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Preliminary *in vivo* Antimalarial Screening of Petroleum Ether, Chloroform and Methanol Extracts of Fifteen Plants Grown in Nigeria

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Abstract: Fifteen plants were screened for *in vivo* antimalarial activity in albino mice. The plants are *Mormodica balsamina*, *Artemisia maciverae*, *Xylopia aethiopica*, *Cyperus articulatus*, *Guiera senegalensis*. *Syzygium aromaticum*, *Zingiber officinale*, *Thonningea sanguinea*, *Sorghum* sp., *Securinega virosa* B, *Chrozophora senegalensis*, *Feretia apodanthera*, *Diospyrous mespiliformis*, *Centaturea perrottetti* and *Acacia nilotica* Del. The petroleum ether, chloroform and methanol extracts from the various parts of the plants were screened for *in vivo* antimalarial activity in mice experimentally infected with *Plasmodium berghei*. Three days after inducing the malaria, the plant extracts were administered intraperitoneally to the mice daily for four days, while chloroquine was used as a standard drug control. Parasitaemia was monitored microscopically in all the groups for four days using thick and thin blood films obtained from tail vein of each mouse. At the end of this study, it was observed that the chloroform extracts of *Artemisia maciverae* (whole plant), *Xylopia aethiopica* (fruits) and *Acacia nilotica* Del (Leaves) have antimalarial activity. The methanol extracts of *Syzygium aromaticum* (cloves) and *Zingiber officinale* (tuber stem) showed slight antimalarial activity, while the rest of the plant extracts earlier listed showed no noticeable activity. These results suggest that many plants used as recipes in ethnomedical preparation for malaria, have no direct antimalarial activity.

Key words: Antimalarial, medicinal plants, *in vivo* study, *Plasmodium berghei*, Nigeria

INTRODUCTION

Malaria is a mosquito-borne disease caused by the parasite plasmodium. Four identified species of this parasite, which cause different types of human malaria include *Plasmodium vivax*, *Plasmodium falciparum*, *Plasmodium ovale* and *Plasmodium malariae*, all of which are transmitted by the female anopheles mosquito (Hardman and Limbird, 2001). Although all the four species of malaria parasites can infect humans and cause illness, only the malaria caused by *Plasmodium falciparum* is known to be potentially life threatening in humans. Infection with *P. falciparum* is therefore a medical emergency. About 2% of persons infected with *Falciparum* malaria die usually because of delayed treatment (Peter and Anatoli, 1998).

The present global situation indicates a recent resurgence in the severity of the disease and that malaria could still be described as one of the most important diseases, with an annual incidence of 300-500 million clinically manifest cases and a death toll of 1-2 million people (Martin *et al*., 2004; Miller *et al*., 1994; More, 2002; David *et al*., 2004). Mortality and morbidity due to malaria are a

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matter of great concern throughout the whole world, especially in tropical and subtropical regions. Even though casualty in children below the age of 5 years is very high, the disease affect all age groups (Bickii *et al*., 2000).

In earlier times, the common oral treatment for uncomplicated *falciparum* malaria was limited to chloroquine and quinine sulfate, mefloquine, doxcycline, sulfadoxine/pyrimethamine (SP) in chloroquine resistant cases (Williams *et al*., 2004; Laxminarayan, 2004).

One of the most important factors limiting success in the treatment of malaria, whether for preventive or for curative purposes, is the varying response of individuals parasites to the drug used. Drug resistance has emerged as one of the greatest challenges facing malaria control today (Trape, 2002). It has been implicated in enhanced mortality from malaria in hyper and holoendemic areas and in the development of new and expanding foci of falciparum malaria, but above all, it has been identified as a factor in the economic constraint of malaria control. Emerging and spreading resistance to an increasing number of antimalarial drugs has been a major concern especially in Asia, Africa and South America (Sibley, 2001). Chloroquine, though effective as a blood schizontocidal drug, is ineffective or partially effective in resistant cases. The emergence of strains of *Plasmodium falciparum* resistant to chloroquine and many other drugs in succession has stimulated efforts to identify new antimalarial agents (Bickii *et al*., 2000).

