Interdisciplinary Journal of Research and Development
ISSN 2410-3411 (online) / ISSN 2313-058X (print)

Vol 10 No 2 / July 2023

Extraction of Bioactive Components from Helichrysim Arenarium

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Received: 05 May 2022 / Accepted: 10 June 2023 / Published: 23 July 2023

Doi: 10.56345/ijrdv10n202

Abstract

The raw material Helichrysum is used for the treatment of various pathological conditions in folk and modern medicine, for the treatment of tropical infections, respiratory diseases and as a detoxifying herbal medicine. Extraction is the most appropriate method for separating the active ingredients from plant materials. Separated bioactive components are mostly used in the food, pharmaceutical and chemical industries. For this purpose, ethanol with different initial alcohol concentration was used as a working solvent: 55%, 70% and 96%, petroleum ether, methanol and methylene chloride. The ethanol solution is prepared from 96% ethanol and deionized water. Calculations were made of the total extract obtained with Soxhlet and ultrasonic extraction. The purpose of this paper is to study the influence of working parameters on the extraction process of working natural material (Helichrysum arenarium). The following separation procedures are applied for the extraction of the working raw material: solid-liquid conventional Soxhlet extraction and unconventional ultrasonic extraction. The plant extracts from this plant have various benefits, like skin care, antioxidant and antimicrobial activities.

Keywords: *ethanol, bioactive components, ultrasonic extraction, Soxlet extraction*

1. Introduction

People around the world today are increasingly trying to use natural phytochemicals that are present in fruit crops, teas, herbs, fruits and vegetables [1]. *Helichrysum* belongs to the *Asteraceae* family which is a very large genus and consists of about 500-600 species worldwide. They are used for the treatment of various types of ailments in traditional medicine [1, 2]. It is believed that this plant have the power to erase the traces of time on the face, which is why the French called it *Immortale*, which means immortal. The botanical name for this plant comes from the Greek words "Helio" and "hrizum" meaning "sun" and "gold" which directly refer to the fact that plant species of this genus usually have a bright yellow colour. The flower of this plant does not lose its colour even after cutting, and that is why the plant got the name eternal [3].

Helichrysum is a perennial Mediterranean herb that grows in dry, stony places and slopes in rock crevices [4], vineyards and roadsides. The height of the plant is 15-40 cm. This species has greyish leaves that are flat and dense, the lower ones are elliptical in shape and the higher ones are straight, with stems that are covered with white trichomes. The flower heads are arranged in a loose cross. They are 3 to 4 mm wide and the colour of the flowers varies from golden-yellow to orange. It flowers in summer and is harvested from June to September. It can be found in a wide geographic area, including Asia, Europe, and China [5, 6, 7].

The most important and most studied species of this genus are *Helichrysum arenarium*, *Helichrysum stoechas*, *Helichrysum graveolens* and *Helichrysum italicum* [3].

Interest in *Helichrysum* is motivated by its traditional therapeutic applications. This plant has many medicinal properties that are used in the treatment and prevention of many diseases. It is traditionally used in Central Europe for the treatment of stomach pains, asthma, arthritis disorders, and is also used as an antiseptic and antispasmodic drug. But today it is best known for its benefits for the skin, as it slows down its aging, can act against free radicals, and improves microcirculation and detoxification of the skin [3, 5, 8-10].

In a large part of the publications it is reported that this plant has biological activities: antimicrobial, antimalarial, antioxidant, antidiabetic, anti-inflammatory, antiviral and anti-tuberculosis activity. The chemical nature of this plant is complex and is composed of various organic compounds such as flavonoids, chalcones, phloroglucinol derivatives, essential oils, fatty acids, α-pyrone derivatives and diterpenes [1,2,6,11-13]. Other compounds besides polyphenols have been isolated from *Helichrysum arenarium*, such as sterols, lignans and aromatic glycosides. The essential oils obtained from *Helichrysum* contain various bioactive substances such as terpenes, aromatic phenol derivatives and aliphatic compounds that act in different ways [14].

