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Phytochemical Profiling and Bioactivity Evaluation of Perilla Species from Thai Nguyen Province, Vietnam

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ABSTRACT

Perilla frutescens, belonging to the Lamiaceae family, serves as a traditional medicinal herb in East Asia, offering benefits such as anti-inflammatory, antimicrobial, and antitumor effects. This study aimed to compare the phytochemical composition and biological activities of two varieties- *P. frutescens* var. *crispa forma* and *P. frutescens* var. *frutescens*—cultivated in Thai Nguyen province, northern Vietnam. Leaf samples were dried and extracted with ethanol using maceration, followed by GC-MS characterization and in vitro assessments of antimicrobial and antitumor effects. GC–MS profiling revealed 39 bioactive compounds, with Perilla ketone dominating in var. *frutescens* (69.41%) and 1-cyclohexene-1-carboxaldehyde, 4-(1-methylethenyl)- (61.48%) in var. *crispa forma*. Var. *crispa forma* exhibited a more diverse composition, particularly of sesquiterpenes such as caryophyllene and nerolidol. Biological assays demonstrated concentration-dependent antimicrobial effects against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Aspergillus flavus*, *Aspergillus brasiliensis*, and *Candida albicans*. Notably, the extract of var. *crispa forma* showed superior inhibitory zones and surpassed the antifungal standard amphotericin B against *C. albicans*. Cytotoxicity testing on AGS gastric cancer cells using the MTT assay showed potent anticancer activity, with var. *crispa forma* achieving an IC_{50} of 10.43 µg/mL, compared to 15.83 µg/mL for var. *frutescens*. These findings suggest that ethanol extracts of *P. frutescens*, particularly var. *crispa forma*, possess strong antimicrobial and anticancer potentials.

Keywords: Antibacterial, antifungal, anticancer activity, ethanol extract, *Perilla frutescens*, *phytochemical profiling.*

Introduction

Perilla frutescens (L.) Britt., widely referred to as perilla, is a member of the Lamiaceae family and holds longstanding cultural and medicinal significance in East Asia [1]. Traditionally, its leaves have been employed in herbal medicine to manage various respiratory and gastrointestinal disorders, such as asthma, influenza, colds, nausea, and abdominal pain [2]. These ethnobotanical applications have been substantiated by contemporary pharmacological studies, which reveal that *Perilla frutescens* possesses a broad spectrum of biological activities, including antioxidant, antibacterial, antiallergic, antidepressant, anti-inflammatory, and anticancer properties [3]. The extraction and isolation of bioactive compounds from *Perilla frutescens* have been widely explored using a variety of solvent systems, each influencing the chemical profile and biological activities of the resulting extracts.

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Recent studies have predominantly employed polar solvents such as ethanol, methanol, and water for initial extractions, followed by partitioning with solvents like ethyl acetate or n-hexane to concentrate specific phytochemical fractions. Samples were subjected to the bioactive compound measurements [4]. Gas chromatography-mass spectrometry (GC-MS) serves as a vital technique for analyzing volatile and semivolatile compounds, linking chemical makeup to functional outcomes. GC-MS thus provides a robust analytical platform to link chemical composition with observed bioactivities [5].

Ethanol extracts of *Perilla frutescens* leaves are of particular interest due to their concentrated phytochemical profile. These extracts are notably rich in volatile mono- and sesquiterpenes, including compounds like limonene and *α*-pinene, which contribute to the characteristic aroma of Perilla and exhibit antibacterial and antifungal properties [6]. Furthermore, perilla aldehyde, often found in higher concentrations in ethanol extracts compared to other extraction methods, is considered a major bioactive compound that underlies the antioxidant and anti-inflammatory effects [2]. Ethanol extracts, especially from purple varieties of *Perilla frutescens*, contain a significant array of phenolic acids, showing strong in vitro antioxidative effects against radicals like ABTS, DPPH, and hydrogen peroxide (H_2O_2) [7]. Rosmarinic acid, a prominent phenolic compound in Perilla, is recognized as a potent natural antioxidant, and alongside luteolin, contributes substantially to the overall antioxidant capacity of the extract [7,8].

The pharmacological activities of *Perilla frutescens* ethanol extracts suggest promising medical applications. Their demonstrated antimicrobial and anti-inflammatory activities suggest promising applications in supporting and accelerating the wound healing process [9]. Emerging research also suggests that these extracts may possess cognitive-enhancing properties. As research progresses, ethanol

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extracts of Perilla leaves continue to reveal a complex composition of bioactive compounds with significant potential in diverse biomedical applications.

