American Journal of Infectious Diseases 5 (3): 259-262, 2009
ISSN 1553-6203

In vitro Anti Plasmodial Activity of *Enicostemma littorale*

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Abstract: Problem statement: Malaria is a prevalent disease in India. The problem of drug resistance is worsening. Hence, new effective and affordable antimalarial drugs are very much needed. The long-established use of quinine and the more recent introduction of artemisinin as highly effective anti malarials demonstrate that plant species are an important resource for the discovery of new anti malarial agents. **Approach:** Majority of the plants belonging to Gentianaceae family were proven as good antimalarials containing swertiamarin as a common marker. *Enicostemma littorale* (Gentianaceae) is also known from traditional knowledge for treatment of visham jwara and is rich in swertiamarin amongst all of the plants belonging to same family. In view of this, initial screening had been undertaken. Our laboratory had also been working on the same for its antidiabetic activity. **Results:** This was first report to demonstrate anti plasmodial activity of *Enicostemma littorale* (Gentianaceae) against *Plasmodium falciparum*. Methanolic extract of plant and swertiamarin isolated from it showed promising results *in vitro* in schizont maturation inhibition assay having IC_{50} of 529.045 and 12 µg mL^{-1} respectively. We also reported a simple and rapid method for isolation of swertiamarin which was applicable at commercial scale. **Conclusion:** The present study represented the potential antimalarial action of plant and its active phytoconstituent, may give new lead to researchers in field of antimalarial drug discovery.

Key words: Antiplasmodial activity, *Enicostemma littorale*, *in vitro* assay, *Plasmodium falciparum*, Swertiamarin

INTRODUCTION

According to an estimate, about 350-500 million people are infected from malarial parasite and approximately one to three million deaths annually-this represents at least one death every 30 sec[3,6]. The vast majority of cases occur in children under the age of 5 years[5,23]. Due to the massive worldwide use of chloroquine, a widely accepted drug in late 1940s resistance has spread to the vast majority of the malaria endemic areas like Africa, Indian subcontinents South East Asia and East Asia. The another choice was a combination antifolate drugs, Sulfadoxine pyrimethamine, widely used inexpensive anti malarial, also faced unacceptable levels of therapeutic failure in many areas in Asia, South America and now in Africa[11]. Resistance to mefloquine is a problem in border areas of Thailand, Combodia and Myanmar whereas in some areas like South East Asia and Brazil, where quinine and tetracycline are used together for the treatment of uncomplicated malaria, sensitivity to quinine is diminishing[4]. Hence, the malaria endemic regions of the world are faced with an unprecedented situation in which the only affordable treatment options are rapidly losing therapeutic efficacy because of some degree of parasite resistance. The uses of artemisinin derivative have been negatively impacted by the observation that high parentral doses of certain compounds can produce a limited, unique selective brain stem neuropathy in laboratory animals[14]. Clinically relevant artemisinin resistance has not been demonstrated, but it is likely to occur since artemisinin resistance has been obtained in laboratory models[15].

In many parts of the world, the worsening problem of resistance makes decision making difficult on the choice of the anti malarial drugs to use. New, more effective and affordable anti malarial drugs are very much needed. However, the pharmaceutical industry is not interested to develop them because it is not commercially attractive areas as malaria-endemic countries are among the poorest in world[2,12].

The success of the natural products quinine and artemisinin, most potent anti malarial drugs provided impetus the study of plants as anti malarial agents. Ethnopharmacological and then phytopharmacological approach for the search of new anti malarial agents from plant sources has proved to be more predictive. Based on the photochemistry and traditional knowledge

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Enicostemma littorale seems to be a viable candidate. Many plants from family Gentianaceae, like *Swertia chirata*, showed good antimalarial activity[1,20].

Our lab has been working on this plant and first time reported antihyperglycemic effect in alloxan induced diabetic rats. Further dose dependent hypoglycemic antioxidant and hypolipidemic effects were reported in alloxan induced diabetic and cholesterol fed rats[7,21,22]. The insulinotropic action of aqueous extract of *E. littorale* was also demonstrated using rat pancreatic islets[9]. Another group reported increased insulin sensitivity, normalized dyslipidemia and nephroprotection in diabetic rats[10] Plant extract was also clinically evaluated in diabetic patients[19,21].

Swertiamarin is a major phytoconstituent present in *Enicostemma littorale*[8,13,24] and reported for gastroprotective, antiulcerogenic , anticholonergic and CNS depressant activity[8].

The previously reported methods for isolation are the use of column chromatography and time consuming. We report a rapid method of isolation of swertiamarin from *Enicostemma littorale* that is less time consuming, simple, convenient and suitable for commercial scale. We also report anti malarial activity of the same screened for first time.

MATERIALS AND METHODS

Plant material: Plant material was collected from local market of Baroda after proper identification. Voucher specimen is kept in the herbarium of Department of Botany MS University of Baroda, Vadodara.

