CHEMICAL COMPOSITIONS AND CYTOTOXIC ACTIVITY OF ESSENTIAL OILS EXTRACTED FROM *HEDYCHIUM CORONARIUM* J.KOENIG GROWN IN VIETNAM

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ABSTRACT

Hedychium coronarium, commonly known as white ginger lily (Zingiberaceae), is a native species in Asian forests. The plant has long been used as a traditional medicine for treating pain and inflammatory conditions and as a food-flavouring spice. In this work, the essential oil samples prepared from *Hedychium coronarium* leaves, rhizomes, and stems were analyzed by gas chromatography coupled with the mass spectrometry (GC/MS) method, which gave information on the main chemical components of leaf essential oil [β-pinene (27.90%), caryophyllene (23.93%), caryophyllene oxide (17.31%)], rhizome essential oil [β-pinene (29.56%), sabinene (12.05%), coronarin E (10.91%), *p*-cymene (10.39%), and α-pinene (10.06%)], and stem essential oil [α-phellandrene (18.71%), *p*-cymene (12.68%), β-pinene (12.18%)]. Three essential oils were also evaluated for their cytotoxic effects against SK-LU-1 cells (lung adenocarcinoma). The leaf essential oil was shown to be the most potent cytotoxic agent with an IC50 value of 80.19 ± 3.32 µg/mL. The cytotoxic activity against SK-LU-1 cells of the essential oils of *Hedychium coronarium* was reported for the first time. The results of this investigation indicated the similarities and differences among the chemical compositions of leaf, rhizome, and stem parts of *Hedychium coronarium* collected in Vietnam, which can be considered a potential and inexpensive resource of zingiberaceous essential oils.

Keywords: *Hedychium coronarium*; essential oil; GC/MS; SK-LU-1.

1. Introduction

Hedychium coronarium (Zingiberaceae plant family) has been found naturally in Asian countries, such as China, Indonesia, Myanmar, India, Nepal, Sri Lanka, Thailand, and Vietnam. The plant has pseudostems (1-3 m), sessile leaves, membranous ligule (2-3 cm), leaf blade with oblong-lanceolate or lanceolate shape (20-30 × 4.5-8 cm), white and fragrant flower (calyx 4 cm split on one side).

Previous phytochemical investigations revealed that the plant rhizomes contained labdane-type diterpenoids [1], [2], [3] and diarylheptanoids [4] as primary secondary metabolites. The rhizome extract exhibited anti-inflammatory and analgesic effects in radiant heat tail-flick and acetic acid-induced writhing tests [5]. The medium-polar ethylacetate-soluble fraction of *H. coronarium* rhizomes also significantly inhibited the enzymatic activities of α-amylase and α-glucosidase [6]. The isolated labdane-type diterpenoids have shown a wide range of bioactivities, including hepatoprotective, anti-inflammatory, cytotoxic [1], [2], [3], and anti-bacterial [7] activities. *H. coronarium* essential oil was also a topic of interest for phytochemists. The essential oils were prepared from flower, leaf, and rhizome parts with interesting bioactivities, including antioxidative [8] and anti-bacterial activities [9]. It is noticeable that the chemical components of volatile oils prepared from samples collected in different areas have shown vast differences in terms of both compositions and their percentages. The details of the chemical composition of essential oils prepared from leaf, flower, and rhizome parts are described in Table 1. Therefore, it is necessary to research the chemical compositions of essential oils from *H. coronarium* sample collected in Vietnam to identify the similarities and differences between Vietnam and other countries samples, paving the way for further studies on sub-species identification, essential oil accumulation, and evaluation of agricultural factors on essential oil quality. Remarkably, this is the first report on volatile compounds of this species against SK-LU-1 cells.

Table 1: *Previous study results on the chemical composition of the essential oils from H. coronarium*

Plant tissue	Sample location	Major components	Ref.
Rhizome	Harinagar (Bheerapani), Nainital, India	linalool (29.3 %), limonene (20.3 %), *trans*-meta-mentha-2,8-diene (12.9 %), γ-terpinene (8.9 %) and 10-epi-γ-eudismol (3.8 %)	[10]
Rhizome	Registro, Brazil	1,8-cineole (34.8%), β-pinene (16.7%) and α-terpineol (13.1%)	[11]
Rhizome	Chapada das Mesas, Carolina, Brazil	1,8-cineole (33.5%), β-pinene (17.0%), α-terpineol (7.7%), α-pinene (7.3%), limonene (5.2%), and *p*-cymene (4.9%)	[12]
Rhizome	Thiruvanathapuram, India	1,8-cineole (41.42%), β-pinene (10.39%) and α-terpineol (8.8%)	[13]
Rhizome	Bhubaneswar, Odisha, India (10 samples)	β-pinene (11.07–42.74%), eucalyptol (11.48-40.59%), linalool (1.56–45.11%), coronarin E (1.01–39.57%), α-pinene (3.80–16.60%), *p*-cymene (1.05–8.89%), γ-terpinene (1.73–5.82%) and 10-epi-γ-eudesmol (1.11–4.86%)	[6]
Rhizome	Ushabali valley, India	eucalyptol (37.52%), p-cymene (11.68%), *p*-menth-1-en-8-ol (9.44%), terpinen-4-ol (5.47%), β-pinene (5.00%)	

