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EVALUATION OF THE YIELDS AND ANTIOXIDANT ACTIVITIES OF ESSENTIAL OILS, FIXED OILS AND EXTRACTS OBTAINED USING CONVENTIONAL METHODS FROM *NIGELLA SATIVA* L. AND *NIGELLA DAMASCENA* L. SEEDS

NIGELLA SATIVA L. VE NIGELLA DAMASCENA L. TOHUMLARINDAN KONVANSİYONEL YÖNTEMLER İLE ELDE EDİLEN UÇUCU YAĞ, SABİT YAĞ VE EKSTRELERİN VERİM VE ANTİOKSİDAN AKTİVİTELERİNİN DEĞERLENDİRİLMESİ

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ABSTRACT

Objective: *Yields and antioxidant properties of essential oils, fixed oils, and extracts from Nigella sativa L. and Nigella damascena L. seeds were evaluated using conventional extraction methods with solvents of different polarity.*
Material and Method: *The seeds from both species were divided into three segments for the production of fixed oil, essential oil, and essential oil from extracts. Fixed oils and extracts were obtained using Soxhlet extraction with n-hexane, petroleum ether, acetone, chloroform, and methanol, followed by hydrodistillation using a Clevenger apparatus to obtain essential oils from the extracts. Essential oils were also obtained from powdered seeds by hydrodistillation. Oils and extract yields were calculated, and antioxidant activities were evaluated using DPPH· and ABTS⁺ assays.*
Result and Discussion: *For both species, the continuous extraction method with n-hexane produced the highest yield and the essential oils were obtained only from these extracts. Notably, extracting the essential oil directly from the seed resulted in low yields. N. sativa exhibited higher essential oil yields than N. damascena; however, the obtained yields were inadequate for activity studies. Antioxidant assays revealed that the ABTS⁺ method was more discriminative, with acetone extracts showing higher activity. Overall, N. sativa demonstrated greater antioxidant activity compared to N. damascena.*
Keywords: *Antioxidant activity, essential oil, fixed oil, Nigella damascene, Nigella sativa*

ÖZ

Amaç: *Farklı polaritedeki solvanlar kullanarak Nigella sativa L. ve Nigella damascena L. tohumlarından konvansiyonel yöntemler kullanılarak elde edilen uçucu yağ, sabit yağ ve ekstrelerin verimleri ve antioksidan aktiviteleri değerlendirilmiştir.*
Gereç ve Yöntem: *Her iki türün tohumları, sabit yağ, uçucu yağ ve ekstrakttan uçucu yağ çıkarmak*

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için üç kısma ayrıldı. Sabit yağlar ve ekstraktlar, n-hekzan, petrol eteri, aseton, kloroform ve metanol ile Soxhlet ekstraksiyonu kullanılarak elde edildi, ardından Clevenger aparatı kullanılarak hidrodistilasyon yapıldı ve ekstraktlardan uçucu yağlar elde edildi. Ayrıca, uçucu yağlar toz tohumlardan hidrodistilasyon yoluyla da elde edildi. Yağ ve ekstre verimleri hesaplanarak antioksidan aktivitelerin değerlendirilmesinde DPPH· Ve ABTS·⁺ testleri kullanılmıştır.

Sonuç ve Tartışma: *Her iki tür için de n-hekzan ile sürekli ekstraksiyon yöntemi en yüksek verimi ulaştı ve uçucu yağlar sadece bu ekstraktlardan elde edildi. Özellikle, uçucu yağın doğrudan tohumdan ekstrakte edilmesi düşük verim ile sonuçlandı. N. sativa, N. damascena'dan daha yüksek uçucu yağ verimi gösterdi; ancak, elde edilen verimler aktivite çalışmaları için yetersizdi. Antioksidan testleri, ABTS.⁺ yönteminin daha ayırt edici olduğunu ve aseton ekstraktlarının daha yüksek aktivite gösterdiğini ortaya koydu. Genel olarak, N. sativa, N. damascena'ya kıyasla daha yüksek antioksidan aktivite gösterdi.*

Anahtar Kelimeler: *Antioksidan aktivite, Nigella damascena, Nigella sativa, sabit yağ, uçucu yağ*

INTRODUCTION

An imbalance between the production and elimination of reactive oxygen and nitrogen species leads to oxidative stress [1,2]. As oxidative stress accumulates over time, it is possible that it may lead to ageing and neurodegenerative disorders by impairing the function of the DNA repair system and mitochondria [2–4]. Specifically, the brain's limited capacity to manage oxidative stress suggests that oxidative stress may contribute to the aetiology of certain neurodegenerative disorders, such as Parkinson's, Alzheimer's, and Huntington's diseases [1,4,5]. Furthermore, it is considered to be one of the causes of the development of many chronic degenerative diseases, such as coronary heart disease and cancer [6,7].

