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# Chemical Content and Biological Activity Spectrum of Nigella sativa Seed Oil

Yusuf SICAK[1], Elif Ayşe ERDOĞAN ELİUZ[2]

[1]Muğla Sıtkı Koçman University, Köyceğiz Vocational School, Department of Medicinal and Aromatic Plants, 48800, Muğla, [2]Mersin University, Technical Sciences Vocational School, Department of Food Technology, 33343, Mersin
[1]https://orcid.org/0000-0003-2339-5837, [2]https://orcid.org/0000-0003-4317-3000
✉: yusufsicak@mu.edu.tr

## ABSTRACT

In this study, it was aimed to investigate the chemical composition and biological activities including antioxidant, anticholinesterase, antimicrobial and urease activity of *Nigella sativa* seed oil growing in Muğla (Köyceğiz). The oil of plant was obtained using Soxhlet apparatus. Thirty-one components of the oil were determined by Gas Chromatography-Mass Spectrometry (GC-MS). The major components of oil were detected as palmitic acid (10.48%), linoleic acid (8.05%), *o*-cyemene (7.11%), 3,5-dimethyl cyclohexanol (6.68%), thymoquinone (6.44%), *p*-tert-buthyl catechol (6.28%) and 8-methyl-1-undecene (3.28%). The findings obtained from biological activity assays showed that *Nigella sativa* oil was a promising candidate that can be used in the discovery of new drugs and the preparation of new natural drug preparations.

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## Çörek Otu Tohumu Yağının Kimyasal İçeriği ve Biyolojik Aktivite Spektrumu

## ÖZET

Bu çalışmada, Muğla (Köyceğiz)'de yetişen *Nigella sativa* (çörek otu) tohumu yağının kimyasal bileşimi ile yağın antioksidan, antikolinesteraz, antimikrobiyal ve üreaz aktivitesinin araştırılması amaçlanmıştır. Bitkinin yağı, Soxhlet aparatı kullanılarak elde edildi. Yağ bileşimi Gaz Kromatografisi-Kütle Spektrometresi (GC-MS) ile belirlendi. GC-MS sonuçlarından, yağın otuz bir bileşeni tespit edilebildi. Yağın ana bileşenleri palmitik asit (% 10.48), linoleik asit (%8.05), o-siemen (% 7.11), 3,5-dimetil sikloheksanol (% 6.68), timokinon (% 6.44), *p*-tert-butil katekol (% 6.28) ve 8-metil-1-undesen (% 3.28) olarak belirlendi. Biyolojik aktivite test sonuçları tıbbi özelliği olan çörek otu yağının yeni ilaçların keşfinde ve yeni doğal drog preparatların hazırlanmasında kullanılabilecek umut verici bir aday olduğunu göstermektedir.

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## INTRODUCTION

Medicinal aromatic plants are cultivated to obtain specialty products including oils and pharmaceuticals (Ali-Shtayeh *et al.* 2018). *Nigella sativa*, annual herbaceous plant, is generally known as black caraway seed and black cumin which belongs to Ranunculaceae family (Forouzanfar *et al.* 2014). It is commonly cultivated in the Mediterranean region, Eastern Europe, Middle East and Western Asia (Noor *et al.* 2012; Venkatachallam *et al.* 2010). It is one of the most common medicinal herbs which is widely used around the world (Ahmad et al. 2013). Seed and oil of *N. sativa* are used traditionally not only as a spice because of its characteristic aroma and bitter taste but also as a medicine to treat asthma, bronchitis, cough, fever, eczema, headache, influenza and rheumatism for more than 2000 years (Forouzanfar *et al.* 2014; Burits and Bucar 2000; Gharby *et al.* 2015).

There is a growing interest in use of natural bioactive compounds purified from plants that have demonstrated to be potent antioxidants, antimicrobials and key enzyme inhibitors related with many diseases as cholinesterase for Alzheimer's disease; tyrosinase for skin disorders and urease for gastric and peptic ulcers/cancer (Llorent-Martinez *et al.* 2018). *N. sativa* seeds are consisted of complicated substances of many compounds which are rich in source of alkaloids, carvacrol, essential oil (0.4-0.45 %), fixed oil (32-40 %), proteins, saponin, terpenoids, quinones (such as thymoquinone, nigellone, and

thymohydroquinone), minerals and vitamins (Forouzanfar *et al.* 2014; Salem 2005; Hosseinzadeh *et al.* 2013). Finally, highly prized nutritious oil was prepared from *N. sativa* seeds (Gharby *et al.* 2015). It is well known that *N. sativa* seed oil has therapeutic potential as an antiatherogenic (Al-Naqeep *et al.* 2011), antihypertensive (Huseini Fallah *et al.* 2013), and anti-obesity (Le *et al.* 2004) agent. *N. sativa* oil was also found to be effective as a supplementary treatment in patients with insulin resistance (Najmi *et al.* 2008).

