



***In vivo* antimalarial activity of the ethanolic leaf extract of *Hyptis suaveolens* on *Plasmodium berghei* in Mice**

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ABSTRACT

The ethanolic leaf extract of *Hyptis suaveolens* was evaluated for antimalarial activity against *Plasmodium berghei* in infected mice. In the 4 day suppressive test, 60 male and female albino mice (weight: 18-23 g) were infected and treatment began immediately (Day 0), while administration started 72 hours post infection in the curative test. For each test, mice were divided into 5 groups of 12 animals; each group was administered one of the following: 10 mg/kg, 25 mg/kg and 50 mg/kg of the crude extract, 5 mg/kg of chloroquine phosphate or 0.2 ml normal saline for four consecutive days. Thin blood smears were prepared and examined microscopically under x 100 objective on day 4 to day 7 and the percentage parasitaemia were recorded. On day 7 the red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb) and packed cell volume (PCV) were evaluated. The extract demonstrated dose-dependent inhibition effect on the parasites with 42.76% and 18.03% at 50 mg/kg, 33.69% and 10.22% at 25 mg/kg for suppressive and curative tests respectively, while at 10 mg/kg, 22.39% and 6.06% suppression were recorded in the suppressive and curative tests respectively. The RBC, HB and PCV values decreased while there was an increase in WBC compared with that of the control.

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INTRODUCTION

Malaria is the world most important parasitic infection (Hay et al., 2004) and it remains a major impediment to health in Africa, south of the Sahara (Snow, 1999). It is reported to be a major cause of morbidity and mortality amongst Nigeria's children population (Ayoola et al., 2005). It is

estimated that 1-2 million people die yearly as a result of malaria (Sudhanshu et al., 2003).

Efforts to reduce the high malaria morbidity and mortality rates have been hampered by the development to resistance, particularly by *falciparum* to long-standing conventional drugs such as chloroquine (Okoyeh et al., 1993, Ejov et al., 1999, Wongsrichanalai et al., 2002). This set back in

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drug control and eradication of malaria has necessitated the identification of new effective alternative drugs that are active against the resistant form of Plasmodium.

Plants form the major part of treatments used by traditional healers in many societies. Thus, many plants have acquired reputation for being useful against malaria (Adesegun and Coker, 2001). It is estimated that 80% of the world population utilize traditional medicines for the treatment of diseases. The majority of African populations use traditional medicine for their health care needs. The medicaments are often bought in places such as open markets and local stores (WHO, 2004).

Ethno-medicines play a central role in the search for and development of new drugs (Kirby, 1997; Heinrich, 2000). Ajaiyeoba et al., (2003) in their ethno-graphic evaluation of ethno-medicine in Nigeria reported that many herbal remedies have been used traditionally for treatment of febrile illnesses in South-Western Nigeria. In categorizing febrile illnesses, alongside their causes and symptoms, ordinary fever and rainy season fever are the two illnesses compactable with malaria infection. Studies have documented over 1,200 plant species from 160 families used in the treatment of malaria or fever (Willcox and Bodeker, 2004). Hermans et al. (2004) identified 85 species of plants used by the people in Benin who cannot resort to allopathic medicine to alleviate malaria symptoms. 30 species of plants belonging to 28 genera in 20 families were commonly used in the preparation of the herbal remedies by the people of Dangme West District of Ghana, because they were more accessible and cost-effective (Asase et al., 2010). Tabuti (2008) reported that out of the twenty-seven species of plants used for the treatment of malaria in Budiope county, Uganda, the most frequently mentioned were *Vernonia amygdalina*, *Momordica foetida*, *Zanthoxylum chalybeum*, *Lantana camara* and *Mangifera indica*. Bray et al. (2006) using crude extracts of *Azadirachta indica*, *Cedrela odorata* and

Guarea multiflora of the meliaceae used traditionally as febrifuges together with 27 limonoids extracted from malicious plants reported that none of the extracts tested showed high antiplasmodial activity and only four compounds were found to be moderately active.