Reactive oxygen species are neutralised by the reaction of compounds known as antioxidants. They act as agents that reduce oxidative stress, preventing or delaying cell damage [5,8]. However, negative results achieved in clinical trials and possible toxic and carcinogenic effects have led to the restriction of the use of antioxidants [3,5,9,10]. Despite these concerns, considering the link between phenolic acids and antioxidant activity, plant oils containing phenolic compounds are expected to act as potent antioxidants [9–11]. For instance, it is claimed that essential oils with antioxidant activity can reduce oxidative stress and can be used for therapeutic purposes [6,7,12].

Choosing an appropriate extraction technique to isolate targeted substances from plants is crucial to preserving the compounds' integrity and avoiding the extraction of unnecessary molecules [13–15]. Bioactive substances can be isolated using a variety of extraction techniques that use solvents with low to high polarity and vice versa [16]. Several traditional techniques, namely decoction, maceration, hydrodistillation, and soxhlet extraction, have been used to extract essential, and fixed oils [17]. The major advantage of these techniques is their suitability for small-scale research settings [13]. Along with choosing the proper extraction technique, choosing an appropriate solvent is essential for achieving a high yield [13,18]. Especially in the extraction of seed oils, the choice of solvent is a critical step. Generally, the most preferred solvents are ethanol, methanol, *n*-hexane and petroleum ether [19].

Soxhlet extraction is a standard method for extracting bioactive compounds from numerous plants and has been used for over a century to evaluate the efficiency of other solid-liquid extraction techniques [18,20]. It has several benefits, especially as it does not require filtering, is cost-effective, and treats the sample to the solvent repeatedly [20]. However, choosing the right solvent for extraction is crucial since different solvents will provide different extracts and extract compositions. [15]. One of the most preferred solvents, especially in the oil extraction, is *n*-hexane [14].

Hydrodistillation is one of the oldest oil extraction techniques [21]. Water or steam is used as a solvent for the technique [18]. Since organic solvents are not used, it is an affordable and highly convenient method, especially in essential oil extraction [13,14]. In this technique, a device known as the Clevenger apparatus is used [18]. The yield is highly dependent on the volume of water, weight, size, and nature of the material [13]. More specifically, it is the most preferred technique for essential oil extraction [22].

Both *Nigella damascena* L. (ND), also called love-in-a-mist, and *Nigella sativa* L. (NS), also called black cumin, are native to the Mediterranean region and are members of the Ranunculaceae family

[23]. NS, which is the most observed one, has many healing effects [24,25]. From a phytochemical point of view, it has polyphenols comprising acidic phenols [26–28]. Thus, its extracts, and especially its essential oil, have strong antioxidant activity [29,30]. Thymoquinone, the main component of its essential oil, has demonstrated antioxidant properties in a number of illnesses, including diabetes, asthma, cancer, and encephalomyelitis, by scavenging free radicals and superoxide and preserving the activity of antioxidant enzymes [31,32]. Similarly, NS extracts containing flavonoids have been found to enhance stomach mucus as well as mucosal immune responses through scavenging free radicals of superoxide and hydroxyl [33]. On the other hand, ND contains aromatic compounds, diterpenes, triperpenes, alkaloids, and flavonoids. The antioxidant action may be attributed to its phenolic content [34,35]. Furthermore, according to a study, ND fixed oil has a greater antioxidant capacity than NS fixed oil despite lacking thymoquinone [36]. Nevertheless, whereas numerous research studies have documented the phytochemical analysis and pharmacological activity of NS, there is scant information regarding ND in the literature [36].

This study examined the seeds of *Nigella sativa* (NS) and *Nigella damascena* (ND) species cultivated at the Medicinal and Aromatic Plants Garden of Inonu University Faculty of Pharmacy. The objective was to extract fixed oil from seeds using continuous extraction using various solvents, to derive extracts, to acquire essential oil from these extracts and seeds via hydrodistillation, and to evaluate the antioxidant properties of these extracts.

