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Antiplasmodial and antiulcer activities of *Melanthera scadens*

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ABSTRACT

Objective: To evaluate the antimalarial and antiulcerogenic activities of leaf extract and fractions of *Melanthera scandens* (*M. scandens*). **Methods:** The crude leaf extract (37-111 mg/kg) and fractions (chloroform, ethylacetate and methanol; 78 mg/kg) of *M. scadens* were investigated for antiplasmodial activity against chloroquine-sensitive *Plasmodium berghei* infections in mice and for antiulcer activity against experimentally-induced ulcers. The antimalarial activity during early and established infections as well as prophylactic was investigated. Artesunate (5 mg/kg) and pyrimethamine (1.2 mg/kg) were used as positive controls. Thin films made from tail blood of each mouse were used to assess the level of parasitaemia of the mice. Antiulcer activity of the crude extract was also evaluated against indomethacin, ethanol and histamine induced ulcers. **Results:** The extract and its fractions dose-dependently reduced parasitaemia induced by chloroquine-sensitive *Plasmodium berghei* infection in prophylactic, suppressive and curative models in mice. These reductions were statistically significant ($P<0.001$). They also improved the mean survival time (MST) from 9.28 to 17.73 days as compared with the control ($P<0.01 - 0.001$). The activities of extract/fractions were incomparable to that of the standard drugs *i.e.* artesunate and pyrimethamine. On experimentally-induced ulcers, the extract inhibited indomethacin, ethanol and histamine induced ulcers. These inhibitions were statistically significant ($P<0.001$) and in a dose-dependent fashion. **Conclusions:** The antiplasmodial and antiulcerogenic effects of this plant may in part be mediated through the chemical constituents of the plant.

1. Introduction

Melanthera scandens (*M. scandens*) (Schumach. & Thonn.) Roberty (Asteraceae) is a perennial herb up to 1 m to 4 m high, with branches quadrangular and scabrid. It distributes geographically in East, West and South Africa[1]. It is known as 'ayara edemerong' by the Ibibios of Akwa Ibom State of Nigeria. The leaves are traditionally used to treat various ailments such as stomach ulcer and sores in Gosomtwi-Atwimakwanwoma district of Ghana[2]. In Nigeria, the leaves are used to treat dysmenorrhoea, diabetes and malaria[3]. It is also used by the Bete people of Issia district of Cote d'Ivoire to treat malaria[4,5]. The antioxidant[6], *in vitro* antiplasmodial[4,5] and antidiabetic[7] activities have been reported. Triterpenoid saponins have been reported in the leaves[8]. In this study, we reported the *in vivo* antiplasmodial and antiulcer activities of *M. scadens* as the plant is traditionally used as remedy for malaria and ulcer.

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2. Materials and Methods

2.1. Plant materials

The leaves of *M. scadens* were collected from a bush in Ukap in Ikono Local Government area of Akwa Ibom State. The leaves were identified and authenticated by Dr. Magaret Bassey, a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo. A voucher specimen of the plant was deposited in the Hebarium of Department of Botany and Ecological Studies, University of Uyo, Uyo.

2.2. Extraction

The plant parts (leaves) were washed and shade-dried for two weeks. The dried leaves were further chopped into small pieces and reduced to powder. The powdered leaf was divided into two parts, one part (1.5 kg) was macerated in 97% ethanol for 72 h to give the crude ethanolic extract while the other part (1.5 kg) was successively and gradiently macerated for 72 h in each of these solvents *i.e.* chloroform, ethyl acetate and methanol to give the corresponding fractions of these solvents. The liquid filtrates were

concentrated and evaporated to dryness in vacuo at 4 ℃ using rotary evaporator. The yield of each extract was determined; crude ethanolic extract (3.98%), chloroform fraction (0.50%), ethyl acetate fraction (0.30%) and methanolic fraction (0.78%). The dried crude extract and fractions were stored in a refrigerator at 4 ℃ until use for the proposed experiment.

2.3. Phytochemical screening

Phytochemical screening of the crude extract was carried out employing standard procedures[9], to reveal the presence of chemical constituents such as alkaloids, flavonoids, tannins, terpenes, saponins, anthraquinones, reducing sugars, cardiac glycosides and others.

2.4. Animals

Swiss albino mice (17-24 g) and rats (120-145 g) of both sexes were used for these experiments. They were obtained from University of Uyo Animal House. The animals were housed in standard cages and were maintained on a standard pelleted feed (Guinea feed) and water was given *ad libitum*.

