Extraction of Oil from *Nigella sativa* Seeds

Subjects: Food Science & Technology

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Many extraction methods, such as cold pressing, supercritical fluid extraction, Soxhlet extraction, hydro distillation (HD) method, microwave-assisted extraction (MAE), ultrasound-assisted extraction, steam distillation, and accelerated solvent extraction (ASE) have been used to extract the oils from black seeds under optimal conditions. Black seed oil contains essential fatty acids, in which the major fatty acids are linoleic, oleic, and palmitic acids.

Keywords: black seed (Nigella sativa) ; extraction methods ; fatty acid composition

1. Introduction

Black seed (*Nigella sativa*) is an annual flowering plant in the Ranunculaceae family and Plantae kingdom. Black seeds are mostly found in western Asia, the Mediterranean North Sea area, and western and southern Europe. The black seed is also described in the Bible as the “healing black seed”, Hippocrates and Discroides termed it as Melanthion, and Pliny coined it Gith [1]. According to the world’s agricultural production, the production of oil seed is 40.29 million metric tons in Pakistan; in all the world, it is 607.3 million metric tons [2]. According to the Unani Tibb medical system, *Nigella sativa.* has proved very helpful in curing many health disorders. *Nigella sativa* has been used since ancient times in various civilizations of the world, and it is recommended as a “miracle cure” because it has the potential to cure several diseases and regulate the process of natural healing in the human body [3]. According to Indian medicinal culture, seeds can be consumed as a bitter, anthelmintic, astringent, jaundice, stimulant, intermittent fever, diuretic, paralysis, emmenagogue, piles, skin diseases, and dyspepsia [4][5]. They can be utilized in the form of an anti-cancerous, -diabetic, -bacterial, hepato-toxic, -parasitic, and -fungal, as well as a therapeutic agent. Black seeds in herbal medicines are consumed directly as an active ingredient or in the form of herbal tea. The black seed extract has the tendency to show anti-oxidant and -inflammatory properties. It has been used by patients to suppress coughs, disintegrate renal calculi, impede the carcinogenic process, treat abdominal pain, diarrhea, flatulence and polio, exert choleretic and uricosuric activities [4][6]. According to former literature, *Nigella sativa* seeds show various properties against different kinds of cancer, such as blood, [7] skin, [8] cervical, [9] colon, [10] hepatic, [11] prostate, [12] breast, and renal [13]. The extract, seeds, and oil of *Nigella sativa* have proved to manage oxidative stress, hypertension, and diabetes, as well as [14] ulcers, [15] epilepsies, [16] fatty liver, [17] asthma, [18] arthritis, [19] inflammatory disorders, [20] cancers, [21], and parasitic diseases [22][23], in humans [24].

Nigella sativa is consumed in folk and Unani medicines in Pakistan. By following the previous literature, *Nigella sativa* has great potential for disease curing and health improvement; more research work has been needed to convert the herbal medicinal culture to new medicine systems. The thymoquinone contains a carbonyl polymer called Nigellon. Oil of *N. sativa* seeds and its active ingredients reveal therapeutic functions such as antiviral, antimicrobial, lowering the blood sugar level, antitumor, anti-oxidation, muscle relaxation, and anti-inflammatory [25][26][27][28]. Formerly, different kinds of chemical compounds were isolated from various species of *Nigella sativa* [29]. Hence, *Nigella sativa* has 84 g fiber, 216 g protein, 45 g ash, 38 g moisture, 406 g fat, 249 g free nitrogen extract, 60 mg zinc, 105 mg iron, 527 mg phosphorus, 15.4 mg thiamin, 18 mg copper, 57 mg niacin, 0.16 mg folic acid, and 1860 mg calcium per kg [30]. *Nigella sativa* is recognized as an annual herbaceous plant, which is included in the family Ranunculaceae and largely cultivated in different regions of southern Europe, as well as a few areas of Asia [31], which includes Saudi Arabia, Pakistan, Syria, India, and Turkey [32]. The colors of its flowers are mainly white, pink, yellow, light blue, or lavender, and its flower makeup has 6–10 petals. The fruity portion of the plant is a bulky and balloon-like capsule, which carries many black seeds with a bitter and aromatic taste [4]. The farming time for *Nigella sativa* falls between November and April, and its germination period is completed two weeks after seed sowing. However, the fruits are usually obtained from plants from January to April [33]. *Nigella sativa* seed oil and their active ingredients have been used in many dishes for chilling and flavoring [34]. About 28–36% fixed oil is present in *Nigella sativa* seeds, and it consists of a diverse range of unsaturated fatty acids, such as linolenic, arachidonic, linoleic, and eicosadienoic acids. In contrast, saturated fatty acids are myristic, stearic, and palmitic acids [35]. The other components of seed oil are citronellyl acetate, cholesterol, carvone, campesterol, α-spinasterol, stigmasterol, p-

