Nat. Volatiles & Essent. Oils, 2015;2(1):32-36

NVEO
Natural Volatiles & Essential Oils

RESEARCH ARTICLE

Geographical Variation of *Ajuga laxmannii* (L.) Bentham Essential Oil

Yavuz Bülent Köse[1,*], Sevim Alan[1], Betül Demirci[2], K. Hüsnü Can Başer[2,3]

[1] Department of Pharmaceutical Botany, Anadolu University, 26470, Eskişehir, TURKEY

[2] Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470, Eskişehir, TURKEY

[3] Department of Botany and Microbiology, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

*Corresponding author. Email: ybkose@anadolu.edu.tr

Abstract

Aerial parts of *Ajuga laxmannii* collected from five different localities in Turkey were hydrodistilled for 3 h using a Clevenger-type apparatus to produce a small amount of essential oil which was trapped in n-hexane. Oils were analysed by gas chromatography (GC) and mass spectrometry (GC/MS). The main components were as follows: sample A: Nonacosane (17.8%), Heptacosane (12%), Hexahydrofarnesyl acetone (11.2%); sample B: Hexadecanoic acid (21.2%), Dodecanoic acid (12.3%), Tetradecanoic acid (8.9%); sample C: Phytol (12.5%), Hexadecanoic acid (10.0%), Hexahydrofarnesyl acetone (8.6%); sample D: Hexahydrofarnesyl acetone (9.1%), Hexadecanoic acid (8.9%), Nonacosane (7.6%); sample E: Hexadecanoic acid (13.6%), Phytol (13.3%), Hexahydrofarnesyl acetone (8.3%).

Keywords: Essential oil, *Ajuga laxmannii*, Turkey

Introduction

The genus *Ajuga* L. (Lamiaceae) consists of about 90 species, mostly distributed in the north temperate zone of the old world. The genus also occurs in South Africa and Australia (Turill, 1934). In the flora of Turkey *Ajuga* is represented by 14 species and altogether 27 taxa (Davis, 1982). Some *Ajuga* species have been widely used as herbal tea for their diuretic, antipyretic, tonic, diaphoretic, astringent properties in the Turkish folk medicine (Baytop, 1999).

Materials and Methods

Plant Sample

Plant samples were collected from five different localities in Marmara region, Turkey (Table 1). A voucher specimen is kept at the herbarium of the Faculty of Pharmacy in Anadolu University, Turkey (YBK 1545, 1546, 1547, 1550, 1552).

Table 1. Localities of Plant Samples

Locality	
A	Kırklareli, between Vize-Pınarhisar, Sergen way, 1. km, N 41 37 20.5 E 27 40 50.4
B	Çanakkale, between Keşan-Gelibolu, Karadağ, road side
C	Kırklareli, between Kıyıköy-Vize, N 41 39 36.2 E 27 53 41.9
D	Bursa, Uludağ, Bağlıca Village, N 40 06 48.1 E 29 04 15.4
E	Kırklareli, Demirköy, N 41 44 57.1 E 27 40 08.2

Isolation of Essential Oil

The essential oils from air-dried plant materials were isolated by hydrodistillation for 3 h, using a Clevenger-type apparatus according to the method recommended in the European Pharmacopoeia 7.0 (2010). A small amount of essential oil which was trapped in *n*-hexane was collected for oil analysis.

GC–MS Analysis

The GC-MS analysis was carried out with an Agilent 5975 GC-MSD system. Innowax FSC column (60 m x 0.25 mm, 0.25 mm film thickness) was used with helium as carrier gas (0.8 ml/min). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, and kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. Split ratio was adjusted at 40:1. The injector temperature was set at 250°C. Mass spectra were recorded at 70 eV. Mass range was from m/z 35 to 450.

GC analysis

The GC analysis was carried out using an Agilent 6890N GC system. FID detector temperature was 300°C. To obtain the same elution order with GC-MS, simultaneous auto-injection was done on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms. The analysis results are given in Table 2.

Identification of components

Identification of the essential oil components were carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to series of n-alkanes. Computer matching against commercial (Wiley GC/MS Library, Adams Library, MassFinder 3 Library) (McLafferty & D.B. Stauffer, 1989; Koenig, Joulain, Hochmuth, 2004), and in-house “Başer Library of Essential Oil Constituents” built up by genuine compounds and components of known oils, as well as MS literature data (Joulain & Koenig, 1998; ESO 2000, 1999; Jennings & T. Shibamoto, 1980) was used for the identification.

