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COMPOSITION OF *HEDYCHIUM MALAYANUM* RHIZOME ESSENTIAL OIL AND ITS ANTIOXIDANT ACTIVITY

(Komposisi Minyak Pati Rizom *Hedychium malayanum* dan Aktiviti Antipengoksidanya)

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Abstract

The *Hedychium malayanum* rhizome oil with a yield of 0.079% was obtained after hydro distillation using a Clevenger-type apparatus. Nineteen compounds representing 99.5% of the total amount of oil were identified using capillary GC and GC-MS with a DB-5 column. The oil was dominated by monoterpenes (99.0%) with 1,8-cineole (37.7%), β-pinene (35.2%) and α-pinene (10.9%) being the most abundant constituents. The oil was studied for its antioxidant activities by three different tests, involving its effect on the 1,1-diphenyl-2-picrylhydrazil (radical scavenging activity, RSA), ferrous ion chelating (FIC) and β-carotene bleaching (BCB) in linoleic acid system. The oil showed fairly good activities toward all the above tests compared to the standards except in the case of FIC, where the standard ascorbic acid possessed weaker chelating ability than the oil. RSA was calculated as IC_{50}, and the respective values for gallic acid, ascorbic acid and the oil were 1.8 ± 0.06, 3.5 ± 0.30 and 140.3 ± 1.46 µg/mL. For BCB, the oil and standard BHT had possessed high and very high capacity (87.2 ± 1.00 and 96.8 ± 0.21% respectively) to prevent the oxidation by linoleic acid. FIC activity showed that the oil and standard ascorbic acid were weaker chelating than EDTA (IC_{50} 86.9 ± 0.70 µg/mL). However, the oil showed a fairly good chelating (IC_{50} 283.3 ± 5.26 µg/mL) whereas the ascorbic acid possessed a weak activity to chelate Fe^{2+} (IC_{50} 1428.6 ± 7.04 µg/mL).

Keywords: *Hedychium malayanum*, essential oil, composition, GC-GC/MS, antioxidant activity

Abstrak

Minyak rizom *Hedychium malayanum* dengan hasil 0.079% diperoleh selepas penyulingan hidro menggunakan radas jenis-Clevenger. Sembilan belas sebatian yang mewakili 99.5% jumlah amaun bagi minyak telah dikenal pasti menggunakan KG dan KG-SJ rerambut dengan turus DB-5. Minyak tersebut didominasi oleh monoterpena (99.0%) dengan 1,8-sineol (37.7%), β-pinena (35.2%) dan α-pinena (10.9%) merupakan juzuk yang paling banyak. Minyak ini dikaji aktiviti antipengoksidanya dengan tiga ujian yang berbeza, melibatkan kesannya terhadap 1,1-difenil-2-pikrilhidrazil (aktiviti penghapus-sisa radikal, APR), pengkelatan ion ferus (PIF) dan pelunturan β-karotena (PBK) dalam sistem asid linoleik. Minyak ini menunjukkan aktiviti yang agak baik terhadap kesemua ujian di atas berbanding dengan piawai kecuali dalam kes ujian PIF, di mana piawai asid askorbik memperlihatkan kebolehan pengkelatan yang lebih lemah daripada minyak. APR dikira sebagai KP_{50}, dan nilai masing-masing bagi asid galik, asid askorbik dan minyak ini adalah 1.8 ± 0.06, 3.5 ± 0.30 dan 140.3 ± 1.46 µg/mL. Bagi PBK, minyak dan piawai HTT memperlihatkan kapasiti tinggi dan sangat tinggi (87.2 ± 1.00 and 96.8 ± 0.21%, masing-masing) untuk mencegah pengoksidaan oleh asid linoleik. Aktiviti PIF menunjukkan bahawa minyak ini dan piawai asid askorbik adalah pengkelat yang lebih lemah daripada EDTA (KP_{50} 86.9 ± 0.70 µg/mL). Namun, minyak tersebut menunjukkan pengkelatan yang agak baik (KP_{50} 283.3 ± 5.26 µg/mL) manakala asid askorbik memperlihatkan aktiviti yang lemah bagi mengkelat Fe^{2+} (KP_{50} 1428.6 ± 7.04 µg/mL).

