
ORIGINAL ARTICLE**IN VIVO ANTIMALARIAL ACTIVITIES OF PLANTS USED IN ETHIOPIAN TRADITIONAL MEDICINE, DELOMENNA, SOUTHEAST ETHIOPIA**

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ABSTRACT

BACKGROUND: Malaria constitutes one of the major health problems in Ethiopia. One of the reasons attributed for the upsurge was the development of resistance of *Plasmodium falciparum* and the emergence of multi-resistant strains of the parasite to antimalarial drugs. A continued search for other effective, safe and cheap plant-based antimalarial agents thus becomes imperative in the face of these difficulties. The objective of the present study was therefore to evaluate *in vivo* antimalarial activities and acute toxicity profiles of the aqueous and methanolic extracts of nine medicinal plants.

METHODS: Nine plants which are commonly used for the treatment of malaria in the community were identified. The nine medicinal plants species *Cissampelos mucronata*, *Clerodendrum myricoides*, *Gnidia stenophylla*, *Vernonia bipontini*, *Euclea scimperi*, *Solanum incanum*, *Plumbago zylanica*, *Warburgia ugandensis* and *Kalanchoe petittiana* were evaluated for their antimalarial activity *in vivo*, in 4-day suppressive assays against *Plasmodium berghei* Anka strain in mice.

RESULTS: No toxic effect or mortality was observed in mice treated orally with any of the extracts as a single dose of 1000mg/kg/day. At oral doses of 400mg/kg/day, the lyophilized aqueous root extract of *Gnidia stenophylla*, leaf extract of *Vernonia bipontini*, root extract of *Euclea scimperi*, *Cissampelos mucronata*, and *Clerodendrum myricoides* and methanolic leaf extract of *Vernonia bipontini* presented relatively high activities, among which three extracts reduced parasitemia by >50% when tested at an oral dose of 400mg/kg/day indicating that the plants are promising for further investigation.

CONCLUSION: The results justify the use of these plants as traditional medicines for the treatment of malaria. Except the leaf extract of *Cissampelos mucronata*, the methanol extract of *Clerodendrum myricoides* and aqueous extract of *Kalanchoe petittiana* have inhibition of parasitemia above 10%. Further detailed pharmacological and toxicological studies are recommended for drug development.

KEY WORDS: Malaria, medicinal plants, *Plasmodium berghei*, traditional medicine

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INTRODUCTION

Despite all the efforts to eradicate, malaria continues to be one of the greatest health problems facing the tropical and subtropical regions. WHO estimates 300-500million clinical cases and more than 2 million deaths each year from malaria, putting malaria one of the three most deadly communicable diseases in the world (1). The majority of those who die from malaria are infants and children living in sub-Saharan Africa due to poor access to effective malaria treatment (2). Antimalaria drug resistance is extending to new geographical areas and affecting species other than *Plasmodium falciparum* (3). The increasing prevalence of drug-resistant strains of *Plasmodium falciparum*, its most widespread etiologial agent, to standard antimalarial drugs necessitates a continual effort to search for new antimalarial drugs with new modes of action, used alone or in combination (3). The search for new drugs can follow phytochemical investigation of medicinal plants. As a matter of fact, plants always have been a rich source of antiplasmodial compounds and many antimalarial drugs still in use were obtained from plant extracts or from structures designed by pharmacomodulation of the lead compounds.

In sub-Saharan Africa, more than 80% of the population relies on traditional medicines and healers as the primary source of health care (4). This figure is much higher for Ethiopia (5). This is mainly because the accessibility and affordability of consulting healers, and their cultural sensitivity (6).

Medicinal plants have in the past been the source of some of the most successful antimalarial agents such as the quinolines and the endoperoxidases/ artemisinin derivatives. Although Ethiopia has an abundance of flora, their potential as sources of malaria remedies or lead compounds for antimalarial drugs has not been sufficiently explored. In this study, the *in vivo* antimalarial activities of nine plants entering the principal medications in the traditional treatment of malaria in Ethiopia have been explored.

