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CHEMICAL COMPOSITION OF ESSENTIAL OIL OBTAINED FROM (*ARTEMESIA ABSINTHIUM* L.) GROWN UNDER THE CLIMATIC CONDITION OF SKARDU BALTISTAN OF PAKISTAN

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Abstract

The common wormwood *Artemisia absinthium* (L.) is an aromatic perennial herb, which is wildly grown in the Skardu Baltistan. Locally its fresh and dried form is used as both medicinal and insecticidal purposes. This study first time reports the chemical composition of hydro-distilled essential oil obtained from aerial parts of *A. absinthium*. Upon hydro-distillation, it gave dark green colored oil with yield 0.41% (w/w) (based on sheltered dried plant parts). The variation in chemical composition and their relative percentage in essential oil of *A. absinthium* are cited to vary qualitatively and quantitatively according to geographical location and environmental conditions. The GC-MS analysis of *A. absinthium* essential oil obtained from the population collected from Skardu Baltistan also showed variation in chemical composition and their relative percentage. The oil was dominated by Sesquiterpenes (61.01%) and Monoterpenes (17.92%). The major dominating constitutes were Guaiol (19.33%), α-Bisabolol (8.83%), Carveol (6.16%), Chamazulene (5.94%), Limonen-6-ol, Pivalate (5.37%) and Geranyl-α-Terpinene (5.63%), which accounts overall 51.62% of total constitutes identified.

Key words: *Artemisia absinthium,* Essential oil, GC-MS, Sesquiterpenes, Guaiol.

Introduction

Common wormwood (*Artemisia absinthium* L.) is an aromatic herb, perennial medicinal plant (Tan *et al.*, 1998; Bora & Sharma, 2010). It is native to temperate region of the world and widely distributed in Afghanistan, China, India, Japan, Kazakhstan, Kyrgyzstan, Pakistan, Russia, North Africa, Europe, North America (Bora & Sharma, 2011). Its extract and oil were traditionally used in ethno-pharmacology and ethno-medicine (Abad *et al*., 2011). From ancient times species of *Artemisia* i.e., *A. absinthium, A. annua*and *A. vulgaris*have been used in folk medicine (Hayat *et al.*, 2009; Bora *et al.*, 2011b; Bano *et al.*, 2014; Milena *et al.*, 2016). It is also used as antiseptic, stomachic, cardiac stimulant, antispasmodic, anthelmintic and to reducing liver inflammation and restoration of mental function and memory improvement (Howes *et al.,* 2003; Guarrera, 2005). Some recent studies have reported that the genus *Artemisia* contained potent monoterpenes, sesquiterpenes and volatile acetylenes components and biological properties such as deterrent, antifeedant, insecticidal, acaricidal, antifungal and cytotoxic (antitumor) activities (Bora *et al.*, 2011a; Tatjana *et al.,* 2014; Milena *et al.,* 2016).

Chemical composition and essential oil yield of wormwood is greatly influenced by geographical distribution and climatic conditions for example sabinyl acetate (18.6%) was reported as dominating constituent of *A. absinthium*essential oil from Belgium by Orav *et al.* (2006), *cis*-sabinyl acetate (26.4%) from Canada by Lopes-Lutz *et al.* (2008), dehydrocostus lactone (41.8%) from Ethiopia by Tariku *et al.* (2011), β-pinene (23.8%) from India by Rezaeinodehi & Khangholi (2008), chamazulene (17.8%) from Turkey by Kordali *et al.* (2005), β-thujone (19.8-63.4%) from Serbia and Montenegro by Blagojevica *et al.* (2006), *cis*-epoxyocimene (39.9%) from Spain by Orav *et al.* (2006), *trans*-thujone (33.1%) from USA by Tucker *et al.,*. (1993).

In Skardu Baltistan *A. absinthium* grows wildly on roadsides and barren lands and locally it is used as both medicinal and insecticidal purposes, the sun-dried leaf and flowers powder are used for stomach aches, anti-inflammatory, anti- migraine and for high blood pressure and diabetics (Hayat *et al.*, 2009; Bano *et al.,* 2014). Due to ethno-pharmacology, ethno-medicine, insecticidal and antimicrobial properties of *A. absinthium*, much investigation yet has been performed and the previous literature indicated that geographical distribution greatly influences the chemical composition and essential oil content. Nature has blessed the Skardu Baltistan region with a diversity of wild herbs and shrubs of economic importance. The aim of this study is to analyze the chemical composition of *A. absinthium* plant grown under the agro-climatic condition of Skardu Baltistan, Pakistan. This study provides the information about the impact of climatic conditions of Skardu Baltistan on chemical composition and oil yield of *A. absinthium*.