MATERIAL AND METHOD

Plant Materials

The dried capsules containing seeds of NS and ND species cultivated at the Medicinal and Aromatic Plants Garden of Inonu University Faculty of Pharmacy were collected by Z. Torun on time seed ripening following shedding of flowers in August 2022 (Voucher Specimen Code: ZT102 and ZT103, respectively). The seeds were thereafter carefully separated from their capsules and stored at +4-8 ºC in amber-coloured glass bottles tightly closed.

Figure 1. Cultivated *Nigella sativa* L. and *Nigella damascena* L. species

Obtaining Fixed Oils and Extracts

The amount of solvent consumed was determined as 10 parts (ml) according to the amount of 1 part (g) of sample. Using a Soxhlet apparatus (İldamcam) for eight hours in total, the fixed oils were obtained from the powdered sample seeds using *n*-hexane (Honeywell), and then the extracts were attained from the same sample seeds using petroleum ether and acetone solvents (Honeywell), respectively. After the procedure, the solvents were removed from the fixed oils and the extracts by using the rotary evaporator (Heidolph Laborota 4000, Germany). Following solvent removal, the fixed oils and the extracts were weighed gravimetrically and stored in the refrigerator at +4°C.

Obtaining Essential Oil from the Extract by Hydrodistillation

The amount of solvent consumed was determined as 10 parts (ml) according to the amount of 1 part (g) of sample. Using a Soxhlet apparatus (İldamcam) for eight hours in total, the extracts were obtained from the powdered sample seeds using *n*-hexane, chloroform, and methanol solvents (Honeywell), respectively. After the procedure, the solvents were removed from the extracts by using the rotary evaporator (Heidolph Laborota 4000, Germany). Following solvent removal, the extract yields were recorded, and the 100 ml distiled water was added to the extracts. Using a Clevenger apparatus (İldamcam), essential oils were obtained by hydrodistillation for at least four hours. Essential oil yield was calculated volumetrically by the graduated column of Clevenger and stored at +4°C.

Obtaining Essential Oil from Samples by Hydrodistillation

The amount of solvent consumed was determined as 10 parts (ml) according to the amount of 1 part (g) of sample. Using a Clevenger apparatus (İldamcam), essential oils were obtained from the powdered sample seeds by hydrodistillation for at least four hours. Essential oil yield was calculated volumetrically by the graduated column of Clevenger and stored at +4°C.

$DPPH^{\cdot}$ Test for Antioxidant Activity

This test was applied modified based on the method presented by Brand-Williams (1995) [37]. Using dimethyl sulfoxide (DMSO; CarloErba), stocks containing 100000 µg/ml were made from apolar extracts. Then dilutions were made at concentrations of 100000, 50000, 25000, 12500, 6250, and 3125 µg/ml. Stock containing 1000 µg/ml was made from gallic acid (Sigma Aldrich) as the standard, and then dilutions were made at concentrations of 1000, 500, 250, 125, 62.5, and 31.25 µg/ml. For this procedure, 0.0001 M $DPPH^{\cdot}$ (2,2-Diphenyl-1-picrylhydrazyl, TCI) was prepared freshly with DMSO.

In the study, a 96-well microplate was used. Triplets of the same sample, each containing 50 µl of samples and standards, were put in the wells. Then 150 µl of freshly prepared $DPPH^{\cdot}$ reagent was added and kept in the dark for 30 minutes. After incubation, absorbance values were measured at 517 nm. $DPPH^{\cdot}$ inhibition percentage was calculated according to the formula below. IC_{50} value was calculated from the calibration curve (ABS control = absorbance of the control group, ABS sample = absorbance of the sample used).

$$DPPH^{\cdot} \text{ scavenging effect \%} = [\text{ABS control} - \text{ABS sample}] / \text{ABS control X } 100$$

$ABTS^{+}$ Test for Antioxidant Activity

This test was applied modified based on the method presented by Re et al. [38]. For $ABTS^{+}$ (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), AFG) reagent, 2.45 mM potassium persulfate ($K_2S_2O_8$) was prepared. Then a 7 mM $ABTS^{+}$ solution was prepared with this solution. It was placed in the dark for one night, and the next day it was diluted with DMSO at a ratio of 1:4. Using dimethyl sulfoxide (DMSO), stocks containing 100000 µg/ml were made from apolar extracts. Then dilutions were made at concentrations of 100000, 50000, 25000, 12500, 6250, and 3125 µg/ml. Stock containing 1000 µg/ml was made from ascorbic acid (Roth) as the standard, and then dilutions were made at concentrations of 1000, 500, 250, 125, 62.5, and 31.25 µg/ml.

