \frac{\text{Number of infected RBCs}}{\text{Total number of RBCs}} \times 100$$

In addition, the average percentage of parasitemia suppression was calculated using the following formula:

### Phytochemical screening

Crude extracts of *L. trifolia* and *P. oligotricha* were evaluated for the presence of secondary metabolites such as flavonoids, alkaloids, triterpenoids, resins, tannins, saponins and steroids, according to the previously established protocols (Debella, 2002; Jones and Kinghorn, 2006).

### Statistical analysis

Data were analyzed using Windows, statistical package for social science (SPSS) Version 22.0. Comparison of percentage suppression, PCV, weight changes and percentage survival with the negative control group was made using One-way Analysis of Variance (ANOVA) followed by Tukey's honest significant difference (HSD) test. P-values less than 0.05 was considered statistically significant.

## RESULTS

### Acute toxicity study

Oral acute toxicity studies on the extracts of *L. trifolia* roots and *P. oligotricha* leaves showed that the extracts were found to be safe up to a maximum dose of 5000 mg/kg. Administration of either of the extracts did not produce mortality, signs of morbidity and a significant behavioral and physical changes in graded doses given to experimental animals. Therefore, the results reveals that the medium lethal doses (LD<sub>50</sub>) of the extracts are greater than 5000 mg/kg body weight.

**Table 1.** The effect of crude extracts of *P. oligotricha* leaf and *L. trifolia* root on survival time, percentage parasitemia and chemosuppression in *P. berghei* infected mice.

Treatment groups	% survival of animal on day 10	% parasitemia	% chemosuppression
<b><i>P. oligotricha</i></b>			
200 mg/kg leaf	80.40±0.50*	48.16±0.86*	26.21±2.05*
350 mg/kg leaf	80.60±0.50*	34.84±0.42*	46.66±0.70*
500 mg/kg leaf	80.60±0.50*	29.84±0.98*	54.29±1.40*
<b><i>L. trifolia</i></b>			
200 mg/kg root	80.40±0.40*	52.07±1.28*	20.26±2.82*
350 mg/kg root	80.60±0.24*	41.74±0.68*	37.12±1.12*
500 mg/kg root	90.00±0.63*	35.68±1.44*	46.93±2.13*
Choloroquine (25 mg/kg)	100.00±0.00*	00.00±0.00*	100.00±0.00*
Negative control	60.40±0.24	65.37±0.94	00.00±0.00

Results are expressed as Means ± SEM, n = 5. \*P <0.05 versus negative control.

**Table 2.** The effect of crude extracts of *P. oligotricha* leaf and *L. trifolia* root on body weight of *P. berghei* infected mice.

Treatment groups	Weight (g) before treatment	Weight (g) after treatment	% change
<b><i>P. oligotricha</i></b>			
200 mg/kg	21.00±0.63	22.00±0.32	+4.54±1.64
350 mg/kg	28.20±0.58	26.40±0.60	-6.81±0.36*
500 mg/kg	24.60±0.24	26.60±1.29	+7.51±1.10*
<b><i>L. trifolia</i></b>			
200 mg/kg	27.80±0.80	28.00±0.80	+0.71±0.00*
350 mg/kg	22.20±0.49	21.20±0.80	-4.71±0.18
500 mg/kg	22.40±0.50	21.60±0.67	-3.70±0.07
Choloroquine (25 mg/kg)	20.80±0.37	19.40±0.24	-7.21±1.30
Negative control	20.40±0.67	18.40±0.40	-10.86±1.27

Results are expressed as means ± SEM, n = 5. \*P <0.05 versus negative control.

### Effect of the extracts on percentage parasitemia and survival of mice

The effects of treatment with crude extracts of *P. oligotricha* leaf and *L. trifolia* root on parasitemia suppression and survival of *P. berghei* infected mice are shown in Table 1. The study exhibited that crude extracts of both plants exhibited a significant (P<0.05) and dose-dependent chemosuppression compared with the negative control. The chemosuppression was maximum at higher doses for both extracts (54 and 46% for *P. oligotricha* and *L. trifolia*, respectively at 500 mg/kg dose) compared with the negative control.

In addition, 10th day assessment of infected and extract treated mice demonstrated that the extracts prevented mortality in 80 to 90% of mice. Accordingly, mice treated with all doses of extract lived longer than

the negative control (Table 1).

### Effect of the extracts on body weight loss

Doses of 350 and 500 mg/kg *P. oligotricha* leaf extract and 200 mg/kg *L. trifolia* root extract demonstrated a significant (P <0.05) weight gain in mice compared with the negative control group (Table 2). However, the 350 mg/kg dose of *P. oligotricha* leaf extract caused significant body weight loss (P <0.001) compared to the negative control group (Table 2).

### Effect of the extracts on PCV

The extracts employed in this study failed to prevent PCV

**Table 3.** The effect of crude extracts of *P. oligotricha* leaf and *L. trifolia* root on PCV of *P. berghei* infected mice.

Treatment groups	PCV before treatment	PCV after treatment	% change
<b><i>P. oligotricha</i></b>			
200 mg/kg	57.32±2.53	53.02±3.04	-8.11±2.20
350 mg/kg	60.60±2.48	59.04±2.43	-2.64±1.40
500 mg/kg	62.38±1.44	56.04±2.11	-11.31±1.42
<b><i>L. trifolia</i></b>			
200 mg/kg	62.32±2.43	57.42±2.26	-8.53±1.18
350 mg/kg	60.42±1.91	56.20±2.28	-7.50±1.00
500 mg/kg	58.74±0.83	62.06±1.04	+5.34±0.24
Choloroquine (25 mg/kg)	60.52±1.44	63.72±1.08	+5.02±0.34*
Negative control	61.88±0.97	54.80±0.91	-12.9±0.15

Results are expressed as means ± SEM, n = 5. \*P <0.05 versus negative control.