2.5. Determination of median lethal dose (LD_{50})

LD_{50} of the extract was determined using albino mice. The extract was administered intraperitoneally (i.p.) and the method of Miller and Tainter[10] was adopted. This involved the administration of different doses of the extract (100 - 1 000 mg/kg) to groups of six mice each. The animals were observed for physical manifestation of signs of toxicity. The number of deaths in each group within 24 h was recorded.

2.6. Parasite inoculation

Each mouse used in the experiment was inoculated intraperitoneally with 0.2 mL of infected blood containing about 1×10^7 *Plasmodium berghei* (*P. berghei*) parasitized erythrocytes. The inoculum consisted of 5×10^7 *P. berghei* erythrocytes per mL. This was prepared by determining both the percentage parasitaemia and the erythrocytes count of the donor mouse and the blood was diluted with isotonic saline in proportions according to both determinations[11].

2.7. Drug administration

The drugs (artesunate and pyrimethamine), extract and fractions used in the antiplasmodial study were orally administered with the aid of a stainless metallic feeding cannula.

2.8. Evaluation of antiplasmodial activity of the extract and fractions

2.8.1. Evaluation of suppressive activity of the extract and fractions (4-day test)

This test was used to evaluate the schizontocidal activity of the extract, fractions and artesunate against early *P. berghei* infection in mice. This was done as described by Antia *et al*[12]. 48 mice were randomly divided into eight groups of six mice each. On the first day (D_0), 42 mice were infected with the parasite and randomly divided into various groups. They were administered with the extract, fractions and artesunate. The mice in group 1 were administered with 37 mg/kg, the group 2 (74 mg/kg) and group 3 (111 mg/kg) of crude extract, groups 4, 5 and 6 were administered with 74 mg/kg of the chloroform, ethyl acetate and methanol fractions, respectively, while group 7 was administered with 5 mg/kg of artesunate (positive control), and 10 mL/kg of distilled water was given to group 8 (negative control) for four consecutive days (D_0-D_3) between 8 am and 9 am. On the fifth day (D_4), thin blood film was made from tail blood. The film was then stained with Leishman's stain to reveal parasitized erythocytes out of 500 in a random field of the microscope. The average percentage suppression of parasitaemia was calculated in comparison with the controls as follows:

$$\frac{\text{Average \% parasitaemia in negative control} - \text{Average \% parasitaemia in positive control}}{\text{Average \% parasitaemia in negative control}}$$

2.8.2. Evaluation of prophylactic or repository activities of extract and fractions

The repository activity of the extract, fractions and pyrimethamine (daraprim) was assessed by using the method described by Okokon and Nwafor[11]. The mice were randomly divided into seven groups of six mice each. Groups 1-3 were administered with 37, 74 and 111 mg/kg/day of the extract, respectively, while groups 4-7 were respectively given 74 mg/kg/day of the acqueous and chloroform fractions, 1.2 mg/kg/day of pyrimethamine (positive control) and 10 mL/kg of distilled water (negative control). Administration of the extract/fraction/drug continued for three consecutive days (D_0-D_2). On the fourth day (D_3) the mice were inoculated with *P. berghei*. The parasitaemia level was assessed by blood smears 72 h later.

2.8.3. Evaluation of curative activities of extract and fractions (Rane's test)

This was used to evaluate the schizontocidal activity of the extract, fractions and artesunate in established infection. This was done as described by Okokon and Nwafor[11]. *P. berghei* was injected intraperitoneally into another 48 mice on the first day (D_0). 72 h later (D_3), the mice were divided randomly into eight groups of six mice each. Different doses of the extract (37, 74 and 111 mg/kg) were orally administered respectively to mice in groups 1-3. 74 mg/kg of chloroform, ethyl acetate and methanol fractions were administered to groups 4, 5 and 6, respectively, 5 mg/kg/day of artesuante to the group 7 (positive control) and group 8 was given 10 mL/kg of distilled water (negative control). The extract, fractions and drugs were administered once daily for 5 days. Leishman's stained thin smears were prepared from tail blood samples collected on each day of treatment to monitor parasitaemia level. The mean survival time (MST) of the mice in each treatment group was determined over a period of 29 days (D_0-D_{28}).

$$\frac{\text{No of days survived}}{\text{Total No of days (29)}}\times100 = \text{MST}$$

2.9. Evaluation of antiulcer activity