Research Article

Phytochemical Analysis and Antioxidant, Antibacterial, and Antifungal Effects of Essential Oil of Black Caraway (Nigella sativa L.) Seeds against Drug-Resistant Clinically Pathogenic Microorganisms

Otmane Zouirech,1 Abdullah A. Alyousef,2 Azeddin El Barnossi,3 Abdelfattah El Moussaoui,3 Mohammed Bourhia,4 Ahmad M. Salamatullah,5 Lahcen Ouahmane,4 John P. Giesy,6,7 Mourad A. M. Aboul-soud,2 Badiaa Lyoussi,1 and Elhoussine Derwich1,8

1Laboratory of Natural Substances, Pharmacology, Environment, Modeling, Health and Quality of Life (SNAMOPEQ), Faculty of Sciences Dhar El Mahraz, University Sidi Mohamed Ben Abdellah, Fez, Morocco
2Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Saud University, P.O. Box 10219, Riyadh 11433, Saudi Arabia
3Laboratory of Biotechnology, Environment, Agrifood, and Health, Faculty of Sciences Dhar El Mahraz, University of Sidi Mohamed Ben Abdellah, Fez 30050, Morocco
4Laboratory of Microbial Biotechnology, Agro-Sciences and Environment (BioMAgE), Cadi Ayyad University, Marrakesh 40000, Morocco
5Department of Food Science & Nutrition, College of Food and Agricultural Sciences, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia
6Department of Veterinary Biomedical Sciences & Toxicology Centre, University of Saskatchewan, Saskatoon, SK, Canada S7N5B3
7Department of Environmental Science, Baylor University, Waco, TX 76798-7266, USA
8Unity of GC/MS and GC-FID, City of Innovation, Sidi Mohamed Ben Abdallah University, Fez, Morocco

Correspondence should be addressed to Otmane Zouirech; otmane.zouirech@usmba.ac.ma, Mohammed Bourhia; bourhiamohammed@gmail.com, and Elhoussine Derwich; elhoussinederwich@yahoo.fr

Received 3 April 2022; Revised 12 June 2022; Accepted 22 June 2022; Published 26 July 2022

Academic Editor: Sanket Kaushik

Copyright © 2022 Otmane Zouirech et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Nigella sativa (NS) is a plant that has long been utilized in traditional medicine as a treatment for certain diseases. The aim of this work was to valorize the essential oil (EO) of this species by phytochemical analysis and antimicrobial and antioxidant evaluation. EO was extracted by hydrodistillation from the seeds of Nigella sativa (EO-NS). Phytochemical content of EO-NS was evaluated by use of gas chromatography coupled to mass spectrometry (GC-MS/MS). Antioxidant ability was in vitro determined by use of three assays: 2.2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing power (FRAP), and total antioxidant capacity (TAC) relative to two synthetic antioxidants: BHT and quercetin. Antimicrobial effect was in vitro determined by use of three strains: Staphylococcus aureus, ATCC 6633; Escherichia coli, K12; Bacillus subtilis, DSM 6333; and Proteus mirabilis, ATCC 29906) and against four fungal strains (Candida albicans, ATCC 10231; Aspergillus niger, MTCC 282; Aspergillus flavus, MTCC 9606; and Fusarium oxysporum, MTCC 9913). Fifteen constituents that accounted for the majority of the mass of the EO-NS were identified and quantified by use of GC-MSMS. The main component was O-cymene (37.82%), followed by carvacrol (17.68%), α-pinene (10.09%), trans-sabinene hydrate (9.90%), and 4-terpineol (7.15%). EO-NS exhibited significant antioxidant activity with IC_{50} = 0.017 ± 0.0002, EC_{50} = 0.01196 ± 0.012, and TAC = 114.059 ± 0.97 mg EAA/g, respectively. Additionally, EO-NS exhibited promising antibacterial activity on all strains under investigation, especially on E. coli K12 resulting in inhibition diameter of 38.67 ± 0.58 mm and a minimum inhibitory concentration...
(MIC) of 1.34 ± 0.00 μg/mL. Also, EO-NS had significant antifungal efficacy, with a percentage of inhibition of 67.45 ± 2.31% and MIC of 2.69 ± 0.00 μg/mL against *F. oxysporum*, MTCC 9913 and with a diameter of inhibition 42 ± 0.00 mm and MIC of 0.67 ± 0.00 μg/mL against *C. albicans*. To minimize development of antibiotic-resistant bacteria, EO-NS can be utilized as a natural, alternative to synthetic antibiotics and antioxidants to treat free radicals implicated in microbial infection-related inflammatory reactions.

1. Introduction

Excessive generation of free radicals damages biological components directly by oxidation of DNA, proteins, lipids, and carbohydrates, as well as causing secondary damage, due to cytotoxic and mutagenic effects of metabolites released [1]. Due to the diversity and severity of medical problems caused by oxidative stress [2], and the fact that use of synthetic antioxidants is no longer recommended because of their carcinogenic potential [3], in order to minimize oxidative stress and its associated pathologies, new antioxidants have been sought [4]. In particular, natural products of plants are regarded as having potential as antioxidant compounds for protection of cells against damage caused by free radicals [5, 6].