Therefore, it is important to characterize *N. sativa* oil with potential biological activities. In this regard, the aim of present study was to characterize the chemical composition, to evaluate the total flavonoid and phenolic contents and to assess the *in vitro* antioxidant, anticholinesterase, antiurease and antimicrobial potential of *Nigella sativa* seed oil.

## MATERIAL and METHODS

### Plant Material and Extraction of Oil

*N. sativa* were collected from Köyceğiz region of Muğla, Turkey, during September-October of 2017, identified at the Herbarium of Biology, Faculty of Science, Muğla Sıtkı Koçman University, Turkey.

Approximately, 100 grams of *N. sativa* seed sample was used for the oil extraction process. Solvent-extraction was performed using a Soxhlet apparatus with hexane for 8 hours. The mixture added to water. After liqiud-liqiud extraction, the aqua in organic phase was dried over anhydrous $Na_2SO_4$. Organic phase was then concentrated under vacuum. Obtained oil was kept in desiccator, protected from sunlight until analysis.

### GC-MS Analysis

The qualitative and quantitative analysis of oil were conducted at Central Research Laboratories Application and Research Center, Giresun University, using a GC-MS 7890A-(5975C inert MSD) instrument equipped with an Agilent HP5-MS column (30 m x 250 µm film, 0.25 µm). The oil was diluted 1:100 (v/v) in hexane prior to the injection, and the injection volume was adjusted as 1.5 mL of the solution. The column temperature was heated from 50°C to 270°C by an increase of 5°C / minutes, and then maintained for 25 minutes at 270°C. The temperature of injection port and detector were 250°C and 260°C, respectively. The carrier gas was helium. Characterization of oil components were surveyed by the Wiley and NIST libraries with comparing the mass spectra of the analysed oil.

### Biological Activity Studies

### Determination of antioxidant activity

Solutions of oil obtained from *N. sativa* seed were prepared at four different concentrations as 200-100-50-25 ppm in EtOH. EtOH was used as the controlThe comparison of the activity tests were evaluated by BHA and α-tocopherol standard antioxidant compounds. Results were expressed as 50% concentration ($IC_{50}$) for $ABTS^{\cdot+}$ scavenging activity, *β*-carotene-linoleic acid and $DPPH^{\cdot}$ assay. CUPRAC assay results are given as $A_{0.5}$.

The spectrophotometric analysis of antioxidant activities were performed by the following methods: $ABTS^{\cdot+}$ scavenging activity (Re *et al.* 1989), *β*-carotene-linoleic acid (Marco 1968; Öztürk *et al.* 2011), CUPRAC assay (Apak *et al.* 2004) and $DPPH^{\cdot}$ scavenging activity (Blois 1958).

### Determination of anticholinesterase activity

The inhibitory activities of AChE and BChE were measured by the spectrophotometric method. Solutions of *N. sativa* seed oil were prepared from the 200-100-50-25 ppm at concentrations. The enzyme sources were AChE and BChE from electric eel and horse serum, respectively. The substrates were acetylthiocholine iodide and butyrylthiocholine chloride. The Ellman's reagent, DTNB (5,50-dithiobis(2-nitrobenzoic)acid, was used for the determination of the anticholinesterase activity (Ellman *et al.* 1961).

### Determination of antimicrobial activity

The antimicrobial activity of oil of *N. sativa* were tested against several pathogens, namely *Bacillus subtilis* (ATCC 6633), *Candida albicans, Candida parapsilosis, Escherichia coli* (ATCC 25293), *Pseudomonas aureginosa* and *Staphylococcus aureus* (ATCC 25925) using modified spectrophotometric microdilution technique. Firstly, the inoculums of microorganisms were prepared in 4 mL of Triptic Soy Broth for bacteria, 4 mL of Sabouraud Dextrose Broth for yeasts and incubated at 37°C, overnight. After 24 hours, the culture suspensions were adjusted to 0.5 mL McFarland Standard Turbidity (~$10^4$ for bacteria, ~$10^3$ for yeasts) and stored at +4°C until use (Mcfarland 1987).

