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Antiplasmodial Activity of Ethanolic Leaf Extracts of *Spilanthes uliginosa, Ocimum basilicum (Sweet Basil), Hyptis spicigera and Cymbopogon citratus on* Mice Exposed to *Plasmodium berghei* Nk 65

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Authors' contributions

Author AJU designed the research with author ANCO, UAI and KNA and wrote the first draft of the manuscript. Authors NAO, PMA and OUO managed literature searches and performed the statistical analysis. Authors FNN and KNA managed experimental procedures. All authors read and approved the final manuscript as edited by author KNA.

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ABSTRACT

In Africa and elsewhere, medicinal plants including *Spilanthes uliginosa, Ocimum basilicum, Hyptis spicigera and Cymbopogon citratus* are still widely used in the treatment of malaria and other ailments. The aim of the present study was to investigate *in vivo* antiplasmodial effect of ethanolic leaf extract of these plants in mice. Oral acute toxicity of the extracts was evaluated in mice using modified Lorke's method and their *in vivo* anti-plasmodial effect against early infection, curative effect against established infection and prophylactic effect against residual infection were studied

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using total WBC count in chloroquine-sensitive *Plasmodium berghei* NK 65-infected mice. The oral median lethal dose of the extract in mice was determined to be greater than 2000 mg kg^{-1} body weight. The results indicated a significant ($P<0.05$) daily increase in the level of parasitaemia in the parasitized untreated groups and a significant ($P<0.05$) dose dependent decrease in the level of parasitaemia in the parasitized groups treated with varying doses of the various medicinal plants and the standard drug. Overall, the dose dependent effects were in the order of: 5mg/kg body weight of chloroquine > 800 mg/kg > 400 mg/kg > 200 mg/kg body weight of the plant extracts with the efficacy of the plants in the order of: *H. Spicigera* > *O. basilicum* > *C. citratus* > *S. uliginosa (Sw)* with minor variations. The implications of these results is that *Spilanthes uliginosa, Ocimum basilicum, Hyptis spicigera and Cymbopogon citratus* ethanolic leaf extracts posses potent antimalarial effects and may therefore serve as potential sources of safe, effective and affordable antimalarial drugs.

Keywords: Medicinal plants; malaria; plasmodium; suppressive; curative; prophylactic.

1. INTRODUCTION

Before the advent of orthodox medicine in the treatment of ailments which include malaria, the Traditional African Society had devised various means of combating such ailments. One of the major ailments that are of concern in the world today is malaria. Malaria is one of humanity's worst diseases and is frequently referred to as the disease of the poor [1]. Malaria kills more than five million people worldwide [2]. The majority of victims who die are children under the age of five and pregnant women. These children die because they are not protected and are not treated quickly enough to prevent their death [1]. Malaria episodes occur every year and result in these deaths. In 2004 Roll Back Malaria developed a Malaria Indicator Survey package for monitoring estimated that 300-500 malaria episodes occur annually and with 18% of deaths in children under the age five year [1].

Approximately 12 million children younger than 5 years of age die every year, most of these children live in developing countries [3]. More than 50% of these deaths are attributed to malaria, diarrhoea, respiratory infections, or measles, conditions that are either preventable or treatable with low cost interventions. Malaria episodes contribute to about 33% of all child deaths [4]. A survey conducted in Malawi estimated that the relative risk of having episodes of malaria in children under the age of five years increased by 2.7 times [5].

Another survey conducted in Ghana showed that frequency of malaria episodes was highest in paediatric population below five years than in other age groups [3]. The same survey showed higher frequency of malaria episodes in children with advanced HIV disease than normal ones [3]. Malaria which can be fatal is transmitted to humans by mosquito vectors of the *Anopheles* species. In children, malaria presents as a febrile illness with other key symptoms, vomiting, loss of appetite, restlessness and in severe forms with convulsions [6]. Even when it is not fatal, malaria produces considerable impact on the health of the African children, mostly during their first five years, increasing the susceptibility to other infections and hampering their development [3].

Malaria is now a global crisis, with one - fifth of the world's population at risk and with more than 233 million clinical cases of malaria in 2000, 225 million in 2009 and 244 million in 2000 [4]. The disease continues to spread due to a combination of factors, which include weak health systems, social, cultural and behavioral practices relating to treatment and prevention of malaria, large population movements, and climatic changes among others [4]. In October 1998, the Roll Back Malaria Initiative was launched as a catalyst for a renewed global commitment to tackle the disease, which puts a brake to development in Africa [1]. Roll Back Malaria is a partnership working worldwide to reduce the burden of malaria by 50% in 2015 [1]. This is in line with the Millennium Development Goal number 6 which puts its emphasis on the need to fight HIV/AIDS, Tuberculosis, Malaria and other diseases.

