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Professional paper

APPLICATION OF THE MACERATION EXTRACTION METHOD FOR OBTAINING BLACK SEED OIL FROM NIGELLA SATIVA

Fjolla LATIFI[1], Mahi LATIFI[2], Ejup LATIFI[3], Nazif JASHARI[4], Edona LATIFI[5]

[1]University of Tetova
[2]Max Zeller Sohne AG Pflanzliche Heilmittel Romanshorm
[3] Public Enterprise for State Roads -- University of Tetova
[4] PUC "Tetova" –Tetovo-- University of Tetova
[5] Swica-Kreuzlingen
Contact: mahi.latifi@hotmail.com

Abstract

Nigella sativa is the plant raw material that is used in almost all branches of medicine because of its composition, which is rich in different types of vitamins and oils. The main product is black seed oil, used widely in medicine. This product has been known since ancient times and has been used for immunity, not knowing that this plant contains a range of main components that are very healthy. Different methods have been used to obtain oils in ancient times as well as in modern times, starting from the preparation of black seed, which was subjected to pressing by directly squeezing black seed oil without knowing exactly about its constituent conductive substances, as well as pressing of undesirable substances that accompany it. This increases the need to carry out a technological process controlled in such a way that the amount of ingredients is precisely known, at the same time, the black seed oil has complete purity without any conductive material. An advanced extraction method would enable a controlled extraction during which the process would be carried out in two stages. The first stage will be the extraction of the black seed with a suitable solvent, and in the second stage, the separation of oil from conducting materials of the black seed, which requires a complex process for separating the phases, and on the other hand, the use of an adequate solvent that has no contraindications during its use. The application of the extraction method by maceration is an excellent method for extraction, with the help of other extraction factors such as temperature, solvent, extraction time, and the rotation rate of the mixer. Good preparation of the raw material goes in favor of extraction, as well as the preliminary removal of moisture, which prevents the separation of the black seed oil phase from undesirable conductive materials.

Keywords: extract, size of granules, extraction coefficient, separation of phases

1. Introduction

The raw material Nigella Sativa is an annual plant of the Ranunculaceae family that grows and is cultivated in Southeast Asia, today, it is also cultivated in the Mediterranean countries as well as in some European countries. The seeds of black seed grow up to 55 mm long, and it is characterized by a special smell that is associated with Oregano, Aris, Nutmeg, and hot pepper, which are characterized by a taste of bitterness. This is a common plant with large leaves and fruits with black seeds. This plant has separated leaves and blue flowers, from which small black seeds emerge. The black seed contains unsaturated acidic acids, essential oils, vitamins, and many other substances necessary for the body. (Gheita TA, Kenawy SA) It has now been proven that the unsaturated acidic oil that black seed contains has a positive effect on the metabolism, increases immunity, and stops allergies.
The seed contains approximately 38% carbohydrates, 35% different oils, 21% albumin, and the rest consists of more than a hundred different substances. The black seed contains oils with about 11% essential oils, oils with conductive substances about 10%, Cymen, Thymoquinone 35% Thujone about 10%, and palmitic acid about 15%. It also contains many conductive elements such as Zn, Cr, V, Mn, Mg, K, Na, Ba, and Se (Prof. Dr. Sigrun Chrubasik-Hausmann).

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The black seed oil contains a lot of vitamins, among them Beta Carotene, as well as vitamins B, B1, C, and E, except for Omega 6 it also contains amino acids such as Arginine, Asparagine, Glycine, Phenylalanine, Serine, Tyrosine, and Valine. Since ancient times, it has been used and dates back 3500 years ago when it was first used in Egypt. It belongs to the family Ranunculaceae, which in science is known by the name Nigella Sativa. (M Burits, F Bucar). This plant grows throughout the year at a height of 20-35 cm, and its maximum growth is reached within three months. It is good to plant a new plant every three months. The uncontrolled growth of leaves requires that they be removed to strengthen the seeds of the plant. (Butt MS, Sultan MT) In ancient times, the black seed was known as the gold of the Pharaohs and was also used as a means to reduce blood sugar, diabetes mellitus, reduce blood pressure, and cholesterol. The properties of acidic oils are the regulation of the body's metabolism, development and regeneration of cells, development of hormones, and creation of a hormonal system for health protection. Black seed oils prevent the creation of allergic substances, have a very positive effect on blood circulation, and reduce heart diseases, lower blood pressure. These oils are not produced in the human body, so they are very necessary to be taken from outside. Except for the effect that it has on the immune system, black seed cleanses the body of toxic substances, so it is simply a natural antibiotic. (Işik H., Cevikbaş A., Gürer US, Kiran B., Uresin Y., Rayaman P., Rayaman E., Gürbüz B.)

