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Alkaloid and terpenoid fractions from the leaves of *Launaea taraxacifolia* (Willd.) repressed the proliferation of *Plasmodium berghei* in mice

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Abstract

Medicinal plants play a major role in the treatment and prevention of malaria in many communities across the world. The aim of the study was to validate the ethno pharmacological claims of *L. taraxacifolia*, traditionally used to treat malaria in western part of Nigeria by evaluating its antiplasmodial efficacies. A maceration of dried leaves of *L. taraxacifolia* was prepared in ethanol. Alkaloid and terpenoid fractions of *L. taraxacifolia* were extracted using solvent-solvent extraction methods. Antiplasmodial activities of crude extract, alkaloid and terpenoid fractions of *L. taraxacifolia* were assessed by suppressive, curative and prophylaxis assays against chloroquine-sensitive *P. berghei* (NK-65) infected mice. Enzyme activities (AST, ALT, ALP and gamma-GT) were evaluated in serum of *P. berghei* infected mice. Radical scavenging activity of the extract was measured using DPPH, hydroxyl radical and FRAP FRPAS assays. Phytochemical analysis of the extract and fractions were done using GC system.

The results of this study showed that the crude extract and fractions inhibited the proliferation of *P. berghei* with varying degrees. Crude extract displayed a significant ($p < 0.05$) chemosuppression effects at 200 and 400 mg/kg body weight. Alkaloid and terpenoid fractions were relatively potent than the crude extract at 10 mg/kg body weight. The extract and fractions of *L. taraxacifolia* significantly ($p < 0.05$) reversed the increase in activities of enzymes caused by *P. berghei* infection in mice. In addition, oral administration of *L. taraxacifolia* extract and fractions significantly normalized the concentration of urea and creatinine in the serum of treated mice. Some known alkaloids and terpenoids identified from the leaves of *L. taraxacifolia* are β -sitosterol 3-O- β -D-glucopyranoside, 16-carbaldehyde, 7a-Hexahydro 6H-indol-6-one, 3,4-Dimethoxyphenyl, octahydro-1-methyl and β -carboline-3-carboxylic acid. The results of this study support the ethno medical use of *L. taraxacifolia* leave as antimalarial remedies. Further studies on the mechanism of actions of the antiplasmodial components of *L. taraxacifolia* may lead to the discovery of a more potent antimalarial drug.

Keywords *Launaea taraxacifolia* · *P. berghei* · Antiplasmodial · Antimalarial · Gas chromatography Adewale Adetutu
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Introduction

Malaria is a parasitic infectious disease caused by protozoa belonging to the genus *Plasmodium*. It is estimated that there are 300–500 million cases of malaria annually across the world with a mortality of 2–3 million deaths. Children and pregnant women are at special risk to malaria (Desai et al., 2007). Malaria has now become a health threat in more than 90 countries, which are inhabited by 40% of the world's population (WHO, 2020). Over 80% of the reported cases occur in Africa, while one million deaths occur in sub-Saharan Africa alone (WHO 1989; WHO 2000; Beiersman, 2007, WHO, 2020). There has also been an increase in the number of reported cases of imported malaria in Europe and North

America, with over 30,000 cases annually. France which has the largest numbers of migrants of African origin, the UK, USA, Italy and Germany were top countries with the highest number of cases of imported malaria (Legros et al., 2007; WHO 2010).

There are arguably no known vaccines for malaria treatment; hence, chemotherapy remains the major approach to combating malaria. Unfortunately, other than the quinoline based antimalarial, the antifolates, and artemisinin and its derivatives, there are few effective antimalarial drugs on the market, as the malaria parasite has grown resistant to most of them (Peters 1998; Irungu et al. 2012). Consequently, drug-resistance *Plasmodium falciparum* malaria continues to be a health problem throughout the world, and in the past three decades malaria control has been set back by the increasing problem of parasite resistance to the existing antimalarial (Trape 2002; WHO 2013). Fortunately, over the past century, approximately 121 pharmaceutical products have been discovered based on information derived from traditional healers (Anesini and Perez 1993). In some Asian and African countries, 80% of the prescriptions are based on traditional medicine. One of the approaches to antimalarial drug discovery is the investigation of medicinal plants used traditionally to treat malaria and malaria-like symptoms (Ezenyi et al. 2020). Previous investigations have shown that some of these herbal remedies may not be effective (Obi et al. 2006). Therefore, verification of safety of the use and efficacy of these plants is essential.

L. taraxacifolia is a plant employed as an herbal medicine for the treatment of skin diseases; eye diseases, such as conjunctivitis; heart burns; diabetes; and malaria fever in Nigeria (Sofowora 1993, Obi et al. 2006, Adinortey et al. 2012). Although many people living in rural part of Nigeria traditionally treat malaria by drinking aqueous leaf extract of *L. taraxacifolia*, there is still paucity of literature on its antimalarial activity. Even though previous study had reported antiplasmodial activities of *L. taraxacifolia* extract (Adetutu et al. 2016), reports on the leave's antiplasmodial constituents are not presented. Based on this, the present study aimed at investigating the antimalarial activity of the fractions of *L. taraxacifolia* in *P. berghei* infected mice in order to provide more scientific evidence for its continuous use in ethno therapeutic management of malaria.

Materials and methods

Collection of plant and extract preparation

The fresh plant of *L. taraxacifolia* was collected from Ladokpe University of Technology Ogbomosho agricultural farm during the raining season (June to July) when the plants are fully grown. The plant was identified and authenticated by a

qualified taxonomist at the Department of Pure and Applied Biology LAUTECH with voucher number LHO 231, and the specimen of the plant was deposited at the Department Herbarium. The leaves of *L. taraxacifolia* (Fig. 1) were air dried for 15 days at room temperature and ground into powder. The powdered materials were stored in air tight containers until the time of use at 4 °C. About 200 g of leaf powder of *L. taraxacifolia* was soaked in 1000 mL of methanol and kept in the orbit shaker for 72 h. After 72 h, it was filtered through Whatman No 1 Filter Paper, and then the volume of supernatant was concentrated at 37 °C using a rotary evaporator. The concentrated methanol extracts of leaf of *L. taraxacifolia* were used for antioxidant, antimalarial assays and biochemical assays.

