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Study to determine the chemical constituents, antibacterial ability, and antioxidant activity of the red pepper (*Piper Nigrum* l.) essential oil in Gia Lai Province, Vietnam

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Abstract

Pepper often contains aromatic components, spicy ingredients, and biologically active ingredients. The common chemical components in pepper are essential oils and alkaloids. Essential oils are abundant in pepper, usually accounting for about 1.5–2.2%. Therefore, this study aimed to primarily analyze the constituents, antioxidant activity, and inhibition test microorganisms which are very essential. By using the gas chromatography-mass spectrometry method, 17 components in red pepper essential oil have been identified, of which nine constituents are hydrocarbons (such as 41.35% monoterpenes and 3.55% sesquiterpenes) and the rest are oxygenated hydrocarbons (36.62% alcohols and 17.75% aldehydes). The red pepper essential oil has strong antibacterial ability against experimental strains of microorganisms such as *Bacillus cereus, Staphylococcus aureus, Listeria monocytogenes, Streptococcus viridians, Escherichia coli, Proteus vulgaris, Salmonella enteritidis,* and *Aspergillus flavus* with an average antibacterial circle diameter of 15.29 mm. Besides, it has also been determined that the antioxidant activity of red pepper essential oils was 37.69±0.12%.

Keywords: antibacterial ability; antioxidant activity; chemical composition; essential oil; *Piper nigrum* L.

Practical Application: This is the scientific basis for the application of red pepper essential oil in food processing.

1. Introduction

Pepper has the scientific name *Piper nigrum* L. and is widely produced and consumed in Viet Nam. The Central Highlands is an area with a lot of potentials in terms of land and climate suitable for growing pepper (Quyen, 2017). This cultivated plant is part of the food culture and composes the nutritional habits of the population of Vietnam, playing an important socioeconomic role in these territories. Moreover, the products derived from processing this pepper are also widely used in the food, cosmetics, and pharmaceutical industries, as well as popularly used for culinary and therapeutic purposes. Due to its physiological and adaptive characteristics and the bioactive potential of the chemical constituents, it can be exploited throughout the year, provided that the necessary agronomic requirements are met. Pepper often contains aromatic components, spicy ingredients, and biologically active ingredients. The common chemical components in pepper are essential oils and alkaloids. Essential oils are abundant in pepper, usually accounting for about 1.5–2.2%. The essential oil of pepper is usually pale yellow or light green, and the components include limonene, camphene, sabinene, and oxygen-containing derivatives of hydrocarbons. Piperine is the major naturally occurring biologically active alkaloid component of black pepper, due to its potential therapeutic properties (Ogbuewu & Mbajiorgu, 2023) such as brain function and increased nutrient absorption (Ashokkumar et al., 2021). The biological activities of pepper are also widely investigated in several areas of knowledge, including prospecting studies of their essential oils. Essential oils are volatile and aromatic compounds in plants that mostly contain terpenes and terpenoids in their chemical structure (Zhang et al., 2017). Identifying these compounds and understanding their roles are very important issues in plant science. Studies have demonstrated the potential of pepper essential oil as an antimicrobial, antibacterial, and cytotoxic agent, among others (Sultana et al., 2022). The components of different varieties of this plant have shown variability. The features and bioactivities of some pepper essential oils have been reported. However, the constituents and microbial inhibition properties of pepper essential oil, in Gia Lai Province, Vietnam, have not been evaluated yet. Therefore, this study aimed to primarily analyze the constituents, antioxidant activity, and inhibition test microorganisms which are very essential. This is the scientific basis for the application of the pepper essential oil in food processing and preservation, with high scientific and practical significance.

2. Materials and Methods

2.1. Materials

The red pepper was harvested from the Chu Se district of the Gia Lai Province, Vietnam, in 2022. The essential oil was obtained by steam distillation after drying with Na_2SO_4. The sample was stored in the Department of Food Science and Technology, University of Science, Vietnam National University, Hanoi.

