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Original scientific paper

THE EFFECT OF GRANULE SIZE OF THE RAW MATERIAL AND THE SOLVENT CONCENTRATION ON THE EXTRACTION OF HERBA SIDERITIS SCARDICA IN MOUNTAIN SHARR

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Abstract

Sideritis Scardica belongs to the group of herbal raw plant materials found in the mountains, mainly in Balkan countries such as Bulgaria, North Macedonia, Montenegro, the northern part of Turkey, and some parts of North Russia. It is characterized by dense leaves attached directly to the main stem. It has been known as a pharmaceutical plant since ancient times it was used in preparing various teas, without knowing the miraculous features of this plant. In recent years, the raw plant material *Sideritis Scardica* has been increasingly used in pharmaceutics in preparing standardised extracts, which have multiple effects due to its composition. It is not that simple to get an extract of *Sideritis Scardica*, it requires adequate adjustment of extraction parameters as well as choosing an adequate extraction method. Within the extraction parameters, despite the extraction method that is chosen, a very important role has the grinding level and the type of solvent for extraction, as well as the concentration of the solvent to get a higher extraction coefficient, and the ratio of the raw material used to the obtained extract should be as low as possible. The extraction parameters must be adjusted in the way that the extraction process is realized till the end, and take out the main substances that are present in the raw material, hindering the extraction of unwanted constituent substances.

Keywords: Extraction, maceration, percolation, extraction coefficient_

1. Introduction

Sideritis scardica is a pharmaceutical plant that grows in the high mountains and has a height of up to 50 cm. It is characterized by a base stem on which very dense leaves are attached, on the top of which dominate the flowers in the form of fruits, which are also supposed to be the source of the largest amount of main substances. In the research that has been made, it is found that there are about 150 types of species, which have very small differences in their form, but in the content of the constituent substances, they differ a lot, and this has to do with the altitude where they grow (Knörle, R. 2012)
Sideritis scardica, also known as mountain tea, is a plant that belongs to the family Lamiaceae, which grows mainly in the Balkan countries, so depending on the altitude where it grows, the amount of its constituent substances also differs. Using this plant in the form of tea, it is a traditional drink that is considered as a tea for curing a lot of diseases. In recent years, there has been initiated research on the pharmacological features of this plant, mainly about the influence of its constituent substances on the central nervous system, and its ability to reduce β-Amyloid, in preventing dementia and Parkinson (Assenov G (1978).
In the last 10 years, the research on the composition and healing features of *Sideritis scardica* has not stopped and includes a wide field of research, starting from its sedative properties and the reaction of its components in the nervous system, which seem to have more special emphasis Serotonin, Dopa-mine and Noradrenaline, these components have a crucial effect in curing

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features, so depending on their quantity, the curing effects are different, too (Österreichisches Lebensmittelbuch, 2007). According to the latest results from the research, there are indications for positive effects on the central nervous system, especially for its antidepressant features, sedative effect, for coughs caused by cold and bronchitis, helps with gastrointestinal problems, inflammation of the stomach mucosa and stomach ulcers, stomach pain, and bloating. According to recent studies, Sideritis tea reduces the formation of plaques in the brain and improves memory. These studies are still in the initial stage, but there will be clinical studies so that this tea can also be used for Alzheimer's disease. (Mahi Latifi, Nexhbedin Beadini, Zekovic Mario, Ejup Latifi 2023). The variability in the chemical composition of this medicinal plant, depending on the origin, requires the preparation of a protocol for its standardization. In practice, it is important to improve the growth conditions to increase the accumulation of main substances and get raw materials of lasting and good quality. This is to insist on the production of this plant in plantations, and there will be performed a continuous, precise control of the substances of main components of this plant, depending on its use in pharmaceuticals. (Milka Todorova, Antoaneta Trendafilova 2014). In the last decade, *Sideritis scardica* from Bulgaria, the Republic of North Macedonia and Greece has been generally studied for the content of phenolic components, terpenoids, hydrocarbons and related compounds, as well as essential oil components. There were suggestions that phenolic and polyphenolic components create the antioxidant activity of extracts obtained with alcohol, while the terpene components from the essential oil and the diterpenoids have anti-inflammatory, analgesic, antiulcer, antibacterial, antifungal, cytotoxic, and antitumor effects.

This research aims to provide an updated and comprehensive overview of the botany, conservation, and cultivation, phytochemistry, traditional uses, and pharmacological activities of Sideritis scardica (E. González-Burgos, ME Carretero, MP Gómez-Serranillos 2011). A recent study performed in mice has shown the anti-inflammatory and gastroprotective effects of Sideritis scardica, as well as the cytotoxic effects on tumour cell lines HL60 and PBMC of extracts and flavones of S. scardica (VM Tadić, I. Jer-emic, S Dobric, A. Isakovic, I. Markovic, V. Trajkovic, I. Arsic 2012).

