NOTE

ISOLATION, PURIFICATION AND IDENTIFICATION OF CHEMICAL CONSTITUENTS FROM *ELATERIOSPERMUM TAPOS*

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Elateriospermum tapos, known as perah tree of the family Euphorbiaceae, is a monoecious canopy species found in Southeast Asian tropical rainforests. In Malaysia, the species is abundant in lowland forests throughout the country especially in northern parts of Peninsular Malaysia. The tree is easily recognized by the reddish pink foliage during a leaf flush. The timber from the tree is hard and heavy and is used for construction and firewood. Fruits and seeds are consumed by native communities though too much of it at one time has been known to induce dizziness. The bark, leaves and fruit stalks exude copious, white and sticky latex which is used for treating cracked sole of the foot (Chai *et al.* 1989). Not much is known about the chemical constituents except for the presence of hydrocyanic acid in the leaves and seeds, and triterpenes such as β-amyrin palmitate, germanicol palmitate, β-amyrin acetate, germanicol acetate, ψ-taraxasterol acetate and lupeol acetate in the bark (Chow & Quon 1970). During the course of our chemical studies of Malaysian medicinal plants, we investigated the perah tree collected from the forest reserve of Forest Research Institute Malaysia (FRIM). In this paper, we report on the isolation, purification and identification of 16 compounds obtained from the leaves and wood of *E. tapos*.

Fresh leaves and wood were separately extracted by repeated soaking in methanol at room temperature, and the methanolic extracts were suspended in water and successively extracted with *tert*-butyl methyl ether and ethyl acetate for the first and diethyl ether for the second. The resulting fractions *tert*-butyl methyl ether soluble portion, ethyl acetate soluble portion and water-soluble portion of the leaves and the water-soluble portion of the wood were each subjected to a combination of column chromatography employing Diaion HP20-SS, Sephadex LH-20, MCI gel CHP 20P, Chromatorex ODS, Toyopearl HW-40F and silica gel which led to the isolation of two flavan-3-ols, four condensed tannins, one biflavone, two cyanogenic glucosides, two aromatic glucosides, one phenylpropane, one glycerol derivative, one megastigmane, and two glycosides of secondary alcohol (Figure 1). These compounds were identified on the basis of spectroscopic and elemental analyses and comparison of their ^{1}H- and ^{13}C-NMR data with literature values or authentic samples.

The two flavan-3-ols were identified as (**1**) catechin (Nonaka *et al.* 1983a) and (**2**) gallocatechin (Nonaka *et al.* 1983b), while the condensed tannins were identified as (**3**) procyanidin B-1 (Morimoto *et al.* 1986), (**4**) epiafzelechin-(4β→8)-catechin (Tanaka *et al.* 1983), (**5**) procyanidin B-3 (Tanaka *et al.* 1983) and (**6**) AC trimer (Nonaka *et al.* 1981), which were formed as a result of condensation and polymerisation of two or three flavan-3-ol units.

Compound **7** was obtained as a yellow amorphous powder. The ^{1}H-NMR spectrum showed 12 aromatic proton signals in the region δ_H 6.27–8.08. The proton signals at δ_H 6.27 and 6.53 appeared as two doublets with a coupling constant of J = 2.1 Hz, which indicated their *meta* arrangement on the ring skeleton. An ABM pattern for three aromatic protons were observed at δ_H 7.28 (J = 8.7 Hz), 8.02 (J = 2.5, 8.7 Hz) and 8.08 (J = 2.5 Hz). An AA'BB'-type spin system, δ_H 6.84 and 7.63 (J = 6.9 Hz) indicated a 1,4-disubstituted aromatic ring. The remaining proton signals were observed as three isolated singlets equivalent to one proton each at δ_H 6.50, 6.66 and 6.73. The ^{13}C-NMR spectrum showed a total of 27 signals, three of which were clearly twice the intensity of the others, giving 30 carbons in the molecule. The spectrum included two carbonyl carbon signals at δ_C 182.9 and 183.3. These results suggested a biflavone structure for **7**. The analyses of ^{1}H-^{1}H COSY, HSQC and HMBC spectra allowed the precise assignments of all protons and carbons. The HMBC correlations observed between H-6'' and C-7'', C-8'' and C-10'', and that between H-2' and C-3', C-4', C-6' and C-8'', allowed us to establish that the two flavonoid units were linked by a bond involving C-3' and C-8''. The deduced structure was similar to that of amentoflavone, and the NMR spectral data are in agreement with those reported in the literature (Markham *et al.* 1987, Hanrahan *et al.* 2003).

