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Antiplasmodial Potential of Traditional Medicinal Plant *Thlaspi arvense*

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Authors' contributions

This work has been done in collaboration between both the authors. Author NSW contributed to collection of plant specimen; identification and herbarium, performed the laboratory work, wrote the protocol, performed analysis of data and wrote the first draft of the manuscript. Author UB designed the study, supervised the laboratory work and contributed to critical reading of the manuscript. Both the authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: Antiplasmodial potential of traditional medicinal plant *Thlaspi arvense* against *Plasmodium falciparum in vitro* has been evaluated. Cytotoxicity of plant extract against *HeLa* cell lines and normal fibroblasts has also been observed.
Place and Duration of the Study: Department of Zoology, Panjab University, Chandigarh, India, between May 2013 to April 2014.
Materials and Methods: Ethanolic whole plant extract of *Thlaspi arvense* (EWETA) was analyzed for its phytochemical constituents. *In vitro* cytotoxicity was determined colorimetrically by MTT assay. WHO protocol, based on assessment of schizont maturation inhibition, was employed for the evaluation of *in vitro* antiplasmodial activity of plant extract.
Results: Phytochemical screening of EWETA revealed the presence of diterpenes, triterpenes, steroids, anthraquinones and phytosterols. EWETA was observed to inhibit schizont maturation of both chloroquine-sensitive (MRC-2) and resistant (RKL-9) strains of *P. falciparum* with IC50<5µg/ml and =5µg/ml respectively. The extract was revealed to be safe against both *HeLa* cells and normal fibroblasts with CC50>1000µg/ml. Selectivity index for *Thlaspi arvense* was calculated to be >200 and =200 both for chloroquine-

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sensitive and chloroquine-resistant strains of *P. falciparum* with both *HeLa* and normal fibroblasts.
Conclusion: Plant extract possesses considerable *in vitro* antimalarial activity with high selectivity index (SI>10) pointing field pennycress to be an active antimalarial. Hence, present study provides scientific evidence for traditional usage of the plant as an antipyretic agent.

Keywords: Malaria; Plasmodium falciparum; traditional medicine; Thlaspi arvense; selectivity index; cytotoxicity.

1. INTRODUCTION

Malaria remains a devastating global health problem and is still a major burden in many parts of the world. The emergence and relentless spread of resistance against all the antimalarial drugs in current use, including the newly introduced artemisinin-based combination therapy, has aggravated the disease burden in endemic regions [1]. Medicinal plants have been a major source of natural therapeutic remedies and are used to treat various infectious diseases in the developing world [2]. Traditional medicines could be an important and sustainable source of treatment in poor areas that are unable to afford and access effective antimalarial drugs [3].

Nowadays, natural products of plant sources have been the center of focus as the main source of new, safer and more effective bioactive compounds with medicinal properties [4]. Since most of the antimalarial drugs such as quinine and artemisinin were originated from medicinal plants, it is quite likely that several plants described in the traditional medicine literature may lead to novel anti-malarials which are plant derived [5].

Thlaspi arvense is commonly called as field penny-cress/ stinkweed. It is native to Europe. Young leaves of field pennycress were used for food by the Cherokee Indians and medicinally by the Iroquois Indians. The entire plant is used as an antipyretic agent. It is also known to act as a blood tonic, blood purifier and increases the secretion of bile. It is also effective against the growth of staphylococci and streptococci.

Pedras et al. [6] isolated isovitexin, a constitutive glycosyl flavonoid from stinkweed, having antioxidant properties. They also reported two phytoalexins (inducible secondary metabolites produced *de novo* in plants to diverse forms of stress), wasalexin A and arvelexin (4-methoxyindolyl- 3-acetonitrile), synthesized by the plant in response to biotic and abiotic elicitation. The phytoalexins exhibited considerable antifungal activity against isolates of *Leptosphaeria maculans*.

The seeds of *Thlaspi arvense* are used by traditional healers in Ladakh for the treatment of kidney and urinary disorders [7]. The primary fatty acids reported in field pennycress oil were composed of erucic and linoleic acids, while sitosterol and campesterol were some of the primary phytosterols elucidated in its oil [8]. Seeds are also used for treating renal inflammation, appendicitis, seminal and vaginal discharges. The plant is known to possess anti-inflammatory properties. In the present study, traditionally used medicinal plant *Thlaspi arvense* has been tested for its *in vitro* antimalarial activity against *Plasmodium falciparum*. The cytotoxicity of the plant has been tested against human cervix cancer cell lines and normal fibroblasts.