Antimicrobial resistance (AMR) is a major and ongoing global challenge and a threat to public health. It is estimated that by 2050, AMR will be responsible for ten million deaths with a total cost of 100 trillion dollars [7]. Faced with this problem, alternative therapeutic strategies, based on natural resources, particularly medicinal plants, have been the subject of extensive research to develop new antibiotics or new therapeutic modalities and to seek alternatives to currently used antibiotics and develop alternative molecules effective against infectious diseases [8–10]. Aromatic and medicinal plants are an important source of bioactive compounds, such as essential oils (EOs) that could be applied as therapies for infectious diseases [11–14]. EOs have been shown to be valuable as a nontraditional sources of natural, bioactive antioxidants and antimicrobials to combat antibiotic-resistant bacteria and harmful reactive oxygen species (ROS) and are involved in inflammatory immune responses associated with infection. EO derived from seeds of black caraway *Nigella sativa*, a flowering plant in the family Ranunculaceae (EO-NS), was recently studied for biological activities [15, 16]. *N. sativa* is also often referred to as black cumin, nigella, or kalonji. The aim of the current study was to examine the chemical composition of EO-NS as well as their antioxidant and antimicrobial potential against antibiotic-resistant pathogenic and phytopathogenic microorganisms.

2. Materials and Methods

2.1. Plant Material. Seeds of the black caraway (*N. sativa*) were collected in the Souk El Arbaa area of Morocco (34°39′57″N 5°58′54″W).

2.2. Extraction and Identification of Constituents of EO-NS. The EO of the crushed seeds was extracted through hydrodistillation for a period of 4 h at 100 °C by use of Clevenger apparatus (Haborne, 1984) [17]. The chemical profiling of EO-NS was conducted by GC coupled to spectrometer. Varian capillary was employed (Model: TR5- CPSIL-5CB) with length, diameter, and film thickness of 50 m, 0.32 mm, and 1.25 μm, respectively. Temperature programming of the column was in the range of 45-290 °C increasing with a steady rate of 4 °C/min. While the injector had a fixed temperature of 280 °C, temperature of the detector (MS-PolarisQ) was 200 °C. The flow rate of helium (carrier gas) was set to 1 mL/min. The injection volume for EO was 1 μL, after having been diluted in organic solvent (hexane) according to the technique of splitless injection. In electronic ionization mode, the ionization energy was 70 eV. The ion source and interface temperatures were 200 °C and 350 °C, respectively. The range employed for scanning mass was 30-650 m/z. Identification of EO-NS phytoconstituents was conducted by the comparison of their Kovats index values, which were calculated compared to the retention times of a group of linear alkanes (C4-C29), with the values of those standard references collected by Adams library and NIST-MS V2.0 search.

2.3. In Vitro Antioxidant Activity of EO-NS

2.3.1. DPPH Assay. Antioxidant effects of EO-NS were evaluated by use of previously published methods [18]. Briefly, 800 μL of DPPH (0.2 mM, in methanol) was added to 200 μL of various serial dilutions of EO-NS ranging from 0 (in the control) to 1 mg/mL. The obtained mixture was then kept in the dark for 30 min at room temperature (RT). Absorbances were measured at 517 nm against a control consisting of 800 μL of DPPH and methanol solution. Positive controls of quercetin or BHT and blank control were prepared under the same conditions. Antioxidant activity was expressed as percent of inhibition (PI) of the absorbance at 517 nm.

\[
PI(\%) = \left(1 - \frac{\text{Sample absorbance}}{\text{Control absorbance}}\right) \times 100. \tag{1}
\]

The IC_{50} is the concentration of either EO-NS or ascorbic acid, necessary to reduce free radicals in the reaction medium by 50%. The abscissa represents the concentration values of the tested compound and the ordinate represents the percentage inhibition, with IC_{50} values obtained by linear regression and interpolation (PI%).

2.3.2. Total Antioxidant Capacity (TAC). The total antioxidant capacity (TAC) of EO-NS was measured by use of the phosphomolybdenum method. Briefly, 100 μl of various concentrations of EO-NS was added to 1000 μL of H₂SO₄, Na,PO₄, and ammonium molybdate reagent mixture such that their concentration was in the range of 0.6 M, 28 mM, and 4 mM, respectively. The tubes were placed at a...
temperature of about 95°C for 90 minutes. After cooling, the absorbance was read at 695 nm. The control consisted of 100 μL of methanol mixed with 1000 μL of reagent mixture [19]. Samples and controls are incubated under identical conditions. The results obtained are represented as mg of ascorbic acid equivalents per gram (mg EAA/g).

2.3.3. Reduced Ferric Assay (FRAP). The ferric reduction process relies on antioxidants to reduce ferric iron to iron salt, which results in formation of a blue solution. Briefly, 200 μL of different concentrations of EO-NS and 500 μL of 0.2 M phosphate buffer (pH 6.6) were added to glass tubes, followed by 500 μL of 1% potassium hexacyanoferrate (K₃Fe(CN)₆) in distilled water. The mixture was heated to 50°C for 20 minutes in a water bath. A 500 μL aliquot of trichloroacetic acid (10%) was pipetted, and the solution was subjected to centrifugation at 3000 rpm for 10 min. A 500 μL aliquot of the supernatant was transferred to another tube to which 500 μL of double-distilled water (ddH₂O) and 100 μL of 1% FeCl₃ freshly prepared, in ddH₂O were added. A blank without an EO sample was also prepared similarly by replacing the EO-NS with methanol. The absorbance was read at 594 nm with reference to the blank, replacing the EO-NS with methanol, which allows calibrating the apparatus (UV-VIS spectrophotometer). Solution of standard antioxidants, either BHT or quercetin, whose absorbances were read in a similar fashion as with the samples, served as positive controls [20].

