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

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

Mohammed Yahya Abdo¹, Wan Yaacob Wan Ahmad¹*, Laily Bin Din¹, Nazlina Ibrahim²

¹School of Chemical Sciences and Food Technology
²School of Biosciences and Biotechnology
Faculty of Science and Technology,
Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia

*Corresponding author: wanyaa@ukm.edu.my

Received: 9 July 2015; Accepted: 23 September 2015

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 IC₅₀, 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 (IC₅₀ 86.9 ± 0.70 µg/mL). However, the oil showed a fairly good chelating (IC₅₀ 283.3 ± 5.26 µg/mL) whereas the ascorbic acid possessed a weak activity to chelate Fe²⁺ (IC₅₀ 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-picrilhidrazil (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 ascorbik memperlihatkan kebolehan pengkelatan yang lebih lemah daripada minyak. APR dikira sebagai KP₅₀ dan nilai masing-masing bagi asid galik, asid ascorbik 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 dan 96.8 ± 0.21%, masing-masing) untuk mencegah pengoksidaan oleh asid linoleik. Aktiviti PIF menunjukkan bahawa minyak ini dan piawai asid ascorbik adalah pengkelat yang lebih lemah daripada EDTA (KP₅₀ 86.9 ± 0.70 µg/mL). Namun, minyak tersebut menunjukkan pengkelatan yang agak baik (KP₅₀ 283.3 ± 5.26 µg/mL) manakala asid ascorbik memperlihatkan aktiviti yang lebih bagi mengkelat Fe²⁺ (KP₅₀ 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 its 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
Hedychium malayanum rhizomes were collected in December 2013 from Fraser Hills, Pahang, Malaysia and kept freshly in the freezer at −20°C. 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 −20°C 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, 50°C for 3 min, then raised at 3°C/min to 200°C, and then held for 5 min; injector and detector temperatures, 250°C; 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.

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:

\[
RSA(\%) = \left(\frac{Ac - As}{Ac}\right) \times 100
\]

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:

\[
R = \frac{\ln (a / b)}{120}
\]
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:

\[
AA\% = \left[ \frac{(R_{\text{control}} - R_{\text{sample/BHT}})}{R_{\text{control}}} \right] \times 100
\] (3)

where, \( R_{\text{sample}} \) and \( R_{\text{control}} \) represent the bleaching rates of \( \beta \)-carotene with or without addition of samples, respectively.

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

\[
\text{ORR} = \frac{R_{\text{sample}}}{R_{\text{control}}}
\] (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 \( \text{Fe}^{2+} \) ability was monitored by measuring the \( \text{Fe}^{2+}-\text{ferrozine} \) complex spectrophotometrically at 562 nm. Stock oil solution of 5000 \( \mu \text{g/mL} \) were prepared by dissolving 20000 \( \mu \text{g} \) 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 \( \mu \text{g/mL} \)) 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 \( \mu \text{L} \) 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 1 mL of methanol to the solution of \( \text{FeSO}_4 \) (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:

\[
\text{Chelating ability \%} = [1 - \left( \frac{A_{\text{sample}}}{A_{\text{control}}} \right)] \times 100
\] (5)

where, \( A_{\text{sample}} \) and \( A_{\text{control}} \) are absorbance of the oil/standard and negative control, respectively. The capacity of oil and standards to chelate \( \text{Fe}^{2+} \) 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 \( \beta \)-caryophyllene (0.5\%) as a hydrocarbon sesquiterpene. The monoterpenes comprised of twelve hydrocarbons (54.4\%) of tricyclene, \( \alpha \)-pinene, camphene, sabinene, \( \beta \)-pinene, \( \beta \)-myrcene, \( \alpha \)-phellandrene, 3-carene, \( \alpha \)-terpinene, \( \gamma \)-terpinene, \( m \)-cymenene and \( \alpha \)-terpinolen, and six oxygenated compounds (44.6\%) of 1,4-cineole, 1,8-cineole, isoborneol, 4-terpineol, \( \alpha \)-terpineol and bornyl acetate.

1,8-Cineole, \( \beta \)-pinene and \( \alpha \)-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], Sukasathan 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\%). \( \beta \)-Pinene (39.1, 46.7\%) and \( \alpha \)-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*

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

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.
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 acid 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

<table>
<thead>
<tr>
<th>sample</th>
<th>DPPH IC$_{50}$ (µg/mL)</th>
<th>β-Carotene bleaching AA (%)</th>
<th>Ferrous ion chelating ORR</th>
<th>Ferrous ion chelating IC$_{50}$ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhizome oil</td>
<td>140.3±1.46</td>
<td>87.2±1.00</td>
<td>0.13±0.01</td>
<td>283.3±5.26</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>1.8±0.06</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>3.5±0.30</td>
<td>-</td>
<td>-</td>
<td>1428.6 ±7.04</td>
</tr>
<tr>
<td>EDTA</td>
<td>-</td>
<td>-</td>
<td>0.00 ±0.00</td>
<td>86.9±0.70</td>
</tr>
<tr>
<td>BHT</td>
<td>-</td>
<td>96.8±0.21</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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).](image)

**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 FeSO₄. 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 Fe²⁺ 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.

**Acknowledgement**
Authors wish to extend our gratitude to the School of Chemical Sciences and Food Technology, Universiti Kebangsaan Malaysia for the provision of laboratory facilities and technical assistance. Authors also wish to thank the Ministry of Higher Education Malaysia and the Universiti Kebangsaan Malaysia for financial support through the Fundamental Research Grant Scheme of UKM-ST-06-FRGS0110-2009, Publication Intensification Fund of DLP-2013-018 and FRGS/1/2014/SG01/UKM/02/2.

**References**