MATERIALS AND METHODS

The nine plants selected were collected from Dolo-Mena District, Bale, Southeast Ethiopia. The botanical names as well as the families to which the collected plants belong are listed in Table 1. These plants were collected based on Ethnobotanical description with the help of traditional healers. A specimen of each collected plant was deposited at the herbarium of Drug Research Department, Ethiopian Health and Nutrition Research Institute.

The collected plants were separated into one of the four batches according to ethnobotanical indications: whole plant, leaves, root or bark. The various batches were dried separately at ambient temperature (23-26°C),

protected from light. The dry plants were grounded to powder using a sample mill and stored at ambient temperature before extraction. For each plant, two types of extracts were prepared (water and methanol) in accordance with the traditional conditions of use. Rotary evaporator (BUCHI, Scientific Equipments, UK) and a freeze dryer (Labconco, Inc, USA) were used to dry the extracts. Water extract yields were obtained by soaking 15g of plant powder in 200ml of double distilled deionized water for 24 hours and the filtrate freeze-dried. The weight of the dry extract was expressed as the total mass of dry powder. The dried extract was kept in a desicator at room temperature for antiplasmodial testing. For the methanol, 35g of plant powder were weighed and 24 hours extraction performed using 300ml of methanol. The extract was concentrated by the rotary evaporator and the yields were determined as described above and extracts stored in a refrigerator at 4°C until use.

A murine model of chloroquine-sensitive malaria, *Plasmodium berghei* (Anka strain), was used to investigate the effects of the water and methanolic extracts of the nine study plants on disease progress. Male Swiss albino mice weighing 20-25 gram were obtained from colonies in the animal unit of the Ethiopian Health and Nutrition Research Institute. The animals were housed in cages in group of five at 22±1°C and relative humidity of 80%. The diet was a standard pellet and there was continuous availability of clean drinking water.

A donor mouse infected with rodent malaria parasite *Plasmodium berghei* (parasitemia of about 20-30%) was killed by a head blow and blood collected through cardiac puncture with a sterile and a pyrogenic disposable needle and syringe. The blood was diluted with normal saline in such a way that 0.2ml of blood contained approximately 1X10⁷ infected red cells. Animals were infected by administration of 0.2ml blood suspension intraperitoneally, an infecting dose of 1X10⁷ parasitized cells. Treatment with the extracts commenced 3 hours after the mice had been inoculated (early infection). The mice received the plant extract (400mg/kg), once daily for 4 days by the oral route using oral needle (gavage) starting from three hours of parasite inoculation. A parallel test was run using chloroquine (Sigma, USA) 10mg/kg to serve as reference. In the positive control mice given chloroquine level of parasitemia gradually decreased to 0% (100% inhibition) on day 4 in agreement with study results of Peters (10). One group was left untreated as a positive control. After five days, thick and thin films were fixed with methanol and stained with 4% Giemsa at pH 7.2 for 45minutes and examined microscopically. Three different fields were examined on each slide and the number of infected and uninfected red blood cells (RBC) counted and the mean taken. The experimental design was similar to the 4-day

suppressive test (7). Percent parasitemia was calculated according to the following formula:

$$\% \text{Paritemia} = \frac{\text{Total number of PRB}}{\text{Total number of RBE}} \times 100$$

Where; PRBC: Parasitized red blood cells; RBC: red blood cells

Average percentage of parasitemia suppression was calculated according to the following formula (8).

$$\text{Average percentage of paritemia} = \frac{\text{Av. \% parasitaemia in control} - \text{Av. \% parasitaemia in test}}{\text{Av. \% parasitaemia in control}} \times 100$$

Acute toxicity study was conducted for the active extracts (Single dose 2000mg/kg) using the method of Weil (9). Albino mice weighing 20-25 g of either sex were divided in to groups of five mice each. Each group received the extracts. Signs of toxicity were observed for the first two hours and in two hours interval for six hours. Mortality was assessed after 24hours.

