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Bioassay-guided fractionation and *in vivo* antiplasmodial effect of fractions of chloroform extract of *Artemisia maciverae* Linn

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ABSTRACT

In the search for new plant-derived anti-malarial compounds, chromatographic fractions of chloroform extract of whole plants of *Artemisia maciverae* were tested *in vivo* using chloroquine resistant and chloroquine sensitive *Plasmodium berghei* NK 65 infected Swiss albino mice. One fraction and a sub-fraction of this were most active at 10/mg and cleared parasitemia in mice within 3 days. The different fractions and sub-fractions were tested with different reagents to determine the broad classes of compounds present. The active fraction tested positive for triterpenes and alkaloids, and the sub-fraction for only triterpenes. These tests suggest that the anti-malarial activities observed with these fractions may be due to these classes of compounds in the chloroform extract of the *A. maciverae*. Further chemical work is however required to characterize the active constituents.

1. Introduction

Malaria is one of most prevalent infections in Nigeria and other countries in the tropics and sub-tropics regions of the world. It is caused by parasites of the genus *Plasmodium*, transmitted by female Anopheles mosquitoes (Breman, 2001), which occur as four species, namely, *Plasmodium vivax*, *Plasmodium falciparum*, *Plasmodium ovale* and *Plasmodium malariae* (Hardman and Limbird, 2001). However, the most severe form of malaria is caused by *P. falciparum*. Infection with *P. falciparum* causes vast majority of morbidity and mortality as evidenced by 300–500 million clinical cases annually, resulting in 1.5–2.7 million deaths (Chaiyaroj et al., 2004; Breman, 2001). In endemic areas, clinical manifestations of falciparum malaria range from asymptomatic infection through uncomplicated disease to severe disease and death. The spread of chloroquine-resistant *P. falciparum* is compounding and severely limiting human ability to treat malarial infection (Dawit et al., 2006).

Chloroquine, though effective as a blood schizontocidal drug, is ineffective or only partially effective in resistant cases (Bickii et al., 2000). Emerging and spreading resistance to an increasing number of anti-malarial drugs has been a major concern especially in Asia, Africa and South America (Sibley, 2001), where the rise in the number of drug resistant parasites has been hampered effective chemotherapy.

This increasing resistance of *P. falciparum* to available anti-malarial agents has made the identification of new active compounds and diversification of anti-malarial drug research into medicinal plants a matter of urgent priority (Ridley, 2002).

For instance, some workers have studied the *in vitro* anti-malarial activity of bioassay-guided fractions of *Artemisia afra* against *P. falciparum* (Abrahams et al., 1996). Similarly, our earlier investigation showed that crude chloroform extracts of both *Artemisia maciverae* and *Artemisia maritima* harvested from Northern Nigeria possess strong anti-plasmodial effect *in vivo* (Ene et al., 2008a), but it is not yet known what broad classes of phytochemicals might be responsible for this activity. Therefore, to characterize the therapeutically active component of *A. maciverae*, the chloroform extract was subjected to bioassay-guided fractionation and analyzed by reagent-based chemical tests.

2. Materials and methods

2.1. Plant collection and sample preparation

A. maciverae plant was collected from Zaria and identified by a Taxonomist at the Herbarium Section of the Department of Biological Sciences, Ahmadu Bello University, Zaria where a voucher

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specimen with reference number 70763 was deposited. This study was carried out at Department of Biochemistry, Ahmadu Bello University Zaria, Nigeria, between July 2007 and March 2008. The whole plants of *A. maciverae* were air dried at room temperature for 2 weeks and ground into powder.

2.2. Extraction procedure

A. maciverae powder (20 g) was first extracted with 250 ml of petroleum spirit (60–80% boiling range) for 6 h, then followed with chloroform for another 6 h. Total extract yield was recorded, percentage yield calculated and extracts stored in the refrigerator at 4 °C until required. The chloroform extract of *A. maciverae* was evaluated *in vivo* for anti-malarial activity using chloroquine resistant *Plasmodium berghei* NK 65 prior to bioassay-guided fractionation.

2.3. Bioassay-guided fractionation of chloroform extract of Artemisia maciverae

Based on the results of exploratory TLC, the chloroform extract was fractionated on activated aluminium oxide and eluted sequentially with 3.0 l of diethyl ether:petroleum ether (1:1), 1.7 l of chloroform and 1.0 l of methanol. The aluminium oxide was activated by heating in the oven at 100 °C for 2 h. Three quarter of the glass column (80.5 cm in length and 1.7 cm in diameter) was filled with the aluminium oxide slurry. Top layer was covered with treated sand followed by slurry of 1.88 g of the chloroform extract of *A. maciverae*. The extract was then eluted with the various solvent combinations. Fifty-seven different fractions of 100 ml each were collected from the column. The fractions were concentrated using rota-evaporator at 40 °C.

