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Original Research Article

Chemical Composition and Biological Activities of Essential Oil from *Plectranthus amboinicus* Collected in Dak Lak, Vietnam

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ABSTRACT

Plectranthus amboinicus (Lamiaceae) is a medicinal plant widely cultivated and naturalized in the tropics of the Old and New World, with a history of use in folk medicine. However, there has been no prior investigation into the chemical composition and biological properties of this species in the Dak Lak Province, Vietnam. In this study, the essential oil extracted from *P. amboinicus*, collected in Buon Ma Thuot City in Dak Lak Provincfe was analyzed using gas chromatography-mass spectrometry (GC-MS). A total of 89 compounds were identified, constituting 0.62% of the oil; the major constituents included 2,3,5,6-tetramethylphenol (67.94%), caryophyllene (9.74%), *trans*-α-bergamotene (5.82%), α-humulene (3.20%), γ-terpinene (2.5%), *o*-cymene (1.90%), and terpinen-4-ol (1.14%). Notably, the carvacrol content exceeded previously reported values. In addition, *P. amboinicus* showed strong antibacterial activity against *Escherichia coli* using the agar disc diffusion method, with approximately 88.35% inhibition and a diameter of 47.3 mm. The antioxidant capacity of the essential oil was evaluated with the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, yielding an antioxidant efficiency of 89.21% and an IC_{50} value of 44.15 µg/mL. This demonstrates the high pharmaceutical potential of the *P. amboinicus* species grown in Vietnam.

Keywords: *Plectranthus amboinicus*, carvacrol, caryophyllene, antimicrobial, antioxidant

Introduction

Plectranthus amboinicus (also known as *Coleus amboinicus*) is a member of the mint family Lamiaceae, which comprises over 200 genera and 3500 species distributed in the tropical regions of the world. Plants of this family are widely cultivated in Vietnam, with more than 40 genera and approximately 145 existing species.[1,2] *Plectranthus* is a genus of approximately 85 species of flowering plants; it comprises various plants with pharmacological potential, which are used to treat different diseases in traditional communities worldwide.[3-7] *Plectranthus amboinicus* (*P. amboinicus*) is known by different names, such as "rau tan day la" and "rau thom lun". In the context of food processing, the leaves of *P. amboinicus* are used as a spicy and aromatic spice, known for their nontoxic nature. Additionally, in folk medicine, these leaves have been used to treat coughs, fevers, diarrhea, and insect bites.[8,9] One of the notable features of *P. amboinicus* is its essential oil content.

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Several research studies have explored the essential oil properties and other aspects of this plant, attracting the interest of international scientists.[10-19] However, despite the long history of using *P. amboinicus* in remedies, healthcare preparations and products derived from it are limited; it has, thus, received little attention for practical use. Notably, no prior research related to the *P. amboinicus* species in Dak Lak has been conducted. Therefore, this study aims to determine the chemical composition and biological properties of *P. amboinicus* to aid in its exploitation and effective utilization.

Material and Methods

Chemicals

Ascorbic acid, DPPH, Tween 80, a homologous series of C7–C30 straight-chain hydrocarbons, and various reference chemicals for identification were procured from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). All other chemicals, including those of analytical grade, were acquired from Merck (Darmstadt, Germany). The culture media and standard antibiotic discs were obtained from Oxoid Ltd. (Basingstoke, Hampshire, UK).

Plant Material

Leaves and stems of *P. amboinicus* were gathered from Tan Tien commune (12°40′34″N 108°2′7″E), Buon Ma Thuot City, Dak Lak Province, Vietnam in January 2023 (Figure 1). To serve as a reference, a voucher specimen (No: HC-BMT-01) was deposited at the Faculty of Natural Science and Technology, Tay Nguyen University, Buon Ma Thuot City.

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Figure 1: The fresh leaves and stems of *P. amboinicus* collected in Buon Ma Thuot City, Dak Lak Province.

Essential Oil Extraction

The leaves and stems of *P. amboinicus* were cleaned, cut into smaller pieces, and subjected to steam distillation using a Clevenger-type apparatus for 4 hours. The obtained essential oil was dehydrated with anhydrous sodium sulfate and stored in a sealed vial at 10 ℃ in the dark before analysis.

Essential Oil Analysis

Gas chromatography-mass spectrometry (GC-MS) analysis of the essential oil from the leaves and stems of *P. amboinicus* was conducted using a Thermo Trace GC Ultra - ITQ900 system (Thermo Fisher Scientific, MA, USA). Data interpretation was performed using MassFinder 4.0 software. Separation was achieved using a fused silica capillary TG-SQC column (30 m x 0.25 mm i.d., 0.25 µm film thickness).

GC Operation Conditions

The GC operation conditions included an injector temperature of 250 ℃, a detector temperature of 260 ℃, and an oven temperature program from 60 to 260 ℃ at a heating rate of 4 ℃/min. Helium served as the carrier gas at a flow rate of 1.0 mL/min. An oil sample (1 µL) was injected using the split mode with a split ratio of 1:10.

MS Operation Conditions

The mass spectrometer was operated in electron-impact (EI) mode, with an ionization energy of 70 eV, interface temperature of 280 ℃, ion source temperature of 230 ℃, MS quadrupole temperature of 200 ℃, and scan range of 35–650 amu. The GC operation conditions were identical to those described in the section above, "GC Operation Conditions".

Identification and Quantification of Essential Oil Constituents

The retention indices of the oil constituents were determined on an HP-5 MS column using standard C7–C30 straight-chain hydrocarbons (Sigma-Aldrich Chemical Company, USA). Individual compounds in the oil were identified by comparing 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 percentages of the separated compounds were computed from GC data without the use of correction factors.