Kata kunci: *Hedychium malayanum*, minyak pati, komposisi, KG-KG/SJ, aktiviti antipengoksida

Introduction

Peninsular Malaysia has 18 genera under Zingiberaceae family that includes *Hedychium* with seven species [1]. Recently, more than 50 *Hedychium* species have been identified from several distributions, mainly eastern Himalayas to south India, south China and Southeast Asia [2]. *Hedychium* is commonly known as ginger lilies and it is distinguished by its flowering plants; its thick, fleshy and branched rhizomes; and its medium-size [3-5]. This species gets it name from two ancient Greek words, hedys that means "sweet" and chios that means "snow" [3]. Holttum [6] described *Hedychium malayanum* by its bracteoles that are tubular, apex acute and hairy; its petiole to about 4 cm in length; its inflorescence from the base of lowest bract to the tip of apical one is 14 cm in length; and its bracts are very broad, overlapping, glabrous and each bract enclosing three or more flowers. In contrast to the wide use of some *Hedychium* species in the traditional medicine and culinary purpose, the uses of *Hedychium malayanum* have not been discovered yet.

There have been a wide range of products in which essential oils are used as functional ingredients. There is also a common need for such natural extracts to possess a pleasant taste or smell and a preservative action. This is essential to avoid lipid deterioration, oxidation and spoilage by microorganisms [7]. As a result of the potential role of essential oils as a source of natural antioxidants and biologically active compounds, they have attracted a great deal of scientific interest [8]. The essential oils rich in monoterpenes are recognized as food preservatives, and monoterpenic essential oils are natural antioxidants that are active against certain cancers such as breast, liver, colon, and prostate cancers, and leukemia [9]. They are also considered to be important in diets or medical therapy for biological tissue deterioration due to free radicals [10].

The study of chemical composition and antioxidant activity of *Hedychium malayanum* leaf and stem essential oils was done in 2015 by the authors [11]. As the synthetic antioxidants were reported as carcinogenic, the demand for antioxidants from the natural sources has increased. In this paper, we identify the chemical constituents and evaluate the antioxidant activity of the rhizome oil of *H. malayanum*. There are several reports on the essential oils of the rhizomes from some *Hedychium* species, but *H. malayanum* is yet to be discussed. The reports were on the rhizome oils of French Polynesia *H. coronarium* [12], *H. acuminatum* [13], *H. gardinarium* [14], *H. cylindricum* [15], *H. larsenii* [16], *H. venustum* [5], *H. spicatum* var. *acuminatum* [5], Indian *H. coronarium* [5], Indian *H. flavescens* [5], Brazilian *H. coronarium* [17], Thai *H. coronarium*, Thai *H. flavescens*, Thai *H. stenopetalum*, *H. neocarneum*, *H. speciosum* [18], Vietnamese *H. coronarium*, Vietnamese *H. stenopetalum*, *H. flavum* and *H. ellipticum* [19].

Materials and Methods

Plant Materials

Hedychiym malayanum rhizomes were collected in December 2013 from Fraser Hills, Pahang, Malaysia and kept freshly in the freezer at –20ºC. A voucher specimen of WAY 538 has been deposited at the Universiti Kebangsaan Malaysia Herbarium.

Extraction of Essential Oil

Fresh rhizomes were subjected to hydro distillation for 3 hours utilizing a Clevenger-type apparatus. The obtained essential oil was dried over anhydrous sodium sulfate, transferred into a sealed vial covered with aluminum foil, and kept in a freezer at –20ºC until analyzed and tested for its antioxidant activities. The oil was dissolved in *n*-hexane prior to analysis using gas chromatography (GC) and gas chromatography mass spectrometry (GC-MS).

Gas Chromatography

GC analyses were performed by using a DB5890 equipped with a flame ionization detector (FID). A fused silica capillary column of DB-5 (30 m × 0.32 mm id, film thickness 1.50 µm) was used. The operating conditions of this column were as follows: initial oven temperature, 50ºC for 3 min, then raised at 3ºC/min to 200ºC, and then held for 5 min; injector and detector temperatures, 250ºC; carrier gas, 1.0 mL/min He; injection volume, 0.2 µL. Peak areas were determined with a DB-chem-software.

Gas Chromatography-Mass Spectrometry

GC-MS analyses were carried out using an Agilent 7890A GC equipped with the mass spectrometer (Agilent 5975C inert MSD, with Triple-Axis Detector). A fused silica capillary column was used: DB-5 (30 m × 0.25 mm id, film thickness 0.25 µm). The operating conditions of this column were as follows: initial oven temperature, 50ºC for 3 min, then to 200ºC at 3ºC/min then held for 5 min; injector and detector temperatures, 250ºC; carrier gas, 1.2 mL/min He; split ratio, 10:1; injection volume, 1.0 µL. Operating parameters: ionization voltage, 70 eV; ion source temperature, 200ºC; scan mass range, 40-350 amu. Peak areas were determined with MSD ChemStation from Agilent Technologies. A library search was carried out for all peaks using the NIST Mass Spectral Library.

