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DETERMINATION OF *ARNICA FOLIOSA* NUTT. FATTY ACIDS CONTENT BY GC/MS METHOD

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Medicinal plants have been considered as an important source for the prevention and treatment of various diseases. The genus Arnica L. is a genus of Asteraceae family, many species of which are used in traditional medicine. Arnica chamissonis Less. and Arnica foliosa Nutt., which belong to plants of the genus Arnica L., are successfully grown in the culture. There is insufficient information in the literature on the biologically active substances of Arnica foliosa Nutt. The presence of sesquiterpene lactones in the leaves and inflorescences is indicated. The flowers contain polysaccharides, monosaccharides, which mainly contain D-glucose and D-xylose, as well as phenolic compounds (quercetin, luteolin, kaempferol) and essential oils.

The aim. *The aim of our study was to identify and determine the quantitative content of fatty acids by gas chromatography/mass spectrometry method (GC/MS) in Arnica foliosa Nutt. herb.*

Materials and methods. *The determination of fatty acids composition of Arnica foliosa Nutt. was carried out by gas chromatograph Agilent 6890N with a mass detector 5973 inert (Agilent Technologies, USA).*

Results. *The analysis of Arnica foliosa Nutt. herb showed a mixture of saturated (1.61 mg/g; 48.79 %) and unsaturated (1.69 mg/g; 51.21 % from total content acids) fatty acids. The main components of Arnica foliosa Nutt. herb were palmitic (1.02 mg/g; 30.91 % from total content acids), linolenic (0.96 mg/g; 29.09 % from total content acids) and linoleic (0.67 mg/g; 20.30 % from total content acids) acids. This raw material is a source of essential fatty acids, such as omega-3 (linolenic acid) and omega-6 (linoleic acid).*

Conclusions. *As a result of Arnica foliosa Nutt. research, the presence of fatty acids is established in its raw material. The dominant fatty acids in the studied raw material were palmitic, linolenic and linoleic acids, the content of which was 30.91 % (1.02 mg/g), 29.09 % (0.96 mg/g) and 20.30 % (0.67 mg/g) from total content acids, respectively. The result shows that Arnica foliosa Nutt. is the source of fatty acids, so the use of this plant raw material for new remedies is possible in the future*

Keywords: *Arnica foliosa Nutt., herb, fatty acids, GC/MS, linolenic acid, linoleic acids*

1. Introduction

The search for interesting new plants with a long history of use, minor side effects and well tolerated by patients, regardless of age, is an important object of interest in society [1, 2]. 25 % of herbal remedies in the modern pharmacopeia are plant-derived whereas many synthetic drugs are made by means of substances isolated from plant [3]. Medicinal plants have become considered an important source for the prevention and treatment of various diseases [4].

The most typical plants for the treatment of diseases are from family *Asteraceae*, which contains more than 23,000 species, widespread in different regions all over the world [5, 6]. The genus *Arnica* L. is from family *Asteraceae*, many species of which are used in traditional medicine [7]. The most common species of the genus are *Arnica montana* L., *Arnica unalaschcensis* Less., *Arnica sachalinensis* A. Gray, *Arnica foliosa* Nutt., *Arnica Lessingei* Green, *Arnica alpina* Olip et Ladau, *Arnica frigida* L. and *Arnica intermedia* Turcz. The plants of the genus *Arnica* L. are most common in North America, less in Eurasia and North Africa [8]. *Arnica chamissonis* Less., which is found in the USA, Eastern Europe, the Far East, and *Arnica foliosa* Nutt., which originates from the steppe regions of North America, are successfully grown in the culture [8, 9].

