Specialty Journal of Pharmacognosy, Phytochemistry, and Biotechnology
ISSN: 3062-441X
2025, Volume 5, Page No: 310-318

Available online at: www.galaxypub.co/page/journals

Galaxy Publication

Galaxy Publication

Phytochemical Characterization and Antioxidant Activity of Nepeta racemosa Lam

Katarzyna Nowak[1]*, Piotr Zieliński[1], Marta Kaczmarek[2]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Jagiellonian University, Kraków, Poland.
[2]Department of Plant Biotechnology, Faculty of Life Sciences, University of Warsaw, Warsaw, Poland.

***E-mail** ✉ k.nowak@outlook.com

Received: 04 September 2025; **Revised:** 04 December 2025; **Accepted:** 05 December 2025

ABSTRACT

Plants belonging to the genus *Nepeta* have long been employed in traditional medicine for treating skin ailments and snake envenomation because of their antiseptic and astringent properties. Despite the recognized phytochemical diversity of this genus, *Nepeta racemosa* has not been thoroughly investigated. The present study aimed to characterize the phytochemical constituents, essential oil profile, and antioxidant potential of this species. Dried aerial parts of *N. racemosa* were extracted sequentially using *n*-hexane, chloroform, and methanol through maceration. The methanolic extract was fractionated by C18 solid-phase extraction using a graded methanol–water system. Selected fractions were further purified by preparative reversed-phase high-performance liquid chromatography, resulting in the isolation of three secondary metabolites. The volatile components of the essential oil were identified by gas chromatography–mass spectrometry. Antioxidant capacity was determined using the DPPH radical scavenging assay, while total phenolic and flavonoid contents were quantified using Folin–Ciocalteu and aluminum chloride colorimetric methods, respectively. Chemical investigation of the 20% and 40% solid-phase extraction fractions led to the identification of an iridoid compound, 8,9-epi-7-deoxy-loganic acid, along with two phenylethanoid glycosides, forsythoside B and verbascoside. Analysis of the essential oil revealed 4aα,7α,7aα-nepetalactone (31.70%) as the predominant constituent, followed by germacrene D (7.39%) and *n*-hexadecanoic acid (6.47%). Among the tested samples, the methanolic extract exhibited marked antioxidant activity and the highest levels of phenolic and flavonoid compounds (IC_{50} = 0.09 ± 0.01 mg/mL; 1581.80 ± 10.28 mg/100 g; 33.01 ± 0.02 mg/100 g). The 40% solid-phase extraction fraction also demonstrated strong radical scavenging ability, accompanied by substantial phenolic and flavonoid contents (IC_{50} = 0.01 ± 0.00 mg/mL; 659.20 ± 40.32 mg/100 g; 22.50 ± 0.37 mg/100 g). This study indicates that phenylethanoid glycosides are key contributors to the antioxidant properties of *Nepeta racemosa*, highlighting this species as a valuable source of bioactive phenolic compounds.

Keywords: GC–MS analysis, Essential oil, Antioxidant, Antimalarial, Nepeta racemosa

How to Cite This Article: Nowak K, Zieliński P, Kaczmarek M. Phytochemical Characterization and Antioxidant Activity of Nepeta racemosa Lam. Spec J Pharmacogn Phytochem Biotechnol. 2025;5:310-8. https://doi.org/10.51847/BxM9Y0WYz4

Introduction

The Lamiaceae genus *Nepeta* comprises nearly 280 species with a wide geographical distribution across Europe, Asia, and Africa, and its highest species diversity is reported from the Mediterranean basin [1]. In Iran, members of this genus are commonly referred to as “Pune-sa,” and floristic surveys indicate the presence of approximately 67 species throughout the country [2]. Several *Nepeta* species have been traditionally utilized for medicinal purposes. Topical applications are mainly associated with antiseptic and astringent effects in skin conditions, whereas oral formulations have been used for diuretic, antipyretic, antiasthmatic, antispasmodic, diaphoretic, antitussive, sedative, and gastrointestinal uses.

Among the best-known representatives of this genus are *Nepeta racemosa*, *N. bulgaricum*, *N. cataria* (catnip or catmint), and *N. cataria* var. *citriodora* (lemon catnip) [3, 4]. Plants belonging to the Labiatae family are recognized as prolific producers of secondary metabolites, including iridoids, phenolic compounds, nepetalactones, diterpenes, and triterpenes. Due to their aromatic characteristics, these plants hold notable

economic importance and are widely exploited as sources of essential oils, which are predominantly composed of monoterpenoid constituents.

