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BOTANY

ARTEMISININ CONTENT IN *ARTEMISIA SCOPARIA*

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Abstract

Artemisinin is considered as the natural, active and potent antimalarial drug *Artemisia annua* Linn. is the only known source for the production of artemisinin reported till date. An attempt was made on a perennial faintly odoratus herb, *A. scoparia* Waldst et Kit. to find out an alternative of *A. annua* for the production of artemisinin. Generation of hairy roots was also tried wih an attempt to increase the concentration of artemisinin in the biofactories. In the present investigation *A. scoparia* was shown to contain artemisinin

Keywords: *A. scoparia*; *Agrobacterium rhizogenes*; antimalarial drug; artemisinin

Introduction

Malaria is one of the world's most important parasitic diseases. There are at least 300 million acute cases of malaria each year globally, resulting in more than a million deaths. Multi-drug resistance of the *Plasmodium* strains to the cheapest and most widely used antimalarials such as chloroquine, mefloquine and sulfadoxine-pyrimethamine is one of the biggest challenges in the fight against malaria.

Artemisia annua L. (sweet wormwood), a herb of the Asteraceae family has been used for centuries for the treatment of fever and malaria. This plant is the only known source for the production of artemisinin;a potent antimalarial. Artemisinin is the new and promising drug which is also active against resistant plasmodium malaria (15).As artemisinin cannot be synthesized chemically in an economically feasible way, *A. annua* is the only practical source of this valuable drug. Extensive work on the production of artemisinin from plant of *Artemisia annua* has been done (2,3,5,6,7,8,9,10,13,18,19). Artemisinin has also been reported from tissue culture of this plant (11,12,13,14,16,17) and biochemical as well as molecular approaches for enhanced production of artemisinin from *A. annua* has also been tried (1,17)

The present investigation has reported *A. scoparia* Waldst et Kit as a new source of artemisinin. Hairy root generation was also attempted in the present study with an objective to increase the content of artemisinin in the tissue culture of *A. scoparia*

Materials and Methods

Unorganized callus of *A. scoparia* was raised from the young nodal stem segments. Callus of the plant were obtained on MS medium supplemented with 2, 4-D (3mg/L), kinetin (0.25mg/L) and proline (100mg/L) Callus initiation started after 15-20 days of inoculation ; 4-6 weeks old callus as well as aerial plant parts were harvested and subjected to extraction procedures for artemisinin separately.

Aerial plant parts and harvested callus were dried and powdered and extracted by continuous percolation over a period of four to six hours using five to ten fold volume of the non-aqueous solvent ethanol .The extraction process was repeated three to five times to ensure maximum extraction of artemisinin from the herb. The resulting extract was concentrated to 1 to 5% of the original volume. The excess of water (four times of the reduced volume of ethanol extract) to be added to the concentrated to make it 80% aqueous followed by partitioning of the contents between water and hexane. For partitioning, the aqueous content and hexane may be used in a ratio of 1:1 or 2:1 v/v. Partitioning of aqueous content with hexane may be repeated three to five times using the same solvent ratio in order to ensure maximum transfer of artemisinin to hexane fraction. The combined hexane fractions were pooled together to obtain 1-5% of its original volume. The concentrated liquid was a light to dark green oily liquid. Ethyl acetate (10-20% v/v) is added to it. To remove the green pigmentation this liquid was treated with 1-3% w/v of activated charcoal. The yellowish liquid obtained after removal of activated charcoal (by filtration) was subjected to the evaporative crystallization yielding substantially pure artemisinin. The purified crystals thus obtained were further analyzed for artemisinin by infra-red spectroscopy, thin layer chromatography and nuclear magnetic resonance

Thin layer chromatography

Thin Layer Chromatography (TLC) was performed as follows:The extract so obtained were dissolved in ethanol and applied separately 1 cms above the edge

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of the activated plates along with the standard reference compound of artemisinin. The glass plates were then developed in an organic solvent mixture of dichloromethane: ethyl acetate in the ratio 50:2 respectively (4).

The plates were sprayed with a spray mixture consist of 1.5ml anisaldehyde, 30 ml glacial acetic acid, 225 ml methanol and 1.5 ml sulphuric acid. The plates were dried for 5 minutes at 110-120°C and after cooling sprayed again for at least 10 seconds and dried for 10 minutes at constant temperature.

Infra red spectroscopy

The IR spectra of isolated and standard artemisinin were developed in KBr(Potassium Bromide) disc. When the spectrums of both the isolated and standard artemisinin were superimposed there was a similarity in the peaks of isolated and standard compounds at corresponding points.

Nuclear magnetic resonance

The NMR of the isolated artemisinin was developed by dissolving the samples in Dimethyl sulphoxide (DMSO) (NMR grade D6).

Generation of hairy roots

Hairy root generation was tried with *Agrobacterium rhizogenes* A 4 Strain (Figure. 5); on an antibiotic containing medium. The culture of *A. rhizogenes* was revived on LB broth and incubated overnight at 37°C. The 4-6 week old and green calli of *A.scoparia* were then infected with with the revived culture of *A. rhizogenes* A4 strain and then inoculated on Sporidex containing plain MS medium; then incubated in dark at 24±2°C.

