Compositions of the essential oils of Ballota nigra subsp. uncinata and subsp. anatolica from Turkey

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ABSTRACT

Background: Ballota nigra L. is a member of Lamiaceae family and is represented in the Flora of Turkey by five subspecies. Subsp. uncinata is a mediterranean element while subsp. anatolica is an Irano-Turanian phytogeographic region. Material and Methods: The essential oils from aerial parts of subsp. uncinata and subsp. anatolica were isolated by hydrodistillation. The analysis was performed by using a gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) systems, simultaneously. Results: Twenty-two compounds were identified from the oil of subsp. uncinata representing 96.9% of the total oil and fourteen compounds were identified from the oil of subsp. anatolica representing 88% of the total oil. The major components were characterized as caryophyllene oxide (21.2%), hexadecanoic acid (19.9%), β-caryophyllene (18.9%) for subsp. uncinata and hexadecanoic acid (40.9%) and β-bisabolene (13.4%) for subsp. anatolica, respectively.

Keywords: Ballota nigra, Essential oil, subsp. uncinata, subsp. anatolica, Turkey.

INTRODUCTION

The genus Ballota L. is a member of Lamiaceae family and comprised of about 90 species and widespread over the World.1 In Turkey, the genus Ballota is represented by 12 species and 8 subspecies.2-3 B. nigra is a perennial herb and bearing simple hairs. It is represented by five subspecies in Flora of Turkey. B. nigra is commonly distributed in Western Europe.4 B. nigra is known as “yalancı ısırgan” in Turkey and aerial parts of some subspecies of B. nigra are used to treat inflammation, as an antiseptic for wounds, and against gastrointestinal disorders.3,5 B. nigra subsp. anatolica is known as “gripotu” and has been used in folk medicine as an antiseptic, anti-inflammatory, anti-rheumatic, antioxidant, and antimicrobial agent, and also for nausea, vomiting, and nervous dyspepsia.1,6 The essential oil compositions of the B. nigra have been well documented in the literature and they mainly consist of sesquiterpenes and oxygenated sesquiterpenes.7-10 According to our literature survey, the volatile constituents of B. nigra subsp. anatolica have been previously investigated from Iran.1 However, no studies regarding B. nigra subsp. uncinata are reported in the literature. Most probably, this could be the first report on the essential oil chemical compositions of B. nigra subsp. anatolica and B. nigra subsp. uncinata from Turkey.

Experimental

Plant material

Plants were collected during the flowering period from Muğla (subsp. uncinata) and Konya (subsp. anatolica) province of Turkey. Voucher specimens are deposited in NEÜ Herbarium.

Isolation of the Essential Oils

The essential oils from air-dried plant materials were isolated by hydrodistillation for 3 h, using a Clevenger-type appa-
ratus. The obtained oils were dried over anhydrous sodium sulphate and stored at +4°C in the dark until analysed and tested.

**GC-MS analysis**

The GC-MS analysis was carried out with an Agilent 5975 GC-MSD system. Innowax FSC column (60 m × 0.25mm, 0.25µm 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. Set for Split less injection. The injector temperature was at 250°C. MS were taken 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. In order to obtain the same elution order with GC/MS, simultaneous injection was done by using the same column and appropriate operational conditions. FID temperature was 300°C. Relative percentages of the separated compounds were calculated from integration of the peak areas in the GC-FID chromatograms.

**Identification of Compounds**

The components of essential oils were identified by comparison of their mass spectra with those in the Baser Library of Essential Oil Constituents, Adams Library,^1^ Mass Finder Library,^12^ Wiley GC/MS Library^13^ and confirmed by comparison of their retention indices. These identifications were accomplished by comparison of retention times with authentic samples or by comparison of their relative retention index (RRI) to a series of n-alkanes.

**RESULTS AND DISCUSSION**

The water-distilled essential oils from aerial parts of *B. nigra* subsp. *uncinata* and *B. nigra* subsp. *anatolica* were characterized by GC-FID and GC-MS in this study. The compounds identified from the essential oils along with their relative percentages are listed in Table 1 A total of 22 and 14 compounds were identified from the essential oils of subsp. *uncinata* and subsp. *anatolica* respectively, which represented 96.9 and 88% of the oils. Components of the oils can be grouped into three and five main chemical classes in subsp. *uncinata* and subsp. *anatolica*, respectively. The oil isolated from subsp. *uncinata* was dominated by sesquiterpene hydrocarbons (34.4%), oxygenated sesquiterpenes (32.6%) and other contents (29.9%) and caryophyllene oxide (21.2%), hexadecanoic acid (19.9%) and β-caryophyllene (18.9%) being the main compounds. However, the oil of subsp. *anatolica* was characterized by a high content of others (59.3%), sesquiterpene hydrocarbons (18.9%) and a low percentage of oxygenated sesquiterpenes (5.4%), diterpene (2.7%), oxygenated monoterpenes (1.7%) and hexadecanoic acid (40.9%) and β-bisabolene (13.4%) being the main compounds.