Results and Discussion

Analyses of the hydrodistilled oils were performed on GC and GC/MS systems, simultaneously. The compositions of the oils of *A. laxmannii* from five different localities with their relative retention indices (RRI) and relative percentages (%) are given in Table 2.

To the best of our knowledge, here we report on the essential oil of *A. laxmannii* for the first time. The main components of the oils were as follows: sample A: Nonacosane (17.8%), Heptacosane (12%), Hexahydrofarnesyl acetone (11.2%); sample B: Hexadecanoic acid (21.2%), Dodecanoic acid (12.3%), Tetradecanoic acid (8.9%); sample C: Phytol (12.5%), Hexadecanoic acid (10.0%), Hexahydrofarnesyl acetone (8.6%); sample D: Hexahydrofarnesyl acetone (9.1%), Hexadecanoic acid (8.9%), Nonacosane (7.6%); sample E: Hexadecanoic acid (13.6%), Phytol (13.3%), Hexahydrofarnesyl acetone (8.3%).

Oils were characterized by low yield of essential oils and the abundance of hydrocarbons such as in Sample A and D. Sample B was dominated mainly by fatty acids and their esters. Sample C and E were also dominated mainly by hydrocarbons and phytol, an acyclic diterpene. It was interesting to observe the paucity of monoterpenoids in the oils.

Table 2. The Composition of the Essential Oils of *Ajuga laxmannii*

RRI	Compound	A (%)	B (%)	C (%)	D (%)	E (%)
1032	α-Pinene	-	2.4	0.7	-	-
1035	α-Thujene	-	-	1.7	-	-
1118	β-Pinene	-	8.2	2.5	1.6	-
1174	Myrcene	-	-	-	-	0.1
1213	1,8-Cineole	-	-	-	-	0.5
1244	Amyl furan (*2-Pentyl furan*)	-	-	0.6	tr	tr
1391	(*Z*)-3-Hexenol	-	-	-	tr	-
1400	Nonanal	-	-	0.3	0.6	0.3
1452	1-Octen-3-ol	-	-	0.6	1.9	0.3
1474	*trans*-Sabinene hydrate	-	-	-	-	1.1
1479	(*E,Z*)-2,4-Heptadienal	-	-	-	0.5	-
1500	Pentadecane	0.1	-	-	0.5	-
1505	Dihydroedulane II [a]	-	-	-	-	0.7
1506	Decanal	-	-	-	0.5	-
1520	3,5-Octadien-2-one	-	-	-	tr	-
1541	Benzaldehyde	-	-	-	tr	-
1553	Linalool	-	-	0.6	0.9	0.3
1562	Octanol	-	-	-	tr	-
1600	Hexadecane	1.0	-	-	0.2	-
1600	β-Elemene	-	-	-	-	2.2
1611	Terpinen-4-ol	-	-	-	-	0.5
1612	β-Caryophyllene	1.2	4.1	1.8	2.4	4.5
1638	β-Cyclocitral	-		-	tr	-
1655	(*E*)-2-Decenal	-	1.0	-	0.9	0.2
1668	(*Z*)- β-Farnesene	-	-	-	-	0.6
1687	α-Humulene	-	-	-	-	0.5
1715	2-Dodecanone	-	-	-	1.2	-
1722	Dodecanal	-	-	-	0.8	-
1726	Germacrene D	-	-	-	-	5.9
1754	*trans*-Piperitone oxide	-	-	-	-	4.3
1755	Bicyclogermacrene	-	-	-	-	0.3
1764	*(E)*-2-undecenal	-	-	-	1.9	0.7
1766	Decanol	-	-	-	0.5	-
1800	Octadecane	-	-	-	0.5	-
1815	2-Tridecanone	-	-	-	0.2	-
1827	(*E,E*)-2,4-Decadienal	-	-	-	0.9	-
1849	Calamenene	-	-	-	0.3	0.7
1868	(*E*)-Geranyl acetone	1.6	1.6	2.9	2.0	1.4

