DETERMINATION OF CHEMICAL COMPONENTS IN THE RHIZOMES OF *Hedychium coronarium*

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Abstract

*Hedychiumcoronarium*or locally known as white ginger lily belongs to Zingiberaceae family. Hydrodistillation of the fresh rhizomes yielded 0.02% of the essential oil. The composition of the essential oil was analyzed both by gas chromatography and gas chromatography-mass spectroscopy. Eleven compounds were identified representing 79.78% of the whole compositions. 1,8-Cineole (39.03%) is the major component in the essential oil. Soxhlet extraction of the dried rhizomes of H. coronarium with chloroform as solvent yielded a crude extract 4.42%. Purification of the extract took place using column chromatography and preparative thin layer chromatography had afforded two diterpenes. Their structures have been identified using spectroscopic methods as two isomers of coronarin D (1.02%) and 14,15-dihydroxylabda-8(17),12-diene-15,16-olide (coronarin G) (0.1%). Antioxidant property was screened using DPPH radical scavenging assay and has been carried out on the chloroform crude extract, essential oil and two pure compounds. The results revealed that the crude extract gave moderate antioxidant property with IC_{50} 275.05 µg/mL. The antibacterial assay was conducted on chloroform crude extract, essential oil and coronarin D. Coronarin D was found to show moderate antibacterial property towards Gram positive bacteria *Bacillus subtilis* (BS) ATCC 6633 at a concentration of 450 ppm.

Keywords: *Hedychium coronarium,* labdane, coronarin, essential oil, bioassay.

INTRODUCTION

H. coronarium which has many common names including butterfly ginger, butterfly lily, cinnamon jasmine, garland flower, and ginger lily, is widely cultivated in India, Southeast Asian countries, South China, Taiwan, Japan, and Brazil. The rhizomes of *H. coronarium* is used in Chinese natural medicine, which has been prescribed for the treatment of headache, lancinating pain, contusion inflammatory, and sharp pain due to rheumatism in Chinese traditional preparations, while it is used as a febrifuge, tonic, excitant, and anti-rheumatic in the Ayurvedic system of traditional Indian medicine [1].

The chemical composition of the essential oils of ginger lily have been identified in the early studies such as α-muurolol (16.8%), α-terpineol (15.9%), 1,8-cineole (11.2%), α-fenchyl acetate (5.6%), citronellal (5.5%) and (*E*)-methyl cinnamate (5.1%). Some of the compounds that are present in the rhizomes of *H. coronarium* are the labdane diterpenes, (*E*)-labda-8(17),12-diene-15,16-dial, coronarin B, coronarin D, isocoronarin D, labda-8(17),11,13-trien-15,16-olide, an ester of labda-8(17),11,13-trien-15-al-16-oic acid and isoconarin D, and 7β-hydroxycoronarin B [2]. This research focused on the study of the rhizomes of *H. coronarium* and the objectives of this study are to extract the essential oil, phytochemical and to evaluate the bioactivity of *H. coronarium*. Purification of the chemical constituents present in the crude extracts of the rhizomes of *H. coronarium* and also to elucidate the structure of the compound using spectroscopic methods.

EXPERIMENTAL

Chemical composition analysis of the essential oil was carried out using a Gas Chromatography (GC) Hewlett Packard 5890 series II. A GC equipped with an Ultra 1 column (25 m long, 0.32 µm thickness and 0.17 mm internal diameter). The chromatogram of gas chromatography-mass spectrometry (GC-MS) was recorded using a Perkin Elmer Gas Chromatograph Clarus 680 equipped with mass Spectrometer Clarus SQ 8 S. All chemicals involved in this experiment are analytical grade. Soxhlet extraction, fractionation and purification were carried out using several types of organic solvents which are *n*-hexane, petroleum ether, diethyl ether, chloroform, methanol and ethyl acetate. Petroleum ether refers to PE with a boiling point 60-80°C and was redistilled before used. Thin layer chromatography (TLC) was carried out on 0.20 mm Merck silica gel plates (60 F254). Samples were spotted on the baseline (0.5 cm) drawn on the TLC. The compounds were visualized under a UV lamp (254 nm) and vanillin sulphuric acid spray. Fractionation and purification of the crude extracts were conducted using gravity column chromatography (CC) and preparative thin layer chromatography (prep TLC) with Merck silica gel 70-230 mesh and silica gel 60 PF254 (10–40 µ) containing gypsum, respectively. Infrared (IR) spectra were recorded on Perkin Elmer 1650 FTIR spectrophotometer using the attenuated total reflection (ATR) for the gummy samples. The 1H and 13C nuclear magnetic resonance spectra (NMR) were recorded on Bruker Avance 400 Spectrometer (400 MHz and 100 MHz respectively). Deuterated chloroform was used as solvent.

