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Study Factors Affecting the Content of Essential oil of *Vernonia amygdalina* and Alphaglucosidase Inhibitory Activity

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Abstract
From the fresh leaves of the *Vernonia amygdalina*, an essential oil was obtained by hydrodistillation. The essential oil was obtained by steam distillation using a plant material-to-water ratio of 1:8 (w/v), conducted over 2.5 hours at a temperature range of 60–80 °C.
The resulting essential oil was pale yellow in color, with a yield of 0.75%.In addition, we also tested the anti-α-glucosidase activity of *Vernonia amygdalina* essential oil. The results showed that the essential oil also exhibited strong activities with an IC_{50} value of 3.31 ± 0.04 mg/ml. These findings underscore the potential of *Vernonia amygdalina* essential oil as a natural source for pharmaceutical applications.

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1. Introduction

The plant kingdom represents an invaluable resource, offering a vast array of natural compounds with diverse biological activities that serve as a foundation for the development of medicine, pharmacy, and various other industries. In Vietnam, where the flora is exceptionally rich and diverse, the potential for exploiting natural medicinal resources is immense. In the context of modern medicine facing major challenges, such as the rising prevalence of chronic diseases, the alarming increase in antibiotic resistance, and the urgent demand for safer and more effective treatment options, the exploration of traditional medicinal plants has become increasingly essential. Natural products, particularly essential oils, are receiving growing attention due to their wide range of pharmacological properties, including antibacterial, antifungal, antioxidant, and anti-inflammatory activities [1, 2].

Bitter leaf (*Vernonia amygdalina* Delile), also known in Vietnam as *"cây mật gấu"*, is a plant species belonging to the Asteraceae family that has a long history of use in traditional medicine across various regions of the world, including Vietnam [3]. Traditionally, it has been used to support the treatment of liver disorders, gastrointestinal conditions, and fever reduction. In addition, *Vernonia amygdalina* has been extensively studied for its pharmacological potential, particularly its antioxidant, anti-inflammatory, antibacterial, and anticancer activities [4]. Global studies have identified the presence of numerous biologically active compounds in *Vernonia amygdalina*, including vernonioside, vernodalin, vernolepin, and various flavonoids [5].

However, most of the published scientific studies on *Vernonia amygdalina* have primarily focused on various extracts, such as aqueous or ethanol extracts. To date, there have been limited reports on the essential oil of *Vernonia amygdalina* cultivated in Vietnam, particularly in Dak Lak Province, a region in the Central Highlands known for its distinct climate and soil conditions that are highly favorable for the growth of numerous medicinal plant species. Ecological factors specific to each geographical area can significantly influence the biosynthesis and accumulation of chemical constituents in plants, potentially resulting in variations in the composition and biological activity of the essential oils [6].

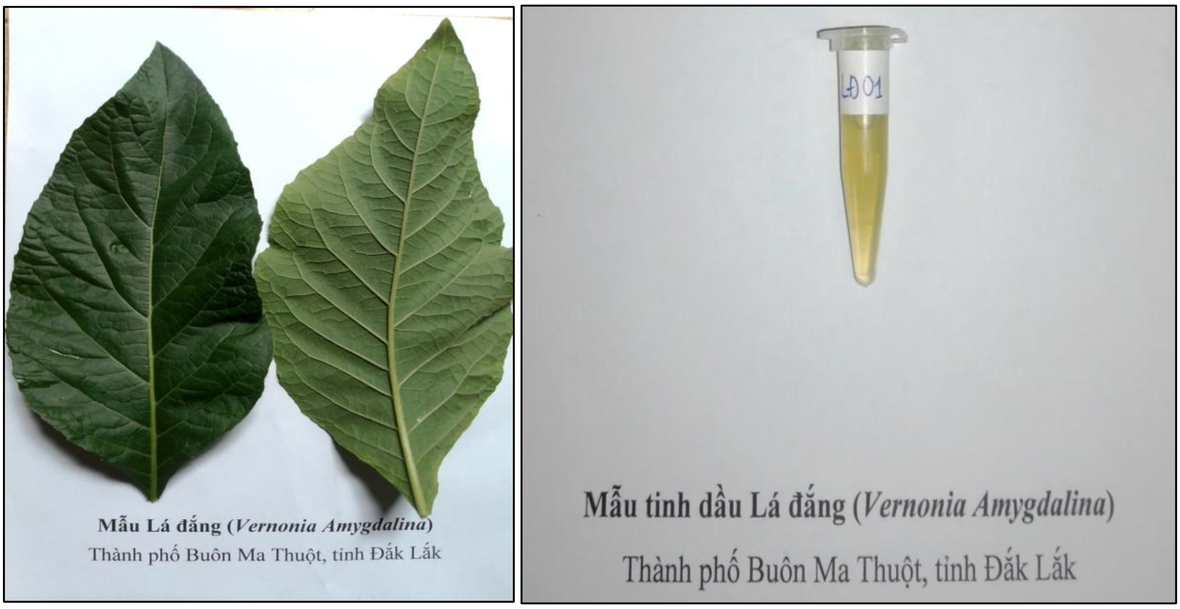

Fig 1: The fresh leaves and of *Vernonia amygdalina* essential oil were collected in Dak Lak Province