Knowing the use of Sideritis Scardica, more detailed research is required to obtain more accurate in-formation on traditional and pharmaceutical uses, as well as the potentially different effects and basic mechanisms of the bioactive components of Sideritis Scardica for different types of extracts. And to investigate in vitro anti-inflammatory effects and isolated polyphenols of S. scardica, focusing on neurodegenerative diseases such as Alzheimer's disease (AD) to discover new bioactive compounds that can be used for the same, and contribute to improving global health challenges related to AD. (Nas-taran Moussavi, Hasina Azizullah, Karl E. Malterud, Kari T. Inngjerdingen, Helle Wangensteen 2022).

Among the constituent components of Sideritis scardica are Terpenes, which belong to the group of organic hydrocarbons found in biodiversity. The so-called diterpenes are interesting to analyse from a pharmacological point of view. This group of substances is known for its anti-inflammatory, antibacterial and antifungal effects. Flavonoids are part of this too, which are presented as bioflavonoids, isoflavonoids and neoflavonoids. It is found that they have antioxidant and anti-inflammatory effects. In particular, the flavonoids of Sideritis species act as selective inhibitors of monoamine oxidases. (MAO). Therefore, these active components are the focus of pharmacologists for producing new drugs for the treatment of mental illnesses, depression, anxiety, and dementia or dementia disease. A study by the University of Nicosias found that *Sideritis Scardica* contains over 20 types of constituent compounds, the main ones were Beta-Caryophyllene (23%), Beta-Pinene (11%), Beta-Bisabolene (8%) and 1-Octen-3 -ol (8%). Which are distinctive components of antioxidants (Yaneva, I.; Balabanski, V.)

2. Body of Manuscript

The purpose of this work is to adjust the optimal size of granules of the ground raw material to achieve an optimal extraction coefficient, and on the other hand to choose an adequate solvent for extraction to fully extract the accompanying constituent components in the raw material *Sideritis scardica,* first the constituent components which are of particular importance, which affect the curing features of *Sideritis scardica.* First, it was required to find the optimal size of granules, therefore there were made two granulometric analyses with different degrees of grinding Table 1 Figure 1 and Table 2 Figure 2. There were weighed 50 grams of *Sideritis scardica* with a grinding sieve of 1 mm for the first sample, and a 1.5 mm sieve for the second sample, the analysis of the sieves was made within a time interval of 10 minutes, and two different fractions of granules size were obtained as can be seen from Table 1, Figure 1 and Table 2, Figure 2, both samples have a different level of grinding we need to monitor the degree of extraction for both samples and the extraction coefficient. The extraction is made with water at a temperature of 50 °C, and the sample that has the highest extraction coefficient was also used for other analyses with different solvents.

2.1 In a percolator in the laboratory, there were weighed 5 grams of the first sample and there were added 100 ml of water at a temperature of 50°C.In a second percolator in the laboratory, there were also weighed 5 grams of the second ground sample there were added 100 ml of water at a temperature of 50 °C, The extraction process with percolation lasts 6 hours for both samples. For different times of percolation, it was taken by one sample of the extract and filtered with a 2 µm microfilter, thedry mass was measured and it was created the extraction curve Table 3, Figure 3 and Table 4 Figure 3. According to the results, which means the dry mass during extraction with hot water, it is found that the first sample has a higher dry mass, that is the size of granules is more suitable for extraction, and the same sample will be used for analysis of the extraction with various solvents. Although the high level of grinding in most cases supports the extraction process, such a thing has not happened, which implies that the highest level of grinding is accompanied by technical obstacles for the realization of percolation, creating solutions with pellets inside, so the active surface contacting granules with the solvent is reduced. There were analyses of three solvents ethyl alcohol with a concentration of 20%, 40% and 60%

2.2 In a percolator in the laboratory, there were weighed 5 grams of ground raw material according to the grinding level Table 1 Figure 1 and there was added to it 100 ml of Ethanol 20%, with a temperature of 50°C, percolation lasted 6 hours. At different times of extraction, there was taken by one sample which underwent a filtration with a 2 µm micro filter and based on it the dry mass was weighed, and the extraction curve was created. Table 5 Figure 4.Based on the results, extraction with Ethanol 20% is ideal for percolation extraction and if we ana-lyse the extraction with water and Ethanol 20%, we can conclude that both solvents are almost equally efficient for extraction, even though the extraction with water in the further technological process is more difficult to be realized precisely in the stage of concentration of the extract. Even though there are obtained satisfactory values of a dry mass, we need to find which constituent substances have been extracted and this depends on the practical pharmaceutical application.

