

Research

Optimization of ultrasound-assisted extraction of *Nigella sativa* seed oil for enhancement of yield and antioxidant activity

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Received: 17 September 2023 / Accepted: 5 February 2024

Published online: 01 March 2024

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Abstract

The seeds of *Nigella sativa* have been shown to contain a number of constituents that have nutritional and therapeutic characteristics. The oil extracted from the seeds of *Nigella sativa* has been traditionally used in food systems with reference to its medicinal properties. The different bioactive compounds present in the *Nigella sativa* seed oil consist of polyphenols which offer a fair amount of antioxidant activity to the oil. Most of these bioactive compounds are generally heat sensitive and prone to oxidation. During the processing and storage, there is loss of antioxidant activity of the oil and the associated bioactivity. This occurs primarily due to the effects of temperature and other environmental factors during processing and storage. Under this background, this study was undertaken with the aim of extracting the *Nigella sativa* seed oil in order to ensure the improved retention of antioxidant and bioactive characteristics in the essential oil. The extraction of *Nigella sativa* seed oil was carried out using ultrasound-assisted extraction technique using hexane as solvent. The process was optimized using response surface methodology in order to enhance the yield and antioxidant activity of the extracted *Nigella sativa* seed oil. The effect of different processing parameters was evaluated on the yield and antioxidant activity. The process variables included the extraction temperature, extraction time and solvent concentration. It was observed that all the factors had significant effect on the yield and antioxidant activity of the extracted *Nigella sativa* oil. The optimized conditions for maximizing the yield and antioxidant activity obtained were a solvent concentration of 42.82 mL/5 g of sample, the extraction temperature of 69.09 °C and extraction time of 86.60 min, which were validated and found close to the experiment values. Under this set of conditions, the yield was maximized to 34.53% with enhanced antioxidant activity, having IC₅₀ value of 203.56 µg/mL.

Article Highlights

1. Cavitation induced by ultrasound enhanced mass transfer and facilitates release of phytochemicals.
2. Ultrasound assisted extraction of *Nigella sativa* oil at lower temperatures preserved heat sensitive compounds.
3. *Nigella sativa* oil mainly contains thymoquinone, thymohydroquinone, and thymol.

Keywords Antioxidant activity · Extraction · *Nigella sativa* · Novel techniques · Valorization

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1 Introduction

There has been a growing interest in the use of natural bioactive compounds due to their potential health benefits. These bioactive compounds are mostly found as secondary metabolites in various plants in the form of phenolic compounds, flavonoids, carotenoids, etc. in grains, fruits, herbs, spices, seed oils, and other components [1]. *Nigella sativa*, commonly called as black cumin, has been used in different medicinal systems to provide various health benefits. The seed and oil of *Nigella sativa* have found numerous applications in providing therapeutic benefits in treating diabetes, cancer, inflammation, ulcers, fatty liver, parasitic diseases, and disorders [2, 3]. The seeds of *Nigella sativa* have been shown to contain a number of constituents that possess medicinal properties like antitumor, antipyretic, analgesic, etc. [4–6]. The bioactivity of black cumin has been proven to be largely attributed to the different fatty acids and polyphenols, antioxidants and other chemical components like thymoquinone. These components form major constituents of the essential oil extracted from the black seeds [7–9]. The essential oil has been traditionally used in food systems with reference to its medicinal properties. The different therapeutic characteristics of this essential oil have been proven through different studies which includes the anticancer [10], antibacterial properties [11]. It has also been shown to possess potential antioxidant activity [12] along with antioxidant and anti-inflammatory properties [13]. In addition to this, it has various antitumor [14], anticonvulsant [15] and antidiabetic properties [16].

The different bioactive compounds present in the black seed oil consist of polyphenols which offer a fair amount of antioxidant activity to the oil. During the processing and storage there is loss of antioxidant activity of the oil and the associated bioactivity. This occurs primarily due to the effects of temperature and other environmental agents during extraction [17, 18]. In this regard, different modifications in the extraction methods are applied in order to have an efficient extraction method which results in the maximum yield with minimum loss in the antioxidant activity and bioactivity of associated oil. Conventionally, the extraction methods like solvent extraction, cold pressing, etc. for extraction of essential oil from plants have a number of disadvantages associated with the process. These include extended extraction time, loss of volatile components, and other drawbacks. Novel extraction methods like supercritical carbon dioxide extraction utilized for extraction of temperature sensitive compounds are quite expensive and involve high costs of extraction. The solvent extraction process, though most common and effective method is time consuming [19].

Modified methods for extraction like the ultrasound assisted extraction (UAE) has been practiced which offer benefits in terms of shortened extraction time, enhanced yield and improved bioactivity of extracted oil. The UAE method has been shown to be an effective method for extraction of essential oils. It consists of exposing the materials in a solvent to high intensity sound wave frequencies, which cause the cavitation phenomenon resulting in the accelerated release of bioactive compounds. It provides improved yield along with the minimal loss of volatile compounds in the extracted oil. The UAE has been shown to increase the yield in pomegranate seed oil, pyrethrines, soyabean oil etc. [20–22]. In this study, the UAE method was applied for the extraction of black seed oil in order to improve the yield of the extracted oil with enhanced antioxidant activity and bioactivity. The process parameters were optimized using response surface methodology (RSM) utilizing the Box-Behnken design (BBD) for improving the yield and antioxidant activity followed by the validation of predicted values.

2 Materials and methods

2.1 Plant materials and chemical reagents

The *Nigella sativa* seeds (black seed) for the extraction of oil were obtained from Department of Unani Medicine, Aligarh Muslim University. The seeds are traditionally used as spice and Unani medicinal formulations for the treatment of many diseases [15]. Ethanol, methanol, Folin–Ciocalteu's phenol reagent, gallic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), deionized water and n-hexane of analytical grade used in the experiments were purchased from Sigma-Aldrich. Food grade carbon dioxide (99.99% purity) was supplied by Praxair Technology, Inc. It was used as solvent for the extraction of *Nigella sativa* seed oil.

