BJP

Bangladesh Journal of Pharmacology

Research Article

Artemisia scoparia: A new source of artemisinin

A Journal of the Bangladesh Pharmacological Society (BDPS) **Bangladesh J Pharmacol 2010; 5: 17-20**
Journal homepage: www.banglajol.info
Abstracted/indexed in Academic Search Complete, Agroforestry Abstracts, Asia Journals Online, Bangladesh Journals Online, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Current Abstracts, Directory of Open Access Journals, EMBASE/Excerpta Medica, Global Health, Google Scholar, HINARI (WHO), International Pharmaceutical Abstracts, Open J-gate, Science Citation Index Expanded and Social Sciences Citation Index
ISSN: 1991-0088

Artemisia scoparia: A new source of artemisinin

Aditi Singh and Renu Sarin

Laboratory of Bioactive Compounds, Department of Botany, University of Rajasthan, Jaipur 302004, India.

Article Info

Received: 27 April 2010
Accepted: 27 April 2010
Available Online: 4 May 2010

DOI: 10.3329/bjp.v5i1.4901

Cite this article:
Singh A, Sarin R. *Artemisia scoparia:* A new source of artemisinin. Bangladesh J Pharmacol. 2010; 5: 17-20.

Abstract

Artemisinin is considered as the most active and potent antimalarial drug. Till date *Artemisia annua* Linn. plant is the only source for its production. The present investigation was carried out with an objective to search a new plant for artemisinin. An attempt was made on a perennial faintly odoratus herb, *Artemisia scoparia* Waldst et Kit. to find out an alternative of *A. annua* for the production of artemisinin. The yield of artemisinin was higher in aerial plant parts (0.015%) in comparison to callus culture (0.001%). The present study concluded that *A. scoparia* contains an 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. The WHO recommends that all countries experiencing resistance to conventional monotherapies should use combination therapies, preferably those containing artemisinin derivatives (ACTs-artemisinin-based combination therapies).

As artemisinin cannot be synthesized chemically in an economically feasible way, *A. annua* is the only practical source of this valuable drug. Artemisinin is the new and promising drug which is also active against resistant plasmodium malaria (McIntosh and Olliaro, 2004). Extensive work on the production of artemisinin from plant of *A. annua* has been done (Actona and Klayman, 1985; Acton et al., 1985; El-Sohly, 1990; Jha et al., 1988; Ferreira and Janick, 1996; Ferreira et al., 1994; Ferreira and Simon, 1995a, 1995b; Theoharides et al., 1988; Titulaer et al., 1990). Artemisinin has also been reported from tissue culture of this plant (Jha et al., 1988; Fulzele et al., 1991; Jaziri, 1995; Martinez and Staba, 1992; Nair et al., 1986; Van Nieuwerburgh et al., 2006) and biochemical as well as molecular approaches for enhanced production of artemisinin from *A. annua* has also been tried (Abdin et al., 2003; Van Nieuwerburgh et al., 2006).

The present investigation has reported *A. scoparia* Waldst et Kit. as a new source of artemisinin.

Materials and Methods

Unorganized callus of *A. scoparia* was raised from the young nodal stem segments. The sterile nodal segments were inoculated on MS medium supplemented with 2, 4-D (3 mg/L), kinetin (0.25 mg/L) and proline (100 mg/L). Callus initiation started after 15-20 days of inoculation and maintained on MS medium by frequent subculturing after 6-8 weeks for a period of 8 months before using it for the biochemical analysis.

Old callus (4-6 weeks) 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

This work is licensed under a Creative Commons Attribution 4.0 License. You are free to copy, distribute and perform the work. You must attribute the work in the manner specified by the author or licensor.

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 by distillation under vacuum. 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 were used in a ratio of 1:1 or 2:1 v/v. Partitioning of aqueous content with hexane were 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 before they were distilled under vacuum (to recover the solvent for using again) 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 (TLC)

Thin glass plates coated with silica gel 'G' were prepared and activated. The extract so obtained were dissolved in ethanol and applied separately 1 cm above the edge of the activated plates along with the standard reference compound of artemisinin. The glass plates were then developed in an 50:2 ratio organic solvent mixture of dichloromethane and ethyl acetate (De Vries et al., 1999).

