

Article

Antioxidant and Antibacterial Activity of *Nepeta × faassenii* Bergmans ex Stearn Essential Oil

Călin Jianu ^{1,*}, Radu Moleriu ², Daniela Stoin ¹, Ileana Cocan ¹, Gabriel Bujancă ^{1,*}, Georgeta Pop ³, Alexandra Teodora Lukinich-Gruia ⁴, Delia Muntean ^{5,6}, Laura-Cristina Rusu ^{7,8} and Delia Ioana Horhat ⁵

- ¹ Faculty of Food Engineering, Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, Calea Aradului 119, RO-300645 Timisoara, Romania; danielastoin@usab-tm.ro (D.S.); ileanacocan@usab-tm.ro (I.C.)
 - ² Faculty of Mathematics and Computer Science, West University of Timisoara, Str. J. H. Pestalozzi 16, RO-300115 Timisoara, Romania; radu.moleriu@e-uvv.ro
 - ³ Faculty of Agriculture, Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, Calea Aradului 119, RO-300645 Timisoara, Romania; georgeta_pop@usab-tm.ro
 - ⁴ OncoGen Centre, County Hospital "Pius Branzu", Blvd. Liviu Rebreanu 156, RO-300736 Timisoara, Romania; gruia_alexandra@yahoo.com
 - ⁵ Faculty of Medicine, "Victor Babes" University of Medicine and Pharmacy, 2nd Eftimie Murgu Square, RO-300041 Timisoara, Romania; muntean.delia@umft.ro (D.M.); horhat.ioana@umft.ro (D.I.H.)
 - ⁶ Multidisciplinary Research Center on Antimicrobial Resistance, "Victor Babes" University of Medicine and Pharmacy, 2nd Eftimie Murgu Square, RO-300041 Timisoara, Romania
 - ⁷ Faculty of Dental Medicine, "Victor Babes" University of Medicine and Pharmacy, 2nd Eftimie Murgu Square, RO-300041 Timisoara, Romania; laura.rusu@umft.ro
 - ⁸ Multidisciplinary Center for Research, Evaluation, Diagnosis and Therapies in Oral Medicine, "Victor Babes" University of Medicine and Pharmacy, Spl. Tudor Vladimirescu 14A, RO-300173 Timisoara, Romania
- * Correspondence: calin.jianu@gmail.com (C.J.); gabrielbujanca@yahoo.com (G.B.)



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Abstract: This study was designed to evaluate, for the first time, the antioxidant and antimicrobial activities of *Nepeta × faassenii* essential oil (NEO). Twenty-six compounds were identified by gas chromatography and mass spectrometry analysis, of which 4α,7α,7aα-nepetalactone (34.12%), elemol (23.23%), spiro(5,6)dodecane (13.73%), and 3,4α-dihydro-4α,7α,7aα-nepetalactone (7.93%) were the major compounds. The NEO exhibited broad-spectrum antibacterial effects, and possesses potent antifungal activity on *Candida albicans* and *Candida parapsilosis*. NEO's antioxidant activity was evaluated against cold-pressed sunflowers oil by peroxide, thiobarbituric acid, 1,1-diphenyl-2-picrylhydrazyl radical, and β-carotene/linoleic acid bleaching methods. The NEO showed strong scavenging (IC₅₀: 0.032 ± 0.005 mg/mL) and relative antioxidant activity (RAA%: 92.31 ± 0.17%) in 1,1-diphenyl-2-picrylhydrazyl radical and β-carotene/linoleic acid bleaching assays, respectively. Moreover, during 24 days of the incubation period, the oil inhibits the primary lipid oxidation significantly better ($p < 0.05$) than butylated hydroxyanisole (BHA). In the case of secondary lipid oxidation, the oil performed significantly better ($p < 0.001$) than BHA from day 8 to day 12 of the incubation period. The biological activities recorded suggest that NEO may represent an antioxidant and antimicrobial agent with applications in medicine or the food industry.

Keywords: *Nepeta × faassenii* Bergmans ex Stearn; essential oil; nepetalactone; elemol; antimicrobial activity; antioxidant activity

1. Introduction

Food spoilage results when microbiological, chemical, or physical changes occur, rendering the food product unacceptable to the consumer [1]. Moreover, food can be contaminated with foodborne pathogens, which pose a great concern to public health [2]. According to the European Food Safety Authority (EFSA), in 2018, a total of 5146 foodborne outbreaks were reported in the European Union (EU), affecting 48,365 EU citizens [3]. *Campylobacter*, *Salmonella* sp., and *Escherichia coli* were the primary triggers of these food outbreaks.

