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Full Length Research Paper

Anti-plasmodial and antioxidant activities of methanol extract of the fresh leaf of *Lophira lanceolata* (Ochnaceae)

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This study was aimed at investigating the anti-plasmodial and antioxidant activities of the extract of the leaf of *Lophira lanceolata,* a traditional medicine recipe. The methanol extract (ME) obtained by 72 h cold maceration was evaluated for acute toxicity test (LD_{50}) and phytochemical constituents. The suppressive and curative anti-plasmodial activities of the extract were investigated using rodent malaria model. Mice (20 to 34 g) infected with 1×10^7 *Plasmodium berghei* parasitized red blood cell were used to test for suppressive and curative anti-plasmodial activities after oral administration of ME (100, 200 and 400 mg/kg) for four and seven days, respectively. The preliminary antioxidant activity of the extract (25, 50, 100, 200 and 400 µg/ml) was evaluated using *in vitro* 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging, hydrogen peroxide scavenging and reducing (power) ability assays. The methanol leaf extract of *L. lanceolata* exhibited a dose-dependent suppression of parasitaemia up to 100% suppression at 400 mg/kg. The suppression produced by the extract was significantly ($P< 0.05$) higher than the chemo suppression produced by 20 mg/kg chloroquine (50.90%). Similarly, the extract at the same doses (100, 200 and 400 mg/kg) exhibited significant ($P<0.05$) but non-dose-related decreases in parasitaemia in the curative model (60.90, 54.69 and 79.69%) which was comparable to the 82.80% decrease caused by chloroquine (20 mg/kg). Phytochemical studies revealed the presence of flavonoids, alkaloids, oils, saponins, glycosides, carbohydrates, acidic compounds, terpenoids and reducing sugar. The LD_{50} test caused no deaths in the treated mice up to 5,000 mg/kg body weight. The DPPH assay, for free radical scavenging effect of the methanol extract was significant ($P<0.001$) as the concentration increases. Hydrogen peroxide scavenging and reducing power assays showed concentration-dependent and significant ($P<0.05$) results. These findings suggest that methanolic extract of the leaf of *L. lanceolata* is safe up to a dose of 5,000 mg/kg body weight and possesses anti-plasmodial and anti-oxidant activities.

Key words: *Lophira lanceolata*, anti-plasmodial, anti-oxidant, plasmodium berghei, DPPH, hydrogen peroxide, albino mice.

INTRODUCTION

Malaria, a global scourge remains a leading cause of morbidity and mortality worldwide, especially in pregnant women and children, and particularly in tropical Africa, where at least 90% of the malaria deaths occur (WHO,

2002). The disease kills about one million people globally each year, or about 3,000 people daily. About 40% of the world's population lives in malaria endemic areas, while nine out of every 10 malaria infection cases occur in sub-Saharan Africa. Despite significant progress in the treatment of malaria, it has staged a comeback in many areas of the world, due to the resistant by parasites (Najera, 2001; Shiff, 2002). Human malaria is caused by various species of plasmodia, with *Plasmodium falciparum, Plasmodium malariae, Plasmodium Ovale, Plasmodium vivax and Plasmodium Knowlesi (*Modupe et al., 2011) out of the 30 species, accounting for more than 95% of the cases of malaria in the world. *P. falciparum* is the most predominant parasite specie accounting for about 98% of malaria cases (Modupe et al., 2011). In Nigeria, malaria transmission occurs all-year round in the South, and is more seasonal in the North. The country accounts for a quarter of all malaria cases in the WHO African region (WHO, 2008). Free radicals, particularly reactive oxygen species (ROS) have a greater impact on humans. Antioxidants can greatly reduce the damage due to oxidants by neutralizing the free radicals before they can attack the cells to prevent damage to lipids, proteins, enzymes, carbohydrates of and DNA. Anti-oxidants play an important role in inhibiting and sca-venging radicals, thus providing protection against infections and degenerative diseases.