**Table 4.** Phytochemical constituents of crude ethanol extract of *P. oligotricha* leaf and *L. trifolia* root.

Secondary metabolite	<i>P. oligotricha</i> leaf	<i>L. trifolia</i> root
Phenol	+	-
Flavanoid	+	-
Tannin	+	-
Steroids	+	+
Terpenoids	+	+
Steroidal glycosides	+	+
Alkaloids	-	+
Quinines	-	-
Saponins	+	+
Resine	-	+
Glycosides	+	+

NB: (+) = presence, and (-) = absence.

loss at any dose ( $P >0.05$ ), compared with the negative control group (Table 3).

### Phytochemical screening

Phytochemical evaluation of the root and leaf extracts of *L. trifolia* and *P. oligotricha*, respectively was carried out following the standard protocols. Steroids, terpenoids, steroidal glycosides, saponins, and glycosides were found in the extracts of both plants (Table 4).

### DISCUSSION

The antimalarial activity of a compound is commonly assessed using the *P. berghei* infected rodent model because of the similarity with human malaria infection,

and the sensitivity of *P. berghei* to chloroquine. The model takes into account the contribution of the immune system in the elimination of infection (Waako et al., 2007).

The study demonstrated the antimalarial activity of crude ethanol extracts of *P. oligotricha* leaves and *L. trifolia* roots which are traditionally used in Ethiopia and elsewhere, against malaria infection. The four-day suppressive test was used to evaluate the impact of treatment with extracts on parasitemia suppression, survival time, PCV and body weight changes. Moreover, the acute toxicity profile and the phytochemical constituents of both plants were determined.

A compound which produces a minimum of 30% parasitemia suppression or greater percent survival compared to infected and non-treated mice is commonly considered to be an active antimalarial agent (David et al., 2004; Fentahun et al., 2017). The four-day

suppressive test showed that the crude extracts of *P. oligotricha* and *L. trifolia* demonstrated a significant and dose-dependent parasitemia suppression. The suppression caused by both plants was greater than 30% at 350 and 500 mg/kg doses.

The results indicates that the crude extracts of both plants are endowed with antimalarial activity with an optimal minimal dose of 350 mg/kg body weight. On the other hand, treatment with the crude extracts of both plants significantly enhance the percent survival of mice on the 10th day relative to the negative control group. The enhanced survival of mice in the extract treated group could be attributed to the suppressive effect of the extracts on parasitemia. The activity of the extracts, in suppressing parasitemia and enhancing survival time of infected mice, was in agreement with previous studies on other plant extracts such as *Echnopsis kebericho* (Toma et al., 2015), *Croton Machrostachyus*, *Dodonaea angustifolia* (Mengiste et al., 2012) and *Nigella sativa* (Dikasso et al, 2006), at a relatively comparable doses.

Phytoconstituents are frequently reported for the therapeutic benefits of herbal preparations (Habtemariam et al., 1991; Ayoola et al., 2008). Secondary metabolites such as alkaloids, triterpenoids, quassinoids, xanthenes, sesquiterpenes, flavonoids, quinines and phenolic compounds have shown significant antimalarial activities (Habtemariam et al., 1991; Nalubega et al., 2013). Therefore, the antimalarial activity of *P. oligotricha* and *L. trifolia* could be as a result of the individual or combined effects of the phytoconstituents present. Herbal remedies with immunomodulatory and antioxidant (Silva et al., 2005; Okokon et al., 2006) activities are shown to possess antimalarial activity. Moreover, inhibition of protein synthesis and prevention of invasion of new red blood cells (RBCs) by plasmodium parasites (Mukungu et al., 2016; Okokon et al., 2013) could also be considered as a possible mechanism of antimalarial activity.

Prevention of anemia and body weight loss in *P. berghei* infected mice is also a crucial criteria to consider as the best compound for antimalarial agent (Okokon et al., 2013). In this study, except chloroquine treated mice, the significant body weight gain was recorded only in 500 and 200 mg/kg doses of *P. oligotricha* and *L. trifolia*, respectively. On the contrary, treatment with all other doses demonstrated variable degrees of body weight loss. This might be due to the presence of appetite-suppressant metabolites in the extracts, which is supported by earlier studies on other plant extracts (Mukungu et al., 2016; Toma et al., 2015; Gebretsadik and Mekonnen, 2016; Verman et al., 2006).

Erythrocyte fragility, reduced PCV, and life-threatening anemia is commonly observed in *P. berghei* infected mice. The 500 mg/kg dose *L. trifolia* enhances the PCV of mice, which was comparable to the effects of chloroquine. However, *P. oligotricha* and the other doses of *L. trifolia* failed to prevent PCV reduction. The reduction of PCV might be associated with the presence

of saponins, which are responsible to cause hemolysis of erythrocytes (Yang et al., 2005).

Mice treated with the ethanolic extract of *P. oligotricha* and *L. trifolia* were found to be safe at an increasing dose of up to 5 000 mg/kg b. wt. Any orally administered test substance with LD<sub>50</sub> greater than 1000 mg/kg or higher than three times the minimum effective dose can be considered as nontoxic, and can be considered for further studies (Toma et al., 2015). Therefore, acute oral exposure to *P. oligotricha* and *L. trifolia*, at 5 g/kg can be considered non-toxic.

## Conclusion

The crude ethanol extracts *P. oligotricha* and *L. trifolia* exhibited significant and dose-dependent antimalarial activity in *P. berghei* infected mice, which may justify the traditional uses of the plants against malaria. Further studies are required to isolate and identify the active compound(s) responsible for the antimalarial activity .

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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