Antimalarial potency of the methanol leaf extract of *Maerua crassifolia* Forssk (Capparaceae)

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1. Introduction

Malaria is still an ever–continuing endemic disease that claims hundreds of thousands of lives in the tropical and subtropical countries each year, and the majority of the malarial deaths are due to *Plasmodium falciparum*. The majority of these disease cases and deaths occur in sub-Saharan Africa where the disease is endemic<sup>1,2</sup>. In some communities in Africa, mortality due to the disease has been reduced by the ability of the local traditional medical practitioners to manage the disease<sup>3</sup>. The burden of this disease is getting worse mainly due to the increasing resistance of *Plasmodium falciparum* against the widely available antimalarial drugs. Medicinal plants have been the focus of many anti–infective drugs and alternative sources of antimalarial agents in various parts of the world.
Studies have been conducted on traditionally claimed medicinal plants in Nigeria and elsewhere for scientific validation. This is because they have been part of human life and a number of plant products have been in extensive use in ethnomedicine.

The search for new drugs based on plants is important due to the emergence and spread of chloroquine-resistant and multidrug-resistant malaria parasites which require the development of new antimalarials. An acquaintance with antimalarial plants may be a springboard for new phytotherapies that could be affordable to treat malaria, especially among the less privileged native people living in endemic areas of the tropics, mostly at risk of this devastating disease.

*Maerua crassifolia* (*M. crassifolia*), which belongs to the Capparaceae family, is mainly found in the sub-Saharan Africa. In Nigeria, the plant is mainly found in Sokoto and some parts of Zamfara and Katsina States (north western part of the country). The plant is called “Jega” in Sokoto and Nigeria, “agagar” in the Republic Niger. The leaf of this plant has long been used for the treatment of gastric ulcer, toothache and intestinal diseases.

The study was aimed at scientifically evaluating the methanol leaf extract of *M. crassifolia* for antimalarial activity. The result is hoped to substantiate or otherwise, the ethnomedicinal use of the leaf extract for the treatment of malaria and possibly pave the way for its wider acceptability as antimalarial agent.

### 2. Materials and methods

#### 2.1. Collection of plant material

Fresh leaves of *M. crassifolia* were collected in the month of March, 2009 from Sokoto, Sokoto State (north west), Nigeria. The plant was identified and authenticated by Dr. (Mrs.) Jemilat A Ibrahim of the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria where a voucher specimen (NIPRD/H/6406) was deposited at the herbarium for reference. The international plant number index is Fl. Aegypt.–Arab. P. Cxiii. 1775 (1 Oct. 1775).

#### 2.2. Extraction of plant material

The leaves were washed, cut into smaller pieces and dried at room temperature for 7 d and pulverized to a coarse powder. The coarse powder (500 g) was macerated in methanol for 24 h. The resultant filtrate was dried on a water bath at reduced temperature to obtain 13.28% (w/w) of methanol extract. The extract was stored in an airtight container and used for the study.

#### 2.3. Phytochemical analysis

The phytochemical screening of methanol extract of *M. crassifolia* leaf was carried out to determine the presence of alkaloids, tannins, saponins, terpenoids, flavonoids, steroids and cardiac glycosides, phlobatannins and anthraquinones with standard procedures.

#### 2.4. Animals

Swiss albino mice (18–22 g) of both sexes obtained from Animal House, Department of Pharmacology, College of Medical Sciences, University of Calabar (South South), Nigeria were used for the study. The animals were housed in cages at room temperature and moisture, under naturally illuminated environment of 12:12 h dark/light cycle. They were fed on standard diet and water ad libitum.

#### 2.5. Acute toxicity study of the extract

The LD$_{50}$ of the leaf extract was tested to determine the safety of the agent according to the guidelines set by Organization for Economic Cooperation and Development (OECD). The study was carried out in two phases. In the first phase, nine mice were randomized into three groups of three rats per group and administered 10, 100 and 1000 mg/kg of the extract orally. The animals were observed for the first 4 h and 24 h for signs of toxicity and mortality. The results of this phase informed the choice of doses for the second phase, in which 2000, 3000 and 5000 mg/kg were administered to another set of three mice per group. The mice were also observed for signs of toxicity such as paw licking, salivation, stretching of the entire body, weakness, respiratory distress, coma and death for 72 h.

#### 2.6. Malaria parasites

The chloroquine-sensitive *Plasmodium berghei* berghei (*P. berghei*) (NK65) was obtained from National Institute for Medical Research Lagos (South West), and kept at the Department of Pharmacology, College of Medical Sciences, University of Calabar (South South), Nigeria. The parasites were maintained by continuous re–infestation in mice.