2.3 In a percolator in the laboratory, there were weighed 5 g of the raw material Sideris scardica with a level of grinding Table1, Figure 1, there were added 100 ml of solvent Ethanol with a concentration of 40% and a temperature of 50°C, making the percolation last 6 hours. During the percolation time, for different times of extraction, there were taken which were filtered with a 2 μm filter and the dry mass of the extract was found, with this it was created the extraction curve Table 6, Figure 5. From the results, we find that the highest concentration of ethyl ethanol is not necessarily more efficient for extraction compared to the extraction with water and 20% ethyl ethanol, but this is only the extraction coefficient and not for the main substances of Sideritis scardica

2.4. The same analysis was made with the same amount of raw material of 5 g and 100 ml of 60% ethyl ethanol at a temperature of 50°C, and based on the weighed dry mass, it was created the extraction curve Table 7 Figure 5. Based on the results, as expected, the concentration of the solvent does not have a very large impact on the extraction.

3. Table Figures

Table 1: Granulometric analysis of *Sideritis scardica* sample 1

Size of strainer	**Measuring vessel [gr]**	**Vessel + raw material [gr]**	**Netto [gr]**
8.00 mm	451.58	451.58	0.00
4.00 mm	430.32	430.32	0.00
2.00 mm	400.32	402.70	2.38
1.00 mm	361.97	385.34	23.37
0.50 mm	322.47	337.80	15.33
0.25 mm	289.58	293.84	4.26
0.125 mm	280.18	282.90	2.72
Sludge	400.88	403.82	2.94

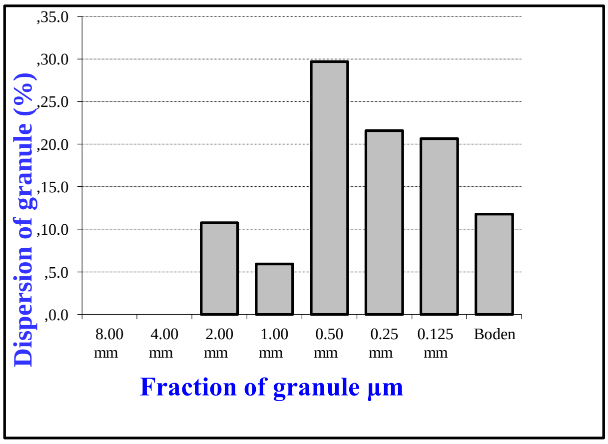

Figure 1. Fraction of granule *Sideritis scardica* **s**ample 2

Table 2 Granulometric analysis of

Sideritis scardica sample 2

size of strainer	**measuring vessel [gr]**	**vessel + raw material [gr]**	**netto [gr]**
8.00 mm	451.58	451.58	0.00
4.00 mm	430.32	430.32	0.00
2.00 mm	400.32	405.78	5.46
1.00 mm	361.97	364.97	3.00
0.50 mm	322.47	337.50	15.03
0.25 mm	289.58	300.50	10.92
0.125 mm	280.18	290.63	10.45
Sludge	400.88	406.85	5.97

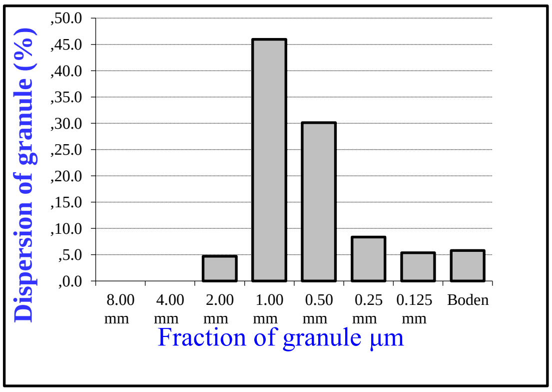

Figure. 2 Fraction of granule *Sideritis scardica* sample 2

Table 3 Results of dry mass in relation to the extraction time *Sideritis scardica* sample 1

Sample 1	**Water**
time min	**Dry content%**
0	0
60	1.38
120	2.13
180	2.87
240	3.49
300	3.93
360	4.22

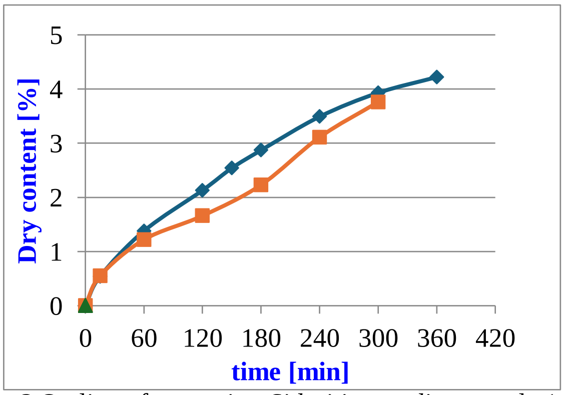

Figure.3 Outline of extraction *Sideritis scardica* sample 1,2

Table 4 Resultsof dry mass in relation to the extraction time *Sideritis scardica* sample 2