The need for an alternative drug initiated intensive efforts for developing new antimalarials from indigenous plants (Wright and Phillipson, 1990). This led to the diversification of drug research into medicinal plants. For example, *in vitro* antimalarial activity of leaf extracts of *Guiera senegalensis* on *Plasmodium yoelii nigeriensis* (Iwalewa *et al*., 1990), as well as *in vivo* and *in vitro* antimalarial activity of two crude extracts of *Cassia occidentalis* leaf (Iwalewa *et al*., 1997) had been reported. In the present study, fifteen plants grown in Nigeria were screened *in vivo* for antimalarial activity.

MATERIALS AND METHODS

Plant Collection and Sample Preparation

Fifteen plants commonly found in Maiduguri, Nigeria were used for this study. The plants are: *Mormodica balsamina*, *Artemisia maciverae*, *Xylopia aethiopica*, *Cyperus articulatus*, *Guiera senegalensis*, *Syzygium aromaticum*, *Zingiber officinale*, *Thomningea sanguinea*, *Sorghum* sp., *Securinega virosa* B, *Chrozophora senegalensis*, *Feretia apodanthera*, *Diospyrous mespiliformis*, *Centaurea perrotteti* and *Acacia nilotica* Del. The various parts of these plants ie leaves, stem bark, roots, fruits and whole plants were collected from Maiduguri, North-Eastern Nigeria. All the plants were identified by a Botanist from the Department of Biological Sciences, University of Maiduguri, Nigeria. This study was carried out at the Biochemistry Department of Ahmadu Bello University Zaria, Nigeria, between April and October, 2006.

All the plant parts were air dried and ground into powdered form before extraction in the following order: ie petroleum ether-chloroform-methanol. The extracts of the various plant parts were kept in a tightly closed glass bottle in a refrigerator at 4°C until used for antimalarial testing.

Plasmodium berghei Parasite

The *Plasmodium berghei* used for this study was obtained from the Biochemistry Department of Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria. The parasite was maintained by sub-passaging into healthy mice on a weekly basis throughout the duration of the study.

The infection of the recipient mice was initiated by needle passage of the above mentioned parasite preparation, from the donor to healthy test animals via an intraperitoneal route (David *et al*.,

2004; Peter and Anatoli, 1998). That is *P. berghei* infected red blood cells were intraperitoneally injected into the mice from the blood diluted with Phosphate Buffered Saline (PBS) so that each 0.2 mL had approximately 10^6-10^7 infected red cells (parasite per kg of body weight). Each mouse was infected with single inoculums of 0.2 mL blood.

***In vivo* Antimalarial Tests**

Tests were performed using a 4-day curative standard test (David *et al*., 2004; Peter and Anatoli, 1998) and employing the rodent malaria parasite- *Plasmodium berghei*.

The mice were divided into groups. Three mice were used for each of the 69 test/treatment groups, chloroquine (CQ) control and negative control groups. All the mice were infected with the malaria parasites as described above.

Seventy two hours after infecting the mice with the malaria parasites, the plant extracts were administered to the test groups at a dose level of 100 mg kg^{-1} body weight daily for four days. Chloroquine was administered to the CQ standard (control) group at the standard dose of 25 mg kg^{-1} b.wt. for four days. The negative control group were not treated. All drug administration was done through the intraperitoneal route. The dose level of 100 mg kg^{-1} b.wt. of the extract was selected from a pilot study carried out in mice and earlier studies (Teferi and Heinz, 2002).

The extracts were dissolved to the indicated suitable dose level in solution and suspension, the later requiring total dissolution in 3% v/v Tween 80. Treatments were performed daily for 4 consecutive days starting 72 h after infection, receiving a total of 4 intraperitoneal doses (David *et al*., 2004).

The parasitemia was monitored in all the groups for four days using thick and thin smears of blood films made from tail vein of mice (David *et al*., 2004; WHO, 1980). The smears were stained with 10% Giemsa at pH 7.2 for 15 min and examined under the microscope to access the level of parasitemia. The percentage parasitemia was calculated according to the method outlined by Iwalewa *et al*. (1997) as:

$$\text{Percentage parasitemia} = \frac{\text{No. of parasitemia in treated}}{\text{No. of parasitemia in control}} \times \frac{100}{1}$$

All the results were compared with the untreated control using student t-test.