Previous studies have demonstrated that numerous compounds isolated from *Nepeta* species possess pronounced biological and pharmacological activities [4]. These activities are largely attributed to nepetalactone, a volatile iridoid commonly present as a major component of *Nepeta* essential oils. On the basis of essential oil composition, species within the genus are generally classified into nepetalactone-rich and nepetalactone-deficient groups [5]. Nepetalactone naturally occurs in several stereochemical forms. Investigations on *Nepeta* species native to Turkey have identified nepetalactone, caryophyllene oxide, and either 1,8-cineole or linalool as the predominant constituents [6]. Considering the ethnomedicinal relevance and chemotaxonomic significance of this genus, the present work was undertaken to examine the chemical composition, antioxidant activity, and in vitro antiplasmodial potential of the aerial parts of *N. racemosa*.

Materials and Methods

Ethical approval

All experimental procedures were conducted following approval from the Ethics Committee of Tabriz University of Medical Sciences (IR.TBZMED.REC.1394.807).

Reagents and chemicals

All solvents and chemicals employed in this study were of analytical purity. Methanol, *n*-hexane, acetone, ethyl acetate, chloroform, DMSO, DMSO-d_6, D_2O, hydrochloric acid (HCl), sodium hydroxide (NaOH), and sodium acetate were supplied by Merck (Germany). Gallic acid, rutin, hematin, anhydrous sodium carbonate (Na_2CO_3), 1,1-diphenyl-2-picrylhydrazyl (DPPH), aluminum trichloride, oleic acid, sodium nitrite, and Folin–Ciocalteu reagent were obtained from Sigma-Aldrich (Germany).

Collection and identification of plant material

Aerial parts of *Nepeta racemosa* were harvested in May 2016 from the Marand region of East Azerbaijan province, Iran. Taxonomic identification was carried out by Dr. Fatemeh Ebrahimi (Faculty of Pharmacy, Tabriz University of Medical Sciences). A voucher specimen (TBZFPH 2639) was deposited in the herbarium of Tabriz University of Medical Sciences.

Extraction, fractionation, and isolation

The air-dried aerial parts (100 g) were ground and sequentially extracted by maceration using *n*-hexane, chloroform, and methanol (1.2 L each solvent). Each extract was filtered and concentrated under reduced pressure at temperatures below 40 °C using a rotary evaporator. A portion of the methanolic extract (4 g) was subjected to C18 solid-phase extraction (Sep-Pak cartridge, 10 g, 35 cc). Elution was performed using a stepwise methanol–water gradient (10:90, 20:80, 40:60, 60:40, 80:20, and 100:0; 200 mL per step). The collected fractions were evaporated to dryness under reduced pressure at 40 °C.

The fraction eluted with 20% methanol was further purified by preparative reversed-phase HPLC using an ODS column (Dr. Mainsch GmbH, 20 µm, 250 mm × 20 mm). Separation was achieved using a methanol–water gradient program (15–75% methanol over 50 min), followed by isocratic elution and re-equilibration at a flow rate of 8 mL/min, yielding one iridoid glycoside (compound 1; tR = 33.31 min).

Similarly, the 40% methanol SPE fraction was subjected to preparative HPLC using a different gradient system (35–60% methanol in water), resulting in the isolation of two phenylethanoid glycosides (compound 2, tR = 14.45 min; compound 3, tR = 18.49 min). Structural elucidation of all isolated compounds was performed using one-dimensional nuclear magnetic resonance spectroscopy (^1H and ^13C NMR).

Determination of flavonoid content

Total flavonoid concentration was evaluated using the aluminum chloride colorimetric assay [7]. Briefly, 0.5 mL of methanolic extract solution (1 mg/mL) was mixed with 0.1 mL of aluminum chloride solution (10%), 0.1 mL of potassium acetate (1 M), 1.5 mL of methanol, and 2.8 mL of distilled water. After incubation at room temperature for 30 min, absorbance was recorded at 510 nm. The formation of a yellow complex indicated the

presence of flavonoids. Quantification was carried out using a rutin standard calibration curve (0.0039–0.125 mg/mL), and results were expressed as milligrams of rutin equivalents per gram of extract.

Determination of total phenolic content

The overall phenolic content of the extracts was quantified using the Folin–Ciocalteu colorimetric method [8]. Briefly, 1 mL of each extract solution (1 mg/mL) was allowed to react with 200 µL of Folin–Ciocalteu reagent for 5 min, after which 1 mL of 2% (w/v) aqueous sodium carbonate was added. The reaction mixture was incubated at room temperature for 30 min, and absorbance was subsequently measured at 750 nm using a spectrophotometer. A calibration curve was constructed with gallic acid standard solutions at concentrations of 5, 10, 15, 25, 50, 75, and 100 mg/L prepared in an acetone–water mixture (60:50, v/v). The total phenolic content was expressed as milligrams of gallic acid equivalents per gram of dry plant material.