Studies have shown that some plants are highly toxic despite their high chemosuppression of parasitaemia. *Morinda lucida* which is top on the list of frequently used plants has shown *in vitro* cytotoxicity and the stem bark was reported to be extremely toxic (Ajaiyeoba et al., 2006). Idowu et al. (2010) in their search for published studies on the level of antimalarial activity and toxicity of the 38 plants in their study, reported that 80% have been documented to have antimalarial property but suppressive and none clearance, while about 65% have toxic effects on the liver and kidney of experimental mice.

Hyptis suaveolens is a strong-scented annual or perennial herb that reaches a height of 1.5 m found as weeds that are tropical and sub-tropical. With quadrate hairy stems and ovate to obovate leaves 3-5 cm long and 2-4 cm wide, the margins serrulate with the lower surfaces densely hairy. Flowers are usually bisexual in small cymes along branch ends with reduced leaves. The calyx is inferior, persistent, bell-shaped and measures 5mm long in flowers, 10 mm long in fruit, corolla is blue and tubular nutlet about 1.2-1.5 mm long, slightly notched at the end (Stone, 1970).

An infusion of the dried leaves of *H. suaveolens* is taken as a fever remedy. According to Dalziel (1955), it is tied round the head against headache or applied to cure boils. In some communities, the plant has been used in traditional medicine for the treatment of various illnesses such as stomach ache, diarrhea, dysentery and as eye remedy. The genus had been known to possess 23 biologically active chemical compounds (Peerzada, 1997). Azevedo et al. (2001) identified a total of 40 compounds from oil samples obtained from *Hyptis suaveolens* in

Brazil, accounting for 91-97% of the volatile constituents. However, there was a correlation between essential oil composition and geographic variations. Sesquiterpenes were mainly produced at lower altitudes where as monoterpenes at higher altitudes. Two triterpenoids obtained from the bark of *Betula utilis* and the root of *H. suaveolens* were reported to be similar to betulin and betulinic acid which have antitumor activity against human melanoma cell culture (Sharma et al., 2010). Aqueous aerial parts of *H. suaveolens* extracts showed promising lymphocytes stimulating activities on non-specific cell-mediated immune responses (Sriwanthana et al., 2007).

Phytochemical investigation of the leaf, stem and root extracts of *Hyptis suaveolens* revealed the presence of flavonoids, steroids, terpenes, saponins, tannins, resins, glycosides. (Akueshi et al. 2002, Anyanwu and Dawet, 2005, Egunyomi et al. 2010) with high amount in leaves, followed by stem and lower in root. The leaf extract was more toxic to mice than that of the stem which had an LD₅₀ 2511.89 mg/kg and 3162.27 mg/kg respectively (Anyanwu and Dawet, 2005). Egunyomi et al. (2010) in a comparative study of the effectiveness of ethnobotanical mosquito repellents reported low repellency in *H. suaveolens* against *Anopheles stephensi*. *H. suaveolens* ethylacetate extracts was reported to significantly reduce probing activity of *Aedes aegypti* (L) (Jaenson et al., 2006). However, Attawish et al. (2005) in a 6 month study of the aqueous leaf extract of *Hyptis suaveolens* in wister rats reported that the plant did not produce any significant dose related changes of haematological parameters, serum biochemistry or histopathology of any internal organs. Studies on the insecticidal activity of the plant showed that it is a potential source of raw material for the control of *Culex* mosquito's larvae and adults (Audu et al., 2002). Amusan et al. (2005) in a comparative toxicity study reported that the ethanolic extract of *H.*

suaveolens showed low toxic effect on larvae of *Ae. aegypti* compared with the effect produced by *Citrus sinensis* oil extract. Similarly, Okigbo et al. (2010) reported that *H. suaveolens* showed little or no larvicidal activity against *Culex* mosquito larvae. Antimicrobial tests of the crude extracts showed that the plant has growth-inhibitory potential against microorganisms (Iwu et al., 1990; Rojas et al., 1992; Akueshi et al., 2002). *H. suaveolens* did not exhibit antifungal activity against *Aspergillus niger* F2723 (Babbarala et al., 2009). Shirwaikar et al., 2003 reported the wound healing activity of the ethanolic leaf extract of *Hyptis suaveolens*. Because of the devastating nature of malaria, there is an urgent need to develop new drugs or vaccines for the treatment, prevention and management of the disease. The present study was carried out to determine the *in vivo* antimalarial effect of *Hyptis suaveolens* against *Plasmodium berghei* in mice.