Materials and Methods

Plant materials: The *A. absinthium* leaves and flowers were collected at "Halqa 2 Skardu" (35.18°N 75.37°E) in August 2016 from Skardu Baltistan, Pakistan. The plant was identified by (Hayat *et al.,* 2009), with voucher specimens PUP, PH004 (ART004) submitted to the Herbarium, University of Peshawar, Pakistan.

Extraction of essential oil: The oven dried plant samples (500g) were put in a Clevenger-type apparatusfor 5 hours for hydro-distillation. The plant material was divided into 2 samples of each 250g, and 400ml of distilled water was added to each sample. The oil was collected and dried over anhydrous sodium sulfate and stored in transparent glass vials (1.5ml) (CNW Technologies (Shanghai) Inc.) and was kept at 4°C for further analysis.

Gas chromatography-mass spectrometry (GC-MS): The *A. absinthium* essential oil was analyzed by using a GC-MS (Agilent 6890N GC, Agilent 5973N MS). The GC-MS was equipped with capillary column DB-1(30 m x 0.22mm i.d., 0.25 µm film thickness). Using helium as a carrier gas at 1.0 mL/min, the injector and detector temperatures were 250°C and 280°C. The oven temperature was programmed from 70°C (2 min) to 250°C (2 °C/min and held for 5 min) and then increased to 270°C (3°C/min, held for 5 min). The injection size was 0.1 mL of 1% solution prepared in *n*-hexane; split ratio was 1:50.MS was taken at 70 eV with mass scan range of 50–800 amu.

Identification of components: The chemical constitutes of oilwere identified by retention index (RIs) determined with relative to the homologous series of *n*-alkanes C_7-C_{40} (Sigma Aldrich), under identicalexperimental conditions (Adams, 2001), the individual peaks were computer accorded with NIST 05 spectral library and their disintegration arrangements were also compared with the previous literature for further confirmation (Li *et al.*, 2010; Joshi *et al.*, 2013; Zhang *et al.*, 2014; Vieira *et al.*, 2017; NCBI, 2017).

Kovats retention index formula:

$$I = 100\left[Z + \frac{\log t'_{Ri} - \log t'_{Rz}}{\log t'_{(z+1)} - \log t'_{Rz}}\right]$$

where:
I = retention index for isothermal GC analysis.
t_{Ri}' = adjusted retention time of sample peak.
t_{Rz}' = adjusted retention time of *n*-alkane peak eluting immediately before sample peak.
$t_{R(z+1)'}$= adjusted retention time of *n*-alkane peak eluting immediately after sample peak.
z = carbon number of *n*-alkane peak eluting immediately before sample peak.

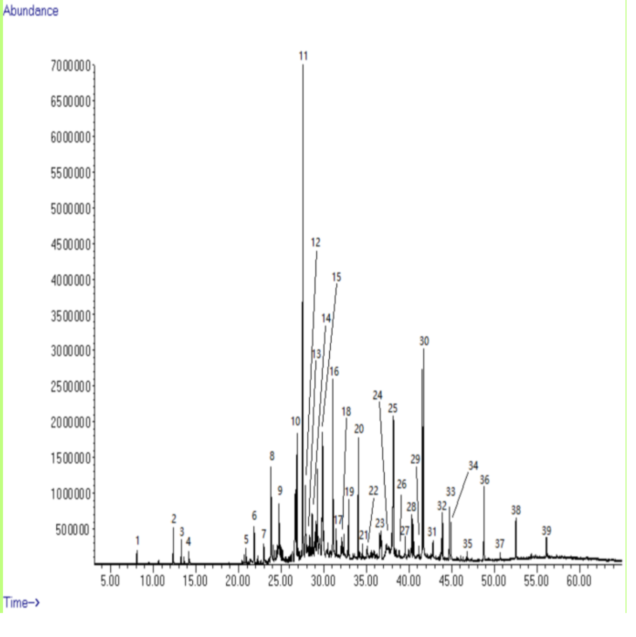

Fig. 1. GC-MS peaks of hydro-distilled *A. absinthium* essential oil obtained from the population grown under the agro-climatic condition of Skardu Baltistan, Pakistan.