Isolation of essential oil

Essential oil was obtained from the aerial parts of air-dried *N. racemosa* (60 g) by hydro-distillation using a Clevenger-type apparatus for a duration of 3 h. Following distillation, residual moisture in the oil was removed using anhydrous sodium sulfate. The dried essential oil was stored at 4 °C until further analysis [9]. The oil yield was calculated and expressed as milliliters of essential oil per 100 g of plant material.

Gas chromatography–mass spectrometry (GC–MS) analysis

Chemical characterization of the essential oil was performed using a Shimadzu GC–MS-QP5050A system equipped with a flame ionization detector (FID) and a DB-1 fused silica capillary column (60 m × 0.25 mm internal diameter, 0.25 µm film thickness). Helium was used as the carrier gas at a constant flow rate of 1.3 mL/min. The oven temperature program was set to hold at 50 °C for 2 min, followed by a gradual increase to 275 °C at a rate of 3 °C/min, and maintained at this final temperature for 3 min. Injector and detector temperatures were set at 250 °C and 280 °C, respectively. Samples (1 µL) were injected using a split ratio of 1:20. Mass spectra were recorded under electron ionization at 70 eV within a mass range of 30–600 amu. The ion source temperature was maintained at 260 °C, with a solvent delay of 2 min. Identification of individual components was achieved by comparing their mass spectra and Kovats retention indices with reference data from Wiley and NIST spectral libraries, as well as with those of authentic standards.

Antioxidant activity assay

Free radical scavenging capacity of the extracts and fractions was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay [10]. Stock solutions of the samples were prepared at a concentration of 10 mg/mL using methanol or chloroform as solvents. Serial dilutions were then obtained from these stock solutions. For each assay, 2.5 mL of DPPH solution (0.08 mg/mL in methanol) was combined with 2.5 mL of each diluted sample solution and incubated in the dark at room temperature for 30 min. Absorbance was measured at 517 nm, corresponding to the maximum absorption wavelength of DPPH. All experiments were conducted in triplicate, and mean absorbance values were calculated. The IC_{50} value, defined as the concentration required to inhibit 50% of DPPH radicals, was determined from plots of percentage inhibition versus sample concentration [11, 12]. Percentage inhibition (IC%) was calculated using the following equation, where AB represents the absorbance of the blank and AA corresponds to the absorbance of the test sample:

$$IC\% = [(AB - AA) / AB] \times 100 \quad (1)$$

Antiplasmodial activity

The antimalarial potential of the extracts and fractions was assessed using a modified hem polymerization inhibition assay based on the method described by Tripathi *et al*. [13], with slight modifications [14]. The activity of the samples was expressed as the percentage inhibition (I%) of heme polymerization relative to the positive control, chloroquine diphosphate. The percentage inhibition was calculated using the following equation:

$$I\% = [(AB - AA) / AB] \times 100 \quad (2)$$

where AB denotes the absorbance of the blank and AA represents the absorbance measured in the presence of the test samples.

Results and Discussion

Fractionation of the methanolic extract of *N. racemosa* using solid-phase extraction followed by reversed-phase preparative HPLC analysis of the 20% and 40% fractions resulted in the isolation of three secondary metabolites, consisting of one iridoid (a) and two phenylethanoid glycosides (b and c) **(Figure 1).** Structural identification of the isolated constituents was achieved through detailed nuclear magnetic resonance spectroscopic analysis. Comparison of the obtained spectroscopic data with previously published reports confirmed that all three compounds were known metabolites. Definitive structural elucidation was performed using ^1H and ^13C NMR spectroscopy.

Compound A exhibited the molecular formula molecular formula: $C_{15}H_{22}O_9$,^{1}H NMR (DMSOd6) δ: 1.01 (3H, d, J= 6.0 Hz, H- 10), 1.20 (1H, m, H-7a), 1.52 (1H, m, H-6a), 1.68(1H, m, H-b7b), 1.90 (1H, m, H-6b), 2.17 (1H, m,H-8), 2.21 (1H, m, H-9), 2.74 (1H, m, H-5), 3.00 (1H, m, H-2'), 3.00-3.50 (1H, m, H-3', H-5'),3.05 (1H, m, H-4'), 3.46 (1H, dd, H-6'a), 3.65(1H, d, H-6'b), 4.47 (1H, d, J= 8.0 Hz, H-1'), 5.22(1H, s, H-1), 7.24 (1H, s, H-3).

