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IDENTIFICATION AND SIEVING ANALYSIS OF PLANTS - A SOURCE OF BIOLOGICALLY ACTIVE SUBSTANCES FROM THE AREA OF DOSPAT MUNICIPALITY, WESTERN RHODOPES, BULGARIA

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Abstract

Bulgaria has a rich diversity of medicinal plants, with around 770 species, or 19% of all plant species in the country. According to the floristic regional division of the country, the Rhodope Mountains are divided into 3 regions: Western, Central, and Eastern. Dospat municipality falls within the area of the Western floristic region. The present study aimed to carry out the identification and sieve analysis of three plants. This study serves as a basis for subsequent research on the content and distribution of biologically active substances in the selected plants and can propose an extraction model for their further optimal extraction.

The plants identified were thyme (*Thymus callieri* Borbás ex Velen), thistle (*Onopordum acanthium* L.), and hawthorn (*Crataegus monogyna*). The cropped plants were examined for the presence of both inorganic and organic impurities. Once impurities were removed, the first quality grade plant material was sorted, dried, and ground. The ground plant material was then subjected to sieve analysis, which involved sorting the material into six classes based on particle size. The density of distribution of the size fractions was determined and analyzed for each of the three crop years studied. The study aimed to investigate the variation in the size distribution of the plant material over three crop years.

The results showed the nature of the variation in the size distribution of the plant material over the studied range of 0 - 2,000 µm. The density of distribution of the size fractions varied between the different crop years, indicating that factors such as weather and other environmental conditions may have influenced the size distribution of the plant material.

The obtained result of the grinding and fractionation can serve as a basis for additional research, contributing complete and qualitative extraction of biologically active composites from the herbal plant source.

Key words: *Identification, Herbal plant source, Sieve analysis, Sieve fractions.*

1. Introduction

Plants are used as sources of biologically active substances. The aromatic and medicinal plants comprise the largest share of the research about plants. This is due to their practical applicability in several industries (food, pharmaceutical, and cosmetics). Wild aromatic and medicinal plants can be cultivated. The biological potential of plants, their essential oil composition, and the possibilities of obtaining various extracts from them have not been fully and thoroughly investigated. This calls for further studies focusing on the biological resources of wild plants worldwide, including Bulgaria [1 - 4].

Bulgaria has a rich diversity of medicinal plants, with around 770 species, or 19% of all plant species in the country, possessing healing properties. Around 760 of these species are wild, and many were used as a source of remedies before the advent of synthetic drugs [5]. Additionally, around 250 of these species are used in large quantities for trade and processing. Around 30 to 40 species of medicinal plants are cultivated currently in Bulgaria. This accounts for one-fifth of the country's mass resource of medicinal plant diversity. Bulgaria is among the countries with the highest percentage of medicinal plants in relation to the total number of plant species.

According to geological studies, the Rhodope Mountain is divided into two zones - Western and Eastern. The Western Rhodope Mountain covers an area of 8732.1 km^2 and is characterized by a higher altitude (on average 1098 m above sea level, with more than half of the area (51.9%) with an altitude of 1000 - 1600 m above sea level, compared to the Eastern part [6].

The plant diversity in the Rhodope Mountains is very rich. According to the floristic regional division of the country, the Rhodope Mountains are divided into 3 regions: Western, Central, and Eastern [7]. Dospat municipality falls within the area of the Western floristic region [6]. Plant species in all three regions are relatively young and the floristic complex is not developed. The richness of endemics, their taxonomic rank, ecology, and preservation are of particular interest. Still, studies in this field are scarce and incomplete [8].

The Dospat area belongs to the southern Bulgarian climatic region, where the Mediterranean influence is strongly pronounced. There are 57 endemic, rare plants in the location of the populations in the Western Rhodope Mountain. They account for 8.93% of the total number of vascular plants (683) found so far on serpentines and are categorized as 40 endemics, among which 31 Balkan (36.5% of the Balkan endemics distributed in the Rhodopes), and 9 Bulgarian (11.25% of the Bulgarian endemics distributed in the Rhodopes) [8]. Studies on the flora of the Dospat Dere locality, which is situated to the right of the Dospat River bank, between the villages of Tukhovishte and Zhizhevo, near the border with Greece have been carried out [9, 10]. The studies found that the marked perimeter contained 197 vascular plant species (excluding moss species). These plants belong to 143 genera and 57 families. Some studies on the diversity of medicinal plant species on the territory of Dospat Municipality have been conducted [6, 11]. The study area covers 282.7 km^2 in the Western Rhodopes subregion. The area is typically mountainous and has the characteristic geomorphological features of the subregion - high altitude, high mountain ridges, and deeply incised valleys. The terrain is highly rugged, defined by a dense hydrographic network [12]. The specific microclimatic conditions and the significant variations in altitude (560 - 1653 m) are prerequisites for the rich floristic diversity.