Many scientists around the world use appropriate and standard methods to extract active ingredients from various plant materials, the use of which is very important in various commercial sectors, such as the pharmaceutical, food and chemical industries [15]. This paper aims to use conventional and non-conventional extraction methods to extract bioactive compounds from the plant raw material (*Helichrysum arenarium*), as well as changing the solvent in order to obtain the highest possible total yield of bioactive components.

2. Materials and Methods

Helichrysum Arenarium was used as a working material (Figure 1) for the extraction of bioactive components. The working raw material was bought in an herbal pharmacy, produced from Alkaloid AD Skopje. Helichrysi flos cone was packaged in a 100 g package intended for commercial use.

In order to achieve homogeneity, the raw material must be mixed and homogenized. The homogenized raw material was placed in a small container and stored in a dark place for further analysis, protected from external influences and at a room temperature of 20 ºC.

Figure 1. Appearance of Helichrysum arenarium

The solvents used in the experimental procedures were purchased from different suppliers in accordance with the Faculty of Technology and Metallurgy, Skopje. Ethanol (96%) and methanol are produced by the pharmaceutical company Alkaloid AD - Skopje. Deionized water was used to obtain 70% and 55% ethanol and it was also used during the overall performance of the experiments. Petroleum ether was produced by Carlo Erba Reagents S.r.l, n-hexane was produced by Merck. Apart from these solvents, methylene chloride was also used as a solvent. The solvents used were of a high degree of analytical purity that meet the need for the realization of the corresponding analyzes and are in accordance with the European Pharmacopoeia [16].

2.1 Granulometric analysis

The granulometric analysis was performed with 6 auxiliary sieves (Prüfsieb) with different hole diameters, which are arranged according to size one after the other. The specification and diameters of the sieve openings are given in the table 1.

Table 1. Specification of sieves used for granulometric analysis

Sieve mark	Opening diameter a_i [mm]
1	3.15
2	2.5
3	1.6
4	1.25
5	0.315
6	0.15

The measured working material is marked with m_T and placed in sieve 1. The sieves are arranged according to the size of the openings, from the largest to the smallest. By oscillating and vibrating the sieve, material that is the size of the sieve openings passes through the sieve openings. After the analysis was completed, the mass of each of the particles was measured on a laboratory scale (Sartorius, model U4600P scale).

The mass of the analyzed amount of the raw material was mathematically defined by the equation1:

$m_T = \sum_{i=1}^{7} m_i$.................................(1)

where m_T is the mass of the analyzed raw material [g], m_i is the mass of the separated fraction [g]. The mass fraction of each part fraction is mathematically expressed by the following equation:

$x_i = \frac{m_i}{m_T} 100$.....................................(2)

where x_i is the mass fraction of particle [%, m/m] m_i is the mass of the initial raw material, while the mean diameter of the particles is defined by the following equation:

$d_i = \frac{a_i + a_{i+1}}{2}$.....................................(3)

where d_i is the mean diameter of the particles of the fraction i [mm] a_i and a_{i+1} are the diameter of the opening of the i and $i+1$ sieve [mm].

For further analyses, a selection of the working material from the fractions between sieves 3 and 5 was determined because they are the most suitable for the needs of the defined experimental procedures.

2.2 Gravimetric analysis

A gravimetric method was used to determine the percentage of moisture in the working raw material.

Procedure: Working material is placed in a pre-measured mass of a porcelain pot on an analytical scale (Tehtnica-Železnik, model 2615, e=1 mg), then the mass of the porcelain pot is weighed together with the raw material and the mass of the material before so the drying is calculated from their difference, where m_v=1.0000±0.005 g. Each weighed sample is then dried in a laboratory dryer (Instrumentaria, model ST-06) at a temperature of 100-110°C. The gravimetric analysis was performed for duration of 120, 180 and 240 min. After drying, the sample are subsequently placed in a desiccator for a period of 10-20 min, with the aim of cooling in the absence of moisture before their re-weighing and determination of the moisture content in the working material. The dry mass content expressed in percent is determined by the following expression:

$$s_w = \frac{m_v - m_s}{m_v} 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

where: s_w is moisture content [%, m/m] in plant material, m_v and m_s are masses [g] of plant material before and after drying. Where the moisture content present in the material for duration of 120, 180 and 240 min was replaced by the following average values from the three analyzes for different drying times. Although according to the prescribed specification in Ph. Eur. 5, the time required for the analysis of the presence of moisture in the plant is 120 min at a temperature of 100-110°C, however, for more security about the degree of moisture in plant, analyzes were made with a longer drying time and obtaining continuity in the results.