Lophira lanceolata (*Ochnaceae)* is widely distributed in the sudano-guinean savannah zone from Senegal through the Central African Republic and northernmost DR Congo to Uganda. Northeast Tropical Africa: Sudan; East Tropical Africa: Uganda; West Central Tropical Africa: Cameroon, Central African Republic and Zaire; West Tropical Africa: Benin, Gambia, Ghana, Guinea, Nigeria, Senegal, Sierra Leone, Cote d'Ivoire, Guinea-Bissau, Togo and Mali (Protabase). *L. lanceolata* is the commonest species in the dry savannah areas while *Lophira procera* is the species found in the forest zone of West Africa. Flowering is from December to February. It is a multipurpose tree; in traditional medicine meni oil is used to treat dermatosis, toothache and muscular tiredness. The sap of the tree is used to treat tiredness by the Dii, Fulbe and Gbaya peoples in Cameroon. In Mali pounded roots, mixed with flour are used to treat constipation, while its concoction is used to cure chronic wounds. A concoction prepared from the roots is drunk by women against menstrual pain, intestinal troubles and malaria. The bark of the roots and trunk is used against pulmonary diseases. The bark is also used to treat fevers and gastro-intestinal problems, and in southern Nigeria the root bark is a remedy for yellow fever. The young stems and sometimes the roots are commonly used as chew-sticks, and an infusion of the bark is used as a mouthwash against toothache in Guinea, Mali and Nigeria.

The present study was designed to investigate the antioxidant and antiplasmodial activities of *L. lanceolata* as acclaimed by the traditional medicine practitioners which might be useful to unravel novel treatment strategies for diseases associated with free radical induced tissue damage.

MATERIALS AND METHODS

Animals

Swiss albino mice (18 to 25 g) of either sex obtained from the Animal Facility centre of the Department of Pharmacology and Toxicology, University of Nigeria Nsukka, were used for the investigation. The animals were kept in cages at room temperature and naturally illuminated environment of 12:12 h dark/light cycle. They were fed on standard diet and had water *ad libitum*. Handling and use of animals were in accordance to the NIH Guidelines for the care and use of laboratory animals (NIH Publication No. 85 to 23, revised 1985).

Chemicals and instruments

1,1-diphenyl-2-picrylhydrazyl (DPPH) Methanol (analytical grade), Ferric chloride, potassium ferricyanide, ascorbic acid, hydrogen peroxide, trichloroacetic acid, phosphate buffer (pH 6.6 and 7.4) were all obtained from Sigma Chemical Company Ltd. (USA). Absorbance measurements were recorded by a Shimadzu UV-160A UV-Visible Reading Spectrophotometer (Shimadzu Corporation, Japan) using disposable cuvettes (Sarstedt, Nümbrecht, Germany) for visible range, and quartz cuvettes for measurements in the ultraviolet (UV) range.

Parasites

Parasitized erythrocytes were obtained from a donor-infected mouse maintained at Animal Facility Centre, Faculty of Veterinary Medicine, University of Nigeria Nsukka. Parasites were maintained by continuous re-infestation in mice. Animals were inoculated intraperitoneally with infected blood suspension (0.2 ml) containing 1×10^7 *P. berghei* parasitized red blood cell.

Plant collection and identification

The fresh leaves of the plant were collected in May, 2013 from Nsukka Enugu Stat, Nigeria. The plant was identified and authenticated by Mr Alfred Ozioko of International centre for Ethnomedicine and Drug development (Inter CED) Nsukka.

Preparation of plant extract

The leaves were air-dried at room temperature and ground into powder using a grinder (ADDIS, Nigeria). The powdered material

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(2370 g) was macerated with 4.5 L of 70 % methanol for 72 h with constant shaking. The resultant mixture was filtered using Whatman (No. 1) filter paper and the filtrate was concentrated to dryness in vacuum at 40°C using rotary evaporator. This gave a yield of 108.81 g (4.59% w/w).

Phytochemical test

Preliminary phytochemical studies were carried out on the extract for the presence of alkaloids, tannins, saponins, terpenes, flavonoids, oils, glycosides, steroids, and carbohydrates using standard procedures (Trease and Evans, 1989).

