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VOLATILE COMPOUNDS AND BIOLOGICAL ACTIVITIES OF ESSENTIAL OIL OF *GLOBBA PENDULA* RHIZOMES COLLECTED AT AN GIANG PROVINCE

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Abstract. The present paper describes chemical composition, NO inhibitory activity against lipopolysaccharide (LPS)-induced nitric oxide release, cytotoxic activity against MCF7 and Hep3B cell lines of the essential oil of *Globba pendula* Roxb. rhizomes. A total of 25 components of the essential oil were identified by using gas chromatography–mass spectrometry (GC-MS), accounting for 89.55 % of the total oil. δ-selinen (36.45 %) and ishwarane (10.76 %) were the main components. The essential oil was found to possess moderate NO inhibitory effect with IC_{50} of 41.68 ± 4.51 µg/ml, and significant cytotoxic activity against MCF7 and Hep3B cell lines with IC_{50} of 28.15 ± 1.08 and 35.24 ± 0.06 µg/ml, respectively. This is the first report on volatile compounds and biological activities of the essential oil of *Globba pendula* Roxb. rhizomes collected in An Giang province, Viet Nam.

Keywords: *Globba pendula*, cytotoxic activity, NO inhibitory activity, essential oil, GC-MS.

Classification numbers: 1.1.3, 1.2.1.

1. INTRODUCTION

Globba pendula Roxb. (called "Ngải mọi" in Viet Nam), a herb belonging to the genus *Globba* (Zingiberaceae), is endemic in Indochina [1]. In folk medicine, the rhizomes of *G. pendula* are used to treat rheumatism and osteoarthritis. In Malaysia, people use the decoction of the rhizomes for postpartum women and deworming. In Indonesia, it has been used to treat the flatulence [2, 3]. However, just only one report on chemical constituents presented the isolation and structural identification of ten compounds including labdane diterpenes and benzofurans [4].

Up to date, no report on biological activities of the plant has ever been published worldwide. Essential oils are oily, hydrophobic, aromatic, and volatile liquids that can be extracted from natural sources, usually plants [5]. Essential oils are valuable plant products, which are used as therapeutic agents in ethno, conventional, and complementary alternative medicines. Particularly they have analgesic, anti-inflammatory, antispasmodic, local anaesthetic, anthelmintic, antipruritic, and antiseptic as well as many other therapeutic uses and they contribute many disease control benefits [6]. It is worth noting that there has been no research on the essential oil of *G. pendula*. Therefore, it is necessary to investigate the chemical composition and biological activities to clarify uses of *G. pendula* rhizomes in folk medicine. In the current paper, we present the results of screening chemical composition by GC-MS analysis and assaying cytotoxic activity, and inhibitory effects on LPS-induced NO production in RAW264.7 cells of the essential oil from the rhizomes of *G. pendula*.

2. MATERIALS AND METHODS

2.1. Chemical and biological materials

Lipopolysaccharides (LPS) from *Escherichia coli* were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS) were obtained from Life Technologies, Inc., (Gaithersburg, MD, USA). Sodium nitrite, sulfanilamide, N-1-napthylethylenediamine dihydrochloride and dimethyl sulphoxide (DMSO) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Other chemicals were purchased from Sigma, GIBCO, Invitrogen, Promega. The murine macrophage cell line (RAW 264.7) was obtained from Prof. Domenico Delfino, Perugia University, Italy.

Human breast carcinoma cell line (MCF-7) and human hepatoma cell line (Hep3B) were obtained from Prof. Jeong-Hyung Lee, Kangwon National University, Korea.

2.2. Plant material and preparation of the essential oil

The fresh rhizomes of *Globba pendula* Roxb. were collected in An Giang province, Viet Nam in February 2019 and were identified by Dr. Nguyen Van Du, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology (VAST). The voucher specimens (NGM-02.2019) were deposited in the Pharmaceutical Chemistry Laboratory, Institute of Natural Products Chemistry, VAST.

