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EVALUATION OF ANTI-MALARIAL ACTIVITY OF *BRASSICA JUNCEA*, *GLINUS OPPOSITIFOLIUS*, AND *BARRINGTONIA ACUTANGULAR* AGAINST *PLASMODIUM FALCIPARUM* STRAIN

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ABSTRACT: Malaria, caused by the *Plasmodium* parasite, contributes to a significant global health burden that disproportionally affects those living in developing nations. The majority of cases are caused by the species *P. falciparum* and responsible for the a million deaths each year worldwide. The aim of the present study was to evaluate extracts of three indigenous medicinal plants- Raishorisha, *Brassica juncea;* Gimma shak, *Glinus oppositifolius* and Hijal, *Barringtonia acutangula In vitro* anti-plasmodial activities against the 3D7 laboratory strain of *Plasmodium falciparum,* which is sensitive to all drug was tested. HRP2 ELISA assay was used to evaluate the *in vitro* inhibitory activity of the extracts. Plant extracts showed moderate to good anti parasitic effects. Highly promising anti-plasmodial activity was found in the extract of *Brassica juncea*. 50% inhibitory concentration (IC_{50}) 3D7: 0.00905 µg/ml (methanol extract), and *Glinus oppositifolius* had a good activity (IC_{50}) 3D7: 13.8 µg/ml (methanol extract). A moderate activity (23.8 µg/ml) was found in the methanol extracts of *B. acutangula*. These results demonstrate that methanol extracts of *B. juncea* and *G. oppositifolius* may have antimalarial agents even in their crude form. The isolation of compounds from these two plants seems to be of special interest for further anti-malarial studies.

Key words*:* Anti-malarial drug, *Brassica juncea*, *Glinus oppositifolius*, *Barringtonia acutangula, Plasmodium falciparum,* Indigenous medicinal plants.

INTRODUCTION

Resistance has developed to several antimalarial drugs, most notably Chloroquine (Wellems *et al*. 2002). Since 2010, mortality rates among children have fallen by 34 per cent. Rates of disease have decreased from 2000 to 2016 by 37%, but increased from 2015 during which there were 214 million cases (WHO 2010). About 35 Anopheline species are recorded in Bangladesh (Ahmed 1998, 2008) of which only seven of these species were documented to be competent malaria vectors until 2009. Among these, four have been considered as the principal

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malaria vectors *i.e. Anopheles baimaii (=A. dirus* D*), A. philippinensis*, *A. sundaicus* and *A. minimuss* Other species, such as *A. aconitus*, *A. annularis* and *A. vagus,* were found to be capable of transmitting malaria during outbreak situations (Alam *et al.* 2010),. This creates an opportunities in the search for new anti-malarial compounds is the study of active constituents (metabolites) of medicinal plants (Alshawsh *et al.* 2007; Bagavan *et al.* 2011, Traore-Keita *et al.* 2000).

The risk is greatest in the east and north-east of the country in areas bordering India and Myanmar (Aregawi *et al,* 2008; Haque *et al.* 2009). The distribution, introduction of rapid diagnosis test (RDT) and to introduce combined therapy with coartem. In the absence of baseline information, these resources could not be equitably distributed among the population (Haque *et al.* 2009, Widywaruyanti *et al* 2014). Malaria is largely seasonal in Bangladesh, with the major incidence occurring during the rainy season from April to October (Haque *et al.* 2009, Vijaya *et al.* 2010). Out of the total 64 districts of Bangladesh, 13 are endemic districts which are located along with the border areas with India and Myanmar (Figure 2) (Rahmatullah *et al.* 2012, WHO. 2009; Haque *et al.* 2010).

The traditional medicinal plants that are employed for treatment of malaria represent a potential for discovery of lead molecules for development into potential antimalarial drugs (Diallo *et.al.,* 2001, Wijiyaratne *et al.* 2004), and since the discovery of artemisinin as an effective antimalarial isolated from the herb plant *Brassica juncea* (Rai-Shorisha)*, Glinus oppositifolius* (Gimma Shak) and *Barringtonia acutangula* (Hijal) are some of the medicinal plants traditionally used to treat malaria in Bangladesh. About 89% of the infections were caused by *Plasmodium falciparum*, 5% by *Plasmodium vivax*, and the remaining by mixed infection (Khan *et al.* 2011). The previous successes of finding potent anti-malarial underscore the importance of medicinal plants in the fight against malaria and as a rich reservoir from which new drugs can be developed.

