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Chemical Composition and Biological Activities of Essential Oil from *Ocimum* basilicum L. Collected in Dak Lak, Vietnam

Dam T. B. Hanh¹, Truong N. Ngu¹*, Phan H. T. Bao¹, Trinh N. T. Vy¹, Nguyen T. V. Dung¹, Dang T. T. My¹, Le T. T. Loan¹, Do T. Lam², Phi H. Nguyen², Nguyen P. Khanh³, Dao C. To⁴*

¹ Tay Nguyen University, Buon Ma Thuot, Dak Lak 630000, Vietnam

² Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet, Cau Giay District, Hanoi 122100, Vietnam
 ³ Department of Biochemistry, Military Hospital 175, 876 Nguyen Kiem Street, Go Vap District, Ho Chi Minh City 70000, Vietnam
 ⁴ Phenikaa University Nano Institute (PHENA), Phenikaa University, Yen Nghia, Ha Dong District, Hanoi 12116, Vietnam

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ABSTRACT

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Ocimum basilicum Linn (Lamiaceae), also known as sweet Basil, is a widely recognized medicinal plant. Although this species has been extensively researched on its biological activities and chemical composition in some Provinces of Vietnam, however, studies on the chemical composition and biological properties of this species growing in Dak Lak Province have not been investigated to date. The chemical composition of the essential oil from O. basilicum, collected in Vietnam, was analyzed using the gas chromatography-coupled mass spectrometry (GC-MS) method. The essential oil content yielded 0.55%, with 34 compounds being determined. Among them, the major constituents (%) were as follows: estragole (73.66%), eucalypto (6.41%), trans-α-bergamotene (3.97%), linalool (2.25%), fenchol (1.64%), 2isopropyl-5-methyl-9-methylene- bicyclo[4.4.0]dec-1-ene (1.47%), 1,3,6-octatriene,3,7dimethyl-(Z) (1.36%), (+)-2-bornanone (1.34%), and methyl eugenol (1.11%). Furthermore, an antibacterial test on O. basilicum essential oil was conducted, and the results demonstrated strong antibacterial activity against *Escherichia coli* with an inhibition zone of 18.6 ± 0.9 mm and the lowest minimum inhibitory concentration (MIC) value ($4.3 \pm 0.2 \text{ mg/mL}$). Additionally, the antioxidant capacity of Basil essential oil was evaluated using the 2,2-diphenyl-1picryhydrazyl (DPPH) method. The antioxidant efficiency of essential oil was determined with an IC₅₀ of 6.5 \pm 0.3 µg/mL, compared with butylated hydroxytoluene (BHT) with an IC₅₀ value of $6.1 \pm 0.1 \ \mu$ g/mL. These findings indicate that O. basilicum grown in Vietnam possesses a high natural potential for pharmaceutical applications.

Keywords: Ocimum basilicum Linn, estragole, eucalypto, essential oil, trans- α -bergamotene, antioxidant, antibacterial

Introduction

Ocimum basilicum L., known as sweet Basil, belongs to the Lamiaceae.¹ The herb is native to India but thrives in tropical climate countries including Vietnam. Traditionally, the herb has been used as spices and as a medicinal plant of traditional medicine. Thereby, the therapeutic potentials of this species are utilized in the treatment of numerous diseases such as headaches, diarrhea, coughs, flu, warts, constipation, worms, diabetes, cardiovascular diseases, or kidney malfunctions.²⁻⁴ Basil is known as a popular culinary herb and the essential oil from this herb is used as flavor foods or as oral and dental products, and in perfumery.⁵ The aromatic properties of each type of Basil depend on the main chemical compounds (phenylpropanoids and monoterpenes).^{6,7}

*Corresponding author. E mail: ntnhan@ttn.edu.vn; cuong.todao@phenikaa-uni.edu.vn Tel: +84-902856011; +84-971886989

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The essential oil, second metabolites, extracts and other products of *O. basilicum* have been discovered to play multiple roles as antioxidant, anti-inflammatory, antimicrobial, antifungal, insect-repelling, anticonvulsant, hypnotic, anticancer activities.^{4,8-16}

Basil has been cultivated and popular in Vietnam since 1975. A number of provinces in Vietnam have grown on a large scale to store essential oils for the domestic and foreign aromatics industries. However, studies on this species' chemical composition and biological properties have not been explored to date. Quynh Anh *et al.* reported the Basil essential oil from Thua Thien Hue Province contained crucial chemical components, namely *p*-allylanisole (49.09%), aromadendrene (8.27%), and *trans*-ocimene (5.71%).¹⁷ The results further revealed that the essential oil had a relatively low antioxidant capacity, with an IC₅₀ value of 35.89 µg/mL.¹⁷ However, it exhibited antibacterial effects by inhibiting the growth of two bacterial strains, *E. coli*, and *Salmonella* sp.