Maximum emphasis on the initiative is to ensure that malaria infection and associated deaths are reduced. People especially children and pregnant women are at the centre of RBM movement. This movement also seeks to link with interventions such as Integrated Management of Neonatal and Childhood Illnesses (IMNCI) to reduce children's deaths from malaria.

Following the high level of threat malaria poses to humanity, with increased level of mortality and morbidity rate as index of its threat, it has become very necessary to look further for an alternative measure for malaria prevention, control and treatment [7]. Conventional drugs used in the treatment of malaria infections are sometimes inadequate and sometimes can have some serious adverse effect [8]. It is therefore, necessary to search for alternative drugs for the treatment of the disease and to replace currently used drugs with uncertain safety and wholesomeness.

The anti-malarial potentials of compounds derived from plants have already been established with examples such as quinine, obtained from *cinchoma* species and *Artemisia* obtained from *artemesia species* [9]. The ethno-medicinal use of plants and plant products for the treatment of malaria could be a promising source of wholesome therapy [8]. In fact, the traditional medicine of this continent constitutes an important source for ethno pharmacological investigations [10]. Locally, the following plants; *Spilanthes uliginosa, Ocimum basilicum, Hyptis spicigera* and *Cymbopogon citratus* have been used tradomedically for the treatment of malaria.

This study was aimed at determining the suppressive, curative and prophylactic effect of leaf extract of *Spilanthes uliginosa, Ocimum basilicum, Hyptis spicigera and Cymbopogon citratus* in malarial cases in order to ascertain the extent of their effectiveness as antimalarial agents.

2. MATERIALS AND METHODS

2.1 Preparation of Leaf Extract

The plants were identified and authenticated by Prof. S. C. Onyekwelu of Department of Applied Biology, Faculty of Biological Science, Ebonyi State University Abakaliki, Ebonyi State, Nigeria. The leaves were cleaned, air-dried and pounded into fine powder using an electric blender. The powder was stored in an airtight container and kept in a cool, dry place.

Exactly 150 g of powder samples of *Spilanthes uliginosa, Ocimum basilicum, Hyptis spicigera* and *Cymbopogon citratus* were soaked in 500 ml of ethanol each for 24 hours. They were filtered into a graduated beaker and exposed to mild heat at 40^{o}c in water bath until a semi solid extracts were obtained.

2.2 Animals

Swiss albino mice aged two months weighing 17-34 g of both sexes were obtained from animal house of Nnamdi Azikiwe University Awka, Anambra State. The animals were acclimatized for seven day under standard environment conditions and fed *ad-libitum* on their normal diets.

2.3 Ethical Approval

Ethical clearance was given by Ebonyi State University Research and Ethics Committee.

2.3 Determination of Acute Toxicity (LD_{50})

The crude leaf extract of *Spilanthes uliginosa, Ocimum basilicum, Hyptis spicigera and Cymbopogon citratus* were evaluated for their toxicity in Swiss mice using, modified Locke method [11]. The test was carried out in two phases with each of the extracts. In phase one of the study, twelve mice were randomized into three groups, namely; A, B and C of four mice each, and were treated orally with 50, 100 and 200mg/kg body weight of the extract for each extract respectively. The mice were observed for changes in physical appearance, gross behavior and death in the first four hours and subsequently daily for seven days. Based on the results obtained from phase one treatment above, phase two treatment was carried out using another fresh set of twelve mice randomized into three groups namely; D, E and F of four mice each, and were given 500, 1000 and 2000 mg/kg body weight of the extracts respectively of each extract orally. These were also observed for signs of toxicity and mortality for the first four hours and thereafter daily for seven (7) days.

2.4 Rodent parasite (*Plasmodium berghei berghei NK65*)

The rodent parasite was sourced from National Institute for Medical Research (NIMR), Lagos, Nigeria and maintained alive in mice by continuous intraperitoneal passage in mice after every 5 days. The reinfected mice were moved to the Animal House of Department of Biochemistry, Faculty of Biological Science, Ebonyi State University Abakaliki where the study was carried out. Prior to the start of the study, one of the infected mice was kept and observed to reproduce signs of diseases similar to human malarial infection.

