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## Research Article

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### Humara Wani

Department of Chemistry, National  
Institute of Technology Srinagar,  
Hazratbal-190006, J & K, India

### Shakeel A. Shah

Department of Chemistry, National  
Institute of Technology Srinagar,  
Hazratbal-190006, J & K, India

### Javid A. Banday

Department of Chemistry, National  
Institute of Technology Srinagar,  
Hazratbal-190006, J & K, India

### Correspondence:

#### Dr. Javid A. Banday

Department of Chemistry, National  
Institute of Technology Srinagar,  
Hazratbal-190006, J & K, India

Tel: +91 9906626635

#### E-mail:

[javidbanday@rediffmail.com](mailto:javidbanday@rediffmail.com)

## Chemical composition and antioxidant activity of the leaf essential oil of *Artemisia absinthium* growing wild in Kashmir, India

Humara Wani, Shakeel A. Shah, Javid A. Banday\*

### Abstract

The essential oil composition of the leaves of *Artemisia absinthium*, growing in Kashmir, India, along with its antioxidant activity, is reported in the present study. Gas chromatography coupled with mass spectrometry (GC-MS) revealed the presence of twelve constituents, representing 99.99% of the total oil. The major constituents of the oil were Chrysanthenyl acetate (49.15%) and L- $\beta$ -pinene (39.62%). The monoterpene content in the oil was found to be 98.76 %, while as sesquiterpene hydrocarbon content was 1.23%. The essential oil was evaluated for antioxidant activity with DPPH radical, exhibiting promising antioxidant profile.

**Keywords:** *Artemisia absinthium*, Essential oil, Monoterpenes, Antioxidant activity.

### Introduction

The genus *Artemisia* is most widely distributed genera of the family Asteraceae, consisting of 522 small herb and shrub species native to the northern hemisphere, South America, Southern Africa and the Pacific Islands.<sup>1, 2</sup>

These herbs have been used worldwide in folk medicine since ancient times.<sup>3</sup> Literature reveals the application of these herbs as tonics, antimalarials, antihelmintics and antidiabetics, and in treating wounds, bronchitis, ulcers, and tuberculosis in traditional Anatolian medicine.<sup>3-6</sup> There are also several reports concerning the antimalarial, antioxidant, cytotoxic, antipyretic, analgesic, antidiabetic, antimicrobial, and antifungal activities of different *Artemisia* species.<sup>1, 7-9</sup> The chemical studies on *Artemisia* species indicate that all classes of compounds are present in the genus with particular reference to terpenoids and flavonoids. The rich accumulation of essential oils and other terpenoids in the genus is responsible for the use of various members for flavouring foods or liqueurs.<sup>1</sup>

*Artemisia* is represented by 45 species in the Indian flora<sup>10</sup>, some of them are naturally distributed in Jammu and Kashmir region.<sup>11</sup> All of these taxa have been the subject of numerous chemical studies. Most of the research pertaining to these plants indicates the variability of the essential oil composition with geographic origin, harvesting time, and environmental edaphic factors.<sup>12-20</sup>

Even though the essential oil chemistry of numerous *Artemisia* species, originating from India, have been reported previously<sup>21-23</sup>, there is no report of the essential oil composition and antioxidant activity of *Artemisia absinthium* from Jammu and

Kashmir region. This study was carried out to investigate the essential oil composition and antioxidant activity of *Artemisia absinthium* collected from the Kulgam area of Kashmir, India.

## Materials and Methods

### Plant material

The aerial part of the plant was collected from Kulgam, Kashmir (India) in June-2013. The plant sample was identified and authenticated by Akhter H. Malik, curator, Centre for Biodiversity and Taxonomy, University of Kashmir and voucher specimen was deposited in the herbarium (voucher specimen no-1938 KASH).

### Isolation procedure

The fresh plant material was finely chopped and the essential oil was obtained by hydro-distillation in a Clevenger type apparatus as recommended by the European Pharmacopoeia. The yield of oil, as calculated on fresh weight basis (v/w), was 0.156%. The oil was dried over anhydrous sodium sulfate and stored in a sealed glass vial in a refrigerator at 4°C prior to analysis.

### Gas chromatography-mass spectrometry (GC/MS)

GC-MS analysis was carried on a Varian Gas Chromatograph series 3800 fitted with a VF-5 ms fused silica capillary column (60m x 0.26mm, film thickness 0.25µm) coupled with a 4000 series mass detector under the following conditions: injection volume 0.20 µl with split ratio 1:60, helium as a carrier gas at 1.0 ml/min constant flow mode, injector temperature 230°C, oven temperature 60°C to 280°C at 3°C/min.

### Antioxidant activity assay

DPPH Free radical scavenging activity was evaluated by measuring the scavenging activity of the leaf essential oil of *Artemisia absinthium* on stable 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH).

A 4 µM solution of DPPH in methanol was prepared and a stock solution of sample (1 mg/mL) in methanol was prepared. Various concentrations (5-100 µg/mL) were added to 1.0 mL (4 µM DPPH) and final volume was made to 3.0 mL with methanol. The mixture was shaken thoroughly and kept standing at room temperature for 20 minutes. Then the absorbance of the mixture was measured at 517 nm on a spectrophotometer. The decrease in the absorbance indicates an increase in DPPH-radical scavenging activity.