The fresh rhizomes (500 g) of *G. pendula* were washed with water, chopped and hydrodistilled using Clevenger apparatus for 6 hours to give the raw essential oil. The oil was then dried with anhydrous sodium sulphate (Na_2SO_4) to remove the remaining water trace. The obtained essential oil (2.6 g) was then used to analyze chemical composition and evaluate biological activities.

2.3. GC-MS analysis

GC-MS analysis of the essential oil was carried out on an Agilent Technologies HP7890A GC equipped with a mass spectrum detector (MSD) Agilent Technologies HP5975C and a HP5-MS column (60 m × 0.25 mm, film thickness 0.25 µm, Agilent Technologies). The injector and detector temperatures were set at 250 and 280 ^oC, respectively. The column temperature progress initiated at 60 ^oC, followed by an increase to 240 ^oC at 4 ^oC/min. The carrier gas was helium at a flow rate of 1 mL/min. The sample was injected by splitting. The split ratio was

100:1. The volume injected was 1 µL of essential oil. The MSD conditions were as follows: ionization voltage 70 eV, emission current 40 mA, acquisitions scan mass range 35-450 amu under full scan. A homologous n-alkane series was used as the standard to calculate retention time indices (RI) of each component. The relative amounts of individual components were calculated based on the GC peak area (MSD response) without correction. MassFinder 4.0 software connected to the HPCH1607, W09N08 libraries, and the NIST Chemistry WebBook was used to match mass spectra and retention indices. To confirm these results, further comparison was made with data of authentic compounds reported in the original literature.

2.4. Assay for NO inhibitory effect using RAW264.7 cells

Cell culture

RAW264.7 cells were cultured in DMEM containing 10 % fetal bovine serum, 2 mM glutamine, 10 mM HEPES, 1 mM sodium pyruvate. Cells were grown after 3 - 5 days with the ratio of 1:3 at 37 °C, 5 % CO_2 in fully humidified air. The assay was performed at Bioassay Group, Institute of Biotechnology, VAST.

Inhibitory activity on NO production assay

RAW264.7 cells were initially grown in 96-well plate (2×10^5 cells/well) and incubated in a humidified atmosphere with 5 % CO_2 at 37 ^oC for 24 hours. After that, the medium was removed and replaced by DMEM (free FBS) for 3 hours. The cells were treated with sample for 2 hours followed by 1 µg/mL of LPS treatment for 24 hours. The nitrite accumulated in culture medium was measured as an indicator of NO production based on the Griess reaction. Briefly, 100 µl of culture medium were incubated with 100 µl of Griess reagent (50 µL of 1 % (w/v) sulfanilamide in 5 % (v/v) phosphoric acid and 50 µL of 0.1 % (w/v) N-1-naphthylethylenediamine dihydrochloride in water) in a 96-well plate, incubated at room temperature for 10 min [7]. After incubation the absorbance was determined using an ELISA reader at 540 nm. The DMEM (free FBS) medium was used for blank-reading in all experiments, and the positive control was N^G-Methyl-L-arginine acetate (L-NMMA). Standard calibration curves were prepared using sodium nitrite as standard. The inhibition percentage was calculated by the Eq. (1):

$$I = 100 - \frac{NO_{sample}}{NO_{LPS}} \times 100 \quad (1)$$

in this equation, I is the inhibition percentage (%), NO_{sample} and NO_{LPS} are the concentrations of NO produced when using studied sample and negative control, respectively. The assay was repeated three times to ensure accuracy. The IC_{50} values were identified using Table Curve 2Dv4.