2.4. In Vitro Antimicrobial Activity of EO-NS

2.4.1. Microbial Strains. The antimicrobial capacity of EO-NS was assessed against four clinically important fungal strains (Candida albicans, ATCC 10231; Aspergillus niger, MTCC 282; Aspergillus flavus, MTCC 9606; and Fusarium oxysporum, MTCC 9913) and four bacterial strain (Staphylococcus aureus, ATCC 6633; Escherichia coli, K12; Bacillus subtilis, DSM 6333; and Proteus mirabilis, ATCC 29906), which were obtained by Sidi Mohammed Ben Abdellah University (Fez, Morocco).

2.4.2. Method for Assessing Antimicrobial Activity. Antimicrobial activity of EO-NS was assessed by use of the disc diffusion method [21]. Briefly, Petri dishes containing Mueller-Hinton and malt extract were inoculated with the four bacterial strains and C. albicans, respectively, using the double-layer method. From fresh cultures grown in Mueller-Hinton and malt extract media, serial dilutions were established in sterilized saline solution (NaCl, 0.9%) until obtaining turbidity of 0.5 McFarland (10⁶ to10⁸ CFU/mL). Then, 100 μL was added to tubes containing 5 mL of soft agar (0.5% agar), and the inoculated tubes were plated into Petri dishes containing Mueller-Hinton and malt extract media. Whatman paper discs No. 4, with a diameter of 6 mm, were impregnated with 20 μL of EO-NS. For the fungal strains A. niger, A. flavus, and F. oxysporum, the antifungal potency was determined by use of the direct confrontation assay in the malt extract medium between EO-NS and

| P | R.T | Name | C.C | Cal | RI | Lit | Area (%) |
|---|-----|------|-----|-----|----|-----|----------|
| 1 | 7.68 | α-Thujene | MO | 902 | 930 | 10.09 |
| 2 | 7.90 | α-Pinene | MO | 948 | 939 | 2.57 |
| 3 | 9.03 | α-Phellandrene | MO | 994 | 1002 | 0.97 |
| 4 | 9.19 | β-Pinene | MO | 972 | 979 | 2.33 |
| 5 | 10.35 | α-Terpinene | MO | 998 | 1017 | 0.95 |
| 6 | 10.59 | O-cymene | MO | 1042 | 1026 | 46.36 |
| 7 | 10.72 | Cis-chrysanthenyl acetate | O | 1256 | 1265 | 2.56 |
| 8 | 11.60 | Limonene | MO | 998 | 1029 | 0.90 |
| 9 | 12.74 | Cis-sabinene hydrate | O | 1040 | 1070 | 0.72 |
| 10 | 12.81 | Linalool | MO | 1082 | 1096 | 0.54 |
| 11 | 13.43 | Trans-sabinene hydrate | O | 1070 | 1098 | 8.71 |
| 12 | 15.26 | Terpinen-4-ol | MO | 1148 | 1177 | 5.98 |
| 13 | 18.56 | Carvacrol | MO | 1274 | 1299 | 14.82 |
| 14 | 21.66 | Longifolene | ST | 1398 | 1390 | 1.95 |
| 15 | 38.23 | Widdrol | ST | 1604 | 1599 | 0.55 |

Chemical classes (C.C)
Monoterpane (MO) 85.51
Sesquiterpane (ST) 2.50
Others (O) 11.99
Total 100
the fungal strains tested. Briefly, Whatman paper discs No. 4 with a diameter of 6 mm were soaked with 20 μL of EO-NS, and an agar plate of the fungal strain was positioned 1 cm from the disc containing EO-NS. To assess the efficacy of EO-NS negative controls and positive controls containing conventional antimicrobial drugs, streptomycin and oxacillin for bacterial strains and fluconazole for fungal strains were performed in the same way as the tests. Petri dishes, which had been inoculated with the strain, were placed in an incubator at 30°C or 37°C for fungi or bacteria and C. albicans, respectively. Diameters and percentages of inhibition were measured after 24 h, bacteria; 48 h, C. albicans; and 7 days, A. niger, A. flavus, and F. oxysporum [22, 23].

2.4.3. Minimum Inhibitory Concentration. Minimum inhibitory concentration (MIC) of EO-NS against each of the four strains of bacteria and fungi was determined by use of previously described methods for microdilution [23]. Briefly, sterile 96-well microplates were premarked, under aseptic conditions; then, 100 μL of EO-NS prepared in DMSO (10%, v/v) was added to the first row of the plate. The following volumes were subsequently pipetted into all remaining wells, 50 μL sterile Mueller-Hinton and 50 μL sterile malt extract for bacterial and fungal strains, respectively. Multichannel pipette was utilized to make serial dilutions. Finally, 30 μL of bacterial or fungal suspensions of each strain was pipetted into each well. Following a 24 h of incubation for bacteria, 48 h for C. albicans, and 7 days for A. niger, A. flavus, and F. oxysporum at 37°C and 30°C, respectively [21–23], the MIC end point was assessed by close observation of the growth inside the wells or via colorimetric determination (0.2% TTC, w/v) [23].

2.5. Statistical Analysis. Results were represented as means of triplicates ± standard deviation (SD). GraphPad Prism (version.8.0.1) was utilized to perform statistical analyses by use of the Shapiro-Wilk tests to verify the normality of the variables as well as Levene’s test to assess the homogeneity of variances. Statistical differences between the means were calculated by analysis of variance (One way-ANOVA)
and Tukey’s test for multiple comparison. Significance of differences was considered at a probability cut-off level of \( p \leq 0.05 \).