Antioxidant Activity

The antioxidant activity of the *P. amboinicus* essential oil extract was assessed using the DPPH assay. Different concentrations of the extract in methanol (ranging from 1 to 30 mg/mL) and a positive control, ascorbic acid, were mixed with 200 µL of a methanolic solution containing DPPH radicals at a concentration of 150 µmol/L. The resulting mixtures were then vigorously shaken and allowed to stand for 30 minutes in the dark for the reactions to run to completion. Subsequently, the absorbance of the solutions was measured using a Shimadzu UV1800 spectrophotometer (Shimadzu Corporation, Japan) at 517 nm against a blank (a control solution with no extract or ascorbic acid). Each test was performed in triplicate to maintain accuracy and reliability. The scavenging ability was calculated as in Equation 1:

$$Scavenging\ acbility\ (\%) = \frac{A_{517}\ of\ control - A_{517}\ of\ sample}{A_{517}\ of\ control}\ x\ 10 \quad (1)$$

Antimicrobial activity

The antibacterial activity of the *P. amboinicus* leaf essential oil was evaluated using a Gram-negative strain – *Escherichia coli* (*E. coli*; ATCC 25922) – obtained from laboratory stock cultures and the agar disc diffusion method. A liquid culture of *E. coli* (at a concentration of 10^7 colony-forming units per milliliter [CFU/mL]) was spread evenly on a solid medium in a Petri dish. Circular pieces of filter paper with a diameter of 6 mm were placed in the center of the dish. The essential oil from *P. amboinicus* was extracted by steaming and dissolved in 10% dimethyl sulfoxide (DMSO); 40 µL of the essential oil was then applied to the filter paper, using 10% DMSO as a negative control. The Petri dishes were then sealed and incubated. The diameter of the inhibition zones formed around the filter paper was measured and used as an indicator of antimicrobial activity, and the entire assay was conducted in triplicate to maintain accuracy. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the *P. amboinicus* essential oil that visibly inhibits the growth of the bacteria.[14] The essential oil was dissolved in ethanol, and two-fold serial dilutions were carried out in a 96-well plate to yield a concentration range of 1.0 to 10.0 mg/mL. A bacterial broth medium (20 µL) was added to each well to produce the different solution concentrations. The pH of the medium was adjusted to a value in the range of 7.4 to 7.6, and the microplates were incubated at 37 ℃ for 24 hours. Each assay was performed in triplicate to ensure the reliability and accuracy of the results. The MIC value is a measure of the potency of the essential oil as an antimicrobial agent against *E. coli*.

Statistical Analysis

All treatments were carried out in triplicate, and the data were subjected to statistical analysis using the analysis of variance (ANOVA) with Statistica 5.5 software (Stat Soft Inc., Tulsa, OK, USA). The results are presented as the mean ± standard deviation (SD).

Results and Discussion

Composition of the Essential Oil of P. amboinicus Leaves and Stems

The essential oil extracted from the leaves and stems of *P. amboinicus* was obtained in a yield of 0.62% (w/w, fresh weight) via hydrodistillation and was characterized as a strongly scented yellow oil. Figure 2 displays the total ion chromatogram obtained from the GC-MS analysis of the oil, and the detailed composition is provided in Table 1. A total of 89 compounds were detected in the essential oil, accounting for 99.48% of the overall oil composition. Of these compounds, 78 were identified by comparing their mass spectra and retention indices with reference data from GC-MS libraries (Supporting information). The peak numbers in the chromatogram were used as a notation system to identify each compound and facilitate further discussions within this report. The structural formulas of the identified compounds are presented in Figure 2 to aid in subsequent computations and analysis.

The essential oil is predominantly composed of terpenes, with nearly equal proportions of the various terpene classes. These include oxygenated monoterpenes, comprising six compounds and accounting for 1.85% of the total oil; monoterpene hydrocarbons, consisting of six compounds and representing 4.63% of the total oil; and sesquiterpenes, the most abundant group, with 15 compounds, constituting 88.45% of the total oil.

Further, 33 nitrogen compounds are present, accounting for 1.13% of the total oil. Notably, the major components of the essential oil, along with their respective percentages, include 2,3,5,6-tetramethylphenol (67.94%), caryophyllene (9.74%), *trans*-α-bergamotene (5.82%), α-humulene (3.20%), γ-terpinene (2.5%), *o*-cymene (1.90%), and terpinen-4-ol (1.14%). These represent the significant constituents of *P. amboinicus* essential oil and provide valuable insights into its potential applications and properties.

