

Antimalarial Efficacy of *Thalictrum foliolosum* (Meadow rue) Against Chloroquine-Resistant *P. falciparum*

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Abstract

Background: Investigation of traditionally used medicinal plants can provide safe and cost effective good antimalarials. The present study explores the *in vitro* antimalarial potential of *Thalictrum foliolosum* (meadow rue) leaf extract against *Plasmodium falciparum*.

Methods: Phytochemical screening of the extract was carried out following standardized methods. WHO method based on schizont maturation inhibition was employed to assess the *in vitro* antiplasmodial activity of plant extract. Colorimetric MTT assay was used to evaluate the *in vitro* cytotoxicity of *Thalictrum foliolosum*. Selectivity Index was also calculated.

Results: Phytochemical screening of the extract revealed presence of alkaloids, phenols, triterpenes, saponins and phytosterols. Ethanolic leaf extract of *Thalictrum foliolosum* (ELETf) exhibited IC₅₀<5 µg/ml against chloroquine (CQ) resistant (RKL-9) strains of *Plasmodium falciparum*, whereas, it was=5.89 µg/ml against chloroquine sensitive (MRC-2) strain. The extract revealed no signs of toxicity with CC₅₀>1000µg/ml against both *HeLa* cells and normal fibroblasts. Selectivity index of ELETf was calculated to be >200 and =169.7 respectively for chloroquine resistant (RKL-9) and sensitive (MRC-2) strains of the parasite.

Conclusions: Based on WHO recommendations ELETf can be classified as highly active antimalarial against CQ-resistant strain and possesses promising activity against CQ-sensitive strain. The high selectivity index (SI>10) also establishes *Thalictrum foliolosum* as an active antimalarial against both the strains of *Plasmodium falciparum*.

Keywords: Traditional medicine; Malaria; *Thalictrum foliolosum*; *Plasmodium falciparum*; Selectivity index

Introduction

Malaria remains one of the most deadly infectious parasitic diseases in the world. Plants have been utilized as major therapeutic resources in the treatment of various ailments especially against malaria. Historically, most of the effective antimalarial drugs (quinine, artemisinin) have been derived from medicinal plants. In view of increasing drug resistance to classical antimalarial drugs, there has been a renewed interest in exploring traditional medicinal plants as safe, low cost and effective antimalarials. *Thalictrum* is an herbaceous perennial flowering plant of the Ranunculaceae (buttercup) family. It is commonly known as “meadow-rue”. Meadow-rues are usually found in shaded or damp locations, native to the temperate regions of the world. It is also found in southern Africa and tropical South America.

The plant is used traditionally as an antipyretic besides being a good remedy for many other ailments. Several secondary metabolites such as isoquinolines: berberine and pseudoberberine; bisbenzylisoquinolines: thalfoetidine, northalfoetidine, aporphine, phenanthrene and benzylisoquinoline alkaloids have been isolated from the roots of *Thalictrum flavum* L. The isolated compounds have also been reported to possess considerable *in vitro* antiparasitic activities with IC₅₀ ranging between 0.5-3.6 µg/ml against *Plasmodium falciparum* and IC₅₀ ranging between 13-27 µg/ml against *Leishmania major* [1]. A triterpenoid, isolated from *Thalictrum fortunei* has also been reported to possess anti-tumour activity [2]. Northalrugosidine, bisbenzyltetrahydroisoquinoline alkaloid, isolated from *Thalictrum alpinum* exhibited considerable *in vitro* antileishmanial with IC₅₀ 0.28 µM. It also exhibited dose dependent reduction of parasite burden in liver and spleen in murine model of visceral leishmaniasis, without any toxicity [3]. Present study reports the antimalarial efficacy of *Thalictrum foliolosum* leaf extract against *P. falciparum* and its cytotoxicity against both cancerous and normal cell lines.

Materials And Methods

Plant material

Thalictrum foliolosum (leaves) were collected from Shimla district of Himachal Pradesh, India. The permission for collection of the plant was obtained from H.P. State Biodiversity Board, State Council for Science Technology and Environment, Kasumpti, Shimla (letter no. SCSTE/SBB-959). Voucher specimen no. 1106 has been deposited at the Botany Department, Panjab University, Chandigarh where the taxonomic identification was carried out. Leaves of *Thalictrum foliolosum* were washed with water, shade-dried at room temperature and then powdered. The ethanolic leaf extract of *Thalictrum foliolosum* (ELETf) was prepared by Soxhlet extraction method.

