DETERMINATION OF ANTIMALARIAL ACTIVITY OF METHANOL EXTRACT OF *Euphorbia hirta* USED LOCALLY AS HERBAL TEA AMONG RURAL DWELLERS IN OVIA NORTH-EAST LGA, EDO STATE, NIGERIA

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Abstract: The research was conducted to determine the antimalarial activity of methanol extract of *Euphorbia hirta* in *Plasmodium-infected* mice. The plant is the first line herbal recipe for the treatment and management of malaria and fever among herbal practitioners in Ovia North East local government area of Edo State, Nigeria. *Euphorbia hirta* (Cat's hair) L, Family-*Euphorbiaceae*, whole plant were collected, dried, pulverised and extracted with methanol solvent in a soxhlet extractor. The extract was concentrated with rotary evaporator and dried over Na_2SO_4. A portion of the extract was used for phytochemical screening according to standard methods. The antimalarial activity analysis of the methanol extract of *E. hirta* was conducted along with quinine to determine the antimalarial potency of the plant extract in *Plasmodium* –infected balb/c albino mice. The results of the phytochemical screening indicated the presence of phenolics, flavonoids, alkaloids, glycosides and terpenoids while steroid was absent. The antimalarial activities revealed that the methanol extract of *E. hirta* exhibit significant suppression ($P<0.05$) of *Plasmodiumfalciparum* which was evident by the 18.71% mean chemo suppression of malaria parasite at day 4 of treatment of *P. falciparum* – infected mice with 84 mg/kg/day. This work corroborates the local use of the plant for the treatment of malaria.

Keywords: *Euphorbia hirta*, phytochemical screening, antimalarial activity, *Plasmodium falciparum*

Introduction

Euphorbiahirta (Cat's hair) belongs to the family-*Euphorbiaceae*, commonly called asin-uloko (Bini), egele-emi-ile (Yoruba), odaneinenemili (Ibo), nonankurchiya (Hausa) and ajemugba among the Akoko-edo people. A survey on the phytochemical analysis of *Euphorbia hirta* hasrevealed that it contains: flavonoids, euphorbianin, leucocyanidol, camphol, menthol, quercitrin, quercitol. Gallic acid, 2,4,6–trio-O-galloyl –D-glucose, 1,2,3,4,6-penta-O-galloyl-β-D-glucose (Chen, 1991; Yoshida, 1988), euphorbins A, B, C, D, E; triterpenes and phytosterols: β-Amyrin, -24-methylene cycloartenol, and β-sitosterol (Martinez, 1999), heptacosane, n-nonacosane and others (Gnecco, 1996). The ethanolic extract of *Euphorbiahirta* have been reported to inhibit the growth of some microorganisms except *Salmonella typhi*.

The antibacterial effect was attributed to the presence of alkaloids, tannins and flavonoids which have been shown to have antibacterial properties. The results support its use in traditional medicine. The study on the antibacterial effect of the methanol extract of *Euphorbia hirta* against dysentery causing *Shigella* spp. showed the extracts to be non-cytotoxic and an effective antibacterial (Ogueke *et al.* 2007). The diuretic studies of *Euphorbia hirta* showed that the leaf extracts increase urine output and electrolytes in rats. Study suggests that active components in the water extract of *E. hirta* leaf had similar diuretic effect as that of acetazolamide results validates its traditional use as a diuretic by the Swahilis and Sukumas (Patricia *et al*., 1999). On anticancer activity, studies of extracts of *E. hirta* showed selective cytotoxicity against several cancer lines, extracts screened showed antiproliferative activities against normal mouse fibre cells (Suni and Kumar, 2010) while on malarial activity, isolated portion of the aerial parts of *E hirta* which contained flavonol glycosides, afzelin and quercimyricitrin indicated inhibition of proliferation of *Plasmodium falciparum* (Liu *et al.*, 2007). Another antimalarial survey of twenty extract including ten ethanol and ten CH_2Cl_2 from different parts of nine African medicinal plants used in Congolese traditional medicine for the treatment of malaria, were submitted to a pharmacological test in order to evaluate their effect an *P. falciparium* growth in vitro of these plant species, 14 (70%) extracts including ethanol and CH_2Cl_2 from *Cassia occidentalis* leaves, *Cryptolepissanguinolenta* root bark, *Euphorbia hirta* whole plant, *Garcina* kola stem bark and seeds, *Marindalucida* leaves and *Phyllanthusniruri* whole plant produced more than 60% inhibition of the parasite growth in vitro. *E. hirta* among others showed a significant chemosuppression of parasitaemia in mice infected with *P. berghei* at orally given doses of 100 – 400 mg/kg/day (Tona *et al*., 2009).