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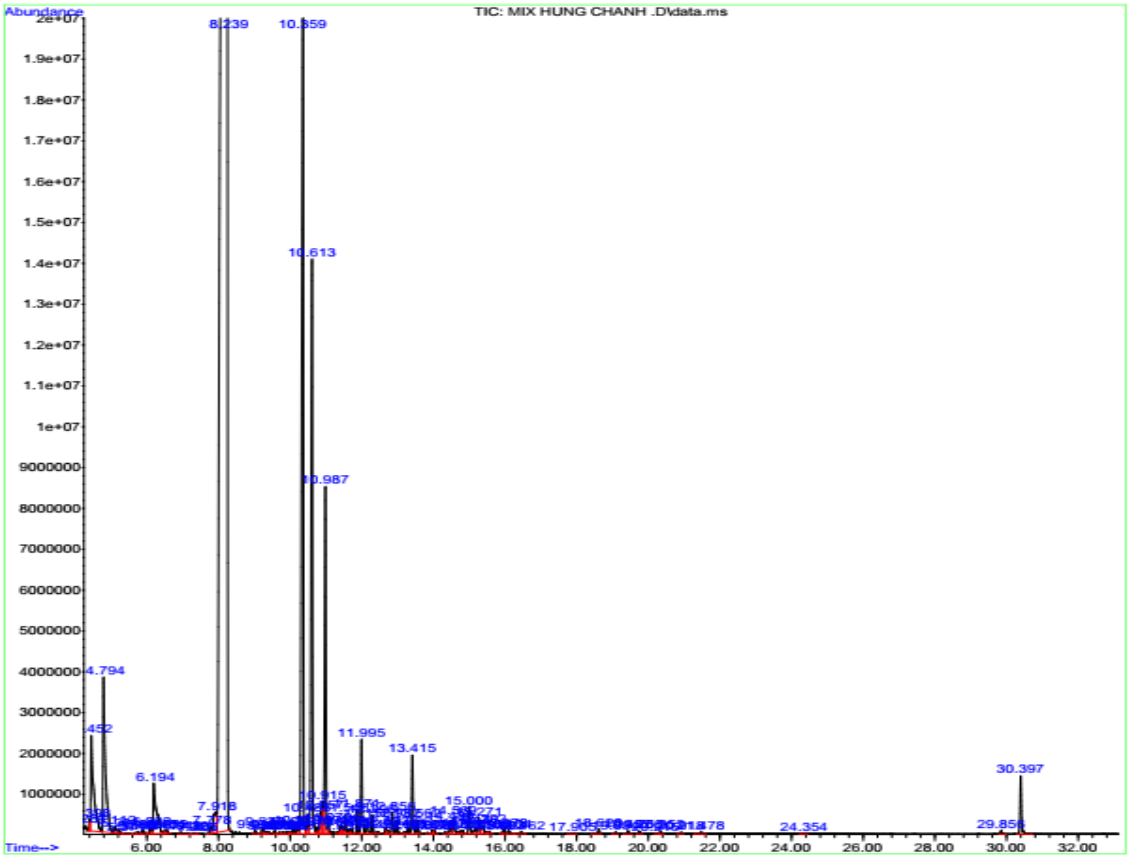

Figure 2: GC-MS total ion chromatogram of *P. amboinicus* leaves and stems essential oil.

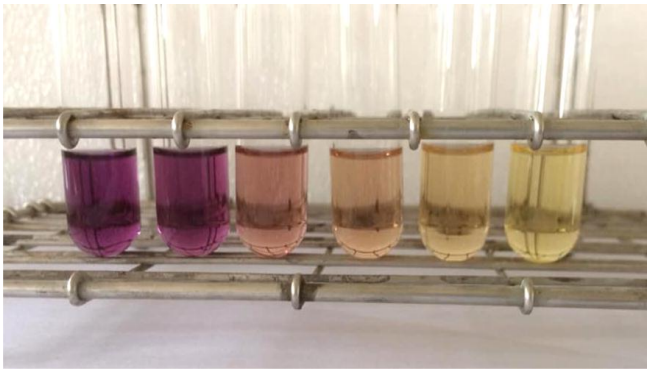

Figure 3: The resistance to oxidation of *P. amboinicus* essential oil in various concentrations.

Several differences were observed when comparing the chemical composition of *P. amboinicus* essential oil from Buon Ma Thuot City, Dak Lak Province, with the survey results conducted by Lu TMT in Cu Chi District, Ho Chi Minh City.[8] The oil from Buon Ma Thuot City has higher levels of carvacrol but lower caryophyllene levels compared to the specimen from Cu Chi District. Furthermore, the oil from Buon Ma Thuot City contains compounds such as α-humulene, *o*-cymene, γ-terpinene, *trans*-α-bergamotene, and terpinen-4-ol, though these are absent from the oil specimen from Cu Chi District.

Nguyen *et al.* studied the chemical composition of *P. amboinicus* essential oil in Can Tho City, Vietnam,[9] which shows some notable differences compared to the composition of the oil from Buon Ma Thuot City. The essential oil from Can Tho City has higher levels of cymene and γ-terpinene but lower levels of carvacrol and caryophyllene compared to the specimen from Buon Ma Thuot City. Furthermore, α-caryophyllene and caryophyllene oxide are present in the essential oil from Can Tho City but are absent from the oil from Buon Ma Thuot City; on the other hand, α-humulene, *trans*-α-bergamotene, and terpinen-4-ol are absent from the former but were detected at relatively high levels in the latter.[9]

Studies have also reported the chemical compositions of *P. amboinicus* essential oil from other parts of the world. The findings of the current study reveal that *P. amboinicus* grown in Buon Ma Thuot City has a lower carvacrol content than that grown in Cuba (71%) but a significantly higher content than that in India (28.65%) and France (23%).[10,11,14] In addition, some substances found in the essential oil samples from Buon Ma Thuot City, such as *trans*-α-bergamotene, caryophyllene, and terpinen-4-ol, are not present in those from France and Cuba.[10,11,14] The chemical composition of *P. amboinicus* leaf essential oil originating from Malaysia is also different from that of Buon Ma Thuot City.[20,21] The carvacrol content in the oil from Malaysia is lower (ranging from 47.0 to 60.0%) compared to that in the oil from Buon Ma Thuot City.[21] Furthermore, in the essential oil from Malaysia, the caryophyllene content (6.00%) and *trans*-α-bergamotene content (ranging from 4.70 to 5.00%) are lower and the γ-terpinene content (ranging from 8.00 to 10.00%) is higher than the corresponding contents in the essential oil from Buon Ma Thuot City.[21] *P. amboinicus* from Malaysia does not contain α-humulene, *o*-cymene, or terpinen-4-ol,[21] compounds that are present in the essential oil from Buon Ma Thuot City. On the other hand, no *p*-cymene or thymoquinone were detected in the oil from Buon Ma Thuot City.[21]

