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# Reassessment of structures of flavonoids isolated from the fruits of *Pongamia pinnata* (L.) Pierre

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**Abstract**
*Pongamia pinnata* (L.) Pierre, syn. *Millettia pinnata* (L.) Panigrahi; (family Fabaceae), is a native to tropical and temperate Asia. Its fruits, seeds and sprouts are used to treat abdominal tumours, bronchitis, fever, whooping cough, lumbago, piles, urinary discharges and diseases of the brain, eye, head and skin. A methanolic extract of the defatted fruits was subjected to silica gel column chromatography using petroleum ether, chloroform and methanol as eluants in order of increasing polarity to isolate the chemical constituents. The structures of the isolated phytoconstituents were elucidated on the basis of the spectral data analysis and chemical reactions. Four new flavonoids characterized as 5,6-furano-7,8-furano-4′-methoxyflavanol (pinnataflavonol, 1), *cis*-,*cis*-13-(3′-methoxy-4′-hydroxy- chalconyl-(13→13′)-3,4-dimethoxychalcone (pinnatabichalcone, 2), 5,7,2′,3′-tetrahydroxy-4′-methoxy-8-ethylflavonyl-(6→8a)-5a,7a,2′a,3′a,4′a-pentahydroxyflavone (pinnatabiflavone, 3) and *cis*,*cis*-13-(3′-methoxy-4′-hydroxychalconyl-(13→13′)-3-*n*-tetradecanyloxy-4-methoxychalcone (pinnatabichalconyl myristate, 6) together with the known compounds lanost-5-en-3β-ol 3β-D-glucopyranoside (4) and glyceryl 1-octadec-9′-enoate 2-tetradecanoate 3-phosphate (5) were isolated. All the phytoconstituents are reported from *P. pinnata* fruits for the first time.

**Keywords:** *Pongamia pinnata* (L.) Pierre, fruits, furano flavanol, bichalcones, biflavone, structure elucidation

## 1. Introduction
*Pongamia pinnata* (L.) Pierre, syn. *Millettia pinnata* (L.) Panigrahi (family Fabaceae), known as karanja, is a native to tropical and temperate Asia including parts of India, China, Japan, Malaysia and Australia[1]. Its fruits, seeds and sprouts are used to treat abdominal tumours, bronchitis, fever, whooping cough, lumbago, piles, urinary discharges and diseases of the brain, eye, head and skin [2-4]. The oil has a high content of triglycerides and its disagreeable taste and odour are due to bitter flavonoid constituents. The fruits and seeds possessed karanjin, kojnone, pongamol, pongapin, pinnatin, pongagalabrone, tannins, karanjachromene and pongamosides [5]. The leaves, root bark and stem of the plant contained several flavone and chalcone derivatives [6]. This manuscript describes the isolation and characterization of two bichalcones, and one each of furanoflavonol and biflavone from the fruits of *P. pinnata* of Delhi region.

## 2. Material and Methods
### 2.1. General procedure
Melting points were determined on a Perfit melting apparatus (Ambala, Haryana, India) and are uncorrected. UV spectra were measured with a Lambda Bio 20 spectrophotometer (Perkin-Elmer-Rotkreuz, Switzerland) in methanol. Infrared spectra were recorded on Bio-Rad FTIR 5000 (FTS 135, Kawloon, Hong Hong) spectrophotometer using KBr pellets; $\gamma_{max}$ values are given in $cm^{-1}$. The $^{1}H$ and $^{13}C$ NMR spectra were screened on Advance DRX Bruker spectrospin 400 and 100 MHz, respectively, instruments (Karlesruthe, Germany) using $CDCl_3$ or DMSO-$d_6$ as a solvent and TMS as an internal standard. Mass spectra were scanned by effecting ionization at 70 eV on a JEOL-JMS-DX 303 spectrometer (Japan) equipped with direct inlet probe system. Column chromatography was performed on silica gel (60-120 mesh; Qualigen, Mumbai, India). TLC was run on silica gel G (Qualigen). Spots were visualized by exposing to iodine vapours, UV radiation and spraying with ceric sulphate solution.