Extraction of alkaloid and terpenoid *L. taraxacifolia* leaves

The extraction of the alkaloid and terpenoids fractions was done using the continuous extraction methods of Abdullahi et al. (2013) and Joshi et al. (2013) respectively with little modification.

Laboratory animals and ethical protocol

A total of seventy (70) male albino mice, with average weight of 21.5 ± 1.4 g, obtained from the Faculty Animal Holding Unit were used for the study. The animals were placed on standard pellet feed and were allowed to have free access to water. They were acclimatized for 2 weeks before the commencement of the study. Ethical protocol was approved by the Research Ethics committee of the Faculty of Basic Medical Science in line with the standard accepted principles for laboratory animal handling and care. Standard scientific guideline and protocol on animal care, handling and treatment were duly followed.



Fig. 1 Leaves of *Launaea taraxacifolia*

Parasite

To test for the antimalarial activity of methanolic extract and fractions of *L. taraxacifolia*, Mouse-infective Chloroquine-sensitive strain of *Plasmodium berghei* NK-65 was used. The parasites were maintained by continuous intraperitoneal passage in mice every 4 days (Adzu et al. 2007). These infected mice were used for the study. Before the study began, few of the infected mice were kept and observed to produce disease symptoms similar to human infection (English et al. 1996). Any rise in temperature of mice was checked with a thermometer through the anus. In addition, malaria was established in mice by checking the parasitaemia in donor mouse with percentage parasitaemia of 59% as standard.

Antiplasmodial assays

Suppressive antimalarial assay (early infected *P. berghei*)

The Peters' 4-day suppressive test was adopted against chloroquine-sensitive *P. berghei* NK-65 infection in mice (Peters 1998). Treatments protocols are detailed in Table 1.

Curative antimalarial assay (established infected *P. berghei*)

The curative potential of *L. taraxacifolia* extracts were done employing the method described by Ryley and Peters (1970). The mice were injected intraperitoneally with standard inoculum of 1×10^{-7} *P. berghei* NK-65 infected erythrocytes on the first day (day 0). After 72 h, and following confirmation of parasitaemia, the mice were divided into 6 groups of six mice per group. These groups were treated with the prepared leaf extract of *L. taraxacifolia* (100, 200, 400 mg/kg body weight) and were also given alkaloid and terpenoid fractions (10 mg/kg body weight), chloroquine (5 mg/kg/body weight) (positive control) and distilled water (negative control) group. The treatment lasted for 5 days at a single dose per day after which blood smears were collected and examined microscopically to

monitor the parasitaemia level. The choice of dose was based on the LD50 value as previously reported by Kuatsienu et al. (2017).

Prophylactic antimalarial assay

The methods of Peters (1998) was adopted in the evaluation of the prophylactic potential of was adopted in the evaluation of the prophylactic potential of *L. taraxacifolia* leave extract. The mice were randomly divided into 6 groups with 6 mice in each group. Negative control group were given distilled water per kg body weight orally. While the positive group was given 5 mg chloroquine per kg body weight, the experimental groups were administered 100, 200 and 400 mg extract per kg body weight and were also given alkaloids and terpenoids fractions at 10 mg/kg body weight respectively. All treatments were initiated on day 0 and continued until day 4; the mice were all infected with the parasite. Blood smears were then made from each mouse 72 h after inoculation. Increase or decrease in parasitaemia was then determined.

Enzyme and urea indices assay

Alanine and aspartate aminotransferases

Alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma-glutamyltransferase (γ -GT) and aspartate aminotransferase (AST) were evaluated following the method described on the manufacturer's kit (Cypress diagnostic). Serum creatinine and urea concentration were determined spectrophotometrically by the method described by Kaplan and Pesce (1964) and Veniamin and Vakirtzi (1970), respectively.

GC analysis of *L. taraxacifolia* extract

Sample extraction

An aliquot of 1 ml of methanol solution of the sample was injected into the column with the injector temperature at 220 °C. GC oven temperature started at 240 °C, holding for 2 min. Holding will be allowed at 270 °C for 2 min with a program initial temperature rate of 50 °C/min. Interpretation of mass spectrum of GC-MS was done using the computer-aided matching of unknown spectra with spectra of known compound. The fragmentation patterns of the identified compounds were examined for consistency with known data from literature (Williams and Howe 1972). In addition, the hit quality (which indicates how closely matched the compound is with the Library data) was used to further verify the identity of the compounds in the sample. The name, the molecular weight and the structure of the components of the test materials were then ascertained; the relative percentage

Table 1 Protocols for the assays

Group	Treatments
I	Uninfected
II	Distilled H ₂ O + <i>P. berghei</i>
III	5 mg/kg of chloroquine + <i>P. berghei</i>
IV	100 mg/kg of <i>L. taraxacifolia</i> + <i>P. berghei</i>
V	200 mg/kg of <i>L. taraxacifolia</i> + <i>P. berghei</i>
VI	400 mg/kg of <i>L. taraxacifolia</i> + <i>P. berghei</i>

All the groups were inoculated by intraperitoneal (IP) injection with standard inoculum of *P. berghei* with 10^{-7} parasitized erythrocytes except group one (uninfected)

composition of each component was calculated by comparing its average peak area to the total area. The software adopted to handle mass spectra and chromatogram was an HP Series.

In vitro antioxidant assays

A 2, 2-diphenyl-2-picryl hydrazyl hydrate (DPPH) radical scavenging effect was determined according to method of Mensor et al. (2001). Ferric reducing antioxidant power and hydroxyl radical scavenging antioxidant activities were measured by the method of Klein et al. (2002).