2.3 Soxhlet extraction

The working raw material with a pre-measured mass is placed in a filter paper in the form of a capsule and placed in the Soxhlet apparatus. The solvent is placed in a round-bottomed flask in a ratio of 1:3 (mass of working raw material: solvent) and immersed in a water bath that has a temperature regulator. According to the type of solvent, the water bath is adjusted to the evaporation temperature of the solvent. The extraction time also depends on the type of solvent. At the end of the conventional Soxhlet extraction in the round bottom flask is left the solvent enriched with dissolved bioactive components of the plant material.

2.4 Ultrasound assisted extraction

In the ultrasound assisted extraction, an ultrasonic bath with a tank capacity of 30 l and a nominal ultrasonic generator with power of 240 W is used, using a constant ultrasonic frequency of 40 kHz. The duration of the extraction is 180 min. The ratio of mass of working raw material and the volume of solvent was 1 : 40.

The resulting mixture consisting of extract and solvent, after the completion of both extraction methods, is taken to separate the solvent from the bioactive components in a rotoevaporator, and the evaporation itself is carried out under vacuum conditions.

3. Results and Discussions

3.1 Granulometric analysis

The granulometric composition of 100g of the raw material was obtained through the granulometric analyses, the results of which are given in table 2 and are shown in figure 2.

Table 2. Separated fractions from the plant raw material

Faction, f_i (mm)	Mean particle diameter, d_i (mm)	Mass fraction, d_i (mm)
> 3,15	> 3,15	2,412
2,5–3,15	2,825	7,352
1,6–2,5	2,05	13,84
1,25–1,6	1,425	28,196
0,315–1,25	0,7825	42,812
0,15–0,315	0,2325	4,957
< 0,15	0,075	0,413

Since in solid-liquid systems, the shape and size of the particles have a great influence on the mass transfer rate, the 0.315–1.6 mm fraction with a total of 71.008 % and an average particle diameter of 0.9575 mm was separated from the whole raw material. Further analyzes were performed on the separated fraction, which is further defined as the working material.

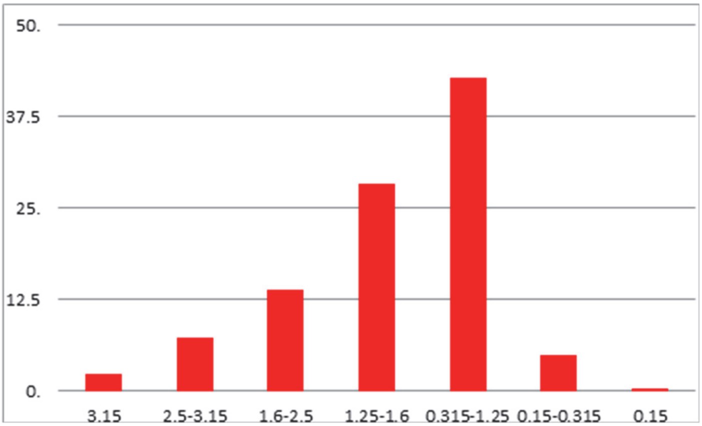


Figure 2. Mass fraction of particles in the plant raw material

3.2 Determination of moisture content

According to the experimental procedure, the moisture content in the working material with di= 0.9575 mm was determined. Table 3 shows the results of the analysis.

According to the European Pharmacopoeia, a moisture content of 10% is allowed. The material is placed in a dryer for 120 min at a constant temperature of 100-110°C. The obtained results from the analysis of the dry material showed an average moisture content of 8.66% (table 3), which shows that this material meets the conditions for further treatment and obtaining extracts from it.

