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IN VIVO EVALUATION OF ANTIMALARIAL ACTIVITY OF *Moringa stenopetala, Withania somnifera* AND *Vernonia amygdalina*

BY

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LIST OF ABBREVIATIONS

ANOVA = Analysis of variance

CQ. = Chloroquine

$CHCl_3$ = Chloroform

Dist. H_20 = Distilled water

F_{1w} = First column fraction obtained from crude methanol extract of leaves of *Withania somnifera* eluted with 2:8 chloroform and methanol respectively.

F_{2w} = Second column fraction obtained from crude methanol extract of leaves of *Withania somnifera* eluted with 5:5 chloroform and methanol respectively.

F_{3w} = Third column fraction obtained from crude methanol extract of leaves of *Withania somnifera* eluted with 8:2 chloroform and methanol respectively.

F_{1m} = First column fraction obtained from crude water extract of roots of *Moringa stenopetala* eluted with 8: 2 chloroform and methanol respectively.

F_{2m} = Second column fraction obtained from crude water extract of roots of *Moringa stenopetala* eluted with 5: 5 chloroform and methanol respectively.

F_{3m} = Third column fraction obtained from crude water extract of roots of *Moringa stenopetala* eluted with 2: 8 chloroform and methanol respectively.

F_{1v} = First fraction obtained from crude water extract of leaves of *Vernonia amygdalina* eluted with 8: 2 chloroform and methanol respectively.

F_{2v} = Second column fraction obtained from crude water extract of leaves of *Vernonia amygdalina* eluted with 5: 5 chloroform and methanol respectively.

F_{3v} = Third column fraction obtained from crude water extract of leaves of *Vernonia amygdalina* eluted with 2: 8 chloroform and methanol respectively.

MeOH = Methanol

PCV = Packed cell volume.

RBC = Red blood cell.

SEM = standard error of the mean.

SPSS = statistical package for social sciences.

% Para. = Percentage of parasitemia.

% Supp. = Percentage of suppression.

WHO = World Health Organization.

ABSTRACT

Two crude methanol and chloroform extracts obtained from leaves of *Withania somnifera* and nine column fractions obtained from the crude methanol extracts of leaves of *Withania somnifera*, water extracts of roots of *Moringa stenopetala* and leaves of *Vernonia amygdalina* were tested *in vivo* for antimalarial activity on Swiss albino male mice. Each mouse in the study was infected interaperitonially with blood samples taken from mice previously infected with chloroquine sensitive *Plasmodium berghei* that was diluted so that 0.2 ml contained 10^6-10^7 infected erythrocytes. The extracts were given to the infected mice interaperitonially starting from three hours following inoculation. Antimalarial activity was evaluated by taking blood smears on day four. This study revealed that methanol extract obtained from leaves of *Withania somnifera* suppressed parasitemia significantly both in crude form (43.2 % at 750 mg/kg and 53.34 % at 900 mg/kg) and in fractionation of the crude extract (F_{3w}, 44.3 % at 200 mg/kg and 57 % at 300 mg/kg respectively). Column fractions obtained from water extract of leaves of *Vernonia amygdalina* also showed significant suppressive effect on the parasitemia level (F_{2v}, 56.8 % at 200 mg/kg and 66.13 % at 300 mg/kg respectively). On the other hand column fractions obtained from water extracts of roots of *Moringa stenopetala* did not show significant effect on parasitemia as compared to the reported activity in its crude form.

Key words: Antimalarial activity, *in vivo,* Medicinal plants, *Moringa stenopetala, Plasmodium berghei, Vernonia amygdalina Withania somnifera.*

1. INTRODUCTION

Malaria is an infectious disease that causes enormous medical, economic, and emotional burden in the world. It is estimated that malaria affects more than 300-500 million people world wide (Olliaro *et al.,* 2001). Epidemic malaria remains a major public health concern in tropical countries. In sub-Saharan countries alone, an estimated 0.9-2.3 million annual child deaths are attributed to malaria (Villamor *et al.,* 2003). Amin *et al.* (2003) reviewed earlier estimates and recent empirical evidence that suggest one million deaths caused by malaria annually and that the burden is increasing rather than declining.

Four species, *Plasmodium malariae*, *Plasmodium ovale*, *Plasmodium falciparum* and *Plasmodium vivax* are recognized as natural malaria parasites of humans. Among these species of *Plasmodium* the most damaging type of malaria is caused by *Plasmodium falciparum*, which represents a growing threat and burden to human health and welfare (Deponte and Becker, 2004; Mayxay *et al.*, 2004).

It has been shown that changes in weather conditions play a major role as the cause of most of the sever epidemics (Abeku *et al.*, 2004). According to Thomas *et al.* (2004) changes in climate has an impact on malaria transmission. The periodicity of malaria epidemics has been linked to cyclical climate patterns (Bouma and Vanderkaay, 1996 cited in Herrel *et al.*, 2004).

In Africa malaria has had a deleterious effect on growth, development, and economic prosperity of the countries. Malaria and poverty are intimately connected and it is most intractable for developing countries Africa. Gallup and Sachs (2001) showed the impact of malaria on the economy of the endemic countries.

Out of more than 150 countries with a population more than one million in 1995, which accounts for more than 99% of the world's population, forty four have intensive malaria. Thirty five of these 44 countries are in Africa. The average purchasing power parity gross domesting product (GDP) per capita in 1995 for the malarious countries was $1,526, compared with an average income of $8,268 in the countries without severe malaria which is more than 5 times higher on ranking the 150 countries by income per capita, almost all the 44 countries with severe malaria were found to be in the bottom of the rank.

It has also been estimated that the economic burden due to malaria accounting for a 1.3% reduction in the annual economic growth rate of malaria endemic countries and that the long term impact of this is a reduction of GNP by more than half (TDR, 2002). Ethiopia is among the countries where malaria is endemic and disastrous epidemic occur frequently.

Malaria stands as the leading cause of morbidity and mortality in Ethiopia , where nearly 48 million people live in malaria risk areas. Three quarters of Ethiopia 's total landmass is regarded as malarious thus malaria in Ethiopia is one of the most costly of communicable disease and impedes socio economic development as well as it is responsible for heavy hospital costs, high incidence of death and absenteeism from work (Hodes,1996).

More than 4 million clinical cases are reported yearly from health facilities and communities, reflecting the magnitude of the problem. Climatic changes, recurrent drought, large-scale population movement; and wide spread multi-drug resistant *falciparum* malaria are some of the major factors that contribute to the worsening malaria situation.

Clinical malaria accounts for 10 - 40% of all outpatient consultations, with corresponding proportional morbidity among children under 5 years being 10% - 20%. An average of 4-6 hundred thousand confirmed malaria cases are treated every year. *P. falciparum* in Ethiopia is resistant to chloroquine and recent studies also showed that resistant to Sulphadoxine-Pyrimethamine (SP), which has been the first line antimalarial drug for falciparum malaria for the past few years, is now highly prevalent. This has triggered a shift to more effective antimalarials, particularly Artemisinin combination therapy (ACT); with the first line treatment for uncomplicated *falciparum* malaria in Ethiopia being Arthemeter Lumefantrine (AL). No ACT resistant *P. falciparum* has been detected and a recent study has shown that AL is fully effective against uncomplicated *falciparum* malaria infection. All non respondence to AL are treated with quinine. As AL is not yet recommended for infants under 5kg body weight and pregnant women, quinine is used to treat uncomplicated *falciparum malaria* in these groups.

P. vivax responds well to chloroquine and chloroquine alone is used to treat confirmed *P. vivax* cases. In the absence of diagnosis non severe case are treated with AL first and then with chloroquine if clinical symptoms are highly suggestive of malaria. Quinine injections are used to treat sever and complicated falciparum malaria cases.

It has been recommended that reducing the impact of a malaria epidemic largely depends on its early detection and timely targeting of appropriate and effective control measures. However, currently, there are no robust forecasting and early warning methods that can guide estimation on the likely burden of malaria with good lead time to ensure preparedness. As a result epidemics capture health facilities and communities almost unnoticed until the capacity of health facilities is overwhelmed (UNCT, 2004)

It has been estimated that *Plasmodium falciparum* alone is responsible for approximately three million deaths per year (Gatton *et al.*, 2003). The incidence and range of endemic malaria caused by *Plasmodium vivax* has also been expanded causing malaria approximately in 80 million people annually (Baird and Rieckmann, 2003). It has also been indicated that since malaria parasite species of humans are usually present in a particular area, infections with more than one species of plasmodium at the same time are possible (Mayxay *et al.*, 2004).

However, mixed infections have been neglected due, in part, to the debate on the existence of different *Plasmodium* species of humans in early time (Snounou and White, 2004), difficult in distinguishing young ring form parasites of *Plasmodium vivax* and *Plasmodium ovalae* liver

hypnozoites and that many infections are at densities below the threshold of detection by microscopy (Mayxay *et al.*, 2004).

Malaria is still the most important parasitic disease having serious impact on health and economy of the affected areas. The resistance of the vector mosquitoes to insecticide and the emergence of *Plasmodium* species resistance to widely used antimalarial drugs, such as Chloroquine (CQ) has made malaria control and treatment much more difficult (O'Neill *et al.*, 1985), which traditional treatment could possibly address (Benoit *et al.*, 1996).

Traditional medicine continues to be the first choice of healthcare for many African people (Rekdal 1999, cited in Bruce, 2002). WHO reported that population throughout Africa, Asia and Latin America use traditional medicine to help meet their primary healthcare needs (WHO, 2002). It has been documented that Among African medicines, plants are often used by native healers to treat disease like malaria (Benoit *et al.*, 1996).

Natural product research has been conducted for centuries worldwide helping in identification of a tremendous amount of new chemicals most of which are isolated from plants. Medical effects of many plants have long been known and more than 75% of the world population relies on medicinal plants and there are more than hundreds of clinically useful prescription drugs that are derived from plants; about 74% of them came to the attention of pharmaceuticals because of their use in traditional medicine (Nicholson, 1990).