According to a 2019 analysis of the chemical composition of *P. amboinicus* grown in Taiwan,[22] carvacrol (50.0%), γ-terpinene (13.1%), and β-caryophyllene (11.3%) were identified as the main components. Compared with the composition of *P. amboinicus* essential oil in Buon Ma Thuot, the carvacrol content is lower while the γ-terpinene content is higher.[22] Additionally, even though the essential oil in Buon Ma Thuot has significantly greater levels of sesquiterpene hydrocarbons (88.45%), the monoterpene hydrocarbon and oxygenated monoterpene contents are much lower than in Taiwan.

In 2023, Gutiérrez *et al.* extracted the essential oil of *P. amboinicus*

grown in Mexico via microwave and GC-MS analysis.[23] In comparison, the content of carvacrol (41.20%) is lower but those of *o*-cymene (11.61%) and caryophyllene (11.45%) are higher in the essential oil from Mexico than in the oil from Buon Ma Thuot. Comparisons with the *P. amboinicus* essential oil from Egypt and Brazil further highlight the significant differences in the chemical composition of the oil from different regions.[12,24] For instance, carvacrol, a major component in the essential oils from Egypt and Brazil, was not detected in the oil from Buon Ma Thuot. Furthermore, while the main compositions of the essential oil from Egypt and Brazil vary with the seasons, they remain distinct from that of the essential oil from Buon Ma Thuot.[12,24] Compounds such as δ-cadinene, β-copaen-4-α-ol, humulene, thymol, and β-caryophyllene are present in varying concentrations in these countries. Further, the essential oil from Buon Ma Thuot contains a significant number (33) of nitrogen-containing compounds, setting it apart from the essential oils in other countries. These variations in chemical composition further emphasize the influence of soil and geographical factors on the chemical profile of *P. amboinicus* essential oil. The environmental conditions in different regions can result in distinct chemotypes of the plant and affect the presence and abundance of specific compounds. Such differences have implications for the potential uses and properties of the essential oil from different locations.

Antioxidant Activity of P. amboinicus Essential Oil

The antioxidant activity of *P. amboinicus* essential oil was evaluated using the DPPH radical scavenging assay, as presented in Table 2 and Figure 3. The test results indicate that the percentage of free radical inhibiting activity of *P. amboinicus* essential oil increases gradually with increasing concentration, ranging from 0.3125 to 10 µg/mL, as shown in Table 2. The highest resistance to oxidation is observed in the sixth sample, with a DPPH free radical inhibiting activity of 89.21%. Furthermore, the IC_{50} value of *P. amboinicus* essential oil is 44.15 µg/mL, while that of ascorbic acid is 39.99 µg/mL. This implies that *P. amboinicus* essential oil exhibits greater antioxidant activity compared to ascorbic acid, which is a well-known antioxidant.

The *P. amboinicus* essential oil in the current study shows a stronger resistance to oxidation than that observed in the study by Bezerra *et al*.[13] However, compared to the study by Manjamalai *et al*., it is weaker in terms of resistance to oxidants, with levels ranging from 5 to 100 µg/mL.[16] These findings highlight that the resistance to oxidation of *P. amboinicus* essential oil can vary depending on the geographical location and soil type. In other words, different environmental conditions and growing regions can lead to variations in the antioxidant activity of the essential oil. This variability has implications for the potential applications of *P. amboinicus* essential oil as a natural antioxidant and can be important in determining its efficacy in different contexts.

Table 1: Chemical compositions from *P. amboinicus* leaves and stems essential oil.

Peak no.	Retention time (min)	Compounds	Molecular formula	Relative amount (%)
1	4.29	*α*-Phellandrene	$C_{10}H_{16}$	0.02
2	4.40	1-methyl-4-(1-methylethyl)-1,3-cyclohexadiene	$C_{10}H_{16}$	0.08
3	4.45	o-Cymene	$C_{10}H_{14}$	1.90
4	4.79	*γ*-Terpinene	$C_{10}H_{16}$	2.50
5	5.11	1-methyl-3-(1-methylethenyl)benzene	$C_{10}H_{14}$	0.08
6	5.20	3-Carene	$C_{10}H_{16}$	0.05
7	5.37	3-(3,4-dimethylphenylsulfonyl)propanamide	$C_{11}H_{15}NO_3S$	0.01
8	5.67	3,5-Dimethylamphetamine	$C_{11}H_{17}N$	0.01
9	5.75	2-Isopropyl-5-methyl-6-oxabicyclo[3.1.0]hexane-1-carboxaldehyde	$C_{10}H_{16}O_2$	0.03
10	5.89	3-Fluorobenzoic acid, 2-fluorophenyl ester	$C_{13}H_8F_2O_2$	0.05
11	6.08	endo-Borneol	$C_{10}H_{18}O$	0.09
12	6.19	Terpinen-4-ol	$C_{10}H_{18}O$	1.14
13	6.49	Metaraminol	$C_9H_{13}NO_2$	0.02
14	6.55	2-Chloroacetamide	C_2H_4ClNO	0.05
15	6.95	2-Fluoroamphetamine	$C_9H_{12}FN$	0.02
16	7.14	1,4-Dibromo-2,3-butanediol	$C_4H_8Br_2O_2$	0.02
17	7.33	Paradrine	$C_9H_{13}NO$	0.01
18	7.40	3-Azabicyclo[3.2.2]nonane	$C_8H_{15}N$	0.01
19	7.78	Carvacrol	$C_{10}H_{14}O$	0.10
20	7.92	Thymol	$C_{10}H_{14}O$	0.46
21	8.24	2,3,5,6-tetramethylphenol	$C_{10}H_{14}O$	67.94
22	9.02	Eugenol	$C_{10}H_{12}O_2$	0.03
23	9.24	Clovene	$C_{15}H_{24}$	0.08
24	9.31	(*S*)-Atomoxetine	$C_{17}H_{21}NO$	0.02
25	9.40	1-(3,5-Dimethyl-1-adamantanoyl)semicarbazide	$C_{14}H_{23}N_3O_2$	0.02
26	9.58	4-tert-butylamphetamine	$C_{13}H_{21}N$	0.03
27	9.70	Amphetamine	$C_9H_{13}N$	0.02