2. Materials and Methods

2.1 Plant material

The fresh leaves of *Vernonia amygdalina* were collected from Ea Tam commune, Buon Ma Thuot city, Dak Lak province, Vietnam in 2024. The sample was identified by Dr. Nguyen Quoc Binh (Vietnam National Museum of Nature, Vietnam Academy of Science and Technology). A voucher specimen, LĐ-BMT-01, is deposited at Faculty of Natural Science and Technology, Tay Nguyen University, Buon Ma Thuot city, Dak Lak province, Vietnam.

2.2 Essential oil Extraction

The fresh leaves of *Vernonia amygdalina* (Fig. 1) were cleaned, cut into small, and subjected to steam-distillation in a Clevenger-type apparatus for 2.5 h. The obtained essential oil was dried over anhydrous sodium sulfate and stored in a sealed vial at 10°C in the dark prior to analysis.

2.3 Inhibition of α-Glucosidase Assay

The anti-α-glucosidase activity of *Vernonia amygdalina* essential oil was evaluated according to the method of Sihvonen *et al*. (1999) [7]. 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 α-glucosidase solution, incubated at 37°C for 10 min. Then 50 µL of 2.5 mM *p*-nitrophenyl-α-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 α-glucosidase inhibition was calculated using equation 1.

$$Inhibition\ (\%) = \frac{A0-A1}{A0}\ x\ 100\% \qquad (1)$$

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) [7].
Each assay was conducted in triplicate.

3. Results and Discussion

3.1 Factors affecting the content of *Vernonia amygdalina* essential oil

3.1.1 Investigation of Plant Material-to-Water Ratio

Table 1: Effect of Plant Material-to-Water Ratio on Essential Oil Yield

Plant Material-to-Water Ratio (g/mL)	**1:6**	**1:8**	1:10	1:13
Essential Oil Yield (%)	0.557	0.591	0.568	0.470

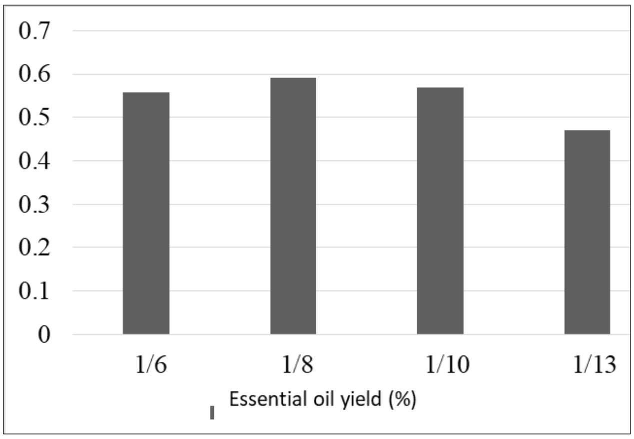

Fig 2: Effect of Plant Material-to-Water Ratio on Essential Oil Yield

Based on the data presented in Table 1 and Figure 2, it was observed that as the volume of water increased relative to the amount of plant material (i.e., as the plant material-to-water ratio decreased from 1:6 to 1:13), the essential oil yield tended to decline after reaching an optimum at the 1:8 ratio. An insufficient amount of water may be inadequate to effectively extract all the essential oil, whereas an excessive amount of water may dilute the oil or reduce extraction efficiency. Therefore, a plant material-to-water ratio of 1:8 was selected as the optimal condition for the distillation of *Vernonia amygdalina* essential oil.

3.1.2. Investigation of Distillation Time

Table 2: Effect of Distillation Time on Essential Oil Yield

Distillation Time (h)	**1.0**	**1.5**	**2.0**	2.5	3.0
Essential Oil Yield (%)	0.419	0.437	0.746	0.751	0.626

