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RESEARCH ARTICLE

Essential oil composition and antioxidant activity of *Reinwardtiodendron cinereum* (Hiern) Mabb. (Meliaceae)

Wan Mohd Nuzul Hakimi Wan Salleh[1,*], Shamsul Khamis[2], Muhammad Helmi Nadri[3], Hakimi Kassim[4] and Alene Tawang[4]

[1]Department of Chemistry, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, 35900 Tanjong Malim, Perak, MALAYSIA
[2]School of Environmental and Natural Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, MALAYSIA
[3]Innovation Centre in Agritechnology (ICA), Universiti Teknologi Malaysia, 84600 Pagoh, Muar, Johor, MALAYSIA
[4]Department of Biology, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, 35900 Tanjong Malim, Perak, MALAYSIA

*Corresponding author. Email: wmnhakimi@fsmt.upsi.edu.my

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Abstract
This study was designed to investigate the chemical composition and antioxidant activity of the essential oil from *Reinwardtiodendron cinereum* (Hiern) Mabb. growing in Malaysia. The essential oil was obtained by hydrodistillation and fully analysed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The analysis led to the identification of thirty-five components representing 83.6% of the total oil. The most abundant components were α-zingiberene (15.4%), pimaradiene (10.1%), (*E*)-caryophyllene (7.5%), and β-elemene (6.3%). The antioxidant activity was determined by DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging, total phenolic content, and β-carotene linoleic acid bleaching assays. The essential oil showed weak activity in DPPH radical scavenging (IC_{50} value of 105.2 µg/mL), phenolic content (45.5 ± 0.2 mg GA/g), and β-carotene linoleic acid bleaching (52.5 ± 0.2%) assays. To the best of our knowledge, this is the first study of the chemical composition and antioxidant activities of the essential oil report concerning the genus *Reinwardtiodendron*.

Keywords: Antioxidant, essential oil, Meliaceae, *Reinwardtiodendron cinereum*, α-Zingiberene

Introduction

Essential oils are chemically characterized as complex mixtures of low molecular weight compounds and some of them are highly volatile and capable of generating flavors (Trombetta et al., 2005). Scientific studies have shown the role of essential oils in biological interactions among plants and they are potential therapeutic including anti-inflammatory, analgesic, anti-tumor, antifungal and antibacterial activities (Osei-Safo et al., 2010; Salleh et al., 2014a, 2016a).

Meliaceae, also called Mahogany family is a flowering plant family of mostly trees and shrubs in the order Sapindales. It is composed of 50 genera and 1400 species. In Southeast Asia, species of Meliaceae are widely found from lowlands to higher elevation highlands, and are one of important components in tropical and subtropical evergreen forests (Perez-Flores et al., 2012). A great variety of plants belonging to the Meliaceae family have been phytochemically investigated and several compounds have been isolated and identified. They were limonoids, triterpenes, steroids, diterpenes, sesquiterpenes, and coumarins (Soares et al., 2012; Scur et al., 2016). *Reinwardtiodendron* is one of the genus of Meliaceae, distributed mainly in Peninsular Malaysia, Borneo, and Sumatra. It is composed of only seven species, which are *R. anamalaiense*, *R. celebicum*, *R. cinereum*, *R. humile*, *R. kinabaluense*, *R. kostermansii*, and *R. merrillii* (Mabberley, 2011).

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Reinwardtiodendron cinereum (Hiern) Mabb. is a tree of up to 27 m height. The leaf is 10-15 cm long, while the bark is smooth with scaly patches and conspicuous knobbly tubercles. The colour for the inner bark is white, while the fruits are globose or glabrous with a yellow colour (Mabberley, 2011). There has been no information of this plant in traditional or folk medicine practice. Most of the species of genus are unexplored, both pharmacologically and phytochemically. Previous phytochemical investigation of *R. cinereum* has resulted in the isolation of onocerane triterpenoids (Nugroho et al., 2018). Besides, the bark extract of *R. cinereum* was found to be cytotoxic against human promyelocytic leukaemia HL-60 cells (Nugroho et al., 2018). Meanwhile, the literature search did not reveal any report on the essential oil composition of the genus *Reinwardtiodendron*. As a continuation part of our systematic evaluation of the aromatic flora of Malaysia (Salleh et al., 2014b, 2015a, 2015b, 2015c), this study reports the chemical composition and antioxidant activities of the essential oil from the leaves of *R. cinereum*.

Materials and Methods

Plant material

Sample of *Reinwardtiodendron cinereum* was collected from Behrang, Perak in January 2019, and identified by Dr. Shamsul Khamis from University Kebangsaan Malaysia (UKM). The voucher specimen (SK165/19) was deposited at UKMB Herbarium University Kebangsaan Malaysia.