Dried fractions were re-dissolved in their respective eluants, spotted on activated pre-coated aluminium oxide TLC plates (Merck), and developed using diethyl ether:petroleum ether (1:1). The plates were viewed under UV light at 254 nm. The fractions with the same Rf values were pooled together. This reduced the number of fractions from 57 to 7. These pooled fractions with Rf values of 0.69, 0.77, 0.83, 0.38, 0.44, 0.54 and 0.40 were screened *in vivo* for anti-malarial activity using chloroquine resistant *P. berghei* NK 65.

The combined fractions (54–57) with Rf values of 0.5 which showed the highest activity was sub-fractionated using the same stationary and mobile phase described earlier to obtain a purer sub-fractions that were also evaluated *in vivo* for anti-malarial activity with both chloroquine resistant and chloroquine sensitive *P. berghei* NK 65

2.4. Chloroquine resistant Plasmodium berghei

The chloroquine resistant *P. berghei* used for this study was developed in our laboratory (Ene et al., 2008b). It was maintained by sub-passaging into healthy mice every 5–7 days through intraperitoneal injection of 0.2 ml/kg blood solution made in Phosphate Buffered Saline (PBS) to contain approximately 10^6 to 10^7 infected red cells (David et al., 2004; Peter and Anatoli (1998). Parasitemia was confirmed in the infected mice after 24 h of infection with the chloroquine resistant *P. berghei*, while infection with chloroquine sensitive *P. berghei* parasitemia was usually confirmed after 72 h, because the chloroquine resistant parasite was found to be more virulent than the chloroquine sensitive parasite.

Table 1
Percentage yield of extracts of *Artemisia maciverae* following extraction with petroleum ether and chloroform.

Solvents	No of extraction (n)	Weight of extracts (g)	% extract yield
Petroleum ether	6	1.41 ± 0.08	7.07 ± 0.06
Chloroform	6	2.14 ± 0.12	10.69 ± 0.10

2.5. In vivo anti-plasmodial evaluation

Tests were performed using the 4-day curative standard test as described by David et al. (2004), Peter and Anatoli (1998) employing the chloroquine resistant and chloroquine sensitive *P. berghei* NK 65.

2.6. Screening of fractions and sub-fractions for anti-malarial activity

For the preliminary screening of the fractions for anti-malarial activity, three Swiss albino mice were used per fraction. After confirmation of parasitemia, pooled fractions or sub-fractions derived from chloroform extract of *A. maciverae* were administered intraperitoneally at a dose of 10 mg/kg for 4 days. Artesunate was administered to the control group at the standard dose of 1.6 mg/kg for 4 days, and the negative control group was left untreated. The fractions were dissolved in 0.3% (v/v) Tween 80 in normal saline.

The parasitemia was monitored in all the groups for 14 days (for the animals that survived) using thick and thin smears of blood films made form tail vein of mice (David et al., 2004; WHO, 1980). To assess the level of parasitemia, the smears were stained with 10% Giemsa at pH 7.2 for 15 min and examined under the microscope at 100×. The percentage parasitemia was calculated according to the method outlined by Iwalewa et al. (1997) as:

$$\text{percentage parasitemia} = \frac{\text{number of parasites in treated}}{\text{number of parasites in untreated control}} \times 100$$

Combined fractions 54–57 found to be most active was then subjected to a comprehensive anti-malarial therapeutic study at different doses. The sub-fraction (G) obtained by further fractionation of combined fractions 54–57 was also subjected to a comprehensive anti-malarial study at doses of 1.6, 5 and 10 mg/kg.

Table 2
Combined yield of fractions with the same Rf value and combined yield as % of total extract fractionated.

S/N	Fractions pooled	Rf values	Combined yield of fractions with same Rf values (g)	Combined fraction yield as % of total fractions collected	Combined fraction yield as % of total crude fractionated
1	1–6	0.69	0.15	12.93	7.98
2	7–16	0.77	0.06	5.17	3.19
3	17–30	0.83	0.05	4.31	2.66
4	31–47	0.38	0.15	12.93	7.98
5	48	0.40	0.13	11.21	6.91
6	49–53	0.44	0.23	19.83	12.23
7	54–57	0.54	0.39	33.62	20.74
Total		–	1.16 g	100%	61.69%