Some natives in Nigeria, particularly the Yorubas and Binis, collect whole plant of *Euphorbia hirta* and blend the stem and leaves of *Stigmaphyllonovatum*, with other leaves to prepare a concoction for the treatment of fevers, stomach ache and particularly as an aid to reduce excessive abdominal heat in pregnant women (Edegbe, 2007). In Ovia north east local government area where major community like Ekiadolor, Utekon, Ugbogui, Odighi, Usen, Okada and Utese are located, some herbal practitioners blend the whole plant of *E. hirta* and prepare decoction tea with it for the treatment of malaria. The dose of the herbal tea is 3 glasses a day (one shot each in the morning, afternoon and evening) to treat malaria (Edegbe, 2007). Further consultation with the herbal practitioners revealed that most indigenes use only *E. hirta* plant for the decoction tea while others have a blend with other medicinal plants like *Phyllanthusamarus*, *Sigmaphyllonovatum* and *Azadirachtaindica* (Neem plant) leaves.

In Nigeria, over 100 million people are at risk of malaria every year and its estimated that about 50 percent of the adult population experience at least one episode of the disease yearly while children of under five years old have up to 2 to 4 attacks of the disease annually (Federal Ministry of Health (FMOH, 2005). Latest report revealed that Africa is the most affected continent: about 90% of all malaria deaths occur there (WHO, 2012). Since antimalarial drug resistance has emerged as one of the greatest challenges facing malaria control today, there is need for identification of new versatile agent to curtail the spread of malaria to new areas and re-emergence in areas where the disease had been eradicated. In Nigeria in the past decades, malaria cases have been aggravated by the increasing spread of drug- resistant *Plasmodium falciparum* strains and this continuing threat by this tropical disease lends urgency to the need to expand the systematic exploration of medicinal plants in the search of

new bioactive molecules (drugs) or their precursors. Thus, the study is aimed at determining the antimalarial activity of *E. hirta* to corroborate local use of the plant in rural areas of Ovia north east local government of Edo State.

Materials and Methods

Chemicals and reagents

Sample collection and treatment

The whole plants of *E. hirta* were collected from the bush in Ekiadolor community in Ovia North East Local Government Area of Edo State, Nigeria. The plant was identified and authenticated by a taxonomist Prof. J. F. Bamidele, with herbarium voucher number (UBHM, 0193) deposited in the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria. The plant leaves were air-dried under shade in the laboratory for four weeks and pulverized to a powdered form. Six hundred grams (600 g) of the powdered leaves were extracted with methanol (Merck, Germany) in a Soxhlet apparatus for 8 hours. The crude extract was dried with Na_2SO_4 (Vickers, England) and then concentrated in a rotary evaporator at 50^0C. A portion of the dried residue (crude extracts) were used for phytochemical screening.

Phytochemical screening

Phytochemical screening was carried out to find the presence of the active chemical constituents such as alkaloids, glycosides, steroids, flavonoids, saponins, terpenoids, phenolics, tannins and eugenols by using the standard procedures (Kokate *et al.,* 2009) and (Evans and Trease, 2002).

Thin layer chromatography

Thin layer chromatography was carried out on the methanol extract of *E. hirta* extract at different solvent ratios (100% hexane, hexane/methanol-1:1 and hexane/methanol-9:1) using the precoated TLC plates.

Acute toxicity test

The acute toxicity assay was performed according to standard procedure (Lorke, 1983), to estimate the lethal dose, LD_{50} values of the methanol extract using Balb/c albino mice before the antimalarial analysis (using *P. falciparum*-infected mice).

