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Chemical Composition and Antioxidant Activity of the Leaf Essential Oil of *Artemisia salsoloides* growing wild in Kashmir Himalayas

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ABSTRACT

The essential oil composition of the leaves of *Artemisia salsoloides*, growing in Jammu & Kashmir, India, along with its antioxidant activity, is reported in present study. Gas chromatography coupled with mass spectrometry (GC-MS) revealed the presence of eight constituents, representing 99.96% of the total oil. The major constituents of the oil were 2,4-Penta diynylbenzene (49.68%), β -trans-ocimene (17.98%), Sabinene (16.68%), 2,5-etheno(4,2,2) propeller-3,7,9-triene (5.58%). The monoterpene content in the oil was found to be 44.70 % while as other hydrocarbon content was 55.26%. The essential oil was evaluated for antioxidant activity with DPPH radical, exhibiting an interesting antioxidant profile.

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Introduction

The genus *Artemisia* is among the largest and most widely distributed genera of the family Asteraceae, consisting of 522 small herb and shrub species native to northern hemisphere, South America, Southern Africa and the Pacific Islands [1-2].

These herbs have been used worldwide in folk medicine since ancient times [3-4]. 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 [5-8]. There are also several reports concerning the antimalarial, antioxidant, cytotoxic, antipyretic, analgesic, antidiabetic, antimicrobial, and antifungal activities of different *Artemisia* species [1,9-12]. "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" [1].

Artemisia is represented by 45 species in the Indian flora [13], some of them are naturally distributed in Jammu and Kashmir region [14]. 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 [5,15-23].

Even though the essential oil chemistry of numerous *Artemisia* species, originating from India, have been reported previously [24-26], there is no report of the essential oil composition and antioxidant activity of *Artemisia salsoloides* from Jammu and Kashmir region. This study was carried out to investigate the essential oil composition and anti oxidant activity of *Artemisia salsoloides* collected from the Zaskar region of Jammu and Kashmir.

Experimental

Plant material

The aerial part of the plant was collected from Zaskar, Ladakh (India) in August-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-1939 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 European Pharmacopoeia. The yield of oil, as calculated on fresh weight basis (v/w), was 1.22%. 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 0.25 μ m) coupled with a 4000 series mass detector under the following conditions: injection volume 0.20 μ l with split ratio 1:60, helium as 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 salsoloides* on stable 2, 2-diphenyl-1-picryl hydrazyl radical (DPPH) (Figure 3).

A 4 μ M solution of DPPH in methanol was prepared and a stock solution of sample (1mg/mL) in methanol was prepared. Various concentrations (5-100 μ g/mL) were added to 1.0 mL (4 μ M 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 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 salsoloides*, analyzed by GC-MS, is presented in Table-1. The GC-MS total ion chromatogram of the oil is shown in Figure 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 [27]. The relative percentages of the individual components were calculated based on GC peak area. GC-MS analysis revealed the presence of 8 constituents representing 99.9% of the total oil. The major constituents of oil were 2,4-pentadienyl benzene (49.68%), β -trans ocimene (17.98%) followed by Sabinene (16.68%) and 2,5-etheno(4,2,2)propeller-3,7,9-triene (5.58%). The percentage yield of the oil calculated was found to be 1.22% (v/w), as per fresh weight basis.

Antioxidant activity assay

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

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

S. No.	RT (min)	Compound	% Composition
1	16.132	Sabinene	16.68
2	18.961	p-Cymene	5.29
3	19.453	β -Trans ocimene	17.98
4	20.757	γ -Terpinene	0.91
5	25.558	Camphor	0.68
6	27.168	4-Terpinol	3.16
7	32.146	3,4-Pentadienylbenzene	49.68
8	41.626	2,5-Etheno(4,2,2)propeller-3,7,9-triene	5.58

Class composition:

Monoterpenehydrocarbons	44.70
Other hydrocarbons	55.26
Total	99.96

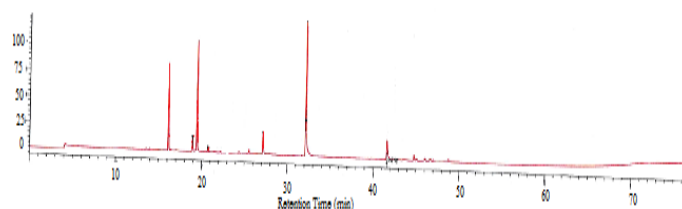


Figure 1. GC-MS total ion chromatogram of essential oil of *Artemisia salsoloides*

The percentage inhibition was calculated by the following equation.

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

Where, Ac is the absorbance of control, As is absorbance of sample. L-Ascorbic acid (Sigma-Aldrich) served as positive control (Figure 2). The experiment was done in triplicate and mean values were recorded. IC₅₀ value was calculated as the concentration of the sample, required to scavenge 50% of DPPH free radicals.

Table 2. DPPH radical scavenging activity (%)

Concentration ($\mu\text{g/mL}$)	Ascorbic Acid	Essential oil
5	76.11	29.51
10	85.32	37.22
20	87.86	47.01
40	89.43	52.27
60	91.61	58.64
80	93.43	62.40
100	94.61	66.07

IC₅₀ Ascorbic Acid: 3.36 $\mu\text{g/mL}$

IC₅₀ Essential oil: 30.56 $\mu\text{g/mL}$

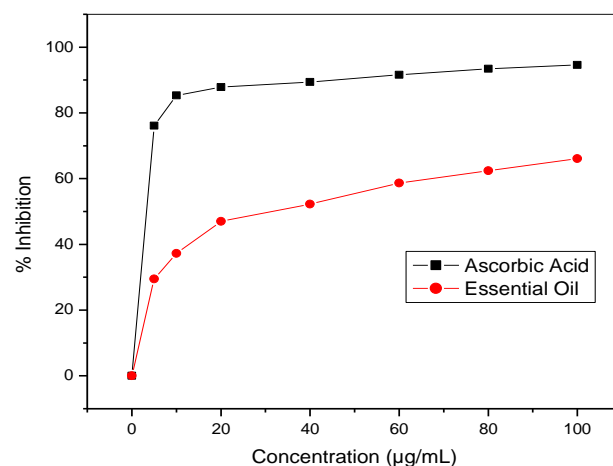


Figure 2: Percentage inhibition of the Essential oil at varying concentrations

Conclusion

The present study reports the essential oil composition of the leaves of *Artemisia salsoloides*, growing in Kashmir. Eight constituents were identified on the basis of GC-MS from the essential oil of the leaves of *Artemisia salsoloides*, major constituents being 2,4-Penta dienylbenzene (49.68%), β -trans-ocimene (17.98%), Sabinene (16.68%), 2,5-etheno(4,2,2) propeller-3,7,9-triene (5.58%). The oil showed marked antioxidant activity with DPPH radical

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