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Essential Oil Components Identification

The essential oil constituents' identification was based on a comparison of their Kovats retention indices relative to C8-C20 *n*-alkanes, with those of the chemical components gathered by Adams [20,21] and Choi [22]. Compounds were further identified using their MS data compared to the NIST mass spectral library.

Antioxidant Activity: DPPH Radical Scavenging Assay (RSA)

Radical scavenging activity (RSA) assay described by Chan et al. [23] was adopted with slight modification. Stock oil solution of 500 µg/mL was prepared by dissolving 2000 µg of the oil in acetone:methanol (3:7) up to 4 mL mark, and stored at 4ºC. Different dilutions of the oil (1, 25, 50, 75 and 100 µg/mL) were prepared by diluting the stock solutions with methanol. DPPH solution was also prepared by dissolving 5900 µg of DPPH in 100 mL methanol. Then, 1 mL from each dilution of oil was added into the test tube containing 2 mL of DPPH. Control was prepared by adding 1 mL of methanol to 2 mL of DPPH solution. Ascorbic acid and gallic acid were used as standards and prepared at concentrations similar to the oil solutions [2000 µg of each standard was dissolved in acetone:methanol (3:7) to 4 mL mark]. Each of the mixtures (DPPH-oil/DPPH-standard) was shaken vigorously and left to stand in the dark for 30 min. The absorbance of the resulting solution was measured at 517 nm using the Varian Cary 100 Conc UV-VIS spectrophotometer. RSA (%) of the oil on DPPH was calculated using the following equation 1:

$$\text{RSA (\%)} = [(Ac - As) / Ac] \times 100 \quad (1)$$

where, Ac is the absorbance of the control (DPPH solution without sample), and As is the absorbance of the sample (oil/standard with DPPH solution). All samples and readings were prepared and measured in triplicate. The half maximal inhibitory concentration (IC_{50}) for the oil and standards was calculated using the equation of "y = ax + b" which was obtained from a plotting curve for each sample based on RSA (%) at different concentrations, where, y is equal to 50 and x is the IC_{50} and b is different for each sample.

β-Carotene Bleaching (BCB) Test

The antioxidant activity of oil was evaluated by the spectrophotometric β-carotene bleaching test proposed by Sacchetti et al. [7], with a modification. A stock solution of β-carotene-linoleic acid mixture was prepared as follows: 10 mg of β-carotene was dissolved in 10 mL of chloroform; this β-carotene-chloroform solution (0.2 mL) was pipetted into a boiling flask containing 20 mg of linoleic acid and 200 mg of Tween 40. Chloroform was completely evaporated by using a rotary evaporator at 40ºC for 5 min. To the residue, 50 mL of distilled water was slowly added with vigorous shaking to form an emulsion. This emulsion (5 mL) was added to a tube containing 0.2 mL of the oil solution prepared by diluting 4 mg of the oil in ethanol to 2 mL and the absorbance was immediately measured at 470 nm (t = 0) against a blank consisting of an emulsion without β-carotene. The tubes were placed in a water bath at 50ºC and the oxidation of the emulsion was monitored spectrophotometrically by measuring absorbance at 470 nm at every 30 min for 120 min. Control contained 0.2 mL of distilled water instead of the oil. Butylated hydroxytoluene (BHT), a stable antioxidant, was used as a standard reference. Its solution was prepared accordingly like the above oil solution whereby the oil was replaced with BHT (4 mg of this was dissolved in ethanol to 2 mL). For each solution, three replicates were recorded. The rate of β-carotene bleaching was calculated using the following equation 2:

$$R = [\text{In } (a / b)] / 120 \quad (2)$$

where, a = absorbance at 470 nm at t = 0, and b = absorbance at 470 nm at t = 120 min.

The antioxidant activity of BHT and the oil was calculated according the following formula equation 3:

$$AA\% = [(R_{control} - R_{sample/BHT}) / R_{control}] \times 100 \quad (3)$$

where, R_{sample} and $R_{control}$ represent the bleaching rates of β -carotene with or without addition of samples, respectively.