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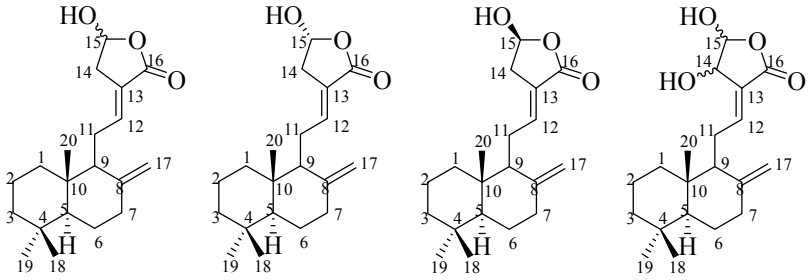
Intensity of the colour change of DPPH for antioxidant assay were recorded on Biotek Epoch Microplate Spectrophotometer. The wavelength was set to 517nm.



(1) **(2)** **(3)** **(4)**

Plant Material

The sample of *H. coronarium* or also known as ginger lily was obtained from Skudai, Johor in 2014.

Extraction and Isolation

The fresh chopped rhizomes (205.0 g) were extracted using hydrodistillation technique in a Dean- Stark apparatus for 8 hours. The essential oil collected was extracted with ether (3×10 mL), dried over anhydrous magnesium sulphate and filtered. The ether was then evaporated at room temperature overnight to give the essential oil (0.039 g, 0.02%) as pale yellow oil with a fragrant scent.

The rhizomes of *H. coronarium* (74.0 g) were air dried, powdered and extracted with chloroform in a soxhlet apparatus for 20 h. The resulting chloroform extract was evaporated to dryness under reduced pressure using rotary evaporator to afford a thick dark yellowish browngum crude extract labeled as HC (3.3 g, 4.5%). The crude extract HC (2.0 g) obtained was purified using column chromatography (CC) (100×3.5 cm length) packed with Merck silica gel 70-230 mesh (60 g). The column was eluted using n-hexane and Et_2O as solvent with increasing polarity gives eight major compounds. Fraction HC 5 was evaporated under reduced pressure to give a mixture of epimers of coronarin D **(1)** (0.0203 g, 1.02%) as a dark yellowish brown gum. Further purification of fraction HC 4 using prep TLC afforded another four compounds using triple development with *n*-hexane and Et_2O (2:1). Fraction HC 4-4 was concentrated to give a compound that was tentatively predicted as 14,15-dihydroxylabda-8(17),12-diene-15,16-olide (Coronarin G) **(4)**.

RESULTS AND DISCUSSION

The essential oil of *H. coronarium* was analysed by GC and GC-MS. The mass spectrum of each peak was compared with mass spectrum from the National Institute of Standards and Technology (NIST). The high percentage matching (more than 80%) was selected as the constituents. The identified constituents of the essential oil of *H. coronarium* are listed in **Table 1**. A total of eleven components were successfully identified from the GC and GC-MS comprising 79.78% of the total. The essential oil consisted of only monoterpenes with 1,8-cineole (39.03%) as the main constituent. The other major components found in the rhizome oil were α-terpineol (21.67%) and β-pinene (8.05%). Previous study showed that the major essential oil components of *H. coronarium* from Mauritius is α-muurolol (16.8%), α-terpineol (15.9%), 1,8-cineole (11.2%), α-fenchyl acetate (5.6%), citronellal (5.5%) and (*E*)-methyl cinnamate (5.1%) [3]. Meanwhile another research from India reported that the major constituents of fresh rhizome oil were 1,8-cineole (41.42%), β-pinene (10.39%), α-terpineol (8.80%) and α-pinene (4.06%) [4]. The chemical composition of rhizome oil of H. Coronarium collected in Johor, Malaysia was closely resemble to the essential oil composition reported by Beena Joy [4], in which 1,8-Cineole is the major component.

Coronarin D epimers **(1)** were obtained as dark yellowish brown gum (0.02 g, 1.02 %) with a R_f 0.38 (*n*-hexane:Et_2O, 1:2). The IR spectrum showed that there is weak hydroxyl bend at the position of 3373 cm^{-1}. The spectrum also showed a sharp bend at 1737 cm^{-1} for carbonyl C=O ester in the furan ring. It could be observed that the bend is shifted to a lower value due to the conjugation of double bond outside the ring causing it to weakened the strain of C=O bond. The ^{1}H NMR spectrum of compound **(1)** revealed the presence of a mixture of coronarin D epimers **(2)** and**(3).** The proton NMR spectrum showed three singlet integrating for three proton