MATERIAL AND METHODS

The present study dealt with the effect of three indigenous medicinal plants as anti-malarial agent expressed in the form of IC50, was done in Department of Pharmaceutical Chemistry, University of Dhaka and Emerging Infections and Parasitology Laboratory, icddr,b on from March, 2017 to January, 2018. Materials for this study are mainly the extracts of the plants, *Brassica juncea* (Indian Mastered or Rai-Shorisha)*, Glinus oppositifolius* (Gimma or Gimma Shak)

and *Barringtonia acutangula* (Indian Oak Hijal). *Preparation of Extracts:* Fresh plant of *B. juncea, G. oppositifolius* and stem of *B. acutangula* were separately cut and sun dried. About 100g of dried and powdered material was soaked in 500 mL methanol at 25 ± 2 °C for 7 days in case of whole plants and 14 days for stem in airtight bottles. All the extracts were filtered and concentrated to dryness and later on water bath.

In- vitroculture techniques: The *Plasmodium* strains: 3D7 strain was received from the American Type Culture Collection (ATCC), MR4, USA. The *Plasmodium* strain was maintained in a continuous culture in sealed flasks (75 cm2) followed after Trager and Jensen (1976).

Cultivation of parasites: The parasites were cultivated/grown in culture medium RPMI 1640 supplemented with 25 mM HEPES, 200 mM L-glutamine, 1 M NaOH, 20 % D-glucose, 10 mg/mL gentamicin, pooled serum and human erythrocytes (blood group 0). The cultures were incubated at 37°C. The parasites were unsynchronized and studied at 2 % and at 5 % parasitemia, and a 2 % hematocrit.

Erythrocyte Preparation: Blood from a healthy volunteer was collected into Na-EDTA BD Vacationer® and centrifuged at 4000 rpm for 5 mins followed by 3 times of wash using RPMI. .

Sorbitol synchronization of growth stage: Parasites are synchronized. The suspension was centrifuged, the supernatant removed the infected erythrocytes were suspended in culture medium to restore the ~5% The suspension was filter and sterilized using 0.45 µn Millipore nylon syringe filters, and hematocrit.

Preparation of drug plate and Assay plate: A batch of drug plates was prepared by adding 75µl of stock solution and 150 µl of RPMI-1640 media. Serial dilutions of each set of plant extracts were made in triplicates in 96-well micro titer plates with concentration ranging from 3.3 mg/ml – 0.0187µg/ml. Assay plate preparation was done by the WWRAN protocol INV03. Antimalarial assay plates were incubated for 72 hours at 37°C in a candle jar. Then the plates were thawed for hemolysis. HRP2 ELISA was done by WWRAN protocol no: INV09. It measures the quantity of histidine-rich protein 2 (HRP2) produced by *P. falciparum* during the 72-hour incubation and its inhibition by antimalarial drugs by following steps.

a. Preparation of antibody-coated ELISA plates: Antibody-coated, 96-well, high-binding ELISA plates were prepared by diluting primary IgM antibody to a final concentration of 1.0 µg/mL in PBS.

b. Addition of samples to ELISA plates and incubation: 20 µL sample + 80 µl dH 2O was transferred from the assay plate to an antibody-coated ELISA plate.

c. Dilution of second antibody conjugate and added to ELISA plate: Second antibody conjugate was prepared by adding diluent and added 100 µl to each well of the ELISA plate.

d. Addition of substrate and read sample absorbance: 100 µl of TMB chromogen was added in each well and incubated for 5 minutes at room temperature in the dark.

Data Analysis: Antimalarial activity of extracted biota was calculated using results from three independent assays, each carried out in triplicate for antimalarial assays.

