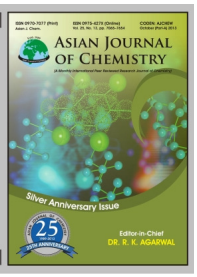
Asian Journal of Chemistry; Vol. 25, No. 16 (2013), 9126-9128

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.15045



Essential Oil Composition from the Aerial Parts of *Ajuga orientalis* L. Growing in Turkey

F. ZEHRA KÜÇÜKBAY[1,*], EBRU KUYUMCU[1] and BAYRAM YILDIZ[2]

[1]Division of Analytical Chemistry, Department of Basic Pharmaceutical Sciences, Faculty of Pharmacy, Inönü University, 44280 Malatya, Turkey
[2]Department of Biology, Faculty of Science and Arts, University of Balikesir, 10100 Balikesir, Turkey

*Corresponding author: Fax: +90 422 341 0037; E-mail: zkucukbay@inonu.edu.tr

(*Received*: 27 December 2012; *Accepted*: 18 September 2013) AJC-14128

The essential oil obtained from the aerial parts of *Ajuga orientalis* L. (Lamiaceae) was analyzed by using GC and GC-MS. Thirty compounds representing 95.4 % of the oil were identified. The main compounds were phytol (36.7 %), *n*-hexadecanoic acid (14.2 %) and dodecanoic acid (12.2 %).

Key Words: *Ajuga orientalis*, Essential oil, Phytol, *n*-Hexadecanoic acid.

INTRODUCTION

The plants of genus *Ajuga* are evergreen, clump-forming rhizomatous perennial or annual herbaceous flowering species, with *Ajuga* being one of the 266 genera of family Lamiaceae. There are at least 301 species of the genus *Ajuga* with many variations. These plants, growing in Europe, Asia, Africa, Australia and North America are used in gardens as ground cover or border for their foliage and beautiful flowers[1]. Several papers have been previously published about the ethnopharmacological uses of some *Ajuga* species. There are some reports on the phytochemical analysis of species belonging *Ajuga* found in the literature but only a small number of these species have so far been studied chemically for their essential oils. Several species of this genus have been reported to be rich sources of bioactive metabolites, including diterpenes[2-4], steroids[5,6] and iridoids[7,8], which display insect antifeedant[4], antibacterial[3], antiplasmodial[9], antimycobacterial[10], cytotoxic[11] and vasoconstrictor activities[12].

In the flora of Turkey, the genus *Ajuga* (Lamiaceae) is represented by 12 species and 22 taxa[13]. This species which has been mentioned as the name of "mayasilotu, yercami, basirotu, soguklama otu or yer selvisi" in the old texts of traditional medicine, is one of the species of the Lamiaceae that has been used in the Turkey traditional medicine for centuries[14]. This plant has long been used as a diuretic, tonic, emmenagogue agent and menser remover and for wound-healing and perspiration[15]. Application of this species to treat scorpion and snake bites, stomachache, jaundice, hemorrhoids, inflammatory diseases, such as gout and joint pains and common colds have also been well documented[14,15]. The purpose of this investigation was to determine the composition of the essential oil of the aerial parts of *Ajuga orientalis* L. growing in Turkey.

EXPERIMENTAL

Aerial parts of *Ajuga orientalis* L. were collected from plants growing in Erzurum (A8) province (Turkey), south of Ovit gateway, from 2200 m altitude, during the flowering in 2008. The plants were identified by B. Yildiz and the Herbarium specimens (collector No: BY 16802) were deposited at the Department of Biology, Faculty of Science and Education, Balikesir University, Turkey.

Isolation of the essential oils: The dried plant sample was subjected to water distillation using a Clevenger-type apparatus (Ildam, Turkey) for 3 h according to the European Pharmacopoeia[16]. One hundred grams of *A. orientalis* was added in a glass 2000 mL flask together with 1000 mL of distilled water. The essential oil obtained was dried over anhydrous sodium sulfate and stored in dark glass vials with Teflon-sealed caps at + 4 ºC before analyses. The yield of essential oil determined on average over three replicates. The percentage yield (%) of the oil calculated on a moisture-free basis was 0.02 % for the essential oil (v/w).

GC-FID and GC-MS analysis: GC-FID analysis of the oil was carried out on an Agilent Technologies 6890N Network system GC apparatus, equipped with a split-splitless injector, an automatic liquid sampler, HP-Innowax column (60 m × 0.25 mm i.d., 0.25 µm film thicknesses) and a flame ionization detector (FID). Helium was used as a carrier gas. The column temperature was initially set at 60 ºC for 10 min and then it was gradually increased to 220 and 240 ºC at rates of

4 and 1 ºC/min, respectively. Finally, the temperature was held at the final level 10 min. Split flow was adjusted at 84.9 mL/min. The split ratio was adjusted to 50:1. The injector and flame ionization detection (FID) detector temperatures were 250 ºC. GC-MS analysis was conducted using an Agilent technologies 5973 inert mass selective detector (Agilent G3180B Two-Ways Splitters with make up gas) system. The same analytical conditions as those mentioned for GC-FID were employed for GC-MS analysis. MS were taken at 70 eV. The mass range was between *m/z* 10 and 425.

