Analysis of Artemisinin in *Artemisia* Species Using High Performance Liquid Chromatography

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Abstract: Malaria a serious global health problem particularly in the developing countries. Recently many efforts have been attempted in the treatment of malaria. Artemisinin isolated from *Artemisia annua* is a promising and potent antimalarial drug, which meets all the challenges posed by drug resistant plasmodium and rapid growth of malarial illness. To-day artemisinin and its derivatives in various combinations remains the main weapon in the fight against this disease. The aim of the present study was to investigate and analyze the content of artemisinin in *Artemisia* species including *Artemisia Vulgaris, Artemisia Maritima, Artemisia Khramensis, Artemisia Absinthium* and *Artemisia annua* collected from different environmental zones of Khyber Pukhtunkhwa, Pakistan, using high performance liquid chromatography (HPLC). The optimizing conditions for the detection parameters with regard to extraction, chemical treatment, partitioning, pH, composition of the elution mixture and suitable wavelength, reproducible results for the test samples were derived. The content of artemisinin was found to be 0.023% in *A. annua*, however in the rest of herbs, presence of the desired compound was not established either due to its trace content or poor accumulation.

Key words: *Artemisia · Artemisinin · High performance liquid chromatography*

INTRODUCTION

Artemisinin, a cadinane-type sesquiterpene lactone containing an endoperoxide group, has been established as an antimalarial component in the plant *Artemisia annua* L [1]. Artemisinin and semisynthetic derivatives, artemether, arteether, artesunate have shown great promise against multidrug resistant stain of plasmodium (the malarial parasite) [2]. They have been increasingly popular in Asia over the last 10 years [3]. Recently artemisinin and its derivatives are considered as part of the ideal strategy for malaria in Africa by WHO [4].

*Artemisia annua* L belongs to the family Asteraceae is an annual herbaceous plant with a strong fragrance endemic to the northern part of chahar and Suiyuan province in China, at 1000-1500 m above sea level [5-6] where it is known as qinghao (green herb) and used as a remedy for chill and fevers since more than 2000 years [7].

It also occurs in northern areas (Skardu) of Pakistan at 5000 m above sea level. *Artemisia* is now growing in many European countries and has been become naturalized in North America. *A. annua* is an interesting source of essential oils [8], however its economical importance is mainly determined by being probably the only source of genus *Artemisia alba* to synthesize artemisinin.

Artemisinin offers impressive effects including characteristics of high efficacy, fast action and low toxicity and is regarded as a breakthrough in the history of antimalarial drugs [9]. It is a potent blood schizonticide.
with minimum inhibitory concentration of $10^{-7}$ [10] and has been found to be effective also against other infectious diseases including hepatitis B [11] and parasite that cause schistosomiasis [12-13]. More recently it has been shown to be effective also against numerous types of tumors, including breast cancer, human leukemia, colon and small cell lung carcinomas [14-15].

In view of the scarcity of artemisinin and its actual high demands in Pakistan and in the world in particular in *Artemisia* species. For this purpose five species of *Artemisia* including *Artemisia Vulgarus, A. Maritima, A. khramensis, A. absinthium and A. annua* were collected from different environmental zone of Khyber Pukhtunkhwa, Pakistan.

**Experimental**

**Determination of Extractive Values:** Members of the genus *Artemisia*, including *Artemisia Vulgarus, A. maritima, A. khramensis, A. absinthium and A. annua*, aerial parts were identified and extractive values (pet ether, Alcohol and water) of the resultant dried samples determined according to procedures detailed in the British Pharmacopoeia [8,9].

**Extraction of Plant Material for Artemisinin:** The method of Zhao and Zeng (1985) was used for estimation of Artemisinin in the herbes [12]. After collection from specified zones were dried, chopped and powdered material 1.5 gm extracted with pet ether (20 mL) in soxlet apparatus for three hours. Pet ether extrac filtered, concentrated under reduced pressure and residue obtained dissolved in (6 mL) 95% ethanol, the solution filtered and clear extract transferred to 10 mL flask, followed by addition of sufficient 95% ethanol to adjust to 10 mL final volume.

**Derivatization of Extracts:** Ethanolic extract (1 mL) was transferred to 10 mL round bottom flasks, standard artemisinin solution (0.4 mL) and 0.2% sodium hydroxide (4 mL) added, the mixture refluxed on water bath at 50°C for 30 min, then cooled to room temperature and final volume adjusted 10 mL with (1 mL) ethanol and 0.2 N hydrochloric acid.

**Derivatization of Artemisinin:** Artemisinin 0.1 g dissolved in (20 mL) 95% ethanol and final volume adjusted to 100 mL with the same solvent. From the stock solution, 0.1 mL, 0.2 mL, 0.3 mL 0.4 mL were transferred to 10 mL flask, sodium hydroxide 0.2% (4 mL) added to each flask, the contents refluxed on water bath at 50°C for 30 min. After the reaction, the contents were allowed to attain room temperature followed by the addition of (1 mL) 95% ethanol and sufficient quantity of 0.2 N hydrochloric acid to 10 mL final volume.

**HPLC and Chromatographic Conditions for Estimation:**

HPLC of standard and tested solutions were performed using Shimadzu LC-6A system equipped with ODSC (Rp-18 Column) and SPD-6AV, UV-Visible detector set at 260 nm. The mobile phase consisted of 0.01 M, 10 μL were injected for peak area measurement.

