Chemical Constituents of the Essential Oil of Stachys fruticulosa M. Bieb. From Iran

Faraz Mojab1*, Neda Khalaj2

1School of Pharmacy and Pharmaceutical Sciences Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
2School of Pharmacy, Islamic Azad University of Medical Sciences, Tehran, Iran.

Abstract

Background: Stachys (Lamiaceae) is a genus with varied effects and applications in traditional medicine. The essential oil (EO) of different species of genus Stachys has received much attention in different studies. According to previous evidence, the components of EO in different species have diversity although there is no previous data regarding investigating Stachys fruticulosa for EO. Considering the importance of the plants of genus Stachys, vast types of compounds in the EO of the genus, the aim of the present study was to evaluate the components of EO of S. fruticulosa M. Bieb.

Methods: The EO of the aerial parts from S. fruticulosa was obtained by hydrodistillation and then the oil was analyzed by gas chromatography/mass spectrometry (GC/MS) and 95% of the oil (17 components) was identified accordingly. The identity of the components was assigned by comparing their mass spectra and retention indices with those of authentic samples.

Results: Most oil components were α-terpinyl acetate (24.6%), 3- n-Butyl phthalide (20.5%), p-cymene (18.2%), and β- phellandrene (18.2%).

Conclusion: The components of essential oil from S. fruticulosa were identified for the first time, and these substances may be responsible for the biological effects of these essential oils.

Keywords: Stachys fruticulosa, Lamiaceae, Essential oil, GC-MS

Introduction

In the flora of Iran, genus Stachys is represented by 34 species while its number is about 300 species in the world. Stachys fruticulosa, with the local name of Sonbole gachdust, is grown in many parts of Iran, Iraq, and Anatolia, and is used as an anti-inflammatory agent in traditional medicine (1). Salvia lavandulifolia is known as mountain tea (Chaye-kuhi in Iran) and its English name is Betony. In addition, it is used as the herbal tea in gastrointestinal disorders (2).

The hydrodistillation of the aerial parts of S. palaestina collected in Lebanon yielded 0.1% (w/w) of EO. Gas chromatography (GC) and GC-mass spectrometry (MS) analyses enabled the identification of 87 compounds representing 90.8% of the total oil. Hexadecanoic acid (10%), hexahydrofarnesyl acetone (6.9%), eugenol (4.3%), and (E)-caryophyllene (4.3%) were the main components (3). According to another report (4), the water distilled EO of the aerial part of S. pubescence was rich in fatty acids like hexadecanoic and linoleic acids in addition to benzaldehyde and spathulenol whereas the steam distilled oil of the plant contained hexadecanoic acid, spathulenol, and eugenol. Both oils were rich in fatty acids (36.6% and 27.9%, respectively).

The chemical composition of the EOs from the aerial parts of two endemic Turkish species (i.e., Stachys amanica and S. petrokosmos) was analyzed by GC and GC-MS. The major components of the EO of S. amanica in Dadagli were found to be α-pinene, α-bisabolol, (E)-β-caryophyllene, and germacrene D. However, the EO from S. amanica in Basyurt contained α-pinene, β-pinene, and (E)-β-caryophyllene. On the other hand, the major components of the EO from S. petrokosmos in Dadagli were reported as α-pinene, β-pinene, α-zingiberene, (E)-β-caryophyllene, and γ-curcumene. Moreover, the EO from S. petrokosmos in Belen included α-pinene, γ-curcumene, α-bisabolol, 16-kaurene, and α-zingiberene (5). In another study (6), characterization by the GC-flame ionization detector and GC/MS analyses of the S. officinalis EO obtained by the hydrodistillation of the aerial parts allowed the identification of 190 components that represented 97.9% of the total oil content. The main identified constituents were germacrene D (19.9%), β-caryophyllene (14.1%), and α-humulene (7.5%). A
Seventeen compounds were analyzed by GC-FID and GC-MS apparatus for 4 hours and 3 minutes after the oil under the same chromatographic conditions.