Antimalarial test

Development of experimental humanized mouse model

Mice: Balb/c mice weighing 20 – 33 g were used. They were housed in standard mosquito-netted metal cages under standard conditions of light and temperature and were maintained on a standard mice diet and water ad libitum. They were acclimatized for 14 days and were treated in accordance with guidelines for animal care approved by the Animal Ethics Committee of the University of Benin, Benin City, Nigeria. The mice were certified medically fit for the experiment by Dr. J. Danjuma, a veterinary Doctor.

Modification of mice: The Balb/c mice were modified by using pharmacological compounds (4 mg aspirin/kg body weight and 4mg anhydrous doxycycline eq./kg body weight) to retard their innate immune responses (Javeed *et al.,* 2011; Bellahsene and Forgren, 1985) followed by engraftment of human blood (Bellahsene and Forgren, 1985; Imade *et al.,* 2012). Infection of experimental humanized mouse model with *Plasmodium falciparum* were done according to the method of Bellahsene and Forgren (1985) and Imade *et al.* (2012).

Antimalarial activity of the P. falciparum-infected mouse model with methanol extract of E. hirta

Plasmodium falciparum-infected humanized immunosuppressed mice were employed to verify the antimalarial principles of each of the test samples. The mice were divided into 5 independent experimental groups (5 mice per group). The negative control drug –DMSO (4 mg/kg body weight administered three time daily for four days) and positive control drug- quinine (73 mg/kg body weight administered three times daily for four days) were used to treat mice in two of the experimental groups (antimalarial validation). The mice received these drugs orally via a gastric cannular. Varying doses (21, 42, 84 mg/kg/day each) of the methanol extract were used to treat the mice in the other three experimental groups. The test samples were administered, by taking into consideration their LD_{50} values.

Upon administration of quinine, DMSO, and the plant extract, the net mean malarial chemo suppression (WHO, 2009) due to drug/extracts administration was calculated according to the formula:

$$Net\ mean\ malarial\ chemosuppression\ (\%) = \frac{100\ (A-B)}{A} - MC0$$

Where: A = Percentage of parasitaemia in the mice administered with DMSO; B = Percentage of parasitaemia in mice treated with quinine/plant extracts;

$\frac{100\ (A-B)}{A}$ = Mean malarial chemosuppression

MC0 = Mean malarial chemosuppression obtained from mice at day 0 of treatment.

Statistical analysis

Values were expressed as mean and standard error of the mean, as well as in percentages. Chi-square test and regression analysis, where appropriate, were used to determine the level of significance, and P-value less than 0.05 ($P < 0.05$) were considered significant. The software, SPSS version 16, was employed for the statistical analysis.

Results and Discussion

The results of the phytochemical screening of the methanol extract of the leaves of *E. hirta* is shown in Table 1. The result indicated that the methanol extract constitutes important phytochemicals like terpenenoids, steroids, saponins, phenolics and alkaloids (Table 1) which have physiological effects on man. Alkaloids have been reported to have anti-inflammatory, antiprotozoal and anti-microbial properties (Kumar and Tandon, 1979). Cardiac glycosides are important class of naturally occurring drugs whose actions helps in the treatment of congestive heart failure (Yukari *et al*., 1995). Steroids have been reported to have antibacterial properties (Raquel, 2007) and they are very important compounds especially due to their relationship with compounds such as sex hormones (Okwu, 2001).

Table 1: Phytochemical constituents of methanol extract of *S. ovatum* leaves

S/N	Phytochemical	Methanol extract
1	Glycosides	+
2	Saponin	+
3	Phenolics	+
4	Flavonoids	+
5	Tannins	+
6	Terpenoids	+
7	Eugenols	+
8	Steroids	-
9	Alkaloids	+

+ = Present; – = Absent

Table 2: Retention factor (Rf) values and colour reaction of *E. hirta* extract

Extracts	Solvent system	Colour of spots (under UV lamp)	Rf values
*E.hirta*M	100% methanol	Light yellow	0.71
*E.hirta*M	Hexane: methanol (1:1)	Blurred yellow	0.55
*E.hirta*M	Hexane: methanol (9:1)	Yellow	0.68

***E.hirta*M =** *Euphorbia hirta* methanol extract

Thin layer chromatography

The pre coated thin layer chromatography results of methanol extracts of *E. hirta* are shown in the following Table 2. The retention factors of 0.55, 0.68 and 0.71 indicated that all the constituents of the extract screened with the selected solvents can be isolated when subjected to further isolation techniques.