Arnica montana L. is an alpine perennial plant, distributed on mountain slopes in Europe, Siberia, America and North Asia [10]. Preparations made from its flowers are widely used as a herbal remedy for medical purposes [11, 12]. *Arnica montana* L. has long been used in pharmacy and cosmetics due to its anti-inflammatory, antioxidant, antibacterial, antiseptic and antifungal activities [13, 14]. The chemical constituents in *Arnica* comprise a complex of chemical compounds including flavonoids, phenolic acids [13], carotenoids, sesquiterpene lactones [15], diterpenes, essential oils [16], coumarins and lignans [10]. It contains large amounts of luteolin, apigenin, kaempferol and quercetin glycosides, sesquiterpene lactones such as isobutyryl, metacryl, methacryloyl and tygloyl [10, 11].

There is not enough information in the literature about biologically active substances of *Arnica foliosa* Nutt. The presence of sesquiterpene lactones in its leaves and inflorescences is indicated [17]. Polysaccharides, monosaccharides are available in its flowers, the compo-

sition of which is presented mainly with D-glucose and D-xylose, and also phenolic compounds (quercetin, luteolin, kaempferol) and essential oils [18].

Having analyzed the available sources of literature on the current state of the studied plants of the genus *Arnica*, it should be noted that *Arnica montana* L. is the most studied and widely used in folk and scientific medicine and homeopathy among the plants of the genus. Other species, in particular *Arnica foliosa* Nutt., which is cultivated in Ukraine, has not been studied in chemical and pharmacological aspects.

Thus the aim of our study was to identify and determine the quantitative content of fatty acids by gas chromatography/mass spectrometry method (GC/MS) in *Arnica foliosa* Nutt. herb.

2. Planning (methodology) of research

Considering the lack of information in the literature about the content of fatty acids of *Arnica foliosa* Nutt. their definition in this raw material is relevant. For the study of fatty acids contexture, the method of gas chromatographic mass spectrometry was selected, which is one of the best appropriate methods for the identification of multicomponent mixtures of volatile substances. This method is based on a combination of two individual methods chromatography and mass spectrometry (the first separate the mixture into components; the second to identify the substance and quantitative content). The quantitative content of fatty acids was investigated by the internal standards method. The method of the internal standard has a preference, because when used it is not needed to determine the complete composition of the mixture and the results of the analysis do not depend on the size of the sample.

3. Material and method

3.1. Plant materials

Arnica foliosa Nutt. herb was selected as the object of study. Plant raw material was collected at the M. M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine in summer 2019. The raw material was authenticated by prof. Svitlana Marchyshyn (TNMU, Ternopil, Ukraine).

3.2. Chemicals and standards

All solvents for metabolite extraction were purchased as HPLC grade, including methanol, heptane and toluene from Sigma-Aldrich (St. Louis, MO). Fatty acids were identified by the reference standard mixture FAME (Supelco, Belle fonte, PA, USA). The internal standard nonadecanoic acid used for metabolite quantification was purchased from Sigma-Aldrich (St. Louis, MO).

3.3. GC/MS determination of fatty acids

GC/MS analysis of fatty acids was performed using gas chromatograph Agilent 6890N with mass detector 5973 inert (Agilent Technologies, USA). Samples were analyzed on a silica capillary column HP-5MS (apolar) length – 30 m, internal diameter – 0.25 mm, the diameter of sorbent grain – 0.25 μm [19]. First, the oven temperature was set at 60 °C for 4 minutes, then at a rate of 4 °C/min was raised to 250 °C and kept at this point for 6 min and maintained at a final temperature for 7 min.

Helium was used as the carrier gas at a constant flow rate of 1.0 ml/min. The sample with a volume of 1 μl was injected in a splitless mode using 7683 series Agilent Technologies injector. Detection was performed in scan mode in the range (38–400 m/z).

0.5 g (accurately mass) of the raw material was refluxed with a 3.3 ml mixture containing (methanol:toluene:sulfuric acid (44:20:2 *v/v*)) and 1.7 ml of internal standard solution (nonadecanoic acid in heptane solution). The sample was maintained in the ultrasonic water bath at 80 °C for 2 h. The resulting mixture was allowed to cool and centrifuged for 10 min at 5000 rpm. Then 0.5 ml of the upper heptane phase with containing methyl esters of fatty acids was selected.