Plants have been used as sources of traditional medicine for the treatment of malaria. They may provide drugs directly or template molecules on which to base further new structure by organic synthesis.

Quinine for instance, the parent compound of contemporary antimalarial, was isolated from the bark of *Cinchona* species (Rubiaceae) in 1820 (Van-Beek and Plett, 2000). In 1820, the French chemist's, Pierre and Joseph successfully isolated two of the active alkaloids, quinine and inchonine. The production of a number of pure alkaloids from Cinchona species soon followed including quinidine and cinchonidine (Leffingwell, 2002).

The plant *Artemisia annua* has been used in China for many years to treat fevers and found to contain artemisinin, which has efficacy against malaria (Mueller *et al.*, 2000). Park *et al.*, (1998) indicated that peroxides from artemisinin, the active principle of the herb, *Artemisia annua*, are important chemical species for antimalarial activity *in vivo*. The promise of these compounds lies in their effectiveness against malaria. Their review showed that artemisinin compounds have several advantages over other antimalarial drugs in rapid clearance of parasites in the periphery, more rapid resolution of fever and a reduction in the transmission potential of *falciparum* malaria. On the other hand, the main clinical disadvantage of artemisinin and its derivatives is the high rate of recrudescence when compared with other antimalarials.

The fact that the use of traditional medicinal plants to treat malaria and many conventional antimalarial drug derivatives originate from natural resources (Jenett-Siems *et al.*, 1991) initiates the screening of medicinal plants for antiplasmodial activity in different parts of the world.

In Africa, among the development of new antimalarial, a study of plants traditionally used against malaria has been in progress. In Ethiopia, some of the medicinal plants used traditionally for the treatment of malaria have been screened for their antimalarial activity. Methanol extracts of *Croton macrostachyus* fruits and *Dodonaea angustfolia* seeds, chloroform extracts of Crocosmia *aurea* leaves and *C. macrostachyus* fruits have been reported to have high antimalarial activity with IC_{50} values of less than 10 μg/ml (Sorrsa, 1992).

According to the report of Asrese and Balcha (1998), acetone and methanol extracts of stem bark of *Combretum molle* showed antiplasmodial activity *in vitro*. Similarly Bogale and Petros (1996) reported the antimalarial activity of *V. amygdalina* and *W. somnifera in vitro* against *P. falciparum*.

Plants traditionally used to heal malaria such as, *Vernonia colorata*, *Guiera senegalens*, in Coted'Ivoire (Benoit *et al.*, 1996), plants traditionally used for fever/malaria treatment in Ghana like, *Cliestophilis patens*, *Uvaria chamae* (Addae-Kyereme *et al.*, 2002), in West Africa extracts obtained from the plants *Azadirachta indica*, *Cinnamonum champhara*, (Ancolio *et al.*, 2002) have been reported to have antimalarial activity. In Kenya alkaloids, isolated from *Teclea trichocarpa* a species that grows in tropical and warm temperate climates and which is used for malaria treatment have been reported to have activity against *Plasmodium berghei* in mice (Murithi *et al.*, 2002).

In Japan leaf extract of *Hydrangea macrophyla*, a common Japanese flower has been reported to have antimalarial activity with the IC_{50} value of 0.18 µg/ml (Kamei *et al.*, 2000).

In central America, *in vitro* anti malarial activity of lipophilic extracts of *S. andina*, *S. tonduziana* and other plants have been shown to have antimalarial activity against *Plasmodium falciparum in vitro* with the IC_{50} values ranging from 3.0 µg/ml to 21.9µg/ml (Jenett-Siems, *et al.*, 1999). *Eurycoma longifera*, a Thai medicinal plant has been reported as a traditional antimalarial medicine and showed to have antimalarial activity both *in vivo* and *in vitro* (Satayavivad *et al.*, 1998).

The development of resistance to antimalarial drugs and the fact that a large number of modern drugs were obtained from traditional medicinal plants force researchers to look for a better antimalarial drugs. The study will validate the hypothesis that some traditionally used Ethiopian medicinal plants have valid antimalarial effects and fractions of crude extracts of plants that have demonstrated *in vitro* antimalarial activity effects may have increased antimalarial effects *in vivo*.

2. OBJECTIVES

2.1. *General objective*

- To validate the antimalarial effects of the crude extracts by testing different fractions.

2.2. *Specific objectives*

- To validate the importance of the antimalarial use of *Withania somnifera*, *Moringa stenopetala* and *Vernonia amygdalina.*

- To evaluate the efficacy of the plant extracts those have a reported *in vitro* anti malarial activity *in vivo.*

3. MATERIALS AND METHODS

3.1. *Plant material collection*

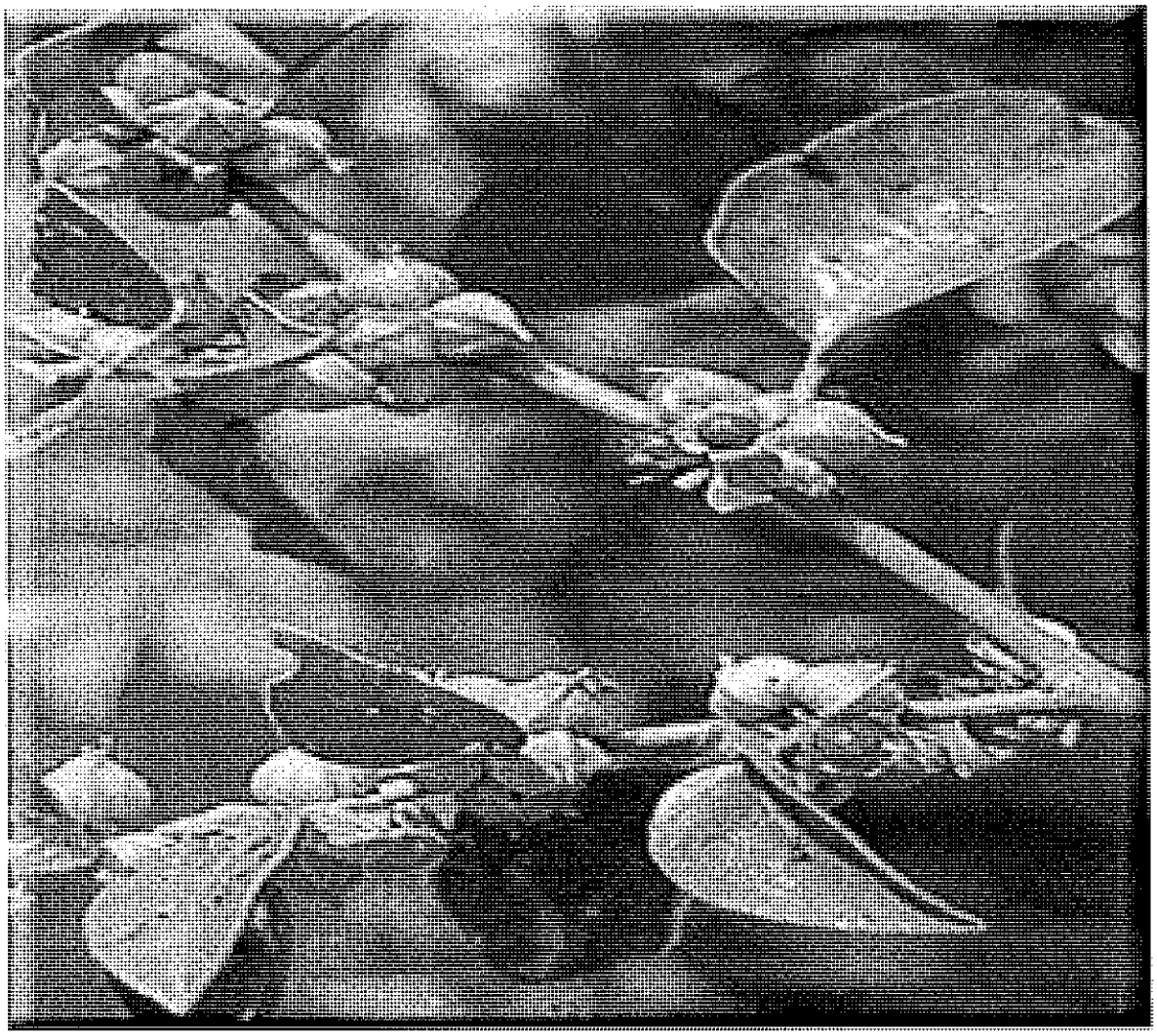
Parts of the plants used in the study were collected from Arbaminch town (Arbaminch University), 502 km south of Addis Ababa, Ziway, 160 km South of Addis Ababa and Addis Ababa in November 2004 and January 2005. Representative samples for each plants were collected and identification done at the National Herbarium, Department of Biology, Addis Ababa University. Specimens of plants were deposited in the herbarium. The plants colleted were *Moringa stenopetala, Withania somnifera and Vernonia amygdalina.*

3.2. *Description of the plants used in the study*

Withania somnifera (Amh. Gizawa), member of the Solanaceae, is an erect branched shrub with greenish or lurid yellow flowers. It grows up to 2 m and distributed between an altitude of 700m and 2700m. The medicinal values of *Withania somnifera* have been well indicated. In India it is used widely in the treatment of many clinical conditions. Its antistressor properties and its ability to alternate the concentrations of neurotransmitters have been investigated in animal studies (Archana and Namasivayama, 1999).

As shown by Bogale and Petros (1996) the plant has antimalarial activity *in vitro*. According to the report chloroform and methanol leaf extracts of the plant have shown an IC_{50} values of less than 20 μg/ml.

