Publications
Essential oil composition of *Artemisia japonica* Thunb. from Kerala

Rashmi T.R, Francis M.S. and Soumya Murali

Abstract

The genus *Artemisia* has a medicinal history reaching back over two millennia and some of its extracts and isolates are of proven efficacy against several diseases. *Artemisia japonica* Thunb. (Syn. *Artemisia parviflora* Buch.-Ham. ex D. Don) is a member of family Asteraceae (Compositae) and is distributed in India, Myanmar, Pakistan, Nepal, Bhutan, Afghanistan and Japan. It is a nonaromatic perennial herb which is reported in the southern Kerala particularly in Munnar. This plant is used in medicine and extracting essential oil, having anti-viral and anthelmintic properties [9]. The plant has been used as a traditional medicine to treat fever and eczema [10]. In traditional medicine, various parts of *Artemisia japonica* (leaves, stem, seeds and fruits) have been widely used by tribal people for its wound healing properties, treatment of skin diseases, febrile, diarrheal properties, digestive and in ethnoveterinary medicine [9]. Antimalarial activity of *A. japonica*, *A. maritima* and *A. nilagirica* had been studied [9].

To our knowledge, there are no reports on the essential oil composition of *Artemisia japonica*. So the components of the essential oil is analysed.

1. Materials and Methods

2.1 Plant material and isolation of essential oil

The plant material was collected from Munnar of Idukki district of Kerala and were identified and the herbarium with voucher specimen *Artemisia japonica*-SHC: 04 was maintained in the College. The leaves were shade dried, crushed and the essential oil was isolated by hydrodistillation using a Clevenger type apparatus for four hours. The essential oil isolated were obtained in glass vials and stored at 4°C for GC-MS analysis.

2.2 Gas chromatography - Mass spectrometry (GC-MS)

GC-MS analysis was carried out by 6850 Network GC system, Agilent Technologies 5975C VLMSD with Triple Axis Detector, using helium as carrier gas with a flow rate of 1.0 ml/min on split ratio 10:1 fitted with HP5MS capillary column (nominal length 30.0 m, nominal diameter 250.00 μm and nominal film thickness 0.25 μm). The temperature was programmed from 60-250 °C, initially 60-180 °C at the rate of 2.50 °C and 180-250 °C at the rate of 5 °C. The injection temperature was 200 °C.

2.3 Identification of essential oil constituents

The constituents of essential oil were identified using spectrometric electronic NIST 98 library.

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3. Results and Discussion
The essential oil yield was 0.1 % and was bright yellow in colour. Fifteen compounds were identified which constitute 34.3 % of which spathulenol has 12 % (fig.2-A), germacrene D with 7.5 % (fig.2-B), β-elemene with 2.8 % (fig.2-C), caryophyllene with 2.4 % (fig.2-D). The essential oil is dominated by sesquiterpenes particularly sesquiterpene hydrocarbons. The essential oil composition is given in table I.

![Fig 1: GCMS Chromatogram of Artemisia japonica leaves](image)

A: Spathulenol  B: Germacrene D
Sujatha et al. [9] reported the essential oil of Artemisia vulgaris L. (ragwort) as slightly greenish in colour, and the yield of essential oil was 0.4% whereas the oil of Artemisia japonica was bright yellow in color and with an intense odor and the yield was 0.3%. The intense odor may be due to the presence of oxygenated mono and sesquiterpenes. Studies on the essential oil of Artemisia herba-alba by Lawrence [7] & Lemberg [9] revealed the presence of oxygenated monoterpenoids such as 1,8-cineole, chrysanthenone, chrysanthenol (and its acetate), α-thujone and camphor as the major components, whereas in the studied essential oil of Artemisia japonica, these components were completely absent. Padalia et al. [9], analyzed and compared the essential oil composition of Artemisia annua growing in Uttarakhand, India, and identified 81 constituents, with linalool (0.1-11.9%), β-caryophyllene (2.9-2.2%), (E)-β-farnesene (1.3-8.5%), germacrene D (0.5-7.3%) as the major components, whereas the oil of Artemisia japonica contained similar constituents such as linalool (0.9%), carophyllene (2.4%), α-farnesene (0.6%) and germacrene D (7.5%).

Joshi et al. [10], investigated the essential oil composition of Artemisia scoparia and showed the presence of phenyl alkanes (61.2-85.5%), γ-terpinene (11.1%), p-cymene (4.5%) and (E)-β-ocimene (4.4%). Li et al. [11] reported for the first time the separation and identification of volatile constituents of Artemisia argyi with main components as bornol and bornyl acetate, whereas, the oil of Artemisia japonica is devoid of these components.