### 2.2. Plant material
The fruits of *P. pinnata* were collected from the Jamia Hamdard campus, New Delhi and identified by Dr. Javed Ahmad, Department of Botany, Jamia Hamdard. A voucher specimen No. PRL-OO1-07 has been retained in the Phytochemistry Research Laboratory, Jamia Hamdard.

### 2.3. Preparation of extract
The dried fruits (2.2 kg) were coarsely powdered, defatted with *n*-hexane and extracted exhaustively in a Soxhlet apparatus with methanol. The methanolic extract was concentrated under reduced pressure to yield a dark brown viscous mass (158 g; 7.2% yield). A small portion of the extract was analyzed chemically to determine the presence of different chemical constituents.

### 2.4. Isolation of phytoconstituents
The viscous dark brown extract (150 g) was dissolved in small quantity of methanol and adsorbed onto silica gel (60-120 mesh) for preparation of a slurry. The slurry was air dried and subjected to chromatography over silica gel column packed in petroleum ether. The column was eluted successively with petroleum ether, mixture of petroleum ether- chloroform (9:1, 3:1, 1:1, 1:3), chloroform and the mixture of chloroform-methanol (99:1, 97:3, 95:5, 92:8, 9:1, 3:1, 1:1, 1:3). Various fractions were collected separately and matched by TLC to check the homogeneity. Similar fractions having the same $R_f$ values were combined and crystallized. The isolated compounds were recrystallized to get the following compounds:

### 2.5. Pinnataflavonol (1)
Elution of the column with petroleum ether-chloroform (1:3) afforded pale yellow crystals of 1, recrystallized from chloroform-methanol (1:1); $R_f$ value: 0.80 (chloroform-methanol, 99:1); m.p. 139-140 °C; 794 mg (0.53% yield); UV $\lambda_{max}$ (MeOH): 219, 260, 331 nm (log ε 5.6, 4.9, 2.5); IR $\nu_{max}$ (KBr): 3553, 3047, 2924, 1662, 1625, 1550, 1448, 1365, 1250, 1180, 1105 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): δ 8.17 (1H, d, *J* = 2.3 Hz, H-12), 8.13 (1H, d, *J* = 2.6 Hz, H-14), 8.12 (2H, d, *J* = 9.0 Hz, H-2′, H-6′), 7.73 (1H, d, *J* = 2.3 Hz, H-12), 7.54 (1H, d, *J* = 2.3, Hz, H-11), 7.15 (2H, dd, *J* = 9.0 Hz, H-3′, H-5′ ), 3.87 (3H, brs, OMe); $^{13}C$ NMR ($CDCl_3$): 145.69 (C-2), 130.93 (C-3), 175.12 (C-4), 159.93 (C-5), 129.13 (C-6), 158.14 (C-7), 129.66 (C-8), 154.89 (C-9), 104.25 (C-10), 110.22 (C-11), 131.60 (C-12), 110.02 (C-13), 130.71 (C-14), 121.85 (C-1′), 117.01 (C-2′), 126.19 (C-3′), 141.80 (C-4′), 129.18 (C-5′), 119.68 (C-6′), 60.24 (OMe); EIMS *m/z* (rel. int.): 348 $[M]^+$ ($C_{20}H_{12}O_6$) (5.3), 333 (10.1), 317 (12.9), 200 (17.1), 148 (11.3).