Zahariev *et al*., [11], studied the species composition of medicinal plants during the growing seasons of 2013 and 2015. The species identification was carried out at the Department of Botany of Paisii Hilendarski University of Plovdiv [13]. The group [11] carried out a floristic analysis, based on which they prepared a systematic list of species, taking into account their membership in botanical families. The nature conservation status of the described medicinal plants was determined according to "Plants and Fungi" [14]; Red list of Bulgarian plants [15]; Conspectus of the higher flora in Bulgaria [16]; Balkan endemics in the Bulgarian flora [15]; Atlas of Bulgarian endemics [16]; Biodiversity Act (2002); Medicinal Plants Act (2000); and Red List of Threatened Species compiled and maintained by the International Union for Conservation of Nature (IUCN), Version 2016-3 [17].

332 plant species occur in the area of Dospat Municipality. The medicinal plants discovered by the teams [11 and 6] belong to 75 families. This represents more than 42% of the plant species listed in the Bulgarian Medicinal Plants Act and 21.68% of all species. The families *Asteraceae, Lamiaceae,* and *Rosaceae* have the highest species diversity, with herbaceous perennials being predominant. It is worth noting that the studies on the plant diversity in the Rhodope Mountains are scarce and incomplete, and the richness of endemics, their taxonomic rank, ecology, and preservation are of particular interest.

Thyme (*Thymus callieri* Borbás ex Velen) is an herbaceous perennial plant that belongs to the *Lamiaceae* family. It is a fragrant plant that is commonly used in cooking and is also known for its medicinal properties. Thyme has antibacterial, antifungal, antiseptic, and expectorant properties, and it is commonly used to treat respiratory infections, bronchitis, and coughs.

The thistle (*Onopordum acanthium* L.) is a tall, prickly herbaceous plant that belongs to the *Asteraceae* family. It has been traditionally used for its medicinal properties and is known to have anti-inflammatory and analgesic properties. Thistle is commonly used to treat digestive disorders such as constipation, diarrhea, and dyspepsia.

Hawthorn (*Crataegus monogyna*) is a small tree or shrub that belongs to the *Rosaceae* family. It has been used for centuries for its medicinal properties and is known to have a positive effect on the cardiovascular system. Hawthorn is commonly used to treat heart failure, angina, high blood pressure, and other cardiovascular conditions. It is also known to have antioxidant and anti-inflammatory properties, making it useful for a variety of health conditions.

This study provides valuable information on the identification and processing of three medicinal plants from the Dospat area in the Western Rhodopes region. Thyme (*Thymus callieri* Borbás ex Velen), thistle (*Onopordum acanthium* L.), and hawthorn (*Crataegus monogyna*) are three medicinal plants that are commonly found in the area of Dospat, Western Rhodopes. The findings of the study could be useful for the production of high-quality herbal medicines and for further research on the properties and uses of these plants. The present study aimed to carry out identification and sieve analysis and to estimate the distribution density of the fractions by size. This study serves as a basis for subsequent research on the content and distribution of biologically active substances in the selected plants and can propose an extraction model for their further optimal extraction.

2. Materials and Methods

2.1 Plant sources

The sieve analysis covers three plants from the area of Dospat, situated in the Western Rhodope Mountain in Bulgaria.

2.2 Identification of plant sources

Plant raw materials - thyme (*Thymus*) and hawthorn (*Crataegus*) were identified at the Department of Plant and Fungal Diversity and Resources of the Institute of Biodiversity and Ecosystem Research of the Bulgarian Academy of Sciences (IBEI-BAS), Sofia, Bulgaria. The identification was conducted at the Institute of Biology and Biological Sciences of the Bulgarian Academy of Sciences (IBE-BSI), Sofia. The identification of the milk thistle (*Onopordum*) was carried out at the Department of Botany, Agricultural University - Plovdiv, Bulgaria.

Table 1 presents data related to the origin and description of the selected plant sources, while Table 2 presents the year of collection and the morphological parts processed.