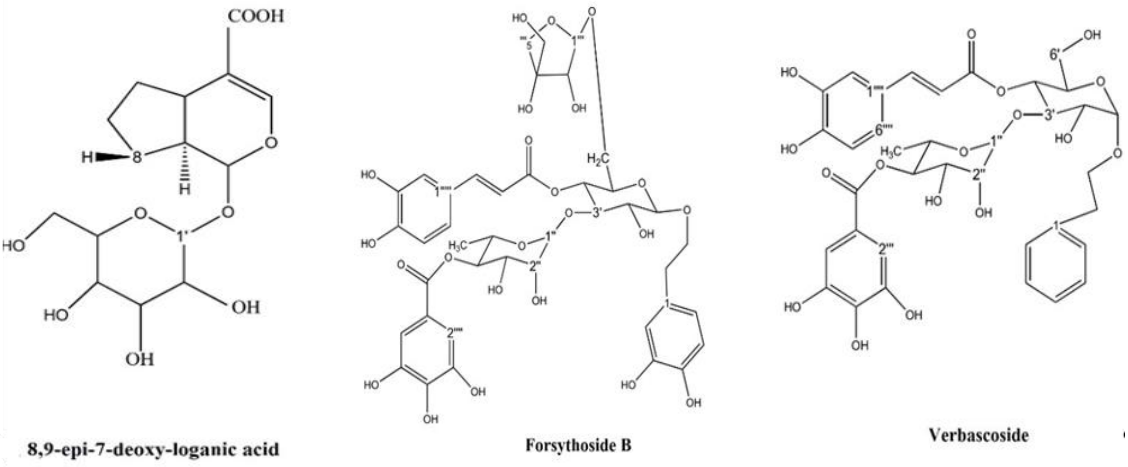
Based on close agreement between these data and previously reported values [15, 16], compound A was identified as 8,9-epi-7-deoxy-loganic acid **(Figure 1a).**

Compound B was assigned the molecular formula $C_{34}H_{44}O_{19}$,^{1}H NMR (D_2O) δ: δ: 0.88 (3H, d, J = 7.2 Hz, H-6''),3.20 (1H, m, H-4''), 3.39 (1H, m, H-2'), 3.49 (1H,m, H-6'a), 3-4 (1H, m, H-2''), 3.55 (1H, m, H-3''),3.58 (2H. s, H-5'''), 3.58 (1H, m, H-6'b), 3.59 (1H,m, H-5''), 3.70-3.74 (1H, H-4'''a), 3.75 (1H, m, H-5'), 3.78 (1H, m, H-3'), 3.80 (1H, d, H-2'''), 4.60 (1H, dd, H-1'), 4.89 (1H, s, H- 1'''), 4.91 (1H, t,H-4'), 5.19 (1H, dd, H-1''), 6.22 (1H, d, H-α'),6.66 (1H, dd, J = 8.4, H-6), 6.72 (1H, d, J = 8.0, H-5), 6.76 (1H, s, H-2), 6.79 (1H, d, H-5''''), 6.95(1H, s, H- 2''''), 6.97 (1H, dd, H-6''''), 7.43 (1H, d,H-β'). ^{13}C NMR(D2O) δ: 18.5 (C-6''), 36.6 (C-α),70.4 (C-5''), 70.9 (C-4'), 71.9 (C-3''), 72.3 (C-2''),72.4 (C-β), 73.7 (C-4''), 74.5 (C-5'), 75.2 (C-4''',5'''), 78.4 (C-2'''), 81.7 (C-3'), 80.7 (C-3'''),103.0 (C-1''), 104.2(C-1'), 111.0 (C-1'''), 114.8(α'), 115.4 (C-2''''), 116.5 (C-5), 116.7(C-5''''),117.3 (C-2), 121.4 (C-6), 123.4 (C-6''''), 127.6(C-1''''), 131.6 (C-1), 144.6 (C-4), 146.0 (C-3),146.8 (C-3''''), 148.2 (β'), 149.8 (C-4''''), 168.4 (C=O).

Comparison with reported spectral data confirmed compound B as forsythoside B **(Figure 1b)** [17, 18].