### 2.6. Pinnatabichalcone (2)
Elution of the column with chloroform-methanol (49:1) yielded a pale yellow crystalline powder of 2, recrystallized from acetone-methanol (1:1); 498 mg, $R_f$ value: 0.89 (chloroform-methanol, 49:1); m.p.:132-133°C; 503 mg (0.33% yield); UV $\lambda_{max}$ (MeOH) 218, 241, 346 (log ε 2.6, 5.2, 4.6); IR$\nu_{max}$ (KBr): 3482, 2927, 2948, 1693, 1586, 1543, 1456, 1259, 1135, 977, 856 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): δ 7.97 (1H, d, *J* = 8.1 Hz, H-5), 7.90 (1H, d, *J* = 2.8 Hz, H-2′), 7.87 (1H, d, *J* = 8.9 Hz, H-8), 7.85 (1H,d, *J* = 8.7 Hz, H-8′), 7.63 (1H, d, *J* = 2.8 Hz H-2), 7.55 (1H, d, *J* = 7.3 Hz, H-5′), 7.52 (1H, m, H-6), 7.50 (1H, m, H-6′), 7.48 (2H, m, H-11, H-11′), 7.45 (2H, m, H-12, H-12′), 7.33 (1H, d, *J* = 8.9 Hz, H-7), 7.28 (1H, d, *J* = 8.7 Hz, H-7′), 7.15 (2H, m, H-14, H-14′), 6.98 (2H, m, H-15, H-15′), 4.11 (3H, brs, OMe), 3.98 (3H, brs, OMe), 3.93 (3H, brs, OMe); $^{13}C$ NMR ($CDCl_3$): δ 136.74 (C-1), 126.47 (C-2), 154.71 (C-3), 159.85 (C-4), 128.69 (C-5), 127.57 (C-6), 106.09 (C-7), 128.76 (C-8), 186.24 (C-9), 144.88 (C-10), 107.09 (C-11), 97.96 (C-12), 133.43 (C-13), 122.16 (C-14), 119.59 (C-15), 135.75 (C-1'), 127.17 (C-2′), 153.79 (C-3′), 158.38 (C-4′), 128.69 (C-5′), 127.21 (C-6′), 105.36 (C-7′), 128.39 (C-8′), 184.29 (C-9′), 144.61 (C-10′), 106.82 (C-11′), 97.91 (C-12′), 132.19 (C-13′), 122.75 (C-14′), 112.43 (C-15′), 59.16, 56.03, 54.25 (3 x OMe); EIMS *m/z* (rel. int.): 520 $[M]^+$ ($C_{33}$ $H_{28}$ $O_6$) (3.2).

### 2.7. Pinnatabiflavone (3)
Elution of the column with chloroform-methanol (97:3) gave green amorphous powder of 3, recrystallized from chloroform-methanol (1:1); 115 mg (0.076%); $R_f$: 0.50 (chloroform-methanol (97:3); m.p. 240-242 °C; UV $\lambda_{max}$ (MeOH): 221, 264, 322 nm (log ε 5.6, 3.2, 2.8); IR $\nu_{max}$ (KBr): 3410, 3257, 3160, 2921, 2852, 1687, 1579, 1503, 1448, 1367, 1288, 1252, 1170, 1069, 1027, 832 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): δ 7.92 (1H, d, *J* = 8.8 Hz, H-5′), 7.77 (1H, d, *J* = 8.8 Hz, H-6′), 7.73 (1H, d, *J* = 8.4 Hz, H-5′a), 7.46 (1H, d, *J* = 8.4 Hz, H-6′), 7.31 (1H, brs, H-3), 6.88 (1H, brs, H-3a), 6.63 (1H, brs, H-6a), 3.86 (3H, brs, OMe), 2.19 (2H, m, $H_2$-1″), 0.84 (3H t, *J*=6.3 Hz, Me-2″); $^{13}C$ NMR (DMSO$d_6$): δ 163.11 (C-2), 104.3 (C-3), 177.61 (C-4), 161.19 (C-5), 109.91 (C-6), 161.06 (C-7), 128.06 (C-8), 158.05 (C-9), 105.03 (C-10), 122.16 (C-1'), 144.04 (C-2'), 146.36 (C-3'), 150.53 (C-4'), 121.27 (C-5'), 117.22 (C-6'), 29.55 (C-1″), 14.53 (C-2″), 162.82 (C-2a), 104.65 (C-3a), 177.48 (C-4a), 161.17 (C-5a), 99.81 (C-6a), 161.05 (C-7a), 127.95 (C-8a), 158.03 (C-9a), 104.85 (C-10a), 122.03 (C-1a′), 145.35 (C-2a′), 146.44 (C-3a′), 147.93 (C-4a′), 119.35 (C-5a′), 116.32 (C-6a′), 56.34 (OMe); EIMS *m/z* (rel. int.): 644 $[M]^+$ ($C_{33}H_{24}O_{14}$) (11.3), 629 (6.3), 615 (8.1), 343 (2.5), 328 (100), 312 (22.1), 301 (80.3).

