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Research Article

Evaluation of *In vitro* Antimalarial Activity of *Hedyotis Herbacea* by Schizont Maturation Inhibition Assay

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Abstract

The present study was designed to evaluate *in vitro* antimalarial activity of different extracts of *Hedyotis herbacea* by schizont maturation inhibition assay. The preliminary phytochemical screening of different extracts of *Hedyotis herbacea* have shown the presence of flavonoids, alkaloids, carbohydrates, saponins, tannins, cardiac glycosides, steroids and triterpenoids. The alkaloid contents in different extracts of *Hedyotis herbacea* were examined and expressed in terms of Atropine equivalent as mg of AE/g of extract. The ethyl acetate extract of *Hedyotis herbacea* has shown dose dependent % Inhibition of schizont maturation of *Plasmodium falciparum* and the highest *in vitro* antimalarial activity by schizont maturation inhibition assay indicated by IC_{50} = 55.50 µg/ml. All other extracts of *Hedyotis herbacea* had showed non-significant activity with $IC_{50} >$ 100 µg/ml.

1. INTRODUCTION

A large number of herbal medicines are presumed to have admirable therapeutic value and can be employed for the management of many ailments. In natural medicine, different local herbs are used, in single or in combined form, to treat different types of infectious diseases, with great success. Even though the exercise of these herbs has a sound practice, and their therapeutic uses are well known to local people, their place has yet to be modernized in therapeutics, using the recent methods. Scientific studies are therefore necessary to evaluate their effectiveness to broaden the scope of these herbs. Natural products from medicinal plants, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug synthesis because of the unmatched availability of chemical diversity.[1] Most of the present investigation modernizing traditional medicine is aimed to confirm the safety and efficacy of certain traditional methods and treatments through scientific experimentation which is designed on the basis of modern scientific theories and approaches. In the development of antimalarial phytomedicines from traditional medicine the emphasis is on the identification of new therapeutic lead over a variety of traditional medicinal plants to be investigated for drug discovery and development. One of the most difficult tasks in the development of new antimalarial drugs from traditional medicine is to select the lead having the highest probability to yield safe and efficacious antimalarial drugs or phytomedicies.[2]

Malaria causes about 400-900 million cases of fever and approximately one to three million deaths annually.[3] This represents at least one death every 30 seconds. The vast majority of cases occur in children under the age of 5 years and pregnant women are especially vulnerable.[4] Precise statistics of incidence are unknown because many cases occur in rural areas where the majority of cases are undocumented. Malaria is more common in rural areas than in cities, this is in contrast to dengue fever where urban areas are more prevalent.[5] It was observed from Ayurvedic literature and ethnopharmacological studies, that various plants are very useful in treating malaria but no scientific investigation has been done in such direction. Literature survey reveals that selected medicinal plant exhibits wide spectrum of medicinal value and they are used in the treatment of malaria and high fever. So, it's imperative to evaluate selected part of medicinal plant scientifically and investigate its active phytoconstituents. Therefore it was thought worthwhile to provide safe and effective phytoconstituents for antimalarial activity from some members of Indian medicinal plants

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2. MATERIALS AND METHODS

Giemsa stain, HEPES and Quinine hydrochloride were purchased from Sigma Chemicals, USA. Solvents for extraction *viz.,* petroleum ether (60-80ºC), ethyl acetate, acetone and ethanol were procured from Loba Chemicals, Mumbai, India. Atropine was generously gifted by Medico remedies Private Limited, Mumbai, India. All other chemicals and reagents used for study in the present investigation were of analytical grade.

2.1 Collection and authentication of *Hedyotis herbacea*

Hedyotis herbacea whole plant was collected from Aamgaon, District Gondia, Maharashtra, India. Herbarium specimens of *Hedyotis herbacea* was prepared and submitted at Department of Botany, Bhawabhuti Mahavidyalaya, Aamgaon, R. T. M. Nagpur University, Nagpur.

