STUDY OF CRUDE EXTRACTS OF *Ajuga remota* BENTH (LABIATAE) AS POTENTIAL ANTI-MALARIAL DRUG

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ABSTRACT
Malaria is among the killer diseases in the tropics and the parasite has been noted to develop resistance to many synthetic drugs. This study screened and tested the efficacy of crude extracts of different parts of *Ajuga remota*. Aqueous crude extracts of *Ajuga remota* which have been traditionally used to treat fevers and malaria, were used *in vivo* against *Plasmodium berghei* malaria infections in mice using the four-day suppressive test. Leaves, stems, roots and flowers either boiled wet in water immediately after collection or dried first before boiling in water were then injected intravenously through the tail vein of mice infected with *Plasmodium berghei* parasite. Chloroquine, a standard antimalarial drug, was used as a control. On day four, parasitized blood smears were made from tail strip for determination of parasitaemia and calculation of percentage suppression. The different preparation showed different suppressive activities against *P. berghei* parasites. The leaves showed the highest antimalarial activity compared to stems, roots and flowers for wet and dry parts, respectively. Thus, *A. remota* has potential antimalarial compounds which need further evaluation to determine their activity against human malaria parasites.
Key words: *Plasmodium berghei, Parasitaemia, Malaria, Chloroquine*

INTRODUCTION
Although malaria has been eradicated or controlled in the developed nations, it still remains a common tropical disease in South East Asia, Africa and Eastern Mediterranean that account for 99% of global malaria cases and deaths (WHO, 2013). In 2010, 219 million cases of malaria were reported as well as 660,000 deaths (WHO, 2012). This is alarming especially in tropical Africa where the disease is endemic with the region accounting for a high percentage of both the incidence of malaria and resulting deaths. According to Farook (2004) Sub-Saharan Africa and countries in tropical Africa account for more than 90% of total malaria incidence and great majority of deaths due to the disease. Together, the Democratic Republic of the Congo and Nigeria account for over 40% of the estimated total of malaria deaths globally (WHO, 2012).

Artemisinin is one of the best anti-malaria drugs in use today and is usually used in combination with other drugs (Krungkrai et al, 2010). By the end of 2011, Artemisinin Combination Therapy (ACTs) had been adopted as national policy for first-line treatment in 79 of 88 countries where *P. falciparum* is endemic and chloroquine is still used in some countries in the Region of the Americas where it remains efficacious (WHO 2012). Recent studies have shown that malaria parasite has started developing resistance to this drug. A decrease in clinical efficiency was noted of the artemisinin derivative in treatment in falciparum malaria patients at the Thai-Cambodian border in 2009, showing that

the parasite clear slowly from the patients' blood after the ACTs treatment without corresponding reduction in-vitro susceptibility testing (Dondorp et al 2009, as cited in Krungkrai et al, 2010).

It is not only the developing of resistance of ACTs to malaria parasite that is a hindrance to treatment of malaria, the affordability is also important. The cost and availability of ACT in the public sector remains a major challenge in Africa. In 2008, ACT coverage in the public sector in high-burden African countries was only 42% and a survey in seven African countries showed that the percentage of fever cases in children < 5 years treated with ACTs was only 16% (WHO, 2009 as cited in Achan et al, 2011).

According to Bloland (2001) as long as drugs are used, the chance of resistance developing to those drugs is commonly noted. This is the case for *P. falciparum* which has developed resistance to nearly all available antimalarial drugs and it is highly likely that the parasite will eventually develop resistance to any drug that is used widely. This shows the need for continued research in the identification and development of new anti-malaria drugs to pre-empt any crisis that may result in the use of current drugs hence the need for the current research. Ideally, new drugs for uncomplicated *P. falciparum* malaria should be efficacious against drug-resistant strains, provide cure within a reasonable time (three days or less) to ensure good compliance, be safe, be suitable for small children and pregnant women, have appropriate formulations for oral use and, above all, be affordable (Thaithong et al, 1981; Peters, 2002 as cited in Fidock 2004).

