Research Journal of Pharmacognosy (RJP) 8(4), 2021: 1–8
Received: 2 May 2021
Accepted: 28 July 2021
Published online: 8 Aug 2021
DOI: 10.22127/RJP.2021.272832.1678

Original article

Phytochemistry and Bioactivity of *Nepeta racemosa* Lam.

Fariba Heshmati Afshar[1], Abbas Delazar[1], Yahya Rahimpour[2], Niloufar Moharrer Navaei[3], Solmaz Asnaashari[4], Parina Asgharian[1,5*]

[1]Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.
[2]Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran.
[3]Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.
[4]Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.
[5]Department of Pharmacognosy, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.

Abstract

Background and objectives: The genus *Nepeta* is used in traditional medicine for the antiseptic and astringent properties in cutaneous eruptions and snake bites. *Nepeta racemosa* was investigated here due to the rich source of phytochemical compounds in the *Nepeta* genus and the lack of any phytochemical studies. **Methods:** The aerial parts were extracted successively using n-hexane, chloroform, and methanol (MeOH) by maceration method, respectively. The MeOH extract was exposed to C18 Sep-Pak fractionation by a step gradient of MeOH-H_2O. Further purification of the fractions by preparative reversed-phase HPLC yielded three compounds. The chemical ingredients of essential oil were determined by GC-MS. Free-radical scavenging activity of the extracts, fractions, as well as their total flavonoid and phenolics contents, were assessed using the DPPH method, $AlCl_3$, and Folin-Ciocalteu reagents, respectively. **Results:** Phytochemical study of 20% and 40% solid phase extraction fractions of MeOH extract yielded one iridoid (8,9-epi-7-deoxy-loganic acid) and two phenylethanoids (forsythoside B and verbascoside), correspondingly. The 4aα,7α,7aα-nepetalactone (31.70 %), germacrene D (7.39 %), n-hexadecanoic acid (6.47 %), were the main compounds of essentials oil. The MeOH extract demonstrated high activity in terms of antioxidant activity, total phenolics content along with total flavonoids content (0.09 ± 0.01 mg/mL, 1581.80 ± 10.28 mg/100g and 33.01 ± 0.02 mg/100g) as well as 40% SPE fraction (0.01 ± 0.00 mg/mL, 659.20 ± 40.32 mg/100g and 22.5 ± 0.37 mg/100g), respectively. **Conclusion:** The presence of phenylethanoid derivatives as phenolic compounds appears to be an important antioxidant compound in *Nepeta racemosa*.

Keywords: antimalarial; antioxidant; essential oil; GC-MS analysis; *Nepeta racemosa*

Citation: Heshmati Afshar F, Delazar A, Rahimpour Y, Moharrer Navaei N, Asnaashari S, Asgharian P. Phytochemistry and bioactivity of *Nepeta racemosa*. Res J Pharmacogn. 2021; 8(4): 1–8.

Introduction

The genus *Nepeta* (Lamiaceae) contains 280 species distributed in Asia, Africa, and Europe. However, it is most abundant in the Mediterranean region [1]. *Nepeta* is called "Pune-sa" in Iran and noticeably, there are around 67 species present in Iran [2]. Some of these species are used in folk medicine for anti-septic and astringent properties in cutaneous eruptions; orally, they are used as diuretic, anti-pyretic, anti-asthmatic, anti-spasmodic, diaphoretic, antitussive, sedative, and stomachic properties. *Nepeta racemosa*, *N. bulgaricum*, *N. cataria* (catmint or catnip), and *N. cataria* var. citriodora (lemon catnip) are common and famous species of the *Nepeta* genus [3,4]. The plants of Labiatae family are main sources of secondary metabolites