#### 2.7. Inocula

Parasitized erythrocytes were obtained from a donor infected mouse by cardiac puncture. This was prepared by determining percentage parasitemia and the erythrocytes count of the donor mouse and diluting them with normal saline in proportions indicated by both determinations. Each mouse was inoculated intraperitoneally with infected blood suspension (0.2 mL) containing $1 \times 10^7$ *P. berghei* parasitized red blood cells.
2.8. Suppressive test

A 4–day suppressive test as described Akuodor et al. and Mbah et al., was employed for the study[11,12], Thirty Swiss albino mice of both sexes weighing (18–22 g) were passaged intraperitoneally with standard inocula of P. berghei containing 1x10^7 infected erythrocytes. After 3 h of inoculation, the infected mice were randomly divided into five groups of six mice per cage and treated for 4–consecutive day (Day 0–Day 3). Group 1 received 0.2 mL of normal saline (drug–free control). Groups 2, 3 and 4 received 100, 200 and 400 mg/kg of the methanol leaf extract respectively, while group 5 received 10 mg/kg of chloroquine diphosphate. All doses were administered orally. On Day 3, thin films were made from the tail blood of each mouse. The films were fixed with methanol, stained with 10% Giemsa and parasite density determined by microscopically counting the parasitized red blood cells on at least 1000 red blood cells in 10 different fields[10].

2.9. Curative test

On Day 0, 30 Swiss albino mice were passaged intraperitoneally with standard inocula of 1x10^7 P. berghei infected erythrocytes. After 72 h, the mice were randomly divided into five groups of six mice per cage. Group 1 received 0.2 mL of normal saline (drug–free control). Groups 2, 3 and 4 received 100, 200 and 400 mg/kg of the methanol leaf extract respectively, while group 5 received 10 mg/kg of chloroquine diphosphate. All doses were administered orally. On Day 3, thin films were made from the tail blood of each mouse. The films were fixed with methanol, stained with 10% Giemsa and parasite density examined by microscopically counting the parasitized red blood cells on at least 1000 red blood cells in 10 different fields[13,14]. The mean survival time of each group was determined by finding the average survival time (d) of the mice in each group over a period of 30 d (Day 0–Day 29).

2.10. Statistical analysis

Results obtained were expressed as mean±SEM. The data were analysed using one–way ANOVA and differences between the means were considered significant at P<0.05[15].

3. Results

3.1. Phytochemical tests

The results of phytochemical screening of the methanol leaf extract of M. crassifolia revealed the presence of alkaloids, saponins, tannins, terpenoids, flavonoids, steroids, phenol and cardiac glycosides, while phlobatannins and anthraquinones were absent.

3.2. Acute toxicity test

There was no mortality observed in mice after oral administration of the methanol extract even at doses as high as 5000 mg/kg signifying that the oral LD₅₀ was more than 5000 mg/kg. Thus the experimental doses used (100, 200 and 400 mg/kg) were within safe margin.

3.3. Suppressive effect

The methanol leaf extract exhibited a dose–dependent effect at different doses employed. Doses of 100, 200 and 400 mg/kg caused 71%, 80% and 86% inhibition of parasitemia respectively. The effect of the extract was significant (P<0.05) when compared with the control. The reference drug, chloroquine (10 mg/kg), caused 95% suppression (Table 1).

Table 1

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Parasitemia (%) (Day 5)</th>
<th>Suppression (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>0.2 mL</td>
<td>32.9±0.6</td>
<td>–</td>
</tr>
<tr>
<td>M. crassifolia extract</td>
<td>100</td>
<td>9.5±0.6</td>
<td>71*</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>7.2±0.4</td>
<td>80*</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>4.6±0.6</td>
<td>86*</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>10</td>
<td>2.5±0.3</td>
<td>93*</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SEM. *: significantly different from control at P<0.05.

3.4 Curative effect

The methanol leaf extract caused a dose–dependent reduction in parasitemia in the extract treated groups similar to the chloroquine–treated group, unlike the control in which there was a consistent increase in the parasite density. The survival values showed that the extract significantly (P<0.05) suppressed established infection at the doses employed. Death was observed in the control group on Day 6 and by Day 9, all mice in the group died. On the other hand, mice in the extract treated groups survived beyond 20 d. However, some of the mice in the 400 mg/kg group survived during the 30–day observation period, while chloroquine treated group recorded no death at all (Table 2).

