A Chemical Study on *Phyllanthus reticulatus*

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Abstract: *A phytochemical study was conducted on the leaves of* Phyllanthus reticulatus obtained *from a riverside in Taman Negara Kuala Koh, Kelantan. The separations of the chemical components were carried out using different chromatographic techniques and structures of compounds were elucidated by spectroscopic methods including nuclear magnetic resonance as well as mass spectrometry. Three compounds were isolated and identified as lupeol acetate, stigmasterol and lupeol.*

Keywords: *Phyllanthus reticulatus,* leaves, lupeol acetate, stigmasterol, lupeol, NMR analysis

1. INTRODUCTION

The *Phyllanthus* genus contains species which have useful medicinal applications. A considerable number of these species have been examined and some effective constituents have been reported. In particular, the isolation of antineoplastic bisabolene glycosides phyllanthoside and phyllanthostatins from *Phyllanthus accuminatus*. *Phyllanthus reticulatus* is a large straggling or climbing shrub growing from 8 to 10 ft in height.[1] The plant is used for a variety of ailments, including smallpox, syphilis, asthma, diarrhea and bleeding from gums.[2] Moreover, it is also claimed the plant has antidiabetic activity in tribal area. In this paper, the isolation and characterization of three known compounds from *P. reticulates* were reported.

2. EXPERIMENTAL RESULTS

Thin layer chromatography (TLC) and preparative TLC were performed using pre-coated aluminium and glass plates with silica gel 60 F_{254}, whereas column chromatography was carried out on silica gels 230–400 mesh. Spots and bands of compounds on TLC were detected using UV light.

V spectra were recorded on a UV-1650PC spectrophotometer. Proton NMR (400 MHz) and carbon-13 NMR (100 MHz) spectra were recorded on JEOL JNM-ECP400 and chemical shifts in ppm were referenced to internal

acetone-d_6 and $CDCl_3$, respectively. ^1H-^1H COSY and NOESY spectra were acquired using the standard JOEL software.

2.1 Plant Material

The leaves of *P. reticulatus* were collected from a riverside in Taman Negara Kuala Koh, Kelantan. Voucher specimens of WYA14 have been deposited at the Herbarium of Universiti Kebangsaan Malaysia.

2.2 Extraction and Isolation

The air-dried powder leaves (960 g) of *P. reticulatus* were extracted (Soxhlet) with methanol (3 times 8 h each) and the combined extracts evaporated to give a brown gummy residue (4 g). This extract was subjected to silica gel flash column chromatography (FCC) with chloroform containing increasing percentage of methanol as eluent, and each collected fraction was 20 ml. Fractions 1–4 were combined and re-chromatographed by radial chromatography to yield three compounds: 3.6 mg of a compound which is identified as lupeol acetate (Fig. 1), R_F 0.65 (hexane-EtOAc 7:3); 2.5 mg of a compound that is identified as stigmasterol (Fig. 2), R_F 0.7 (hexane-EtOAc 8:2), and the last constituent is identified as lupeol (Fig. 3), 3.1 mg, R_F 0.73 (hexane-EtOAc 7:3). Lupeol acetate, stigmasterol and lupeol, were identified by comparison with data from previous NMR and mass spectra.[3,4,5]

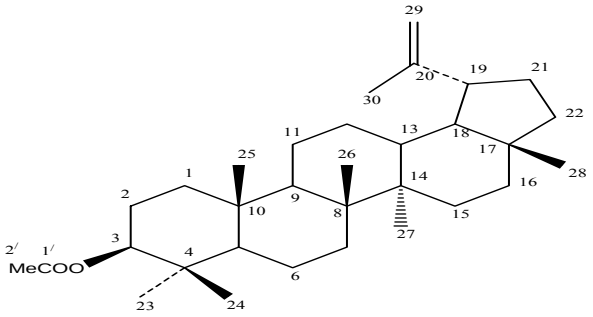

Figure 1: Lupeol acetate.