β-caryophyllene, a constituent present in the essential oil of Artemisia japonica is a common sesquiterpene which is widely distributed in plants and possesses anti-inflammatory and anticarcinogenic activities while also playing a role in plant defense [12]. Artemisia globella essential oils have pronounced anti-inflammatory properties and slight anti-proliferative and analgesic properties [13].

The chemical composition of essential oils from the Artemisia genus has been extensively studied in several species from around the world. Many studies have shown that Artemisia species display significant intra-specific variations in the terpene constituents of their essential oils. In some cases, the variation in the volatile components of these plants may occur during plant ontogeny or growth at different altitudes. The quality and yield of essential oils from Artemisia species is influenced by the harvesting season, fertilizer and pH of soils, the choice and stage of drying conditions, the geographic location, chemotype or

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Table 1: Chemical composition of the essential oil of Artemisia japonica from Kerala.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>RT</th>
<th>Compound</th>
<th>% composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11.652</td>
<td>Linalool</td>
<td>0.9</td>
</tr>
<tr>
<td>2</td>
<td>24.897</td>
<td>α-cubebene</td>
<td>0.6</td>
</tr>
<tr>
<td>3</td>
<td>25.325</td>
<td>β-eudesmol</td>
<td>0.6</td>
</tr>
<tr>
<td>4</td>
<td>25.745</td>
<td>β-elemene</td>
<td>2.8</td>
</tr>
<tr>
<td>5</td>
<td>26.994</td>
<td>Caryophyllene</td>
<td>2.4</td>
</tr>
<tr>
<td>6</td>
<td>28.949</td>
<td>Trans-β-farnesene</td>
<td>0.9</td>
</tr>
<tr>
<td>7</td>
<td>30.006</td>
<td>Germaacrene D</td>
<td>7.5</td>
</tr>
<tr>
<td>8</td>
<td>31.373</td>
<td>α-farnesene</td>
<td>0.6</td>
</tr>
<tr>
<td>9</td>
<td>31.516</td>
<td>Naphthalene1,2,4,5,6,8a-hexahydro-4,7-dimethyl-1,1-(1-methylethyl)</td>
<td>0.7</td>
</tr>
<tr>
<td>10</td>
<td>31.994</td>
<td>γ-cadinene</td>
<td>1.3</td>
</tr>
<tr>
<td>11</td>
<td>34.637</td>
<td>Spathulanol</td>
<td>12</td>
</tr>
<tr>
<td>12</td>
<td>36.650</td>
<td>γ-elemene</td>
<td>0.6</td>
</tr>
<tr>
<td>13</td>
<td>36.893</td>
<td>Aromadendrene</td>
<td>0.6</td>
</tr>
<tr>
<td>14</td>
<td>37.380</td>
<td>γ-murolene</td>
<td>1.4</td>
</tr>
<tr>
<td>15</td>
<td>40.735</td>
<td>6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol;</td>
<td>0.8</td>
</tr>
</tbody>
</table>

% Identification: 34.3
Grouped Components: 20.5
Monooxygenated sesquiterpene: 12.9
Oxygenated monoterpenes: 0.9

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Fig 2: Chemical Structures of Major Compounds of Essential Oil of Artemisia japonica.
subspecies, choice of plant part or genotype, or extraction method\textsuperscript{13}. In nature, essential oils play an important role in the protection of the plants as antibacterials, antivirals, antifungals, insecticides and also against herbivores by reducing their appetite for such plants. They may also attract some insects to favour the dispersion of pollens and seeds, or repel undesirable others\textsuperscript{13}. These activities may be due to the synergistic action of various components present in the plant. Spathulenol, the major component of essential oil of \textit{Artemisia japonica} has various biological activities and hence the oil could be exploited for various purposes.

4. Acknowledgement
The author\textsuperscript{11} thank Kerala State Council for Science, Technology and Environment (KSCSTE) for providing Junior Research Fellowship for the work.