2.2 Extraction of *Hedyotis herbacea*

Selected plant materials were shade dried, cleaned and pulverized using a milling machine to obtain coarse powder which was separated on sieves. Soxhelet apparatus of capacity (5L) were used for extraction. Coarse powder of *Hedyotis herbacea* was exhaustively defatted using petroleum ether (60-80ºC). After deffating, plant material were removed from assembly, dried and again inserted into using soxhelet apparatus. Remaining residue of plant material was further extracted successively in order of increasing polarity of solvents such as ethyl acetate, acetone and

ethanol. Hydro-alcoholic extract [absolute ethanol: distilled water (1:1)] was obtained by maceration after 15 days. All the extracts were collected, filtered through Whatman filter paper (No. 44), concentrated over thermostat water bath. Dried extracts were kept in tight container, preserve in BOD incubator till further use.

2.3 Preliminary phytochemical screening

All the extracts were screened for presence of phytoconstituents viz. alkaloids, flavonoids, tannins, steroids, saponins, triterpenoids, proteins and sugars.[6]

2.4 Phytochemical standardization of different extracts of *Hedyotis herbacea*

Determination of total alkaloid content

The different extracts of *Hedyotis herbacea* (1 mg) was dissolved in dimethyl sulphoxide (DMSO), added 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10 ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of Atropine (20, 40, 60, 80 and 100 µg/ml) were prepared. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer.[7-9]

2.5 Evaluation of *in vitro* antimalarial activity of different extracts of *Hedyotis herbacea* by schizont maturation inhibition assay[10-11]

Different extracts of *Hedyotis herbacea* were evaluated for antimalarial activity by schizont maturation inhibition assay. Parasites were incubated with different test extracts and then parasitemia of control and treated groups are compared by counting Giemsa stained parasites using light microscopy. This technique relies on a morphological criterion of response. This is a reliable technique because in this method parasite inhibition can be seen visually.

Strain of *Plasmodium falciparum* was obtained from National Institute of Malaria Research (NIMR), New Delhi and was cultured and maintained by candle jar method of Trager and Jensen (1976) in vitro. The medium used was RPMI-1640 (Sigma-Alderich) supplemented with 25 mM HEPES, 0.2 % $NaHCO_3$ and 10 % human serum.

Stock solutions of extracts were prepared by dissolving known quantities of different extracts of *Hedyotis herbacea* in DMSO. These were further diluted with RPMI-1640 to achieve the required concentration before testing with the culture.

Each concentration of extracts along with 10 µM Quinine dihydrochloride (positive control) and respective solvent (negative control) were run in triplicate 96 well microtitre plates. Each well contained 20 µl of diluted extract and 200 µl RPMI-1640 supplemented with parasitized RBCs (1:10 ratio of extract and medium). These plates were incubated at 37ºC in the candle jar. After 24 h of incubation, these smears from each well were prepared and stained with Giemsa stain. The morphological features of erythrocytic blood stages of parasites including rings, tropozoites and schizonts were observed under microscope. The number of parasitized RBCs per 100/500 cells were counted and IC_{50} value of each extract was estimated. IC_{50} (50 % inhibiting concentration) is the drug concentration corresponding to 50 % inhibition of schizont development as compared to control. Percent schizont maturation inhibition was calculated by using this equation:

$$\% \text{ Schizont maturation inhibition} = 100 \times \frac{(A-B)}{A}$$

A - Average schizont maturation in untreated control well

B - Average schizont maturation in extract/ standard treated well

2.6 Statistical analysis

Data were presented as mean ± SD. Statistical analysis was carried out using one way ANOVA using Graph Pad Prism Software Version 6.04. Differences were considered statistically significant at $P \leq 0.05$.

3. RESULTS AND DISCUSSION

Different extracts of *Hedyotis herbacea* have shown presence of flavonoids, alkaloids, carbohydrates, saponins, tannins, cardiac glycosides, steroids and triterpenoids.

The alkaloid contents were examined in different extracts of *Hedyotis herbacea* and expressed in terms of Atropine equivalent as mg of AE/g of extract (the standard curve equation: y = 0.004x + 0.044, R^2 = 0.980). The total Alkaloid contents in different extracts of *Hedyotis herbacea* expressed in terms of Atropine equivalent (mg of AE/g of extract) are shown in Table 1.The highest concentration of alkaloid was measured 50.48 mg of AE/g of ethyl acetate extracts of *Hedyotis herbacea.*

IC_{50} value was determined by plotting a graph between various concentrations of extracts and percentage of schizont maturation inhibition corresponding to that concentration are shown in Table 2 and Figure 2.The ethyl acetate extract of *Hedyotis herbacea* has shown dose dependent % Inhibition of schizont maturation of *Plasmodium falciparum* and the highest *in vitro* antimalarial activity by schizont maturation inhibition assay indicated by IC_{50} = 55.50 µg/ml. All other extracts of *Hedyotis herbacea* had showed non-significant activity with IC_{50} > 100 µg/ml.