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

Infra red spectroscopy

The IR spectra of isolated and standard artemisinin were developed in KBr disc on a Perkin Elmer, 337, Grating infra red spectrophotometer (4000 to 400 cm-1). 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 sulfoxide (NMR grade D6).

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 0.7 corresponding with that of standard compound was observed.

The IR spectrum of the extracts of callus tissue and aerial plant parts showed considerable over lapping with the IR spectra of standard artemisinin (Figure 1 and 2). 1H NMR spectra of the plant extract were done in DMSO (D6, NMR grade) matched considerably with that of artemisinin spectra provided in literature (extraction of ionic liquids; Bioniqs Ltd) indicating the presence of artemisinin in the extracts of *A. scoparia*

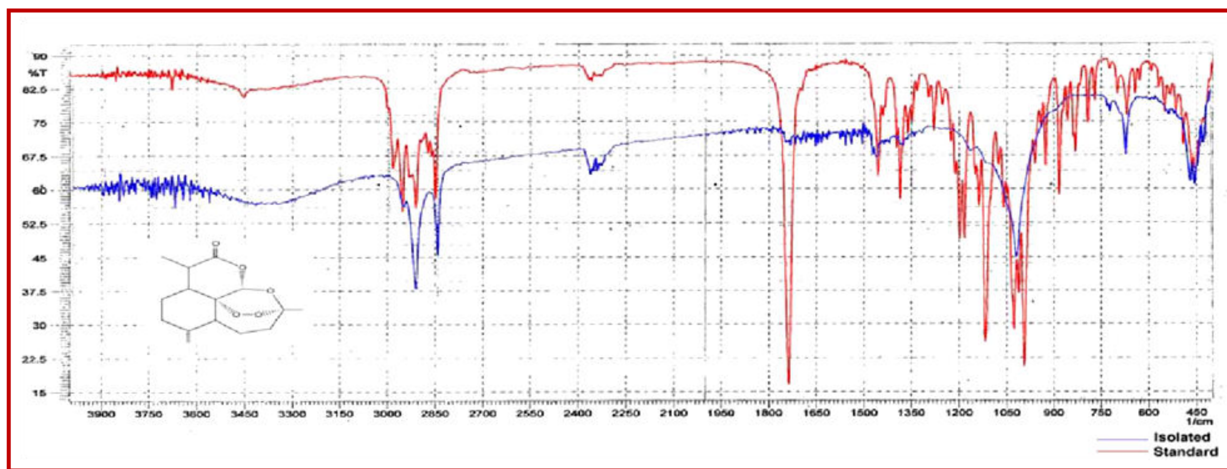

Figure 1: IR Spectra of standard and isolated artemisinin from *Artemisia scoparia* Waldst et Kit. tissue culture

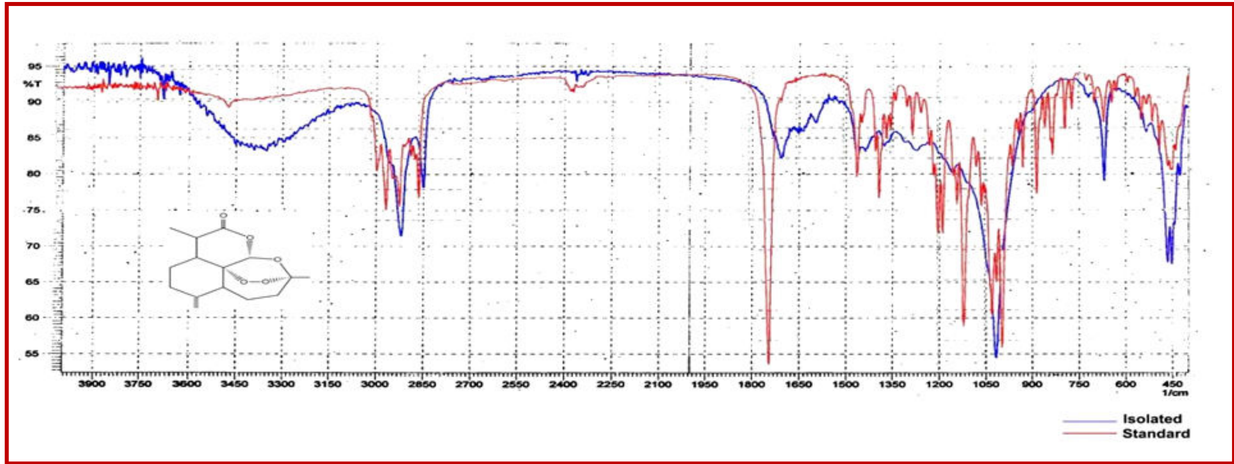

Figure 2: IR spectra of standard and isolated artemisinin from aerial plant parts of *Artemisia scoparia* Waldst et Kit