The plant has anticancerous properties that have been attributed to various classes of Withanoid compounds as shown by Negi *et al.*, (2000). Siddique *et al.* (2004) reported that in Bangladesh *Withania somnifera* is a widely used medicinal species useful in the treatment of inflammatory conditions, tuberculosis, rheumatism, as a tonic, or as an anti-tumor agent, the reproductive and nervous systems, having a rejuvenative effect on the body and is used to improve vitality and aid recovery after chronic illness.



Withania somnifera

Moringa stenopetala (Wol. Aleko, Amh. Shiferaw), commonly known as cabbage tree, belongs to the family *Moringaceae.* It is branched tree that grows 6-10 m tall, thick at base; bark with pale gray or silvery coloration. It grows wide in elevation between 1000 and 1800m.

It has been reported that *Moringa stenopetala* has medicinal value for stomach pain and to expel retained placenta following childbirth (Mekonnen, 1999), antitrypanosomal activity (Mekonnen *et al.*, 1999) and antileshimanial activity (Mekonnen and Gessese, 1998). The plant has been also reported to have some other medicinal uses traditionally. A tea of dried leaves is reported to be very efficient in treating light cases of diabetes and it is said that the extract of fresh leaves can cure indigestion and even the cure of an amoebic dysentery has been reported. Eye inflammations are treated and a root extract helps against unconsciousness (Engels *et al.*1991).

Moringa stenopetala

Vernonia amygdalina (Amh. Grawa), commonly known as bitter leaf, is a popular African vegetable. Is a shrub or small tree usually branched from near the base, 2-10m high, bark rough with dense black streaks, grows under a range of ecological zones in Africa and produces large mass of forage and is drought tolerant (Hutchioson and Daizein, 1963 cited in Bonis *et al.*, 1995).

Vernonia amygdalina and other species of the genus *Vernonia* are have significant ethno medical value in Africa. According to the report of Cousins and Huffman (2002) the plants are used in the treatment of nasopharyngeal illness and are considered as cures for pulmonary infections.

The review by Ogboli *et al.* (2000) showed that the plant leaf extract has broad-spectrum antimicrobial activity and it has been used to treat malaria and gastrointestinal ailments, and aqueous leaf extract has been shown to reduce blood sugar levels in rabbits. They also reported that the leaf extracts of *V. amygdalina* has been shown to have a significant anti schistosomial activity on experimental mice.

In addition, the plant has been used in treatment of cattle against helminth infections and reported to be cost- saving replacement of commercial anthelimentics in cattle (Mathias, 2004).

Vernonia amygdalinja

3.3. *Plant Extraction*

Roots of *Moringa stenopetala*, leaves of *Vernonia amygdalina* and *Withania somnifera* were ground in to pieces using grinding mill (Straub Model 4E, Philadelphia USA). The powder obtained from roots of *Moringa stenopetala* and *Vernonia amygdalina* was extracted using water and that obtained from leaves of *Withania somnifera* was extracted using methanol and chloroform.

The roots of *Moringa stenopetala* and leaves of *Vernonia amygdalina* in 1:10 (W/V) in distilled water and the leaves of *Withania somnifera* in 1:10 (W/V) in chloroform and methanol were mixed in separate Erlenmeyer flasks and placed on orbital shaker (GFL, model 3020, Germany) at room temperature for 24 hrs. The extracts were then filtered through cotton and subsequently with Whatman filter paper (15.0 cm size). The organic solvents, methanol and chloroform, were removed from the filtrate by rotary evaporator (Buchi RE 121, Switzerland) and the water extracts were freeze dried by using centrifugal freeze dryer (Vacuubrand GMBH, Germany)

The extracts obtained were fractionated by means of column chromatography by using silica gel and eluting with chloroform and subsequently with chloroform containing increasing concentration of methanol. Fractions were collected and examined by thin layer chromatography and fractions having similar substances were pooled.

3.4. *Antimalarial activity studies*

Swiss albino mice 5-7 weeks of age weighing 24-31 gm obtained from the animal house, Department of Biology, Addis Ababa University, were used in the study. They were fed with standard mice pellet and given water *ad libitum*. The test animals were put randomly in to five groups each containing five mice.

Blood samples taken from donor mice previously infected with chloroquine-sensitive *P. berghei* and with parasitaemia of about 20%. The blood samples were then diluted with saline so that 0.2 mL contained 1×10^6 - 10^7 infected erythrocytes. Each mice used in the study was infected interaperitonially on day 0.

Test extracts and Chloroquine were administered interaperitonially based on their weight. Group 1 mice in each of the test were the negative control groups and were given the vehicle (3% w/v Tween80) for water insoluble extracts and 0.5 ml distilled water for water soluble extracts following the procedure used by Satayavivad *et al.*, (1998). The test groups were administered with different doses of the plant extracts. The last groups of mice in each experiment were the positive controls that were given Chloroquine (10 mg/kg).

To evaluate the safety of the extracts, crude methanol and chloroform extract of *W. somnifera* leaves were given at a dose of 1000 mg/kg to the non-infected mice. Similarly fractions of methanol extract of leaves of *W. somnifera*, water extract of roots of *M. stenopetala* and leaves of *C. amygdalina* were given at a dose of 500mg/kg following the procedure used by Tchoumboughang, *et al.* (2004).

For the four day suppressive test, the test compounds were given once daily continuously for 4-days starting from day 0 to day 3 and % parasitemia was recorded on day 4 following procedure used by Satayavivad *et al.*, (1998). Of the two *Withania somnifera* leaf extracts, chloroform and methanol, extracts showing significant suppressive effect on parasitemia level during the four day suppressive test and water extracts of *Moringa stenopetala* root and *Vernonia amygdalina* leaf were studied by fractionation of the crude extract.

4. DATA ANALYSIS

Results obtained from the study were presented as mean plus or minus standard error of the mean (M ± SEM). Statistical significance was determined by one-way ANOVA and Posthoc's Scheffe test using SPSS Version 13.0. Students paired t-test was used to compare parameters within groups. For all the data obtained, statistical significance was set at P = 0.05. Percent parasitemia and percentage of suppression were calculated using formulae proposed by Li *et al.* (2003) and Devi. *et al.* (2001) respectively.

$$\% \text{ Para.} = \frac{\text{Number of infected RBC}}{\text{Number of infected RBC + Number of uninfected RBC}}$$

$$\% \text{ of supp} = \frac{\text{Para. in negative control - parasitemia with extracts}}{\text{Parasitemia in negative control.}}$$

5. RESULTS

Examination of thin smears of blood from mice of each experimental and control groups showed the percentage of parasitemia was lower in all the mice administered with the extracts from the three plants, *W. somnifera*, *M. stenopetala* and *V. amygdalina*, as compared to the negative control groups that were given the vehicle(Tween 80 or water) only. Although the positive control groups administered with Chloroquine cleared completely the parasite on day four, parasitemia of the mice treated with the extracts did not clear completely the parasite. In the study the highest suppressive effect shown was 66.13 % and the lowest was only 4.4 % respectively.

Statistical analysis using Scheffe's procedure indicated that group three and four mice treated with 750 mg/kg and 900 mg/kg of *Withania somnifera* leaf methanol extract showed statistically significant difference on day four parasitemia level ($P< 0.01$) as compared to the negative control group. On the other hand at a dose of 500 mg/kg, the suppressive effect of the same extract on day four-parasitemia level was not statistically significant (Table 1).

Table 1. *In vivo* suppressive test of *W. somnifera* leaf Methanol and Chloroform crude extracts gainst *P. berghe*i in mice.

Solvent	Days	Parameters	0.5ml 3% Tween80 (-ve control)	500mg/kg	750mg/kg	900mg/kg	10 mg/kg CQ. (+ve control)
MeOH	Day 0	Wt.	28.25 ± 0.59	28.75 ± 0.52	26.5 ± 1.55	27.5 ± 0.45	27.37 ± 0.62
		PCV	48.87 ± 1.54	50.86 ± 0.85	51.56 ± 0.67	51.9 ± 0.4	50.97 ± 0.7
	Day 4	Wt.	31.22 ± 0.9	28.12 ± 1.07	27.65 ± 1.1	26.9 ± 1.43	27.8 ± 1.43
		PCV	49.37 ± 0.23	49.75 ± 0.48	48.5 ± 1.04	49.45 ± 0.21	49.87 ± 0.43
		%para.	9.88 ± 0.82	8.82 ± 1.05	5.61 ± 0.43**[a]	4.61 ± 0.24***[a]	0
		%supp	0	10.63	43.2	53.34	100
		Mean sur. time (days)	7.25± 0.25	8.25± 0.38	9 ± 0.71	10.5 ± 0.65*	ND
$CHCl_3$	Day 0	Wt.	28.25 ± 0.56	25.35 ± 1.12	24.4 ± 0.57	25.82 ± 1.27	28.0 ± 0.91
		PCV	48.87 ± 1.54	50.5 ± 0.6	50.0 ± 0.41	50.5 ± 0.5	50.9 ± 0.81
	Day 4	Wt	26.55 ± 1.3	26.35 ± 1.71	30.17 ± 0.73	26.8 ± 0.7	27.9 ± 0.9
		PCV	49.75 ± 0.5	49.4 ± 0.8	49.87 ± 0.43	49.87 ± 0.43	50.12 ± 0.42
		%para.	10.0 ± 0.71	8.06 ± 0.87	7.9 ± 0.52	8.6 ± 1.2	0
		%supp.	0	18.42	17.63	14	100
		Mean sur. time (days)	7.25± 0.25	7.5 ± 0.5	7.75 ± 0.25	7.78 ± 0.25	

Key: * = P < 0.05, ** = P< 0.01, *** = P< 0.001

N. B: a = Comparison against –ve control, b = differences between day 0 and day 4.

The percentage suppression of parasitemia on day four in mice treated with 750 mg/kg and 900 mg/kg of the methanol extract of the leaves of *Withania somnifera* was found to be about 43.2 and 53.34 respectively (Fig. 1).