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28	9.79	Ephedrine	$C_9H_{13}NO$	0.02
29	9.90	1,3-Adamantanediacetamide	$C_{14}H_{22}N_2O_2$	0.04
30	10.01	(*R*)-Atomoxetine	$C_{17}H_{21}NO$	0.02
31	10.08	[1*R*-(1R^*,4*Z*,9S^*)]-4,11,11-trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene	$C_{15}H_{24}$	0.07
32	10.22	Di-*epi*-*α*-cedrene	$C_{15}H_{24}$	0.10
33	10.36	Caryophyllene	$C_{15}H_{24}$	9.74
34	10.48	10,10-Dimethyl-2,6-dimethylenebicyclo[7.2.0]undecane	$C_{15}H_{24}$	0.18
35	10.61	*Trans*-*α*-Bergamotene	$C_{15}H_{24}$	5.71
36	10.77	2,6-dimethyl-6-(4-methyl-3-pentenyl)-bicyclo[3.1.1]hept-2-ene	$C_{15}H_{24}$	0.06
37	10.86	9,10-dehydroisolongifolene	$C_{15}H_{22}$	0.23
38	10.91	*β*-Longipinene	$C_{15}H_{24}$	0.32
39	10.99	Z,Z,Z-1,5,9,9-Tetramethyl-1,4,7-cycloundecatriene,	$C_{15}H_{24}$	3.20
40	11.07	(1S-exo)-2-methyl-3-methylene-2-(4-methyl-3-pentenyl)-Bicyclo[2.2.1]heptane	$C_{15}H_{24}$	0.10
41	11.35	3-Chloro-N-methylpropylamine	$C_4H_{10}CIN$	0.03
42	11.41	1-Methyl-4-(6-methylhept-5-en-2-yl)cyclohexa-1,3-diene	$C_{15}H_{24}$	0.08
43	11.47	N-Desmethyltapentadol	$C_{13}H_{21}NO$	0.06
44	11.55	*Cis*-*β*-Farnesene	$C_{15}H_{24}$	0.17
45	11.64	2,4-Dimethylamphetamine	$C_{11}H_{17}N$	0.05
46	11.72	*Cis*-*α*-Bergamotene	$C_{15}H_{24}$	0.05
47	11.87	*α*-Muurolene	$C_{15}H_{24}$	0.21
48	12.00	Butylated Hydroxytoluene	$C_{15}H_{24}O$	0.86
49	12.16	*Cis*-*α*-Bisabolene	$C_{15}H_{24}$	0.15
50	12.30	*β*-sesquiphellandrene	$C_{15}H_{24}$	0.18
51	12.46	*α*-Methyl-benzenepropanamine	$C_{10}H_{15}N$	0.02
52	12.67	4-[(1*E*)-1,5-Dimethyl-1,4-hexadien-1-yl]-1-methyl-cycclohexene	$C_{15}H_{24}$	0.06
53	12.72	2-(adamantan-1-yl)-1-methyl-ethylamine	$C_{13}H_{23}N$	0.03
54	12.86	*Trans*-3,4,4a,5,8,8a-hexahydro-4a-methyl-2(1H)-naphthalenone	$C_{11}H_{16}O$	0.23
55	12.98	(1*Z*,7*E*)-5-dodecatriene	$C_{12}H_{20}$	0.14
56	13.20	[*R*-(*R**,*R**)]-*α*-(1-aminoethyl)benzenepropanoic acid	$C_{11}H_{15}NO_2$	0.02
57	13.30	N,5-dimethyl-1*H*-Imidazole-4-ethanamine	$C_7H_{13}N_3$	0.08
58	13.42	(1*Z*,5*E*)-7-dodecatriene	$C_{12}H_{20}$	0.71
59	13.56	2-(4a,8-Dimethyl-2,3,4,4a,5,6-hexahydronaphthalen-2-yl)propan-1-ol	$C_{15}H_{24}O$	0.14
60	13.83	3-Methoxyamphetamine	$C_{10}H_{15}NO$	0.02
61	13,97	3-Phenylpiperidine	$C_{11}H_{15}N$	0.03
62	14,02	2,4-Dimethylamphetamine	$C_{11}H_{17}N$	0.04
63	14.34	1-[*α*-(1-adamantyl)benzylidene]thiosemicarbazide	$C_{18}H_{23}N_3S$	0.02
64	14.49	8-ethenyl-3,4,4a,5,6,7,8,8a-octahydro-5-methylene-2-Naphthalenecarboxylic acid	$C_{14}H_{18}O_2$	0.12
65	14.57	N,N-dimethyl-N'-phenyl-Caryophylla-4(12),8(13)-dien-5*α*-ol	$C_{15}H_{24}O$	0.2
66	14.73	Methanimidamide	$C_9H_{12}N_2$	0.03
67	14.81	2-Amino-1-(o-methoxyphenyl)propane	$C_{10}H_{15}NO$	0.04
68	15.00	1,5-diethenyl-3-methyl-2-methylene-(1*α*,3*α*,5*α*)cyclohexane	$C_{12}H_{18}$	0.27
69	15.18	6-(2-Aminopropyl)benzofuran	$C_{11}H_{13}NO$	0.09
70	15.27	τ-Muurolol	$C_{15}H_{26}O$	0.18
71	15.39	5-methoxy-3-(2-methylamino)ethylndole	$C_{12}H_{16}N_2O$	0.14

