Microwave-assisted extraction of *Nigella sativa* L. essential oil and evaluation of its antioxidant activity

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Abstract It has been previously reported that the essential oil of *Nigella sativa* L. seeds and its major active component, thymoquinone (TQ), possess a broad variety of biological activities and therapeutic properties. In this work, microwave-assisted extraction (MAE) of the essential oil from *Nigella sativa* L. seeds and its antioxidant activity were studied. Response surface methodology based on central composite design was used to evaluate the effects of extraction time, irradiation power and moisture content on extraction yield and TQ content. Optimal parameters obtained by CCD and RSM were extraction time 30 min, irradiation power 450 W, and moisture content 50%. The extraction yield and TQ content of the essential oil were 0.33 and 20% under the optimum conditions, respectively. In contrast, extraction yield and TQ amount of oil obtained by hydrodistillation (HD) were 0.23 and 3.71%, respectively. The main constituents of the essential oil extracted by MAE and HD were *p*-cymene, TQ, α -thujene and longifolene, comprising more than 60% of total peak area. The antioxidant capacity of essential oils extracted by different methods were evaluated using 2,2-diphenyl-1-picrylhydrazyl and Ferric reducing antioxidant power assays, and compared with traditional antioxidants. The results showed that MAE method was a viable alternative to HD for the essential oil extraction from *N. sativa* seeds due to the excellent extraction efficiency, higher thymoquinone content, and stronger antioxidant activity.

Keywords Microwave-assisted extraction · *Nigella sativa* L. · Essential oil · Antioxidant activity · Response surface methodology

Introduction

Nigella sativa L., commonly known as black cumin, is an indigenous herbaceous plant belonging to the Ranunculaceae family growing in Mediterranean countries, Middle East, Eastern Europe and Western Asia. Its taste is a little bitter and peppery with a crunchy texture. Seeds are angular, generally small in size (1–5 mg) and dark grey or black in color (Cheikh-Rouhou et al. 2007; Ahmad et al. 2013). They have been used for thousands years as a food preservative, spice and remedy (Bachir and Benattouche 2013).

Nigella sativa seeds and its constituents have been reported to possess many biological activities, including antitumor, antiinflammatory, bronchodilator, gastroprotective, antimicrobial, antidiabetic, antihypertensive, immunomodulator, analgesic and hepatoprotective (Ramadan 2007; Ahmad et al. 2013). Many studies have proven that most of the biological activities and therapeutic properties of the *N. sativa* seeds are due to the presence of thymoquinone (2-methyl-5-isopropyl-1, 4-benzoquinone), a monoterpenoid quinone, which is a major active component of the essential oil or volatile oil (Schneider-Stock et al. 2014; Majdalawieh and Fayyad 2015; Darakhshan et al. 2015). The yellow crystalline molecule thymoquinone (TQ) possesses a basic quinone structure comprising of a para-substituted dione conjugated to a benzene ring to which methyl and isopropyl side chain groups are attached (Schneider-Stock et al. 2014).

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Essential oil has been utilized for many years for food flavorings, food preservatives, pharmaceuticals, and non-traditional medicine (Tisserand and Young 2013). Essential oil of *N. sativa* seeds has been proved to have a wide range of pharmaceutical activities, such as anticancer (Edris 2009), antibacterial (Harzallah et al. 2011), antioxidant activity (Erkan et al. 2008), antiinflammatory (Hajhashemi et al. 2004), antitumor (Ait Mbarek et al. 2007), anticonvulsant (Hosseinzadeh and Parvardeh 2004) and antidiabetic (Al-Hader et al. 1993). Therefore, applying an effective extraction method for the preparation of high quality and high yield volatile compounds of *N. sativa* is important. Conventional methods for extracting essential oils from plants are organic solvent extraction, hydrodistillation and steam distillation (El Asbahani et al. 2015). However, these techniques have various drawbacks, such as high energy expense, long extraction time, low extraction efficiency, high volume solvent, toxic organic solvents, losses of some volatile components and few modifiable factors to control the selectivity of the process (Qi et al. 2014). Supercritical fluid extraction (SFE) is a quick and selective technique for the extraction of essential oils from aromatic plants especially *N. sativa* (Piras et al. 2013). However, the expensive electronic equipment problems and the high cost of producing specific products have limited its use (Reverchon 1997; Li et al. 2012).