Chemical food spoilage, such as lipid oxidation of foods, increases the formation of unhealthy compounds, which adversely affects the sensory properties of foods and contributes significantly to decreased shelf life and food waste. Multiple studies have also shown that oxidized lipids in the diet could affect major diseases (e.g., heart disease and cancer) of food consumers [4,5].

Various techniques, such as vacuum packaging, modified atmosphere, refrigeration, or inactivation of enzymes catalyzing oxidation, have been developed to control the rate of lipid oxidation in foods. Still, the addition of food additives remains the most effective method to increase the shelf-life of food products without adverse effects on their sensory or nutritional qualities [6]. Synthetic antioxidants such as butylated hydroxyanisole (BHA), propyl gallate (PG), octyl gallate (OG), dodecyl gallate (DG), and butylated hydroxytoluene (BHT) are currently authorized for use in controlling lipid oxidation in foodstuffs. Moreover, sorbic acid and its salts, benzoic acid, and its derivatives are widely used as food preservatives to inhibit bacterial and antifungal growth. However, in recent years, food consumers have been concerned about exposure to synthetic additives in their daily diet, with an increasing preference for natural ones. New sources of antioxidants and preservatives, such as natural extracts and essential oils (EOs), were investigated in the last decades to meet the growing demands of food consumers for cleaner label products [7,8].

The genus *Nepeta* (*Lamiaceae*) is native to Asia, Africa, Europe, and North America [9] and includes over 200 species. In Romania, the *Nepeta* genus is represented by four species: *N. cataria* L., *N. nuda* L. ssp. *nuda*, *N. parviflora* Bieb., and *N. ucranica* L. The last two are rare species located only in the Transylvanian plain (*N. ucranica* L.) and Dobrogea (*N. parviflora* Bieb.) [10]. *Nepeta* species are EO abundant taxa and are defined by the presence of nepetalactone isomers, the biochemical markers of this genus [9,11]. Various genus species have been recorded for their biological activities, including antibacterial, antifungal, antioxidative, anti-inflammatory, insecticidal, and antidepressant [9]. However, some of the genus members remain insufficiently explored, such as *Nepeta* × *faassenii* Bergmans ex Stearn, a sterile hybrid of *N. mussinii* Spreng., and *N. nepetella* L. [12]. Until now, no data regarding the antioxidant and antimicrobial properties of the *N. faassenii* essential oil (NEO) are reported.

Based on these considerations, the aim of our study was (a) to investigate the chemical composition and (b) to evaluate the antioxidant and antimicrobial activity of NEO.

2. Materials and Methods

2.1. Plant Material

N. faassenii was harvested manually, at the plant maximum flowering stage (July 2018) from the experimental fields of Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara (BUASVMT). A voucher specimen (VSNH.BUASTM—91/1) was deposited after identification in the BUASVMT Herbarium.

2.2. Isolation of the Essential Oil

The fresh plant material (flowers, leaves, and stems) of *N. faassenii* was subjected to steam distillation (yielding at 0.31% *v/w*) according to the method described by Muntean, D. et al. [13]. After separation of the NEO by decantation, the traces of water were removed with anhydrous sodium sulphate. The oil was stored in sealed dark glass vials at $-18\text{ }^{\circ}\text{C}$ until analysis.

2.3. Gas Chromatography and Mass Spectrometry (GC-MS) Analysis

The NEO composition was analyzed by the gas chromatography technique. Here, 1 μL of oil diluted 1:1000 in hexane was injected in an HP6890 GC coupled with HP5973 MS in splitless mode. The injected sample passed through a Bruker Br-5MS capillary column (30 m × 0.25 mm id × 0.25 μm), with a helium gas flow rate of 1 mL/min. The column was heated in the gas chromatograph oven at a temperature range between 50 $^{\circ}\text{C}$ and 300 $^{\circ}\text{C}$ with a rate of 6 $^{\circ}\text{C}/\text{min}$, the final hold was 5 min, and the solvent delay was 3 min. The

mass spectrometer had the source set up at 230 °C, the MS Quad at 150 °C, and ionization energy at 70 eV. The mass of compounds ranged between 50 and 550 amu. The retention indices (RIs) were calculated based upon a linear equation obtained from serial dilutions of a C₈–C₂₀ alkane standard mixture ($r^2 > 0.9973$) injected in conditions equal to the sample one. Identification of NEO components was based on comparisons of the calculated RIs with the data published in the literature [14] and their mass spectra compared with those from the NIST2.0 library (USA National Institute of Science and Technology software).