The essential oil of several *Ballota nigra* has been previously studied. In 2003, Bader *et al.*^7^ reported β-caryophyllene (25.1%) and germacrene D (24.2%) as the main compounds of *B. nigra* subsp. *foetida* from Jordan. Monoterpenes were little represented, while sesquiterpenes were present in large amounts in their study. In a study of Semnani *et al.*,^8^ 42 components were identified in Iranian *B. nigra* oil, which presented about 95.4% of the total composition of the oil. Caryophyllene oxide (7.9%), epi-α-murolol (6.6%), δ-cadinene (6.5%), and α-cadinol (6.3%) were found to be the main constituents. A literature survey has shown that there is one report on the volatile constituents of *B. nigra* subsp. *anatolica* growing in Iran.^1^ Twelve compounds were identified, representing 91.8% of the total oil and germacrene D (18.1%), nerolidol epoxyacetate (15.4%), selareol oxide (12.1%), linalyl acetate (11.5%), and β-caryophyllene (10.5%) were found to be the main constituents. This oil consisted of oxygenated monoterpenes (18.1%), sesquiterpene hydrocarbons (32.5%), and oxygenated sesquiterpenes (41.2%). Oil composition of our *B. nigra* subsp. *anatolica* was found to be quite different from those already reported. According to Vukovic *et al.***8* *B. nigra* produces two types of essential oils: the first was associated with the stem and leaf and the second was typical of the root. The first type of the oil should be considered a sesquiterpene hydrocarbon-rich oil, dominated by β-caryophyllene and germacrene D. In contrast to the stem and leaf oils, the root produced an oxygenated monoterpenes-rich oil in which the 17 monoterpenes accounted for more than 43% of the oil. In 2014, Fraternale and Ricci^10^ reported β-caryophyllene (22.6% and 21.8%), caryophyllene oxide (18.0% and 20.5%) and germacrene-D (16.5 and 13.1%) as major compounds of *B. nigra* subsp. *foetida*. However, hexadecanoic acid and β-bisabolene are main compounds in oil of *B. nigra* subsp. *anatolica* and these main compounds were not seen other *B. nigra*. The difference in the oil composition of the present study and previous research may be due to the collection time, chemotypes, drying conditions, mode of distillation, and geographic and climatic factors.8
CONCLUSION

The major components were characterized as caryophyllene oxide (21.2%), hexadecanoic acid (19.9%), β-caryophyllene (18.9%) for subsp. *uncinata* and hexadecanoic acid (40.9%) and β-bisabolene (13.4%) for subsp. *anatolica*, respectively.

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CONFLICT OF INTEREST
Kaya et al.: Composition of the essential oil of subspecies of Ballota nigra

None

ABBREVIATIONS USED

GC: Gas chromatography; GC-MS: Gas chromatography–mass spectrometry; FID: Flame Ionization Detector; GC-FID: Gas chromatography with Flame Ionization Detector; ID: Identification method RRI: Relative retention indices; TR: Trace; tR, identification based on the retention times; tR: of genuine compounds on the HP Innowax column; ms: identified on the basis of computer matching of the mass spectra with those of the Wiley and MassFinder libraries and comparison with literature data.

REFERENCES


SUMMARY

• The genus Ballota L. is a member of Lamiaceae family. B. nigra is a perennial herb and aerial parts of some subspecies of B. nigra are used to treat inflammation, as an antiseptic for wounds, and against gastrointestinal disorders.
• Our objectives are to find out the presence of phytochemical constituents in aerial parts of the plant extracts using GC and GC-MS.
• Caryophyllene oxide (21.2 %), hexadecanoic acid (19.9 %), β-caryophyllene (18.9 %) for subsp. uncinata and hexadecanoic acid (40.9 %) and β-bisabolene (13.4 %) for subsp. anatolica were found main components.

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