Variation in Volatiles of *Astragalus gombiformis* Pomel

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The essential oils obtained by hydrodistillation (Clevenger apparatus) from aerial parts of *Astragalus gombiformis* were analysed by gas chromatography coupled with mass spectrometry (GC/MS). This study showed that the *A. gombiformis* essential oils are complex mixtures of important natural compounds, which varied qualitatively and quantitatively between cultivated and wild plants and between phenological stages of development. All analysed oils are characterized by the constant presence of phytol, 6,10,14-trimethyl-2-pentadecanone, 4-terpineol, and γ-terpinene. This study is the first report on the chemical composition of essential oils from *A. gombiformis* and indicates that these oils should be more studied.

Key words: Essential Oils, GC/MS, Phenological Stages

Introduction

Astragalus, genus of Fabaceae, comprises more than 1500 species that are distributed in the Orient and North Africa (Ozenda, 1991). Many *Astragalus* species are toxic. In contrast, several therapeutic properties are assigned to other plants of this genus. In folk and modern medicine, *Astragalus* species such as *Astragalus mongholicus* Bunge and *Astragalus membranaceus* (Fisch) Bunge are used against various diseases and for cancer therapy (Ríos and Waterman, 1997; Lei *et al.*, 2003; Yesilada *et al.*, 2005; Yin *et al.*, 2006). In the last decades, many plant essential oils were studied to discover compounds of possible interest for medicinal and environmental applications due to their biological activities, such as fungicidal, antimicrobial, insecticidal, and antiproliferative effects against cancer cells (Conforti *et al.*, 2009; Batish *et al.*, 2008). To our knowledge, few studies related to essential oils of *Astragalus* species have been reported. Wild and cultivated *Astragalus corniculatus* contains essential oils that possess cytotoxic activity (Krasteva *et al.*, 2008). The volatiles from *Astragalus glycyphyllos*, *Astragalus hamosus*, *Astragalus cicer*, and *Astragalus spruneri*, and their application for the chemotaxonomy were studied by Platikanov *et al.* (2005). In Tunisia, the flora contains many *Astragalus* species such as *A. epiglottis* L., *A. sesameus* L., *A. baeticus* L., and *A. caprinus* usually distributed in desert regions (Le Floc'h, 1983). Except the studies of Semmar *et al.* (2001, 2002, 2005) on *Astragalus caprinus*, no previous phytochemical investigations related to other *Astragalus* species of Tunisian flora have been reported. *Astragalus gombiformis* Pomel, taking recently the taxonomy of *Astragalus gombo* subsp. *gomboeformis* (Pomel) Ott, is a sort spread in the big oriental Erg of Tunisia under Saharan climate characterized by different stresses. This plant, presenting an important biomass, is moderately appetible by animals, probably for its toxicity or its rankness. The present study aims at investigating the chemical composition of volatile compounds from aerial parts of *Astragalus gombiformis* at different phenological phases.

Material and Methods

Plants collection

Wild plants have been collected from Bir Soltane (33° 28' 10'' N, 09° 23' 50'' E, 107 m above sea level) and cultivated plants from the experimental field of medicinal and aromatic plants of the Arid Land Institute of Medenine (Elgordhab) in Southern Tunisia. Cultivation was done in 2006 using seeds collected from Bir Soltane and stored in the seed bank of the Range Ecology Labora-

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tory. The experimental design was a completely randomized block that was irrigated using drip irrigation with 6 l/s flow and 2.3 g/l water salinity. For both wild and cultivated plants, the aerial parts have been collected at leaf development (January 2009), flowering (February 2009), and fructification (April 2009) seasons. Voucher specimens were deposited at the herbarium of Range Ecology Laboratory of the Arid Land Institute of Medenine, Tunisia.

Extraction and analysis of essential oils

Fresh samples have been submitted to hydrodistillation for 4 h, using a Clevenger apparatus. The essential oils were taken up in hexane (HPLC grade), dried over anhydrous sodium sulfate, and then stored at 4 °C until analysis.