Acute toxicity test

The safety of the extract orally was evaluated by determining its LD_{50} using the Lorke's (1983) method. Dose levels used were from 10 to 5,000 mg/kg. All the animals were kept under the same condition and observed for signs of acute intoxication and mortality for 24 h. The LD_{50} was calculated as the geometric mean of the highest non-lethal dose and the lowest lethal dose.

Experimental design and suppressive antiplasmodial assay

Evaluation of suppressive activity of the extract of *L. lanceolata* (4-days test) was performed as described by Knight and Peters (1980). Twenty five Swiss albino mice of either sex weighing (18 to 25 g) were inoculated by intra-peritoneal (i.p.) injection with 0.2 ml infected erythrocytes. The animals were divided into five groups of five per group and treated for four consecutive days. Group 1 and 2 received 3% Tween 80 (0.2 ml/kg) and chloroquine (20 mg/kg) daily, while groups 3, 4 and 5 received daily doses of the extract (100, 200 and 400 mg/kg), respectively. All administrations were by oral route. On day five of the study, thick and thin films were prepared with blood collected from the tail of each mouse. The films were fixed with methanol stained with Giemsa and parasitaemia was determined by counting the number of infected and uninfected red blood cells in 5 different fields. The percentage suppression of parasitaemia was calculated for each dose level by comparing the parasitaemia in infected controls with those of treated mice.

Curative antiplasmodial assay

Evaluation of curative potential of aqueous methanol extract of *Lophira lanceolata* was done by adopting the method described by Ryley and Peters (1970). Twenty five mice were selected and intra-peritoneally injected with 1 x 10^7 *Plasmodium berghei* infected erythrocyte on the first day. 72 h after, the animals were divided into five groups of six per group. Group 1 received 3% Tween 80 (0.2 ml/kg) and chloroquine (20 mg/kg) daily by orally. While, group 3, 4 and 5 received daily doses of the extract orally (100, 200 and 400 mg mg/kg, respectively). Treatment continued until the fifth day when thick and thin films were prepared with blood collected from the tail of each mouse. The films were fixed with methanol, stained with Giemsa and parasitaemia was determined by microscopic examination in 5 different fields. The mean survival time for each group was determined by finding the average survival time (days) of the mice in each group was calculated.

In vitro antioxidant tests

Tests on reducing power

The reducing power of the extracts was evaluated as described by Oyaizu (1986). Briefly, 1 ml of the test sample is mixed with 2.5 ml of phosphate buffer (200 mM, pH 6.6), 2.5 ml of potassium ferricyanide (30 mM) and incubated in a water bath at 50°C for 20 min. Trichloroacetic acid solution (2.5 ml: 600 mM) was added to the mixture and centrifuged at 3000 rpm for 10 min. The upper layer (2.5 ml) was carefully removed and combined with 2.5 ml of distilled water and 0.5 ml of 5 mM ferric chloride and the absorbance of the reaction mixture were measured at 700 nm. Ascorbic acid diluted in methanol was used as a standard.

Hydrogen peroxide scavenging assay

The ability of the *L. lanceolata* extracts to scavenge hydrogen peroxide was determined according to the method of Ruch et al. (1989). A solution of hydrogen peroxide (43 mM) was prepared in phosphate buffer (pH 7.4). Different concentration of extracts (25, 50, 100, 200, 400 µg/ml) were added to a hydrogen peroxide solution (1 ml, 43 mM). Absorbance of hydrogen peroxide at 230 nm was determined 10 min later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenged by both extract and standard compounds were calculated.

DPPH radical scavenging assay

DPPH radical scavenging activity was measured using the method of Blois (1958). The extract (1 ml) was added to 1.0 ml of DPPH in methanol (0.3 mM) and mixed with 1.0 ml of 5 mM DPPH in methanol. The reaction mixture was then kept in dark at room temperature for 10 min. Positive control used was ascorbic acid. The absorbance of the resulting solution was measured at 517 nm. The decrease in absorbance at 517 nm was calculated as the percentage of inhibition using the following equation.

$$\% \text{ Inhibition} = [(A_0 - A_1)/A_0] \times 100$$

A_0 = absorbance of control, A_1 = absorbance of the tested sample.