Recently, microwave-assisted extraction (MAE) method has been defined as an effective tool for the extraction of essential oil (Lucchesi et al. 2004; Petrakis et al. 2014). It has many advantages, such as rapid energy transfer, effective heating, short time extraction, high yield extraction, organic solvent-free, high recovery of bioactive compounds and low operating costs (Gavahian et al. 2015). MAE has already been used for extraction of essential oil from a number of aromatic plants in pharmaceutical and food processing, with a high potential for future applications (Qu et al. 2013; Petrakis et al. 2014).

To the best of our knowledge, a few studies have been conducted on MAE of essential oil from *N. sativa* seeds. Benkaci-Ali et al. (2006, 2007) and Liu et al. (2013) applied MAE for the extraction of *N. sativa* L. essential oil, and many volatile compounds were identified by GC–MS. However, in these studies, the main operating parameters of MAE were optimised, using the one-factor-at-a time approach.

Response surface methodology (RSM) includes a body of statistical and mathematical methods for obtaining optimum operating conditions and is used to investigate the relationship between several quantitative experimental variables and one or more response variables. It needs relatively few tests and the least amount of time and manpower. The methodology comprises three steps including experimental design using well-established statistical

experimental designs such as the central composite design (CCD); response surface modeling using regression analysis; and process optimization through the response surface models. In comparison with other methods (such as one-factor-at-a time and full-factors experimentation), RSM provides a quicker and more economical method for gathering research results (Myers et al. 2016). Furthermore, it has been successfully showed that RSM can be applied in optimizing process factors, and it has been used in many areas such as chemicals, foods and biological processes (Xie et al. 2010).

In the present study, the process of MAE of essential oil from *N. sativa* seeds was optimized by a central composite design (CCD) and response surface methodology (RSM). The chemical composition of the essential oil was characterized by GC–MS. Moreover, the antioxidant activity of the essential oil obtained under optimized conditions were assessed using DPPH, FRAP tests. Additionally, the results were compared with those acquired by conventional hydrodistillation (HD).

Materials and methods

Plant materials and chemical reagents

Nigella sativa seeds were obtained from a local market in Tehran, Iran. Prior to the extraction process, the seeds were grounded by a food chopper (Hamilton, FH-140 N Chopper, Canada) and sieved through a standard sieve of 0.6 mm pore size.

2,2-Diphenyl-1-picrylhydrazyl (DPPH 95%), thymoquinone (99%), 2,4,6-tripyridyl- S-triazine (TPTZ 98%), and alpha-tocopherol (VE 95.5%) were purchased from Sigma–Aldrich (St. Louis, MO, USA), ferric chloride (97%), ferrous sulfate (99%), sodium ascorbate (99%), hydrochloric acid (37%), n-hexane (HPLC grade), and methanol (HPLC grade) were purchased from Merck (Darmstadt, Germany).

Microwave-assisted extraction

The extraction was performed in a domestic microwave oven (Daewoo Electronics KOC-154KWR Microwave Oven) operating at 2450 MHz. The modification of the microwave and basic experimental set-up was carried out according to the method of Liu et al. (2013). The maximum power of the oven was 900 W. Microwave oven was changed by drilling a 4 cm diameter hole at the top center of the cavity. A sample flask (500 mL) was placed in the oven and connected to a Clevenger apparatus through the hole (Fig. 1). The Clevenger apparatus was placed outside the oven to condense and collect the volatile constituents.

Fig. 1 Schematic diagram of microwave-assisted extraction

The front panel buttons were used to control the irradiation power and extraction time.

