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. The objective of the present study was to screen and test for the efficacy of the crude extracts of different parts of *Ajuga remota*. Aqueous crude extracts of *Ajuga remota*, which has been traditionally used to treat fevers and malaria, were used in vivo against *Plasmodium berghei* malaria infections in mice using 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 suppression percentage. The different preparations had different percent suppressive activities against *P. berghei* parasites. The leaves had the highest antimalarial activity compared to stems, roots and flowers for wet and dry parts, respectively. The antimalarial activity of the leaves was higher than for chloroquine, a conventional drug currently being phased out. These results show that *A. remota* has potential antimalarial compounds, which need further evaluation to determine activity against human malaria parasites.
Keywords: Chloroquine, Crude plant extracts, Malaria, *Plasmodium berghei*, Parasitaemia,

INTRODUCTION

Malaria remains a common tropical disease in developing countries, although it has been eradicated or controlled in the developed nations. It remains a common disease in southeast 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 deaths. Farooq (2004) reported that sub-Saharan Africa and countries in tropical Africa account for more than 90% of total malaria incidences and great majority of deaths due to the disease. The Democratic Republic of the Congo and Nigeria account for over 40% of the estimated total 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 (ACT) 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 regions 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 of falciparum malaria patients at the Thai-Cambodian border in 2009, showing that the parasite cleared slowly from the patients' blood after the ACT treatment without corresponding reduction in in-vitro susceptibility testing (Dondorp *et al*., 2009, as cited in Krungkrai *et al*., 2010).

It is not only the development of resistance by malaria parasite to ACT that is a hindrance to treatment of malaria, but ACT availability is also a problem. 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 fever cases in children aged less than 5 years treated with ACT 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 development 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 result reinforces the need for continued research to identify and

develop new anti-malaria drugs to pre-empt any crisis that may result during use of current drugs. 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, suitable for small children and pregnant women, have appropriate formulations for oral use and, above all, be affordable (Peters, 2002 as cited in Fidock, 2004).

Ajuga remota is a herb which grows widely in East Africa. In Kenya, the leaves are pounded and steeped in cold water and the infusion drunk as a remedy for fever, malaria, toothache, dysentery and treatment of high blood pressure (Kokwaro, 1976). Traditionally, it is believed by many communities in East Africa to be antimalarial. *Ajuga remota* is reported as the most used traditional medicine in the treatment of malaria in Kenya (Kuria *et al.,* 2001 as cited in Muthaura *et al*., 2007). In Kenya, the Luhya call it *Mataliha* and the Kikuyu call it *Wanjiru wa Rurii* (Kokwaro, 1976)

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* that 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 that the *A. remota* isolated ajugarin IV structure determined by spectroscopic and chemical data means has insecticidal activity against the insect *Bombyx mori* at 500 ppm, but only growth inhibitory activity against the 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.

Antihypertensive 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 10 mg/L in drinking water to experimentally hypertensive rats lowered blood pressure by 40 mmHg and 50 mmHg, respectively.

The present study aimed at the development of new antimalarial drugs from indigenous plants by testing the antimalarial activity of the crude extracts from the various morphological parts of the *A. remota* species.

MATERIAL AND METHODS

Ecology of the Plant in Kenya

Ajuga remota (Figure 1) grows in many parts of the Kenya in low lying areas with humid soils. It has been found growing in Nakuru, Kisumu, Kirinyaga, Nairobi and Nyandarua Counties (Kariuki, 2014, personal communication).

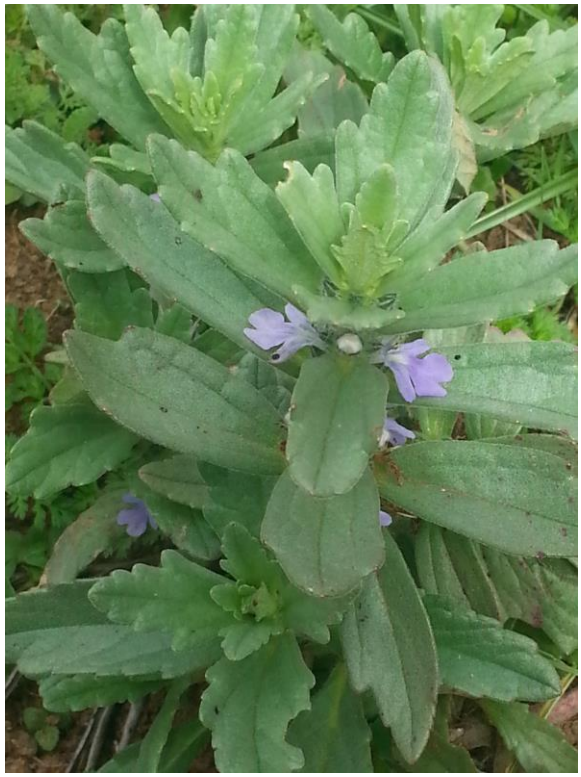

Figure 1: *Ajuga remota* plant (Source: Author 2014)

Extract Preparation

The plant materials were collected from Limuru (Kiambu County) and Njoro (Nakuru County) where they readily grow in great amounts. They were detached off their individual parts and the experiment was then divided into two parts.