While previous studies have explored the bioactivity of *Perilla frutescens* in other regions, little is known about the phytochemical profiles and bioactivity of varieties cultivated in northern Vietnam, particularly under specific environmental conditions. This work aims not only to identify chemotype-specific bioactive markers but also to provide foundational data supporting the development of natural antimicrobial and anticancer agents derived from perilla leaf extracts. To our knowledge, this is the first comparative study characterizing the bioactivity and phytochemistry of these two *Perilla* varieties from northern Vietnam, contributing novel insights into their pharmacological relevance and potential therapeutic applications.

Materials and Methods

Chemicals

This study utilized analytical grade chemicals and reagents, which were obtained from Merck (Darmstadt, Germany). Ethanol (≥99.5%, absolute), ascorbic acid (≥99%, ACS reagent), gallic acid (≥98%, standard grade), sodium carbonate (≥99.5%, ACS reagent), and so on were used as received without further purification.

Plant collection and identification

In July 2024, two botanical varieties of *Perilla frutescens-var. crispa forma* and *var. frutescens*-were collected from mature populations naturally cultivated in Thai Nguyen Province, located in northern Vietnam. The collection site is positioned at an altitude of 597 meters above sea level, with precise geographic coordinates of 21°35.11′N and 105°52.31′E. Taxonomic authentication of the plant materials was conducted by Associate Professor Danh Thuong Sy, Head of the Department of Botany, Faculty of Biology, Thai Nguyen University of Education. Representative voucher specimens were curated and deposited in the Biological Museum of the Faculty of Biology under accession codes TNUE-PF2023-001 and TNUE-PF2023-002 for future reference.

Freshly collected leaves were immediately washed under running tap water to eliminate surface contaminants. Subsequently, the clean samples were oven-dried at 40°C for 48 hours in a Memmert IN110 unit to protect their phytochemical compounds. Dried leaves were subsequently cut into uniform segments (0.5–1.0 cm) and ground into a coarse powder using a mechanical grinder. The resulting powdered material was stored in airtight containers under dry, cool conditions prior to extraction and further analysis.

Perilla leaf extraction method

The focus of the *Perilla* leaf extract preparation was the maceration method. A total of 100 g of blended, dried Perilla leaf powder was extracted using 500 mL of 90% ethanol, maintaining a solvent-to-solid ratio of 5:1 (v/w), in a round-bottom flask. The mixture was then refluxed for four hours at a temperature ranging from 80 °C to 90 °C in a water bath AHYQ HH4. Leaf removal from the heated mixture was achieved by direct filtration with Whatman filter paper. The filtrate underwent solvent removal through rotary vacuum evaporation with an EYELA N1210 rotary vacuum evaporator. Percentage yield of the extract = $\frac{\text{Weight of extract (g)}}{\text{Weight of dried plant material (g)}}$ x 100. The extract was stored in glass bottles at a temperature of 4°C for subsequent experiments.

Phytochemical screening by GC–MS method

GC-MS analysis was conducted using a Hewlett-Packard HP5890 Series II gas chromatograph coupled with a quadrupole mass spectrometer (HP MSD5971), operated with an electron ionization source at 200°C. Separation was carried out using an HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness) with a non-polar stationary phase. The chromatographic parameters were identical to those used for GC analysis. Electron impact spectra were recorded at an ion voltage of 70 eV within a mass range of 30-600 amu. The GC-MS analysis was conducted at the Faculty of Chemistry, Hanoi University of Science, VNU.

Antibacterial activity

The antibacterial activity of the ethanol extracts was assessed using the disc agar diffusion method, with the diameter of the zone of inhibition serving as the metric for their capacity [10]. Tested bacterial strains included *Escherichia coli* (*E. coli* - ATCC 25922), *Pseudomonas aeruginosa* (*P. aeruginosa* - ATCC 9027), and *Staphylococcus aureus* (*S. aureus* - ATCC 25923). The ethanol extract was diluted to specified concentrations using DMSO (25 µg/mL, 50 µg/mL, and 100 µg/mL). To assess the antimicrobial activity, the discs were incubated with the bacterial cultures at 37°C for 24 hours. The diameter of the resulting inhibition zones was subsequently measured. The experimental setup included both a negative control (DMSO) and a positive control (50 µg/mL ampicillin). Each experiment was conducted in triplicate. All bacterial strains were sourced from the Department of Biology, Thai Nguyen University of Education, TNU.