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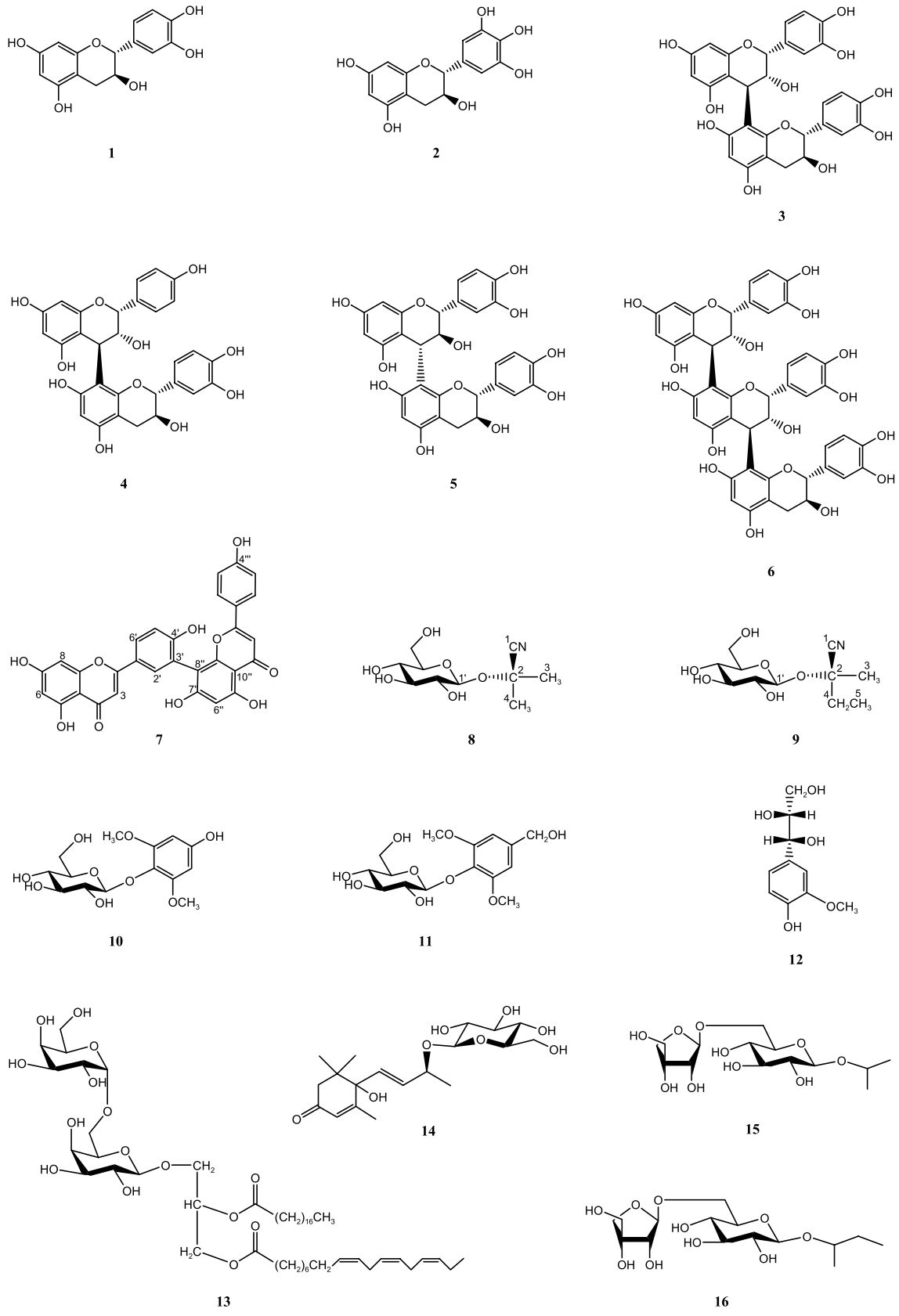

