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ORIGINAL ARTICLE

Chemical Composition of Hexane Extract of Different Parts of *Anthemis talyschensis* and its Potential to Use in Sunscreen Products

Alireza Motavalizadehkakhky*[1], Ali Shafaghat[2], Jamshid Mehrzad[1], Masoud Shafaghatlonbar[3], Farzaneh Azimi[4], Zohreh Ebrahimi[1], Abollfazl Sakeri[5]

[1] Department of Chemistry, Neyshabur Branch, Islamic Azad University, Neyshabur, Iran

[2] Department of Chemistry, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran

[3] Department of Chemistry, Tehran North Branch, Islamic Azad University, Tehran, Iran

[4]Agriculture Research Center, Ardabil, Iran

[5] Department of Pharmacognosy, Mashhad University of Medical Sciences, School of Pharmacy, Mashhad, Iran

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KEYWORDS

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ABSTRACT: In this study, both the presence and concentration of some unsaturated compounds in hexane extracts of different parts of *Anthemis talyschensis* showing absorption at wavelength 280-450 nm were surveyed, with the view of possibly using extracts of this plant in new formulations of sunscreen creams. The hexane extracts of flower, leaf and stem of *A. talyschensis*, collected from Northwest Iran, were obtained using a Soxhlet apparatus. The fatty acids were derivatized to methyl esters and were determined by gas chromatography/flame ionization detector (GC/FID) and gas chromatography/mass spectrometry (GC/MS) systems. The chemical analysis resulted in identification of 14, 9 and 29 constituents, comprising about 99.5, 97.1 and 98.2% of the total constituents in hexane extracts of flower, leaf and stem, respectively. The main unsaturated constituents in the hexane extract of *A. talyschensis* flower were 9, 12-octadecadienoic acid, 9-octadecenoic acid and 6, 9, 12-octadecatrienoic acid; while the leaf's extract contained 9, 12-octadecadienoic acid and 9-octadecenoic acid; no unsaturated compounds were detected in the stem. The ratios of unsaturated fatty acid /saturated fatty acid were 13.6, 9.3 and 0 in extracts of the flower, leaf and stem, respectively, but the total amounts in the leaf were much greater. It can be concluded the leaf extract is more likely to be suitable for producing sunscreens creams than others.

* Corresponding author: amotavalizadeh@yahoo.com (A. R. Motavalizadeh).

INTRODUCTION

Species of the *Anthemis* genus (Asteraceae) are commonly used in pharmaceutics, cosmetics and foodstuffs. Some of the main components of the flowers of *Anthemis* species, natural flavonoids and essential oils, have been as disinfectants and healing herbs [1-3]. Some *Anthemis* essential oils contain anti-aging activity [4]. *Anthemis* L., the second largest genus of the tribe *Anthemideae*, consists of approximately 210 species [5, 6] distributed widely in the Mediterranean, South Africa and Southwest Asia [7] and all around Iran, in particular [3]. Thirty-nine species of the *Anthemis* genus have been observed in Iran, including 15, which are endemics, *A. talyschensis* (known as Babone Taleshi in Iran), is one of these [8]. So far, there have been studies of the essential oils of *A. mazandranica* [9], *A. altissima* [4, 10,11], *A. auriculata, A. chia, A. cotula, A. melanolepis, A. tomentosa, A. werneri* [4], *A. gayana* [12], *A. nobilis* [13-20], *A. ruthenica* [21], *A. carpatica* [22], *A. montana* L., *A. cretica* L. [23], *A. melampodina* [24], and *A. tinctoria* L. [4, 25, 26]. Information on the essential oil and extracts of *A. talyschensis* has not been reported previously.

Ultraviolet (200 to 400 nm), visible (400 to 800 nm) and infrared (greater than 800 nm) radiation comprise the sunlight that reaches the Earth's surface [27]. UV radiation can be divided into three regions: UVC (200 to 290 nm), UVB (290 to 320 nm) and UVA (340 to 400 nm). In addition, the UVA portion is subdivided into UVA1 (320 to 400 nm) and UVA2 (320 to 340 nm) [28, 29]. The UVC radiation is absorbed by the protective ozone layer, but the UVB portion, as well as part of UVA, reaches the Earth's surface. Of the radiation reaching the surface of Earth, 6% is in the UVB and 94% in the UVA region. Both UVA and UVB radiation are harmful to the skin and can cause sunburn and skin cancers, which have become an increasingly significant public health problem in recent years, although the UVB portion is one thousand times more damaging than UVA. Skin cancer occurs mostly on the areas of the body that are most frequently exposed to the sunlight, such as the face, neck, and head and back of the hands [30, 31].

