Anti-malarial investigation of Acorus calamus, Dichapetalum gelonioides, and Leucas aspera on Plasmodium falciparum strains

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Abstract

Introduction: Malaria is a significant global health concern and adversely affects people in developing countries including Bangladesh. The causative agent Plasmodium falciparum is resistant to several currently available anti-malarial drugs, such as mefloquine, chloroquine, and artemisinin-based combination therapy (ACT), and this has been a major global challenge towards the control of the disease. There is urgent need for novel anti-malarial chemotherapeutic agents.

Methodology: The present study aimed to evaluate antimalarial activity of methanolic extracts of three Bangladeshi medicinal plants - Acorus calamus, Dichapetalum gelonioides and Leucas aspera - against both chloroquine sensitive (3D7) and resistant (Dd2) strains of P. falciparum. Histidine-rich protein 2 (HRP2) based ELISA was used to evaluate the in vitro inhibitory activity of the extracts.

Results: D. gelonioides extract showed moderate (IC50 = 19.15 µg/mL) and promising activity (IC50 = 10.43 µg/mL) against 3D7 and Dd2 strains respectively. A. calamus remained inactive against both 3D7 (IC50 = 72.29 µg/mL) and Dd2 strain (IC50 = 67.81 µg/mL). L. aspera initially remained inactive against 3D7 strain (IC50 = 60.51 µg/mL), but displayed promising activity (IC50 = 7.693) against Dd2 strain.

Conclusions: This is the first time these plant materials have been assessed for their in vitro antimalarial properties. It is pivotal to conduct further phytochemical analysis of D. gelonioides and L. aspera to evaluate the presence of potential novel antimalarial drug compounds.

Key words: Plasmodium; in vitro; anti-malarial resistance; 50% inhibitory concentration (IC50).
Leucas aspera (common leucas; locally known as ghal ghas) for antimalarial properties. All the plant materials were collected from Bandarban, Chattogram. These plants were selected based on their ethnomedicinal values. Leaves and rhizomes of A. calamus are used in medicinal preparations to treat various diseases [13]. D. gelonioide has traditionally been used to treat amenorrhea and mouth ulcers, and L. aspera is used to cure cold, cough, and skin disorders [14,15]. The prime objective of the study is to evaluate antimalarial efficacy of methanolic extracts of these plants.

**Methodology**

**Study site and period**

The current study was performed at the Emerging Infections and Parasitology Laboratory, International Centre for Diarrhoeal Disease Research, Bangladesh from November 2020 to June 2021.

**Plant materials**

Acorus calamus (sweet flag), Dichapetalum gelonioide (gelonium poison-leaf) and Leucas aspera (common leucas) were collected from Bandarban, Chattogram.

**Preparation of methanolic extract**

Fresh leaves of A. calamus and entire plants of D. gelonioide and L. aspera were collected and sun dried. The leaves of A. calamus were cut into small pieces and sun dried for 7 days, while the D. gelonioide and L. aspera plants took 10 and 12 days respectively to dry. The dried plant parts were converted into powder using a laboratory grinder. 250 g of dried and powdered plant material was soaked in 1000 mL methanol at 25 ± 2°C for 7 days in the case of leaves and 14 days in the case of whole plant in airtight bottles and the mixture was stirred every 18 hours using a sterile glass rod. Thereafter, the solution was vacuum filtered using Whatman Grade 1 filter paper. All the extracts were concentrated and dried in a rotary evaporator, followed by a water bath. The extracts were then stored in airtight containers and kept in a refrigerator at 4°C to protect against light and humidity until used.