Antifungal activity

The pour plate method, as applied in the antifungal test, was conducted in accordance with established protocols [10]. The antifungal activity of the extract against *Aspergillus brasiliensis* and *Aspergillus flavus* was quantified by measuring the radius of the inhibition zone, which reflects the area of prevented fungal growth. The culture medium used was Potato Dextrose Agar (PDA). The test samples were assessed at three concentration levels (25 µg/mL, 50 µg/mL, and 100 µg/mL) during the bioassays. Amphotericin B (50 µg/mL) served as the positive control, and DMSO 2% was the negative control. The zone radius was calculated by subtracting the agar plug diameter from the sterile ring diameter. All fungal strains were sourced from the Department of Biology, Thai Nguyen University of Education, TNU.

Anticancer evaluation

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was employed to assess the cytotoxicity of the *Perilla frutescens* ethanol extract against the human gastric cancer cell line AGS [11]. The cell culture medium, obtained from Sigma-Aldrich (St. Louis, MO, USA), was composed of a mixture of Dulbecco's modified Eagle's medium and Eagle's minimal essential medium, enriched with 10% fetal bovine serum. The cell line incubation conditions consisted of 5% CO_2, 95% air, 37°C in a CB 220 incubator (Thermo Scientific). Optical density (OD) measurements were performed at 540 nm on an ELISA Plate Reader. The cytotoxicity of ethanol extract from the Perilla plant was expressed as IC_{50} value using Table Curve 2Dv4 software.

Statistical analysis

All experiments were carried out in three replicates. The resulting data were statistically analyzed using one-way ANOVA, followed by Duncan's multiple range test at a significance level of $p < 0.05$. The statistical software used for the analysis was SPSS version 26.0.

Results and Discussion

Determination of phytochemical components by GC-MS

The extraction of dried *Perilla frutescens* leaves using 90% ethanol at 90°C for 2 hours resulted in a relatively high yield of crude extract. The extraction yield was recorded at 15.2% (w/w) (*Perilla frutescens* var. *frutescens*) and 14.6% (w/w) (*P. frutescens* var. *crispa forma*) based on the dry weight of the plant material, indicating the efficiency of the method in recovering bioactive constituents. The phytochemical composition of *Perilla* extract was analyzed by GC-MS (Table 1).

Based on the GC-MS table, *Perilla frutescens* var. *frutescens* is dominated by *Perilla ketone* ($C_{10}H_{14}O_2$) at 69.413%, followed by *Hexadecanoic acid* (5.807%), *Linoleic acid* (5.961%), *Phytol* (2.883%), and *Farnesol* (2.059%). In contrast, *Perilla frutescens* var. *crispa forma* is primarily characterized by *1-Cyclohexene-1-carboxaldehyde, 4-(1-methylethenyl)-* ($C_{10}H_{14}O$) at 61.479%, alongside *2-Propenal, 2-methyl-3-phenyl-* (9.378%), *Phytol* (3.794%), *Caryophyllene* (3.593%), and *Nerolidol* (2.561%). This stark difference highlights distinct chemical profiles, with *P. frutescens* var. *frutescens* focusing on a single prominent ketone compound, while *P. frutescens* var. *crispa forma*

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Table 1: Compounds showed by GC-MS analysis of ethanol extract of *P. frutescens* var. *frutescens and P. frutescens* var. *crispa forma*