RESULT AND OBSERVATIONS

The study was carried on from March, 2017 to January, 2018. Plants were collected from local markets and Shariatpur and those are *B. juncea, G. oppositifolius* and *B. acutangula* respectively. The yields of extracts after drying and extraction are given in Table 2 below. The percentage yield was calculated by dividing the mass of powder with the original mass of plant extract multiplied by a 100. Overall, the methanolic extracts of *B. acutangula* resulted in the highest quantity of crude extract, while the *G. oppositifolius* extract gave the least quantity.

Table 1. Yield of extracts of *Barringtonia acutangula, Brassica juncea,* and *Glinus oppositifolius*

Plant	Solvent	Weight of plant (powdered) (g)	Weight of extract (g)	Yield (%)
Barringtonia acutangula	Methanol	100	14.6	14.6
Brassica juncea	Methanol	100	11.7	11.7
Glinus oppositifolius	Methanol	100	8.4	8.4

ELISA (enzyme-linked immunosorbent assay) reading at 450nm: The HRP2 assay generates the optical density (OD) value for each well. OD values were proportional to the quantity of produced HRP2. The plants denoted in the table are as following: *Brassica juncea, Glinus oppositifolius* and *Barringtonia acutangula.* The curve indicates that the 50% inhibitory concentration is residing between the 6th and 7th concentration of drug. The concentration at which growth of parasites was inhibited by 50 % (IC50) was estimated from the graph drawn on the percentage (%) growth inhibition data. The concentration of drug corresponding to 50 % growth inhibition (IC50) for chloroquine was 1.82 nM. By using the Graphpad prism 7software a non-linear regression graph with a sigmoidal dose response curve is obtained, where concentrations of

Chloroquine are expressed as logarithmic numbers in x-axis and O.D values are normalized to express as percentage inhibition values. From the above curve we saw, initially when the drug concentration is high it inhibits all the parasite to growth, then the gradual reduction of drug concentration the survival rate of parasite is increased. The curve indicates that the 50% inhibitory concentration is residing between the 6th and 7th concentration of drug (Figure 1).The concentration at which growth of parasites was inhibited by 50 % (IC50) was estimated from the graph drawn on the percentage (%) growth inhibition data. The concentration of drug corresponding to 50 % growth inhibition (IC50) for chloroquine was 1.82 nM.

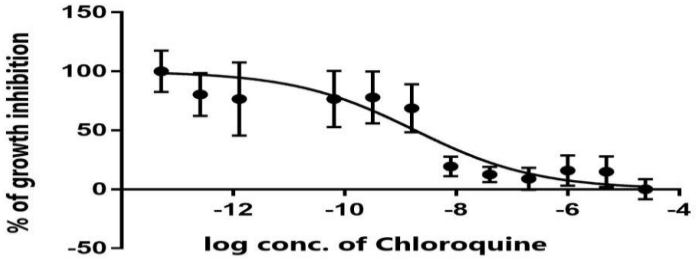

Fig.1. Dose response curve of Chloroquine.

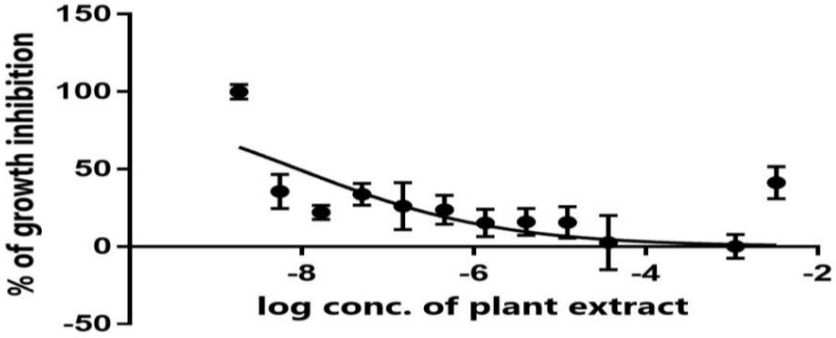

Fig. 2. Dose response curve of *Brassica juncea.*

The curve (Figure 2) indicates that the 50% inhibitory concentration is residing between the 6th and 7th concentration of plant extract. The concentration at which growth of parasites was inhibited by 50 % (IC50) was estimated from the graph drawn on the percentage (%) growth inhibition data. The concentration of plant extracts corresponding to 50 % growth inhibition (IC50) for *B. juncea* was 0.00905µg/ml. Initially when the plant extract concentration was high, it inhibited all the parasite to growth, then the gradual reduction of plant extract concentration the survival rate of parasite is