2. MATERIALS AND METHODS

2.1 Plant

Whole plant of *Thlaspi arvense* was collected from Shimla District of Himachal Pradesh. Voucher specimen of *Thlaspi arvense* (voucher No. 8478) has been deposited at the Botany Department, Panjab University, Chandigarh, India, where the taxonomic identification of the plant was carried out by an expert taxonomist. The permission for collection of the plant was obtained from H.P. State Biodiversity Board, State Council for Science Technology and Environment, Kasumpti, Shimla, Himachal Pradesh, India (letter no. SCSTE/SBB-959). The plant specimen was washed thoroughly with water, dried at room temperature and then powdered.

2.2 Extraction

Dried and powdered whole plant of *Thlaspi arvense* exhibited maximum solubility in ethanol. Thus, ethanolic whole plant extract (EWETA) was prepared by Soxhlet extraction method. Approximately 300ml of ethanol was added to 100g dried and powdered plant specimen in a glass flask. The extraction was carried out till the solvent turned colorless in the siphon. The solvent extract thus obtained was concentrated under vacuum in a rotary evaporator. The residue obtained was lyophilized and stored at –4ºC till further use.

2.3 Phytochemical Screening

Phytochemical screening of *Thlaspi arvense* was carried out by employing standard procedures [9-11]. Qualitative tests were performed to detect alkaloids, saponins, phenols, anthraquinones, steroids, diterpenes, triterpenes, flavonoids, cardiac glycosides, tannins and phytosterols.

2.4 *In vitro* Antiplasmodial Activity against *Plasmodium falciparum* and *Plasmodium berghei* (NK-65)

The chloroquine (CQ)-sensitive (MRC-2) and chloroquine (CQ)-resistant (RKL-9) strain of *Plasmodium falciparum* was obtained from National Institute of Malaria Research (NIMR), New Delhi, India. *Plasmodium falciparum* culture was maintained in A+ human erythrocytes using RPMI-1640 as culture medium supplemented with 10% human AB+ serum by modified method of Trager and Jenson [12].

The antiplasmodial activity of EWETA was checked according to WHO method, which is based on assessing the inhibition of schizont maturation [13]. The stock solution of *Thlaspi arvense* was prepared by dissolving known quantity of extract (10mg/ml) in 1% DMSO. The stock solution was further diluted in RPMI-1640, to make various concentrations (5, 10, 20, 40, 60, 80 and 100µg) of extract. Chloroquine (10µM) was used as positive control. Negative control contained solvent alone.90µl complete medium was added to each well in 96 well plates along with different extract concentrations in duplicate. Parasite culture synchronized at ring stages was then added (1% parasitaemia and 1.5% final hematocrit) and plates were maintained for 48h at 37ºC, under 5% CO_2 atmosphere.

After 48h of incubation, Giemsa-stained thin blood films were prepared for each well and the percentage of inhibition of parasite growth was determined microscopically by comparison of

the number of schizonts in the extract/drug treated well with that of control wells. The percent inhibition of schizont maturation was determined using the formula:

$$100 - \frac{\text{Number of schizonts in the treated well}}{\text{Number of schizonts in the control well}} \text{x } 100$$

In vitro antimalarial efficacy of EWETA was also assessed against lethal rodent malarial parasite *Plasmodium berghei* (NK-65) using the same method [13]. Here, 1ml complete medium (RPMI-1640 supplemented with 10% foetal calf serum and mixture of normal and infected mouse erythrocytes at 2% parasitaemia) contained either 10µl of extract (different concentrations (5-100µg) in duplicate or standard drug in each well. After 21h of incubation the percent schizont maturation inhibition was calculated using the above mentioned formula.

2.5 *In vitro* Cytotoxicity of *Thlaspi arvense*

HeLa cells were purchased from National Center for Cell Science (NCCS), Pune, India. Normal human fibroblasts were obtained from Department of Dermatology, Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, India. Both fibroblasts and *HeLa* cells were maintained at 37ºC in 5% CO_2, in DMEM medium (Debaco's Modified Eagle's Medium) supplemented with 10% foetal calf serum and glutamine 2mM. *In vitro* cytotoxicity was assessed employing the MTT assay [14]. The cells in DMEM were seeded into 96-well culture plate at 5000-10000 cells per well. After 24h, cells were incubated with different concentrations of EWETA (10-1000µg) for 48h. (3-(4, 5- Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was dissolved in incomplete medium to make 1mg/ml stock solution. 10µl of tetrazolium reagent was added into each well followed by further incubation at 37ºC for 2h. The supernatant was decanted, and DMSO (100µl/ well) was then added to allow Formosan solubilization. The optical density of each well was detected using a Microplate Readerat 450nm.

