HPU2 Journal of Sciences:
Natural Sciences and Technology

journal homepage: https://sj.hpu2.edu.vn

Article type: Research article

Chemical composition and anti-acetylcholinesterase, nitric oxide suppressing activities of *Piper betle* leaves oil

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Abstract

Essential oil of *Piper betle* leaves in Quang Nam-Da Nang do obtain via the steam distillation method with oil collection efficiency reaching 0.83%. The chemical composition of the essential oil *from Piper betle* leaves in Quang Nam-Da Nang is determined via the GC-MS method that includes 21 components (99.68%), among which the main components are eugenol acetate (32.33%), eugenol (24.56%), 4-allyl-1,2-diacetoxybenzene (19.8%), germacrene D (5.98%), caryophyllene (3.32%), and γ-muurolene (2.45%). The essential oil of *Piper betle* leaves in Quang Nam-Da Nang also showed moderate anti-acetylcholinesterase activity with IC_{50} (µg/mL) = 65.94±5.96 and moderate nitric oxide suppressing activity with IC_{50} (µg/mL) = 46.69±3.57.

Keywords: *Piper betle* oil, *Piper betle*, steam distillation, anti-acetylcholinesterase, nitric oxide suppressing;

1. Introduction

Piper betle (Piperaceae) is one of the essential thermophilic plants in Asia zone, widely cultivated in India, Sri Lanka, Malaysia, Thailand, Taiwan, and other Southeast Asian countries (Karak S *et al*., 2018). In Vietnam, *Piper betle* leaves are used for many different purposes like chewing betel to strengthen teeth, curing foot ulcers teeth, and decoction of betel leaves to wash or apply to treat wounds, burns, sores, boils, eczema (Loi DT, 2003).

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https://doi.org/10.56764/hpu2.jos.2022.1.2.91-96

Received date: 19-12-2022 ; Revised date: 25-12-2022 ; Accepted date: 28-12-2022

There are several studies on the chemical composition and biological activities of *Piper betle* in the world (Ganguly S *et al*., 2007; Karak S *et al*., 2018; Biswas P *et al*., 2022; Devi A *et al*., 2022; Seo J *et al*., 2022) and in Vietnam (Chinh PT *et al*., 2009; Chi NT *et al*., 2016). However, there are no published anti-acetylcholinesterase and nitric oxide suppressing activities of *Piper betle* leaves oil in Vietnam.

The present study aimed to determine the chemical composition and anti-acetylcholinesterase, nitric oxide suppressing activities of *Piper betle* leaves oil collected from Quang Nam-Da Nang. The result, the essential oil from *Piper betle* leaves in Quang Nam-Da Nang includes 21 components (99.68%), and the oil showed moderate anti-acetylcholinesterase activity and moderate nitric oxide suppressing activity.

2. Materials and methods

2.1. Materials

The fresh leaves of *Piper betle* 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. PB001 was deposited at the Department of Chemistry, The University of Danang-University of Science and Education.

2.2. The steam distillation method

Piper betle leaves oil was obtained by steam distillation with light Clevender with 200 g *Piper betle* leaves/400 mL distilled water in 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 betle* leaves oil, d = 0.920; m (g): Weight of the fresh leaves of *Piper betle*.

2.3. Analysis of the chemical composition

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

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.

2.5. Determine the nitric oxide suppressing activity

The test was performed according to the method of Cheenpracha S *et al*., 2010 with RAW 264.7 cell line by Prof. Dr. Domenico Delfino, University of Perugia, Italy, and Prof. Dr. Chi-Huang, National Yang-Ming University, Taiwan.

3. Results and discussion

3.1. The oil collection efficiency

The result of the *Piper betle* leaves oil collection efficiency is presented in **Table 1**. The essential oil of *Piper betle* leaves in Quang Nam-Da Nang was obtained via the steam distillation method with an oil collection efficiency of 0.83% in the conditions of 200 g *Piper betle* leaves/400 mL distilled water in three hours.

