

## Chemical Composition, Antioxidant and inhibition $\alpha$ -Glucosidase Activity of (*Amomum villosum* Lour.) Essential Oil

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**Abstract:** The *Amomum villosum* is widely used to treat intestinal diseases, abdominal pain, helminth infections, digestion, stimulates digestive enzyme secretion, enhances appetite, reduces bloating, and indigestion. However, the chemical composition and biological properties of the essential oil of *Amomum villosum* collected in Thanh Hoa, Vietnam, have not been investigated. Gas chromatography-mass spectrometry (GC-MS) was used to identify the chemical composition of *Amomum villosum* essential oil. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and inhibition of  $\alpha$ -Glucosidase activities of the essential oil, respectively. Experimental results revealed that all parts of the plant, including the leaves, stems, and roots, contain essential oil. Among them, the leaves exhibited the highest oil content, ranging from 1.0 to 4.0%, while the stems and roots showed comparatively lower yields. The major constituents of the essential oil obtained from different plant parts were  $\alpha$ -pinene (11.6–22.0%) and  $\beta$ -pinene (6.6–48.1%), which are characteristic monoterpenes contributing to the distinctive aroma of the oil. The strong antioxidant and antibacterial effects of *Amomum villosum* essential oil were demonstrated with an  $IC_{50}$  value of  $6.91 \pm 0.2$   $\mu$ g/mL and anti- $\alpha$ -glucosidase activities with an  $IC_{50}$  value of  $4.16 \pm 0.32$  mg/mL, respectively. Our results indicate that the essential oil of *Amomum villosum* collected in Thanh Hoa, Vietnam, has great potential in the pharmaceutical industry.

**Keywords:** Thanh Hoa, *Amomum villosum*, essential oil, antioxidant, antibacterial.

### INTRODUCTION

*Amomum villosum* belongs to a genus of medicinal plants with many valuable species, widely used in traditional medicine and as a flavoring agent (Do, H. B., et al., 2003). The plant grows 1–2 meters tall, with a swollen green stem base. The leaves are lanceolate, measuring 15–30  $\times$  4–8 cm, sessile, with a ligule 1–3 mm long. The inflorescence stalk is 4.0–7.0 cm in length, with bracts about 0.8–1.2 cm long. Flowers arise from the base of the stem. The calyx tube is narrowly funnel-shaped, 1.4–1.8 cm long. The corolla tube measures 1.6–2.0 cm, with lobes 1.4–1.6 cm long, white in color. The labellum is suborbicular, about 2.3–2.5 cm wide, white with a yellow midrib bearing reddish-purple spots, and its apex is 3-lobed. The filament is approximately equal to the anther length (5–6 mm); the anther is 3-lobed. The lateral staminodes appear as two ridges at the base of the labellum. The ovary is cylindrical, white-hairy. The capsule fruit is globose, 1.3–1.5 cm in diameter, green turning yellow when ripe, and covered with soft spines. The seeds are angular and aromatic (Do, T. L. 1999; Dao, L. P. 1995).

The flowering season occurs from April to June, and the fruiting season from June to September. The plant grows under humid forest canopies at elevations of 100–1,000 m. In the herb layer, it is commonly found together with species such as *Alpinia macroura*, *Alpinia menghaiensis*, *Amomum muricarpum*, *Amomum xanthioides*, *Piper sp.*, *Pronephrium megacuspis*, and *Athyrium*

*boryanum*. The shrub layer includes *Goniothalamus sp.*, *Streblus sp.*, *Lasianthus sp.*, *Glycosmis craibii*, *Clausena sp.*, and *Boehmeria sp.* The tree layer consists of *Alphonsea tonkiensis*, *Knema sp.*, *Ficus sp.*, *Lagerstroemia sp.*, and *Goniothalamus macrocalyx* (Do, H. B., et al., 2003; Nguyen, X. M. A. 2009).