The oxidation rate ratio (ORR) is based on the following equation 4:

$$ORR = R_{sample} / R_{control} \quad (4)$$

Ferrous Ion Chelating Assay (FIC)

The ferrous ion-chelating potential of the oil was investigated according to the method of Chan et al. [23] and Singh et al. [24] with modification. This Fe^{2+} ability was monitored by measuring the Fe^{2+}-ferrozine complex spectrophotometrically at 562 nm. Stock oil solution of 5000 µg/mL were prepared by dissolving 20000 µg of the oil in acetone:methanol (3:7) to 4 mL mark, and stored at 4ºC. Working extract solutions (250, 500, 750, 1000, and 1250 µg/mL) were prepared by diluting the stock solution with methanol. Ferrous sulfate (2 mM) and 5 mM of ferrozine were diluted for 20 times by taking 50 µL of each solution and added with distilled water to 1 mL. The diluted ferrous sulfate (1 mL) was mixed with 1 mL of oil from each dilution, followed by 1 mL of the diluted ferrozine. Ethylenediaminetetraacetic acid (EDTA) and ascorbic acid were used as synthetic chelating references. Control was prepared by adding I mL of methanol to the solution of $FeSO_4$ (1 mL) and ferrozine (1 mL). The mixture was shaken well and incubated for 10 min at room temperature. For EDTA or ascorbic acid or oil, three replicates were recorded. Absorbance was measured at 562 nm using the Varian (Cary 100 conc) UV-VIS spectrophotometer. The ability of samples (oil and standards) to chelate ferrous ions was calculated using the following equation:

$$\text{Chelating ability } \% = [1 - (A_{sample} / A_{control})] \times 100 \quad (5)$$

where, A_{sample} and $A_{control}$ are absorbance of the oil/standard and negative control, respectively. The capacity of oil and standards to chelate Fe^{2+} was also calculated from a calibration curve of the chelating ability (%) versus the concentrations of oil or standard solutions and the data are expressed as IC_{50}.

Results and Discussion

Chemical Composition

Hydrodistillation of the rhizomes of *Hedychium malayanum* produced colorless viscous oil in 0.079% yield (w/w). GC-GC/MS analysis (Table 1) resulted in the identification of 19 compounds accounting for 99.5% of the oil in which eighteen were monoterpenes (99.0%) and one of β-caryophyllene (0.5%) as a hydrocarbon sesquiterpene. The monoterpenes comprised of twelve hydrocarbons (54.4%) of tricyclene, α-pinene, camphene, sabinene, β-pinene, β-myrcene, α-phellandrene, 3-carene, α-terpinene, γ-terpinene, *m*-cymenene and α-terpinolen, and six oxygenated compounds (44.6%) of 1,4-cineole, 1,8-cineole, isoborneol, 4-terpineol, α-terpineol and bornyl acetate.

1,8-Cineole, β-pinene and α-pinene were the major components, amounting to 37.7%, 35.2% and 10.9%, respectively. The contribution of the three main compounds to the total constituent percentages of the oil is 83.8%. The results of the present study regarding the three major constituents are in agreement with previous studies reported by Dan et al. [25], Joshi et al. [26], Suksathan et al. [18] and Uribe et al. [27] in the rhizome oils of *Hedychium* species. The previous study on the leaf and stem oils of *Hedychium malayanum* [11] found that they were not rich in 1,8-cineole (4.5%, 17.7%) as compared to the current study on the rhizome (37.7%). β-Pinene (39.1, 46.7%) and α-pinene (22.3, 16.9%) were also found as major components of the respective leaf and stem oils. Their percentages are more than that in the present study (35.2 and 10.9%).

Table 1. Percentages of the chemical constituents of the rhizome oil obtained from *Hedychium malayanum*

