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Luteolin and 4-Hydroxybenzoic Acid from the Leaves of *Vitex negundo* L.

W. C. Ling[1], F. Ahmad[1] and R. Mat Ali[2]

[1]Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, 81310, Skudai, Johor, Malaysia
[2]Medicinal Plant Division, Forest Research Institute Malaysia (FRIM), 52109, Kepong, Kuala Lumpur, Malaysia

ABSTRACT Chemical investigation on the leaves of medicinal plant *Vitex negundo* or laggundi had been carried out. The sample of this species was extracted with petroleum ether, chloroform, ethyl acetate and ethanol consecutively with a soxhlet apparatus. The individual crude extract was fractionated with vacuum liquid chromatography and further purified by column chromatography. The crude ethyl acetate extract yielded two pure compounds, which were characterized as 4-hydroxybenzoic acid and luteolin. The structures of the pure compounds were elucidated using various spectroscopic techniques such as NMR, FTIR, UV-VIS and GC-MS.

ABSTRAK Kajian kimia ke atas daun tumbuhan traditional *Vitex negundo* atau laggundi telah dijalankan. Sampel telah diekstrak dengan petroleum eter, klorofom, etil asetat dan etanol dengan alat radas soxhlet. Setiap ekstrak mentah telah diperingkatkan dengan kromatografi cecair vakum dan seterusnya ditulenkan dengan kaedah kromatografi turus graviti. Dua komponen tulen iaitu, asid 4-hidroksibenzoik dan luteolin telah berjaya dipisahkan daripada ekstrak mentah etil asetat dengan kaedah kromatografi turus graviti. Sebatian tulen yang dipisahkan dicirikan strukturnya dengan menggunakan kaedah spektroskopi seperti RMN, FTIR, UL dan KG-SJ.

(*Vitex negundo*, Verbenaceae, luteolin, 4-hydroxybenzoic acid)

INTRODUCTION

The genus of *Vitex* is arranged under the Verbenaceae family. There are about 140 species throughout the tropics and subtropics but only 16 species in Malaysia, mostly in the lowlands. The Malaysian species of *Vitex* are evergreen. *V. negundo* or locally called lagundi is well known for its medicinal value. The leaves of *V. negundo* helped ease the pain after childbirth. Besides that, the drink is also said to cure coughs and reduce phlegm [1, 2]. Previous studies of this plant reveal the presence of variety of compounds such as 2'-*p*-hydroxybenzoyl mussaenosidic acid, 6'-*p*-hydroxybenzoyl mussaenosidic acid, gardenin A, gardenin B etc. [3 – 5]. In this present paper, we wish to report the isolation and characterization of two compounds from the ethyl acetate extract of the leaves of *V. negundo*.

EXPERIMENTAL

General

^{1}H and ^{13}C NMR spectra were recorded at 300 MHz and 75 MHz using Bruker Avance. The IR and UV/VIS spectra recorded using FTIR Shimadzu 8000 and Spectrophotometer Visible Varian CARY 3, respectively. The Mass spectra were obtained with Spectrometer Lucy Version 2.22. VLC (vacuum liquid chromatography) and CC (column chromatography) were carried out using silica gel Merck (230-400 mesh) and silica gel Merck (70-230 mesh), respectively.

Extraction and isolation

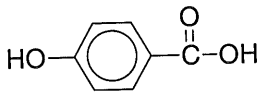
The powdered leaves of *V. negundo* (747 g) were extracted with petroleum ether, $CHCl_3$, EtOAc and EtOH consecutively with a soxhlet apparatus. The individual extract was evaporated to dryness to yield a residue. The EtOAc extract (40.5 g) was fractionated by VLC on a silica gel column (230-400 mesh) and eluted with hexane, $CHCl_3$,

EtOAc, acetone followed by a gradient of MeOH up to 100%. Fraction 28, which was eluted with $CHCl_3$-EtOAc (90:10) and concentrated to give compound **1** as colourless needles (168.1 mg, 0.21 %), m.p. 211.0 – 212.0°C, lit [6] 211°C, R_f 0.56 (EtOAc-MeOH 4:1); IR γ_{max} cm^{-1} (KBr): 3391.6 (-OH), 1674.1 (C=O), 1595.0, 1423.4 (C=C aromatic) and 1318.3, 1245.9 (C-O); ^1H NMR δ ppm (Me_2CO-d_6): 6.9 (2H, *d*, *J* = 8.7 Hz, H-3 & H-5), 7.9 (2H, *d*, *J* = 8.7 Hz, H-2 & H-6); ^{13}C NMR δ ppm (Me_2CO-d_6): 115.06 (C-3 & C-5), 121.84 (C-1), 131.82 (C-2 &C-6), 161.68 (C-4) δ 166.72 (C=O); MS: *m/z* 138 [M^+, $C_7H_6O_3$], 121, 93, 81, 65, 62, 53.