Figure 1 Chemical structures of compounds isolated from *Elateriospermum tapos*

Compounds **8** and **9** were obtained as white amorphous solids which displayed very similar signals in their ^{1}H- and ^{13}C-NMR spectra attributable to two methyl groups, two quaternary carbons and a β-glucopyranosyl moiety in each molecule, except that 9 showed an addition of one methylene unit. The chemical shifts of one of the quaternary carbons in each compound (δ_C 122.2 and 121.9) suggested nitrile functions. These spectral features closely resembled those of linamarin and lotaustralin (Hübel *et al.* 1981), which were the cyanogenic glucosides present in some food plants such as *Manihot esculenta* (cassava), *Phaseolus lunatus* (butter bean) and *P. vulgaris* (French/kidney bean) (Jones 1998). Therefore, compounds **8** and **9** were deduced as linamarin and lotaustralin respectively. The hydrocyanic acid reported to be present in the leaves and seeds could be the hydrolysis products of these compounds.

The structures of the two aromatic glycosides were determined to be the same as that of (**10**) 4-hydroxy-2, 6-dimethoxyphenol 1-*O*-β-D-glucopyranoside (leonuriside A) (Otsuka *et al.* 1989) and (**11**) 3,5-dimethoxy-4-hydroxybenzyl alcohol 4-*O*-β-D-glucopyranoside (Kitajima *et al.* 1998) respectively, by comparison with published spectral data. The phenyl propane was identified as (**12**) guaiacyl glycerol (Ishimaru *et al.* 1987) and the glycerol derivative as (**13**) linolenyl stearyl 3-*O*-(α-D-galactopyranosyl-(1''→6')-*O*-β-D-galactopyranosyl)-*sn*-glycerol (Murakami *et al.* 1991). The megastigmane glycoside **14** was identified as roseoside by comparison of NMR spectral data with those reported in the literature (Bhakuni *et al.* 1974). The remaining two glycosides were identified as (**15**) 2-((6-*O*-(β-D-apiofuranosyl)-β-D-glucopyranosyl)oxy)propane and (**16**) 2-((6-*O*-(β-D-apiofuranosyl)-β-D-glucopyranosyl)oxy)butane, which were first isolated from the fresh root cortex of *Manihot esculenta* (Prawat *et al.* 1995).

All the compounds isolated from *E. tapos* were known compounds. Phenolics were found to be the major constituents present in the leaves. Many of the compounds have been evaluated for biological and therapeutic properties and significant results were reported. Examples are the protective activity against glutamate-induced neuronal death, anti-inflammatory and anti-mutagenesis activities of catechin (Monteith 1990, Shimada *et al.* 2001, Nardi *et al.* 2003), anti-oxidant and hair-growth stimulating activities of procyanidin B-3 (Takahashi *et al.* 1999, Kamimura & Takahashi 2002, Stevens *et al.* 2002) and anti-inflammatory, anti-depression, anti-viral, and Ca^{2+} releasing activity in skeletal muscle sarcoplasmic reticulum of amentoflavone (Suzuki *et al.* 1999, Ma *et al.* 2001, Banerjee *et al.* 2002; Butterweck *et al.* 2002, Kim *et al.* 2002). Although *E. tapos* is not classified as a major medicinal plant, the results of this study provided some understanding on the chemical constituents of the plant and also showed that the plant could be a potential source of some useful phytochemicals.