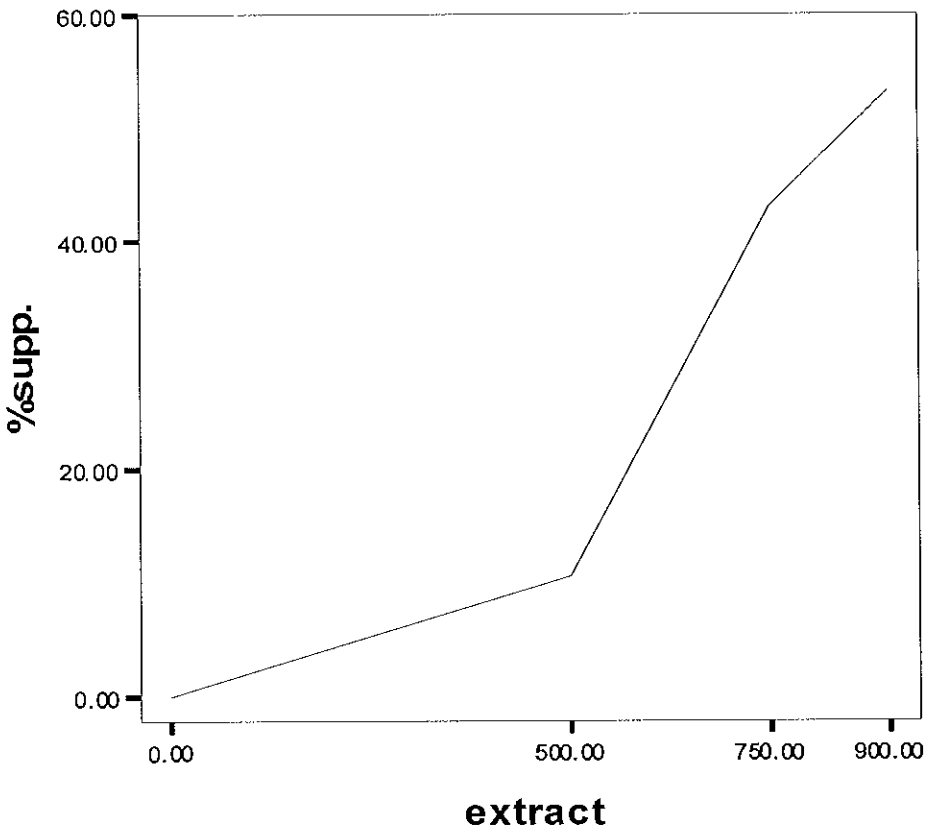


Fig.1. *In vivo* suppressive effect of crude methanol extract of leaves of *Withania somnifera* on *Plasmodium berghei* at a dose of 500, 750 & 900 mg/kg.

The mean survival time of mice treated with 750 mg/kg and 900 mg/kg of methanol extract of leaves of *W. somnifera* was 9 ± 1.84 and 10.5 ± 0.64 respectively. Where as that of the negative control group was 7.25 ± 0.25. On the other hand the mean survival time of mice administered with the chloroform extract of the leaves of *W. somnifera* at a dose of 750mg/kg and 900mg/kg were 7.0 ± 0.25 and 7.75 ± 0.25 respectively as compared to the negative control group of 6.5 ± 0.29 (Table 1).

As compared to the methanol extract, chloroform extract of leaves of *Withania somnifera* showed no significant suppressive effect on parasitemia of the mice administered with the extract. The statistically analyzed result indicated that effect induced by the chloroform extract of the leaves of the plant was not significant ($P>0.05$) at all doses administered to the experimental group parasitemia level compared to the negative control (Table 1).

In further study of methanol extracts of leaves of *W. somnifera*, fractions of the crude extracts showed different suppressive effect on the four-day parasitemia level of the treated mice. Fraction 2 (F_{2w}) and 3 (F_{3w}) Showed significant suppressive effect ($P<0.01$) and ($P<0.001$) at a dose of 200 mg/kg respectively. On the other hand the first fraction of the extract (F_{1w}) showed no significant effect on the parasitemia level of the treated mice as compared to the negative control mice (Table 2)

Table 2. *In vivo* suppressive test of fractions of methanol extracts of leaves of *Withania somnifera* against *P. berghei* in mice

Dose	Days	Parameters	0.5ml 3% Tween80 (-ve con.)	F_{1w}	F_{2w}	F_{3w}	10 mg/kg CQ. (+ve con.)
200mg/kg	Day 0	Wt.	29.12 ± 0.82	28.25 ± 0.14	29.8 ± 0.34	29.75 ± 0.32	29.3 ± 0.18
		Pcv.	51.5 ± 0.64	50.25 ± 0.32	50.12 ± 0.31	50.25 ± .433	50.62 ± 0.4
	Day 4	Wt.	29.75 ± 0.48	29.42 ± 0.22	29.35 ± 0.22	30.8 ± 0.52	30.0 ± 0.41
		Pcv	49.5 ± 0.54	48.22 0.3*[b]	48.77 ± 0.41*[b]	49.45 ± 0.63	50.87 ± 0.66
		%para.	9.75 ± .433	9.37 ± 0.24	7.13 ± 0.3**[a]	5.5 ± 0.65***[a]	0
		%supp	0	4.45	28.14	44.33	100
		Mean sur. time (days)	7.25 ± 0.25	8.0 ± 0.41	8.5 ± 0.29	9.25 ± 0.84	ND
300mg/kg	Day 0	Wt.	28.25 ± 0.9	ND	29.12 ± 0.9	28.37 ± 0.7	29.25 ± 0.6
		pcv	50.25 ± 0.32	ND	50.12 ± 0.31	49.9 ± 0.43	50.0 ± 0.4
	Day 4	Wt	27.8 ± 0.6	ND	29.3 ± 0.9	28.62 ± 0.24	29.5 ± .03
		Pcv	47.87 ± 0.43	ND	49.5 ± 0.3	49.5 ± 0.9	50.0 ± 0.4
		%para.	9.87 ± 0.61	ND	6.8 ± 1.5**[a]	4.25 ± 0. 3***[a]	0
		%supp.	0	ND	29	57	100
		Mean sur. time (days)	6.5 ± 1	ND	8.75 ± 0.3	10.5 ± 0.3*	ND

Key: * = P < 0.05, ** = P< 0.01, *** = P< 0.001

N. B: a = Comparison against –ve control 1, b = differences between day 0 and day 4.

ND = not determined

The percent suppression effects of mice treated with second (F2w) and third (F3w) fractions of the crude extract at a dose of 200 mg/kg were found to be about 28.14% and 44.33% respectively. However, the suppressive effect induced by the first fraction was only 4.45% (Fig. 2).

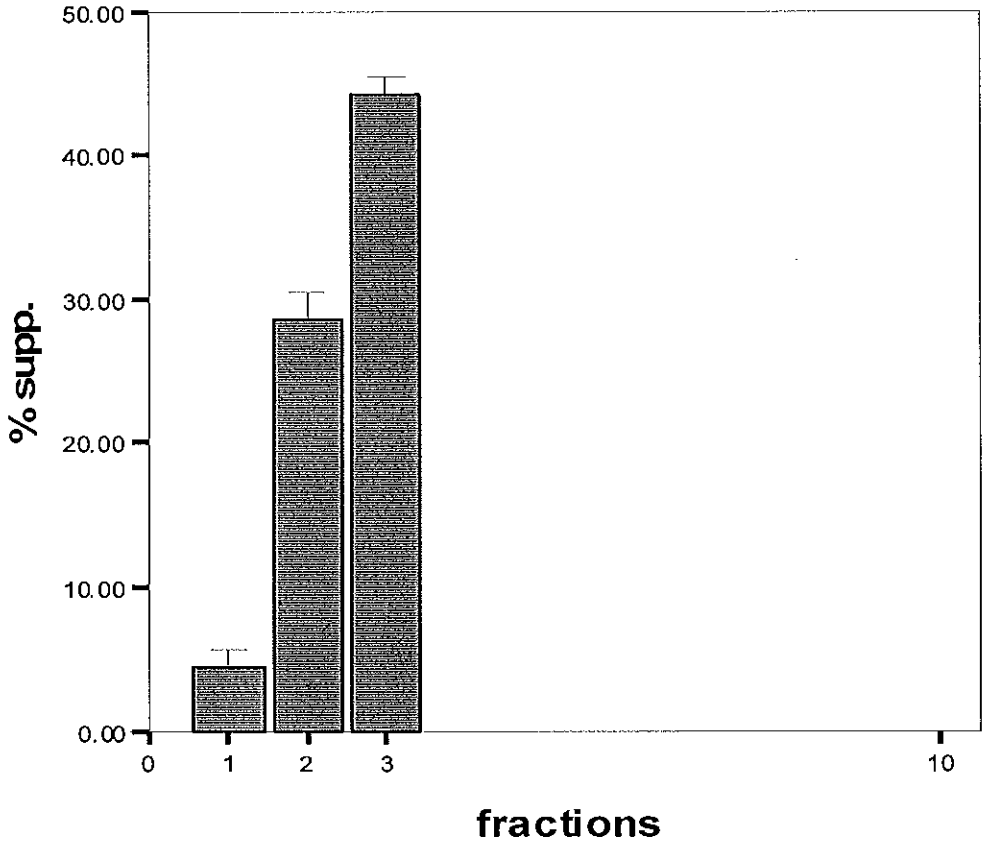


Fig. 2. *In vivo* suppressive effect of fractions F_{1w} (1), F_{2w} (2) and F_{3w} (3), of methanol extract of of *Withania somnifera* leaves against *P. berghei* in mice at a dose of 200 mg/kg.