*Corresponding author: asgharianp@tbzmed.ac.ir

including nepetalactones, phenols, iridoids, diterpenes and triterpenes. These aromatic plants are economically important and include most of the essential oil (mainly monoterpenoids) that occurs in the genera of Labiatae family. Moreover, several compounds from the Nepeta species have shown to have potent bioactivity [4]. Biological and pharmacological activities are typically credited to the occurrence of nepetalactone as a volatile compound, mainly found in *Nepeta* oils. *Nepeta* species in terms of essential oil compositions can be separated into two groups nepetalactone-containing and nepetalactone-less species [5]. Nepetalactone is an iridoid and is found in various isomeric forms in nature. The main compounds isolated from different species of *Nepeta* growing in Turkey were nepetalactone, caryophyllene oxide, and 1,8-cineole/linalool [6]. The chemotaxonomic and ethnopharmacological prominence of the genus *Nepeta* motivated us to carry out phytochemical studies as well as investigating in vitro anti-plasmodial activity, and antioxidant capacity of the aerial parts of *N. racemosa*.

Materials and Methods

Ethical considerations

This study was approved by Ethics Committee of Tabriz University of Medical sciences (IR.TBZMED.REC.1394.807).

Chemicals

All materials used were of analytical grade. Methanol, n-hexane, acetone, ethyl acetate, chloroform, DMSO, DMSO-d6, D_2O, hydrochloric acid (HCl), sodium hydroxide (NaOH), and sodium acetate were attained from (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.

Plant material

Nepeta racemosa wascollected from Marand in Eastern Azerbaijan province during May 2016. The identification of the plants was approved by Dr. Fatemeh Ebrahimi**,** a botanist of the Faculty of Pharmacy, Tabriz University of Medical Science. A voucher specimen of the plant (TBZFPH 2639) was kept at the Herbarium of Tabriz University of Medical Science.

Extraction

The ground and dried aerial parts of *N. racemosa* (100 g) were extracted by maceration successively, with *n*-hexane, chloroform, and MeOH (1.2 L each). All the extracts were concentrated via a rotary evaporator at a maximum temperature of 40 ºC. Four g of the dried MeOH extract was fractionated by solid-phase-extraction (SPE) on Sep-Pak (C18, 10 g, 35 cc cartridge) via a step gradient of MeOH-water mixture (10:90, 20:80, 40:60, 60:40, 80:20 and 100:0), 200 mL of each. All SPE fractions were dried by a rotary evaporator at a temperature of 40 ºC. The preparative HPLC (Dr. Mainsch GmbH ODS column 20 µm, 250 mm × 20 mm; mobile phase: 0-50 min, MeOH from 15% to 75% in water; 50-62.5 min, 75% MeOH in water, 62.5-67.5 min MeOH from 75% to 15% in water, 67.5-75 min 15% MeOH, flow rate = 8 mL/min) purification of the 20% MeOH SPE fraction yielded one iridoid glycosides**:** 1 (t_R = 33.31 min). Subsequently, the preparative HPLC (mobile phase: 0-50 min, MeOH from 35% to 60% in water; 50-62.5 min, 60% MeOH in water, 62.5-67.5 min MeOH from 60% to 35% in water, 67.5-75 min 35% MeOH, flow rate = 8 mL/min) for purification of the 40% MeOH SPE fraction yielded the two phenylethanoid glycosides: 2 (t_R = 14.45 min) and 3 (t_R = 18.49 min). Structures of 1–3 were determined conclusively by 1D (^{1}H and ^{13}C NMR).

Determination of total flavonoids content

The content of flavonoids was determined based on the aluminum chloride colorimetric method [7]. Briefly, 0.5 mL of each plant extracts (1 mg/mL) in methanol was separately mixed with 0.1 mL of 1 M potassium acetate, 0.1 mL of 10% aluminum chloride, 1.5 mL of methanol, and 2.8 mL of distilled water. It stayed at room temperature for 30 min, and the absorbance of the reaction blend was quantified spectrophotometrically at 510 nm. The yellow color indicated the existence of flavonoids. Total flavonoids contents were calculated as rutin equivalents (mg/g extract) (with different concentrations (0.0039, 0.0078, 0.0156, 0.0312, 0.0625, 0.125) from a calibration curve.

