Survey some Factors Affecting the Content of Essential Oil of *Eupatorium Odoratum* L and Alphaglucosidase Inhibitory Activity

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Abstract
From the fresh leaves of the *Eupatorium odoratum* L, an essential oil was obtained by hydrodistillation. The essential oil of *Eupatorium odoratum* L. was extracted in Buon Ma Thuot City using steam distillation, with a plant material to water ratio of 1:10, carried out 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 approximately 0. 19%. In addition, we also tested that anti-*α*-glucosidase activity of *Eupatorium odoratum* L essential oil. The essential oil also exhibited strong anti-*α*-glucosidase activities with an IC_{50} value of 9. 05 ± 0. 61mg/mL. These findings underscore the potential of *Eupatorium odoratum* L essential oil as a natural source for pharmaceutical applications.

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Keywords: *Eupatorium odoratum* L*, Essential oil, GC-MS,* anti-*α*-glucosidase

1. Introduction

Eupatorium odoratum L., commonly known in Vietnam as "Cỏ lào", belongs to the Asteraceae family (Compositae). It is also referred to by various local names such as "*cộng sản"*, "*bớp bớp"*, "*bù xích"*, "*yêu bạch"*, "*chùm hôi"*, "*nhả Nhật"* (in Tày language), and "*muồng mung phia"* (in Dao language). Its English common names include *Fragrant thoroughwort* and *Bitter bush*. *Eupatorium odoratum* L. is a wild plant species widely distributed throughout Vietnam, commonly found in lowland areas, midlands, and lower mountainous regions. [1].

According to traditional medicine, *Eupatorium odoratum* L. possesses a slightly bitter taste, a pungent odor, and warm properties. It is traditionally used for its antiseptic effects and in the treatment of diarrhea, musculoskeletal pain, scabies, constipation, and for preventing or treating leech bites. In addition, several studies have demonstrated that the essential oil of *Eupatorium odoratum* L. contains various bioactive compounds with notable pharmacological properties, including antibacterial, antioxidant, anti-inflammatory, detoxifying, suppuration-inhibiting, hemostatic, and anti-ulcer activities. [2, 3]. These findings suggest that the essential oil of *Eupatorium odoratum* L. holds significant potential for medicinal and pharmaceutical applications.

According to traditional medicine, *Eupatorium odoratum* L. has a bitter taste and warm nature. It has been traditionally used for promoting blood circulation, hemostasis, antisepsis, detoxification, anti-inflammation, and pain relief [4].

Phytochemical investigations, both domestic and international, have revealed that *Eupatorium odoratum* L. contains a variety of bioactive compounds. The plant is particularly rich in essential oils, which are predominantly found in the fresh leaves. In addition, it contains pyrogallic-type tannins, various classes of flavonoids—including flavonols, flavanols, chalcones, and dihydroflavonols [5, 6, 7], as well as coumarins, alkaloids (mainly concentrated in the roots), anthraquinones, glucosides, and saponins. Among these constituents, essential oils and flavonoids are considered the primary bioactive components contributing to the plant's pharmacological activities [8, 9, 10].

Both *in vitro* and *in vivo* studies have demonstrated that the essential oil of *Eupatorium odoratum* L. exhibits inhibitory effects against several Gram-positive and Gram-negative bacterial strains, including *Staphylococcus aureus*, *Escherichia coli*, and

Pseudomonas aeruginosa [11]. The major constituents of the essential oil such as β-caryophyllene, germacrene D, caryophyllene oxide, and α-pinene are believed to contribute significantly to its potent antibacterial activity. In particular, β-caryophyllene and caryophyllene oxide have been shown to disrupt bacterial cell membranes and inhibit biofilm formation, which is considered one of the primary mechanisms underlying bacterial resistance to antibiotics [12, 13]. In addition, the essential oil of *Eupatorium odoratum* L. has demonstrated notable anti-inflammatory activity. Compounds such as α-humulene, δ-cadinene, and spathulenol have been reported to inhibit the release of key inflammatory mediators, including prostaglandin E2 (PGE_2) and nitric oxide (NO), thereby contributing to the suppression of both acute and chronic inflammatory responses [14]. Although several studies on *Eupatorium odoratum* L. have been conducted both domestically and internationally, no research to date has specifically focused on the chemical composition and biological activities of the essential oil derived from *Eupatorium odoratum* L. cultivated in Buon Ma Thuot City, Dak Lak Province.