The compositions of the product obtained were identified by comparison of their mass-spectrums with data obtained from National Institute Standard and Technology (NIST, 2008) database. The quantitative content of fatty acids was done using internal standard of nonadecanoic acid in heptane solution added to the sample.

The amount of fatty acids in mg/g was calculated according to the following equation:

$$X = \frac{Sx \times Minst \times 1000}{Sinst \times m},$$

where S*x* is a peak area of each fatty acid,

M*inst* is a mass of the internal standard,

S*inst* is a peak area of the internal standard,

m is a mass of a plant material [20–22].

3.4. Statistical analysis

Statistical processing and data analysis were performed using Statistica v 10.0 program package for Microsoft Office for Windows. The level of significance was set at $^{*}p<0.05$ for all statistical analyses.

4. Results

In total, nine fatty acids were determined in the *Arnica foliosa* Nutt. herb, including palmitic, stearic, nonadecanoic, arachidic, behenic, lignoceric, 9-lauroleic, linoleic and linolenic acids by means of the GC/MS method (Fig. 1, Table 1).

The quantitative content of fatty acids is presented in Table 1.

The quantitative content of saturated and unsaturated fatty acids is almost the same. As shown in Table 1, *Arnica foliosa* Nutt. herb contains a mixture of fatty saturated (1.61 mg/g; 48.79 % from total content acids) and unsaturated (1.69 mg/g; 51.21 % from total content acids) acids.

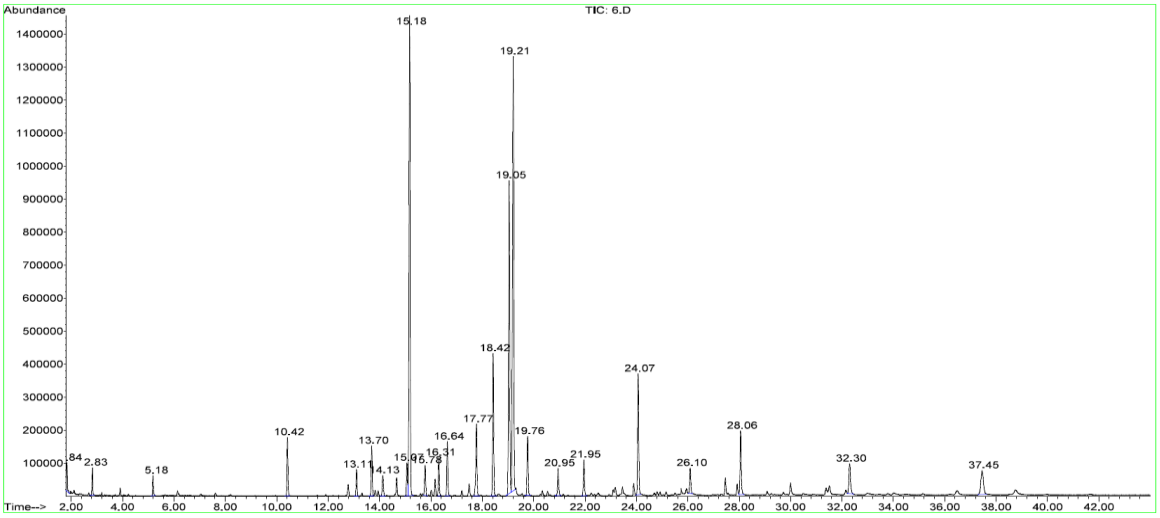

Fig. 1. GC/MS chromatogram of fatty acids in *Arnica foliosa* Nutt. herb

Table 1

The results of fatty acids determination in *Arnica foliosa* Nutt. herb.