Results and Discussion

The hydro-distillation of *A. absinthium* aerial parts gave dark green-colored oil with a yield of 0.41% (w/w) (based on sheltered dried plant parts). The essential oil chromatogram and composition analyzed by GC-MS are listed in (Table 1, Fig. 1). Analyses revealed that the ecotype is contained of thirty-nine compounds, representing 99.99% of the essential oil. The chemical constitutes were identified by NIST 05 MS spectral library andby retention index (RI) determined with relative to the homologous series of *n*-alkanes C_7-C_{40} and also compared with previous literature for further confirmation (Joshi, 2014; Zhang *et al.*, 2014; Vieira *et al.*, 2017). The GC-MS analysis revealed that the *A. absinthium* grown under the climatic condition of Skardu Baltistan was enriched in sesquiterpenes (61.01%) and monoterpenes (17.92%) and their derivatives. The major dominating constitutes were guaiol (19.33%), α-bisabolol (8.83%), carveol (6.16%), chamazulene (5.94%), limonene-6-ol, pivalate (5.37%) and geranyl-α-terpinene (5.63%). Their structures can be seen in Fig. 2. The major six dominating constituent accounts for 51.62% of the total constitutes identified, and their economic importance and uses are presented (Table 2). While other 33 minor components make up the balance are camphor (0.826%), terpinen-4-ol (0.619%), caryophyllene (1.683%), santolinatriene (0.940%), α-copaene (3.505%), germacrene-D (0.450%), β-bisabolene (2.669%), caryophyllene oxide (2.102%), (-)-spathulenol (1.936%), α-santalol (3.481%), cedrol (2.693%), 4-*epi*-cubedol (0.676%), cubenol (1.886%), **γ**-eudesmol (1.187%), 8-*epi*-**γ**-eudesmol (1.136%), geranylisobutyrate (2.755%), longifolenaldehyde (0.925%), methyl hinokiate (0.953%), tetrakis (1-methyl)-pyrazine (2.261%), cubedol (1.159%), geranyl-*p*-cymene (1.629%), nerolidol-epoxyacetate (1.123%), spathulenol (0.734%), heneicosane (1.601%), phytol (1.212%), 1-ethyl-4-methoxy-benzene (0.633%), carvacrol (1.293%), tricosane (1.483%), 1-heptatriacotanol (1.027%), pentacosane (2.102%), heptacosane (1.203%) andnonacosane (0.796%) respectively.

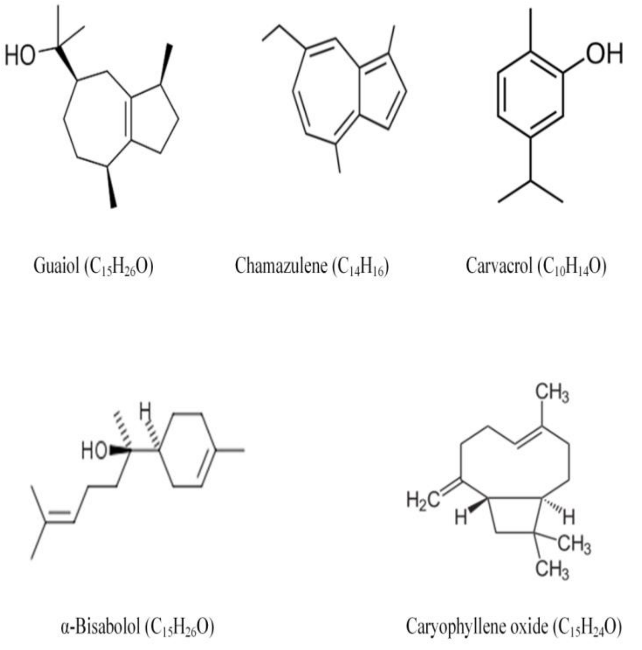

Fig. 2. Chemical structures of some economically important constituents found in *A. absinthium* essential oil.