No.	**Retention Time (min.)**	***Name of the compound***	***Formula of the compound***	***RI***	**% of Total**	
					P. frutescens* var. *Frutescens	***P. frutescens* var. *crispa forma***
1	12.337	Perilla ketone	$C_{10}H_{14}O_2$	1280	69.413	
2	12.696	1-Cyclohexene-1-carboxaldehyde, 4-(1-methylethenyl)-	$C_{10}H_{14}O$	1300		61.479
3	12.884	2-Propenal, 2-methyl-3-phenyl-	$C_{10}H_{10}O$	1315		9.378
4	13.6	Linalool	$C_{10}H_{18}O$	1350	0.956	
5	13.668	Limonene	$C_{10}H_{16}$	1355		0.656
6	14.217	Caryophyllene	$C_{15}H_{24}$	1415		3.593
7	14.22	*α*-Humulene	$C_{15}H_{24}$	1415	1.038	
8	14.505	Perillaldehyde	$C_{10}H_{14}O$	1435		0.973
9	14.679	*β*-Pinene	$C_{10}H_{16}$	1450		0.807
10	14.691	Camphene	$C_{10}H_{16}$	1455	1.466	
11	15.013	*α*-Terpinene	$C_{10}H_{16}$	1480		0.951
12	15.014	*γ*-Terpinene	$C_{10}H_{16}$	1480	0.733	
13	15.423	Germacrene D	$C_{15}H_{24}$	1505		1.138
14	15.598	*β*-Bisabolene	$C_{15}H_{24}$	1520		0.782
15	16.113	*α*-Farnesene	$C_{15}H_{24}$	1565		0.565
16	16.117	*β*-Farnesene	$C_{15}H_{24}$	1565	0.777	
17	16.365	Spathulenol	$C_{15}H_{24}O$	1585	1.306	
18	16.367	Caryophyllene oxide	$C_{15}H_{24}O$	1585		0.951
19	16.834	Humulene epoxide	$C_{15}H_{24}O$	1620		0.666
20	16.845	*τ*-Cadinol	$C_{15}H_{26}O$	1625	0.353	
21	17.07	*α*-Cadinol	$C_{15}H_{26}O$	1650		0.455
22	17.299	*τ*-Muurolol	$C_{15}H_{26}O$	1665		0.62
23	17.299	*β*-Eudesmol	$C_{15}H_{26}O$	1665	1.145	
24	17.496	Farnesol	$C_{15}H_{26}O$	1670	2.059	
25	17.497	Farnesol isomer	$C_{15}H_{26}O$	1670		2.049
26	17.565	Nerolidol	$C_{15}H_{26}O$	1675		2.561
27	17.616	Nerolidol isomer	$C_{15}H_{26}O$	1680	1.61	
28	18.055	Phytone	$C_{18}H_{36}O$	1840		0.729
29	18.267	Phytol	$C_{20}H_{40}O$	2115	2.883	
30	18.269	Phytol	$C_{20}H_{40}O$	2115		3.794
31	18.489	Isophytol	$C_{20}H_{40}O$	2125		2.355
32	18.493	Phytol isomer	$C_{20}H_{40}O$	2125	2.082	
33	18.569	Squalene	$C_{30}H_{50}$	2130	1.886	
34	18.998	Stearic acid	$C_{18}H_{36}O_2$	2175		0.927
35	19.001	Palmitoleic acid	$C_{16}H_{30}O_2$	2175	0.527	
36	19.446	Hexadecanoic acid	$C_{16}H_{32}O_2$	2210	5.807	
37	19.453	Hexadecanoic acid	$C_{16}H_{32}O_2$	2210		2.671
38	19.934	Linoleic acid	$C_{18}H_{32}O_2$	2245	5.961	
39	19.938	Oleic acid	$C_{18}H_{34}O_2$	2245		1.9

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exhibits greater diversity with aldehydes and terpenoids. *Perilla ketone* is the hallmark of *P. frutescens* var. *frutescens*, accounting for an overwhelming 69.413%, whereas *1-Cyclohexene-1-carboxaldehyde, 4-(1-methylethenyl)-* dominates *P. frutescens* var. *crispa forma* at 61.479%. This reflects differing biosynthetic pathways, with *P. frutescens* var. *frutescens* favoring ketones and *P. frutescens* var. *crispa* leaning toward cyclic aldehydes. *P. frutescens* var. *crispa forma* shows a rich presence of sesquiterpenes such as *Caryophyllene* (3.593%), *Nerolidol* (2.561%), and *Germacrene D* (1.138%), while *P. frutescens* var. *frutescens* contains fewer, including *Farnesol* (2.059%) and *Phytol* (2.883%). This suggests var. *crispa forma* tends to produce a more diverse essential oil profile. *P. frutescens* var. *frutescens* has higher levels of *Hexadecanoic acid* (5.807%) and *Linoleic acid* (5.961%) compared to *P. frutescens* var. *crispa forma* (2.671% for *Hexadecanoic acid* and negligible *Linoleic acid*), indicating a potentially higher lipid content in *P. frutescens* var. *frutescens*. *Perilla frutescens* var. *frutescens* is commonly classified as the *perilla ketone* chemotype, with this compound typically constituting 30–60% of leaf essential oils, depending on cultivation conditions and extraction methods. The observed 69.413% in the analyzed data exceeds this range, likely due to ethanol extraction enhancing *perilla ketone* yield compared to steam distillation, which is frequently employed in essential oil studies [12]. *Perilla ketone* is recognized as a key compound in this variety, contributing to its distinctive flavor and certain bioactivities, though it poses potential toxicity risks in livestock [13]. *Perilla frutescens* var. *crispa* is typically characterized by high *perillaldehyde* content in its essential oils, with levels ranging from 40–50%, particularly in red-leaf varieties. However, in the analyzed data, *perillaldehyde* constitutes only 0.973% in var. *crispa forma*, while 1-Cyclohexene-1-carboxaldehyde, 4-(1-methylethenyl)- predominates. This marked deviation may indicate the presence of a distinct chemotype or result from ethanol extraction favoring other volatile compounds over *perillaldehyde* [13]. Variability in volatile compounds has been observed between purple and green varieties of var. *crispa*, but 1-Cyclohexene-1-carboxaldehyde has not been reported as a primary component, suggesting that the current data may represent a rare chemical variant [14]. *Caryophyllene*, *nerolidol*, and *phytol* are present in both *P. frutescens* var. *frutescens* and var. *crispa*, with higher prevalence in var. *crispa*. Previous research has identified *caryophyllene* (2–10%) and *nerolidol* (1–5%) as significant components in var. *crispa* essential oils from China and Japan, consistent with the current findings of 3.593% and 2.561%, respectively [15].

