

Full Length Research Paper

In vivo antiplasmodial effect of chloroform extracts of *Artemisia maciverae* Linn and *Artemisia maritima* Linn

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Accepted 7 August, 2009

Chloroform extracts of *Artemisia maciverae* and *Artemisia maritima* (whole plants) were tested *in vivo* for anti-malarial activity in Swiss albino mice experimentally infected with chloroquine resistant *Plasmodium berghei* NK 65 at a dose of 100 mg/kg. The 2 plant extracts showed high anti-malarial activity. The parasitemia in the infected mice treated with the extracts were significantly reduced ($P < 0.05$) when compared with the untreated negative control and the pre-treatment period (Day 0). The plant extracts were also screened for phytochemicals and secondary metabolites. Some phytochemicals like flavonoids, steroids, terpenoids, tannins, phlobatannins, alkaloids and anthraquinones were detected in the two plant extracts. The anti-malarial activity of these extracts might be attributed to these phytochemicals/secondary metabolites. This study suggests that antiplasmodial activity of *Artemisia* may be widely distributed within the genus.

Key words: Malaria therapy, *Plasmodium berghei*, *Artemisia maciverae*, *Artemisia maritima*, medicinal plants.

INTRODUCTION

Malaria is one of the most prevalent infections in the world. It constitutes one of the main causes of death in much of the tropics. Malaria is caused by parasites of the genus *Plasmodium*, transmitted by female *Anopheles* mosquitoes. The most severe form of malaria is caused by *Plasmodium falciparum* and this species is responsible for over a million deaths each year (Breman, 2001). In tropical and subtropical countries, malaria is the most important parasitic disease of man. A delay in diagnosis and treatment can have fatal consequences. The present global situation indicates a recent resurgence in the severity of the disease, that malaria could still be described as one of the most important diseases, with an annual incidence of 247 million clinically manifest cases and a death toll of 881,000 people (Martin et al., 2004; Miller et al., 1994; More, 2002; David et al., 2004; Breiger, 2009). Mortality and morbidity due to malaria are

a matter of great concern throughout the world, especially in tropical and subtropical regions. Though casualty is heaviest among children below the age of 5 years, the disease remains a major cause of death in all age groups in the affected geographical areas (Bickii et al., 2000). Part of the reason for the failure to control malaria is the emergence and spread of resistance to first-line anti-malarial drugs, cross-resistance between the members of the limited number of drug families available and in some areas, multi-drug resistance (Bickii et al., 2000).

A major breakthrough of the past decades has been the discovery by Chinese researchers of artemisinin (qinghaosu), an endoperoxide sesquiterpene lactone, as the active component of *Artemisia annua*, a herbal remedy used in Chinese folk medicine for over 2000 years. This molecule and its oil-soluble artemether and arteether and water soluble artesunate semi-synthetic derivatives have shown excellent antiplasmodial efficacy *in vivo* and are being used increasingly, especially in combination with traditional anti-malarials (Ernesto et al., 2002). Few other species of *Artemisia* especially *Artemisia absinthium* (Zafar et al., 1990) *Artemisia parviflora*

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Table 1. Yield of *A. maciverae* and *A. maritima* extracts.

Plants	Solvents	Weight of extracts (g)	Yield (%)
<i>Artemisia maciverae</i>	Petroleum ether	1.39 ± 0.11	6.95
<i>Artemisia maciverae</i>	Chloroform	2.14 ± 0.08	10.70
<i>Artemisia maritima</i>	Petroleum ether	0.86 ± 0.04	4.30
<i>Artemisia maritima</i>	Chloroform	0.47 ± 0.12	2.35

(Badam et al., 1988), *Artemisia nilagarica* and *Artemisia japonica* have also been shown to have antimalarial activities (valecha et al., 1994).

Artemisia maciverae Linn is a small herbaceous plant belonging to the family Asteraceae and is found in the northern part of Nigeria where it is locally known as "Tazargade" in Hausa. The Hausa people of northern Nigeria use this plant in treating malaria when it is boiled in water with lemon and red potash or soaked in local gin to treat malaria (Ene et al., 2008a). Our preliminary work suggested that this plant possesses high anti-malarial activity (Ene et al., 2008c). Another species known as *Artemisia maritima* as the name suggests is found in the riverine areas. It is locally known in Hausa as "Babamore". and used in treating ailments like malaria and ulcer. This plant also belongs to the family Asteraceae.