It is widely recommended that sunscreens such as creams and lotions and other hair and skin preparations be used in particular to reduce the possibility of harmful effects due to UV radiation [32, 33]. Sunscreens work by either reflecting or absorbing the UV radiation before it reaches the skin. In particular, the effects of UVB radiation are cumulative, which suggests a need for sun protection from early childhood [28, 34].

However, it is necessary that a very efficient sunscreen substance be used in the formulation of creams [35, 36]. The ideal creams are defined as those that can protect and filter both UVA and UVB from UV radiation .Many chemical compounds absorb UV light at various wavelengths but most of them are synthetic and many are harmful to the skin, and /or are carcinogenic. Various components of fruits and plants, including chemical compounds found in different parts of plants e.g. flowers, fruits, leaves, stems, roots and seeds, show important biological activities and have been used in different industries, especially the medicinal, food, cosmetics and chemical industries. Some of the substances found in plants that show remarkable herbal effects are unsaturated fatty acids and flavonoids [44]. This project is part of an overall effort to identify plant sources of valuable materials.

In this study, the chemical compositions of the hexane extracts of the flowers, leaves and stems of *A. talyschensis* were investigated for the first time. The specific aim of this study was to identify and determine the amounts of compounds showing absorption at

wavelength 280-450 nm that could potentially be used to produce new sunscreen cream formulations.

MATERIALS AND METHODS

Plant material

The flowers, leaves and stems of *A. talyschensis* were collected separately along the Khalkhal-Ardabil road (in Ardabil Province, Iran) at an altitude of 1650 m (latitude +37º58' N, longitude +48º32'E), in August 2012. A voucher specimen (A-215) is kept at the Herbarium of Agricultural Research in Ardabil Center, Iran.

Extraction of plant material

Dried and powdered materials (flower, leaf or stem) were extracted with hexane (95 % hexane, Merck, Germany) using a Soxhlet apparatus (70 ºC, 4h) to obtain the fatty acids and the other apolar components. The extracts were concentrated under vacuum at 45 ºC using rotary evaporator.

Methylation of fatty acids in the hexane extract

After removal of hexane using a rotary evaporator, the fatty acids in the oily mixtures were converted to their methyl esters according to procedures described in the International Olive Oil Council (IOOC) (2001) and IUPAC (1992) reports. In this process, dried hexane extracts were dissolved in hexane and then extracted with 2M methanolic KOH at room temperature for 30 S. The upper phase was analyzed by GC/FID and GC/MS systems.

Analysis of the essential oils

Gas Chromatography

The extracts were diluted (1/100 in hexane, v/v), and 1.0 µL was injected manually, and run in the splitless mode. GC-MS analyses of the extracts was done on an Agilent Technologies 7890A GC system coupled to a 5975C VLMSD mass spectrometer equipped with series 7683B injector. An Agilent (9091) 413 °C HP-5 column (320 µm diameter x 30 m long; 0.25 µm film thickness) was used with helium as the carrier gas at a flow rate of 3.35 mL/min. The GC oven was programmed for an initial temperature of 50 °C (hold for 1 min) and a final temperature of 300 °C (hold for 5 min) and an increase of temperature at a rate of 8 °C/min; while the trial temperature was 37 °C.

The column heater was set at 250 °C in the splitless mode at a pressure of 10.2 psi and an average velocity of 66.5 cm/sec, whit a hold-up time of 0.75 min. Mass spectrometry was run in the electron impact mode (EI) at 70eV. The percentage compositions were obtained from electronic integration measurements using a flame ionization detector (FID) set at 250 °C.

Gas Chromatography-Mass Spectrometry

The extracts were analyzed by GC-MS on an Agilent Technologies 7890A GC system coupled to a 5975C VLMSD mass spectrometer with a series 7683B injector. An Agilent (9091) 413 °C HP-5 column (320 µm diameter x 30 m long; 0.25 µm film thickness) was used with helium as carrier gas at a flow rate of 3.35 mL/min. The GC oven temperature and conditions were as described above. The injector temperature was 250 °C. Mass spectra were recorded at 70 eV over a mass range from m/z 30 to m/z 500. Aliquots (1.0 µL) of the diluted (1/100 in hexane, v/v) extracts were injected manually and run in the splitless mode.