Hydroxyl radical scavenging antioxidant activity

Hydroxyl radical scavenging antioxidant activity was measured using the method of Halliwell and Gutteridge, (1989) with some modification. 1.5 mL of varying concentration of extract, 60 μ of (1 mM) ferrous chlorides, 90 μ of (0.2 M) phosphate buffer (pH 7.8) and 150 μ of (0.17 M). Hydrogen peroxide was added and incubated at room temperature for 5 min. Absorbance was read at 560 nm. Percentage inhibition was calculated as follows:

$$\text{AA\%} = \frac{100 - (\text{Abs sample} - \text{Abs blank})}{\text{Abs control}} \times 100$$

Statistical analysis

The results obtained were expressed as mean $\pm$ SEM. The data was analysed with Graph Pad Prism version 5, using one-way ANOVA, and differences between mean were considered significant if $p < 0.05$.

Results

Evaluation of antiplasmodial potential of *L. taraxacifolia* leaf extract

Suppressive antimalarial assay (early infected *P. berghei*)

The in vivo antiplasmodial activity of extracts of *L. taraxacifolia* was assessed in *P. berghei* NK-65 parasitized mice. There was a significant ($p < 0.05$) decrease in the parasitaemia level of the *L. taraxacifolia* and chloroquine treated *P. berghei* mice when compared with the untreated *P. berghei* infected mice (Fig. 2). However, the decrease in parasitaemia level of the *L. taraxacifolia* treated at 400 mg/kg body weight was equally significant as chloroquine treated mice at 5 mg/kg body weight.

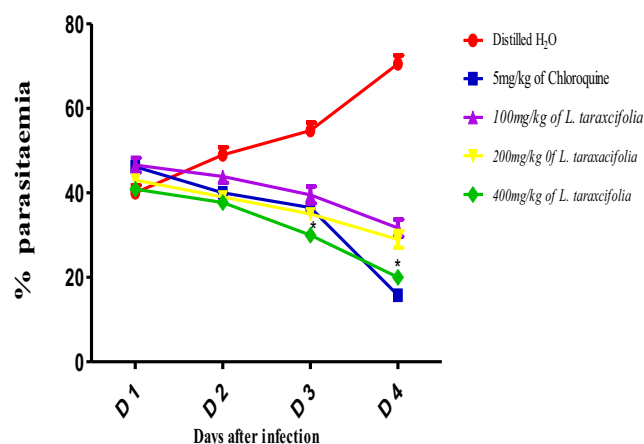


Fig. 2 Percentage parasitaemia in animal mice infected with *P. berghei* and treated with extract (suppressive model). Values are significantly different from the untreated group at $p < 0.05$

Curative antimalarial assay (established infected *P. berghei*)

In the curative antimalarial test, there was a significant ($p < 0.05$) decrease in the chloroquine and extract treated *P. berghei* infected mice when compared with the untreated infected *P. berghei* mice (Fig. 3) at days 0–4. Extract treated at 200 and 400 mg/kg body weight showed a significant ($p < 0.05$) decrease in parasitaemia level when compared with treatment at 100 mg/kg body weight of extract. There was no significant difference in the level parasitaemia in the extract treated at 100 mg/kg body weight and 200 mg/kg body weight after a 4-day treatment.

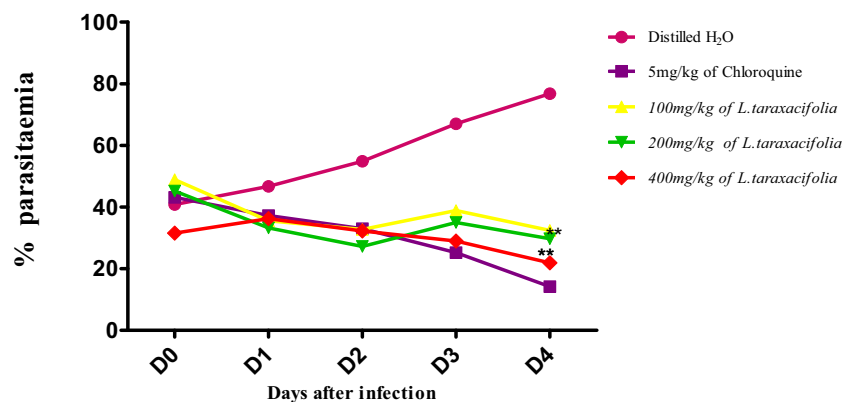
Prophylactic antimalarial assay

In the prophylactic antimalarial test, there was a significant ($p < 0.05$) decrease in the parasitaemia level of *L. taraxacifolia* and chloroquine treated *P. berghei* infected mice when compared with the untreated *P. berghei* mice (Fig. 4). The level of decrease in the percentage parasitaemia in extract treated at 400 mg/kg body weight was more pronounced with the treatment at 100 mg/kg and 200 mg/kg body weight of the extract. However, the level of parasitaemia in the extract treated at 400 mg/kg body weight compared favourably with the standard drug (chloroquine) treated.

Comparison of suppressive, curative and prophylactic models after 4 days of infections and antiplasmodial potentials of fractions

Considering the 200 and 400 mg/kg body weight of the extracts, the prophylactic model of *L. taraxacifolia* had the best activity on the 4th day. The entire model had a similar activity at 100 mg/kg body weight. The activity of the standard drug (chloroquine) was significantly ($p < 0.05$) better than those treated with the extracts in the three models. This scatter plot

Fig. 3 Percentage parasitaemia in animal mice infected with *P. berghei* and treated with extract (curative model). Values are significantly different from the untreated group at $p < 0.05$



(Fig. 5) showed that the chloroquine drug and (alkaloids and terpenoids) fractions of *L. taraxacifolia* in suppressive, curative and prophylaxis assay are in the same range; hence, further test was carried out to know the effect of the fractions on *P. berghei* infected mice, whereas the residues of the *L. taraxacifolia* extract were not studied further. The alkaloid and terpenoid fractions of *L. taraxacifolia* exerted a significantly ($p < 0.05$) suppressive and curative effect when compared with the untreated *P. berghei* infected mice (Fig. 5).