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The tested bacterial strains (i.e., *Bacillus cereus, Staphylococcus aureus, Listeria monocytogenes, Streptococcus viridians, Escherichia coli, Proteus vulgaris, Salmonella enteritidis*, and *Aspergillus flavus*) were obtained from the Institute for Quality Testing and Inspection.

2.2. Methods

2.2.1. Gas chromatography-mass spectrometry

For identification of the phytochemical constituents present in the essential oil, gas chromatography-mass spectrometry (GC-MS)-QP2010 ULTRA (Shimadzu) equipment, equipped with a column Rxi-1MS 30 m×0.25 mm×0.25 µm (Restek) was used.

The column temperature was programmed from 80 to 150°C in 23.5 min at a rate of 3°C/min and then from 150 to 220°C in 8.85 min at a rate of 8°C/min. The used injector temperature was 230°C. The MS conditions were as follows: ionization voltage was 70 eV, transfer temperature was 250°C, the carrier gas was helium used at a flow rate of 0.5 mL/min, and the split ratio of the injector was 1:5.

The quantification of red pepper essential oil constituents was performed using gas chromatography on an HP 7820A Gas Chromatograph (Agilent) equipped with a capillary column with dimensions of 30 m×0.32 mm×0.25 µm (Agilent), with a temperature of 50°C, 3°C/min, up to 220°C. Samples containing 1 µL of essential oil diluted (1%) in chloroform with an initial temperature of 200°C in the split ratio (1:50) were injected. The gas chromatography with flame ionization detector worked at a temperature of 220°C. Drag gas was helium at 3 mL/min. The data acquisition software was Ezchrom Elite Compact Agilent (Don et al., 2019; Purwaniati et al., 2021; Sayed et al., 2021).

2.2.2. Method to determine the antibacterial ability of red pepper essential oil

Antibacterial activity was roughly determined by the agar diffusion method. A volume of 50 µL red pepper essential oil was placed in wells on plates containing the tested bacterial strains. The activity was roughly estimated by the diameter of the antibacterial round (mm), which was calculated by the formula: D-d (mm), where D is the diameter of the antibacterial round (mm) and d is the hole diameter (mm) (Zhang et al., 2017).

2.2.3. Method for determining the antioxidant activity of red pepper essential oil

The free radical scavenging activity of red pepper essential oil was measured using 1,1-diphenyl-2-picrylhydrazol. A volume of 0.5 mM solution of 1,1-diphenyl-2-picrylhydrazol in methanol and 0.005 M acetate buffer (pH 5.5) were prepared. An aliquot of 0.1 mL of red pepper essential oil solution was added to the tube containing 2 mL of acetate buffer, 1.9 mL of methanol, and 1 mL of 1,1-diphenyl-2-picrylhydrazol solution. In the blank tube, 1,1-diphenyl-2-picrylhydrazol was removed; in the control tube, 1 mL of 1,1-diphenyl-2-picrylhydrazol was added to the tube containing 2 mL acetate buffer and 2 mL methanol. The mixture was shaken immediately after adding 1,1-diphenyl-2-picrylhydrazol and allowed to stand at room temperature in the dark. The decrease in absorbance at 517 nm was measured after 32 min until the reaction reached a plateau. Ascorbic acid with a concentration of 0.5 mM was used as a positive control, and its free radical scavenging activity was performed in parallel in the same experiment. These experiments were run in duplicate.

The inhibitory percentage of 1,1-diphenyl-2-picrylhydrazol was calculated as follows (Equation 1):

$$\text{Scavenging effect (\%)} = [(A_o-(A-A_b))/A_o]\times 100\% \quad (1)$$

where A_o is the value of absorbance of the control at the wavelength of 517 nm, A is the value of absorbance of the sample at the wavelength of 517 nm, and A_b is the value of absorbance of the blank at the wavelength of 517 nm (Lee et al., 2020).

3. Results and Discussion

3.1. Constituents of red pepper essential oil

The constituents of red pepper essential oil, identified by GC-MS and quantified by GC-FID, are presented in Table 1 and Figure 1.