For a typical MAE process, 50 g of ground *N. sativa* seeds was soaked in 50 mL distilled water in a 500 mL round-bottomed flask for 30 min. Then, the Clevenger apparatus was attached to the flask and heated using 450 W power for 30 min. The essential oil obtained was eluted out by *n*-hexane. The solvent was removed under vacuum conditions with nitrogen gas. The essential oil extracted was stored at 0 °C until analysis. The extraction yield of essential oil was calculated from Eq. (1):

$$EY = A / B \times 100 \quad (1)$$

where EY (% w/w) is the extraction yield. A and B are the mass of obtained essential oil (g) and the mass of sieved seeds (g), respectively.

Hydrodistillation

Conventional hydrodistillation (HD) was performed with a Clevenger apparatus to compare with MAE method. 100 g ground seeds were put into a sample flask and mixed with 300 mL deionized water. The mixture was distilled for 3–4 h until no more essential oil was obtained. The essential oil was collected and stored at 0 °C until use. This method was carried out in triplicate.

Experimental design and statistical analysis

For determining the best combination of process variables for the extraction of essential oil from *N. sativa* seeds, a central composite design (CCD) combined with response surface methodology (RSM) was employed. The independent variables were extraction time (ET: 15–30 min), irradiation power (P: 270–720 W) and moisture content (M: 30–80%, wet weight basis), while the response variables were the extraction yield (YE, g/100 g seeds) and thymoquinone quantity (TQ, g/100 g oil) of essential oil. The CCD includes a three-level full factorial design (+1, −1), overlying by the center points (coded 0), and the star points (+ α , − α). The star points establish new extremes for the low and high settings for all parameters and provide estimation of the curvature in the model. The range of these three variables and central point were chosen based on the preliminary experiments. In this experimental design, for fitting the full quadratic equation model, 20 randomized experiments including six replicates as the center points were applied. Specific protocols for experimental conditions were shown in Table 1.

The data obtained from CCD were analyzed statistically with a response surface analysis procedure (Design-Expert 8.0.5, Minneapolis, USA). Analysis of variance (ANOVA) was used for calculating and modeling of the optimum conditions for MAE of essential oil from *N. sativa* seeds. After obtaining optimal conditions, verification experiments were performed six times under these conditions.

Scanning electron micrographs (SEM)

Microstructure analysis of samples with different extraction methods was performed by a Philips XL 30 ESEM field emission scanning electron microscope. The remaining ground seeds were air dried, and fixed on an adhesive tape, then sputtered with gold. All the samples were assessed under high vacuum conditions. SEM was executed at 5 kV with magnification of 2000 $\times$.

Gas chromatography–mass spectrometry identification

GC–MS analysis of essential oil was immediately performed on an Agilent 7890A gas chromatograph with a HP-5 ms capillary column with a non-polar stationary phase (60 m $\times$ 0.25 mm $\times$ 0.25 μ m film thickness, J&W Scientific Products, Santa Clara, CA, USA) equipped with an Agilent 5975C mass selective detector in the electron impact mode. The GC–MS was carried out using the following operating conditions: carrier gas helium; flow velocity 1.0 mL/min; split ratio 1:40; injection volume 1 μ L; injection temperature 280 °C; oven temperature

Table 1 Results of the central composite design for the extraction of essential oil from *N. sativa* seeds

Runs	Parameters			Responses variables	
	ET ^a (min)	P ^b (W)	M ^c (%)	Extraction yield (%)	TQ (%)
1	45	630	30	0.12	14.53
2	45	270	80	0.18	13.01
3	10	450	50	0.20	17.76
4	30	720	50	0.20	16.27
5	30	450	100	0.14	21.76
6	45	630	80	0.25	16.61
7	30	450	50	0.30	19.43
8	15	270	30	0.25	14.86
9	15	630	30	0.30	17.86
10	30	450	50	0.32	18.17
11	15	630	80	0.20	20.95
12	30	450	50	0.33	19.42
13	30	180	50	0.18	17.49
14	45	270	30	0.20	15.65
15	15	270	80	0.15	18.29
16	5	450	50	0.16	18.23
17	30	450	50	0.32	19.83
18	15	450	15	0.20	16.32
19	30	450	50	0.32	20.41
20	30	450	50	0.31	19.59
HD ^d	–	–	–	0.25	3.71

