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Original Article

Qualitative and Quantitative Analysis of *Nepeta leucostegia* Essential Oil Components by GC–MS/FID Along With Antioxidant Activity

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Abstract

Background: *Nepeta* is a large genus belonging to the Lamiaceae family. So far, no study has been reported on *Nepeta leucostegia* compounds and biological effects.
Objectives: In the present study, we aimed to evaluate the qualitative and quantitative analysis of *Nepeta leucostegia* essential oil components along with antioxidant activity.
Methods: Essential oil compounds of *N. leucostegia* were qualitatively and quantitatively identified and analyzed using gas chromatography–mass spectrometry (GC-MS) and GC with flame ionization detection (GC-FID) analysis. Besides, the evaluation of the antioxidant property was performed using DPPH assay.
Results: After analysis, 96.63% of essential oil compounds were identified. Among the 36 identified compounds, 1,8-cineole (39.1%), epi-α-cadinol (6.9%), α-Terpineol (6.0%), α-fenchene (5.8%), and Camphene (5.5%) were the main compounds, respectively. The essential oil showed a significant antioxidant effect (IC_{50} = 75 μg/mL).
Conclusion: The results of this study complete the information on the *Nepeta* genus and can be used in the chemotaxonomic study of the *Nepeta* genus. Due to its antioxidant effect, *N. leucostegia* has the potential for use in pharmaceutical and food industries.
Keywords: *Nepeta leucostegia*, Medicinal plant, Essential oil, Antioxidant, DPPH, GC-MS

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Background

Medicinal plants and natural compounds are among the main sources for discovering new drugs. Recently, due to their availability and fewer side effects, natural products have gained significant popularity among people. Plants, especially edible ones, are more important in terms of daily use. In addition to nutrition, plants are suitable sources of useful compounds such as antioxidants, antibacterials, and anti-inflammatory agents (1,2).

Free radicals produced by various factors in the body can cause various problems such as inflammation and cancer. For this reason, preventing the formation of free radicals in the body or removing them is one of the most important ways to maintain health and prevent diseases. Natural compounds found in plants such as phenols, flavonoids, and terpenes have shown significant antioxidant effects due to their structure and functional groups. Therefore, the oral use of plants as well as natural products such as medicine and nutritional supplements containing antioxidant compounds plays an important role in controlling and eliminating free radicals (3,4).

Nepeta is a large genus belonging to the Lamiaceae family and comprises 280 species. In previous studies, different medicinal properties of the species of this genus, such as sedative, diuretic, anti-spasm, anti-asthma, anti-allergic, diaphoretic, and febrifugal effects, were reported (5-8). Roughly 67 species of the genus *Nepeta* are found in Iran. *Nepeta leucostegia* is indigenous to Kurdistan province (Sanandaj) in western Iran. The phytochemical and biological evaluation of *N. leucostegia* essential oil was done for the first time in this study.

Materials and Methods

Preparation of Plant Material

The aerial parts of *N. leucostegia* were gathered in June 2021 from Kurdistan province (Hawraman mountains), Iran, and identified by Hiva Ghaderi according to voucher number 3104 in Herbarium of the Research Institute of Forests and Rangeland, Sanandaj, Iran.

Preparation of Essential Oil

The extraction of the essential oil was done by distillation using a Clevenger apparatus. For this purpose, 100 g of the aerial parts of the plant was transferred into the flask containing water, and the distillation process was carried out for 3 hours. In the end, the resulting essential oil was collected and its water was removed using sodium sulfate (9).