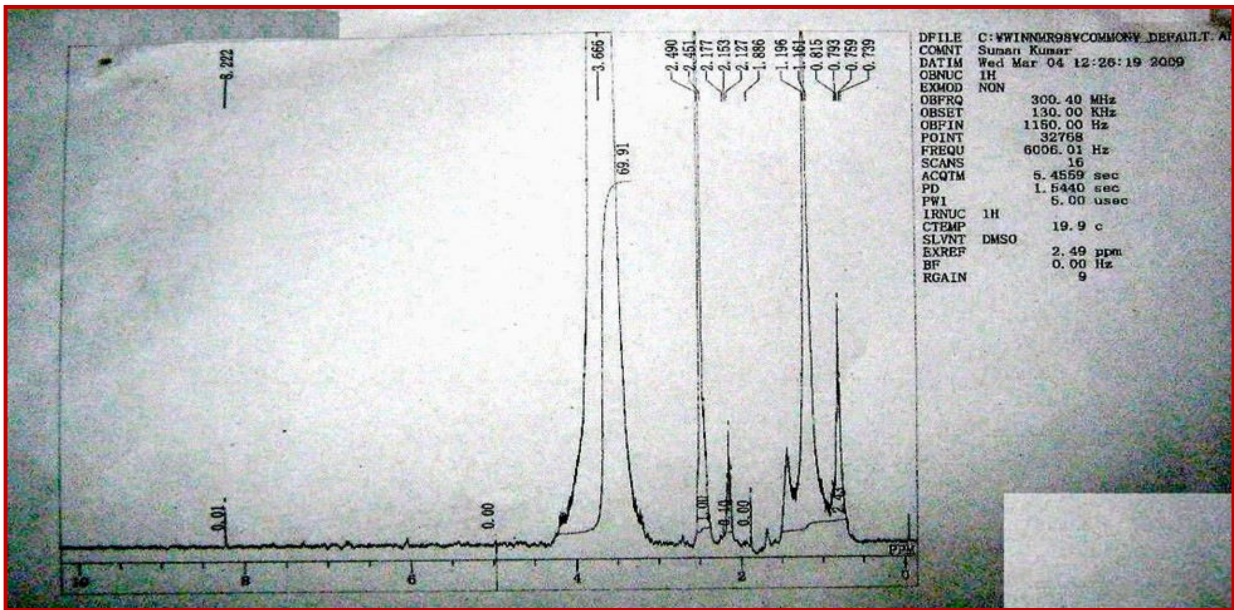

Figure 3: 1H NMR spectra of artemisinin isolated from aerial plant parts of *Artemisia scoparia* Waldst et Kit

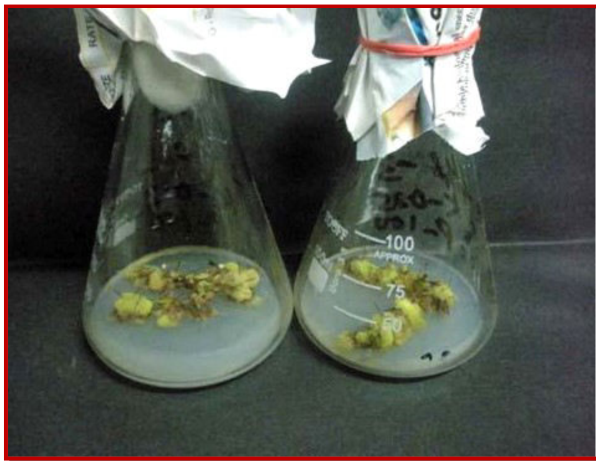

Figure 4: Unorganised callus of *Artemisia scoparia*

(Figure 3). The yield of artemisinin was higher (0.015%) in aerial plant parts in comparison to that of callus cultures (0.001%; Figure 4).