In Northern Nigeria, *Artemisia maciverae* and *A. maritima* are used interchangeably in the treatment of malaria. Therefore, these 2 species of *Artemisia* grown in Nigeria, were compared *in vivo* for antiplasmodial activity.

MATERIALS AND METHODS

Animal and animal husbandary

Swiss albino mice weighing between 20 – 30 g were used for this study. The animals were obtained from Faculty of Pharmaceutical sciences, Ahmadu Bello University, Zaria and housed in the animal house of Department of Biochemistry. The animals were fed ad libitum on water and a standard diet (Vital Feeds, Jos, Nigeria).

Plants

The whole plants of *A. maciverae* and *A. maritima* used for this study were collected in Zaria and identified by a Taxonomist at the Herbarium Section of the Department of Biological Sciences, Ahmadu Bello University, Zaria. Zaria lies within Latitude 11°3'N and Longitude 7°42' E.

Extract preparation

The whole plants of *A. maciverae* and *A. maritima* were air dried in the laboratory for about 2 weeks and ground into powder. 20 g each of the powdered samples was weighed into extraction tumbler and placed in a soxhlet extractor. The plant samples were extracted with petroleum ether for 6 h and with chloroform for another 6 h. The percentage yield of each of the plant sample was calculated (Table 1). The chloroform extracts were then stored in the refrigerator at -4°C, ready for use.

Chloroquine resistant *Plasmodium berghei* parasite

The chloroquine resistant *P. berghei* NK 65 used for this study was developed at the Post graduate Laboratory of the Department of Biochemistry of Ahmadu Bello University, Zaria using the method of Ene et al. (2008b). The parasite was maintained by sub-passaging into healthy mice every 5 - 7 days.

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). Briefly *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 that was injected per kg body weight of mice contained approximately 10 (Breiger, 2009) – 10 (Bickii et al., 2000) infected red blood cells.

In vivo antiplasmodial tests

Tests were performed using a 4 – day curative standard test (David et al., 2004; Peter and Anatoli, 1998; WHO, 1980) and employing the chloroquine resistant *Plasmodium berghei* NK 65.

The mice were divided into 5 groups of 6 mice each. The groups consisted of the 2 chloroform extract/treatment groups, artesunate standard control, chloroquine standard control and untreated control groups. All the mice were infected with the malaria parasites as described above.

24 h after infecting the mice with the malaria parasites and parasitemia confirmed, the plant chloroform extracts were administered to the 2 test groups at a dose level of 100 mg/kg body weight (b.wt) for 4 days. Artesunate was administered to the standard control group at the standard dose of 1.6 mg/kg b.wt for 4 days. The untreated control groups were not treated. All drug administration was done through the intraperitoneal route. The dose level of 100 mg/kg b.wt of the extract was selected from a preliminary study carried out in mice (Ene et al., 2008c).

The extracts were dissolved to the indicated suitable dose level of 100 mg/kg in suspension, which requires total dissolution in 0.3% V/V Tween 80. Treatments were performed daily for 4 consecutive days starting 24 h after infection, receiving a total of 4 intraperitoneal doses (David et al., 2004).

Estimation of parasitemia

The parasitemia was monitored in all the groups starting from day 0 to day 7 using thick and thin smears of blood films made from the tail vein of the mice (David et al., 2004; Ene et al., 2008b). The smears were stained with 10% Giemsa at pH 7.2 for 15 min and examined under the microscope at x100 to assess the level of parasitemia. The percentage parasitemia was calculated according to the method outlined by Iwalewa et al. (1997) as: Percentage parasitemia = (No of parasite in treated / No of parasite in control) x 100.