Experimental

General: Optical rotations were measured on a JASCO DIP-370 digital polarimeter. ^{1}H- and ^{13}C-NMR spectra were recorded in ppm (δ) in CD_3OD, acetone-d_6, or DMSO-d_6 with TMS as the internal standard, employing Varian Unity plus 500 and Varian Gemini 300 spectrometers operating at 500 and 300 MHz for ^{1}H, and 125 and 75 MHz for ^{13}C. Column chromatography was performed with MCI gel CHP 20P (75–150 μm, Mitsubishi Chemical Co., Ltd.), Diaion HP20-SS (75–150 μm, Mitsubishi Chemical Co., Ltd.), Chromatorex ODS (100–200 mesh, Fuji Silysia Chemical Ltd.), Sephadex LH-20 (25–100 μm, Pharmacia Fine Chemical Co., Ltd.), Toyopearl HW-40F (Tosoh Co., Ltd.) and silica gel 60 (0.040–0.063 mm, 0.063–0.200 mm, Merck). TLC was performed on precoated silica gel 60 F_{254} plates (0.2 mm thick, Merck) with chloroform:methanol:water (9:1:0.1, or 8:2:0.1, or 7:3:0.5, or 6:4:0.5 v/v), or benzene:ethyl formate:formic acid (1:7:1 v/v) and spots were detected by UV illumination, and by spraying with 10% sulphuric acid (H_2SO_4) followed by heating, or by spraying with 2% ethanolic iron (III) chloride ($FeCl_3$) reagent.

Plant materials: Leaves and wood of *E. tapos* were collected from the forest reserve at the Forest Research Institute Malaysia (FRIM), Kepong in July 1999. A voucher specimen (FRI 45993) was deposited at the herbarium of FRIM.

Extraction and isolation: Fresh leaves (2.0 kg) were cut into small pieces and extracted with methanol (MeOH) by soaking at room temperature for three times. In the case of wood, the sample (3.0 kg) was chopped and ground into fine particle which was then extracted following the same procedure as the leaves. The solvent was evaporated under reduced pressure to obtain the dry crude extracts of leaves and wood (139.0 g and 65.0 g respectively). The MeOH extract of the leaves was suspended in water (H_2O) and successively partitioned with *tert*-butyl methyl ether (*t*-BuOMe) and ethyl acetate (AcOEt) to obtain the *t*-BuOMe fraction (75.1 g), AcOEt fraction (9.2 g) and H_2O fraction (50.6 g). The H_2O fraction was subsequently column-chromatographed over Diaion HP20-SS eluted with H_2O with increasing proportions of MeOH to obtain three fractions. Fraction 2 (11.6 g) was further fractionated and purified using a combination of column chromatography employing Sephadex LH-20, MCI gel CHP 20P, Chromatorex ODS and silica gel to afford **1** (200.0 mg), **2** (54.5 mg), **3** (635.0 mg), **4** (24.2 mg), **5** (381.9 mg), **6** (29.5 mg), **8** (8.1 mg), **9** (28.3 mg), **10** (14.8 mg) and **12** (4.8 mg). Fraction 3 (20.6 g) was column-chromatographed over Sephadex LH-20 (40 → 60% MeOH), followed by Chromatorex ODS (H_2O →100% MeOH) and silica gel ($CHCl_3$:MeOH:H_2O-9:1:0.1 → 7:3:0.5) to afford **14**

(17.9 mg). The AcOEt fraction was chromatographed over Sephadex LH-20 and eluted with ethanol (EtOH) (100 → 95%), followed by further purification using MCI gel CHP 20P and Chromatorex ODS to give **3** (55.4 mg), **4** (88.8 mg) and **5** (74.8 mg). The *t*-BuOMe fraction was fractionated using silica gel column chromatography with $CHCl_3$:MeOH:H_2O-9:1:0.1 → 5:5:1, MeOH, to afford four fractions. Fraction 3 (18.7 g) was further purified by silica gel column chromatography eluted with the same solvent system to give another four sub-fractions. Sub-fraction 1 (7.8 g) was purified by repeated silica gel column chromatography ($CHCl_3$:MeOH:H_2O-10:0:0 → 5:5:1; AcOEt:MeOH-9:1; $CHCl_3$:MeOH:H_2O-95:5:0 → 6:4:1; and $CHCl_3$:MeOH:H_2O-10:0:0 → 7:3:0.5) to afford **7** (368.3 mg) and **13** (139.7 mg). The MeOH extract of the wood was suspended in H_2O, followed by filtration to remove the precipitates. The filtrate was partitioned with diethyl ether (Et_2O) to give the Et_2O fraction and H_2O fraction. The H_2O fraction was column-chromatographed over Sephadex LH-20 (H_2O → 100% MeOH) to afford four fractions. Fraction 1 (36.7 g) was fractionated using Diaion HP20-SS (80 → 100% MeOH), followed by further purification using MCI gel CHP 20P (H_2O → 100% MeOH), silica gel ($CHCl_3$:MeOH:H_2O-8:2:0.2 → 5:5:0.5), Chromatorex ODS (H_2O → 40% MeOH), and Toyopearl HW-40F (H_2O → 10% MeOH) to give **11** (24.0 mg), **15** (401.0 mg) and **16** (70.0 mg).