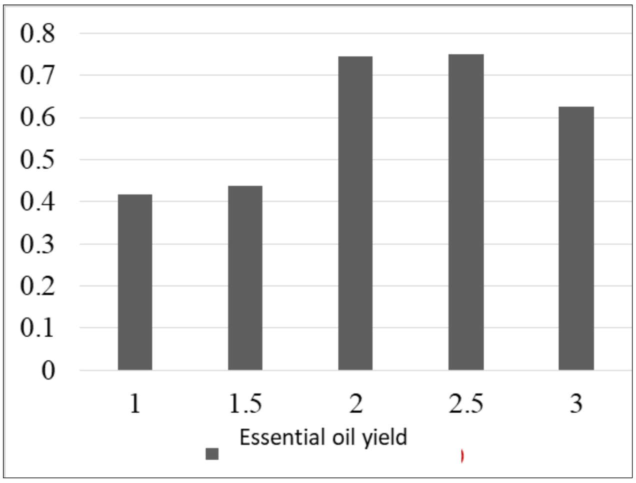

Fig 3: Distillation time is a critical factor influencing the yield of essential oil

As shown in Table 2 and Figure 3, the essential oil yield increased progressively with longer distillation times and reached its peak at 2.5 hours. This trend may be attributed to the requirement of sufficient time for the essential oil to be released from the plant matrix and carried by the steam during the initial stages of the process. However, when distillation continued beyond 2.5 hours, the oil yield began to decrease. This reduction could be due to thermal degradation or chemical transformation of the essential oil components, or loss of volatile compounds through prolonged exposure to heat and excessive condensation. Therefore, it is essential to optimize the distillation time to ensure maximum extraction of essential oil while minimizing losses caused by extended processing. Based on these findings, a distillation time of 2.5 hours was selected as the optimal condition for essential oil recovery.

3.2. *α*-Glucosidase Inhibitory Activity of *Vernonia amygdalina* Essential Oil

The *α*-glucosidase inhibitory activity of *Vernonia amygdalina* essential oil was evaluated using spectroscopy, and the results are presented in Table 3.

Table 3: α-Glucosidase inhibitory activity of *Vernonia amygdalina* essential oil

Essential oil (mg/mL)	**% Inhibiting**			IC_{50} (mg/mL)
	1	**2**	**3**	
25	94.36	96.87	95.25	3.31 ± 0.04
10	71.23	72.17	71.38	
5	55.01	54.95	54.88	
2.5	46.28	46.98	57.27	
1.25	41.23	42.65	40.38	
Acarbose^{a}				0.013 ± 0.01

^{a}Positive control for anti-*α*-glucosidase

The essential oil of *Vernonia amygdalina* demonstrated a significant inhibitory effect on α-glucosidase activity. The percentage of inhibition increased from 41.23% to 95.49% as the concentration of the essential oil rose from 1.25 mg/mL to 25 mg/mL. A clear dose-dependent relationship was observed, with the highest concentration (25 mg/mL) yielding an average inhibition rate of over 95%. The experimental results showed that the average IC_{50} value of the essential oil was 3.31 ± 0.04 mg/mL. Although this inhibitory potency is lower than that of the positive control, Acarbose (IC_{50} = 0.013 mg/mL), the findings still indicate promising potential for *Vernonia amygdalina* essential oil in supporting blood glucose regulation via α-glucosidase inhibition. These results highlight the need for further investigation into the chemical constituents and biological activities of *Vernonia amygdalina* essential oil for potential applications in medicine.

The investigation of *Vernonia amygdalina* essential oil from Dak Lak has preliminarily demonstrated its α-glucosidase inhibitory potential, with an average IC_{50} value of 3.31 mg/mL. When compared with other studies evaluating extracts from different parts of the plant, such as aqueous, ethanol, or methanol extracts from roots and leaves, the inhibitory activity of the essential oil was relatively lower. Specifically, the study by Uthman (2024) [8] reported an IC_{50} of 45 µg/mL for aqueous extracts, while other studies using leaf and root extracts also demonstrated significantly stronger activity [9]. In particular, ethanol leaf extracts obtained via ultrasound-assisted extraction exhibited highly potent inhibition [10]. Nevertheless, the notable contribution of the present study lies in being the first to evaluate the α-glucosidase inhibitory activity of essential oil from *Vernonia amygdalina* cultivated in Dak Lak. This opens a new research direction into the potential use of essential oil as a natural agent for blood glucose regulation. It also provides an important foundation for further optimization of extraction processes and the development of essential oil-based products for the prevention and supportive treatment of diabetes.

4. Conclusion

Vernonia amygdalina essential oil collected in Buon Ma Thuot city, Dak Lak Province, the essential oil is light yellow in color and has a slight fragrance. The investigation showed that essential oil obtained by steam distillation under optimal conditions, using a plant material-to-water ratio of 1:8, for 2.5 hours at a temperature range of 60–80°C, resulted in a pale yellow oil with a yield of 0.75%. Additonaly, the essential oil also exhibited strong anti-*α*-glucosidase activities with an IC_{50} value of 3.31 ± 0.04 mg/mL. These findings underscore the potential of *Vernonia amygdalina* essential oil as a natural source for pharmaceutical applications, particularly in alpha glucosidase therapies.

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