The 50 µL (400 µg/mL) of *N. sativa* seed oil was dissolved with 1 mL of dimethyl sulfoxide (10 % DMSO). The experiment was performed on 96-well microtiter plates and firstly 50 µL of Mueller Hinton Broth (MHB) medium were added into all wells. Two-fold serial dilutions of 50 µL of oil was made on all x-axis along of Elisa plate. Columns 11 and 12 were used as negative and positive controls. Finally, 10 µL culture of microorganisms was inoculated on all wells except medium control wells. All of the plates were incubated at 37°C for 24 hours, the growth (turbidity) was measured at 600 nm for bacteria, 415 nm for yeasts. For MIC analysis, the optical density was read both before, T0 and after 24 hours-incubation, T24. For each plate, MIC were calculated using the following formula: The OD for each replicate at T0 was

subtracted from the OD for each replicate at T24.

$$\text{The Percent Growth} = \frac{\text{ODtest}}{\text{ODcontrol}} x100$$

$$\text{Percent Inhibition} = 1 - \frac{\text{ODtest well}}{\text{ODcontrol well}} x100$$

for each row of the 96-well plate. The dose-response curves obtained from plotting the linear of the concentration of the oils against the resulting percent inhibition of microbial growth were evaluated with the regression analysis, giving an $R^2$ value. MIC (the lowest concentration of test material which results in 99.9% inhibition of growth) were calculated using the $R^2$ formula on inhibition curve (Patton *et al.* 2006; Erdoğan Eliuz *et al.* 2017).

### Determination of urease activity

The spectrophotometric analysis of urease inhibition was performed according to the indophenol method of Weatherburn (1967) by measuring ammonia production.

### Statistical Analysis

All data on biological activity assay studies were the averages of triplicate analyses. Antioxidant, anticholinesterase and urease activity assays were conducted at four concentrations, while antimicrobial activity conducted at ten concentrations. All the results are presented as 50% concentration ($IC_{50}$) (%). Results are presented as mean ± standard error of the mean (SEM). Significant differences between means were determined by Student's-t test and considered to be significant at a level of $p \leq 0.05$.

The SPSS-one-way analysis of variance (ANOVA), Tukey test were performed for the % inhibition values to detect ($p \leq 0.01$.) the differences. The experiments were conducted with a minimum of 3 times.

## RESULTS and DISCUSSION

### Chemical Composition of the Seed Oil

The investigation of the quantitative and qualitative composition of natural products is a key parameter to explore their potential in various applications including functional food, nutraceutical or pharmaceutical. In this study, totally thirty-one components were detected in *N. sativa* seed oil and were given in Table 1. The major components detected from seed of N. sativa were palmitic acid (10.48%), linoleic acid (8.05%), *o*-cyemene (7.11%), 3,5-dimethyl cyclohexanol (6.68%), thymoquinone (6.44%), *p*-tert-buthyl catechol (6.28%) and 8-methyl-1-undecene (3.28%). Whereas, El-Dakhakhny and coworkers (1963) characterized thymoquinone (27.8%-57.0%), *ρ*-cymene (7.1%-15.5%), carvacrol(5.8%-11.6%), t-anethole (0.25%-2.3%), 4-terpineol (2.0%-6.6%) and longifoline (1.0%-8.0%) as major components. Burits and Bucar (2000) reported that the major components in *N. sativa* commercial seeds oil were thymoquinone (30%-48%), *p*-cymene (7%-15%), carvacrol (6%-12%), 4-terpineol (2%-7%), *t*-anethole (1%-4%) and the sesquiterpene longifolene (1%-8%). Piras *et al.* (2013) showed that the major constituents in the samples from *N. sativa* seed of Turkey and Egypt were thymoquinone (77.2%-86.2%), *o*-cymene (5.4%-11.0%) and the lower terpinen-4-ol, methyl chavicol, trans-sabynil acetate, *α*-thujene, limonene and *γ*-terpinene. A study using the soxhlet extraction method reported that the oil contents of *Nigella sativa* seeds collected from Turkey, India, U.S.A, Egypt are quite similar and the basic components were to be myristic, palmitic, stearic, oleic, linoleic, linolenic, arachidic, behenic, eicosadienoic acids (Ustun *et al.* 1990).

