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ANALYSIS OF THE FATTY ACID CONTENT OF *SIDERITIS SCARDICA* GRIS. USING *GC-FID*

SUMMARY

In large parts of the world, various plants are used in treatment and cures of various diseases. *Sideritis* species are plentiful of important metabolites such as terpenes, flavonoids, essential oils and sterols. Fatty acids are known as important bio-composites that participate in complex metabolic pathways, thus they possess key biological roles. Considering that increasing collection and consumption of plants and extracts of *S. scardica* for therapeutic purposes, it is crucial to accomplish qualitative chemical analysis of extracts, to determine the content of fatty acids into them. Analysis and extraction of fatty acids was done by GC-FID gas chromatography. The fatty acid content into the plant extract from *S. scardica* Griseb. was done according to the modified AOAC Official Method 996.06 (2005). Based on the results achieved we can conclude that the fatty acid profile into the plant extract of *S. scardica* contains saturated fatty acids (SFA) and, in larger amounts, unsaturated fatty acids (UFA), respectively oleic and linoleic acid (as representative of omega-6 essential acids).

INTRODUCTION

Throughout the ages, people have mainly relied on plants as a source of food, fragrances and medicines. Even nowadays an immense number of people use medicinal plants that contain compounds as an essential resource in the prevention and treatment of various diseases or in addition to improve health in general (Rakotoarivelo *et al.,* 2015; Carović-stanko *et al.,* 2016). Every plant contains important bio-components which can be used in medi-

cine and they also can be involved in the production of different types of herbs. In large parts of the world, various plants are used in treatment and cures of various diseases (Yuan *et al.,* 2016).

Plant metabolites are vitally important for plant development, strength and protection. Various studies have been performed on the identification, biochemical characterization, location and their health benefits (Park *et al.,*2019). *Sideritis scardica* Griseb. (mountain tea) is considered as an endemic plant of the Balkan Peninsula, with an abundance in the high mountains of Bulgaria, NorthMacedonia, Albania and Greece. The aerial parts of this plant are traditionally known for their anti-inflammatory, antimicrobial, antibacterial, anti-rheumatic and gastro-protective properties. *S. scardica* may be used as an agent in alleviating bronchitis and bronchial asthma, in common colds as well as in cases of pulmonary emphysema (Tadić*et al.,* 2012). *Sideritis* plants species are plentiful of important metabolites such as terpenes, flavonoids, essential oils and sterols. According to existing references, various metabolites have been isolated from *Sideritis* species from Mediterranean region, same as the our samples from Balkan area (Ema / Hmpc, 2016).

Fatty acids are known as important bio-composites that participate in complex metabolic pathways, thus they possess a key biological roles. They are obtained from various dietary sources which determine the type of fat consumed and the health outcome (Tvrzicka *et al.,* 2011). Essential fatty acids are essential for the normal functioning of the body, but they must be obtained through the plant-based diet (Glick and Fischer, 2013). Considering that increasing collection and consumption of plants and extracts of *S.scardica* for therapeutic purposes, it is crucial to accomplish qualitative chemical analysis of extracts, to determine the content of fatty acids into them.

The main objective of this study was to identify and determine the content and type of fatty acids that are present in the extract of *Sideritis scardica* Griseb.

MATERIAL AND METHODS

Aerial parts of plants were collected from Sharr Mountain, North Macedonia during the periodJune - July 2021. After determination, plant material was dried in natural conditions, at an airy, dry and shadowedplace and a temperature of 20-25° C, until there was no more change in mass. A considered amount of dried plant material was ground with a mixer (PRIMAX, Keine G Nummer, Germany) for around 10 minutes. The finely chopped herbs were weighed and separated by 10 g each into jars, each of them were usedfor extraction.

Analyze and extraction of fatty acids usually is done by gas chromatography. Preparation of the samples for a gas chromatography includes two different procedures: extraction and metilation.

Extraction and metilation

The fatty acid content of the plant *Sideritis scardica* Griseb. was determined according to the modified AOAC Official Method 996.06 (2005). About 30 g (3 tubes x 10 g) of finely chopped and homogenized plant was dissolved in 60 ml (20 ml in each tube) of isopropane : hexane (40:60). The samples were then centrifuged for 10 min, at 4000 rpm, at + 4°C. The fat layer was transferred to a 10 ml glass test tube and evaporated to dryness under Nitrogen vapor. 100 mg of the extracted fat was transferred to another 22 ml dark vessel, where 0.1 ml of Sodium methanoate, 1.0 ml of 14% BF3 reagent and 1.0 ml of toluene were added to convert the fatty acid esters (FAMEs).

