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Chemical compositions and anti-acetylcholinesterase, nitric oxide suppressing activities of *Piper longum* fruits oil

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Abstract

Piper longum fruits oil in Binh Dinh, Vietnam was obtained via steam distillation with oil collection efficiency reaching 1.01%. The chemical compositions of the essential oil from *Piper longum* fruits in Binh Dinh, Vietnam is determined via the GC-MS method that includes 35 components (99.68%), among which the main components are caryophyllene (10.78%), 3-heptadecene (9.95%), zingiberene (9.54%), germacrene D (8.96%), pentadecane (8.76%), heptadecane (8.73%), β-bisabolene (5.98%), humulene (5.80%), (*E*)-5-tetradecene (2.73%), α-bisabolene (2.47%), tridecane (2.35%). The essential oil of *Piper longum* in Binh Dinh, Vietnam also showed weak anti-acetylcholinesterase activity with IC_{50} (µg/mL) = 164.55±13.79 compared to galantamine as a positive control and potent nitric oxide suppressing activity with IC_{50} (µg/mL) = 13.02±0.29 compared to dexamethasone as a positive control.

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

1. Introduction

Piper longum (Piperaceae) is native to the Indo-Malaya region and widely distributed in the tropical and subtropical world, including the Indian subcontinent, Sri Lanka, the Middle East, and America (Manoj P *et al*., 2004). In Vietnam, *Piper longum* is commonly known as long pepper, tarpaulin, or purple pepper leaf (Loi DT, 2003). The fruits are mainly used as culinary spices, preservatives, and potent remedies in various traditional medicinal systems against bronchitis, cough, cold, snakebite, scorpion-sting, and contraceptives. Various bioactive phytochemicals, including

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alkaloids, flavonoids, esters, and steroids, were identified from the plant extracts. Essential oils from the roots and fruits were reported as antimicrobial, antiparasitic, anthelminthic, mosquito-larvicidal, anti-inflammatory, analgesic, antioxidant, anticancer, neuro-pharmacological, antihyperglycaemic, hepato-protective, antihyperlipidaemic, antiangiogenic, immunomodulatory, antiarthritic, antiulcer, antiasthmatic, cardioprotective, and anti-snake-venom agents (Zaveri M *et al*., 2010; Grover M *et al*., 2021). Many of its pharmacological properties were attributed to its antioxidative and anti-inflammatory effects and its ability to modulate several signaling pathways and enzymes (Biswas P *et al*., 2022).

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, this 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 galanthamine derived from natural products are commonly prescribed cholinergic enhancers as acetylcholinesterase (AChE) inhibitors (Xiang CP *et al*., 2017). Nitric oxide is an endogenous free radical species that is synthesized from L-arginine by nitric oxide synthase in various animal cells and tissues. Small amounts of nitric oxide are essential regulators of physical homeostasis, whereas more significant amounts have been closely correlated with the pathophysiology of various diseases and inflammation. After exposure to inducers, such as lipopolysaccharide from gram-negative bacteria, inducible nitric oxide synthase can be induced in various cells, such as macrophages, Kupffer cells, smooth muscle cells, and hepatocytes, thereby triggering cytotoxicity, tissue damage, inflammation, sepsis, and stroke. Thus, measuring NO production may be a method for assessing the anti-inflammatory effects of plant extracts (Tung YT *et al*., 2010). Aromatic plants have been used to cure neuronal ailments and anti-inflammatory for centuries by different cultures worldwide. Such plant's essential oils and volatile compounds might be potential drugs for Alzheimer's disease therapies and nitric oxide suppressing.

Many species of *Piper* plants are widely used as dietary spices in cuisine worldwide due to their delicious and unique taste (Xiang CP *et al*., 2017). There are several studies on the chemical compositions and biological activities of *Piper longum* fruits oil worldwide (Shankaracharya NB *et al*., 1997; Tewtrakul S *et al*., 2000; Varughese T *et al*., 2016) and Vietnam (Lan TTN, 2012). However, there have been no published on *Piper longum* fruits oil's anti-acetylcholinesterase and nitric oxide suppressing activities both in the world and Vietnam.

The present study aimed to determine the chemical compositions and anti-acetylcholinesterase, nitric oxide suppressing activities of *Piper longum* fruits oil collected from Binh Dinh, Vietnam.

2. Materials and methods

2.1. Materials

The fruits of *Piper longum* were collected at Binh Dinh, Vietnam in June 2020. The sample used for steam distillation is of uniform quality and without spoilage and stored in a cool place. After harvesting, the 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, University of Danang-University of Science and Education. A voucher specimen No. PL001 was deposited at the Department of Chemistry, University

of Danang-University of Science and Education.