Based on the retention times and molecular weights of the sample and the standards, 17 constituents and their percentages of red pepper essential oil were evaluated and are shown in Table

Table 1. Constituents of red pepper essential oil.

No.	Constituents	Retention time (min)	Proportion(%)
Monoterpenes			41.35
1	α-Pinene	10.93	1.23
2	Camphene	12.33	3.12
3	Sabinene	12.93	2.76
4	Limonene	14.15	34.24
Sesquiterpenes			3.55
5	α-Copaene	22.24	0.63
6	β-Elemene	23.53	0.58
7	α-Humulene	24.93	0.61
8	β-Bisabolene	26.98	0.67
9	δ-Cadinene	27.67	1.06
Alcohols			36.62
10	Terpinen-4-ol	15.10	11.46
11	p-Cymen-8-ol	16.06	1.31
12	α-Terpineol	18.95	22.07
13	Eugenol	21.06	0.96
14	Copaen-4-α-ol	25.53	0.82
Aldehydes			17.75
15	Neral	17.58	1.14
16	Citral	18.60	15.29
17	Cinnamaldehyde	19.37	1.32
Total			99.27

%: calculated by chromatographic peak area.

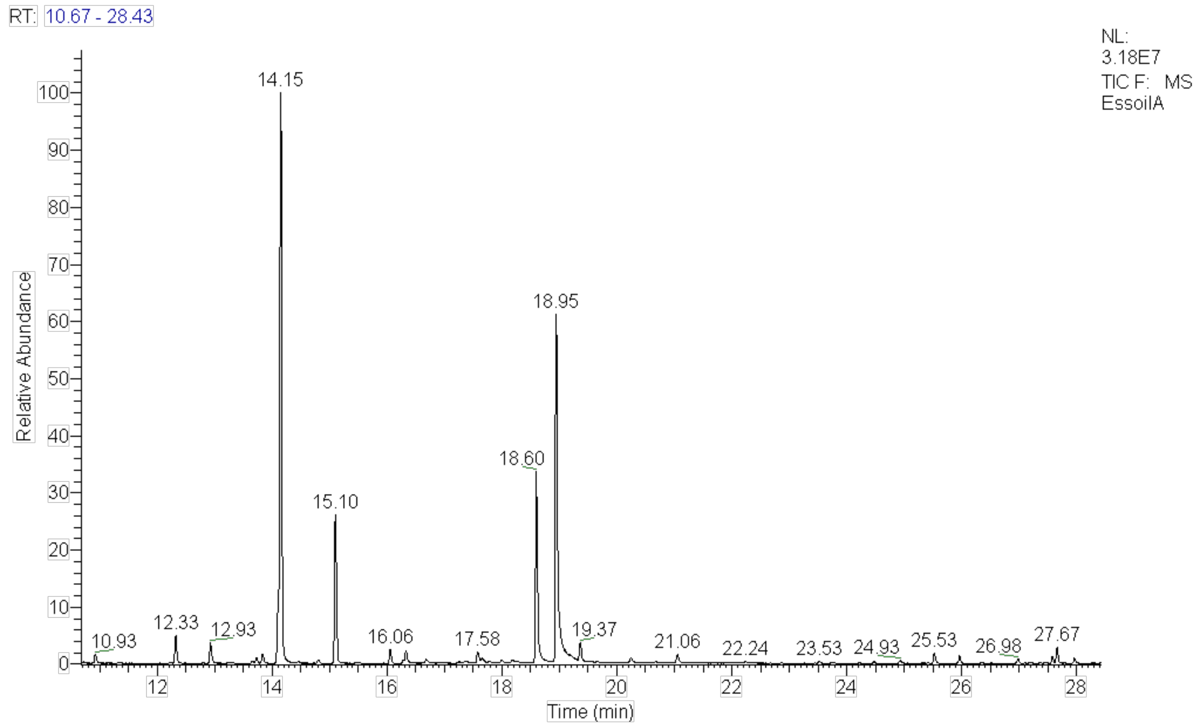

Figure 1. The content of the constituents is calculated as a percentage of the chromatographic peak area.

