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RESEARCH PAPER

Improving the Efficiency of Extracting *Nigella sativa* Oil through Pretreatment: Effects on Yield and Active Constituents

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ABSTRACT

The plant known as black seed, or *Nigella sativa*, is well-known for its small, jet-black seeds, which are bursting with health benefits. Because of the oil's potent therapeutic properties, there has been a lot of interest in its extraction from these seeds. The effectiveness of steam and microwave pretreatments on *Nigella sativa* seed oil extraction is examined in this study, along with their impact on oil yield and active ingredients. Two extraction techniques, hydrodistillation and supercritical fluid extraction, are used to investigate the effects of different pretreatment times on seed samples. The results show that utilizing hydrodistillation, microwave pretreatment for three minutes (HM3) produces the maximum oil extraction (0.892%), outperforming untreated samples (H0) at 0.436%. In the same direction, samples that have been microwave-pretreated for three minutes (HS3) in supercritical fluid extraction show the highest yield (9.44%) when compared to their untreated counterpart (SO), which shows a yield of 4.10%. Microwave-treated samples exhibit a more noticeable increase in oil yield, even if steam pretreatment also improves it. Furthermore, H0 has a 19.40% Thymoquinone level, which is crucial for the active ingredients in the oil, but pretreatment HM3 samples have an increased 26.73% Thymoquinone content. On the other hand, the Thymoquinone content in supercritical fluid-extracted S0 is 8.35%, while pretreatment SM3 samples show 11.53%. Thymoquinone content lags despite supercritical fluid extraction's greater yield; this could be because the technique has a lower operating pressure (70 atm). The results of this study highlight how pretreatment techniques can increase oil yield without lowering oil quality or antioxidant activity. Prospective enhancements in process parameters, namely in the operating pressure of supercritical fluid extraction, could potentially augment Thymoquinone content and augment oil yield. The results validate the feasibility of pretreatments in maximizing the extraction efficiency of *Nigella sativa* oil while preserving its antioxidant qualities and oil quality.

HIGHLIGHTS

- Balancing oil yield and quality through pretreatment techniques.
- Effect of microwave pretreatment on *Nigella sativa* oil extraction efficiency.

Keywords: Fixed oil, saturated fatty acids, thymoquinone, hydrodistillation

The achievement of agricultural sustainability is a prime concern of today's agriculture and to achieve that goal it is important to adopt suitable cropping system and crop diversification (Maitra *et al*. 2023; Sahoo *et al*. 2023; Sairam *et al*. 2023). The investigation of herbal medicines and medicinal plants has attracted significant interest because of their possible medical advantages. Of these, *Nigella sativa*, also referred to as black cumin or black seed, has garnered significant attention due to its historical relevance and its health benefits. The key

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to its therapeutic effectiveness is its seed oil, which is abundant in bioactive substances, including thymoquinone. The diverse uses of *Nigella sativa* seed oil in conventional medicine, coupled with the increasing curiosity surrounding its pharmacological properties, have encouraged scientists to further explore the plant's characterization, extraction process, and possible therapeutic benefits.

Commonly referred to as "Kalonji," *Nigella sativa* L., or black cumin, is a good source of several nutritionally important ingredients. Many tribes and civilizations have utilized *Nigella sativa* seeds as a natural medicine to treat and prevent a variety of illnesses. According to recent study, *Nigella sativa* seeds were found at Uli Burun, off the southwest coast of Turkey, about 3,000 years ago (Sultan *et al*., 2009). The Prophet Muhammad's proverb, "Hold on to use of the black cumin seed, for it has a remedy for every illness except death," is another reason for its notoriety. There are lower concentrations of saturated (12–25%) and monounsaturated (18–29%) fatty acids in the fixed oil that is produced from *Nigella sativa* seeds. Unsaturated fatty acids, especially polyunsaturated ones, make up 48–70% of the oil's overall composition and are its main constituent.