Phytol (1–5%), associated with chlorophyll degradation, has also been reported in perilla leaf extracts, and the observed values of 2.883–3.794% align with this range [3]. *Hexadecanoic* acid and *linoleic acid*, common fatty acids in *Perilla frutescens* leaf ethanol extracts, typically range from 2–10% depending on plant part and solvent [8]. The current data show 5.807% (*hexadecanoic acid*) and 5.961% (*linoleic acid*) in var. *frutescens*, fitting within this range, while the lower 2.671% in var. *crispa* forma indicates potential differences in lipid content between the variants.

Most prior studies have utilized steam distillation for essential oil analysis, whereas the current data derive from ethanol extraction [12,13]. Ethanol extraction captures both volatile and non-volatile compounds, which may account for the elevated levels of *perilla ketone* (69.413%) and the dominance of 1-Cyclohexene-1-carboxaldehyde over Perillaldehyde (0.973%) in var. *crispa forma*. This broader chemical profile in ethanol extracts compared to essential oils has been noted in previous research [16]. Specifically, var. *frutescens* is characterized by high *perilla ketone* and fatty acid content, while var. *crispa forma* exhibits a greater proportion of terpenoids. The unusually high perilla ketone levels and the prominence of 1-Cyclohexene-1-carboxaldehyde in var. crispa forma represent significant deviations from typical profiles. These differences may arise from a distinct chemotype, environmental factors, or the ethanol extraction method. Further studies are needed to investigate these factors and confirm the representativeness of these findings.

Antibacterial activity

Research on the antibacterial activity of extracts from *Perilla frutescens* leaves was conducted to evaluate their ability to inhibit the growth of three bacterial strains: *E. coli*, *P. aeruginosa*, and *S. aureus*. The data presented in Figure 1 and Table 2 indicate that the ethanol extracts from both *P. frutescens* var. *frutescens* and *P. frutescens* var. *crispa* forma possess notable antibacterial properties. The extracts showed efficacy against both Gram-negative strains (*E. coli* and *P. aeruginosa*) and a Gram-positive strain (*S. aureus*). The antibacterial effect is generally concentration-dependent, as evidenced by the increasing diameter of the inhibition zone with increasing extract concentration from 25 µg/mL to 100 µg/mL for both varieties and across all bacterial strains tested.