3. Results and Discussion

3.1. Extraction of EO-NS. The yield of EO achieved by the hydrodistillation, expressed on mass of seed, was about \( 0.8 \pm 0.02\% \), with characteristic transparent yellow color with aromatic odor. This yield was similar to that of \( 0.832 \pm 0.025\% \) found previously for EO-NS from Beni Mellal, Morocco [24]. Several studies have the EO-NS content of \( N. \) sativa seeds [25] and revealed that EO-NS extracted by hydrodistillation was about \( 0.08\% \), while EO-NS extracted by microwave distillation was approximately \( 0.11\% \). Yields of EO-NS of seeds of \( N. \) sativa from five different countries, namely, Saudi Arabia, Syria, Morocco, India, and France, were achieved by hydrodistillation ranging from \( 0.047\% \) to \( 1.7\% \) [26, 27]. Variability among yields of EO-NS can be ascribed for slight differences in extraction procedures as well as other factors, such as geographic origin, ecological factors, agronomic practices, and storage conditions [27–29].

3.2. GC-MS/MS Studies. Based on GC-MS/MS analysis of EO-NS extracted from seeds of Moroccan origin, the following compounds and respective proportions of the total mass of EO-NS were determined. Among the 15 compounds
identified, 10 were basic monoterpenoids, which accounted for 85.51% of total masses of constituents. These are mainly terpenoid hydrocarbons, including α-pinen, β-pinene, sandine, γ-terpinene, α-terpinene, and O-cymene. Terpenoid alcohols represented 6.52% of total constituents EO-NS, with terpinene-4-ol and linalool comprising 5.98% and 0.54%, respectively. Terpenoid phenols, including carvacrol comprised 14.82%. Other components of EO-NS were chrysanthenyl acetate, trans and cis-sabinene hydrate, two sesquiterpenoids, which represented 2.50% of total mass of EO-NS, including longifolene and widdrol that accounted for 0.55% and 1.95%, respectively. Monoterpenes were dominant, with O-cymene being the major component in EO-NS of Moroccan origin. The chemical components of EO-NS and structures of main constituents of EO-NS are presented (Table 1 and Figure 1) and GC-MS/MS chromatograms (Figure 2).

3.3. Antioxidant Activity of EO-NS

3.3.1. DPPH Assay. EO-NS exhibited significant antioxidant activity against the DPPH free radical, which was used to evaluate its antiradical efficacy (Figures 3 and 4), exhibiting an IC₅₀ value of 0.017 ± 0.001 mg/mL, compared to BHT or quercetin which exhibited IC₅₀ values of 0.0118 ± 0.007 and 0.035 ± 0.004 mg/mL, respectively. In comparison, the IC₅₀ values of the EO-NS studied showed a greater

![Figure 6: In vitro antibacterial activity of essential oil extracted from seeds of black caraway Nigella sativa (EO-NS).](image1)

![Figure 7: Antibacterial potency of essential oil extracted from seeds of black caraway Nigella sativa (EO-NS). Means (± SD, n = 3) with the same letter denote no evident significant differences based on Tukey’s multiple range tests p ≤ 0.05.](image2)
Generally, EOs with greater terpene content possess power-
ful antioxidant potential [41]. Indeed, EO-NS seem to be
attributed to the monoterpene compounds in essential oils [41].

The antioxidant activity is probably attrib-
uted to the TAC assay, EO-NS exhibited antioxidant activity equiv-
alent to that of BHT with EC50 of 6333 μg/mL. Di-

terpenes, and hydrocarbons (α-caryophyllene, α-copaene, α-
dihydrocaryophyllene, α-farnesene, 1,8-cineole, and α-

dihydrocarveol), can operate synergistically with EOs, such as alcohols (linalool),
ethers, and hydrocarbons (α-terpinene and γ-terpinene), can contribute to their antioxidant potency [37–39].

3.3.3. Total Antioxidant Capacity (TAC). Based on results of the TAC assay, EO-NS exhibited antioxidant activity equivalent to 114.059 ± 0.972 μg EAA/mg EO-NS (Figure 5). This result can be attributed to the presence of active antioxidant substances [40]. The antioxidant activity is probably attributed to the monoterpene compounds in essential oils [41]. Generally, EOs with greater terpene content possess powerful antioxidant potential [42]. Indeed, EO-NS seem to be effective antioxidant [31]. However, minor compounds are more likely than the major compounds to provide a pivotal role in the observed antioxidant potency [43]. It is well doc-
umented that synergies between various chemicals must be

3.4. Antibacterial Activity of EO-NS. The N. sativa EO-NS exhibited measurably antibacterial efficacy against all bacte-
rial strains tested (Figures 6 and 7) (Table 2) and showed
promising antibacterial activity compared to two commer-
cially available antibiotics (streptomycin and oxacillin),
especially against E. coli K12 with a diameter of inhibition of
30.77 ± 0.58% c 21 mm against B. subtilis (ATCC 10231)
and a MIC of 1.34 ± 0.00 μg/mL. Differences in inhibition diameters obtained could be due to dif-
ferences in chemical compositions of the EO, and the
antibacterial activity could be due mainly to the majority
compound (O-cymene) or to a combination of less predom-
inant compounds found in EO-NS. Results of several studies have indicated that EOs from N. sativa and their single com-
ponents are effective against infections caused by bacteria.
Results of the current study reported here are in agreement
with those of Harzallah et al. [46], which demonstrated that
EO-NS from N. sativa collected in Tunisia and its bioactive
compound, thymoquinine, had significant antibacterial
activity. Other studies have found that the seed extract
derived from N. sativa has strong antibacterial potency
against B. subtilis, IMG 22 with an inhibition diameter of
27 mm, against E. coli DM with an inhibition diameter of
19.3 mm [47]. Another study found that N. sativa extract has antibacterial activity against S. aureus (ATCC 103207)
with an inhibition diameter of 19 mm and against B. subtilis
(ATCC 27853) with an inhibition diameter of 26 mm and against E. coli (ATCC 12079), with an inhibition diameter of
21 mm [48]. The results presented here demonstrate
greater than that or the previous studies [49], which found
that ethyl alcohol extract of N. sativa exhibited antibacterial

