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PRELIMINARY EVALUATION OF ANTIMALARIAL ACTIVITY OF PLANTS USED IN TRADITIONAL MEDICINE IN INDIA

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Malaria is one of the most important tropical diseases and the greatest cause of morbidity and mortality in India. The search for new antimalarial compounds has been necessitated by *Plasmodium falciparum* resistance to almost all antimalarial drugs. In this study the *in vitro* antimalarial activity of ethanolic leaf extract of *Momordica charantia* (Cucurbitaceae), *Momordica dioica* (Cucurbitaceae), *Solanum lycopercicum* (Solanaceae) and *Annona squamosa* (Annonaceae) were evaluated against chloroquine sensitive *Plasmodium falciparum* (3D7 strain). All four plants showed promising antimalarial activity with IC_{50} ranged between 48.31 to 143.8 µg/ml. Further work is suggested to isolate, identify and characterize the active principle for more potent antimalarial activity.

Keywords: *Annona squamosa;* Antimalarial; *Momordica charantia; Momordica dioica; Plasmodium falciparum; Solanum lycopercicum.*

Malaria represents the world's greatest public health problem in terms of number of people affected, levels of morbidity and mortality[1]. Each year, malaria affects about 400 - 500 million people worldwide, with at least 2-3 million deaths[2]. The alarming rate at which *Plasmodium falciparum* has developed resistance to chloroquine and other synthetic antimalarial drugs makes it necessary to search for more effective antimalarial compounds[3,4]. Developing countries, where malaria is endemic, depend strongly on traditional medicine as a source for inexpensive treatment of this disease[5]. However, scientific data to validate the antimalarial properties of these herbal remedies are scarce. The present study provided evidence for the rational exploration of indigenous Indian medicinal plants as a source of antiplasmodial agents. Earlier claims show that these plants are used in traditional medicine and cultivated throughout India[6].

Plant material : Fresh leaves of *Momordica charantia, Momordica dioica, Solanum lycopercicum* and *Annona squamosa* Linn. were collected from Rajasthan, washed thoroughly 2 to 3 times with running tap water and then with sterile water followed by shade-dried, powdered and used for extraction.

Preparation of solvent extractions-For each plant 50 g powder of the plant leaves were extracted with ethanol in soxhlet extractor for 72 hours. All the extracts were concentrated to dryness on a water bath and weighed. The extracts were then stored in closed containers and kept in a refrigerator at 4°C to protect from light and moisture till used.

Preparation of culture media-Plasmodium falciparum 3D7 strain was procured from National Centre for Cell Sciences, Pune. Culture medium was prepared by dissolving 10.43 g RPMI 1640 powder (Gibco), 6 g of HEPES, 2 g of $NaHCO_3$ (Sigma Aldrich) in 1 liter of distilled-deionised water. The medium was filtered using 0.22µm membrane filter and 0.5 ml gentamycin (from 50 mg/ml stock) was added and stored at 4°C in aliquots of 90 ml. Before cultivation, every aliquot was supplemented with 10 ml of 5 % Albumax II.

In vitro cultivation of Plasmodium falciparum and sensitivity test-The *in vitro* activity of crude leaf extracts against *P. falciparum* intra-erythrocytic stages was evaluated by means of the Mark III test, as developed by the WHO[7]. Stock solutions of extracts were prepared by dissolving known quantities of the dried ethanol extracts in methanol, di-methyl sulfoxide (DMSO), and distilled water (1:1:3 by volume). The stock solutions were further diluted with RPMI 1640 to achieve the required concentrations. The culture, before testing was synchronized by treatment with 5% D-sorbitol[8]. Titer plates were incubated in CO_2 condition at 37°C in candle jar for 24-30 hours[9]. After incubation, thin smears were made from duplicate wells, fixed in methanol, and stained with Giemsa stain. An area of stained thin blood film where the erythrocytes were evenly distributed, was observed

Table 1. IC_{50} value of leaf extracts against *Plasmodium falciparum* 3D7strain.

S. No.	Name of the plants	IC_{50} µg/ml
1.	*Solanum lycopersicum*	48.31
2.	*Momordica charantia*	74.59
3.	*Momordica dioica*	129.0
4.	*Annona squamosa*	143.8

IC_{50}: Concentration inhibiting 50 percent parasitemia

using 100 X objective (under oil immersion).The control parasite culture freed from extracts was considered as 100% growth. The average suppression of parasitemia in comparison to control was calculated to estimate percent inhibition for each concentration. Fifty percent inhibitory concentration (IC_{50}) was calculated.

The basic measurement of antimalarial activity used in this study was the reduction in number of parasitized cells in the test cultures compared to control at 24-30 hours of incubation (Fig.1). Of the four leaf extracts tested, the extract of *Solanum lycopercicum* showed the highest antimalarial activity (IC_{50} of 48.31 µg/ml) followed by *Momordica charantia* (IC_{50} of 74.59 µg/ml) *Momordica dioica* (IC_{50} of 129.0 µg/ml) and *Annona squamosa* (IC_{50} of 143.8 µg/ml). The IC_{50} values are presented in Table 1.

In India plants have always been used for the treatment of malaria in traditional medicine[10]. Consequently, it is important that antimalarial medicinal plants are investigated, in order to establish their efficacy and to determine their potential as sources of new antimalarial drugs[11]. In this study, the *in vitro* antimalarial activity of ethanolic leaf extracts of *Momordica charantia, Momordica dioica, Annona squamosa* and *Solanum lycopercicum* Linn. plants used in traditional medicine in India is reported. *Solanum lycopercicum* had the highest antimalarial activity (IC_{50} 48.31 µg/ml) followed by Momordica chirantia (IC_{50} 74.59µg/ml). This may be indicative of a significant potential for isolating purer compounds with much higher antimalarial activity from these plants; crude plant extracts with moderate activity have yielded purer compounds with potent antimalarial activity[12]. The results justify the traditional use of the plants in the treatment of malaria. Further work is suggested to isolate, identify and characterize the active principles from these plants.

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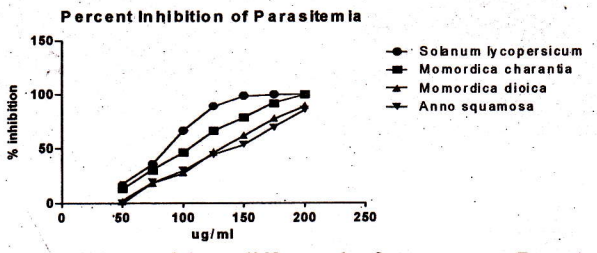

Fig.1. Effect of four different leaf extracts on Percent inhibition of parasitemia.

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