Plant tissue	Sample location	Major components	Ref.
Flower	Hangzhou, Zhejiang, China	β-*trans*-ocimenone (28.05%), linalool (18.52%), 1,8-cineole (11.35%), α-terpineol (7.11%), 10-epi-γ-eudesmol (6.06%), sabinene (4.59%) and terpinen-4-ol (3.17%)	[14]
Flower	Tohseien, Chiba, Japan	linalool (29.27%), methyl benzoate (5.70%), (*E*)-isoeugenol (18.35%), jasmin lactone (3.46%), indole (6.98%)	[15]
Flower	Havana, Cuba	(*E*)-β-ocimene (28.7%), linalool (19.3%) and 1,8-cineole (14.5%)	[16]
Flower	Sato Farm, Chiba, Japan	linalool (13.17%), methyljasmonate (17.16%), eugenol (3.00%)	[17]
Leaf	Registro, Brazil	β-caryophyllene (43.0%), caryophyllene oxide (12.1%), and β-pinene (11.6%)	[8]

2. Materials and methods

2.1. Plant materials

The leaves, rhizomes, and stems of *H. coronarium* were collected from Bidoup Nui Ba National Park, Lac Duong District, Lam Dong Province, in May 2021 (12°7'1.12”N, 108°32'55.32”E). The scientific name of the plant was authenticated by Assoc. Prof. Dr. Van-Son Dang from the Institute of Tropical Biology, Vietnam Academy of Science and Technology. The voucher specimen (HC03-05.2021) has been deposited at the Department of Chemistry, Vinh University, Nghe An, Vietnam.

2.2. Essential oil isolation

The hydro distillation process of *H. coronarium* leaves, stems, and rhizomes was carried out on a Clevenger-type apparatus in 4 hours, according to the Vietnamese Pharmacopoeia [18]. The obtained volatile oil was then separated from the mixture, exhaustively dried with anhydrous sodium sulfate, and stored in a refrigerator at the temperature of 4°C before analysis.

2.3. Determination of cytotoxic assay

The cytotoxic activity of the essential oils and their main components are evaluated against the human lung carcinoma SK-LU-1 cell line from Dr. J. M. Pezzuto's lab (Long Island University, NY, USA). Our study replicates the in vitro testing protocol described in the method section of Skehan *et al.*'s study [19], confirmed by the National Center Institute (NCI) to be the standard cytotoxicity test to screen and detect substances capable of inhibiting growth or killing TBUT under in vitro conditions. The test determines the total cellular protein content based on the optical density (OD) measured when the protein composition of the cells was stained with Sulforhodamine B (SRB). The measured OD value is directly proportional to the amount of SRB attached to the protein molecule. During the experiment, we trypsinized the experimental cells to separate cells and count

them in a counting chamber to adjust the density to suit the experiment. We first put 190 µL of cells in a 96-well plate. The test sample was dissolved in 100% DMSO to a stock concentration of 20 mM. The sample was then diluted on a 96-well plate with cell culture medium (without FBS) into four concentration ranges from high to low. Diluted reagents at different concentrations (volume 10 mL) were introduced into the wells of the cell-prepared 96-well plate. Wells without reagent but with TBUT (190 mL) + DMSO 1% (10 mL) are used as day 0 control. After 1 hour, cells in day 0 control wells are fixed with Trichloracetic acid - TCA 20% before being incubated for 72 hours. After 72 h, cells were fixed with TCA for one h, stained with SRB for 30 min at 37^{o}C, washed 3 times with acetic acid, and dried at room temperature. 10 mM unbuffered Tris base is used to dissolve the SRB residue. The sample is gently shaken for 10 min before the OD result is obtained at 540 nm using an ELISA Plate Reader (Biotek). The percentage inhibition of cell growth in the presence of reagents is determined through the following formula:

$$\%\ \text{inhibition} = 100\% - \frac{\text{OD(sample)} - \text{OD(day 0)}}{\text{OD(DMSO)} - \text{OD(day 0)}}$$

To ensure accuracy, the test is repeated 3 times. Ellipticine at concentrations of 10-2-0.4-0.08 mg/mL is used as reference control; 1% DMSO is always used as a negative control (the final concentration in the test well is 0.05%). The IC50 value (concentration that inhibits 50% of growth) is determined using TableCurve 2Dv4 computer software. An extract with IC50 £ 20 µg/ml and a purified substance with IC50 £ 5 µM are considered high activity, according to the NCI standard [20].