2.6 Selectivity Index

The selectivity index is defined as the ratio of the CC50 determined on *HeLa* cells/Normal fibroblasts to the IC50 determined on *Plasmodium falciparum*.

2.7 Data Analysis

All results including IC50 (concentration of the extract/drug corresponding to 50% schizont maturation as compared to control) are reported as mean±standard deviation (SD) of two independent experiments. Determination of CC50 (concentration of the extract/drug causing 50% inhibition of cell growth as compared to control) is represented as the average mean of three replicates. Each extract concentration was tested in duplicate in a 96-well plate for a single experiment. For antiplasmodial and cytotoxicity activities, the 50% inhibitory concentration (IC50/CC50) was calculated by probit regression analysis using Sigmaplot software.

3. RESULTS

3.1 Phytochemical Screening

Qualitative phytochemical screening of EWETA revealed the presence of various secondary plant metabolites (Table 1). *Thlaspi arvense* tested positive for anthraquinones, steroids, diterpenes, triterpenes and phytosterols.

Table 1. Phytochemical screening of ethanolic whole plant extract of *Thlaspi arvense* (EWETA)[a]

Plant metabolites	EWETA
Phenols	-
Alkaloids	-
Saponins	-
Anthraquinones	+
Cardiac glycosides	-
Flavonoids	-
Steroids	+
Diterpenes	+
Triterpenes	+
Phytosterols	+
Tannins	-

[a] *'+' indicates presence of active component, '-' indicates absence of active component*

3.2 *In vitro* Antiplasmodial Activity against *Plasmodium falciparum* and *Plasmodium berghei* (NK-65)

Ethanolic whole plant extract of *Thlaspi arvense* (EWETA) exhibited considerable activity against both chloroquine- sensitive and resistant strain of *P. falciparum* with IC50< 5µg/ml and 5µg/ml respectively (Fig. 1A, B). The standard drug chloroquine (10µM) exhibited 75% inhibition against the sensitive (MRC-2) strain, while no inhibition of schizont maturation was observed against the resistant strain (RKL-9) at this concentration. In case of chloroquine-sensitive MRC-2 strain, a chemo suppression of 87.49% was observed at a very low concentration of 5µg/ml with complete inhibition of schizont maturation (100%) above 20µg/ml concentration of the extract. In case of chloroquine-resistant strain, maximum chemosuppression of 96.15% was observed at 100µg/ml concentration of EWETA, which was greater than the standard drug chloroquine in which the schizont development was comparable to 48h control.

The ethanolic leaf extract of *Thlaspi arvense* (EWETA) was found to inhibit *P. berghei* schizont maturation in dose dependent manner with IC50<5µg/ml (Fig. 2). The standard drug chloroquine exhibited 95.24% chemosuppression at the tested concentration of 10µM. Different concentrations of EWETA (5-100µg/ml) exhibited 66.67%, 76.2%, 78.58%, 83.34%, 88.1%, 90.48% and 92.86% chemo suppression respectively.