2.2 Extraction of *Nigella sativa* seed oil (NSO)

2.2.1 Preparation of sample

The seeds of *Nigella sativa* were cleaned, sorted and graded. The fine grade seeds were then crushed finely in mixer and sieved. These powdered samples were subjected to three methods of extraction for obtaining oil. The extraction methods used were conventional soxhlet extraction, supercritical carbon dioxide extraction and ultrasound-assisted extraction.

2.2.2 Conventional extraction

The conventional soxhlet extraction (CSE) was done in soxhlet apparatus (Pelican Equipments, Chennai, SCS-6) as per the method explained by Dinakaran et al. [23]. with some modifications. 5 g of powdered samples were loaded in each of the thimbles inside the soxhlet apparatus for extraction using 70 ml of n-hexane as the solvent. The process was carried out for 4 h at a temperature of 80 °C. After the extraction, the solvent was evaporated in a rotary vacuum evaporator (KSHITIJ Instruments-2189, 2020) at 45 °C, leaving behind the essential oil as residue. The extracted oil was collected and stored in the refrigerator at 5 °C for further use.

2.2.3 Supercritical carbon dioxide extraction

The supercritical carbon dioxide extraction (SCE) was carried out in Speed SFE-2 system (Crescent Scientific Ltd., Scientific and Analytical Instruments, Mumbai, India) as per the method explained by Mohammed et al. [24] with some modifications. The *Nigella sativa* seeds powder of weight approximately 50 g was placed inside the load cell. The supercritical carbon dioxide was flushed through it at a pressure of 350 bar and a temperature of 45 °C for 60 min at a flow rate of 100 mL/min. The extracted oil was obtained in the collecting vessel after constant depressurization through automatic back-pressure regulator. The extracted oil was then stored in dark and refrigerated condition for further use.

2.2.4 Ultrasound-assisted extraction

The ultrasound-assisted extraction (UAE) of *Nigella sativa* seed oil (NSO) was undertaken as per the method explained by Moghimi et al. [25] with some modifications. The extraction was carried out in batches using n-hexane as solvent. The sample of 5 g was mixed with selected volumes of solvent n-hexane in order to have the different sample to solvent ratios from 1:5 to 1:10 in 100 mL glass beakers. The beakers loaded with samples were subjected to ultrasound treatment in the sonicating bath (Ultrasonic cleaner, LMUC-12) set at the fixed ultrasonic frequency of 40 kHz and ultrasonic power of 100W. The sample was ultrasonicated for 60 min, 90 min, and 120 min in the water bath maintained at temperatures of 40 °C, 60 °C and 80 °C. The different combinations of time, temperature and sample-to-solvent ratio were set out according to the experimental design using response surface methodology (RSM). The range of each independent variable was presented in Table 1.

Table 1 Levels of the three independent variables used in the Box–Behnken design

Independent variables	Coded symbol	Levels		
		– 1	0	+ 1
Solvent concentration per 5 g sample (mL)	A	10	30	50
Extraction temperature (°C)	B	40	60	80
Extraction time (min)	C	30	60	90

After extraction, the contents were filtered through Whatman filter paper no. 1. The filtrate containing extracted oil and the solvent was subjected to evaporation in a vacuum evaporator set at a temperature of 45 °C. The extracted oil was then collected, weighed, and stored in refrigerated condition at 5 °C for further use.

2.3 Experimental design for ultrasound-assisted extraction and its optimization

For the UAE process optimization, the Box–Behnken design (BBD) in conjunction with response surface methodology (RSM) was used to determine the optimized combination of process factors for the extraction of essential oil from *Nigella sativa* seeds with enhanced antioxidant activity. The data was analyzed using the Stat-Ease's Design-Expert v13 software. The independent variables included solvent Concentration per 5 g sample (mL), extraction temperature (°C) and extraction time (min). The effect of these parameters was analyzed on the response variables which included the oil yield and the antioxidant activity of extracted oil.

2.4 Analytical studies of extracted oil

2.4.1 Yield of extracted oil

The weighing of samples was carried out using a standard scientific weighing electronic balance. The weight of the extracted oil in grams obtained after extraction from known weight of the seed sample provided the oil yield. The yield of the extracted oil was calculated by dividing mass of oil extracted with weight of the *Nigella sativa* seed sample, using the formula Eq. (1) [26].

$$\text{Yield(\%)} = \frac{O}{M} \times 100 \quad (1)$$

where O is the mass of oil extracted in grams and M is the mass of sample in grams.

2.4.2 Antioxidant activity

The antioxidant activity of the oil samples was determined using DPPH radical scavenging assay, as per the method of Baliyan et al. [27] with some modifications. The oil samples of different concentrations (5, 10, 20, 60, 80 and 100 mL) were prepared in methanol. A 0.1 mM methanolic solution of DPPH was prepared by dissolving 2.4 mg in 100 ml of methanol. 800ul of this DPPH solution was mixed with 200 μL of methanolic solutions of different oil concentrations. The mixtures were shaken and kept in dark for 30 min. Subsequently, the absorbance of these mixtures was measured at 517 nm with methanol as blank in a spectrophotometer. The percent of antioxidant activity was calculated as inhibition ratios by means of equation Eq. (2)

$$\text{Antioxidant activity (DPPH inhibition\%)} = \left(\frac{A_0 - A_1}{A_0} \right) \times 100 \quad (2)$$

where A_0 is the absorbance of blank and A_1 is the absorbance of samples to be tested.