2.4. Determination of Antioxidant Activity

NEO's antioxidant activity was compared to BHA and BHT by carrying out *in vitro* tests, including peroxide, thiobarbituric acid value, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, and β -carotene bleaching assays. The NEO, BHA, and BHT were added individually at the concentration of 200 mg/L to cold-pressed sunflowers oil, acquired from the local market. An equal quantity of cold-pressed sunflowers oil without any additives was taken as a negative sample for peroxide and thiobarbituric acid value assays.

2.5. Peroxide Value (PV)

The PV measured the total peroxide and hydroperoxide oxygen content of the edible oil system [15]. This parameter was determined in triplicate on at least three separate experiments at days 0, 4, 8, 12, 16, 20, and 24 of storage, according to ISO 27107:2010 [16]. The PV was expressed as milliequivalents of peroxide per kilogram of the sample.

2.6. Thiobarbituric Acid (TBA) Value

The assay is based on the property of TBA to react with malondialdehyde (MDA) and the production of a pink fluorescent complex, whose concentration can be calculated by measuring the absorbance at 540 nm. This parameter was determined in triplicate on at least three separate experiments at days 0, 4, 8, 12, 16, 20, and 24 of storage, according to the previous investigation described by Jianu et al. [17]. The TBA was expressed as micrograms of malondialdehyde per gram of the sample.

2.7. Scavenging Effect on 1,1-diphenyl-2-picrylhydrazyl Radical (DPPH) Assay

The ability of NEO to scavenge (DPPH \cdot) was determined according to a Brand-Williams adapted method [18,19]. Briefly, stock solutions (1 mg/mL each) of NEO and the positive controls used (alpha-tocopherol, BHA, and BHT) were prepared in methanol. Serial dilutions are done to obtain concentrations ranging from 1.5 to 0.093 mg/mL. All samples were transferred in triplicate in a 96-well plate for 30 min incubation (at room temperature in the dark) before their absorbance readings at 515 nm (Tecan i-control spectrophotometer, 1.10.4.0 infinite 200Pro). Methanol was used as a negative control. Each test was performed in triplicate on at least three separate experiments. The DPPH free radical inhibition percent (I%) was calculated with the equation: $I\% = (A_{\text{methanol}} - A_{\text{sample}} / A_{\text{methanol}}) \times 100$, where A_{methanol} is the absorbance of the methanol and A_{sample} is the absorbance of the tested sample. BioDataFit 1.02 software (Chang Broscience Inc., Fremont, CA, USA) was used to determine the IC₅₀.

2.8. β -Carotene Bleaching Assay

The assay described previously by Jianu et al. [17] was adopted with slight modifications. Briefly, a stock solution was prepared from β -carotene (0.5 mg), chloroform (1 mL), linoleic acid (25 μ L), and Tween 40 (200 mg). The chloroform was evaporated under vacuum, and on the residue, distilled water saturated with oxygen (100 mL) was added and it was vigorously shaken to form an emulsion. Aliquots (2.5 mL) from this emulsion were added in the test tubes among 350 μ L of NEO methanolic solution (2 g/L concentration). The test tubes were incubated for 48 h (room temperature) together with a test tube that contained BHT (positive control) before their absorbance readings at 490 nm. Each test was performed in triplicate on at least three separate experiments.

2.9. Determination of Antimicrobial Activity

2.9.1. Bacterial Strains

Nine bacterial strains, *Staphylococcus aureus* (ATCC 25923), *Streptococcus mutans* (ATCC 35668), *Streptococcus pyogenes* (ATCC 19615), *Enterococcus faecalis* (ATCC 51299), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 700603), *Salmonella enterica* serotype Typhimurium (ATCC 14028), *Shigella flexneri* serotype 2b (ATCC 12022), and *Pseudomonas aeruginosa* (ATCC 27853), and two fungus strains, *Candida albicans* (ATCC 10231) and *Candida parapsilosis* (ATCC 22019), were used to determine the NEO antimicrobial activity.