Sample 2	**Water**
time min	**Dry content%**
0	0
60	1.22
120	1.66
180	2.23
240	3.11
300	3.76
360	3.91

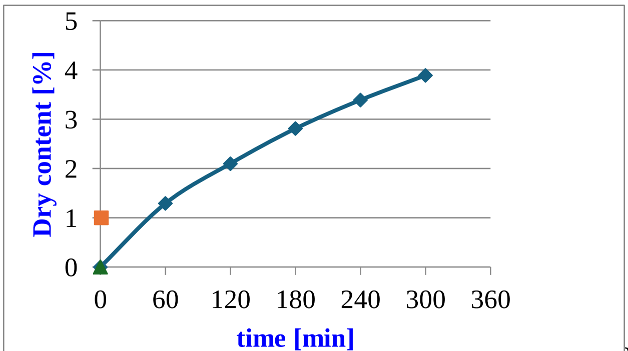

Figure 4. Outline of extraction *Sideritis scardica* sample 3

Table 5 Results of dry mass in relation to the extraction time *Sideritis scardica* sample 3

Sideritis Sc.Eth.20%	**Sample 3**
time min	**Dry content%**
0	0
60	1.29
120	2.1
180	2.81
240	3.39
300	3.89
360	4.13

Table 6 Results of dry mass in relation to the extraction time *Sideritis scardica* sample 4

Sideritis Sc.Eth.40%	**Sample 4**
time min	**Dry content%**
0	0
60	1.18
120	1.96
180	2.57
240	3.01
300	3.64
360	4.03

Table 7 Results of dry mass in relation to the extraction time *Sideritis scardica* sample 5

Sideritis Sc.Eth.60%	**Sample 5**
time min	**Dry content%**
0	0
60	1.34
120	2.13
180	2.66
240	3.12
300	3.48
360	4.29

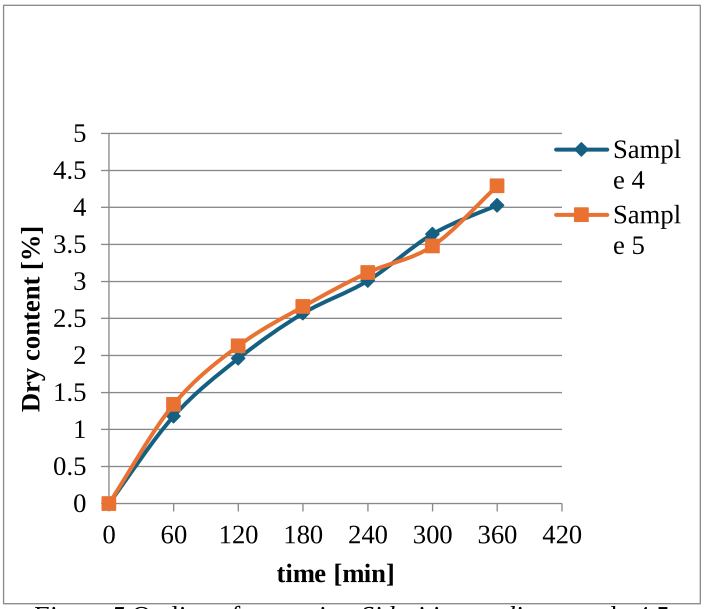

Figure.5 Outline of extraction *Sideritis scardica* sample 4,5

4. Conclusion

Based on the analyses made for different levels of grinding of the raw material *Sideritis s*cardica and the use of different types of solvent and their concentration, we can conclude that the level of grinding is a key factor for the realization of the extraction process, with the higher level of grinding we get a lower extraction coefficient, as a result of the creation of a non-homogeneous solution, by creating a solution in the form of pellets, which reduce the contact ability of the raw material and the solvent. We may conclude that the use of water as a solvent has a good level of extraction with a good dry mass, but in a technological process, it is not the most preferred solvent due to the difficulties that occur during further processing to get the extract, firstly during the process of densification by evaporation. Very good results were achieved during the extraction with ethyl ethanol with a concentration of 20%. The use of the solvent ethyl ethanol with a concentration of 40% and 60% gives good results and a very high extraction coefficient, but the possibilities are very real that some of the main components, which are constituents in this raw material, remain without being extracted as a result of their transformation into other modifications which are not necessarily preferable in pharmaceutical use. A general conclusion for all the analysed samples is that according to the place where the samples were taken, more specifically in Mountain Sharr, does not really present the dry mass, as we expected that the dry mass has higher values and a higher extraction coefficient, this is related with harvesting this plant at different heights, having not so high content of the composing substances that are constituent to this pharmaceutical raw plant material.

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