The IC_{50} values for each oil sample were calculated from the plotted graph between the inhibitory ratios against the concentration of oil sample.

2.4.3 Total phenolic content

The determination of the total phenolic content (TPC) in *Nigella sativa* oil (NSO) samples was conducted using a modified version of the method described by Singleton et al. [28], which is a widely employed technique for quantifying phenolic compounds in a sample. This method also enables the evaluation of the sample's antioxidant potential. Firstly, the phenolic compounds were extracted from the NSO samples using a mixture of methanol and water. A 0.025 mL sample of the extract was then combined with distilled water to make a final volume of 1 mL. Subsequently, 0.1 mL of Folin–Ciocalteu reagent and 2 mL of a 10% sodium carbonate solution were added to the mixture. After a 30-min incubation period, the resulting blue color was measured at 760 nm using a UV–VIS spectrophotometer

(UV5704SS). To determine the TPC values, the obtained results were compared to a standard curve of gallic acid equivalents (GAE). The TPC values were expressed as milligrams of gallic acid equivalents (GAE) per 100 g of sample weight.

2.5 Statistical analysis

The experiments for each combination of the factors were carried out in triplicates. The SPSS software (v.24, SPSS, Chicago, IL, USA) was used for conducting the data Analysis of Variance (ANOVA) for the data. The data were reported as means \pm standard deviation (SD). The statistical significance between the means was evaluated at the significance level of p value less than 0.05 using Duncan's multiple range test.

3 Results and discussion

3.1 Optimization of the extraction process

The extraction of essential oils is affected by various factors and conditions. These parameters have a notable impact on the bioactive characteristics of the extracted oil. To address the primary objective of the present study, the extraction of *Nigella sativa* seed oil was conducted using the ultrasound-assisted extraction (UAE) method. The study's findings were then compared with outcomes obtained from alternative extraction methods to evaluate the enhancement of the extracted oil's bioactivity.

The objective of the study was to optimize the process parameters to improve both the yield and antioxidant activity of *Nigella sativa* seed oil. The study evaluated the impact of various process parameters, namely solvent concentration (mL/5 g), extraction temperature ($^{\circ}$ C), and extraction time (min), on the responses, which included oil yield and antioxidant activity. A summary of the model parameters and corresponding responses can be found in Table 2.

Table 2 Experimental design (BBD) for the extraction of *Nigella sativa* seed oil

Run	Process factors			Observed responses	
	Factor 1 A: Solvent concentration per 5g sample (mL)	Factor 2 B: Extraction temperature ($^{\circ}$ C)	Factor 3 C: Extraction time (min)	Response 1 Yield (%)	Response 2 Antioxidant activity (IC ₅₀) (μ g/mL)
1	50	40	60	21.27	285.36
2	10	80	60	30.13	292.37
3	30	60	60	30.56	203.56
4	10	60	30	24.93	266.52
5	50	80	60	32.07	238.65
6	30	40	30	19.07	316.23
7	50	60	30	28.31	224.45
8	30	80	30	30.95	262.43
9	30	60	60	30.56	203.56
10	30	40	90	21.44	310.37
11	10	60	90	29.31	260.67
12	50	60	90	32.99	203.56
13	10	40	60	18.31	342.43
14	30	80	90	34.92	257.46
15	30	60	60	30.56	203.56
16	30	60	60	31.82	198.14
17	30	60	60	28.94	200.79

3.2 Influence of UAE parameters

3.2.1 Effect of the factor variables on the yield (%)

The UAE method utilizes the cavitation phenomenon which creates high pressure and temperature bubbles inside the substrate followed by the rupture of them which results in the enhanced extraction of components due to breakdown of the cells, thereby facilitating effective extraction of components. The acoustic cavitation due to the application of ultrasound waves leads to fragmentation and localized erosion. The effect of factor variables viz. solvent concentration (mL/5 g), extraction temperature (°C) and extraction time (min) on the yield (%) is shown graphically through the contour plots and 3-D surface plots in Fig. 1a–f and summarized in Table 3.

It was observed that the yield increased significantly with the increase in solvent concentration with p-value less than 0.05 (Table 3). The yield increased from 27 to 30% with the increase in solvent concentration from 10 to 30 mL per 5 g of sample. Afterwards there was no significant increase in the yield, which remained almost constant (Fig. 1). The increased solvent volume facilitates improved mass transfer due to larger concentration gradient and enhanced interaction between the solvent and solutes. The presence of the solvent in increased concentration results in the improved solubility of oil leading to the higher yield. This effect was in agreement with the observations made by Zhao et al. [29], while studying the Ultrasound-assisted extraction of favela (*Cnidocolus quercifolius*) seed oil using ethanol as a solvent.

The yield of extracted oil was most significantly affected by the extraction temperature among the three factors. It was observed that the yield increased from 21 to 33%, as the extraction temperature increased from 40 to 72 °C. Afterwards the yield remained almost constant. The increased temperature facilitates the diffusion of solvent and also there is improved oil solubility in the solvent which increases the yield of extracted oil. There is also a progressive reduction in the surface tension which enhances the solubility oil, thereby increasing the oil yield. However, this increase in yield was observed up to an optimum level, after which the effect of temperature is negligible. This effect was in agreement with the observations made by Tian et al. [30] while studying the extraction of pomegranate (*Punica granatum L.*) seed oil using ultrasonic-assisted extraction method.