cymene, β-sitosterol, palmitoleic, oleic, citronellol, nigellone, and limonene [36]. The fixed oil contains 12.5% of oleic, linoleic, and palmitic acids; the volatile oils contain carvone, trans-anethole, limonene, and p-cymene [37]. The oil also contains considerable amounts of carbohydrates, amino acids, fixed or volatile oils, and proteins [32]. However, the versatility in the pharmacological properties of seeds is mainly due to the presence of quinine constituents, the most abundant of which is thymoquinone. The volatile oils of black seeds have larger quantities of thymoquinone. Gali-Muhtasib et al. [38] explained that thymoquinone, flavonoids, alkaloids, and tannins are the active ingredients of black seeds, extracted with ethyl alcohol and cold water [39]. Nowadays, *Nigella sativa* oil is categorized as functional oil because it has a high content of omega-9 (oleic, 15–24%) and -6 (linoleic, 54–70%) fatty acids, as well as others found in minor amounts [40]. This crude oil has a protective effect, mostly on nerve cells [41] and the liver [42]. In addition to other biological functions, *Nigella sativa* crude oil a carries small amount of volatile oil and exhibits functional properties, due to thymoquinone. This crude oil is safe and largely utilized in dietary supplements because it has less toxic effects [43]. The key constituent of oil is thymoquinone, which performs its function as an anti-epileptic agent [44]. This extract also contains tannins, terpenes, alkaloids, glycosides, saponins, steroids, and flavonoids [45]. Biologically active compounds of *Nigella sativa* are not stable during different chemical reactions, and their prescribed amount was not appropriate for clinical research. The *Nigella sativa* seeds also have unsaturated fatty acid esters with nigellimin, terpene alcohols, saponin, and the alkaloid nigellidine [46][47]. According to Agbaria et al. [48], the unroasted seeds have less anti-proliferative activity than the pretreated heated seeds (50–150 °C, about 10 min) for the milling process. Initially, the oxidation process was low; it was enhanced during storage (about 55 days) and leveled off [49]. To overcome all of the above-mentioned issues, different encapsulation techniques have been used for black seed oil and their active components. It is the most successful method for protecting thymoquinone (**Figure 1**). Today's black seed oil is microencapsulated with emulsification processes spray-drying and nanoprecipitation. This encapsulated black seed oil has high phytochemical content, which improves the nutritional status of food items. *Nigella sativa* oil is consumed as a functional ingredient in food systems. It can be utilized in the form of flavoring and seasoning agents during food product development [50][51].