Antimalarial potential

Table 3 represents the malarial chemo suppressive activities obtained during therapy of *P. falciparum*-infected mice with the test sample (methanol extract) and positive control (quinine). Quinine-treated mice had the lowest parasite counts (mean parasite counts at day 4 of therapy) with 0.27±0.01%, while the lowest parasite count for methanol extract of *E. hirta* was recorded as 1.36±0.02 (day 4, 84 mg/kg/day) and in the mice treated with DMSO as 1.67±0.32%; thus indicating that the methanol extract has antimalarial effect on *P. falciparum* parasite more than the negative control group but less than quinine treated group. Mean chemo suppression of quinine treated *P. falciparum* –infected mice increased successively from day 0, 2 and 4 with 1.18, 60.67 and 83.82%, respectively while the methanol extract at day 0, 2 and 4 (84 mg/kg/day) had mean chemo suppression of 2.75, 1.37, and 18.71%, respectively. All the doses of the methanol extract exhibited significant suppression ($P < 0.05$).

MCS indicates mean malarial chemosuppression; NCS indicates net mean malarial chemosuppression; NC indicates the negative control group of *P. falciparum*-infected mice which received dimethyl sulfoxide; PC indicates the positive control group of *P. falciparum*-infected mice which were administered with quinine; T1 *E. hirta*, T2 *E. hirta*, T3 *E. hirta*, indicate the experimental groups of *P. falciparum*-infected mice which were administered with varying doses of *E. hirta* methanol extracts.

Table 3: Malarial Chemosuppressive activities of *E.hirta*against *P. falciparum*

Days of Therapy	Experimental groups of mice	Dose of therapeutic agents given (mg/kg/day)	Mean parasite counts ($\times 10^3$ cells/µl)	Mean parasite counts (%)	MCS (%)	NCS (%)
0	NC group	12	77.05±3.18	1.54±0.06	-	-
	PC group	219/mg/kg/day	76.14±4.45	1.52±0.09	1.18	0
	T1 *E. hirta*	21	76.96±1.10	1.54±0.02	0.11	
	T2 *E. hirta*	42	78.50±1.50	1.57±0.03	{1.89}	0
	T3 *E. hirta*	84	79.16±1.11	1.58±0.02	{2.75}	0
2	NC group	12	80.79±2.60	1.62±0.05	-	-
	PC group	219/mg/kg/day	31.78±1.28	0.64±0.03	60.67	59.49
	T1 *E. hirta*	21	83.39±8.67	1.67±0.17	{3.21}	{3.32}
	T2 *E. hirta*	42	74.11±0.38	1.48±0.01	8.28	
	T3 *E. hirta*	84	79.69±6.33	1.59±0.13	1.37	
4	NC group	12	83.58±15.94	1.67±0.32	-	-
	PC group	219/mg/kg/day	13.52±0.57	0.27±0.01	83.82	82.65
	T1 *E. hirta*	21	94.2±20.65	1.88±0.41	{12.70}	{12.81}
	T2 *E. hirta*	42	71.21±0.99	1.42±0.01	14.80	16.69
	T1 *E. hirta*	84	67.94±0.90	1.36±0.02	18.71	21.46

