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GC-MS analysis of methanolic and dichloromethane extracts of *Scutellaria pinnatifida* A. Hamilt. ssp. *alpina* roots

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Abstract

Background: *Scutellaria pinnatifida* A. Hamilt. ssp. *alpina* is an Iranian species of *Scutellaria* genus. This genus is used for the treatment of hypertension, arteriosclerosis, inflammation, hepatitis, allergy, cancer, anxiety and sleeplessness. The present investigation evaluated the chemical composition of the methanolic and dichloromethane extracts of *S. pinnatifida*.

Materials and Methods: Identification of the components of the methanolic and dichloromethane extracts of *S. pinnatifida* was done by GC-MS using NIST and WILEY libraries.

Results: GC-MS analysis clearly showed the presence of 23 compounds in the methanolic extract and 22 compounds in dichloromethane extract.

Conclusion: Compounds such as diacetin, docosane, bis (2-ethylhexyl) phthalate, and tetratetracontane were abundantly present in the methanolic extract and compounds like trans-squalene, tridecane, docosane, pentadecane and palmitoyl glycol were the major components of dichloromethane extract of *S. pinnatifida*.

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Introduction

The genus *Scutellaria* belongs to the family Lamiaceae family and comprises over 300 species (Evans, 1989; Rechinger, 1982) of which 20 species are found in Iran (Ghahreman, 1999) and 10 species and two hybrids are endemic to this country (Emami and Aghazari, 2008). One of the Iranian species of *Scutellaria* is *Scutellaria pinnatifida* that has two subspecies namely, *S. pinnatifida* A. Hamilt. ssp. *pinnatifida* and *S. pinnatifida* A. Hamilt. ssp. *alpina* (Mozaffarian, 1996; Rechinger, 1982). Locally, this plant is called "Boshghabi" (Mozaffarian, 1996). This genus is well-known for its uses in the treatment of hypertension, arteriosclerosis, inflammation, hepatitis, allergy, cancer, anxiety and sleeplessness (Mozaffarian, 1996). Also, the plants of this genus have sedative, tonic, and anti-spasmodic properties (Awad et al, 2003; Hirotani et al, 1998). Dried roots of the plants of this genus are administered in Chinese traditional herbal medicine (Hosokawa et al, 2000) due to their antioxidant (Gabrielska et al, 1997), anti-inflammatory (Lin et al, 1999) and sedative (Ying and Guo, 1994) activities. Flavonoids are the main chemicals found in this genus (Parajuli et al, 2009). For example, flavonoids such as baicalin, baicalein and wogonin have been isolated from *Scutellaria* species (Parajuli et al, 2009). In recent years, the flavonoids isolated from *Scutellaria* plants have been widely studied for their chemical properties and biological activities (Shi et al, 2011). The hydrodistilled essential oil of the aerial parts of *S. pinnatifida* collected from Khorasan province, Iran was analyzed and the major components of the oil were germacrene-D and beta-caryophyllene (Ghannadi and Mehregan, 2003). Free-radical-scavenging and antibacterial activity and brine shrimp toxicity of different extracts of the aerial parts of *S. pinnatifida* were assessed and dichloromethane and methanolic extracts exhibited free-radical-scavenging property (Sauvage et al, 2010). Also, dichloromethane extract of *S. pinnatifida* showed the most marked cytotoxic effects among other extracts on cancer cell lines including K562 and HL-60 and a normal cell line (Boozari, 2015).

The main qualitative and quantitative analytical methods used to isolate compounds from plants, are high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) (Yanget al, 2012; Domingues et al, 2010; Leipoldet al, 2010; Martelancet al, 2009; Kontogianniet al, 2009). The present investigation was carried out to determine the chemical composition of the methanolic and dichloromethane extracts of *S. pinnatifida* A. Hamilt. ssp. *alpina* by GC-MS.

Materials and Methods

Gas chromatography apparatus

GC-MS analysis was performed by a Shimadzu QP2010SE (Japan) equipped with RTX-5MS column (30.0 m, 0.25 mm, 0.25 µm film thickness) composed of 100% dimethyl polysiloxane. For GC-MS evaluations, an electron ionization energy system with ionization energy of 70 eV was used. Helium gas was used as the carrier gas, the injector temperature was set at 300°C and the ion-source temperature was 300°C. The temperature was programmed to start from 70°C and increase to 300°C in 10 min. GC running time was 15 min.

Plant material

The roots of *S. pinnatifida* were collected from the Tighbal Mountain (2700 meters above sea level) in Darkesh valley, Bojnurd, North Khorasan province, Iran, in June 2014. A voucher specimen (voucher No. 11868) was deposited at the Herbarium of the School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran.