MATERIALS AND METHODS

Collection of experimental plant

Hyptis suaveolens was obtained from Bwai in Mangu L.G.A, of Plateau State, Nigeria and identified by Prof. S.H.W. Hussaini, in the Department of Botany, University of Jos, Nigeria. A voucher specimen reference UJ/PGNS/HSP/1001 has been deposited at the Herbarium in the Department of Pharmacognosy, University of Jos, Nigeria. The leaves were collected and dried in the open air under shade and pulverized using mortar and pestle.

Extraction of the plant material

This was carried out as described by Anyanwu and dawet (2005). Sixty (60) grams of the powdered leaf was extracted with the aid of a soxhlet extractor using 350 ml of 99% ethanol for 72 hours. The extract was concentrated to dryness using a rotary evaporator and the residue stored in the freezer at 4 °C. Prior to use, the extract was dissolved in normal saline so that the doses

required was prepared by necessary dilutions and given according to weight of the animal.

Drug

Chloroquine phosphate (Drug field, Nigeria) was diluted in normal saline to final doses of 5 mg/kg body weight.

Animals

Ethical approval was obtained from the ethical committee of the Animal House, University of Jos, Jos, Nigeria. Animals were treated as approved by international standard for the treatment of experimental animals. Healthy male and female albino mice, weighing 18-23 g were obtained from the Nigerian Institute for Trypanosomiasis Research (NITR), Vom near Jos, and were kept in the animal house of the University of Jos in cages (25cm × 20cm × 10cm) with perforated metal cover for free passage of air, under approximately 12 hours periodicity of light / dark cycle. The animals were left to acclimatize for two weeks prior to their use for the experiment. They were fed on standard pellet diet with water *ad libitum*.

Parasite

Chloroquine sensitive *Plasmodium berghei* were obtained from the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. The parasites were maintained by intraperitoneal inoculation of mice with diluted blood obtained by cutting the tip of the tail of *Plasmodium berghei* infected mice.

Parasite inoculum

The inoculum consisted of 1.2×10^7 *Plasmodium berghei* parasitized red blood cells per millilitres. This was done by determining both the percentage of parasitaemia and the red blood cells count of the donor mice using an improved Neubauer haemocytometer and diluting the blood with normal saline.

Drug administration

Chloroquine phosphate was administered intraperitoneally using syringe and needle while the extract was orally administered using a feeding cannula.

Antimalarial activity

4-day suppressive test

The suppressive test was conducted in a similar method followed by Mesia et al. (2005) and Iwalokun (2008). 60 animals were divided into 5 groups of 12 animals each. Three of the 5 groups was each administered one of the following: 50 mg/kg, 25 mg/kg or 10 mg/kg of the extract. A group was administered chloroquine phosphate (5 mg/kg) as positive control, while the other group (negative control) received 0.2 ml normal saline. Each mouse was inoculated on the first day (Do), intraperitoneally, with infected blood containing *Plasmodium berghei* parasitized red blood cells. The mice were treated daily from day 0 (immediately after infection) to day 3. Thin smears fixed in methanol stained with Giemsa stain were prepared from the tail blood of each mouse daily from day 4 to day 7, examined microscopically under oil immersion and the percentage parasitaemia was determined by counting the parasitized erythrocytes out of 200 erythrocytes in random fields. The mean parasite count for each group was determined and the average percentage chemosuppression for each dose was calculated as $[(A-B)/A]$, where A is the average percentage of parasitaemia in the negative control (normal saline group) and B is the average percentage of parasitaemia in the test groups.

Rane (curative) test

This was conducted in a similar method adopted by Tona et al. (2001) and Iwalokun (2008). Another set of 60 albino mice were infected with parasitized erythrocytes. Seventy-two hours after infection, the mice were divided into 5 groups of 12 animals. Three of the 5 groups was each administered one of the following: 50 mg/kg,

25 mg/kg or 10 mg/kg of the leaf extract. The fourth group was given 5 mg/kg of chloroquine phosphate while the last group (negative control) received 0.2 ml normal saline. Each mouse was treated orally once daily with the dose for 4 consecutive days (D4-D7) post inoculation during which the parasitaemia level was monitored daily.