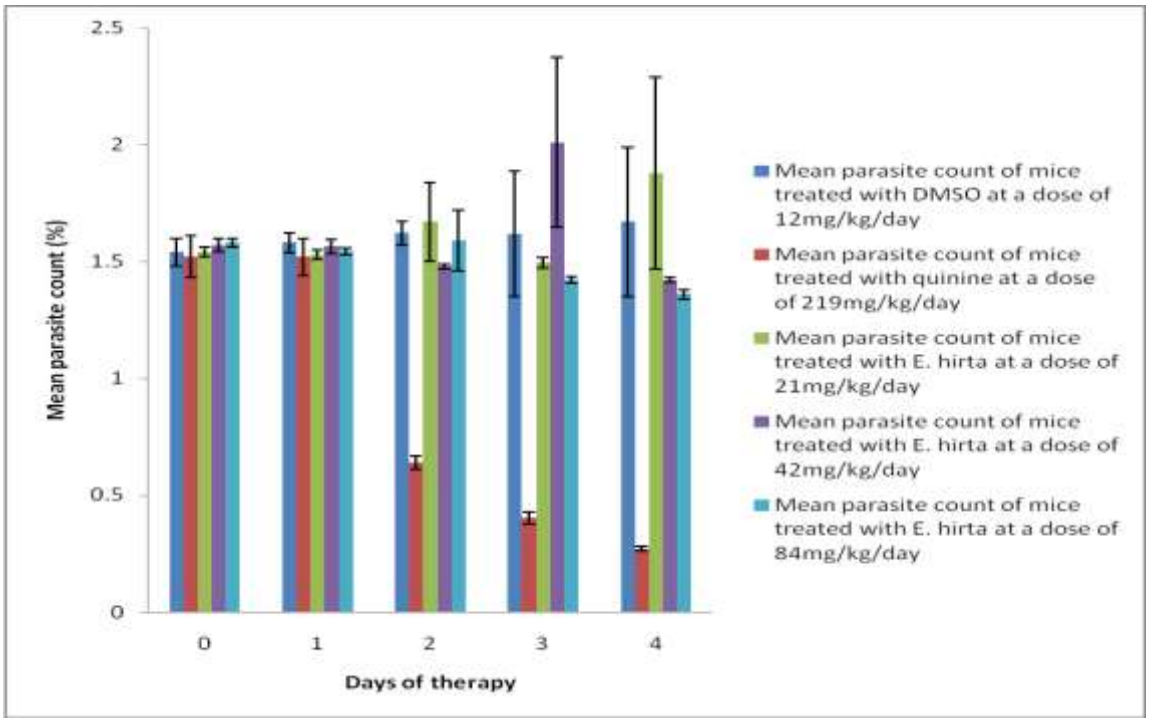

Fig. 1: *P. falciparum* densities obtained from quinine-validatedinfected mice treated with methanolic extract of *E. hirta*