This species is distributed sporadically in Vietnam, and also found in China, India, Thailand, Laos, Cambodia, and Myanmar (Nguyen, X. M. A. 2009). Currently, the domestic demand for dried fruits and seeds of *Amomum villosum* is increasing rapidly, particularly for pharmaceutical production and essential oil extraction (Le, T. H. 2015). *Amomum villosum* is recognized for its high medicinal value and has been traditionally used in folk remedies for centuries. Numerous studies have reported the chemical composition of *A. villosum*, with particular emphasis on its essential oil constituents (Nguyen, D. C. et al., 2017; Dung, N. X. et al., 1990; Do, N. D. et al., 2015; Gilani, S. R. et al., 2006).

Up to now, studies have also shown that the raw material from different regions and different extraction methods also greatly affect the content of *Amomum villosum*. Therefore, when testing its chemical activity, it is possible to discover different active constituents according to the above factors. This study aims to analyze and characterize the chemical composition and

biological properties *Amomum villosum* essential oil collected in Thanh Hoa, Vietnam, in support of its potential exploitation and effective utilization. Determination of the chemical composition of *Amomum villosum* essential oil was conducted using gas chromatography-mass spectrometry (GC-MS). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and the agar disc diffusion method were used to evaluate the antioxidant and antibacterial activities, respectively.

## MATERIAL AND METHODS

### Chemicals

Butylated hydroxytoluene (BHT), C7–C30 straight-chain hydrocarbons, reference chemicals

for identification, Tween 80, and DPPH were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Other chemical analytical grades, the culture media, and standard antibiotic discs were procured from Merck (Darmstadt, Germany) and Oxoid Ltd. (Basingstoke, Hampshire, UK), respectively.

### Plant Material

The *Amomum villosum* were collected from Thanh Hoa, Vietnam, in January 2023. A voucher specimen (No. SN-TH-01) was prepared and deposited at the Faculty of Natural Science and Technology, Hong Duc University, Thanh Hoa Province, Vietnam.



**Figure 1:** The *Amomum villosum* collected in Thanh Hoa Province.

### Extraction of Essential Oil

The *Amomum villosum* were collected and cleaned, cut into small pieces, and subjected to steam distillation for 4 hours by a Clevenger-type apparatus. The essential oil obtained was then dehydrated using anhydrous sodium sulfate and stored in a sealed vial at 10 °C in the dark prior to subsequent experiments.

### Analysis of Essential Oil by GC-MS

To analyze the composition of the essential oil from the of *Amomum villosum*, a Trace GC Ultra-ITQ900 system (Thermo Fisher Scientific, MA, USA) was used. Data were interpreted by MassFinder 4.0 software. The separation was performed on a fused silica capillary TG-SQC column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The GC operational parameters included injector temperature 250 °C, detector temperature 260 °C, oven temperature program 60 to 260 °C at a heating rate of 4 °C/min, carrier gas

helium at a flow rate of 1.0 mL/min, and sample injection volume 1 µL in split mode with a split ratio of 1:10. The mass spectrometer was operated in electron ionization (EI) mode with the following parameter values: ionization energy 70 eV, interface temperature 280 °C, ion source temperature 230 °C, MS quadrupole temperature 200 °C, and scan range 35–650 amu (Kitajima, J., & Ishikawa, T. 2003). The retention indices of the essential oil constituents were determined using an HP-5 MS column and standard C7–C30 straight-chain hydrocarbon reference standards (Sigma-Aldrich Chemical Company, USA). The mass spectra and retention indices of individual compounds were identified by comparing them with those in GC-MS libraries (National Institute of Standards and Technology-NIST 08 and Wiley 09th version) and/or with published data. The relative percentages of the identified compounds were calculated based on GC peak areas without applying correction factors.