Compound C possessed the molecular formula $C_{27}H_{36}O_{15}$,^{1}H NMR (D_2O) δ:0.88 (3H, d, H-6''), 2.76 (2H, m, H-β), 3-4 (1H, m, H-2', 3', 5', 6'a, 6'b, 2'', 3'', 4'',5'',), 3.61(1H, dd, H-αa), 3.79 (1H,dd, H-αb), 4-5(1H, d, H-1'), 4-5 (1H, t, H-4'), 5.21 (1H, s, H-1''),6.17 (1H, d, J = 16 Hz, H-α'), 6.65 (1H, d, H-6),6.75 (1H, d, H-5), 6.76 (1H, s, H-2), 6.76 (1H,d,H-5'''), 6.89 (1H, d, J = 8 Hz, H-6''') 6.98 (1H, s,H-2'''), 7.37 (1H,d, J = 16 Hz, H-β'). ^{13}CNMR(D2O) δ: 15.68 (C-6''), 36.72 (C-β), 60.54(C-6'), 69.30 (C-4'), 73.05 (C-α), 76.24 (C-5'),76.37 (C-2'), 102.09 (C-1''), 104.47 (C-1'),114.45 (C-α'), 115.02 (C-2'''), 116.07 (C-5'''),116.14 (C-5), 117.11 (C-2), 121.7 (C-6), 122.63(C-6'''), 126.95 (C-1'''),130.15 (C-1), 144.17 (C-3), 146.98 (C-4'''),146.98 (C-4), 147.01 (C-3'''),147.01 (C-β'), 162 (CO),168 (CO). Further supported the structure, showing resonances corresponding to sugar moieties, aromatic carbons, and ester carbonyls. Comparison with published data led to the identification of compound C as verbascoside **(Figure 1c)** [19–21].

Based on structural features, the isolated metabolites from *N. racemosa* were categorized into two major phytochemical groups: one iridoid and two phenylethanoid derivatives. Quantitative analysis of total phenolic and flavonoid contents in the methanolic extract and its corresponding fractions is summarized in **Table 1.**



a) b) c)

Figure 1. Chemical structures of compounds isolated from *Nepeta racemosa*.

Table 1. Total flavonoid content, total phenolic content, and antioxidant capacity of the *n*-hexane, chloroform, and methanolic extracts, along with their corresponding fractions, obtained from *Nepeta racemosa*.

Sample	Phenolic content (gallic acid equivalent, mg/100 g)	Flavonoid content (rutin equivalent, mg/100 g)	DPPH scavenging capacity (RC_{50}, mg/mL)
Chloroform extract	Not detected	Not detected	0.16 ± 0.05
Methanolic extract	1518.80 ± 10.28	33.01 ± 0.02	0.09 ± 0.04
n-Hexane extract	Not detected	Not detected	0.41 ± 0.01
SPE fraction (10% MeOH–water)	20.60 ± 0.73	1.30 ± 0.12	0.32 ± 0.13
SPE fraction (20% MeOH–water)	58.10 ± 1.16	1.20 ± 0.08	0.19 ± 0.02
SPE fraction (40% MeOH–water)	659.20 ± 40.32	22.50 ± 0.37	0.01 ± 0.00
SPE fraction (60% MeOH–water)	197.70 ± 3.81	12.70 ± 0.42	0.09 ± 0.04
SPE fraction (80% MeOH–water)	37.40 ± 0.75	0.80 ± 0.04	0.42 ± 0.02
SPE fraction (100% MeOH)	117.60 ± 5.18	1.10 ± 0.10	0.34 ± 0.04
Quercetin (reference compound)	—	—	2.78×10^{-5}

Values represent the mean ± SD, n=3

Quantitative analysis revealed that phenolic compounds were most abundant in the methanolic extract, followed in decreasing order by fractions Fr 40, Fr 60, Fr 100, Fr 20, Fr 80, and Fr 10. A similar trend was observed for flavonoid content, with the highest concentration detected in the methanolic extract, followed by Fr 40, Fr 60, Fr 10, Fr 20, Fr 100, and Fr 80. Among all fractions, the 40% methanol–water fraction exhibited markedly elevated levels of total phenolics (659.20 ± 40.32 mg/g) and flavonoids (22.50 ± 0.37 mg/g).

Measurement of phenolic and flavonoid contents is of particular importance, as the antioxidant activity of many plant extracts has been attributed to these constituents, especially flavonoids, which are known for their wide range of biological activities [22]. Previous studies have demonstrated a strong correlation between total phenolic concentration and radical scavenging capacity in plant materials [23, 24]. Phenolic compounds contribute to antioxidant effects through their ability to neutralize free radicals and chelate metal ions [25, 26]. Consistent with these findings, the results of the present study showed that the 40%, 20%, and 60% methanol–water fractions possessed higher levels of total phenolics and flavonoids, which corresponded well with their enhanced free radical scavenging activities. These observations suggest that flavonoid constituents play a major role in the antioxidant potential of *N. racemosa* extracts.

