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PHYTOCHEMICAL STUDY ON THE STEM BARK OF *Mallotus leucodermis* Hook F.

(Kajian Fitokimia ke atas Kulit Ranting *Mallotus leucodermis* Hook F.)

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Abstract

The stem barks of *Mallotus leucodermis* Hook F. (Euphorbiaceae) was studied for its chemical constituents. The air dried and pulverized of stem bark of *M. leucodermis* (1.3 kg) was extracted successively with acetone for three days at room temperature yielding 66.0 g of crude extract. The crude extract was fractionated using vacuum liquid chromatography (VLC) to afford six fractions. Fraction 6 was further washed and recrystallized to afford bergenin (1). Fraction 3 was subjected to multiple purification using radial chromatography ($CHCl_3$: acetone) with different ratio 9:1, 8:2, 6:4 and 5:5 to yield a flavonoid compound, epicatechin (2). These compounds were elucidated based on spectroscopic analysis (Ultra Violate, Infra-Red, Mass Spectrometry and Nuclear Magnetic Resonance) as well as comparison with literatures.

Keywords: euphorbiaceae, *Mallotus*, bergenin, epicatechin

Abstrak

Kulit ranting *Mallotus leucodermis* Hook F. (Euphorbicaeae) telah dikaji untuk sebatian kimianya. Kulit ranting *M. leucodermis* (1.3 kg) yang telah dikeringkan dan dikisar, direndamkan dalam aseton selama tiga hari pada suhu bilik bagi memperolehi 66.0 g bahan ekstrak. Fraksinasi telah dilakukan ke atas ekstrak tersebut dgn menggunakan hampagas kromatografi cecair dan menghasilkan enam fraksi. Fraksi 6 telah dicuci beberapa kali dan dihablurkan semula untuk menghasilkan bergenin (1). Manakala fraksi 3 ditulenkan beberapa kali menggunakan radial kromatografi untuk mendapatkan sebatian flavonoid, epicatechin (2). Dua sebatian tulen ini dikenal pasti berdasarkan analisis spektroskopi (Ultralembayung, Inframerah, Spektrometri Jisim and Resonans Magnet Nukleus) dan juga perbandingan data.

Kata kunci: euphorbiaceae, *Mallotus*, bergenin, epicatechin

Introduction

Mallotus leucodermis Hook F., is a species under genus of *Mallotus* from Euphorbiaceae family. Euphorbiaceae is also commonly known as spurge family. The family of Euphorbiaceae consists of 283 genera and 7300 species which have been distributed in tropical region around the world. The main genus of Eurphobiaceae family consist of *Euphorbia*, *Phyllantus*, *Mallotus*, *Macaranga* and many more [1]. *Mallotus* is a genus of shrubs, trees and climbers with approximately 150 species were distributed in tropical and sub-tropical regions in Asia [2]. The genus of *Mallotus* belongs to the Malphighiales order, the Euphorbiaceae family, Acalyphoideae subfamily, Acalypeae pro parte and Rotterinae subtribe [3]. Research on *Mallotus* species revealed that this genus were very rich with active

compounds such as flavonoids, phenolic compounds, terpenoids and phloroglucinol derivatives [5]. Moreover, the pharmaceutical research has found that some of chemical constituents of *Mallotus* have the capability to possess several biological activities such as antioxidant, anti-inflammatory, antimicrobial, antiviral, cytotoxic and antitumor activities [3]. Therefore, the outcomes from this study will improve and enhance the application of natural product in the development of drug discovery.

Some species of the genus of *Mallotus* in Malaysia are used as traditional medicines. The roots of *M. paniculatus* are boiled and drunk after child birth, the leaves of *M. macrostachyus* are used as an antidote against snake poison whereas the roots and fruits of *M. barbatus* are used against muscle stiffness [2]. *Mallotus leucodermis* Hook f., is also commonly known as "balik angin bopeng" in Malaysia, is used to treat skin complaints [4]. In this study, two compounds namely bergenin (1) and epicatechin (2) have been isolated from the stem bark of *Mallotus leucodermis*. The structure elucidation of these two pure compounds will be discussed based on according to NMR (1D and 2D) analysis and also based on the comparison with the literature data.