Statistical analysis

Results were expressed as mean ± S.E.M. The data was analyzed using Student's t-test and one way ANOVA: LSD post hoc compared to control using SPSS version 16 software. $P<0.05$ and $P<0.001$ were considered statistically significant.

RESULTS

Phytochemical analysis

In the preliminary phytochemical screening, the methanol leaf extract of *L. lanceolata* gave positive test for flavonoids, alkaloids, oils, saponins, glycosides, carbohydrates, acidic compounds, terpenoids and reducing sugars.

Acute toxicity

In the acute toxicity test, no death was recorded even up to 5,000 mg/kg; hence the LD_{50} of the *L. lanceolata* is above 5 g/kg.

Table 1. Suppressive effect of methanol leaf extract of *L. lanceolata* against *P. berghei* in mice.

Treatment	Dose (mg/kg)	Parasitaemia count	% Inhibition
Control	2 ml/kg	11.0 ± 3.27	
Lophira lanceolata	100	2.60 ± 1.96	23.63
Lophira lanceolata	200	5.80 ± 1.96*	52.72
Lophira lanceolata	400	8.80 ± 1.96*	80.00
Chloroquine	20	5.60 ± 1.96*	50.91

Results are Mean count ± S.E.M. (n = 5). * $P < 0.05$.

Table 2. Curative effect of methanol leaf extract of *L. lanceolata* against *P. berghei* in mice.

Treatment	Dose (mg/kg)	Parasitaemia count	% Inhibition
Control	2 ml/kg	12.8 ± 1.24	
Lophira lanceolata	100	4.6 ± 0.87*	64.06
Lophira lanceolata	200	5.8 ± 1.43*	54.69
Lophira lanceolata	400	2.6 ± 0.51*	79.69
Chloroquine	20	2.2 ± 0.49*	82.81

Results are Mean count ± S.E.M. (n = 5). * $P < 0.05$.

Table 3. Reducing power effect of methanol leaf extract of *L. lanceolata*.

Concentration (µg/ml)	Absorbance (700 nm)	
	Extract	Ascorbic acid
25	0.7690 ± 0.0015*	0.2530 ± 0.0006
50	0.8597 ± 0.0015*	0.2660 ± 0.0000
100	1.0183 ± 0.0007*	0.2847 ± 0.0003
200	1.0167 ± 0.0015*	0.2807 ± 0.0003
400	1.1120 ± 0.0040*	0.3673 ± 0.0007

Values are represented as Mean ± S.E.M (n=6); *$P<0.05$

Antiplasmodial activities

Suppressive effect

The methanol leaf extract of *L. lanceolata* exhibited a dose-dependent suppression of parasitaemia with 100% suppression at 400 mg/kg. The suppression produced by the extract was significantly ($P< 0.05$) higher than that produced by chloroquine 50.90% (Table 1).

Curative effect

The methanol extract, at the same doses (100, 200 and 400 mg/kg) exhibited significant ($P<0.05$) but non-dose-related decrease in parasitaemia (60.90, 54.69 and 79.69%) which was comparable to that of chloroquine (20 mg/kg, 82.80%) (Table 2).

Antioxidant assays

Test on reducing power

The reducing power of ascorbic acid and methanol extract increased in a concentration-dependent manner. The extract exhibited a significantly ($P< 0.05$) higher reducing power activity than ascorbic acid used as standard at same concentrations (25, 50, 100, 200 and 400 µg/ml). The highest reducing power was seen at 400 µg/ml of the extract (1.1120±0.0040) compared to the standard, ascorbic acid at same concentration (0.3673±0.0007) (Table 3 and Figure 1).