Fig 1: The *Eupatorium odoratum* L were collected in Dak Lak Province

2. Materials and methods

2.1. Plant Material

The fresh leaves of *Eupatorium odoratum* l were collected from Tan Lap 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, CL-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 *Eupatorium odoratum* L (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 *Eupatorium odoratum* L essential oil was evaluated according to the method of Sihvonen *et al*. (1999) [15]. 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 2.

$$Inhibition\ (\%) \frac{A0-A1}{A0}\ x\ 100\% \quad (2)$$

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).
Each assay was conducted in triplicate.

3. Results And Discussion

3.1. Factors affecting the content of *Eupatorium odoratum* L 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)	1:8	1:10	1:12	1:14
Essential Oil Yield (%)	0. 135	0. 206	0. 177	0. 113

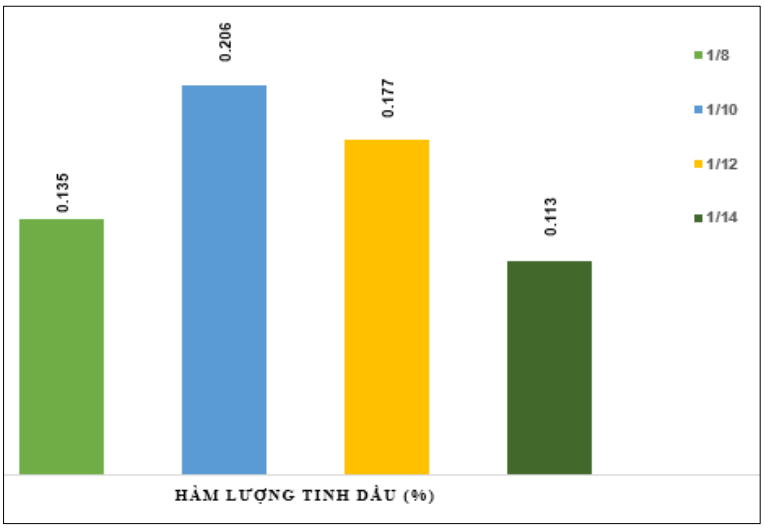

Fig 2: Effect of Plant Material-to-Water Ratio on Essential Oil Yield

Based on the above results, it is evident that determining an appropriate plant material-to-water ratio is a critical factor influencing the essential oil yield. An excessive amount of water negatively affects the mass yield of the essential oil. When the solvent volume is too high, the diffusion of the essential oil becomes inefficient, leading to lower amounts of oil being carried by the steam. This not only reduces the yield but also increases energy consumption and may compromise the quality of the essential oil. Therefore, based on the experimental findings, we selected the plant material-to-water ratio of 1:10 (g/mL) as the optimal condition for the distillation process.

3.1.2 Distillation Time Investigation

Table 2: Effect of Distillation Time on Essential Oil Yield

Distillation Time (h)	1. 0	1. 5	2	2. 5	3. 0
Essential Oil Yield (%)	0. 018	0. 0400	0. 107	0. 189	0. 189