^a ET is expressed as the extraction time (min)^b P is expressed as the irradiation power (W)^c M is expressed as the percentage of moisture content of the matrix (wet weight basis)^d Hydrodistillation

program from 40 to 240 °C at 5 °C/min, and 240 to 290 °C at 30 °C/min; the ionization mode used was electronic impact at 70 eV, and spectra were acquired from *m/z* 50 to 550. The percentage composition of essential oil was produced by the GC peak areas. Identification of essential oil constituents was carried out by direct comparison their retention indices and mass spectral patterns in NIST02 Mass Spectral Library.

Antioxidant activity

Free radical scavenging activity (DPPH)

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of the essential oil was compared to that of traditional antioxidants such as α -tocopherol (VE) and ascorbic acid (VC), according to the method of Ma et al. (2012) with minor modification. Briefly, a 200 μ L methanolic solution of samples with different oil concentration (5, 20, 40, 60, 80 and 100 μ L/ml) was mixed with 800 μ L methanolic solution of DPPH (0.1 mM). The mixture was lightproof shaken and incubated in the dark at

room temperature for 30 min. The absorbance of the resulting solution was measured at 517 nm against methanol as the blank in a spectrophotometer (UV-7200, CECIL, UK). Lower absorbance of the reaction mixture signified higher free radical scavenging activity. The percent of antioxidant activity of the samples was calculated according to the formula (Eq. (2)):

$$SC : (A_0 - A) / A_0 \times 100 \quad (2)$$

where SC (%) is the scavenging activity. A_0 is the absorption of blank sample (containing all reagents except the test compound), and A is the absorption of tested samples. The IC₅₀ of DPPH scavenging activity was calculated from the graph with percentage inhibition plotted against oil against oil concentration. Experiments were carried out in triplicate, and VC and VE with the same concentrations mentioned above were used as positive controls.

Ferric reducing antioxidant power (FRAP)

The FRAP assay was determined according to the method of Li et al. (2012) with minor modification. The FRAP

reagent was prepared from 10 mM 2,4,6-tripyridyl-S-triazine made up in 40 mM hydrochloric acid, 20 mM ferric chloride and 300 mM acetate buffer (pH 3.6). The solutions were mixed together in the ratio of 10:1:1 (v/v/v). The temperature of the solution was raised to 37 °C before use. A 10 µL of samples with different oil concentration (5, 20, 40, 60, 80 and 100 µL/ml) were allowed to react with 190 µL of the FRAP solution in a 96-well plate for 30 min in the dark. Colorimetric readings of the product ferrous tripyridyltriazine complex were examined at 593 nm using a micro-plate reader (BioTek, ELX808, USA). The standard curve was linear between 200 and 1000 µM FeSO₄. A higher absorbance indicates a higher ferric reducing power. Experiments were performed in triplicate and repeated with VC and VE with the same concentrations. Results were expressed as µM Fe(II)/g dry mass of samples and compared with those of the positive controls (VC and VE).

Result and discussion

Optimization by central composite design

Table 1 indicates the responses achieved in the CCD experiments and the overall design. The results indicated considerable variations in the extraction efficiency of essential oil and TQ content. These variations reflected the importance of optimization to attain higher productivity of essential oil and TQ.

Table 2 comprises the equation of dependent variables and the analysis of variance (ANOVA). Quadratic (second-order) polynomial models applied to express essential oil extraction yield (YE) and TQ content. The R-squared statistic indicated that the model explains 97.04 and 92.26% of the variability in YE% and TQ%, respectively. The adjusted R-squared statistic was 0.93 for YE% and 0.83% for TQ%. The *p* value (*p* < 0.05) showed the model terms were significant. The “lack of fit *p*-values” more than 0.05 implied the lack of fit was not significant corresponding to the pure error, which indicated that the models were accurate and satisfactory. The significance of each coefficient was determined by *p*-value and F-value presented in Table 2. The data indicated that irradiation power (P) and moisture content (M) had significant effects on both YE% and TQ%, but extraction time (ET) influenced YE% only.