On the other hand further study on the fractions (F_{2w} and F_{3w}) at a dose of 300 mg/kg showed improved suppressive effect (Fig. 4) on the parasitemia level of the mice administered with the fractions (Fig. 3). Both fractions (F2w and F_{3w}) sowed significant ($P<0.01$) and ($P< 0.001$) suppressive effect respectively (Table 2).

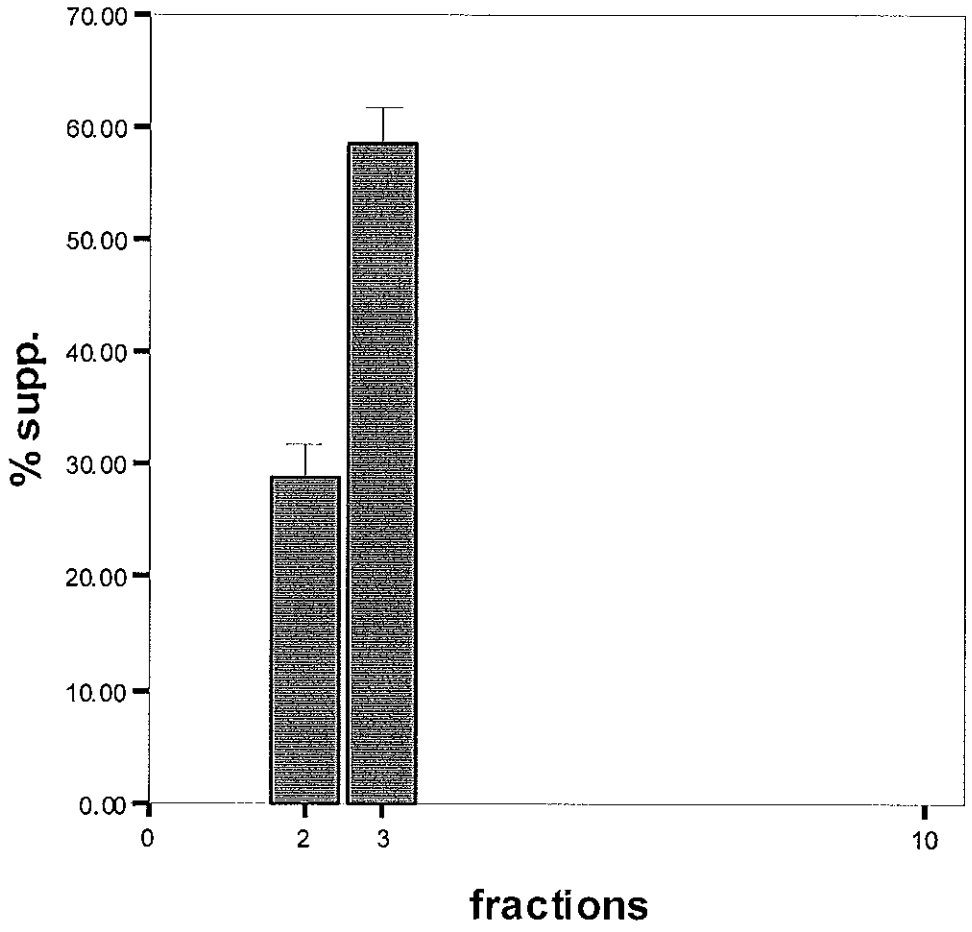


Fig. 3. *In vivo* suppressive effect of fractions F2w (2) and F_{3w} (3), of methanol extract of *Withania somnifera* leaves against *P. berghei* in mice at a dose of 300 mg/kg.

The mean survival time of mice treated with fractions F_{2w} and F_{3w} at a dose of 200 mg/kg was 8.5 ± 0.29 and 9.25 ± 0.85 respectively. Where as that of the negative control mice was 7.25 ± 0.25 (Fig. 4).

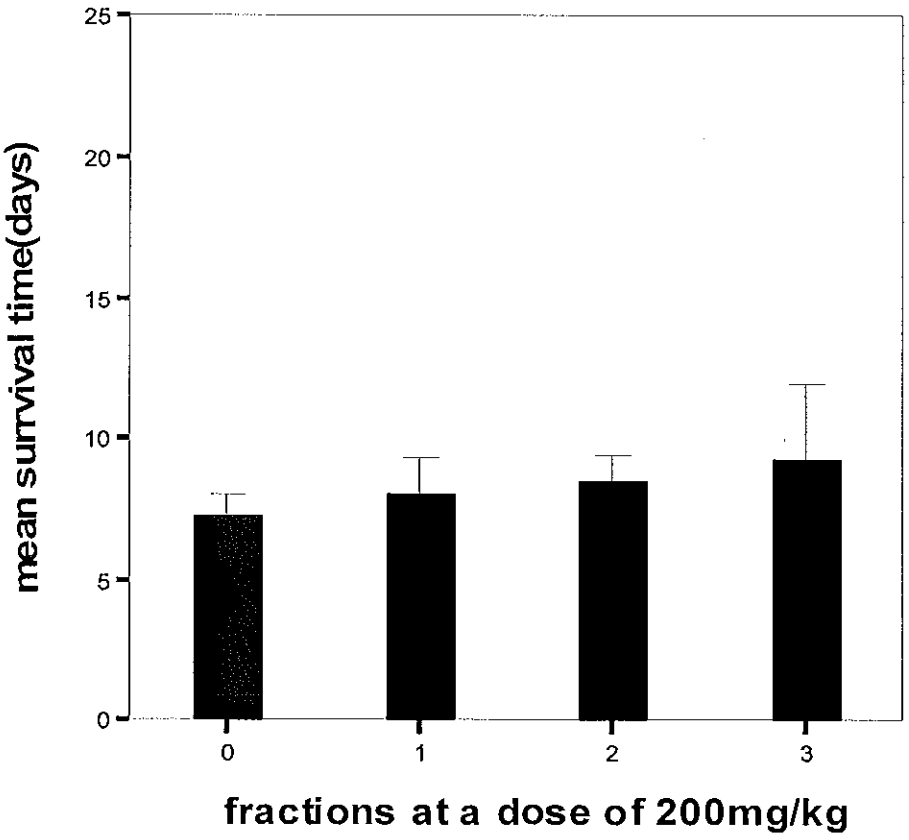


Fig. 4. The effect of fractions F_{1w} (1), F_{2w} (2) and F_{3w} (3), 0f methanol extract of *Withania somnifera* leaves on survival time in mice infected with *P. berghe*i.

On the other hand at a dose of 300 mg/kg the mean survival time of the mice treated with fractions (F_{2w} and F_{3w}) was found to be a bout 8.5 ± 3 and 10.5 ± 3 and that of the negative control was 6.5 ± 1 respectively. (Fig. 5)

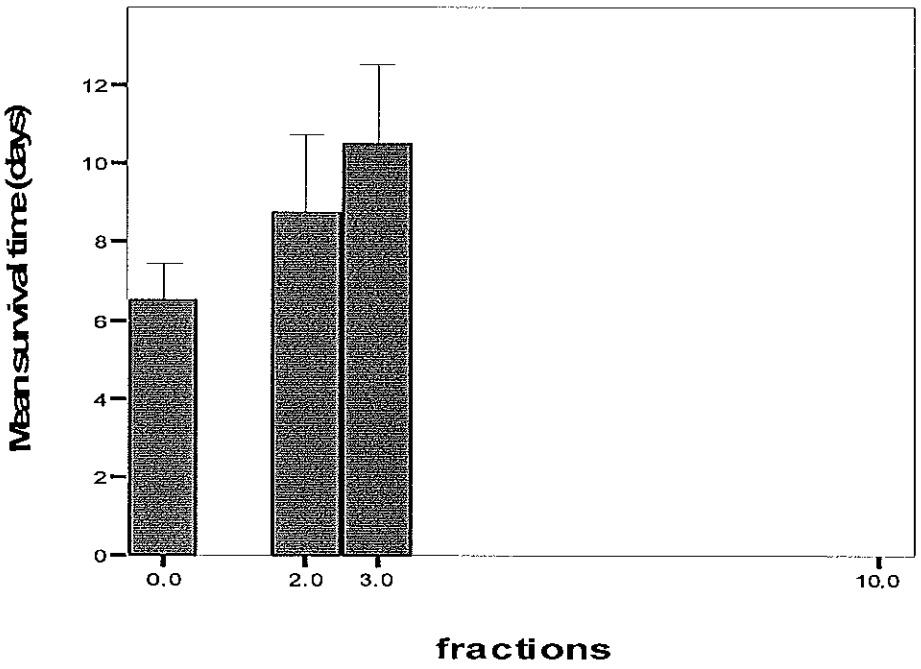


Fig. 5. The effect of fractions F_{2w} (2) and F_{3w} (3) from methanol extract of leaves of *Withania somnifera* on survival time in mice infected with *P. berghei* at a dose of 300 mg/kg.

In the in vivo suppressive test result of fractions from water extract of roots of *Moringa stenopetala*, no significant difference in the mean parasitemia level were observed among the groups that were given the three fractions of the extract at a dose of 200 mg/kg and in that of the untreated control group.

Table 3. *In vivo* suppressive test of fractions from water extract of roots of *Moringa stenopetala* against *Plasmodium berghei*.