2.3 Inspection, sorting, drying

Plant raw material (crops from 2020 and 2022) was collected, and sorted out, and impurities were removed. The raw plant material was spread in a thin layer and dried in a ventilated dark room, at a temperature of 22 - 25 ^{0}C, until humidity of 11 - 12%. The dried mass was stored in tightly closed paper envelopes in a dry place before analysis. Inert and organic foreign impurities were classified according to Stoyanova *et al.*, [18].

2.4 Grinding of dry plant sources and sieve analysis of ground plant raw material

Grinding was carried out with a laboratory pin grinder "Schule" - Germany at a measured peripheral pin speed of 64 m/s by repeatedly passing each of the samples through the crusher.

The collection of the average sample and the sieve analysis of the milled product was carried out according to BNS 754:1980 [19] "Ground products. Sampling rules and test methods".

Table 1. Characteristics (origin and description) of the medicinal plants used

Trivial name	*Thymus*	*Onopordum acanthium*	*Crataegus*
Scientific name	*Thymus Linnaeus*, 1753	*Onopordum Linnaeus*,1753	*Crataegus Linnaeus*, 1753
Description	perennial herbaceous plants and small shrubs	biennial herbaceous plant	bushes and small trees
Kingdom	Plants (*Plantae*)	Plants (*Plantae*)	Plants (*Plantae*)
Division/phylum	Vascular (superior) (*Tracheophyta*)	Vascular (superior) (*Tracheophyta*)	Vascular (superior) (*Tracheophyta*)
(unranked):	Cover crops (*Angiosperms*)	Cover crops (*Angiosperms*)	Cover crops (*Angiosperms*)
(unranked):	Eudicotti (*Eudicots*)	Eudicotti (*Eudicots*)	Eudicotti (*Eudicots*)
(unranked):	Asterides (*Asteridc*)	Asterides (*Asteridc*)	Rosidi (*Rosids*)
Class:	Bicentennial (*Magnoliopsida*)	–	Bicentennial (*Magnoliopsida*)
Order:	*Lamiales*	*Asterales*	Rosaceae (*Rosales*)
Family:	Woody plants (*Lamiaceae*)	Compound flowers (*Asteraceae*)	(*Rosaceae*)
Subfamily	*Nepetoideae*	*Cynareae*	*Maloideae*
Genus:	*Thyme* (*Thymus*)	*Plain Ginger* (*Onopordum*)	*Crataegus* (*Crataegus*)

Table 2. Year of collection, location, and processed morphological part

№	Medicinal plants	Morphological parts	Harvests	Location
1	Thyme (*Thymus*)	stems with flowers	2020 2022	Town of Dospat
2	Milk thistle (*Onopordum*)	flowers	2020 2022	Town of Dospat
3	Hawthorn-fruit (*Crataegus*)	fruits	2020 2022	Village of Satovcha

Sieve analysis of the obtained milled product was carried out with a laboratory plansifter LP-200 with five sieve frames of overall dimensions 200 x 200 mm and sieve clear aperture sizes as follows: 2000; 1000; 670; 450, 280, and 132 μm. The sieving time was 5 min with a rotation speed of 180 x min^{-1}. To aid the sieving process, two rubber balls were placed in each frame.

The results were processed and distribution density diagrams of the obtained fractions by size were plotted for each of the samples. The distribution density of the shredded and fractionated plant mass was determined according to DIN 66 141 methodology [20].

2.5 Statistical data processing

Statistical processing of the data was performed, including the determination of standard deviation (± SD), with triplicate replication for each analysis. The statistical data processing was carried out using the Excel 2007 program (Microsoft Corporation, USA).

3. Results and Discussion

The primary processing of the collected herbs was done to preserve their quality and the biologically active components in them. Other objectives were to block changes in their appearance, smell, and taste, and also to avoid possible contamination by external impurities and microorganisms.

The herbs analyzed were hand-cropped, so an inspection was carried out in each sample immediately after collection to identify and promptly remove unwanted impurities.

The absence of inorganic foreign impurities such as soil, sand, pebbles, dust, and metal particles was found in both crops analyzed (2020 and 2022).

The data regarding the presence of undesirable organic impurities for the collected plant sources from the 2020 and 2022 crops are presented in Tables 3 and 4.