Solvents and chemicals

β-Carotene, linoleic acid, DPPH$^{\bullet}$, gallic acid and butylated hydroxytoluene (BHT) were obtained from Sigma-Aldrich Chemie (Steinheim, Germany). Analytical grade methanol, ethanol, and dimethylsulfoxide (DMSO), HPLC grade chloroform, Folin-Ciocalteu's reagent, anhydrous sodium sulfate, sodium carbonate, polyoxyethylene sorbitan monopalmitate (Tween-40) were purchased from Merck (Darmstadt, Germany).

Extraction of the essential oil

The fresh leaf (500 g) was subjected to hydrodistillation in Clevenger-type apparatus for 4 hours. The essential oil obtained and was dried over anhydrous magnesium sulfate then stored at 4-6 °C. The yield (v/w) of the obtained essential oil was 1.8%, expressed as a percentage of absolute dry weight.

Gas chromatography (GC) analysis

GC analysis were performed on an Agilent Technologies 7890B and an Agilent 7890B FID equipped with DB-5 column. Helium was used as a carrier gas at a flow rate of 0.7 mL/min. Injector and detector temperature were set at 250 and 280 °C, respectively. The oven temperature was kept at 50 °C, then gradually raised to 280 °C at 5 °C/min and finally held isothermally for 15 min. Diluted samples (1/100 in diethyl ether, v/v) of 1.0 µL were injected manually (split ratio 50:1). The injection was repeated three times and the peak area percents were reported as means ± SD of triplicates. Calculation of peak area percentage was carried out by using the GC HP Chemstation software (Agilent Technologies).

Gas chromatography-mass spectrometry analysis

GC-MS chromatograms were recorded using Agilent Technologies 7890A and Agilent 5975 GC MSD equipped. The GC was equipped with HP-5MS column (30 m × 0.25 mm × 0.25 µm film thickness). Helium was used as carrier gas at a flow rate of 1 mL/min. Injector temperature was 250 °C. The oven temperature was programmed from 50 °C (5 min hold) to 250 °C at 10 °C/min and finally held isothermally for 15 min. For GC-MS detection, an electron ionization system, with ionization energy of 70 eV was used. A scan rate of 0.5 s (cycle time: 0.2 s) was applied, covering mass range was from 40–400 amu.

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Identification of chemical components

For the identification of essential oil components, co-injection with the above standards were used, together with correspondence of retention indices and mass spectra with respect to those occurring in ADAMS, NIST 08 and FFNSC2 libraries (Adams, 2007). Semi-quantification of essential oil components was made by peak area normalization considering the same response factor for all volatile components. Percentages values were the mean of three chromatographic analyses.

Antioxidant activity

DPPH radical scavenging

The free radical scavenging activity was measured by the DPPH method with minor modifications (Salleh et al., 2014c). Each sample of stock solution (1.0 mg/mL) was diluted to a final concentration of 1000−7.8 µg/mL. Then, a total of 3.8 mL of 50 µM DPPH• methanolic solution (1 mg/50 mL) was added to 0.2 mL of each sample solution and allowed to react at room temperature for 30 min. The absorbance of the mixtures was measured at 517 nm. A control was prepared without a sample or standard and measured immediately at 0 min. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity and vice versa. The percent inhibitions (I %) of DPPH radical were calculated as follow: I % = [$A_{blank} - A_{sample} / A_{blank}$] ×100; where A_{blank} is the absorbance value of the control reaction and A_{sample} is the absorbance values of the test samples. The sample concentration providing 50% inhibition (IC_{50}) was calculated by plotting inhibition percentages against concentrations of the sample. All tests were carried out in triplicate and IC_{50} values were reported as means ± SD of triplicate.

Total phenolic content (TPC)

Total phenolic contents of the essential oil were determined as described previously (Salleh et al., 2014c). A sample of stock solution (1.0 mg/mL) was diluted in methanol to final concentrations of 1000 µg/mL. A 0.1 mL aliquot of sample was pipetted into a test tube containing 0.9 mL of methanol, then 0.05 mL Folin-Ciocalteu's reagent was added, and the flask was thoroughly shaken. After 3 min, 0.5 mL of 5 % Na_2CO_3 solution was added and the mixture was allowed to stand for 2 h with intermittent shaking. Then, 2.5 mL of methanol was added and left to stand in the dark for 1 h. The absorbance measurements were recorded at 765 nm. The same procedure was repeated for the standard gallic acid solutions. The concentration of total phenolic compounds in the oil was expressed as mg of gallic acid equivalent per gram of sample. Tests were carried out in triplicate and the gallic acid equivalent value was reported as mean ± SD of triplicate.