### Table 2: Minimum inhibition concentration (MIC) of essential oil extracted from seeds of black caraway Nigella sativa (EO-NS).

|                          | Staphylococcus aureus ATCC 6633 | Escherichia coli K12 | Bacillus subtilis DSM 6333 | Proteus mirabilis ATCC 29906 |
|--------------------------|---------------------------------|----------------------|---------------------------|-----------------------------|
| EO-NS (μg/mL)            | 2.69 ± 0.00^a                    | 1.34 ± 0.00^b        | 1.34 ± 0.00^b             | 2.69 ± 0.00^a               |
| Streptomycin (μg/mL)     | 1.56 ± 0.00                      | Rs                   | Rs                        | Rs                          |

Means (± SD, n = 3) labeled with different letters in same row are considered significantly different according to one-way ANOVA and Tukey’s test, p ≤ 0.05.

### Table 3: Antifungal activity and the MIC of essential oils extracted from seeds of black caraway Nigella sativa (EO-NS).

|                          | Candida albicans ATCC 10231 | Aspergillus niger MTCC 282 | Aspergillus flavus MTCC 9606 | Fusarium oxysporum MTCC 9913 |
|--------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| EO-NS                    |                             |                             |                             |                             |
| Antifungal activity      | 42 ± 0.00 mm^a              | 0.0 ± 0.00^b                | 0.0 ± 0.00^b                | 67.45 ± 2.315%^c            |
| CMI (μg/mL)              | 0.67 ± 0.00^a               | —                           | —                           | 2.69 ± 0.00^b               |
| Fluconazole              |                             |                             |                             |                             |
| Antifungal activity      | 0.0 ± 0.00 mm^a             | 8.20 ± 2.02%^b             | 0.0 ± 0.00^b                | 30.77 ± 0.58%^c            |
| CMI (μg/mL)              | —                           | 7.125^a                     | —                           | 3.125^b                     |

Means (± SD, n = 3) labelled by different letters within the same row are considered significantly different (one-way ANOVA; Tukey’s test, p ≤ 0.05).
potency against *B. subtilis* with an inhibition diameter of 7 mm. The results of the study presented here are also more potent than that reported previously [32], which indicated that the antibacterial potency of EO-NS was greater on *S. aureus* (MTCC 9542) with an inhibition diameter of 16 mm than *Vibrio harveyi* (MTCC 7771) with an inhibition diameter of 5 mm for the 10 mg/mL concentration. EO-NS exhibits potent antibacterial activities against antibiotic-resistant bacterial strains (gram-negative and gram-positive), thereby advocating the utility of the bioactive molecules contained in EO-NS as an alternative to commercially available antibiotics to combat bacterial resistance.

The antibacterial potency of EO-NS on the bacterial strains that we have highlighted in this study is supported by literature data showing the action of EOs rich in carvacrol, whose antimicrobial efficacy is explained by the actual position of the hydroxyl group on the phenolic structure of these molecules [50–52] and which modify permeability and cause leaking of intracellular components through the specific binding to the amine and hydroxylamine groups of bacterial membrane-bound proteins [53]. Because of their cheap cost, biocompatibility, antibacterial and resistance reversal potential, lack or low toxicity to eukaryotic cells, and decreased toxicity to eukaryotic cells and the environment, these volatile compounds are termed green antimicrobials [54]; as a result, they are regarded as an efficient approach for addressing AMR in underdeveloped nations as well as in bacterial strains, including ESKAPEE members [54].

### 3.4.1. Antifungal Activity of EO-NS

Evaluation of the *in vitro* antifungal activity of EO-NS against *A. niger*, *A. flavus*, *F. oxysporum*, and *C. albicans* by the disc diffusion method has revealed promising antifungal activity with an inhibition percentage of 67.45 ± 2.31% and MIC of 2.69 ± 0.00 μg/mL against *F. oxysporum*, MTCC 9913 and with an inhibition diameter of 42 ± 0.00 mm and MIC of 0.67 ± 0.00 μg/mL against *C. albicans*, compared to the control and the antibiotic fluconazole (Table 3 and Figure 8). Furthermore, EO-NS exhibits fungicidal efficacy against both *F. oxysporum* and *C. albicans*.
and C. albicans. However, EO-NS did not exhibit significant antifungal potency against A. niger, MTCC 282 or A. flavus, MTCC 9606. The current results indicated that EO-NS has an inhibitory effect against pathogenic and phytopathogenic fungi, which might be attributed for its chemical composition, especially the presence of O-cymene, carvacrol, α-thujene, and trans-sabinene hydrate, all of which exhibit significant antifungal efficacies [55, 56]. The significant antifungal activity against C. albicans might be due to the presence of O-cymene, a conclusion that is in agreement with results of previous studies [57, 58], which reported that O-cymene component of EO-NS presents antifungal potency on C. albicans and other pathogenic fungal strains. The results reported here are different from those of Khosravi et al. [59], which showed that EO-NS exhibits antifungal activity against A. flavus. For cytotoxicity of EO-NS, the study of Mahmoud et al. [60] demonstrates that EO-NS did not show significant cytotoxicity on macrophages, and results of the current study indicate promising antifungal activity of EO-NS without causing cytotoxicity, which suggests its usage as a good alternative to substitute commercially available antifungals to combat fungal resistances.