Furthermore, dihomolionolenic acid (1.9–2.3%) is recognized for its ability to decrease cholesterol and act as an antioxidant (Butt and Sultan, 2010). In addition to having a balanced fatty acid composition, it has significant levels of related bioactive compounds including tocopherols. These phytochemicals play a key role in decreasing the body's total antioxidant capacity and lowering the modification of low-density lipoproteins (LDL) brought on by the generation of free radicals (Bjørklund and Chirumbolo, 2017; Jiang *et al*. 2021). Additionally, its hypoglycemic and hypercholesterolemic aspects are strengthened by the presence of phytosterols in levels ranging from 0.33 to 0.36% (Selin *et al.* 2017). The predominant component of sterol contents among phytosterols is α sitosterol, which accounts for 32.2–34.1%. This is followed by Δ 5-avenasterol (27.8–27.9%) and Δ 7-avenasterol (18.5–22.0%). The combined contributions of stigmasterol, campesterol, and lanostero account for 17.6–19.5% (Atta 2003; Mazaheri *et al.* 2019). Because of its rich nutritional profile, *Nigella sativa* and its constituents have a multifaceted role against numerous illnesses. Since local black cumin oil is just getting started, it is imperative to make sure the quality is on par with that of commercial black cumin oil. Determining the ideal conditions for extracting *Nigella sativa* oil is crucial, since the right parameter may result in a higher oil output. The most effective method for obtaining black seed oil is still being investigated. Numerous studies are currently being conducted globally to examine the potential for improving oil extraction yield, oxidative stability, and nutraceutical content using pretreatments before oil extraction. Pretreatments can take many different forms, such as microwave treatment, enzymatic pretreatment, steam conditioning, and high-pressure steam explosion (Sarker *et al.* 2021). These pretreatments are applied to the seeds in the current study before they are extracted for oil using two different techniques: the traditional hydrodistillation method and the sophisticated supercritical fluid extraction method. An effort has been made to look into the potential for raising the oil output as well as how various pretreatments affect the active components of the oil like thymoquinone are studied.

MATERIALS AND METHODS

The indigenous variety of *Nigella sativa* seeds was procured from the local market in Bangaluru, India ensuring a consistent and region-specific sample pool for analysis. The research utilized reagents of analytical and HPLC grade, sourced from reputable suppliers such as Sigma-Aldrich (Sigma-Aldrich Tokyo, Japan) and Merck (Merck KGaA, Darmstadt, Germany). This ensured the use of high-quality materials in the extraction and analysis processes, maintaining the integrity of the study's results.

The pretreatment phase involved subjecting *Nigella sativa* seeds to different durations in a microwave oven (Kenstar appliances limited, model no. OM26DCF), operating at a stable frequency of 2450 MHz, power of 1400±5%, and voltage of 230V with a 50Hz AC supply. Specifically, seeds underwent pretreatment durations of 1 minute, 2 minutes, and 3 minutes in the microwave, standardized for consistency in the extraction process. Additionally, steam pretreatment was employed at durations of 5 minutes, 20 minutes, and 35 minutes before the oil extraction phase.

Two different techniques were used to extract the essential oil: supercritical fluid extraction (SFE) and hydro-distillation. These methods sought to assess the effectiveness of oil extraction with respect to the different pretreatment times used on *Nigella sativa* seeds. Through a methodical investigation of microwave and steam pretreatment times in conjunction with various extraction methods, the study aimed to identify the best conditions for optimizing oil yield and preserving critical active ingredients in the extracted oil.

Hydrodistillation

The extraction of essential oil was done using Janeš *et al.* (2009) method. A round bottom flask (2L) containing 250g of ground black cumin seeds was filled with water (1:6 v/v), and the mixture was boiled in a hydro distillation apparatus for 4 hours at 100°C. The essential oil was decanted after collecting on the water's surface. The oil's moisture content was eliminated using anhydrous sodium sulfate. The essential oil yield was calculated and kept for later use at 4°C.

Supercritical fluid extraction

The extraction was conducted in SFE mode using an SFE–1000 equipment (Thar Technologies, Germany) at the Food Engineering and Properties Laboratory. In this investigation, 50 g of fully crushed seeds were added to an extraction vessel to carry out the extraction process. After that, the plant was extracted using SFE for 15 minutes of static and 20 minutes of dynamic pressure at 70 atm and 40°C temperature. In the procedure, ethanol is employed as a co-solvent. The SFE system employed a Duraflow manual variable restrictor (Suprex) to gather the extracted analytes. About 25/g was the SFE flow rate that passed past the Duraflow restrictor. The extracted analytes were collected in a volumetric flask. In order to have better collection efficiency, volumetric flask was placed in an ice bath during the dynamic extraction stage. Yield of essential oil was determined and stored at 4ºc for further use.

$$\text{Yield of oil} = \frac{\text{Amount of oil extracted}}{\text{Amount of sample taken}} \times 100$$