### Antioxidant Assay

The DPPH assay was used to evaluate the antioxidant activity of the essential oil from *Amomum villosum* leaf and stems (Choi, J. W. *et al.*, 2009). The essential oil was dissolved in methanol to different concentrations (0.3125, 0.625, 1.25, 2.5, 5.0, and 10 µg/mL), and the positive control BHT was mixed with 200 µL of a methanolic solution (containing DPPH radicals at a concentration of 150 µmol/L). The mixture was then vigorously shaken and left in the dark for 30 minutes to complete the reaction. The absorbance solutions were measured using a Shimadzu UV1800 spectrophotometer (Shimadzu Corporation, Japan) at 517 nm. A blank sample (a control solution without extract or BHT) was used for comparison. The scavenging ability of the essential oil was calculated as in Equation 1.

$$\text{Scavenging ability (\%)} = \frac{A_{517 \text{ of control}} - A_{517 \text{ of sample}}}{A_{517 \text{ of control}}} \times 100 \quad (1)$$

### Inhibition of $\alpha$ -Glucosidase Assay

The anti- $\alpha$ -glucosidase activity of *B. variegata* essential oil was evaluated according to the method of Sihvonen, *et al.*, (1999) (Hanh, D. T. *et al.*, 2023). Take 50 µL of solution prepared by dissolving essential oil in 0.1 M phosphate buffer (pH 6.8) with different concentrations (10, 5, 2.5, 1.25, and 0.625 mg/mL) containing 30 µL of 2U/mL  $\alpha$ -glucosidase solution, incubated at 37°C for 10 min. Then 50 µL of 2.5 mM *p*-nitrophenyl-

$\alpha$ -D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.8) was added and continued to be incubated at 37°C for 30 min. After incubation, the absorbance was measured at 405 nm using an ELISA (BIO-RAD iMark microplate reader). The measured results were compared with the control sample (containing only 50 µL of solvent). The  $\alpha$ -glucosidase inhibition was calculated using equation 2.

$$\text{Inhibition (\%)} = \frac{A_0 - A_1}{A_0} \times 100\% \quad (2)$$

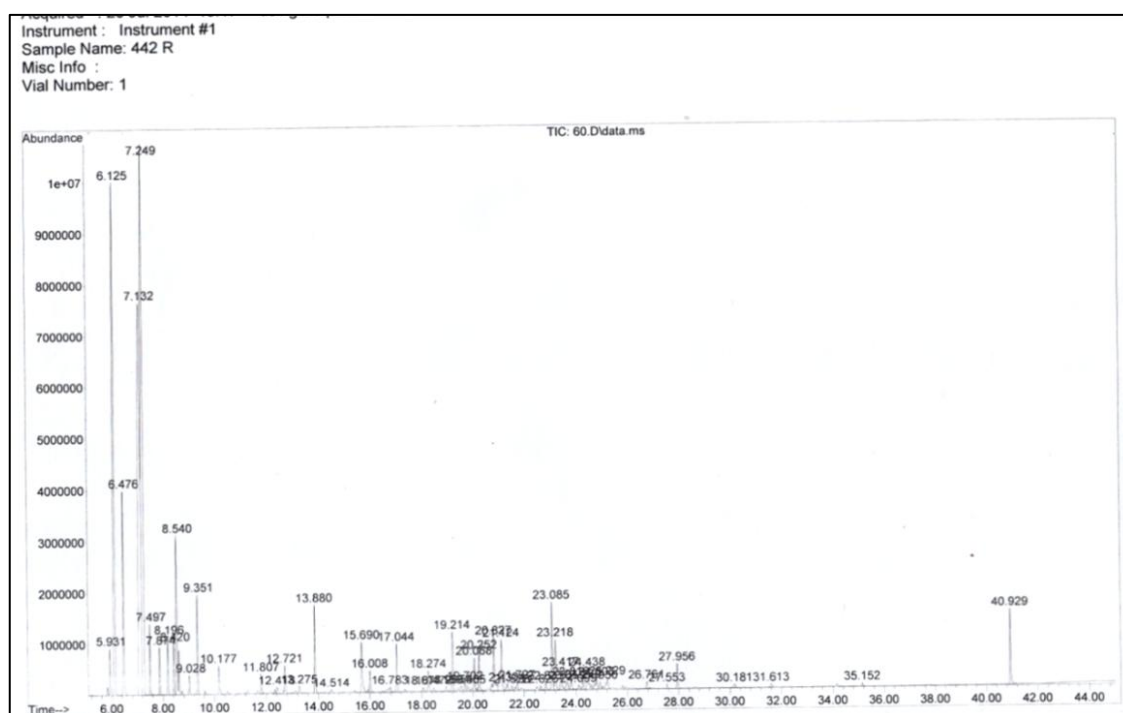
where A0 is the absorbance of the control sample (containing only solvent) at the initial time. A1 is the absorbance of the test sample (Sihvonen *et al.* 1999).