MTT assay for cell viability

To evaluate the cytotoxic effect of sample in RAW 264.7 cells in the assay condition, MTT assay was performed. Briefly, cells were treated with studied sample in 96-well plate in a humidified atmosphere with 5 % CO_2 at 37 ^oC for 72 hours then added 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). After 4 hours of incubation, the medium was discarded and the formazan blue formed in the cells was dissolved in DMSO. The optical density (OD) was measured at 540 nm. The percentage of cell viability (P) was calculated by the Eq. (2):

$$\text{P (\%)} = \frac{\text{OD}_{\text{sample}} - \text{OD}_{\text{plank}}}{\text{OD}_{\text{DMSO}} - \text{OD}_{\text{plank}}} \times 100 \quad (2)$$

2.5. Cytotoxic evaluation procedure

MTT assay, as previously described by Skehan *et al.* [8] was performed to assess the cytotoxicity of essential oil of *G. pendula*. The cells were cultured in RPMI-1640 or DMEM medium supplemented with 10 % foetal bovine serum (FBS), 100 U/ml penicillin and 100 µg/ml streptomycin, incubated in a humidified atmosphere with 5 % CO_2 at 37 ^oC for 48 h. After that, the cells were plated in a 96-well microtiter plate (2.5×10^4 cells/well) and cultured for 24 h. The cells were then treated with the studied sample dissolved in dimethyl sulfoxide (DMSO) at different concentrations. After 72 h of incubation at 37 ^oC, 5 % CO_2, 20 µL MTT reagents (5 mg/mL) were added to each well and incubated at 37 ^oC for a further 4 h. Then the medium was removed and the precipitate formazan dissolved in isopropanol. The absorbance was measured at 570 nm. Camptothecin was used as positive control. CS (%) (% cell survival) was calculated by the Eq. (3):

$$\text{CS (\%)} = \left[\frac{\text{OD}_{\text{sample}} - \text{OD}_{\text{day 0}}}{\text{OD}_{\text{DMSO}} - \text{OD}_{\text{day 0}}} \text{ x } 100\right] \pm \sigma \quad (3)$$

σ (standard deviation) was calculated by the formula: $\sqrt{\frac{\sum(x_i - \overline{x})^2}{n-1}}$ (4)

in this equation, x_i is the OD value at well number i, $\overline{x}$ is the mean OD value, n is the number of repeated wells. Sample with potential activities ($CS_{50} \leq 50$ % ± σ) will be selected for the next step test to determine IC_{50} value.

3. RESULTS AND DISCUSSION

3.1. Chemical composition

The yield of the essential oil of fresh rhizomes of *G. pendula* was 0.52 % based on the fresh weight of the sample. A total of 25 components of the essential oil were identified by using GC-MS method, accounting for 89.55 % of the total oil. The two main components were δ-selinene (36.45 %) and ishwarane (10.76 %). 14 of the 25 compounds were identified as sesquiterpenoids, corresponding to 72.63 % of the whole oil, while 6 of the 25 constituents were monoterpenoids (6.81 % of the essential oil). Aromatic compounds and other components were 6.52 and 3.59 %, respectively. Sesquiterpenoids were also found to be the main class in the essential oils of several *Globba* species such as *G. sessiliflora* (95.0 %), *G. schomburgkii* (72.9 %), and *G.ophioglossa* (64.3 %) [9, 10].

δ-selinene is a member of the class of compounds known as eudesmane, isoeudesmane or cycloeudesmane sesquiterpenoids with a structure based on the eudesmane skeleton. δ-selinene can be found in all spice, lovage, and wild celery, which makes it a potential biomarker for the consumption of these food products [11]. Ishwarane belonging to sesquiterpenoid class was first isolated from the plants *Aristolochia indica* and *Cembopentalum penduliflorum*. It has been isolated from the essential oils of *Bixa orellana*, *Corallocarpus epigaeus* and *Piper fulvescens* [12]. Ishwarane exhibited antifungal activity against *C. albicans* and *C. cladosporioides* [12, 13]. However, δ-selinene and ishwarane have not been seen in other *Globba* species. Therefore, these two compounds may be biomarkers for the identification of *G. pendula*from *Globba* species.