Extraction procedure: The plant material was dried in hot air oven at a temperature not exceeding 50°C. The material was powdered and extracted with methanol. The solvent was removed under reduced pressure.

Isolation of swertiamarin: The methanolic extract was reconstituted in water and fractionated successively with chloroform, ethyl acetate and butanol. The butanol fraction was concentrated under reduced pressure and excessive amount of ethyl acetate was added to it. The precipitates so obtained were of swertiamarin. The compound was confirmed by TLC pattern, melting point and UVmax.

Anti plasmodial assay: Strain of *P. falciparum* was cultured continuously according to the candle jar method of Trager and Jensen[17] *in vitro* in human red blood cells (blood type B^+) with 5% hematocrit in LIQUID RPMI 1640 medium (HIMEDIA) supplemented with 25 mM HEPES (Sigma), 0.2% sodium bicarbonate (Sigma) and 10% human B^+ serum. Slides of culture were observed after 3, 6 and 24 h for regular development of parasite stages.

Chloroquine sensitive *P. falciparum*, MRC 20 was obtained from the National Institute of Malaria Research (NIMR), New Delhi. The culture was synchronized using sorbitol and parasitaemia was adjusted to 1-1.5% by diluting with fresh human erythrocytes. The cells were diluted with complete media to make 8% haematocrit. Again the slides of culture were prepared and observed for the calculation of parasitemia, particularly for young trophozoites or ring stages. One mg of each compound/extract was dissolved in 100 µL DMSO and 900 µL RPMI-1640 to obtain a stock of 1 mg mL^{-1} (stock solution). A series of eight concentrations were prepared from the stock solutions by 2 fold dilutions. After 24 h, thin films of the contents of each well were prepared and examined under the microscope.

Parasite count for each blood film was made using a compound microscope under oil immersion with X 100 objective after staining the film with eosin yellow and methylene blue. Each film was observed at three different visual fields. The number of schizonts per 200 parasites were noted and compared between control and test wells for the determination of the % inhibition. All doses were studied in cultures and the mean was observed for purposes of inferences. The inhibition of parasite growth in the drug-treated groups was calculated as follows: Parasitaemia in the control (non-treated) group minus parasitaemia in the drug-treated group, divided by parasitaemia in the control (non-treated) group, expressed as percentages.

All values are expressed as percentage growth inhibition. Dose response curves of the fractions were obtained by plotting percentage inhibition against log concentration. The values of the compounds provided a mid-point value where parasite growth would be 50%. Linear regression analysis was applied to the linear portion of the sigmoidal curve was plotted and IC_{50} values were derived for each fraction.

RESULTS

Methanolic extract of *Enicostemma littorale* was found active by inhibiting formation of schizonts from trophozoits showing 50 % inhibition at 529.04 µg mL^{-1}. Swertiamarin (Fig. 1) is a major compound present in *Enicostemma littorale*. The isolated compound was showing a single band at Rf 0.55 when spotted on a precoated TLC plate and chromatographed in Ethyl acetate: Methanol: Water (7.7: 1.5: 0.5)[16].

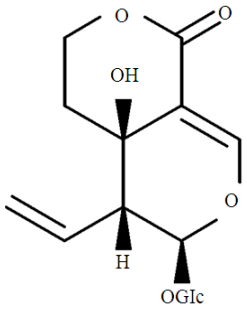

Fig. 1: Structure of swertiamarin

Melting point of the compound was 190-192°C[18]. The UVmax of the compound was 240-245 nm. Swertiamarin was active in a dose dependent manner. The 50% inhibition was observed at 12 µg mL^{-1}.

DISCUSSION

Bitter herbs from Gentianaceae family like *Swertia chirata* are reported traditionally useful in all types of fevers especially for chronic and intermittent fevers. Ethanol extract of *Swertia chirata* has been reported for antimalarial activity[1]. Ayush-64 an Ayurvedic coded compound formulation consisting "*Kiratatikta-Swertia chirata*" was found to be effective in malaria[20]. Swertiamarin is present in *Swertia chirata*. Swertiamarin may be responsible for the antimalarial activity of *Swertia chirata* and Ayush-64. *Enicostemma littorale*, a coastal herb, belonging to family Gentianaceae also contains bitter glycosides and irridoids. Swertiamarin is a major active principle of *Enicostemma littorale*. The reported amount of Swertiamarin in *E. littorale* (7.7%)[16] is much more higher than that in *Swertia chirata* (0.94%)[24] which favors more commercial viability of the plant when swertiamarin is to be isolated. The IC_{50} value of Chloroquine and Quinine[25] is around 10 and 29 µg mL^{-1} respectively which indicate that swertiamarin lies between the scales.

CONCLUSION

The present study represents the potential antimalarial action of the plant and its active phytoconstituent, may give new lead to researchers in field of antimalarial drug discovery.

ACKNOWLEDGEMENT

We are thankful to Dr. C.R. Pillai, Former Director, National Institute of Malaria Research (NIMR) for providing parasite culture and valuable guidance.

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