Determination of total phenolics content

The total content of phenolic compounds was

determined with the Folin-Ciocalteu reagent [8]. The 1 mL of extract samples (1 mg/mL) were mixed with Folin-Ciocalteu reagent (200 µL) for 5 min and 1 mL aqueous sodium carbonate 2 % (w/v) was added. The mixture was permitted to stay for 30 min and the total phenolics were determined by calorimetry at 750 nm. The standard curve was prepared using 5, 10, 15, 25, 50, 75, and 100 mg/L solutions of gallic acid in acetone: water mixture (60:50, v/v). Total phenol values were declared in terms of gallic acid equivalent (mg/g of dry mass) as the reference compound.

Isolation of the essential oil

The aerial parts of the air-dried *N. racemosa* (60 g) were submitted to hydro-distillation in a Clevenger type apparatus for 3 h for isolation of essential oil. The oil was dehydrated over anhydrous sodium sulfate and kept at 4 °C for further analysis [9]. The volume (ml) of essential oil per 100 g of plant material was recorded.

Gas chromatography mass spectrometry (GC-MS) analysis

The analysis of the essential oil was undertaken via a Shimadzu GC-MS-QP5050A, equipped with flame ionization detector (FID), and DB-1 capillary column (60 m × 0.25 mm id, 0.25 µm film thickness). Operating conditions were as follows: carrier gas, helium with a flow rate of 1.3 mL/min; column temperature, 2 min in 50 °C, then, the temperature was increased from 50 to 275 °C at 3.0 °C/min rate and finally it remained 3 min in 275 °C. The temperature of the injector and detector were 250 and 280 °C, respectively; the volume injected, and split ratio were adjusted to 1 µL and 1:20, respectively. Mass spectra were taken at 70 eV over the mass range 30-600 amu, ion source temperature at 260 °C, and solvent delay 2.0 min. The constituents of the essential oil were assessed by the comparison of their mass spectra and Kovats indices with those of the spectrophotometer database using the Wiley and NIST mass spectral databases or with authentic compounds.

Antioxidant assay

The ability for inhibiting the free radicals of the extracts and fractions were measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical quenching [10]. The extracts/fractions were dissolved in MeOH/chloroform to obtain the stock concentration of 10 mg/mL. Serial dilutions were obtained from the stock solutions of the plant extracts and fractions. A volume of 2.5 mL of DPPH solution (0.08 mg/mL in MeOH) was mixed with diluted solutions (2,5 mL each) and remained at room temperature in the dark for 30 min for any reaction to happen. The UV absorbance was recorded at 517 nm which corresponds to the maximum absorption of DPPH. The tests were repeated in triplicate and the average absorption was noted for each concentration. The inhibitory capacity IC_{50} values that presents scavenging 50% of DPPH free radicals was computed from the graph plotting inhibition percentage against test sample concentrations [11,12]. Inhibitory capacity (IC%) was calculated according to the following formula where AB is the absorbance of the blank and AA is the absorbance of test samples.

$$IC\% = [(AB–AA)/AB] \times 100$$

Anti-plasmodial activity

The potential anti-malarial activity of samples was evaluated using a modified assay by Tripathi et al. [13] with some alterations [14]. The results were reported as % inhibition (I%) of hem polymerization/crystallization compared to the positive control (chloroquine diphosphate) by the following formula:

$$I\% = ((AB - AA)/AB) \times 100$$

AB is absorbance of the blank, and AA is the absorbance test samples.

Results and Discussion

A blend of solid-phase extraction and reversed-phase preparative HPLC analysis of the 20% and 40% fractions of *N. racemosa* MeOH extract guided to the identification of one iridoid (A) and two phenylethanoids (B, C) structures (Figure1). The chemical structures of the constituents have been recognized through nuclear magnetic resonance spectroscopy. Three compounds were known by comparison of their chemical analysis with those reported in the literature. The chemical structures of the isolated constituents were elucidated unequivocally via ^{13}CNMR and ^{1}HNMR.

Compound A: 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).

From the comparison of these data with those reported in the literature [15,16], the compound was identified as 8,9-epi-7-deoxy-loganic acid (Figure 1A).

