The Journal of Phytopharmacology 2014; 3(4): 234-237
Online at: www.phytopharmajournal.com

Research Article

ISSN 2320-480X
JPHYTO 2014; 3(4): 234-237
July- August

Mahpara Qadir
Department of Chemistry, University of Kashmir, Srinagar, Hazratbal-190006, J & K, India

Javid A. Banday
Department of chemistry, National Institute of Technology, Srinagar, Hazratbal-190006, J & K, India

Wajaht A. Shah
Department of Chemistry, University of Kashmir, Srinagar, Hazratbal-190006, J & K, India

Correspondence:
Dr. Wajaht A. Shah
Department of Chemistry, University of Kashmir, Srinagar, Hazratbal-190006, J & K, India
E-mail: doctorwajaht@gmail.com

GC-MS analysis and Antioxidant activity of essential oil of *Artemisia amygdalina* from Kashmir, India

*Mahpara Qadir, Javid A. Banday, Wajaht A. Shah**

Abstract

The essential oil composition of the leaves of *Artemisia amygdalina*, 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 28 constituents representing 95.58% of the total oil. Sabinene, p-Cymene, Eucalyptol and L-Borneol were the major constituents present in the oil. The monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons and oxygenated sesquiterpene content in the oil was found to be 58.08%, 31.14%, 5.58% and 0.78%, respectively. The essential oil was evaluated for antioxidant activity through DPPH assay, exhibiting prominent antioxidant profile.

Keywords: *Artemisia amygdalina*, Essential oil, Monoterpenes, Sesquiterpenes, Antioxidant activity.

Introduction

Artemisia is one of the largest genera of the family Asteraceae. The genus belongs to a useful group of medicinal and aromatic plants comprising of about 400 species most of which are distributed in North America, Europe, Asia and North Africa. In India, about 37 species have been reported which are mostly confined to North Western Himalayan region.[1, 2] *Artemisia* species are popular from chemical, pharmacological and taxonomic point of view and have been used for the treatment of diseases like hepatitis, cancer, inflammation and infections by fungi, bacteria and viruses.[3] The genus *Artemisia* is known to contain many bioactive compounds such as artemisinin, which possess anti-malarial and cytotoxic activity against tumor cells[4] and arglabin, another bioactive molecule, is used for treating certain types of cancers.[5]

Artemisia amygdalina Decene, locally known as "veer tethven" in Kashmir, is a critically endangered and endemic species of the Himalayan region of Pakistan and Kashmir. In an additional system of medicine, the plant is used by the locals for the treatment of cough, cold, worms, etc. It is also used as a vermifuge.[6] Literature reveals the identification of 25 components in the leaf essential oil of *A. amygdalina*, dominated by monoterpene hydrocarbons (38.2%) and oxygenated monoterpenes (43.8%) together constituting 82.0% of total oil composition, the principal components being sabinene (10.2%), p-cymene (14.7%), 1,8 cineole (17.5%) and borneol (19.8%). The stem essential oil of the same plant has been reported to possess 32 components, the monoterpene hydrocarbons, oxygenated monoterpenes and sesquiterpene hydrocarbons being in the ratio of 66.1%: 12.8%: 11.2%. The major constituents were

α-pinene (6.0%), camphene (10.4%), β-pinene (40.2%) and borneol (5.7%).[7] This study was carried out to investigate the essential oil composition and antioxidant activity of *Artemisia amygdalina* collected from the botanical garden of Kashmir University, India.

Materials and Methods

Plant material

The aerial part of the plant was collected from the University of Kashmir, Srinagar, India in Nov-2013. The specimen 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. 1911 KASH).

Essential oil extraction

The fresh plant material was finely chopped and subjected to hydro-distillation separately in a Clevenger type apparatus for three hours as recommended by the European Pharmacopoeia. The yield of oil as calculated on fresh weight basis (v/w) was 0.12%. The oil obtained was dried over anhydrous sodium sulphate and stored at 4^0C in sealed vials in a refrigerator 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 (60 m × 0.25 mm, film thickness 0.25 µm) coupled with a 4000 series mass detector under the following conditions: injection volume 0.5 µl with split ratio 1:60, helium as a carrier gas at 1.0 ml/min constant flow mode, injector temperature 230 ^{0}C, the oven temperature was programmed from 60 to 280 ^{0}C at 3 ^{0}C/min. Mass spectra: electron impact (EI+) mode, 70 eV and ion source temperature 250 ^{0}C. Mass spectra were recorded over 50–500 amu range.