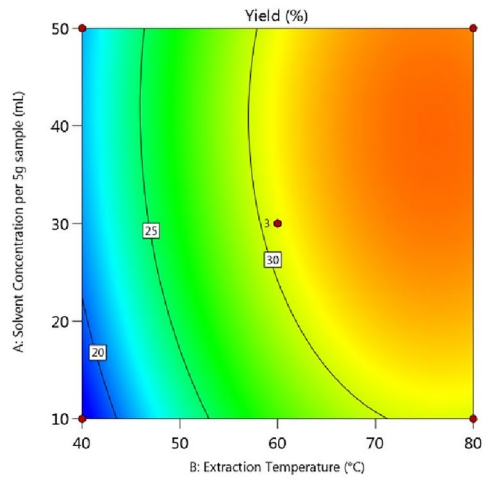
The extraction time, which is equivalent to the exposure time of ultrasound treatment, also increased the yield proportionately. With the increase in extraction time from 30 to 90 min, a continuous increase in the yield was observed from 28 to 32%. This phenomenon might be due to the longer exposure of ultrasonication waves to the sample dissolved solvent which leads to the improved oil solubility that results in increased yield. Similar trend of increase in essential oil from apple seed was observed with increase of extraction time [31]. The comparable results were reported by Zhang et al. [32] while studying the Ultrasound-assisted extraction of oil from flaxseed.

The enhancement of yield of the oil extracted using ultrasound assisted methods has been attributed to the combined effects of several factors. The improved mass transfer occurs due to the cavitation phenomenon which creates localized turbulence and micro-streaming thereby facilitating the release of oil molecules within the matrix of the seed and consequently increasing the extraction yield. The ultrasound waves cause disruption of the cell walls and membranes of the plant material, thereby facilitating the release of oil trapped within the cells. The acoustic streaming and agitation caused by ultrasound waves promotes the movement of solvent molecules within the extraction system which causes accelerated diffusion resulting in the increased yield of extracted oil [33–36]. In ultrasound assisted extraction, the energy of ultrasound waves causes the mechanical disruption within the solid matrix of cells releasing the oil. This presents UAE method as a better alternative for extracting the oil from variety of seeds. The flexibility in operating conditions including the process temperature and intensity of ultrasound waves further provides capability of this method of extraction for variety of oils [3, 37].

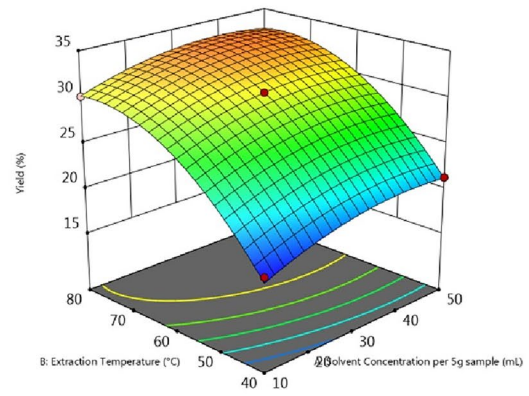
3.2.2 Effect of process factor on antioxidant activity of UAE extracted oil

The ultrasound application involved in the UAE method improves the yield of oil and also composition of bioactive components in the extract. The effect of the factor variables viz. Solvent Concentration (mL/5 g), Extraction temperature (°C) and Extraction time (min) on Antioxidant Activity (IC₅₀ value, µg/mL) of extracted oil is shown graphically through the 3-D surface plots and contour plots in Fig. 2a–f and summarized in Table 4.

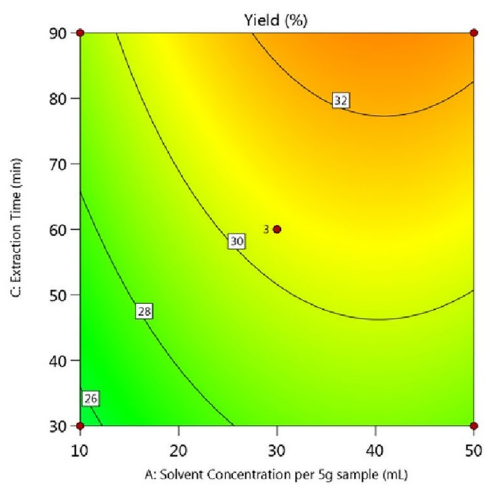
It was noticed that the increase in solvent concentration resulted in a continuous decrease in IC₅₀ values which represented an enhancement in the antioxidant activity (improved radical scavenging capacity) of the extracted



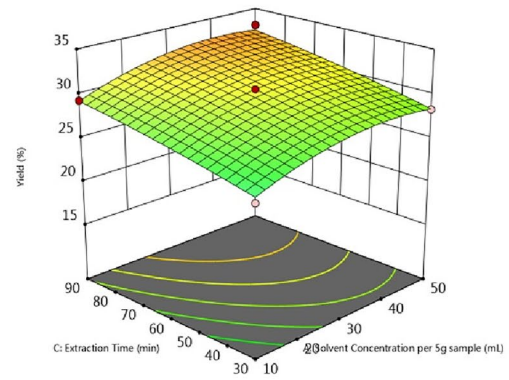
(a)



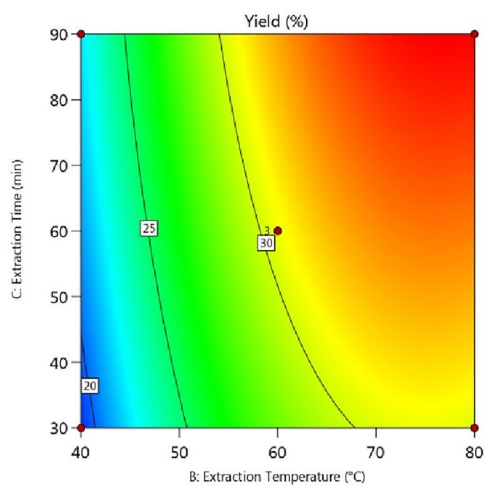
(b)