### Statistical Analysis

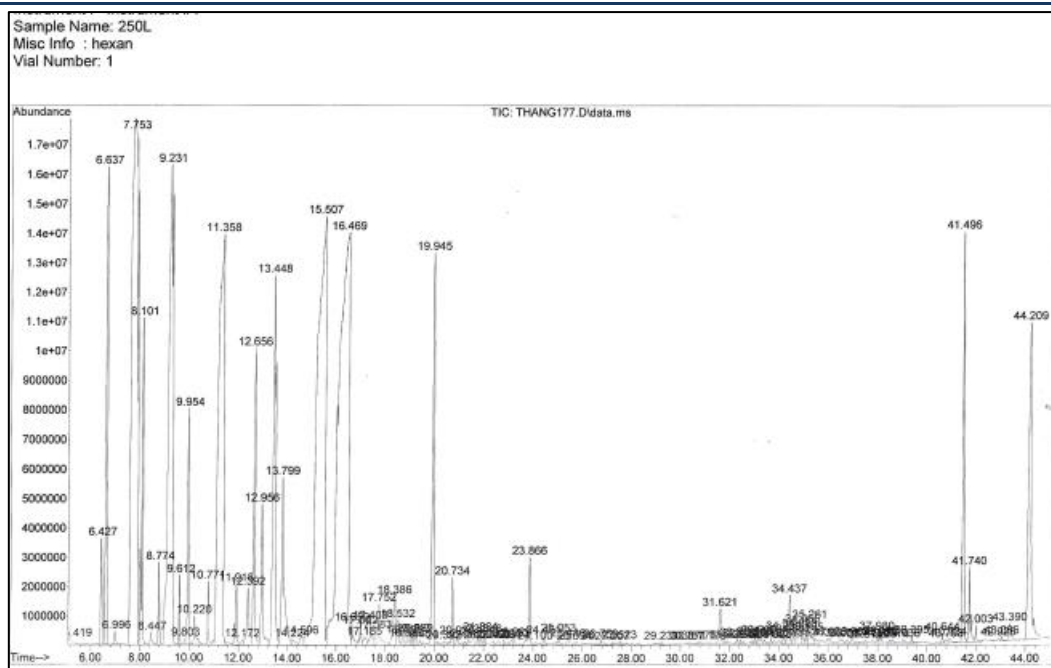
All experiments were carried out in triplicate. Analysis of variance (ANOVA) and Statistica 5.5 software (StatSoft Inc., Tulsa, OK, USA) were utilized to analyze the results. The results are presented as the mean  $\pm$  standard deviation (SD).

