IN VIVO ANTIMALARIAL ACTIVITY OF *AJUGA REMOTA* WATER EXTRACTS AGAINST *PLASMODIUM BERGHEI* IN MICE

John N Gitua[1], Daniel R Muchiri[2] and Xuan-An T Nguyen[1]

[1]Department of Chemistry, Drake University, Des Moines, IA, USA;
[2]Department of Chemistry, Egerton University, Njoro, Kenya

Abstract. We investigated the *in vivo* activity of crude water extracts of *Ajuga remota* Benth (Labiatae) against *Plasmodium berghei* in mice using plants harvested from two areas in Kenya where the plant is commonly used to treat malaria. The extract was tested using a 4-day test at a dose of 30 mg/kg/day (equivalent to 0.2 ml solution per mouse). Wet leaf extract was the most effective with 90.4% suppression of parasitemia. Extract from air - dried and powdered flowers were the least effective with 17.2% suppression of parasitemia.

Keywords: *Ajuga remota*, medicinal plant, *in vivo*, *Plasmodium berghei*

INTRODUCTION

Despite malaria control programs, the control and eradication of malaria in Africa has been made difficult by mosquito resistance to insecticides and rising parasite resistance to clinically useful drugs. Between 300-500 million new cases of malaria occur every year resulting in 1.5-2.8 million deaths annually (Guadalupe *et al*, 2007). The severity of malaria caused by *Plasmodium falciparum* depends on a complex interplay between the infecting parasite and the immune status and genetic background of the host (Sharma *et al*, 2004). The use and misuse of chloroquine to prevent and treat falciparum malaria has led to widespread resistance to chloroquine in Kenya and other countries (Njoroge and Bussmann, 2006) and has become a great obstacle toward preventing and curing malaria. The lack of affordability of antimalarial drugs by the majority of the population has forced many to seek alternative sources of treatment using traditional herbal remedies, such as weeds, plants and bark. We evaluated the antimalarial activity of *Ajuga remota* Benth (Labiatae) roots and other parts against *Plasmodium berghei* parasites in mice. *Ajuga remota* is an erect rhizomatous pubescent herb commonly found in East Africa. It is used to treat pneumonia and liver problems (Kokwaro, 1993) and has been found to reduce blood pressure experimentally in hypertensive rats (Odek-Ogunde *et al*, 1993). A decoction made from the leaves of *Ajuga remota* has long being used by Kenyan herbalists to treat stomach ache, malaria and other problems (Kuria and Muriuki, 1984).

Correspondence: John N Gitua, Department of Chemistry, Drake University, 2507 University Avenue, Des Moines, IA 50325, USA.
Tel: 1 515 2713760; Fax: 1 515 2711928
E-mail: John.Gitua@drake.edu

MATERIALS AND METHODS

Plant materials

Ajuga remota was collected from Limuru and Mutukanio (Njoro) in Kenya

where the plant is commonly used as a traditional treatment for malaria. The specimen's authenticity was confirmed by the Department of Botany, Egerton University and the Botany Department Herbarium, University of Nairobi, Kenya. The roots and other parts of *Ajuga remota* were divided into two batches: wet and air-dried plant parts before extraction. The plant was air-dried for 21 days at room temperature until a constant weight was obtained. The powdered air-dried and wet plant parts (20.0 g) were each extracted by soaking them separately in 300 ml of water for two hours at 90°C with regular stirring. The decoction was then removed from the hot plate and allowed to cool to room temperature with regular stirring. The resulting respective aqueous crude extracts were filtered and filtrate concentrated under vacuum at 55°C followed by freeze drying at 250 milliter pressure and -40°C temperature. The freeze dried extracts were kept in tightly closed bottles in a refrigerator until used for antimalarial testing.

Animals

Swiss mice (20-25 g each) bred locally at the animal house of the National Public Health Laboratory Services (NPHL), Kenyatta National Hospital in Kenya were used. The animals were placed in wired cages and given a pelleted diet (mice cubes) along with free access to water. The animals were allowed to acclimatize to the laboratory at a controlled temperature of 22°C for 30 days before being subjected to the experiments. The mice were divided into ten groups of eight each, and all infected with malaria parasites. Four of the groups received the wet extract treatment, four other groups received the air-dried powdered plant extract treatment, one group received chloroquine (standard antimalarial drug) and one tenth group was a control (no drug administered).

Parasite inoculation

A single donor mouse infected with *Plasmodium berghei* parasites was bled into sterile heparinized culture medium and the blood was diluted with RPMI 1640 medium. The healthy experimental mice were infected intravenously via a tail vein with 0.2 ml of the diluted blood containing 1×10^7 parasitized (*Plasmodium berghei*) red blood cells on day one.

Evaluation of antimalarial activity

The antimalarial activity tests were performed using the four-day suppressive tests described by Peters *et al* (1975). The two extracts (wet and dry) were administered intravenously to two groups of mice at a dose of 30 mg/kg/day (equivalent to 0.2 ml solution per mouse) for four consecutive days. Parallel tests with chloroquine were conducted for reference purposes at the same dose in one group and with an equivalent volume of distilled water (0.2 ml/mouse/day) in the control group. Thin smears were obtained from the tail vein of each mouse on day five after infection. The smears were fixed with methanol and stained with Giemsa stain. The percent parasitemia suppression was determined by counting the number of parasitized erythrocytes out of 500 red blood cells on random fields under the microscope. The average percent suppression of parasitemia was calculated using the following formula:

$$\% \text{ suppression} = \frac{A - B}{C}$$

where A = % parasitemia in untreated controls, B = % parasitemia in treated groups, and C = % parasitemia in untreated controls

The data were analyzed using the *F*-test. A p-value < 0.05 was considered significant.