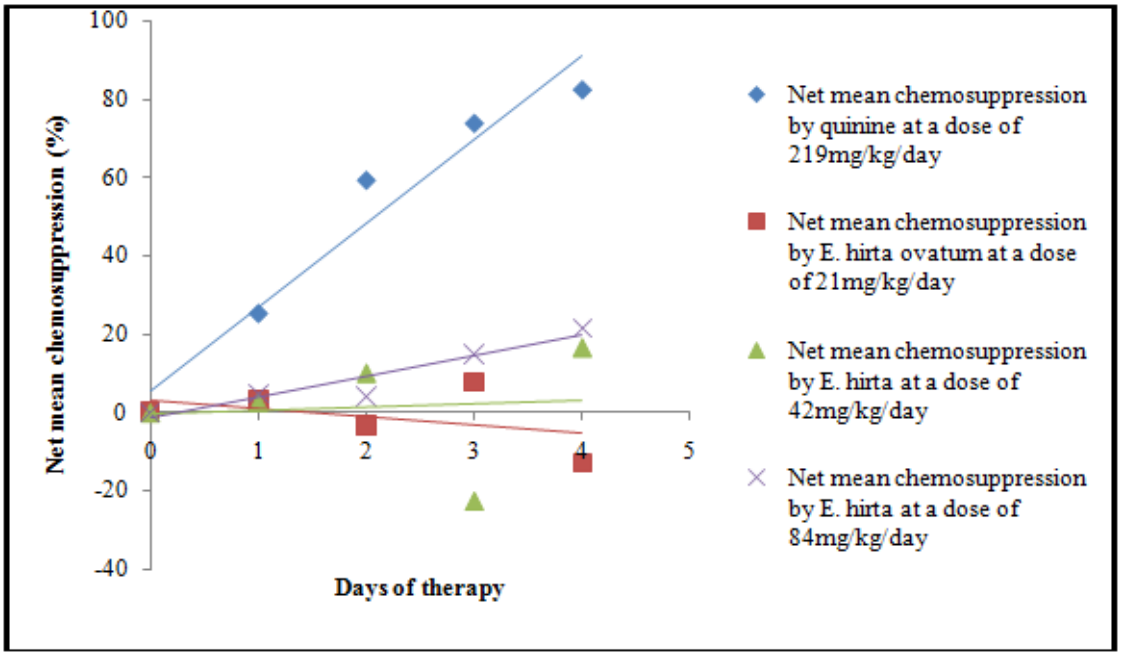

Fig. 2: Effects of treatment with methanol extracts of *E. hirta* and quinine on *P. falciparum* suppression in the *P. falciparum*-infected mice

The mean malaria chemo suppression of the extract of *E. hirta* is further represented in the bar chat of Fig. 1; while the net mean malaria chemo suppression between *E. hirta* and standard drug quinine is shown in Fig. 2.

In Fig. 1 above, the degree of chemosuppression of *P. falciparum* upon treatment with varying doses of the methanol extracts and control were compared. Chemosuppression of *P. falciparum* parasites by the methanol extract was dose dependent. Parasite suppression was highest in the infected mice treated with 84 mg dose of *E. hirta* extracts/kg body weight/day and lower in the mice treated with 21 mg dose of*E. hirta* extracts/kg body weight/day. From Fig. 2 above, the net mean chemosuppression of *P. falciparum* parasites by the 84 mg dose of *E. hirta* extracts/kg body weight/day as well as the 219 mg dose of quinine/kg body weight/day were estimated at 24.46, and 82.65%, respectively.

From the work of Imade *et al.* (2012), *in-vitro* culture *P. falciparum*-infected human blood sample usually show proliferation of the parasites after 92 h incubation. For this research, the balb/c mice were inoculated with clinical isolates of *P. falciparum* that had been previously cultured before treatment started. At the end of treatment of the infected mice with the standard drug quinine, post-mortem evaluation of the parasitological responses in the peripheral blood of the mice were assessed and indicated in the photomicrographs showed in Plate 1. The photomicrograph of quinine-treated mice showed an absence of knobs on the surface of their erythrocytes, with a relatively low parasitaemia (Plate 2).

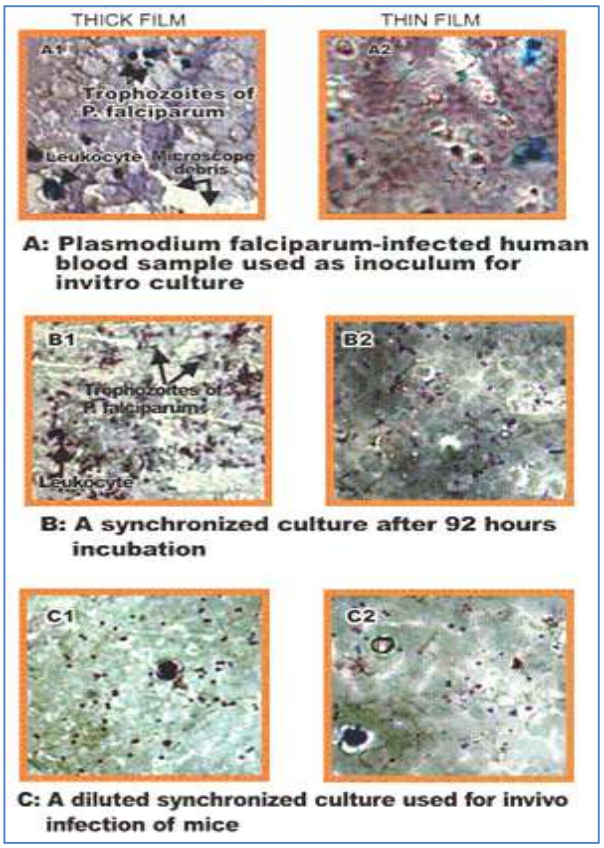

Plate 1: Photomicrographs obtained from the in-vitro culture of *Plasmodium falciparum* (Giemsa, × 1000; original magnification)