Table 2

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Pre (Day 3)</th>
<th>Post (Day 7)</th>
<th>Mean survival time (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>0.2 mL</td>
<td>30.27±0.53</td>
<td>41.45±0.59</td>
<td>9.33±1.20</td>
</tr>
<tr>
<td>M. crassifolia extract</td>
<td>100</td>
<td>29.40±0.66</td>
<td>11.47±0.45</td>
<td>20.17±0.60</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>30.03±0.55</td>
<td>9.80±0.31</td>
<td>22.33±0.67</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>29.65±0.55</td>
<td>6.75±0.16</td>
<td>27.17±0.95</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>10</td>
<td>32.97±0.65</td>
<td>2.97±0.25</td>
<td>30.0±0.0</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SEM. *: significantly different from control at P<0.05. All mice treated with chloroquine survived until Day 30.
4. Discussion

The results show that the methanol leaf extract of *M. crassifolia* exhibited various degrees of antimalarial activity in mice infected with *P. berghei*. The traditional use of *M. crassifolia* for the treatment of malaria could be attributed to the presence of certain phytochemicals which constitute the bioactive principles in the plant. Various plants with a wide variety of phytochemicals as their bioactive principle have shown antiplasmodial activities[16–18]. Alkaloids, flavonoids and terpenes which have been implicated in antiplasmodial activities of plant substances are found in the extract studied. Flavonoids and phenolic compounds have also been shown to exert antiplasmodial activity by elevating the red blood cell oxidation and inhibiting the parasite’s protein synthesis. This activity counteracts the oxidative damage induced by the malaria parasite[19–21]. Thus, the antimalarial activity of *M. crassifolia* can similarly be related to the presence of these same phytochemicals.

The 4–day suppressive test is a standard method used for antimalarial screening, and the determination of percentage inhibition of parasite growth is the most reliable parameter. The mean group parasitemia levels of less than or equal to 90% of the mock–treated control animals usually indicate that the test agent is active in standard screening studies[22]. The methanol leaf extract of *M. crassifolia* exhibited a dose–dependent activity against *P. berghei* in infected mice.

The plant extract also exerted significant curative activity during an established infection. The observed antimalarial activity of the leaf extract is consistent with the traditional use of the plant as herbal medication against the disease and indicative of its potential as a chemotherapeutic antimalarial agent. This was confirmed by the mean survival time values which at doses used were more than those of the control group. In untreated mice, the parasite count increased daily until the death of the animal, which was also observed in our previous studies[23].

However, as the *P. berghei* strain is sensitive to chloroquine, this drug was used as standard drug in the study. Chloroquine has been used for suppressive and curative antimalarial activities. In early and established infection, chloroquine interrupts the heme polymerization by forming a Pf–chloroquine complex. This complex is responsible for the disruption of the parasite’s cell membrane function and ultimately leads to auto–digestion.

The methanol leaf extract of *M. crassifolia* showed no lethality to mice at 5000 mg/kg and no gross behavioural and physical changes were revealed. Therefore, the fact that no death was observed with up to an oral dose of 5000 mg/kg could indicate that the tests extract is not toxic.

This could also explain the safe use of the plant by local people, who have been using it in the treatment of diseases.

The results of this study have shown that the methanol leaf extract of *M. crassifolia* possesses antimalarial activity as seen in its ability to suppress chloroquine sensitive *P. berghei* infection in two evaluated models. We recommend that further chemical studies should be done to identify, properly characterize and develop the actual compounds responsible for the observed activity. It is therefore hoped that the screening of locally used medicinal plants can fully be investigated with a view to establishing their efficacy and to determine their potential as sources of new antimalarial agents.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Related reports

The traditional use of *M. crassifolia* for malaria is also reported from other African countries, including Mauritania.
and Mali. However, bibliographic research, using the key words “malaria” and “Maerua” in PubMed and google did not yield any result. Therefore, it seems that no previous study has evaluated the antimalarial activity of M. crassifolia.

Innovations & breakthroughs

As mentioned above, there seems to be no previous study on the antimalarial activity of M. crassifolia. Although the concentration of M. crassifolia crude extract used in the study is at least 10 times higher than that of chloroquine, it may be a good starting point. Moreover, toxicity was not found in mice at 5000 mg/kg.

Applications

The results suggested that M. crassifolia may have an antimalarial activity. Further studies are required to identify which component(s) in the crude extract is/are active against malaria.

Peer review

Since M. crassifolia is used in traditional medicine to treat malaria in several African countries, this will be a preliminary study requiring further confirmation (evaluation of fractions, purification, characterization of natural products that are active against malaria).

References