### 2.8. Lanosteryl glycoside (4)
Elution of the column with chloroform-methanol (9:1) furnished colourless crystal of 4, recrystallized from chloroform-methanol (1:1); 159 mg (0.106% ); $R_f$ value: 0.30 (chloroform: methanol, 9:1); m.p. : 258-260 °C; UV $\lambda_{max}$ (MeOH); 217 nm (log ε 4.3); IR $\nu_{max}$ (KBr): 3485, 3409, 3350, 2930,1644, 1464, 1373, 1258, 1163, 1071, 1022; 1644 $cm^{-1}$; $^1H$ NMR (DMSO$d_6$): δ 5.24 (1H, brs, H-6), 4.73 (1H,d, *J* = 4.8 Hz, H-1′α ), 4.67 ((1H, d, *J* = 4.8 Hz, H-2′), 4.18 (1H, dd, *J* = 8.8,4 Hz, H-4′), 3.61 (1H, m, H-5′) 3.44 1H,dd, *J* = 4.8, 7.2 Hz, H-3β ), 3.14 (1H, m, H-3′), 3.10 (1H, d, *J* = 8.0 Hz, $H_2$-6′a), 3.03 (1H, d, *J*=8.0 Hz, $H_2$-6′b), 1.16 (3H, brs, Me-19), 0.90 (3H, brs, H-28), 0.84 (3H, d, *J* = 6.4 Hz, Me-21), 0.77 (3H, d, *J* = 7.2 Hz, Me-26), 0.75 ( 3H, d, *J* = 7.2 Hz, Me-27), 0.71 (3H, brs, Me-29), 0.69 (3H, brs, Me-30), 0.59 (3H, brs, Me-18); $^{13}C$ NMR (DMSO$d_6$): δ 36.67 (C-1), 34.09 (C-2), 77.73 (C-3), 42.28 (C-4), 140.63 (C-5), 121.64 (C-6), 24.33 (C-7), 43.51 (C-8), 50.05 (C-9), 36.31 (C-10), 21.67 (C-11), 29.06 (C-12), 45.65 (C-13), 57.31 (C-14), 31.84 (C-15), 37.28 (C-16), 50.71 (C-17), 12.02 (C-18), 20.75 (C-19), 31.79 (C-20), 19.27 (C-21), 40.72 (C-22), 23.19 (C-23), 38.81 (C-24), 29.71 (C-25), 19.49 (C-26), 12.13 (C-27), 28.64 (C-28), 27.08 (C-29), 18.94 (C-30), 101.64 (C-1′), 76.85 (C-2′), 73.81 (C-3′), 70.52 (C-4′), 77.18 (C-5′), 61.72 (C-6′); EIMS *m/z* (rel. int.): 590 $[M]^+$ ($C_{36}$ $H_{62}$ $O_6$) (3.7), 428 (48.9), 315 (100), 220 (3.7), 208 (3.9), 192 (4.8), 180 (6.3), 152 (7.2), 100 (66.7).

### 2.9. Glyceryl 1-oleiyl 2-myristyl 3-phosphate (5)
Elution of the column with chloroform-methanol (17:3) mixture produced a dull yellow amorphous mass of 5, recrystallized from methanol; 121 mg (0.08% yield); $R_f$: 0.90 (chloroform-methanol, 17:3); m.p. 157-160 °C; IR $\nu_{max}$ 3409, 2923, 2852, 1740, 1644, 464, 1258, 1091, 1025, 807, 721 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): 5.19 (2H, brs, H-9′, H-10′), 3.50 (1H, m, H-2), 3.45 (2H, d, *J* = 6.8 Hz, $H_2$-3), 3.39 (2H, d, *J* = 6.9