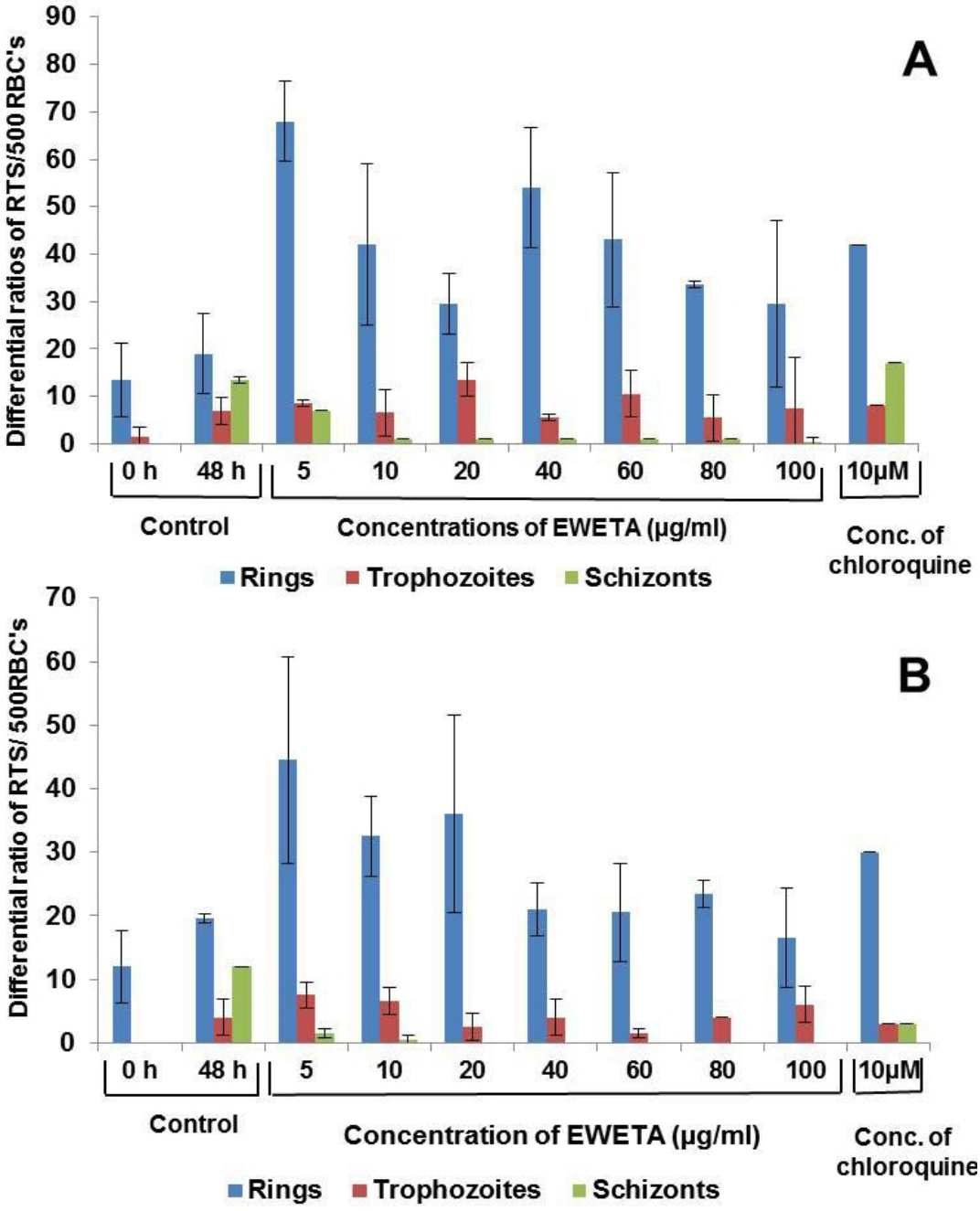

Fig. 1. Effect of different concentrations (5-100µg/ml) of EWETA on schizont maturation of chloroquine-sensitive [A] and chloroquine-resistant [B] strains of *P. falciparum in vitro*

RBC-Red Blood Cells, EWETA-Ethanolic whole plant extract of Thlaspi arvense

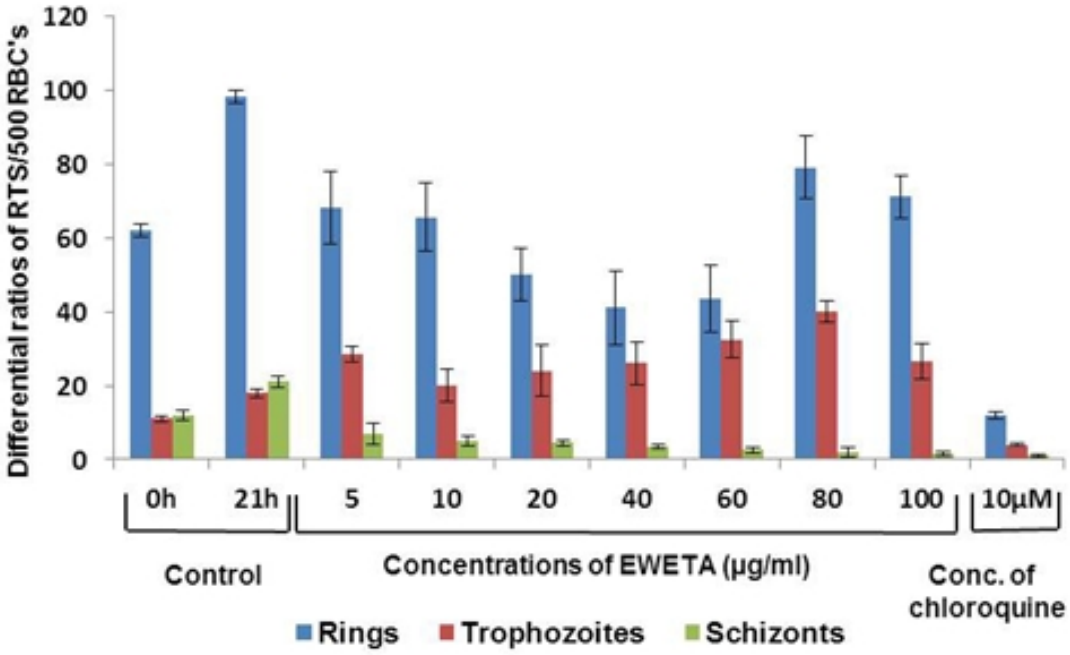

Fig. 2. Dose-dependent effect of different concentrations of EWETA (5-100µg/ml) on intraerythrocytic stages of *P. berghei in vitro* after 21h of incubation