increased. The curve (Fig. 3) indicates that the 50% inhibitory concentration was residing between the 5th and 6th concentration of plant extract. The concentration of plant extracts corresponding to 50 % growth inhibition (IC50) for *G. oppositifolius* was 13.8 µg/ml. From the above curve (Figure 4) we saw, initially when the plant extract concentration is high it inhibits all the parasite to growth, then the gradual reduction of plant extract concentration the survival rate of parasite is increased. The curve indicates that the 50% inhibitory concentration is residing between the 9th and 10th concentration of plant extract. The concentration at which growth of parasites was inhibited by 50 % (IC50) was estimated from the graph drawn on the percentage (%) growth inhibition data. The concentration of plant extracts corresponding to 50 % growth inhibition (IC50) for *B. acutangula* was 23.8 µg/ml.

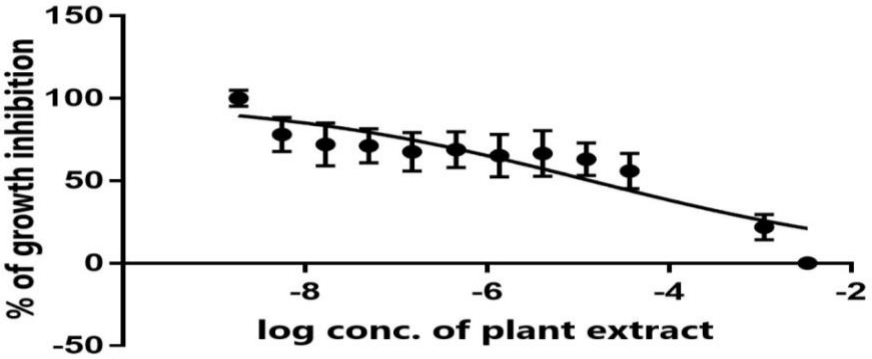

Fig.3. Dose response curve of *Glinus oppositifolius*

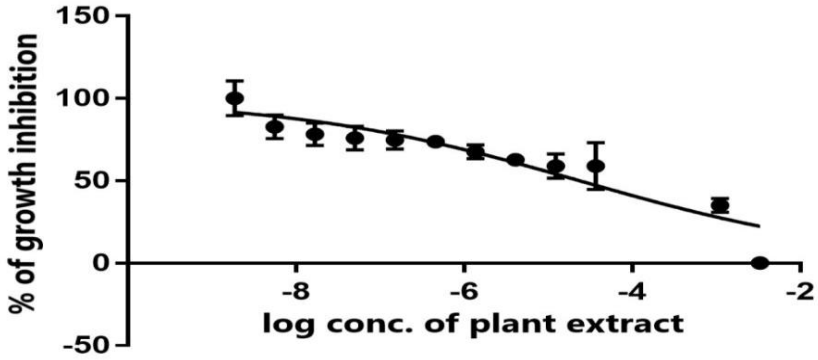

Fig. 4. Dose response curve of *Barringtonia acutangula.*

By observing the dose response curve of DMSO it can be anticipated that the concentration that is provided was non-toxic to the parasite and did not have any inhibitory effect on the growth of all drug sensitive 3D7 strain. Three of the plant extracts, belonging to several families, screened for their potential antimalarial properties against all drug-sensitive *P. falciparum* 3D7 strain using HRP2 assay. Extract of *Brassica juncea* (whole plant), has the highest potential antimalarial activity using IC50 as a parameter of activity. The IC50 values from these three extract showed that *B. juncea* has the lower IC50 value than the other two (table 6). IC50 values of the three extracts indicated that concentration

