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CHEMICAL COMPOSITION AND ACETYLCHOLINESTERASE INHIBITORY ACTIVITY OF ESSENTIAL OIL FROM RHIZOMES OF *DISTICHOCHLAMYS BENENICA*

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Abstract. Twenty-seven constituents were identified by using GC/MS, representing 99.57% of the rhizome oil of *Distichochlamys benenica*. The major constituents of the essential oil are 1,8-cineole (54.39%), β-pinene (7.50%), (*E*)-citral (7.26%), and (*Z*)-citral (6.79%). The rhizome essential oil has anti-acetylcholinesterase activity with an IC_{50} value of 136.63 ± 2.70 μg/mL.

Keywords: *Distichochlamys benenica*, essential oil, acetylcholinesterase, 1,8-cineole

1 Introduction

Essential oils, which are complex mixtures of volatile compounds, mainly terpenes, are extracted from plants by using steam distillation and various solvents [1, 2]. All over the world, around 3000 essential oils have been extracted from at least 2000 plant species, out of which approximately 300 essential oils are considered important in commerce [1]. Since ancient times, essential oils have been used in traditional medicines to treat inflammatory disease, pain relief, gastrointestinal disease, or the reduction of stress. Modern pharmacological studies have shown that essential oils exhibit biological activity such as antifungal, antibacterial [3-6], anti-inflammatory [7-9], cytotoxicity, cancer chemoprotective [10, 11], cardiovascular effects [12, 13], anticonvulsant [14], and anti-insect [15, 16]. With a broad spectrum of biological activity and aromatic properties, essential oils are increasingly popular, especially in cosmetics, food products and pharmaceuticals.

Distichochlamys, a genus belonging to Zingiberaceae family, was first discovered in 1995 by Newman [17]. Up to now, only four species of this genus have been identified, all endemic to Vietnam. These include *D. benenica* Q.B. Nguyen & Skornick [18], *D. citrea* M. F. Newman [17], *D. orlowii* K. Larsen & M. F. Newman [19], and *D. rubrostriata* W. J. Kress & Rehse [20]. The composition of rhizome essential oils from *D. rubrostriata*, *D. citrea*, and *D. orlowii* has been reported. 1,8-Cineole (13.2–22.0%), α-citral (18.5–22.1%), β-citral (14.2–22.3%), *trans*-geraniol (12.5–12.8%), and geranyl acetate (6.6–14.9%) are the main constituents in *D. rubrostriata* [21]. The phytochemical investigation of the rhizome essential oil of *D. citrea* indicates that 1,8-cineole is the main component (30.71–43.67%) [22], while high contents of geranyl acetate (16.5%), β-elemene (9.2%), β-pinene (9.0%), and β-caryophyllene (7.9%) are present in the rhizome essential oil of *D. orlowii* [23]. To the best of our knowledge, the phytochemical analysis and biological activity of

D. benenica have not been performed yet. This article aims to report the chemical composition from the rhizome essential oil of *D. benenica* as well as its acetylcholinesterase (AChE) inhibitory activity.

2 Material and methods

2.1 Plant collection and extraction of essential oil

D. benenica Q.B.Nguyen & Škorničk species was collected in Tay Giang district, Quang Nam province, Vietnam in February 2020. A voucher specimen (B.En.01) was deposited at the Faculty of Fundamental Sciences, University of Agriculture and Forestry, Hue University, Vietnam.

Fresh rhizomes of *D. benenica* (0.2 kg) were shredded and their essential oil obtained was by hydrodistillation for 6 hours. The oil was dried with Na_2SO_4 and kept under refrigeration (4 °C) until analysis. The experiments were performed in triplicates.

2.2 Analysis of essential oil

A Shimadzu Technologies GCMS-QP2010 Plus chromatograph fitted with a fused silica Equity-5 capillary column (30 m × 0.25 mm, film thickness 0.25 μm, Supelco, USA) and coupled with a mass spectrometer (MSD QP2010 Plus) was used for GC-MS analysis. The analytical conditions are as follows: carrier helium (1.78 mL/min), injector temperature of 250 °C, interface temperature of 250 °C, and a column temperature programmed from 40 °C (1 min hold) to 285 °C (5 min hold) at 3 °C/min. Samples were injected using a split ratio of 30:1. The injected volume is 1.0 μL, and the inlet pressure is 100 kPa.