Effect of administration of *L. taraxacifolia* extract on enzyme activity of *P. berghei* infected mice (curative)

The administration of alkaloid and terpenoid fractions (10 mg/kg) of *L. taraxacifolia* resulted in a significant decrease ($p < 0.05$) in ALT, AST, Γ -GT and ALP compared with the untreated *P. berghei* infected mice; the standard drug chloroquine (5 mg/kg) significantly ($p < 0.05$) decreased when compared with the untreated *P. berghei* infected mice. However, the elevated concentrations were significantly brought to near normal in the extract treated mice. The activity of the extract is favourably compared with the control (Table 2).

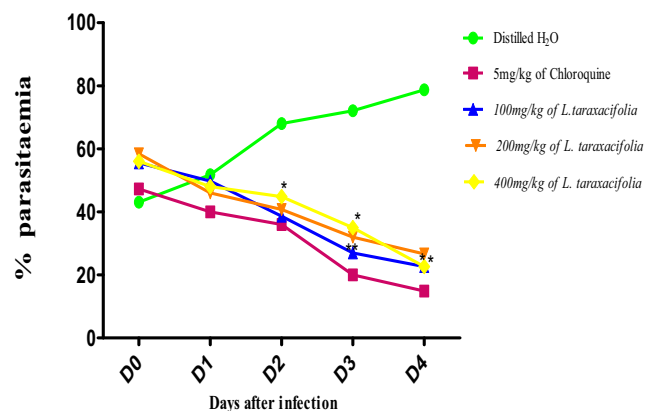


Fig. 4 Percentage parasitaemia in animal mice infected with *P. berghei* and treated with extract (prophylaxis model). Values are significantly different from the untreated group at $p < 0.05$

Effect of alkaloid and Terpenoid fractions and crude extract of *L. taraxacifolia* on the creatinine and urea concentration

The effect of alkaloid and terpenoid fraction of *L. taraxacifolia* extract on the concentration of urea and creatinine after a period of treatment revealed a significant decrease ($p < 0.05$) at doses 10 and 10 mg/kg body weight in treated *P. berghei* infected mice as compared with the untreated *P. berghei* infected mice in creatinine, but urea showed a general decrease at all doses but no statistically significant difference when compared with the untreated *P. berghei* infected mice (Table 3).

GC-MS analysis

GC-MS system was used to identify the constituents of phytochemical compounds in *L. taraxacifolia*. Analysis by GC-MS of *L. taraxacifolia* fractions lead to the identification of some compounds (Figs. 6–7).

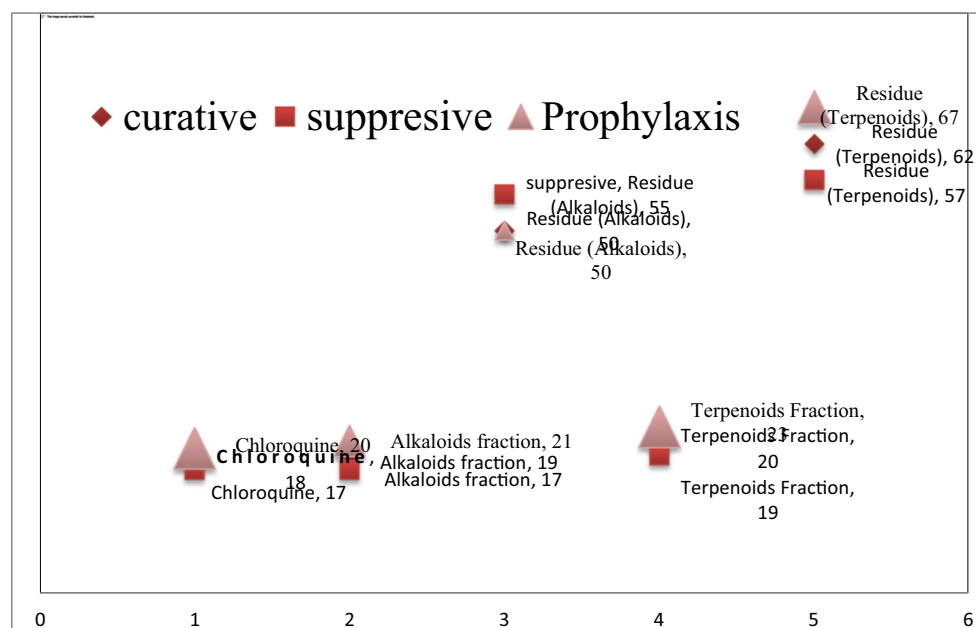
Antioxidant assays

The antioxidant activity of the methanolic leaves' extract of *L. taraxacifolia* was estimated in vitro using 2, 2-diphenyl-2-picryl hydrazyl hydrate (DPPH), hydroxyl radical scavenging activity and ferric reducing antioxidant power (FRAP). The results showed that the extract has strong DPPH, FRAP and hydroxyl radical scavenging activity as compared with the standard antioxidant compounds, garlic acid and ascorbic acid (Table 4).

Discussion

The resistance to malaria drug by *Plasmodium* parasites is a major problem in malaria chemotherapy especially in most endemic developing countries. The rate of resistance to malaria drug and currently efficacious artemisinin combine

Fig. 5 Scatter plots of antiplasmodial activities of *L. taraxacifolia* alkaloids, terpenoids fractions with residues and chloroquine



therapy give emphasis to the need to discover and develop new antimalarial drugs from other natural sources. Many herbs are used traditionally and known to cure malaria since time immemorial.