Table 1
Efficacy of extracts of *Ajuga remota* against *Plasmodium berghei* infected mice.

Drug/extract	Wet plant parts		Dried plant parts	
	Average % parasitemia	Average % suppression	Average % parasitemia	Average % suppression
Control	0.850	-	0.850	-
Leaves	0.082	90.35	0.146	82.82
Flowers	0.565	33.53	0.704	17.18
Stems	0.553	34.94	0.621	26.94
Roots	0.476	44.00	0.586	31.06
Chloroquine	0.300	84.71	0.300	84.71

RESULTS

The wet plant extracts of the leaves resulted in a 90.4% suppression in parasitemia, the flowers extract caused a 33.5% reduction, the air-dried leaf extracts caused a 82.8% reduction and the air-dried flowers extract caused a 17.2% reduction in parasitemia (Table 1).

DISCUSSION

The present study was undertaken to evaluate the antimalarial activity of a widely used traditional treatment for malaria in Kenya. Traditional remedies are common in regions where patients cannot afford to use chemically synthesized drugs. Poverty, traditional beliefs and lack of available health centers have caused plants to be used as the major source for treatment of various ailments (Tshibangu *et al*, 2002). The ethanol and water extracts of *Ajuga remota* leaves have been shown to have significant *in vitro* antimalarial activity against both chloroquine sensitive and resistant strains of *Plasmodium falciparum* (IC_{50} = 441 g/ml) (Kuria *et al*, 2001). Therefore, we undertook an *in vivo* antimalarial study of the water extracts of *Ajuga remota* against *Plasmodium berghei* in mice.

The rodents have been used to study the antimalarial effects of chloroquine, mefloquine and artemisinin derivatives (David *et al*, 2004). *Plasmodium berghei* has been used to predict treatment outcomes and is an appropriate parasite for this study. Since this parasite is sensitive to chloroquine, this drug was used as the standard treatment drug in this study. When a standard antimalarial drug is used in mice infected with *Plasmodium berghei*, it suppresses parasitemia to non-detectable levels (Kiseko *et al*, 2000), which is in agreement with the effects of chloroquine in this study.

The 4-day suppressive test is a standard test commonly used for antimalarial screening, and the determination of percent suppression of parasitemia is the most reliable parameter. The observed antimalarial activity is consistent with the traditional use of this plant as a herbal medication against malaria in the Central and Rift Valley Provinces of Kenya. The highest antimalarial activity was observed with the leaves which indicates there is a higher concentration of active antima-

larial ingredients in the leaves than other parts of the plant. These findings confirm the traditional use of this plant's leaves for treatment of malaria, as opposed to other parts of the plant.

From the present study, it can be concluded: 1) water extract of *Ajuga remota* has parasite suppressive effects against *Plasmodium berghei* in infected Swiss mice; 2) the antimalarial activity of the extracts vary depending on the part of the plant, with the leaf extracts having the highest activity.

ACKNOWLEDGEMENTS

The authors are thankful for the financial support from both Drake University and Egerton University, Kenya. We are grateful to K.E.M.R.I. for the supply of *Plasmodium berghei* used in this study and Kenyatta National Hospital for the supply of experimental animals.

REFERENCES

David AF, Phillip JR, Simon LC, Reto B, Solomon N. Antimalarial drug discovery: Efficacy models for compound screening. *Nature Rev* 2004; 3: 509-20.

Guadalupe E, Garcia L, Rodriguez JB. Current status and progress made in malaria chemotherapy. *Curr Med Chem* 2007; 14: 289-314.

Kiseko K, Hiroyuki M, Syun-ichi F, *et al*. Antimalarial activity of leaf – extract of *Hydrangea macrophyla*, a common Japanese plant. *Acta Med Okayama* 2000; 54: 227-32.

Kokwaro JO. Medicinal plants of East Africa. 2nd ed. Nairobi: Kenya Literature Bureau, 1993: 118.

Kuria, KAM, Muriuki G. A new cardiotonic agent from *Ajuga remota* benth (Labiatae). *East African Med J* 1984; 61: 533-8.

Kuria KAM, De Coster S, Muriuki G, *et al*. Antimalarial activity of *Ajuga remota* benth (Labiatae) and *Caesalpinia volkensii* Harms (Caesalpiniaceae): *in vitro* confirmation of ethnopharmacological use. *J Ethnopharmacol* 2001; 74: 141-8.

Njoroge GN, Bussmann RW. Diversity and utilization of antimalarial ethnophytotherapeutic remedies among the Kikuyus (central Kenya). *J Ethnobiol Ethnomed* 2006; 2: 8.

Odek-Ogunde M, Rajah MS, Migwi GJ, Ndegwa JM. Blood pressure response to an extract of *Ajuga remota* in experimentally hypertensive rats. *Planta Med* 1993; 59: 573-4.

Peters W, Portus JH, Robinson BL. The chemotherapy of rodent malaria, XXII the value of drug-resistant strains of *P. berghei* in screening for blood schizotocidal activity. *Ann Trop Med Parasito* 1975; 69: 155-71.

Sharma SK, Chattopadhyay R, Chakrabarti K, *et al.* Epidemiology of malaria transmission and development of natural immunity in a malaria-endemic village, San Dulakudar, in Orissa State, India. *Am J Trop Med Hyg* 2004; 71: 457-65.

Tshibangu JN, Chifundera K, Kaminsky R, Wright AD, Konig GM. Screening of African medicinal plants for antimalarial inhibitory activity. *J Ethnopharmacol* 2002; 80: 25-35.