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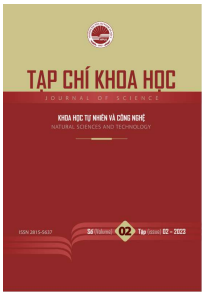

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Chemical components and anti-acetylcholinesterase activity of *Piper lolot* leaves oil

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Abstract

Essential oil of *Piper lolot* leaves in Quang Nam-Da Nang do obtain via the steam distillation method with oil collection efficiency reaching 0.68%. The chemical components of the essential oil *from Piper lolot* leaves in Quang Nam-Da Nang are determined via the GC-MS method that includes 20 compositions (99.97%), among which the main compositions are myristicin (72.70%), asarone (5.16%), β-bisabolene (3.59%), γ-asarone (3.52%), caryophyllene (2.63%), and α-copaene (1.93%). The essential oil of *Piper lolot* leaves in Quang Nam-Da Nang also showed potent anti-acetylcholinesterase activity with IC_{50} (µg/mL) = 28.72±2.80 compared to galantamine (a positive control) and essential oils, extract from *Piper* species.

Keywords: *Piper lolot* leaves oil, *Piper lolot*, steam distillation, chemical components, anti-acetylcholinesterase;

1. Introduction

Vietnam is located in the tropical monsoon region where natural conditions are favorable for the formation and development of plants, especially high-value essential oil-bearing plants. The essential oils have a unique aroma and fragrance that can improve relaxation, comfort, and pleasure. These distillates possess many medicinal properties which can be applied to medicine production and

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antimicrobial and antiviral agents (Vergis *et al*., 2015; Tariq *et al*., 2019). *Piper* species are widely used in folk medicine to heal wounds and reduce swelling and skin irritation (Gardner RO, 2010). *Piper lolot* (Piperaceae), a popular vegetable in Vietnam, is also a special spice and effective medicine. The entire plant possesses anti-inflammatory and anodyne properties. It is used in the treatment of rheumatism, ostealgia, lumbago, headache, hyperhidrosis of the extremities, dyspepsia, vomiting, flatulence, colic, diarrhea, toothache, chronic catarrhal rhinitis and edema (Dung NX, 2013). *Piper lolot* leaves contribute positive health effects on exhibiting warmth and pain relief (Loi DT, 2003; Lau TV, 2017).

Alzheimer's disease is a devastating neurodegenerative disorder with grave concerns in the elderly. The disease is characterized by the deposition of amyloid-β plaques and neurofibrillary tangles in the brain, accompanied by synaptic dysfunction and neurodegeneration. Due to the required long-term care and management, these seriously impacts the patient's health and quality of life and burdens the family and society. It has been demonstrated that the neuropsychological impairments of Alzheimer's disease are attributed, at least partially, to cholinergic disturbance. Rivastigmine and galantamine derived from natural products are commonly prescribed cholinergic enhancers as acetylcholinesterase (AChE) inhibitors (Xiang CP *et al*., 2017). Aromatic plants have been used to cure neuronal ailments for centuries by different cultures worldwide. Such plant's essential oils and volatile compounds might be potential drugs for Alzheimer's disease therapies.

Several studies have examined *Piper lolot* leaves oil's chemical compositions and biological activities (Dung NX *et al*., 2013; Thuyen NTB *et al*., 2020; Phong HX *et al*., 2022). However, there have been no published on *Piper lolot* leaves oil's anti-acetylcholinesterase activity.

The present study aimed to determine the chemical components and anti-acetylcholinesterase activity of *Piper lolot* leaves oil collected from Quang Nam-Da Nang.

2. Materials and methods

2.2. Materials

The fresh leaves of *Piper lolot* were collected at Quang Nam-Da Nang, Vietnam, in June 2022. The sample used for steam distillation is of uniform quality and without spoilage and stored in a cool place. After harvesting, the fresh sample is processed with preliminary treatment, removed impurities washed, and pureed before essential oil extraction.

Its scientific name was identified by Dr. Quang-Dan Tran, Department of Biology&Environmental Science, The University of Danang-University of Science and Education. A voucher specimen No. PL001 was deposited at the Department of Chemistry, The University of Danang-University of Science and Education.