4. Conclusion

The results of the present study show that EO-NS was effective as antioxidants, antibacterial, and antifungal that could be used as an alternative to currently available synthetic molecules. Notably, GC/MS-MS analysis of EO-NS revealed the richness of these oils in potentially bioactive compounds with a dominance of O-cymene and carvacrol. The antioxidant activity of EO-NS was confirmed by three tests (DPPH, FRAP, and TAC) even at low concentrations. EO-NS acts as a promising antibacterial agent on almost all the studied strains, with a more pronouncing effect on E. coli (K12). Antifungal activity indicated that EO-NS had broad spectrum of action against almost all the studied strains. In further studies, the focus will be on testing purified compounds of EO-NS along with investigating their mode of action. Prior to any prospective application of EO-NS as a natural agent to control microorganisms, we expect to assess the possible adverse consequences on nontarget creatures, as well as clinical trials on both humans and nonhuman primates.

Data Availability

Data used to support the findings are included within the article.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Acknowledgments

The authors extend their appreciation to the Researchers Supporting Project number (RSP-2022R437) King Saud University, Riyadh, Saudi Arabia. Giesy was supported by a Discovery Grant from the Natural Science and Engineering Research Council of Canada, the Canada Research Chair Program, and a Distinguished Visiting Professorship in the Department of Environmental Sciences, Baylor University, Waco, TX, USA.

References

[1] A. Favier, Le stress oxydant Intérêt conceptuel et expérimental dans la compréhension des mécanismes des maladies et potentiel thérapeutique.
[2] A. Favier, “Oxidative stress in human diseases,” Annales Pharmaceutiques Françaises, vol. 64, no. 6, pp. 390–396, 2006.
[3] C. Kaur and H. C. Kapoor, Review antioxidants in fruits and vegetables ± the millennium’s health.
[4] J. P. Pokorny, Preparation of natural antioxidants.
[5] J. K. Willcox, S. L. Ash, and G. L. Catignani, “Antioxidants and prevention of chronic disease,” Critical Reviews in Food Science and Nutrition, vol. 44, no. 4, pp. 275–295, 2004.
[6] I. Kivrak, M. E. Duru, M. Öztürk, N. Mercan, M. Harmandar, and G. Topçu, “Antioxidant, anticholinesterase and antimicrobial constituents from the essential oil and ethanol extract of Salvia potentillifolia,” Food Chemistry, vol. 116, no. 2, pp. 470–479, 2009.
[7] N. Pas, Etat des lieux de la recherche, pp. 1–27, 2011.
[8] J. Njoroge and V. Sperandio, “Jamming bacterial communication: new approaches for the treatment of infectious diseases,” EMBO Molecular Medicine, vol. 1, no. 4, pp. 201–210, 2009.
[9] M. Der Torossian Torres and C. De La Fuente-Nunez, “Reprogramming biological peptides to combat infectious diseases,” Chemical Communications, vol. 55, no. 100, pp. 15020–15022, 2019.
[10] S. J. Jeon, M. Oh, W. S. Yeo, K. N. Galvão, and K. C. Jeong, “Underlying mechanism of antimicrobial activity of chitosan microparticles and implications for the treatment of infectious diseases,” PLoS One, vol. 9, no. 3, article e92723, 2014.
[11] “Chemical composition and in vitro antimicrobial and mutagenic activities of seven lamiaceae essential oils,” Molecules, vol. 14, no. 10, pp. 4213–4230, 2009.
[12] M. H. Jang, X. L. Piao, J. M. Kim, S. W. Kwon, and J. H. Park, “Inhibition of cholinesterase and amyloid-β aggregation by resveratrol oligomers from Vitis amurensis,” Phytotherapy Research, vol. 22, no. 4, pp. 544–549, 2008.
[13] A. Bouyahya, “Determination of phenol content and antibacterial activity of five medicinal plants ethanolic extracts from north-west of Morocco,” Journal of Plant Pathology & Microbiology, vol. 7, no. 4, 2016.
[14] A. El-Touyy, A. Bouyahya, H. Fellah et al., “Antileishmanial activity of medicinal plants from Africa: a review,” Asian Pacific Journal of Tropical Disease, vol. 7, no. 12, pp. 826–840, 2017.
[15] M. Burits and F. Bucar, Antioxidant activity of Nigella sativa essential oil.
[16] S. Cheikh-Rouhou, S. Besbes, B. Bentati, C. Blecker, C. Deroanne, and H. Attia, “Nigella sativa L.: chemical composition and physicochemical characteristics of lipid fraction,” Food Chemistry, vol. 101, no. 2, pp. 673–681, 2007.
[17] J. B. Harborne, “Methods of Plant Analysis,” Phytochemical Methods, vol. 1973, pp. 1–32, 1973.
[18] Y. El Atki, I. Aouam, F. El Kamari et al., “Phytochemistry, antioxidant and antibacterial activities of two Moroccan Teucrium polium L. subspecies: preventive approach against nosocomial
infections,” *Arabian Journal of Chemistry*, vol. 13, no. 2, pp. 3866–3874, 2020.

[19] M. Petkovč, J. Schiller, M. Müller et al., "Detection of individual phospholipids in lipid mixtures by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry: phosphatidycholine is the predominant compound in further species," *Analytical Biochemistry*, vol. 289, no. 2, pp. 202–216, 2001.