2.5 Parasite Inoculation

The inoculums consisted of 1×10^7 of *P. berghei* parasitized erythrocytes per ml. This was carried out by determining both the percentage parasitaemia and erythrocytes count of the donor mouse and diluting the blood with phosphate buffer saline in proportions indicated by both determinations. Each mouse was inoculated on first day with 0.2 ml of infected blood containing $\times10^7$ *P. berghei* parasitized red blood cells.

2.6 Anti - Plasmodial Studies

2.6.1 Preparation and administration of *Plasmodium berghei*

The swizz mice were all inoculated by intraperitoneal (IP) injection with standard inoculums of *Plasmodium berghei* NK 65 with 1×10^7 infected erythrocytes [12]. The *P. berghei* was subsequently maintained in the laboratory by serial blood passage from mice to mice every 5 days. Ten animals at a time were used as infection donors and as parasite reservoir. The donor mice were monitored for signs of infection which included lethargy, anorexia, ruffled appearance, shivering and heat-seeking behavior. Blood was taken on the fifth day, to confirm level of parasitaemia in the donor mice, using the White Blood Cell (WBC) count method [13]. Blood collected from the tail of the infected donor mice was diluted with normal saline to produce standard inoculums of 0.2 ml containing $1x10^7$ *P. berghei* infected erythrocytes [14].

2.6.2 Determination of suppressive test

The Peters 4 day suppressive test against chloroquine sensitive *Plasmodium berghei* NK 65 injection in rats was employed [12]. The swiss mice were inoculated by intraperitoneal (IP) injection with standard inoculums of *Plasmodium berghei* with 1×10^7 infected erythrocytes. Exactly seventy two (72) swiss mice of both sexes were randomly divided into six groups namely; A, B, C, D, E and F, of twelve mice per group. Groups B, C, D and E were subdivided into three (3): B_1, B_2, B_3, C_1, C_2, C_3, D_1, D_2, D_3, E_1, E_2 and E_3. The subgroups were treated with the extracts of *Spilanthes uliginosa (Sw), Ocimum basilicum, Hyptis spiligera* and *Cymbopogon citratus* each for five (5) consecutive days with 200, 400 and 800 mg/kg body weight via oral intubation daily respectively. Two control groups, A and E were used. The negative control (A) was treated daily with 5ml/kg normal saline while positive control group (F) was treated with 5 mg/kg body weight of chloroquine. All groups were given water and fed *ad libitum*. On day six of the experiment, blood was collected from the tail of each mouse and smeared on to a microscope slide to make a thick film. The blood films were stained with10% Giemsa at pH 7.2 for 10 minutes and examined microscopically. The number of parasites per field was counted for ten fields on each slide. The percentage suppression of parasitaemia was calculated for each dose level by comparing the parasitaemia in infected controls with those of treated mice and the results multiplied by 100.

2.6.3 Determination of curative test (schizontocidal activity)

Evaluation of the curative potentials of the leaf extracts was carried out according to the method of Peter and Anatoli [15]. Fresh seventy two (72) swiss mice were treated the same way as described for suppressive test. On each day of treatment, about 3 drops of blood were collected from the tail of each mouse smeared unto a microscope slide to make a thick film, stained with 10% Giemsa stain and examined microscopically to monitor the parasitaemia level.

2.6.4 Determination of prophylactic activity (respository test)

Investigation of prophylactic potential was carried out according to the method described by Iyiola et al. [12]. Another seventy two (72) swiss mice were grouped as described for suppressive test. The treatments were initiated on the first day and continued till day five when the rats were all infected with the parasite. Blood was collected from the animals seventy two hours after treatment and smears were then made. Parasitaemia was determined microscopically.

2.7 Statistical Analysis

All the results obtained were expressed as mean ± S.D of 6 rats in each group. All the tested parameters were subjected to statistical analysis using ANOVA. Differences between means were regarded significant at $P<0.O5$.

3. RESULTS

3.1 Results of Acute Toxicity Study

The gross physical and behavioural signs of toxicity observed in mice given 1000 mg/kg body weight of the extract and above include; jerking,

paw licking, hair erection, ear coiling, stretching, reduced activity and reduction in feeding activities. However, there was no mortality at all dose levels of *S. uliginosa (Sw), H. spicigera* and *C. citratus* leaves used up to 2000 mg/kg except at only 2000 mg/kg of the extract of *O. basilicum* where one died.