2.3. The steam distillation method

Piper lolot leaves oil was obtained by steam distillation with light Clevender with 250 g *Piper lolot* leaves/500 mL distilled water for three hours at the Chemistry laboratory, The University of Danang-University of Science and Education. The experiment was repeated three times.

The oil collection efficiency is calculated according to the amount of essential oil in the raw materials, which is determined by the formula:

$$Y\ (\%) = \frac{V \times d}{m} \times 100$$

In which: Y(%): The oil collection efficiency; V (mL): Volume of essential oil; d (g/cm^3): Specific gravity of *Piper lolot* leaves oil, d = 0.995 g/cm^3; m (g): Weight of the fresh leaves of *Piper lolot*.

2.3. Analysis of the chemical components

The chemical components of the essential oil from *Piper lolot* leaves are determined via the GC-MS method with GC-MS equipment (GC 7890A, MS 5975C-Agilent).

For GC: Operating temperature: 35°C-450°C, temperature resolution: 1°C, maximum heating speed: 0.1°C-120°C/min, the maximum run time for sample: 999.99 minutes, speed line: 1-13 mL/min for Helium, column type: HP-5MS (Length: 30 m, diameter: 0.25 mm, film: 0.25 µm).

For MS: EI with m/z: 20 – 500 amu, retention time repeatability with a trace: < 0.0012 min, area repeatability with a trace: < 2.0 RSD. The percentage of a compound is based on the ratio of compound pick area to total pick area.

2.4. Determine the anti-acetylcholinesterase activity

The test was performed according to the method of Ellman GL *et al*., 1961.

The method is carried out according to the principle: Acetylcholinesterase (AChE) is a catalyst for the hydrolysis reaction acetylthiocholine iodide (ACTI) produces thiocholin. The thiocholin will react with DTNB (acid 5-5'-dithiobis-2-nitrobenzoic) to form a yellow 5-thio-2-nitro benzoic acid. The amount of this color compound is proportional to AChE activity. The experiment was repeated three times. As follows:

- The experimental sample was dissolved in 100% DMSO solvent, then diluted to different concentrations with H_2O (deionized distilled water).

- The experimental well contains a mixture of 140 µL of phosphate buffer solution (pH: 8), 20 µL of the test sample at various concentrations, and 20 µL of AChE enzyme 0.25 IU/mL, mixed well and incubated at 25°C for 15 minutes.

- Next, 10 µL of 2.5 mM DTNB and 10 µL of 2.5 mM ACTI were added to the experimental wells and incubated for 10 minutes at 25°C.

- Then, the solution is measured for absorbance at a wavelength of 405 nm (the standard is 412 nm).

- Galantamine was used as a positive control. A blank well is a well that does not contain enzymes. A negative control well is a well that does not contain experimental samples. The percentage inhibition of AChE enzyme activity (%I) was calculated according to the formula: %I = ((Ac-At)/(Ac))*100; In which: %I: percentage of AChE activity inhibited; Ac: absorbance of the control sample (without 20 µL of test solution) minus the absorbance of the blank well; At: absorbance of the test sample minus the absorbance of the blank well.

- Data are processed by Excel and expressed as mean ± SD/SE. The 50% inhibitory value of AChE enzyme activity (IC_{50}) of the samples was determined using TableCurve 2Dv4 computer software.

3. Results and discussion

3.1. The oil collection efficiency

The result of the *Piper lolot* leaves oil collection efficiency is presented in **Table 1**. The essential oil of *Piper lolot* leaves in Quang Nam-Da Nang was obtained via steam distillation with an oil collection efficiency of 0.68% in 250 g *Piper lolot* leaves/500 mL distilled water for three hours.

The oil collection efficiency of the *Piper lolot* leaves oil in Quang Nam-Da Nang was higher than that in Can Tho (0.61%) (Thuyen NTB *et al*., 2020) and lower than that in Thai Nguyen (0.82%) (Hue PT *et al*., 2021). Differences in seed quality, growing method, climatic conditions, soil, and growing period can lead to differences in the amount of oil between localities.

