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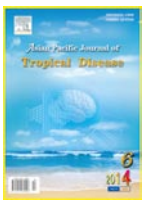

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Antimalarial activity of *Evolvulus alsinoids* Linn.-an *in vitro Plasmodium falciparum* specific lactate dehydrogenase enzyme inhibition assay

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PEER REVIEW

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Comments

E. alsinoids is commonly known as *Shankhpushpi* and used in traditional medicine for antimalarial treatment by several ethnic populations of India. Although there is no any scientific report towards the antimalarial activity of this plant, the present study provides evidence of its antimalarial potential.
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ABSTRACT

Objective: To investigate the effect of standardized methanol extract of *Evolvulus alsinoids* Linn. (*E. alsinoids*) on *Plasmodium falciparum* specific lactate dehydrogenase (*Pf*LDH) enzyme inhibition.

Methods: To carry out enzyme inhibition studies, lactate dehydrogenase was cloned from *Plasmodium falciparum* 3D7 strain using expression vector pET28a and expressed in *Escherichia coli* BL21 (DE3). Protein purification was carried out by Ni-affinity chromatography. This protein was used for the inhibition study. Methanol extract of *E. alsinoids* was standardized by high performance thin layer chromatography described by previous literature and screened for *Pf*LDH enzyme inhibitory activity and compared with gossypol (a known *Pf*LDH enzyme inhibitor).

Results: It was found that *E. alsinoids* possesses a compond scopoletin and potentially inhibits *Pf*LDH [(25.04±0.51)%].

Conclusions: Methanol extract of *E. alsinoids* showed significant *Pf*LDH inhibition as evidenced from the experiments performed. The activity may be attributed to the presence of various polyphenolic and flavanoids compounds.

KEYWORDS

Enzyme inhibition, Malaria, *Plasmodium falciparum*, Lactate dehydrogenase

1. Introduction

The *Plasmodium falciparum* lactate dehydrogenase enzyme (*Pf*LDH) has been considered as a potential molecular target for antimalarials because it is an essential metabolic enzyme in the malarial parasite, responsible for energy production during glycolysis and its inhibition leads to parasite death[1]. Gossypol, found in cotton seed, was identified as a potent inhibitor of *Pf*LDH[2]. However, its side effects of hypokalaemia and possibly permanent infertility make it unacceptable as an antimalarial drug[3]. *Evolvulus alsinoides* Linn. (*E. alsinoides*) (Convulvulaceae) is commonly known as *Shankhpushpi* and used in traditional medicine for antimalarial treatment by several ethnic populations of India[4]. The most widespread application of *E. alsinoides* is for mental problems[5]. *E. alsinoides* has been proved to possess scientific potential in central nervous system depression, anxiolytic, tranquillizing, antidepressant, antistress, neurodegenerative, antiamnesic, antioxidant, hypolipidemic, immunomodulatory, analgesic, antifungal, antibacterial, antidiabetic, antiulcer, anticatatonic and cardiovascular activity[6]. *E. alsinoides* is reported to

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contain several types of alkaloids, steroids, flavanoids and coumarins as active chemicals that bring about its biological effects[7]. In the present investigation, we screened the plant for its specific inhibitory effect on *Pf*LDH enzyme. To this end, we obtained *Plasmodium* species specific lactate dehydrogenase enzyme by cloning recombinant enzyme from *Plasmodium falciparum* of Indian origin, in *Escherichia coli*.

2. Materials and methods

2.1. Plant material and preparation of extract

E. alsinoides was collected from Vadodara (Gujarat, India) and identified in the Department of Botany, The M. S. University of Baroda, Vadodara (Gujarat, India). Voucher specimens of plant (No. Pharmacy/EA/09-10/10/NS) has been deposited in Herbal Drug Technology Department, The M. S. University of Baroda, Vadodara (Gujarat, India). Extraction was done according to the method described in our previous study[5]. Plant material was shade dried and ground into a coarse powder. Powdered material (120 g) was first defatted with petroleum ether (500 mL). The marc was extracted with methanol to obtain the methanol extract (yield 4.47%, w/w).

2.2. Standardizations of extract

High performance thin layer chromatographic analysis on silica gel G precoated plates was performed for standardization of extract. Chloroform : methanol : toluene (7:2:1) was found to be the best solvent system. The detecting reagent was anisaldehyde in sulphuric acid followed by heating at 110 °C for 5 min to obtain the fingerprint.

2.3. Screening of E. alsinoids extracts for PfLDH inhibitory activity

To carry out enzyme inhibition studies, lactate dehydrogenase was cloned from *Plasmodium falciparum* 3D7 strain using expression vector pET28a and expressed in *Escherichia coli* BL21 (DE3). The gene coding for *Pf*LDH was cloned in *Escherichia coli* and recombinant protein was purified by Ni-affinity chromatography and characterized as described previously[8]. Recombinant *Pf*LDH and bovine lactate dehydrogenase were diluted to 18 kU/L concentration in phosphate buffered saline (pH 7.4) containing 1 mg/mL bovine serum albumin and 1 mmol/L phenylmethanesulfonyl fluoride, and from this stock, 30 mL enzyme was used in the assay. Effect of *E. alsinoides* extract on *Pf*LDH was determined by examining variation in the enzyme activity by adding 15 mL extract (to get final concentration 50 mg/mL) in the assay mix. The phosphate buffer, used for redissolving plant extract, was tested as vehicle control. Gossypol (Sigma Aldrich, India) was also tested as positive control along with the extracts.