RBC-Red Blood Cells, EWETA-Ethanolic whole plant extract of Thlaspi arvense

3.3 *In vitro* Cytotoxicity of *Thlaspi arvense*

Thlaspi arvense was found to be non-toxic to both *HeLa* cell lines and normal fibroblasts even at higher concentrations. Cell viability was observed to be 79.3% at a concentration of 1000µg/ml of EWETA against *HeLa* cells. The CC50 of EWETA was determined to be >1000µg/ml for both *HeLa* cells and normal fibroblasts. The selectivity index was calculated to be >200 and =200 for both chloroquine sensitive (MRC-2) and resistant (RKL-9) strain *P. falciparum* respectively for both normal as well as cancerous cells.

4. DISCUSSION

Medicinal plants have been reported to possess strong antiplasmodial activity [15]. A number of studies have evaluated the inhibitory effects of various plant extracts on *P. falciparum* [16]. But spread of multidrug resistant strain of *Plasmodium* and the adverse effects of the existing antimalarial drugs have necessitated the search for novel, well tolerated and more effective antimalarial drugs [17].

Phytochemical compounds such as alkaloids, quinones, flavonoids and terpenoids are commonly implicated in the antiplasmodial activity of many plants [18-20]. The phytochemical screening of EWETA has pointed towards the presence of various secondary plant metabolites *viz.* anthraquinones, steroids, diterpenes, triterpenes and phytosterols. The presence of these secondary metabolites in EWETA might be responsible for its antimalarial activity.

The present study describes the antiplasmodial activity of ethanolic whole plant extract of *Thlaspi arvense in vitro*. The IC50 of EWETA was found to be <5µg/ml for chloroquine-sensitive strain of *P. falciparum* (MRC-2) and *P. berghei* (NK-65). In case of resistant strain of *P. falciparum* IC50 was observed to be=5µg/ml. The results of *in vitro* antiplasmodial activity of the plant extract have been classified based on WHO recommendations and previous works [21,22], which classify extracts with IC50 <5µg/ml as highly active, promising activity at 5-15µg/ml, moderate activity at 15-50µg/ml and inactivity at IC50>50µg/ml. Hence, EWETA can be classified as highly active antimalarial against CQ sensitive strains (MRC-2 and NK-65) and possesses promising antimalarial activity against CQ-resistant strain (RKL-9). It is noteworthy that the *Thlaspi arvense* exhibited greater efficacy than chloroquine against both the strains of *P. falciparum*. As complete parasite clearance was observed above 20µg/ml of EWETA against sensitive (MRC-2) strain, whereas, chloroquine (10µM) exhibited 75% chemo suppression. In case of resistant strain (RKL-9), chloroquine exhibited no activity at this concentration and was comparable to control, while EWETA caused more than 90% inhibition of schizont maturation above a concentration of 10µg/ml.

The cytotoxicity studies reveal the safety of extract for human host. The selectivity index (SI) was determined to be >200 and =200 both for CQ sensitive and CQ resistant strains of *P. falciparum* with both *HeLa* cells and normal fibroblasts. Hence, *Thlaspi arvense* can be classified as an active antimalarial according to Valdes et al. [23], which classifies extracts with SI>10 as active. The higher selectivity index of the plant also signifies that the pharmacological activity of EWETA is because of its specific antiplasmodial efficacy and not due to its general *in vitro* cytotoxicity [24].

5. CONCLUSION

Present study reports the antiplasmodial efficacy of EWETA for first time. The extract possesses the ability to suppress both chloroquine sensitive (MRC-2) and resistant (RKL-9) strains of *Plasmodium in vitro* with no cytotoxicity against human cell lines. Since *Thlaspi arvense*is used by the local communities in the Himalayan region for its fever reducing properties, it was hypothesized that the plant could possess some activity against the malarial parasite, as fever is one of the major clinical manifestation observed in malaria infection. Hence, the traditional use of this plant as an antipyretic is confirmed with scientific evidence. Further studies are being carried out to explore its antimalarial potential *in vivo.*

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the institutional animal ethics committee (Reg. No. 45/1999/CPCSEA, Panjab University, Chandigarh, India) and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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