The research highlights that the chemical compositions and biological activities of essential oils can vary significantly depending on the geographical locations and soil conditions where the plants are grown. This emphasizes the importance of considering the origin and environment when studying essential oils and their potential applications. In this study, the experimental work focused on extracting essential oil from *O. basilicum*. Following the extraction process, GC-MS was used to analyze and characterize the chemical composition of the essential oil. In addition, the antioxidant and antibacterial activities of this Basil oil were also investigated.

Chemicals

Butylated hydroxytoluene (BHT), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Tween 80, homologous series of C7-C30 straight-chain hydrocarbons, and various reference chemicals used for identification were obtained from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). All other chemicals and analytical grades were purchased from Merck (Darmstadt, Germany). All culture media and standard antibiotic discs were purchased from Oxoid Ltd. (Basingstoke, Hampshire, UK).

Plant material

The leaves and stems of *O. basilicum* were collected from Tan Tien commune (12°40'34"N 108°2'7"E), Buon Ma Thuot City, Dak Lak Province, Vietnam in January 2023. A voucher specimen (No: HQ-BMT-01) was deposited at the Faculty of Natural Science and Technology, Tay Nguyen University, Buon Ma Thuot City, Dak Lak Province, Vietnam.

Essential oil extraction

The fresh leaves and stems (2.5 kg) of *O. basilicum* were cleaned, cut into smaller pieces, and subjected to steam distillation in a Clevenger-type apparatus for 4h. The obtained essential oil was dehydrated by anhydrous sodium sulfate and stored in a sealed vial at 4°C in the dark prior to analysis.

Essential oil analysis

GC-MS analysis of *O. basilicum* leaves and stems essential oil was carried out on a Thermo Trace GC Ultra - ITQ900 gas chromatography. Data interpretation was performed using the MassFinder 4.0 software. A fused-silica capillary TG-SQC column (30 m x 0.25 mm i.d., 0.25 μ m film thickness) was used for separation.

GC operation conditions: injector temperature = 250°C; detector temperature = 260°C; oven temperature programme: from 60 to 260°C at 4°C.min⁻¹. Helium was used as carrier gas at a flow rate of 1.0 mL.min⁻¹. An oil sample (1 μ L) was injected using split mode with a split ratio of 1:10.

GC-MS operation conditions: mass spectrometer was operated in electron-impact (EI) mode; ionization energy was 70 eV; interface temperature was 280°C; ion source temperature was 230°C; MS quadrupole temperature was 200°C; scan range was 35-650 amu. GC operation conditions were identical to those described above for GC.

Identification and quantification of essential oil constituents

Retention indices of oil constituents were determined on the HP-5 MS column using standard C7-C30 straight-chain hydrocarbons. Individual compounds in the oil were identified by comparison of their mass spectra and retention indices with those in GC-MS libraries (NIST 08, Wiley 09th Version) and/or with those reported in the literature. The relative percentage amounts of the separated compounds were computed from GC data without the use of correction factors.

Antioxidant activity

The antioxidant activity of the *O. basilicum* essential oil was assessed by measuring its scavenging ability to the DPPH stable radicals. The DPPH assay was performed according to the methods previously described.¹⁸ The samples (from 0.5 to 15.5 μ g mL⁻¹) were mixed with 1 mL of 90 μ M DPPH solution and filled up with 95% methanol, to a final volume of 4 mL. The absorbance of the resulting solutions and the blank were recorded after 1 h at room temperature. BHT was used as a positive control. The disappearance of DPPH was read spectrophotometrically at 515 nm using a spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan). Inhibition of free radicals by DPPH in percent (%) was calculated in the following way:

I (%) = $100 \times (A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}})$

where A_{blank} is the absorbance of the control reaction mixture excluding the test compounds, and A_{sample} is the absorbance of the test compounds. IC₅₀ values, which represented the concentration of essential oils that caused 50% neutralization of DPPH radicals, were calculated from the plot of inhibition percentage against concentration.