Materials and Methods

Chemicals and plant materials

The industrial grade solvent was used for extraction of sample while the analytical grade solvents such as acetone, chloroform ($CHCl_3$), ethyl acetate (Ea), hexane (Hex), and methanol (MeOH), were used in the isolation and purification of compounds. The stem bark of *M. leucodermis* Hook f., was collected from Kuala Keniam, Pahang National Park.

Extraction procedure

Air dried and pulverized stem bark of *M. leucodermis* (1.3 kg) was successively extracted with 95 % acetone for 72 hours at room temperature. The extract was then filtered and evaporated to dryness under reduced pressure using rotary evaporator. The maceration was repeated three times to obtain optimum yield of crude extract. The crude extract (124.6 g) was subjected to removal of tannin by using diethyl ether yielded less tannin crude extract 67.8 g.

Fractionation and isolation

The crude extract was subjected to vacuum liquid chromatography (VLC) over silica gel eluted with hexane: ethyl acetate (3:7 – 1:9) and $CHCl_3$: acetone (7:3 – 3:7) to yield several fractions. All fractions were spotted on thin layer chromatography (TLC) plate and the similar patterns of separation were combined together into six fractions (F1 – F6). The last fraction from VLC was further purified by repeated washing using acetone and methanol to give white crystal ML1 (0.66 g). Fraction 3 (800 mg) was then subjected to radial chromatography on silica coated plate with $CHCl_3$: acetone (9:1 – 5:5) to give seven subfractions. Subfraction 7 (F3.7) was further purified by radial chromatography (RC) using $CHCl_3$: acetone (8:2, 6:4, 5:5) and washing method to give ML2 (17 mg).

Structure elucidation

The pure compounds were identified by using NMR spectroscopy on a Bruker AC 300 instrument, UV-Vis spectrophotometer, Infra-red, FTIR and gas chromatography – mass spectrometry, GC-MS. The spectra were measured on the spectrometers. Structures identified after analysis of the data obtained from spectra.

Results and Discussion

Structural elucidation of isolated compounds

The isolated compounds were characterized and identified via spectroscopic analyses including 1D and 2D NMR spectroscopy, combined with values reported in the literature. The structures of these pure compounds were shown in Figure 1.

The pure compound ML1 is a white crystals, isolated from VLC and recrystallized in methanol. Compound ML1 gave a $[M]^+$ at m/z 328 in the EI-mass spectrum, which corresponds to the molecular formula $C_{14}H_{16}O_9$. The melting point for this compound is consistent with the reported melting point of bergenin which is 236 – 238 °C [6]. The ^1H NMR spectrum showed a signal for an aromatic proton at δ_H 6.99 (1H, s) and a signal for methoxy protons at δ_H 3.77 (3H, s). The ^{13}C APT spectrum showed the total of 14 resonances representing 14 carbons. The carbonyl group signal appeared at downfield region, δ 163.8 (C-2) whereas the signal for methoxy carbon appeared at δ 60.3

(C-15). The methylene carbon (C-16) showed a signal at δ_C 61.5 while the five peaks of methine carbons in glucose ring appeared at δ_C 71.3 (C-12), 72.5 (C-9), 74.1 (C-13), 80.2 (C-14) and 82.2 (C-11). Table 1 summarised the ^{1}H and ^{13}C NMR data for ML1 in comparison with the literature data. Based on the spectral data and comparison with literature [7] and [8] respectively, ML1 was determined to be a bergenin.

