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# Chemical Constituents of *Vitex ovata* (Verbenaceae)

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**ABSTRAK**

Tiga sebatian, luteolin, asid ursolik dan asid *meta*-hidroksibenzoik telah diasingkan daripada daun *Vitex ovata*. Struktur sebatian tersebut telah dikenalpasti melalui analisis spektroskopi.

**ABSTRACT**

Three compounds, luteolin, ursolic acid and *meta*-hydroxybenzoic acid were isolated from the leaves of *Vitex ovata*. The structures of the compounds were identified using modern spectroscopic techniques.

**Keywords: luteolin, ursolic acid, *meta*-hydroxybenzoic acid, *Vitex ovata*, Verbenaceae**

## INTRODUCTION

*Vitex* is a shrub of family Verbenaceae commonly found throughout the Asia Pacific. There are approximately 140 species distributed in the tropics or sub-tropics of which 16 have been identified in Peninsular Malaysia (Ng 1978). Some species of this genus such as *Vitex negundo, V. cannabifolia,* and *V. stricker* have been extensively studied (Dutta *et al.* 1983; Zhang *et al.*1992; Iwagawa *et al.* 1993). *Vitex ovata is* a small shrub usually found in the lowland forest. It is known locally as *tetuban* and used for treating dysentery and stomach discomfort (Burkill 1936).

## MATERIALS AND METHODS

### *Plant Materials*

*Vitex ovata* was collected from Kuantan, Malaysia and the voucher specimen was deposited at the herbarium of the Biology Department, Universiti Pertanian Malaysia.

### *General*

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. The UV spectra were recorded on Shidmazu UV-VIS 160 and IR spectra on Perkin Elmer 1600 FTIR spectrometers. Mass spectra were recorded on a Finnigan MAT SSQ 710 spectrometer with ionization being induced by electron impact at 70 eV. $^{1}$H- and $^{13}$C-NMR spectra were recorded on a JEOL JNM 500 spectrometer at 500 and 125 MHz, for $^{1}$H and $^{13}$C, respectively. Column chromatography and analytical TLC utilized Merck 7734 and Merck DC-Plastikfollen 60 $F_{254}$, respectively.

### *Extraction of Plant Materials*

The leaves of *Vitex ovata* (5 kg) were soaked in methanol for 72 hours. The solvent was removed by filtration and fresh methanol was then added. The methanol extracts were combined and evaporated under reduced pressure to give a greenish mass (125 g). The crude extract was then partitioned between petroleum ether and water. The aqueous layer was extracted successively with chloroform, ethyl acetate and then butanol. The crude ethyl acetate extract was then subjected to column chromatography on silica gel and eluted with chloroform followed by $CHCl_3$:MeOH mixture in increasing polarity. Thirty-six fractions were collected and fractions with a similar pattern on analytical TLC were combined and further purified before the pure compounds were isolated.

### *Isolation of Luteolin [1]*

The combined fractions 13-16 (40 mg) from the column chromatography were subjected to preparative thin layer chromatography on silica gel using $CHCl_3$/MeOH (70:30) as solvent to afford luteolin (12 mg), m.p. 345-348°C (lit. m.p. 328-330°C, Buckingham 1994).
UV $\lambda_{max}$ nm (MeOH, log ε): 266.0 (1.22), 333 (0.56); IR $\nu_{max}$ cm$^{-1}$(KBr disk): 3424, 2928, 1656, 1612; $^{1}$H-NMR (500 MHz, $CDCl_3$+ $CD_3OD$): 7.37 (dd, 1H,$J_{6',5'}$ = 9.0 Hz, $J_{6',2'}$ = 2.4 Hz, H-6'), 6.94 (d, 1H, $J_{2',6'}$ = 2.3 Hz, H-2'), 6.93 (d, 1H, $J_{5',6'}$ = 9.0 Hz, H-5'), 6.51 (s, H-3), 6.44 (d, 1H, $J_{8,6}$ = 2.4 Hz, H-8), 6.28 (d, 1H, $J_{6,8}$ = 2.3 Hz, H-6); $^{13}$C-NMR (125 MHz, $CDCl_3$+ $CD_3OD$, DEPT Experiment): 182.4 (C-4), 164.5 (C-7), 163.9 (C-2), 161.3 (C-5), 157.8 (C-9), 148.8 (C-4'), 144.9 (C-3'), 122.5 (C-1'), 119.1 (C-6'), 115.2 (C-5'), 112.7 (C-2'), 104.3 (C-10), 102.9 (C-3), 99.1 (C-6), 94.1 (C-8); MS m/z (%): 286 ($M^+$, 100), 134 (7), 152 (5);