The composition of the essential oil, including retention indices and relative percentages of individual components, is summarized in **Table 2.** Compounds are presented according to their elution order on the chromatographic column. GC–MS analysis led to the identification of 53 constituents, representing 77.1% of the total essential oil content. The dominant components were identified as 4aα,7α,7aα-nepetalactone (31.70%),

germacrene D (7.39%), and *n*-hexadecanoic acid (6.47%). Oxygenated monoterpenes constituted the largest chemical class (33.53%), followed by sesquiterpene hydrocarbons (12.64%), fatty acid derivatives (12.89%), oxygenated sesquiterpenes (9.41%), and monoterpene hydrocarbons (0.18%). In addition, non-terpenoid compounds such as ketones and aldehydes accounted for 8.47% of the total oil composition.

Based on the high proportion of nepetalactone, *N. racemosa* can be classified among *Nepeta* species in which nepetalactones are the principal volatile constituents. In contrast, nepetalactone-free species are typically characterized by higher contents of compounds such as 1,8-cineole and caryophyllene oxide [27]. In the present study, these compounds were detected only in minor amounts, accounting for 0.33% and 3.98% of the oil, respectively. Among the known nepetalactone isomers—namely 4aα,7α,7aα-nepetalactone, 4aβ,7α,7aβ-nepetalactone, 4aα,7α,7aβ-nepetalactone, and 4aβ,7α,7aα-nepetalactone—the first is generally reported as the predominant form, while other isomers such as 4α-dihydronepetalactone, 4aα,7β,7aα-nepetalactone, and 4aα,7β,7aβ-nepetalactone occur in lower proportions [28]. In this investigation, only 4aα,7α,7aα-nepetalactone was detected. This compound has also been reported as a major constituent in the essential oils of several *Nepeta* species, including *N. racemosa* [1, 2, 29], *N. cadmea* [30], *N. cephalotes* [31], *N. govaniana* [32], and *N. teydea* [33].

The antioxidant activities of the extracts and solid-phase extraction fractions were evaluated using the DPPH free radical scavenging assay **(Table 2)** [10]. Among all tested samples, the 40% methanol–water SPE fraction demonstrated the strongest radical scavenging effect, with an RC_{50} value of 0.01 ± 0.00 mg/mL. Comparable antioxidant activities were observed for the methanolic extract and the 60% methanol–water SPE fraction, both showing RC_{50} values of approximately 0.09 mg/mL. In contrast, the weakest scavenging activity was recorded for the *n*-hexane extract (0.41 ± 0.01 mg/mL) and the 80% methanol–water SPE fraction (0.42 ± 0.02 mg/mL).

Table 2. Chemical composition of the essential oil obtained from the aerial parts of *Nepeta racemosa*.

Identified compound	Relative abundance (%)	Experimental KI	Literature KI[a]
β-Pinene	0.19	972	971
(E,E)-2,4-Heptadienal	0.10	984	981
(E)-2-Hexenal	0.23	831	825
1-Octen-3-ol	0.07	967	964
Linalool	0.39	1088	1086
trans-Pinocarveol	0.33	1129	1125
1,8-Cineole	0.33	1028	1021
Nonanal	0.18	1080	1083
Pinocarvone	0.10	1146	1140
Terpinen-4-ol	0.14	1169	1164
Myrtenal	0.16	1172	1171
4aα,7α,7aα-Nepetalactone	31.70	1370	1367
α-Copaene	0.24	1381	1380
α-Terpineol	0.24	1177	1175
Myrtenol	0.14	1189	1181
β-Bourbonene	0.67	1389	1388
β-Elemene	0.79	1395	1391
α-Humulene	0.13	1456	1455
α-Amorphene	0.27	1477	1475
β-Caryophyllene	2.09	1427	1423
β-Cubebene	0.23	1439	1431
γ-Cadinene	0.21	1513	1510
δ-Cadinene	0.39	1526	1518
Dodecanoic acid	0.21	1550	1549
1,5-Epoxysalvial-4(14)-ene	0.39	1567	1562
Germacrene D	7.39	1485	1482
α-Farnesene	0.23	1496	1497
(–)-Spathulenol	0.61	1573	1571
Caryophyllene oxide	3.98	1579	1577
Salvial-4(14)-en-1-one	0.93	1592	1585