2.9.2. Antimicrobial Screening

According to the Clinical and Laboratory Standards Institute (CLSI), the antibacterial and antifungal activity of the NEO was performed by the disk diffusion agar method [20]. All bacterial cultures were adjusted with NaCl 0.85% (bioMérieux, Marcy-l'Etoile, France) to a concentration of 0.5 McFarland. A blank paper disk (6 mm in diameter) (BioMaxima, Lublin, Poland) was placed on the surface of the Mueller–Hinton plate (Sanimed, Bucharest, Romania), which was previously inoculated with the microbial suspension, and 10 µL of NEO was added. The plates inoculated with bacteria strains were incubated for 24 h at 35–37 °C and 48 h at 28 °C for yeasts. Then, the inhibition zones were measured in millimeters using a ruler. Levofloxacin (5 µg) and fluconazole (25 µg) disks (BioMaxima, Lublin, Poland) were used as positive control. All experiments were performed in triplicate. As a negative control, a blank paper disk impregnated with dimethylsulphoxide (DMSO) was used.

2.9.3. Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) of NEO was performed by the broth microdilution method according to the CLSI guidelines M07-A10 for bacteria [21] and European Committee on Antimicrobial Susceptibility Testing (EUCAST) definitive document E.Def 7.2 for yeasts [22]. The microbial suspension was prepared using a dilution of the standardized suspension (0.5 McFarland meaning 10^8 CFU/mL for bacterial strains and 2×10^6 CFU/mL for *Candida* strains) to yield 5×10^5 CFU (colony forming units)/mL. Serial dilutions of NEO in DMSO were prepared to obtain concentrations ranging from 400 to 12.5 mg/mL. In six test tubes, 0.1 mL of each dilution of the NEO, 0.4 mL Mueller Hinton broth, and 0.5 mL microbial suspension was added, obtaining a final inoculum of 0.5×10^5 CFU/mL. After 24 h incubating at 35–37 °C for bacteria and 48 h at 28 °C for yeasts, the lowest concentration without visible growth was considered the MIC. All experiments were performed in triplicate.

2.10. Minimum Bactericidal Concentration and Minimum Fungicidal Concentration

The minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) was performed according to the method described by Danciu C. et al. and Brezoiu A.M. et al. [23,24] with minor modifications. Here, 1 µL from each test tubes with no growth in the MIC test was inoculated on Columbia agar supplemented with 5% blood (for bacteria) or on Sabouraud with chloramphenicol (for yeasts). After 24 h of incubation at 35–37 °C (for bacteria) and 28 °C (for yeasts), the lowest concentration without visible growth in this new medium was considered the MBC or MFC. All experiments were performed in triplicate.

2.11. Statistical Analysis

Statistical analysis was carried out using the SPSSv25 software (SPSS Inc., Chicago, IL, USA). Because of the small sample sizes (<50 data points), the Shapiro–Wilcoxon test was performed to observe the data distribution for each time point (day 0, day 4, day 8, day 12, day 16, day 20, and day 24). The Shapiro–Wilcoxon test showed that the data distribution is not normal. Therefore, nonparametric (or distribution-free) tests were applied. In order to

make comparisons between three or more data sets, the Kruskal–Wallis test was employed as an overall omnibus test in the case of independent samples (synthetic antioxidants and NEO). Multiple pairwise comparisons were performed via Mann–Whitney tests to detect statistically significant differences between specific samples. All the above mentioned tests were applied for each time point (level of incubation period). Regarding the evolution in time of the analyzed samples (over the incubation period levels), the Friedman test for dependent samples (repeated measurements) was used. Each statistical method was applied separately for peroxide and TBA values. The Tukey test was applied to detect significant differences ($p \leq 0.05$) between the mean values obtained from three replicates performed in antimicrobial analysis. All statistical tests were employed to detect statistically significant differences at the 95% significance level ($p < 0.05$).

3. Results and Discussion

3.1. NEO Chemical Composition

Steam distillation of the fresh plant material of *N. faassenii* gave a yellowish oil with 0.31% (*v/w*) yield, similar to those reported in the literature [12,25]. The chemical composition of EO isolated from *N. faassenii* was investigated using gas chromatography and mass spectrometry analysis (GC-MS). Table 1 presents the NEO chemical compounds according to their elution order on a Br-5MS capillary column, together with their calculated retention indexes.