In the study, a 96-well microplate was used. Absorbance values were measured at 734 nm after 20 µl of the samples and standard, and 200 µl of $ABTS^{+}$ reagent were placed in the wells. The The experiments were performed in triplicate. The $ABTS^{+}$ reduction rate was determined according to the formula below. The IC_{50} value was determined from the calibration curve.

$$ABTS^{+} \text{ scavenging effect \%} = [\text{ABS control - ABS sample}] / \text{ABS control X } 100$$

RESULT AND DISCUSSION

Yields Obtained

The yields of fixed oils obtained using *n*-hexane and petroleum ether through the continuous

extraction method in the Soxhlet apparatus, as well as the yields of the extracts obtained using acetone of moderate polarity, are given in Table 1.

Table 1. The yields of fixed oil and extracts obtained by Soxhlet extraction

Species	Solvents	Total Powdered Seeds (g)	Total Fixed Oils/ Extracts (g)	% Yields (g/g)
NS*	*n*-Hexane	21.4227	1.7332	8.0904
NS	Petroleum Ether	21.4227	0.2181	1.0180
NS	Acetone	21.4227	0.2336	1.0904
ND*	*n*-Hexane	23.0061	2.3198	10.0834
ND	Petroleum Ether	23.0061	0.1924	0.8362
ND	Acetone	23.0061	0.3512	1.5265

*NS: *Nigella sativa*; ND: *Nigella damascena.*

The yields of essential oils obtained from the extracts prepared using *n*-hexane, chloroform, and methanol through the continuous extraction method and connected to the Clevenger apparatus are given in Table 2.

The yields of essential oils obtained directly from the powdered seeds using hydrodistillation method are given in Table 3.

Table 2. The yields of essential oil obtained from the extract by using hydrodistillation

Extracts	Total Powdered Seeds (g)	Total Extracts (g)	Total Essential Oils (ml)	% Yields (ml/g)
NS-*n*-Hexane*	25.8441	1.8154	0.05	2.7542
NS-Chloroform**	25.8441	0.5060	NA***	NA
NS-Methanol**	25.8441	0.9854	NA	NA
ND-*n*-Hexane*	22.9867	3.2062	0.03	0.9356
ND-Chloroform**	22.9867	0.8903	NA	NA
ND-Methanol**	22.9867	1.3851	NA	NA

* The measured amount of essential oil is considered inadequate for antioxidant activity studies. ** The amount of essential oil extracted is inadequate to be measured. ***NA: not available; NS: *Nigella sativa*, ND: *Nigella damascena*

Table 3. The yields of essential oils obtained directly from powdered seeds by hydrodistillation

Species	Total Powdered Seeds (g)	Total Essential Oils* (ml)	% Yields (ml/g)
NS	25.8910	0.3	1.1587
ND	25.8630	0.15	0.6560

* The measured amount of essential oil is considered not adequate for antioxidant activity studies

For fixed oil extraction using Soxhlet extraction, the highest yields were obtained when *n*-hexane was used as solvent (8.0904% for NS and 10.0834% for ND). Petroleum ether and *n*-hexane possess identical polarity values in scientific expression. Despite the implementation of an 8-hour technique, the poor yield achieved in the extraction process using petroleum ether subsequent to *n*-hexane indicates that not all fixed oils were extracted with *n*-hexane throughout this operation. It is anticipated that

prolonging the procedure duration will enhance the yield.

Furthermore, for obtaining essential oil from the extracts by hydrodistillation, the highest yields were obtained from *n*-hexane extracts. *n*-Hexane is a nonpolar, volatile organic solvent. With this solvent, which is generally used in the extraction of steroidal compounds, fixed oils are obtained. At the same time, chloroform and acetone, the other solvents used, are easily evaporable organic solvents. Based on the extraction here, it can be considered that in the essential oil extraction process, essential oils entrained with water vapour are actually obtained together with fixed oil. The fact that a significant essential oil yield was obtained despite the use of other extracts compared to the *n*-hexane extract proves this hypothesis. In the comparison of essential oil yields, NS (2.7542%) showed superiority over ND (0.9356%). Likewise, some research reports favourable yields with *n*-hexane [39], while some others also exhibit superior yields with ethanol as the solvent [40].

Attempts to obtain essential oil from extract by using hydrodistillation and directly from the seed resulted in low yields and were considered inadequate for evaluation. Hence, fixed oils and extracts derived from Soxhlet extraction were chosen for antioxidant activity assessments.