Table 1. Composition of *N. sativa* seed oil

| RT | Component | Quantity(%) |
|---|---|---|
| 3.115 | 8-methyl-1-undecene | 3.28 |
| 9.003 | *α*-Thujene | 1.26 |
| 9.289 | *α*-Pinene | 0.28 |
| 10.983 | Sabinene | 0.16 |
| 11.121 | 2-ß-Pinene | 0.33 |
| 13.306 | *o*-Cyemene | 7.11 |
| 13.467 | *D*-Limonene | 0.34 |
| 14.897 | *γ*-Terpinene | 0.47 |
| 17.781 | *Trans*-methoxy thujane | 1.11 |
| 20.619 | 4-terpinol | 0.20 |
| 21.849 | 1,2-epoxy-menth-4-ene | 0.19 |
| 23.978 | Thymoquinone | 6.44 |
| 26.410 | Carvacrol | 0.87 |
| 28.206 | *α*-Longipinene | 0.28 |
| 30.564 | Longifolene | 1.38 |
| 37.196 | *p*-tert-buthyl catechol | 6.28 |
| 45.378 | Myristic acid | 0.51 |
| 51.077 | 4-undecyl phenol | 1.31 |
| 51.523 | Borneol | 0.95 |
| 52.170 | Oleic acid | 1.33 |
| 52.771 | Palmitic acid | 10.48 |
| 54.190 | *N*-(3-methylphenyl)pentanamide, | 0.71 |
| 3.115 | 8-methyl-1-undecene | 3.28 |
| 9.003 | *α*-Thujene | 1.26 |
| 54.327 | 4-octyl phenol | 0.81 |
| 54.688 | *m*-Tolylacetamide | 0.44 |
| 55.346 | 6-acetamido-2,2-dimethyl-2*H*-1-benzopyran-4-one | 0.80 |
| 56.112 | Linoleic acid methyl ester | 1.49 |
| 56.267 | Elaidic acid methyl ester | 0.66 |
| 56.484 | 3,5-dimethyl cyclohexanol | 6.68 |
| 57.022 | *N*-(3-methylphenyl)hexanamide | 0.78 |
| 57.200 | *p*-Propionotoluidide | 0.68 |
| 57.938 | Linoleic acid | 8.05 |

RT: Retention Time

### Biological Activities of the Seed Oil

### Antioxidant Activity

To the best of our knowledge, this study is the first attempt to highlight the *in vitro* antioxidant activity of

oil obtained from the seed of *N. sativa* collected from Köyceğiz-Turkey. The antioxidant activity results of *N. sativa* seed oil given in Table 2. According to the *β*-carotene/ linoleic acid assay results, the oil exhibited the lipid peroxidation inhibitory activity value of ($IC_{50}$) 10.15±0.13 µg/mL. In the $ABTS^{\cdot+}$ assay, oil ($IC_{50}$:4.77±0.19 µg/mL) showed better cation radical scavenging activity than standard *α*-TOC ($IC_{50}$=54.97±0.99 µg/mL). The oil demonstrated activity with an $IC_{50}$ value of 52.61±0.22 µg/mL in DPPH free scavenging activity. The oil indicated higher CUPRAC activity with an $A_{0.5}$ value of 31.27±0.02 µg/mL, than α-TOC ($A_{0.5}$=40.55±0.04 µg/mL) using as a pharmaceutical standard. The antioxidant activity of seed oil is related to its composition. Among the bioactive compounds of *N. sativa* seed oil, especially the presence of thymoquinone has positive effect on the antioxidant activity (Beckstrom-Sternberg and Duke, 1994; Bourgou et al.,2010). Additionally, carvacrol, cymene, 4-terpineol and α-pinene thymol are the most important bioactive compounds of *N. sativa* seed oil (Islam et al., 2017).