The various detached parts of the wet plants' leaves 578.95 g, stems 300.00 g, roots 219.02 g and flowers 100 g were extracted by boiling separately in water for two hours at 90^{o}C. The crude extracts were then filtered and the various filtrates were freeze-dried.

The remaining detached parts were dried under shade in the laboratory on clean trays placed on benches for 21 days until consecutive constant weights, namely leaves 362.96 g, stems 596.05 g, roots 423.52 g and flowers 66.75 g were obtained. They were then exhaustively extracted by boiling separately in water for a duration of two hours at 90^{o}C. After filtration, the various filtrates were then freeze-dried.

In Vivo Antimalarial Test

Swiss mice weighing 20-25 g each and bred locally in the animal house of Kenyatta National Hospital Laboratory (KNHL) were used. 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 and some water.

A single donor mouse was bled into sterile heparinized culture medium and centrifuged for five minutes at 350 g's. After aspirating the supernatant, 0.4*PRBC volume of Glycerolyte '57' was added over two minutes interval with gentle shaking. It was then frozen at -70^{o}C in 0.4 ml aliquots. This was then thawed rapidly in hand and placed in 15 ml centrifuge tube, while slowly adding 0.1 ml of 12% NaCl and then left to stand for two minutes.

Thereafter, 10 ml of 1.6% NaCl was slowly added over two minutes period, vortexed gently and then left to stand for five minutes. After centrifuging at 350 g's for five minutes, the supernatant was aspirated and 10 ml of 0.9% NaCl, 0.2% Dextrose slowly added and left to stand for five minutes followed by centrifuging for five minutes. The supernatant was aspirated and the desired volume for infection made with RPML 1640 medium.

The 0.2 ml diluted blood, containing 1 x 10^7 parasitized (*P. berghei*) red blood cells, were injected intravenously via a tail vein into healthy mice. A single donor mouse was used to infect all the animals to minimize variability in the induced parasitaemia. The day of infection was termed "D0" and subsequent days were identified as "D1", "D2" etc. Each drug was administered in 0.2 ml solution per mouse as a single daily dose intravenously for four days. The Chloroquine was crushed into powder and diluted before intravenous injection.

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 red blood cells (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 mouse were calculated using the following formula:

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

Evaluation of antimalarial Activity

The four day technique employed here was similar to that described by Peters *et al*. (1975). Infected mice were divided into groups of eight mice. The crude aqueous extracts from *A. remota*, both wet and dry were given intravenously to the mice. Each mouse received a dose of 30 mg/kg day^{-1} (equivalent to 0.2 ml solution per mouse) for four consecutive days. Parallel tests with Chloroquine (a standard antimalarial drug) were conducted for reference purposes. The drugs were administered intravenously (injections with microlitre syringes). Tail blood film was taken from each mouse on D4. These were stained with Giemsa stain and percentages suppression of parasitaemia in relation to the control was calculated using the following formula:

$$\text{Av \% suppression} = \frac{\text{Av \% P in untreated ctrls} - \text{AV. \% P treated groups}}{\text{Av. \% P in untreated ctrls}} \times 100$$

Where: Av % P = Average % Parasitaemia

RESULTS AND DISCUSSIONS

The crude aqueous extracts of *Ajuga remota* had higher antimalarial activity than Chloroquine in this four-day test using sensitive strain of *P. berghei* in mice. The different parts of *A. remota* with the wet and dry plant preparations had different suppressive activities against *P. berghei* parasites in mice. The leaves had the highest antimalarial activity compared to the stems, roots and flowers for both wet and dry parts. Chloroquine suppression of parasitaemia was less that of the wet leaves extract.

The F-test was used to test the difference in parasitaemia level of the plant parts and the control (no drug) at 5% level of significance. The F-values for the dry plant parts were: 20.00 (leaves), 2.06 (stems), 2.78 (roots) and 0.71 (flowers). For the wet parts the F values were: 32.98 (leaves), 2.41 (stems), 5.03 (roots) and 2.92 (flowers).

Table 1: The parasitaemia suppression levels of *P. berghei* in mice by crude extract preparation of *A. remota*

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

The parasitaemia of dry and wet leaves and wet roots were significantly different from the control as the F values were higher than the tabulated F (4.54). The results indicated 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 Chloroquine.

This study confirmed the potency of the plant as noted by Kubo *et al*. (1981) against insects and hypertension. It also confirmed the knowledge of the communities of Kenya who use it against malaria as reported by Kokwaro (1976). It compares well with the activity of *A. bracteosa* against *P. berghei* parasite (Chandel, and Bagai, 2010). Kasa *et al*. (1998) also reported effectiveness of ethanolic extract of *A. remota* against *P. falciparum* in in-vitro studies.

CONCLUSION

The present study with the crude aqueous extracts of *A. remota* indicates that the plant has considerable antimalarial potential. This is a confirmation of the knowledge of malaria treatment by traditional practitioners in Kenya.

RECOMMENDATIONS

It is recommended that more research 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 using *A. remota*.

Also more research should be done to produce a product which can be used as an alternative to synthetic conventional drugs in the fight against malaria as has already happened with Artemisia.

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