The RSM is interested as a convenient tool to obtain the highest amount of complex information and the best method to predict the influence of the independent factors on the dependent one. Three-dimensional response surface plots were applied to explain the interactions between extraction time, moisture content and irradiation power on

Table 2 ANOVA of response surface quadratic model for extraction yield and thymoquinone of essential oil from *N. sativa* seeds

Source	Yield extraction		Thymoquinone	
	F value	P value	F value	P value
Model	68.07	<0.0001	13.26	0.0007
E—Extraction time	75.94	<0.0001	3.02	0.1206
P—Power	3.33	<0.0001	7.93	0.0227
M—Moisture content	28.25	0.0143	32.43	0.0005
Lack of Fit	1.59	0.3260	1.95	0.2405
R ²	0.97		0.92	
Adjusted R ²	0.93		0.83	
Model	YE = + 32 - 0.014A + 0.024B + 0.051C - 0.02AB + 0.097AC + 7.7BC - 0.055A ² - 0.13B ² - 0.12C ²		TQ = + 20.09 - 0.065A + 0.46B + 2.55C - 2.6AB + 0.10AC - 0.80BC1.35A ² 2.75B ² - 1.19C ²	
			Significance	
			Significant	
			Non-Significant	
			Significant	
			Non-Significant	

the YE% and TQ% (Fig. 2a–d). Figure 2a and c depict the interaction between irradiation power and extraction time on the essential oil extraction yield and its TQ content. Increasing the extraction time from 15 to 30 min with irradiation power from 180 to 450 W enhanced YE% and TQ%, while both started decreasing when the extraction time and irradiation power were increased up to 45 min and 720 W respectively. This might be the reason for the volatilization and decomposition of essential oil and its constituents when the irradiation power and extraction time increased (Qi et al. 2014). The effect of extraction time on TQ% was less than YE%. The results showed that a longer irradiation time and power were not suitable for essential oil extraction. Figure 2b shows the interaction between extraction time and moisture content on the extraction yield of essential oil. The increase of extraction time from 15 to 30 min with an increase of moisture content from 15 to 50% significantly accelerated essential oil extraction, and extraction yield of essential oil decreased when moisture

content was higher than 60%. At low moisture content, the evaporation rate was low, resulting in an incomplete extraction. On the contrary, a high proportion of water might lead to hydrolysis of some volatile components (Li et al. 2012).

As illustrated in Fig. 2d, increasing the moisture content from 15 to 100% along with extraction time from 15 to 30 min led to an increase in TQ%, which did not showed significant change after 45 min. Those results suggested that increasing moisture content from 30 to 50% along with an increase of extraction time from 15 to 30 min, YE% achieved a peak value with a good recovery of TQ. The results indicated that the content of oil extracted increased, initially when the power was increased, but started to lower when the power exceeded 450 W. This revealed that the mass transfer ratio was enhanced until a certain value with an increase in power irradiation, thus increasing the extraction yield. However, YE% lowered slightly at higher irradiation power. This can be due to a rapid change of

Fig. 2 Response surface modeling of extraction yields and thymoquinone recoveries versus the affecting parameters in microwave-assisted extraction: **a** Effect of varying extraction time and irradiation power on yield extraction; **b** effect of varying extraction time and

moisture content on yield extraction; **c** effect of varying extraction time and irradiation power on thymoquinone recovery; **d** effect of varying extraction time and moisture content on thymoquinone recovery

temperature, leading to partially thermal degradation of volatiles (Qi et al. 2014).