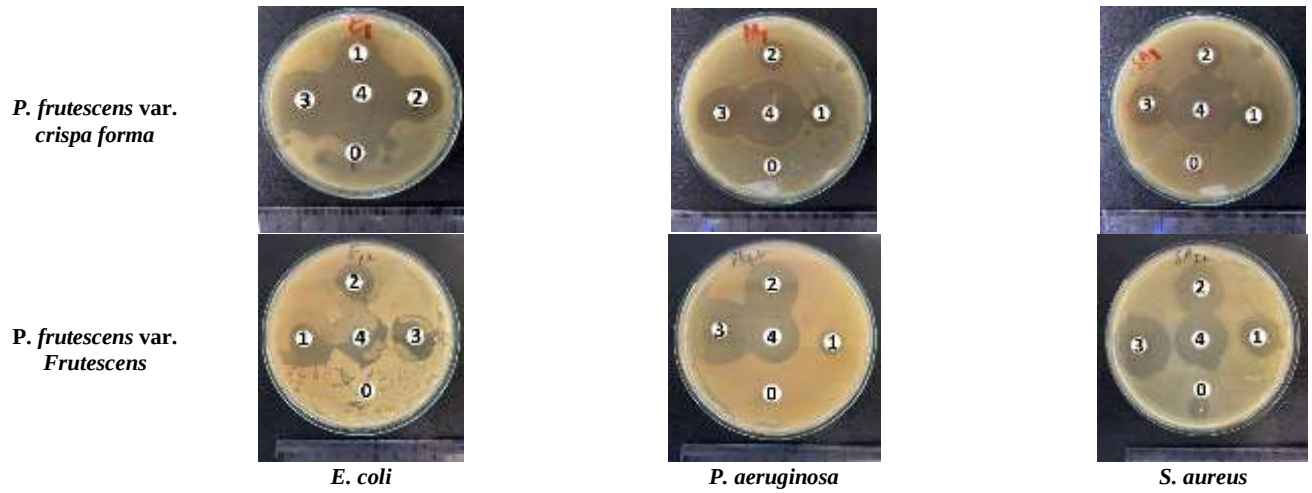

Figure 1: Zone of inhibition for the different Perilla extracts against bacterial strains activity

Note: 0: Negative control (DMSO); 1: Conc. 25 µg/mL; 2: Conc. 50 µg/mL; 3: Conc. 100 µg/mL; 4: positive control (ampicillin 50 µg/mL)

Notably, *P. frutescens* var. *crispa forma* appears to demonstrate a slightly stronger antibacterial effect than var. *frutescens* at equivalent concentrations, particularly at 100 µg/mL against *P. aeruginosa* (20.3 mm vs 16.2 mm) and *S. aureus* (14.7 mm vs 14.5 mm), although this difference is less pronounced against *E. coli* at higher concentrations (both 22.5 mm). However, when compared to the antibiotic ampicillin at 50 µg/mL, both Perilla extracts, even at 100 µg/mL, generally show a comparable or slightly reduced zone of inhibition, especially against *E. coli* and *P. aeruginosa*. This suggests that while *Perilla* ethanol extracts possess significant antibacterial properties, as also highlighted

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in other studies mentioning terpenes and perilla aldehyde as key antibacterial components, their potency might be lower than that of a standard antibiotic like ampicillin at the tested concentration. Perilla extracts demonstrate efficacy against a wide range of bacteria, including both Gram-positive and Gram-negative strains. This finding is consistent with the broad-spectrum antibacterial properties reported in previous studies.

Table 2: Average diameter of zone of inhibition of bacterial growth (mm) against extracts of *Perilla* extract

Sample	Conc. µg/mL	Diameter ring (mm)		
		E. coli	*P. aeruginosa*	*S. aureus*
Ampicillin	50	$14.6^{a} \pm 0.3$	$13.4^{b} \pm 0.2$	$14.5^{a} \pm 0.4$
	25	$10.5^{a} \pm 0.3$	$8.3^{b} \pm 0.3$	$6.3^{c} \pm 0.2$
P. *frutescens* var. *frutescens*	50	$22.5^{a} \pm 0.5$	$16.2^{b} \pm 0.4$	$14.5^{c} \pm 0.3$
	100	$22.5^{a} \pm 0.6$	$16.2^{b} \pm 0.5$	$14.5^{c} \pm 0.2$
	25	$9.7^{a} \pm 0.2$	$7.3^{b} \pm 0.3$	$6.3^{c} \pm 0.2$
P. frutescens var. *crispa forma*	50	$12.3^{a} \pm 0.4$	$8.7^{b} \pm 0.2$	$7.3^{c} \pm 0.3$
	100	$20.7^{a} \pm 0.5$	$20.3^{b} \pm 0.4$	$14.7^{c} \pm 0.2$

Note: Values are means ± standard deviation (SD) of triplicate readings expressed in mm. Values with different letters with in the same row differ significantly at $p < 0.05$ according to Duncan's multiple range test