Compound	KI	KI*	Rhizome	Identification methods
Monoterpene hydrocarbons				
tricyclene	923	921[a]	0.5	K, MS
α-pinene	932	932[a]	10.9	K, MS
camphene	946	946[a]	1.2	K, MS
sabinene	970	969[a]	1.0	K, MS
β-pinene	977	979[a]	35.2	K, MS
β-myrcene	984	988[a]	1.2	K, MS
α-phellandrene	1000	1002[a]	0.9	K, MS
3-carene	1007	1008[a]	0.6	K, MS
α-terpinene	1020	1017[a]	0.8	K, MS
γ-terpinene	1054	1054[a]	1.2	K, MS
m-cymenene	1082	1082[a]	0.4	K
α-terpinolen	1092	1089[a]	0.5	K, MS
Sub-total (%)			54.4	
Oxygenated monoterpenes				
1,4-cineole	1013	1012[a]	0.6	K, MS
1,8-cineole	1031	1031[a]	37.7	K, MS
isoborneol	1165	1162[a]	0.9	K, MS
4-terpineol	1175	1174[a]	1.8	K, MS
α-terpineol	1188	1189[a]	3.3	K, MS
bornyl acetate	1283	1284[a]	0.3	K, MS
Sub-total (%)			44.6	
Sesquiterpene hydrocarbons				
β-caryophyllene	1427	1428[b]	0.5	K, MS
Sub-total (%)			0.5	
Total (%)			99.5	

KI = Kovats index on DB-5 capillary column, KI* = Kovats index in literature, K = Comparison of retention data with published data, MS = Identification based on mass spectral data, a = [20,21] and b = [22].

In term of the main component of 1,8-cineole in our rhizome oil (37.7%) and other *Hedychium* rhizome oils, it was also found as an important component in the French Polynesia *H. coronarium* (40.2%), *H. acumnatum* (76.0%), *H. gardinarium* (48.7%), *H. larsenii* (14.4%), *H. venustum* (45.4%), *H. spicatum* var. *acuminatum* (44.3%), Indian *H. coronarium* (48.7%), Brazilian *H. coronarium* (34.8%), Thai *H. coronarium* (33.8%), Thai *H. flavescens* (17.9%), *H. neocarneum* (14.2%), *H. speciosum* (32.0%) and *H. flavum* (13.5%). It should be noted here that the *H. malayanum* rhizome oil (37.7%) ranks the sixth among the above *Hedychium* rhizome oils having high percentages of 1,8-cineole. The second major compound of β-pinene in the *H. malayanum* rhizome oil was found in high amount (35.2%) compared to those from French Polynesia *H. coronarium* (24.8%), *H. cylindricum* (5.6%), Brazilian *H. coronarium* (16.7%), Thai *H. coronarium* (13.6%), Thai *H. flavescens* (23.1%), *H. neocarneum* (11.2%), *H. speciosum* (23.4%), Vietnamese *H. coronarium* (23.6%), Vietnamese *H. stenopetalum* (12.3%), *H. flavum* (21.8%) and *H. ellipticum* (11.0), but in lower percentage (35.2%) as compared to those from *H. gardinarium* (43.6%) and Indian *H. flavescens* (43.6%). α-Pinene (10.9%) was found in low percentage in the *H. malayanum* rhizome oil among the three major components that also includes 1,8-cineole (37.7%) and β-pinene

(35.2%). This is also true for the other *Hedychium* rhizome oils of Polynesia *H. coronarium*, Thai *H. coronarium*, Thai *H. flavescens*, Thai *H. stenopetalum*, *H. neocarneum*, *H. speciosum*, Vietnamese *H. coronarium*, Vietnamese *H. stenopetalum* and *H. flavum* that contained α-pinene in the range of 4.3 to 10.5% which were lower than their percentages of 1,8-cineole and β-pinene.

Antioxidant Activity: Radical scavenging Activity

The *Hedychium malayanum* rhizome oil and the two standard references of ascorbic acid and gallic acid exhibited RSA results (Table 2) that are concentration-dependent. According to the IC_{50}, gallic aid and ascorbic acid gave higher RSA (1.8±0.06 and 3.5±0.30 µg/mL, respectively) than the oil (140.3±1.46 µg/mL). The antioxidant activity of *H. malayanum* rhizome oil might be due to the presence of 4-terpineol (1.8%), γ-terpinene (1.2%) and α-terpinolen (0.5%) in this oil [28]. These compounds show activities comparable to α-tocopherol and phenols that were reported to reduce the DPPH radicals [29].

Although γ-terpinene, α-terpinolen and 4-terpineol were not the main compounds of essential oil of *Hedychium malayanum* but the presence of these compounds may contribute to the free radicals scavenging activity. This is because the minor compounds play a critical role in biological activities to produce a synergistic effect between the compounds [30]. 1,8-Cineole, β-pinene and α-pinene were identified as the major compounds in the *Hedychium malayanum* rhizome oil, but it seems that they did not contribute in DPPH radical scavenging activity [31]. Many studies previously done on the individual component from essential oils revealed that these three compounds possess low antioxidant activity.