Haematological parameters

On day 7, blood was obtained from the experimental mice by cutting the tip of the tail and collected in heparinised tubes. Blood parameters were determined using the method of Dacie and Lewis as described by Ibu and Adeniyi (1989). White blood cell (WBC) and Red blood cell (RBC) were determined using haemocytometer. The haemoglobin (Hb) concentration was measured with C₂10 digital calorimeter in optical density (Å) with drabkins solution as reference for zero adjustment. The packed cell volume (PCV) was determined using microhaematocrit.

Statistical analysis

Student's t-test was used to compare the treated groups and the untreated groups, where P values < 0.05 were considered significant.

RESULTS

Suppressive test

The changes in the parasitaemia in mice administered *Hyptis suaveolens* leaf extract is shown in Table 1. The groups treated with 50 mg/kg, 25 mg/kg and 10 mg/kg leaf extract, chloroquine phosphate and normal saline had percentage parasitaemia of 24.42±1.38, 28.67±1.2, 33.3±1.52, 21.17±0.94 and 37.87±1.36 respectively. The percentage parasitaemia in groups administered the doses of the leaf extract and normal saline increased throughout the period of observation, while a decrease was observed in the positive control group administered the chloroquine phosphate. The percentage parasitaemia was higher in the control group followed by 10 mg/kg, 25 mg/kg, 50 mg/kg leaf extract and 5

mg/kg chloroquine phosphate with 67±2.8, 53.17±2.08, 49.36±2.34, 47.25±1.41 and 0.56±0.15 respectively on day 7. However, even though the mean parasitaemia in mice given the ethanolic leaf extract decrease, it was significantly (P<0.05) lower than that in the negative control (normal saline group) only in the 50 mg/kg group.

Curative test

In this study the mean percentage parasitaemia on day 4 was 27.67±1.34, 31.58±1.92, 32.20±1.45, 19.25±1.02 and 32±2.08 for 50 mg/kg, 25 mg/kg, 10 mg/kg leaf extract, 5 mg/kg chloroquine phosphate and normal saline respectively (Table 2). The percentage parasitaemia gradually increased in groups treated with the doses of the leaf extract and normal saline with 52.25±2.1, 59.74±2.06, 61.92±2.91 and 68.92±4.66 in the 50 mg/kg, 25 mg/kg, 10 mg/kg leaf extract and normal saline groups respectively. While there was a decrease in parasitaemia in the groups administered chloroquine phosphate from 19.25±1.02 on day 4 to 0.65±0.21 on day 7. The decrease in percentage parasitaemia in groups administered leaf extract 72 hours post infection was not significant (P>0.05) compared with the normal saline groups.

The mean percentage parasitaemia through out the period of observation was high at 10 mg/kg dose with 42.6±4.35 and 47.41±6.49 for the 4-day suppressive and curative test respectively. (Table 3). As the dose increased, the mean parasitaemia decreased, which at 50 mg/kg was 31.42±3.44 and 41.37±5.27 in the suppressive and curative test respectively. The mean parasitaemia in the normal saline (negative control) rose to 54.89±6.48 and 50.47±8.11 in the suppressive and curative groups respectively, while that of the positive control group (chloroquine Phosphate) fell to 8.97±4.89 and 8.09±4.4 in suppressive and curative tests respectively.

Hyptis suaveolens extract produced a dose-dependent effect at the doses employed in this study. At 50 mg/kg dose the chemosuppression was 42.76% and 18.03% in

suppressive and curative test respectively while 10 mg/kg dose produced the least chemosuppression of 22.39% and 6.06% in suppressive and curative tests respectively amongst the extract treated groups as against 83.66% and 83.97% suppression induced by the standard drug chloroquine phosphate in suppressive and curative test respectively. In observing the relationship between the treated and untreated groups, students t-test showed that the decreased in parasitaemia in extract treated groups were not significant ($P>0.05$), while the standard drug chloroquine phosphate significantly ($P<0.01$) reduced the level of parasitaemia compared with that of the normal saline (negative control) group.

Haematological parameters

In the suppressive test, infection of mice with *Plasmodium berghei* reduced RBC from 5.47 ± 0.29 to 3.62 ± 0.81 in the chloroquine phosphate and normal saline group respectively (Table 4). The WBC rose from 5.46 ± 0.81 in chloroquine phosphate treated (positive control) groups to 7.52 ± 1.03 in the normal saline (negative control) group.