5. References
13. Seidakhmetova RB, Bejsenbaeova AA, Atazhanova GA, Sulejmenov IM, Pak RN, Kuliasov AT \textit{et al}. Chemical composition and biological activity of the essential oil of

Determination of Artemisinin In Selected *Artemisia* L. Species By HPLC

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**ABSTRACT**
Malaria is an infectious disease that has been the cause of mortality of hundreds of people around the world. Through screening of many traditional Chinese medicinal plants, the Chinese team of experts discovered, the powerful antimalarial artemisinin, from *Artemisia annua*. This study deals with the quantification of artemisinin using standard HPLC procedure in selected *Artemisia* sp. (*Artemisia vulgaris* and *Artemisia japonica*). Mobile phase for HPLC analysis was 45% (v/v) methanol and 55% 0.01M sodium phosphate buffer (pH 7.0). Chromatographic separation was performed with Shimadzu LC 2010 AHT/2010 CHT system and the samples were analysed with a reverse phase phenomenex Luna 5u C18 (2) 100A column (250x4.6mm). Isocratic elution was carried out with a flow rate of 1.0ml/min. The temperature was set at the range of 20-25°C. Artemisinin was detected at 260 nm. Seasonal and Geographical variations in the concentration of artemisinin was also studied to know whether these factors have any influence in the concentration of artemisinin. The presence of artemisinin in the studied *Artemisia* sp. ranges from 0.0040-0.01%. Out of the two species studied, *A. japonica* possess the higher artemisinin content (0.01%) in summer and monsoon seasons.

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Introduction

Many natural products in plants are multifunctional molecules that protect them from infections of bacteria, viruses, and other microorganisms, or from herbivores such as insects, worms and mammals [1]. Artemisinin, also called Qingshaosu (QHS), is a naturally occurring sesquiterpene lactone endoperoxide in the terpenoid family of secondary metabolites isolated from Artemisia annua L. of family Asteraceae and chemically characterised by Chinese scientists during the 1970's [2]. It has an empirical formula of C₁₅H₂₀O₅ and possesses a peroxide bridge (C=O-O-C) to which its antimalarial properties are attributed (Figure 1). It has been proposed that the endoperoxide bridge is cleaved by a heme group to form a free radical that causes selective alkylation of parasite proteins, leading to parasite death. Artemisinin forms a complex with heme that possibly interrupts the parasite's hemoglobin catabolism [3]. Despite the fact that its chemical action on the human organism has not yet completely clarified, artemisinin offers impressive effects including characteristics of high efficacy, fast action and low-toxicity, which is regarded as a breakthrough in the history of antimalarial drugs [4]. Artemisinin is now available commercially in China, Vietnam and other countries as an antimalarial drug and, although its total synthesis has been achieved (the total organic synthesis gives low yields and is uneconomical due to its complex structure), its extraction from the plant still seems to be the only reliable resource [5,6,7].

![Figure 1: Structure of Artemisinin](image)

Artemisinin is a new antimalarial drug that has been shown to be effective against the erythrocytic stages of the plasmodial parasite, even against strains that have developed resistance to other currently available drugs such as chloroquine. This is particularly important since malaria is still a major disease in many areas of the world [9]. Artemisinin or its derivatives have been demonstrated to be novel anti-tumour agents for some of the deadliest cancers known to man. For example, artemisinin derivatives have been shown to be very effective against radiation-resistant breast cancer cells in vitro [10], drug-resistant small cell lung carcinoma cells [11], human leukaemia cell lines [12] and colon cancer and active melanomas [13]. Artemisinin is active against diverse plant pathogenic fungi including Gliomastix sp., Cylindrocarpon, Rhizoctonia cerealis, Gerlachia rivas and Verticillium dahliae [14].

Analysis of artemisinin is challenging as the compound is unstable, present in the plants is low, the intact molecule has poor staining characteristics, and other constituents in the plant interfere with the detection. For the methods developed so far, HPLC with UV detection was the most suitable method to determine artemisinin content in crude plant extracts [15]. Since the compound lacks chromatographic properties, it has to be derivatized to Q260 prior to HPLC analysis. The current study deals with the quantitative estimation of artemisinin in the selected species of Artemisia, namely Artemisia vulgaris and Artemisia japonica. Artemisia vulgaris L. (mugwort) syn. A. milagística (Clarke) Pamp. belongs to the family Asteraceae and is a tall aromatic perennial herb, which grows in the hilly districts of India in areas up to 2400m elevation. In traditional medicine, this plant is widely used for the treatment of diabetes and extract of the whole plant is used for epilepsy and in combination for psychoses, depression, irritability, insomnia, anxiety and stress [16]. Infusion of the leaves is given as a vermifuge. Mugwort is commonly used in traditional European medicine as a chlortic and for amenorrhea and dysmenorrhea [17]. In herbal medicine, aerial parts of A. vulgaris are being used as an anthelmintic, an anti-inflammatory, an antispasmodic, and a tonic for vital organs and for various disorders including hepatitis [18]. In various studies, A. vulgaris showed antibacterial activity and showed efficacy in the correction of breath presentation [19]. Its crude extract has been used as an antimalarial agent for thousands of years, and it was found that artemisinin extracted from A. vulgaris had antitumor activity [20]. A paste or powder of the leaves is applied over skin diseases. It is used as an inferior substitute of chinchona for treating fever [21]. Artemisia japonica Thunb. (Syn. Artemisia parviflora Buch.-Hamm. ex D. Don) is also a member of the family Asteraceae (Compositae) and is distributed in India, Myanmar, Pakistan, Nepal, Bhutan, Afghanistan and Japan. It is a nonaromatic perennial herb which is reported in the southern Kerala particularly in Munnar. This plant is used in medicine and extracting essential oil, having anti-viral and anthelmintic properties [22]. The plant has been used as a traditional medicine to treat fever and oedema [23]. In traditional medicine, various parts of Artemisia japonica (leaves, stem, seeds and fruits) have been widely used by tribal people for its wound healing properties, treatment of skin diseases, febrifuge, depurative properties, digestive and in ethnovernetary medicine [24].