Phytochemical screening

Phytochemical screening of ethanolic leaf extract of *Thalictrum foliolosum* (ELETf) was carried out by employing standard procedures [4-6]. Qualitative tests were performed to detect alkaloids, saponins, phenols, anthraquinones, steroids, diterpenes, triterpenes, flavonoids, cardiac glycosides, tannins and phytosterols (Table 1).

In vitro antimalarial activity against *P. falciparum*

The chloroquine-sensitive (MRC-2) and chloroquine-resistant (RKL-9) strain of *Plasmodium falciparum* were obtained from National Institute of Malaria Research (NIMR), New Delhi, India. *Plasmodium falciparum* culture was maintained *in vitro* by modified method of

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Trager and Jensen (1976) in A+ human erythrocytes using RPMI-1640 as culture medium supplemented with 10% human AB+ serum [7]. The antiplasmodial activity of ELETf was assessed by schizont maturation inhibition assay [8]. 10 mg/ml of ELETf was dissolved in 1% DMSO to prepare stock solution of extract. The stock solution was further diluted in RPMI-1640, to make various concentrations (5-100 µg) of extract. 90 µl complete medium was added in duplicate in each well in 96 well plates along with different extract concentrations. Chloroquine (10 µM) was used as positive control, while negative control contained solvent alone. Parasite culture synchronized with ring stages were then added at 1% parasitaemia and 1.5% final hematocrit. The plates were kept for 48 h at 37°C, under 5% CO₂ atmosphere. After 48 h of incubation, thin blood smears were prepared from each well, fixed in methanol and stained with Giemsa stain. Inhibition of schizont development in comparison to the control wells was determined by following formula:

$$100 - \frac{\text{Number of schizonts in drug treated well}}{\text{Number of schizonts in control well}} \times 100$$

IC₅₀ is defined as concentration of the extract/drug corresponding to 50% schizont maturation as compared to control. It was obtained by probit regression analysis using Sigmaplot software.

In vitro cytotoxicity against cancerous (HeLa) and normal cell lines

HeLa cells were purchased from National Center for Cell Science (NCCS), Pune, India. Primary culture of dermal fibroblasts was obtained from Department of Dermatology, P.G.I.M.E.R, Chandigarh. The cells were maintained at 37°C in 5% CO₂ in DMEM medium (Dulbecco's Modified Eagle's Medium) supplemented with 10% foetal calf serum and glutamine 2 mM. Colorimetric MTT assay was employed to assess *in vitro* cytotoxicity of ELETf [9]. 96-well culture plates were seeded with 100 µl of complete medium containing 5000- 10000 cells per well. After 24 h incubation, cells were treated with different concentrations of ELETf (10-1000 µg) in duplicate for 48 h. Stock solution (1 mg/ml) of (3-(4, 5- Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was prepared in incomplete medium. 10 µl of this tetrazolium reagent was added into each well followed by further incubation for 2 h at 37°C. The supernatant was removed and 100 µl DMSO was then added to each well to allow formosan solubilization. CC₅₀ values, indicating the concentration of drug needed to obtain 50% inhibition of cell growth, were then obtained by plotting % cell viability versus concentration of extract used.

Phytochemical Test	Plant Metabolites	ELETf
Ferric Chloride Test:	Phenols	+
Wagner's Test:	Alkaloids	+
Froth Test:	Saponins	+
Borntrager's Test:	Anthraquinones	-
Keller-Killiani Test:	Cardiac glycosides	-
Alkaline Reagent Test:	Flavonoids	-
Acetic anhydride test	Steroids	-
Copper Acetate Test:	Diterpenes	-
Salkowski's Test:	Triterpenes	+
Liebermann Burchard's Test:	Phytosterols	+
Gelatin Test:	Tannins	-

'+' indicates presence of active component, '-' indicates absence of active component

Table 1: Phytochemical screening of *Thalictrum foliolosum* ELETf^a.

Selectivity index

The selectivity index (SI) has been depicted as the ratio of the CC₅₀ determined on *HeLa* cells /dermal fibroblasts to the IC₅₀ determined on *Plasmodium falciparum*.

Results

Phytochemical screening

Phytochemical screening of ethanolic leaf extract of (ELETf) revealed the presence of various secondary plant metabolites (Table 1). *Thalictrum foliolosum* tested positive for phenols, alkaloids, saponins, triterpenes and phytosterols.