Table 1. Chemical composition of *Nepeta × faassenii* essential oil (NEO) analyzed by gas chromatography and mass spectrometry analysis (GC-MS).

No	Compounds	RI ^a	AI ^b	%
1.	alpha-Pinene	919	932	0.16
2.	beta-Pinene	959	974	0.67
3.	3-Octanone	964	-	0.17
4.	para-Cymene	1005	1020	0.64
5.	Limonene	1011	1024	0.19
6.	1,8-cineole	1014	-	1.39
7.	Terpinen-4-ol	1178	1174	0.34
8.	alpha-Terpineol	1194	1186	0.23
9.	(S)-Carvone	1248	1239	0.27
10.	Hydrocinnamic acid, methyl ester	1280	-	0.12
11.	Cyclopropane, (2-methylenebutyl)	1307	-	0.61
12.	4a alpha,7alpha,7a alpha-Nepetalactone	1381	1386	34.12
13.	Naphthalene, 1-isocyano	1392	-	1.61
14.	beta-Bourbonene	1403	1387	0.97
15.	3,4 alpha-dihydro-4a alpha,7alpha,7a alpha-Nepetalactone	1438	-	7.93
16.	Caryophyllene	1440	1417	1.68
17.	Germacrene-D	1480	-	1.88
18.	2,9-Dimethyl-5-decyne	1487	-	1.14
19.	gamma-Muurolene	1496	1500	0.84
20.	Spiro(5,6)dodecane	1526	-	13.73
21.	Elemol	1568	-	23.23
22.	Caryophyllene oxide	1602	1582	1.96
23.	alpha-Cadinol	1671	1652	0.91
24.	cis-Z-alpha-Bisabolene epoxide	1809	-	1.23
25.	4,4,8-Trimethyltricyclo[6.3.1.0(1,5)]dodecane-2,9-diol	1865	-	1.29
26.	4-Oxo-beta-isodamascol	2339	-	2.14
Total:				99.45

^a The retention index (RI) was calculated upon a C₈–C₂₀ alkane standard mixture calibration curve obtained by injecting in the same conditions as samples. ^b The retention index (AI) described by Adams.

Twenty-six compounds representing 99.45% of the total oil were identified in NEO. The major components were 4a alpha, 7alpha, 7a alpha-nepetalactone (34.12%), elemol (23.23%), spiro(5,6)dodecane (13.73%), and 3,4 alpha-dihydro-4a alpha,7 alpha,7a alpha-nepetalactone (7.93%) (Table 1). Nepetalactone isomers were the main component of the EOs obtained from species of the *Nepeta* genus [26]. Three chemotypes were previously reported: nepetalactone chemotype, caryophyllene oxide chemotype, and 1,8-cineole/linalool chemotype [27]. *N. faassenii* evaluated in our study belongs to nepetalactone chemotype. Our results are in accordance with the previous investigation reporting large amounts of nepetalactone in EOs isolated from *N. faassenii* [12,25]. Besides, our study reports for the first time the presence of elemol (23.23%) in the chemical composition of *N. faassenii* oil. This sesquiterpenoid has also been recorded as a major compound in other oils of the *Nepeta* genus, such as *N. elliptica*, *N. govaniiana*, *N. crassifolia*, *N. nuda*, and *N. cataria* [11,28].

3.2. Antioxidant Activity

Peroxide value (PV) is widely accepted as a parameter to determine the primary lipid oxidation, indicating the amount of peroxides formed in fats and oils throughout oxidation [29]. PV evolution of the samples treated with NEO, BHA, and BHT recorded during 24 days of the storage period is presented in Figure 1a.

The experimental data are not normally distributed ($p < \alpha = 0.05$), according to the Shapiro–Wilcoxon test. Consequently, three different non-parametrical tests, Mann–Whitney, Kruskal–Wallis, and Friedman tests, were run to observed differences that can be considered statistically significant. The Kruskal–Wallis test was performed to observe the statistical differences between tested samples during 24 days of the storage period. After the initial day, extremely significant differences between the mean values of the tested samples were observed. Thus, the Mann–Whitney test performed at each storage period in this interval (from day 4 to 16) reveals significantly different PVs ($p < 0.001$) between the samples treated with NEO and those treated with the antioxidants BHA and BHT. During the storage period of 24 days, the PV of the sample treated with NEO increased from 1.89 meq/kg to 10.32 meq/kg, smaller than the sample containing BHT, except for days 20 and 24, with statistically significant differences after day sixteen ($p < 0.05$). For BHA, the same tendency was recorded, with insignificant differences ($p = 0.136 > 0.05$) for day zero, extremely significant differences ($p < 0.001$) for days 4 to 16, and significant differences ($p < 0.05$) after this time point. Friedman’s test reveals extremely significant differences between the mean values of our data in all tested moments ($p < 0.001$).

