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Isolation and Characterization of Gallic Acid, Quercetin and Methyl Dehydrochebulate from *Phyllanthus niruri* (Dukung Anak)

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ABSTRACT Three natural compounds, gallic acid, methyl dehydrochebulate and quercetin were isolated from the methanolic extract of the leaves of *Phyllanthus niruri*. All the isolated compounds were identified on the basis of UV, IR, ^{1}H NMR, ^{13}C NMR, mass spectra and elemental analysis.

(*Euphorbiaceae*; gallic acid; leaves; methanolic extract, *Phyllanthus niruri*)

INTRODUCTION

Phylanthus niruri Linn. (Euphorbiaceae), a small plant which grows mainly in tropical and subtropical regions in Central and South American countries, India, and East Asia. It is one of the most important medicinal plants used by people in these countries for treatment of jaundice, asthma, hepatitis, urolithic disease, fever, malaria, stomachache, and tuberculosis [1]. Extensive chemical examinations of this plant have been carried out and several constituents were isolated such as lignans, alkaloids, flavonoids, tannins, phthalic acid, gallic acid and terpenoids [2-6]. In this paper, we describe the isolation and structural elucidation of a new compound and another two known compounds from the methanolic extract of *Phyllanthus niruri*. All the isolated compounds were identified by their spectral data.

MATERIALS AND METHOD

General

Melting points were determined using an electrothermal melting point apparatus (Gallenkamp). IR spectra were recorded (KBr discs) on a FT-IR spectrophotometer, validation (ν_{max} in cm^{-1}). ^{1}H-NMR spectra were recorded on a Bruker R-32 (300 MHz) instrument in acetone-d_6 with TMS as an internal standard (chemical shifts δ, ppm). UV spectra were recorded on HITACHI, U-2000 spectrophotometer Ultrospeck in methanol (λ_{max} in nm). Mass spectra were recorded using a Hewlett-Packard HP 5989 mass engine with HP UX workstation in electron impact ionization mode at 70 eV.). The entire chemical used is analytical reagent grade. TLC was performed using silica gel GF_{254}.

Plant Materials

The leaves of *Phyllanthus niruri* were collected from the Jawa Island, Indonesia. The plant was identified and voucher specimen was deposited in the herbarium of the School of Biology, University Sains Malaysia.

Extraction

Dried aerial parts of the plant (1900 g) were powdered and extracted by petroleum ether, chloroform and finally methanol (36 hrs) using Soxhlet extractor. The extract was evaporated in a rotatory evaporator and dried by vacuum pump. The methanolic extract (100 g) was suspended on water and extracted successively with ethyl acetate, butanol and water to yield ethyl acetate (21 g), butanol (44 g) and water-soluble (30 g) fractions, respectively. Ethyl acetate soluble fraction (10 g) was subjected to chromatography on silica gel (60-120 mesh, Merck) eluted with chloroform and increasing a mount of methanol to give 24 fractions (15 ml each).

The fractions (4-7) were combined and concentrated. The concentrated fraction was further chromatographed over Sephadex LH-20 (10 g) and eluted with methanol to give compound **1**, which was crystallized from methanol- chloroform to give white crystals (11mg); m.p. 197-200 °C; MS (M^+, 396); UV: 331.5, 271 nm; IR: 3396, 3261, 1761, 1731, 1710, 1622, 1495, 1450, 1390, 1369, 1310, 1280,

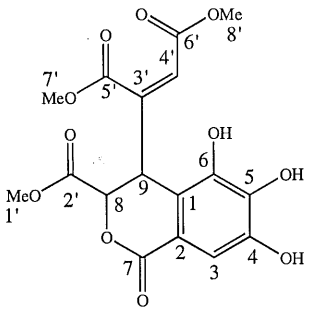
1115, 1078, 1160 cm^{-1}; ^{1}H-NMR (acetone-d_6): 3.62-3.67 (3s, -OCH_3), 5.27(s, H-8), 5.40 (s, H-9), 6.82 (s, H-4'), 7.12 (s, H-3), 8.53 (s, OHx3). [Found: C, 51.48; H, 4.09 Calculated for $C_{17}H_{16}O_{11}$, C, 51.51; H, 4.04 %].