2.4. Chemical characterization of essential oil

The chemical components of *H. coronarium* essential oils were analyzed by an Agilent Technologies 7890B GC System equipped with a 5977B MSD model. The GC/MS analytical conditions were set as follows: column: HP-5MS Ultral Inert (30 m × 0.25 mm × 0.25 µm); injection volume: 1 µL with the split ratio of 50:1; carrier gas: helium with flow rate 1.00 mL/min (7.65 psi); injector temperature: 300°C, MS Quad temperature: 150°C and MS source: 230°C. The oven temperature program was set as follows: 0-2 min: 50°C; 2-22 min: 50-150°C (rate: 5°C/min); 22-32 min: 150°C; 32-45 min: 150-280°C (rate: 10°C/min); 45-55 min: 280°C. As for MS conditions, the ionization voltage was set at 70 eV, and acquisitions scan mass was set in the *m/z* 50-550 range at 2.0 scan/s. The identification of chemical composition was conducted based on comparing retention indices (RIobsd.) concerning a homologous series of *n*-alkanes (C8-C30, Sigma-Aldrich) and the matching of the mass spectral fragmentation patterns with those of MS library (NIST 17, Adams book) [21], [22].

3. Results and discussion

The essential oils of *H. coronarium* leaves, stems, and rhizomes were pale yellow and lighter than water. The yield results of the essential oil from leaves, stems, and rhizomes of *H. coronarium* were 0.56, 0.21, and 0.42% (w/w, fresh weight), respectively. The GC/MS analysis showed the presence of 38 components in these essential oils, accounting for 93.02-98.24% of the total content, in which all compounds occupying more than 0.05% area were considered for analysis (Table 2).

Chemical profiles of essential oils prepared from leaves, stems, and rhizomes of *H. coronarium* shared some similarities. Specifically, β-pinene was present in all samples with a relatively high content (27.9% for leaf, 29.56% for rhizome, and 12.18% for stem). Two other components, α-pinene and sabinene, were also found in three samples with contents ranging from 5.76-12.05%. However, there are some characteristic components present in each essential oil sample. In the stem essential oil, α-phellandrene occupied 18.71% of the total content, while the monoterpene was only considered a minor component in leaf or rhizome essential oil. The significant components of leaf essential oil were identified as caryophyllene (23.93%) and caryophyllene oxide (17.31%), while the plant stem part only contained 6.98 and 2.26%, respectively. Coronarin E, a characteristic oxygenated diterpene from *H. coronarium*, was abundant in only rhizome essential oil.

Table 2: *Chemical constituents of H. coronarium leaf, rhizome, and stem essential oils*

No.	RT (min)	RI (obsd.)	RI (lit.)	Compounds	Percentage (%)		
					Leaves	Rhizomes	Stems
1	6.82	926	925	Tricyclene	0.14	-	-
2	6.96	931	929	*α*-Thujene	0.6	1.96	0.74
3	**7.14**	**938**	**937**	***α*-Pinene**	**9.67**	**10.18**	**8.37**
4	7.55	953	952	Camphene	1.96	1.69	0.96
5	**8.26**	**978**	**974**	**Sabinene**	**5.81**	**12.19**	**6.91**
6	**8.36**	**981**	**979**	***β*-Pinene**	**28.16**	**29.9**	**12.94**
7	8.75	993	991	*β*-Myrcene	0.66	1.56	3
8	**9.13**	**1005**	**1005**	***α*-Phellandrene**	0.45	0.24	**19.88**
9	9.30	1012	1010	3-Carene	0.11	1.11	3.43
10	9.50	1020	1017	*α*-Terpinene	-	-	0.5
11	**9.72**	**1028**	**1025**	***p*-Cymene**	0.84	**10.51**	**13.47**
12	**9.84**	**1032**	**1030**	**Limonene**	2.96	3.26	**6.22**
13	9.93	1035	1032	Eucalyptol	-	4.14	0.51
14	10.43	1053	1037	*β*-Ocimene	-	-	0.12
15	10.73	1063	1060	*γ*-Terpinene	0.11	0.19	2.52
16	11.61	1090	1088	Terpinolene	-	0.15	1.21
17	11.94	1100	1099	Linalool	-	2.83	-
18	12.59	1125	1122	*cis*-1-methyl-4-(1-methylethyl)-2-cyclohexen-1-ol	-	0.11	-
19	13.09	1143	1139	L-*trans*-Pinocarveol	-	0.36	-
20	13.80	1167	1164	Pinocarvone	0.11	0.18	-
21	13.87	1170	1166	(1*S*,2*R*,4*S*)-Borneol	-	0.14	-