When analyzing the results of the 2020 crops, it was found that fruits and leaves of weedy and other plants were detected during the inspection only in the case of thyme (*Thymus*) - 13.70%. Milk thistle (*Onopordum*) and hawthorn fruit (*Crataegus*) showed purity in this indicator.

Damage from steaming, rotting, or mould was found in all three plant sources. In percentage terms, they were highest in hawthorn-fruit (*Crataegus*) - (6.1%), while thyme (*Thymus*) and milk thistle (*Onopordum*) showed significantly lower levels of this damage, with the percentage (0.6%) being slightly higher in thyme (*Thymus*) compared to milk thistle (*Onopordum*) - 0.4%.

The indicators 'green fruit' and 'overripe fruit' were found only for hawthorn (*Crataegus*), as this was the only source where fruit is used. The percentage of green fruit found in this species (6.3%) was slightly higher than that found for overripe fruit (5.1%).

All impurities, damage, and fruit found to be of poor technological maturity from the plant sources inspected were removed so that the first quality plant material could be cropped.

Table 4 indicates the established amount of organic foreign impurities for the 2022 crops. During the inspection carried out, 12.60% of organic impurities consisting of fruits and leaves of weeds and other plants were found in the cropped thyme (*Thymus*). This type of impurity was absent in the collected plants of milk thistle (*Onopordum*) and hawthorn (*Crataegus*). All three plant sources were found to be

Table 3. Presence of organic foreign impurities (%) in the analyzed plant sources for the crops from 2020

Herbs	Impurities			
	Fruits and leaves of weeds and other plants	Damaged by steaming, rotting, or mould	Green fruits	Overripe fruit
Thyme (*Thymus*)	13.7	0.6	-*	-
Milk thistle (*Onopordum*)	-	0.4	-	-
Hawthorn-fruit (*Crataegus*)	-	6.1	6.3	5.1

Legend: -* Not found.

Table 4. Presence of organic foreign impurities (%) in the analyzed plant sources for the crops from 2022

Herbs	Impurities			
	Fruits and leaves of weeds and other plants	Damaged by rotting or mould	Green fruits	Overripe fruit
Thyme (*Thymus*)	12.6	0.4	-*	-
Milk thistle (*Onopordum*)	-	0.3		-
Hawthorn-fruit (*Crataegus*)	-	3.2	5.7	4.1

Legend: -* Not found.

damaged by decay, rot, or mould. In thyme (*Thymus*) and milk thistle (*Onopordum*) this indicator was found in low percentages, 0.4% and 0.3%, respectively, while hawthorn (*Crataegus*) showed a slightly higher percentage of this damage - 3.2%. The third and fourth indicators for organic impurities - 'green fruits' and 'overripe fruits' - were found only in the hawthorn (*Crataegus*), which was understandable since it was the only one where fruits were collected. These impurities were 5.7% and 4.1% respectively.

Comparing the crops from 2020 and 2022, we can say that in both years the presence of fruits and leaves of weeds and other plants was found only in thyme (*Thymus*), with a 1.1% higher percentage in the 2020 crops. Damage by rot or mould was found for all plant sources examined in both crops and for this indicator, the 2020 crop also showed higher levels than the 2021 crops. Thus, in the case of thyme (*Thymus*) in the 2020 crops, they were 0.2% higher than in the 2021 crops; in the case of milk thistle (*Onopordum*), by 0.1%; and hawthorn-fruit (*Crataegus*) showed almost twice as much damage in the 2020 crops, where they were 2.9% higher than in the 2022 crops. On the green fruit indicator, hawthorn-fruit (*Crataegus*) in the 2020 crop showed 0.6% more underripe fruit detected, compared to the 2022 crops, and overripe fruit detected in hawthorn-fruit (*Crataegus*) in the 2020 crops was 1% more, compared to 2022.

Bulgaria is among the European countries with the richest biodiversity. For this reason, it was necessary to identify cropped and pre-processed plant raw materials.

The Herbarium of the Department of Plant and Fungal Diversity and Resources, Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences (IBEI-BAS) is the largest and most representative source of information about Bulgarian plant diversity, as well as one of the main sources of information for the flora of the Balkans. It is registered in Index Herbariorum as an internationally recognized herbarium with the acronym SOM.

The selected Bulgarian medicinal plants were from the genera *Thymus*, *Onopordum,* and *Crataegus*. They are typical for the area of Dospat and have a long traditional use. These native plants are morphologically similar to other species, so it is necessary to establish their authenticity. The botanical identity of thyme and hawthorn fruit was established by the preparation of herbariums from the collected medicinal plants, according to the requirements of IBEI-BAS.