The MS conditions are as follows: ionization voltage 70 eV, detector voltage 0.82 kV, and acquisition scan mass range 40–350 amu at a sampling rate of 0.5 scans/s. The MS fragmentation patterns were checked against those of other essential oils of known compositions by using NIST 11 and WILEY 7 Libraries (on ChemStation HP) and by comparison of mass spectra of the separated constituents with the data reported in the literature [24]. The relative percentage of particular components in the essential oils was calculated from the area percent report (Uncalibrated calculation procedure) generated in the GC software.

2.3 Acetylcholinesterase inhibition assay

The AChE inhibition assay was determined with a modified version of the Ellman colorimetric method [25]. Each of the reaction mixtures contains 140 μL of Tris-HCl buffer (pH 8.0), 20 μL of the tested sample solution, and 20 μL of the AChE solution (0.25 units/mL). After incubation for 15 min, the reaction was initiated by adding 10 μL of 0.24 mM 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) and 10 μL of 0.24 mM acetylthiocholine iodide (ACTI). The final mixture then was incubated at ambient temperature for 15 min. The same reaction mixture without sample was used as a negative control. The optical density was measured at 405 nm on an ELISA microplate reader (EMR-500, Labomed Inc.) and the percentage inhibition was calculated. Galanthamine was used as a positive control. All tested samples and the positive control, galanthamine, were dissolved in 10% DMSO (analytical grade). The reaction was performed in triplicates in 96-well microplates. The percentage inhibition (I%) was calculated according to the following equation:

$$I\% = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

where A_{sample} is the absorbance of the sample solution; A_{control} is the absorbance of the negative control. Each sample was assayed at 5

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concentrations (60, 80, 100, 120, and 140 μg/mL) so that the IC_{50} value could be calculated from the logarithmic dose-inhibition curve.

3 Results and discussion

3.1 Essential oils composition

The rhizome essential oil of *D. benenica* is a pale yellow liquid with a characteristic aromatic odor and lighter than water. The yield of essential oil is 0.2% (v/w), calculated on a fresh weight basis. A total of 27 components are identified, representing 99.57% of the oil content. Oxygenated monoterpene derivatives are the major type of compounds present in the rhizome essential oil with 75.89%, followed by monoterpene hydrocarbons (19.97%), sesquiterpene hydrocarbons (0.5%), and other compounds (3.21%). Moreover, 1,8-cineole (54.39%), β-pinene (7.50%), (*E*)-citral (7.26%), (*Z*)-citral (6.79%), and α-pinene (4.51%) are found in this oil as main constituents (Table 1).

Table 1. Chemical composition of the rhizome essential oil of *Distichochlamys benenica*

No.	Compound	Class	Percentage composition (%)
1	(*Z*)-3-Propylidenecyclopentene	NT	0.04
2	α-Thujene	MH	0.12
3	α-Pinene	MH	4.51
4	Camphene	MH	2.53
5	2,4,4-Trimethyl-2-penten-1-ol	NT	0.04
6	Isopropyl glycolate	NT	0.06
7	Sabinene	MH	0.93
8	β-Pinene	MH	7.50
9	6-Methylhept-5-ene-2-one	NT	2.97
10	(5*S*,8*R*)-5-Isopropyl-8-methyl-2-methylene-3,9-decadien-1-ol	NT	0.09
11	α-Phellandrene	MH	0.77
12	Limonene	MH	2.93
13	1,8-Cineole	OM	54.39
14	γ-Terpinene	MH	0.59
15	Terpinolene	MH	0.09
16	Linalool	OM	2.10
17	Borneol	OM	1.33
18	Terpinene-4-ol	OM	1.68
19	Arthole	OM	0.67
20	α-Terpineol	OM	0.73
21	Fenchyl acetate	OM	0.56
22	(*Z*)-Geraniol	OM	0.23
23	(*Z*)-Citral	OM	6.79
24	β-Farnesene	SH	0.50

No.	Compound	Class	Percentage composition (%)
25	(*E*)-Citral	OM	7.26
26	Neryl acetate	OM	0.14
27	Isovalerone	NT	0.01
	Total		**99.57**
	MH (Monoterpene Hydrocarbons)		**19.97**
	OM (Oxygenated Monoterpenes)		**75.89**
	SH (Sesquiterpene Hydrocarbons)		**0.5**
	NT (Non-Terpenes)		**3.21**