The granulometric and gravimetric characterization of the material show that the working material with d_i = 0.9575mm and s_W = 8.66% meets the necessary requirements according to the set standards. Accordingly, the second part of the experimental plan was further approached, which covers the processes of separation of bioactive components with the defined techniques.

Table 3. Moisture content in plant material

Drying time, (min)	Medium moisture content, s_W (%)	Medium moisture content, s_W (%)
120	8,35	8,66
180	8,79	
240	8,84	

3.3 Conventional Soxhlet extraction

In order to maximally extract the bioactive components from the working material, the Soxhlet extraction was performed with 6 siphonings of the solvent, which lasts approximately 180 minutes. The obtained results are shown in table 4. During Soxhlet extraction as a continuous and exhaustive technique, organic solvents of different polarity were used, 55%, 70% and 96% solution of ethanol. Besides ethanol, other solvents were also used: methanol, methylene chloride and petroleum ether.

The particle size of the plant material was d_i = 0.9575 mm, worked at a modulus of m:V=1.5 : 50 (g/mL).

From table 4 it can be seen that the highest yield with Soxhlet extraction is obtained when was used petrol ether as a solvent with total extract of 34.2%, followed by 55% solution of ethanol with a total extract of 27.33%, 70% solution of ethanol with 26,67% of the total extract and 25.17% of the total extract is obtained when it was used 96% solution of ethanol, while the lowest yield was obtained when methanol (6.86%) and methylene chloride (2.68%) were used as a solvents. These results are all in accordance with the predictions, which can be explained by the fact that the percentage of water in the ethanol solution increases the polarity of the solvent and makes it more selective. The polarity of ethanol increases with an increase in the proportion of water. Also, this suggests that the working material contains a higher proportion of polar bioactive components.

The change in the total yield of separated substances from the extract (y_e) with time (t) for this extraction technique has not been defined, and this technique has only been used to determine the potency of the solvents used.

Table 4. Yield of total extract obtained from Soxhlet extraction with organic solvents

Solvent	Yield (%)
Ethanol (96 %)	25,17
Ethanol (70 %)	26,67
Ethanol (55 %)	27,33
Methylene chloride	2,68
Methanol	6,86
Petoleum ether	34,2

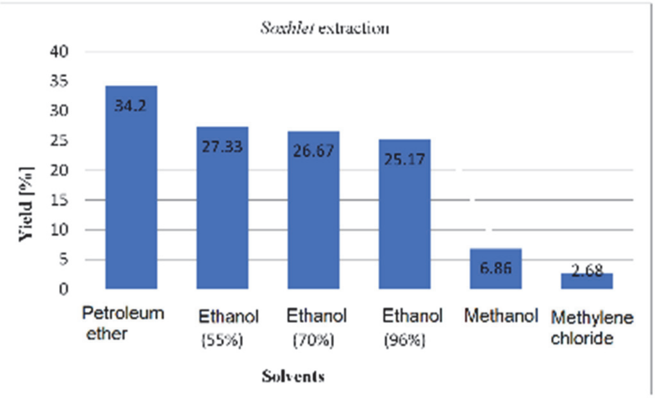


Figure 3. Graphic representation of total extract yield obtained at different initial concentrations of ethanol and with other solvents with Soxhlet extraction

3.4 Ultrasound assisted extraction

Ultrasound assisted extraction was applied for the separation of bioactive components from *Helichrysum arenarium* with d_i = 0.9575 mm, in the form of an extract. Experiments were performed to investigate the effect of different parameters affecting the yield of the extract, such as different solvents.

The advantage of using ultrasonic extraction to determine the influencing parameters on the extraction process is that this technique is more simpler than Soxhlet extraction and other conventional techniques, and it also represents an alternative extraction technique that is gaining more and more application in industrial processes.

The operating conditions during the ultrasound assited extraction are described in the experimental section. Table 5 and Figure 4 show the total extracts obtained during ultrasound assisted extraction. From table 5 and figure 4 it can be seen that the highest extract yield as in the Soxhlet extraction is obtained with petroleum ether (32%) and with 55% solution of ethanol (27%), while the lowest yield is obtained with 96% solution of ethanol (9%), methanol (4%) and methylene chloride (1.16%).