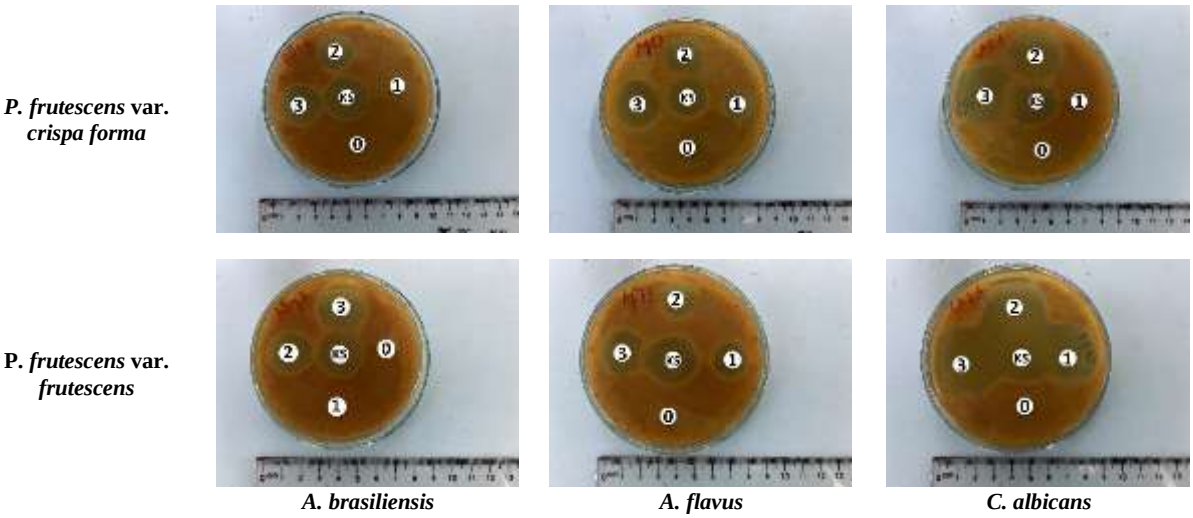

Figure 2: Zone of inhibition for the different Perilla extracts against fungal strains activity

Note: 0: Negative control; 1: Conc. *20 µg/mL; 2:* Conc. *50 µg/mL; 3:* Conc. *100 µg/mL; KS: positive control (amphotericin B 50 µg/mL)*

Antifungal activity

For antifungal evaluation, three fungal strains were examined: *A. brasiliensis*, *A. flavus*, and *C. albicans*. Based on the data presented in Figure 2 and Table 3, the ethanol extracts of both *Perilla frutescens* var. *frutescens* and *P. frutescens* var. *crispa forma* demonstrate antifungal activity against the tested fungal strains, *A. brasiliensis*, *A. flavus*, and *C. albicans*.

Similar to the antibacterial activity, the antifungal effect is generally concentration-dependent, with the diameter of the inhibition zone increasing as the extract concentration rises from 25 µg/mL to 100 µg/mL. *Perilla frutescens* var. *crispa forma* appears to exhibit a slightly stronger antifungal effect than var. *frutescens* in most cases, particularly at higher concentrations against *A. brasiliensis* and *C. albicans*. Notably, *C. albicans* appears to be the most sensitive fungal strain to both Perilla extracts, with the largest inhibition zones observed, even surpassing the effect of amphotericin B at 50 µg/mL for var. *crispa forma* at 100 µg/mL (25.3 mm vs 15.5 mm). However, against *A. brasiliensis* and *A. flavus*, amphotericin B at 50 µg/mL generally shows superior or comparable activity to the Perilla extracts, especially at lower concentrations. The absence of inhibition zones for var. *frutescens* and var. *crispa forma* at 25 µg/mL against *A. brasiliensis* suggests a lower susceptibility of this fungal strain to Perilla extracts at lower concentrations. These findings align with existing research indicating that *Perilla frutescens* extracts possess antifungal properties, likely attributed to similar bioactive compounds as perillaldehyde [17], perilla ketone, and isoegomaketone [18]. The antifungal activity of these active constituents is attributed to their ability to damage fungal cell membranes, prevent spore germination, and affect key enzymatic pathways involved in fungal development. Such mechanisms may explain the observed inhibitory effects of *P. frutescens* extracts against *A. brasiliensis*, *A. flavus*, and *C. albicans* in the present study.