Out of 27 compounds identified in the *D. benenica* oil, 19 of them are also found in the rhizome essential oils of *D. rubrostriata, D. citrea*, and *D. orlowii*. The most abundant class of the rhizome essential oil of *D. benenica* is oxygenated monoterpenes (75.89%), similar to those of *D. rubrostriata* (64.92–94.06%) [21] and of *D. citrea* (79.47–90.73%) [22, 23]. However, this finding is very different from that of the essential oil of *D. orlowii*, which comprises oxygenated monoterpenes (29.4%), monoterpene hydrocarbons (23.9%), sesquiterpene hydrocarbons (33.7%), and oxygenated sesquiterpenes (11.2%) [23]. Furthermore, 1,8-cineole presented in rhizome essential oil of *D. benenica*, *D. rubrostriata*, and *D. citrea* as the major component with 54.39%, 13.2–22.0% and 23.00–43.67%, respectively [21-23]. Surprisingly, this compound is not identified in *D. orlowii* [23]. Similarly, a remarkable amount of (*E*)-citral is found in the rhizome essential oil of *D. benenica*, *D. rubrostriata*, and *D. citrea* but conspicuously absent in *D. orlowii*. Besides, a significant quantity of β-pinene (7.50%), (*Z*)-citral (6.79%), and α-pinene (4.51%) in *D. benenica* is previously reported on three other *Distichochlamys* species [21-23]. All of the data in the present and previous studies indicate a similarity in the chemical composition of the rhizome essential oil of *D. rubrostriata*, *D. citrea*, and *D. benenica*.

3.2 Acetylcholinesterase inhibition

The essential oil is tested for AChE inhibitory activity at various concentrations. Galanthamine is used as a positive control. The essential oil exhibits moderate AChE inhibition with an IC_{50} value of 136.63 ± 2.70 μg/mL. However, this oil displays a much weaker activity compared with galanthamine (IC_{50} = 0.33 ± 0.01 μg/mL) (Table 2). The potency of *D. benenica* essential oil is stronger than that of *Lavandula officinalis* (IC_{50} = 820 μg/mL) and *Ocimum sanctum* oils (IC_{50} = 1600 μg/mL) [26], but slightly weaker than that of *Artemisia maderaspatana*, *Artemisia dracunculus*, *Pinus heldreichii* subsp. *leucodermis*, and *Pinus nigra* subsp. *nigra* oils with IC_{50} values of 31.33, 58, 51.1, and 94.4 μg/mL, respectively [26-28]. A literature survey indicates that 1,8-cineole, α-pinene, and β-pinene possess a potent AChE inhibitory effect with IC_{50} values of 0.06 ± 0.01, 0.09 ± 0.005, and 0.2 ± 0.004 mg/mL, respectively [29]. These components are found in the essential oil of *D. benenica* with a high content (4.51–54.39%). Therefore, it is reasonable to believe that these compounds contribute significantly to the AChE inhibition of *D. benenica* oil.

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Table 2. AChE inhibitory activity of rhizome essential oil of *Distichochlamys benenica*

Samples	Concentration (μg/mL)	Percentage of AChE inhibiton (%)	IC_{50} (μg/mL) ± SD
Essential oil	140	51.56 ± 0.58	136.63 ± 2.70
	120	45.72 ± 0.53	
	100	39.89 ± 0.38	
	80	33.67 ± 0.91	
	60	25.69 ± 0.62	
Galanthamine[#]	0.5	57.86 ± 1.79	0.33 ± 0.01
	0.4	52.88 ± 0.75	
	0.3	47.61 ± 0.34	
	0.2	42.44 ± 1.09	
	0.1	28.37 ± 1.23	

[#]Positive control

4 Conclusion

In this study, we report the phytochemical composition and AChE inhibitory activity of the rhizome essential oil of *D. benenica* for the first time. The oil is a pale yellow liquid with a characteristic aromatic odor. Twenty-seven constituents are present in the oil, in which 1,8-cineole (54.39%), β-pinene (7.50%), (*E*)-citral (7.26%), (*Z*)-citral (6.79%), and α-pinene (4.51%) are major compounds. In addition, the essential oil has moderate AChE inhibitory activity with an IC_{50} value of 136.63 ± 2.70 μg/mL. The obtained results contribute positively in the establishment of the database on Vietnamese endemic plants.

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