The oil collection efficiency of *Piper betle* leaves oil in Quang Nam-Da Nang was higher than Hau Giang (0.63%) (Chi NT *et al*., 2016) and lower than Hai Duong (1.01%) (Chinh PT *et al*., 2009). 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 betle* leaves oil collection efficiency

m (g)	V[a] (mL)	Y (%)
200	1.8	0.83

[a]*The experiment was repeated three times, and the average volume was calculated.*

3.2. The chemical composition

The result of the chemical composition of *Piper betle* leaves oil is presented in **Table 2**. The chemical composition of the essential oil *from Piper betle* leaves in Quang Nam-Da Nang is determined via the GC-MS method that includes 21 components (99.68%), among which the main components are eugenol acetate (32.33%), eugenol (24.56%), 4-allyl-1,2-diacetoxybenzene (19.8%), germacrene D (5.98%), caryophyllene (3.32%), γ-muurolene (2.45%). This result is consistent with the published chemical composition of *Piper betle* leaves oil in Vietnam (Chinh PT *et al*., 2009; Chi NT *et al*., 2016) and in the world (Karak S *et al*., 2018; Biswas P *et al*., 2022).

Table 2. The chemical composition of *Piper betle* leaves oil.

Retention (R_T)	Compounds	Area (%)
10.808	Eucalyptol	0.25
11.510	*trans*-β-Ocimene	0.28
12.918	Linalool	0.29
19.403	Phenol, 4-(2-propenyl)-, acetate	1.93
20.088	Eugenol	24.56
20.937	α-Cubebene	0.68
21.275	β-Elemene	0.57
21.961	Caryophyllene	3.32
22.770	Humulene	0.90
23.315	γ-Muurolene	2.45
23.412	Germacrene D	5.98

23.793	Eugenol acetate	32.33
24.115	β-Bisabolene	0.63
24.338	δ-Cadinene	1.35
24.817	Germacrene B	0.25
25.075	(-)-Globulol	0.46
25.149	Viridiflorol	0.42
25.359	4-Allyl-1,2-diacetoxybenzene	19.8
25.555	tau-Muurolol	0.71
25.579	Epicubenol	0.73
25.642	α-Cadinol	1.63
	Total	99.68

3.3. The anti-acetylcholinesterase activity

The result of the anti-acetylcholinesterase activity of *Piper betle* leaves oil is presented in **Table 3**. The essential oil of *Piper betle* leaves showed anti-acetylcholinesterase activity with IC_{50} (µg/mL) = 65.94±5.96. Compared with the results about the anti-acetylcholinesterase activity of *Piper betle* leaves oil (Karak S *et al*., 2018) and essential oils from *Piper* species (Xiang CP *et al*., 2017), it was found that *Piper betle* leaves oil in Quang Nam-Da Nang had moderate anti-acetylcholinesterase activity.

Table 3. The anti-acetylcholinesterase activity of *Piper betle* leaves oil.

	Piper betle leaves oil		Galantamine	
Concentration (µg/mL)	Percentage of inhibition	Error	Percentage of inhibition	Error
500	88.24	2.93	91.07	1.31
100	59.80	1.85	56.56	1.69
20	28.00	1.08	21.83	0.93
4	9.59	0.53	9.07	0.42
IC_{50}	65.94±5.96		1.70±0.12	

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

3.4. The nitric oxide suppressing activity

The result of the nitric oxide suppressing the activity of *Piper betle* leaves oil is presented in **Table 4**. The essential oil of *Piper betle* leaves showed nitric oxide suppressing activity with IC_{50} (µg/mL) = 46.69±3.57. Compared with the results about the nitric oxide suppressing activity of some extracts and oil of *Piper betle* leaves in reference materials (Ganguly S *et al*., 2007; Biswas P *et al*., 2022; Devi A *et al*., 2022; Seo J *et al*., 2022), it was found that *Piper betle* leaves oil in Quang Nam-Da Nang had moderate nitric oxide suppressing activity.

Table 4. The nitric oxide suppressing activity of *Piper betle* leaves oil.

Concentration (µg/mL)	*Piper betle* leaves oil				L-NMMA			
	Percentage of NO inhibition		Percentage of living cells		Percentage of NO inhibition		Percentage of living cells	
	Average	Error	Average	Error	Average	Error	Average	Error
100	91.18	1.66	92.18	1.44	95.55	2.30	87.58	1.71
20	25.88	2.50	98.65	1.37	78.37	1.78	92.80	1.30
4	13.24	1.25			20.28	0.41		
0.8	4.71	0.33			10.13	0.63		
IC_{50}	46.69±3.57		-		8.42±0.41		-	

L-NMMA: The positive control, which acts stably in the experiment.