Peroxides, primary products of lipid oxidation, play a central role in lipid auto-oxidation by their decomposition into carbonyls and other compounds that may catalyze further oxidation [15]. One of the most important products of peroxide decomposition is malondialdehyde (MDA), which reacts with thiobarbituric acid (TBA) at low pH and high temperature and produces a pink fluorescent MDA–TBA complex with an absorption maximum between 530 and 540 nm [30]. TBA values are widely accepted as an indicator for assessing the degree of secondary lipid oxidation [31]. The evolution of TBA values of the samples treated with NEO, BHA, and BHT recorded during 24 days of the storage period is presented in Figure 1b. The data distribution tested by Shapiro–Wilcoxon does not reveal a normal distribution ($p < \alpha = 0.05$), which suggests the usage of non-parametrical tests, Mann–Whitney, Kruskal–Wallis, and Friedman tests to observed differences that can be considered statistically significant. Kruskal–Wallis test was run for each time point and showed significant differences ($p < 0.05$) for day zero and extremely significant differences ($p < 0.001$) in the other six moments. The Mann–Whitney test was performed at each time point of the storage period to compare the TBA values of the samples treated with NEO and BHA, respectively, as well as BHT. During the storage period of 24 days, TBA values ranged from 2.70 to 15.01 malonaldehyde equivalents/kg for the sample treated with NEO, which is significantly smaller ($p < 0.001$) than the samples containing BHA from day 8 to day 12. However, the TBA values for the BHT sample are significantly ($p < 0.001$) better

after day 4. Friedman's test was performed with significant differences ($p < 0.001$) in data dynamics for all-time points of storage period and for all samples.