Hz, $H_2$-1), 2.32 (2H, t, *J* = 7.2 Hz, $H_2$-2′), 2.25 (2H, t, *J* = 7.5 Hz, $H_2$-2″), 2.05 (2H, m, $H_2$-8′), 2.01 (2H, m, $H_2$-11′), 1.67 (2H, m, $CH_2$), 1.55 (4H, m, 2 x $CH_2$), 1.43 (2H, brs, $H_2$-2), 1.12 ( 36H, brs, 18 x $CH_2$), 1.14 (3H, t, *J* = 7.2 Hz, Me-14″), 0.74 (3H, t, *J* = 6.8 Hz, Me-18′); $^{13}$C NMR (DMSO-$d_6$): 171.32 (C-1′), 170.55 (C-1″), 72.45 (C-2), 63.60 (C-1′), 63.27 (C-3), 57.23 (C-2′), 55.17 (C-2″), 33.41 ($CH_2$), 31.81 ($CH_2$), 29.66 (10 x $CH_2$), 29.44 (5 x $CH_2$), 29.23 (5 x $CH_2$), 27.13 ($CH_2$), 24.59 ($CH_2$), 22.68 ($CH_2$), 14.42 (Me-14″), 14.13 (Me-18′); EIMS *m/z* : 346 $[M]^+$ ($C_{35}H_{67}O_8$ P) (3.8).

### 2.10. Pinnatabichalconyl myristate (6)

Elution of column with chloroform-methanol (41:9) mixture afforded a light green amorphous powder of **6**, recrystallized from methanol, 187 mg (0.12% yield); $R_f$ : 0.40 (chloroform-methanol, 41:9); m.p.: 219-220 ºC; UV $\lambda_{max}$ (MeOH): 217, 238, 350 nm (log ε 5.3, 6.2, 4.1); IR $\nu_{max}$ (KBr): 3436, 2921, 2851, 1721, 1708, 1597, 1463, 1260, 1097, 1061, 871, 801, 744 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$): δ 7.93 (1H, d, *J* = 7.6 Hz, H-5), 7.88 (1H, d, *J* = 2.6 Hz, H-2′), 7.78 (1H, d, *J* = 8.8 Hz, H-8), 7.72 (1H, d, *J* = 8.8 Hz, H-8′), 7.61 (1H, *J* = 2.6 Hz, H-2), 7.56 (1H, m, H-5′), 7.53 (1H, brs, H-6), 7.50 (1H, m, H-6′), 7.47 (2H, m, H-11, H-11′), 7.42 (2H, m, H-12, H-12′), 7.35 (1H, d, *J* = 8.8 Hz, H-7), 7.26 (1H, d, *J* = 8.6 Hz, H-7′), 7.12 (2H, m, H-14, H-14′), 7.01 (1H, m, H-15), 6.89 (1H, m, H-15′), 4.14 (3H, brs, OMe), 4.11 (3H, brs, OMe), 2.12 (2H, t, *J* = 6.8 Hz, $H_2$-2″), 1.91 (2H, m, H-3), 1.45 (2H, brs, $CH_2$), 1.20 (18H, brs, 9 x $CH_2$), 0.78 (3H, t, *J* = 6.4 Hz, Me-14″); $^{13}$C NMR (DMSO-$d_6$): δ 135.36 (C-1), 127.75 (C-2), 153.23 (C-3), 158.77 (C-4), 129.01 (C-5), 128.38 (C-6), 105.96 (C-7), 129.82 (C-8), 186.07 (C-9), 145.69 (C-10), 106.72 (C-11), 97.85 (C-12), 132.63 (C-13), 124.35 (C-14), 119.02 (C-15), 136.03 (C-1'), 126.41 (C-2′),15 4.09 (C-3′), 154.01 (C-4′), 129.01 (C-5′), 127.95 (C-6′), 105.96 (C-7′), 131.16 (C-8′), 184.04 (C-9′), 144.38 (C-10′), 107.81 (C-11′), 97.85 (C-12′), 133.12 (C-13′), 121.68 (C-14′), 113.31 (C-15′), 172.35 (C-1″), 34.19 (C-2″), 31.79 (C-3″), 29.56 (C-4″), 29.51 (C-5″), 29.46 (C-6″), 29.42 (C-7″), 29.38 (C-8″), 29.32 (C-9″), 29.16 (C-10″), 27.09 (C-11″), 24.94 (C-12″), 22.60 (C-13″), 14.21 (C-14″), 61.02, 54.36 (2 x OMe); EIMS *m/z* (rel. int.): 716 $[M]^+$ ($C_{46}H_{52}O_7$) (3.2), 701 (5.5), 506 (8.1), 210 (16.2).