No.	Retention time	Common name of fatty acid (IUPAC)	Chemical nomenclature	Quantitative content of methyl esters of fatty acids	
				mg/g	% of the total
Saturated acids					
1	15.18	Palmitic (hexadecanoic)	C 16:0	1.02±0.04	30.91
2	19.76	Stearic (octadecanoic)	C 18:0	0.12±0.01	3.64
3	21.95	Nonadecanoic	C 19:0	internal standard	
4	24.07	Arachidic (eicosanoic)	C 20:0	0.24±0.02	7.27
5	28.06	Behenic (docosanoic)	C 22:0	0.14±0.01	4.24
6	32.30	Lignoceric (tetracosanoic)	C 24:0	0.09±0.01	2.73
Monounsaturated acids (ω-9)					
7	15.07	9-lauroleic ((*E*)-dodec-9-enoic acid)	C 12:1	0.06±0.01	1.82
Polyunsaturated acids (ω-3 and ω-6)					
8	19.05	Linoleic (octadecadienic, ω-6)	C 18:2	0.67±0.01	20.30
9	19.21	Linolenic (octadecatrienic, ω-3)	C 18:3	0.96±0.02	29.09
The amount of saturated fatty acids				1.61	48.79
The amount of unsaturated fatty acids				1.69	51.21
Total				3.3	100

5. Discussion

The biological role of saturated fatty acids is that they are a source of energy for the human body. They are involved in the transfer and absorption of vitamins and trace elements, hormone synthesis and construction of cell membranes. Unsaturated fatty acids also play an important role in the body's vital functions.

The results of the study showed that the major components of *Arnica foliosa* Nutt. herb were palmitic (1.02 mg/g; 30.91 % from total content acids), linolenic (0.96 mg/g; 29.09 % from total content acids) and linoleic (0.67 mg/g; 20.30 % from total content acids) acids. This raw material is a source of essential fatty acids, such as omega-3 (linolenic acid) and omega-6 (linoleic acid), which must be in the diet, as the body needs them, but cannot synthesize. Linolenic and linoleic acids are the starting point for the synthesis of other unsaturated fatty acids.

Linoleic acid is the most consumed polyunsaturated fatty acid found in human nutrition [23]. This poly-

unsaturated acid provides normalization of hormonal balance and metabolic processes, production of bile acids in liver, production of prostaglandins and, also, improves the work of peptic enzymes [21]. Linoleic acid functions as a structural component to support a membrane liquidity level of epidermis transdermal water barrier [23]. Linoleic acid is converted in the body to γ-linolenic acid, which is the most active, and is converted to prostaglandin E1, which increases immunity. Prostaglandins provide suppress inflammatory processes, reduce the likelihood of cardiovascular disease, normalize the nervous system, regulate insulin levels, brain function and metabolism. α-Linolenic acid is also polyunsaturated fatty acid found mostly in plant food. Linolenic acid performs a number of vital functions, such as producing prostaglandins, normalizing blood cholesterol levels and blood pressure [21]. Furthermore, in the body human cardioprotective and other health effects of this acid have been characteristic to its precursor role in converting to eicosapentaenoic acid [24].

The fatty acids profile plays an important role in chemical properties, so it is useful information for further research.

Study limitations. Some of the acids compounds were not identified during the study. Also, for the statistical importance of the study, it would be expedient to investigate, even wild samples of *Arnica foliosa* Nutt. from various regions of Ukraine.

Prospects for further research. The obtained results might be used in the standardization and quality assurance of new remedies containing *Arnica foliosa* Nutt. herb.

6. Conclusion

As a result of *Arnica foliosa* Nutt. research, the presence of fatty acids is established in its raw material. The qualitative composition and quantitative content of fatty acids were studied by GC/MS method. Nine fatty acids were determined in *Arnica foliosa* Nutt. herb. The content of unsaturated fatty acids was 51.21 % (of the total amount of all detected fatty acids), and of saturated – 48.79 %. The dominant fatty acids in the studied raw material were palmitic, linolenic and linoleic acids, the content of which was 30.91 % (1.02 mg/g), 29.09 % (0.96 mg/g) and 20.30 % (0.67 mg/g) from total content acids, respectively. The result shows that *Arnica foliosa* Nutt. is the source of fatty acids, so its raw material can be used as a source for new remedies in the future.

Conflict of interests

The authors declare that they have no conflicts of interest.

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