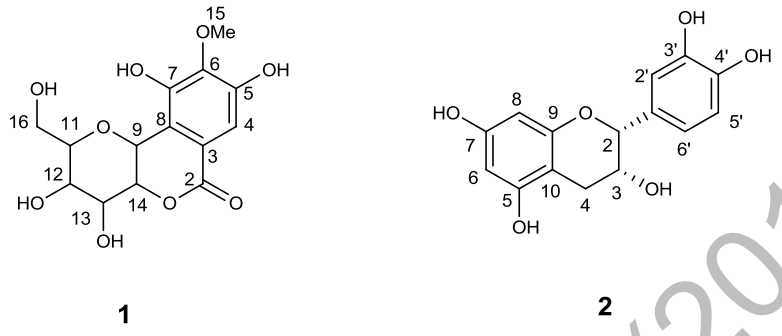

Figure 1. Structure of isolated compounds (1) bergenin and (2) epicatechin

Bergenin is a colourless crystalline polyphenol, which contains five hydroxyl groups that played an important role in its pharmacological activity [5]. This compound is reported in literature to exhibit various biological activities such as antifungal, antiulcer, burn wound healing effects and also immunomodulatory [6]. Bergenin was previously isolated from *Mallotus philippinensis*, *Mallotus japonicus*, and *Bergenial crassifolia* [5].

Table 1. The comparison of ^{1}H and ^{13}C NMR for ML1 with the literature data

No.	δH	*δH	δC	**δC
1	-	-	-	-
2	-	-	163.8	164.6
3	-	-	118.5	118.2
4	6.99 (1H, s)	6.99 (1H, s)	109.9	109.9
5	9.76 (1-OH, s)	9.75 (1-OH, s)	151.4	151.1
6	-	-	141.0	141.1
7	8.45 (1-OH, s)	8.45 (1-OH, s)	148.5	148.2
8	-	-	116.4	116.1
9	4.98 (1H, d, J= 10.8 Hz)	4.99 (1H, d, J= 10.5 Hz)	72.5	73.1
10	-	-	-	-
11	3.58 (1H, t, J=8.1 Hz)	3.57 (1H, t, J= 8.8 Hz)	82.2	81.9
12	3.22 (1H, ddd, J= 8.1, 5.1 Hz)	3.20 (1H, ddd, J= 8.8, 6.6 Hz)	71.1	70.7
13	3.67 (1H, ddd, J=5.4,3.3,5.4Hz)	3.65 (1H, ddd, J=8.8,5.6,5.6Hz)	74.1	74.4
14	4.00 (1H, dd, J=10.2, 9.6 Hz)	4.00 (1H, dd, J= 10, 9.6 Hz)	80.2	80.2
15	3.77 (3H, s)	3.78 (3H, s)	60.3	59.8
16	3.83 (1H, dd, J= 10.8 Hz)	3.84 (1H, dd, J= 11.6, 4.4 Hz)	61.5	61.5

* ^{1}H (400 MHz, DMSO-d_6) [7]
**^{13}C (500 MHz, CD_3OD-d_4) [8]

The pure compound ML2 was obtained as yellow powder, after several purification using RC eluted with $CHCl_3$: acetone (9:1 – 5:5). Compound ML2 gave a $[M]^+$ at m/z 313.2 in the EIMS, corresponding to the molecular formula $C_{15}H_{14}O_6$. The melting point obtained 224-226 °C which is acceptable with the literature melting point of epicatechin [9]. The 1H NMR spectrum showed a broad singlet peak at δ_H 4.88 (1H, s, H-2) which indicate a cis-orientation between H-2 and H-3. An ABD system was observed at region by protons at δ_H 6.81 (d_o), 6.83 (d_m) and 7.06 (dd). The spectrum also revealed two doublets at δ_H 6.04 (H-8) and δ_H 5.93 (H-6) which were indicated as proton with meta-subtitution in ring A. The ^{13}C APT spectrum showed the total of 15 resonances representing 15 carbons. The methylene carbon (C-4) showed a signal at very upfield region at δ_C 28.1 whereas the signal at δ_C 78.5 represents C-2. The total of seven signals of quarternary carbons at δ_C 157.0, 157.2, 156.6, 99.6, 131.7, 144.9 and 145.1 representing as C-5, C-7, C-9, C-10, C-1', C-3', and C-4', respectively.

Table 2 summarized the ^1H and ^{13}C NMR data for ML2 in comparison with the literature data. Based on the spectral data and comparison with literature [10], ML2 was confirmed to be an epicatechin. Epicatechin is a flavan-3-ol, a type of natural phenol and antioxidant. It belongs to the family of flavonoids. This major flavanol in food is known to possess antioxidant, antiulcer and anti-inflammatory [7].