Extraction, isolation and purification

Plant's roots were dried in the shade at room temperature and then cut into small pieces. About 100 g of dried roots was macerated in methanol and dichloromethane (each one contained 50 g of dried roots) at room temperature for 24 hr. Each solvent was allowed to remain in contact with the plant material for 24 hr, and replaced with fresh solvent everyday for 4 days. Removal of the solvents under vacuum at 40 °C yielded 10.0 and 0.5 g of the methanolic and dichloromethane extracts, respectively (Prachayasittikul et al, 2008).

GC-MS studies

GC-MS analysis of the methanolic and dichloromethane extracts was done by the method of Deattu (Deattu et al, 2013). About 1 g of the methanolic and dichloromethane extract was taken into a vial and 10 ml of methanol was added. Then, it was sonicated for 1 hr and kept for 12 hr. Identification of compounds by GC-MS was done using NIST and WILEY libraries. Chemical name, retention time, peak area % and structure of the components were determined.

Results

GC-MS studies of the extracts

Yields of the methanolic and dichloromethane extracts were 10.1% and 0.5%, respectively. The methanolic and dichloromethane extracts were evaluated by GC-MS method (Figures 1 and 2) and compounds relevant to the peaks were identified based on NIST and Wiley libraries (Tables 1 and 2). GC-MS analysis clearly showed the presence of 23 and 22 compounds in the methanolic and dichloromethane extracts, respectively. The

retention time (RT) and concentration (peak area %) of identified compound are presented in Tables 1 and 2.

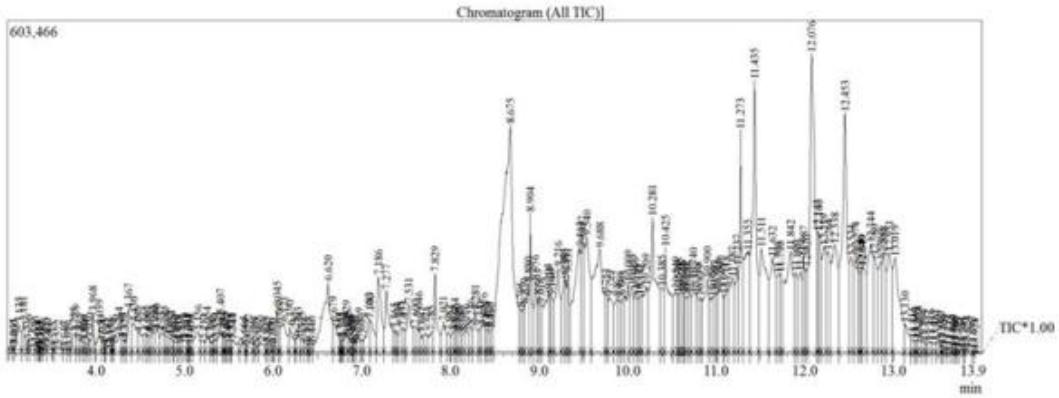

Figure 1. GC-MS chromatogram of the methanolic extract of *S. pinnatifida* roots.

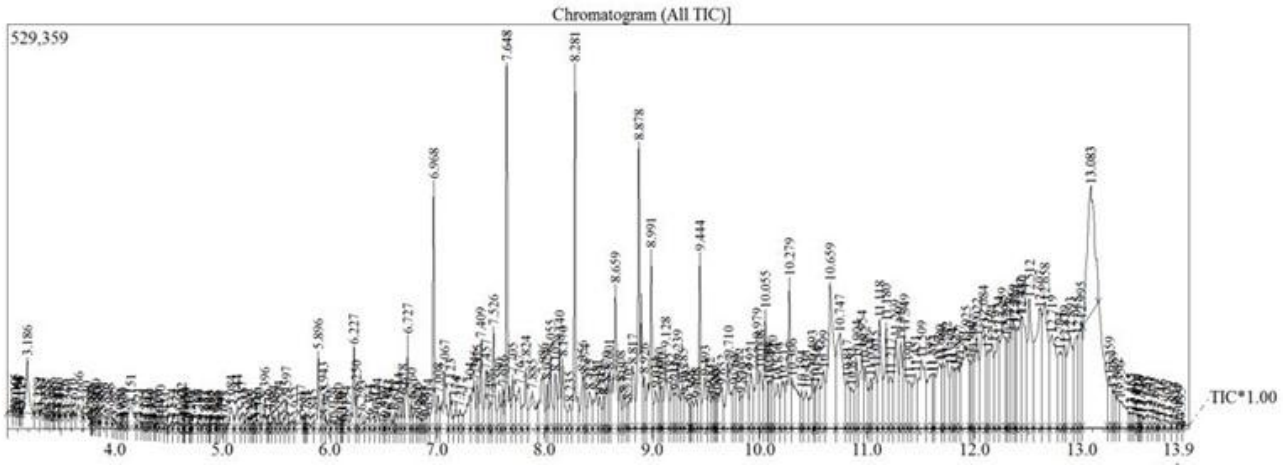

Figure 2. GC-MS chromatogram of the dichloromethane extract of *S. pinnatifida* roots.

