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Phytochemical Investigation of the Leaves of *Flaveria trinervia*

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Abstract – Phytochemical study of the methanolic extract of *Flaveria trinervia* (Asteraceae) leaves has led to the isolation of three constituents characterised as 3,5,7,4' tetrahydroxy-6-methoxy flavone (6-methoxy kaempferol), oleanolic acid and β-sitosterol-β-D-glucoside. The identities of the compounds were confirmed by the physical and spectroscopic data and by comparison with authentic samples.
Keywords – *Flaveria trinervia*, 6-methoxy kaempferol, oleanolic acid, β-sitosterol-β-D-glucoside

Introduction

Flaveria trinervia (Spreng.) C. Mohr, Syn: *F. australasica* HK (Asteraceae) is an dichotomously branched herb. Leaves are opposite, oblong, auricled at base, dentate. In Tamil it is called as Namakayyan The plant is native to Australia, and is widely distributed in Chengalpattu, Coimbatore, Dharmapuri, Salem, Tiruchirappalli and Tirunelveli (Tamil Nadu). It is reported that the leaf juice of the plant is used to overcome jaundice and in skin diseases (Yoganarasimhan *et al.*, 2000) and the methanolic extract of the plant leaf is proved as hepatoprotective (Umadevi *et al.*, 2004). Patuletin-3-*O*-β-glucoside (Wagner *et al.*, 1971) and sulfur compounds (Downum *et al.*, 1988) are reported in the plant. As part of our continuing phytochemical study of the plant, we now report the isolation of three constituents from the methanolic extract of the plant leaves.

Experimental

Plant material – The leaves of the plant was collected from Tirunelveli, Tamil Nadu, in January 2003 and authenticated by Dr. V. Chelladurai, Survey of Medicinal Plants Unit (CCRAS), Palayamkottai, Tamil Nadu. A voucher specimen of the plant has been deposited in the Department of Pharmacy, Annamalai University, Tamil Nadu.

Extraction – Dried and coarsely powdered leaves of the plant (2 Kg) were extracted with methanol in the cold (72 hr). The methanolic extract was concentrated under reduced pressure and dried in vacuum (Yield - 13.5%).

Preliminary phytochemical test – The preliminary investigation of the extract showed the presence of flavonoid, sterol, triterpenoid and saponin.

Extraction and isolation – The extract was chromatographed over silica gel (100-200 mesh) and eluted with solvents of increasing polarity, in the order of hexane, benzene, chloroform, and ethylacetate, collecting 100 ml each. The corresponding eluates were combined depending on TLC profiles.

3,5,7,4'- tetrahydroxy -6 -methoxy flavone (6-methoxy kaempferol) (**1**). Elution of the column with Chloroform : ethylacetate 19:1 gave a flavonoid, which was crystallized from methanol (mp 269-271°C) as pale yellow needles. It gave positive ferric reaction and reddish pink color on Shinoda test (Mg/HCl). TLC over silica gel G gave a single spot with benzene: ethylacetate 4:1 as the developing system (R_f = 0.12).

UV λ_{max} (MeOH) nm: 269, 294 (sh), 344 (sh), 367; (+NaOAc) 272, 298, 312 (sh), 378; (+NaOAc/H_3BO_3) 270, 344 (sh), 368; (+$AlCl_3$) 271, 306 (sh), 375 (sh), 426; (+$AlCl_3$/HCl) 271, 305 (sh), 367, 425; (+NaOMe) 280, 316 (sh), 423. IR υ_{max} (KBr) cm^{-1}: 3306, 2900, 2852, 1640, 1602, 1464, 1376, 1262, 1091, 877, 801. ^{1}H-NMR (400 MHz, CD_3OD) δ ppm: 6.40 (1H, s, H-8), 7.98 (2H, d, *J* = 8.6 Hz, H-2' and H-6'), 6.80 (2H, d, *J* = 8.6 Hz, H-3' and H-5'), 3.79 (3H, s, -OMe). ^{13}C-NMR (100 MHz, CD_3OD) δ ppm: 156.6 (C-2), 134.9 (C-3), 175.7 (C-4), 151.7 (C-5), 130.2 (C-6), 156.6 (C-7), 92.8 (C-8), 151.0 (C-9), 103.0 (C-10), 121.8 (C-1'), 128.8 (C-2'), 114.3 (C-3'), 158.6 (C-4'), 114.3 (C-5'), 128.8 (C-6'), 59.0 (-OCH_3).