Table 1. Chemical composition of *A. absinthium* essential oil.

Peak #	RT[a]	Compounds name[b]	Relative %	KI (Exp)[c]	KI (Lit)[d]	ID[e]
1	12.351	Camphor	0.862	1147	1148	MS,RI
2	13.291	Terpinen-4-ol	0.619	1172	1179	MS,RI
3	21.838	Caryophyllene	1.683	1416	1427	MS,RI
4	22.976	Santolinatriene	0.940	1452	---	MS
5	23.811	α-Copaene	3.505	1478	1470	MS,RI
6	24.054	Germacrene-D	0.450	1486	---	MS
7	24.754	β-Bisabolene	2.669	1508	1505	MS,RI
8	26.599	Caryophyllene oxide	2.102	1570	---	MS,RI
9	26.737	(-)-Spathulenol	1.936	1575	1575	MS,RI
10	26.861	α-Santalol	3.481	1579	1582	MS,RI
11	27.559	Guaiol	19.33	1602	1596	MS,RI
12	27.837	Cedrol	2.693	1612	1610	MS,RI
13	28.356	4-*epi*-Cubedol	0.676	1631	1627	MS,RI
14	28.64	Cubenol	1.886	1641	1652	MS,RI
15	29.011	γ-Eudesmol	1.187	1654	1652	MS,RI
16	29.113	8-*epi*-γ-Eudesmol	1.136	1657	1653	MS,RI
17	29.269	Geranylisobutyrate	2.755	1663	1685	MS
18	29.844	α-Bisabolol	8.833	1683	1684	MS,RI
19	31.067	Chamazulene	5.943	1728	1710	MS,RI
20	31.455	Longifolenaldehyde	0.925	1742	---	MS
21	32.355	Methyl hinokiate	0.953	1776	---	MS
22	32.92	Tetrakis(1-methyl)-Pyrazine	2.261	1797	1797	MS,RI
24	36.568	Cubedol	1.159	1941	1939	MS,RI
25	36.748	Geranyl-*p*-Cymene	1.629	1948	---	MS
26	37.999	Nerolidol-epoxyacetate	1.123	1999	---	MS
27	38.176	Geranyl-α-terpinene	5.636	2007	---	MS
28	39.549	Spathulenol	0.734	2066	2071	MS,RI
29	40.341	Heneicosane	1.601	2100	2100	MS,RI
30	40.507	Phytol	1.211	2107	---	MS
31	41.557	Limonen-6-ol, Pivalate	5.371	2154	---	MS
32	41.721	Carveol	6.167	2161	---	MS
33	43.784	1-ethyl-4-methoxy-benzene	0.633	2256	---	MS
34	43.922	Carvacrol	1.293	2262	2252	MS,RI
35	44.735	Tricosane	1.483	2300	---	MS,RI
36	44.931	1-Heptatriacotanol	1.027	2309	---	MS
37	48.786	Pentacosane	2.102	2500	---	MS
38	52.539	Heptacosane	1.203	2700	---	MS
39	56.106	Nonacosane	0.796	2899	---	MS
		Total identified	99.9			
		Oil yield (%)	0.41			
		Monoterpenes	17.92			
		Sesquiterpenes	61.01			
		Others	21.06			

[a]Retention time
[b]Compounds are listed in order of their retention time
[c]Linear retention index on DB-1 (30 m x 0.22 mm i.d., 0.25 µm film thickness), experimentally determined using homologous series of C_7-C_{40} *n*-alkanes
[d]Linear retention index taken from Adams (2007) and/or NIST 08 (2008)
[e]Identification methods: based on comparison with authentic compounds, NIST 08 MS databases; RI, based on comparison of calculated RI with those reported in Adams or NIST 08

Table 2. Some economically important constitutes identified in *A. absinthium* essential oil obtained from the population collected from Skardu Baltistan.