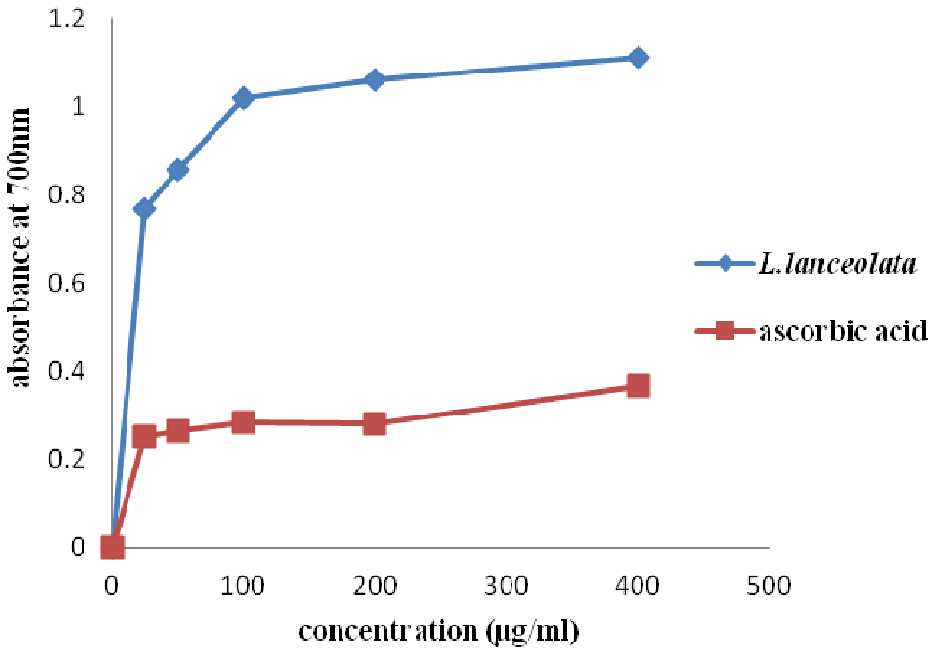

Figure 1. Reducing power ability of *L. lanceolata* extract and ascorbic acid at various concentrations.

Table 4 DPPH Radical Scavenging effect of *L. lanceolata.*

Conc. (µg/ml)	Absorbance (517 nm)		% Inhibition	
	Extract	Ascorbic acid	Extract	Ascorbic acid
25	0.2010±0.0020*	0.2025±0.0005*	87.14	87.14
50	0.1250±0.0100*	0.1860±0.0010*	92.00	88.11
100	0.1045±0.0250*	0.1825±0.0005*	93.31	88.33
200	0.0895±0.0035*	0.1680±0.0010*	94.27	89.25
400	0.0870±0.0010*	0.1660±0.0010*	94.43	89.38
Control	1.5640±0.0057			

Values are represented as Mean ± S.E.M (n=6); *P<0.001.

DPPH radical scavenging assay

DPPH radical scavenging activity of methanol leaf extract of *L. lanceolata* was compared with ascorbic acid. The extract exhibited a significantly (P< 0.001) higher DPPH radical scavenging activity compared to the standard; ascorbic acid at the same concentrations (25, 50, 100, 200 and 400 µg/ml) in a dose dependent manner. At concentration of 400 µg/ml, the DPPH radical scavenging activity of the extract was found to be 94.43% and that of ascorbic acid was 89.38% (Table 4 and Figure 2).

Hydrogen peroxide scavenging assay

The free radical scavenging activity of the methanol leaf extract of *L. lanceolata* showed significant (P<0.001) concentration dependent activity with the hydrogen peroxide scavenging effect of the extract of 400 µg/ml (95.19%) higher than that of ascorbic acid 400 µg/ml (88.01%) used as standard (Table 5 and Figure 3).