Results and Discussion

In the present investigation presence of artemisinin in *A. scoparia* plant as well as in callus tissue was confirmed by TLC, IR and MNR studies.

By Thin layer chromatographic studies a brown spot with Rf value of 0.67 corresponding with that of standard compound was observed. The IR spectrum of the extracts of callus tissue and aerial plant parts showed considerable overlapping with the IR spectra of standard artemisinin (Figure 1 and 2). 1H NMR spectra of the plant extract done in DMSO (D6, NMR grade) matched considerably with that of artemisinin spectra provided in literature (Extraction of ionic liquids; Bioniqs Ltd; Page 17) indicating the presence of artemisinin in the extracts of *A. scoparia.* The yield of artemisinin was higher (0.015%) in aerial plant parts in comparison to that of callus cultures (0.001%). [Figure 4].But even after the incubation period of 6-8 weeks no signs of hairy root formation appeared on the calli of *A. scoparia* .

Figure 1: IR Spectra of standard and isolated artemisinin from *Artemisia scoparia* Waldst et Kit tissue culture

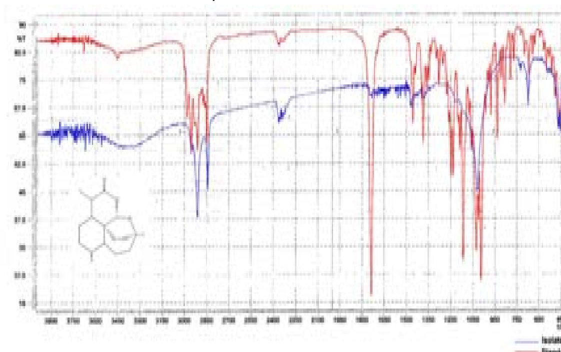

Figure 2: IR spectra of standard and isolated artemisinin from aerial plant parts of *Artemisia scoparia* Waldst et Kit

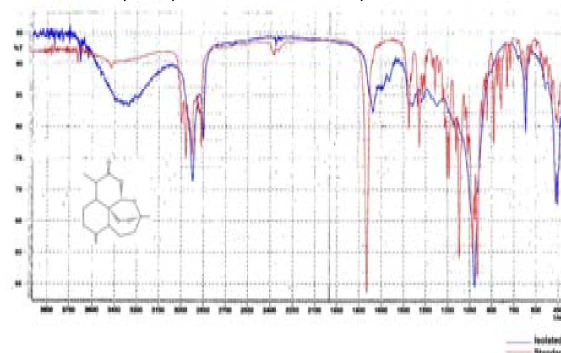

Figure 3: 1H NMR Spectra of artemisinin isolated from aerial plant parts of *Artemisia scoparia* Waldst et Kit

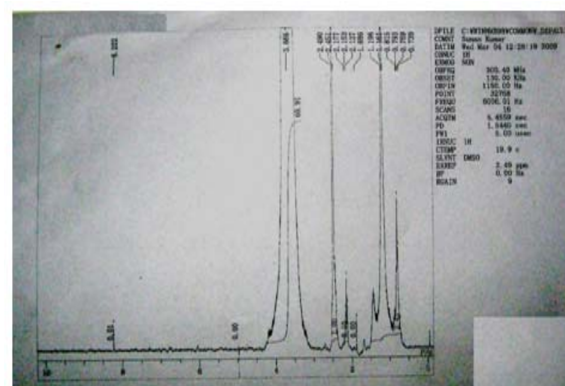

Figure 4: Unorganised callus of *Artemisia scoparia* Waldst et Kit

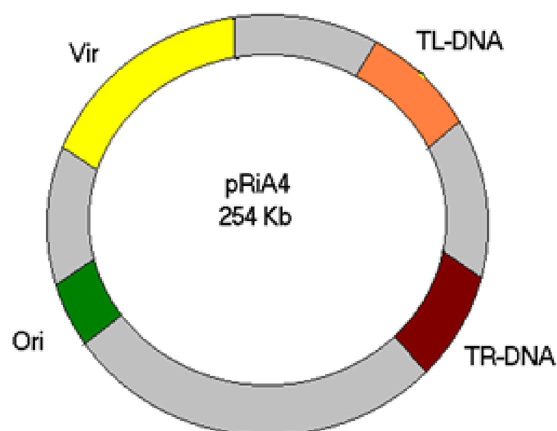

Figure 5. Circular Ri plasmid of A. rhizogene strain A4 showing the different genetic regions.

The present investigation confirms that aerial plant parts and callus cultures of *A. scoparia* Waldst et Kit. has the potentiality to produce artemisinin. This study concluded that *A. scoparia* besides possessing antibacterial and insecticidal properties also contains artemisinin. Thus, the plants of *A. scoparia* hold a new promise to the field of medicinally and economically important bioactive compounds which should be explored further to open new vistas for mankind.

But being unable to induce hairy root formation in the calli it could be concluded that for hairy root induction some elicitor like acetosyringone is required or it could be attempted with a different strain of *A.rhizogenes*. Thus, with the help of advanced techniques there is a probability that the amount of artemisinin in *A. scoparia* could be increased and used in future for curing the deadly parasitic disease in humans.

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