Pre-treatments used in the study

H_0 – Hydrodistillation control

HM_1 – Hydrodistillation microwave pretreated sample at 1 min

HM_2 – Hydrodistillation microwave pretreated sample at 2 min

HM_3 – Hydrodistillation microwave pretreated sample at 3 min

HS_1 – Hydrodistillation steam pretreated at 5 min

HS_2 – Hydrodistillation steam pretreated at 20 min

HS_3 – Hydrodistillation steam pretreated at 35 min

S_0 – Supercritical fluid extraction (SCFE) control

SM_1 – SCFE microwave pretreated at 1 min

SM_2 – SCFE microwave pretreated at 2 min

SM_3 – SCFE microwave pretreated at 3min

SS_1 – SCFE steam pretreated at 5 min

SS_2 – SCFE steam pretreated at 20 min

SS_3 – SCFE steam pretreated at 35 min

RESULTS AND DISCUSSION

An evaluation of the proximate composition is essential for assessing raw material quality. *Nigella sativa* seeds were analyzed in this study in order to identify different quality features. The percentages of moisture 7.46 ± 0.12, proteins 21.40 ± 0.50, fat 32.52 ± 0.74, fiber 7.42 ± 0.14, and ash 4.80 ± 0.12 were found in the data. Furthermore, the extract free of nitrogen was determined to be 32.45 ± 0.80% (Table 1). The results of this study's characterization closely matched the values reported in the literature, with a few minor deviations; environmental variables including location and climate may have contributed to these discrepancies. Furthermore, since indigenous variety was evaluated in the research, genetic differences may also have played a role.

Table 1: Proximate composition of *Nigella sativa* seeds

Sl. No.	Proximate composition	(%)
1	Moisture	7.46 ± 0.12
2	Crude Protein	21.40 ± 0.50
3	Crude Fat	32.52 ± 0.74
4	Crude Fiber	7.42 ± 0.14
5	Ash	4.80 ± 0.12
6	Nitrogen free extract	32.45 ± 0.80

**Values expressed are means ± standard deviation.*

Effect of pretreatments on yield of oil extracted by hydrodistillation method

When *Nigella sativa* oil was extracted using the hydrodistillation method without any pretreatments, the yield of oil increased significantly to approximately 0.436% (Fig. 1).

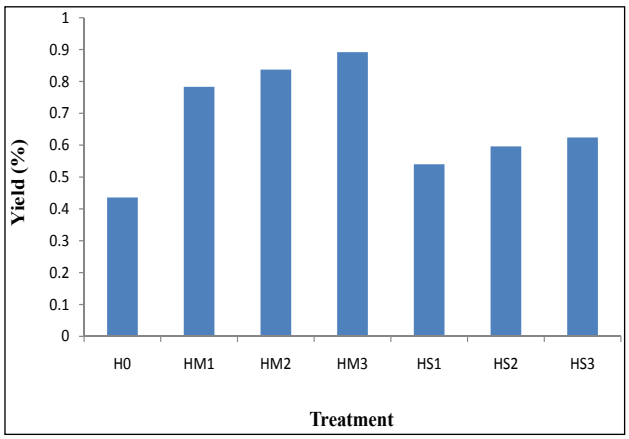

Fig. 1: Effect of pretreatments on yield of oil extracted by hydrodistillation method

This may be deduced from the extraction data since the samples HM1, HM2, and HM3 that were microwave-pretreated yielded yields of around 0.783, 0.837, and 0.892, respectively, indicating increases of approximately 79.58, 91.97, and 104.5%, respectively, in comparison to the H0 sample. The results of this study are corroborated by studies conducted by Azadmard-Damirchi *et al.* (2010), Yang *et al.* (2013) and Wroniak *et al.* (2016). The possible reason for higher extraction yield might be due to cell membrane rupture obtained using microwave radiation and permanent pores can be generated, enabling the oil to move through the permeable cell walls.

When compared to microwave pretreatment, the samples that were treated to steam pretreatment also had shown a little increase in oil output. In comparison to the H0 sample, the samples HS1, HS2, and HS3 displayed yields of roughly 0.540, 0.596, and 0.624, respectively. This represents increases of roughly 23.85, 36.69, and 43.11%, respectively. Research studies by Jacquet *et al.* (2015) and Sarker *et al.* (2021) corroborate the current findings. Steam pretreatments also easily break down the cell walls, increasing to better oil yields. Currently, studies are being conducted to examine the use of high pressure steam explosion treatments to boost oil output.