[20] D. Cando, D. Morcuende, M. Utrera, and M. Estévez, “Phenolic-rich extracts from Willowherb (*Epilobium hirsutum* L.) inhibit lipid oxidation but accelerate protein carbonylation and discoloration of beef patties,” *European Food Research and Technology*, vol. 238, no. 5, pp. 741–751, 2014.

[21] A. El Barnossi, F. Moussaid, and A. I. Housseini, “Antifungal activity of Bacillussp. Gm-A11-18isolated from decomposing solid green household waste in water and soil against Candida albicans and Aspergillus Niger,” *E3S Web of Conferences*, vol. 150, article 02003, 2020.

[22] K. Chebbac, H. K. Ghneim, A. el Moussaoui et al., “Antioxidant and antimicrobial activities of chemically-characterized essential oil from *Artemisia aragonensis* lam. Against drug-resistant microbes,” *Molecules*, vol. 27, no. 3, p. 113, 2022.

[23] S. D. Sarker, L. Nahar, and Y. Kumarasamy, "Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals," *Methods*, vol. 42, no. 4, pp. 321–324, 2007.

[24] T. Ainane, Z. Askaoui, M. Elkouali et al., "Chemical composition and antibacterial activity of essential oil of *Nigella sativa* seeds from Beni Mellal (Morocco): what is the most important part, essential oil or the rest of seeds?," *Journal of Materials and Environmental Science*, vol. 5, pp. 2017–2020, 2014.

[25] M. Barzalona and J. Casanova, "Chemical variability of the leaf oil of 113 hybrids from," *pp. 152–163*, 2008.

[26] M. Dalli, S. E. Azizi, H. Benouda et al., "Molecular composition and antibacterial effect of five essential oils extracted from *Nigella sativa* L. seeds against multidrug-resistant bacteria: a comparative study," *Evidence-Based Complementary and Alternative Medicine*, vol. 2021, Article ID 6643765, 9 pages, 2021.

[27] I. Hamrouni-sellami, M. E. Kchouk, and B. Marzouk, *Lipid and aroma composition of black cumin*, vol. 32, pp. 335–352, 2007.

[28] M. B. Atta, “Some characteristics of nigella (*Nigella sativa* L.) seed cultivated in Egypt and its lipid profile,” *Food Chemistry*, vol. 83, no. 1, pp. 63–68, 2003.

[29] L. Afaf, *Activités antioxydante et anticoagulante des huiles essentielles des graines de* 2011.

[30] A. S. Abedi, M. Rismanchi, M. Shahdoostkhany, A. Mohammad, and A. M. Mortazavian, "Microwave-assisted extraction of *Nigella sativa* L. essential oil and evaluation of its antioxidant activity," *Journal of Food Science and Technology*, vol. 54, no. 12, pp. 3779–3790, 2017.

[31] M. Kazemi, "Phytochemical composition, antioxidant, anti-inflammatory and antimicrobial activity of *Nigella sativa* essential oil," *Journal of Essential Oil Bearing Plants*, vol. 17, no. 5, pp. 1002–1011, 2014.

[32] M. Ş. Karaçil Ermumucu and N. Şanlıer, "Black cumin (*Nigella sativa*) and its active component of thymoquinone: effects on health," *Journal of Food and Health Science*, vol. 3, pp. 170–183, 2017.

[33] V. Hajhashemi, A. Ghannadi, and H. Jafarabadi, "Black cumin seed essential oil, as a potent analgesic and antiinflammatory drug," *Phytotherapy Research*, vol. 18, no. 3, pp. 195–199, 2004.

[34] G. Singh, P. Marimuthu, C. S. De Heluani, and C. Catalan, "Chemical constituents and antimicrobial and antioxidant potentials of essential oil and acetone extract of *Nigella sativa* seeds," *Journal of the Science of Food and Agriculture*, vol. 85, no. 13, pp. 2297–2306, 2005.

[35] M. B. Gholivand, M. Rahimi-Nasrabad, H. Batooli, and A. H. Ebrahimabadi, "Chemical composition and antioxidant activities of the essential oil and methanol extracts of *Pammogeton canescens*," *Food and Chemical Toxicology*, vol. 48, no. 1, pp. 24–28, 2010.

[36] M. Hazzit, A. Baaliouamer, A. R. Verissimo, M. L. Faleiro, and M. G. Miguel, "Chemical composition and biological activities of Algerian *Thymus* oils," *Food Chemistry*, vol. 116, no. 3, pp. 714–721, 2009.

[37] E. I. Blejan, D. E. Popa, T. Costea et al., “The in vitro antimicrobial activity of some essential oils from aromatic plants,” *Farmácia*, vol. 69, no. 2, pp. 290–298, 2021.

[38] K. P. Anthony, S. A. Deolu-Sobogun, and M. A. Saleh, "Comprehensive assessment of antioxidant activity of essential oils," *Journal of Food Science*, vol. 77, no. 8, pp. C839–C843, 2012.

[39] V. Lagouri, G. Blekas, M. Tsimidou, S. Kokkini, and D. Boskou, "Composition and antioxidant activity of essential oils from oregano plants grown wild in Greece," *Zeitschrift für Lebensmittel-Untersuchung und -Forschung*, vol. 197, no. 1, pp. 20–23, 1993.

[40] A. Durazzo, "Study approach of antioxidant properties in foods: update and considerations," *Food*, vol. 6, no. 3, pp. 1–7, 2017.

[41] S. Bouhdid, S. N. Skali, M. Idaomar et al., "Antibacterial and antioxidant activities of *Origanum compactum* essential oil," *African Journal of Biotechnology*, vol. 7, pp. 1563–1570, 2008.