## RESULTS AND DISCUSSION

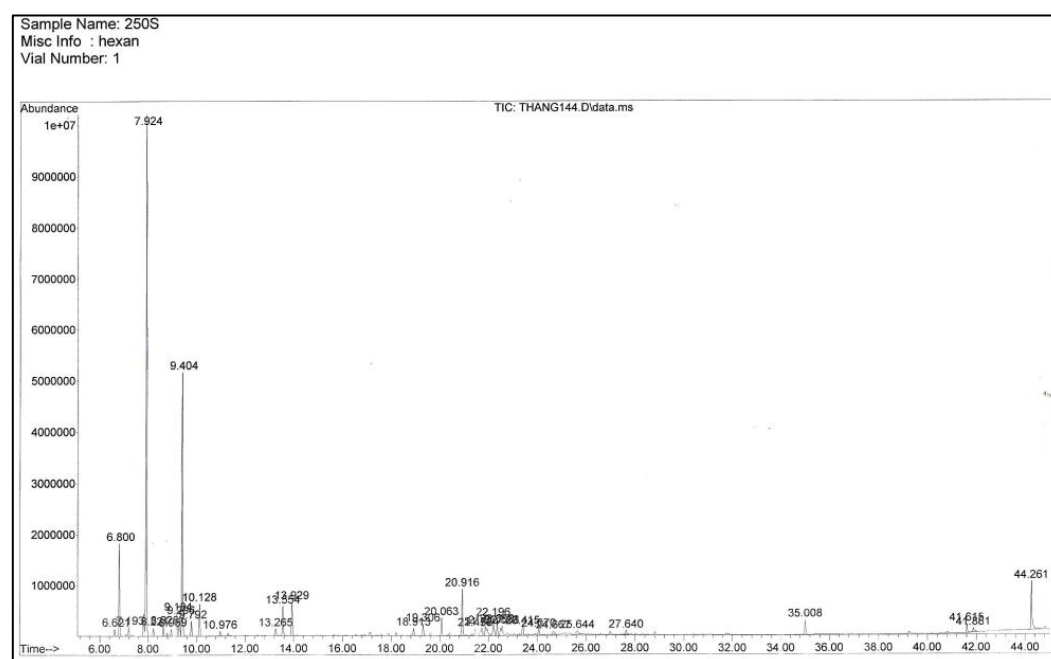
Essential oil was extracted from the leaves and stems of *Amomum villosum* hydrodistillation, with a 0.1-0.18% yield (w/w, based on fresh weight). The total ion chromatogram obtained from GC-MS analysis is shown in Figure 2,3 and 4.



**Figure 2.** GC-MS total ion chromatogram from leaves of *Amomum villosum* essential oil.



**Figure 3.** GC-MS total ion chromatogram from stems of *Amomum villosum* essential oil.



**Figure 4.** GC-MS total ion chromatogram from roots of *Amomum villosum* essential oil.

**Table 1.** Chemical compositions from stems *Amomum villosum* essential oil

|                        |        |                                                   |
|------------------------|--------|---------------------------------------------------|
| <i>Amomum villosum</i> | Leaves | $\beta$ -pinen (6.6%) và $\alpha$ -pinen (22.0%)  |
|                        | Stems  | $\beta$ -pinen (48.1%) và $\alpha$ -pinen (16.0%) |
|                        | Roots  | $\beta$ -pinen (34.7%) và $\alpha$ -pinen (11.6%) |

All parts of the plant, including the leaves, stems, and roots, contain essential oils, among which the leaves have the highest content, ranging from 1–4%, while the stems and roots contain lower amounts. The main constituents of the essential oil in various plant parts, particularly in the bark, are  $\alpha$ -pinene (11.6%–22.0%) and  $\beta$ -pinene (6.6%–

48.1%). The fruits of *Amomum villosum* are used in traditional medicine as a digestive stimulant and to treat stomach pain, vomiting, enteritis, dysentery, and threatened miscarriage. The juice extracted from the rhizome is used to relieve coughs. The plant has a pungent taste and a warm nature, commonly employed to alleviate

abdominal pain, indigestion, nausea, diarrhea, and uterine disorders. The rhizomes are also used as a spice, and the whole plant yields essential oil of medicinal and aromatic value.

Based on the above comparisons and analyses, it can be affirmed that the essential oil extracted from *Amomum villosum* chemical profile, reflecting its unique ecological signature. It is particularly rich in oxidized sesquiterpenoids and nitrogen-containing heterocyclic compounds components that are rarely reported in samples from other regions. These compounds not only contribute to the oil's promising biological activities, such as anti-inflammatory, antioxidant, and antibacterial effects, but also serve as important indicators for guiding the standardization of cultivation zones. This

highlights the high application potential of raw materials from the Central Highlands in the development of herbal medicines and natural cosmetic products.

#### Antioxidant Activity of *Amomum villosum* Essential Oil

The antioxidant activity was evaluated by the DPPH radical scavenging assay, with the results summarized in Table 2. The IC<sub>50</sub> value of *Amomum villosum* essential oil was determined to be  $6.91 \pm 0.2$  µg/mL, compared to  $6.1 \pm 0.1$  µg/mL for BHT, a well-known synthetic antioxidant. These results indicate that the essential oil of *Amomum villosum* collected in Thanh Hoa has strong antioxidant activity comparable to BHT, highlighting its potential as a natural antioxidant agent.