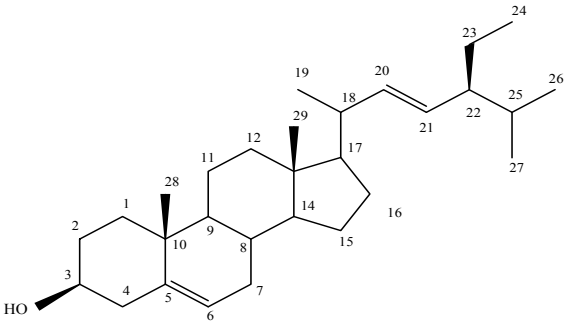

Figure 2: Stigmasterol.

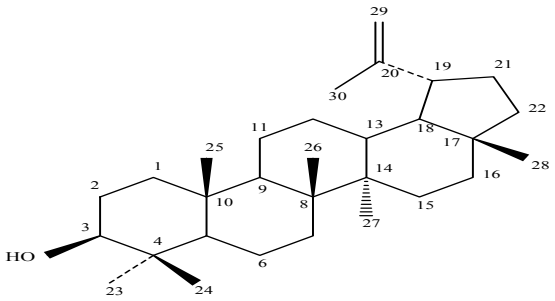

Figure 3: Lupeol.

Lupeol acetate (1). White needles (3.6 mg). EIMS for $C_{32}H_{52}O_2$ *m/z* (rel. int.): 468 [M^+] (17.2%), 453 (2.9%), 408 (1.7%), 357 (3.9%), 218 (15.2%), 189 (46.4%), 109 (29.1%), 43 (100%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.69 (1H, *s*, H-29b), 4.57 (1H, *s*, H-29a), 4.47 (1H, *dd, J* = 4.4, 12.8 Hz, H-3), 2.05 (3H, *s*, H-2′), 1.69 (3H, *s*, H-30), 1.03 (3H, *s*, H-25) 0.94 (3H, *s*, H-28), 0.85 (3H, *s*, H-23), 0.84 (3H, *s*, H-24), 0.83 (3H, *s*, H-26), 0.79 (3H, *s*, H-27). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.3 (C-1'), 151.2 (C-20), 109.6 (C-29), 81.2 (C-3), 55.6 (C-5), 50.5 (C-9), 48.5 (C-18), 48.2 (C-19), 43.2 (C-17), 43.0 (C-14), 41.0 (C-8), 40.2 (C-22), 38.6 (C-1), 38.0 (C-4), 37.3 (C-10), 36.2 (C-13), 35.8 (C-16), 34.4 (C-7), 30.0 (C-21), 28.2 (C-2'), 27.6 (C-23), 25.3 (C-15), 24.0 (C-12), 21.7 (C-2), 21.1 (C-11), 19.5 (C-30), 18.4 (C-6), 18.2 (C-28), 16.7 (C-24), 16.4 (C-25), 16.2 (C-26), 14.7 (C-27).

Stigmasterol (2). White powder (2.5 mg). EIMS for $C_{29}H_{42}O$ *m/z* (rel. int.): 412 [M^+] (39.7%), 351 (13.5%), 314 (7.0%), 300 (25.5%), 271 (38.4%), 229 (8.6%), 213 (1.6%), 55 (100%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.68, 0.79,

0.82, 0.86, 0.92, 1.02 (each 3H, s, Me × 6), 3.53 (1H, m, H-3), 5.36 (1H, t, H-6), 5.15 (1H, s, H-22), 5.01 (1H, s, H-23). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 140.9 (C-5), 138.5 (C-22), 129.5 (C-3), 121.9 (C-6), 72.0 (C-3), 57.0 (C-14), 56.1 (C-17), 51.4 (C-24), 50.3 (C-9), 46.0 (C-25), 42.4 (C-13), 40.7 (C-20), 39.8 (C-12), 37.5(C-4), 37.4 (C-1), 36.7 (C-10), 32.1 (C-8), 31.9 (C-7), 29.2 (C-16), 28.4 (C-2), 25.6 (C-28), 24.5 (C-15), 21.4 (C-21), 21.3 (C-11), 20.0 (C-27), 19.6 (C-26), 19.1 (C-19), 12.2 (C-29), 12.1 (C-18).