In this study, *L. taraxacifolia* extract suppressed the growth of established *P. berghei* parasite. Chemosuppression produced by *L. taraxacifolia* extract may be associated with its inherent ability to clear parasite in infected cells. In addition to suppression and curative action of *L. taraxacifolia* extract, it also exhibited prophylaxis values in infected mice showing ability to ameliorate malaria. The clearance of parasitaemia by alkaloids and terpenoids fractions showed a better and more significant reduction in multiplication of parasites than the crude extract. This implies that the alkaloids and terpenoids fractions may have a better and direct action on the parasites. Phytochemical compounds such as alkaloids are commonly implicated in the antiplasmodial activity of many plants (Waako et al. 2005). It has been reported that several

plant alkaloids and terpenoids possess protein-binding and plasmodial-inhibiting properties (Woodrow et al. 2005). Terpenoids have been identified as active antimalarial agents in many pharmacological studies (Delazar et al. 2004). These classes of compounds which were found in *L. taraxacifolia* extracts may be acting singly or in synergy with one another to exert the observed antiplasmodial activity. The likely mechanism of action of this alkaloids and terpenoids are synonymous with quinine action. The mode of action of alkaloids on the *Plasmodium* parasites appears to be due to the interference of the compound with the haemozoin formation (Soares et al. 2009). Similarly, terpenoids are said to act through interaction with *Plasmodium* DNA or inhibition of its DNA synthesis (Goijman et al. 1984; Kirby et al. 1989).

The role of oxidative stress as an important biochemical mechanism of disease pathogenesis is very relevant to malaria (Nanda et al. 2004). Investigation on the antioxidant activity of *L. taraxacifolia* extract using DPPH, hydroxyl radicals and

Table 2 Effect of oral administration of crude and fractions of *L. taraxacifolia* extract on activity of ALT, AST, ALP and Γ -GT in *P. berghei* infected mice (curative)

Parameter (U/L)	Treatment (kg body weight)					
	Uninfected	Control (distilled H ₂ O)	Chloroquine (5 mg)	Crude extract (200 mg)	Alkaloids (10 mg)	Terpenoids (10 mg)
ALT	34.00 $\pm$ 1.98	582.75 $\pm$ 20.06	337.50 $\pm$ 29.02	273.75 $\pm$ 21.21**	264.00 $\pm$ 27.54	301.25 $\pm$ 42.23
AST	23.67 $\pm$ 4.44	158.00 $\pm$ 16.58	84.00 $\pm$ 1.87	84.00 $\pm$ 3.41	80.250 $\pm$ 3.568**	94.00 $\pm$ 24.75**
ALP	32.76 $\pm$ 0.99	350.00 $\pm$ 19.33	243.25 $\pm$ 11.77	147.75 $\pm$ 14.81	155.50 $\pm$ 37.4**	172.75 $\pm$ 41.96**
Γ -GT	17.55 $\pm$ 0.12	43.88 $\pm$ 1.74	29.40 $\pm$ 2.28	29.87 $\pm$ 1.70	12.75 $\pm$ 0.82*	13.62 $\pm$ 4.18*

Values are expressed as mean $\pm$ SEM ($n = 5$); ** $p < 0.05$ as compared with the infected group (distilled H₂O) were observed between the test and the infected group with respect to the serum parameter tested

Table 3 Effect of administration of crude and fractions of *L. taraxacifolia* extract on the creatinine and urea concentration in *P. berghei* infected mice (curative)

Parameter	Treatment (kg body weight)					
	Uninfected	Control (distilled)	Chloroquine (5 mg)	Crude extract (200 mg)	Alkaloids (10 mg)	Terpenoids (10 mg)
Urea (mg/dl)	3.00 ± 1.23	18.07 ± 0.23	5.00 ± 1.178	5.75 ± 0.21	4.12 ± 0.95	4.72 ± 0.60
Creatinine (mg/dl)	9.77 ± 2.48	57.75 ± 1.25	44.25 ± 1.65	39.50 ± 2.21	16.52 ± 2.16**	20.20 ± 2.51**

Values are expressed as mean $\pm$ SEM ($n = 5$); $**p < 0.05$ as compared with the infected group (distilled H₂O) were observed between the test and the infected group with respect to the serum parameter tested

FRAP revealed a strong radical scavenging capacity comparable with that of ascorbic acid. The potent antioxidant capacity exhibited by the *L. taraxacifolia* extract may be due to the known antioxidant components such as stigmasterol, sabandinol, 1 coumarins, phylloclades and quercetin identified in the leaf extract of the plant in this study. The antioxidant effect of *L. taraxacifolia* extract may represent another mechanism that contributes to its antimalarial activity. *L. taraxacifolia* extract may have decreased nitric oxide (NO) production in macrophages (Adinortey et al. 2012; Adejuwon et al. 2014). According to Däubener and Hadding (1997), the inhibition of NO starves the parasite of an essential amino acid, leading to its death by increasing tryptophan degradation through indolamine deoxygenase induction in human peritoneal macrophages. The formation of reactive oxygen species by malaria parasites can be assumed to be countered by antioxidants and phytochemicals in *L. taraxacifolia* extract. Hence, the extracts are thought to prevent the oxidative damage that could have been affected by *plasmodial* infection.

Multiplication of *Plasmodium* in mice was observed by aggregate level of parasite concentration in the blood of mice infected with *P. berghei* alone, which could cause injury to organs like the kidney and liver. The infection of the liver by the sporozoites and the subsequent proliferation of the merozoites in the blood cells may account for these injuries (Sharkoori et al. 1990). The results of this study showed that *L. taraxacifolia* extract possessed a significant antimalarial activity, which was evident from the curative, chemosuppression and prophylaxis activities exhibited in mice. The results of this study showed that there was a stark increase in the concentration of urea and creatinine of the mice infected with *P. berghei* alone which could be an indication of renal impairment. Increase in urea and creatinine level has been observed in acute and chronic renal disease (Biyani et al. 2003). Alkaloids and terpenoids known for antiplasmodial activity identified from the extract of the *L. taraxacifolia* extract may be responsible for the renal protective activities exhibited by extract of *L. taraxacifolia* extract.