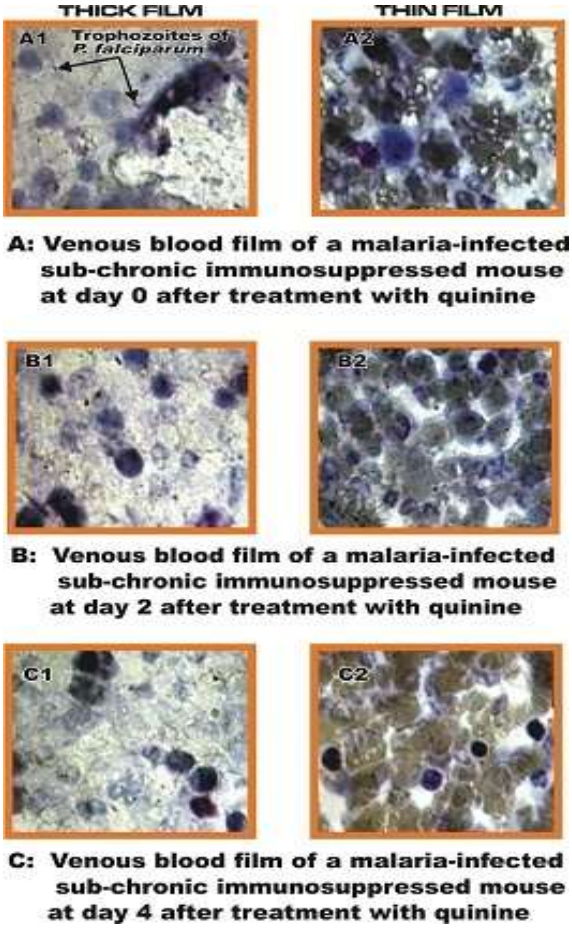

Plate 2: Peripheral blood of sub-chronic immunosuppressed mice after treatment with quinine (Giemsa × 1000; original magnification)

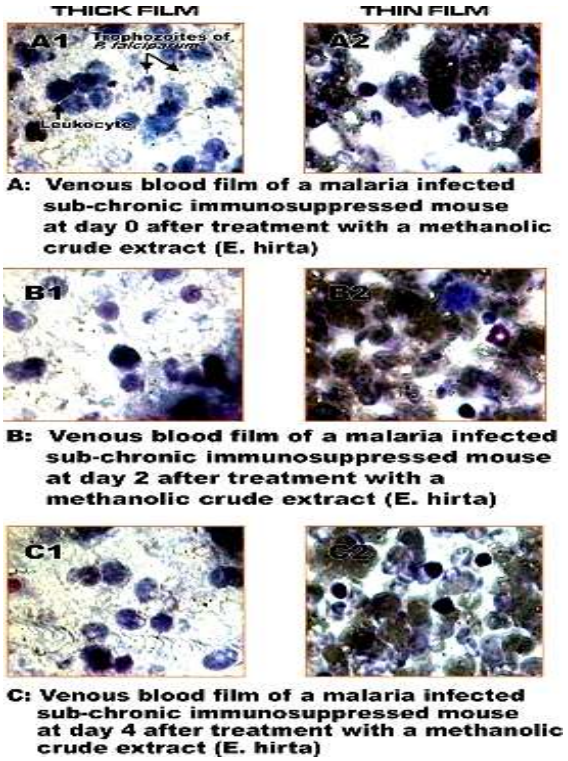

Plate 3: Peripheral blood of sub-chronic immunosuppressed mice after treatment with methanol extract of *E. hirta* (Giemsa × 1000; original magnification)

From Plate 3, there was a significant reduction in *P. falciparum* parasites from the methanol extract sample of *E. hirta* upon therapy with varying doses. This is an indication of antimalarial potency.

Conclusion

The phytochemical screening had shown that methanol extract of *E. hirta* contains alkaloids, phenolics and flavonoids which are of pharmacological importance in medicine. The antimalarial activity revealed that methanol extract of the plant have antimalarial potency and this result support the local use of the plant as malaria herbal tea.

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Conflict of Interest

Author has declared that there is no conflict of interest reported in this work.

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