To our knowledge, there were no reports on the concentration of artemisinin in Artemisia vulgaris and Artemisia japonica using HPLC. Seasonal and geographical variations were also considered.

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Materials and Methods

Collection of plant materials and authentication

The leaves of *Artemisia vulgaris* were collected randomly from wild populations, of three different locations namely Munnar (Idukki), Palakkad and Wayanad districts of Kerala in three seasons namely winter (November - January), summer (February - May) and monsoon (June-October), whereas the leaves of *Artemisia japonica* were collected from Munnar (Idukki district), because of its restricted distribution in Kerala in three seasons. The plant materials were identified and authenticated and the voucher specimens were deposited in the Herbarium of Department of Botany, Sacred Heart College, Thiruvananthapuram.

Chemicals and Reagents

Artemisinin standard (98%) was purchased from Sigma-Aldrich, USA. All the reagents and chemicals used were of HPLC grade.

Methods

Artemisinin was extracted from leaves of *Artemisia vulgaris* and *Artemisia japonica* following a reported methodology [25] using HPLC technique with some modifications as follows:

Preparation Of Working Standards

The stock solution was prepared using 5mg of standard artemisinin, diluted with toluene (Merek) to furnish standard solutions of 10-20 μg/ml for calibration. The calibration curve was obtained from triplicate injections of three different by plotting the peak area (y) against the concentration (x). (Figure. 2).

![Figure 2. Calibration graph of artemisinin](image)

Sample Preparation

The fresh leaves of the plant were placed in hot air oven at 60°C for three days. They are then reduced in powders by crushing in a mixer.

Preparation of extracts

The dry weights of the leaves were measured. One gram of the dried powder was taken for analysis. The tissues were mixed with 5ml of toluene (Merek) to make a homogenous mixture. These mixtures were centrifuged at 2000 rpm for 20 minutes (Eppendorf centrifuge). Supernatant obtained were decanted and pooled in vials. Pellets were resuspended in 5ml toluene, centrifuged and decanted as above. Pellets were discarded, both supernatants were pooled; extracts were air dried before storing under refrigeration for further analysis.

Prederivatization

The compound lacks chromophores, so it has to be derivatized first to Q292 compound and then to Q260 compound which can be detected at 260nm. The dried extracts and dried dilutions of standard artemisinin were converted to Q260 derivative of artemisinin as follows: each extract was solubilized in 400μl of methanol (Spectrochem) and 1600μl of 0.2% (v/v) NaOH (Sigma-Aldrich); then hydrolyzed for 45 min at 50°C. The reaction was stopped by adding 1600μl of 0.2M acetic acid (Merek) and placing the test tube on ice. To make a final volume of 4 ml, 400μl of methanol was added. Samples at this stage contained Q260 derivative of artemisinin, and were filtered through Whatman’s 0.2μm pareude PVDF syringe filter before injecting into HPLC for analysis. All samples were hydrolyzed just before use.

For HPLC analysis, mobile phase was prepared by combining 45% (v/v) methanol and 55% 0.01 M sodium phosphate buffer (pH 7.0) (Sigma-Aldrich). Chromatographic separation was performed with Shimadzu LC 2010 AHT/2010 CHT system and the samples were analysed with a reverse phase phenomenex Luna 5u C18(2) 100A column (250×4.6 mm). Isocratic elution was carried out with the flow rate of 1.0ml/min. The temperature was set at the range of 20-25°C. Artemisinin was detected at 260 nm absorbance and retention time was 12 ± 0.5 min. Data acquisition, integration and instrument control were performed using LC solutions software.