Based on the results, the high content of TQ (20%) in the highest overall yield of essential oil (0.33%) was obtained through MAE extraction conditions of extraction time 30 min, irradiation power 450 W, and moisture content 50%. Verification experiments were conducted six times under these conditions. The resulting mean extraction yield and thymoquinone percent were 0.32 and 19.47% with a RSD (relative standard deviation) of 3.16 and 3.78% respectively.

Comparison of MAE with HD

Compared with MAE method, HD was applied as a reference method for the extraction of essential oil from *N. sativa* seeds (Table 1). The results revealed that the extraction yields of MAE for 30 min ($0.316 \pm 0.01\%$, w/w) were higher than HD for 3 h ($0.23 \pm 0.035\%$, w/w). Also, as seen in Table 1, the content of TQ obtained from HD dropped drastically (3.71%) compared with MAE. In contrast to HD, MAE could decrease the rate of oxidation and hydrolysis of bioactive compounds by reducing the extraction time (Qi et al. 2014). In the HD process, the samples were heated by the thermal conductivity from the outside to the inside of samples. However, in the MAE process, heat transfer arises from the samples center to the external colder environment. Moreover, the internal heating of the in situ water produces areas of compression in the plant, resulting in the serious rupture of glands and oleiferous receptacles (Lucchesi et al. 2007). This might cause the considerable difference in the extraction yield between two extraction methods. Hence, MAE is an environmentally friendly, energy-saving and efficient extraction method. Consequently, MAE is a suitable alternative to extract essential oils from aromatic plant.

Structural changes after extraction

SEM was employed to evaluate the structural changes of *N. sativa* seeds when subjected to different oil extraction procedures. Figure 3a is a SEM image of the untreated seeds (before extraction), and Fig. 3b and c show the micrographs of samples that have treated by HD (3 h) and MAE (30 min), respectively. As illustrated in Fig. 3a, the large number of cells containing essential oil with a full balloon shape is observed. Most of them became rupture, atrophic and seemed wrinkled after extraction by HD (Fig. 3b). In HD process, the heat transfer is principally executed by convection and conduction only, while in the procedure of MAE, it is implemented in three ways including convection, conduction and radiation (Ma et al. 2012). Therefore, in MAE procedure, heat is generated from inside the glands as well as

Fig. 3 SEM images of *N. sativa* seeds: **a** without any treatment; **b** after treatment by HD; and **c** after treatment by MAE. The arrows indicate balloon shaped oil cells

from the outside. When the glands were exposed to more acute thermal stresses and localized high pressures, as in the case of microwave heating, the pressure build-up inside the glands could have exceeded their capacity for expansion, and caused their rupture more quickly and completely than in conventional extraction (Lucchesi et al. 2007; Qi et al. 2014). After MAE, most of cells appeared completely disrupted

explaining that all the cell walls are finally damaged and collapsed, and have resulted into undefined boundaries (Fig. 3c).

Chemical composition of the essential oil

The constituents of essential oil from *N. sativa* seeds obtained by MAE and HD were analyzed by GC–MS. The found constituents, their relative percentages and retention indices are given in Table 3. Thirty components were identified in the essential oil obtained by MAE and HD. The number of identified compounds was lower than that reported by Benkaci-Ali et al. (2007) and Liu et al. (2013). The various origin of the seeds and/or the lower sample injection volume may be the reason for this. The essential oils contained mainly of monoterpenes hydrocarbons (59.93% for MAE and 76.36% for HD) together with noticeable contents of oxygenated monoterpenes (24.61 for MAE and 8.2% for HD) and smaller amounts of sesquiterpenes hydrocarbons (5.57 for MAE and 5.69% for HD). In both methods, the monoterpene hydrocarbons were noticeably dominated by *p*-cymene (41.99% for MAE and 52.82% for HD) while α -thujene, α -pinenes, β -pinene, sabinene and terpinene were present at lower amounts. Among the oxygenated monoterpenes, TQ was the major bioactive constituent especially in oil isolated by MAE (20.41%), together with lower contents of linalool, terpinen-4-ol and carvacrol. In the HD method, the oxygenated monoterpene content of the essential oil was substantially lower than that obtained with MAE, because of the decomposition or hydrolysis of thymoquinone (see Table 3). These results are similar to those of Benkaci-Ali et al. (2007) who suggested that the amount of oxygenated monoterpenes of the oil extracted by MAE was higher than that by HD for *N. sativa* seeds. Li et al. (2012) suggested that during MAE process, microwave irradiation extremely accelerates the extraction process without considerable effect on the essential oil composition, though the percentages of some constituents depend on the method used. Moreover, Benkaci-Ali et al. (2006) reported that MAE can reduce the time of extraction of *N. sativa* seeds essential oil to less than 10 min. However, the composition of the major products presented a fluctuation according the extraction time.