Effect of pretreatments on yield of oil extracted by supercritical fluid extraction

Supercritical fluid extraction of *Nigella sativa* oil without any pretreatment resulted in an oil yield of around 4.10%, however, pretreatments significantly increased the oil yield. The extraction results suggest this since the samples SM1, SM2, and SM3 that were microwave pretreatment yielded yields of around 7.96, 8.18, and 9.44%, respectively (Fig. 2).

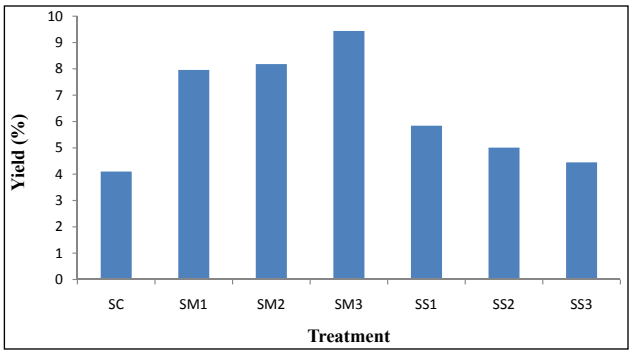

Fig. 2: Effect of pretreatments on yield of oil extracted by supercritical fluid

This indicates an increase of approximately 94.14, 99.51, and 130.24%, respectively, in comparison to the S0 sample (Table 2). The results of this study are corroborated by studies conducted by Nehmeh *et al.* (2022) and Abedinzadeh *et al.* (2023). The possible reason for higher extraction yield might be due to cell membrane rupture obtained using microwave radiation and permanent pores can be generated, enabling the oil to move through permeable cell walls.

When compared to microwave pretreatment, the samples that were treated to steam pretreatment also showed a little increase in oil output. In comparison to the S0 sample, the samples SS1, SS2, and SS3 showed yields of around 5.84, 5.01, and 4.45%, respectively. This represents increases of approximately 42.43, 22.19, and 8.5%, respectively. However, as pretreatment times increased, the oil output decreased. This could be because the tissues were overly softened by water absorption, making it more difficult to extract during the supercritical fluid extraction process. Research studies by Naviglio *et al.* (2019) and Jha and Sit (2022) corroborate the current findings. Steam treatments also easily break down the cell walls, increasing to larger oil yields.

Table 2: Effect of pretreatments on yield of oil extracted by hydrodistillation and supercritical fluid

Sl. No.	Treatments	Yield (%)
1	H_0 – Hydrodistillation control	0.436
2	HM_1 – Hydrodistillation microwave pretreated sample at 1 min	0.783
3	HM_2 – Hydrodistillation microwave pretreated sample at 2 min	0.837
4	HM_3 – Hydrodistillation microwave pretreated sample at 3 min	0.892
5	HS_1 – Hydrodistillation steam pretreated at 5 min	0.540
6	HS_2 – Hydrodistillation steam pretreated at 20 min	0.596
7	HS_3 – Hydrodistillation steam pretreated at 35 min	0.624
8	S_0 – Supercritical fluid extraction (SCFE) control	0.410
9	SM_1 – SCFE microwave pretreated at 1 min	7.96
10	SM_2 – SCFE microwave pretreated at 2 min	8.18
11	SM_3 – SCFE microwave pretreated at 3min	9.44
12	SS_1 – SCFE steam pretreated at 5 min	5.84
13	SS_2 – SCFE steam pretreated at 20 min	5.01
14	SS_3 – SCFE steam pretreated at 35 min	4.45

GC-MS Analysis of *Nigella sativa* Oil

Nigella sativa oil is evaluated for its active constituents by using GC-MS analysis. Samples for GC-MS analysis are selected based on the increase in the yield of the oil in the sample after pretreatment. Two samples H0 and HM3 from hydrodistillation method and two samples S0 and SM3 from supercritical fluid extraction are taken for GC-MS analysis to evaluate any effect on the constituents of oil after pretreatments. The pretreated samples were compared with their respective untreated control samples.

GC-MS analysis of 'H_0' sample

The following compounds were identified through quantitative analysis of the hydrodistillation-extracted *Nigella sativa* essential oil. Linalool (8.47%), Thymoquinone (19.40%), Longifolene (9.69%), and n-propyl 9,12 octadecadienoate (6.55%) are the main ingredients. Other constituents in significant proportions include α-longipinene, palmitic acid ethyl ester, terpinen-4-ol, ethyl oleate, and 9-octa decenoic acid. There are about 35.33% minor components found. A research conducted by Saleh et al. (2018) provide support for these findings. To look into any effects on the oil contents, the results of this untreated or control sample are compared to those of the pretreated sample.