Table 2. Antioxidant activities of *Hedychium malayanum* rhizome oil and the standards used for the antioxidant tests

sample	**DPPH**	**β-Carotene bleaching**		**Ferrous ion chelating**
	IC_{50} (µg/mL)	AA (%)	ORR	IC_{50} (µg/mL)
Rhizome oil	140.3±1.46	87.2±1.00	0.13±0.01	283.3±5.26
Gallic acid	1.8±0.06	-	-	-
Ascorbic acid	3.5±0.30	-	-	1428.6 ±7.04
EDTA	-	-	-	86.9±0.70
BHT	-	96.8±0.21	0.00 ±0.00	-

Value of each data was mean ± SD (n = 3).

β-Carotene Bleaching (BCB)

The greater the efficiency of an antioxidant, the slower will be the discoloration of β-carotene. A smaller decrease in the β-carotene absorbance, as a result of lower rate of oxidation of linoleic acid, indicated better antioxidant activity of the oil. This information is used in the antioxidant activity evaluation of the *Hedychium malayanum* rhizome oil in comparison with the conventional synthetic antioxidant of BHT. Fig. 1 shows the averages of absorbance values of the *H. malayanum* rhizome oil and the synthetic antioxidant BHT against the time intervals (0 -120 min).

Smallest decrease in the β-carotene absorbances with BHT and the rhizome oil came between the time intervals of 90 and 120 min. Therefore, the antioxidant activity percentages (AAs) and oxidation rate ratios (ORRs) (Table 2) were calculated at t = 120 min. The smaller reduction in the β-carotene absorbance with BHT indicated that the BHT had the higher antioxidant activity (96.8% inhibition and 0.00 ORR) than that of the rhizome oil (87.2% and 0.13). From our previous publication in 2015, we have established the BCB activity of the leaf and stem oils from the same plant. The rhizome oil showed better BCB activity (87.2%) compared to the stem oil (79.8%) and leaf oil (54.7%). Obviously, the ability of essential oils to hinder of β-carotene bleaching depends on their chemical

composition which is able to neutralize linoleate-free radical. Terpenic components and their synergistic effects are presumably responsible to the BCB activity of the essential oil as previously reported by Sacchetti et al. [7].

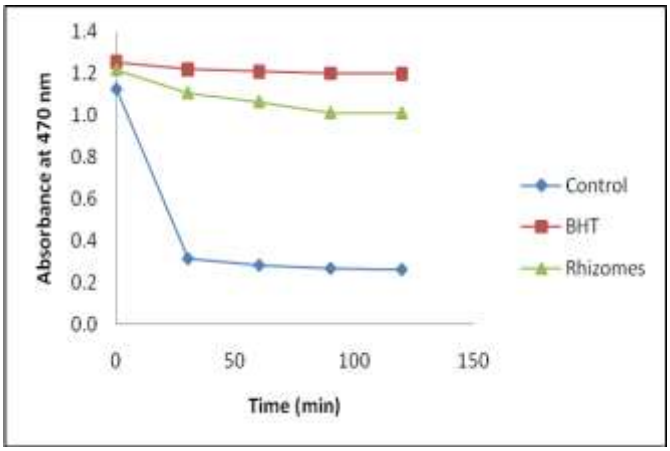

Figure 1. The averages of absorbance values of the *Hedychium malayanum* rhizome oil and the synthetic antioxidant BHT against the time intervals (0 - 120 min).

Ferrous Ion Chelating

The acetone:methanol (3:7) solutions of the *Hedychium malayanum* rhizome essential oil, EDTA and ascorbic acid were tested at different concentrations in the presence of ferrozine and $FeSO_4$. All these solutions gave uneven FIC activities toward ferrozine (Table 2), as monitored by the change in the absorbance of the ferrozine-complex. The oil showed a good chelating power (283.3 ± 5.26 µg/mL) after that of EDTA (86.9 ± 0.70 µg/mL), whereas the ascorbic acid had possessed the worst one (1428.6 ±7.04 µg/mL).

The obtained data showed that the *Hedychium malayanum* rhizome essential oil can be considered as good in FIC activity, and thus function as Fe^{2+} chelating agent. This oil provides a pathway to avoid free-radical generation and iron-overload by either directly binding the metal ions or indirectly suppressing their chelating reactivity by occupying their coordination sites. As a result, it can be deduced that the *H. malayanum* rhizome oil is an active chelator, and could afford protection against oxidative damage and iron-overload.