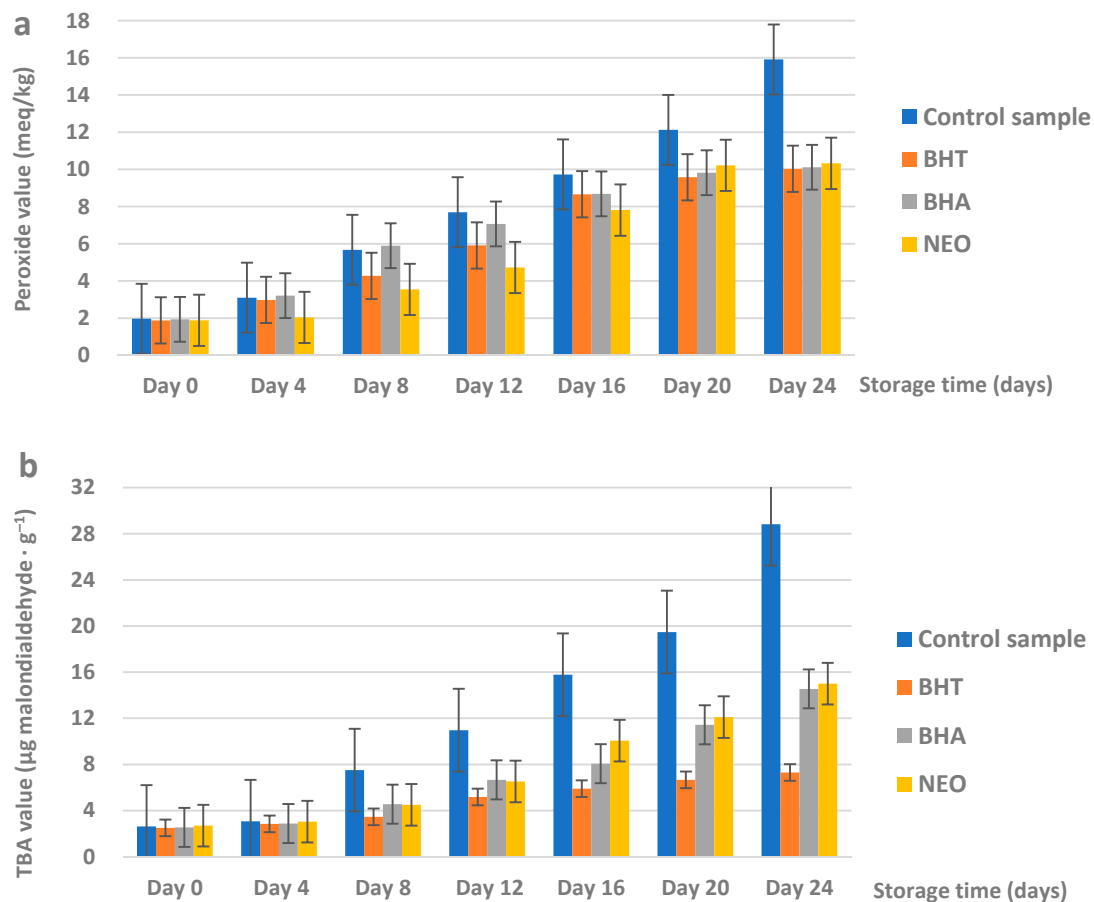


Figure 1. Antioxidant activity of samples treated with NEO, butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) in terms of peroxide values (PVs) (a) and thiobarbituric acid (TBA) values (b) of cold-pressed sunflowers oil during 24 days of the storage period.

The antioxidant activity in the DPPH test is due either to the hydrogen-donating ability or the radical scavenging activity of the analyzed compound. The NEO exhibited strong activity to reduce the stable radical DPPH into yellow-colored diphenylpicrylhydrazine, with an IC₅₀ value of 0.032 ± 0.005 mg/mL (Table 2). The radical scavenging activity of NEO was significantly stronger ($p < 0.001$) than that of the alpha-tocopherol (IC₅₀: 0.142 ± 0.011 mg/mL) and BHA (0.086 ± 0.006 mg/mL). Only BHT exhibits a significantly ($p < 0.001$) better antioxidant activity than NEO (IC₅₀: 0.013 ± 0.009 mg/mL) (Table 2). Moreover, the relative antioxidant activity percentage (RAA %) of NEO ($92.31 \pm 0.17\%$) in the β -carotene-linoleic acid assay was comparable to that of BHT (100%) (Table 2). The RAA% of NEO was determined with the following equation: $RAA = A_{NEO} / A_{BHT}$, where A_{BHT} is the absorption of BHT (positive control used) and A_{NEO} is the absorption of NEO.

To the best of our knowledge, this is the first study regarding NEO antioxidant activity. The present results are in discordance with previous studies that report weak antioxidant activity of EOs isolated from other *Nepeta* species [32–35]. Generally, *Nepeta* species EOs are rich in nepetalactone isomers known to be weak antioxidants [32,36]. However, significant antioxidant activity was recorded in the case of *N. nuda* subsp. *nuda* oil, with $4\alpha\beta,7\alpha,7\beta$ -nepetalactone and 1,8-cineole as main components. In this case, the antioxidant activity was attributed to the relatively high presence of 1,8-cineole in the oil composition [37]. The presence of elemol (23,23%) in our oil composition, a sesquiterpene recognized for its antioxidant capacity [38], can partially explain the strong antioxidant capacity

recorded. Moreover, the literature survey revealed that α -pinene [39], beta-pinene [40], 1,8-cineole [37], caryophyllene [38], and caryophyllene oxide [40], minor compounds of our oil, possess a powerful antioxidant capacity. Based on these considerations, the NEO's shown antioxidant capacity might be associated with their terpenoid compounds.

Table 2. Antioxidant activities of the NEO by 1,1-diphenyl-2-picrylhydrazyl (DPPH) and β -carotene bleaching assay.

Samples Tested	Parameters	
	DPPH, IC ₅₀ (mg/mL)	β -Carotene Bleaching (RAA ^c) (%)
NEO	0.032 ± 0.005	92.31 ± 0.17
BHA ^a	0.086 ± 0.006	Nt ^d
BHT ^b	0.013 ± 0.009	100
alpha-Tocopherol	0.142 ± 0.011	Nt ^d

^a BHA—butylated hydroxyanisole; ^b BHT—butylated hydroxytoluene; ^c RAA—relative antioxidative activity; ^d Nt—not tested.