DISCUSSION

The results indicate that the methanol leaf extract of *L. lanceolata* possessed antiplasmodial activity as shown in the significant chemo suppression data obtained from the early and established infection. In the acute toxicity test there was no deaths in mice within 24 h up to 5000 mg/kg oral dose. This suggest that the extract is relatively nontoxic acutely. The chemosuppression obtained in the

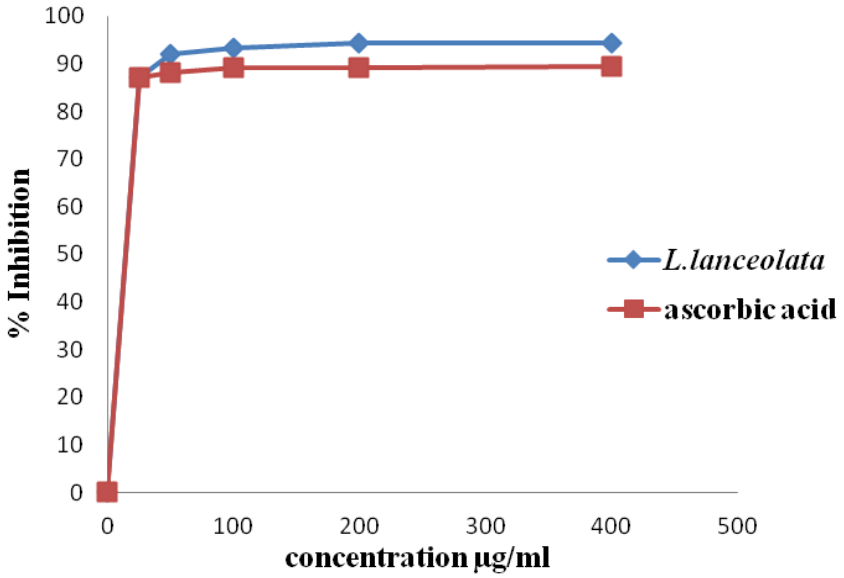

Figure 2. DPPH Radical scavenging activity of methanol extract of *L. lanceolata.*

Table 5 Hydrogen peroxide scavenging effect of *L. lanceolata*

Concentration (µg/ml)	Absorbance (230 nm)		% Inhibition	
	Extract	Ascorbic acid	Extract	Ascorbic acid
25	0.0670±0.0006*	0.1980±0.0005*	93.47	80.70
50	0.0657±0.0007*	0.1697±0.0010*	93.56	83.53
100	0.0657±0.0003*	0.1540±0.0005*	93.56	84.99
200	0.0543±0.0003*	0.1347±0.0010*	94.74	86.84
400	0.0510±0.0006*	0.1233±0.0010*	95.19	88.01
Control	1.0263±0.0003			

Values are represented as mean ± S.E.M (n=6); *P<0.001.

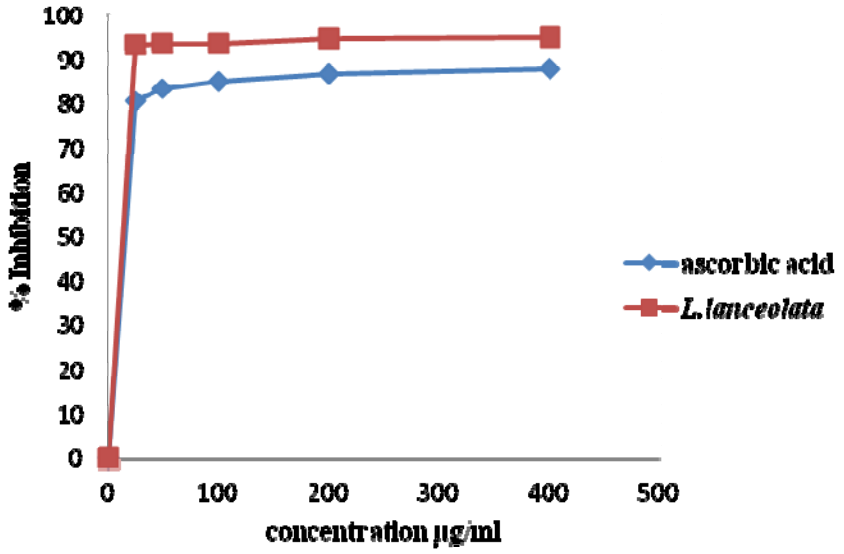

Figure 3. Hydrogen peroxide radical scavenging of *L. lanceolata* compared to ascorbic acid at various concentrations.