Dose	Days	Parameters	0.5ml Distilled water(-ve. Control)	F_{1m}	F_{2m}	F_{3m}	10mg/kg CQ.(+ve control)
200mg/kg	Day 0	Wt.	27.9 ± 2.04	29.1 ± 1.79	29.7 ± 2.18	28.28 ± 1.92	30.13 ± 0.43
		Pcv	50.22 ± 0.88	50.3 ± 0.33	49.65 ± 0.79	50.5 ± 0.87	50.0 ± 0.4
	Day 4	Wt.	27.25 ± 0.52	28.0 ± 0.41	27.62 ± 0.24	28.5 ± 0.29	29.0 ± 0.41
		Pcv	47.75 ± 0.52	47.63 ± 0.24*[b]	48.5 ± 0.29	50.6 ± 0.22	49.8 ± 0.3
		%para.	9.63 ± 0.63	9.13 ± 0.3	8.37 ± 0.24	8.12 ± 0.31	0
		%supp	0	5.5	12.8	15.9	100
		Mean sur. Time(day)	6.75 ± 0.48	7.25 ± 0.25	7 ± 0.41	7.25 ± 0.25	ND
400mg/kg	Day 0	Wt.	28.5 ± 0.4	27.12 ± 0.9	27.62 ± 0.24	27.3 ± 0.3	27.0 ± 0.5
		pcv	50.13 ± 0.9	50.0 ± 0.5	50.4 ± 0.38	49.5 ± 0.6	49.8 ± 0.5
	Day 4	Wt	27.13 ± 0.3	27.13 ± 0.5	26.6 ± 0.6	26.9 ± 0.3	26.8 ± 0.3
		Pcv	48.3 ± 0.6	49.9 ± 0.43	48.87 ± 0.43*[b]	48.9 ± 0.4	49.2 ± 0.4
		%para.	10.0 ± 0.7	9.0 ± 0.4	8.5 ± 0.3	7.7 ± 1*[a]	0
		%supp	0	10	15	22.5	100
		Mean sur. Time(day)	6.25 ± 1	7.0 ± 2	7.25 ± 0.2	8.25 ± 0.2	ND

Key: * = P < 0.05, ** = P< 0.01, *** = P< 0.001

N. B: a = Comparison against –ve control, b = differences between day 0 and day 4.

ND = not determined

However, the mean parasitemia level of group four mice that were treated with fraction number three (F_{3m}) of *Moringa stenopetala* water extract at a dose of 400 mg/kg is significantly ($P<0.05$) different from the untreated control group (Table 3).

The effect of water extracts of leaves of *Vernonia amygdalina* was studied by fractionation of the crude extracts. The suppressive effect induced by the second and third fractions (F2v and F_{3v}) of the crude extract was statistically significant ($P<0.001$) at a dose of 200 mg/kg and 300 mg/kg as compared to the negative control mice. On the other hand suppressive effect of the first fraction (F_{1v}) was not significant ($P>0.05$) in comparison with the negative control (Table 4)

Table 4. *In vivo* suppressive test of fractions from water extract of Leaves of *Vernonia amygdalina* against *Plasmodium berghei*.

Dose	Days	Parameters	0.5ml 3% Tween80(-ve control)	F_{1v}	F_{2v}	F_{3v}	10mg/kg CQ. (+ ve control)
200mg/kg	Day 0	Wt.	26.5 ± 1.42	27.5 ± 0.5	28 ± 0.5	26.5 ± 0.9	27 ± 0.33
		Pcv.	49.1 ± 0.3	49.4 ± 0.7	50 ± 0.7	49.3 ± 0.6	49.1 ± 0.8
	Day 4	Wt.	26 ± 1.06	26.42 ± 0.5	26.9 ± 0.4	26 ± 0.7	26 ± 0.3
		Pcv	48 ± 0.5	48 ± 0.8*[b]	48 ± 0.5*[b]	48 ± 0.6*[b]	48 ± 0.5
		%para.	1 2.8 ± .1.4	11.7 ± 0.6	5.52 ± 0.3***[a]	11.3 ± 0.6	0
		%supp	0	8.2	56.8	11.7	100
		Mean sur. time (days)	6.77 ± 0.25	6.75 ± 0.63	11.75 ± 0.48*	8.0 ± 0.8	ND
300mg/kg	Day 0	Wt.	24.5 ± 0.6	ND	25.25 ± 0.9	25.25 ± 1.2	25.5 ± 0.9
		pcv	50.5 ± 0.6	ND	49 ± 0.7	48.9 ± 0.9	49.4 ± 0.6
	Day 4	Wt	22.25 ± 0.5	ND	24.1 ± 0.9	24.4 ± 0.9	24.5 ± 0.6
		Pcv	48.37 ± 0.3	ND	46 ± 0.13*[b]	47.5 ± 0.6*[b]	47.7 ± 0.3
		%para.	12.4 ± 0.4	ND	4.2 ± 0.1***[a]	10.1 ± 0.6***[a]	0
		%supp.	0	ND	66.13	17.4	100
		Mean sur. time (days)	6.5 ± 0.3	ND	11 ± 0.4*	9 ± 0.4*	ND

Key: * = P < 0.05, ** = P< 0.01, *** = P< 0.001

N. B: a = Comparison against –ve control, b = differences between day 0 and day 4.,

ND = not determined

The percent suppression effect of mice treated with second (F_{2v}) and third (F_{3v}) fractions of the crude extract at a dose of 200 mg/kg was found to be about 56.8 % and 11.7 % respectively. How ever the suppressive effect induced by the first fraction was 8.2 % (Fig. 6)

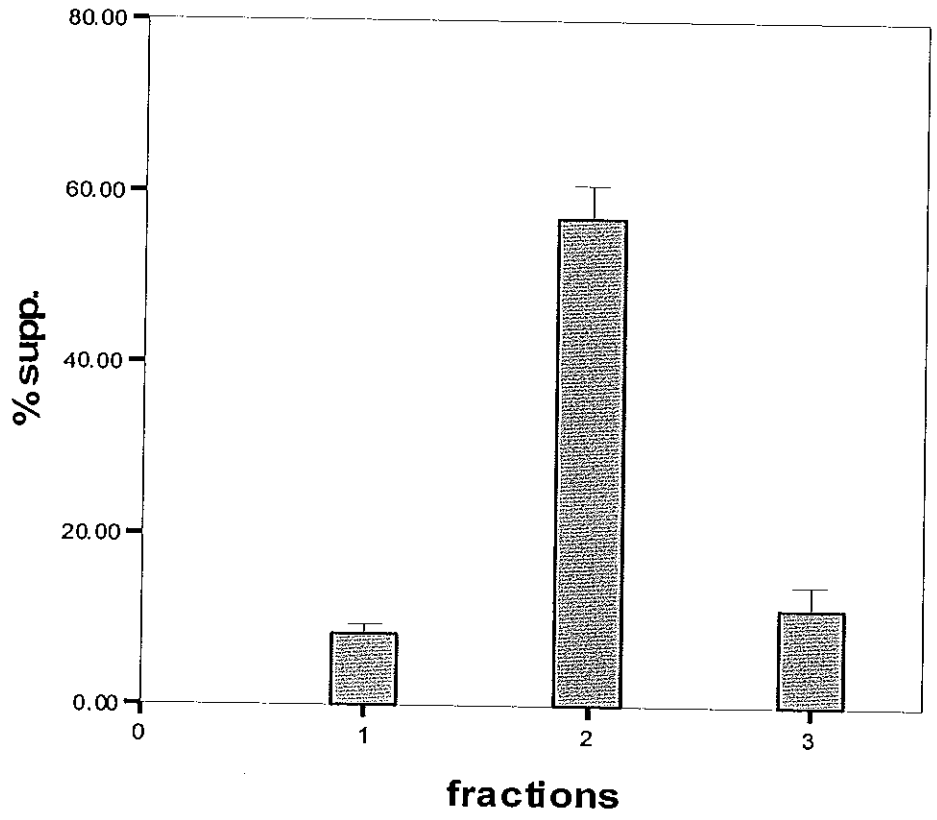


Fig. 6. *In vivo* suppressive effect of fractions F_{1v} (1), F_{2v} (2) and F_{3v} (3), of water extract of *Vernonia amygdalina* leaves against *P. berghei* in mice at a dose of 200 mg/kg.

The percent suppression effect of mice treated with second (F_{2v}) and third (F_{3v}) fractions of the crude extract, at a dose of 300 mg/kg were found to be improved and found to be about 66.13 % and 17.4 % respectively. (Fig.7).

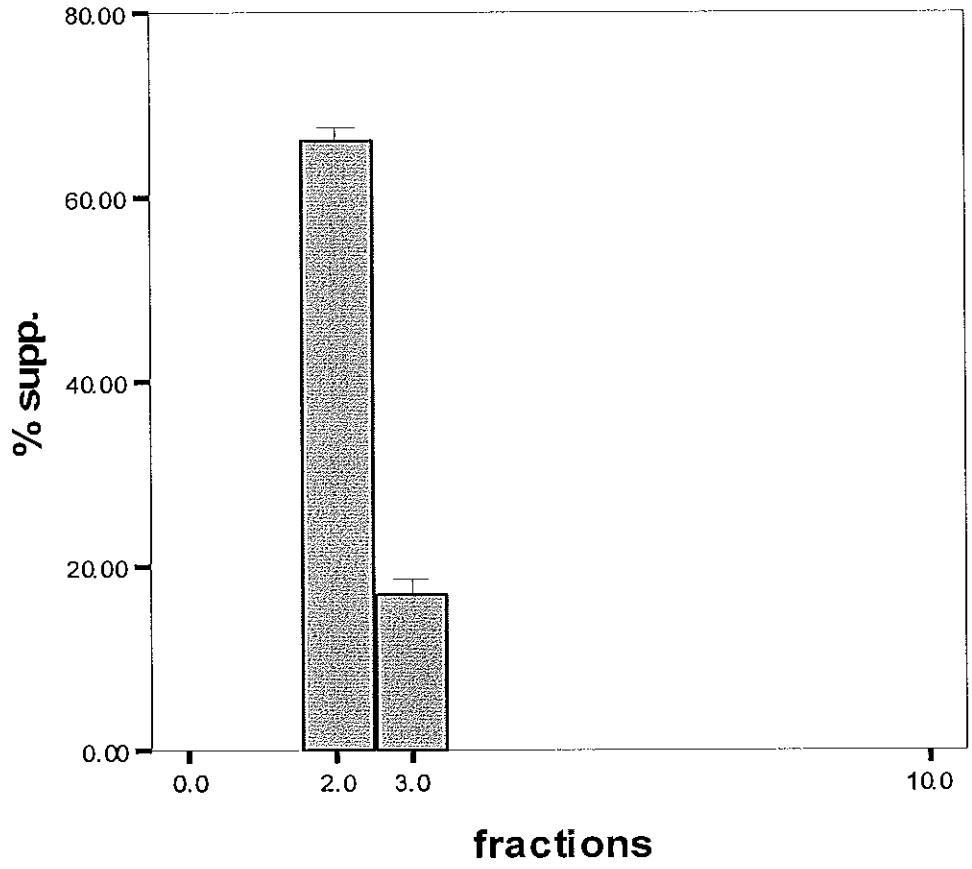


Fig. 7 *In vivo* suppressive effect of fractions F_{2v} (2) and F_{3v} (3) of water extract of *Vernonia amygdalina* leaves against *P. berghe*i in mice at a dose of 300 mg/kg.