S. No.	Compounds name		Uses	Source
1.	Guaiol	1.	Toxicity against *Plutella xylostella* with LD_{50} values of 0.07 and 8.9 mg/larva and against *Musca domestica*, with LC_{50} values of 3.5 µL/L and 16.9 µL/L	Tao *et al.*, 2013
		2.	Act as amelanogenesis inhibitor in human skin	Baschong *et al*., 2008
		3.	Complete feeding inhibition in aphids occurs at concentration of 70 mg/L	Liu *et al*., 2013
		4.	Showed larvacidal and mosquito biting-deterrent at concentration of 25 µL/L	Ali *et al*., 2015
2.	Chamazulene	1.	Anti-inflammatory properties and inhibits the CYP1A2 enzyme.	Safayhi *et al., 1997*
		2.	Knock down effect against *Rhizoctonia solani* at 12.5mL/20mL.	Bouzenna *et al*., 2013
		3.	LC_{50} *Rhysopertha dominica* occur at 50 mg/L topical appliaction	Bouzenna *et al*., 2013
		4.	Phytotoxic activity against *Arvense persicaria, Chenopodium album* at 100 mg/L	Solymosi, 2000
3.	α-Bisabolol	1.	Glioma cells treated with high concentration of α-bisabolol (10 µM) resulted in a 100% cell death	Cavalieri *et al.*, 2004
		2.	α-Bisabolol at the dose of 25 g/L caused 100 mortality after 72 hours of application against *Bemisia argentifolii*	Corpas-Lopez *et al*., 2015
		3.	*α*-Bisabolol at oral doses of 50 and 100 mg/kg markedly in rats reduced gastric damage up to 87% and 96%, respectively	de Andrade *et al.*, 2004
4.	Carvacrol	1.	Used in food industries for their flavoring and preservative properties	Marchese *et al*., 2016
		2.	Soya sauce containing a combination of thymoland carvacrol (<0.0157%) killed > 7 log CFU/ml of foodborne pathogen	Moon *et al*., 2016
		3	Showed antioxidant, anti-inflammatory, local anesthetic, cicatrizing, antiseptic, and especially antibacterial and antifungal activities	Marchese *et al*., 2016
		4	LC_{50} values 28.52 mg/L against *Pochazia shantungensis* adults and nymphs using leaf dip bio assay	Park *et al*., 2017
5.	Caryophyllene oxide	1	It alters several key pathways for cancer development i.e. I3K/AKT/mTOR/S6K1 and STAT3 pathways	Nagappan *et al*., 2016
		2	Antitumor and apoptotic effects on MG-63 human osteosarcoma cells	Pan *et al*., 2016

According to the previous literature, the chemical composition and essential oil content within the same species of *A. absinthium* were greatly influenced by the geographical distribution (Telci *et al.*, 2006; Okut *et al.*, 2017; Fatima *et al.*, 2018), climate conditions and time of harvesting (Joshi, 2013). Similarly, the chemical constituents and oil yield of coriander (*Coriandrum sativum* L.) collected from two different locations of Tunisian were not similar (Sangwan *et al*., 2001). According to the previous literature the *A. absinthium* essential oil yield and its chemical composition collected from different geographical locations of the world showed great variations (Telci *et al.,* 2006), for example, sabinyl acetate (18.6%) was the dominant constitutes of wormwood collected from Belgium (Orav *et al.*, 2006), *trans*-verbenol (11.6%) from Lithuania (Judzentiene *et al.*, 2009), 1, 8-cineol (3.3%) from Germany (Orav *et al*., 2006), *cis*-linalool oxide (34.2%) from Spain (Bailen *et al.*, 2013), and borneol (18.7%) from India (Joshi, 2014). The geographical distribution and climatic condition greatly influenced the chemical composition and their relative percentage in the oil, as our result revealed that the chemical composition of *A. absinthium* grown under the agro-climatic condition of Skardu Baltistan have variation in chemical composition and their relative percentage as compared to the previous literature.

Conclusions

It was observed in the current investigation that *A. absinthium* grown in the Skardu Baltistan, Pakistan showed considerable variation in chemical composition and in the percentage of essential oil yield. The reported yields for essential oil obtained from *Artemisia* sp. range from 0.1 to 1.46 % (w/w) (Msaada *et al*., 2015). However, in the current investigation greater amount of oil was present 0.41% (w/w). Its composition resembles a previous report and differs from others suggesting the existence of chemo-types also in this plant. The geographical distribution and climatic conditions of Skardu Baltistan influenced the chemical composition and their relative percentage in essential oil as the oil was dominated by guaiol (19.33%).

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