3.2 Results of Anti-plasmodial Study of the Treated and Untreated Mice

3.2.1 Results of suppressive activity (test on early malarial infection)

The results of 4-day suppressive study showed that there was a dose-dependent significant decrease in the parasite count at P<0.05 in mice infected with *Plasmodium berghei* malaria parasite in all the plant leaf extracts. The highest suppression (reduction) of parasitaemia was observed at the doses of 800 and 400 mg/kg body weight of mice treated with *C. citratus* and *H. spicigera* when compared to the negative control group. The extract of *C. citratus* at 400 mg/kg did not show any significant change (P>0.05) when compared to 800 mg/kg. The standard drug, chloroquine (Positive control) caused higher chemosuppression than those of the extract treated groups while the extract of *H. spicigera* caused higher chemosuppression than those of the other extracts (Table 2).

3.2.2 Results of schizontocidal activities of treated and untreated mice (test on established infection)

On established infection, it was observed that there was a daily significant (p<0.05) increase in parasitaemia level of the control group from day 0 to day 8. However, a daily reduction in the parasitaemia levels of the extract treated groups as well as that of positive control (chloroquine) was also observed. The highest extract suppression of parasitaemia was observed at the dose of 800 mg/kg body weight of mice when compared to the negative control group. The extract of *Hyptis spicigera* at 400 and 800 mg/kg body weight exerted highest chemosuppression of parasitaemia (Table 3).

3.2.3 Results of prophylactic activities of treated and untreated mice

The results showed that the extracts of *S. uliginosa (Sw), O. basilicum, H. spiligera* and *C. citratus* produced a significant (P < 0.05) dose-dependent prophylactic activity at the different doses with a reduction in the level of parasitaemia. The extracts of *H. spicigera* and *C. citratus* at 800 mg/kg body weight exerted the highest suppression of parasitaemia. Though, the effects are not significant from each other at P<0.05 except only in *O. basilicum* (Table 4).

4. DISCUSSION

The menace of multidrugs resistant malaria parasite and the absence of a functional safe and widely available malaria vaccine have necessitated research in the direction of development of new antimalarial drugs. In this crusade, it is very important that chemical components derived from plants used in traditional medicine for treatment of malaria are investigated.

The results obtained from this study showed that the gross physical and behavioral signs of toxicity observed in mice given 1000 mg/kg body weight of the extracts and above include; jacking, paw licking, hair erection, ear coiling, stretching, reduced activity and reduction in feeding activities. However, there was no mortality at 2000 mg/kg body weight (Table 1). The median lethal dose LD50 was estimated to be ≥2000 mg/kg body weight. The results of acute toxicity test showed that the plant extracts possess central depressant effect. The absence of death following oral administration of the above mentioned extracts up to 2000 mg/kg body weight as observed on the mice suggests that the extracts are practically non-toxic in the short term [16]. The safety and wholesomeness of these plant extracts is responsible for their widespread use in different ethno-therapeutic interventions without acute toxic symptoms and complications.

Although, primate models provide a better prediction of anti- malaria efficacy in human than the rodent models, the latter have also been validated though the identification of several conventional antimalaria such as chloroquine, amalar, laridox, maldox, lonart D.S and more recently artemisinin derivations [12] and [17]. This could also explain the safe use of the plants by the local people, who have been using them in traditional treatment of malaria, in Southern Nigeria.

Table 1. The acute toxicity study after 172 hours of administration of extracts *S. uliginosa (Sw), O. basilicum, H. spicigera* and *C. citrates*