The black seed may also be used in combination with different types of other pharmaceutical plants or with other products such as honey, which represents a great combination for use since on the one hand it improves the taste of the oil and on the other hand it completely completes the healing activity, but it is especially important to study well the composition of black seed oil, so we have the most rational combination. To get black seed oil, there are used fruits (seeds) are used, which are well-ripened, after fatty acids, vitamins, and minerals have been deposited in them; therefore, it is of particular importance that the raw material must have its own growth time. (Kalus U., Pruss A., Bystron J., Jurecka M., Smekalova A., Lichius JJ, Kiesewetter H.) Premature harvesting reduces the amount of oils and also the healing abilities of black seed oil. A recent study by American scientists concluded that black seed oil has antitumor abilities, but these are studies in the initial phase, and much more medical research is expected to reach a definite conclusion. The black seed oil has a dark yellow color, the color of which also signals a sign of quality during its extraction. Cold-pressed oils are generally cleaner and have a brightening dark yellow color, while hot-pressed oils are characterized by a darker yellow color as a result of the undesirable components that are created during the hot press. (Nikahlagh S., Rahim F., Aryani FH, Syahpoush A., Brougerdnya MG, Saki N.)

The quality of black seed oil consists of several main components of black seed, such as unsaturated fatty acids, alkaloids (Nigelicin and Nigelidine), Saponin, and essential oil. The key main component in the black seed is Thymoquinone as a component of essential oils. The higher the amount of this key component, the higher the quality of the black seed oil will be. (Karola Berger)

Black seed oil is used in almost all fields, such as in the pharmaceutical, food, cosmetic industry, etc., industries. It is used alone or combined with other products that complement each other according to the ingredients that they contain. Today, black seed oil has a considerable place for its use, especially in the food industry, and this happens because of the large healing abilities of this oil, but they are consumed without doing any detailed analysis of the main components of the black seed, which requires qualitative and quantitative analyses, which are expensive. Mainly in the food industry, cold-pressed oil is used, but this does not apply to the pharmaceutical industry, where the process is fully controlled. (Gürbüz B., Büyüköztürk S)

2. Body of Manuscript

Although black seed oil has been obtained by cold and hot pressing, the control of the process is not as good as it should be, as the entire process takes place without adjustment, and gives a possibility to use another method, such as extraction, where the process will be controlled and coordinated. In this particular case, it is used for extraction by maceration. At first, it is performed grinding of the black seed was performed with different degrees of grinding, where two granulometric analyses were made Table 1, Figure 1, and Table 2 Figure 2.The first sample was ground with the highest level of grinding, and the second sample with the lowest level of grinding. For both samples, 50 g of black seed fruit were weighed for one analysis. The main principle for extraction is to first define the grinding level, which is the main factor for the development of the technological process of extraction and directly affects the coefficient of extraction. It is not always a principle that the higher the grinding level is, the higher the extraction coefficient will be, but this depends on the type of pharmaceutical raw material that requires adjusting the degree of grinding in the way that the extraction process is developed without technical problems, and on the other hand to keep the highest extraction coefficient, and develop the process to the end, and not to reach equilibrium prematurely. After the gravimetric analysis, the maceration process started, where 94% ethyl ethanol was used as a solvent. In a laboratory vessel of 2000 ml, there are weighed 200 g of ground black seed and then 1398 g of 94% ethyl ethanol was added, undergoing consistent mixing with a mixer at about 400 rpm. This prepared solution is placed on a gas ring where the temperature is adjusted to 50°C under consistent mixing for 90 min. At this point of extraction, the mixer is turned off, and such a homogeneous solution is left to sit for 20 min to create a sediment, and the extract is left in the upper part. About 2/3 of the extract of the upper phase is removed by a pipette into another laboratory vessel of 1000 ml, which is considered a pure extract after maceration. 70 gr of ethyl ethanol 94% is added to the remaining mixture together with the sediment. At the same time, the mixer is turned on with a rotation speed of 400 rot/min, while the temperature is kept constant at 50°C, and the maceration continues for 30 min. This is done because during the 90 minutes of maceration, it is noticed that the solution already is in equilibrium, creating a supersaturated solution, and the extraction reaction does not continue further, therefore, a variant of shifting the reaction from equilibrium, is adding pure solvent, which shifts it from equilibrium, and the extraction process continues for a certain time. This is done in such a way that even the remains of the main components, which have not been extracted up to this point, are subjected to the black seed for extraction. After the maceration, the solution is subjected to filtration with a filtration paper, so the obtained extraction is combined with the second vessel of 1000 ml. The same procedure is used for the second sample with a lower degree of grinding to compare which degree of grinding is the ideal one for a higher extraction coefficient. During maceration, one sample is taken at different times, and the dry mass is filtered and weighed at the same time as maceration. Table 2 Figure 2. Based on the results measured at different times, we may conclude that the second sample with a lower level of grinding presents a higher dry mass during maceration, and this should be used. Table 4 Figure 4 and Table 5 Figure 5.