An Agilent Technologies 6890N gas chromatograph coupled with an Agilent 5973 B mass-spectrometer, which was operating (full scan mode) in the EI mode at 70 eV and an ionization energy of 1294 V, and an HP-5 MS capillary column (30 m x 0.25 mm ID, 0.25 μm film thickness, fused capillary column) were used. The column temperature was programmed at 50 °C for 1 min, then at 7 °C/min to 250 °C for 5 min. The injection port and detector temperatures were, respectively, 240 °C and 250 °C. Helium was used as carrier gas at a rate of 1.2 ml/min. The compounds of the essential oil were identified by comparing their mass spectra data with spectra available from the Wiley 275 and NIST 0.5 mass spectra libraries. Retention indexes (RI) were calculated using a standards series of *n*-alkanes (C_6–C_{30}) (Dohou *et al.*, 2005) and compared with previously published RI.

Results and Discussion

The chemical composition of the essential oils extracted from *A. gombiformis* Pomel (cultivated and wild) aerial parts collected at three phenological stages (leaf development, flowering, and fructification) is reported for the first time. A total of 86 compounds were detected in the analysed sample oils, and they are arranged in Table I according to their order of elution from a HP-5 MS capillary column.

At leaf development stage, the major compounds of the essential oil of cultivated *A. gombiformis* were dillapiole (50.80%), thymyl methyl ether (15.10%), and phytol (14.96%), and the largest chemical groups were aromatic compounds and diterpenes. At the vegetative phase, wild *A. gombiformis* essential oil was mainly constituted by diterpenes, aldehydes, oxygenated monoterpenes, and esters, with a considerable quantity of oxygenated sesquiterpenes. The most abundant components were phytol (27.97%), ethyl linoleolate (6.50%), 2,5-diformyl thiophene (5.46%), and 4-terpineol (3.89%). In the *Astragalus* species studied by other authors, phytol was detected at amounts of 8.50%, 10.00%, 0.10%, and 0.90%, respectively, in *A. glycyphyllos*, *A. hamosus*, *A. cicer*, and *A. spruneri* at the leaf development phase (Platikanov *et al.*, 2005). Essential oil of wild *A. gombiformis* seems to be more diversified than oil of cultivated plants, which is in agreement with the results of Krasteva *et al.* (2008) on *A. corniculatus*.

At flowering stage, the major component of wild *A. gombiformis* essential oil was also phytol (35.82%), with others such as ethyl linoleolate

Table I. Chemical composition (%) of essential oils from wild and cultivated *Astragalus gombiformis* Pomel.

RI[a]	Compound	Wild			Cultivated		
		Vegetative	Flowering	Fructification	Vegetative	Flowering	Fructification
765	Toluene	-	-	-	0.17	-	-
800	Octane	-	0.67	-	-	-	-
801	Hexanal	-	-	0.26	-	0.58	1.09
854	*trans*-2-Hexenal	0.57	0.44	0.38	-	-	1.41
861	Ethylbenzene	-	-	-	0.14	-	0.26
869	*p*-Xylene	-	-	-	0.22	-	1.08
898	*m*-Xylene	-	-	-	-	-	0.32
935	α-Pinene	-	0.24	0.47	-	-	1.78
951	Camphene	-	0.18	0.27	-	-	0.70
965	Benzaldehyde	-	-	-	-	0.54	-
992	1-Octen-3-ol	-	0.36	-	-	-	-
993	2-Pentyl-furan	-	0.82	0.89	-	1.22	1.53

Table I (continued).