Compared to Soxhlet extraction, the obtained yield is twice as low, which indicates saturation of the solvent with bioactive components already in the middle of the process. In Soxhlet extraction, during the entire process, a pure solvent is used, while in ultrasonic extraction, the solvent is gradually saturated and reaches its maximum extractive power when only half of the bioactive components are extracted, or there is a decrease in the amount of bioactive components in the solid material below a certain concentration, which allows expression of diffusion influences and the process to slow down significantly. However, this should be ascertained during the analysis of the kinetics of the extraction process. The solubility of the solute in the solvent plays an important role in the extraction process. The polarity of the solvent is also a significant factor affecting the extraction yield which is in accordance with these results.

By increasing the quantity of water in ethanol, its polarity also increases, which can be seen from the obtained extract yield.

Table 5. Yield of total extract obtained from Ultrasound assisted extraction with organic solvents

Solvent	**Yield (%)**
Ethanol (96 %)	9
Ethanol (70 %)	14
Ethanol (55 %)	27
Methanol	4
Methylene chloride	1,16
Petroleum ether	32

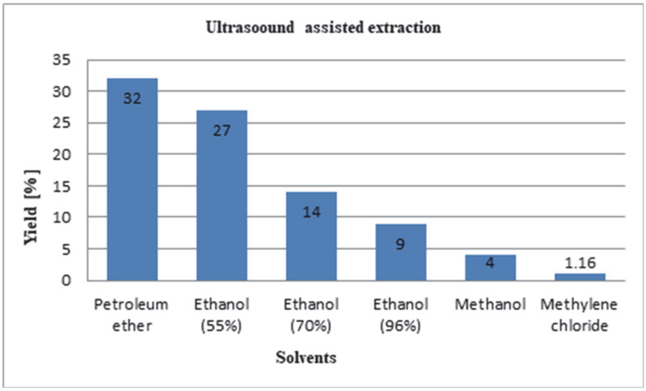


Figure 4. Graphic representation of total extract yield obtained at different initial solvent concentrations of ethanol and with others solvents with ultrasonic extraction

3.5 Comparative analysis of the results

Figure 5 shows the results obtained with Soxhlet and ultrasound assisted extraction, the comparison of the yields obtained when 96% solution of ethanol was used as solvent.

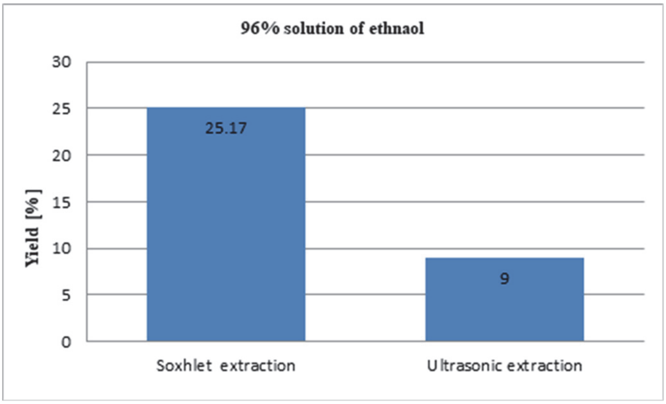
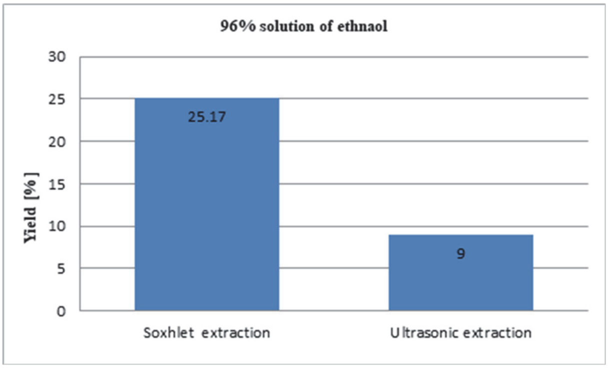


Figure 5. Graphical representation of total extract yield obtained by different extraction techniques when 96% solution of ethanol was used

Figure 6 shows the comparison of the obtained yields with Soxhlet and ultrasound assisted extraction when 70% solution of ethanol was used as solvent.