In vitro antimalarial activity against Plasmodium falciparum

Thalictrum foliolosum (ELETf) exhibited considerable *in vitro* antimalarial activity against both chloroquine- sensitive (MRC-2) and resistant (RKL-9) strains of *Plasmodium falciparum*. 50% inhibitory concentration (IC₅₀) was calculated to be <5 µg/ml and 5.89 µg/ml respectively for RKL-9 and MRC-2 strains of *P. falciparum* (Figure 1). The extract exhibited considerable efficacy against the chloroquine resistant (RKL-9) strain with more than 77% inhibition at 10 µg/ml and complete parasite clearance at 80 µg/ml concentration of extract. In case of chloroquine-sensitive MRC-2 strain, more than 87.5% inhibition was observed above a low concentration of 10 µg/ml.

In vitro cytotoxicity against cancerous (HeLa) and normal cell lines

Thalictrum foliolosum (ELETf) was found to be non-toxic to *HeLa* cell lines and dermal fibroblasts with CC₅₀>1000 µg/ml. Cell viability

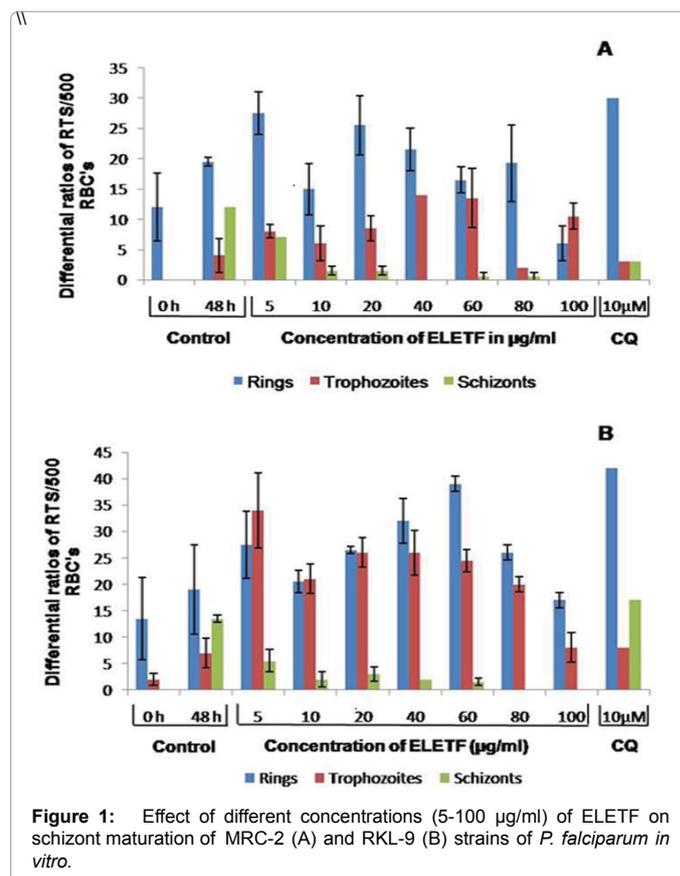


Figure 1: Effect of different concentrations (5-100 µg/ml) of ELETf on schizont maturation of MRC-2 (A) and RKL-9 (B) strains of *P. falciparum* in vitro.