Conclusion

The good antioxidant activity shown by the rhizome oil of *Hedychium malayanum* has been related to the presence of monoterpenes with high abundance in the oil. Hence *H. malayanum* is a potential source of natural antioxidants which can be further utilized in cosmetic and pharmaceutical industries, and as well as natural food preservatives.

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References

1. Turner, I. M. (1995). A catalogue of the Vascular Plants of Malaya. *Gardens' Bulletin Singapore*, 47(2): 642-655.

2. Wongsuwan, P. and Picheansoonthon, C. (2011). Taxonomic Revision of the Genus *Hedychium* J. König (Zingibeaceae) in Thailand (Part 1). *The Journal of the Royal Institute of Thailand*, 3: 126-149.
3. Bhaigyabati, T. H., Grihanjali Devi, P. and Bag, G. C. (2014). Total Flavonoid Content and Antioxidant Activity of Aqueous Rhizome Extract of Three *Hedychium* Species of Manipur Valley. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 5(5): 970-976.
4. Picheansoonthon, C. and Wongsuwan, P. (2011). A new Species and A new Record of *Hedychium* J. König (Zingiberaceae) from Thailand. *Taiwana* , 56(3): 231-239.
5. Sabulal, B., George, V., Dan, M. and Pradeep. N. S. (2007). Chemical Composition and Antimicrobial Activities of the Essential Oils from the Rhizomes of Four *Hedychium* Species from South India. *Journal of Essential Oil Research*, 19: 93 - 97.
6. Holttum, R. E. (1950). The Zingiberaceae of the Malay Peninsula. *Gardens' Bulletin Singapore*, 13(1): 1-249.
7. Sacchetti, G., Maietti, S., Muzzoli, M., Scaglianti, M., Manfredini, S., Radice, M. and Bruni, R. (2005). Comparative Evaluation of 11 Essential Oils of Different Origin as Functional Antioxidants, Antiradicals and Antimicrobials in Foods. *Food Chemistry*, 91(4): 621-632.
8. Hussain, A. I., Anwar, F., Hussain Sherazi, S. T. and Przybylski, R. (2008). Chemical Composition, Antioxidant and Antimicrobial Activities of Basil (*Ocimum basilicum*) Essential Oils Depends on Seasonal Variations. *Food Chemistry*, 108(3): 986-995.
9. Mario, R. M. J., Thomaz, A. A. R. S., Gilberto, C. F., Alexander, N., Glaucia, M. P. and Stephen, H. (2009). Antioxidant Potential of Aroma Compounds Obtained by Limonene Biotransformation of Orange Essential Oil. *Food Chemistry*, 116: 8-12.
10. Singh, G., Maurya, S., Lampasona, M. P. and Catalan, C. (2006). Chemical Constituents, Antifungal and Antioxidative Potential of *Foeniculum vulgare* Volatile Oil and its Acetone Extract. *Food Control*, 17: 745-752.
11. Mohammed Yahya Abdo, W. A. Yaacob, Laily B. Din and I. Nazlina (2015). Chemical Composition and Antioxidant Activity of *Hedychium malayanum* Essential Oils. *Malaysian Journal of Analytical Sciences*, 19(1): 194-201.
12. Lechat-Vahirua, I., Francois, P., Menut, C., Lamaty, G. and Bessiere, J. M. (1993). Aromatic Plants of French Polynesia. I. Constituents of the Essential Oils of Rhizomes of Three Zingiberaceae: *Zingiber zerumbet* Smith, *Hedychium coronarium* Koeing and *Etlingera cevuga* Smith. *Journal of Essential Oil Research*, 5(1): 55-59.
13. Weyerstahl, P., Marschall, H., Schneider, S. and Subba, G. C. (1995). Constituents of the Essential Oil from the Rhizomes of *Hedychium acumnatum* Roscoe. *Flavor Fragrance Journal*, 10(3): 179-185.
14. Weyerstahl, P., Marschall, H., Thefeld, K. and Subba, G. C. (1998). Constituents of the Essential oil from the Rhizomes of *Hedychium gardnerianum* Roscoe. *Flavor Fragrance Journal*, 13(6): 377-388.
15. bin Ahmad, F., bin Jantan, I. and Jalil, J. (2004). Constituents of the Rhizome Oil of Hedychium cylindricum Ridl. Journal of Essential Oil Research, 16 (4): 299-301.
16. Gopanraj, Dan, M., Shiburaj, S., Sethuraman, M. G. and George, V. (2005). Chemical Composition and Antibacterial Activity of the Rhizome Oil of *Hedychium larsenii*. *Acta Pharmaceutica*, 55: 315-320.
17. Santos, B. C. B., Barata, L. E. S., Marques, F. A., Baroni, A. C. M., Karnos, B. A. C., Oliveria, P. R. and Guerrero, P. G. (2010). Composition of Leaf and Rhizome Essential Oils of *Hedychium coronarium* Koen. from Brazil. *Journal of Essential Oil Research*, 22(4): 305-306.
18. Suksathan, R., Sookkhee,S., Anuntalabhochai, S. and Chansakaow, S. (2013). Chemical Composition and Antibacterial Activity of Rhizome Oils from Five *Hedychium* species. *Natural Product Communications*, 8(4): 519-522.
19. Thanh, B. V., Dai, D. N., Thang, T. D., Binh, N. Q., Anh, L. D. N. and Ogunwande, I. A. (2014). Composition of Essential Oils of Four *Hedychium* Species from Vietnam. *Chemistry Central Journal*, 8(54): 1-5.
20. Adams, R. P. (2001). *Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy*. 3ed. Carol Stream, IL: Allured Publishing Corp.
21. Adams, R. P. (2007). *Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy*. 4ed. Carol Stream, IL: Allured Publishing Corp.
22. Choi, H. S. (2003). Character Impact Odorants of Citrus Hallabong [(C. unshiu Marcov x C. sinensis Osbeck) x C. reticulata Blanco] Cold-pressed Peel Oil. *Journal Agricultural and Food Chemistry*, 51: 2687-2692.
23. Chan, E. W. C., Lim, Y. Y. & Omar, M. (2007). Antioxidant and Antibacterial Activity of Leaves of *Etlingera* Species (Zingiberaceae) in Peninsular Malaysia. *Food Chemistry*, 104(4): 1586-1593.