The results were expressed as mean \pm SEM and significance of differences between control and treated groups were determined using the student's *t*-test.

For the purpose of this study, any extract having a parasitemia inhibition of 10% or above is regarded as active whereas value less than 10% was considered as having no activity (10).

RESULTS

The extraction yields for the various parts of the study plants classified by extraction solvent showed that, the highest percentage yields were obtained for the MeOH extracts (Table 2)

Table 1. Traditional uses of the selected medicinal plants

Species	Families	Parts used	Indigenous uses
<i>Clerodendrum myricoides</i> (Hochst) R.Br.ex Vatke	Verbenaceae	root	Gonorrhea, colic, gout, swelling, malaria, wound dressing, rabies
<i>Cissampelos mucron</i> A.Rich in Guill perrot and A.Rich	Menispermaceae	leaf & root	Dysmenorrhagia, infertility, menorrhagia
<i>Gnidia steiopylla</i> Gilg	Thymelaeacea	root	Malaria, ascaris, rabies; gnicoumarin
<i>Vernonia bipontini</i> Vatke	Compositae	bark & leaf	Antispasmodic, malaria, snake bite, venereal disease, purgative, vermifuge
<i>Wargurgia ugandensis</i> Sprague <i>Plumbago zeylanica</i> L.	Cannaleceaceae Plumbaginaceae	bark & leaf root	<i>Plasmodium falciparum</i> ; muzigadial Antiprotozoal, plumbagin; antifungal, eczema, wound and analgesic
<i>Kalanchoe petitiiana</i> A.Rich. <i>Solanum incanum</i>	Crassulaceae Solanaceae	whole plant root	Gonorrhoea, syphilis, tapeworm, trachoma
<i>Euclea scimperi</i> (DC) Dandy	Ebenaceae	root	Amoebic dysentery; vermifuge, protozoa and antifungal <i>Tinea nigra</i> ; antifungal, immunostimulant action

Table 2. Plant parts and extract yield of the study plant species

Species	Local names	Part used	Solvent	% Yield
<i>Cissampelos mucronata</i>	Gure toke(Or.)	Root	MeOH	17.3
		Root	Aqueous	12.0
<i>Clerodendrum myricoides</i>	Haware(Or.)	Leaf	MeOH	5.4
		Leaf	Aqueous	3.0
	Root	MeOH	12.7	
	Root	Aqueous	10.0	
	Root	MeOH	10.0	
<i>Solanum encanum</i>	Enboay (Am)	Root	Aqueous	8.2
		Root	MeOH	13.3
<i>Vernonia bipontini</i>	Reji (Or.)	Root	Aqueous	7.3
		Leaf	MeOH	10.8
<i>Waburgaria ugandensis</i>	Befi (Or.)	Leaf	Aqueous	7.0
		Bark	MeOH	18.2
	Bark	Aqueous	6.6	
	Leaf	MeOH	20.3	
<i>Plumbago zylonica</i>	Dhigagi (Or.)	Leaf	Aqueous	13.2
<i>Eculea shimperi</i>	Dedeho(Am.)	Root	MeOH	14.3
		Root	Aqueous	7.9
<i>Kalanchoe pettitana</i>	Endawla (Am.) (Hanchura Or.)	Root	MeOH	16.3
		Root	Aqueous	12.5
		Leaf	Aqueous	8.3