In addition, the retention indices for all components were determined using n-alkanes as the standard. Then, the constituents were identified by comparing retention indices (i.e., RRI & RTx-1) with those reported in the literature, along with comparing their mass spectra with those held in the Wiley library of mass spectra or with the published mass spectra (14).

Results

The EO of S. fruticulosa was obtained by hydrodistillation-solvent extraction using a Clevenger-type apparatus.

The yield of the oil was 0.25% and the S. fruticulosa oil was examined by GC and GC-MS (Figure 1). Table 1 presents the list of compounds that were identified in the oil of S. fruticulosa. Seventeen compounds were identified, representing 98.2% of the EO, in which the major components were p-cymene (18.2), β-phellandrene (18.2), α-terpinyl acetate (24.6), and 3-n-butyl phthalide (20.5). The other compounds included β-myrcene (2.6%), α-terpineol (1.5%), Z-butyldienephthalide (1.5%), E-butyldienephthalide (1.2%), palmitic acid (1.2%), carvacrol (1.1%), thymol (0.9%), γ-terpinene (0.7%),

Methods

Plant Material and Isolation Procedure

The plant material was collected from the Savejbolagh area in the north-west of Iran as well. The EO was extracted by hydrodistillation from the selected plants, and its chemical composition was determined by the GC-MS system. The mass fraction of the oil on the dry weight base was 0.4%. Altogether, 21 compounds were identified corresponding to 87.9% of the total oil. The main components were germacrene-D (9.6%), menthone (6.9%), 1,8-cineole (14.8%), α-terpineol (7.8%), cubenol (9.9%), α-cadinol (6.8%), and linalool (12.9%). Additionally, the EO composition of the aerial parts of S. parviflora was analyzed by GC-FID and GC-MS apparatus, followed by characterizing 23 compounds representing 99.9% of the oil. Muurolol (48.4%) and Z-caryophyllene (11.2%) were the major components of the oil, and oxygenated sesquiterpenes (71.4%) were the major fraction of the EO (8).

In similar research (9), the EO of S. lavandulifolia Vahl (Lamiaceae) was isolated by the hydrodistillation of the aerial parts of the plant with a yield of 0.25%. Then, the chemical composition of the volatile oil was analyzed by capillary GC and GC/MS. Finally, the main components were germacrene-D (13.2%), β-phellandrene (12.7%), β-pinene (10.2%), myrcene (9.4%), α-pinene (8.4%), and Z-β-ocimene (5.8%). Some biological and pharmacological activities were reported from Stachys species as well. According to previous reports, the hydroalcoholic extract of the aerial parts of other species (S. inflate) shows potent anti-inflammatory activity in the rat and the methanolic extract of the tuber of S. sieboldii has anti-anoxia actions in mice (10,11).

A literature survey has shown that S. fruticulosa has not previously been investigated for EO. But, the methanol extracts of its aerial parts were evaluated for their antioxidant activity and the total phenolic content using ferrie ion reducing antioxidant power (FRAP) and Folin-Ciocalteu assays. The FRAP value and phenol content were reported 62.09 ± 4.527 mmol Fe 2+ /100 g dry weigh plant and 4450.368 ± 280.0766 mg gallic acid equivalent 100 g dry weigh plant, respectively (12). The obtained extracts from the aerial parts of S. fruticulosa were examined for its antibacterial activities against G- and G+ strains and the results revealed that S. fruticulosa methanolic extract inhibited the growth of Staphylococcus aureus and Bacillus cereus (13).