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72	15.50	(*R*)-Phenylephrine	$C_9H_{13}NO_2$	0.02
73	15.55	N-Acetyl-2-ethoxyamphetamine	$C_{12}H_{17}NO_2$	0.03
74	15.90	3-Ethoxyamphetamine	$C_{11}H_{17}NO$	0.01
75	15.98	2,4-Dimethylamphetamine	$C_{11}H_{17}N$	0.05
76	16.11	3-Methoxyamphetamine	$C_{10}H_{15}NO$	0.04
77	16.46	(*S*)-Phenylephrine	$C_9H_{13}NO_2$	0.02
78	17.90	6-amino-2-methyl-2-heptanol	$C_8H_{19}NO$	0.01
79	18.62	2-Oxo-3-methyl-cis-perhydro-1,3-benzoxazine	$C_9H_{15}NO_2$	0.04
80	19.09	3-Methyl-3,5-(cyanoethyl)tetrahydro-4-thiopyranone	$C_{12}H_{16}N_2OS$	0.02
81	19.43	4-Fluoroamphetamine	$C_9H_{12}FN$	0.03
82	19.76	4-Methoxyamphetamine	$C_{10}H_{15}NO$	0.02
83	20.22	N-(2-aminoethyl)-1,3-propanediamine	$C_5H_{15}N_3$	0.01
84	20.35	2,5-Dimethoxy-4-(methylsulfonyl)amphetamine	$C_{12}H_{19}NO_4S$	0.03
85	20.92	N,N-Dimethyl-N'-phenymethanimidamide	$C_9H_{12}N_2$	0.01
86	21.48	N-methyl-1-pentanamine	$C_6H_{15}N$	0.02
87	24.35	1-(4-tert-butylphenyl)-3-(1-methyl-2-phenylethyl)-urea	$C_{20}H_{26}N_2O$	0.01
88	29.86	dl-Alanyl-dl-asparagine	$C_7H_{13}N_3O_4$	0.03
89	30.40	Diisooctylphthalate	$C_{24}H_{38}O_4$	0.59

Total number of constituents	89
Number (%) of constituents identified	78 (99.48%)
Number (%) of monoterpene hydrocarbons	6 (4.63%)
Number (%) of oxygenated monoterpenes	6 (1.85%)
Number (%) of sesquiterpene hydrocarbons	15 (88.45%)
Number (%) of oxygenated sesquiterpenes	5 (1.48%)
Number (%) of nitrogen compounds	33 (1.13%)
Number (%) of different compounds	11 (1.94%)

Table 2: Antioxidant activity results.

Essential oil (µg/mL)	% Inhibiting			IC_{50} (µg/mL)
	1	2	3	
10	89.25	89.14	89.24	
5.0	58.13	58.10	58.14	
2.5	40.13	40.11	40,15	44.15 ± 0.15
1.25	31.64	31.72	31.63	
0.625	23.97	23.91	23.93	
0.3125	21.92	21.79	21.84	
Ascorbic acid				34.99 ± 0.01

The values were expressed as mean values ±S.D of three parallel measurements

Antibacterial Activity of P. amboinicus Essential Oil

The antimicrobial activity of *P. amboinicus* essential oil against *E. coli* is evident from the results presented in Table 3. At a dilution concentration of 1.0 mg/mL, the oil shows an inhibitory ability of 12.94%, as indicated by the diameter of the antibacterial ring (7.2 mm) measured against the resistance ring of *E. coli*. As the concentration of the essential oil increases gradually from 1.0 to 8.5 mg/mL, its resistance toward *E. coli* also increases. At 10 mg/mL, the bacteria do not exhibit any growth on the agar plate, indicating the complete inhibitory effect of the oil at this concentration. The maximum inhibitory ability of the oil against *E. coli* (88.35%) is observed at 8.5 mg/mL, with a large antibacterial ring measuring 47.3 mm in diameter.

These results align with the findings of Lu's study on the resistance ability of *P. amboinicus* essential oil against *E. coli* in Ho Chi Minh City and Nguyen *et al.*'s study in Can Tho City, suggesting similarities in the antibacterial properties of the essential oil in different regions.[8,9] Several international reports have also supported the strong antibacterial ability of *P. amboinicus* against various tested bacterial strains.[10,14,15,20] The main component of the essential oil, carvacrol, is a phenolic compound known for its potent antibacterial activity.[25,26] This explains why *P. amboinicus* essential oil has the potential to act as a natural antibacterial agent. Indeed, the experimental observations indicate that the antibacterial activity of *P. amboinicus* essential oil against *E. coli* is more likely due to enzymatic inhibition rather than the mechanism of biological oxidation. This conclusion is supported by the