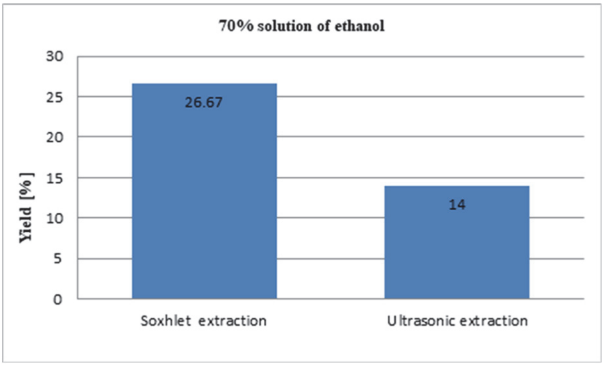


Figure 6. Graphical representation of total extract yield obtained by different extraction techniques when 70% solution of ethanol was used

Figure 7 shows the comparison of the obtained yields with Soxhlet and ultrasonic extraction when 55% solution of ethanol was used as solvent.

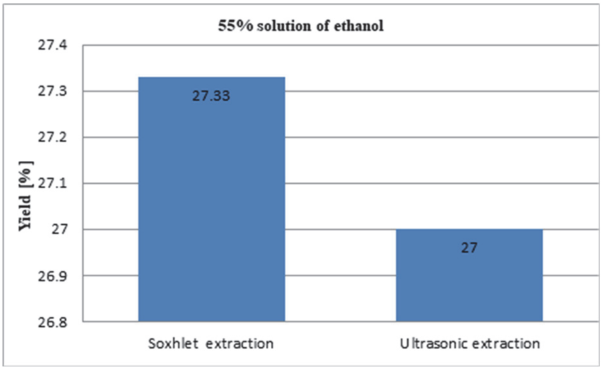


Figure 7. Graphical representation of total extract yield obtained by different extraction techniques when 55% solution of ethanol was used

From Figures 5, 6 and 7 it can be observed that with the conventional Soxhlet extraction a higher yield is obtained compared to the ultrasonic extraction method. The difference can be clearly seen in Figure 5 where the conventional Soxhlet extraction yielded three times higher yield than the unconventional ultrasonic extraction using 96% solution of ethanol. When using 70% solution of ethanol the yield is almost twice as high with the conventional Soxhlet extraction as compared to the non-conventional ultrasonic extraction (Figure 6). While from figure 7 it can be seen that the difference in yield is much smaller and it is only 4% when the conventional Soxhlet extraction was used in relation to the non-conventional ultrasound assisted extraction when 55% solution of ethanol was used as solvent.

The polarity of ethanol increases with an increase in the proportion of water in ethanol, this can be seen from the obtained extract yield.

Figure 8 shows the comparison of the obtained yields with Soxhlet and ultrasound assisted extraction when methylene chloride was used as solvent.

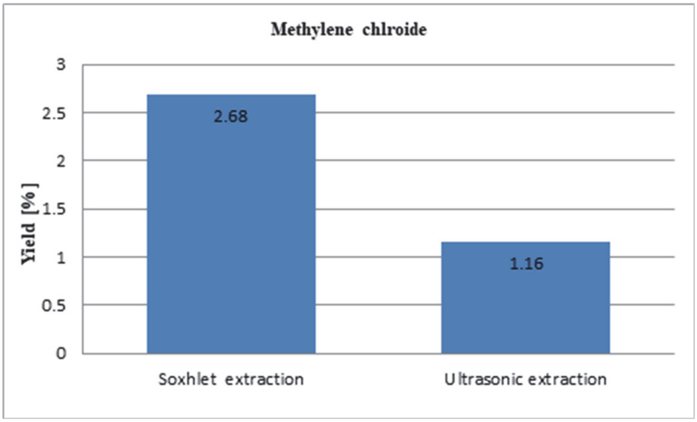


Figure 8. Graphical representation of total extract yield obtained by different extraction techniques when methylene chloride was used as solvent.