Analysis of the Essential Oil

Briefly, 1 mL of essential oil extracted by water distillation was injected into a gas chromatography device coupled with a mass spectrometer (GC/MS) to identify compounds. The column of this device was 60 m in length, 0.25 mm in diameter, and 0.25 µm in thickness in the inner layer. The carrier gas of this device was helium with a flow of 1.1 mL/min. The temperature program of the column was set from 60 to 250°C at a rate of 5°C/min and was kept at 250°C for 2 minutes. The detector of the device had a power of 70 eV. The temperature of the injection site and detector was set at 250 and 300°C. The separated compounds were identified using reference libraries (the Adams Library and Wiley Library) as well as the calculation of the Kovats index and comparison with the sources. The Kovats index was obtained based on the retention index of normal alkanes (C6-C30) under the same temperature and injection conditions as the sample (5,10). GC-FID was used to determine the amount of essential oil compounds. The temperature program, injection amount, column, and other parameters were similar to GC-MS device. The peak area percentages of GC-FID were calculated using DB-5 column without using correction factors (11).

Antioxidant Properties

The antioxidant activity of the essential oil was evaluated by measuring free radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH). The samples with different concentrations were mixed with 1 mL of 90 µM DPPH° solution, made up to 4 mL with 95% methanol, and stirred for one hour in the dark. The absorbance of the samples and control solutions was read after one hour at a wavelength of 517 nm using a spectrophotometer. Tests were carried out in triplicate and butylated hydroxytoluene was used as positive control (12).

Results

The yield of the essential oil of *N. leucostegia* aerial parts was reported to be 0.4% v/w of the plant. According to the result of gas chromatography–mass spectrometry (GC-MS) and *GC with flame ionization detection* (GC-FID) analysis of *N. leucostegia* essential oil, 36 compounds were identified which comprise 96.63% of total essential oil (Figure 1). The essential oil of the aerial parts of *N. leucostegia* was