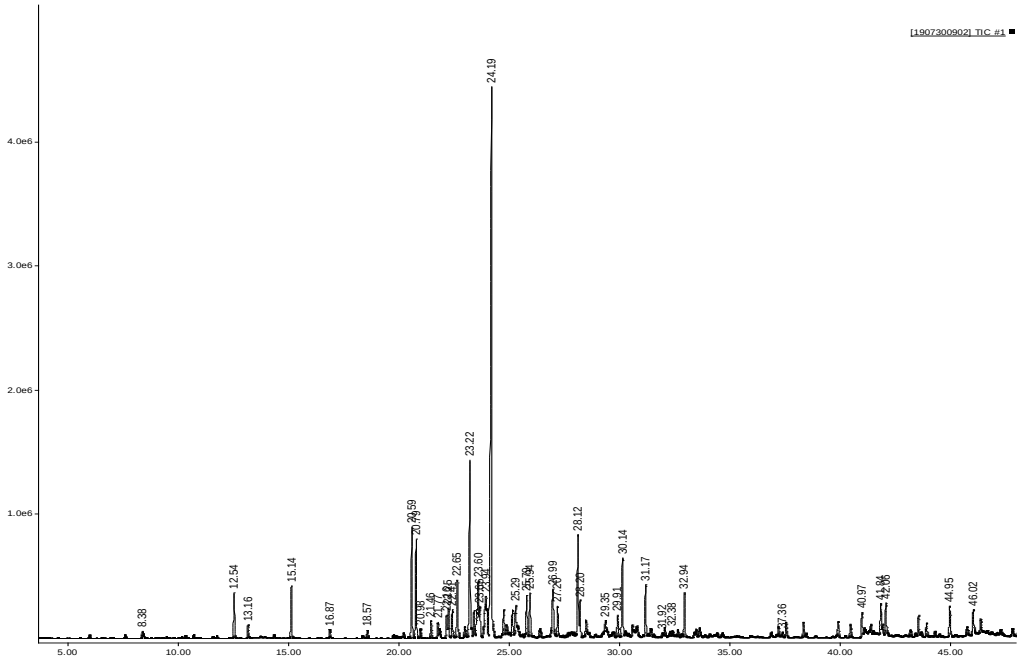

Figure 1. GC-MS chromatogram of the essential oil.

Table 1. Chemical constituents of the *G. pendula* essential oil.

No	**RI_{Exp}**	**RI_a**	**Chemical name**	**$\%_b$**
1	1040	1031	1,8-Cineole	0.17
2	1169	1162	Isoborneol	2.31
3	1187	1169	Borneol (endo-Borneol)	0.74
4	1246	1239	Isobornyl formate	2.72
5	1297	1296	3-Thujyl acetate	0.44
6	1348	1349	α-Terpinyl acetate	0.43
7	1415	1399	Cyperene	5.46
8	1421	1410	α-Gurjunene	0.58
9	1436	1419	*E*-Caryophyllene (=β-Caryophyllene)	0.96
10	1446	1437	γ-Elemene	0.89
11	1459	1443	Guaia-6,9-diene	1.35
12	1461	1445	Cubeb-11-ene	1.75
13	1466	1450	Spirolepechinene	1.68
14	1474	1473	Drima-7,9(11)-diene	3.58
15	1492	**1467**	**Ishwarane**	**10.76**
16	1506	1485	Germacrene D	2.08
17	1515	1494	Bicyclogermacrene	4.47
18	1524	**1493**	**δ-Selinene**	**36.45**

19	1576	1563	*E*-Nerolidol	2.69
20	1656	1602	*α*-Corocalene	6.04
21	1719	1671	Daucalene	1.42
22	1792	1740	Oplopanone	0.26
23	1809	1760	(*E*)-Ethyl *p*-methoxycinnamate	0.48
24	2004	1993	Ethyl palmitate	0.26
25	2156	2092	Benzyl Cinnamate	1.58
			Total identified	89.55
			Monoterpenoids	6.81
			Sesquiterpenoids	72.63
			Aromatic compounds	6.52
			Others	3.59

a: HPCH1607 and MassFinder 4.0 libraries; b: according to GC-MS with HP5-MS column.