Or-Oromiffa; Am-Amaharic

The parasitemia and parasite growth inhibition expressed as percentages are given for each of the extract/drug. The results of the 4 day suppressive antimalarial screening of the 19 extracts of the nine selected medicinal plants at 400mg/kg/day in albino mice parasitized with *P. berghei* showed that parasitemia in all the mice given any of the plant extracts except for the aqueous leaf extracts of *Cissampelos mucronata* and *Kalanchoe pettitana* as well as methanolic leaf extract of *Cissampelos mucronata* were significantly lower ($p < 0.05$) on day 4 than on day 0 compared to the control mice. The suppressive activities

of the aqueous extracts of *Cissampelos mucronata*, *Gnidia stenophylla* and *Euclaea shimperi* are more than two-fold higher than the suppressive activities of their methanolic extracts. Aqueous root extract of *Clerodendrum myricoides* suppressive activity is six-fold higher than its methanolic root extract. Chemosuppression activity of methanolic leaf extract of *Vernonia bipontini* is significantly lower ($p < 0.05$) than its aqueous leaf extract. Similarly, aqueous and methanolic extracts of the 4 study plants exhibited antiplasmodial activity ranging from 20.4 to 34.6%.

Table 3. Percent parasitemia inhibition by the MeOH and Aqueous extracts of study plants *in vivo*.

Plant species	Part used	Extract type	% parasitemia ± SEM	% Inhibition
<i>Cissampelos mucronata</i>	Root	MeOH	27.7 ± 1.80	20.4
	Root	Aqueous	24.5 ± 0.93	47.5
	Leaf	MeOH	40.6 ± 0.93	0
	Leaf	Aqueous	41.3 ± 0.76	0
<i>Clerodendrum myricoides</i>	Root	MeOH	32.1 ± 0.86	7.5
	Root	Aqueous	21.4 ± 0.51	44.0
<i>Gnidia stenophylla</i>	Root	MeOH	26.8 ± 1.30	22.7
	Root	Aqueous	19.2 ± 0.65	55.4
<i>Solanum encanum</i>	Root	MeOH	28.4 ± 30.00	20.4
<i>Vernonia bipontinii</i>	Leaf	MeOH	18.1 ± 0.06	40.0
<i>Waburgia ugandensis</i>	Leaf	Aqueous	18.7 ± 0.75	52.7
	Bark	MeOH	22.8 ± 1.13	34.6
	Bark	Aqueous	21.25 ± 1.25	28.3
	Leaf	Aqueous	21 ± 2.50	30.0
<i>Plumbago zylonica</i>	Root	MeOH	24.1 ± 1.52	30.5
	Root	Aqueous	30.1 ± 1.33	29.5
<i>Eculea shimperi</i>	Root	MeOH	21.6 ± 1.35	21.6
	Root	Aqueous	27.7 ± 1.34	51.4
<i>Kalanchoe peltata</i>	Leaf	Aqueous	43 ± 1.53	0
<i>Chloroquine</i> (10mg/kg)			0.33 ± 0.24	100

The level of parasitemia in the untreated control mice increased throughout the period of observation, the highest parasitemia attained on day 4 was 34% (Fig.1).

For untreated mice death was first observed on the six day post-infection and all mice had died by the eighth day. In all cases death was preceded by convulsions, rapid breathing and high parasitemia.

0.33±0.24 100

Acute toxicity study conducted in order to determine the safety margin of the extracts showed the LD₅₀ to be above 2000mg/kg in mice with the exception of extracts of *Clerodendrum myricoides* for which the LD₅₀ value is above 1000mg/kg. Although single dose treatments were conducted, there was no sign of conventional toxicity and mortality in the observation period.