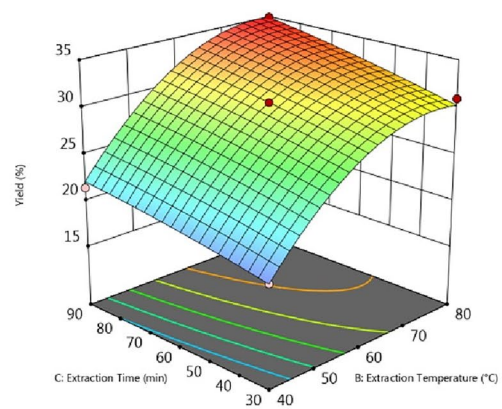
(c)



(d)



(e)



(f)

Fig. 1 Contour plots and RSM modelling for the combined effects of factor variables on Yield

Table 3 Analysis of variance (ANOVA) for response surface quadratic model of yield

Source	Sum of squares	df	Mean square	F-value	P Value	
<i>Model</i>	391.59	9	43.51	88.92	<0.0001	Significant
A-Solvent concentration per 5g sample	17.88	1	17.88	36.54	0.0018	
B-Extraction temperature	287.76	1	287.76	588.12	<0.0001	
C-Extraction time	29.65	1	29.65	60.59	0.0006	
AB	0.2601	1	0.2601	0.5316	0.4986	
AC	0.0225	1	0.0225	0.0460	0.8387	
BC	0.6400	1	0.6400	1.31	0.3045	
A ²	7.37	1	7.37	15.06	0.0116	
B ²	50.62	1	50.62	103.45	0.0002	
C ²	0.2544	1	0.2544	0.5200	0.5031	
<i>Residual</i>	2.45	5	0.4893			
Lack of fit	2.45	3	0.8155			
Pure error	0.0000	2	0.0000			
<i>Cor total</i>	394.03	14				

oil. The IC₅₀ values showed a decreasing trend from 248 to 195 µg/mL with the increase in solvent concentration from 10 to 40 mL per 5 g of sample. After that further increase in the solvent concentration did not result in any significant change in the IC₅₀ values which remained almost constant. The improvement in antioxidant activity was primarily attributed to the enhancement in the solubility of different bioactive components (antioxidants) with the increase in solvent concentration which promotes improved solubility and extraction. Comparable observations were made by Santos et al. [38] while studying the extraction of seed oil from favela (*Cnidioscolus quercifolius*) by Ultrasound-assisted technique.

The effect of extraction temperature was significant on the antioxidant activity of the extracted oil. It is because of the fact that temperature affects the solubility of bioactive compounds and their extraction yield. With the increase in extraction temperature there was improvement observed in the antioxidant activity with the IC₅₀ values showing a decreased trend from 295 to 203 µg/mL with increase in extraction temperature from 40 to 60 °C. Afterwards further increase in the extraction temperature resulted in the decrease in antioxidant activity with IC₅₀ values showing an increasing trend from 203 to 245 µg/mL. This can be attributed to the fact that the increase in extraction temperature beyond some specific limit results in the diminishing of antioxidant activity due to deterioration of bioactive compounds particularly contributing to the antioxidant activity. Similar trend was observed by Samaram et al. [39] while studying the RSM optimization of extraction of oil using the ultrasound-assisted technique from papaya seeds.

As the extraction time increased, an increase in the antioxidant activity was observed initially and a decrease afterwards. The IC₅₀ values showed a decreasing trend from 224 to 203 µg/mL with increase in extraction time up to 60 min. Afterwards, the IC₅₀ values showed a slight increase up to IC₅₀ value of 214 µg/mL at the extraction time of 90 min. The increase in the time of exposure to ultrasound treatment results in the improved extraction of the bioactive compounds with antioxidant activity which resulted in the improved antioxidant activity with more extraction time. However, the extended exposure results in the deterioration of antioxidant compounds due to increased temperature levels in the cavitation bubbles which leads to the slight decrease in antioxidant activity. Similar trend was observed by Sanwal et al. [40] while studying the effects of ultrasound-assisted extraction on efficiency, antioxidant activity, and physicochemical properties of sea buckthorn (*Hippophae salicifolia*) seed oil.

The improvement in the antioxidant activity of the extracted oil became evident with the increase in the process parameters in ultrasound assisted extraction. It was attributed to the enhanced extraction of bioactive compounds with potential antioxidant activity. From the results it was observed that the ultrasound treatment along with suitable changes in the process parameters presents a better extraction method for extraction of sensitive compounds which are susceptible to degradation [41, 42].