The identification of the thistle was made at the Agricultural University of Plovdiv, Department of Botany. The identification of the species *Onopordum* was made by analyzing the type and size of the seeds located under the mossy part of the flower.

The results obtained from the identification process, i.e. species identified and specimen identification numbers, are given in Table 5.

For sieve analysis, the samples were dried and grounded. Sieve analysis was performed and results were analyzed for three crop years (2018, 2020, and 2022), with the 2018 crops including preliminary data from Parzhanova *et al*., [21], which was included in the data to comprise a three-year comparison.

Sieving through mesh sieves with selected aperture sizes allows for the separation of particles based on their size, with smaller particles passing through the sieve and larger particles being retained. The use of sieves with a uniform distribution of aperture sizes helps to ensure that the fractions obtained have consistent particle size ranges, which can be important for quality control and standardization of medicinal plant products.

The process of grinding medicinal plant material into a milled product resulted in different particle sizes, which were typically categorized into grades or fractions based on the type of plant material, its structure, and composition. Sieving was then used to separate the milled product into fractions with specific particle diameter ranges, typically using mesh sieves with specific aperture sizes. In this case, it appeared that six fractions were produced, with particle diameters ranging from 0 to 2,000 µm, and distributed in classes based on their size ranges: Class I - 132/0 µm; Class II - 280/132 µm; Class III - 450/280 µm; Class IV - 670/450 µm; Class V - 1,000/670 µm; Class VI - 2,000/1,000 µm.

Data from sieve analysis of thyme (*Thymus callieri* Borbás ex Velen) and the size density distribution of fractions of ground thyme are presented in Table 6 and Figure 1.

Table 5. Identified species and specimen identification numbers

Species	Voucher specimens
***Thymus callieri* Borbás ex Velen**	SOM 176971
***Crataegus monogyna* - fruits**	SOM 176970
***Onopordum acanthium* L.**	Recognized by analyzing the type and size of seeds

Table 6. Sieve analysis of ground thyme for three crop years (2018*, 2020, and 2022)

Class of coarseness	Dimensions of light openings, µm	Thyme /*Thymus callieri* Borbás ex Velen/ M ± SD**		
		2018*	2020	2022
I	132/0	2.04 ± 0.5	13.38 ± 1.3	14.33 ± 1.0
II	280/132	22.74 ± 1.1	22.02 ± 0.4	32.09 ± 1.3
III	450/280	20.78 ± 1.0	20.02 ± 1.0	19.78 ± 0.9
IV	670/450	17.11 ± 1.6	15.38 ± 0.6	12.21 ± 1.1
V	1000/670	21.68 ± 0.9	17.91 ± 1.1	12.61 ± 1.2
VI	2000/1000	15.65 ± 2.2	11.28 ± 1.8	8.98 ± 1.7
Average diameter d_{cp}, µm		454.3 ± 2.3	597.2 ± 2.9	670.1 ± 2.9

Legend: *Data from Parzhanova *et al.*, [21]; ** M (mean); SD (standard deviation).

When comparing the sieve analysis results of ground thyme (*Thymus callieri* Borbás ex Velen) from three crops in 2018, 2020, and 2022, it was found that the sieve analysis profile of ground thyme was similar in the number of fractions within the particle size ranges studied (0 - 2,000 µm). The fractions in the 670 - 1,000 µm range for the 2018 and 2020 crops were the highest in relative abundance. Relatively, the most even in terms of quantity were the fractions from the 2020 crops. The average diameter of the fractions obtained during milling varied from 454 µm (2018) to 670 µm (2022), which was an indicator of likely structural changes that had occurred as a result of herb mass storage or other causes.

Figure 1 presents graphically, the variations in the density of distribution of ground thyme fractions by particle size for each of the crop years studied.

The graphical curves give an idea of the composition and distribution density of the milled fractions by particle size in the size range of 0 - 2,000 µm after sieve analysis. In the course of the curves, one distinct maximum in the particle densities was found in different years, i.e. the distribution was considered as monomodal, except for the 2022 crops where a second smaller maximum at 132 µm stood out. The area with the highest distribution density (670 µm) was located to the right of the plot, i.e. it appeared asymmetric. In the studied region of fraction sizes, the shape of the curves in different years was similar, but the values of the distribution densities qi in the zone of maximum density varied in a range between 0.85 and 1.46.