Our results showed that *N. sativa* seeds purchased from Iran belong to the *p*-cymene/thymoquinone chemotype, which is in agreement with the results of Hajhashemi et al. (2004). A diversity of chemotypes has been explained in the literature. Burits and Bucar (2000) reported the chemical composition of the essential oils from *N. sativa* from Austria. They found thymoquinone

(27–57%) and *p*-cymene (7–15.7%) as the major compounds. Another Iranian *N. sativa* essential oil was observed to be dominated by phenylpropanoid compounds and exhibited a trans-anethole chemotype (Nickavar et al. 2003). *N. sativa* essential oil from Bangladesh (Liu et al. 2013), India (Singh et al. 2005) and Algeria Benkaci-Ali et al. (2007) was found to be a *p*-cymene/thymoquinone chemotype. A chemotype with 33.0% *p*-cymene and 26.8% thymol and the majority of monoterpenes was reported for *N. sativa* essential oil from Morocco (Moretti et al. 2004; D'Antuono et al. 2002) and a chemotype with 60.2% *p*-cymene and 12.9% α -terpinene was reported by Wajs et al. (2008) for *N. sativa* from Poland. This shows that the contents of compositions of essential oils of *N. sativa* seeds were dependent on the extraction method as well as the species.

Antioxidant activity

Because of the complicity of essential oils, the antioxidant activities cannot be assessed by only a single technique, but at least two experiment systems have been proposed for determining antioxidant activity to establish authenticity (Schlesier et al. 2002). In the present study, the antioxidant activity of *N. sativa* seeds essential oils obtained by MAE and HD were evaluated in vitro by DPPH scavenging activity and ferric reducing antioxidant power assays, and compared with traditional antioxidants.

Free radical scavenging activity (DPPH)

DPPH assay, an electron-transfer-based assay, is often used as an indicator of free radical scavenging capacity (Bayramoglu et al. 2008). As shown in Fig. 4a, DPPH radical-scavenging activity (SC %) increased when the oil concentration increased. A low IC₅₀ value shows strong antioxidant activity in a sample. The IC₅₀ values of the essential oil, extracted by MAE and HD, were 28.10 and 36.90 μ g/ml, respectively. In brief, the DPPH scavenging effect decreased in the order: VC > MAE > HD > VE. The results showed that essential oil obtained by MAE possessed a higher free radical scavenging capacity than HD.

Ferric reducing antioxidant power (FRAP)

The reduction capacity of an extract or oil may use as an important indicator of its potential antioxidant activity (Ma et al. 2012). A higher absorbance indicated a higher ferric reducing power. As shown in Fig. 4b, both the essential oil by different extraction methods and standards showed increased ferric reducing power with the increased concentration. Essential oil extracted by MAE at the

Table 3 Volatile compounds in *N. sativa* seeds obtained by SFME and HD methods followed by GC–MS