Table 1: The *Piper lolot* leaves oil collection efficiency

m (g)	V[a] (mL)	Y (%)
250	1.7	0.68
[a]*The experiment was repeated three times, and the average volume was calculated.*		

3.2. The chemical components

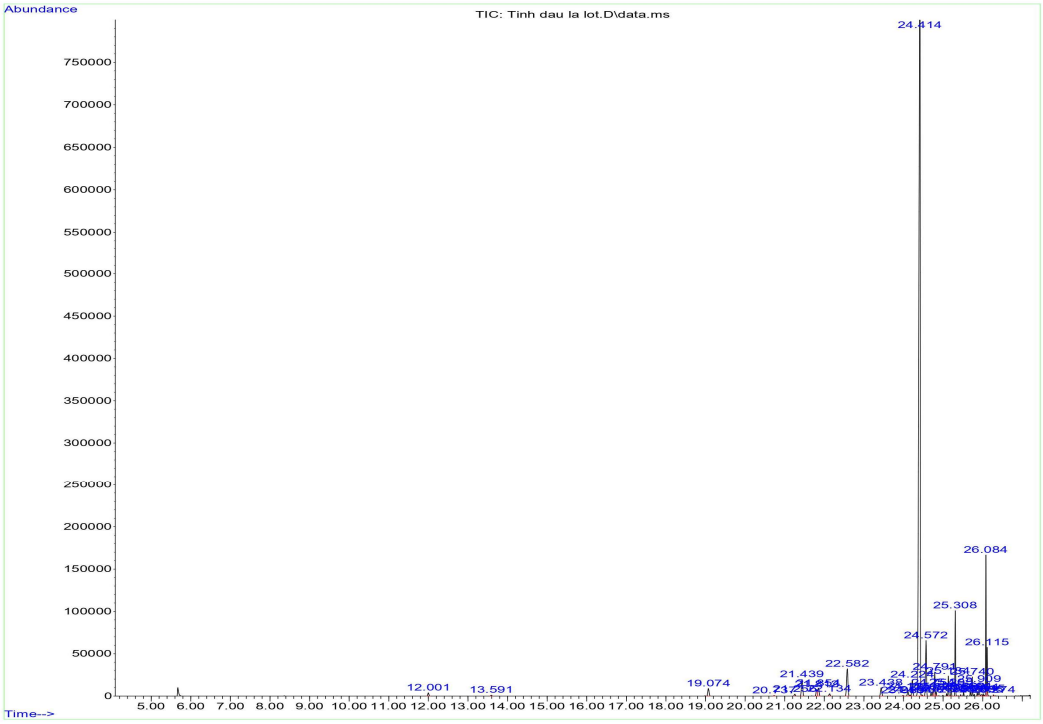

Figure 1: GC-MS spectrum of *Piper lolot* leaves oil

The chemical components of the *Piper lolot* leaves oil are presented in **Figure 1** and **Table 2**. The chemical components of the essential oil from *Piper lolot* leaves in Quang Nam-Da Nang are determined via the GC-MS method that includes 20 compositions (99.97%), among which the main

compositions are myristicin (72.70%), asarone (5.16%), β-bisabolene (3.59%), γ-asarone (3.52%), caryophyllene (2.63%), and α-copaene (1.93%). This result is consistent with the published chemical components of the *Piper lolot* leaves oil (Dung NX *et al*., 2013; Thuyen NTB *et al*., 2020; Phong HX *et al*., 2022), although some differences may be due to seed quality, growing method, climatic conditions, soil, and growing period.

Table 2: The chemical components of *Piper lolot* leaves oil

Retention (R_T)	Compounds	Area (%)
12.001	β-Ocimene	0.32
19.074	Safrole	0.78
21.439	α-Copaene	1.93
21.812	β-Cubebene	0.16
21.854	β-Elemene	0.84
22.134	Methyl eugenol	0.18
22.582	Caryophyllene	2.63
23.438	Humulene	0.79
24.115	Germacrene D	0.44
24.224	β-Eudesmene	1.21
24.414	Myristicin	72.70
24.572	β-Bisabolene	3.59
24.791	δ-Cadinene	1.26
24.826	Phenol, 2-methoxy-4-(1-propenyl)-, acetate	0.39
25.134	Elemicin	0.99
25.308	γ-Asarone	3.52
25.691	β-Asarone	0.59
25.909	Copaene	0.69
26.084	Asarone	5.16
26.115	Apiol	1.80
	Total	**99.97**