β-Carotene-linoleic acid bleaching assay

The β-carotene-linoleic acid bleaching assay was determined as described previously with minor modifications (Salleh et al., 2016b). A mixture of β-carotene and linoleic acid was prepared by adding together of 0.5 mg β-carotene in 1 mL chloroform 25 µL linoleic acid and 200 mg Tween 40. The chloroform was then completely evaporated and 100 mL of oxygenated distilled water was subsequently added to the residue and mixed gently to form a clear yellowish emulsion. The essential oil (2 g/L) was in methanol and 350 µL of each sample solution was added to 2.5 mL of the above mixture in test tubes and mixed thoroughly. The test tubes were incubated in a water bath at 50 °C for 2 h together with two blanks, one contained BHT (positive control) and the other contained the same volume of methanol. The absorbance was measured at 470 nm on an ultraviolet-visible (UV–Vis) spectrometer. Antioxidant activities (inhibitions percentage, I %) of the samples were calculated using the following equation: I % = [$A_{\beta\text{-carotene after 2 h}} / A_{\text{initial }\beta\text{-carotene}}$] × 100; where $A_{\beta\text{-carotene after 2 h}}$ assay is the absorbance value of β-carotene after 2 h assay remaining in the samples and $A_{initial}$

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$_{\beta\text{-carotene}}$ is the absorbance value of β-carotene at the beginning of the experiment. All tests were carried out in triplicate and inhibition percentages were reported as means ± SD of triplicate.

Statistical analysis

Data obtained from essential oil analysis and antioxidant were expressed as mean values. The statistical analyses were carried out by employing one way ANOVA ($p<0.05$). A statistical package (SPSS version 11.0) was used for the data analysis.

Results and Discussion

The essential oil was obtained by hydrodistillation as a pale yellow colour from the fresh leaves of *R. cinereum*. The chemical compositions of the essential oil were analysed by GC, GC-MS and Kovats Indices (KI). The identified components are listed in Table 1 according to their elution on a DB-5 column in parallel with their KI and their relative's percentage.

Table 1. Chemical composition of *Reinwardtiodendron cinereum* essential oil

No.	Components	KI[a]	KI[b]	Percentage (%)[c]	Identification methods
1	α-Patchoulene	1455	1457	0.2	RI, MS
2	α-Cubebene	1458	1460	0.3	RI, MS
3	4,5-di-*epi*-Aristolochene	1468	1467	0.3	RI, MS
4	δ-Elemene	1470	1468	0.5	RI, MS
5	α-Copaene	1490	1491	1.7	RI, MS
6	α-Gurjunene	1530	1529	0.2	RI, MS
7	*trans*-Cadina-1,4-diene	1535	1532	0.5	RI, MS
8	α-*cis*-Bergamotene	1562	1559	0.2	RI, MS
9	β-Copaene	1580	1579	0.3	RI, MS
10	α-Cedrene	1585	1582	0.5	RI, MS
11	(*Z*)-Caryophyllene	1589	1588	4.6	RI, MS
12	β-Elemene	1590	1590	6.3	RI, MS, Std
13	(*E*)-Caryophyllene	1595	1598	7.5	RI, MS, Std
14	Aromadendrene	1625	1620	0.5	RI, MS
15	Pogostol	1650	1651	0.5	RI, MS
16	α-Humulene	1665	1666	2.1	RI, MS
17	γ-Muurolene	1690	1689	1.8	RI, MS
18	Germacrene D	1705	1708	4.0	RI, MS
19	β-Selinene	1715	1716	2.7	RI, MS
20	α-Zingiberene	1720	1720	15.4	RI, MS, Std
21	α-Muurolene	1725	1723	1.0	RI, MS
22	(*E*, *E*)-α-Farnesene	1745	1743	2.4	RI, MS
23	Xanthorrhizol	1751	1751	1.3	RI, MS
24	Geranyl acetate	1752	1752	0.7	RI, MS
25	δ-Cadinene	1756	1755	5.9	RI, MS, Std
26	γ-Cadinene	1765	1763	2.4	RI, MS
27	(*E*)-α-Bisabolene	1775	1775	0.4	RI, MS
28	Germacrene B	1825	1823	0.5	RI, MS

29	Pimaradiene	1910	1909	10.1	RI, MS
30	Elemol	2075	2078	0.3	RI, MS
31	Globulol	2080	2082	2.7	RI, MS
32	1-*epi*-Cubenol	2086	2088	1.9	RI, MS
33	Spathulenol	2020	2126	0.8	RI, MS
34	α-Cadinol	2222	2227	3.0	RI, MS
35	Manool oxide	2075	2376	0.3	RI, MS
	Sesquiterpene hydrocarbon			**72.6**	
	Oxygenated sesquiterpenes			**11.0**	
	Total (%)			**83.6**	

[a]Linear retention index experimentally determined using homologous series of C6-C30 alkanes. [b]Linear retention index taken from Adams (2007) or NIST 08 (2008) and literature. [c]Relative percentage values are means of three determinations ± SD. Identification methods: Std, based on comparison with authentic compounds; MS, based on comparison with Wıley, Adams, FFNSC2 and NIST 08 MS databases; RI, based on comparison of calculated RI with those reported in Adams, FFNSC 2 and NIST 08.