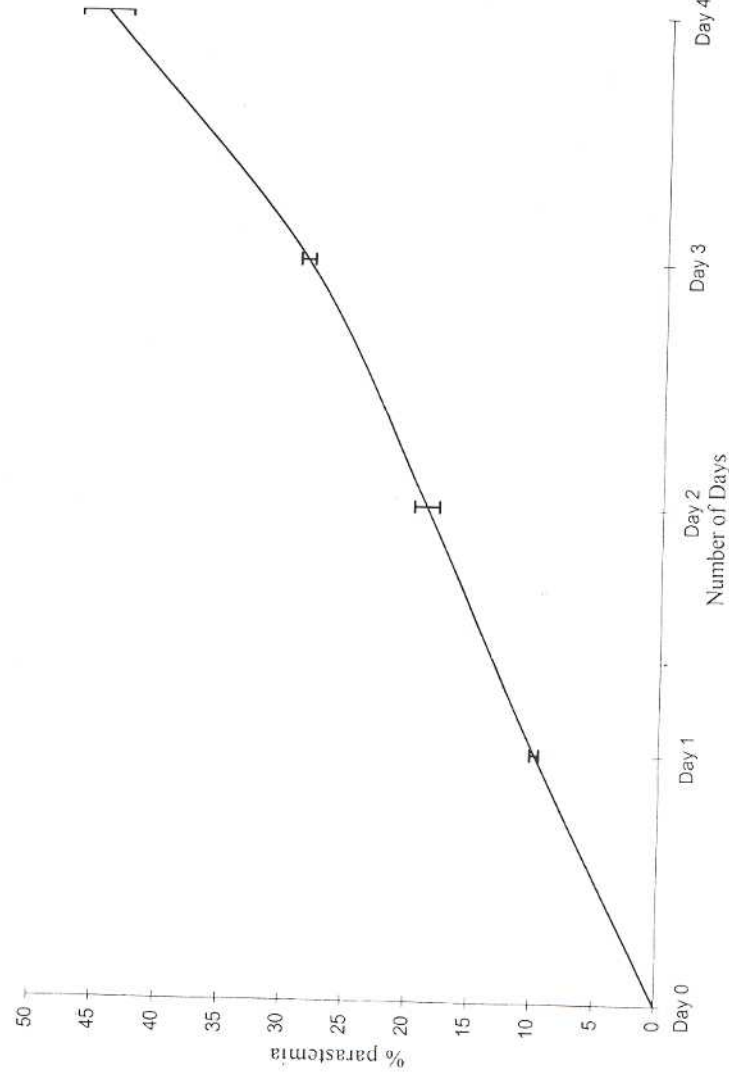


Figure 1. The development of Progress of in *Plasmodium berghei* (Anka) parasitemia infected mice ($\sim 10^7$), $n = 5 \pm$ SEM.

DISCUSSION

These results showed that six extracts presented relatively high activities. These were the aqueous root extract of *G. stenopylla*, leaf extract of *V. bipontini*, root extract of *E. schimperi*, *C. mucronata*, and *C. myricoides* and methanolic leaf extract of *V. bipontini* among which three extracts had very high percent parasitemia inhibition values (>50%). Nine extracts from the nine studied plants exhibited moderate activities. In this case, the extracts concerned the methanolic root extracts of *C. mucronata*, *G. stenopylla*, *S. incanum*, *P. zyleneica*, *E. schimperi* and bark of *W. ugandensis* as well as aqueous root extract of *P. zyleneica*, the root, bark and leaf extracts of *W. ugandensis*. The percent parasitemia inhibition of other extracts were less than 10% and hence of no interest.

Generally, the aqueous extracts showed a higher antimalarial activity against *P. berghei* compared to the methanolic extracts. This may be an indication that the antiplasmodial activity is due to compounds, probably glycosides, which are better extracted with polar solvents (11). In this study, the aqueous extracts showed an enhanced antiplasmodial activity compared to the methanolic extracts, this was in agreement with the traditional claim as concoction and decoction were the

methods used in traditional preparation of herbal remedies.

Three plants, though often used in traditional medicine, appeared to have little antiplasmodial activity. They are perhaps useful for treating associated symptoms, as fever, or to enhance the immune system. This situation concerns plants such as aqueous leaf extracts of *C. mucronata* and *K. petitiana* and methanolic leaf and root extracts of *C. mucronata* and *C. myricoides*, without activity against *Plasmodium* but with very high antispasmodic and anti-inflammatory properties (16). Another explanation for their lack of activity is perhaps the solvent used. We studied here only plants whose aqueous or methanolic extracts exhibited an activity, because only these solvents are used in traditional medicine. However, plants showing no activity with these solvents can appear active against malaria when extracted with non-polar solvents (12, 13).