RESULTS

Results obtained showed that normal mice infected with *P. berghei* and not treated died within 7-10 days. While infected mice treated with chloroquine survived. On the other hand, all infected mice treated with the chloroform medicinal plant extracts of *Artemisia maciverae* (whole plant), *Xylopia aethiopica* (fruits) and *Acacia nilotica* Del (Leaves) showed clearance of the parasite to a higher level when compared with the untreated control (Table 2), though the parasitemia was not completely cleared (Table 1). This did not lead to their survival, they died after about four weeks of drug administration.

The methanol extracts of *Syzyguim aromaticum* (cloves) and *Zingiber officinale* (tuberstem) showed slight antimalarial activity when compared with untreated control (Table 3). Their extracts cleared the parasites to a minimal level. The remaining plant extracts showed no activity at all (Table 1, 2, 3).

Table 1: *In vivo* screening of petroleum ether extract for anti-malarial activity

Plant species	Part	Extract (Solvent)	Drug dose (mg kg^{-1})	Parasitemia (%)	Activity/ Inference
Mormodica balsamina	Whole plant	Pet. ether	100	4.20±0.12	Not active
Artemicia maciverae	Whole plant	Pet. Ether	100	4.10±0.06	Not active
Xylopia aethiopica	Fruits	Pet. ether	100	3.80±0.19	Not active
Cyperus atrticulatus	Fruits	Pet. Ether	100	4.20±0.15	Not active
Guiera senegalensis	Leaves	Pet. ether	100	4.10±0.06	Not active
Syzygium aromaticum	Cloves	Pet. Ether	100	3.70±0.06	Not active
Zingiber offcinale	Tuber stem	Pet. ether	100	3.40±0.15	Not active
Thonningea sanguinea	Fruits	Pet. Ether	100	4.20±0.06	Not active
Sorghum sp.	Leaves	Pet. ether	100	3.80±0.15	Not active
Sorghum sp.	Roots	Pet. Ether	100	4.10±0.06	Not active
Securinega virosa B.	Leaves	Pet. ether	100	3.90±0.09	Not active
Securinega virosa B.	Stem bark	Pet. Ether	100	3.80±0.06	Not active
Securinega virosa B.	Roots	Pet. ether	100	4.20±0.00	Not active
Chrozophora sengalensis	Whole plant	Pet. Ether	100	4.10±0.06	Not active
Feretia apodanthera Del	Stem bark	Pet. ether	100	3.70±0.06	Not active
Feretia apodanthera Del	Roots	Pet. ether	100	3.90±0.06	Not active
Diospyrous mespiliformis H	Leaves	Pet. Ether	100	3.90±0.06	Not active
Diospyrous mespiliformis H	Stem bark	Pet. ether	100	4.10±0.07	Not active
Diospyrous mespiliformis H	Roots	Pet. Ether	100	3.80±0.06	Not active
Centaturea perrotteti	Whole plant	Pet. ether	100	4.20±0.20	Not active
Acacia nilotica Del	Stem bark	Pet. Ether	100	3.70±0.06	Not active
Acacia nilotica Del	Leaves	Pet. ether	100	3.60±0.12	Not active
Acacia nilotica Del	Roots	Pet. Ether	100	3.80±0.12	Not active
Untreated control				4.50±0.17	
Chloroquine standard (Control)				0.00	