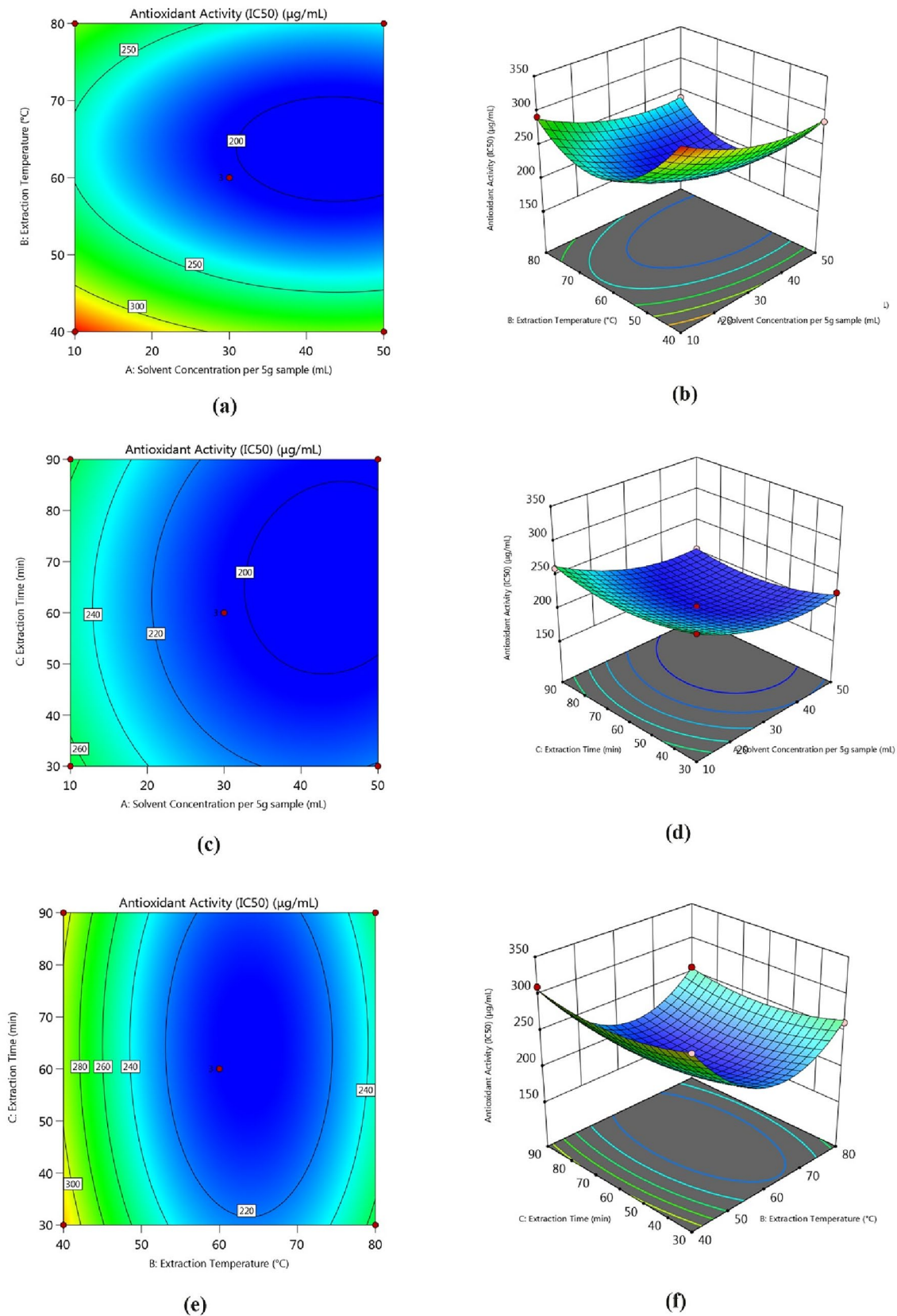


Fig. 2 Contour plots and RSM modelling for the combined effects of factor variables on antioxidant activity (IC₅₀ value, μg/mL) of extracted oil

Table 4 Analysis of variance (ANOVA) for response surface quadratic model of antioxidant activity (IC₅₀ value)

Source	Sum of squares	df	mean square	F-value	p Value	
<i>Model</i>	28,585.22	9	3176.14	261.02	< 0.0001	Significant
A-Solvent concentration per 5g sample	5510.93	1	5510.93	452.90	< 0.0001	
B-Extraction temperature	5175.51	1	5175.51	425.33	< 0.0001	
C-Extraction time	176.44	1	176.44	14.50	0.0125	
AB	2.81	1	2.81	0.2306	0.6514	
AC	56.55	1	56.55	4.65	0.0836	
BC	0.1980	1	0.1980	0.0163	0.9035	
A ²	1355.47	1	1355.47	111.40	0.0001	
B ²	16,566.11	1	16,566.11	1361.44	< 0.0001	
C ²	954.71	1	954.71	78.46	0.0003	
<i>Residual</i>	60.84	5	12.17			
Lack of fit	60.84	3	20.28			
Pure error	0.0000	2	0.0000			
Cor total	28,646.06	14				

3.3 Optimization of UAE factor variables and validation of model

The RSM model was constructed as per Box–Behnken design. The summary of the model parameters and responses is presented in Table 2. The yield and antioxidant activity were analyzed using a second-order quadratic model, as indicated by the R-squared (R²) statistic. The significance of the process variables on the responses was evaluated through ANOVA, and the summarized results can be found in Table 3 and 4. The ANOVA tables revealed that the quadratic polynomial model provided a satisfactory fit for both responses. The R² statistics for yield and antioxidant activity were 0.90 and 0.96, respectively (Table 5). It indicates that the quadratic model could explain 90% and 96% of variability in the estimation of yield and antioxidant activity respectively. The model F values for the yield and antioxidant activity were 88.92 and 261.02 respectively which implied the model to be highly significant ($p < 0.001$) in respect of both the responses. The p value for all the factor variables was less than 0.05 in case of both the responses which indicated that the all the factor variables have notable effect on the responses. This illustrated the good adequacy of the model to explain the influence of the factor variables viz. solvent concentration, extraction temperature and extraction time on yield and antioxidant activity of extracted oil.

The optimized conditions obtained for maximizing the yield and antioxidant activity were the solvent concentration (mL/5 g) of 42.82 mL, the extraction temperature (°C) of 69.09 °C and extraction time (min) of 86.60 min. Under this set of extraction conditions, the extraction process resulted in a yield of 34.53% and antioxidant activity (IC₅₀ value) of 203.56 µg/mL, both of which were found close to the respective predicated values (Fig. 3). This corroborated the good adequacy of fitted quadratic models for both the responses.

3.4 Comparison of UAE method with CSE and SCE methods

The ultrasound assisted extraction (UAE) method was compared with the conventional soxhlet extraction (CSE) and supercritical carbon dioxide extraction (SCE) methods in order to assess the applicability of UAE method for extraction of bioactive rich *Nigella sativa* seed oil. The comparison was done on the basis of following parameters.