The obtained and filtered extract for the second sample is subjected to further processing, where it first undergoes evaporation in a rotavapor to a 39.2% dry mass, creating optimal conditions for the separation of phases. The obtained extract is placed in a laboratory vessel of 100 ml and is set still, without moving it, so that the process of layering of the phases happens within 24 hours. It is noted that the creation of two phases, in the upper part is the extract, and in the lower part is the sediment with undesirable substances and impurities that conduct the black seed. The obtained extract, specifically the upper phase, is again subjected to evaporation in a rotavapor to 78.5%. Dry mass. To have a better division of the phases, the upper part undergoes a centrifugation process in a laboratory centrifuge, and the upper part is considered a pure extract,

respectively, black seed oil. This obtained extract is collected in a laboratory vessel of 50 ml. The obtained extract, specifically black seed oil, after a long time, decants, and a thin layer is created at the bottom of the glass; it is minimal in quantity, but it creates a highly pure black seed oil. Although there is a possibility that this oil can be rinsed once more with ether and then the ether is removed, in this case, this was not tried, because when we look at the obtained oil, it looks very pure. The purpose of this work is to try to obtain black seed oil by extraction, and the goal has been achieved and the quality of this black seed oil is high and it is very rich in oils that are pure with no conductive components. This is not the case when we obtain the same with pressing, and black seed oil contains conductive impurities.
After obtaining this oil, there was balancing during the technological process Table. 6 where it can be noticed that the ratio of the raw material of the black seed to the obtained extract is 4.5:1, which represents a very substantial ratio for obtaining oils. It will be considered in the future to try to reduce this ratio even more by adjusting the extraction parameters such as temperature, extraction time, and concentration of the solvent, and of course, this remains to be done in another work.

3. Table and Figures

Size of strainer	Measuring vessel [gr]	Vessel + raw material [gr]	Netto [gr]
8.00 mm	448.08	448.08	0.00
4.00 mm	430.32	430.32	0.00
2.00 mm	400.30	400.36	0.06
1.00 mm	362.00	366.08	4.08
0.50 mm	322.69	339.30	16.61
0.25 mm	290.12	303.67	13.55
0.125 mm	279.70	287.95	8.25
Sludge	400.76	408.12	7.36

Table 1: Granulometric analysis of Nigella Sativa sample 1

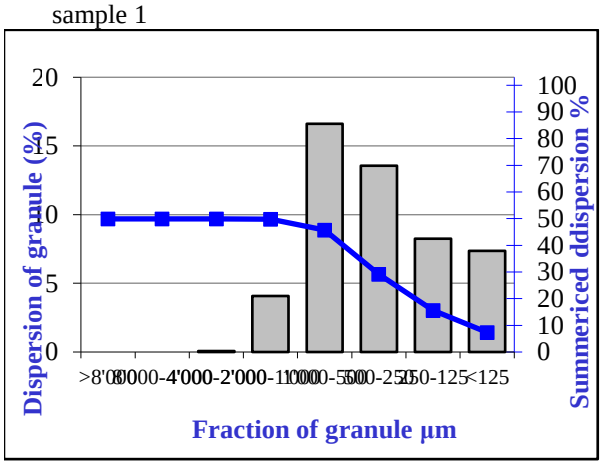

Figure 1: Fraction of granule Nigela Sativa sam**ple1**

Table 2: Granulometric analysis of Nigella Sativa sample 2

Size of strainer	Measuring vessel[gr]	Vessel + raw material [gr]	Netto[gr]
8.00 mm	448.1	449.13	1.03
4.00 mm	430.82	434.32	3.5
2.00 mm	399.7	49.72	10.02
1.00 mm	362.8	376.35	13.55
0.50 mm	322.5	334.76	12.26
0.25 mm	290.1	292.84	2.74
0.125 mm	279.48	283.14	3.66
Sludge	400.88	404.13	3.25