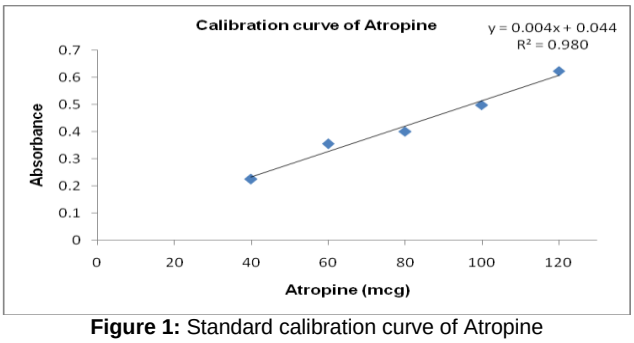

Figure 1: Standard calibration curve of Atropine

Table 1: Alkaloid contents in different extracts of *Hedyotis erbacea* expressed in terms of Atropine equivalent (mg of AE/g of extract)

***Hedyotis herbacea* extracts**	**Absorbance**	**mg of AE/g of extract**
Petroleum ether extract	0.060	03.91
Ethyl acetate extract	0.246	50.48
Acetone extract	0.071	06.71
Ethanol extract	0.166	30.52
Hydro-alcoholic extract	0.130	21.42

Table 2: Effect of different extracts of *Hedyotis herbacea* on schizont maturation of *Plasmodium falciparum*

***Hedyotis herbacea* extracts**	**Dose (µg/ml)**	**% Inhibition**	**IC_{50} (µg/ml)**
Petroleum ether extract	10	6.98 ± 0.15	> 100
	25	7.80 ± 0.50	
	50	10.38 ± 0.47	
	100	10.89 ± 0.48	
Ethyl acetate extract	10	34.63 ± 0.46	55.50***
	25	41.24 ± 0.38	
	50	50.29 ± 1.57	
	100	62.72 ± 2.37	
Acetone extract	10	8.66 ± 0.37	> 100
	25	12.93 ± 0.34	
	50	16.26 ± 1.14	
	100	20.78 ± 0.33	
Ethanol extract	10	10.24 ± 0.12	> 100
	25	16.58 ± 0.52	
	50	28.14 ± 2.60	
	100	42.02 ± 1.08	
Hydro-alcoholic extract	10	9.73 ± 0.35	> 100
	25	14.70 ± 0.39	
	50	21.41 ± 1.07	
	100	25.66 ± 1.00	
Standard (Quinine dihydrochloride)	0.005	40.48 ± 0.28	0.0096
	0.01	45.33 ± 0.30	
	0.015	65.07 ± 0.42	
	0.020	83.14 ± 2.25	

*N = 3, Values are expressed in Mean ± SD. * p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001.*

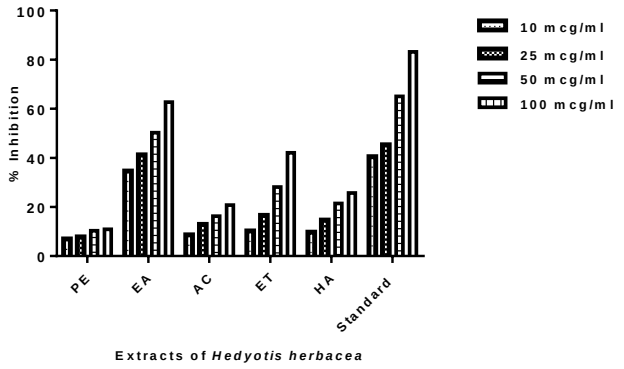

Figure 2: Effect of different extracts of *Hedyotis herbacea* on schizont maturation of *Plasmodium falciparum*

4. ACKNOWLEDGEMENT

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