Table 5 Second-order polynomial model equations for the factor responses

Factor response	Model equation	R ²
Yield (Y), %	$Y = 30.56 + 1.49A + 6.00B + 1.93C - 2.55 \times 10^{-1}AB + 7.50 \times 10^{-2}AC + 4.00BC - 1.41A^2 + 3.70B^2 - 2.63 \times 10^{-1}C^2$	0.90
Antioxidant Activity (AA), IC ₅₀ value (µg/mL)	$AA = 203.56 - 26.25A - 25.43B - 4.70C + 8.37 \times 10^{-1}AB - 3.76AC + 2.23 \times 10^{-1}BC + 19.16A^2 + 66.98B^2 + 16.08C^2$	0.96

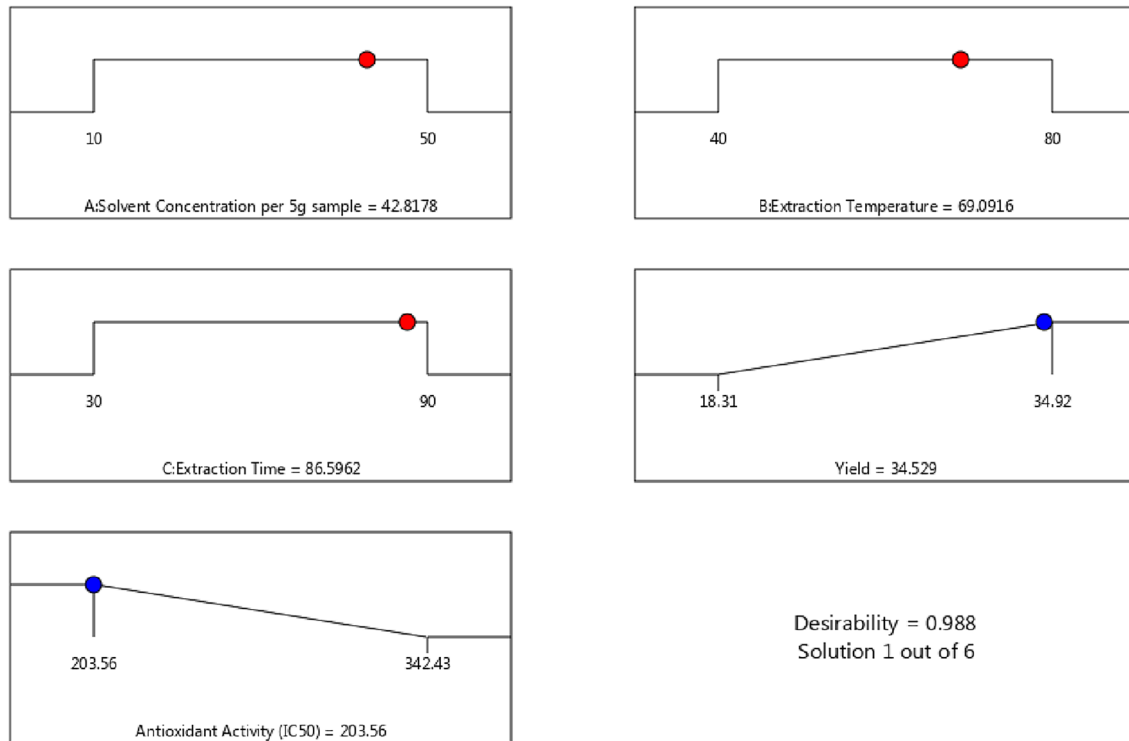


Fig. 3 Point prediction of extraction factors viz. Solvent Concentration (mL/5 g), Extraction Temperature ($^{\circ}$ C) and Extraction Time (min) on process responses viz. Yield and Antioxidant Activity (IC_{50} value, μ g/mL) by numerical optimization

Table 6 Nutritional composition of *Nigella sativa* seed obtained after proximate analysis

Physico-chemical constituent	Mean value (wb,%)
Moisture	9.61 ± 0.14
Crude protein	20.69 ± 0.84
Crude fiber	5.83 ± 0.63
Ash content	4.04 ± 0.17
Crude fat	40.37 ± 0.38
Carbohydrates	25.29 ± 0.16

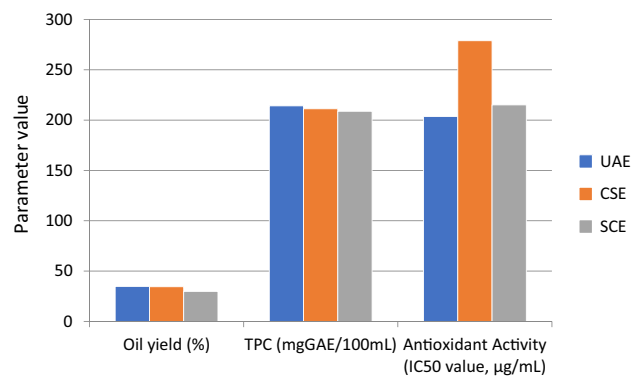
Table 7 Yield, TPC and antioxidant activity of the *Nigella sativa* seed oil extracted using different methods

Parameter	Mean value as per the extraction method		
	UAE	CSE	SCE
Oil yield (%)	34.73 ± 0.28	34.43 ± 0.32	29.85 ± 0.43
TPC (mgGAE/100 mL)	214.17 ± 0.74	211.32 ± 0.76	208.78 ± 0.68
Antioxidant activity (IC_{50} value, μ g/mL)	203.56 ± 0.84	278.96 ± 0.71	215.10 ± 0.93

3.4.1 Yield

The physico-chemical composition of the oil extracted from *Nigella sativa* seeds, as obtained on proximate analysis is presented in Table 5. The oil composition was approximated around 40% on wet basis, presented in Table 6 along with other parameters. It depicts the *Nigella sativa* seeds to be a good source of essential oil. The results of oil yield, antioxidant activity and total phenolic content are presented in Table 7 and illustrated in Fig. 4. It was observed that the yield of oil obtained by the UAE method at optimized parameters ($34.73 \pm 0.54\%$) did not differ significantly from CSE method ($34.43 \pm 0.32\%$). However, it was significantly higher than the extraction using SCE method ($29.85 \pm 0.76\%$). Chemat et al. [43] reported comparable

Fig. 4 Comparison of Yield, TPC and Antioxidant Activity of the *Nigella sativa* seed oil extracted using different methods



findings in their investigation comparing conventional and ultrasound-assisted extraction of carvone and limonene from caraway seeds.