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EIMS *m/z* 316 $[M]^+$, 301 $[M - Me]^+$, 298 $[M - 18]^+$, 273 $[M - COMe]^+$, 183 $[A_1 + H]^+$, 167 $[A_1 - Me]^+$, 121 $[B_2]^+$, 93 $[B_2 - CO]^+$. The structure was further confirmed by comparison of spectroscopic data with the reported values (Rodriguez *et al.*, 1972; Michel Goudard *et al.*, 1978; Denis Barron *et al.*, 1987).

Oleanolic acid (**2**). Elution of the column with $CHCl_3$: ethyl acetate 9:1, gave a triterpenoid which was crystalised from acetone as colorless crystals (mp 306°C). It gave a single spot on TLC over silica gel G with chloroform : methanol 19:1 as the developing system (R_f = 0.60). It gave positive Lieberman Burchard reaction for triterpenoid. The compound was identified as oleanolic acid by comparison with an authentic sample (mp, mmp, Co-TLC and superimposable IR).

IR υ_{max} (KBr) cm^{-1}: 3421, 2900, 2852, 1690, 1630, 1464, 1386, 1304, 1271, 1180, 1032, 820, 762. ^{1}H-NMR (400 MHz, CD_3OD) δ ppm: 0.71-1.16 (7 x CMe), 2.76 (1H, br, H-18), 3.10 (1H, br, H-3), 5.14 (1H, t, *J* = 3 Hz, H-12). ^{13}C-NMR (100 MHz, CD_3OD) δ ppm: 38.54 (C-1), 26.83 (C-2), 77.70 (C-3), 38.54 (C-4), 54.75 (C-5), 18.80 (C-6), 32.0 (C-7), 40.72 (C-8), 47.43 (C-9), 36.17 (C-10), 22.52 (C-11), 121.56 (C-12), 143.20 (C-13), 40.72 (C-14), 28.59 (C-15), 24.41 (C-16), 45.62 (C-17), 40.72 (C-18), 46.37 (C-19), 31.59 (C-20), 32.97 (C-21), 31.60 (C-22), 28.69 (C-23), 14.33 (C-24), 15.71 (C-25), 17.51 (C-26), 25.86 (C-27), 179.86 (C-28), 34.47 (C-29), 32.96 (C-30). The structure was further confirmed by comparison of spectroscopic data with the reported values (Wagner *et al*, 1984., Tori *et al*, 1974., Aoki *et al*, 1982).

β-sitosterol-β-D-glucoside (**3**). Elution of the column with ethylacetate gave a sterol glycoside which was crystalised from acetone (mp 284°C). TLC over silica gel G gave a single spot with benzene : ethylacetate 9:1 as the developing system (R_f = 0.7). It gave green color on LB reaction and gave positive anthrone/sulfuric acid test for glycoside. The compound was identified as β-sitosterol-β-D-glucoside by comparision with an authentic sample (mp, mmp, Co-TLC and superimposable IR).

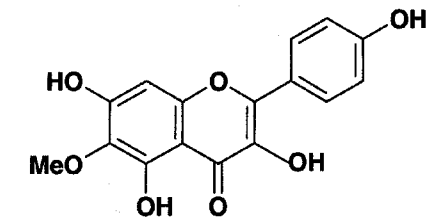
Results and Discussion

The compound 1 was obtained as a pale yellow needle. It analyzed for $C_{16}H_{12}O_7$. The IR spectrum of 1 revealed absorptions due to a hydroxyl groups appeared as a broad band at 3306 cm^{-1}. Flavonoid carbonyl appeared at 1640 cm^{-1} and the presence of aromatic system showed by the bands at 1602, 877, 801 cm^{-1}.