**Malaria parasites and culture**

P. falciparum strain 3D7 is sensitive to all anti-malarial drugs available in the market; and P. falciparum strain Dd2 is resistant to chloroquine. These two strains were used in the study. They were collected from the Malaria Research and Reference Reagent Resource Center (MR4), which includes the American Type Culture Collection (ATCC). The Trager and Jensen in vitro culture technique was used with some modification to maintain the continuous culture of the asexual blood stage [16]. Briefly, the parasites were cultivated in O +ve erythrocyte and maintained in RPMI-1640 media (Gibco by Life Technologies, Grand Island, NY, USA). In addition, 0.5% Albumax II (Gibco by Life Technologies, Grand Island, NY, USA) serum supplement powder, 25 mM HEPES, 11 mM glucose, 23.81 mM NaHCO3, 200 µM hypoxanthine, and 20 mg/L gentamicin solution were also added to the media. The cultivated parasites were kept in a 25 cm2 Corning® culture flask (Corning Inc., NY, USA) with 2% hematocrit at 37 °C inside a candle jar to maintain anaerobic condition. Routine microscopy was performed to monitor and ensure parasite growth at < 5% every 24 hours with the daily change to fresh culture medium.

**Evaluation of in-vitro antimalarial activity**

Worldwide Antimalarial Resistance Network (WWARN) protocols were used for the experiment. Assay plate preparation were done by the WWARN protocol INV03 and Histidine-rich protein 2 (HRP2) Enzyme-linked immunosorbent assay (ELISA) was done by WWARN protocol no: INV09 (WWARN, Oxford, United Kingdom) [17]. A batch of drug plates were prepared by adding 40 µL of stock solution and 160 µL of RPMI 1640 (Roswell Park Memorial Institute) media. Serial dilutions of each set of plant extracts were made in triplicates in 96 well microtiter plates with concentration ranging from 0.003-1.67E-08 gm/mL). In each well, 8 µL of diluted plant extract and 192 µL of parasitized culture was added in concentrations ranging from 200 µg/mL–0.0976 µg/mL. Parasitized red blood cell cultures with Chloroquine (CQ) were used as a positive control; the last well was drug free and was used as the negative control. The assay plates were incubated for 72 hours at 37°C in a candle jar. After the 72 hour incubation period, the plates were removed from the incubator and stored at −20°C until all the wells was completely frozen. Then the plates were thawed for hemolysis. HRP2 ELISA technique measures the quantity of HRP2 produced by P. falciparum during the 72 hour incubation and its inhibition by anti-malarial drugs.

The percentage inhibition values were calculated from normalized activities (activity expressed as percentage of solvent control) for assessing the antimalarial activity. The concentration of extracts that caused 50% inhibition of P. falciparum (IC50 values) was also calculated using the GraphPad Prism Software Version 8.4.3 (La Jolla, CA 92037 USA).
Results

Anti-malarial activity of extracted biota was calculated using results from three independent anti-malarial assays, each carried out in triplicate. The Graphpad prism 8.4.3.686 software was used to construct a graph of non-linear regression of the optical density values of the chloroquine and plant extracts. Dose versus response curves (Figures 1 and 2) were obtained, where concentrations of chloroquine and plant extracts were expressed as logarithmic numbers in the x axis and O.D. values were normalized and expressed as percentage inhibition values. The concentration at which parasite growth was inhibited by 50% (IC50) was calculated from the graph representing the percentage growth inhibition data.

IC50 values for chloroquine drug (positive control) were 17.79 nM and 59.64 nM respectively for 3D7 and Dd2 strain. Based on previous studies, anti-malarial activity can be characterized as high (IC50 < 5 µg/mL), promising (5 < IC50 < 15 µg/mL), moderate (15 < IC50 < 50 µg/mL) and inactive (IC50 > 50 µg/mL) [18-20]. IC50 values of Dichapetalum gelonioides were 19.15 µg/mL against 3D7 (CQ-sensitive strain) and 10.43 µg/mL against Dd2 (CQ-resistant strain) exhibiting moderate and promising antimalarial activity respectively. IC50 values of Leucas aspera against Dd2 was 7.693, showing promising activity. However, L. aspera against 3D7 and A. calamus against both the strains remained inactive (Table 1).