3.4.2 Antioxidant activity and Total Phenolic Content

The total phenolic content (TPC) and antioxidant activity (IC_{50} value) of the *Nigella sativa* seed oil extracted by the three methods is presented in Table 7. The TPC of oil extracted by UAE method was found higher than both CSE and SCE methods. The TPC expressed as Gallic Acid Equivalents observed in *Nigella sativa* seed oil extracted by UAE method was 214.17 ± 0.24 mg GAE/100 mL which was significantly higher than that of CSE and SCE methods with the TPC values of 211.32 ± 0.36 and 208.78 ± 0.28 respectively. The phenolic compounds present in the oil contribute to the antioxidant nature and impart bioactive characteristics. They constitute a number of antioxidant compounds with a number of health benefits. Therefore, this study presents the UAE methods effective over conventional extraction methods for the extraction of essential oil composed with bioactive compounds having prominent antioxidant activity.

In this study, the antioxidant activity was evaluated using the DPPH radical scavenging method. The *Nigella sativa* seed oil extracted using the ultrasound-assisted extraction (UAE) method exhibited the highest antioxidant activity, with an IC_{50} value of 203.56 ± 0.14 µg/mL. It was significantly higher than that of CSE and SCE methods with the IC_{50} values of 278.96 ± 0.21 and 278.96 ± 0.21 respectively. The bubbles formed due to the acoustic cavitation in ultrasonication might have accelerated the dissolution of polyphenols into the oil and resulted in higher antioxidant activity while the Soxhlet extraction occur only by the heating of solvent for longer time [44]. Comparable result of higher antioxidant activity in terms of IC_{50} was reported for the ultrasound assisted extraction (106.60 mg/mL) then Soxhlet extraction (810.40 mg/mL) during extraction oil from mahua (*Madhuca longifolia*) seed Thilakarathna et al. [45]. The results were similar to the observations made by Da Porto et al. [46] while comparing the ultrasound-assisted extraction with conventional extraction methods for extraction of oil and polyphenols from grape (*Vitis vinifera* L.) seeds also reported superior results for ultrasound assisted extraction method. Higher antioxidant activity of 80.13% DPPH inhibition was reported for the extract obtained by ultrasound assisted extraction method than the antioxidant activity of extract obtained for the supercritical extraction method 79.41% DPPH inhibition during phytochemical extraction from bhimkol banana blossom [47]. The solvent (CO_2) used in supercritical extraction method was costlier and this method uses higher energy when compared with the ultrasound assisted extraction method [48].

The synergistic effect of the different parameters associated with the ultrasound enhances the penetration and diffusion of the solvent or enzymes thereby, facilitating the extraction of a broader range of antioxidant compounds. The increased antioxidant activity has been primarily attributed to the improved extraction efficiency and enhanced release of antioxidant compounds. The UAE also improves the solubility and dispersibility of antioxidants in the oil matrix which further increases their ability to scavenge free radicals and exhibit antioxidant activity. The combined action of all these processes leads to a more comprehensive extraction of antioxidants with enhanced antioxidant activity and consequently increasing the overall antioxidant activity of the oil [39, 40, 49, 50].

4 Conclusion

The extraction method used for the extraction of black seed oil was ultrasound assisted solvent extraction technique. The process was optimized using RSM for enhancing the yield and antioxidant activity of the extracted oil. The optimized conditions obtained through RSM were a solvent concentration (mL/5 g) of 42.82 mL, the Extraction temperature of 69.09 °C and Extraction time of 86.60 min. Under the set of extraction conditions, the extraction process resulted in the maximum yield of 34.53% with improved antioxidant activity (IC_{50} value of 203.56 $\mu\text{g/mL}$). From the results, it was inferred that the yield was significantly affected by all the three extraction parameters. However, it was more pronounced by the effect of temperature. Similar effect was observed in case of the antioxidant activity, where temperature had most significant effect on the antioxidant activity of extracted oil. The antioxidant activity of the extracted oil was found out using DPPH method and compared with the conventional soxhlet extraction and supercritical carbon dioxide extraction method. It was observed that the UAE method produced the oil with enhanced quantities of antioxidants and thus provides an alternative method for efficient extraction of essential oil from black seeds composed of rich bioactive compounds. Further studies are needed to evaluate the effect of other parameters like ultrasound frequency and power which are expected to impose significant effect on yield and antioxidant activity.

Author contributions IHD, PMJ, SA, RS: Conceptualization, Methodology, Software, Data curation, Writing—Original draft preparation, Investigation, Validation, Writing—Reviewing and Editing;KKD: Conceptualization, Methodology, Software, Data curation, Writing—Original draft preparation, Investigation, Supervision, Validation, Writing- Reviewing and Editing;AMS, KB: Supervision, Validation, Writing—Reviewing and Editing. All authors reviewed the manuscript.

Funding Open access funding provided by University of Debrecen. Funding Project No. TKP2021-NKTA-32 has been implemented with support from the National Research, Development and Innovation Fund of Hungary, financed under the TKP2021-NKTA funding scheme.

Availability of data and materials The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Competing interests The authors declare no competing interests.

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