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Table 1. GC-MS analysis of the methanolic extract of *S. pinnatifida* roots.

NO	Retention Time	Compound	% Peak area
1	6.62	3,5-dihydroxy-2-Methyl-5, 6-DihydroPyaran-4-one	2.325
2	8.675	Diacetin	10.98
3	9.216	Nonacosane	1.62
4	9.465	Nerolidol	2.85
5	9.54	3-deoxy-d-mannoic lactone	2.64
6	9.688	3--d-mannonic Acid Deoxy	3.325
7	10.281	γ-Hydroxy isoeugenol	3.325
8	10.425	Nootkatane	2.115
9	10.9	Nuoplaz	1.5
10	11.273	Palmitic acid	2.235
12	11.435	Docosane	8.115
13	11.511	Propionyl filicinic acid	3.09
16	12.076	Bis(2-ethylhexyl)phthalate	6.07
18	12.338	N-Hentetracontane	3.05
19	12.453	Tetratetracontane	6.825
20	12.574	Hexacontane	1.56
21	12.744	Tritetracontane	2.385
22	12.951	Ditetradecyl ether	2.175
23	13.019	Nona hexacontanoic acid	2.665

Table 2. GC-MS analysis of the dichloromethane extract of *S. pinnatifida* roots.

NO	Retention Time	Compound	% Peak area
1	5.89	Octane-5-Ethyl-2-methyl	0.7
2	6.725	Methylcyclohexyl dimethoxysilane	0.28
3	6.970	Dodecane	1.68
4	7.065	2,6-dimethylundecane	0.9
5	7.525	5-isobutylnonane	0.84
6	7.645	Tridecane	4.82
7	8.140	Phytane	0.63
8	8.28	Tetradecane	2.56
9	8.660	Octadecane	1.90
10	8.880	Pentadecane	3.44
12	8.990	2,4-di-tert-butyl-phenol	1.78
13	9.71	2,6,10-trimethyl-penthadecane	1.34
16	9.980	Nonadecane	1.23
18	10.280	Coniferol	1.6
19	10.660	Docosane	3.74
20	11.35	Butyl phthalate	1.21
21	12.510	Palmitoyl glycol	2.73
22	13.085	Trans-squalene	18.68

Discussion

Compounds like diacetin, docosane, bis (2-ethylhexyl)phthalate, tetratetracontane and gamma-hydroxy isoeugenol are abundantly present in the methanolic extract and compounds like trans-squalene, tridecane, docosane, pentadecane and palmitoylglycol are the major constituents of the dichloromethane extract. Diacetin, a volatile acetylated glycerol is used as a plasticizer, softening agent, and solvent for resins and cellulose derivatives such as nitrocellulose (Hua et al, 2015). Squalene is a triterpene and an intermediate chemical in the biosynthesis of sterols in plants and animals world (Psomiadou and Tsimidou, 1999). This compound is widely found in vegetable oils and is the main component of skin surface polyunsaturated lipids with some advantages for the skin (Tuberoso et al, 2007; b Huang et al, 2009). Moreover, Conforti et al. reported antioxidant effect of squalene (Conforti et al, 2005). In another study, the hexane and $CHCl_3$ extracts of *S. comosa*, contained 77 compounds and the major constituents of the hexane extracts were hydrocarbons whereas in the $CHCl_3$ extract of the roots, (Z)-14-methyl-8-hexadecenal (44.85%), 1-(4-hydroxy-3 methoxyphenyl) ethanone (%11.49), 7-methoxycoumarin (%5.65), butyl 8-methylnonylphthalate (%5.26) and di-i-octylphthalate (96.97%) were the most abundant chemicals (Karimov et al, 2015). Because of high polarity, low volatility and poor thermal stability, gas chromatography is not widely employed for the determination of flavonoids including baicalin, baicalein or wogonin. In this study, these compounds were not extracted and determined by this method. However, in another study, these flavonoids were identified in the methanolic extract of *S. baicalensis* by GC-MS (Lin et al, 1999).

Conclusion

In conclusion, in the present study using GC-MS analysis, 22 and 23 chemicals were identified in the dichloromethane and methanolic extracts of roots of *S. pinnatifida*, respectively. Further studies are needed to understand the chemical structures of the compounds identified in this study.

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Conflict of interest

The authors have no competing interests to declare.

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