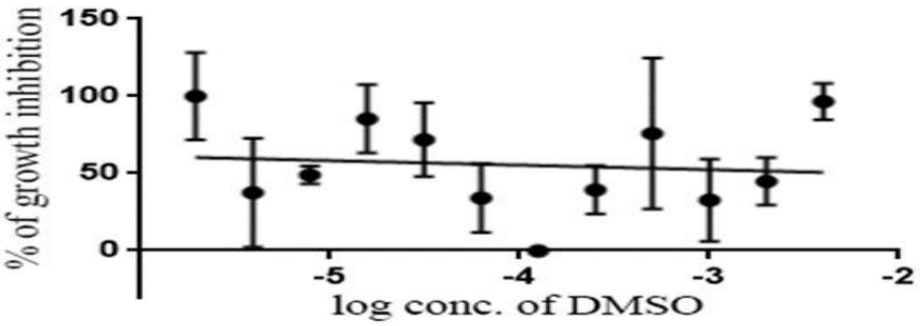

Figure 5 . Dose response curve of DMSO

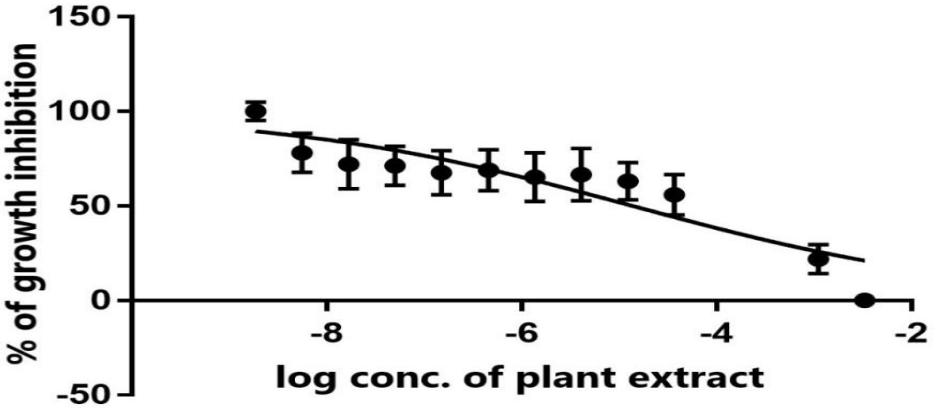

Fig. 6. Dose response curve of DMSO

Table 2. Inhibitory Concentration (IC50) of methanolic extracts of plants *Brassicajuncea, Glinus oppositifolius,* and *Barringtonia acutangula.*

Methanol extract of	Inhibitory concentration(IC_{50}) of	Result

Plants	plant extract IC50 (µg/ml)	
Brassica juncea	0.00905	Highly active
Glinus oppositifolius	13.8	Active
Barringtonia acutangula	23.8	Moderately active

It can also be said that the amount of *B. acutangula* required for effective *Plasmodium* inhibition activity was 2630 times more than that of *B. juncea*. Chemical substances of *B. juncea* and *G. oppositifolius* as active extract were considered to take effect in antimalarial activity, such as Sinigrin and Gluconapin. (Nancé *et al.* 2012, Kuluye *et al.* 2015, Kilame and Ntoumi. 2009) spergulagenic acid, sapogenin, spergulageninA and a tri-hydroxy ketone, glycoside, mollugo glycoside A, α-Spinasterol and β-sitosterolglucoside, bis-nortriterpenesapogenol, spergulatriol, spergulagenol, oleanolic acid, methyl spergulagenate and spergulagenin A (www.mpbd.info/plants/glinus-oppositifolius.php). Extract of *Brassica juncea* (whole plant), has the highest potential antimalarial activity using IC50 as a parameter of activity. The IC50 values from these three extract showed that *B. juncea* has the lower IC50 value than the other two (table 6).

IC50 values of the three extracts indicated that concentration of the extract did not have a linear relationship with the inhibition percentage, but rather to follow the curve of the sigmoid function. It can also be said that the amount of *B. acutangula* required for effective *Plasmodium* inhibition activity was 2630 times more than that of *B. juncea*. Chemical substances of *B. juncea* and *G. oppositifolius* as active extract were considered to take effect in antimalarial activity, such as Sinigrin and Gluconapin. (Nancé *et al.* 2012) spergulagenic acid, sapogenin, spergulageninA and a tri-hydroxy ketone, glycoside, mollugo glycoside A, α-Spinasterol and β-sitosterolglucoside, bis-nortriterpenesapogenol, spergulatriol, spergulagenol, oleanolic acid, methyl spergulagenate and spergulagenin A.

