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Study on the components and biological properties of the essential oil of *Amomum aromaticum* Roxb. in Ha Giang, Vietnam

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Abstract

The essential oil of *Amomum aromaticum* Roxb. in Ha Giang was obtained by steam distillation and dried with Na_2SO_4. Using the gas chromatography-mass spectrometry (GC -MS) method, 26 components in the essential oil were predicted by comparing their retention times and determining molecular weights. In particular, there were 13 hydrocarbons such as monoterpenes (33.03%), sesquiterpenes (1.08%), and 13 oxygenated components such as aldehydes (52.04%), alcohols (10.42%), ketones (1.98%), and oxide (0.14%). Antimicrobial activity against *Staphylococcus aureus, Escherichia coli, Enterococcus faecalis* and *Pseudomonas aeruginosa* of the essential was identified by the agar diffusion method. The antioxidant activity was determined by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical percentage inhibition and the observable result was 46.12±0.15%.

1. Introduction

The *Amomum aromaticum* Roxb. is planted in mountainous areas of the Xin Man, Hoang Su Phi, Vi Xuyen, Quang Ba, Bac Me districts of the Ha Giang province. *Amomum aromaticum* Roxb. possesses medicinal properties. It has been used to weld and increases digestion and reduce swelling pain and fever. In particular, in Southeast Asia, *Amomum aromaticum* Roxb. is used to treat skin diseases, dyspepsia, some digestive tract symptoms, flu, malaria, rheumatoid arthritis and other kinds of infections. *Amomum aromaticum* Roxb. has also been used to produce medicines to treat fever, malaria, colic and lousy breath (Loi *et al*., 2004). The components of different varieties of this plant have shown variability (Diao *et al.*, 2014). The features and bioactivities of some *Amomum aromaticum* Roxb. have been reported (Cui *et al.*, 2017). However, the components and biological properties of *Amomum aromaticum* Roxb. in Ha Giang- Vietnam have not been evaluated yet.

Therefore, this study is aimed to primarily analyze the components, antioxidant activity and antibacterial activity of the essential oil of *Amomum aromaticum* Roxb. in Ha Giang- Vietnam, as a basis for applying this essential oil in food processing and preservation.

2. Materials and methods

2.1 Materials

The *Amomum aromaticum* Roxb. was harvested from the Xin Man district of the Ha Giang province in 2020. The essential oil was obtained by steam distillation after drying with Na_2SO_4. The sample was stored in the Department of Biotechnology and Food Processing, Hanoi University of Industry, Vietnam.

The tested bacterial strains (*Staphylococcus aureus, Escherichia coli, Enterococcus faecalis* and *Pseudomonas aeruginosa*) were obtained from the School of Biotechnology and Food Technology, Hanoi University of Science and Technology.

2.2 Essential oil extraction

The essential oil was extracted by using the rhizome of the plant with water in essential oil distillation equipment Clevenger (Germany) in the ratio of 1:5 (w/v) respectively for 180 mins (Loi *et al.*, 2021).

2.3 Gas chromatography-mass spectrometry (GC-MS)

The sample and standards were run parallelly in the GC-MS experiment. Gas chromatography (GC) analysis was performed by using Agilent Technologies HP 6890 Plus Gas chromatograph system equipped with Flame

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Ionization Detector (FID) and fitted with HP-5MS columns (30 m × 0.25 mm, film thickness 0.25 µm). The temperature was programmed as followed, the column temperature was programmed from 80 to 150°C in 23.5 mins at a rate of 3°C/mins and then from 150 to 220°C in 8.85 mins at a rate of 8°C/mins. 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/mins, and the split ratio of the injector was 1:5 (Teresita *et al.*, 2000; Choi and Sawamura 2002; Wang *et al.*, 2014). The *MS* fragmentation patterns were compared with known patterns of other essential oils and those in the literature by using Wiley (Wiley 9thVersion), NIST 08Libraries (on ChemStation HP). The content of the components is calculated as a percentage of the chromatographic peak area.

2.4 Determination of antibacterial activity using agar diffusion method

Antibacterial activity was roughly determined by the agar diffusion method. A volume of 50 µL of the essential oil was put into wells on the plates containing 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), wherein D was the diameter of the antibacterial round (mm) and d was the hole diameter (mm) (Cui *et al.*, 2017).

2.5 Determination of antioxidant activity using free radical scavenging activity

The free radical scavenging activity of the essential oil was measured by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Teresita *et al.*, 2000; Molyneux *et al.*, 2004; Satyal *et al.*, 2012). A 0.5 mM solution of DPPH in methanol and 0.005 M acetate buffer (pH 5.5) were prepared. An aliquot of 0.1 mL of the essential oil solution was added to the tube containing 2 mL of acetate buffer, 1.9 mL of methanol and 1 mL of DPPH solution. In the blank tube, DPPH was removed, in the control tube, 1 mL of DPPH was added to the tube containing 2 mL acetate buffer and 2 mL methanol. The mixture was shaken immediately after adding DPPH and allowed to stand at room temperature in the dark. The decrease in absorbance at 517 nm was measured after 32 mins until the reaction reached a plateau. Vitamin E with the 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 DPPH was calculated as follows:

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

Wherein 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.