4-day early test (suppressive) was dose dependent comparable to the standard drug; chloroquine. Agents with suppressive activity against *P. berghei* were known for antimalarial activity, (Calvalho et al., 1991). Also, chemo suppression of parasitaemia recorded in established infection (curative) was comparable to the standard drug; chloroquine but not dose dependent. The chemosuppression of parasitaemia seen in early infection (suppressive) exhibited a dose-dependent suppression of parasitaemia with 100% suppression at 400 mg/kg which was significantly ($P< 0.05$) higher than that produced by chloroquine 50.90%. Similarly, the methanol extract at the same doses (100, 200 and 400 mg/kg) exhibited significant ($P<0.05$) but non-dose-related decreases in parasitaemia (60.90, 54.69 and 79.69%) recorded in established infection (curative) which was comparable to that of chloroquine (20 mg/kg, 82.80%).

The mechanism of action of the extract for antiplasmodial activity could be either by causing red blood cell oxidation (Etkin, 1997) or by inhibiting protein synthesis (Kirby et al., 1989) depending on their phytochemical constituents. The extract could have exerted its action through either of the two mechanisms mentioned above or by some other unknown mechanism. There are numerous antioxidant methods for evaluating of antioxidant activity. For *in vitro* antioxidant screening, (DPPH) free radical scavenging, metal ion chelating, hydrogen peroxide scavenging, superoxide anion radical scavenging and ferric thiocyanate reducing activities are most commonly used. However, the total antioxidant activity of an antioxidant cannot be evaluated by using one single method, due to oxidative processes. Therefore, at least two methods should be employed in order to evaluate the total antioxidant activity (Ilhami et al., 2005). The decrease in absorbance of DPPH radical caused by methanol leaf extract of *L. lanceolata* was due to the reaction between antioxidant molecule and radical which results in the scavenging of the radical by hydrogen donation (Soares et al., 1997). It is visually noticeable as a discoloration from purple to yellow in addition of the extract in a concentration-dependent manner. However, Fe (III) reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action (Nabavi et al., 2009). In the reducing power assay for the measurements of the reductive ability the Fe^{3+} to Fe^{2+} transformation in the presence of the methanol extract was investigated and found to have significant reducing ability. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.

The methanol leaf extract of *L. Lanceolata* showed moderate scavenging effects. As shown in Figure 3, the extract demonstrated hydrogen peroxide decomposition activity in a concentration dependent manner. Scavenging activity of H_2O_2 by the extract may be attributed to their phenolic content which can donate electrons to H_2O_2 thereby neutralizing it into water (Mathew and Abraham, 2006). Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. It rapidly transverses cell membrane and once inside the cell interior, H_2O_2 can probably react with Fe^{2+} and possibly Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects (Contreras-Guzman and Strong, 1982). Thus, the removal of H_2O_2 is very important for antioxidant defence in cell or food systems. H_2O_2 is highly important because of its ability to penetrate biological membranes. H_2O_2 itself is not very reactive, but it can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells. The results showed that *L. lanceolata* has an effective H_2O_2 scavenging activity.

The antioxidant activity of compound has been attributed to various mechanisms among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging. Phytochemical analysis revealed the presence of anthroquinones, flavonoids, carbohydrates, oils glycosides, phenols, saponins, steroids, tannins and free reducing sugar. Generally, flavonoids are the important class of antioxidants; hence the medicinal plants containing flavonoids and phenolic compounds are repeatedly screened for antioxidant activity. In addition to flavonoids and phenolic compounds, some of the alkaloids, saponins and triterpenoids are reported to possess antioxidant activity (Rai et al., 2006). The presence of flavonoids, alkaloids and terpenoids in *L. lanceolata* has been reported (Audu et al., 2007) and the results of the phytochemical investigation in the present study also further substantiated this. Hence, the results suggest that the methanol leaf extract of *L. Lanceolata's* antioxidant activity may be due to the presence of flavonoids and tannins and the observed *in vitro* antioxidant activity may be because of these phytoconstituents.

Conclusion

The outcome of this investigation revealed that, the methanol leaf extract of *L. lanceolata* is safe and possess potent antimalarial and antioxidant activity.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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