The UV spectrum in methanol showed maxima at 269 and 367 nm for band II and band I respectively. The effect of shift reagents on the UV spectrum was studied (Judd, 1962). On addition of sodium acetate, band II shifts from 269 nm to 298 nm ($\triangle\lambda$ = 29 nm). This showed the presence of 7 OH. Addition of boric acid did not cause any changes, showed the absence of *ortho* dihydroxy function. The shift of 58 units of band I with aluminium chloride and hydrochloric acid showed the presence of both 3 and 5 OH groups. Addition of sodium methoxalate caused the band I to shift by 56 nm. This showed the presence of 4' OH. The datas were comparable with the reported value for the reference compound 6-methoxy kaempferol.

The ^{1}H-NMR also confirmed the structure, H-8 appeared as a singlet 6.40, the two sets of *ortho* coupled protons in the ring B which form an A_2, B_2 pattern appeared as two 2H doublets (*J* = 8.6 Hz) at δ 7.98 (H-2' and H-6') and 6.8 (H-3' and H-5'). The methoxy groups at C-6 appeared as 3-proton singlet at δ 3.79.

The ^{13}C-NMR spectrum also confirmed the structure. The assignments were based on model compounds. The mass spectrum gave $[M]^+$at *m/z* 316. The main fragments due to cleavage of C1-C2 and C3-C4 bonds appeared at *m/z* 183 and 121 [A_1 and B_2]. The loss of methyl group together with hydrogen from the $[A_1 + H]$ fragment gave



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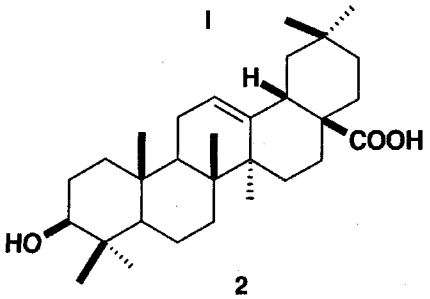

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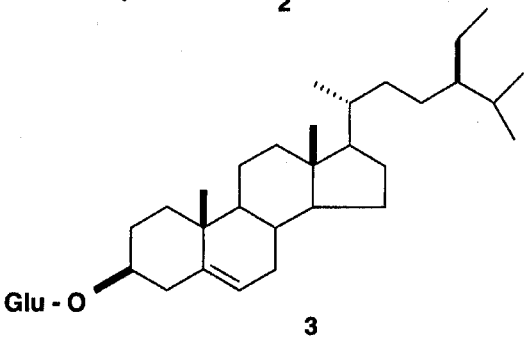

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the fragment at *m/z* 167. This confirmed the presence of OMe group in ring A at C-6. The presence of OH group in the ring B was proved by the fragment *m/z* 121.

The compound 2 was obtained as colorless crystals. It analyzed for $C_{30}H_{48}O_3$. The IR spectrum of 2 revealed absorptions due to a hydroxyl 3421 cm^{-1}, the acid carbonyl appeared at 1690 cm^{-1}, the trisubstituted double bond was showed by peak at 1630 and 820 cm^{-1} and *gem* dimethyl appeared at 1386 cm^{-1}. ^{1}H-NMR spectrum indicated 7 tertiary methyl groups δ 0.71-1.16, the axial proton at H-3 appeared as a broad multiplet at δ 3.10, the olefinic proton H-12 appeared as a distorted triplet at δ 5.14, the axial proton H-18 at the D/E junction appeared as a distorted quardret at δ 2.76 characteristics of oleanane triterpenoids. ^{13}C-NMR spectrum also confirmed the structure as oleanolic acid by showing all the relavant signals. β-sitosterol-β-D-glucoside was also identified in this plant.

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