No.	RT (min)	RI (obsd.)	RI (lit.)	Compounds	Percentage (%)		
					Leaves	Rhizomes	Stems
22	14.21	1181	1182	(-)-Terpinen-4-ol	-	1.2	0.23
23	14.77	1198	1193	Myrtenal	0.17	0.42	-
24	15.83	1238	1235	2-methoxy-4-methyl-1-(1-methylethyl)benzene	-	0.11	-
25	17.28	1289	1285	Bornyl acetate	1.17	0.3	0.49
26	18.97	1354	1350	*α*-Terpinyl acetate	-	0.17	0.34
27	19.69	1381	1376	Copaene	-	0.11	0.25
28	19.83	1386	1379	*trans*-Methyl cinnamate	-	0.48	-
29	**20.87**	**1427**	**1419**	**Caryophyllene**	**24.15**	0.18	**7.41**
30	20.92	1428	1432	*trans*-4-hydroxy-3-methyl-6-(1-methylethyl)-2-cyclohexen-1-one	-	0.13	0.28
31	21.70	1460	1454	Humulene	2.11	-	0.6
32	23.54	1525	1524	Cadina-1(10),4-diene	-	-	0.24
33	**25.57**	**1587**	**1581**	**Caryophyllene oxide**	**17.47**	-	2.4
34	26.53	1613	1606	Humulene epoxide II	0.85	-	-
35	27.63	1637	1644	10,10-Dimethyl-2,6-dimethylene-bicyclo [7.2.0]undecan-5*β*-ol	0.38	-	-
36	35.03	1797	1809	Ambrial	-	0.24	-
37	**40.59**	**2135**	**2136**	**Coronarin E**	0.36	**11.04**	-
Total identified					**98.24**	**95.08**	**93.02**
Monoterpene hydrocarbons (No. 1-12; 14; 5)					51.47	72.79	79.36
Oxygenated monoterpenes (No. 13; 16-26; 30)					1.45	10.24	3.06
Sesquiterpene hydrocarbons (No. 27; 29; 31; 32)					26.26	0.29	8.5
Oxygenated sesquiterpenes (No. 33-36)					18.7	0.24	2.4
Oxygenated diterpenes (No. 37)					0.36	11.04	-
Benzenoid compound (No. 28)					-	0.48	-

RT: Retention time (min); RI (obsd.): Retention indices calculated in this study; RI (lit.): Retention indices from literature; "-": Not determined.

Comparing the result analysis of *H. coronarium* essential oils between the present and previous reports of the world indicates significant differences in chemical composition and relative content, as presented in Table 1. For Vietnamese oils, *H. coronarium* from Bat Xat District, Lao Cai Province, afforded oils whose significant components were β-pinene

(20.0%), linalool (15.8%), α-pinene (10.1%), 1,8-cineole (10.7%) and α-terpineol (8.6%) in the leaf. In comparison, the root consists mainly of β-pinene (23.6%), α-humulene (17.1%), β-caryophyllene (13.0%), α-pinene (6.9%) and elemol (6.9%) [23]. The variations can be explained by various influences, such as when the crop is harvested, the conditions in which it grows, and where it is cultivated.

The essential oils of *H. coronarium* leaves, stems, and rhizomes were also evaluated for their cytotoxic activities against the SK-LU-1 cell line (human lung carcinoma). The samples were tested in concentrations of 0.8-100 µg/mL with positive control, ellipticine, which was tested in the concentration range of 0.08-10 µg/mL. Among three essential oil samples, the leaf essential oil showed the most potent cytotoxic activities against the SK-LU-1 cell line with an IC50 value of 80.19 ± 3.32 µg/mL, while those figures for stem and rhizome essential oils were over 100 µg/mL.