Conclusion

A. scoparia besides possessing antibacterial and insecticidal properties also contains artemisinin.

Financial Support

Self-funded

Conflict of Interest

Authors declare no conflict of interest

Acknowledgement

The authors are grateful to Head, Department of Botany and Head, Department of Chemistry for providing the lab facilities to carry out this work.

References

Abdin MZ, Israr M, Rehman RU, Jain SK. Artemisinin, a novel antimalarial drug: Biochemical and molecular approaches for enhanced production. Planta Med. 2003; 69: 289-99.

Acton N, Klayman DL. Artemisinin, a new sesquiterpene lactone endoperoxide from *Artemisia annua.* Planta Med. 1985; 51: 441-42.

Acton N, Klayman DL, Rollman IJ. Reductive electrochemical HPLC assay for artemisinin (qinghaosu). Planta Med. 1985; 51: 445-46.

de Vries P, Dien TK. Clinical pharmacology and therapeutic potential of artemisinin and its derivatives in the treatment of malaria. Drugs 1996; 52: 818–36.

ElSohly HN, Croom EM, El-Feraly FS, El-Sherei MM. A large scale extraction technique of artemisinin from *Artemisia annua*. J Nat Prod. 1990; 53: 1560-64.

Ferreira JFS, Charles DJ, Wood K, Janick J, Simon JE. A comparison of gas chromatography and high performance liquid chromatography for artemisinin analyses. Phytochem Anal. 1994; 5: 116-20.

Ferreira JF, Janick J. Roots as an enhancing factor for the production of artemisinin in shoot cultures of *Artemisia annua*. Plant Cell Tissue Organ Cult. 1996; 44: 211-17.

Ferreira JF, Simon JE, Janick J. Developmental studies of *Artemisia annua*: Flowering and artemisinin production under greenhouse and field conditions. Planta Med. 1995a; 61: 167-70.

Ferreira JF, Simon JE, Janick J. Relationship of artemisinin content of tissue-cultured, greenhouse-grown, and field-grown plants of *Artemisia annua*. Planta Med. 1995b; 61: 351-55.

Fulzele DP, Sipahimalani AT, Heble MR. Tissue cultures of *Artemisia annua*: Organogenesis and artemisinin production. Phytother Res. 1991; 5: 149-53.

Jazir M, Shimomura K, Yoshimatsu K, Fauconnier ML, Marlier M, Homes J. Establishment of normal and transformed root cultures of *Artemisia annua* L. for artemisinin production. J Plant Physiol. 1995; 145: 175-77.

Jha S, Jha TB, Mahato SB. Tissue culture of *Artemisia annua* L.: A potential source of an antimalarial drug. Curr Sci. 1988; 57: 344-46.

Martinez BC, Staba EJ. The production of artemisinin in *Artemisia annua* L. tissue cultures. Adv Cell Cult. 1988; 6: 69-87.

McIntosh HM, Olliaro P. Artemisinin derivatives for treating severe malaria. The Cochrane library 2. Oxford, Update Software, 2004.

Nair MS, Acton N, Klayman DL, Kendrick K, Basile DV, Mante S. Production of artemisinin in tissue cultures of *Artemisia annua*. J Nat Prod. 1986; 49: 504-07.

Theoharides AD, Smyth MH, Ashmore RW, Halverson JM, Zhou ZM, Ridder WE, Lin AJ. Determination of dihydro-qinghaosu in blood by pyrolysis gas chromatography/mass spectrometry. J Anal Chem. 1988; 60: 115-20.

Titulaer HA, Zuidema J, Kager PA, Wetsteyn JC, Lugt CB, Merkus FW. The pharmacokinetics of artemisinin after oral, intramuscular and rectal administration to volunteers. J Pharm Pharmacol. 1990; 42: 810–13.

Van Nieuwerburgh FC, Vande Casteele SR, Maes L, Goossens A, Inzé D, Van Bocxlaer J, Deforce DL. Quantitation of artemisinin and its biosynthetic precursors in *Artemisia annua* L. by high performance liquid chromatography-electrospray quadrupole time-of-flight tandem mass spectrometry. J Chromatogr A. 2006; 1118: 180-87.

Author Info
Aditi Singh (Principal contact)
e-mail: aditisingh_21@yahoo.co.in