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each at δ 0.72, δ 0.82 and δ 0.89 which were attributed to three methyl groups. Two broad singlet proton resonated at δ 4.40 and δ 4.82 was assigned to exomethylene protons at C-17. These suggested that compound **(1)** has a labdane type skeleton. The epimers are assigned as coronarin D (A) **(2)** and coronarin D (B) **(3)**. The position of the first set exomethylene could be given to compound (**2**), meanwhile for compound **(3)** two weak doublet protons resonated at δ 4.35 and δ 4.82 with a value of proton coupling J = 0.8 Hz. As for the ^{1}H-^{1}H COSY spectrum for compound **(1)** showed the correlation between H-11, H-14 and H-17. The ^{13}C NMR supported the presences of 23 peaks with three extra peaks due to the epimeric mixture that contains in compound **(1)**. The duplicate peaks were at the position of C-8, C-12 and C-17 which can be assigned to one set to compound **(2)** while the other set belongs to compound **(3)**. Analysis of DEPT spectrum showed the existing of three methyl group at δ 33.58 (C-18), δ 21.73 (C-19) and δ 14.35 (C-20); one exomethylene at δ 107.36 (C-17) for compound **(2)** while the other at δ 107.65 (C-17) for compound **(3)** and seven methylene at δ 39.21 (C-1), δ19.32 (C-2), δ 41.99 (C-3), δ 24.09 (C-6), δ 37.78 (C-7), δ 25.51 (C-11) and δ 124.48 (C-14); and four methine groups at δ 55.33 (C-5), δ 56.12 (C-9), δ 96.46 (C-15) and δ 143.55 (C-12) for compound **(2)** while the other at δ 143.64 (C-12) for compound **(3)**. The complete assignments of the carbons were accomplished by the HMQC spectrum**.** The complete ^{1}H NMR and ^{13}C NMR parameters for coronarin D **(1)** epimers are listed in **Table 2.**

Table 1: Chemical composition of the essential oil of *H. coronarium*

No.	Compounds	Kovats Index	Percentage of Composition (%)
1	α-Pinene	928	1.81
2	β-Pinene	964	8.05
3	Myrcene	980	2.77
4	α-Phellandrene	997	0.47
5	1,8-Cineole	1017	39.03
6	*E*-Sabinene hydrate	1055	0.89
7	Linalool	1094	1.49
8	Camphor	1117	0.31
9	Pinocarvone	1132	0.90
10	Terpinen-4-ol	1160	2.39
11	α-Terpineol	1171	21.67
Total amount identified (%)			79.78

Table 2: ^{1}H and ^{13}C NMR data of compound **(1)**

Carbon	^{1}H (δ ppm)	^{13}C (δ ppm)
1	1.00-2.10	39.21
2	1.00-2.10	19.32
3	1.00-2.10	41.99
4	-	33.58
5	1.00-2.10	55.33
6	1.00-2.10	24.09
7	2.30-2.40 m	37.78
8	-	147.91/148.12
9	1.00-2.10	56.12
10	-	39.44
11	2.20/2.35	25.51
12	6.73 m	143.55/143.64
13	-	124.48
14	2.73 dd J = 2, 15.2/ 3.00-3.06 m	33.56
15	5.95 m	96.46
16	-	170.66
17	4.35/4.82 d J = 0.8 Hz 4.40/4.82 s	107.36/107.65
18	0.89 s	33.58
19	0.82 s	21.73
20	0.72 s	14.35

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Preparative TLC of HC4 using *n*-hexane:Et_2O (1:1) afforded a minor constituent HC4-4 as a pale yellow oil (2.0×10^{-3} g, 0.1 %) with R_f0.5 (*n*-hexane:Et_2O, 1:1). The ^{1}H NMRspectrum HC4-4 revealed the presence of three singlet each integrating for three proton at δ 0.71, δ 0.82 and δ 0.89 and exomethylene proton at δ 4.57 and δ 4.89 suggested that HC4-4 has the labdane skeleton. Two deshielded protons were observed at δ 6.82 and δ 6.11 which were assign to be β-olefinic proton at C-12 and methine proton at C-14 respectively. Another broad singlet at δ 3.67 was an oxymethine proton at C-14. Due to insufficient amount of sample the compound (4), the ^{13}C NMR was not obtained to support the structure of compound. Therefore tentatively the compound was assigned as 14,15-dihydroxylabda- 8(17),12-diene-15,16-olide (Coronarin G) **(4)**.

Antioxidant activity was screened using DPPH radical scavenging assay and has been carried out on the chloroform crude extract, essential oil and two pure compounds. The results revealed that the crude extract gave moderate antioxidant property with IC_{50} 275.05 µg/mL. The antibacterial assay was conducted on chloroform crude extract, essential oil and coronarin D. Coronarin D was found to show moderate antibacterial properties towards Gram positive bacteria *Bacillus subtilis* (BS) ATCC 6633 at a concentration of 450 ppm.

CONCLUSION

The extraction of the fresh rhizomes by hydrodistillation afforded essential oil in 0.02% yielded. By GC and GC-MS analyzed revealed eleven compounds which contributed 79.78% of the total oil.Meanwhile, Soxhlet extraction of *H. coronarium* yielded chloroform extract (4.42%). Purification of chloroform extract have resulted in the isolation of two labdane diterpene compounds. The compounds were identified by using spectroscopic techniques and also by comparison with the literature value and the first compound has be proposed as epimers of coronarin D **(34)**. As for the second compound, it was tentatively identified as 14,15-dihydroxylabda-8(17),12-diene-15,16-olide (coronarin G) **(51)**. The DPPH free radical scavenging activity screening showed that the crude chloroform extract gave positive antioxidant, whereas epimer of coronarin D was active towards *Bacillus subtilis* (Gram positive bacteria).

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