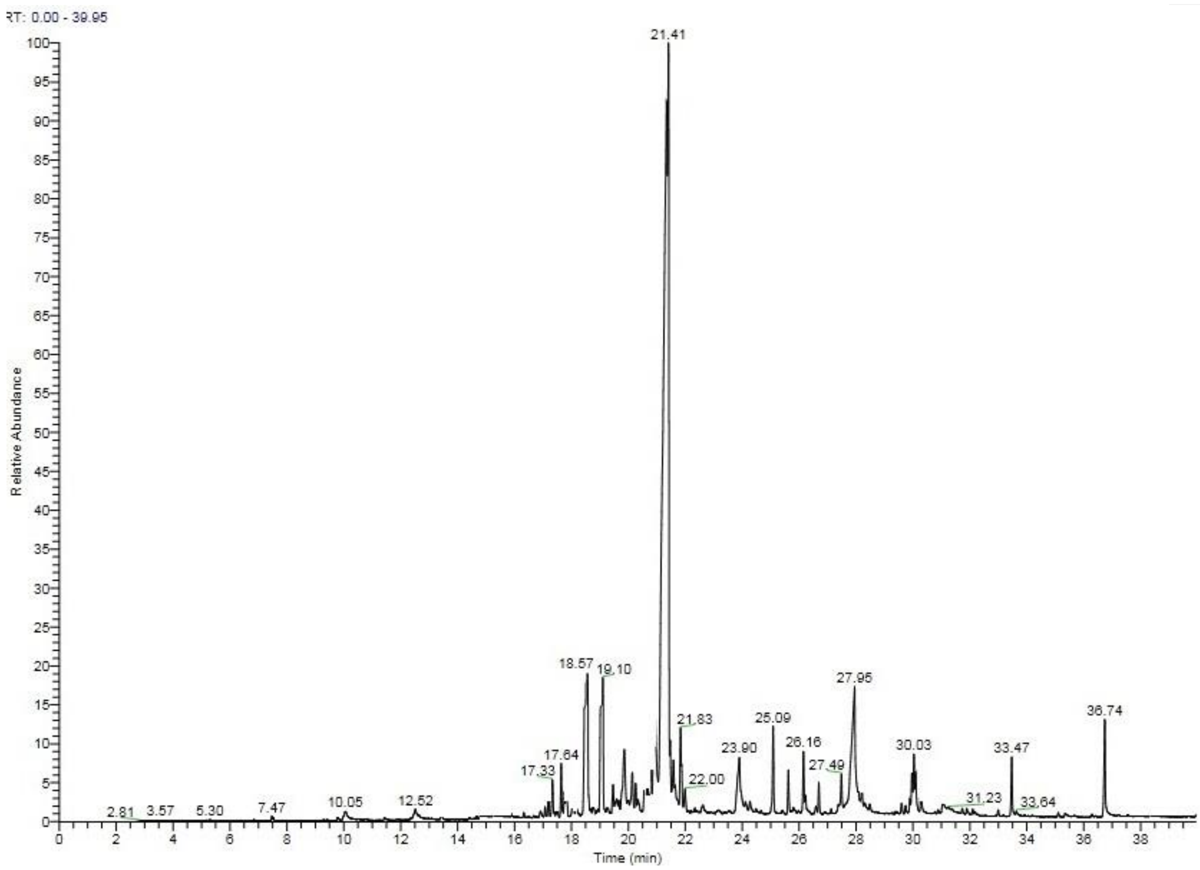

Figure 1. GC-MS Chromatogram of the Essential Oil of *Nepeta leucostegia*

rich in oxygenated monoterpenes. The major component was 1,8-Cineole (39.1%) and the other compounds with almost high percentages were epi-α-cadinol (6.9%), α-terpineol (6.0%), α-fenchene (5.8%), and camphene (5.5%), respectively (Table 1, Figure 2). Essential oil compounds mostly included oxygenated monoterpenes, which comprise 57.3% of the total compounds. The most abundant constituents of essential oil were oxygenated sesquiterpenes, followed by monoterpene hydrocarbons The sesquiterpene hydrocarbons and other compounds formed the smallest group of essential oil compounds. According to DPPH assay, the essential oil showed a significant antioxidant effect (IC_{50} = 75 μg/mL).

Discussion

In terms of efficiency, *N. leucostegia* essential oil is in the range of ordinary plants, and its density is lower than water. 1,8-cineole is the main compound of essential oil, which is one of oxygenated monoterpenes according to its structure. 1,8-cineole has a special structure that, due to its oxygen bridge and stereochemistry, has the ability to interact with different receptors, and as a result, it is effective. Due to the high content of 1,8-cineole (40%) in the essential oil of the plant as well as its special structure, *N. leucostegia* is suggested as a potential source of 1,8-cineole.

According to a review on *Nepeta* genus, there are two main essential oil chemotypes in *Nepeta* species including nepetalactone and 1,8-cineole and/or linalool chemotype. In other words, *N. leucostegia* essential oil belongs to 1,8-cineole and/or linalool chemotype, indicating that it can be used in the chemotaxonomic study of *Nepeta* genus (13,14). The essential oils of herbal medicine, which showed extensive pharmacological properties including anti-inflammatory and antioxidant and were used for the treatment of respiratory diseases, cardiovascular diseases, and so on, are rich in 1,8-cineole. Therefore, *N. leucostegia* can be used in medicine and food industry as an antioxidant and anti-inflammatory agent (15).

The monoterpene compounds make up the largest percentage of essential oils and consist of two isoprene units. Due to their lower molecular weight compared to other terpenoids, monoterpenes comprise a higher percentage of plant essential oils. For this reason, perfume and cosmetic products are widely used in aromatherapy. Additionally, various biological effects such as antioxidant, antimicrobial, and anti-inflammatory activities have been reported for them (16,17).