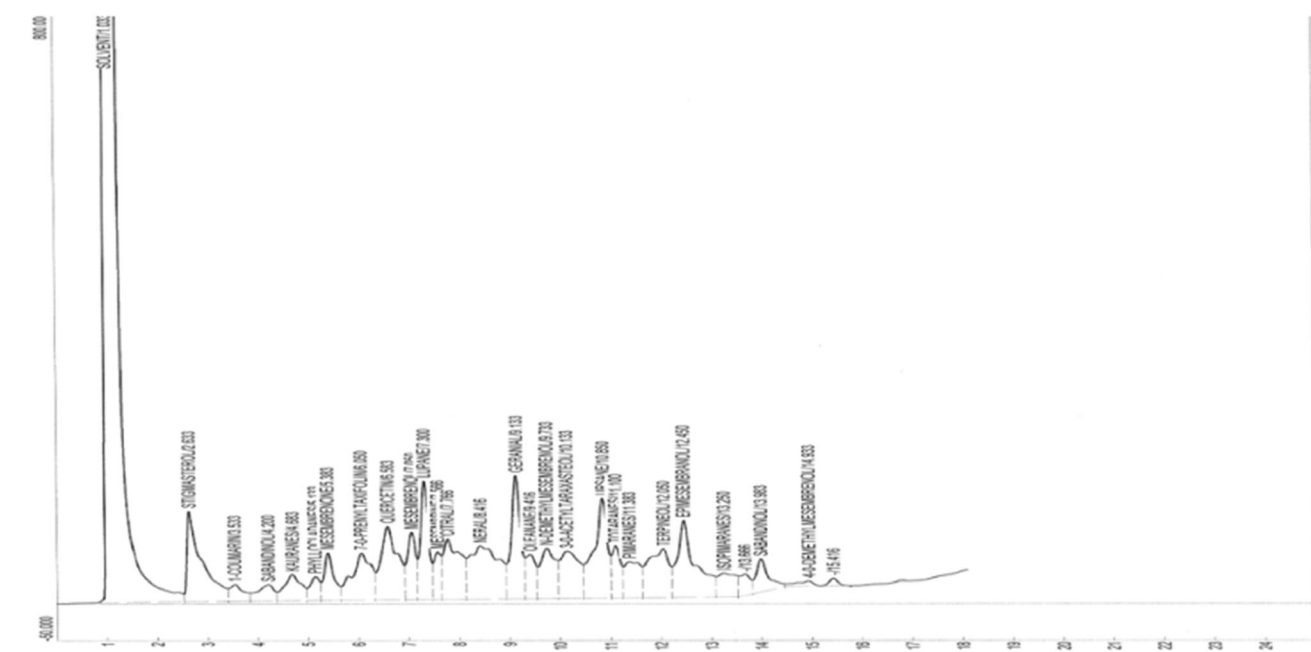


Fig. 6 Chromatogram of alkaloids fraction of methanolic extract of *L. taraxacifolia*

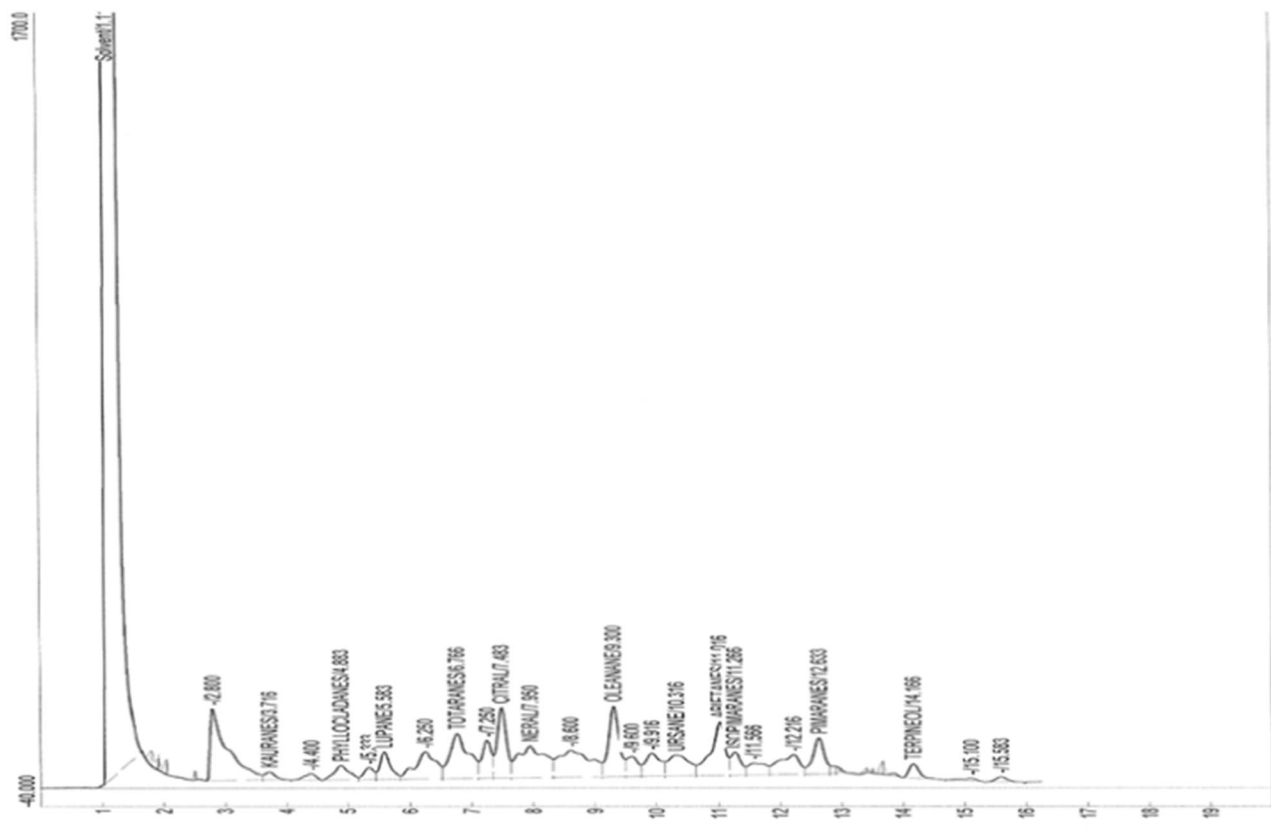


Fig. 7 Chromatogram of terpenoids fraction of methanolic extract of *L. taraxacifolia* leaf