Antimicrobial activity

Microbial strain: the antibacterial activity of *O. basilicum* essential oil was evaluated using a Gram-negative strain - *E. coli* (ATCC 25922) obtained from laboratory stock cultures. Bacterial strains were cultured overnight at 37 °C in nutrient agar (NA, Oxoid).

Disc diffusion method: the antimicrobial activity of O. basilicum essential oil was performed according to the methods previously described.¹⁹ Briefly, 100 µL of suspension of tested microorganisms, containing 10⁸ colony-forming units (CFU)/mL of bacteria cells spread on nutrient agar (NA). The filter discs (6 mm in diameter) were individually soaked with 15 µL of essential oil and placed on the agar plates which had previously been inoculated with the tested microorganisms. Discs without samples were used as a negative control. Amoxycillin (30 µg/disc) was used as a positive reference. The Petri dishes were kept at 4 °C for 2 h. The plates were incubated at 37°C for 24 h. Antimicrobial activity was evaluated by measuring the diameter of the growth inhibition zones in millimeters (including a disc diameter of 6 mm) for the test organisms and comparing them to the controls. The measurements of inhibition zones were carried out for three sample replications, and the values are the average of three replicates.

Determination of minimum inhibitory concentration (MIC): for the determination of MIC, a micro-dilution broth susceptibility assay was used.²⁰. All tests were performed in nutrient broth (NB) supplemented with Tween 80 detergent to a final concentration of 0.5% (v/v). Bacterial strains were cultured overnight at 37°C in NB. Dilutions series were prepared from 0.07 to 72.0 mg/mL of the essential oil in a 96-well microtitre plate, 160 μ L of NB was added onto microplates, and 20 μ L of tested solution. Then, 20 μ L of 5 × 10⁵ CFU/mL of standard microorganism suspension were inoculated onto microplates. Plates were incubated at 37°C for 24 h. The same test was performed simultaneously for the growth control (NB + Tween 80) and sterility control (NB + Tween 80 + test oil). Amoxycillin was used as a reference compound for antibacterial activity.

Statistical analysis

All treatments were conducted in triplicate and statistical analysis of the data was performed by analysis of variance (ANOVA) using STATISTICA 5.5 (Stat Soft Inc., Tulsa, OK, USA) software. Data are presented as the mean \pm standard deviation (S.D).

Results and Discussion

Composition of the essential oil of O. basilicum leaves and stems

The essential oil of O. basilicum from its leaves and stems yielded 0.55% (w/w, 1.375g essential oil/2.5kg fresh leaves and stems) through hydro distillation, resulting in a strong-smelling yellow oil. The GC-MS analysis produced a total ion chromatogram as shown in Figure 1, and the composition of the essential oil is summarized in Table 1. A total of 34 compounds were detected, accounting for 99.33% of the total oil, and were identifiable. The identification of these 34 compounds was achieved by comparing their mass spectra and retention indices with those present in GC-MS libraries (Supporting information). Each compound was assigned a notation based on its peak number for further discussion in this manuscript. Additionally, the structural formulae of these compounds were provided in Figure 1 as input for further computations and analysis. In our analysis of the chemical composition of Basil essential oil, a total of 34 compounds were identified by comparing their mass spectra and retention indices with those in GC-MS libraries as shown in Table 1. The majority of the oil's components are terpenes, and they are approximately evenly distributed among three main groups: oxygenated monoterpenes (13 compounds, accounting for 88.22% of the total oil), monoterpene hydrocarbons (8 compounds, accounting for 2.73% of the total oil), and sesquiterpenes (12 compounds, accounting for 8.18% of the total oil). Among these, the oxygenated compounds are the most dominant, making up 88.22% of the total oil. The essential oil's major components include estragole (73.66%), eucalypto (6.41%), trans-α-bergamotene (3.97%), linalool (2.25%), (1.64%), 2-isopropyl-5-methyl-9-methylenefenchol bicyclo[4.4.0]dec-1-ene (1.47%), 1,3,6-octatriene,3,7-dimethyl-(Z)

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(1.36%), (+)-2-bornanone (1.34%), and methyl eugenol (1.11%) (Table 1). These components collectively make up a significant portion of the essential oil's composition. The presence of various terpenes, especially oxygenated monoterpenes, contributes to the unique aroma and potential therapeutic properties of the Basil essential oil.