Anticancer activity

The anticancer activity of ethanol extracts from *P. frutescens* var. *crispa forma and P. frutescens* var. *frutescens* was evaluated against AGS cancer cells using the MTT assay. The results demonstrate a dose-dependent inhibitory effect of both *P. frutescens* var. *crispa forma* and var. f*rutescens* extracts on AGS cell viability (Table 4). The extracts from var. *crispa* forma and var. *frutescens* exhibited IC50 values of

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Table 3: Average diameter of zone of inhibition of fungal growth (mm) against extracts of *Perilla* extracts

Sample	Conc. µg/mL	Diameter ring (mm)		
		A. brasiliensis	*A. flavus*	*C. albicans*
Amphotericin B	50	$16.4^{a} \pm 0.4$	$12.7^{c} \pm 0.3$	$15.4^{b} \pm 0.5$
	25	0^{b}	$6.6^{a} \pm 0.2$	0^{b}
P. *frutescens* var. *frutescens*	50	$9.5^{c} \pm 0.3$	$11.4^{b} \pm 0.2$	$20.9^{a} \pm 0.6$
	100	$13.0^{c} \pm 0.4$	$16.5^{b} \pm 0.5$	$23.5^{a} \pm 0.4$
	25	0	$9.0^{b} \pm 0.3$	$15.5^{a} \pm 0.5$
P. *frutescens* var. *crispa forma*	50	$11.9^{b} \pm 0.5$	$10.4^{c} \pm 0.2$	$22.5^{a} \pm 0.3$
	100	$20.0^{b} \pm 0.4$	$12.3^{c} \pm 0.3$	$25.3^{a} \pm 0.6$

Note: Values are means ± standard deviation (SD) of triplicate readings expressed in mm. Values with different letters with in the same row differ significantly at $p < 0.05$ according to Duncan's multiple range test

Table 4: Antiproliferative activity of Perilla extract on AGS cancer cells evaluated by MTT assay

No.	Sample	IC_{50} (µg/mL)
1	Ellipticine	0.53
2	*P. frutescens* var. *crispa forma*	10.43
3	*P. frutescens* var. *frutescens*	15.83

10.43 µg/mL and 15.83 µg/mL, respectively. These values represent the concentration needed to inhibit 50% of cell proliferation. According to National Cancer Institute guidelines, compounds with IC_{50} values below 20 µg/mL are considered to possess high cytotoxic potential, thereby classifying the extract from P. *frutescens* var. *crispa forma* as a potent anticancer agent. These results align with recent studies reporting strong anticancer activity from Perilla phytochemicals, especially phenolic acids, flavonoids, and terpenoids [19]. The anti-proliferative activity observed may be linked to the presence of these bioactive compounds, which are known to induce apoptosis, arrest the cell cycle, and counter oxidative stress [20]. The findings of this study suggest that alcoholic extracts of *Perilla frutescens*, particularly var. *crispa forma*, exhibit significant anti-proliferative activity against AGS human gastric adenocarcinoma cells. These results warrant further investigation into the potential of Perilla leaf extracts as a promising source of natural anti-cancer agents.

Conclusion

This investigation offers thorough comparison of chemical makeup and functional effects in *P. frutescens* var. *crispa forma* and var. *frutescens* from Thai Nguyen, Vietnam. The use of GC–MS revealed distinct chemical profiles between the two varieties, with var. *frutescens* characterized by a high concentration of perilla ketone, while var. *crispa forma* displayed a broader diversity of bioactive compounds, including cyclic aldehydes and sesquiterpenes such as caryophyllene and nerolidol. These chemical differences were reflected in their biological performances, as var. *crispa forma* consistently exhibited stronger antibacterial, antifungal, and anticancer activities. Notably, its ethanol extract demonstrated significant inhibitory effects on both Gram-positive and Gram-negative bacteria and surpassed the antifungal efficacy of amphotericin B against *Candida albicans* at the highest tested concentration. Moreover, the cytotoxic potential of var. *crispa forma* against AGS human gastric cancer cells was particularly remarkable, with an IC_{50} value of 10.43 µg/mL, classifying it as a potent anticancer agent according to NCI standards. These results suggest that *P. frutescens*, especially var. *crispa forma*, holds substantial promise as a source of natural compounds with therapeutic applications. These results lay the groundwork for future research aimed at isolating and characterizing individual compounds, elucidating mechanisms of action, and evaluating in vivo efficacy. Furthermore, this study contributes novel insights into the varietal-dependent bioactivity of *Perilla frutescens*, supporting its potential application in the development of plant-based antimicrobial and anticancer agents.

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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