C. mucronata and its other species are largely used as traditional antimalarials and muscle relaxants (14). Although aqueous and methanolic leaf extracts of *C. mucronata* failed to show any antimalarial activity, the aqueous root extract revealed good antimalarial activity against *P. berghei*, Table 3. In a precedent study, ethanol, petroleum ether, ethyl acetate and aqueous root extracts of *C. mucronata* showed the highest antimalarial activity in vitro with IC_{50} values of 1.3 μ g/ml, 8 μ g/ml, 0.38 μ g/ml

and 1.2µg/ml, respectively (15). *Cissampelos* spp. are known to contain bisbenzylisoquinoline alkaloids such as hayatin, isularine, cissamparine and cyclanoline(14). Bisbenzylisoquinoline alkaloids are known to be active against *P.falciparum* in vitro (16).

C. myricoides was one of the plants displaying the most interesting activity in vivo, the aqueous root extract inhibited 51% of the parasite growth at 400mg/kg. *C. myricoides* is commonly encountered in many parts of Ethiopia. People use it and prepare it in form of decoction against malaria, diarrhea, relapsing fever, and abdominal colic (17). Other traditional uses of *Cleodendrum* plants include treatment for gonorrhea, colic, gout, swelling, wound dressing and rabies (18). *C. myricoides* is known to contain cleromyrin I and II; myricoidine and dihydromyricoidine (18). The activity displayed by this species in vivo as well as the presence of this type of alkaloids suggest that the antiplasmodial activity observed might be due to such compounds (19). Ethanol, petroleum ether, ethyl acetate and aqueous root bark extracts of *C. myricoides* exhibited antimalarial activities in vitro with IC₅₀ values of 300µg/ml, 47µg/ml, 11µg/ml and 300µg/ml, respectively (15).

Of all the given plants tested in this study, the second most active was *V. bipontini*. The leaves aqueous extract showed a relatively high activity with 52.7% parasitemia suppression values at 400mg/kg. *V. bipontini*, although not yet studied, belongs to a genus whose other representatives have been well studied, even for antimalarial properties. Many members of the genus are known to contain sesquiterpene lactones, a group of terpenoids, which have a strong antiplasmodial activity and cytotoxic effects (11,20). *V. amygdalina*, for instance, exhibits antiplasmodial activity in vitro as well as in vivo and contains many molecules: flavonoids, sesquiterpenoids, alkaloids, triterpenoids, glycosides and steroids. *Vernonia* spp. have antipyretic, analgesic, anti-inflammatory activities (21-23).

The in vivo tests showed that the aqueous leaf and bark and methanolic root extracts of *W. ugandensis* (400mg/kg.day) reduced parasitemia at the fourth day (28.3-34.6% reduction, p<0.05) in relation to the non-treated control mice. *W. ugandensis* is traditionally used to treat malaria, microbial infections. Its other indigenous uses have been shown Table 1. Two drimane sesquiterpens polygodal and warburganol, tannin, muzigadial, from the bark, with cytotoxic properties against *Plasmodium falciparum* has been reported (24,25).

In this study, *P. zeylanica* is only moderately active in vivo against *Plasmodium berghei* (30%). In Ethiopia, this plant is highly prized for its application against eczema, rheumatic pain, scabies, toothache, and abdominal colic (16). Phytochemical investigation revealed the presence of plumbagin, and epi-isoshinanalone in *Plumbago* spp. Plumbagin is reported to have antibacterial, antifungal, analgesic and immunostimulant activities whereas epi-isoshinanalone exhibited anti-

inflammatory activity (26). Despite its relative abundance and much poplar use, no information was recovered about its antiplasmodial activity. Our study confirms the antiplasmodial activity of its leaves and roots. To our knowledge, this is the first study of this kind on antimalarial in vivo activity, but it should be worthwhile to undertake further studies, in order to elucidate the compounds responsible for antimalarial activity observed.