1900	Nonadecane	-	-	-	0.8	0.7
1958	(*E*)- β-Ionone	0.8	-	0.7	1.7	1.2
1973	Dodecanol	-	-	-	2.2	-
1983	Piperitenone oxide	-	-	-	-	0.9
1992	Neophytadiene	-	-	-	0.3	-
2000	Eicosane	0.5	1.1	1.2	0.3	0.5
2008	Caryophyllene oxide	1.2	1.3	2.4	0.6	0.8
2036	2-Pentadecanone	2.0	1.1	2.7	2.3	0.7
2100	Heneicosane	1.8	4.3	4.3	2.2	1.5
2131	Hexahydrofarnesyl acetone	11.2	2.6	8.6	9.1	8.3
2179	3,4-Dimethyl-5-pentylidene-2(5H)-furanone	-	-	1.5	1.0	0.9
2179	Tetradecanol	-	-	0.9	1.2	-
2200	Docosane	0.4	0.9	0.9	1.1	0.3
2200	3,4-Dimetil-5-pentyl-5H-furan-2-one	-	-	-	1.0	-
2300	Tricosane	2.4	3.1	2.2	4.4	0.2
2384	Farnesyl acetone	-	-	2.3	2.7	1.5
2400	Tetracosane	-	-	-	1.1	-
2500	Pentacosane	6.0	1.2	1.9	3.6	2.5
2503	Dodecanoic acid	-	12.3	-	-	-
2524	Abietatriene	-	-	-	0.4	0.8
2607	Octadecanol	-	-	-	0.3	-
2622	Phytol	7.3	1.7	12.5	4.1	13.3
2670	Tetradecanoic acid	-	8.9	1.2	1.0	-
2700	Heptacosane	12.0	2.3	3.9	3.4	5.0
2795	Eicosanol	1.2	-	-	-	-
2900	Nonacosane	17.8	8.3	7.4	7.6	7.7
2931	Hexadecanoic acid	3.0	21.2	10.0	8.9	13.6
	Monoterpene Hydrocarbons	-	10.6	4.9	1.6	0.1
	Oxygenated Monoterpenes	1.6	1.6	3.5	2.9	9.0
	Sesquiterpene Hydrocarbons	1.2	4.1	1.8	2.7	14.7
	Oxygenated Sesquiterpenes	1.2	1.3	4.7	3.3	2.3
	Fatty acid+esters	3	42.4	11.2	9.9	13.6
	Diterpenes	7.3	1.7	12.5	4.8	14.1
	Alkanes	42.0	21.2	21.8	25.7	18.4
	Others	15.2	4.7	15.9	29.2	13.3
	Total	**71.5**	**87.6**	**76.3**	**80.1**	**85.5**

RRI: Relative retention indices calculated against *n*-alkanes, % calculated from FID data, tr: Trace (< 0.1 %)
[a]Correct isomer not identified

REFERENCES

Baytop, T. (1999). *Therapy with Medicinal Plants in Turkey, Past and Present.* 2nd ed., Istanbul, Turkey: Nobel Tıp Basımevi.

Davis, P.H. in: Davis, P.H. (Eds) (1982).*The Flora of Turkey and East Aegean Islands,* pp. 42, Edinburgh, UK: Edinburgh Univ. Press.

ESO 2000 (1999). *The Complete Database of Essential Oils*, the Netherlands: Boelens Aroma Chemical Information Service.

European Pharmacopoeia, Council of Europe (2010). 7th ed., Strasbourg: European Directorate for the Quality of Medicines & HealthCare.

Jennings, W.G., Shibamoto, T. (1980). *Quantitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary GC,* New York: Academic Press.

Joulain, D., Koenig, W.A. (1998). *The Atlas of Spectra Data of Sesquiterpene Hydrocarbons*, Hamburg: EB-Verlag.

Koenig, W.A., Joulain, D., Hochmuth, D.H. (2004). *Terpenoids and Related Constituents of Essential Oils. MassFinder 3*. Hochmuth DH (ed). Convenient and Rapid Analysis of GCMS, Hamburg, Germany.

McLafferty, F.W., Stauffer, D.B. (1989). The Wiley/NBS Registry of Mass Spectral Data, New York: J Wiley and Sons.

Turill, W.B. (1934). The Correlatıon of Morphological Variation with Distribution in Some Species of *Ajuga*, *The New Phytologist*, *3*, 218-230.