Fraction 29 to 32 which were collected from the VLC process were combined and further fractionated by column chromatography. Fractions 152–230, which were eluted with 100% $CHCl_3$ were combined and concentrated to yield compound **2** (23.1 mg, 0.03 %) as yellowish needles, m.p 328.0 – 329.0°C, lit [6] 330°C, R_f 0.51 (EtOAc 100%); UV λ_{max} (MeOH) nm: 351.0, 270.0; (+NaOMe): 399.0, 271.4; (+NaOAc): 365.4, 271.6; (+NaOAc+H_3BO_3): 365.4, 269.6; (+$AlCl_3$): 406.6, 272.0; (+$AlCl_3$+HCl): 381.6, 272.8; IR (KBr) γ_{max} cm^{-1}: 3419.6 (-OH), 1654.8 (C=O), 1607.6 (-C=C-), 1508.2 and 1435.9 (C=C aromatic), 1366.5, 1268.1 and 1160.1 (C-O); ^1H NMR δ ppm (Me_2CO-d_6): 6.26 (1H, *d*, *J* = 2.1 Hz, H-6), 6.54 (1H, *d*, *J* = 2.1 Hz, H-8), 6.59 (1H, *s*, H-3), 7.01 (1H, *d*, *J* = 8.4 Hz, H-5'), 7.46 (1H, *d*, *J* = 2.1 Hz, H-2'), 7.51 (1H, *dd*, *J* = 2.1 and 8.4 Hz, H-6'), 13.03 (1H, *s*, 5-OH); ^{13}C NMR δ ppm (Me_2CO-d_6): 93.81 (C-8), 98.82 (C-6), 103.29 (C-3), 104.43 (C-10), 113.28 (C-2'), 115.79 (C-5'), 119.21 (C-6'), 122.84 (C-1'), 145.63 (C-3'), 149.24 (C-4'), 157.89 (C-9), 162.46 (C-5), 164.04 (C-2), 164.26 (C-7), 182.78 (C-4).

RESULTS AND DISCUSSION

Compound **1**, 4-hydroxybenzoic acid was obtained as a colourless needles (168.1 mg, 0.21 %), m.p. 211.0 – 212.0°C, lit [6] 211°C, R_f 0.56 (EtOAc-MeOH 4:1). The IR, ^1H and ^{13}C NMR, DEPT and mass spectra confirmed that compound **1** is 4-hydroxybenzoic acid.



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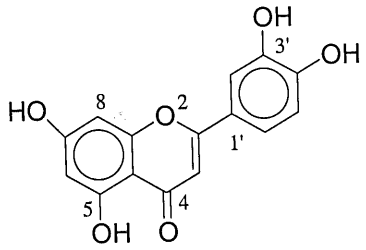

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Compound **2** was obtained as yellowish needles (23.1 mg, 0.03 %), m.p. 328.0 – 329.0°C, lit [6] 330°C, R_f 0.51 (EtOAc 100%). The UV spectrum of compound **2** displayed two absorptions bands at λ_{max} 351 and 270 nm, which were attributed to the skeleton of flavone derivatives [7]. The band at λ_{max} 351 nm was shifted to 399 nm with the addtion of NaOMe. This bathochromic shift confirmed the appearance of 4'-OH at B ring. The bathochromic shift around 14.4 nm with an addition of NaOAc and NaOAc/H_3BO_3 also confirmed the presence of a hydroxyl group, which were located at C-7 and C-3' & C-4' for A and B ring, respectively. The position of OH at C-5 was confirmed with an addition of $AlCl_3$/HCl. The bathochromic shift (406.6, 272.0) was observed with the addition of $AlCl_3$ and this confirmed the presence of di-hydroxyl group, which were located at C-3' and C-4' [7].

The IR spectrum of compound **2** exhibited bands for hydroxyl group at 3419.6 cm^{-1}. The presence of an absorption band at 1654.8 cm^{-1} was attributed to a carbonyl group. Moreover, bands for C=C aromatic were also observed at 1508.2 and 1435.9 cm^{-1}. The C-O group was indicated by bands at 1366.5, 1268.1 and 1160.1 cm^{-1}

The ^1H NMR spectrum of compound **2** exhibited signals resonated at δ 6.26 (1H, *d*, *J* = 2.1 Hz) and 6.59 (1H, *d*, *J* = 2.1 Hz) attributed to H-6 and H-8, respectively. Another doublet resonated at δ 7.01 (*J* = 8.4 Hz) was assigned to an aromatic proton, H-5'. Moreover, a signal at δ 7.46 which appeared as doublet (*J* = 2.1 Hz) was indicated to H-2'. A signal appeared as singlet at δ 6.59 was due to H-3. A double of doublet signals resonated at δ 7.51 (*J* = 2.1 and 8.4 Hz) was attributed to

H-6'. A very sharp singlet at δ 13.03 was due to hydroxyl group at C-5.

The ^{13}C NMR and DEPT spectra showed the presence of 15 signals corresponding to 15 carbons in the molecules. The signals were assigned as one carbon carbonyl (182.78), 6 methine carbons (93.81, 98.82, 103.29, 113.28, 115.79 and 119.21) and 8 quaternary carbons (104.43, 122.84, 145.63, 149.24, 157.89, 162.46, 164.04 and 164.26). Based on the spectroscopy data, compound **2** was assigned as luteolin.

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REFERENCES

1. Whitmore, T. C. (1972). *Tree Flora of Malaya*. Longman Malaysia Sdn. Bhd. **2**: 34-35, 123.
2. Zakaria, M and Mohd, M. A. (1994). *Traditional Malay Medicinal Plants*. Fajar Bakti Sdn. Bhd. **47**: 124, 128.
3. Sehgal, C. K., Taneja, S. C., Dhar, K. L. and Atal, C. K. (1983). *Phyochemistry*. **22**: 1036-1038.
4. Sehgal, C. K., Taneja, S. C., Dhar, K. L. and Atal, C. K. (1983). *Phyochemistry*. **21**: 363-366.
5. Banerji, J., Das, B. and Chakrabarty, R. (1988). *Indian Journal of Chemistry*. **27 B**: 597-599.
6. Devon, T. K. and Scott, A. I. (1975). *Handbook of Naturally Occuring Compound, Volume I - Acetogenins, Shikimates and Carbohydrates*. Academic Press Inc.
7. Markham, K. R. (1982). *Techniques of Flavonoid Identification*. Academic Press. 42-44.