3.3. Antimicrobial Activity

The results of antimicrobial activity testing of the NEO are shown in Table 3. NEO exhibits broad-spectrum antimicrobial effects on the microorganism strains tested with inhibition zones between 22.33 ± 0.57 mm and 29.66 ± 1.52 mm in diameter, respectively. MIC, MBC, and MFC values ranged from 5 to 10 mg/mL. The results revealed that *S. pyogenes* and *C. albicans* were the most susceptible tested strains to the NEO action (Table 3). Similar antifungal activity was previously reported with other EOs *Nepeta* species, such as *N. leucophylla*, *N. elliptica*, and *N. erecta* [41], while *N. ucrainica* [42] and *N. sintonensis* [32] EOs displayed higher activity against *C. albicans*. Furthermore, the NEO's antimicrobial effects recorded against *S. aureus*, *K. pneumoniae*, and *S. enterica* were higher than those previously reported for *N. rtanjensis* [43], *N. persica* [44], and *N. menthoides* [45]. Additionally, our results revealed that NEO presents antibacterial potential against *P. aeruginosa*. In contrast, other studies on *Nepeta* species' EOs antimicrobial activity recorded low inhibitory effects or no activity against *P. aeruginosa* [43,46]. However, a direct comparison of previously reported MIC, MBC, and MFC data is limited by inter-study variations, such as the EOs isolation method, the growth stage of inoculum, incubation duration, and culture medium used [42].

Table 3. Antimicrobial activity of the NEO by disk diffusion, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum fungicidal concentration (MFC).

Bacterial and Yeast Strains	Disk Diffusion (mm)	MIC Value (mg/mL)	MBC Value (mg/mL)	MFC Value (mg/mL)
<i>Streptococcus mutans</i>	25.66 ± 0.57	5	5	-
<i>Streptococcus pyogenes</i>	29.66 ± 1.52	5	5	-
<i>Staphylococcus aureus</i>	26.33 ± 0.57	10	10	-
<i>Enterococcus faecalis</i>	25.33 ± 0.57	10	10	-
<i>Escherichia coli</i>	23.66 ± 1.52	10	10	-
<i>Klebsiella pneumoniae</i>	26.33 ± 0.57	10	10	-
<i>Salmonella enterica</i>	26 ± 1.00	10	10	-
<i>Shigella flexneri</i>	25.33 ± 0.57	10	10	-
<i>Pseudomonas aeruginosa</i>	22.33 ± 0.57	10	10	-
<i>Candida albicans</i>	28.33 ± 1.52	5	-	5
<i>Candida parapsilosis</i>	26.66 ± 1.52	5	-	5

The diameter of the zone of inhibition is presented as mean (n = 3) ± standard deviation, and the mean value for MIC, MBC, and MFC; no significant difference ($p > 0.05$) was observed by applying the Tukey test.

The mechanisms of antimicrobial action of the EOs still are not completely understood. Several studies report that terpenoid compounds by their lipophilic character penetrate through bacterial membranes and exhibit inhibitory activity on the functional properties of the cell, and finally cause leakage of the internal contents of the cell [47–49]. Based on these considerations, NEO's antimicrobial activity may be attributed to the high content of terpenoid compounds. These assumptions are supported by previous studies that reported nepetalactones as the active agents responsible for antimicrobial activities of several *Nepeta* species such as *N. menthoides* [45], *N. elliptica* [41], *N. rtanjensis*, and *N. sibirica* [50]. Moreover, the literature reports described elemol as a well-known chemical with pronounced antimicrobial effects [51–54].

However, EOs are complex mixtures of active principles with different biological properties, and for these reasons, it is challenging to reduce their biological activities to one or several active principles.

4. Conclusions

In conclusion, our study's findings indicated that the EO isolated from the aerial parts of *N. faassenii* is rich in the terpenoid compounds, mainly 4a alpha, 7alpha, 7a alpha-nepetalactone, elemol, and 3,4 alpha-dihydro-4a alpha,7alpha,7a alpha-nepetalactone. Biological evaluations revealed that the NEO exhibited broad-spectrum antibacterial and antifungal effects and possessed significant antioxidant properties. In light of these findings, NEO may represent a new source of natural antioxidants and antimicrobial agents with applications in medicine or the food industry. Further investigations are needed to identify the volatile compounds responsible for the observed biological effects and their synergistic, additive, or antagonistic effects.

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