The dishes were transferred to a water bath at 100°C for 45 min. Every 15 minutes the dishes were lightly mixed. After heating, the dishes were kept to room temperature (20-25°C) and 5.0 ml of distilled water, 1.0 ml of hexane and 1.0 g of anhydrous Sodium sulfate were added. The samples were mixed in the mixer for around 1 min. After separating the layers, the top layer was transferred to another 2 ml test tube. FAME determinations were performed using a GC-FID 5890 (Agilent, USA). Sigma (Sigma-Aldrich, Germany) provided individual standards of methylated esters of fatty acids: capricacid (C10: 0), myristic acid (C14: 0), palmitic acid (C16: 0), palmytoleic acid (C16: 1), margaric acid (C17: 0), heptadecenoic acid (C17: 1), stearic acid (C18: 0), oleic acid (C18: 1n9c), linoleic acid (C18: 2nc6), arachidic acid (C20: 0), γ linolenic acid (C18: 3n6), and α-linolenic acid (C18: 3n3). Identification and determination of fatty acids was done by GC-FID 5890 (Agilent, USA) according to the procedure described by Hajrulai-musliu *et al.* (2015).

Data processing of the achieved results was performed using Microsoft Office Excel (2007) and SPSS version

26. Variables are expressed as mean ± standard deviation (SD) and relative frequency (%).

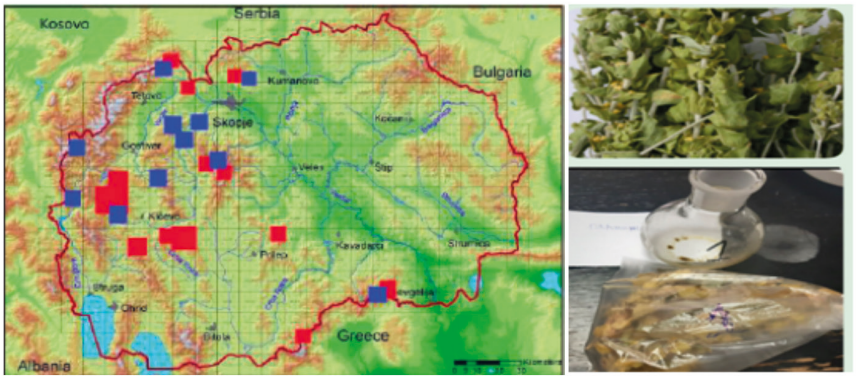

Fig. 1. The plant *Sideritis scardica* and its geographic distribution in North Macedonia ■ Herbarium data ■ Literature data (Matevski, 2019).

RESULTS AND DISCUSSION

Data from the previous studies of the chemical profile of fatty acids in the plant type of *Sideritis scardica* Griseb. resulted that this type of plant contains saturated fatty acids (SFA) and unsaturated fatty acids (UFA). The identification and content of fatty acids are presented in table 1.

Tab. 1. The composition of fatty acids into the plant type *S. scardica* extract(pAs-peak area, Amt/Area, Norm –defining data for peak area) [a] saturated fatty acids; [b] monounsaturated fatty acids; [c] polyunsaturated fatty acids.

Name	Retention time Min	Area [pAs]	Amt/Area	Norm %
C 10:0 (capric acid)[a]	13.647	4.62170	7.29156e-3	1.258
C 14:0 (myristic acid)[a]	18.401	6.25667	6.39684e-3	1.494
C 16:0 (palmytic acid)[a]	20.721	103.11915	5.12219e-3	19.719
C 16:1 (palmytoleic acid)[b]	21.395	7.80041	5.96558e-3	1.737
C 17:0 (margaric acid)[a]	22.280	9.21938	7.85065e-3	2.702
C 17:1 (heptadecenoic acid)[b]	22.716	7.71667	5.38841e-3	1.552
C 18:0 (stearic acid)[a]	23.327	35.18482	5.60533e-3	7.363
C 18:1n9c (oleic acid)[b]	24.050	192.96011	5.31906e-3	38.318
C 18:2n6c (linoleic acid)[c]	25.231	92.84067	5.87517e-3	20.363
C 20:0 (arachidic acid)[a]	26.245	8.06275	1.11920e-2	3.368
C 18:3n6 (γ-linolenic acid)[c]	26.678	8.24690	3.85215e-3	1.186
C 18:3n3 (α-linolenic acid)[c]	27.018	3.18996	7.85814e-3	0.935