Table 2. Antioxidant activity of *N. sativa* seed oil[a]

| Sample | Antioxidant Activity | | | |
|---|---|---|---|---|
| | *β*-carotene/ linoleic acid assay $IC_{50}$ (µg/mL) | $ABTS^{\cdot+}$ assay $IC_{50}$ (µg/mL) | $DPPH^{\cdot}$ assay $IC_{50}$ (µg/mL) | CUPRAC assay $A_{0.50}$ (µg/mL) |
| **Oil** | 5.18±0.45 | 4.77±0.19 | 52.61±0.22 | 31.27±0.02 |
| **BHT[b]** | 2.31±0.11 | 2.97±0.05 | 54.80±0.78 | 3.92±0.04 |
| ***α*-TOC[b]** | 4.48±0.17 | 4.95±0.30 | 12.21±0.06 | 40.44±0.03 |

[a]Value represent the means ± standard deviation of three parallel measurements ($p$<0.05)
[b]Reference compound

## Anticholinesterase Activity

Acetylcholine is a neurotransmitter, requisite in the attention, memory and learning functions. Acetylcholine inactivation can cause many neurodegenerative diseases (e.g. Alzheimer's disease). Cholinesterase inhibitors have a key role on delaying the acetylcholine inactivation and finally supporting neuroprotection. Natural products are extensively investigated for their potential application as cholinesterase inhibitors. In spite of the anticholinesterase and butyrylcholinesterase activity of various plant compounds has been verified, *in vitro* laboratory studies using *N. sativa* seed oils are very limited (Kannan *et al.* 2019).

The anticholinesterase activity results of *N. sativa* seed oil was given in Table 3. *N. sativa* seed oil showed significant activity against AChE with an $IC_{50}$ value of 7.32±0.41 µg/mL. On the other hand, *N. sativa* seed oil against BChE exhibited the highest activity with an $IC_{50}$ value of 35.48±0.83 µg/mL that was higher than galantamine ($IC_{50}$=46.03±0.14 µg/mL) using as a pharmaceutical standard.

Table 3. Anticholinesterase and urease inhibition activities of *N. sativa* seed oil

| Sample | Anticholinesterase Inhibitory Activity | | Urease Inhibitory Activity |
|---|---|---|---|
| | AChE assay $IC_{50}$ (µg/mL) | BChE assay $IC_{50}$ (µg/mL) | Urease assay $IC_{50}$ (µg/mL) |
| **Oil** | 7.32±0.41 | 35.48±0.83 | 30.21±037 |
| **Galantamine[b]** | 4.48±0.78 | 46.03±0.14 | NT |
| **Thiourea[b]** | NT | NT | 23.08±0.19 |

[a]Value represent the means ± standard deviation of three parallel measurements ($p$<0.05), [b]Reference compound, NT: Not tested

## Urease Activity

The urease activity results of *N. sativa* seed oil was also given in Table 3. According to urease activity assay result, *N. sativa* seed oil indicated significant activity with an $IC_{50}$ value of 30.21±0.37 µg/mL.

## Antimicrobial Activity

The 24 hours incubation of *N. sativa* seed oil with microorganisms was found to be statistically significant in terms of the resultant inhibition ($p$<0.01) (Table 4). Accordingly, the highest average inhibitory effect of volatile oil was against *C. albicans* (86.07%), while the lowest inhibition was against *E. coli* (-42.31).

Table 4. Statistical analysis of average % inhibition variation of microorganisms incubated with *N. sativa* seed oil for 24 hours. The difference between the average % inhibition groups level were compared according to the Tukey test.

| Microorganisms | % Inhibition | |
|---|---|---|
| | Average | Std± |
| ***E. coli*** | -42.31[a] | ±21.03 |
| ***P. aureginosa*** | -0.82[b] | ±13.1 |
| ***B. subtilis*** | -9.13[c] | ±7.9 |
| ***S. aureus*** | -17.8[d] | ±12.9 |
| ***C. albicans*** | 86.07[a,b,c,d,e] | ±5.2 |
| ***C. parapsilosis*** | -13.14[e] | ±19.3 |

Values with the same letters are significantly different from each other ($p$<0.01).

