

GC-MS analysis of apolar extract of *Astragalus sibthorpianus*

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Abstract

Astragalus species are widely distributed in Turkey. There are 445 species in the Flora of Turkey. Plenty of species of *Astragalus* are used in traditional medicine in many countries such as European Countries, Turkey, China, India etc. They are used for treatment of diabetes, nephritis, leukaemia, and uterine cancer. In addition, they are used as an antiperspirant, diuretic, and tonic drug.

Astragalus sibthorpianus is endemic to Uludağ mountain in Turkey. In this study for the first time, the chemical composition of n-hexane extract of plant material was investigated using GC-MS. The whole plant material was dried and powdered and macerated with MeOH at room temperature. The concentrated MeOH layer was extracted with n-hexane for the apolar constituents. The crude n-hexane extract was derivatized and identification of each component was exhibited by Wiley library data search.

Keywords: *Astragalus sibthorpianus*, GC-MS, Hexane extract

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1. Introduction

The genus *Astragalus*, which is part of the Fabaceae family, generally known as 'milk-vetch' or 'locoweed'. It is especially distributed through the world's temperate regions. The genus, which is the largest genus in Turkey, is exemplified by 445 species of which 224 are endemic in Flora of Turkey [1,2].

Astragalus species are very important medicinal plants especially for Far East developing countries such as China and India, because of encouraging low-cost alternatives in primary health support program. Plant's secondary metabolites are bioactive and they are used as diuretic, tonic, immune stimulant and an anticancer agent. Also they have diuretic, antibacterial, antiviral, anti-inflammatory and antioxidant (which protect cells against the free radical's damage) activities. Furthermore, some species are well known in remedy for animal bites and poisons, wounds and burns, eye diseases, hypertension, nephritis, diabetes, throat diseases, cirrhosis, uterine and leukaemia cancer. [3-5].

Although 32 species have been identified as of use in foods, as substitutes for drinks, cosmetics and medicines, or as sources of vegetable gums, the most known use of *Astragalus* species is as food for livestock [6]. However, some species are toxic for livestock and in many situations, the toxins may be transferred to humans by means of meat or milk. Thus, *Astragalus* species can be classified in two groups: the toxic species and the medicinal plants. According to the toxins and their effects on animals, the poisonous *Astragalus* species can be categorized into three main groups: (a) species that contains aliphatic nitro compounds; (b) species inducing locoweed poisoning; and (c) species that can amass selenium [7,8].

Astragalus genus are also known to be rich in biologically active compounds such as polysaccharides, saponins, flavonoids, indolizidine type alkaloids and the nitro compounds [9-11].

Our plant material in this study is *Astragalus sibthorpianus* which is endemic to Uludağ Mountain in Turkey. As a part of our ongoing project on the analysis of chemical components of some *Astragalus* genus, we analyzed the content of apolar n-hexane extracts of *A. sibthorpianus* by GC-MS.

2. Material and Methods

2.1. Plant Material

A. sibthorpianus (Endemic) was collected from ~2100 m, Kırkpınarlar locality, Uludağ Mountain National Park, Bursa, Turkey in June 2012 identified by Prof. Dr. Gürçan Gülerüz.



Figure 1. The photos of *A. sibthorpianus*



Figure 2. *A. sibthorpianus* distribution in Turkey

2.2. Sample Preparation

The whole plant material, which was air-dried and powdered, was exhaustively macerated with MeOH at room temperature. The crude extract was yielded after evaporation of the solvent in vacuum and then extracted apolar parts was successively with n-hexane.

After removing solvent by rotary evaporator system, the apolar oily mixtures were derived to their methyl esters by trans-esterification process according to the International Olive Oil Council (IOOC) and International Union of Pure and Applied Chemistry (IUPAC) reports [12,13]. In this process, dried apolar oily extracts were dissolved in hexane and extracted with 2 M KOH in MeOH at room temperature for 30 s. The upper phases were investigated by GC-MS.

2.3. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Apolar extract and methyl esters of fatty acids were analyzed by Agilent 7890A and Agilent 5975C inert XL MSD combined system with HP-5MS chromatography column (30m × 0.25mm × 0.25µm). Pure helium gas (99.999%) was used as a carrier gas at a steady flow rate of 1 mL/min. The oven temperature was programmed as 60 °C for 5 min. then 5 °C/min to 220 °C and held there for 5 min. 1 µL of the sample in hexane was injected in the split mode with 20:1 split ratio. The compounds were identified by NIST–Wiley library data search.

3. Results

The identified compounds in apolar hexane extract of *A. sibthorpianus* are represented in Table 1. The major compounds in hexane extract were found as Alpha-Linolenic Acid Methyl Ester (ALA- ω -3) (39.94%), Palmitic Acid Methyl Ester (17.75%), Linoleic Acid Methyl Ester (LA- ω -6) (14.82%), Phytol (9.85%) and Stearic Acid Methyl Ester (6.26%).

Table 1. Chemical compounds of apolar hexane extract of *A. sibthorpianus*

RT	Compound Name	%
29.253	Myristic Acid Methyl Ester	0.58
31.370	Pentadecanoic Acid Methyl Ester	0.50
31.645	Benzoic Acid, N-Hexyl Ester	0.30
31.780	6,10,14-Trimethyl-2-Pentadecanone	0.39
32.071	Benzoic Acid, Cyclohexyl Ester	0.20
32.294	Phthalic Acid, Diisobutyl Ester	0.19
32.979	Palmitoleic Acid Methyl Ester (ω -7)	0.50
33.430	Palmitic Acid Methyl Ester	17.75
35.314	Margaric Acid Methyl Ester	1.13
36.637	Linoleic Acid Methyl Ester (ω -6)	14.82
36.813	Alpha-Linolenic Acid Methyl Ester (ω -3)	39.94
36.969	Phytol	9.85
37.187	Stearic Acid Methyl Ester	6.26
37.529	Tricosanoic Acid, Methyl Ester	1.22
38.302	1-Octadecene	0.68
38.494	Phthalic Acid, Decyl Methyl Ester	0.26
40.450	Behenic Acid Methyl Ester	2.44
41.156	11,14,17-Eicosatrienoic Acid, Methyl Ester	0.76
41.836	Arachidic Acid Methyl Ester (ω -6)	2.17

4. Conclusion

Essential fatty acids (EFAs), which are significant for survival of humans and some mammals, play considerable roles in preventing many diseases. They cannot be produced by human body, therefore, they have to be get by the nutrition. Especially fish (salmon, tuna etc.) and fish oils are very rich sources of EFAs. However, vegetable resources are needed to get the fatty acids necessary for the nutrition of vegans and vegetarians. There are two types of EFAs that are called as omega-3 (ω -3) derived from alpha-linolenic acid (ALA) and omega-6 (ω -6) derived from cis-linoleic acid (LA). The deficiency of EFAs causes several anomalies in the body, such as obesity, atherosclerosis and cardiovascular diseases, hypertension, Alzheimer's disease, schizophrenia and some types of cancer. Also, the composition of EFAs can be used as a taxonomic marker for higher plants [14–20].

Based on the above information, our results show that total EFAs content (ALA (ω -3) and LA (ω -6)) (56,93 %) of apolar hexane extract are meaningful amount. Thus, it seems that *A. sibthorpianus* can be used as dietary source for EFAs, Moreover, these results show that it can be used as a taxonomic marker in a large family such as *Astragalus*.

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