In *S. scardica* extract the phytochemical profile of fatty acids includes: capric, myristic, palmytic, palmytoleic, margaric, heptadecenoic, stearic, oleic, linoleic, arachidic, γ-linolenic and α-linolenic acids. The table above gives the data for retention time and concentration expressed in % for each of the fatty acids encountered in this type. From these data we have concluded that in the composition of *S. scardica* extract in a higher percentage was found oleic acid (38.31%), linoleic acid 20.36%) and palmitic acid (19.71%). Data which correlate with the previous study conducted by Hajdari *et al.,* 2020, fatty acids and hydrocarbons are the main components in the composition of the aerial parts of mountain tea (22.25% and 20.72%) (Hajdari *et al.,* 2020).

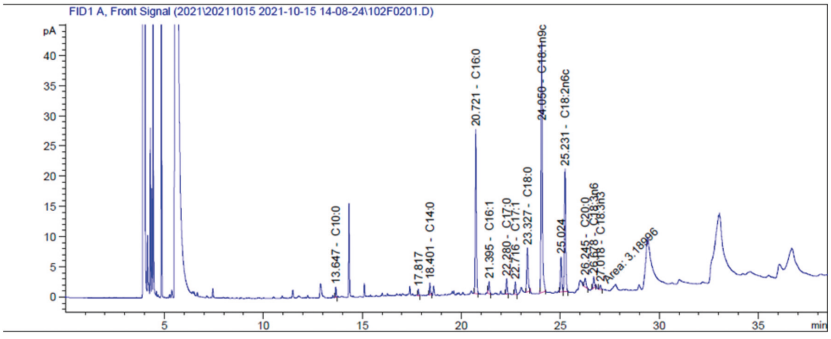

Fig. 2. GC-FID chromatogram of methylated fatty acids.

Figure 2 shows the chromatogram obtained from the fatty acid analysis with GC-FID. This chromatogram shows the fatty acids identified in the composition of the plant *S. scardica* which corresponds to the data presented in Table 1. In total, 12 fatty acids have been identified.

Tab. 2. Tabular presentation of relative percentages of saturated fatty acids (SFA) and unsaturated fatty acids (UFA).

	N	Range	Minimum	Maximum	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
SFA1	6	18.46	1.25	19.71	5.9783	2.89006	7.07917	50.115
MUFA	3	36.76	1.55	38.31	13.8633	12.22344	21.17163	448.238
PUFA	3	19.43	.93	20.36	7.4900	6.43540	11.14645	124.243
Valid N (listwise)	3							

From the extraction of fats with hexane-isopropane of the *S. scardica* extract the average value of saturated fatty acids was 5.97 ± 7.07, with a minimum value of 1.25% (capric acid) and a maximum value of 19.71% (palmitic acid). Unsaturated acids (MUFA) were encountered with an average value of 13.86 ± 21.17. This group includes fatty acid which are found

in the highest percentage in the extract of *S. scardica* oleic acid (38.31%), while PUFA are encountered with an average value of 7.49 ± 11.14. From the obtained results we confirm that in the composition of this plant extract in a higher percentage were found unsaturated fatty acids (64.06%) compared to saturated fatty acids (35.93%). From the group of essential fatty acids which can not be synthesized in the human body omega-6 (linoleic acid) of the type *S. scardica* is present in a percentage of 20.36%, γ-linolenic acid 1.18% while the group of omega 3 fatty acids (α-linolenic acid) is found in traces. The focus of much worldwide research is on studies related to the nutritional deficiency of essential fatty acids and the special roles of omega-6 and omega-3 because it has been proven that PUFAs are necessary for the normal development and functioning of the brain and heart, as well as for the balance of all tissues and organs (Sokola wysoczańska *et al.,* 2018).

CONCLUSION

During this study we have determined, identified and compared the types of fatty acids that were present at the plant extract of *Sideritis scardica* Griseb. Based on the results achieved we can conclude that the fatty acid profile into the plant extract of *S. scardica* contains saturated fatty acids (SFA) and, more abundantly, unsaturated fatty acids (UFA), respectively oleic and linoleic acid (as representative of omega-6 essential acids).

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