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Identification was made by comparison of the retention times and the absorbance spectra with that of the standard references. The experimental results obtained after the HPLC analyses were subjected to statistical analyses using Graph Pad InStat 3 software.

Results and discussion

The content of the active ingredient in *Artemisia annua* varies and is reported to range from 0.01% to 1.5% [26, 27]. From the estimation of artemisinin using HPLC technique, it was observed that the presence of artemisinin was too low in all the studied *Artemisia* samples. Here, the presence of artemisinin ranges from 0.0046 - 0.01%. The mean and standard deviation of the six independent analysis is given in the table using the Instat software (Table 1). The HPLC chromatograms of the plant samples is given (Fig: 3-4). The mean concentration of artemisinin in *A. vulgaris* shows that, it varies from 0.00465 % - 0.00965 %, i.e. *A. vulgaris* collected from Munnar and Wayanad in summer seasons respectively. The concentration of artemisinin in *Artemisia japonica* which is confined to the Southern part of Kerala, particularly Munnar was collected in three seasons ranges from 0.00725 % - 0.01%. From the observation, minor variations can be seen in the concentration of artemisinin with respect to seasons and locations. The highest concentration of artemisinin is seen in *A. japonica* during summer and monsoon, i.e. 0.01 %. (Fig 4- E, F). Artemisinin content in the plant varies depending upon the seasons, geographical changes, method of extraction, type of plant material used. There are reports [28] on the antimarial activity of *Artemisia japonica, Artemisia maritima* and *Artemisia nilgerica*. A study [29] conducted to analyse the content of artemisinin using HPLC in Artemisia species including *A. vulgaris, A. maritima, A. absinthium* and *A. annua* collected from different environmental zones of Pakistan, reported the content of artemisinin in *A. annua* was 0.023 %, other species were devoid of artemisinin. In another study [30] in ten species of Artemisia namely *A. santonicum* L., *A. taurica* Willd., *A. spicigera* K. Koch, *A. herba alba* Asso, *A. haussknechtii* Boiss., *A. campestris* L., *A. araratica* Krasch., *A. armeniaca* Lam., *A. austriaca* Jacq., and *A. absinthum* L. by HPLC method, collected from different localities throughout Turkey, showed the absence of artemisinin in all species.

Table 1 Concentration (%) of Artemisinin in *A. vulgaris* & *A. japonica* during winter, summer and monsoon.

<table>
<thead>
<tr>
<th>Species Place of collection</th>
<th>Munnar</th>
<th>Palakkad</th>
<th>Wayanad</th>
<th>Munnar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter</td>
<td>0.00435±0.00080</td>
<td>0.00760±0.00059</td>
<td>0.00068±0.000346</td>
<td>0.008725±0.00070</td>
</tr>
<tr>
<td>Summer</td>
<td>0.00465±0.000459</td>
<td>0.00828±0.00075</td>
<td>0.00965±0.00045</td>
<td>0.01005±0.00064</td>
</tr>
<tr>
<td>Monsoon</td>
<td>0.00660±0.000827</td>
<td>0.00605±0.00073</td>
<td>0.00696±0.00037</td>
<td>0.01091±0.00047</td>
</tr>
</tbody>
</table>
Figure 3: HPLC Chromatogram showing artemisinin concentration in A. vulgaris from Munnar. A. winter B. summer C. monsoon.

HPLC Chromatogram showing artemisinin concentration in A. vulgaris from Palakkad. D. winter E. summer F. monsoon.

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Figure 4: HPLC Chromatogram showing the artemisinin concentration in A. vulgaris from Wayanad in A. winter B. summer C. monsoon.

HPLC Chromatogram showing the artemisinin concentration in A. japonica in D. winter E. summer F. monsoon.
Conclusion
Artemisinin is a sesquiterpene lactone with an endoperoxide bridge having a wide range of medicinal applications. The results of present study confirm the presence of artemisinin in *A. vulgaris* and *A. japonica*. The concentration of artemisinin in the plant samples is low. Out of the two species studied, *A. japonica* possesses the higher artemisinin content (0.01 %) in summer and monsoon. In *A. vulgaris*, the highest concentration of artemisinin content is seen in summer season (0.009 %), which was collected from Wayanad. The content of artemisinin in monsoon, irrespective of the locations, remain more or less the same in *A. vulgaris* samples. The medicinal property of these plants may be due to the bioactive compounds present in these plants. The synergistic activities of these compounds makes them excellent candidate in the treatment of various ailments. Further studies on the isolation and pharmacological activities of the compounds will explore more potential uses of the plant.

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Authors’ Statements
Competing Interests
The authors declare no conflict of interest.

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