## 3. Results and Discussion

Compound **1,** designated as pinnata flavonol, showed UV absorption maxima at 260 and 331 nm typical to a flavonol moiety [7, 8] and IR absorption bands for hydroxyl group at 3553 cm$^{-1}$ and carbonyl group at 1662 cm$^{-1}$. On the basis of its mass and $^{13}$C NMR spectra the molecular ion peak of **1** was determined at *m/z* 348 corresponding to a difuran substituted flavone $C_{20}H_{12}O_6$. The ion peaks arising at *m/z* 148 $[C_9H_8O_2]^+$ and 200 $[C_{11}H_4O_4]^+$ formed due to RDA fragmentation suggested the presence of two furanic rings in unit A and methoxy group in unit B of the flavones [7]. The ion fragments generated at *m/z* 333 $[M\text{-}Me]^+$ and 317 $[M\text{-}OMe\text{-}]^+$ also supported the existence of a methoxy group in the molecule. The $^1$H NMR spectrum of **1** showed four one-proton doublets at δ 8.17 (*J* = 2.3 Hz), 8.13 (*J* = 2.6 Hz), 7.73 (*J* = 2.3 Hz) and 7.54 (*J* = 2.3 Hz) and two two-proton doublets at δ 8.12 ( *J* = 9.0 Hz,) and 7.15 (*J* = 9.0 Hz) ascribed correspondingly to furanic H-12, H-14, H-12 and H-11 protons and flavanol B-ring H-2′, H-6′ and H-3′, H-5′ protons. A three-proton broad singlet at δ 3.87 was accounted to the methoxy protons [9]. The $^{13}$C NMR spectrum of **1** exhibited important signals for C-4 carbonyl carbon at δ 175.12, aromatic carbons between δ 158.14-104.25 and methoxy carbon at δ 60.24. The absence of any carbon signal near δ 163.0 in the $^{13}$C NMR spectrum supported the location of the furan moieties at C-5 and C-7. The $^1$H-$^1$H COSY spectrum of **1** showed correlations of H-11 with H-12; H-13 with H-14; H-3′ and H-6′ with H-2′; and H-3′ and H-5′ with OMe. On the basis of the spectral data analysis and chemical reactions the structure of **1** has been established as 5,6-furano-7,8-furano-4′-methoxyflavanol. This is a new flavanol isolated from a natural source.