The main chemical components of *H. coronarium* leaf essential oil were α-pinene (9.58%), β-pinene (27.90%), caryophyllene (23.93%), caryophyllene oxide (17.31%), which accounted for 78.72% of total content. In previous pharmacological studies, α-pinene was shown to possess anti-cancer properties against several cancer cell lines, including human ovarian cancer [24], hepatocellular liver carcinoma [25], and N2a neuroblastoma cells [26]. These monoterpenes also showed synergistic effects with paclitaxel, a well-known anti-cancer drug, in the cytotoxic tests against non-small-cell lung carcinoma, which increased paclitaxel-induced apoptosis and mitotic cell cycle arrest [27]. As for caryophyllene and caryophyllene oxide, the two bicyclic sesquiterpenes were also found to be anti-cancer agents in various cancer cells with different IC50 values in the range of 0.87-58.2 µg/mL [28]. In terms of structure, caryophyllene oxide was shown to have more potential due to possessing an epoxide exocyclic functionality, making the compound bind covalently to DNA bases and proteins to initiate signalling in cancer cells [29]. Caryophyllene and caryophyllene oxide could also potentiate the efficacy of anti-cancer drugs, such as doxorubicin [30] and paclitaxel [31], by increasing the drug concentrations inside the cancer cells.

Table 3: *Cytotoxic effects against SK-LU-1 cell line of the essential oils from H. coronarium*

Concentration (µg/mL)	% Inhibition			
	Leaf essential oil	Stem essential oil	Rhizome essential oil	Ellipticine
100	57.55 ± 1.12	39.29 ± 1.36	28.26 ± 1.81	-
20	21.15 ± 1.37	24.27 ± 0.13	13.45 ± 0.87	-
4	7.20 ± 0.74	11.61 ± 1.28	1.54 ± 0.15	-
0.8	3.91 ± 0.22	5.78 ± 0.39	0.74 ± 0.06	-
IC_{50}	80.19 ± 3.32	>100	>100	0.37 ± 0.04
IC_{50}: the half maximal inhibitory concentration; Mean ± SD; n = 3.				

4. Conclusion

In conclusion, the GC/MS analysis identified the major chemical compositions in the essential oils of *H. coronarium* leaves, rhizomes, and stems growing in Vietnam. In addition, the three essential oil samples were also evaluated for their cytotoxicity against the SK-LU-1 cell line (human lung carcinoma), with the most potent cytotoxic effect belonging to the leaf essential oil (IC50 = 80.19 ± 3.32 µg/mL). This is the first study about cytotoxicity against the SK-LU-1 cell line of essential oils extracted from this plant sample. The results obtained in this work would encourage further chemical research experimental efforts and more in-depth biological testing of the most potent compounds.

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TÓM TẮT

THÀNH PHẦN HÓA HỌC VÀ HOẠT TÍNH GÂY ĐỘC TẾ BÀO CỦA TINH DẦU CHIẾT XUẤT TỪ *HEDYCHIUM CORONARIUM* J.KOENIG Ở VIỆT NAM

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Hedychium coronarium, thường được gọi là lily gừng trắng (họ Gừng), là một loài đặc hữu trong các khu rừng ở châu Á. Loài cây này từ lâu đã được sử dụng như một loại thuốc cổ truyền để điều trị đau nhức và viêm, cũng như là một loại gia vị tạo hương vị cho thực phẩm. Trong nghiên cứu này, các mẫu tinh dầu được chưng cất từ lá, thân rễ và thân giả của *Hedychium coronarium* đã được phân tích bằng phương pháp sắc ký khí kết hợp với phổ khối lượng (GC/MS), cho thông tin chi tiết về các thành phần hóa học chính của tinh dầu lá [β-pinene (27,90%), caryophyllene (23,93%), caryophyllene oxide (17,31%)], tinh dầu thân rễ [β-pinene (29,56%), sabinene (12,05%), coronarin E (10,91%), *p*-cymene (10,39%), và α-pinene (10,06%)], và tinh dầu thân giả [α-phellandrene (18,71%), *p*-cymene (12,68%), β-pinene (12,18%)]. Ba loại tinh dầu cũng được đánh giá về tác dụng gây độc đối với tế bào SK-LU-1 (ung thư biểu mô tuyến phổi). Trong đó, tinh dầu lá được chứng minh là tác nhân gây độc tế bào mạnh nhất với giá trị IC50 là 80,19 ± 3,32 µg/mL. Hoạt tính gây độc tế bào đối với tế bào SK-LU-1 của tinh dầu *Hedychium coronarium* được báo cáo lần đầu tiên. Kết quả của nghiên cứu trong bài báo này chỉ ra sự tương đồng và khác biệt giữa thành phần hóa học của lá, thân rễ và thân giả của *Hedychium coronarium* ở Việt Nam, có thể được coi là một nguồn tài nguyên tiềm năng và rẻ tiền của tinh dầu họ Gừng.

Từ khóa: *Hedychium coronarium;* tinh dầu; GC/MS; SK-LU-1.