2.2. The steam distillation method

Piper longum fruits oil was obtained by steam distillation with light Clevender with 100 g *Piper longum* fruits/400 mL distilled water in three hours at the Chemistry laboratory, 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 longum* fruits oil, d = 0.8452; m (g): Weight of the fruits of *Piper longum.*

2.3. Analysis of the chemical compositions

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

For GC: Operating temperature: 35^{o}C-450^{o}C, temperature resolution: 1^{o}C, maximum heating speed: 0.1^{o}C-120^{o}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.

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 longum* fruits oil collection efficiency is presented in **Table 1**. The essential oil of *Piper longum* fruits in Binh Dinh, Vietnam was obtained via the steam distillation method with an oil collection efficiency of 1.01% in 100 g *Piper longum* fruits/400 mL distilled water for three hours.

The oil collection efficiency of *Piper longum* fruits oil in Binh Dinh, Vietnam was higher than that in Binh Duong, Vietnam (an oil collection efficiency of 0.43% in 200 g *Piper longum* fruits/1000 mL distilled water for ten hours) (Lan TTN, 2012). 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 longum* fruits oil collection efficiency

m (g)	V[a] (mL)	Y (%)
100	1.2	1.01

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

3.2. The chemical compositions

The result of the chemical compositions of *Piper longum* fruits oil is presented in **Figure 1** and **Table 2**. The chemical compositions of the essential oil *from Piper longum* fruits in Binh Dinh, Vietnam is determined via the GC-MS method that includes 35 components (99.68%), among which the main components are caryophyllene (10.78%), 3-heptadecene (9.95%), zingiberene (9.54%), germacrene D (8.96%), pentadecane (8.76%), heptadecane (8.73%), β-bisabolene (5.98%), humulene (5.80%), (*E*)-5-tetradecene (2.73%), α-bisabolene (2.47%), and tridecane (2.35%). This result is consistent with the published chemical compositions of *Piper longum* fruits oil in Vietnam (Lan TTN, 2012) and the world (Shankaracharya NB *et al*., 1997; Tewtrakul S *et al*., 2000; Varughese T *et al*., 2016).

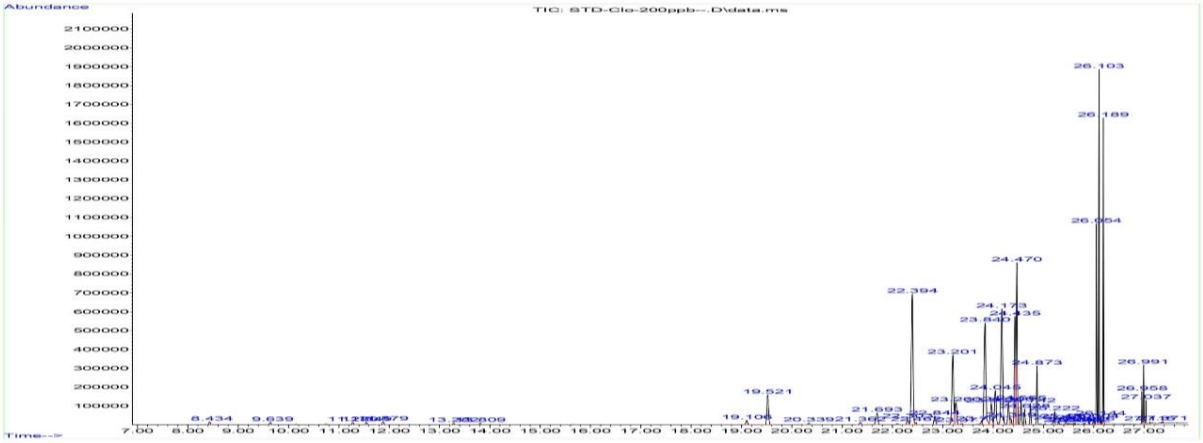

Figure 1. GC-MS spectrum *of Piper longum* fruits oil

Table 2. The chemical compositions of *Piper longum* fruits oil

Retention *(R_T)*	***Compounds***	***Area (%)***
8.434	α-Pinene	0.20
9.639	β-Pinene	0.17
11.280	D-Limonene	0.18
11.549	*trans*-β-Ocimene	0.19
11.879	β-Ocimene	0.25
19.106	(*E*)-5-Tridecene	0.34
19.521	Tridecane	2.35
20.339	δ-Elemene	0.15