Compound B: 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).

From the comparison of these data with those reported in the literature [17,18], the compound was identified as forsythoside B (Figure 1B).

Compound C: 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}C NMR(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). From the comparison of these data with those reported in the literature [19-21], the compound was identified as verbascoside (Figure 1C).

Based on their chemical structures, the three compounds isolated from *N. racemosa* can be classified into two phytochemical subclasses including one iridoid and two phenylethanoids. Total polyphenols and flavonoids in the methanolic extract and methanolic fractions of the aerial parts of *N. racemosa* extracts are presented in Table 1

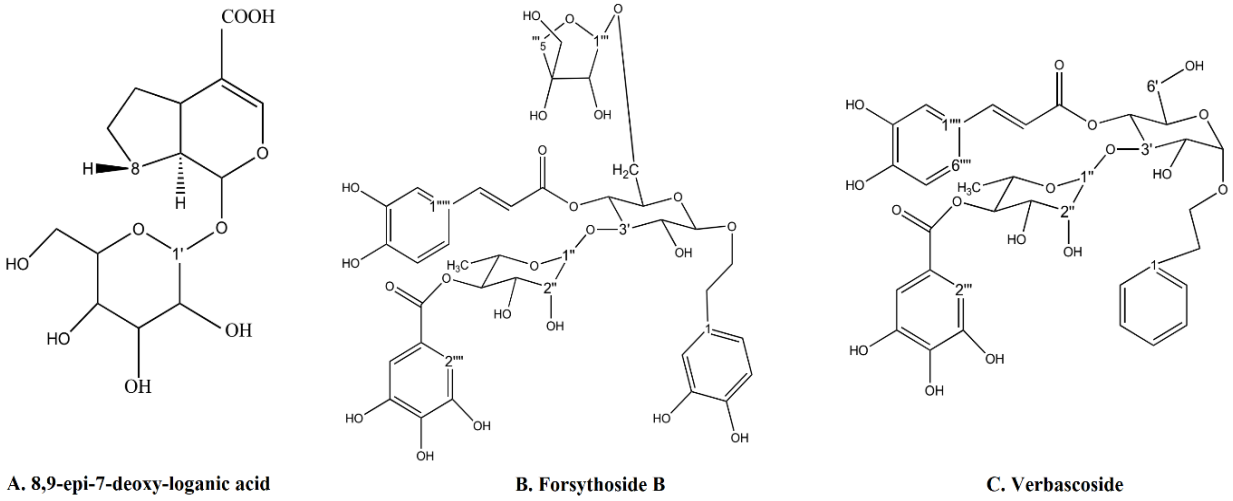

Figure 1. Structures of compounds isolated from *Nepeta racemose*

Table 1. Total flavonoids content, total phenolic content, and antioxidant activity of the chloroform, n-hexane, and MeOH extract and respective fractions of *Nepeta racemosa*

Extracts/fractions	Total flavonoids content as rutin (mg/100g)	Total phenolics content as galic acid (mg/100g)	Antioxidant activity (RC_{50}) (mg/mL)
Chloroform extract	-	-	0.16 ± 0.05
Methanol extract	33.01 ± 0.02	1518.80 ± 10.28	0.09 ± 0.04
n-Hexane extract	-	-	0.41 ± 0.01
SPE fraction 10% MeOH-water	1.30 ± 0.12	20.60 ± 0.73	0.32 ± 0.13
SPE fraction 20% MeOH-water	1.20 ± 0.08	58.10 ± 1.16	0.19 ± 0.02
SPE fraction 40% MeOH-water	22.50 ± 0.37	659.20 ± 40.32	0.01 ± 0.00
SPE fraction 60% MeOH-water	12.70 ± 0.42	197.70 ± 3.81	0.09 ± 0.04
SPE fraction 80% MeOH-water	0.80 ± 0.04	37.40 ± 0.75	0.42 ± 0.02
SPE fraction 100% MeOH	1.10 ± 0.10	117.60 ± 5.18	0.34 ± 0.04
Quercetin (positive control)	-	-	2.78×10^{-5}

Values represent the mean ± SD, n=3

. The order of phenolics content in the extracts was methanol extract > Fr 40 > Fr 60 > Fr 100 > Fr 20 > Fr 80 > Fr 10 and the order of flavonoids was methanol extract > Fr 40 > Fr 60 > Fr 10 > Fr 20 > Fr 100 > Fr 80. The Fr 40 % represented significantly higher phenolic contents (659.20 ± 40.32 g/mg) and flavonoids content (22.50 ± 0.37 g/mg).