Analysis of the essential oil had successfully characterized thirty-five components, accounting for 83.57 % of the total composition. They were grouped into sesquiterpene hydrocarbons and oxygenated sesquiterpenes. Sesquiterpene hydrocarbons were the most dominant components which constituted 27 components, accounting for 72.6% of the total composition. Meanwhile, oxygenated sesquiterpenes were present in appreciable amounts which comprised 8 components, accounting for 11 % of the total composition. The most abundant components in the essential oil were α-zingiberene (15.4%), pimaradiene (10.1%), (*E*)-caryophyllene (7.5%), β-element (6.3 %), δ-cadinene (5.9%), (*Z*)-caryophyllene (4.6%), germacrene D (4.0%), and α-cadinol (3.4%). In addition, the other minor components detected in the essential oil in more than 2 % were β-selinene (2.7%), globulol (2.7%), (*E*,*E*)-α-farnesene (2.4%), γ-cadinene (2.4%), and α-humulene (2.1%). The essential oil was screened for their possible antioxidant activity by DPPH radical scavenging, total phenolic contents, and β-carotene/linoleic acid bleaching assays. The results are shown in Table 2. The essential oil exhibited weak DPPH radical scavenging activity (IC_{50} of 105.2 µg/mL) compared to standard antioxidant, BHT (IC_{50} of 18.5 µg/mL). The low activity of the essential oil was attributed to the low phenolic content of the essential oil (25.5 ± 0.2 mg GA/g) which is responsible for antioxidant activity (Salleh et al., 2016a). This was supported by the results of the Folin-Ciocalteu assay on the essential oil, which showed a low amount of oxygenated sesquiterpenes. In addition, β-carotene/linoleic acid bleaching assay was evaluated by measuring the inhibition of conjugated diene hydroperoxides starting from linoleic acid oxidation. The essential oil gave 52.5 ± 0.2% which were lower compared to BHT, 95.5 ± 0.1%.

Table 2. Antioxidant activity of the *Reinwardtiodendron cinereum* essential oil

Samples	**β-carotene/linoleic acid (%)**	**DPPH IC_{50} (µg/mL)**	**TPC, Gallic acid equivalent (mg GA/g)**
Essential oil	52.5 ± 0.2	105.2 ± 0.2	25.5 ± 0.2
BHT	95.5 ± 0.1	18.5 ± 0.1	ND

Data represent mean±standard deviation of three independent experiments; ND – not determined

α-Zingiberene is a monocyclic sesquiterpene that is the main flavour component of ginger. Previous findings showed that α-zingiberene exhibits apoptotic effects on SiHa cells (Lee, 2016). In addition, the purified α-zingiberene from the essential oil of *Casearia sylvest*ris showed a cytotoxic activity against HeLa, U-87, SiHa, and HL60 cell lines, with IC_{50} values of 63.2, 153.0, 48.0, and 98.7 µg/mL, respectively (Bou et al., 2013). Similar to our results, α-zingiberene has been reported to be the major component from various species of

Zingiberaceae family, including *Zingiber officin*ale (India: rhizomes 46.71%) (Sharma et al., 2016), *Curcuma longa* (China: rhizomes 25.05%) (Zhang et al., 2017) and *Amomum muricarpum* (Vietnam: fruits 6.3%) (Huong et al., 2015). In addition, α-zingiberene was found to be rich in the essential oils of *Senecio selloi* (aerial parts 54.0%) (Silva et al., 2013), *Casearia sylvestris* (leaves 48.31%) (Bou et al., 2013), *Guarea kunthiana* (aerial parts 34.48%) (Pandini et al., 2018), *Piper lucaeanum* (leaves 30.4%) (Marques et al., 2015), and *Polyalthia longifolia* (leaves20.0%) (Ouattara et al., 2014). Thus, these components are considered to be worth furthermore study to develop it as chemotherapeutics.

In conclusion, the genus *Reinwardtiodendron* is still poorly discovered an unidentified as far as its essential oil composition is concerned. This research is the first report on the essential oil composition of *R. cinereum* growing in Malaysia. The high concentration of α-zingiberene makes it a good candidate for a chemical marker for *Reinwardtiodendron* species. In addition, the preliminary evaluation of its antioxidant activity is the first step described in the literature for this species and taken together, the data obtained here inspire more studies supporting the possibility of linking the chemical contents with particular biological properties.

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