21.362	Copaene	0.20
21.693	β-Elemene	1.06
22.303	*trans*-α-Bergamotene	0.45
22.394	Caryophyllene	10.78
22.462	α-Santalene	0.19
22.844	*cis*-α-Bergamotene	0.69
23.201	Humulene	5.80
23.260	*cis*-β-Farnesene	1.76
23.377	β-Santalene	0.13
23.84	Germacrene D	8.96
23.963	α-Selinene	1.79
24.045	(*E*)-5-Tetradecene	2.73
24.173	Zingiberene	9.54
24.306	*cis*-α-Bisabolene	1.90
24.435	β-Bisabolene	5.98
24.470	Pentadecane	8.76
24.555	α-Panasinsene	1.32
24.628	Sesquisabinene	0.81
24.742	γ-Bisabolene	0.99
24.873	α-Bisabolene	2.47
25.222	Caryophyllene oxide	0.67
26.054	1-Heptadecene	7.15
26.103	3-Heptadecene	9.95
26.189	Heptadecane	8.73
26.958	9-Nonadecene	0.82
26.991	1-Nonadecene	1.54
27.037	Nonadecane	0.68
	Total	99.68

3.3. The anti-acetylcholinesterase activity

The result of the anti-acetylcholinesterase activity of *Piper longum* fruits oil is presented in **Table 3**. The essential oil of *Piper longum* fruits showed anti-acetylcholinesterase activity with IC_{50} (μg/mL) = 164.55±13.79. Compared with the results about the anti-acetylcholinesterase activity of galantamine,

Piper longum fruits extract (Ranjan N *et al*., 2018), and essential oils from *Piper* species (Xiang CP *et al*., 2017) (**Table 4)**, it was found that *Piper longum* fruits oil in Binh Dinh, Vietnam had weak anti-acetylcholinesterase activity.

Table 3. The anti-acetylcholinesterase activity of *Piper longum* fruits oil

	***Piper longum* fruits oil**		**Galantamine**	
Concentration (µg/mL)	**Percentage of inhibition**	**Error**	**Percentage of inhibition**	**Error**
500	75.44	2.26	87.38	2.83
100	42.48	1.89	52.80	1.66
20	16.66	1.08	23.58	1.98
4	6.05	0.12	8.26	0.28
IC_{50}	164.55±13.79		1.87±0.11	

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

Table 4. The anti-acetylcholinesterase activity of the *Piper* oils and *Piper longum* fruits extract

***Piper* species**	**Parts**	**100 µg/mL**	**IC_{50} (µg/mL)**
		Percentage of inhibition	
Piper austrosinense	Leaves and stems oil	102.00±3.00	12.40±0.13
Piper puberulum	Leaves and stems oil	148.00±9.60	4.47±0.37
Piper flaviflorum	Leaves and stems oil	96.10±3.70	13.90±1.85
Piper betle	Leaves and stems oil	108.00±1.90	14.00±0.01
Piper hispidimervium	Leaves and stems oil	95.40±4.60	1.51±0.05
Piper longum	Fruits extract (methanol)	42.23±0.28	-

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). Caryophyllene and β-bisabolene are also the main sesquiterpenes of the essential oil of *Piper longum* fruits oil in Binh Dinh, Vietnam, which may be the cause of the anti-acetylcholinesterase activity.

3.4. The nitric oxide suppressing activity

The result of the nitric oxide suppressing activity of *Piper longum* fruits oil is presented in **Table 5**. The essential oil of *Piper longum* fruits showed nitric oxide suppressing activity with IC_{50} (µg/mL) = 13.02±0.29. Compared with the results about the nitric oxide suppressing activity of dexamethasone, it was found that *Piper longum* fruits oil in Binh Dinh, Vietnam had potent nitric oxide suppressing activity. Caryophyllene, zingiberene, germacrene D, β-bisabolene, humulene, and α-bisabolene are the main sesquiterpenes of the essential oil of *Piper longum* fruits oil in Binh Dinh, Vietnam, which may

be the cause of nitric oxide suppressing.

Table 5. The nitric oxide suppressing activity of *Piper longum* fruits oil

Concentration (µg/mL)	*Piper longum* fruits oil				Dexamethasone			
	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	90.12	5.94	6.82	0.31	89.54	1.96	86.51	2.15
20	72.84	2.44	80.21	1.94	52.50	1.65	93.71	1.87
4	20.99	0.35			39.59	1.56		
0.8	9.38	0.00			28.24	0.75		
IC_{50}	13.02±0.29		-		14.20±0.54		-	

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

4. Conclusions

The chemical compositions of the essential oil *from Piper longum* fruits in Binh Dinh, Vietnam includes 35 components (99.68%), among which the main components are caryophyllene (10.78%), 3-heptadecene (9.95%), zingiberene (9.54%), germacrene D (8.96%), pentadecane (8.76%), heptadecane (8.73%), β-bisabolene (5.98%), humulene (5.80%), (*E*)-5-tetradecene (2.73%), α-bisabolene (2.47%), and tridecane (2.35%).

The essential oil of *Piper longum* fruits in Binh Dinh, Vietnam showed weak anti-acetylcholinesterase activity with IC_{50} (µg/mL) = 164.55±13.79 and potent nitric oxide suppressing activity with IC_{50} (µg/mL) = 13.02±0.29.

Declaration of Competing Interest

The authors declare no competing interests.

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