Determination of the contents of flavonoids and phenolic compounds is important because the free radicals scavenging activity of numerous plant extracts is ascribed to the incidence of these constituents which have biological activities, chiefly the flavonoids [22].

According to literature, total phenolics content and free radical scavenging activity of the plant material are in line with one another [23,24]. Phenolic components are responsible for the decreasing amount of free radicals and chelating characteristics [25,26]. Based on our current outcomes in the present manuscript, total flavonoids and total phenolics of 40%, 20%, and 60% MeOH-water fractions were higher than the other fractions in corresponding to the free radical scavenging activity of these fractions. Thus, the antioxidant potential of *N. racemosa* extracts may be attributed to the flavonoid compounds. The components of the essential oils, their retention indices (RI), and percentage compositions are presented in Table 2. All compounds are listed in turn of their retention time on the column. Fifty-three constituents were identified by GC-MS, which accounted for 77.1% of the total oil of the plant material. Among these compounds 4aα,7α,7aα-nepetalactone (31.70 %), germacrene D (7.39 %), and n-hexadecanoic acid (6.47 %), were the most dominant ingredients of the essential oil. Oxygenated monoterpene, monoterpene hydrocarbons, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, and fatty acid derivatives presented 33.53 %, 0.18 %, 12.64 %, 9.41 %, and 12.89 % of the essential oil respectively. Other common ingredients (8.47%) were non-terpenoid derivatives such as ketones and aldehydes. According to the nepetalactone-content, *N. racemosa* could be categorized in the group in which nepetalactones are the major component. 1,8-cineole and caryophyllene oxide are the most component of the volatile oils of nepetalactone-free *Nepeta* species [27] and in this study, they were in low amounts (0.33 %) and (3.98), respectively. The 4aα,7α,7aα-nepetalactone, 4aβ,7α,7aβ-nepetalactone, 4aα,7α,7aβ-nepetalactone, 4aβ,7α,7aαnepetalactone are the four main nepetalactone compound which present in greater amount than other isomers like 4α dihydro nepetalactone, 4aα,7β,7aα-nepetalactone, and 4aα,7β,7aβ nepetalactone [28]. The 4aα,7α,7aα-nepetalactone was the only nepetalactone obtained in this paper. It is also found as a major compound of essential oil of many *Nepeta* species such as *N. racemosa* [1, 2, 29], *N. cadmea* [30], *N. cephalotes* [31]**,** *N. govaniana* [32], and *N. teydea* [33].

The free-radical-scavenging activity of the SPE fractions and the extracts were examined via the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) free-radical scavenging assay (Table 2) [10]. The 40% MeOH-water SPE fraction exhibited the highest level of free-radical scavenging activity with an RC_{50} value of (0.01 ± 0.00 mg/mL) amongst all extracts. The MeOH extract and The SPE fraction 60% MeOH-water showed similar activity (0.09 ± 0.04 mg/mL). The lowest activity was obtained for the n-hexane extract (0.41 ± 0.01mg/mL) and SPE fraction 80% MeOH-water (0.42 ± 0.02 mg/mL).