The mean survival time of mice treated with fractions two (F_{2v}) and three (F_{3v}) at a dose of 200 mg/kg was 11.7 ± 0.5 and 8 ± 0.8 respectively. Where as that of the negative control mice was 6.77 ± 0.25 (Fig. 8).

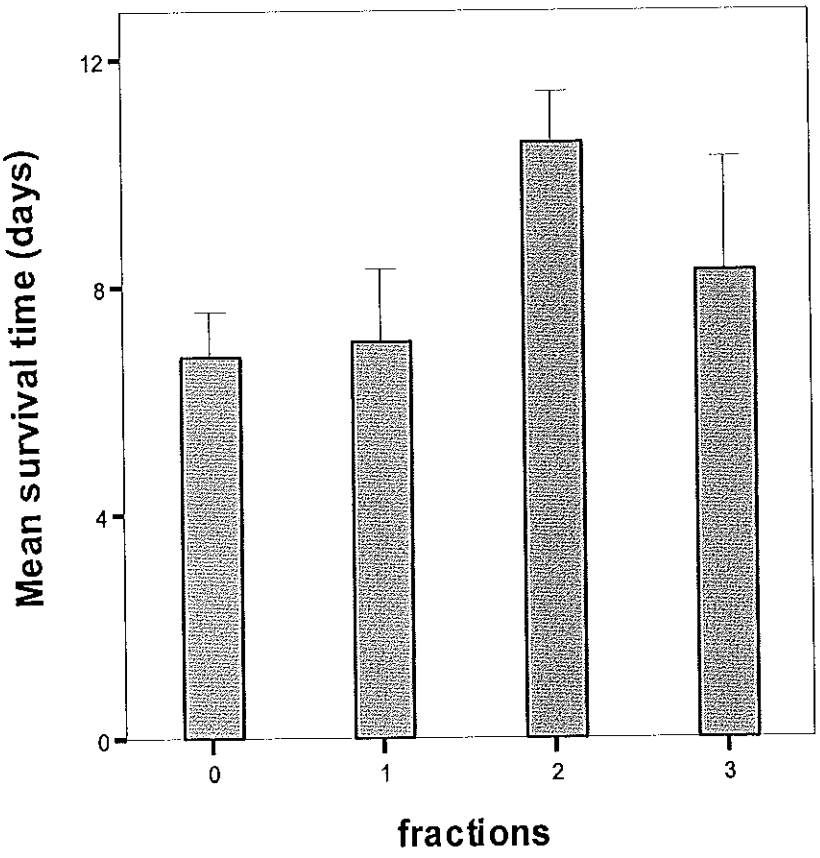


Fig. 8. The effect of fractions F_{1v} (1), F_{2v} (2) and F3v (3), of water extract of *Vernonia amygdalina* leaves on survival time in mice infected with *P. berghei* at a dose of 200 mg/kg.

On the other hand at a dose of 300 mg/kg the mean survival time of the mice treated with the same fractions (F_{2v} and F_{3v}) was found to be a bout 11 ± 0.4 and 9 ± 0.4 and that of the negative control was 6.5 ± 0.3 respectively. (Fig. 9)

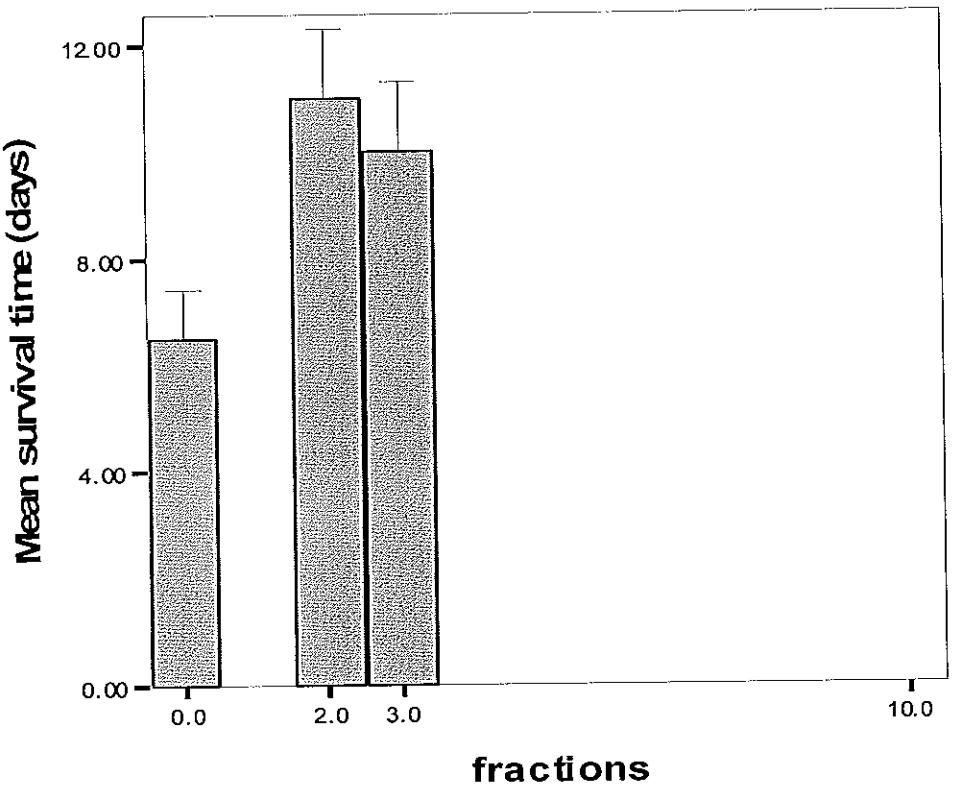


Fig. 9 The effect of fractions F_{2v} (2) and F_{3v} (3), of water extract of *Vernonia amygdalina* leaves on survival time in mice infected with *P. berghei* at a dose of 300 mg/kg.

6. DISCUSSION

Traditional medicine has a long history of serving people all over the world. The long tradition of herbal medicine continuous to the present day in China, India and many countries in Africa and South America. In many village market places, medicinal herbs are sold along side with vegetables and other wares (Kong *et al.*, 2003).

It has been indicated that the origins of most modern drugs are traditional medicinal plants. For instance, quinine the major drug for the treatment of malaria, has been produced from the bark of *Cinchona* species; Artemisinin was isolated as an active principle from the traditional herbal remedy, *Artemisia annua* (Liue *et al.*, 1979 cited in O'Neil, *et al.*, 1985). The success of the antimalarial drug quinine and the discovery of Artemisinin were good reasons for continuing the study of plants as antimalarial agents.

Although indigenous plants play an important role in the treatment of many diseases and 80% of the world population is estimated to use herbal remedies, only limited information is available on their efficiency and safety. The likelihood that a herbal medicine produces an adverse effect depends on the herbal medicine and its dosage and on some other consumer-related parameters, like age. It has been reported that some adverse reactions are pharmacologically predictable and dose dependent, which implies that they can be anticipated and that they could be prevented by dose reduction (Peter, 1996).

The extracts of the plants used in the present study were tested for acute toxicity and no visible toxic effects were detected up to 1000 mg/kg dose of the extract for the leaves of *W. somnifera* chloroform and methanol crude extracts, 500 mg/kg for fractions of methanol extracts of leaves of *Withania somnifera* and fractions of water extracts from roots and leaves of *M. stenopetala* and *V. amygdalina.* On the other hand acute toxicity study on water extract of roots of *Moringa stenopetala* (Asnake, 2004 ; Nibret, 2004), ethanol extracts of leaves of *W. somnifera* (Asnake, 2004), were shown to be non toxic to the mice up to 900 mg/kg dose. Water extracts of leaves of *V.amygdalina* has also been reported to have no acute toxicity on the mice administered with the extract (Animut, 2002)

The non-toxic property of *Moringa stenopetala* extracts has been reported from previous studies. Animut (2002) and Asnake (2004) have shown that water extracts of the plant root and the leaves exhibited no toxic effects in mice. Furthermore, the edibility of the plant parts shows their, safety (Price, 2000) whereby the leaves are one of the best vegetables that can be found in the locality. All parts of the tree, except the wood, are edible, providing a highly nutritious food for both humans and animals. It has also been recommended that pregnant and breast-feeding women, can benefit from consuming *Moringa* leaves and pods. One 100 g portion of *M. stenopetala* leaves could provide a woman with over a third of her daily need of calcium and provides her with adequate quantities of iron, protein, copper, sulfur and B-vitamins (Price, 2000).

According to Aphale *et al.* (1998) extracts from *Withania somnifera* are generally safe when taken in the prescribed dosage and large doses have been shown to cause gastrointestinal upset, diarrhea, and vomiting. Mishra *et al.* (2000) reported that studies have shown various constituents of *Withania somnifera* to exhibit a variety of therapeutic effects with little or no associated toxicity.