Glinus oppositifolius the herb plant commonly known as Gimma, Gimashakin Bangladesh. Its leaves contain spergulagenic acid, a saturated triterpenoidsapogenin, spergulagenin A and a tri-hydroxy ketone. Roots contain a glycoside, mollugo glycoside A. α-Spinasterol and β-sitosterolglucoside, *bis*-nortriterpenesapogenol, spergulatriol, spergulagenol, oleanolic acid, methyl spergulagenate and spergulagenin A have also been isolated from roots. The plant is stomachic, aperient and antiseptic; used in skin diseases and for suppression of the lochia. Aerial parts of *G. oppositifolius* have anti-diarrhoeal activity in rodents (Pattanayak *et al.* 2012).Antifungal, larvicidal, molluscicidal, antioxidant and radical scavenging activities are reported from it (Diallo *et al.* 2001). It has anti-protozoal and anti leishmanial activity and also antibacterial properties (Martin-Puzon *et al.* 2015, Dondorp *et al.* 2010).

Barringtonia_acutangula, the Indian oak or commonly known as Hijal is used tointoxicate fish. Antimicrobial, cytotoxic and antioxidant activities (Nance *et al.* 2012; Faruk *et al.* 2016). Leaf of it contains Terpenoids, Flavonoids, Steroids, Carbohydrates, Glycosides, Ouinones Alkaloids, Phenols, successful extraction of bioactive compounds from plants, is largely dependent on the type of solvent used in the extraction procedure.

Organic extractions were carried out in order to isolate both polar and non-polar compounds because organic solvents showing higher levels of the compounds isolated (Willcox and Bodeker. 2004). Three plant species, *B. juncea, G. oppositifolius,* and *B. acutangula* were tested for anti-plasmodial activities at twelve different concentrations ranging from 333 µg/ml – 0.00187 µg/ml. From the evaluation, two plant species demonstrated antimalarial activity against the *P. falciparum* all drug-sensitive strain, 3D7.

The present study has identified the anti-plasmodial activity in selected plant extracts by HRP2-based assay or HRP2 ELISA technique. Briefly, the HRP2-based assay is a very sensitive and specific measure of *P. falciparum* growth by quantifying parasite specific biomolecule, HRP2. The HRP2-based assay is comparable with other techniques because the result produced by). The extract of *B. juncea* exhibited the highest anti-plasmodial activity, then *G. oppositifolius* showed moderate activity, and the least activity was shown by *B. acutangula.*

The experiment was dosage-dependent with 333 µg/ml concentration the most effective against the *Plasmodium* parasites for all the extracts. The IC50 for the plant extracts of *B. juncea, G. oppositifolius,* and *B. acutangula* were 0.00905 µg/ml, 13.8 µg/ml, 23.8 µg/ml respectively. Based on this classification, the present findings indicate that methanolic extract of *B. juncea* (IC50=0.00905 µg/ml)had a strong anti-plasmodial activity and*G. oppositifolius* (IC50=13.8 µg/ml) plant had a good anti-plasmodial activityon all drug sensitive 3D7 laboratory strain. On the other hand, the methanolic extract of *B. acutangula* (IC50 = 23.8 µg/ml) is moderately active against 3D7 strain. In this study, the ethno pharmacological approach was used and the results obtained proved that the plants uses are rational. The 3 plants that were identified and collected all showed the presence of antimalarial compounds and the 2 that were tested showed a good anti-plasmodial activity against the *P. falciparum* strain present study confirms hypothesis that plants used traditionally to treat symptoms of malaria exhibit biological activity with anti-plasmodial effects.

CONCLUSION

Screening of anti-malarial activity with HRP2 from Bangldesh plants showed varying results, the highest yield was found in extracts of *Barringtonia acutangula* (Hijal). It may be concluded that the methanolic extracts of *Brassica juncea* (Rai Shorisha) and *Glinus oppositifolius* (Gimma Shak) possess significant suppressive effects on *in vitro* cultures of all drug sensitive 3D7 laboratory strain of *P. falciparum*. These plants could serve as useful sources for new antimicrobial agents.

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