Ajuga remota is a herb which grows widely in East Africa. Locally, the leaves are pounded and steeped in cold water and the infusion drunk as a remedy for fever, malaria, toothache, dysentery and for the treatment of high blood pressure (Kokwaro, 1976). Traditionally, it is believed to be antimalarial by many communities in East Africa. As part of this study towards the development of new antimalarial drugs from indigenous plants, the antimalarial activity of the crude extracts from the various morphological parts of the *A. remota* species were examined.

The previous work done on the *A. remota* plant has shown it to have insecticidal activity and antihypertensive properties. Kubo et. al. (1981) isolated the chemical compound phytoecdysones from the *A. remota* which is reported as insect ecdysis inhibitor and feeding deterrent. He further reported the insect ecdysis inhibition by cyasterone and ecdysterone both extracted from the leaves and roots of the same species. Study on the antimalarial activity of a plant in the same genus *Ajuga bracteosa* on *Plasmodium berghei* using its ethanolic leaf extract was not only found to inhibit parasitaemia in dose dependent manner but also enhanced the mean survival time period of treated mice (Chandel and Bagai, 2010). Kassa et al (1998) found ethanolic aerial extracts of *Artemisia afra*, *Artemisia rehan* and *Ajuga remota* to have significant in-vitro activity against *P. falciparum*. Kubo et. al. (1981) has also reported the ajugarin IV isolated from *A. remota* , structure of which was determined by spectroscopic and chemical data means, to have insecticidal activity against the insect *Bombyx Mori* at 500ppm but only growth inhibitory activity against insect *Pectinophora gossypiella*. Similarly, Kubo et al (1976) established the structure of Ajugarin V previously isolated from *A. remota* using spectroscopic and chemical data means. The anti-hypertensive studies on the crude extract of *A. remota* and its major component ajugarin I, *clerodane diterpene* by Odek-Ogude and Rajab (1994), revealed that administration of the crude extract and ajugarin I at 10mg/l in the drinking water of experimentally hypertensive rats lowered their blood pressure by 40mmHg and 50mmHg respectively.

MATERIAL AND METHODS

Extraction preparation

The plant materials were collected from Limuru (Kiambu county) and Njoro (Nakuru County), Kenya. They were detached off their individual parts separately and the experiment was then divided into two parts. The various individual detached parts of the wet plants; leaves 578.95g, stem 300.00g, roots 219.02g and flowers 100.00g were extracted by boiling them separately in water for a duration of two hours at 90^0C. The crude extracts were then filtered and the various filtrates were freeze dried. The remaining various detached parts of the wet plant were dried under shade in the laboratory where the plant parts were laid on clean trays on benches for 21 days until consecutive constant weights were obtained; leaves 362.96g, stem 596.05g, roots 423.52g and flowers 66.75g. They were then exhaustively extracted by boiling them separately in water for a duration of two hours at 90^0C. After filtration, the various filtrates were then freeze dried.

In vivo anti-malarial test

The animals used were Swiss mice weighing 20-25g each and bred locally in the animal house of Kenyatta National Hospital Laboratory (K.N.H.L.). They were divided into groups of N=8 with 4 males and 4 females per group. Each group of mice was kept in wired cages and provided with pelleted diet together with some water. A single donor

mouse was bled into sterile heparinized culture medium and centrifuged for five minutes at 350g's. After aspirating the supernatate, 0.4*PRBC volume of glycerolyte '57' was added over two minutes interval with gentle shaking. It was then frozen at -70^{0}C in 0.4 ml aliquots. This was then thawed rapidly in hand and placed in 15ml centrifuge tube while slowly adding 0.1ml of 12% NaCl then left to stand for two minutes. 10ml of 1.6% NaCl was slowly added over a two minutes period, vortexed gently and then left to stand for five minutes. After centrifuging for five minutes at 350g's, the supernatate was aspirated and 10ml of 0.9% Nacl, 0.2% Dextrose slowly added and left to stand for five minutes followed by centrifuging for five minutes. The supernatate was aspirated and the desired volume for infection made with RPML 1640 medium. The diluted blood (0.2ml) containing 1 x 10^7 parasitized (*P. berghei*) red blood cells was injected intravenously via a tail vein into normal mice. A single donor mouse was used to infect all the animals in order to minimize variability in the induced parasitaemia. The day of infection was termed "D0" and subsequent days "D1", "D2" etc. Each drug was administered in 0.2ml solution per mouse as a single daily dose intravenously for four days.