The haemoglobin levels fell from 17.81 ± 1.07 to 12.43 ± 0.74 in chloroquine phosphate and normal saline groups respectively. The PCV level was reduced from 49 ± 1.53 to 40.53 ± 1.84 in chloroquine phosphate and normal saline groups respectively. However, haemoglobin parameters in the groups administered the extracts showed a dose depended effect. Similarly, the curative test showed that the presence of *Plasmodium berghei* reduced the RBC, Hb and PCV of mice while it increased the WBC (Table 5). The ethanolic leaf extract showed a dose dependent effect on the haemological parameters in the *Plasmodium berghei* infected mice with more reduction in the RBC, Hb and PCV in groups given 10 mg/kg followed by groups that received 25 mg/kg and 50 mg/kg. High WBC was recorded in groups that receive 10 mg/kg than 25 mg/kg and 50 mg/kg with 6.74 ± 0.55 , 6.42 ± 0.81 and 6.02 ± 0.16 respectively against 5.75 ± 0.14 and 7.03 ± 1.21 in the chloroquine phosphate and normal saline respectively. However, the changes in these haematological parameters were not statistically significant ($P>0.05$).

Table 1: Changes in parasitaemia in mice infected with *Plasmodium berghei* on day 0, following daily treatment on day 0-3 with ethanolic leaf extracts of *Hyptis suaveolens*.

Day	50 mg/kg	25 mg/kg	10 mg/kg	CQ 5 mg/kg	Normal saline
4	24.42±1.38	28.67±1.20	33.30±1.52	21.17±0.94	37.87±1.36
5	26.58±1.35	30.50±1.14	38.25±1.50	12.58±1.09	52.58±3.33
6	37.42±1.20	37.08±1.92	45.67±1.50	1.58±0.15	62.32±2.03
7	47.25±1.41	49.36±2.34	53.17±2.08	0.56±0.15	67.00±2.80

CQ=Chloroquine phosphate

Data are mean ± standard error for twelve mice per group.

Table 2: Changes in parasitaemia in mice infected with *Plasmodium berghei* on day 0, treated daily on day 3-6 with ethanolic leaf extract of *Hyptis suaveolens*.

Day	50 mg/kg	25 mg/kg	10 mg/kg	CQ 5 mg/kg	Normal saline
4	27.69±1.34	31.58±1.92	32.20±1.45	19.25±1.02	32.00±2.08
5	29.35±1.53	40.00±1.50	42.07±1.74	11.25±0.98	43.10±1.81
6	46.20±1.21	49.92±1.07	53.40±2.04	1.22±0.51	57.83±2.52
7	52.25±2.10	59.74±2.06	61.92±2.91	0.65±0.21	68.92±4.66

CQ= Chloroquine phosphate

Data are mean ± standard error for twelve mice per group.

Table 3: *In vivo* anti-malarial activity of ethanolic leaf extract of *Hyptis suaveolens* and Chloroquine phosphate in mice infected with *Plasmodium berghei*.

Test	Drug/Extract	Dose (mg/kg)	Parasitaemia (Mean±SE)	Chemosuppression (%)
4-day (Suppressive)	Leaf	50	31.42±3.44*	42.76
	Leaf	25	36.40±4.68*	33.69
	Leaf	10	42.60±4.35*	22.39
	Chloroquine Phosphate	5	8.97±4.89**	83.66
	Normal saline	0.2 ml	54.89±6.48	-
Rane (Curative)	Leaf	50	41.37±5.27	18.03
	Leaf	25	45.31±6.10	10.22
	Leaf	10	47.41±6.49	6.06
	Chloroquine Phosphate	5	8.09±4.40	83.97
	Normal saline	0.2 ml	50.47±8.11	-

S.E. = Standard Error.

*significant at p<0.05 compared with the normal saline (control).

**significant at p<0.01 compared with the normal saline (control).

Table 4: Haematological level of mice infected with *Plasmodium berghei* in the 4-day suppressive test of the ethanolic leaf extract of *Hyptis suaveolens*.