In this study, elevation of serum ALT, γ -GT AST and ALP was observed in infected mice. The ability of *L. taraxacifolia* extract to reverse the serum ALT, γ -GT AST and ALP activity in infected mice to normal could be suggesting that *L. taraxacifolia* extract may be protective against *P. berghei* induced hepatic abnormality and injury in mice. The lymphocytic infiltration observed in *P. berghei* infected mice is denser when compared with the treated group which showed no infiltration of inflammatory cell. The protection of liver in *P. berghei* infected mice may be attributed to the antiplasmodial terpenoid and alkaloid components of the extract.

Alkaloids and terpenoids are known as phytochemical with an established significant antiplasmodial activity. For instance, the famous antimalarial drug quinine is alkaloid. Therefore, the alkaloid and terpenoid components of *L. taraxacifolia* extract were analysed for possible identification of antiplasmodial components. GC analysis of alkaloid

fractions of *L. taraxacifolia* extract established five known alkaloids, namely, β -sitosterol 3- β -D-glucopyranoside, 16-carbaldehyde, 7 α -Hexahydro 6H-indol-6-one, 3,4-Dimethoxyphenyl, octahydro-1-methyl and β -carboline-3-carboxylic acid (Jayaprakasha and Patil 2007). A lot of compounds were identified from terpenoid fraction, but specifically kaurane, 16 (CH)-phyllocladane, lupanine, isosorbide 5-nitrate and -3,7-dimethyl-2,6-octadienal are known to have antiplasmodial and anti-inflammatory property. Conventional artemisinin combine therapy has been confirmed to be terpenoids' derivatives (Kar 2008). These compounds are known to have anti-inflammatory and possibly antiplasmodial activities which could account for the antimalarial activities of *L. taraxacifolia* extract. The main active compound is yet to be identified, although the antimalarial activity of *L. taraxacifolia* extract could be attributed to a single metabolite or a combination of its secondary

Table 4 Antioxidant activities of *L. taraxacifolia* extract

Fractions	Antioxidant Assay (IC ₅₀) μ g/ml		
	DPPH	Hydroxyl radical scavenging Assay	FRAP
<i>L. taraxacifolia</i> extract	348.7	371.6	223.1
Ascorbic acid	287.5	126.6	127.8

metabolites such as alkaloids, flavonoids, terpenoids, and phenolic compounds (Noster and Kraus 1990).

The results of this study suggest that the local people in rural Africa have through sustained use discovered that the leaves of *L. taraxacifolia* are potent in treating malaria. Therefore, this result of this investigation might justify the use of *L. taraxacifolia* extract for the treatment of malaria in most rural areas of Africa. The importance of this study lies not only in promoting the significance of the African plants and the search for discovery of antimalarial ingredients from potential antimalarial plants but likewise the need for traditional healthcare to complement a conventional one.