When comparing the chemical composition of Basil essential oil from various sources, including different regions in Vietnam and other countries, significant differences were observed. The Basil essential oil from Buon Ma Thuot City had lower levels of estragole but higher levels of *trans-α*-bergamotene compared to the samples from Ho Chi Minh City and Thanh Hoa Province.^{21,22} Additionally, specific compounds like eucalypto, fenchol, 2-isopropyl-5-methyl-9-methylene-bicyclo[4.4.0]dec-1-ene, 3,7-dimethyl-(*Z*)-1,3,6-octatriene, were found only in Buon Ma Thuot Basil, while τ-cadinol, 1,8-cineole, and 3-carene were absent in Buon Ma Thuot Basil. In addition, the Basil essential oils from Binh Dinh and Binh Duong samples showed

variations in their main components.^{23,24} Binh Dinh and Binh Duong samples had high levels of methyl chavicol, while Thua Thien Hue samples had p-allylanisole as a significant component. estragole, which was a major component in Buon Ma Thuot Basil oil, was not found in these samples.^{25,24} Compared to other Basil essential oils, it is worth noting that the plants from Ethiopia produced estragole, similar to Buon Ma Thuot Basil, but at a significantly lower concentration. The Ethiopian sample did not contain any other major components found in Buon Ma Thuot Basil.²⁵ The samples, from other countries like India, Egypt, Saudi Arabia, Algeria, and Oman showed significant variations compared to Buon Ma Thuot Basil. Each region had its specific set of major components, and compounds like estragole, methyl cinnamate, β -elemene, methyl chavicol, eugenol, and others were either present or absent in different proportions. These variations in the chemical compositions of Basil essential oil can be attributed to differences in climatic and soil conditions in various regions, as well as genetic variations in the Basil plants.²⁶



Figure 1: GC-MS total ion chromatogram of O. basilicum leaves and stems esstential oil.

Table 1: Chemical compositions from the essential oil of O. basilicum leaves and stems.

Peak no.	Retention (min)	time Compounds	Molecular fomular	Relative amount (%)
1	4.45	α-Pinene	$C_{10}H_{16}$	0.08
2	5.06	Sabinene	$C_{10}H_{16}$	0.11
3	5.08	β -Pinene	$C_{10}H_{16}$	0.24
4	5.17	1-Octen-3-ol	$C_{10}H_{16}O$	0.30
5	5.30	β -myrcene	$C_{10}H_{16}$	0.48
6	5.71	Eucalyptol	$C_{10}H_{18}O$	6.41
7	5.83	<i>trans-β</i> -Ocimene	$C_{10}H_{16}$	0.18
8	5.92	3,7-Dimethyl-(Z)-1,3,6-octatriene	$C_{10}H_{16}$	1.36
9	6.03	γ-Terpinene	$C_{10}H_{16}$	0.09
10	6.26	L-Fenchone	$C_{10}H_{16}O$	0.20
11	6.29	Terpinolene	$C_{10}H_{16}$	0.19
12	6.37	Linalool	$C_{10}H_{18}O$	2.25
13	6.48	Fenchol	$C_{10}H_{18}O$	1.64
14	6.67	Camphor	$C_{10}H_{16}O$	1.34
15	6.88	Sabinol	C ₁₀ H ₁₈ O	0.31