In traditional medicine of different countries including Iran, India, Pakistan, China, Nepal, and Turkey, different species of *Nepeta* genus are used for treating various diseases, including Asthma, respiratory disorders, influenza, pneumonia, tuberculosis, chicken pox, and eye irritation (18). *N. leucostegia* is used in traditional and ethnobotanical medicine to treat stomach problems, colds, and coughs. According to DPPH assay, the essential oil showed a significant antioxidant effect (IC_{50} = 75 μg/

Table 1. Composition of the Essential Oil of *Nepeta Leucostegia*

Compound[c]	**RI[a]**	**RI[b]**	**Area% (±SD[c])**	**Method of Identification**
2E-Hexenal	845	846	0.18 ± 0	RI, MS[d]
α-Thujene	925	924	0.25 ± 0	RI, MS
α–Pinene	934	932	3.2 ± 0.3	RI, MS
α-Fenchene	**946**	**945**	**5.8 ± 0.5**	RI, MS
Camphene	**948**	**946**	**5.5 ± 0.4**	RI, MS
Myrcene	1000	988	0.9 ± 0.1	RI, MS
P-Cymene	1023	1020	0.1 ± 0	RI, MS
1,8-Cineole	**1027**	**1026**	**39.1 ± 1.6**	RI, MS
Linalool	1097	1095	0.5 ± 0.1	RI, MS
α-Campholenal	1124	1122	0.3 ± 0	RI, MS
cis-β-Terpineol	1142	1140	0.5 ± 0.1	RI, MS
Trans-Verbenol	1143	1140	2.0 ± 0.3	RI, MS
Pinocarvone	1160	1160	2.1 ± 0.2	RI, MS
Umbellulone	1168	1167	0.1 ± 0	RI, MS
Terpinen-4-ol	1173	1174	1.9 ± 0.1	RI, MS
α-Terpineol	**1187**	**1186**	**6.0 ± 0.5**	RI, MS
Myrtenol	1195	1194	0.5 ± 0.1	RI, MS
Myrtenal	1196	1195	2.2 ± 0.2	RI, MS
Verbenone	1204	1204	0.5 ± 0.1	RI, MS
cis-Carveol	1228	1226	0.4 ± 0	RI, MS
Carvone	1240	1239	0.2 ± 0	RI, MS
Perilla alcohol	1295	1294	0.1 ± 0	RI, MS
Aromadendrene	1440	1439	0.4 ± 0.1	RI, MS
Germacrene D	1486	1484	0.2 ± 0	RI, MS
α-Selinene	1499	1498	0.5 ± 0.1	RI, MS
γ-Cadinene	1515	1513	1.7 ± 0.1	RI, MS
δ-Cadinene	1525	1522	0.1 ± 0	RI, MS
Germacrene D-4-ol	1577	1574	2.6 ± 0.2	RI, MS
Spathulenol	1579	1577	3.8 ± 0.3	RI, MS
Caryophyllene oxide	1585	1582	0.2 ± 0	RI, MS
Globulol	1594	1590	0.9 ± 0.1	RI, MS
Viridiflorol	1597	1592	2.1 ± 0.2	RI, MS
Ledol	1604	1602	1.3 ± 0.1	RI, MS
epi-α-Cadinol	**1641**	**1638**	**6.9 ± 0.8**	RI, MS
Cubenol	1647	1645	0.7 ± 0.1	RI, MS
α-Cadinol	1655	1652	2.0 ± 0.3	RI, MS
Monoterpene hydrocarbons			15.75	
Oxygenated monoterpenes			57.3	
Sesquiterpene hydrocarbons			2.9	
Oxygenated sesquiterpenes			20.5	
Others			0.18	
Total identified			96.63	

[a] RI = retention indices calculated from the homologous series (C6 – C30).
[b] RI = retention indices according to literature.
[c] Standard deviation for three replications.
[d] MS = library (Adams and Wiley) of the mass spectrometer.