Chemicals

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical was purchased from Sigma-Aldrich, Madrid, Spain. Anhydrous sodium sulphate and all other reagents were of analytical grade (SISCO, Mumbai, India).

Antioxidant activity

DPPH free radical scavenging activity was evaluated by measuring the scavenging activity of the essential oil on stable 2,2-diphenyl- 1-picryl hydrazyl radical. A 0.5 mM solution of DPPH in methanol was prepared and a stock solution of oil sample (1 mg/mL) in methanol was prepared. Various concentrations (20–100 µg/mL) were added to 1mL (0.5 mM DPPH) and final volume was made to 3 mL with methanol. The mixture was shaken thoroughly and kept standing at room temperature for 10 min. Then, the absorbance of the mixture was measured at 517 nm on a spectrophotometer. A decrease in the absorbance indicates an increase in DPPH-radical scavenging activity.

The percentage inhibition was calculated by the following equation:

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

Where, Ac is the absorbance of the control and As is the absorbance of the sample.

L-ascorbic acid served as positive control.

Results and Discussion

Chemical composition

The chemical composition of the essential oil isolated from the aerial parts of *Artemisia amygdalina*, analyzed by GC-MS are shown in Table 1, in order of their election from the RTX-5 column. The total ion chromatogram of the essential oil is shown in Figure 1. Identification of essential oil constituents was done on the basis of MS library search (NIST 98 and WILEY), by comparison with MS literature data.[8] The relative percentage of the individual components was calculated based on GC peak area. GC-MS analysis led to the identification of 28 components in the essential oil. The major constituents of the oil were Sabinene (14.3%), p-cymene (12.5%), Eucalyptol (16.7%) and L-borneol (12%). The yield of oil as calculated on fresh weight basis (v/w) was 0.12%.

Table 1: Chemical composition of the essential oil from the aerial part of *Artemisia amygdalina* growing in Kashmir

S. No.	RT (min)	Compound	% Composition
1	12.19	Santolina triene	1.22
2	13.556	*α*- Thujene	0.33
3	13.834	*α*-Pinene	3.89
4	14.834	δ-Camphene	0.41
5	14.929	Camphene	1.59
6	16.138	**Sabinene**	**14.3**
7	16.454	β-Pinene	5.52
8	16.953	L-β-pinene	3.69
9	17.964	α–Phellandrene	1.60
10.	18.958	***p*-Cymene**	**12.5**
11.	19.204	Limonene	5.18
12.	19.411	**Eucalyptol**	**16.7**
13.	20.044	Trans-Ocimene	1.02
14.	20.751	γ-Terpenene	3.53
15.	25.566	Camphor	0.64
16.	26.225	Cis-Verbinol	0.55
17.	26.804	**L-Borneol**	**12.0**
18.	27.165	4-Terpineol	1.10
19.	27.868	α-Terpineol	3.30
20.	30.799	Piperitone	0.15
21.	35.220	α-Longipinene	0.23
22.	36.289	Valencene	0.68
23.	36.681	β-Bourbonene	0.14
24.	38.287	β-Caryophyllene	2.17
25.	39.824	α-Caryophylene	1.24
26.	42.269	δ-Cadinene	1.12
27.	45.050	Caryophyllene oxide	0.49
28.	48.773	Cedr-8-ene	0.29

Class composition:

Monoterpene hydrocarbons	58.08
Oxygenated monoterpenes	31.14
Sesquiterpene hydrocarbons	5.58
Oxygenated sesquiterpenes	0.78
Total	**95.58**

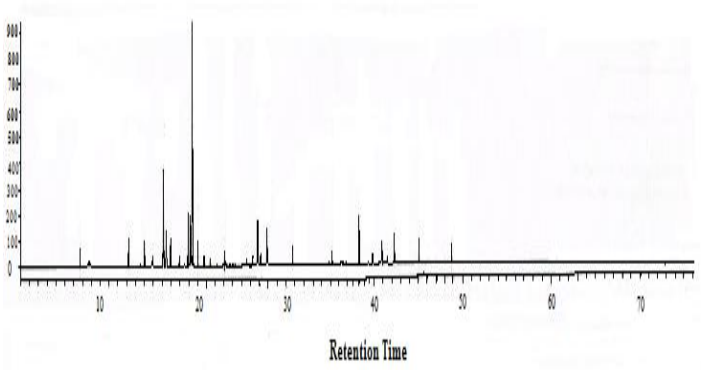

Figure 1: Total ion chromatogram of essential oil of *Artemisia amygdalina*

The difference in the essential oil composition than previous report may be due climatic, seasonal, geographical or genetic differences.