Amentoflavone (**7**): Yellow powder; $[\alpha]_D^{24}$+5.1° (*c* 0.3, acetone); ^{1}H-NMR (500 MHz, acetone-d_6) δ_H 8.08 (1H, d, *J* = 2.5 Hz, H-2'), 8.02 (1H, dd, *J* = 2.5, 8.7 Hz, H-6'), 7.63 (2H, d, *J* = 6.9 Hz, H-2''', 6'''), 7.28 (1H, d, *J* = 8.7 Hz, H-5'), 6.84 (2H, d, *J* = 6.9 Hz, H-3''', 5'''), 6.73 (1H, s, H-3), 6.66 (1H, s, H-3''), 6.53 (1H, d, *J* = 2.1 Hz, H-8); 6.50 (1H, s, H-6''), 6.27 (1H, d, *J* = 2.1 Hz, H-6); ^{13}C-NMR (125 MHz, acetone-d_6) δ_C 183.3 (C-4''), 182.9 (C-4), 165.1 (C-2, 2'')*, 165.0 (C-7)*, 162.7 (C-5, 5''), 162.0 (C-7''), 161.9 (C-4'''), 160.3 (C-4'), 158.7 (C-9), 155.9 (C-9''), 132.4 (C-2'), 129.0 (C-2''', 6'''), 128.6 (C-6'), 122.8 (C-1', 1'''), 121.0 (C-3'), 117.1 (C-5'), 116.6 (C-3''', 5'''), 105.0 (C-10, C-10''), 104.7 (C-8''), 104.0 (C-3), 103.5 (C-3''), 99.6 (C-6), 99.5 (C-6''), 94.8 (C-8).

Linamarin (**8**): White amorphous solid; ^{1}H-NMR (300 MHz, CD_3OD) δ_H 4.60 (1H, d, *J* = 8.0 Hz, H-1'), 3.85 (1H, dd, *J* = 2.0, 12.0 Hz, H-6a'), 3.65 (1H, dd, *J* = 5.0, 12.0 Hz, H-6b'), 3.30-3.40 (3H, m, H-3', 4', 5'), 3.20 (1H, t, *J* = 8.0 Hz, H-2'), 1.65 (6H, s, H-3, 4); ^{13}C-NMR (75 MHz, CD_3OD) δ_C 122.2 (C-1), 99.8 (C-1'), 79.9 (C-2)*, 77.0 (C-5')*, 76.4 (C-3'), 73.7 (C-2'), 70.4 (C-4'), 61.5 (C-6'), 27.7 (C-4), 27.1 (C-3).

Lotaustralin (**9**): White amorphous solid; ^{1}H-NMR (300 MHz, CD_3OD)δ_H 4.60 (1H, d, *J* = 8.0 Hz, H-1'), 3.85 (1H, dd, *J* = 2.0, 12.0 Hz, H-6a'), 3.70 (1H, dd, *J* = 5.0, 12.0 Hz, H-6b'), 3.30-3.40 (3H, m, H-3', 4', 5'), 3.20 (1H, t, *J* = 8.0 Hz, H-2'), 1.90 (2H, m, H-4), 1.60 (3H, s, H-3), 1.10 (3H, t, *J* = 7.0 Hz, H-5); ^{13}C-NMR (75 MHz, CD_3OD) δ_C 121.9 (C-1), 99.5 (C-1'), 77.0 (C-5')*, 76.7 (C-2)*, 76.5 (C-3'), 73.7 (C-2'), 70.4 (C-4'), 61.5 (C-6'), 33.9 (C-4), 24.1 (C-3), 8.7 (C-5).

*These assignments are interchangeable within each spectrum.

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