Conclusion

Therefore, it is imperative to develop suitable herbal formulations for local production and administration of *L. taraxacifolia* extract and other herbal extracts in collaboration with health regulatory agency in rural areas of Africa that are highly marginalized and are not easily reached by the healthcare facility.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Abdullahi MN, Ilyas N, Ibrahim H (2013) Evaluation of phytochemical screening and analgesic activity of aqueous extract of the leaves of *Microtrichia perotitii* dc (Asteraceae) in mice using hotplate method med. Plant Res 3(2013):37–43
- Adejuwon A.S., Femi-Akinlosotu, J.O. Omirinde, O.R. Owolabi and A.M. Afodun (2014). *Launaea taraxacifolia* ameliorates cisplatin-induced hepato-renal injury
- Adetutu A, Olorunnisola OS, Owoade AO, Adegbola P (2016) Inhibition of *in vivo* growth of *Plasmodium berghei* by *Launaea taraxacifolia* and *Amaranthus viridis* in mice. Malaria Res Treatment 2016:1–9. <https://doi.org/10.1155/2016/9248024>
- Adinortey MB, Sarfo JK, Quayson ET, Weremfo A, Adinortey CA, Ekloh W, Ocran J (2012) Phytochemical screening, proximate and mineral composition of *Launaea taraxacifolia* leaves. Res J Med Plant 6:171–179
- Adzu B, Haruna A, Salawu OA, Katsayal UM, Nyan A (2007) In vivo antiparasmodial activity of ZS-ZA: A fraction from chloroform extract of *Zizyphus spina-christi* root bark against *Plasmodium berghei* in mice. International Journal of Biological and Chemical Sciences 1(3):281–286
- Anesini C, Perez C (1993) Screening of plants used in argentine folk medicine for antimicrobial activity. J Ethnopharmacol 39:19–128
- Beiersmann C, Sanou A, Wladarsch E, Allegri, M, Kouyate B, Muller O (2007) Malaria in rural Burkina Faso: local illness concepts, patterns of traditional treatment and influence on health-seeking behaviour. Malar J 6:106
- Biyani MK, Banavalikar MM, Suthar AC, Shahani S, Sivakami S, Vidri J (2003) Antihyperglycemic effects of three extracts from *Momordica charantia*. J Ethnopharmacol 88:107–111
- Däubener W, Hadding U (1997) Cellular immune reactions directed against *Toxoplasma gondii* with special emphasis on the central nervous system. Med Microbiol Immunol 185:195–206
- Delazar A, Reid, RG, Sarker SD (2004) GC-MS Analysis of the Essential Oil from the Oleoresin of *Pistacia atlantica* var. *mutica*. Chem Nat Compd 40:24–27. <https://doi.org/10.1023/B:CONC.0000025459.72590.9e>
- English ME, Waruiru C, Lightowler C, Murphy SA, Kirigha G, Marsh K (1996) Hyponatraemia and dehydration in severe malaria. Arch Dis Child 74:201–205
- Ezenyi IC, Verma V, Singh S, Okhale SE, Adzu B (2020) Ethnopharmacology-aided antiplasmodial evaluation of six selected plants used for malaria treatment in Nigeria. J Ethnopharmacol. <https://doi.org/10.1016/j.jep.2020.112694>
- Gojman SG, Turens JF, Marini-Bettolo OB, Stoppani AO (1984) Medicina:44(361)
- Irunu BN, Mbabu MJ, Kiboi DM, Moindi E, Kinyua J, Romano M (2012) In vivo antimalarial and acute toxicity properties of hexane and chloroform extracts from *Clausena anisata* (Willd.) Benth. Afr J Pharmacol Ther 1:24e29
- Jayaprakasha GK, Patil BS (2007) In vitro evaluation of the antioxidant activities in fruit extracts from citron and blood orange. Food Chem 1(101):410–418. <https://doi.org/10.1016/j.foodchem.2005.12.038>
- Joshi A, Bhohe M, Saatarkar A (2013) Phytochemical investigation of the roots of *Grewia microcos* Linn J. Chem Pharm Res 5(2013):80–87
- Kaplan LA and Pesce AJ (1964) Clinical Chemistry: Theory, Analysis, Correlation Fifth Edition. Published by Mosby/Elsevier, St Louis, USA
- Kar A (2008) Pharmacognosy and pharmacobiotechnology, Second edn. Anshan Ltd., Kent
- Kirby GC, O'Neill MJ, Phillipson DJ, Warhurst DC (1989) In vitro studies on the mode of action of quassinoids with activity against chloroquine-resistant *Plasmodium falciparum*. Biochem Pharmacol 38(24):4367–4374
- Klein SM, Cohen G, Cederbaum AI (2002) Production of formaldehyde during metabolism of dimethyl sulfoxide by hydroxyl radical-generating systems. Biochemistry 20:6006–6012
- Kuatsienu LE, Ansah C, Adinortey MB (2017) Toxicological evaluation and protective effect of ethanolic leaf extract of *Launaea taraxacifolia* on gentamicin induced rat kidney injury. Asian Pac J Trop Biomed 7(7):640–646
- Legros F, Bouchaud O, Ancelle T, Arnaud A, Cojean S, Le Bras J, Danis M, Fontanet A, Durand A, Epidemiology Autochthonous Malaria (2007) Risk factors for Imported Fatal Malaria, France, 1996–2003. Emerg Infect Dis 13(6):883–888
- Mensor LL, Menezes FS, Leitão GG, Reis AS, Santos TC, dos Coube CS, Leitão SG (2001) Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother Res 15(2):127–130
- Nanda R, Mishra PK, Das UK, Rout SB, Mohapatra PC, Panda A (2004) Evaluating role of oxidative stress in determining the pathogenesis of falciparum malaria induces acute renal failure. Indian J Clin Biochem 19:39–96
- Noster S, Kraus L (1990) In vitro antimalarial activity of *Coutarea latiflora* and *Exostema caribaeum* extracts on *Plasmodium falciparum*. Planta Med 56:63–65
- Obi RK, Iroagba II, Ojiako OA (2006) Virucidal potential of some edible Nigerian vegetables. Afr J Biotechnol 5:1785–1788
- Peters W (1998) Drug resistance malaria parasites of animals and man. Adv Parasitol 41:1–4

- Riley JF, Peters W (1970) The anti-malarial activity of some quinolone esters. *Ann Trop Med Parasitol* 84:209–222
- Sharkoori AR, Alam J, Ali SS (1990) Toxic effects of Talstar: a new synthetic pyrethroid, on blood and liver of rabbit. *Pakistan J Zool* 22(3):289–300
- Soares, J. B. R. C., Menezes, D., Vonier-Santos, M. A., Ferreira-Pereira, A., Almeida, G. T., Venancio, T. M. and Verjoviski-Almeida, S. (2009). Interference with hemozoin formation represents an important mechanism of schistosomicidal act of antimalarial quinoline methanols. *PLOS Neg Trop Dis*, 3(7)
- Sofowora A (1993). Medicinal plants and traditional medicine in Africa. 2nd edn., Spectrum books Ltd., Ibadan, Nigeria, ISBN-13: 9782462195, pages: 289
- Trape JF (2002) Combating malaria in Africa. *Trends Parasitol* 18:224–230
- Veniamin MP, Varkirtzi C (1970) Chemical basis of the carbamidodi-acetyl micro-method for estimation of ure, citrulline and carbamyl derivatives. *Clin Chem* 16:3–6
- Waako PJ, Gumede B, Smith P, Folb PI (2005) The in vitro and in vivo antimalarial activity of *Cardiospermum halicacabum* L. and *Momordica foetida* Schumch, EtThonn. *J Ethnopharmacol* 99: 137–143
- WHO (1989) Scientific group on the chemotherapy of Malaria & World Health organization. Practical chemotherapy of malaria : report of a WHO scientific group [meeting held in Geneva from 5 to 12 June 1989]. World Health Organization. <https://apps.who.int/iris/handle/10665/39778>. Accessed 15 Jan 2019
- WHO (2000). *General guidelines for methodologies on research and evaluation of traditional medicine*. Geneva, World Health Organization, (document reference WHO/EDM/TRM/2000.1)
- Williams OH and Howe I (1972). Principles of organic mass spectrometry McGraw-Hill UK Ltd p1
- World Health Organization (WHO) (2010). World Malaria Report, pp. 13–15
- World Health Organization (WHO) (2020). World Malaria Report, 2019
- WHO (2013) World Malaria Report for year 2013. https://www.who.int/malaria/publications/world_malaria_report_2013/en/. Accessed April, 2019
- Woodrow CJ (2005) Artemisinins. *Postgra Med J* 81(952):71–78

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