Antioxidant activity

The radical scavenging activity of the essential oil *Artemisia amygdalina* was measured by the DPPH assay in-vitro (Table 2). This is the first report on its antioxidant activity to the best of our knowledge. The highest activity (80.7%) was found at a concentration of 100 µg/mL as is shown in the Figure 2. The Ascorbic acid, which is used as a standard showed 78.7% radical scavenging activity at a conc. of 100 µg/mL. The DPPH radical scavenging assay is commonly employed in evaluating the ability of antioxidants to scavenge free radicals. This method has been used extensively to predict the antioxidant activity because of the relatively short time for analysis. The change in absorbance at 517 nm is used as a measure of the scavenging effect of a particular sample for DPPH radicals. The more rapidly the absorbance decreases, the more potent the antioxidant activity of the sample in terms of its hydrogen atom-donating capacity.[9, 10]

Table 2: DPPH radical scavenging activity (%)

Concentration (µg/mL)	Essential oil	Ascorbic Acid
20	46.6	52.3
30	49.3	60.8
50	55.7	65.6
70	67.4	75.4
100	77.8	80.7

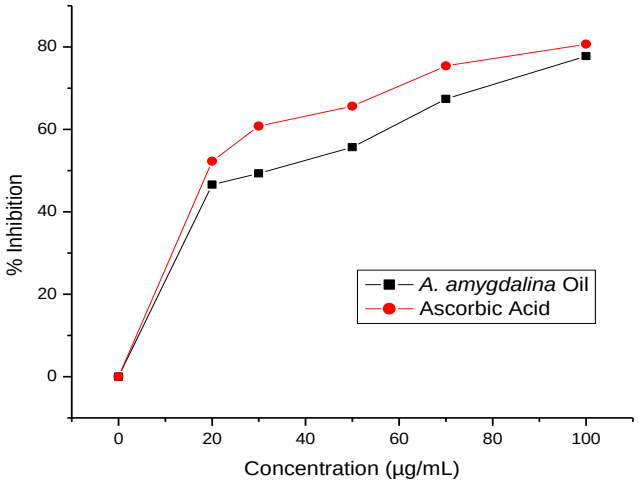

Figure 2: Percentage inhibition of the essential oil of *A. amygdalina* at varying concentrations

Conclusion

The essential oil of *Artemisia amygdalina* displayed significant antioxidant activity that can be of great interest to both pharmaceutical and food industries because of their possible use as natural additives to replace toxic synthetic food additives.

References

1. Tutin T.G., and Persson K., IN Flora Europea, T.G. Tutin, N.A. Burges, D.M. Moure, D.H.Valentine, S.M.Walters, D.A.Webb and V.H. Heywood, Cambridge University press, Cambridge. 1979; 4:178-186.

2. Anonymous., Wealth of India, Raw materials. Vol. IA, 1985: 434-445.

3. Efferth T., Antiplasmodial and antitumor activity of artemisinin from bench to beside. Planta Med. 2007; 73: 299-309.

4. Wons H., and Brown G.D., Germacranolides from *Artemisia myriantha* and their conformation. Phytochem. 2002;59: 529-536.

5. Kim J.H., Kim H.K., Jeon S.B., Son K.H., Kans E.H., Suns N.D., and Kwon B.M., New sesquiterpene- monoterpene lactone, artemisolide, isolated from *Artemisia argyi*. Tetrahedron lett.2002;43: 6205-6208.

6. Qaisar M., Asteraceae, Flora of Pakistan. 2006;27:120-121.

7. M. A. Rather, S.N. Sofi, B.A.Dar, B.A.Ganaie, Akbar Masood, M.A.Quireshi and A.S. Shawl. Comparative GC-FID and GC-MS analysis of the leaf and stem essential oil constituents of *Artemisia amygdalina* Decene from Kashmir. J. Pharmacy Research. 2011;4(6):1637-1639.

8. Adams R.P. Identification of essential oil components by Gas Chromatography/ Mass Spectrometry, Allured Publishing Corp., Carol Stream, IIIinois, USA. 2007.

9. Alasalvar, C., Magdalena, K., Agnieszka, K., Rybarczyk, A., Shahidi, F., & Amarowicz, R.. Antioxidant activity of hazelnut skin phenolics. Journal of agriculture and food chemistry. 2009;57:4645-4650.

10. Shahidi, F., Alasalvar, c., & Liyana-Pathirana, C. M. Antioxidant phytochemicals in hazelnut kernel (*Corylus avellana* L.) and hazelnut by-products. Journal of agriculture and food chemistry. 2007; 55(4): 1212-1220.