3. Results

3.1. Standardization of E. alsinoides

The chromatographic analysis of the methanol extract brought the fingerprint and identification of one marker compound, *i.e.* scopoletin (Figure 1).

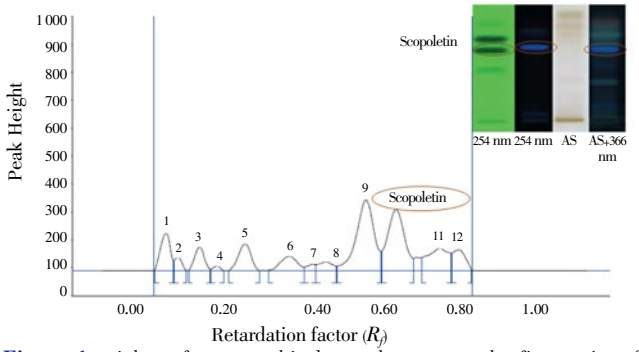

Figure 1. High performance thin layer chromatography fingerprint of methanol extract of *E. alsinoids*.
AS: anisaldehyde sulfuric acid reagent.

3.2. Inhibition studies of PfLDH

Methanol extract of *E. alsinoides* was tested at 50 mg/mL concentration for *Pf*LDH inhibitory activity. Methanol extract of *E. alsinoides* reduced *Pf*LDH activity to (25.04±0.51)%. Effects of *E. alsinoides* were statistically significant at 0.001 levels (Figure 2).

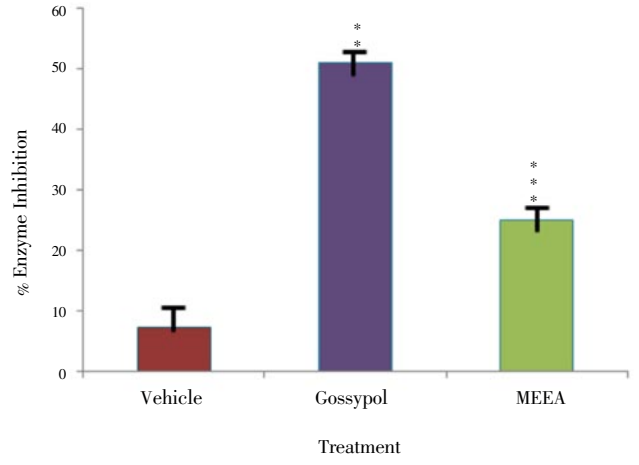

Figure 2. Inhibition of *Pf*LDH by methanol extract of *E. alsinoids*.
Data are expressed in mean±SEM, n=3; $^{***}P<0.001$, $^{**}P<0.01$.
MEEA: methanol extract of *E. alsinoids*.

4. Discussion

Methanol extract of *E. alsinoides* was prepared, standardized as per previous report[5]. Preliminary phytochemical and high performance thin layer chromatography studies on *E. alsinoides* showed the presence of flavonoids and coumarins in the methanol extract as shown previously. Methanol extract of *E. alsinoides* reduced *Pf*LDH

activity. The findings of the present study clearly indicate that the methanol extract of *E. alsinoides* significantly inhibit *Pf*LDH, thus demonstrating antimalarial activity. It is reported previously that the intraerythrocytic malaria parasite is under constant oxidative stress originating both from endogenous and exogenous processes. The parasite is endowed with a complete network of enzymes and proteins that protect it from those threats, but it also uses redox activities to regulate enzyme activities. Thus antioxidant may play an important role for inhibiting lactate dehydrogenase enzymes and Bozdech and Ginsburg elaborated various biochemical processes of antioxidant defense on malaria parasite in their paper[9]. The presence of scopoletin, a furanocoumarin in *E. alsinoides* further strongly support the view of involvement of antioxidant mode of action. The presence of scopoletin in *E. alsinoides* can thus be correlated to the antimalarial activity of *E. alsinoides*. Further identification and analysis of effective molecules from *E. alsinoides* extract may lead to new therapeutic molecule with known mode of action. Nevertheless this study is more economical for screening antimalarials and useful for post-drug development studies on determination of target and mode of action.

Conflict of interest statement

Author does not have any conflict of interest.

Acknowledgements

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Comments

Background

E. alsinoids Linn. is used as antimalarial in traditional medicine by several ethnic populations of India. Recent research identified *Pf*LDH enzyme as a suitable drug target for antimalarial studies, which is utilized in present studies for antimalarial screening.

Research frontiers

The data obtained from the present experiments are in agreement with its use in traditional medicine.

Related reports

It was reported that inhibition of this enzyme may leads to death of parasite and further parasite cycle stop, which is the basis of antimalarial activity.

Innovations & breakthroughs

This is the first scientific report on the antimalarial activity of the plants. The present report serves as the first hand information on search of potent antimalarial compound from this plant.

Applications

There are several medicinal plants identified to treat malaria, which ultimately provide quality leads towards identifying novel antimalarial drugs. Here we combined this approach with target based drug discovery and explored *Plasmodium* specific lactatede hydrogenase inhibitory activity of *E. alsinoids*.

Peer review

E. alsinoides is commonly known as *Shankhpushpi* and used in traditional medicine for antimalarial treatment by several ethnic populations of India. Although there is no any scientific report towards the antimalarial activity of this plant, the present study provides evidence of its antimalarial potential.

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