high antibacterial activities observed in experimental work,[10,14,15,20] along with the low antioxidant activities of the essential oil.[13]
In addition, the results obtained from using the disc diffusion method and the measurement of the MIC indicate that higher concentrations of the essential oil exhibit greater antimicrobial activity against *E. coli*. The presence of high-content components, particularly 2,3,5,6-tetramethylphenol (67.94%), is strongly correlated with the observed antibacterial activities. Oxygenated monoterpenes, including carveol, are known for their relation to antibacterial properties, further supporting the essential oil's potential to serve as an effective natural antibacterial agent. *E. coli* was selected as the target in this study due to its importance as a member of the normal intestinal microflora in humans and other mammals. Additionally, *E. coli* is known to be a versatile and potentially dangerous pathogen that can cause a wide range of intestinal and extraintestinal diseases.[26-29] Overall, the experimental findings provide strong evidence for the antimicrobial activity of *P. amboinicus* essential oil against *E. coli*, which is likely based on enzymatic inhibition. The high-content components, particularly carveol, contribute to the observed antibacterial properties, making the essential oil a promising candidate as a natural antibacterial agent for potential therapeutic applications.

Conclusion

In conclusion, this study is the first to report on the essential oil extracted from the whole plant of *P. amboinicus* in Buon Ma Thuot City, Vietnam. GC-MS analysis revealed a diverse chemical composition, with 78 identified natural components and 11 unknown compounds. The major components of the essential oil were 2,3,5,6-tetramethylphenol (67.94%), caryophyllene (9.74%), *trans*-α-bergamotene (5.82%), α-humulene (3.20%), γ-terpinene (2.5%), *o*-cymene (1.90%), and terpinen-4-ol (1.14%). The essential oil demonstrated promising biological activity. It exhibited normal resistance to oxidation, with a significant ability to inhibit DPPH free radicals, showing inhibitory activity with an IC_{50} value of 44.15 ± 0.15 µg/mL. Additionally, the essential oil demonstrated effective inhibition against *E. coli* bacteria, with an inhibitory ability of 88.35% and a large antibacterial diameter of 47.3 mm. These results indicate the potential of *P. amboinicus* essential oil for use in pharmaceutical applications. Further research and development in this area could lead to the utilization of this essential oil as a natural and effective agent for various medicinal and therapeutic purposes.

Table 3: Antibacterial activity results.

Bacterial density	Concentration of *P. amboinicus* (mg/mL)	Diameter of sterile ring (mm)	Resistance (%)
10^7 CFU/mL	10.0	53.0, 54.0, 54.0	100 (bacteria do not grow all over the agar plate)
10^7 CFU/mL	8.5	47.5, 47.5, 47.0	88.35
10^7 CFU/mL	7.0	38.5, 38.0, 38.0	70.87
10^7 CFU/mL	5.5	32.5, 32.0, 32.0	59.27
10^7 CFU/mL	4.0	22.0, 22.0, 22.5	40.44
10^7 CFU/mL	2.5	17.0, 17.0, 16.5	31.37
10^7 CFU/mL	1.0	7.5, 7.0, 7.0	12.94

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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References

1. Do TL. Vietnamese medicinal plants and herbs. Hanoi: Medicinal Publishing House; 2006. 1294 p.
2. Moi LD, Cu LD, Hoi TM, Thai TH, Ban NK. Essential-oil plants in Vietnam. Hanoi: Agriculture Publishing House; 2000. 368 p.
3. Dwita LP, Hikmawanti NPE, Husniah J, Hazraj M, Bela SN, Aryanti E. *Plectranthus scutellarioides* leaf extract protective effects against isoniazid and rifampicin-induced hepatotoxicity in Wistar rats. Trop J Nat Prod Res. 2022; 6(12):1936-1940.
4. Djunarko I, Fakhrudin N, Nurrochmad A, Wahyuono S. In vivo anti-inflammatory activity of *Coleus atropurpureus* leaves extract and fractions. Trop J Nat Prod Res. 2022; 6(1):40-43.
5. Ahamed AN, Yaser SM, Idhris SM, Padusha MSA, Sherif NA. Phytochemical and pharmacological potential of the genus *Plectranthus* - A review. S Afr J Bot. 2023; 154:159-189.
6. Jaramillo-Colorado BE, Vega-Díaz R, Pino-Benítez CN. Volatile chemical composition of Colombian *Plectranthus amboinicus* (Lour.) Spreng essential oil and its biocidal action against *Tribolium castaneum* (Herbst). Rev Colomb Cienc Hortic. 2022; 16(1):e13413.
7. Almalki A, Abutaha N, Al-Doaiss AA, Mohammed AZ, Wadaan MA. Cytotoxicity, in vivo toxicity, and chemical composition of the hexane extract of *Plectranthus amboinicus* (Lour.) Spreng. Environ Sci Pollut Res. 2021; 28(35):48141-48153.
8. Lu TMT. The study on the extraction of *Plectranthus amboinicus* (Lour.) Spreng essential oil by the steam distillation. HUFI, J Sci Technol Food. 2016; 10:14-17.
9. Nguyen TBT, Nguyen TDT, Chau TTH. Study on chemical composition and some anti-microorganism activity of *Plectranthus amboinicus* L. essential oil. CTU, J Sci. 2012; 21a:144-147.
10. Senthilkumar A, Venkatesalu V. Chemical composition and larvicidal activity of the essential oil of *Plectranthus*