RI[a]	Compound	Wild			Cultivated		
		Vegetative	Flowering	Fructification	Vegetative	Flowering	Fructification
1019	*α*-Terpinene	0.55	0.61	0.46	-	-	0.45
1027	*p*-Cymene	0.26	-	0.71	0.13	-	1.05
1030	Limonene	-	-	-	-	-	1.07
1033	1,8-Cineole	1.56	1.12	0.17	-	0.53	0.97
1060	*γ*-Terpinene	0.97	1.08	2.35	1.71	1.16	0.99
1071	*cis*-Sabinene hydrate	0.84	0.97	-	0.36	-	0.11
1090	Terpinolene	0.37	0.23	0.20	-	-	-
1101	*trans*-Sabinene hydrate	-	0.71	-	0.31	-	-
1105	Nonanal	1.67	-	0.33	0.63	1.45	1.76
1121	Unidentified	-	-	-	-	0.43	-
1126	1-Methyl-4-(1-methyl-ethyl)-2-cyclohexen-1-ol	0.41	-	-	-	-	-
1145	Benzene acetonitrile	1.33	-	-	0.46	-	-
1150	Camphor	2.24	1.00	-	-	-	1.08
1154	1-(1,4-Dimethyl-3-cyclohexen-1-yl)-ethanone	-	0.38	0.56	0.22	-	-
1162	Nonenal	-	-	-	-	1.10	-
1173	Borneol	0.39	0.97	-	-	-	1.18
1177	Thujen-2-one	0.36	0.44	-	-	-	-
1183	4-Terpineol	3.89	6.22	2.75	1.87	5.04	2.30
1190	Naphthalene	1.24	-	-	-	-	-
1201	2,5-Diformyl thiophene[b]	5.46	-	0.77	0.79	1.30	-
1206	Decanal	-	-	-	0.34	0.94	-
1225	*β*-Cyclocitral	0.26	0.13	-	-	0.53	0.26
1230	Unidentified	1.27	-	0.59	0.96	-	-
1238	Thymyl methyl ether	-	-	0.28	15.10	-	-
1245	Cinnamylnitrile[b]	0.40	-	-	-	-	-
1289	Bornyl acetate	-	-	-	-	-	0.45
1294	Dihydroedulan II	0.51	0.48	-	-	-	-
1297	Unidentified	-	-	-	-	0.67	0.53
1298	Dihydroedulan I	0.54	0.35	-	-	0.90	-
1308	Undecanal	-	-	-	-	0.51	1.17
1320	*trans,trans*-2,4-Decadienal	-	-	1.84	-	3.02	2.62
1323	2-Methoxy-4-vinyl phenol	2.56	-	-	1.43	-	-
1358	1,2-Dihydro-1,1,6-trimethyl naphthalene[b]	1.25	-	-	-	0.70	-
1389	*β*-Damascenone	0.39	-	0.26	0.21	-	-
1405	Dodecanal	-	-	-	0.35	1.65	1.21
1450	*trans*-*β*-Ionon-5,6-epoxide[b]	0.31	-	-	-	0.53	-
1455	Geranyl acetone	0.44	0.34	-	-	0.78	0.65
1461	Unidentified	-	0.98	-	-	2.17	-
1476	Unidentified	-	-	-	-	1.09	0.85
1492	*β*-Ionone	1.15	1.78	0.39	-	2.55	2.11
1514	Tridecanal	-	-	-	-	0.89	0.56
1530	Myristicin	-	-	-	2.72	-	-
1562	Elemicin	-	-	-	0.33	-	-
1570	*trans*-Nerolidol	-	-	-	-	-	2.82
1572	Megastigmatrienone[b,c]	0.32	-	0.74	-	-	-
1576	Unidentified	0.57	-	-	-	-	-
1588	Megastigmatrienone[b,c]	0.77	-	-	-	-	-
1601	Diethyl phthalate	-	-	-	-	-	3.45
1614	Tetradecanal	0.42	0.77	0.35	-	0.78	0.70
1641	Dillapiole	-	0.77	-	50.80	1.17	4.97
1637	Megastigmatrienone[b,c]	0.91	-	-	-	-	-

Table I (continued).