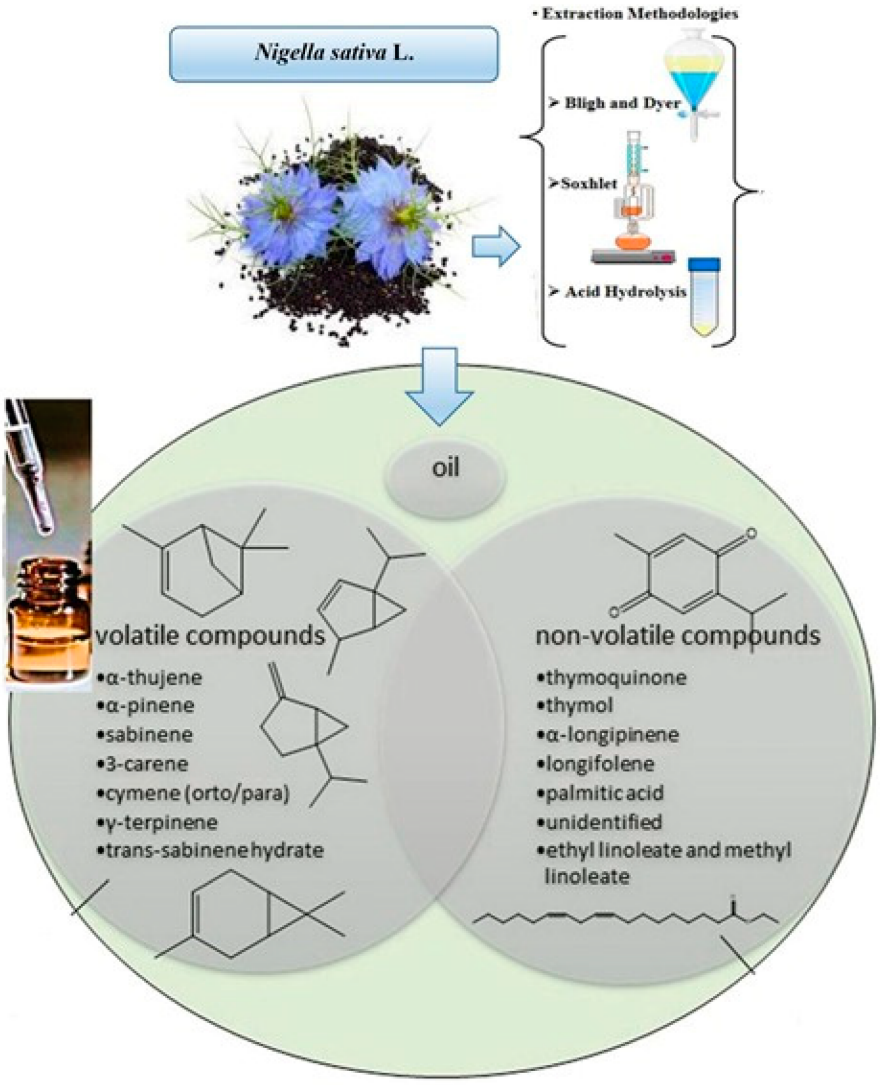

Figure 1. *Nigella sativa* oil extraction and its chemical composition.

2. Extraction of Oil from *Nigella sativa* Seeds by Using Different Novel Techniques

2.1. Cold Pressing

Oil can be extracted from *Nigella sativa* seeds by using the different methods. According to the Kiralan et al. [52], the cold pressing method is suitable for extracting *Nigella sativa* oil from seeds. In this method, mechanical pressing was used for the pressing of seeds at a temperature of 25 °C. Furthermore, the separation of oil and crushed seed fiber has been performed by soaking the solution for one night at a 25 °C temperature. After that, filtered oil was obtained by using a glass funnel and Watman #4 filter paper (0.45 µm, Vivascience AG, Hannover, Germany).

2.2. Supercritical Fluid Extraction

Another innovative method for the extraction of *Nigella sativa* oil from seeds was used by Mohammed et al. [53]. The supercritical fluid extraction equipment (FeyeCon Development B.V. Weesp, Netherlands) was used for *Nigella sativa* seed oil extraction, by using a stainless steel grinder (Waring Commercial, Torrington, CT, USA) for 3–4 min; the crushed dried seeds were obtained, placed the material in a 50-L container of extractor, and sealed tightly. The system used an automatic back pressure regulator for maintaining the temperature at 40 °C for 1 h; the pressure was 600 bar, and the flow rate of injected liquid carbon dioxide (CO_2) was 150 L/h.

Rao et al. [54] also chose the supercritical fluid extraction method for *Nigella sativa* seed oil extraction. In its instrumentation, it contained a syringe pump with 260 mL capacity, controller system (ISCO 260D), and ISCO series 2000 SCF extraction system (SFX 220), consisting of a dual chamber extraction module with two 10 mL stainless steel vessels. Hence, about 5 g of ground black seeds were added in a stainless steel cell (10 mL). Then, the standard quantity of supercritical carbon dioxide (SC CO_2) (50–400 mL) was flushed into the cell at a 1 mL/min flow rate. The final concentration of the extract was collected in the cold trap. After optimization of supercritical fluid extraction conditions, the lower yield of 0.84% (508 °C, 400 bar, and 100 mL) and higher yield of 31.7% (508 °C, 100 bar, and 200 mL) were obtained at optimum levels.

2.3. Soxhlet Extraction

Dinagaran et al. [55] used the soxhlet apparatus for *Nigella sativa* oil extraction from black seeds. For this purpose, *Nigella sativa* seeds were collected from different regions of India, including Tamil Nadu, Triplicane, and Chennai. During the sieving process, the small and contaminated seeds were removed at room temperature. In this process, the seeds were first ground using a tabletop mixture, hexane was used for extraction of seed oil for approximately 2 h in a soxhlet apparatus, and the extracted oil was stored at room temperature in a selected amber glass bottle until use. *Nigella sativa* seed has 28–35% fixed oil, which mainly consists of unsaturated fats. Through gas chromatography–mass spectrometry (GC-MS) analysis, 32 different compounds were found in black seeds.

2.4. Hydro Distillation (HD) Method

Kokoska et al. [56] selected the hydro distillation (HD) method for the extraction of oil from *Nigella sativa* seeds. In the first step, the seeds were ground at 25 °C. Then, they weighed the 70 g sample to be used for further analysis. The average yields were achieved and figured on a dry weight basis. For attaining essential oil through the HD method, they used a water holding flask for placing the material. It is called a Clevenger-type apparatus because the flask is directly connected to the condenser. After 2 h of continuous processing, a yield of 0.29 wt/wt of pale-yellow oil was obtained.