16	6.95	Terpinen-4-ol	$C_{10}H_{18}O$	0.24	
17	7.09	Estragole	$C_{10}H_{12}O$	73.66	
18	7.21	Fenchyl acetate	$C_{12}H_{20}O_2$	0.50	
19	7.57	Bornyl acetate	$C_{12}H_{20}O_2$	0.19	
20	7.65	Thymol	$C_{10}H_{14}O$	0.07	
21	8.06	Copaene	$C_{15}H_{24}$	0.06	
22	8.11	Methyl eugenol	$C_{11}H_{14}O_2$	1.11	
23	8.26	Caryophyllene	$C_{15}H_{24}$	0.25	
24	8.31	trans-a-bergamotene	$C_{15}H_{24}$	3.97	
25	8.36	Germacrene D	C15H24	0.15	
26	8.41	Humulene	C15H24	0.14	
27	8.45	cis-Muurola-4(15),5-diene	C15H24	0.15	
28	8.52	Unknow			
29	8.56	Alloaromadendrene	$C_{15}H_{24}$	0.08	
30	8.62	a-Bulnesene	$C_{15}H_{24}$	0.86	
31	8.65	a-Amorphen	$C_{15}H_{24}$	0.86	
32	8.92	Aristolediene	$C_{15}H_{22}$	0.06	
33	9.06	Epicubenol	$C_{15}H_{24}O$	0.20	
34	9.15	Bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene-	$C_{15}H_{24}$	1.47	
35	9.20	Alloisolongifolene	$C_{15}H_{24}$	0.13	
Total number	of constituents	35			
Number (%)	of constituents identif	34 (99.33%)			
Number (%) of monoterpene hydrocarbons			8 (2.73%)		
Number (%)	of oxygenated monote	13 (88.22%)			
Number (%) of sesquiterpene hydrocarbons			12 (8.18%)		
Number (%) of oxygenated sesquiterpenes			1 (0.2%)		
Number (%) of others					

Indeed, the comparison of Basil essential oil compositions from different regions worldwide reveals significant variations in their chemical profiles. In the study conducted in Turkey, the main components of Basil essential oil varied among its different parts. The flowers had the highest content of estragole (58.26%), followed by the leaves (52.60%), and the stems (15.91%).²⁷ However, in the Buon Ma Thuot Basil sample, the level of estragole was lower, and limonene and *p*-cymene were not detected.^{4,28} Basil essential oil from the Western Ghats of South India primarily contained methyl cinnamate (70.1%), linalool (17.5%), β -elemene (2.6%), and camphor (1.52%).²⁹⁻³⁰ In Buon Ma Thuot Basil, the linalool level was higher, but methyl cinnamate, β -elemene, and camphor were absent. In another sample from Karnataka, India, the main components were methyl eugenol (39.5%) and methyl chavicol (38.3%), differing from the Buon Ma Thuot Basil, which had estragole (73.66%) as the main composition.²⁹⁻

Basil essential oil from Egypt was mainly composed of methyl chavicol (27.82%) and linalool (25.35%), while the Saudi Arabian sample contained eugenol (25.85%) and linalool (13.41%). In Buon Ma Thuot Basil, neither methyl chavicol nor eugenol was detected, but there was a higher level of linalool.²⁹ Another Algerian Basil essential oil had significant levels of linalool (32.83%) and myrcene (6.12%), which differed from Buon Ma Thuot Basil.³⁰ Compounds such as linalyl acetate, elemol, geranyl acetate, allo-ocimene, α -terpineol, (*E*)- β -ocimene, and neryl acetate were not found in the Buon Ma Thuot Basil sample. Interestingly, the Basil essential oil from Owan was primarily composed of linalool (69.87%), geraniol (9.75%), *p*-allylanisole (6.02%), 1,8-cineole (4.90%), *trans-* α -bergamotene (2.36%), and neryl acetate (1.24%).³¹ In contrast, Buon Ma Thuot

Basil had estragole (73.66%) as the dominant component and did not contain geraniol and neryl acetate. However, the levels of *p*-allylanisole, 1,8-cineole, and *trans-* α -bergamotene were higher in Buon Ma Thuot Basil. These significant variations in the chemical compositions of Basil essential oil from different regions indicate the strong influence of diverse climatic and soil conditions on the plant's development and oil production. Each region's specific environmental factors contribute to the unique aromatic and medicinal properties exhibited by basil essential oil, making it a subject of great interest for further research and exploration.