Discussion

Research is needed to develop plant-based complementary medicine for malaria since malarial parasites have developed resistance to the synthetic drugs like chloroquine, and ACT [21,22]. We found no studies on antimalarial activity, in vitro or in vivo, of A. calamus, D. gelonioides and L. aspera; however, there are reports on the antioxidant and antihepatotoxic activities of A. calamus [23], nematicidal and antifungal activities of compounds extracted from D. gelonioides [24], and antioxidant activity of L. aspera [25].
Table 1. Inhibitory concentration (IC50) and antimalarial activity of methanolic extracts of *Acorus calamus*, *Dichapetalum gelonioides* and *Leucas aspera* against 3D7 and Dd2 strains.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Plant extract</th>
<th>IC50 (µg/ml)</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>3D7</td>
<td>A.C1 Methanol</td>
<td>72.29</td>
<td>Inactive</td>
</tr>
<tr>
<td></td>
<td>D.G2 Methanol</td>
<td>19.15</td>
<td>Moderate activity</td>
</tr>
<tr>
<td></td>
<td>L.A3 Methanol</td>
<td>60.51</td>
<td>Inactive</td>
</tr>
<tr>
<td>Dd2</td>
<td>A.C1 Methanol</td>
<td>67.81</td>
<td>Inactive</td>
</tr>
<tr>
<td></td>
<td>D.G2 Methanol</td>
<td>10.43</td>
<td>Promising activity</td>
</tr>
<tr>
<td></td>
<td>L.A3 Methanol</td>
<td>7.693</td>
<td>Promising activity</td>
</tr>
</tbody>
</table>

1 *Acorus calamus*; 2 *Dichapetalum gelonioides*; 3 *Leucas aspera*.

Our study was designed to evaluate anti-plasmodial activity on two *P. falciparum* strains in the selected plant extracts by HRP2 ELISA technique. The effectiveness of all the extracts against *P. falciparum* parasites was dose-dependent; 0.003 gm/mL was the most effective dose. The initial IC50 value of the plant materials suggested that *D. gelonioides* has moderate and promising activities against 3D7 and Dd2 strains, respectively. *L. aspera* showed promising activity against the Dd2 strain, whereas *A. calamus* remained inactive against both the strains. The results indicate that two of the studied species of plants, *D. gelonioides* and *L. aspera*, possess active components capable of inhibiting *P. falciparum* in vitro, which is in agreement with its traditional use.

Plant materials may contain phenolics that may be simple (e.g., phenolic acids, anthocyanins) or highly polymerized substances (e.g., tannins). The type of solvent used in the extraction procedure has a big impact on the success of extracting bioactive compounds from plants [26]. Methanol has proven to be a good solvent for extracting the bioactive compounds from the plant materials. Previous studies have shown that the growth of *P. falciparum* in the schizont stage was inhibited by a methanolic leaf extract of the chikadoma plant [27]. Another study reported that the methanolic crude extract of *Syzygium cumin* had promising effect against 3D7 (IC50 = 6.28 g/mL), Dd2 (IC50 = 13.42 g/mL) [28].

Certain plant extracts can prove to be a good resource for antimalarial properties. *Vitex negundo* leaf extract showed effective anti-malarial interaction against the 3D7 and K1 strains, with IC50 values of 7.21 g/mL and 7.43 g/mL, respectively [29]. Similarly, *Acacia nilotica* plant extracts had antimalarial properties with initial IC50 values of leaves, pods and bark extracts of 1.29, 4.16 and 4.28 µg/mL respectively [30]. The activity of *D. gelonioides* and *L. aspera* against *P. falciparum* strains indicate that these plants can be vital sources of antimalarial agents. The results of the phytochemical investigation of these plants warrants further investigation to determine the active ingredient responsible for their antimalarial activity.

Conclusions

Antimalarial efficacy of plant extracts should be justified in both in vitro and in vivo settings. We used in vitro experiments only due to lack of resources and laboratory settings. However, this is the first ever report of antimalarial activity of *Dichapetalum gelonioides* against both CQ-sensitive and resistant strains, and *Leucas aspera* against CQ-resistant strain. These plants may have some valuable bio-active compounds and further phytochemical analysis of *Dichapetalum gelonioides* and *Leucas aspera* to isolate, purify, and identify the active compounds is recommended for using them as a source of potential drug candidates in the fight against malaria.

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Authors’ Contributions

MSA, MRHH, HK and PB participated in the design of the study. AB and SAS collected and extracted the plants. SAS and MFZ carried out the laboratory experiments and data analysis. MFZ drafted the manuscript. All authors read and approved the final manuscript.

References


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**Conflict of interests:** No conflict of interests is declared.