Identification was performed by comparison of mass spectra with the database library (NIST 05 and Wiley 7n, comparison quality > 90 %), as well as by comparison of retention indices for alkenes C_7-C_{29} with the ones reported by Adams[17]. Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

RESULTS AND DISCUSSION

Many studies have been performed on the chemical composition of essential oil from *Ajuga* species. In the literature revision, some qualitative and quantitative differences between the components of the essential oil from the same species were observed. These differences both in the oil content and genetic factors, agronomical practices or plant chemotype or nutritional status.

The percentage of the volatile of the dried aerial parts from *Ajuga orientalis* L. was light yellow with yield of 0.02, V/W, on the dry weigh basis. The yields in essential oil of *Ajuga bombycina* (0.1 %)[18], *A. chamaepitys* (trace), subsp. *cuneatifolia* (0.1-0.4 %), subsp. *laevigata* (0.1-0.4 %)[19], subsp. *mesogitana* (0.1-0.4 %), subsp. *chia var. chia* (0.046 %) and subsp. *chia var. ciliata* (0.09 %)[20]. GC-FID and GC-MS analyses of the essential oil led to separation of 11 peaks accounting for 98 % of the total oils. Out of 21 separated peaks, 16 compounds (95.4 %) could be identified (Table-1). The major constituents detected were phytol (36.7 %), *n*-hexadecanoic acid (14.2 %) and dodecanoic acid (12.2 %). Other components such as tetradecanoic acid (6.7 %), carvacrol (3.0 %), β-terpineol (2.1 %) and 1-octen-3-ol (3.8 %) were detected in lower amounts. Germacrene-D (24.2 %), β-cubebene (18.3 %), β-caryophyllene (16.9 %) and α-cubebene (5.3 %) have been reported as the main constituent of the essential oil of *A. orientalis* growing in Iran[21].

In literature, compositions of the essential oils of *Ajuga* species have showed variation. *Ajuga bombycina* is very rich in β-pinene, α-pinene and germacrene D[18], *A. chamaepitys* subsp. *chia var. chia-in* β-caryophyllene, β-pinene and germacrene D and *A. chamaepitys* subsp. *chia var. ciliate-in* β-pinene and germacrene D[20]. In the essential oil of *A. chamaecistus* Ging., from Iran, *p*-cymene, β-pinene, α-phellandrene and α-pinene were found to be the major components[22]. Hydrodistilled oil from *A. austro-iranica* Rech. From Iran were investigated by Javidnia *et al.* and *trans*-verbenol, caryophyllene oxide, myrtenol, 1-octen-3-ol and β-pinene were identified as the main components among the 39 compound characterized[23]. Mazloomifar *et al*. reported that *A. chamaecistus* Ging. ssp *chamaecistus* contained β-pinene (15.0 %) and linalool (14.5 %)[24]. In the essential oil of *A. chamaecistus* Ging. ssp *chamaecistus* (L.) Schreber. ssp. *chamaepitys* from Spain γ-muurolene (40.3 %), limonene (20.5 %) and germacrene (7.8 %) were found to be major components[25]. When results from literature were compared to those in Table-1, oils contents showed differences and similarities. Several applications of *Ajuga* species in folk medicine and recent activities of their compounds offer that *A. orientalis* essential oil may have a potential to be of great use in next pharmacological and biological screening tests.

TABLE-1
COMPOSITION OF THE ESSENTIAL OIL OF AJUGA ORIENTALIS FROM TURKEY (YIELD PERCENTAGE ≈ 0.02 %)

RRI	Compound	Composition (%)
1019	α-Pinene	1.6
1117	β-Pinene	2.2
1515	1-Octen-3-ol	3.8
1526	α-Cubebene	0.2
1595	α-Gurjunene	0.3
1602	β-Cubebene	0.1
1606	Linalool	0.3
1603	β-Terpineol	2.1
1657	β-Caryophyllene	0.8
1738	α-Terpineol	1.4
2036	Hexahydrofarnesyl acetone	9.8
2094	Carvacrol	3.0
2268	Dodecanoic acid	12.2
2400	Phytol	36.7
2481	Tetradecanoic acid	6.7
2760	*n*-Hexadecanoic acid	14.2
		95.4

RRI, relative retention indices; tr, trace (< 0.1 %).

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