RI[a]	Compound	Wild			Cultivated		
		Vegetative	Flowering	Fructification	Vegetative	Flowering	Fructification
1657	Isoelemecin [b]	0.32	-	-	-	-	-
1679	Unidentified	1.11	1.07	-	-	-	-
1716	Pentadecanal	3.20	2.46	1.35	-	2.32	1.97
1735	Unidentified	-	-	-	-	-	0.45
1770	Tetradecanoic acid	-	-	1.10	-	-	-
1777	Unidentified	0.39	-	-	-	-	-
1815	Hexadecanal	0.52	-	-	-	-	-
1835	Neophytadiene	0.32	-	-	-	-	-
1844	6,10,14-Trimethyl-2-pentadecanone	1.15	3.68	0.60	0.43	1.16	1.30
1871	Versalide[b]	-	-	-	0.39	-	-
1880	Hexadecanol	0.83	0.91	-	-	0.90	-
1893	Ethyl linoleolate	6.50	7.79	1.89	-	1.80	1.60
1920	Hexadecanoic acid methyl ester	-	0.36	-	-	0.61	-
1966	Hexadecanoic acid	1.38	7.58	27.05	-	-	9.64
1999	Eicosane	-	-	-	-	-	0.35
2066	Unidentified	-	-	-	-	1.08	-
2080	9,12,15-Octadecatrienoic acid methyl ester	0.82	1.73	-	-	1.60	-
2094	Phytol	27.97	35.82	31.99	14.96	37.52	17.87
2118	9,12-Octadecadienoic acid	-	-	9.97	-	-	1.87
2138	Octadecanoic acid	-	-	1.79	-	-	1.08
2159	Unidentified	0.63	-	-	-	-	-
2397	9-Octadecenamide	1.79	-	-	0.43	-	1.87
2544	1,2-Benzenedicarboxylic acid mono (2-ethylhexyl) ester	0.44	-	3.61	-	-	9.35
Aliphatics							
Alkanes		-	0.67	-	-	-	0.35
Ketones		0.75	0.72	0.56	0.22	1.31	0.65
Aldehydes		11.84	3.67	5.28	2.11	15.08	12.49
Alcohols		3.39	1.27	-	1.43	0.90	-
Fatty acids		1.38	7.58	39.91	-	-	12.59
Esters		7.76	9.88	1.89	-	4.01	14.40
Terpenoids							
Monoterpene hydrocarbons		2.15	2.34	4.46	1.84	1.16	6.04
Oxygenated monoterpenes		9.95	11.56	2.92	2.54	6.10	6.35
Oxygenated sesquiterpenes		4.59	2.61	1.39	0.21	3.45	4.93
Diterpenes		29.44	39.50	32.59	15.39	38.68	19.17
Aromatics		4.54	0.77	0.28	69.94	1.87	6.63
Others		1.79	0.82	0.89	0.82	1.22	3.40
Unidentified		3.97	2.05	0.59	0.96	5.44	1.83
Total		81.55	83.44	94.37	95.46	79.22	88.83

[a] Retention indexes relative to C_6–C_{30} *n*-alkanes and justified by comparison with previously published data.
[b] Compound identified only by comparing the mass spectra data with spectra available from the Wiley 275 and NIST 0.5 mass spectra libraries.
[c] Correct isomer not identified.

(7.79%), hexadecanoic acid (7.58%), and 4-terpineol (6.22%). Diterpenes, aldehydes, oxygenated monoterpenes, esters, and fatty acids were the main metabolite classes of this oil. In cultivated *A. gombiformis*, the essential oil was also characterized by a large presence of diterpenes at the flowering stage, with smaller amounts of aldehydes, and oxygenated monoterpenes. Phytol

(37.52%) and 4-terpineol (5.04%) were the major compounds of this oil. At flowering, phytol represented 26.00%, 3.10%, and 2.30%, respectively, in *A. glycyphyllos*, *A. hamosus*, and *A. cicer*, but it was not present in *A. spruneri* (Platikanov *et al.*, 2005). It was also the major compound (9.50%) of volatiles in wild *A. corniculatus* at the flowering phase (Krasteva *et al.*, 2008).