Figure 9 shows the comparison of the obtained yields with Soxhlet and ultrasound assisted extraction when methanol was used as solvent.

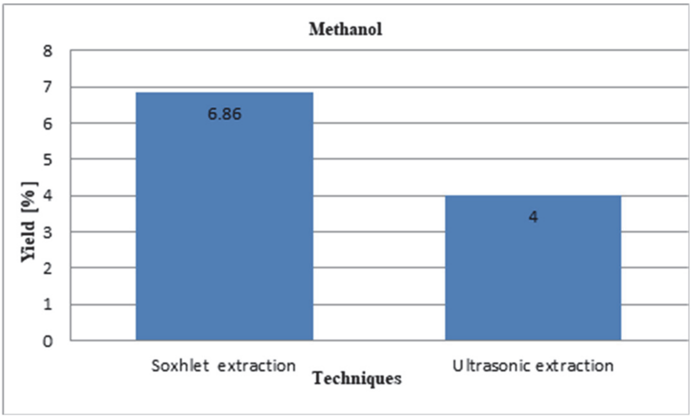


Figure 9. Graphical representation of total extract yield obtained by different extraction techniques when methanol was used as solvent.

Figure 10 shows the comparison of the obtained yields with Soxhlet and ultrasound assisted extraction when petroleum ether was used as a solvent.

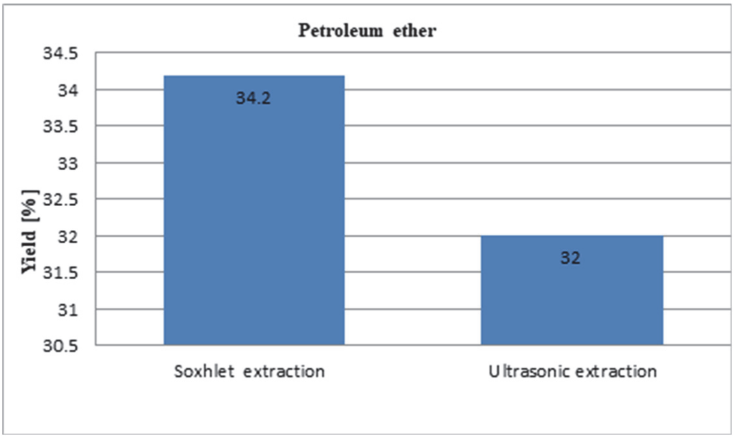


Figure 10. Graphical representation of total extract yield obtained by different extraction techniques when petroleum ether was used as solvent

4. Conclusions

From the overall work, the extraction of bioactive components from the plant material was successfully done using ultrasonic and Soxhlet extraction. In order to obtain the maximum yield, the influence of various parameters was investigated.

Based on these researches, it is important to draw the following conclusions:

- The separated working fraction with mean particle diameter d_i=0.9575 mm and mass partipication $\dot{x}_i$=71.008 % in the primary raw material, with mean moisture content s_W=8.66 % meets the needs for performing the defined separation experimental procedures, as well as the prescribed specifications by the European Pharmacopoeia.
- With both techniques Soxhlet and Ultrasound assisted extraction the highest extract yield was obtained with petroleum ether followed by 55% and 70 % solution of ethanol, while the lowest yield is obtained with 96% ethanol, methanol and methylene chloride.
- The benefits of the extraction obtained from the ultrasound assisted extraction were compared to the conventional Soxlet extraction. The extraction with the help of the conventional process gave a greater yield of the extract from the sample. The most pronounced difference in yields is given at 96% ethanol, when the conventional Soxhlet extraction yield is nearly three times higher than the non-conventional ultrasound assisted extraction.
- When using 70% solution of ethanol, the yield with Soxhlet-extraction is almost twice as high as compared to the non-conventional ultrasound assisted extraction, while using 55% solution of ethanol the difference in the yield of extraction is almost similar when conventional extraction is used compared to non-conventional ultrasound assisted extraction, while when it was used petroleum ether, methanol and methylene chloride the difference in the yield is very low between both techniques.

Within the framework of this work, the results are generally in good agreement with experimental data.

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