Antioxidant activity of O. basilicum essential oil

The test results indicate that the antioxidant activity of O. basilicum essential oil from Dak Lak Province is very strong (Table 2). The results showed that the essential oil exhibited an increased free radical scavenging capacity in a concentration-dependent manner. Comparing the essential oil's antioxidant capacity with the positive control, BHT, it was found that the essential oil's IC_{50} value was 6.5 \pm 0.3 $\mu g/mL,$ while the IC₅₀ value for BHT was $6.1 \pm 0.1 \ \mu g/mL$. This indicates that the antioxidant capacity of the essential oil demonstrates comparable antioxidant activity. Furthermore, when comparing the antioxidant capacity of O. basilicum essential oil from Dak Lak Province with that of essential oil from Algeria,²⁸ it was found that the Dak Lak essential oil exhibited much higher antioxidant activity (IC₅₀ = 6.5 μ g/mL) compared to the Algerian essential oil (IC₅₀ = $83.4 \ \mu g/mL$). These findings highlight the significant antioxidant potential of O. basilicum essential oil from Dak Lak Province and suggest promising opportunities for its future applications as a natural antioxidant agent. The strong antioxidant activity of the essential oil makes it a valuable candidate for further research and development in various industries, including pharmaceuticals, cosmetics, and food products. **Table 2:** Antioxidant activity of *O. basilicum* essential oil

Samples	$IC_{50} \left(\mu g/mL\right)^{a}$
Essential oil	6.5 ± 0.3
BHT^b	6.1 ± 0.1

^{*a*} The values were expressed as mean values \pm S.D of three parallel measurements; ^{*b*} Positive control.

 Table 3: Antibacterial activity of O. basilicum essential oil against E. coli.

Samula	Antibacterial activity	
Sample	IZD (mm)	MIC (mg/mL)
DMSO	6.0 ± 0.1	
Essential oil	18.6 ± 0.9^{b}	4.3 ± 0.2
Amoxicillin ^c	24.5 ± 0.6	0.3 ± 0.1

^{*a*} Not test; ^{*b*} The values were expressed as mean values \pm S.D of three parallel measurements; ^{*c*} Positive control; IZD (Inhibition Zone Diameters); MIC (Minimum Inhibitory Concentration).

Antibacterial activity of O. basilicum essential oil

Table 3 presents the antimicrobial activity of O. basilicum essential oil against E. coli bacteria. The essential oil of O. basilicum exhibited strong antimicrobial activity against E. coli tested with inhibition zone $(18.6 \pm 0.9 \text{ mm})$ and lowest MIC value $(4.3 \pm 0.2 \text{ mg/mL})$. In this study, amoxicillin, used as a positive control, had an inhibition zone of 24.5 \pm 0.6 mm and the lowest MIC values (0.3 \pm 0.1 mg/mL) (Table 3). It was revealed that O. basilicum essential oil showed greater activity against bacterial strains. Comparing these results with the studies by Quynh Anh et al. and colleagues in Hue City (E. coli: antibacterial circle of 5.08 mm) and Thanh Tuyen et al. in Binh Dinh (E. coli: antibacterial circle of 16.0 mm), it is evident that the antibacterial ability of Basil essential oil from Dak Lak Province is stronger.^{17,23} This finding aligns with the study by Sienkiewicz et al. in 2013, which highlights Basil essential oil as a potential natural antibacterial agent.³² Additionally, the combination of Basil essential oil with other essential oils can further enhance its antibacterial efficacy. Overall, the results demonstrate the significant antimicrobial potential of O. basilicum essential oil against E. coli bacteria, making it a promising candidate for further exploration and potential applications in the field of natural antibacterial agents.

Conclusion

In conclusion, the essential oil from O. basilicum collected in Buon Ma Thuot City, Vietnam, has been thoroughly analyzed using GC-MS, revealing a diverse chemical composition. A total of 34 natural components have been identified. The major constituents include estragole (73.66%), eucalypto (6.41%), trans-α-bergamotene (3.97%), linalool (2.25%), fenchol (1.64%), 2-isopropyl-5-methyl-9-methylenebicyclo[4.4.0]dec-1-ene (1.47%), 1,3,6-octatriene,3,7-dimethyl-(Z) (1.36%), (+)-2-bornanone (1.34%), and methyl eugenol (1.11%). The essential oil has demonstrated remarkable antioxidant capacity with an IC_{50} value of 6.5 ± 0.3 µg/mL. Furthermore, it exhibited significant antibacterial activity, inhibiting E. coli with an inhibition zone of 18.6 \pm 0.9 mm and the lowest MIC value of 4.3 \pm 0.2 mg/mL. These findings highlight the promising pharmaceutical applications of O. basilicum essential oil, given its diverse chemical composition and potent antioxidant and antibacterial properties. The results encourage further research and development of this essential oil for potential use in various industries, including pharmaceuticals and natural health products.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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