Many studies about different *N. sativa* extracts showed that they had a potent effect on twenty-one pathogenic bacteria such as *E. coli, S. aureus, P. aeruginosa* and some yeasts (Hanafy and Hatem 1991; Nair *et al.* 2005; Hannan *et al.* 2008; Chaieb *et al.* 2011). In our study, *N. sativa* oil has been studied for its antimicrobial activity against a several range of microorganisms by using the spectrophotometric microdilution assay. Generally, all tested microorganisms were sensitive to *N. sativa* oil at MIC range of 8.36-21.11 µg/mL. The MIC of *N. sativa* oil results for E. coli was 13.62 µg/mL, *B. subtilis* was 21.11 µg/mL, *S. aureus* was 16.14 µL/mL, *C. albicans* was 8.36 µL/mL, *C. parapsilosis* and *P. aureginosa* were 8.9 µg/mL (Figure 1). Therefore, the maximum antimicrobial activity was determined against *C. albicans* (8.36 µg/mL) while the minimum activity was determined against *B. subtilis* (21.11 µg/mL). According previous studies, *N. sativa* oil was reported to have a broad spectrum of activity against *Salmonella typhi*, *Staphylococcus albus*, *Shigella niger, Escherichia coli* and *Vibrio cholera* (Agrawal *et al.* 1979) and *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa* (El-Kamali *et al.* 1998), using the plate diffusion method (Hosseinzadeh *et al.* 2007). *İn vivo* conditions, chloroform and methanol extracts, and the essential oil of *N. sativa* inhibited strongly *E. coli* and *S. aureus* at a range of 0.38-33 mg/kg. In addition, it was reported that the *N. sativa* seed oil was active against multiple drug-resistant (ampicillin, tetracycline and co-trimoxazole etc.) isolates of *Vibrio chloerae*, *E. coli* and Shigella spp. *in vitro* (Ferdous *et al.* 1992).

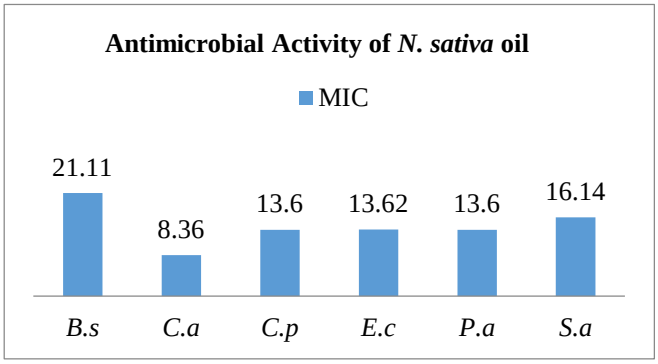


Figure 1. The comparison of MIC (µg/mL) values of *N.sativa* oil against *B. subtilis* (*B.s*), *C. albicans* (*C.a*), *C. parapsilosis* (*C.p*), *E. coli* (*E.c*), *P. aureginosa* (*P.a*) and *S. aureus* (*S.a*).

The pharmacological actions of *N. sativa* have been reported include antipyretic, antimicrobial, antineoplastic activity and protection against nephrotoxicity, depending on its chemical content (Ali and Blunden, 2003). Similarly, it is appear that the beneficial effects of *N. sativa* might be related to its components which have strong antimicrobial, antioxidant, urease, and anticholinesterase actions in this study.

## CONCLUSION

In complementary medicine concept, herbal drugs extracted from natural products are being increasingly preferred around the world. Furthermore, the extracts are evaluated as important ingredients in several applications ranging from functional food to active packaging industries. *N. sativa* is one such herb with numerous beneficial effects. In this study, the chemical content of oil derived from the *N. sativa* seed collected from Köyceğiz region were investigated and antioxidant, anticholinesterase, urease and antimicrobial activity of oil were determined *in vitro*. Among the identified thirty-nine compounds, high amounts of palmitic acid (10.48%) and linoleic acid (8.05%) were detected in the *N. sativa* oil.

The results of *N. sativa* seed oil biological activities can be summarized as follows:

- It can be used as a natural antioxidant supplements to be beneficial in preventing diseases.
- In particular, for the BChE test result, it can be evaluated as anticholinesterase agent to inhibit the action against butyrylcholinesterase enzymes.
- The seed oil can be potent urease inhibitors which will be explored for patients to prevent urease-induced symptoms or diseases.
- The antimicrobial performance of *N. sativa* seed oil suggests that it is a good candidate for antimicrobial practices.

Hence, the findings can be a valuable for biotechnology, biodiversity, medicines and medical studies for prospective studies of *N. sativa* seed oil. Moreover, they will help to understand the significance of the biological diversity and the conservation efforts of plant.

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