The combined fractions (9-17) were further chromatographed over Sephadex LH-20 (10g) and eluted with methanol, 30 fractions were collected. Fractions (5-11), which showed the same TLC profile as pure compound **2**, which was crystallized from methanol as amorphous yellow powder (9 mg), m.p 307-309°C (lit. m.p.308°C); MS (M^+, 302); UV: 255.4, 271.83 (MeOH), 317.52, 428.63 (MeOH + NaOMe); 268.42, 318.56, 432.53 (MeOH+$AlCl_3$); 254.57, 317.45, 422 MeOH +$AlCl_3$+ HCl); 359.83, 315.28, 386.3 (MeOH + NaOAc + H_2BO_3); IR: (KBr): 3405 (-OH), 1665 (C=O), 1611, 1561, 1516, 1467 (C=C), 1096 (C-O) cm^{-1}; ^{1}H-NMR (DMSO-d_6): 7.53 (s, H-8), 7.72 (s, H-6), 9.02 (d, 1H, *J*=2Hz, H-2'), 8.24 (d, *J*= 8.5 Hz, H-5'), 8.88 (dd, J=8.5, 2.2 Hz, H-6'); ^{13}C-NMR (DMSO-d_6); 148.55 (C-2), 136.57 (C-3), 176.68 (C-4), 161.56 (C-5), 161.56 (C-6), 164.74 (C-7), 94.19 (C-8), 156.98 (C-9), 103.84 (C-10), 122.79 (C-1'), 115.9 (C-2'), 145.9 (C-3'), 147.64 (C-4'), 116.4 (C-5'), 120.05 (C-6'); [Found: C, 59.60; H, 3.31. Calculated for $C_{15}H_{10}O_7$, C, 59.51; H, 3.64 %].

Combined fractions (16-21) were concentrated under vacuum to give compound **3**, which was crystallized from acetone as white needles (30 mg). Compound **3**, was crystallized from acetone as fine needles m.p. 250-252°C; MS (M^+, 170); UV: 271, 274nm; IR (KBr): 3488, 3367, 3288, 3066, 2661, 1703, 1619, 1540, 1467, 1384, 1384 cm^{-1}; ^{1}H-NMR (DMSO-d_6): 12.22 (s, -OH), 9.18 (s, -OHx2), 8.28 (s, -OH), 6.91 (s, H-5/H-5'). ^{13}C-NMR (DMSO-d_6): 168.21 (C-7), 146.46 (C-2 & C-2'), 138.97 (C-3), 121.66 (C-4), 109.1(C-5 & C-5').

RESULTS AND DISCUSSION

Dried sample of the plant (1900 g) were powdered and extracted by petroleum ether, chloroform and finally methanol (36 hrs) using Soxhlet extractor. The extract was evaporated in a rotatory evaporator and dried by vacuum pump. The methanolic extract (100 g) was suspended on water and extracted successively with ethyl acetate, butanol and water to yield ethyl acetate (21 g), butanol (44 g) and water-soluble (30 g) fractions, respectively. The ethyl acetate fraction was purified by a series of column chromatography and three compounds were obtained. On the basis of spectral data, they were identified as methyl dehydrochebulate **1**, quercetin **2** and gallic acid **3**.



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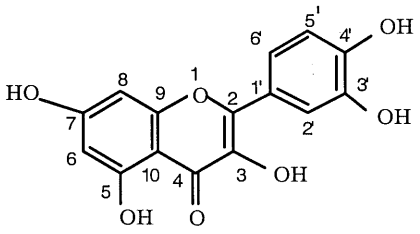

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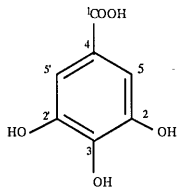

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Compound **1** (11 mg) was isolated from ethyl acetate fraction as white crystals, m.p. 197-200 °C. The UV spectrum showed absorption bands at 331.5 and 271 nm. The IR spectra exhibited bands at 3396 cm^{-1} (-OH), 1761, 1731, 1710 cm^{-1} (-$COOCH_3$) and 1622 and 1495 cm^{-1} (aromatic rings). The MS of compound **1** displayed a molecular ion peak at *m/z* 396 corresponding to $C_{17}H_{16}O_{11}$. In ^{1}H-NMR spectrum three signals at δ 3.62-3.67 indicated the presence of three-methyl groups in the molecule. Three singlets at δ 5.27, 5.40, and 8.53 indicating the presence of H-8 and H-9 and three -OH protons respectively [7].