3.3. The anti-acetylcholinesterase activity

The anti-acetylcholinesterase activity of the *Piper lolot* leaves oil is presented in **Table 3**. The essential oil of *Piper lolot* leaves showed anti-acetylcholinesterase activity with IC_{50} (μg/mL) = 28.72±2.80. Compared to galantamine and the results about the anti-acetylcholinesterase activity of the essential oils and extract from *Piper* species (Xiang CP *et al*., 2017; Ranjan N *et al*., 2018; Van DTT *et al*., 2022; Bich TTN *et al*., 2023) (**Table 4**) it was found that *Piper lolot* leaves oil in Quang Nam-Da Nang had potent anti-acetylcholinesterase activity.

Table 3: The anti-acetylcholinesterase activity of *Piper lolot* leaves oil

Concentration (µg/mL)	***Piper lolot* leaves oil**		**Galantamine**	
	Percentage of inhibition	**Error**	**Percentage of inhibition**	**Error**
100	79.66	1.55	86.43	3.22
20	42.57	2.49	55.89	0.26
4	22.82	0.92	26.79	1.31
0.8	8.02	1.75	8.41	0.69
IC_{50}	**28.72±2.80**		**1.67±0.08**	

Galantamine: The positive control, which acts stably in the experiment.

Sesquiterpenes and phenylpropanoids were rich in *Piper* species oils, of which asaricin, caryophyllene, caryophyllene oxide, isospathulenol, (+) spathulenol and β-bisabolene are the primary constituents. The active compound was isolated and identified from *Piper* species oils as asaricin, which showed potent anti-acetylcholinesterase activity (Xiang CP *et al*., 2017). β-Bisabolene and caryophyllene are also the main sesquiterpenes of the essential oil of *Piper lolot* leaves oil in Quang Nam-Da Nang, which may be the cause of the anti-acetylcholinesterase activity.

Table 4: The anti-acetylcholinesterase activity of the *Piper* oils and extract

***Piper* species**	**Parts of *Piper* species**	**100 µg/mL**	**IC_{50} (µg/mL)**
		Percentage of inhibition	
Piper austrosinense (Xiang CP *et al*., 2017)	Leaves and stems oil	102.00±3.00	12.40±0.13
Piper puberulum (Xiang CP *et al*., 2017)	Leaves and stems oil	148.00±9.60	4.47±0.37
Piper flaviflorum (Xiang CP *et al*., 2017)	Leaves and stems oil	96.10±3.70	13.90±1.85
Piper betle (Xiang CP *et al*., 2017)	Leaves and stems oil	108.00±1.90	14.00±0.01
Piper betle (Van DTT *et al*., 2022)	Leaves oil	59.80±1.85	65.94±5.96
Piper hispidimervium (Xiang CP *et al*., 2017)	Leaves and stems oil	95.40±4.60	1.51±0.05

Piper longum (Bich TTN *et al*., 2023)	Fruits oil	42.48±1.89	164.55±13.79
Piper longum (Ranjan N *et al*., 2018)	Fruits extract	42.23±0.28	-

4. Conclusions

The essential oil of *Piper lolot* leaves in Quang Nam-Da Nang was obtained via steam distillation with an oil collection efficiency of 0.68% in 250 g *Piper lolot* leaves/500 mL distilled water for three hours.

The chemical components of the *Piper lolot* leaves oil in Quang Nam-Da Nang include 20 compositions (99.97%), among which the main compositions are myristicin (72.70%), asarone (5.16%), β-bisabolene (3.59%), γ-asarone (3.52%), caryophyllene (2.63%), and α-copaene (1.93%).

The *Piper lolot* leaves oil in Quang Nam-Da Nang presented potent anti-acetylcholinesterase activity with IC_{50} (µg/mL) = 28.72±2.80.

Declaration of Competing Interest

The authors declare no competing interests.

Author contributions

"Experiments: Thi Thuy-Van Do; analysis chemical components and anti-acetylcholinesterase activity: Thi Thuy-Van Do, Ngoc-Linh Nguyen, Anh-Hung Nguyen; writing original draft: Thi Thuy-Van Do; review and editing. All authors have read and agreed to the published version of the manuscript."

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