Table 2. The essential oil composition of the aerial parts of *Nepeta racemosa*

Compound	Oil composition (%)	Reported KI [a]	Calculated KI
2-Hexenal, (E)-	0.23	825	831
1 Octen 3 ol	0.07	964	967
β-Pinene	0.19	971	972
Trans, Trans-2,4-Heptadienal	0.10	981	984
1,8-Cineole	0.33	1021	1028
Nonanal	0.18	1083	1080
Linalool	0.39	1086	1088
Trans-Pinocarveol	0.33	1125	1129
Pinocarvone	0.10	1140	1146
4-Terpineol	0.14	1164	1169
Myrtenal	0.16	1171	1172
α-Terpineol	0.24	1175	1177
Myrtenol	0.14	1181	1189
4aα,7α,7aα-Nepetalactone	**31.70**	1367	1370
α-Copaene	0.24	1380	1381
β-Bourbonene	0.67	1388	1389
β-Elemene	0.79	1391	1395
β-Caryophyllene	2.09	1423	1427
β-Cubebene	0.23	1431	1439
α-Humulene	0.13	1455	1456
α-Amorphene	0.27	1475	1477
Germacrene D	**7.39**	1482	1485
α-Farnesene	0.23	1497	1496
γ-Cadinene	0.21	1510	1513
δ-Cadinene	0.39	1518	1526
Dodecanoic acid	0.21	1549	1550
1,5-Epoxysalvial-4(14)-ene	0.39	1562	1567
(-)-Spathulenol	0.61	1571	1573
Caryophyllene oxide	3.98	1577	1579
Salvial-4(14)-en-1-one	0.93	1585	1592
1-Tetradecanol	0.51	1628	1630
Widdrol	0.33	1635	1636
Cycloisolongifolene, 8-hydroxy-, endo-	1.13	1644	1648
β-Copaen-4 α-ol	0.50	1669	1670
6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthalene-2-ol	1.82	1675	1673
Octadecanal	0.08	1697	1697
Tetradecanoic acid	0.33	1746	1750
Hexahydrofarnesyl acetone	1.12	1833	1836
n-Pentadecanoic acid	0.14	1844	1846
1-Hexadecanol	0.14	1861	1862
7,9-di-tert-butyl-1-oxaspiro [4.5] deca-6,9-diene-2,8-dione	0.16	1874	1875
Pentadecanoic acid, 14-methyl-, methyl ester	0.20	1911	1912
n-Hexadecanoic acid	**6.47**	1951	1950
1-Octadecanol	0.34	2066	2064
Methyl linolenate	0.27	2081	2079
Phytol	3.96	2105	2098
Methyl-11,14,17-eicosatrienoate	1.46	2290	2285
Hexadecanal diallyl acetal	0.12	2295	2293
Hexadecanoic acid, butyl ester	0.18	2174	2169
Nonadecanoic acid	2.82	2236	2228
Hexadecanamide	1.18	2350	2348
Hexanedioic acid, bis(2-ethylhexyl) ester	0.53	2361	2360
Heneicosane	0.27	-	2378
Total identified	**77.12**		
Terpene	**55.76**		
Non-terpene	**21.36**		

[a] Compounds listed in order of elution from a DB-1 column.

The in vitro β-hematin formation assay was applied for testing the anti-malarial activity of total extracts of *N. racemosa* aerial parts. The extracts exhibited no inhibitory results on heme biocrystallization properties.

Conclusion

In this study, 40% SPE fraction of MeOH extract from the aerial part of *N. racemosa* could be a valuable source for future studies due to strong free-radical-scavenging activity as well as high amounts of total phenolic and flavonoid content and the main components such as forsythoside B, and verbascoside.

Acknowledgments

The present research was financially supported by Vice chancellor for Research, Tabriz University of Medical sciences (IR.TBZMED.REC.1394.807).

Author contributions

Fariba Heshmati Afshar and Abbas Delazar designed and supervised the study; Yahya Rahimpour prepared the manuscript the article; Niloufar Moharrer Navaei performed the experiments; Solmaz Asnaashari and Parina Asgharian analyzed the data.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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Abbreviations

HPLC: high performance liquid chromatography; GC-MS: gas chromatography mass spectrometry; DPPH: 2,2-diphenyl-1-picryl-hydrazyl; MeOH: methanol; UV: ultraviolet; RI: retention indices; SPE; solid-phase extraction; TPC: total phenolics content; TFC: total flavonoids content