K. petitiiana had never been studied for antiplasmodial activity. Ethiopian traditional medicine uses *K. petitiiana* in the treatment of abdominal dropsy, rabies, stomach distention, tapeworm, constipation, and ascariis (16). It enters the medication of various diseases including gonorrhea, tumorous growth, syphilis and tapeworm (17,27). Gallic (bacteriostatic and antitumor), steroids and triterpenes have been reported from *Kalanchoe* spp. (17,28).

S. incanum methanol root extract has been evaluated for its in vivo antimalarial activity, in the 4-day-suppressive test against *P.berghei* in mice. The chemosuppression of parasitemia, with oral administration (400mg/kg.day), was 20.5% for the methanolic extract. *Solanum* spp. are administered in Ethiopia to treat malaria, rabies, diarrhea, tapeworm, toothache, and febrifuge (16). Use of various *Solanum* spp. in traditional medicine for several therapeutic purposes such as gonorrhea, colic, gout, swelling, malaria, wound dressing, and rabies has been reported (17). Phytochemical investigations of several members of the genus revealed the presence of tomatidine and tomatine. These compounds exhibit strong antiprotozoal and antimicrobial properties (17).

Of the two extracts of the root of *E. schimperi* that were investigated in the present study, the aqueous extract appeared to have the greatest antimalarial activity, although the methanolic extract showed good activity in vivo (Table 3). *E. schimperi* is used in traditional medicine in Ethiopia as a treatment for acne, eczema, scabies and tinea capitis (16). Other *Euclea* spp. are used as a treatment for gonorrhoea, uterine prolapse, haemostatic, gastritis, diarrhea, cataract, constipation rabies vitiligo and epilepsy (17). The results of previous phytochemical studies of *Euclea* have revealed the presence of ramentacene, euclain, biranacetanone, ramentone, aromadendrin, xylopyrin, isidiospyrin and natalenone (17,28). The present results appear to be the first confirmation that the roots of *E.schimperi* have antimalarial activity, especially when tested as aqueous extract.

G. stenopylla is the most interesting plant in this study. Its activity was especially revealed by the aqueous root extract. The methanolic root extract also has an interesting antiplasmodial activity. To our knowledge, no study on antiplasmodial activity had yet been reported on this plant. In traditional medicine, *Gnidia* enters various preparations as a treatment for gonorrhea, leprosy, syphilis, malaria, ascariis, rabies, toothache, heart pain rheumatic pain, breast cancer and tumors (28). Numerous species of this plant contain daphnetin (antibacterial

agent), daphenin (analgesic), diterpenes, gnidicin, gnididin and gnidtrin (17,28).

The aqueous root extract of *G. stenopylla*, leaf extract of *V. bipontini*, root extract of *E. schimperi*, *C. mucronata*, and *C. myricoides* and methanolic leaf extract of *V. bipontini* presented relatively high activities, among which three extracts reduced parasitemia by >50% when tested at an oral dose of 400mg/kg.day. Further investigation is being undertaken to fractionate the extract and isolation of the active ingredient. In conclusion, the results of the present study appear to justify the use of these plants as traditional medicines for the treatment of malaria. The study warrants further investigation the plants will contribute in the development of malaria drug from plant origin.

In Conclusion, the results justify the use of these plants as traditional medicines for the treatment of malaria. Except the leaf extract of *Cissampelos mucronata*, the methanol extract of *Clerodendrum myricoides* and aqueous extract of *Kalanchoe peltiata* have inhibition of parasitemia above 10%. We recommend detailed pharmacological and toxicological studies before drug development.

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