According to the report of Ibrahim *et al.* (2001) elemental analysis of the leaves of *Vernonia amygdalina* revealed adequate concentrations of some elements, such as iron and calcium that are essential for normal growth. The plant was evaluated in relation to tissue damage, in sixteen male rats that were fed orally for 65 days with amended diets containing, 50% (w/w), 25% (w/w) 5%(w/w) powdered *V. amygdalina* leaves. Histological examinations at the end of the showed all the various tissues to be of normal architecture with good cellular morphology study. On this basis *V. amygdalina* could be regarded as relatively safe for use as medicine.

The chloroform extracts of leaves of *Withania somnifera* did not induce significant *in vivo* suppression on *Plasmodium berghei* in mice at doses tested. However, this result is in contradiction with the *in vitro* results reported by Bogale and Petros (1996) on *Plasmodium falciparum*, which showed significant antimalarial activity at IC_{50} = 2.04 µg/kg.

This shows that antiplasmodial activity of plants *in vitro* may not necessary imply the same *in vivo* effect since compounds may either act as prodrugs, which must undergo metabolic activity, as febrifuges or immunemodulaters (Muregi *et al.* (2003). Thus, plant extracts may not posses the same antiparasite activity both *in vivo* and *in vitro*..

On the other hand, the results obtained from *in vivo* antimalarial activity of methanol extract of the leaves of *Withania somnifera* were in agreement with the reported antimalarial activities of the same extracts *in vitro* by Bogale and Petros (1996). Similarly previous work on the plant provided information on its antimalarial activity (Asnake, 2004) showing that repeated treatment with water extract of the leave of *Withania somnifera* would reduce the parasite to a significant level at a dose of 750 mg/kg and 900 mg/kg on day nine of treatment. In addition Animut (2002) had also reported the *in vivo* antimalarial activity of the same plant in mice infected with *P. berghei.*

In the present study of the antimalarial activity of methanol extract of the leaves of *W. somnifera* up on fractionation showed a better antiplasmodial activity *in vivo.* The observation is a confirmation of the *in vitro* effects reported by Bogale and Petros (1996) in which the antimalarial activity of the same extract improved up on fractionations *in vitro.* The level of suppression induced by the third fraction (F_{3w}) at a dose of 300 mg/kg (57%) is within the limits of what may be considered a good antimalarial activity for a medicinal plant extract, which is a suppression of $\geq$ 50 % at a dose of 250 mg/kg (Bertain *et al.*, 2005).

The suppressive effect of the same fraction (F_{3w}) is also comparable to the reported antimalarial activity of *Cassia occidentalis* root bark and *Phyllanthus niruri* that produced suppression of parasitemia (60%) *in vivo* (Fennel *et al.*, 2004), essential oil of *C. citrates* produced 62.1 % of suppression at 200 mg/kg *in vivo* (Techoumbougnang *et al.*, 2004). The improved antimalarial activity of the fractions suggests that the observed suppression of parasitemia was not due to synergetic effect of the compounds present in the crude extracts.

The suppression induced by the extracts might be associated with the presence of chemical ingredients that have antimalarial properties. The presence of alkaloids in the extracts of the plant has been reported (Owais, *et al.*, 2003), which are known to have antimalarial activity Andrade-Neto, *et al.*, 2003), for instance alkaloidal extracts of *Golipa longifora* has been indicated to have *in vivo* antiplasmodial activity at a dose of 50 mg/kg (Saxena *et al.*, 2003).

The therapeutic value of *W. somnifera* has been extensively reported. Recently the plant was demonstrated to possess strong antifungal activity and was found effective against treatment of murine aspergillosis and has also been reported that both its aqueous and methanolic extracts of leaves and roots possess strong antibacterial properties against various pathogens including *Salmonella typhimurium* (Owais *et al.*, 2005).

In vivo antimalarial evaluations of fractions of water extracts of *Moringa stenopetala* root extracts exhibited no significant (P>0.05) antimalarial activity against *Plasmodium berghei* in mice. However, the results obtained from the study will not disprove the traditional use of the plant to treat malaria. Since the same extract of the plant in its crude form showed antimalarial activity in mice infected with *Plasmodium berghei* (Asnake, 2004). The fact that the fractions were much less active could be due to possible synergism between different compounds in the crude extracts that are lost during fractionation. Similar findings have been reported in different experimental results. Madureira *et al.* (2002) had shown crude extracts from two medicinal plants, *P. angolensis and M. lucida* to have marked antiplasmodial activities but the fractions were much less active, this could be due to possible synergism between the complex and heterogeneous mixture of different compounds in the crude extracts and the instability of the fractionated and/or purified molecules.

Hilda *et al.*, (1994) reported the improved antiplasmodial activity of ajoene, a product initially isolated from extracts of garlic, *Alium sativum, in vivo*. The results showed that when used alone, ajoene displays a moderate anti *Plasmodium berghei* activity. However, when used in combination with noneffective dose of Chloroquine, ajoene synergistically enhances the susceptibility of the parasite to this drug.

According to Mariga *et al.* (2004) had studied *in vitro* activity of the prodrug amodiaquine and its metabolite monodesethyl-amodiaquine for three strains of *Plasmodium falciparum*: LS-2, LS-3, and LS-1 and both compounds showed significant activity against all three strains.

However, interaction studies with both compounds yielded evidence of significant synergism, which improved the activity of the fractions.

Study on the interaction of extracts from plants like *Vernonia lasiopus* had also showed to have effect on the efficacy of chloroquine by reversing its resistance (Muregi *et al.*, 2003)

It has also been indicated that the low antiplasmodial activity in some traditionally used plants could partly be explained by the circumstances that many plants are used in the treatment of malaria not for anti- parasitic effect (that is curing the disease) but because of other activities (like reducing fever, easing convulsions and headache, and as immunostimulatory agents) with therapeutic value for a patient with the disease. Another possible reason is that traditional healers give a mixture of money plants homogenates for the treatment of a diseases (Wanyoike, 2004).

The obtained significant suppression of parasitemia by fractions of water extract of leaves of *Vernonia amygdalina* on day four is in agreement with the repot of Muregi *et al.* (2003) showing the *in vitro* antimalarial activity of chloroform, ethyl acetate and methanol extracts from *Vernonia lasiopus*. The finding is also consistent with the work of Animut (2002), which indicated the antiplasmodial activity of water extracts of leaves of *V. amygdalina* in mice infected with *P. berghei* in its crude state. The finding is also a confirmation of the reported *in vitro* antimalarial activity of the plant (Bogale and Petros, 1996) against *Plasmodium falciparum.*

A number of other studies have also showed the antimalarial activity of the genus *Vernonia*. In study done by Oketch-Rabah (1996), *in vitro* antiplasmodial activity of *Vernonia* species such as, *Vernonia brachycalyx* chloroform: ethyl acetate (1:1) crude extract of leaves have shown to be effective. Further more, *Vernonia brasiliana* aqueous extract (Sexana *et al.*, 2003) and n-hexane extract (Tona *et al.*, 2004) have been shown to have antiplasmodial activity *in vivo* and *in vitro*.

The reported compounds of its leaves further evidence the exhibited antimalarial activity of *V. amygdalina*. According to Masaba (2000) the bioactive compounds of *V. amygdalina* sesquiterpene lactones have been isolated from its leaves and found to have bioactivity against a number of protozoan, helminthes, bacteria and antitumer activities.

It has also been indicated that a number of bioactive compounds such as flavonids, saponins and alkaloids, have been isolated from leaves of *V. amygdalina* (Masaba, 2000). Yenesaw *et al* (2004) reported that flavonoids from ethyl acetate extract of the stem bark of *Erythrina abyssinica* showed *in vitro* antiplasmodial activity against the chloroquine sensitive (D_6) and chloroquine resistant (W2) strains of *P. falciparum* with IC_{50} values of 7.9 and 5.3 µg/ml respectively. According to the report of O'Neill (1985) flavonoids have been isolated from *Artemisia annua*, Chinese medicinal plant from which the antimalarial drug Artemisinin was isolated. On the other hand the observed antimalarial activity of *V. amygdalina* could also be associated with the existence of alkaloids in its leaves. The antimalarial activity of alkaloids has been demonstrated by the work of others (Saxena *et al.*, 2003). Quinine for instance, is an

instance, is an alkaloid first identified and isolated from the bark of the Peruvian plants *Cinchona calisaya* and *Cinchona succirubra* (Andrade-Neto, 2003).

7. CONCLUSIONS

The resistance of malaria parasites to available drugs continues to be a serious concern as it increasingly limits our ability to control this serious disease. Despite this fact chemotherapy remains one of the key control measures against the intolerable burden of malaria. The search of Plant-derived compounds offers important approach to chemotherapy. Importantly, this approach can benefit from knowledge of medicinal plants among natives of malarious regions, where the appreciation of the use of plant products to treat febrile illnesses has grown over many generations

Antimalarial drug development is constrained by the same factors as any drug development program in that new agents must demonstrate efficacy, be safe and have additional properties important for the specific disease indication. It is also challenged by little investment by pharmaceutical companies and lack of specialized expertise

This study has demonstrated the presence of antimalarial activities in fractions of water extract of leaves of *Vernonia amygdalina* and methanol extract of leaves of *Withania somnifera*. However, the antiplasmodial activity of fractions from water extract of roots of *Moringa stenopetala* was lower as compared to the other plants.

On the basis of the results obtained from the study the following conclusions may be made.

i. In screening plant-derived compounds for antimalarial activities, discrepancy between *in vivo* and *in vitro* results must be pointed out.

ii. The antiplasmodial activities of *Withania somnifera* and *Vernonia amygdalina* may partly explain and lends support to the traditional use of the plants for malaria treatment.

iii. After detailed antimalarial evaluations, phytochemical analysis and thorough toxicological studies, these plants may find use as antimalarials in known dosages where the conventional drugs are unaffordable or unavailable.

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