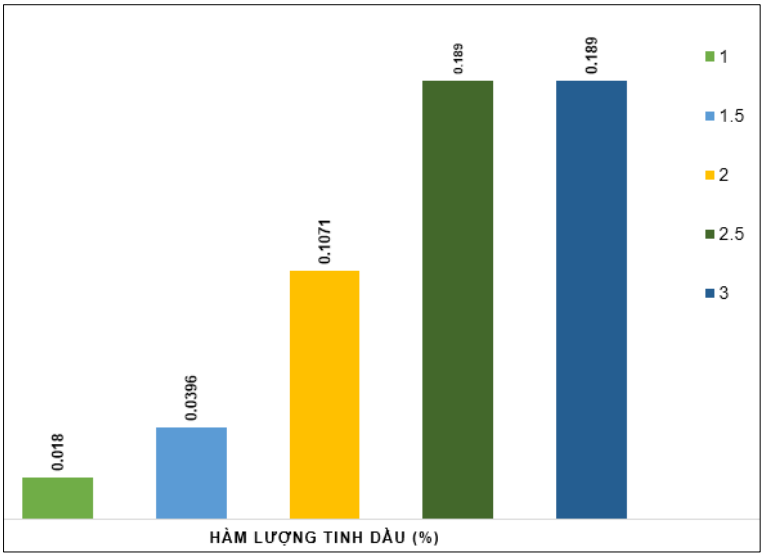

Fig 3: Effect of Distillation Time on Essential Oil Yield

The results indicate that the essential oil yield from *Eupatorium odoratum* L. varies with different distillation times. The oil yield increased from 0. 018% to 0. 189% as the distillation time increased from 1. 0 to 2. 5 hours. Distillation time plays a crucial role in determining the amount of essential oil recovered. A short distillation duration may result in incomplete extraction of the oil, while prolonged distillation leads to unnecessary time and energy consumption. Moreover, extended distillation may negatively affect oil quality due to emulsification of fatty components. Between 2. 0 and 3. 0 hours, the essential oil yield remained unchanged, suggesting a saturation point. Therefore, to optimize both energy efficiency and processing time, a distillation duration of 2. 5 hours was selected for subsequent experiments. ss

3.2 α-Glucosidase Inhibitory Activity of *Eupatorium odoratum* L essential Oil

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

Table 3: α-Glucosidase inhibitory activity of *Eupatorium odoratum* L essential oil

Essential oil (mg/mL)	% Inhibiting			IC_{50} (mg/mL)
	1	2	3	
40	94. 73	95. 37	95. 03	9. 05 ± 0. 61
20	66. 48	67. 37	66. 92	
10	54. 72	55. 58	54. 37	
2. 5	42. 08	42. 35	43. 28	
1. 25	38. 28	38. 94	38. 46	
Acarbose[a]				0. 013 ± 0. 01

[a]Positive control for anti-α-glucosidase

The results presented in Table 3 demonstrate that the α-glucosidase inhibitory activity of *Eupatorium odoratum* L. essential oil increased progressively from 38. 46% to 95. 03% as the oil concentration increased from 2. 5 to 40 mg/mL. This indicates that the essential oil possesses α-glucosidase inhibitory potential. However, its activity is relatively moderate, as evidenced by the IC_{50} value, which was higher than that of the positive control, acarbose (IC_{50} = 0. 013 mg/mL). To date, no published scientific studies have specifically focused on the α-glucosidase inhibitory activity of *Eupatorium odoratum* L. essential oil. Therefore, this study provides a novel approach to exploring the biological potential of this essential oil in the modulation of enzymes associated with type 2 diabetes, thereby contributing to the scientific foundation for the potential use of this plant in pharmaceutical and medicinal applications.

4. Conclusion

The essential oil of *Eupatorium odoratum* L. was extracted in Buon Ma Thuot City using steam distillation. The process was carried out with a plant material-to-water ratio of 1:10 (w/v), over a period of 2. 5 hours at a temperature range of 60–80°C. The obtained essential oil was pale yellow in color, with a yield of approximately 0. 19%. Additonaly, the essential oil also exhibited strong anti-α-glucosidase activities with an IC_{50} value of 9. 05 ± 0. 61 mg/ml. These results indicate the potential of *Eupatorium odoratum* L essential oil for use in pharmaceutical.

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