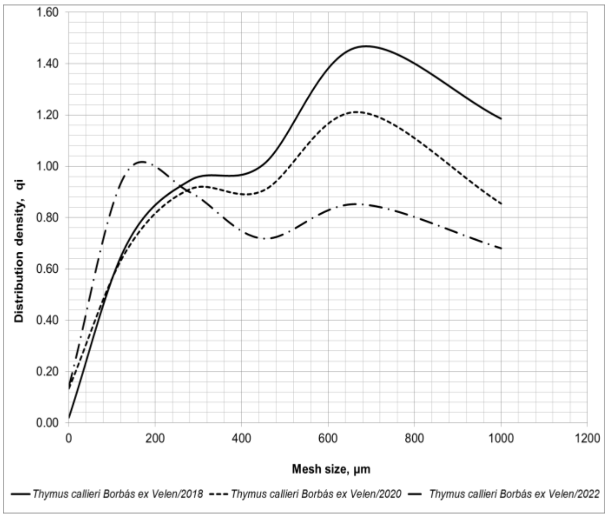

Figure 1. Size of distribution density of ground thyme fractions

The harder and more robust structure of the fibrous vegetative parts of the thyme stems the lower their grinding rate on impact, leaving these fractions with larger final sizes than the others.

Table 7. Sieve analysis of ground hawthorn fruit from three crops (2018*, 2020, and 2022)

Class of coarseness	Dimensions of light openings, µm	Hawthorn fruit /*Crataegus monogyna - fruits*/ M ± SD**		
		2018*	2020	2022
I	132/0	33.9 ± 2.9	26.1 ± 1.6	29.1 ± 3.5
II	280/132	65.3 ± 3.1	40.9 ± 1.3	41.2 ± 2.2
III	450/280	35.4 ± 1.5	36.1 ± 2.0	36.1 ± 2.2
IV	670/450	42.8 ± 2.0	49.9 ± 2.4	49.7 ± 2.1
V	1000/670	108.5 ± 2.2	128.0 ± 2.2	123.8 ± 2.6
VI	2000/1000	38.3 ± 2.1	39.6 ± 2.5	47.7 ± 2.3
Average diameter d_{cp}, µm		511.1 ± 4.9	438.9 ± 2.9	442.8 ± 2.9

Legend: *Data from Parzhanova *et al.*, [21]; ** M (mean); SD (standard deviation).

Data from sieve analysis of hawthorn fruit (*Crataegus monoguna*-fruits) and the distribution density by size fraction are presented in Table 7 and Figure 2.

When comparing the sieve analysis profile of ground hawthorn-fruit (*Crataegus monogyna* - fruits) from the crops in 2018, 2020, and 2022, it was found that there were similar numbers of fractions in the studied area of particle sizes (0 - 2,000 μm). The composition and structure of medicinal plant fruits could affect their strength and impact behavior during grinding, which in turn determines the degree of grinding and the size of the obtained fractions. This means that the anisotropic (having different properties or characteristics in different directions) nature of the fruits could impact the grinding process and the resulting particle sizes. It appeared that the fractions in the 670 - 1,000 μm range had the highest relative quantitative share for all three crop years, indicating that they were stronger compared to other fractions. This suggested that these fractions may had a higher resistance to grinding and were less likely to break or shatter during the grinding process, resulting in larger particle sizes. The average diameter of the obtained fractions during grinding varied from 443 μm in 2022 to 511 μm in 2018, which indicated that the particle size distribution obtained from grinding was relatively consistent and fell within a narrow range. This suggested that the grinding process used for these medicinal plant fruits was well-controlled and resulted in consistent particle sizes across different crop years.

Figure 2 shows graphically in the form of a line diagram the changes in the distribution density of the milled fractions by particle size for each of the studied years.