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Table 2. *In vivo* effect of crude chloroform extracts of *Artemisia maciverae* and *Artemisia maritima* on chloroquine resistant *Plasmodium berghei* NK 65

Drug	n	Drug dose (mg/kg)	Average parasitemia (%)							
			Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
<i>Artemisia maciverae</i>	6	100	0.53±0.07 ^c	0.60±0.09	0.63±0.03	0.43±0.06 ^a	0.30±0.04	0.30±0.04	0.27±0.04	0.27±0.04 ^b
<i>Artemisia maritima</i>	6	100	0.50±0.04 ^c	0.60±0.01	0.60±0.07	0.57±0.08	0.40±0.05	0.40±0.05	0.37±0.06	0.37±0.06 ^b
Artesunate standard (control)	6	1.6	0.43±0.03 ^c	0.13±0.04	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Chloroquine standard (Control)	6	25	0.49±0.04 ^c	3.67±0.10	4.17±0.10	7.97±0.17 ^{bc}	7.93±0.32	8.03±0.62	8.03±0.62	8.03±0.62 ^{ef}
Untreated control	6		0.50±0.07 ^c	2.37±0.17	4.27±0.12	6.90±0.35 ^{bc}	7.93±0.53	8.20±0.60	8.20±0.60	8.20±0.60 ^{ef}

All values were compared with each other on days 0, 3 and 7 at P = 0.05.

n = number of animals in a group.

Values with different superscripts vertically differ statistically (P < 0.05).

Phytochemical analysis of the chloroform extracts

Phytochemical screening of the chloroform extracts of *A. maciverae* and *A. maritima* was carried out to determine the secondary metabolites present therein using standard procedures (Brain and Turner, 1975; Sofowara, 1982; Trease and Evans, 1983). The following were determined; flavonoids, cardiac glycosides, anthraquinones derivatives, carbohydrates, tannins, saponins, phlobatannins and alkaloids.

Statistical analysis

Results are expressed as mean ± standard deviation and the data were compared using Analysis of Variance (ANOVA).

RESULTS

The percentage yield of extracts showed that for *A. maciverae*, more extract was produced with chloroform than with petroleum ether, but for *A. maritima*, more extract was produced with petroleum ether than with chloroform.

Normal mice infected with chloroquine resistant *P. berghei* but not treated died within 4 - 7 days of infection, while infected mice treated with artesunate (1.6 mg/kg b.w) survived (Table 2). On the

other hand, all the infected mice treated with the chloroform extracts of *A. maciverae* and *A. maritima* showed clearance of the parasite to a higher level (that is, from 53 ± 0.07 to 27 ± 0.04 for *A. maciverae* and from 50 ± 0.04 to 37 ± 0.06 for *A. maritima*) when compared with the untreated controls (Table 2)

A statistically significant difference (P < 0.05) was observed between the percentage parasitemia of the infected mice treated with the chloroform extract of *A. maciverae* and untreated infected mice (Table 2). The comparisons were made between days 0, 3 and 7. There was also a statistically significant difference (P < 0.05) between the percentage parasitemia of the infected mice treated with the chloroform extract of *A. maritima* and the infected mice not treated (Table 2). There was however no statistically significant difference (P > 0.05) observed when the infected mice treated with chloroquine was compared with untreated infected mice (Table 2).

Though, the parasitemia was not completely cleared in the tests groups, it was drastically reduced as stated in Table 2. However, this did not lead to their survival as they died within two weeks of extract administration (the parasitemia rose again after being suppressed, when treat-

ment with extract was withdrawn) as opposed to the group on artesunate which survived.

In this study, parasitemia was confirmed in the infected mice after 24 h of infection with the chloroquine resistant *P. berghei*, while in infection with chloroquine sensitive *P. berghei*, parasitemia is usually confirmed after 72 h. This shows that the chloroquine resistant parasite is more virulent than the chloroquine sensitive parasite.

The results of the phytochemical analysis of the extracts showing the presence of some secondary metabolites are presented in Table 3. The results show that the chloroform extract of *A. maciverae* contains flavonoids, steroids, terpenoids, tannins, phlobatannins and alkaloids, while the chloroform extract of *A. maritima* in addition to anthraquinones also contains flavonoids, steroids, terpenoids and alkaloids.

DISCUSSION

There was *in vivo* anti-malarial activity observed with the chloroform extracts of the whole plants of *A. maciverae* and *A. maritima*. The anti-malarial activity observed with the chloroform extracts of these plants might be attributed to the presence of some active ingredients/secondary metabolites

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Table 3. Phytochemical screening of chloroform extracts of *A. maciverae* and *A. maritima*.