*Isolation of Ursolic acid [2]*

This combined fractions 1-4 from the column chromatography gave a whitish powder, m.p. 269-271°C (lit. m.p. 266-267 °C, Takagi *et al.* 1979). UV $\lambda_{max}$ nm (MeOH, log ε): 470 (0.14), 441 (1.18), 421 (0.17); IR $\nu_{max}$ $cm^{-1}$ (KBr disk): 3424, 1693, 1540, 1272, 1092, 996: $^{1}$H-NMR (500 MHz, $CDCl_3$ + $CD_3OD$): 5.24 (m, 1H, H-12), 3.38 (s, 1H, H-3), 2.18 (d, 1H, $J_{18,19}$ = 11.3 Hz, H-18), 2.02 - 1.15 (m, 22H), 1.14 (s, H-27, Me), 1.08 (s, H-25, Me), 0.96 (s, H-23, Me), 0.95 (s, H-30, Me), 0.90 (d, H-29, Me), 0.78 (s, H-24, Me), 0.77 (s, H-26, Me); $^{13}$C-NMR (125 MHz, $CDCl_3$ + $CD_3OD$, DEPT Experiment): 181.0 (C-28), 138.4 (C-13), 125.6 (C-12), 79.0 (C-30), 55.4 (C-18), 53.0 (C-5), 47.7 (C-17), 46.6 (C-9), 42.2 (C-14), 41.9 (C-9), 41.4 (C-20, C-19), 39.6 (C-22), 39.4 (C-22), 38.6 (C-1), 34.0 (C-7), 31.0 (C-21), 29.8 (C-15), 28.1 (C-2), 26.0 (C-16), 24.4 (C-11), 23.6 (C-27), 21.3 (C-30), 18.4 (C-6), 17.1 (C-29), 17.0 (C-26), 16.9 (C-25), 15.7 (C-24); MS m/z (%): 456 ($M^+$), 248 (100), 207 (27), 203 (42.6)

*Isolation of meta-hydroxybenzoic acid [3]*

This minor component from combined fractions 14-16 was identified as *meta*-hydroxybenzoic acid, melting point 210-213°C (lit. m.p. 202 °C, Buckingham 1992). The $^{13}$C-NMR are similar to those reported in the literature (Scott 1972).

## RESULTS AND DISCUSSION

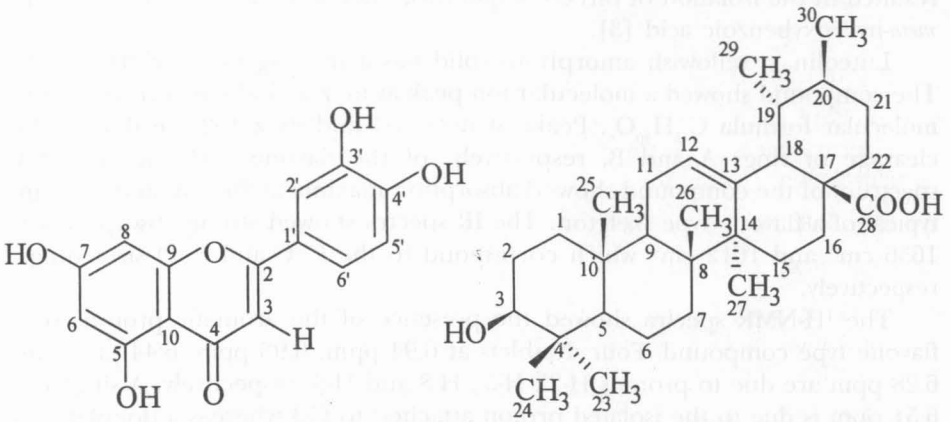
Chemical investigations of the crude ethyl acetate extract of *Vitex ovata* have resulted in the isolation of three compounds, luteolin [1], ursolic acid [2] and *meta*-hydroxybenzoic acid [3].

