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

Md Fahad Zamil1,2, Saiful Arefeen Sazed1, Muhammad Riadul Haque Hossainey1,3, Anik Biswas4, Mohammad Shafiul Alam1, Hamida Khanum2, Priyanka Barua2

1 International Centre for Diarrhoeal Disease Research Bangladesh (icddr,b), Mohakhali, Dhaka, Bangladesh
2 Department of Zoology, University of Dhaka, Dhaka, Bangladesh
3 Department of Biological Sciences, George Washington University, Washington, DC, United States
4 Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka, Bangladesh

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).

J Infect Dev Ctries 2022; 16(11):1768-1772. doi:10.3855/jidc.16741

(Received 25 April 2022 – Accepted 05 September 2022)

Copyright © 2022 Zamil et al. This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Introduction

Malaria is a fatal disease caused by Plasmodium, a genus of protozoan parasite [1]. Each year, it affects between 200 and 400 million people, killing nearly 400,000 people and adversely affecting children in Sub-Saharan Africa [2]. The region with the highest malaria burden is Sub-Saharan Africa, where P. falciparum is the predominant form [3]. Bangladesh is a densely populated nation with endemic malaria. Most of the cases are found in the thirteen endemic districts near or bordering India and Myanmar [4,5]. The Chittagong Hill Tracts (Bandarban, Rangamati and Khagrachhari) have the highest malaria burden accounting for nearly 90% of all malaria cases in Bangladesh [6].

Resistance to antimalarial drugs is a major concern in the global fight against malaria. Chloroquine and sulfadoxin-pyrimethamine resistance were reported in Bangladesh as early as 1970 and 1985, respectively [7,8]. Despite the fact that no clinical or molecular resistance to the current artemisinin-based chemotherapy (ACT) treatments have been documented in the country, it has been discovered that this is ineffective in the countries bordering on the east [9]. This highlights the need to identify new antimalarials in the near term, as a backup in case of ACT failure.

Plants have been investigated as antimalarial agents as a direct outcome of the two potent antimalarial drugs, quinine and artemisinin, both of which are derived from plants [10]. The anti-malarial activity in plants is attributed to a variety of phytoconstituents such as alkaloids, terpenes, steroids, and flavonoids [11]. Traditional healthcare practitioners in Bangladesh have a long history of using medicinal plants [12].

In this study, we investigated three plants that grow locally in Bangladesh, Acorus calamus (sweet flag; locally known as bach), Dichapetalum gelonioides (gelonium poison-leaf; locally known as moacurra) and
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. gelonioides 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 gelonioides (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. gelonioides 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. gelonioides 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 antimalarial 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 anti-malarial 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.

| Plant Extract | Strain | IC50 (µg/ml) | Activity |
|---------------|--------|--------------|----------|
| A.C             | 3D7    | 72.29        | Inactive |
| D.G             | 3D7    | 19.15        | Moderate activity |
| L.A             | 3D7    | 60.51        | Inactive |
| A.C             | Dd2    | 67.81        | Inactive |
| D.G             | Dd2    | 10.43        | Promising activity |
| L.A             | Dd2    | 7.693        | Promising activity |

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 cymosum 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 is recommended for using them as a source of potential drug candidates in the fight against malaria.

Acknowledgements

MFZ was supported by NST fellowship from the Ministry of Science and Technology, Government of the People’s Republic of Bangladesh.

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

1. World Health Organization (2021) Malaria 2021. Available: https://www.who.int/news-room/fact-sheets/detail/malaria. Accessed: 26 July 2022.
2. World Health Organization (2018) World malaria report 2018. Available: http://apps.who.int/iris/bitstream/handle/10665/275867/978924156563-eng.pdf. Accessed: 19 November 2018.
3. Kelly-Hope LA, McKenzie FE (2009) The multiplicity of malaria transmission: a review of entomological inoculation rate measurements and methods across sub-Saharan Africa. Malar J 8: 1-16.
4. Haque U, Ahmed SM, Hossain S, Huda M, Hossain A, Alam MS, Mondal D, Khan WA, Khalequzzaman M, Haque R (2009) Malaria prevalence in endemic districts of Bangladesh. PLoS One 4: e6737.
5. Haque U, Overgaard HJ, Clements AC, Norris DE, Islam N, Karim I, Roy S, Haque W, Kabir M, Smith DL, Glass GE
(2014) Malaria burden and control in Bangladesh and prospects for elimination: an epidemiological and economic assessment. Lancet Glob Health 2: e98-e105.

6. Noé A, Zaman SI, Rahman M, Saha AK, Aktaruzzaman M, Maude RJ (2018) Mapping the stability of malaria hotspots in Bangladesh from 2013 to 2016. Malar J 17: 1-21.

7. Ahmed SM, Haque R, Haque U, Hossain A (2009) Knowledge on the transmission, prevention and treatment of malaria among two endemic populations of Bangladesh and their health-seeking behaviour. Malar J 8: 1-11.

8. World Health Organization (2012) World Malaria Report 2012. Available: https://www.who.int/malaria/publications/world_malaria_report_2012/wmr2012_full_report.pdf. Accessed: 19 November 2012.

9. Alker AP, Lim P, Sem R, Shah NK, Yi P, Bouth DM, Tsuyuoka R, Maguire JD, Fandeur T, Arieu F, Wongsrichanalai C (2007) Pfmdr1 and in vivo resistance to artesunate-mefloquine in falciparum malaria on the Cambodian–Thai border. Am J Trop Med Hyg 76: 641-647.