**Artemisinin Content in Extracts Was Quantified According to the Equation:**

\[ W = (C1-C2) \times 10 \times X/100/Z \]

\[ C1 = \text{Conc. of std. Artemisinin (mg/mL)} \]

\[ C2 = \text{Conc. of test solution (mg/mL)} \]

\[ C3 = \text{Additional quantity of Artemisinin} \]

**RESULTS AND DISCUSSION**

**Extractive Values and Artemisinin Content of Artemisia Species:** Whilst the diagnostic features enable the nature and origin of the material but in order to determine the quality of products, these are often subjected to extraction procedures, which normally give quantitative information based on the determination of one or more active constituents. The extractive values are designed to indicate the percentage of a material, which is soluble in particular, solvent rather than the percentage of therapeutically active constituents. The observed data regarding extractive values (Table 1) displayed variability, which suggests that accumulation of active ingredient in *Artemisia* species depend on the intrinsic factor.

Studies concerning structure, biosynthesis, accumulation and functions of artemisinin, an endoperoxide sesquiterpene lactone extracted from *Artemisia annua* have been reported [16]. Artemisinin occurs in the herb along with small amount of other sesquiterpenes, which interfere during its quantitative estimation in the crude extracts. Zhao and Zeng (1985) [17] have developed a reliable estimation procedure for assaying artemisinin content in various parts of the herb through partial chemical treatment of crude extract for creation of suitable chromophore necessary for UV absorption.
Table 1: *Artemisia* species extractive values

<table>
<thead>
<tr>
<th>S #</th>
<th>Species</th>
<th>Origin</th>
<th>Pet. ether</th>
<th>Alcohol</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>A. Vulgaris</em></td>
<td>Ayubia</td>
<td>4.7</td>
<td>7.0</td>
<td>7.8</td>
</tr>
<tr>
<td>2</td>
<td><em>A. maritima</em></td>
<td>Gilgit</td>
<td>5.1</td>
<td>11.2</td>
<td>15.1</td>
</tr>
<tr>
<td>3</td>
<td><em>A. khuransis</em></td>
<td>Parsinar</td>
<td>4.0</td>
<td>13.8</td>
<td>13.8</td>
</tr>
<tr>
<td>4</td>
<td><em>A. absinthium</em></td>
<td>Parsinar</td>
<td>5.2</td>
<td>9.5</td>
<td>10.5</td>
</tr>
<tr>
<td>5</td>
<td><em>A. absinthium</em></td>
<td>QHS</td>
<td>5.1</td>
<td>10.1</td>
<td>9.0</td>
</tr>
<tr>
<td>6</td>
<td><em>A. absinthium</em></td>
<td>QHS Tissue Cul</td>
<td>5.6</td>
<td>8.5</td>
<td>14.2</td>
</tr>
<tr>
<td>7</td>
<td><em>A. annua</em></td>
<td>Skardu</td>
<td>4.7</td>
<td>8.7</td>
<td>12.6</td>
</tr>
</tbody>
</table>

Table 2: Artemisinin content in the chosen species

<table>
<thead>
<tr>
<th>S #</th>
<th>Species</th>
<th>Origin</th>
<th>Artemisinin %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>A. Vulgaris</em></td>
<td>Ayubia</td>
<td>--</td>
</tr>
<tr>
<td>2</td>
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<td>Gilgit</td>
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<td>--</td>
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<tr>
<td>6</td>
<td><em>A. absinthium</em></td>
<td>QHS Tissue Cul</td>
<td>--</td>
</tr>
<tr>
<td>7</td>
<td><em>A. annua</em></td>
<td>Skardu</td>
<td>0.023</td>
</tr>
</tbody>
</table>

Fig. 1: Conversion of artemisinin into precursor

Fig. 2: Partial derivatization of artemisinin
Optimizing the detection parameters with regard to extraction, chemical treatment partitioning, pH, composition of elution mixture and suitable wavelength reproducible results for standard and test samples were derived. As evident from Table 2, artemisinin content (0.023%) was found and the yield was consistent with reported findings [18].

In the rest of herbs, presence of the desired compound was not established either due to its trace content or poor accumulation. Detection of artemisinin in other herb is also subject to constituent variability [19]. Some indicate the appropriate time for detection would be before flowering whereas other prefer at full flowering. During the present investigation, herbs were collected during the flowering season. Concerning biological activity, together with other flavonoids especially ecaisin and artemisinin reported to occur in *Artemisia* species, administration of artemisinin has significantly increased antimalarial activity against *Plasmodium falciparum*. These findings [20] suggested that synergistic activity with herbal product might not be just a question of enhanced absorption, excretion or bio-availability. Artemisinin is still regarded as highly potent blood schizontocide, active against both chloroquine and mefloquine resistant stains of *P. falciparum* with a fast onset of action and lesser side effects. However occurrence of malaria in a few cases due to artemisinin therapy has also been reported which suggest that probably due to lesser absorption through the blood brain barrier layers, not all the parasites had killed. To overcome this problem and develop drugs with enhanced efficacy [21] derivatives have been prepared by reduction of lactone carbonyl followed by preparation of ether or ester derivatives detailed as under.

The methyl ether, artemether is lipophilic and is administered by intramuscular injection, while esters such as sodium artesunate or sodium artelinate are hydrophilic and given orally or by intravenous route. An unusual feature of the artemisinin derivatives is that they are also active against the gametocyte from of the malaria parasite which is responsible for the transmission of the disease from man to mosquito during feeding, thus these drugs not only cure the patient but also help to reduce transmission of the disease. Chemical synthesis of artemisinin has also been achieved but the process is not economically viable therefore it is extracted from *A. annua* and then derivatized as required.

ACKNOWLEDGEMENTS

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REFERENCES