1, of which, nine constituents are hydrocarbons (such as 41.35% monoterpenes and 3.55% sesquiterpenes) and the rest are oxygenated hydrocarbons (36.62% alcohols and 17.75% aldehydes). Study of the composition of red pepper essential oil carried out in Chu Se district of the Gia Lai Province, Vietnam, revealed 17 different compounds, the main components being limonene 34.24%, α-terpineol 22.07%, citral 15.29%, terpinen-4-ol 11.46%, camphene 3.12%, and sabinene 2.76%. By methods of GC-MS, we found that pepper essential oil in South India contains limonene constituents of 15.13–20.78%; pepper essential oil in Bangladesh contains limonene of 16.16%; pepper oil in Sri Lanka contains limonene constituents of 19.2%; and pepper essential oil in Malaysia contains limonene constituents of 29.9% (Ashokkumar et al., 2021; Morshed et al., 2017). These results show that pepper varieties grown under different ecological and edaphoclimatic conditions have a direct influence on their composition. Limonene was identified as the major constituent of the essential oil pepper under conditions in which this study was conducted. This result is in agreement and corroborates with other investigations that have been developed with pepper essential oil in different phases of its phenological stage and in several regions of the world. Limonene is a monoterpene of biotechnological interest, mainly because of its widely researched bioactive potential. This difference can be justified by the climatic and land conditions that the plants were subjected to during their growth and development. From the results of the analysis of the essential oil pepper by GC-MS, we also found limonene as the main component (34.24%). In addition, the other compounds identified were 22.07% α-terpineol, 15.29% citral, and 11.46% terpinen-4-ol, which are also the major constituents of red pepper essential oil. This result is consistent with the results of Purwaniati et al.'s (2021) and Asadi's (2022) analysis of white pepper essential oil components using GC-MS.

3.2. Antibacterial activity of red pepper essential oils

The antimicrobial activities of red pepper essential oils determined by the agar disk diffusion assay are listed in Table 2.

In general, Gram-positive pathogens were highly sensitive to red pepper essential oil than fungi and gram-negative pathogens. *B. cereus* was the most sensitive bacterium, with an inhibition diameter longer than 23.18±0.12 mm, followed by *S. aureus* with an antibacterial circle diameter of 21.15±0.17 mm, *L. monocytogenes* with an antibacterial circle diameter of 18.13±0.19 mm, and *S. viridians* with an antibacterial circle diameter of 17.24±0.21 mm. The results of the research show that *E. coli* was the most inhibited gram-negative bacterium with an inhibition diameter longer than 11.19±0.14 mm, followed by *S. enteritidis* with an antibacterial circle diameter of 11.17±0.12 mm and *P. vulgaris* with an antibacterial circle diameter of 9.26±0.17 mm. Besides, the research results also show that red pepper essential oil also has strong antibacterial ability against *A. flavus*, with an antibacterial circle diameter of 11.06±0.15 mm. The results of this study are also consistent with the results of Singh et al. (2013) and Zhang et al. (2017).

Interestingly, red pepper essential oil showed a broad spectrum of antimicrobial activity with a diameter of 15.29 mm on average. Furthermore, MICs of red pepper essential oils against eight microbes were also tested, as listed in Table 3.

MICs of red pepper essential oils against most microorganisms were 6.05 μL/mL, peaking at 18.25 μL/mL. The antimicrobial activity of red pepper essential oil was predominantly controlled by the amount of active compound with high diffusivity in an agar medium. The antimicrobial activities of red pepper essential oils were apparently related to a large amount of α-terpineol, citral, terpinen-4-ol, cinnamaldehyde, p-cymen-8-ol, neral, eugenol, copaen-4-α-ol, and especially limonene. Taking both of the results from MICs and inhibition zone diameter into consideration, *B. cereus, S. aureus, L. monocytogenes, S. viridians*, and *E. coli* were the appropriate target bacteria with higher sensitivity. These results revealed that the active compounds from essential oil might bind to the cell surface and then penetrate into the target sites, which could destroy the structure of cell walls. It should be noted that, as shown in Tables 2 and 3, the cell walls changed much to different extents after being treated with the essential oil, while pepper essential oil showed the strongest inhibitory effect against the strains of experimental microorganisms mentioned above. The results of this study are also consistent with the results of Singh et al. (2013) and Zhang et al. (2017).