Evaluation of Parasitaemia

Blood films were made from the cut tail vein of animals infected with *P. berghei*. These were stained with Giemsa stain after fixing with methanol. Parasite counts were done under oil immersion with the x1000 objective x10 eye piece of compound microscope. The number of microscopic fields counted was obtained by dividing 10^4 rbc by the mean of rbc in two fields. The total number of parasitized rbc were then counted in the above number of fields. Percentage parasitaemia was assessed for each field and the mean percentage parasitaemia for each field and the mean percentage parasitaemia for each mouse were then calculated as:

$$\% \text{ Parasitaemia} = \frac{\text{No. of infected Rbc} \times 100}{10{,}000}$$

Evaluation of antimalarial Activity

The four day technique employed here was similar to that described by Peters and Porter (1975).

Infected animals were divided into groups of eight mice. The crude aqueous extracts from *A. remota* , both wet and dry were given intravenously to the animals. Each animal received a dose of 30mg/kgday $^{-1}$ (equivalent to 0.2ml solution per mouse) for four consecutive days. Parallel tests with chloroquine (a standard anti malarial drug) were conducted for reference purposes. The drugs were administered intravenously (injections with microlitre syringes). Tail blood film was taken from each animal on D4. These were stained with Giemsa stain and percentages suppression of parasitaemia in relation to the control was calculated as follows:

AV. % = Parasitaemia – AV. % Parasitaemia

AV. % = (in untreated ctrls in treated groups/ Suppression Av. % Parasitaemia in untreated ctrls) X 100

RESULTS

The results of the study are presented in the table below.

Table 1: Parasitaemia suppression levels of *Plasmodium berghei* in mice by crude extract preparation of *A. remota*

		Average (N=8)	Average (N=8)
Drug	Nature of Drug	Parasitaemia (%)	Suppression
Untreated ctrl	-	0.850	-
Leaves extract	Wet	0.820	90.35
	Dry	0.146	82.82
Stem extract	Wet	0.565	33.53
	Dry	0.621	26.94
Roots extract	Wet	0.476	44.00
	Dry	0.586	31.06
Flowers extract	Wet	0.553	34.94
	Dry	0.704	17.18
Chloroquine	Powder	0.300	84.71

DISCUSSION

The crude aqueous extracts, of *Ajuga remota* were shown to possess higher antimalarial activity than Chloroquine in this four-day test using sensitive strain of *P. berghei* in mice. The different parts of *A. remota* showed different suppressive activities against *P. berghei* parasites in mice with the wet and dry plant preparations. The leaves showed the highest antimalarial activity (90.4%, 82.8%) compared to the stems (33.5%, 26.9%), roots (44.0%, 31.1%) and the

flowers (34.9%, 17.2%) for wet and dry parts respectively. Chloroquine showed 84.7% suppression of parasitaemia. The results indicate that the activity is reduced with drying of the plant which is true for all the parts of the plant. Among the plant parts, the leaves had the highest activity which was even higher than that of the control which was Chloroquine. The present study with the crude aqueous extracts of *A. remota* is indicative that the plant has considerable antimalarial potential. This is a confirmation of the knowledge of malaria treatment by traditional practitioners. From this study it is recommended that more should be done to advice the traditional practitioners on the parts of the plants to use, the mode of preparation and the dosage to be administered in the treatment of malaria by use of *A. remota*.

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