		Toxicity observed							
Groups	**Doses (mg/kg)**	***S. uliginosa (Sw)***	**T/D**	***O. basilicum***	**T/D**	***H. spicigera***	**T/D**	***C. citratus***	**T/D**
A	50	No toxic changes observed.	4/0	No toxic changes observed.	4/0	No toxic changes observed.	4/0	No toxic changes observed.	4/0
B	100	No toxic changes observed.	4/0	No toxic changes observed.	4/0	No toxic changes observed.	4/0	No toxic changes observed.	4/0
C	200	No toxic changes observed.	4/0	No toxic changes observed.	4/0	No toxic changes observed.	4/0	No toxic changes observed.	4/0
D	500	No toxic changes observed.	4/0	No toxic changes observed.	4/0	No toxic changes observed.	4/0	No toxic changes observed.	4/0
E	1000	Paw licking, stretching, jerking, hair erection, ear coiling dullness, reduction in feeding activities and sleep were observed.	4/0	Paw licking, stretching, jerking, hair erection, ear coiling dullness, reduction in feeding activities and sleep were observed.	4/0	Paw licking, stretching, jerking, hair erection, ear coiling dullness, reduction in feeding activities and sleep were observed.	4/0	Paw licking, stretching, jerking, hair erection, ear coiling dullness, reduction in feeding activities and sleep were observed.	4/0
F	2000	Paw licking, stretching, jerking, hair erection, ear coiling dullness, reduction in feeding activities and sleep were observed.	4/0	Paw licking, stretching, jerking, hair erection, ear coiling dullness, reduction in feeding activities and sleep were observed.	4/1	Paw licking, stretching, jerking, hair erection, ear coiling dullness, reduction in feeding activities and sleep were observed.	4/0	Paw licking, stretching, jerking, hair erection, ear coiling dullness, reduction in feeding activities and sleep were observed.	4/0

T/D: Number of rats treated/number of deaths

Table 2. Effect of ethanolic leaf extract of *Spilanthes uliginosa, Ocimum basilicum, Hyptis spicigera* and *Cymbopogon citratus* on early malaria infection

Groups	***Spilanthes uliginosa (Sw)***		***Ocimum basilicum***		***Hyptis spicigera***		***Cymbopogon citratus***		
	Parasite count	Suppression (%)	Parasite count	Suppression (%)	Parasite count	Suppression (%)	Parasite count	Suppression (%)	Suppression (%)
I	6.02 ± 0.02^{e}	0	6.02 ± 0.02^{e}	0	6.02 ± 0.02^{d}	0	6.02 ± 0.02^{d}	0	
II	3.39 ± 0.16^{d}	43.69	3.01 ± 0.03^{d}	50	2.67 ± 0.06^{c}	55.65	3.11 ± 0.13^{c}	48.34	48.34
III	2.65 ± 0.05^{c}	55.98	2.49 ± 0.10^{c}	58.64	0.98 ± 0.08^{b}	83.72	1.08 ± 0.04^{b}	83.06	82.06
IV	1.80 ± 0.04^{b}	70.1	1.41 ± 0.04^{b}	76.58	0.36 ± 0.02^{a}	94.02	0.94 ± 0.05^{b}	84.39	84.39
V	0.30 ± 0.02^{a}	99.5	0.30 ± 0.02^{a}	99.5	0.30 ± 0.02^{a}	99.5	0.30 ± 0.02^{a}	99.5	99.5

Values are expressed as Mean±SD, n = 6 animals per group. Values in same row with different superscripts are significantly different from each other at P<0.05

Table 3. Effect of ethanolic leaf extract of *Spilanthes uliginosa, Ocimum basilicum, Hyptis spicigera* and *Cymbopogon citratus* on established infection

Groups	*Spilanthes uliginosa (Sw)*		*Ocimum basilicum*		*Hyptis spicigera*		*Cymbopogon citratus*		
	Parasite count	Suppression (%)	Parasite count	Suppression (%)	Parasite count	Suppression (%)	Parasite count	suppresssion (%)	Suppression (%)
I	12.03±0.03^{e}	0	12.03±0.03^{e}		12.03±0.03^{c}		12.03±0.03^{e}	0	
II	4.83±0.61^{d}	59.85	4.58±0.02^{d}	61.93	2.06±0.05^{b}	82.88	4.00±0.02 ^{d}	66.75	66.75
III	3.54±0.13^{c}	70.57	3.72±0.11^{c}	69.08	0.48±0.03^{a}	96.01	3.01±0.03 ^{c}	74.98	74.98
IV	2.30±0.04^{b}	80.88	2.14±0.12^{b}	82.21	0.28±0.02^{a}	96.51	2.01±0.05^{b}	83.29	83.29
V	0.28±0.02^{a}	97.67	0.28±0.02^{a}	97.67	0.28±0.02^{a}	97.67	0.28±0.02 ^{a}	97.67	97.67

Values are expressed as Mean±SD, n = 4 animals per group. Values in same row with different superscripts are significantly different from each other at P<0.05

Table 4. Prophylactic Effect of *Spilanthes uliginosa, Ocimum basilicum, Hyptis spicigera* and *Cymbopogon citratus* leaf extract against *P. berghei berghei* infection in mice