3. Results and discussion

3.1 Components of the essential oil of Amomum aromaticum Roxb. in Ha Giang-Vietnam

GC-MS of the sample was performed to determine the components of the essential oil. Based on the retention times and molecular weights of the sample and the standards (the GC profile was not shown here), 26 components and their percentages in the essential oil were evaluated and shown in Table 1. Table 1 showed that twenty-six components were predicted in the essential oil of *Amomum aromaticum* Roxb. in Ha Giang -Vietnam. Thirteen out of twenty-six were hydrocarbons (such as monoterpenes: 33.03% and sesquiterpenes: 1.08%) and the rest were oxygenated hydrocarbons (aldehydes: 52.04%, alcohols: 10.42%, ketones: 1.98% and oxide: 0.14%). The results provided additional evidence to show varied percentages of the components of the essential oils of *Amomum aromaticum* Roxb. in Ha Giang-Vietnam. Notably, the amounts of aldehydes and alcohols in the essential oil were higher than those of the oil in Nepal (Satyal*et al.*, 2012). The differences were probably due to geographical conditions such as the soil factors, weather, climate, growing conditions and harvesting time (Diao *et al.*, 2014).

3.2 The antibacterial activity of the essential oil of Amomum aromaticum Roxb.in Ha Giang-Vietnam

The agar diffusion method was used to estimate the antibacterial potentials of the essential oil of *Amomum aromaticum* Roxb. in Ha Giang-Vietnam. The tested microorganisms used in this experiment were *S. aureus, E. coli, E. faecalis* and *P. aeruginosa.* The diameters of antibacterial activity rounds of the essential oil against these bacteria were shown in Table 2. The results showed that the essential oil of *Amomum aromaticum* Roxb. in Ha Giang-Vietnam possessed antibacterial activity against all of the four microorganisms tested. Among them, the antibacterial activity against *Staphylococcus aureus* was the highest. The activity of the essential oil of *Amomum aromaticum* Roxb. in this research is similar to that of the essential oils of the leaves of *Liquidambar formosana* Hance in Bac Giang as these essential oils were found to possess antibacterial activities against all of the four tested microorganisms

Table 1.The components of the essential oil of *Amomum aromaticum* Roxb. in Ha Giang-Vietnam

No.	Components	Retention time (mins)	Proportion (%)
	Monoterpenes		33.03
1	*α*-pinene	7.092	5.88
2	camphene	7.564	0.13
3	*β*-pinene	8.358	7.94
4	*β*-myrcene	8.928	3.46
5	*α*-phellandrene	9.677	10.78
6	*β*-ocimene	11.030	1.89
7	*ϒ*-terpinene	11.389	1.95
8	2-carene	12.282	1.00
	Sesquiterpenes		1.08
9	*α*-isopropenyltoluene	12.425	0.37
10	*ϒ*-muurolene	25.851	0.21
11	*α*-muurolene	26.077	0.13
12	*α*-copaene	26.651	0.23
13	*β*-bisabolene	26.364	0.14
	Aldehydes		52.04
14	2-octenal	11.543	1.83
15	neral	17.923	12.51
16	citral	19.338	20.82
17	2-isopropylbenzaldehyde	19.800	11.37
18	cinnamaldehyde	22.220	0.04
19	7-tetradecenal	25.328	5.47
	Alcohols		10.42
20	eucalyptol	10.487	6.58
21	carveol	13.954	0.01
22	*α*-terpineol	16.395	3.05
23	geraniol	20.630	0.08
24	*α*-acorenol	24.700	0.70
	Ketones		1.98
25	tetracyclo [3.3.1.0.1(3,9)] decan-10-one	20.446	1.98
	Oxide		0.14
26	longipinene epoxide	32.159	0.14
	Total		98.69

Table 2. The diameters of antibacterial activity rounds of the essential oil of *Amomum aromaticum* Roxb. in Ha Giang-Vietnam

No.	Tested microorganisms	Diameter of antibacterial round (mm)	Antibacterial activity (%)
1	*Staphylococcus aureus*	46.34±0.15	67.96±0.55
2	*Escherichia coli*	42.29±0.23	53.28±0.83
3	*Enterococcus faecalis*	39.06±0.25	41.57±0.91
4	*Pseudomonas aeruginosa*	37.43±0.17	35.67±0.62

(Loi *et al.*, 2015). This result is also consistent with the research result by Diao *et al*. (2014).

3.3 The antioxidant activity of the essential oil of Amomum aromaticum Roxb. in Ha Giang-Vietnam

The results of the study are shown in Table 3. The DPPH free radical scavenging activity of the essential oil of *Amomum aromaticum* Roxb. was 46.12±0.15% and this value was slightly higher than that of 0.5 mM vitamin E (41.18±0.26%). These activities of the essential oils of the leaves of *Liquidambar formosana* Hance in Bac Giang and *Citrus sinensis* peel were found to be 41.13±0.22% and 45.32±0.18%, respectively (Matook *et al.*, 2006; Loi *et al.*, 2015). Therefore, the DPPH free radical scavenging activity of the essential oil of *Amomum aromaticum* Roxb. is higher than the leaves of *Liquidambar formosana* Hance in Bac Giang and *Citrus sinensis* peel. This research result was also consistent with the research result of Teresita *et al.* (2000).

Table 3. The antioxidant activity of the essential oil of *Amomum aromaticum* Roxb. in Ha Giang-Vietnam

No.	Tested substances	Result (%)
1	Essential oil	46.12±0.15
2	Vitamin E	41.18±0.26

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4. Conclusion

By Gas chromatography-mass spectrometry (GC-MS) method, twenty-six components in the essential oil were predicted by comparing their retention times and molecular weights with the standards. In particular, there were thirteen hydrocarbons such as monoterpenes (33.03%), sesquiterpenes (1.08%), and 13 oxygenated components like aldehydes (52.04%), alcohols (10.42%), ketones (1.98%), and oxide (0.14%). Antimicrobial activity against *S. aureus, E. coli, E. faecalis* and *P. aeruginosa* of the essential was identified by the agar diffusion method. The antioxidant activity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical percentage inhibition and 46.12±0.15%.

Conflict of interest

The author declares the research results in this article to be completely honest. The data has never been used or rotated from other research projects in any form.

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