Luteolin, a yellowish amorphous solid has a melting point of 345-348°C. The compound showed a molecular ion peak at m/z 286 which corresponds to molecular formula $C_{15}H_{10}O_6$. Peaks at m/z 134 and m/z 152 are due to the cleavage of rings A and B, respectively, of the flavone agylcone. The UV spectrum of the compound showed absorption maxima at 266 nm and 333 nm, typical of a flavone type skeleton. The IR spectra showed strong absorptions at 1656 $cm^{-1}$ and 1612 $cm^{-1}$ which correspond to the C=C and C=O stretchings, respectively.

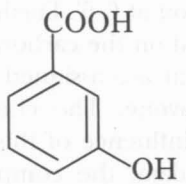
The $^{1}$H-NMR spectra showed the presence of the aromatic protons of a flavone type compound. Four doublets at 6.94 ppm, 6.93 ppm, 6.44 ppm and 6.28 ppm are due to protons H-2', H-5', H-8 and H-6, respectively. A singlet at 6.51 ppm is due to the isolated proton attached to C-3 whereas a doublet of a doublet at 7.37 ppm is due to a proton at C-6'. Further information to establish the structure was accomplished based on the carbon-13 data of the compound. Signals at 163.9 ppm and 102.9 ppm are assigned to C-2 and C-3 which are characteristic of a dehydro-type flavone. The chemical shift of a carbonyl carbon at 184.2 ppm is due to the influence of this double bond carbon, C-2 and C-3. Based on these spectral data, the compound is concluded to be luteolin [1] (Harborne and Mabry 1981; Markham 1982).

The UV spectrum of the second compound showed absorptions at 421, 427, and 444 nm and the IR spectrum exhibited absorptions at 3424 $cm^{-1}$ (hydroxyl) and 1693 $cm^{-1}$ (carbonyl). The compound displayed a molecular ion peak at m/z 456 which corresponds to molecular formula $C_{30}H_{48}O_3$. A base peak at m/z 248 is a typical retro Diels Alder cleavage of an α- or β- type triterpene. Comparison of the carbon-13 with the literature values for the signals at C-12, C-13, C-18, C-19 and C-20 indicated that the compound is an α-type triterpene (Doddrell *et al.* 1974). This conclusion was further supported by the $^1$H-NMR spectrum of the compound with the doublet at 2.20 ppm having J value of 11.3 Hz indicating that the protons at C-18 and C-19 are *trans* to each another. This observation further suggests that the triterpene is of the α-type and not the - type. Based on these spectroscopic data and also by comparison with those reported in the literature, the compound was assigned as ursolic acid [2] (Romeo *et al.* 1977; Kriwacki and Pitner 1989) .

*Meta*-hydroxybenzoic acid [3] is a white crystalline compound which melted at 210-213°C. The mass spectrum showed a molecular ion peak at m/z 138 which corresponds to molecular formula $C_7H_6O_3$. The IR spectrum showed strong absorptions at 3414 $cm^{-1}$ and 1683 $cm^{-1}$ for the hydroxyl and carbonyl stretchings, respectively. The $^1$H-NMR spectra showed the presence of two doublets at 7.86 ppm and 6.80 ppm. The former is due to H-6 coupled to the proton attached to C-5 (*ortho* coupling) and the later also shows *ortho* coupling between H-4 and H-5. A multiplet at 3.31 ppm is due to proton H-5 which is



[1] luteolin [2] ursolic acid



[3] *meta*-hydroxybenzoic acid

coupled to both H-4 and H-6. The proton attached to C-2 resonates as a singlet at 4.87 ppm. The $^{13}$C-spectral data were consistent with those of the literature (Scott 1972).

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