Burits and Bucar [57] also chose the same technique for oil isolation, and an Austrian pharmacopoeia (Clevenger apparatus) was used as standard apparatus in the whole process. The results were not satisfactory because the oil extracted had lower quantities of essential oil, with only 3% thymoquinone content, while Soxhlet extraction yielded 48% thymoquinone content.

2.5. Microwave-Assisted Extraction (MAE)

Abedi et al. [58] performed the oil extraction through a domestic microwave oven (Daewoo Electronics KOC-154KWR Microwave Oven) with a frequency of 2450 MHz. Initially, they took 50 g of ground seeds and selected a 500 mL round-bottomed flask for the soaking of seeds in 50 mL of water for about half an hour. After that, the Clevenger apparatus was fixed with a flask and utilized 450 W of power for heating (30 min). However, the essential oil was leached out in the n-hexane solvent. Only 0.33% essential oil yield was achieved by using MAE extraction conditions (power 450 W, moisture content 50%, and time 30 min).

2.6. Ultrasound-Assisted Extraction

Moghimi et al. [59] used an ultrasound-assisted extraction method for oil extraction. For one treatment, a sample of 500 g was transferred to the 1.5-l container that was placed in the ultrasonic bath. Several optimization conditions were selected, including the time (30, 45, and 60 min) and ultrasound pretreatment power (30, 60, and 90 W) at a fixed frequency of 25 kHz. After completing this process, the oil was isolated by using a screw press at 33 rpm speed. The maximum results of 39.93% extraction efficiency were achieved at power of 90 W and time of 60 min, while the minimum results of 27.29% extraction efficiency were achieved at power of 30 W and time of 30 min.

2.7. Steam Distillation

For the prevention of the side effects of degradation, steam distillation was performed at a low temperature. In 100 mL of distilled water, 10 g of seeds were added and mixed. This mixture was quantitatively transferred into the separatory funnel. This process of extraction was performed three times; a total of 10 mL of diethyl ether was added at every step, and the funnel was shaken vigorously. Sodium sulfate was used to dry the organic layer, and 0.4% was the obtained yield after evaporation in the water bath [60]. The steam distillation process was used by Kokoska et al. [56]. A glass column-containing material was interpolated between the condenser and flask. The yield of oil that was extracted by steam distillation was 0.39%, and the color of the oil was pale yellow.

2.8. Accelerated Solvent Extraction (ASE)

A 1 g sample of black seeds in powdered form was taken in a stainless steel cell with a 34 mL capacity. The conditions were set: 100 atm pressure, 10 min static time, 20% rinse volume, 2 extraction cycles, 30 s purge time, and 26 mL of solvent volume. P1-P9 black seed samples from Pakistan, Indian, and Saudi Arabian were treated with n-hexane as P1-P3, methanol (MeOH), and dichloromethane (DCM) at 40 °C, P4-P6 with MeOH, DCM, and n-hexane at 50 °C; the same procedure was performed for P7-P9 at 70 °C. The results reveal that the solvent with high yield, following n-hexane, was MeOH, whereby the yield and recovery observed was 2.5 g (12.5%) for Saudi Arabia, 2.2 g (11%) for Pakistan, and 2.04 g (10.2%) for Indian black seed sample [61] (**Table 1**).

Table 1. Different extraction methods of *Nigella sativa* seeds oil.

Extraction Method	Solvent Used	Advantage	Disadvantage	Yield/Efficiency	Source
Cold pressing	Hexane	Involves no heat or chemical treatments during oil extraction	Provides low yield	27%	[62]
Supercritical fluid extraction	SC CO_2	Rich in antioxidants	High cost	31.7%	[54]
Soxhlet extraction	Methanol	Low in cost	Residues of solvent has been left behind in the extracted oil	29.9%	[55]
Hydro distillation (HD) method	Water	Very simple method and instrument, shorter extraction time, free from organic components, less labor consumption, good in quality, lower cost with good efficiency	High energy is required for extraction	0.29%	[56]
Microwave-assisted extraction (MAE)	n-hexane	Free from organic solvent, less time with maximum yield	Additional filtration or centrifugation required to remove the solid residue	0.33%	[58]
Ultrasound-assisted extraction	Hexane	Less energy and solvent consumption, reduced time of extraction		39.93	[59]
Steam distillation	Sodium sulphate	Performed at a low temperature to prevent from degradation	More time consuming, due to the low pressure of rising steam	0.40%	[60]
Accelerated solvent extraction	MeOH, DCM, and n-hexane	A latest and efficient method for extraction		2.5 g, 2.2 g, and 2.04 g	[61]

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