All values were compared with the untreated control at p = 0.05, n = 3

Table 2: *In vivo* screening of chloroform extract for anti-malarial activity

Plant species	Part	Extract (Solvent)	Drug dose (mg kg^{-1})	Parasitemia (%)	Activity/ Inference
Mormodica balsamina	Whole plant	Chloroform	100	3.40±0.12	Not active
Artemicia maciverae	Whole plant	Chloroform	100	0.20±0.06^{a}	Active
Xylopia aethiopica	Fruits	Chloroform	100	0.40±0.06^{a}	Active
Cyperus atrticulatus	Fruits	Chloroform	100	4.10±0.17	Not active
Guiera senegalensis	Leaves	Chloroform	100	4.40±0.23	Not active
Syzygium aromaticum	Cloves	Chloroform	100	3.40±0.12	Not active
Zingiber offcinale	Tuber stem	Chloroform	100	4.10±0.06	Not active
Thonningea sanguinea	Fruits	Chloroform	100	3.80±0.06	Not active
Sorghum sp.	Leaves	Chloroform	100	4.10±0.06	Not active
Sorghum sp.	Roots	Chloroform	100	3.70±0.06	Not active
Securinega virosa B.	Leaves	Chloroform	100	4.20±0.12	Not active
Securinega virosa B.	Stem bark	Chloroform	100	3.90±0.12	Not active
Securinega virosa B.	Roots	Chloroform	100	4.10±0.06	Not active
Chrozophora sengalensis	Whole plant	Chloroform	100	3.80±0.12	Not active
Feretia apodanthera Del	Stem bark	Chloroform	100	4.10±0.06	Not active
Feretia apodanthera Del	Roots	Chloroform	100	3.80±0.23	Not active
Diospyrous mespiliformis H	Leaves	Chloroform	100	4.00±0.00	Not active
Diospyrous mespiliformis H	Stem bark	Chloroform	100	3.90±0.12	Not active
Diospyrous mespiliformis H	Roots	Chloroform	100	4.10±0.06	Not active
Centaturea perrotteti	Whole plant	Chloroform	100	4.20±0.12	Not active
Acacia nilotica Del	Stem bark	Chloroform	100	3.80±0.06	Not active
Acacia nilotica Del	Leaves	Chloroform	100	0.60±0.06^{a}	Active
Acacia nilotica Del	Roots	Chloroform	100	3.90±0.06	Not active
Untreated control				4.30±0.21^{bc}	
Chloroquine standard (control)				0.00	

All values were compared with the untreated control at p = 0.05, n = 3. All values with the superscript a are statistically different from the untreated control bc

Table 3: *In vivo* screening of methanol extract for anti-malarial activity

Plant species	Part	Extract (Solvent)	Drug dose (mg kg^{-1})	Parasitemia (%)	Activity/ inference
Mormodica balsamina	Whole plant	Methanol	100	3.80±0.15	Not active
Artemicia maciverae	Whole plant	Methanol	100	3.60±0.12	Not active
Xylopia aethiopica	Fruits	Methanol	100	3.20±0.06	Not active
Cyperus atrticulatus	Fruits	Methanol	100	4.20±0.12	Not active
Guiera senegalensis	Leaves	Methanol	100	4.30±0.06	Not active
Syzygium aromaticum	Cloves	Methanol	100	1.00±0.12[d]	Slightly active
Zingiber officinale	Tuber stem	Methanol	100	1.00±0.16[d]	Slightly active
Thonningea sanguinea	Fruits	Methanol	100	4.20±0.12	Not active
Sorghum sp.	Leaves	Methanol	100	3.80±0.12	Not active
Sorghum sp.	Roots	Methanol	100	3.90±0.06	Not active
Securinega virosa B.	Leaves	Methanol	100	4.10±0.06	Not active
Securinega virosa B.	Stem bark	Methanol	100	4.20±0.12	Not active
Securinega virosa B.	Roots	Methanol	100	3.90±0.06	Not active
Chrozophora sengalensis	Whole plant	Methanol	100	4.10±0.00	Not active
Feretia apodanthera Del	Stem bark	Methanol	100	3.90±0.06	Not active
Feretia apodanthera Del	Roots	Methanol	100	4.00±0.00	Not active
Diospyrous mespiliformis H	Leaves	Methanol	100	4.20±0.06	Not active
Diospyrous mespiliformis H	Stem bark	Methanol	100	3.80±0.31	Not active
Diospyrous mespiliformis H	Roots	Methanol	100	4.00±0.12	Not active
Centaturea perrotteti	Whole plant	Methanol	100	4.10±0.15	Not active
Acacia nilotica Del	Stem bark	Methanol	100	3.70±0.06	Not active
Acacia nilotica Del	Leaves	Methanol	100	3.60±0.12	Not active
Acacia nilotica Del	Roots	Methanol	100	4.00±0.29	Not active
Untreated control				4.40±0.12[ef]	
Chloroquine standard (control)				0.00	

All values were compared with the untreated control at p= 0.05, n = 3. All values with the superscript d are statistically different from the untreated control ef