## Results and discussion

### Chemical Composition

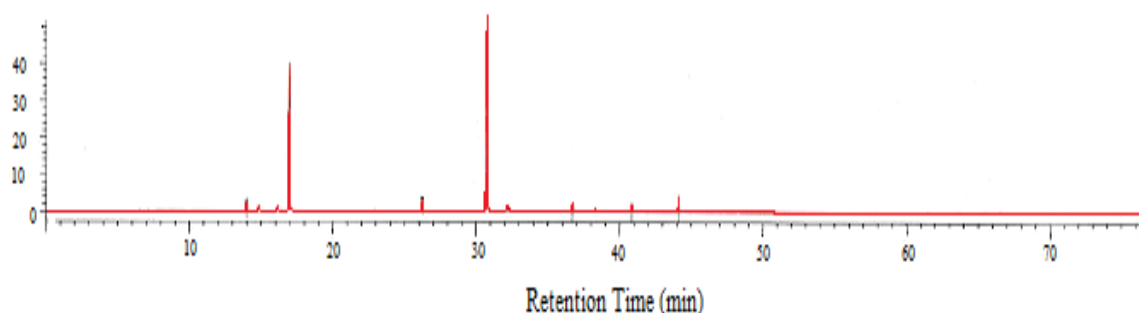
The chemical composition of the essential oil isolated from the aerial part of *Artemisia absinthium*, analyzed by GC-MS, is presented in Table-1. The GC-MS total ion chromatogram of the oil is shown in Fig 1. Identification of the essential oil constituents was done on the basis of MS Library search (NIST 98 and WILEY), by comparing with the MS literature data.<sup>24</sup> The relative percentages of the individual components were calculated based on GC peak area. GC-MS analysis revealed the presence of 12 constituents representing 99.99% of the total oil. The major constituents of the oil were Chrysanthenyl acetate (49.15%) and L-β-pinene (39.62%). The percentage yield of the oil calculated was found to be 0.156%. (v/w), as per fresh weight basis.

**Table 1:** Chemical composition of the essential oil from the aerial part of *Artemisia absinthium*

S. No.	RT (min)	Compound	% Composition
1	13.982	α- Pinene	2.38
2	14.814	Camphene	1.05
3	16.128	Sabinene	1.28
4	16.444	β- Pinene	0.15
5	16.935	L- β- Pinene	39.62
6	26.229	Cis-Verbenol	1.38
7	30.724	Chrysanthenyl acetate	49.15
8	32.177	Sabinyll acetate	3.41
9.	36.677	β- Bourbonene	0.12
10.	38.276	Caryophyllien	0.37
11.	40.852	Germacone	0.73
12.	44.097	Nerol acetate	0.32

**Class composition:**

Monoterpene hydrocarbons	98.76
Sesquiterpene hydrocarbons	1.23
<b>Total</b>	<b>99.99</b>



**Figure 1:** GC-MS total ion chromatogram of the oil from the aerial part of *Artemisia absinthium*

**Antioxidant activity assay**

DPPH Free radical scavenging activity was evaluated by measuring the scavenging activity of the leaf essential oil of *Artemisia absinthium* on stable 2, 2-diphenyl-1-picryl hydrazyl radical (DPPH). (Table 2)

Where, Ac is the absorbance of control, As is absorbance of the sample. L-Ascorbic acid (Sigma-Aldrich) served as positive control. The experiment was done in triplicate and mean values were recorded. IC<sub>50</sub> value was calculated as the concentration of the sample, required to scavenge 50% of DPPH free radicals.

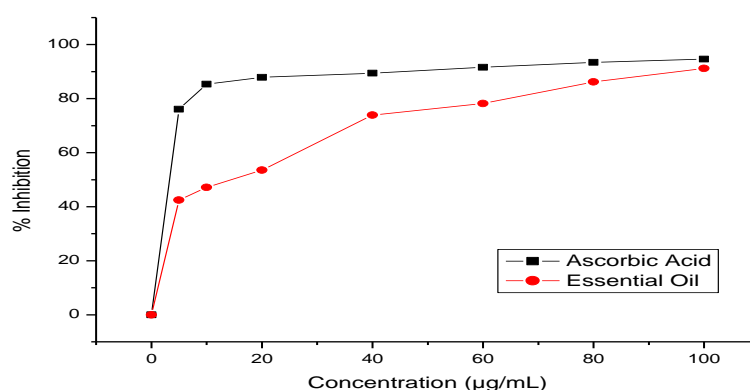
The percentage inhibition was calculated by the following equation.

IC<sub>50</sub> Ascorbic Acid: 3.27 µg/mL  
 IC<sub>50</sub> Essential oil: 14.14 µg/mL

$$\text{DPPH radical scavenging (\%)} = \{(Ac-As) / Ac\} \times 100$$

**Table 2:** DPPH radical scavenging activity (%)

Concentration (µg/mL)	Ascorbic Acid	Essential oil
5	76.11	42.45
10	85.32	47.19
20	87.86	53.54
40	89.43	73.89
60	91.61	78.19
80	93.43	86.18
100	94.61	91.21



**Figure 2:** Percentage inhibition of the Essential oil at varying concentrations

## Conclusion

The present study reports the essential oil composition of the leaves of *Artemisia salsoloides*, growing in Kashmir. Twelve constituents were identified on the basis of GC-MS from the essential oil of the leaves of *Artemisia absinthium*, major constituents being Chrysanthenyl acetate (49.15%) and L- $\beta$ -pinene (39.62%). The oil showed marked antioxidant activity with DPPH radical

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