Table 2. The comparison of ^1H and ^{13}C NMR for **ML2** with the literature data

No.	δH	*δH	δC	*δC
1	-	-	-	-
2	4.88 (1H, s)	4.88 (1H, s)	78.5	79.1
3	4.21 (1H, m)	4.24 (1H, m)	66.1	66.6
4β	2.72 (1H, dd, J=16.5, 3.6 Hz)	2.72 (1H, dd, J=16.7, 3.5 Hz)	28.1	28.5
4α	2.89 (1H, dd, J=16.5, 4.5 Hz)	2.87 (1H, dd, J=16.7, 4.5 Hz)		
5	-	-	156.7	157.0
6	5.93 (1H, d, J= 1.5 Hz)	5.96 (1H, d, J=2.3 Hz)	95.3	96.1
7	-	-	156.7	157.2
8	6.04 (1H, d, J= 1.5 Hz)	6.06 (1H, d,J= 2.3Hz)	94.8	95.4
9	-	-	156.2	156.6
10	-	-	98.9	99.6
1'		-	131.3	131.7
2'	7.06 (1H, s)	7.05 (1H, s)	114.4	115.1
3'	-	-	144.5	144.9
4'	-	-	145.6	145.1
5'	6.83 (2H, m)	6.83 (2H, m)	114.6	115.1
6'			118.4	119.1

*(300 MHz, acetone-d_6) [10].

Spectroscopic data

Bergenin (**1**): $C_{14}H_{16}O_9$, white crystals (0.66 g), m.p 236-238 °C. ESI-MS (positive mode) m/z 328.0 $[M+Na]^+$. ^1H NMR (DMSO-d_6, 300 MHz) δ: 3.22 (1H, ddd, J=8.1 Hz, 5.1 Hz, H-12), 3.58 (1H, t, J=8.1 Hz, H-11), 3.67 (1H, ddd, J=5.4Hz, 3.3Hz, 5.4Hz, H-13), 3.77 (3H, s, OCH_3), 3.83 (1H, dd, J=10.8Hz, H-16), 4.00 (1H, dd, J=10.2Hz, 9.6Hz, H-14), 4.98 (1H, d, J=10.5Hz, H-9), 6.99 (1H, s, H-4). 13C NMR (DMSO-d_6, 300 MHz) δ: 163.8 (C-2), 151.4 (C-5), 148.5 (C-7), 141.0 (C-6), 118.5 (C-3), 116.4 (C-8), 109.9 (C-4), 82.2 (C-11), 80.2 (C-14), 74.1 (C-13), 72.5 (C-9), 71,1 (C-12), 60.3 (C-15), 61.5 (C-16).

Epicatechin (**2**): $C_{15}H_{14}O_6$, yellowish powder (17 mg), m.p. 224-226 °C. ESI-MS (positive mode) m/z 313.2 $[M+Na]^+$, 291.2 $[M+H]^+$. ^{1}H NMR (Acetone-d_6, 300MHz) δ: 2.72 (1H, dd, J= 16.5 Hz, 3.6 Hz, H-4β), 2.89 (1H, dd, J= 16.5 Hz, 4.5 Hz, H-4α), 4.21 (1H, m, H-3), 4.88 (1H, s, H-2), 5.93 (1H, d, J= 1.2 Hz, H-6), 6.04 (1H, d, J= 1.5 Hz, H-8), 6.83 (2H, m, H-5', H-6'), 7.06 (1H, s, H-2'). ^{13}C NMR (Acetone-d_6, 300MHz) δ: 28.5 (C-4), 66.6 (C-3), 79.1 (C-2), 95.4 (C-8), 96.1 (C-7), 99.6 (C-10), 115.1 (C-2'), 115.5 (C-5'), 119.1 (C-6'), 131.2 (C-1'), 144.9 (C-3'), 145 (C-4'), 156.6 (C-9), 157.0 (C-5), 157.2 (C-7).

Conclusion

The isolation and identification of bergenin (**1**) and epicatechin (**2**) from the stem bark of *Mallotus leucodermis* Hook f., was the first ever to be reported from this plants. These compounds were elucidated based on spectroscopic analysis (UV-Vis, Infra-Red, Mass Spectra and Nuclear Magnetic Resonance) as well as comparison with literatures.

Acknowledgement

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