1-Tetradecanol	0.51	1630	1628
Widdrol	0.33	1636	1635
Cycloisolongifolene, 8-hydroxy-, endo-	1.13	1648	1644
β-Copaen-4α-ol	0.50	1670	1669
6-Isopropenyl-4,8a-dimethyl-octahydronaphthalen-2-ol	1.82	1673	1675
Hexahydrofarnesyl acetone	1.12	1836	1833
n-Pentadecanoic acid	0.14	1846	1844
1-Hexadecanol	0.14	1862	1861
Octadecanal	0.08	1697	1697
Tetradecanoic acid	0.33	1750	1746
7,9-di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	0.16	1875	1874
Pentadecanoic acid, 14-methyl-, methyl ester	0.20	1912	1911
n-Hexadecanoic acid	6.47	1950	1951
1-Octadecanol	0.34	2064	2066
Methyl linolenate	0.27	2079	2081
Phytol	3.96	2098	2105
Methyl-11,14,17-eicosatrienoate	1.46	2285	2290
Nonadecanoic acid	2.82	2228	2236
Hexadecanamide	1.18	2348	2350
Hexadecanal diallyl acetal	0.12	2293	2295
Hexadecanoic acid, butyl ester	0.18	2169	2174
Hexanedioic acid, bis(2-ethylhexyl) ester	0.53	2360	2361
Heneicosane	0.27	2378	—
Non-terpenoid fraction	**21.36**		
Total identified components	**77.12**		
Terpenoid fraction	**55.76**		

[a] Compounds are arranged according to their elution sequence on the DB-1 capillary column.

The antimalarial potential of the crude extracts obtained from the aerial parts of *N. racemosa* was assessed using an in vitro β-hematin formation assay. None of the tested extracts demonstrated inhibitory activity against heme crystallization.

Conclusion

The findings of the present investigation indicate that the 40% MeOH–water SPE fraction derived from the methanolic extract of *N. racemosa* aerial parts represents a promising candidate for further research. This fraction exhibited pronounced free-radical scavenging capacity, along with elevated levels of total phenolics and flavonoids, and contained major bioactive constituents, including forsythoside B and verbascoside.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

References

1. İşcan G, Köse YB, Demirci B, Can Başer KH. Anticandidal activity of the essential oil of Nepeta transcaucasica Grossh. Chem Biodivers. 2011;8(11):2144–8.
2. Dabiri M, Sefidkon F. Chemical composition of the essential oil of Nepeta racemosa Lam. from Iran. Flav Fragr J. 2003;18(2):157–8.
3. Kraujalis P, Venskutonis PR, Ragazinskiene O. FOODBALT: Proceedings of the 6th Baltic Conference on Food Science and Technology; 2011 May 5–6; Jegava, Latvia.