DISCUSSION

The antimalarial activity observed with the chloroform extracts of the whole plant of *Artemisia maciverae*, fruits of *Xylopia aethiopica* and Leaves of *Acacia nilotica* Del might be attributed to the presence of some active ingredients in these plants. Also the slight antimalarial activity observed with the methanol extracts of *Syzyguim aromaticum* (cloves) and *Zingiber officinale* (tuber stem) might be due to the presence of minute quantity of some active ingredients. Some phytochemicals have been reported to be present in the above mentioned plant extracts. In the extracts of the *Artemisia maciverae*, the presence of artemisinin and 1,2,4- trioxane analogs have been reported (Rajendra *et al*., 2002). Diterprene kaurenoids have been reported to be present in *Xylopia aethiopica* fruits (Samova *et al*., 2001). Saponins and triterpenes have equally been reported to be present in the extracts of *Acacia nilotica* Del (Kamalijit *et al*., 2002). In *Syzygium aromaticum* clove, the presence of eugenol, thymol and benzyl alcohols were reported (Lee and Shibamoto, 2001), while *Zingiber officinale* was found to contain antioxidant cyclic diaryheptanoid (He, 2001). The presence of these phytochemicals in these plants might be responsible for the antimalarial activity exhibited by them. This can be supported by the studies carried out by Badman *et al*. (1988) and Klayman (1985) which show conclusively that artemisinine the antipyretic principle/ingredient of plant *Artemisia annua*, possess antimalarial activity.

The slight antimalarial activity shown by the methanol extract of *Syzyguim aromaticum* (cloves) after about 10 days of drug administration has stopped might be attributed to the fact that the extract boosted the immune response of the animals thereby giving the capacity to handle the parasites (Abebe *et al*., 2003; Kiseko *et al*., 2000).

In a similar study carried out by Ajaiyeoba *et al*. (1999) on two Nigerian simaroubaceae plants, *Quassia amara* L. and *Quassia undulata* were screened *in vivo* in mice for antimalarial properties using

hexane and methanol extracts. The extracts at a concentration of 100 mg kg^{-1} body weight (b.wt.) of mouse showed significant antimalarial activities in the 4 day suppressive *in vivo* antimalarial assay in mice inoculated with red blood cells parasitized with *Plasmodium berghei*. This is in support of the antimalarial activity observed with the chloroform extracts of the whole plant of *Artemisia maciverae*, fruits of *Xylopia aethiopica*, leaves of *Acacia nilotica* Del and methanol extracts of *Syzygium aromaticum* (cloves) and *Zingiber officinale* at a dose concentration of 100 mg kg^{-1} b.wt.

Mice infected with the parasite and not treated, showed fulminant parasitemia which resulted in death 7-10 days later. This is in agreement with the work of Agomo *et al*. (1992).

The results suggest that many plants used as recipes in ethnomedical preparation for malaria, have no direct antimalarial activity and have also indicated that some plants grown in Nigeria were not previously in the list of antimalarial plants are now known to have antimalarial effect. That plant may be sources of potent antimalarial agents have previously been established by other workers (Iwalewa *et al*., 1997).

There may be geographical, regional and specie differences in medicinal efficacy of plants. This justifies our inclusion of the plant *Artemisia maciverae* in our list of plants, although *Artemisia annua*, another specie within the genus has been extensively studied in Asia, particularly in China. Currently we are conducting a detailed study to establish the antimalarial potency, toxicity and possible mechanism of action of *Artemisia maciverae* and other plants that have showed significant antimalarial activity in this study.

CONCLUSION AND RECOMMENDATIONS

The results of the preliminary studies carried out with the above mentioned plants are encouraging. The scope for developing antimalarials from indigenous plants depends on screening of a large number of medicinal plants from different geographical regions, especially from the tribal/rural areas, where usage of these medicinal plants is more common. Once the anti-plasmodial effect of the plant is confirmed, the active ingredients could be isolated by different extraction methods.

Since the chloroform extracts of *Artemisia macivae* (whole plant), *Xylopia aethopica* (fruits) and *Acacia nilotica* Del (Leaves) showed a high level of antimalarial activity, more studies are currently being undertaken by our team to establish the antimalarial component ie active ingredient distribution and quantity and toxicological potential in malaria therapy.

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