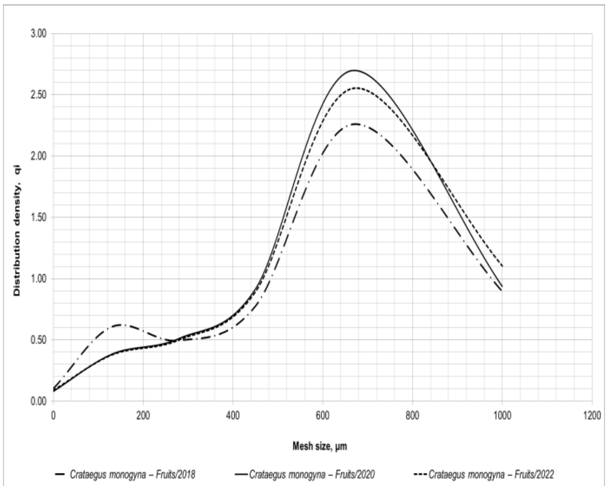

Figure 2. Size of distribution density of ground hawthorn-fruit fractions

These results, which were obtained under the same fixed conditions, showed a different degree of grinding, which was a function of the specific structure and composition of their morphological parts. Herbs as raw materials belong to polydisperse systems with complex structures and composition. This determined great differences in the strength and density of their structure both in the native state and in the dried form.

In the course of the curves, in the different years, clearly expressed maxima in the distribution densities of the particles were established, i.e. the distribution was considered to be monomodal. The area with the highest distribution density (670 μm) was located to the right of the graph, i.e. it appeared asymmetric. In the studied area of fraction sizes, the course of the curves in different years was close, the values of the distribution densities qi varied between 2.26 and 2.70.

The harder and stronger structure of the hawthorn fruit shells resisted the grinding working organs, which resulted in a lower degree of grinding, compared to other anatomical parts.

Sieve analysis of the flowers of *Onopordum acanthium* could not be performed due to the morphological characteristics of the plant. The flowers cannot be ground by the method described for the other plants.

The contact surface between the processed mixture of the plant material and the extracting agent is a significant factor that could impact the amount of extracted substances. A larger contact surface area allows for more efficient extraction of bioactive compounds from the plant material, as it provides more surface area for the extracting agent to come into contact with the plant material and extract the desired substances.

Comminution, or the process of grinding and reducing the size of the plant raw material, plays a crucial role in increasing the contact surface area between the plant material and the extracting agent. By breaking down the plant material into smaller particles, comminution increases the overall surface area, which in turn enhances the contact between the plant material and the extracting agent. A higher degree of comminution, resulting in smaller particle sizes, can lead to a more efficient extraction process, as it exposes more surface area of the plant material to the extracting agent. This allows for better penetration of the extracting agent into the plant material, facilitating the extraction of the desired bioactive compounds.

One of the stages in the preparation of the raw material for extraction is the crushing and chopping of the solid phase. The particle size affects the overall process. In the extraction of solid mixtures, this is achieved by crushing them (saturation, grinding) and mixing them with the solvent. It was found that the larger the particle size, the more extractable components remain in them after a certain equilibrium time. It was also found that as the particle size decreases, the amount of extracted component increases, and the kinetics of the process intensifies. The concentration of extracted useful substances was greatest when using particles (0.2 - 0.4 mm) and lowest in the case of the largest particles (0.8 - 1.25 mm) [22]. As the size decreases, the equilibrium concentration of target components in the liquid phase increases. Unfortunately with very small particles, the mass sticks together, which worsens the penetration of the solvent into it.

4. Conclusions

The following conclusions could be made from the conducted research:

- Identification of three plant sources from the area of the town of Dospat, Western Rhodopes, Bulgaria - thyme, hawthorn fruit, and milk thistle was carried out. The identification process proved that the collected thyme was of the species *Thymus callieri* Borbás ex Velen, the hawthorn fruit was identified as *Crataegus monogyna* - fruits, and the milk thistle as *Onopordum acanthium* L.

- Millings of thyme and hawthorn fruit from crops in 2018, 2020, and 2022 were carried out and the resulting ground mass was fractionated by sieve analysis into six determined size classes (in the range 0 - 2,000 μm). The nature of the distribution of the fractions was determined by determining the density of distribution by size indicator. The results obtained by year were compared and it was found that for all the examined samples the highest distribution density was recorded in the 670 - 1000 μm class. Thyme was distinguished by a higher degree of grinding (443 μm - 511 μm), which had a smaller average equivalent particle diameter than hawthorn (454 μm - 670 μm).

- The obtained result of the grinding and fractionation could serve as a basis for additional research, contributing to the creation of an effective model for the saturation of the two plant sources (thyme and hawthorn fruit), which ensures subsequent extractions with guaranteed high-quality extraction process; complete and qualitative extraction of biologically active composites from the herbal plant source; maximum utilization of the biological composition of the plant source.

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