Identification of components

The identification of the components was based on their Retention Indices data determined by reference to a homologous series of n-alkenes (C_9-C_{28}), and by comparison of their mass spectral fragmentation patterns with those of authentic compounds or with data

published in the literature as described by Adams [37]. Further identification was made by matching their recorded mass spectra with those stored on the MS library (NIST 08.L database/ ChemStation data system). Determination of percentage composition was based on peak area normalization without correction factors.

Table 1. Chemical composition (%) of the hexanic extract from flower, leaf and stem of *A. talyschensis*

Compound * (Related Fatty acid)	RT (min)	Flower (%)	Leaf (%)	Stem (%)
Octane	3.3	1.2	-	1.6
Octane, 3-methyl	4.7	-	-	0.2
Nonane	5.2	0.7	-	1.2
1-Ethyl-4-methylcyclohexane	5.5	-	-	0.4
Octane, 2,6-dimethyl	6.1	0.4	-	6.7
Octane	6.3	0.3	-	-
Heptane, 3-ethyl-2-methyl	6.3	-	-	0.4
Nonane, 4,5-dimethyl	6.7	-	-	0.5
Nonane, 4-methyl	6.9	-	-	4.4
Nonane, 2-methyl	7.0	-	-	0.4
Nonane, 3-methyl	7.1	0.3	-	-
Cyclohexane, (1-methylpropyl)	7.6	-	-	0.4
Decane	7.9	3.2	-	13.9
Undecane	8.4	-	-	11.5
Decane, 4-methyl	8.6	-	-	4.6
Decane, 3,7-dimethyl	8.8	-	-	0.3
Cyclohexane, butyl	8.9	-	-	1.9
Tetradecane	9.0	-	-	0.2
Dodecane, 2,6,10-trimethyl	9.1	-	-	0.7
2-Hexyl-1-octanol	9.5	-	-	0.5
4-Butyl-cyclohexanone	10.5	-	-	0.4
Undecane	10.9	1.1	-	-
trans-Decalin, 2-methyl	11.2	-	-	1.0
Cyclododecane	12.1	-	-	0.9
Undecane, 5-methyl	12.5	-	-	2.1
Undecane, 4-methyl	12.7	-	-	8.7
Germacrane B	13.7	-	-	0.3
Dodecane	13.8	1.4	-	12.9
Undecane, 2,6-dimethyl	14.2	-	-	10.6
Tridecane	16.6	-	-	2.5
Tetradecane	26.6	-	-	2.7
Hexadecanoic acid, methyl Ester (Hexadecanoic acid)	31.1	0.8	2.8	-
9,12,15-Octadecatrienoic acid, methyl ester(9,12,15-Octadecatrienoic acid)	32.8	-	0.6	-
Bis(2-ethylhexyl) phthalate	33.8	71.9	-	-
9,12-Octadecadienoic acid, methyl ester(9,12-Octadecadienoic acid)	34.2	3.2	76.7	-
9-Octadecenoic acid methyl Ester (9- Octadecadienoic acid; linoleic acid)	34.3	5.1	9.7	-
Eicosane	34.4	-	-	6.3
Octadecanoic acid, methyl Ester (Octadecanoic acid)	34.8	0.5	4.1	-
Eicosanoic acid, methyl Ester (Eicosanoic acid)	35.5	-	1.7	-
6,9,12-Octadecatrienoic acid, methyl Ester (6,9,12-Octadecatrienoic acid)	37.0	9.4	-	-
Docosanoic acid, methyl Ester (Docosanoic acid)	38.1	0.6	-	-
Tetracosanoic acid, methyl Ester (Tetracosanoic acid)	40.6	0.2	-	-
Squalene	42.0	0.7	-	-
Number of identified compound		17	6	29
Total		99.5	95.6	98.2

*The composition of the extracts was determined by comparison of the mass spectrum of each component with Wiley GC/MS library data and also from its retention times (RT). RT= Retention time.