Compound **2** was obtained as a yellow crystal. It had a melting point 307-309°C. High-resolution

mass spectrometer indicated the molecular formula $C_{15}H_{10}O_7$ (M^+, found 302.8, calculated 302.23) IR spectrum showed frequencies at 3405 and 1665 cm^{-1} indicating the presence of a hydroxyl group and a conjugated keto group respectively. Absorption peaks at 1611 and 1561 cm^{-1} indicated the presence of unsymmetric ethylenic double bond and the aromatic rings, respectively. In ^{1}H-NMR spectrum showed two singlets at δ 7.53 and 7.72 indicated the presence of H-8 and H-6 protons on the aromatic rings. Two doublets at δ 9.02 (*J*= 2 Hz) and 8.24 (*J*= 8.5 Hz) indicated the presence of H-2' and H-5' respectively. A doublet doublet at δ 8.8 (J=8.5, 2.2 Hz) indicated the presence of H-6'. Singlet at δ 10.93 indicated the presence of H-3. The other aromatic protons and hydroxyl group have their usual chemical shift values. Comparing their data with those of literature [8, 9], identified the known compound **2** as quercetin.

Compound **3** (30 mg) was crystallized from acetone as fine needles, m.p. 250-252°C. The UV spectra showed absorption at λ_{max} 270 nm in methanol indicated the presence of conjugated double bond in the structure. The IR spectrum of showed the absorption frequency at 3488 cm^{-1} indicating the presence of hydroxyl groups and the absorption peaks at 1703, 1619 and 1540 cm^{-1} indicated the presence of carboxyl group and the conjugated ethylene double bond and the aromatic rings respectively. The mass spectrometer indicated the molecular formula $C_7H_6O_5$ (M^+ found 170.0, calculated 170.12). In ^{1}H-NMR spectra showed singlet at δ 12.23 ppm indicated the presence of carboxyl proton (>C-OH). Two sharp singlets at δ 9.18 ppm and δ 8.29 indicating the presence of three hydroxyl group for C-2, C-2'and C-3. Other sharp singlets at δ 6.91 ppm indicated the presence of two methine protons on the aromatic ring. When D_2O was added to the compound **3** in DMSO-d_6 solution, the proton signals at δ 12.22, 9.18 and 8.28 ppm were disappeared which confirmed their identity as hydroxyl protons. The elemental analysis for C and H showed satisfactory results (within 0.4%). Comparing their data with those in the literature [10] identified the known compound **3** as gallic acid.

CONCLUSION

Three natural compounds, methyl dehydrochebulate **1**, quercetin **2** and gallic acid **3** were isolated from the methanolic extract of the aerial parts of *Phyllanthus niruri*. methyl dehydrochebulate is now reported for first time from *Phyllanthus niruri*. All the isolated compounds were identified on the basis of UV, IR, ^{1}H-NMR, ^{13}C-NMR, mass spectra and elemental analysis [7-10].

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REFERENCES

1. Unander, D. W, Webster G. L. and Blumberg, B. S. (1991). *J. Ethnopharmacology*. **34**: 97-133.
2. Balawant, S. J. William S. P., Goppinath, K. and Krishna, B. (1986). *J. Nat. Prod.* **49**(4): 614-620.
3. Calixto, J. B., Adair, R. S. Santos, Valdir, C. F. and Yunes, R. A. (1998). *Med Res Rev. Jul.* **18**(4): 225-85.
4. Ying, J. Z., Abe, T., Takashi, T., Chong, R. Y. and Isao, K. (2001). *J. Nat. Prod.* **64**: 1527-1532.
5. Singh, B., Agrawal, P. K., Thakur, R. S (1986b). *Indian J. Chem.* **28**: 319-321.
6. Qian-Cutrone, J., Huang, S., Trimble, J., Li, H., Lin, P. F., Alam, M., Kalhor, S. E. and Kado, K. F. (1996). *J. Nat. Prod.* **59**: 196-199.
7. Zhong, Y., Zuo, C., Li, F., Ding, X., Yao, Q., Wu, K., Zhang, Q., Wang, Z., Zhou, L. and Wang, J. (1998). *Studies on chemical constituents of Phyllanthus urinaria L. And its antiviral activity against hepatitis B virus. Institute of Materia Medica*, Shandong Academy of Medical Sciences, Jinan, Peop. Rep. China. Zhongguo Zhongyao Zazhi. 363-364.
8. Mabry, T. J., Markham, K. R. and Thomas M. B. (1970). *The systematic identification of flavonoids*. Springer-Verlage, New York, Heidelberg, Berlin, p. 294.
9. Akowuah, G.A, Sadikum, A. and Aariam, A. (2001). *Trop. Med. Plants*. **2**(2).
10. Nawwar, M. A., Buddrus, J. and Bauer, H. (1982). *Phytochemistry*. **21**(7): 1755-1758.