4. Formisano C, Rigano D, Senatore F. Chemical constituents and biological activities of Nepeta species. Chem Biodivers. 2011;8(10):1783–818.
5. Javidnia K, Mehdipour A, Hemmateenejad B, Rezazadeh S, Soltani M, Khosravi A, et al. Nepetalactones as chemotaxonomic markers in the essential oils of Nepeta species. Chem Nat Compd. 2011;47(5):843–7.
6. Baser K, Kirimer N, Kurkcuoglu M, Demirci B. Essential oils of Nepeta species growing in Turkey. Chem Nat Compd. 2000;36(4):356–9.
7. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal. 2002;10(3):178–82.
8. Chlopicka J, Pasko P, Gorinstein S, Jedryas A, Zagrodzki P. Total phenolic and total flavonoid content, antioxidant activity and sensory evaluation of pseudocereal breads. LWT Food Sci Technol. 2012;46(2):548–55.
9. Abd El-Gaber AS, El Gendy ANG, Elkhateeb A, Saleh IA, El-Seedi HR. Microwave extraction of essential oil from Anastatica hierochuntica (L): comparison with conventional hydro-distillation and steam distillation. J Essent Oil Bear Plants. 2018;21(4):1003–10.
10. Ghosh T, Zarif Morshed M, Islam N, Al Masud KN, Akter M, Islam R. In vitro investigation of antioxidant activity of Cissus adnata in different fractions. J Pharmacogn Phytochem. 2018;7(2):2625–8.
11. Otang WM, Grierson DS, Ndip RN. Phytochemical studies and antioxidant activity of two South African medicinal plants traditionally used for the management of opportunistic fungal infections in HIV/AIDS patients. BMC Complement Altern Med. 2012;12(1):1–7.
12. Ammor K, Bousta D, Jennan S, Bennani B, Chaqroune A, Mahjoubi F, et al. Phytochemical screening, polyphenols content, antioxidant power, and antibacterial activity of Herniaria hirsuta from Morocco. Sci World J. 2018;Article ID 7470384.
13. Tripathi AK, Gupta A, Garg SK, Tekwani BL. In vitro β-hematin formation assays with plasma of mice infected with Plasmodium yoelii and other parasite preparations. Life Sci. 2001;69(23):2725–33.
14. Afshar FH, Delazar A, Janneh O, Nazemiyeh H, Pasdaran A, Nahar L, et al. Evaluation of antimalarial, free-radical scavenging and insecticidal activities of Artemisia scoparia and A. spicigera. Rev Bras Farmacogn. 2011;21(6):986–90.
15. Dinda B, Debnath S, Banik R. Naturally occurring iridoids and secoiridoids. An updated review, part 4. Chem Pharm Bull. 2011;59(7):803–33.
16. Dinda B, Debnath S, Harigaya Y. Naturally occurring secoiridoids and bioactivity of naturally occurring iridoids and secoiridoids: a review, part 2. Chem Pharm Bull. 2007;55(5):689–728.
17. Toth E, Toth G, Mathe I, Blunden G. Martynoside, forsythoside B, ladanein and 7α-acetoxyroyleanone from Ballota nigra L. Biochem Syst Ecol. 2007;35(12):894–7.
18. Sahpaz S, Garbacki N, Tits M, Bailleul F. Isolation and pharmacological activity of phenylpropanoid esters from Marrubium vulgare. J Ethnopharmacol. 2002;79(3):389–92.
19. Noiarsa P, Ruchirawat S, Kanchanapoom T. Acanmontanoside, a new phenylethanoid diglycoside from Acanthus montanus. Molecules. 2010;15(12):8967–72.
20. Kim SR, Kim YC. Neuroprotective phenylpropanoid esters of rhamnose isolated from roots of Scrophularia buergeriana. Phytochemistry. 2000;54(5):503–9.
21. Santos JD, Lanza AMD, Fernández L, Rumbero A. Isoangoroside C, a phenylpropanoid glycoside from Scrophularia scorodonia roots. Z Naturforsch C. 2000;55(5-6):333–6.
22. Sousa EO, Miranda CM, Nobre CB, Boligon AA, Athayde ML, Costa JG. Phytochemical analysis and antioxidant activities of Lantana camara and Lantana montevidensis extracts. Ind Crops Prod. 2015;70:7–15.
23. Abdille MH, Singh R, Jayaprakasha G, Jena B. Antioxidant activity of the extracts from Dillenia indica fruits. Food Chem. 2005;90(4):891–6.
24. Asgharian P, Delazar A, Lotfipour F, Asnaashari S. Bioactive properties of Eremostachys macrophylla rhizomes growing in Iran. Pharm Sci. 2017;23(3):238–43.
25. Juan MY, Chou CC. Enhancement of antioxidant activity, total phenolic and flavonoid content of black soybeans by solid-state fermentation. Food Microbiol. 2010;27(5):586–91.
26. Sharififar F, Dehghn-Nudeh G, Mirtajaldini M. Major flavonoids with antioxidant activity from Teucrium polium L. Food Chem. 2009;112(4):885–8.
27. Sajjadi SE. Analysis of the essential oil of Nepeta sintenisii Bornm. from Iran. Daru J Pharm Sci.

2005;13(2):61–4.
28. Sharma A, Cannoo DS. Phytochemical composition of essential oils isolated from different species of genus Nepeta: a review. Pharmacophore. 2013;4(6):181–211.
29. Rustaiyan A, Khosravi M, Larijany K, Masoudi S. Composition of the essential oil of Nepeta racemosa Lam. from Iran. J Essent Oil Res. 2000;12(2):151–2.
30. Baser K, Demircakmak B, Altintas A, Duman H. Composition of the essential oils of Nepeta cadmea Boiss. J Essent Oil Res. 1998;10(3):327–8.
31. Rustaiyan A, Komeilizadeh H, Monfared A, Nadji K, Masoudi S, Yari M, et al. Volatile constituents of Nepeta denudata and N. cephalotes from Iran. J Essent Oil Res. 2000;12(4):459–61.
32. Thappa R, Agarwal S, Srivastava T, Kapahi B. Essential oils of four Himalayan Nepeta species. J Essent Oil Res. 2001;13(3):189–91.
33. Velasco-Negueruela A, Perez-Alonso MJ, Rodriquez AB. Essential oil analysis of Nepeta teydea. Flav Fragr J. 1989;4(4):197–9.