**Table 2.** Antioxidant activity of *Amomum villosum* essential oil

| Essential oil (mg/mL) | % Inhibition |       |       | IC <sub>50</sub> (mg/mL) |
|-----------------------|--------------|-------|-------|--------------------------|
|                       | 1            | 2     | 3     |                          |
| 30                    | 96.21        | 94.53 | 95.16 | $6.91 \pm 0.2$           |
| 15                    | 66.35        | 67.48 | 66.72 |                          |
| 7.5                   | 51.75        | 52.67 | 52.18 |                          |
| 3.75                  | 48.28        | 47.09 | 47.09 |                          |
| 1.875                 | 43.51        | 42.59 | 42.67 |                          |
| BHT <sup>a</sup>      |              |       |       | $6.10 \pm 0.1$           |

#### Butylated hydroxytoluene was used as positive control

Glucosidase Inhibitory Activity of *Amomum villosum* Essential Oil

The  $\alpha$ -glucosidase inhibitory activity of *Amomum villosum* essential oil was evaluated using spectroscopy, and the results are presented in Table 3.

**Table 3.**  $\alpha$ -Glucosidase inhibitory activity of *Amomum villosum* essential oil

| Essential oil (mg/mL) | % Inhibiting |       |       | IC <sub>50</sub> (mg/mL) |
|-----------------------|--------------|-------|-------|--------------------------|
|                       | 1            | 2     | 3     |                          |
| 20                    | 93.68        | 94.31 | 94.12 | $4.16 \pm 0.32$          |
| 10                    | 63.87        | 64.79 | 67.13 |                          |
| 5                     | 51.12        | 52.65 | 51.74 |                          |
| 2.5                   | 39.36        | 38.09 | 38.43 |                          |
| 1.25                  | 34.56        | 33.15 | 34.79 |                          |
| Acarbose <sup>a</sup> |              |       |       | $0.013 \pm 0.01$         |

#### Positive control for anti- $\alpha$ -glucosidase

The results in Table 3 show that the  $\alpha$ -glucosidase inhibitory activity of *Amomum villosum* essential oil increased from 33.15% to 94.31% as the concentration of the essential oil increased from 1.25 to 20 mg/mL, indicating that the oil can inhibit  $\alpha$ -glucosidase. However, this activity is not very strong. The IC<sub>50</sub> results showed that the  $\alpha$ -glucosidase inhibitory activity of *Amomum villosum* essential oil (IC<sub>50</sub> =  $4.16 \pm 0.32$  mg/mL) is lower than that of the positive control, acarbose, with an IC<sub>50</sub> value of 0.013 mg/mL.

#### CONCLUSION

This study is the first to investigate the essential oil from the of *Amomum villosum* collected in Thanh Hoa, Vietnam. GC-MS analysis identified a diverse chemical composition.

All parts of the plant (leaves, stems, and roots) contain essential oils, with the leaves showing the highest content, ranging from 1.0–4.0%, while the stems and roots contain lower amounts. The major components of the essential oil in different parts of the plant, particularly in the bark, are  $\alpha$ -pinene

(11.6%–22.0%) and  $\beta$ -pinene (6.6%–48.1%). The essential oil exhibited notable biological activities, demonstrating strong antioxidant activity with an  $IC_{50}$  value of  $6.91 \pm 0.2 \mu\text{g/mL}$ , surpassing the reference compound, BHT ( $6.1 \pm 0.1 \mu\text{g/mL}$ ). Additionally, the essential oil also exhibited strong anti- $\alpha$ -glucosidase activities with an  $IC_{50}$  value of  $4.16 \pm 0.32 \text{ mg/mL}$ . These findings underscore the potential of *Amomum villosum* essential oil as a natural resource for pharmaceutical applications particularly in antioxidant and  $\alpha$ -glucosidase therapies.

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