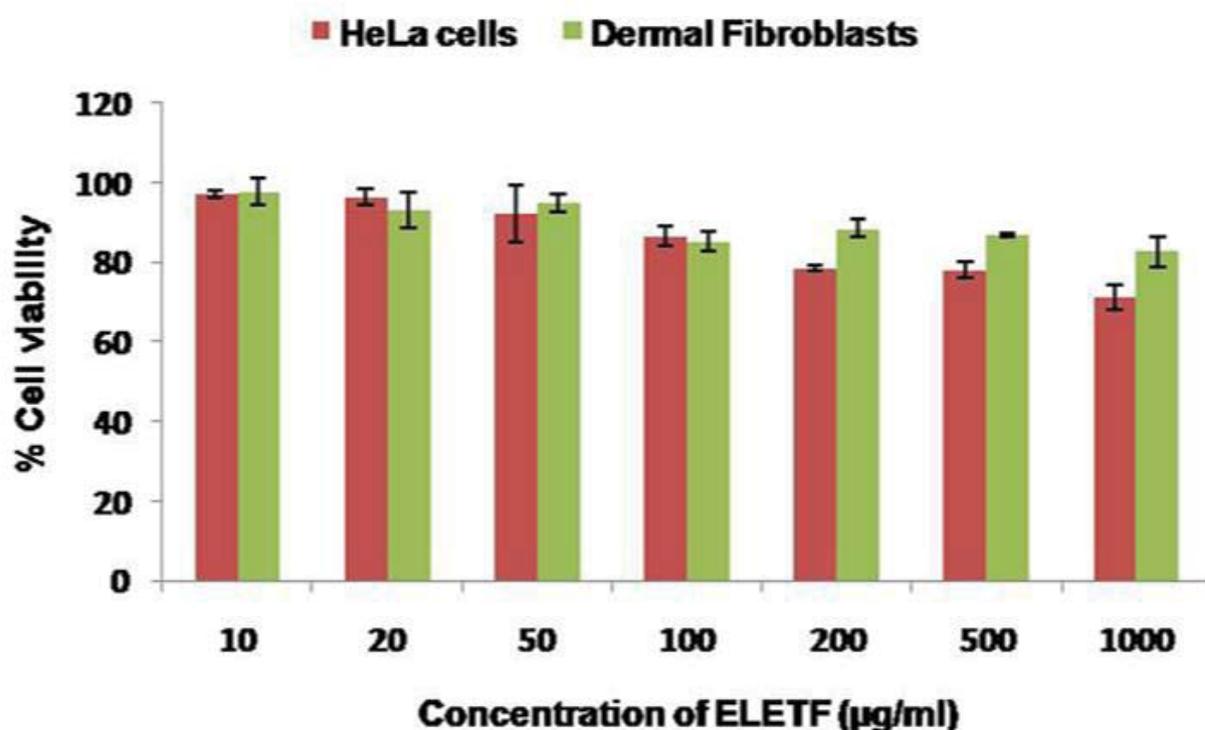


Figure 2: Cell viability (%) of *HeLa* cells and normal dermal fibroblasts upon treatment with various concentrations (10–1000 µg/ml) of ELETF *in vitro*.

was observed to be >80% in all the tested concentration (10-1000 µg/ml) (Figure 2). The selectivity index was calculated to be >200 and =169.7 for both chloroquine sensitive (MRC-2) and resistant (RKL-9) strains of *P. falciparum* respectively.

Discussion

Traditional system of medicine forms an integral part of the indigenous culture of many developing countries in the world. Use of traditional herbal remedies seems to be an alternative choice for malaria treatment in endemic countries [10]. The present study has been designed to evaluate the *in vitro* antimalarial activity of *Thalictrum foliolosum* against *Plasmodium falciparum*. Medicinal plants possess several bioactive compounds, which have been used in the treatment of various human diseases [11]. Terpenoids exhibit various important pharmacological activities i.e., anti-inflammatory, anticancer, antimalarial, anti-viral and anti-bacterial activities [12]. Alkaloids are used as anaesthetic agents [13] and are also implicated in antimalarial activity [14-16]. Presence of these phytochemical constituents in ELETF might be responsible for its antimalarial activity.

The extract exhibited very good *in vitro* antimalarial activity against both CQ-sensitive (MRC-2) and resistant (RKL-9) strains of *P. falciparum* as evident from the IC₅₀ values. According to the WHO recommendations, extracts with IC₅₀<5 µg/ml are classified as highly active, promising activity at IC₅₀ of 5-15 µg/ml, moderate activity at IC₅₀ of 15-50 µg/ml and inactive above IC₅₀>50 µg/ml [17]. Thus, ELETF can be classified as highly active antimalarial against CQ-resistant strain (RKL-9) and possesses promising activity against CQ sensitive strain (MRC-2). The cytotoxicity studies indicate the safety of extract for human use. High selectivity indices (SI>10) establishes

ELETF as an active antimalarial against both the strains [18].

Conclusions and Recommendations

The study establishes that *Thalictrum foliolosum* possess considerable *in vitro* antiplasmodial potential without any general cytotoxicity. It provides a scientific basis for the traditional use of plant as antipyretic agent. The presence of alkaloids and triterpenoids in the extract might be responsible for its significant *in vitro* antiplasmodial efficacy. It is noteworthy that the extract is more active against the resistant strain of one of the deadliest species causing fatal cerebral malaria in humans. Further studies are being carried out to explore its *in vivo* antimalarial efficacy along with isolation of active phyto-constituents responsible for the observed antimalarial activity.

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