Dose (Mg/kg/day)	RBC x 10 ⁶ /mm ³	WBC x 10 ³ /mm ³	Hb (g/dl)	PCV (%)
50 Ext.	4.64±0.32	5.84±0.24	15.33±0.85	44.00±2.63
25 Ext.	4.01±0.57	6.01±0.28	14.09±0.67	42.09±2.21
10 Ext.	3.91±0.21	6.51±0.52	14.50±1.02	1.90±0.74
5 CQ	5.47±0.29	5.46±0.81	17.81±1.07	49.00±1.53
0.2 ml Ns	3.62±0.81	7.52±1.03	12.43±0.74	40.53±1.84

RBC=Red blood cell; WBC= White blood cell; Hb= Haemoglobin; PCV= Packed cell volume.

Ext = Extract; CQ = Chloroquine phosphate; Ns = Normal saline.

Data are mean ± standard error for twelve mice per group.

Table 5: Haematological levels of mice infected with *Plasmodium berghei* in the Rane (curative) test of the ethanolic leaf extracts of *Hyptis suaveolens*.

Dose (Mg/kg/day)	RBC x 10 ⁶ /mm ³	WBC x 10 ³ /mm ³	Hb (g/dl)	PCV (%)
50 Ext.	4.23±0.76	6.02±0.16	14.60±0.81	43.13±1.11
25 Ext.	3.70±0.15	6.42±0.81	13.82±1.32	41.00±8.04
10 Ext.	3.48±0.21	6.74±0.55	13.05±0.73	40.05±0.05
5 CQ	5.27±0.85	5.75±0.14	17.40±1.04	47.00±4.36
0.2 ml Ns	3.12±0.49	7.03±1.21	12.84±0.42	39.02±0.53

RBC= Red blood cell; WBC= White blood cell; Hb=Haemoglobin; PCV= Packed cell volume

Ext = Extract; CQ = Chloroquine phosphate; Ns = Normal saline.

Data are mean ± standard error for twelve mice per group.

DISCUSSION

The extract showed dose-dependent effects, with the higher doses resulting in greater reduction in the parasite load than the lower doses which gave 22.39% and 6.06% at 10 mg/kg for suppression and curative tests respectively. The dose effect observed in this study is in accord with the finding of Awe and Makinde (1998), Tona et al. (2001) and Iwalokun (2008), who separately reported greater reduction in the parasitaemia at higher doses than lower. The reduction in the percentage parasitaemia was more in the suppressive test than the curative test. This observation was supported by the haematological test which showed slight reduction in the RBC, Hb and PCV and increase in WBC at higher doses than lower compared with the normal saline group. The reduction in RBC, Hb and PCV and the increase in WBC of infected mice was more observed in the curative than suppressive tests. This is consistent with work of Okochi et al. (1999) who reported increase in WBC and decrease in RBC, Hb and PCV in rats infected with *Trypanosoma brucei*.

The result of this study showed that the ethanolic leaf extract of *Hyptis suaveolens* may not have a therapeutic effect; however, it could probably be a potential prophylactic agent. The reduction in parasitaemia of the groups treated with the extract compared with the normal saline (negative control) group could probably be due to the bioactive composition of the plant. Mesia et al. (2005) attributed the antimalarial activities of *Croton mubango* to the presence of alkaloids and terpenoids detected in the plant extracts. Tona et al. (2001) reported that *Cassia occidentalis*, *Morinda morindoides* and *Phyllanthus niruri* significantly reduced parasitaemia in *Plasmodium berghei* infected mice.

A study conducted by Bala et al. (2006) showed that *Aloe vera* and *Coriandrum sativum* were not generally effective in eliminating *Trypanosoma brucei brucei* infection in albino rats. Iwalokun (2008) reported that *Vernonia amygdalina*, aqueous

leaf extract has the inherent ability to work in favour of the restoration of chloroquine efficacy as a prophylactic and chemotherapeutic agent against chloroquine sensitive and resistant *Plasmodium berghei* infection in mice. Nwabuisi (2002) reported the prophylactic effect of multi-herbal preparation "Agbo-Iba" against *Plasmodium yoelii nigeriensis* in mice and reported no significant side effects.

The result of this study indicates that *Hyptis suaveolens* ethanolic leaf extract have antimalarial potentials. The plant extracts should be extensively investigated to isolate and identify their antimalarial active constituents.

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