Table 2. Class compositions and yield of the hexanic extract from flower, leaf and stem of *A. talyschensis*.

Class composition	Flower (%)	Leaf (%)	Stem (%)
Essential oil	0	0.7	0.3
Saturated fatty acid	1.3	9.4	0
Unsaturated fatty acid	**17.7**	**87.0**	**0**
Other compounds	80.5	0	97.9
Yield	2.2	3.4	1.8
UFA/SFA*:	**13.6**	**9.3**	0

* UFA= Unsaturated fatty acid; SFA= Saturated fatty acid

RESULTS AND DISCUSSION

The results obtained on analysis of the hexane extracts of the flowers, leaves and stems of *A. talyschensis* are listed in Table 1, which shows the retention time and of percentage of each component of the extracted oil. This study found that the hexane extract yields for parts of *A. talyschensis* were 2.2 % (flowers), 3.4 % (leaves) and 1.8% (stems) based on the dry weight of the plant materials. The highest total percentage was detected in leaves. The total fatty acid (saturated and unsaturated) content of the hexane extracts varied from 0% to 96.4% (Table 2). The major saturated and unsaturated components are shown in the Table 1. The major polyunsaturated fatty acid was 9, 12-octadecadienoic acid.

As can be seen in Table 1, about 99.5% (17 components) of the extract from flower, 95.6% (6 components) of the extract from leaves and 98.2 % (29 components) from stem extract were identified. There were some differences in the fatty acid profiles of the different part of this plant. In flower and leaf extracts, the unsaturated fatty acid contents were higher than saturated ones, whereas the fatty acids were not observed at all in stem extract of this plant. In fact, leaf extracts contain mainly unsaturated fatty acids, predominantly linoleic acid and oleic acid (76.7% and 9.7%, respectively), while the flower extract showed lesser amounts (3.2% and 5.1%) and the stem hexanic extract did not show any saturated or unsaturated fatty acid. One of the essential fatty acids was the predominant component in the leaf of *A. talyschensis*. Linolenic acid is an omega-6 fatty acid investigated only in the leaf extract in this study. The ratios of unsaturated fatty acid /saturated fatty acid (UFA/ SFA) were 13.6, 9.3 and 0 in extracts from flowers, leave and stem, respectively (Table 2). Although leaf extract of *A. talyschensis* had a higher total amount of UFA compared to flower (87.0% vs 17.7%), the UFA/SFA ratio was less for the leaf than for the flower extract. Hexadecanoic acid, octadecanoic acid and eicosanoic acid were the major saturated fatty acid in the leaf extract. Except for germacrane B (a terpene) other identified compounds in stem extract are alkanes, such as dodecane, undecane and decane.

The association of natural products with antioxidant activity in sunscreens can help to improve their photoprotective activity [38]. In addition to being bioactive, natural products are, in general, not harmful for humans, inexpensive, capable of being used in a wide range of products, and obtainable from renewable sources. However, there are few data in the literature about clinical research, functional substances, concentration and the combined effects of these antioxidants and synthetic sunscreens [39].

Indeed, byproducts from the UV photodegradation of synthetic sunscreens have been a matter of concern for more than a decade [40]. Thus, interest in natural products as active agents in sunscreens is growing [39]. For instance, some plant extracts such as those from tea, lutein, tamarind [39], propolis [41], *Passiflora incarnate* L. and *Plantago lanceolata* extracts [28] and others [39], have been reported to protect the skin against UV radiation-induced damage. In addition to health, the search for beauty aids has spurred the cosmetics industry to explore natural products from renewable source for bioactive and innovative formulations [42, 43].

CONCLUSIONS

Extracts of *A. talyschensis* were characterized with the aim of identifying compounds with potential use in health care products. It is suggested that the hexane extract of the leaves of *A. talyschensis* might be useful

in sunscreen production since a high percentage of unsaturated fatty acids was identified among the constituents of the hexane extract. Due to its higher ratio of UFA to SFA, the second priority for use is the hexane extract of the flowers of *A. talyschensis*. The use of a mixture of leaves and flowers extracts is a third priority, but use of the stem extract is not recommended. As far as we know no previous study on this herb and its application has been done before. These results suggest that extracts of *A. talyschensis* are promising natural product for use in sunscreen formulations by decreasing the concentration of synthetic chemicals in such formulations.

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