GC-MS analysis of 'HM_3' oil sample

The quantitative analysis of sample HM3 has revealed numerous functional components. Notably, thymoquinone constitutes a major component at 26.73%, surpassing the untreated sample's concentration of 19.40%. Other elements, including 5-ethyl-3-(1-methylethylidene-4-hexen-2-one), Terpinen-4-01, 3, 7, 11-Trimethyl-2, 10-dodecadien-1-ol, and others, exhibit higher concentrations compared to the untreated sample. Additionally, significant constituents such as Isolongifolene (8.95%), Bicyclo (6.1.0), nonane-9, 9-dicarboxylic acid (8.06%), α-Longipinene (3.92%), Camphor (2.55%), Linalool (2.34%), and Phytane (1.13%) have been identified. Minor components constitute approximately 20.5% of the composition. Saleh *et al.* (2018) findings align with the results of the current study.

GC-MS analysis of 'S_0' sample

The quantitative analysis of *Nigella sativa* oil sample SM3, extracted using supercritical fluid extraction, revealed various constituents. Major functional compounds identified include 9,12 Octadecadienoic acid (39.25%), Thymoquinone (8.35%), palmitic acid (4.34%), Methyl 10-trans12 cis octa deca dienoate (12.97%), and 4-Pentenyl alcohol (5.88%). Some compounds not found in other extractions include E, Z-1, 3, 12 Nonadecatriene (5.83%), Widdrol hydroxyether (3.07%), and Cis 11,14 Eicosadienoc acid (1.54%). Other minor components constitute approximately 11.84%. In comparison to the S0 sample, the thymoquinone content has increased, possibly due to prior pretreatment. Further optimization of process conditions, such as temperature and pressure, may result in higher quantities of thymoquinone and other compounds. Currently, there is insufficient data to validate these observations.

GC-MS analysis of 'SM_3' sample

The following ingredients were revealed by the quantitative analysis of the *Nigella sativa* oil sample

"SM3," which was extracted by supercritical fluid extraction. Methylhexadecadienoate (16.37%), palmitic acid ethyl ester (13.38%), ethylocta decenoate (13.91%), and thymoquinone (11.53%) are the main functional chemicals found. Significant quantities of other ingredients, such as octa decadienoic acid, α-longipinene, and linalool, are present. There have been reports of novel chemicals found in this oil that were not previously discovered, such as α-Glyceryl Linoneate and Eicosanoic Acid. S0 has a lower thymoquinone concentration than the H0 sample. This might be a result of the extraction process circumstances, thus we can investigate the thymoquinone content further by adjusting the process parameters, such as raising the pressure and temperature. Supercritical fluid extraction of *Nigella sativa* oil does not require a lot of work to support these findings.

CONCLUSION

The hydrodistillation method exhibited notable improvements in oil yield when employing microwave pretreatment, particularly with sample HM3 achieving the highest yield at 0.892%, surpassing the untreated H0 sample at 0.436%. Similarly, in supercritical fluid extraction, microwaved-treated samples (HS3) demonstrated a superior yield of 9.44%, compared to the untreated SO sample at 4.10%. Although steam pretreatment resulted in an increase in oil yield, the quantity remained comparatively lower than that achieved with microwave pretreatment. Overall, microwave pretreatment emerged as an effective strategy for enhancing the extraction of *Nigella sativa* oil. GC-MS analysis supported these findings by confirming the retention of antioxidants post-pretreatment. Thymoquinone content in the untreated H0 sample was 19.40%, while the pretreated sample HM3 exhibited a higher content of 26.73%. Similarly, the untreated S0 sample displayed a thymoquinone content of 8.35%, while the pretreated sample SM3 showed an increased content of 11.53%. However, despite the increased yield in supercritical fluid extraction, the retained thymoquinone content was comparatively lower than that of the hydrodistillated sample, possibly attributed to the low operating pressure (70 atm) used in supercritical fluid extraction. It is suggested that refining process conditions, such as pressure, could further enhance both oil yield and thymoquinone content. In summary, the employed pretreatments have proven to be valuable in augmenting oil yield without compromising antioxidant activity or the overall quality of the oil.

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