24. Singh, G., Kapoor, I. P. S., Singh, P., De Heluani, C. S., De Lampasona M. P. and Catalan, C. A. N. (2010). Comparative Study of Chemical Composition and Antioxidant Activity of Fresh and Dry Rhizomes of Turmeric (*Curcuma longa* Linn.). *Food and Chemical Toxicology*, 48(4): 1026-1031.
25. Dan, M., Sabulal, B., George, V. and Pushpangadan, P. (2007). Studies on the Rhizome Oils from Four *Hedychium* Species of South India: A chemotaxonomic Approach. *Gardens' Bulletin Singapore*, 59: 57-64.
26. Joshi, S., Chanotiya, C. S., Agarwal, G., Prakash, O., Pant, A. K. and Mathela, C.S. (2008). Terpenoid Compositions, and Antioxidant and Antimicrobial Properties of the Rhizome Essential Oils of Different *Hedychium* Species. *Chemistry & Biodiversity*, 5: 299-309.
27. Uribe, S., Ramirez, J. and Pena, A. (1985). Effects of β-pinene on Yeast Membrane Functions. *Journal of Bacteriology*, 161: 1195-1200.
28. Abd El-Baky, H. H. and El-Baroty, G. S. (2008). Chemical and Biological Evaluation of the Essential Oil of Egyptian Moldavian Balm (*Dracocephalum moldavica* L). *International Journal of Integrative Biology*, 3(3): 202-208.
29. Ruberto, G. and Baratta, M. T. (2000). Antioxidant Activity of Selected Essential Oil Components in Two Lipid Model Systems. *Food Chemistry*, 69(2): 167-174.
30. Bounatirou, S., Smiti, S., Miguel, M. G., Faleiro, L., Rejeb, M. N., Neffati, M., Costa M. M., Figueiredo, A. C., Barroso, J. G. and Pedro, L. G. (2007). Chemical Composition, Antioxidant and Antibacterial Activities of the Essential Oils Isolated from Tunisian *Thymus capitatus* Hoff. Et Link. *Food Chemistry*, 105(1): 146-155.
31. Kordali, S., Cakir, A., Mavi, A., Kilic, H. & Yildirim, A. (2005). Screening of Chemical Composition and Antifungal and Antioxidant Activities of the Essential Oils from Three Turkish *Artemisia* species. *Journal of Agricultural and Food Chemistry*, 53(5): 1408-1416.