3.2. NO production inhibitory assay

Table 2 shows the inhibitory activity of NO production by the *G. pendula* essential oil in LPS-activated macrophages. The essential oil was found to possess moderate NO inhibitory activity with IC_{50} of 41.68 ± 4.51 µg/ml and have little cytotoxic activity against RAW 264.7 cells. The positive control L-NMMA operated stably in the assay.

Table 2. Inhibitory effects on NO production of the *G. pendula* essential oil.

Dose (µg/ml)	***G. pendula* essential oil**		**L-NMMA**	
	NO inhibition rate (%)	**Viability rate (%)**	**NO inhibition rate (%)**	**Viability rate (%)**
100	86.87	82.27	94.87	93.55
20	32.02	95.73	74.36	99.62
4	5.07	99.53	35.90	
0.8	0.79	98.53	21.77	
IC50	**41.68 ± 4.51**		**6.51 ± 0.31**	

3.3. Cytotoxic evaluation

The essential oil was screened for its cytotoxic activity against MCF-7 and Hep3B cell lines. The studied sample which has potential effects with the percentage of cell survival less than 50 % at tested concentrations of 50 and 100 µg/ml (Table 3) was further studied to determine IC_{50} values. The results showed that the *G. pendula* essential oil has significant cytotoxic activity against MCF7 and Hep3B cell lines with IC_{50} of 28.15 ± 1.08 and 35.24 ± 0.06 µg/ml, respectively (Table 4).

Table 3. The result of screening cytotoxicity of the *G. pendula* essential oil.

Sample	Concentration (µg/ml)	Viability rate (%)	
		Hep3B	MCF7
Control		100 ± 1.2	100 ± 0.79
G. pendula essential oil	50	48.13 ± 0.43	49.66 ± 0.73
	100	41.51 ± 1.84	39.04 ± 0.53
Camptothecin*	10	35.03 ± 1.36	43.84 ± 1.34
	40	25.34 ± 0.79	31.87 ± 0.86

*Camptothecin was used as positive control (µM).

Table 4. Cytotoxicity of the*G. pendula* essential oil (IC_{50} in µg/ml).

Sample	IC_{50} (µg/ml)	
	Hep3B	MCF7
G. pendula essential oil	35.24 ± 0.06	28.15 ± 1.08
Camptothecin*	0.59 ± 0.19	6.46 ± 0.81

4. CONCLUSION

In conclusion our study is the first research on the chemical composition of volatile compounds and biological activities of the essential oil from the fresh rhizomes of *Globba pendula* Roxb. A total of 25 components of the essential oil were identified by using GC-MS method, accounting for 89.55 % of the total oil. The two main components were δ-selinene (36.45 %) and ishwarane (10.76 %). It is important to mention that there have been no reports on GC-MS analysis of the essential oil of *G. pendula* so far. The *G. pendula* essential oil exhibited moderate inhibitory effects on LPS-induced NO production in RAW264.7 cells with IC_{50} values of 41.68 ± 4.51 µg/ml and significant cytotoxic activity against MCF7 and Hep3B cell lines with IC_{50} values of 28.15 ± 1.08 and 35.24 ± 0.06 µg/ml, respectively. This is the first report on volatile compounds and biological activities of the essential oil of *Globba pendula* Roxb. collected in An Giang province, Viet Nam. The study contributes more scientific evidence about *G. pendula*, and also indicates that the essential oil from the fresh rhizomes of *G. pendula* has potential for the development of natural products with anti-inflammatory or cytotoxic activity.

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