Phytochemical	Specific test	<i>A. maciverae</i>	<i>A. maritima</i>
Flavonoids	Shinoda's test	-	-
	Sodium hydroxide test	-	-
	Lead acetate test	+	+
	Ferric chloride test	+	+
Cardiac glycosides	Lieberman Burkard test	+	+
	Salkowskii test	+	+
	Killer-kilianin test	+	+
Anthraquinones derivatives	Test for combined anthraquinones	-	+
	Test for free anthraquinones	-	+
Carbohydrates	Molisch reagent test	+	+
	Barfoed's test	+	-
	Fehling's test	+	+
	Test for combined reducing sugar	+	+
Tannins	Test with ferric chloride solution	+	-
Saponins	Frothing test	-	-
Phlobatannins	Test with dilute hydrochloric acid	+	-
Alkaloids	Dragendorff's tests	+	+
	Wagner's test	+	+
	Mayer's test	+	+

+ = Present; - = absent.

in these plants (Peter and Anatoli, 1998). The chloroform extract of *A. maciverae* was found to contain flavonoids, steroids, terpenoids, tannins, phlobatannins and alkaloids, while in the chloroform extract of *A. maritima*, flavonoids, steroids, terpenoids, anthraquinones and alkaloids were found to be present. The presence of these phytochemicals in these plants, especially alkaloids and terpenoids (Hoet et al., 2004) might be responsible for the anti-malarial 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 artemisinin, the antipyretic principle/ingredient of the plant *A. annua*, possess anti-malarial activity. This artemisinin belongs to sesquiterpene lactone (Ubaleet et al., 1999).

In a similar study carried out by other workers (Ajaiyeoba et al., 1999) on two Nigerian plants belonging to the family simaroubaceae, *Quassia amara* L. and *Quassia undulata* anti-malarial properties were demonstrated when hexane and methanol extracts at a dose of 100 mg/kg body weight (b.wt) of mouse showed significant anti-malarial activities in the 4 – day suppressive *in vivo* anti-malarial assay. Thus is in support of the anti-malarial activity observed with the chloroform extracts of the whole plants of *A. maciverae* and *A. maritima* at a dose concentration of 100 mg/kg b.wt in this study.

In another study (Dikasson et al., 2006) on the plant *Asparagus africanus* Lam using the hydroalcoholic

extracts *in vivo* in *P. berghei* infected mice, the extracts from the roots and aerial parts of the plant were observed to inhibit *P. berghei* parasitemia in the Swiss albino mice by 46.1 and 40.7% respectively. This is also in support of the anti-malarial activity observed with the two plant extracts in this study. It is likely that specific alkaloids and terpenoids are present in all these plants studied by other workers to show their anti-malarial activities.

A. maciverae showed more anti-malarial activity than *A. maritima* in this study. This might be because *A. maciverae* contain specific alkaloids and terpenoids which have better anti-malarial activities compared to *A. maritima*.

Other workers (valecha et al., 1994) have studied the anti-malarial activity of some members of the genus *Artemisia*. Their results show that the ethanol extracts of the aerial parts of *A. Japonica* and *A. nilegarica* inhibited parasitemia at 87.5 and 84.4% respectively. The parasitemia was not completely cleared by these crude ethanol extracts of these plants. This is in agreement with the results obtained with the crude chloroform extracts of *A. maciverae* and *A. maritima* in this study. The crude chloroform extracts of the whole plants of *A. maciverae* and *A. maritima* inhibited parasitemia at 73.0 and 63.0%, respectively. Parasitemia was not completely cleared.

Mice infected with the parasite but not treated, showed fulminant parasitemia which resulted in death 4 – 7 days later. This is in agreement with the work of Ene et al.

(2008a).

Conclusion

From this study, it can be concluded that the chloroform extracts of *A. maciverae* and *A. maritima* (whole plants) possess significant suppressive effects on *P. berghei* infection in Swiss albino mice. Therefore, the chloroform extracts of these two plants are currently under study to establish the active ingredient responsible and its quantitative distribution using bioassay guided isolation. Also under study are their toxicological potentials.

ACKNOWLEDGEMENT

We wish to express our sincere gratitude to Mr. Apeh Y. E. O. of Biochemistry Department and Mr N. Namani of the Department of Mathematics, Ahmadu Bello University Zaria, Nigeria for their assistance during the laboratory work and the statistical analysis, respectively.

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