Antioxidant Activities

The $ABTS^+$ and $DPPH^\cdot$ methods were used to measure the antioxidant activities of the extracts, which had sufficient quantities. While ascorbic acid was used as a reference substance for the $ABTS^+$ test, gallic acid was used as a reference substance for the $DPPH^\cdot$ test. The IC_{50} values obtained were presented in mg/ml. The antioxidant results for NS and ND with standard compounds are given in Table 4.

Table 4. Antioxidant Activities of NS and ND

Method	Continuous Extraction (IC_{50} mg/ml ± Standard Deviation)							
	n-Hexane		Petroleum Ether		Acetone		Standard	
	NS	ND	NS	ND	NS	ND	Ascorbic acid	Gallic acid
$DPPH^\cdot$*	6.4931± 0.12	22.6176± 0.44	219.0383± 0.21	233.5359± 0.39	15.6957± 0.46	64.7884± 0.39	-	0.3231± 0.016
$ABTS^+$*	1.1287± 0.12	40.7551± 0.44	14.4290± 0.21	94.1345± 0.39	0.6754± 0.46	4.9893± 0.39	0.4385± 0.005	-

* Statistically significant differences ($p < 0.05$, n=3)

In our study, for both $DPPH^\cdot$ and $ABTS^+$ methods, a statistical comparison of IC_{50} values of all extracts with the standard compound as positive control used was made using an independent sample t-test by Excel. As a result of statistical analysis, $p<0.05$ was determined. This demonstrates a statistically significant difference. The extracts show antioxidant activity at higher concentrations compared to the standard compounds. Noticeably, $ABTS^+$ method was found to be a more specific method in comparison to $DPPH^\cdot$ method in the light of the antioxidant results obtained. Our results revealed that NS showed higher antioxidant activity compared to ND. Also, the polar acetone extract displayed higher activity than the other extracts. From these results, it can be concluded that more polar extracts contain more active antioxidant compounds.

When previous studies were examined, Goga et al. (2012) used Soxhlet apparatus, ethanol and *n*-hexane as solvents for the oil of NS seeds. The researchers showed that the $DPPH^\cdot$ method gave better results than $ABTS^+$ method to determine the antioxidant activity of the extracts obtained [41]. Dinagaran et al. (2016) used Soxhlet apparatus and *n*-hexane as solvent to extract oil from NS seeds. The researchers showed potent results with the $ABTS^+$ method [42]. Toma et al. (2015) showed that ND's antioxidant activity could be higher [26]. Alrashidi et al. (2022) used a Soxhlet apparatus and six solvents with different polarities to extract oil from NS seeds. They correlated antioxidant activity to high polarity [40].

Differences that we found in the literature suggest that the yields and antioxidant activities obtained from *Nigella* species are heavily dependent on the locations where they are cultivated, the

extraction methods, and the solvents used. Due to the morphological structure of the seed, fixed oils are usually carried in the secretory ducts, and aromatic essential oils, which are usually carried in the secretory hairs in the flower and leaf organs, are not present in the seed structure, which makes the comparison of essential oil yield and fixed oil yield meaningful. While NS, which is usually consumed as food, has been widely studied by researchers, studies on ND have been limited. This may be explained by the fact that ND seeds do not differ from NS seeds externally, and although they have a refreshing floral odour, they are not suitable for consumption as food, leaving a bitter taste in the mouth when consumed. Furthermore, it has been noted that the essential oil derived from ND seeds in our study possesses a stench so unpleasant that it resembles the scent of soiled nappies and disperses swiftly around the study area. Although the studies on ND are limited, the fact that it exhibits significant antioxidant activity emphasises the need for further research on this species.

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AUTHOR CONTRIBUTIONS

Concept: B.E.Ç., F.S., Z.T.; Design: B.E.Ç., F.S., Z.T.; Control: B.E.Ç., F.S., Z.T.; Sources: B.E.Ç., F.S., Z.T.; Materials: Z.T.; Data Collection and/or Processing: B.E.Ç., F.S., Z.T.; Analysis and/or Interpretation: B.E.Ç., F.S., Z.T.; Literature Review: B.E.Ç., Z.T.; Manuscript Writing: B.E.Ç.; Critical Review: B.E.Ç.; Other: -

CONFLICT OF INTEREST

The author declares that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The author declares that the ethics committee approval is not required for this study.

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