No.	RT ^a	Compounds ^b	RI ^c	Relative area percentage ^d %	
				MAE	HD
1	16.22	n-Nonane	900	0.02	0.04
2	17.45	α-Thujene	928	7.89	11.60
3	17.81	α-Pinene	931	1.66	2.49
4	18.55	Camphene	943	0.04	0.06
5	19.26	Sabinene	964	1.14	1.42
6	19.58	β-Pinene	961	2.27	2.81
7	19.82	n-Decane	1000	0.80	1.13
8	20.51	α-Phellandrene	997	0.02	0.02
9	20.84	α-Terpinen	1008	0.70	0.82
10	21.27	p-Cymene	1025	41.99	52.82
11	21.35	Cineole	1023	0.05	0.07
12	22.29	γ-Terpinen	1047	4.28	4.40
13	23.23	Bicycloheptan-3-ol	1125	0.10	0.14
14	23.51	Linalool	1137	1.27	1.26
15	24.31	Terpinolene	1146	7.03	6.65
16	25.68	Camphor	1153	0.03	0.03
17	25.84	Trans-2-Caren-4-ol	1160	0.30	0.25
18	26.55	Terpinen-4-ol	1172	0.57	0.60
19	26.81	p-Cymen-8-ol	1196	0.05	0.02
20	27.06	β-Cyclocitral	1210	1.18	1.00
21	28.78	Thymoquinon	1216	20.41	3.71
22	30.12	Carvacrol	1275	2.36	2.63
23	31.61	α-Longipinene	1353	1.21	1.10
24	31.96	α-Ylangene	1392	0.03	0.01
25	33.09	Caryophyllene	1424	0.02	0.02
26	33.46	Longifolene	1432	4.34	4.57
27	34.86	2-Tridecanone	1481	0.04	0.06
28	35.38	β-Bisabolene	1500	0.03	0.04
29	42.20	2-Phenyldodecane	1791	0.06	0.09
30	45.77	Biformene	1962	0.10	0.16
Oxygenated monoterpenes				24.61	8.20
Monoterpene hydrocarbons				59.93	76.36
Sesquiterpene hydrocarbons				5.57	5.69

MAE microwave-assisted extraction; HD hydrodistillation

^a Essential oil retention time listed in order of elution^b Essential oil compounds listed in order of elution^c Retention index^d Relative area percentage (peak area relative to the total peak area %)

highest concentrations analyzed, showed the highest ferric reducing capacity in terms of Fe (II) concentrations (1670 μM Fe (II)/g) with statistical differences with control VC and VE (1400 and 300 μM Fe (II)/g, respectively). The reducing power of essential oil extracted by HD was slightly lower than that of MAE (1580 μM Fe (II)/g). It can be due to the reduction of thymoquinone compound

which is a major active chemical component of the essential oil studied. As mentioned above the antioxidant activities of *Nigella sativa* L. essential oil could be mainly due to the action of thymoquinone existing in the essential oil studied. In brief, the reducing power of essential oils and antioxidants revealed the descending order of: MAE > HD > VC > VE.

Fig. 4 Results of antioxidant activity. **a** Free radical scavenging activity. **b** Ferric reducing antioxidant power. *MAE* microwave-assisted extraction, *HD* hydrodistillation, *VC* ascorbic acid, *VE* α -tocopherol

Conclusion

Microwave-assisted extraction method (MAE) was considered for extracting essential oil from *Nigella sativa* L. seeds. Response surface methodology was successfully executed for optimizing the extraction yield of essential oil

and its thymoquinone content. The optimum parameters were extraction time 30 min, irradiation power 450 W and moisture content 50%. Thymoquinone content of oil obtained by HD was reduced substantially due to the long time extraction of HD. The antioxidant activity of essential oils obtained by MAE and HD were evaluated by DPPH

and reducing power tests, and compared with traditional antioxidants. Based on the results, we conclude that MAE method is an appropriate alternative to conventional HD for the essential oil extraction from *Nigella sativa* L. seeds owing to the excellent extraction efficiency, higher thymoquinone content and higher antioxidant activity of the essential oil. Further study is recommended to evaluate the antimicrobial activity and other bioactive properties of *N. sativa* essential oil extracted by MAE and compare this method with other extraction methods. Furthermore, essential oil from *Nigella sativa* L. seeds would be a novel nature resource for the usage in food and healthy fields.

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