10. Saxena S, Pant N, Jain D, Bhakuni R (2003) Antimalarial agents from plant sources. Curr Sci 85: 1314-1329.

11. Uzor PF (2020) Alkaloids from plants with antimalarial activity: a review of recent studies. Evi Compl and Alt Med 2020: 8749083.

12. Ocvirk S, Kistler M, Khan S, Talukder SH, Hauner H (2013) Traditional medicinal plants used for the treatment of diabetes in rural and urban areas of Dhaka, Bangladesh – an ethnobotanical survey. J Ethnobiol Ethnomed 9: 1-8.

13. Khwairakpam AD, Damayenti YD, Deka A, Monisha J, Roy NK, Padmavathi G, Kunnunakkara AB (2018) Acorus calamus: a bio-reserve of medicinal values. J Basic Clin Phys and Pharma 29: 107-122.

14. Priyanka G, Bharadwaj NA, Sachin M, Kekuda TP (2018) Antibacterial, antifungal and antioxidant activity of Dichapetalum gelonioides (Roxb.) Engl. (Dichapetalaceae). J Drug Del and Ther 10: 263-267.

15. Chaitanya M, Dhanabal S, Rajan S (2013) Pharmacodynamic and ethnomedicinal uses of weed species in Tamil Nadu State, India: a review. AJF of Agri Res 8: 3505-3527.

16. Trager W, Jensen JB (1976) Human malaria parasites in continuous culture. Science 193: 673-675.

17. Worldwide Antimalarial Resistance Network (2011) Estimation of Plasmodium falciparum drug susceptibility ex vivo by HRP2 ELISA v1.0. Available: https://www.wwarn.org/sites/default/files/attachments/procedures/inv09-estimation-of-plasmodium-falciparum-drugsusceptibility-ex-vivo-by-hrp2-elisa.pdf. Accessed: 12 August 2017.

18. Jonville MC, Kodja H, Humeau L, Fournel J, De Mol P, Cao M, Angenot L, Frederich M (2008) Screening of medicinal plants from Reunion Island for antimalarial and cytotoxic activity. J Ethnopharmacol 120: 382–386.

19. Lekana-Douki JB, Bougii JB, Oyegue Liabague SL, Zang Edou SE, Zatra R, Bisvigou U, Druihle P, Lebibi J, Toure Ndouo FS, Kombila M (2011) In vitro antiplasmodial activity and cytotoxicity of nine plants traditionally used in Gabon. J Ethnopharmacol 133: 1103–1108.

20. Lima RBS, Rocha e Silva LF, Melo MRS, Costa JS, Picanço NS, Lima ES, Vasconcellos MC, Boleti APA, Santos JMP, Amorim RCN, Chaves FCM, Coutinho JP, Tadei WP, Kreteli AU, Pohlit AM (2015) In vitro and in vivo anti-malarial activity of plants from the Brazilian Amazon. Malar J 14: 508.

21. Trape J-F, Pison G, Spiegel A, Enel C, Rogier C (2002) Combating malaria in Africa. Trends in Parasitol 18: 224-230.

22. Wang M-W, Hao X, Chen K (2007) Biological screening of natural products and drug innovation in China. Phil Trans of Royal Soc Bio Sci 362: 1093-1105.

23. Palani S, Raja S, Kumar RP, Venkadesan D, Devi K, Sivaraj A, Kumar BS (2009) Therapeutic efficacy of antihepatotoxic and antioxidant activities of Acorus calamus on acetaminophen-induced toxicity in rat. Int J of Integ Biol 7: 39-44.

24. Jing S-X, Luo S-H, Li C-H, Hua J, Wang Y-L, Niu X-M, Li XN, Liu Y, Huang CS, Wang Y, Li SH (2014) Biologically active dichapetalins from Dichapetalum gelonioides. J of Nat Products 77: 882-893.

25. Chew AL, Jessica JJA, Sasidharan S (2012) Antioxidant and antibacterial activity of different parts of Leucas aspera. Asi J of Trop Biomed 2: 176-180.

26. Dai J, Mumper RJ (2010) Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules 15: 7313-7352.

27. Okolo C, Eban L, Amazu L, Chukwu L, Ohadoma S, Osuala F (2020) In vitro anti-malarial activity of Chikadoma plant from the rainforest of Southern Nigeria. J Drug Del and Ther 10: 251-254.

28. Hossainey MRH, SazedSA, Nima MK, Rahman MS, Ashraf T, Chowdhury AA, Rashid MA, Haque R, Alam MS (2020) Investigation of antimalarial activity and cytotoxicity profiling of a Bangladeshi plant Syzygium cymosum. J Infect Dev Ctries 14: 924-928. doi: 10.3855/jidc.12740.

29. Dwivedi MK, Shukla R, Sharma NK, Manhas A, Srivastava K, Kumar N, Singh PK (2021) Evaluation of ethnopharmacologically selected Vitex negundo L. for in vitro antimalarial activity and secondary metabolite profiling. J Ethnopharmacol 275: 114076.

Corresponding author
Priyanka Barua, PhD.
Department of Zoology,
University of Dhaka, Dhaka 1000, Bangladesh.
Tel: +8801866728886
Fax: (8802) 9667222
Email: baruap@du.ac.bd, baruap7@gmail.com

Conflict of interests: No conflict of interests is declared.