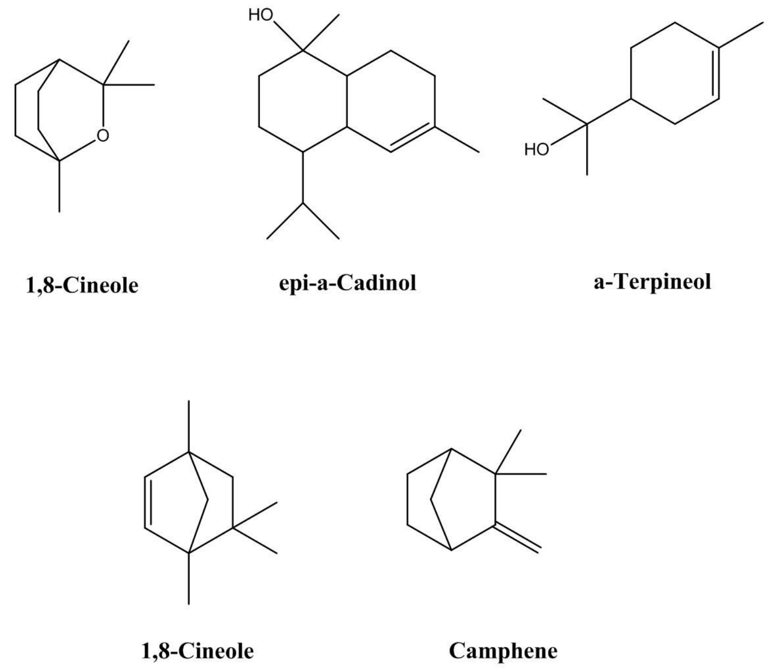

Figure 2. The Major Compounds of the Essential Oil of *Nepeta leucostegia*

mL). It should be noted that the IC_{50} value for the BHT standard was reported to be 26 (Table 2). Due to the side effects of synthetic antioxidant compounds, the use of natural compounds with antioxidant properties in the pharmaceutical, cosmetic, and food industries is widely accepted (19). Compared to the essential oil of other *Nepeta* species, it has significant antioxidant properties. In previous studies, essential oils of *N. cataria*, *N. sintenisii*, and *N. curviflora* species showed antioxidant effects with IC_{50} of 80.62, 716.0, and 180.0 µg/mL, respectively (20-22). Recent studies on the antioxidant activity of essential oils reported radical scavenging activity that occurs through their constituents (23). The antioxidant effect is caused by the components of the essential oil, especially 1,8-cineole and epi-α-cadinol. In the study conducted by Ciftci et al, 1,8-cineole showed antioxidant activity in rats and eliminated TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin)-induced oxidative stress (24).

Conclusion

The phytochemical and biological evaluation of *N. leucostegia* essential oil was done for the first time in this study. The yield of plant essential oil was 0.4% v/w and 96.63% of the total essential oil compounds were identified. The main group of the essential oil belonged to oxygenated monoterpenes and the main composition of the essential oil was 1,8-Cineole. The results of this study complete the information on the *Nepeta* genus and can be used in the chemotaxonomic study of the *Nepeta* genus. Due to its antioxidant effect (IC_{50} = 75 µg/mL), *N. leucostegia* has the potential for use in pharmaceutical and food industries. Therefore, other properties of this plant include its nutritional values and it is suggested that its interaction with other foods should be studied in the future.

Table 2. Antioxidant Activity of the Essential Oil of *Nepeta leucostegia*

Sample	DPPH assay IC_{50} (µg/mL)
Essential oil	75 ± 1.2
BHT	26 ± 0.7

All tests were performed in triplicate.

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Authors' Contribution

Conceptualization: Dara Dastan.
Data curation: Dara Dastan.
Formal analysis: Dara Dastan, Baram Hamah Ameen.
Funding acquisition: Dara Dastan.
Investigation: Dara Dastan.
Methodology: Dara Dastan.
Project administration: Dara Dastan.
Resources: Hiva Ghaderi.
Supervision: Dara Dastan.
Validation: Dara Dastan.
Visualization: Dara Dastan.
Writing–original draft: Dara Dastan.
Writing–review & editing: Baram Hamah Ameen.

Competing Interests

The authors declare no conflict of interests regarding the publication of this paper.

Ethical Approval

Not applicable.

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