At fructification, the essential oil of the wild and cultivated plants were mainly constituted by fatty acids and diterpenes and characterized by phytol as the major compound. Concerning other *Astragalus* species, phytol was absent in *A. glycyphyllos*, *A. hamosus*, *A. cicer*, and *A. spruneri* at fructification (Platikanov *et al.*, 2005). It was present in wild *A. corniculatus* at fructification, but at a low amount (0.90%) (Krasteva *et al.*, 2008). This compound was found above 14% in all analysed samples of *A. gombiformis*.

The chemical composition of the analysed oils differed between cultivated and wild plants, and between phenological stages. It is known that the stage of the life cycle influences the essential oils composition. The portions of essential oils constituents can vary in the different developmental phases of plants, and these variations can be very important (Bruneton, 1999). For example, the amount of dillapiole, representing 50.80% at the leaf development stage of cultivated plants, decreased to 1.17% and to 4.97%, respectively, at flowering and fructification. For other species, Gauvin and Smadja (2005) showed that considerable differences were found in the composition of essential oils of *Psiadia boivinii* between different phases of plant development. Qualitative difference between cultivated and wild plants essential oils can be related to the variation of soil characteristics and climatic conditions. In Bir Soltane, wild plants grow on sandy soil. The experimental field is characterized by rendzic gypsies encrust. These areas are arid to semi-arid with a typical Mediterranean climate, characterized by irregular rainfall events (less than 200 mm per year) and a harsh dry summer and cold winter periods. But, the cultivated plants were irrigated with a water flow of 6 l/s. In fact, Abu-Darwish and Abu-Dieyeh (2009) showed that the composition of *Thymus vulgaris* L. essential oil is mainly affected by variable natural climatic conditions.

Other factors can also modify the chemical composition of essential oils, such as temperature, period of insolation, and hydrodistillation procedure (isomerization, hydrolyse of esters,…) (Bruneton, 1999).

When comparing the chemical composition of the essential oils within the same genus, many similarities are obvious. Many volatile compounds such as tetradecanoic acid, hexadecanoic acid, and octadecanoic acid methyl ester were detected in the *Astragalus* species studied by other authors (Platikanov *et al.*, 2005; Krasteva *et al.*, 2008). At all phenological stages, *A. gombiformis* essential oil is characterized by the constant presence of four compounds: phytol, 6,10,14-trimethyl-2-pentadecanone, 4-terpineol, and γ-terpinene. These four compounds can constitute the chemotype of *A. gombiformis* essential oil. Except phytol, the other three compounds are not detected in the other studied *Astragalus* species.

Numerous sesquiterpenes of essential oils have repellent effects against insects (Bruneton, 1999). The essential oil hydrodistilled from *Teucrium leucocladum* Boiss., mainly constituted by oxygenated sesquiterpenes (53.63%), has a marked effect against *Culex pipiens*, *Musca domestica*, and *Ceratitis capitata* (El-Shazly and Hussein, 2004). The presence of oxygenated sesquiterpenes in essential oils of *A. gombiformis* can explain, in part, the low palatability of this plant. On the other hand, these oils can constitute a source of natural insecticides and contribute to the efforts to find safe and effective products regarding the problems associated with synthetic insecticides. Among the volatile compounds of *A. gombiformis*, *n*-hexadecanoic acid is a potent mosquito larvicide and dillapiole is active against larvae of *Aedes aegypti* (Rahuman *et al.*, 2000; Morais *et al.*, 2007). In addition, some components of *A. gombiformis* essential oils were found to exhibit biological activities. Dillapiole is an inhibitor of biosynthesis of aflatoxin G_1 in *Aspergillus parasiticus* (Razzaghi-Abyaneh *et al.*, 2007). 1,8-Cineole in combination with camphor has shown high antimicrobial effects (Viljoen *et al.*, 2003). Camphene, α-pinene, limonene, and 4-terpineol possess antibacterial and anti-inflammatory activities (Yan *et al.*, 2009). Thus, *A. gombiformis* essential oils comprise several molecules recognized for their biological activities. The determination of the oil composition provides also information on the chemotaxonomic significance in the genus *Astragalus*.

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