3. Conclusions

The chemical composition of the essential oil *from Piper betle* leaves in Quang Nam-Da Nang includes 21 components (99.68%), among which the main components are eugenol acetate (32.33%), eugenol (24.56%), 4-allyl-1,2-diacetoxybenzene (19.8%), germacrene D (5.98%), caryophyllene (3.32%), γ-muurolene (2.45%).

The essential oil of *Piper betle* leaves in Quang Nam-Da Nang showed moderate anti-acetylcholinesterase activity with IC_{50} (µg/mL) = 65.94±5.96 and moderate nitric oxide suppressing activity with IC_{50} (µg/mL) = 46.69±3.57.

Declaration of Competing Interest

The authors declare no competing interests.

Author contributions

"Experiments: Kim-Chi Nguyen, Thi Ngoc-Hiep Nguyen, and Thi Thuy-Van Do; analysis chemical composition and biological activities: Anh-Hung Nguyen, Thi Thuy-Van Do; writing original draft: Thi Thuy-Van Do; review and editing:. All authors have read and agreed to the published version of the manuscript."

References

[1] Biswas P *et al.*, "Betelvine (*Piper betle* L.): A comprehensive insight into its ethnopharmacology, phytochemistry, and pharmacological, biomedical and therapeutic attributes," *J Cell Mol Med*, vol. 26, p. 3083, 2022, doi: 10.1111/jcmm.17323.

[2] Cheenpracha S *et al.*, "Inhibition of nitric oxide (NO) production in lipopolysaccharide (LPS)-activated murine macrophage RAW 264.7 cells by the norsesterterpene peroxide, epimuqubilin A," *Mar Drugs*, vol. 8, no. 3, p. 429, 2010, doi: 10.3390/md8030429.

[3] Chi NT *et al.*, "Surveying the chemical composition and antibacterial of *Piper betle* (Piperaceae)," *Can Tho University Journal of Science*, vol. 45, p. 28, 2016, doi: 10.22144/ctu.jvn.2016.508.

[4] Chinh PT *et al.*, "Chemical composition of *Piper betle* in Hai Duong," *TNU Journal of Science and Technology*, vol. 72, no. 10, p. 48, 2009.

[5] Devi A *et al.*, "A review on anti-inflammatory potential of *Piper betle*," *WJPMR*, vol. 8, no. 2, p. 83,

2022, doi: 10.17605/OSF.IO/HZMTP.

[6] Ellman GL *et al*., "A new and rapid colorimetric determination of acetylcholinesterase activity," *Biochem Pharmacol*, vol. 7, no. 2, p. 88, 1961, doi: 10.1016/0006-2952(61)90145-9.

[7] Ganguly S *et al.*, "An ethanol extract of *Piper betle* Linn. Mediates its anti-inflammatory activity via down-regulation of nitric oxide," *JPP*, vol. 59, p. 711, 2007, doi: 10.1211/jpp.59.5.0012.

[8] Karak S *et al.*, "Essential oil of *Piper betle* L. leaves: Chemical composition, anti-acetylcholinesterase, anti-β-glucuronidase and cytotoxic properties," *JARMAP*, vol. 10, p. 85, 2018, doi: 10.1016/j.jarmap.2018.06.006.

[9] Loi DT, *Vietnamese medicinal plants and herbs*. Medical publisher, Ha Noi, 2003.

[10] Seo J *et al.*, "Anti-inflammatory and antioxidation activities of methanol extract of *Piper betle* leaves and stems by inhibiting NF-KB/MAPK/Nrf2 signaling pathways in RAW 264.7 macrophages," *Biomed Pharmaco*, vol. 155, no. 113734, p. 1, 2022, doi: 10.1016/j.biopha.2022.113734.

[11] Xiang CP *et al.*, "Chemical Composition and Acetylcholinesterase Inhibitory Activity of Essential Oils from *Piper* species," *J Agric Food Chem*, vol. 65, no. 18, p. 3702, 2017, doi: 10.1021/acs.jafc.7b01350.