Identification of Oil Components

The analytical GC method was carried out using a ThermoQuest 2000 GC coupled with the Thermo/Finnigan mass system and an RTx-1 glass capillary column (methyl phenyl siloxane 30 m X 0.32 mm, 0.25 μm film thickness), N2 as the carrier gas with a flow rate of 1.5 mL/minute, the split ratio of 1:10, and a flame ionization detector. Temperature programming was performed from 50-250°C at 3/minute with injector and detector temperatures to 270°C. The quadrupole mass spectrometer operated at 70 eV ionization energy and electron ionization mass spectral spectra were obtained in the scan mode at the m/e range of 35-400 amu. Next, retention indices were determined by using the retention times of n-alkanes, which was injected after the oil under the same chromatographic conditions.

Identification of Oil Components

Methods

Plant Material and Isolation Procedure

The plant material was collected from the Savejbolagh area in the north-west of Tehran in July 2006. A voucher specimen was deposited in the Herbarium of the School of Pharmacy, Shahid Beheshti University of Medical Sciences. The aerial parts of the plant were air-dried and the oil was obtained by hydrodistillation-solvent extraction using a Clevenger-type apparatus for 4 hours and n-hexane.
α-phellandrene (0.7%), sabinene (0.6%), β-pinene (0.6%), α-pinene (0.5%), and linalool (0.3%). This oil mainly composed of monoterpenic hydrocarbons (41.9%), monoterpenic alcohols (3.9%), Monoterpenic acetate (24.6%), and phthalides (23.2%).

Discussion

Stachys fruticulosa M. Bieb. was found to contain 17 compounds, in which monoterpenic hydrocarbons (41.9%) and phthalides (23.2%) were the major classes. To the best of our knowledge, it is the first report on the chemical constituents of S. fruticulosa.

The main components of the oil from the other Stachys species were reported to be germacrene-D (6,7,10,11), muurolol (8), and α-pinene (5), which completely contradicts the findings of the present study, in which only 0.5% α-pinene was present in the oil. Hexadecanoic acid reported in the S. fruticulosa EO (1.2%) was found in S. palaestina (3) and S. pubescence as well (4).

Renda et al (15) investigated the volatile components of three species of Stachys and revealed that carvacrol (28.8%), p-cymene (18.2%), and α-pinene (11.2%) in S. macrantha, as well as, limonene (37.0%), α-cedrene (11.2%), and γ-muurolene (10.2%) in S. sylvatica, and (Z)-β-ocimene (24.8%), β-pinene (23.1%), and α-pinene (11.4%) in S. annua ssp. annua var. annua. are the major components of EO. Although the reported compounds in these species were different from those of the present study, as a whole, the major constituents in S. annua ssp. annua var. annua and S. sylvatica (65.8% and 49.8%, respectively) were monoterpenic hydrocarbons. This finding is in accordance with the results of the present study. It is noteworthy that S. macrantha mainly contained oxygenated monoterpenes (42.1%).

Likewise, Sarikaya (16) reported that benzaldehyde (46.34%), β-caryophyllene (11.23%), and (E)-2-hexenal (8.50%) were the major constituents of Stachys cretica subsp. anatolica. They also evaluated the volatile oil of S. lavandulifolia and revealed that the main components were β-phellandrene (27.71%), myrcene (11.56%), and α-pinene (11.20%).

Eventually, Javidnia et al (17) found that the main components of S. lavandulifolia Vahl were germacrene-D (13.2%), β-phellandrene (12.7%), β-pinene (10.2%), myrcene (9.4%), α-pinene (8.4%), and Z-β-ocimene (5.8%). The findings of these studies contradict the results of the present study.

Conclusion

Literature survey and investigation of the results of different studies was induced by considering the results of the present study and the comparison of the findings of other studies. Thus, it seems that many factors are involved in a wide diversity of chemical constituents of the EOs, and the investigation of volatile oils is a subject of pharmacognostical studies at all times.

Acknowledgement

We are thankful to Mohammad Kamali-Nejad, Medicinal Plant Laboratory, Shahid Beheshti University of Medical Sciences, Iran for the collection and identification of plant specimens.

References

5. İlçim A, Alma MH, Karaogul E. Investigation of volatile