[42] K. Svoboda and J. Hampson, "Bioactivity of essential oils of selected temperate aromatic plants: antibacterial, antioxidant, antiinflammatory and other related pharmacological activities," *Spec. Chem. 21st*, pp. 1–17, 1999.

[43] M. F. N. N. Carvalho, S. Leite, J. P. Costa, A. M. Galvão, and J. H. Leitão, "Ag(I) camphor complexes: antimicrobial activity by design," *Journal of Inorganic Biochemistry*, vol. 199, p. 110791, 2019.

[44] G. Guha, V. Rajkumar, R. A. Kumar, and L. Mathew, "Antioxidant activity of Lawsonia inermis extracts inhibits chromium(Ⅵ)-induced cellular and DNA toxicity," *Evidence-Based Complementary and Alternative Medicine*, vol. 2011, Article ID 576456, 9 pages, 2011.

[45] N. Turkmen, Y. S. Veliglu, F. Sari, and G. Polat, "Effect of extraction conditions on measured total polyphenol contents and antioxidant and antibacterial activities of black tea," *Molecules*, vol. 12, no. 3, pp. 484–496, 2007.

[46] H. Jrah Harzallah, B. Koudhi, G. Flaminì, A. Bakhrouf, and T. Mahjoub, "Chemical composition, antimicrobial potential against cariogenic bacteria and cytotoxic activity of Tunisian *Nigella sativa* essential oil and thymoquinone," *Food Chemistry*, vol. 129, no. 4, pp. 1469–1474, 2011.

[47] M. Arici, O. Sagdic, and U. Geçgel, "Antibacterial effect of Turkish black cumin (*Nigella sativa L.*) oils," *Grasas y Aceites*, vol. 56, no. 4, pp. 259–262, 2005.

[48] M. M. Alam, M. Yasin, J. Nessa, and C. R. Ahsan, "Antibacterial activity of chloroform and ethanol extracts of black cumin seeds (*Nigella sativa*) against multi-drug resistant
human pathogens under laboratory conditions," *The Journal of Medicinal Plants Research*, vol. 4, pp. 1901–1905, 2010.

[49] A. R. Khan, "Wide spectrum antibacterial activity of Nigella sativa L. seeds," *IOSR Journal of Pharmacy (IOSRPFR)*, vol. 6, no. 7, pp. 12–16, 2016.

[50] J. U. S. Erkedjieva, D. I. D. Aferera, M. E. G. Ulluce et al., "In vitro antioxidant, antimicrobial, and antiviral activities of the essential oil and various extracts from herbal parts and callus cultures of Origanum acutidens," 2004.

[51] F. Senatore, F. Napolitano, N. A. Arnold, M. Bruno, and W. Herz, "Composition and antimicrobial activity of the essential oil of Achillea falcata L. (Asteraceae)," *Flavour and Fragrance Journal*, vol. 20, no. 3, pp. 291–294, 2005.

[52] M. B. Pilotto, A. Ludwig, S. H. Alves, R. A. Zanette, and J. M. Santurio, "In vitro activity of carvacrol and thymol combined with antifungals or antibacterials against Pythium insidiosum," *Journal de Mycologie Médicale*, vol. 25, no. 2, pp. e89–e93, 2015.

[53] Q. Zhang, K. Fan, P. Wang et al., "Carvacrol induces the apoptosis of pulmonary artery smooth muscle cells under hypoxia," *European Journal of Pharmacology*, vol. 770, pp. 134–146, 2016.

[54] M. Lahou, "Methods to study the phytochemistry and bioactivity of essential oils," vol. 448, pp. 435–448, 2004.

[55] V. Mmbengwa, A. Samie, M. Gundidza, V. Matikiti, N. J. Ramalivhana, and M. L. Magwa, "Biological activity and phytoconstituents of essential oil from fresh leaves of Eriosema englerianum," *African Journal of Biotechnology*, vol. 8, pp. 361–364, 2009.

[56] H. Saghrouchni, A. El Barnossi, H. Chefaou et al., "Study the effect of carvacrol, eugenol and thymol on Fusariums sp responsible for Lolium perenne fusariosis," *Ecology, Environment and Conservation*, vol. 26, pp. 1059–1067, 2020.

[57] A. Arasu, V. Pingley, N. Prabha et al., "Impact and fungitoxic spectrum of Trachyspermum ammi against Candida albicans, an opportunistic pathogenic fungus commonly found in human gut that causes Candidiasis infection," *Journal of Infection and Public Health*, vol. 14, no. 12, pp. 1854–1863, 2021.

[58] W. Luo, Z. Du, Y. Zheng et al., "Phytochemical composition and bioactivities of essential oils from six Lamiaceae species," *Industrial Crops and Products*, vol. 133, pp. 357–364, 2019.

[59] A. Khosravi, M. Minooeianhaghighi, H. Shokri, S. Emami, S. Alavi, and J. Asili, "The potential inhibitory effect of Cuminum cyminum, Ziziphora clinopodioides and Nigella sativa essential oils on the growth of Aspergillus fumigatus and Aspergillus flavus," *Brazilian Journal of Microbiology*, vol. 42, no. 1, pp. 216–224, 2011.

[60] H. Mahmoudvand, A. Sepahvand, S. Jahanbakhsh, B. Ezatpour, and S. A. Ayatollahi Mousavi, "Evaluation of antifungal activities of the essential oil and various extracts of Nigella sativa and its main component, thymoquinone against pathogenic dermatophyte strains," *Journal de Mycologie Médicale*, vol. 24, no. 4, pp. e155–e161, 2014.