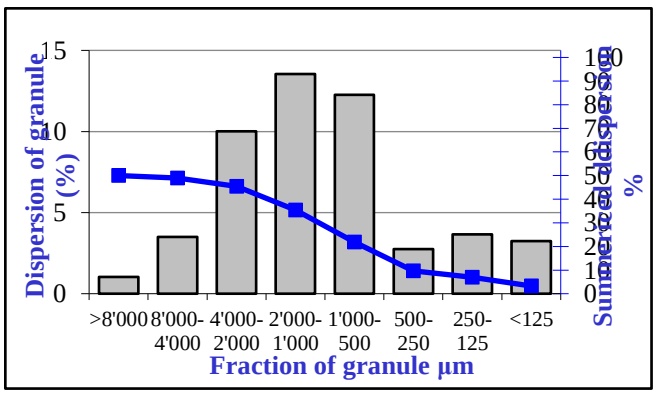

Figure 2: Fraction of granule Nigela Sativa sample 2

Table 3: Results of dry mass in relation tothe extraction time of Nigela Sativa Sample 1

Time [min]	**Dry content [%]**
1	0.8
15	1.52
30	1.96
45	2.36
60	2.82
70	3.11
90	3.49
110	3.62
120	3.74

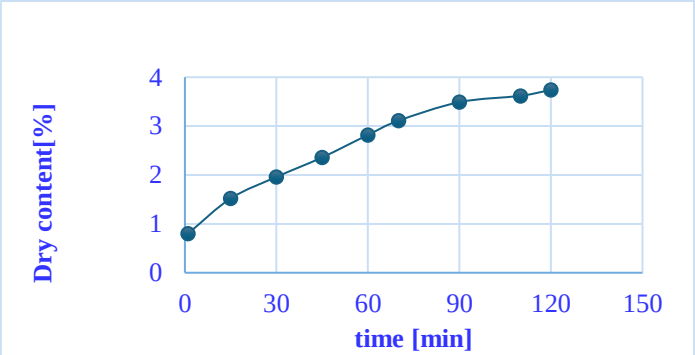

Figure.3 Outline of extraction Nigella Sativa sample 1

Table 4: Results of dry mass in relation to extraction time of Nigella Sativa Sample 2

Time min	**Dry content [%]**
1	0.4
10	1.36
30	2.18
45	2.56
60	3.2
70	3.53
90	4.12
110	4.53
120	4.73

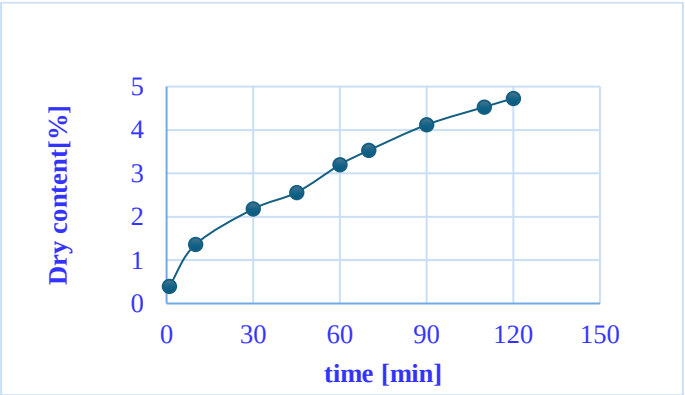

Figure.4 Outline of extraction Nigella Sativa sample 2

Table 5: Numerical balance of the process

Sample No 2		**Dry content [%]**	**Ext.100% [gr]**
Quantity of solvent Ethanol gr	**1468**		
Quantity of black seed gr	**200**		
Extr., quantity after maceration gr	**1401.5**	4.73	66.3
Extraction quantity after evaporation gr	**166.8**	39.92	66.58
Extr. Quantity after separating the phases gr	**56.55**	78.5	44.39
Raw material/ extract ratio X:1	**4.5**		

4. Conclusion

From the analysis and the results for the first sample, we may conclude that grinding with a higher level of grinding is not preferable due to low dry mass. From the analysis and the results for the second sample, we may conclude that the low level of grinding gives better results with a higher dry mass. It is relevant that in the two samples during the maceration, they balance out soon, so this requires additional solvents to quit the equilibrium and further extract the extract obtained after maceration is a homogeneous but impure solution that requires filtration before the evaporation process. The separation of phases after evaporation is not clear and requires that the extract be subjected to centrifugation. The extract - black seed oil is a product with a very high purity, and it is preferred to obtain the same with maceration extraction. The ratio of the raw material to the extract, black seed oil, is very low, which makes the technological process cheaper, but with a very high extraction coefficient. From the results, we may consider that the losses in the extract during the separation of the phases are about 25%

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