amboinicus (Lour.) Spreng against *Anopheles stephensi*: A malarial vector mosquito. Parasitol Res. 2010; 107(5):1275-1278.
11. Monzote L, Scherbakov A, Scull R, Gutiérrez Y, Satyal P, Cos P, Shchekotikhin A, Gille L, Setzer W. Pharmacological assessment of the carvacrol chemotype essential oil from *Plectranthus amboinicus* growing in Cuba. Nat Prod Commun. 2020; 15(10):1-12.
12. El-Hawary SS, El-sofany RH, Abdel-Monem AR, Ashour RS, Sleem AA. Seasonal variation in composition of *Plectranthus amboinicus* (Lour.) Spreng essential oil and its biological activities. Am J Essent Oils Nat Prod. 2013; 1(2):11-18.
13. Bezerra RD, Neto FB, Silva FF, Bertini LM, Alves LA. Seasonal effect in essential oil composition and antioxidant activity of *Plectranthus amboinicus* leaves. Biosci J. 2017; 33(6):1608-1616.
14. Hassani MS, Zainati I, Zrira S, Mahdi S, Oukessou M. Chemical composition and antimicrobial activity of *Plectranthus amboinicus* (Lour.) Spreng essential oil from archipelago of Comoros. J Essent Oil-Bear Plants. 2012; 15(4):637-644.
15. Manjamalai A, Alexander T, Grace VMB. Bioactive evaluation of the essential oil of *Plectranthus amboinicus* by GC-MS analysis and its role as a drug for microbial infections and inflammation. Int J Pharm Pharm Sci. 2012; 4(3):205-211.
16. Manjamalai A, Grace B. Volatite constituents and antioxidant property of essential oil from *Plectranthus amboinicus* (Lour). Int J Pharma Bio Sci. 2012; 3(4):445-458.
17. Rahmawati, Eltivitasari A, Romadhonsyah F, Gemantari BM, Nurrochmad A, Wahyuono S, Astuti P. Effect of light exposure on secondary metabolite production and bioactivities of *Syncephalastrum racemosum* endophyte. Trop J Nat Prod Res. 2021; 5(2):312-318.
18. Kumar P, Singh S, Kumar N. *Plectranthus amboinicus*: A review on its pharmacological and pharmacognostical studies. Am J Physiol Biochem Pharmacol. 2020; 10(2):55-62.
19. dos Santos Silva JM, da Silva Almeida JRG, dos Santos Cerqueira Alves C, Nery DA, Damasceno LMO, de Souza Araújo C, Rolim LA, de Oliveira AP. Antimicrobial activity from species *Plectranthus amboinicus* (Lour.) Spreng, a review. European J Med Plants. 2020; 31(18):1-14.
20. Amer HM, El-Gohary AE, Salem SH, Hussein MS. Foliar application of selenium and humic acid changes yield, essential oil, and chemical composition of *Plectranthus amboinicus* (Lour.) plant and its antimicrobial effects. Egypt Pharm J. 2019; 18(4):356-367.
21. Ashaari NS, Mohamad NE, Afzinizam AH, Ab Rahim MH, Lai KS, Ong Abdullah J. Chemical composition of hexane-extracted *Plectranthus amboinicus* leaf essential oil: Maximizing contents on harvested plant materials. Appl Sci (Basel). 2021; 11(22):10838.
22. Hsu KP, Ho CL. Antimildew effects of *Plectranthus amboinicus* leaf essential oil on paper. Nat Prod Commun. 2019; 14(7):1-6.
23. Antonio-Gutiérrez O, Alvízar-Martínez JA, Solano R, Vásquez-López A, Hernández-Valladolid SL, Lustre-Sánchez H, Flores-Moctezuma HE, de Jesús de Luna-Santillana E, Lagunez-Rivera L. Microwave-assisted hydrodistillation of essential oil from *Plectranthus amboinicus*: Evaluation of its antifungal effect and chemical composition. Life. 2023; 13(2):528.
24. Da Costa JGM, Pereira CKB, Rodrigues FFG, de Lima SG. Chemical composition, antibacterial and fungicidal activities of leaf oil of *Plectranthus amboinicus* (Lour.) Spreng. J Essent Oil Res. 2010; 22(2):183-185.
25. Pinheiro PF, Costa AV, Alves Td, Galter IN, Pinheiro CA, Pereira AF, Oliveira CM, Fontes MM. Phytotoxicity and cytotoxicity of essential oil from leaves of *Plectranthus amboinicus*, carvacrol, and thymol in plant bioassays. J Agric Food Chem. 2015; 63(41):8981-8990.
26. Vasconcelos SE, Melo HM, Cavalcante TT, Júnior FE, de Carvalho MG, Menezes FG, de Sousa OV, Costa RA. *Plectranthus amboinicus* essential oil and carvacrol bioactive against planktonic and biofilm of oxacillin- and vancomycin-resistant *Staphylococcus aureus*. BMC Complement Altern Med. 2017; 17(1):462.
27. Hussain AI, Anwar F, Shahid M, Ashraf M, Przybylski R. Chemical composition, and antioxidant and antimicrobial activities of essential oil of spearmint (*Mentha spicata* L.) from Pakistan. J Essent Oil Res. 2010; 22(1):78-84.
28. Lopez-Romero JC, González-Ríos H, Borges A, Simões M. Antibacterial effects and mode of action of selected essential oils components against *Escherichia coli* and *Staphylococcus aureus*. Evid Based Complement Alternat Med. 2015; 2015:795435.
29. El Abdouni Khayari M, Jamali CA, Kasrati A, Hassani L, Leach D, Markouk M, Abbad A. Antibacterial activity of essential oils of some Moroccan aromatic herbs against selected food-related bacteria. J Essent Oil-Bear Plants. 2016; 19(5):1075-1085.