Lupeol (3). White powder (3.1 mg), mp 215°C–216°C. EIMS for $C_{30}H_{50}O$ *m/z* (rel. int.): 426 [M^+] (33.4%), 365 (14.5%), 207 (51.3%), 189 (25.8%), 161 (22.9%), 135 (71.0%), 107 (100%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.68, 4.56 (2H, *s*, H-29a, 29b), 3.16 (1H, *dd*, *J* = 4.76, 11.00 Hz, H-3), 0.75, 0.78, 0.82, 0.93, 0.95, 1.02, 1.25 (each 3H, *s*, Me × 7). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 151.1 (C-20), 109.5 (C-29), 79.1 (C-3), 55.5 (C-5), 50.6 (C-9), 48.5 (C-18), 48.1 (C-19), 43.2 (C-17), 43.0 (C-14), 41.0 (C-8), 40.2 (C-22), 39.0 (C-13), 38.9 (C-4), 38.2 (C-1), 37.3 (C-10), 35.8 (C-16), 34.5 (C-7), 30.0 (C-21), 28.2 (C-23), 27.6 (C-15), 27.5 (C-12), 25.3 (C-2), 21.1 (C-11), 19.5 (C-30), 18.5 (C-6), 18.2 (C-28), 16.3 (C-25), 16.2 (C-26), 15.6 (C-24), 14.7 (C-27).

3. RESULTS AND DISCUSSION

The concentrated methanol extract of the leaves of *P. reticulatus* was repeatedly fractionated using silica gel FCC, and compounds (1)–(3) were eluted in the order of increasing polarity. The ^{1}H and ^{13}C NMR spectral data for these compounds revealed that (1) and (3) belong to the lupine group. Compound (2) was identified as stigmasterol from its physical constants and spectral data.

Compound (1) was isolated as white needles. The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) showed the presence of eight tertiary methyl singlets at δ 0.79, 0.83, 0.84, 0.85, 0.94, 1.03, 1.69 and 2.05. Two protons appeared at δ 4.57 and 4.69 as singlets, representing the exocyclic double bond protons H-29a and H-29b, respectively. ^{13}C NMR spectrum showed a carbonyl group at δ 171.3, C-3 at δ 81.2 and the alkene carbons at δ 151.20 and 109.6. Lupeol acetate has never been isolated before from *P. reticulatus.* It was found in deertongue leaf,[6] *Erythroxylum leal costae*,[7] stem-bark of *Artocarpus chaplasha*[8] and *Ficus hispida.*[9]

Compound (2) was isolated as white powder. The mass spectral data of the compound gave a molecular formula $C_{29}H_{42}O$, [*m/z* 412 (M^+)]. ^{1}H NMR (400 MHz, $CDCl_3$) spectra showed the presence of six methyls that appeared at δ 0.68, 0.79, 0.82, 0.86, 0.92 and 1.02. The proton of H-3 appeared as a multiplet at δ 3.53. It also showed olefinic protons at δ 5.36, 5.15 and 5.01. ^{13}C NMR and APT showed 29 carbon signals including six methyls, nine methylenes, 11 methane

and three quaternary carbons. The alkene carbons appeared at δ 140.9, 138.5, 129.5 and 121.9. Stigmasterol, isolated from *P. reticulatus* for the first time, was reported in many plants such as *Ambroma augusta*,[10] *Strychnos potatorum*,[11] and *Dalbergia volubilis* flowers.[12]

Compound (3) is a pentacyclic triterpene. It was white powder. The EI-mass spectrum of (3) showed the molecular ion at *m/z* 426 [M^+] corresponding to the formula $C_{30}H_{50}O$, and in agreement with other spectroscopic data. The ^1H NMR spectrum showed seven tertiary methyl singlets and one secondary hydroxyl group. It also showed olefinic protons at δ 4.68 and 4.56. ^{13}C NMR of the compound showed 30 signals for the terpenoid of lupine skeleton which was represented by seven methyl groups. The carbon bonded to the hydroxyl group C-3 appeared at δ 79.1, while the alkenic carbons appeared at δ 151.1 and 109.5. The presence of lupeol in the *P. reticulatus* was not reported before the current study. The lupeol was reported earlier from the seeds of bark of *Heritiera utilis*[13] and *Euphorbia lateriflora.*[14]

4. CONCLUSION

The isolation and identification of compound (1), (2) and (3) from the leaves of *P. reticulatus* was the first ever to be reported from this plant. The work was carried out by means of various physical (solvent extraction, radial chromatography) and spectral techniques.

5. ACKNOWLEDGEMENT

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