	Spilanthes uliginosa (Sw)		*Ocimum basilicum*		*Hyptis spicigera*		*Cymbopogon citratus*		
Groups	Parasite count	Suppression (%)	Parasite count	Suppression (%)	Parasite count	Suppression (%)	Parasite count	suppression (%)	Suppression (%)
I	8.92±0.15^{e}	0	8.92±0.15^{e}		8.92±0.15^{d}		8.92±0.15^{e}	0	
II	6.02±0.03^{d}	32.51	5.82±0.16^{d}	34.75	4.17±0.15^{c}	53.25	5.02±0.17^{d}	43.72	43.72
III	4.72±0.11^{c}	47.09	4.85±0.06^{c}	45.63	3.80±0.10^{c}	57.4	3.87±0.15^{c}	56.61	56.61
IV	3.07±0.12^{b}	65.58	3.50±0.05^{b}	60.76	2.67±0.06^{b}	70.07	2.67±0.06^{b}	70.07	70.07
V	0.93±0.11^{a}	89.57	0.93±0.11^{a}	89.57	0.93±0.11^{a}	89.57	0.93±0.11^{a}	89.51	89.57

Values are expressed as Mean±SD, n = 4 animals per group. Values in same row with different superscripts are significantly different from each other at P<0.05

The extracts of *S. uliginosa (*Sw*), O. basilicum, H. spicigera* and *C. citratus* showed a moderate anti-plasmodic activity with a dose dependent inhibition against *P. berghei* infection on mice (Table 2). The observed higher level of antimalaria activity by the standard drug, chloroquine, may be due to inhibition of hemozoin biocrystallization, which gives rise to toxic free heme accumulation that is responsible for the death of the parasites [18]. The observed low efficacy of the plant extracts may be due to non-selectivity of the extracts or slow absorption and poor bioavailability of the crude extracts as well as interplay of phytochemicals in the extract which are not all selective against the malaria parasite. The chemo-suppressive activity of the standard drug may be due to its active ingredient, aminoquinoline phosphate which an integral component of alkaloids. These findings are similar to those reported for *Zizyphus spina-christi* [19] and *Alstonia boonei* [12].

The results showed that *S. uliginosa (Sw), O. basilicum, H. spicigera and C. citratus* produced dose dependent reduction in parasitaemia levels in the extract treated groups (Table 3), with a similar reduction in the chloroquine treated group (positive control). The decrease in parasitaemia produced by the extracts in the established infection test was higher than the suppressive test probably due to non-selectivity of the extracts against the proliferative processes of the parasite. The presence of the parasite alone in the blood does not induce disorder, but the response of the host immune system against foreign autogenic organism via free radical generation, activation of phospholipase cascade series and generation of prostaglandins and other haemolytic principles such as free fatty acids does [16].

The pronounced anti-malarial activity of the extract observed in the established infection test may be due to inhibitory effect of the extract on generation of free radicals and haemolytic principles such as free fatty acids resulting from high parasitaemia level [14]. It may also be due to the direct plasmocidal effect of the extract as shown by the decrease in parasite count produced by the extract. This study is similar to the reports of Idowu et al. [20], Titanji et al. [21], Odeku et al. [6] and Iyiola et al. [12]. The probable components of these extracts that may be responsible for these actions are alkaloids and saponins which have bactericidal and hemolytic effects [16].

This study revealed dose dependent general reduction in the level of parasitaemia and possesses blood schizontocidal activity which is in line with the suppressive test (Table 4). When anti-malarial drugs are used in mice infected with *P. berghei*, it suppresses parasitemia to almost non-detectable levels [22]. The low activity of the extract observed in the repository test when compared to the effect against early and established infections may be due to rapid hepatic clearance of the active component. However, the mechanism of action of the extract has not been elucidated, some plants are known to exert antiplasmodial activity either by causing red blood cell oxidation [14] or by inhibiting protein synthesis [23] depending on their phytochemical constituents. Vitamin A, C, E and flavonoids have been suggested to act as primary antioxidant or free radicals scavengers that can counteract the oxidative damage induced by the malaria parasite. These plant extracts having appreciable high quantities of these phytochemicals might have explored any of these mechanisms in their antiplasmodial activities.

5. CONCLUSION

In conclusion, the results of the study suggest that the ethanol leaf extract of *Spilanthes uliginosa, Ocimum basilicum, Hyptis spicigera and Cymbopogon citratus* is relatively safe and possesses potent anti-malarial activity. This may partly explain their use in traditional medicine as an antimalarial remedy.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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