3.3. Antioxidant activity of red pepper essential oils

Antioxidant activity is an important parameter to evaluate the quality of red pepper essential oils. The results of determining the antioxidant activity of red pepper essential oils are shown in Table 4.

The antioxidant activity of red pepper essential oils was 37.69±0.12%, and this value was a bit higher than that of ascorbic acid at 32.46±0.17%. The results of the study by Chandel et al. (2020) showed that the free radical scavenging DPPH activity ranged from 9.91% to 36.55% with highest in AVPP 9980-S pepper essential oil and lowest in AVPP 9820-S pepper essential oil with 32.94±3.61% and 11.64±1.73% as their genotype mean, respectively. Compared with the research results of Chandel et al. (2020), red pepper essential oil in Chu Se district, Gia Lai Province, Vietnam, has higher resistance to free radical scavenging DPPH, 4.75%. The antioxidant activity of red pepper essential oils was apparently related to the large amount of α-terpineol, citral, terpinen-4-ol, cinnamaldehyde, p-cymen-8-ol, neral, eugenol, copaen-4-α-ol, and especially limonene. This research result is also consistent with the research results of Lee et al. (2020) and Zhang and Xu (2015).

Table 2. Antibacterial activity diameters of red pepper essential oils.

No.	Tested microorganisms	Diameter of an antibacterial round (mm)
1	*Bacillus cereus*	23.18±0.12
2	*Staphylococcus aureus*	21.15±0.17
3	*Listeria monocytogenes*	18.13±0.19
4	*Streptococcus viridians*	17.24±0.21
5	*Escherichia coli*	11.19±0.14
6	*Proteus vulgaris*	9.26±0.17
7	*Salmonella enteritidis*	11.17±0.12
8	*Aspergillus flavus*	11.06±0.15

Table 3. MICs (μL/mL) of red pepper essential oils against various microorganisms

No.	Tested microorganisms	MICs (μL/mL) of red pepper essential oils
1	*Bacillus cereus*	6.05±0.18
2	*Staphylococcus aureus*	6.05±0.24
3	*Listeria monocytogenes*	6.05±0.35
4	*Streptococcus viridans*	6.05±0.48
5	*Escherichia coli*	12.15±0.26
6	*Proteus vulgaris*	18.25±0.35
7	*Salmonella enteritidis*	12.15±0.36
8	*Aspergillus flavus*	12.15±0.45

Table 4. Antioxidant activity of red pepper essential oils.

No.	Tested substances	Result (%)
1	The pepper essential oils	37.69±0.12
2	Ascorbic acid	32.46±0.17

4. Conclusion

From the above research results, some conclusions are drawn as follows: By the GC-MS method, 17 components in red pepper essential oil have been identified, of which nine constituents are hydrocarbons (such as 41.35% monoterpenes and 3.55% sesquiterpenes) and the rest are oxygenated hydrocarbons (36.62% alcohols and 17.75% aldehydes). The red pepper essential oil has strong antibacterial ability against experimental strains of microorganisms such as *B. cereus, S. aureus, L. monocytogenes, S. viridians, E. coli, P. vulgaris, S. enteritidis*, and *A. flavus* with an average antibacterial circle diameter of 15.29 mm. Besides, it has also been determined that the antioxidant activity of red pepper essential oils was 37.69±0.12%. In the past, pepper was used as a seasoning mainly in its raw form. However, through the production of pepper essential oil, especially red pepper, it will be applied in the production of stronger beverages, functional foods, pharmaceuticals, and cosmetics.

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