Compound 2, named pinnatabichalcone, showed UV absorption maxima at 241 and 346 nm typical to chalcones [7, 10] and IR typical absorption bands for hydroxyl group (3482 cm$^{-1}$), carbonyl group (1693 cm$^{-1}$) and aromatic rings (1586 1543, 977 cm$^{-1}$). On the basis of mass and $^{13}$C NMR spectra the molecular ion peak of 2 was determined at *m/z* 520 consistent to a molecular formula of a bichalcone, $C_{33}H_{28}O_6$. The $^1$H NMR spectrum of 2 displayed two one-proton doublets at δ 7.97 (*J* = 8.1 Hz) and 7.90 (*J* = 2.8 Hz) assigned correspondingly to *ortho*-coupled H-5 and *meta*-coupled H-2′ protons adjacent to the methoxy groups, four one-proton doublets at δ 7.87 (*J* = 8.9 Hz), 7.85 (*J* = 8.7 Hz), 7.33 (*J* = 8.9 Hz), 7.28 (*J* = 8.7 Hz) ascribed to *cis*-oriented vinylic H-8, H-8′, H-7 and H-7′ protons, respectively, other aromatic protons as one-proton doublets at δ 7.63 (*J* = 2.8 Hz, H-2) and 7.55 ( *J* = 7.3 Hz, H-5′), as one-proton multiplets at δ 7.52 (H-6), 7.50 (H-6′) and as two-proton multiplets at δ 7.48 (H-11, H-11′), 7.45 (H-12, H-12′), 7.15 (H-14, H-14′) and 6.98 (H-15, H-15′). Three broad singlets at δ 4.11, 3.98 and 3.93 integrating for three protons each were associated with the methoxy protons. The $^{13}$C NMR spectrum of **2** displayed signals for carbonyl carbons at δ 186.24 (C-9) and 184.29 (C-9′), aromatic carbons between δ 154.71-97.96, vinylic carbons at δ 106.09 (C-7), 128.76 (C-8), 105.36 (C-7′) and 128.39 (C-8′) and methoxy carbons at δ 59.16, 56.03 and 54.25. The absence of any carbon signal near δ 163.0 in the $^{13}$C NMR spectrum suggested the attachment of chalconyl units between δ C-13 and C-13′. There was no signal between δ 0.5-3.94 in $^1$H NMR spectrum and from δ 10.0 to 54.27 in the $^{13}$C NMR spectrum supporting the absence of an aliphatic chain in the molecule. On the basis of the foregoing discussion, the structure of 2 has been established as *cis-,cis*-13-(3′-methoxy-4′-hydroxychalconyl-(13→13′)-3,4-dimethoxychalcone. This is a new bichalcone derivative. An isomer of compound 2 possessing the hydroxyl group at C-3′ was reported from *P. pinnata* [11].

Compound 3, named pinnatabiflavone, showed UV spectrum absorption maxima at 264 and 322 nm typical to flavones [7, 12]. The IR spectrum of 3 displayed characteristic absorption bands for hydroxyl (3410, 3257, 3160 cm$^{-1}$) and carbonyl (1687 cm$^{-1}$) groups. Its molecular ion peak was established at *m/z* 644 on the basis of mass and $^{13}$C NMR spectra corresponding to a molecular formula of a biflavone, $C_{33}H_{24}O_{14}$. The prominent ion fragments generating at *m/z* 629 $[M\text{-}Me]^+$ and 615 $[M\text{-}C_2H_5]^+$ indicated the presence of an ethyl group in the molecule. The ion peaks arising at *m/z* 301 $[C_{15}H_9O_7]^+$ and 343 $[M - 301]^+$ due to fission of the $C_6$-$C_{8a}$ linkage indicated the existence of ethyl and methoxy groups on one of the flavone unit. The $^1$H NMR spectrum of 3 showed four one-proton doublets at δ 7.92 (*J* = 8.8 Hz), 7.77 (*J* = 8.8 Hz), 7.73 (*J* = 8.4 Hz) and 7.46 (*J* = 8.8 Hz) assigned to *ortho*-coupled aromatic H-5′, H-6′, H-5′a and H-6′a protons, respectively. Three one-proton singlets at δ 7.3, 6.88, and 6.63 were ascribed to flavone H-3, H-3a and H-6a

protons, respectively. Two three-proton signals at δ 3.86 (brs) and 0.84 (t, *J*=6.3 Hz) and a two-proton multiplet at δ 2.19 were attributed to methoxy, primary C-2″ methyl and methylene $H_2$-1″ protons, respectively. The $^{13}C$ NMR spectrum of **3** exhibited signals for carbonyl carbons at δ 177.61 (C-4) and 177.46 (C-4a), aromatic carbons between δ 163.11-104.33, methoxy carbon at δ 56.34, methylene carbon at δ 29.55 and methyl carbon at δ 14.53. The absence of a carbon signal near δ 95.0 supported the attachment of a flavone moiety by a ($C_6 \rightarrow C_{8a}$) linkage to another flavone unit. On the basis of these evidences, the structure of 3 has been established as 5,7,2′,3′- tetrahydroxy – 4′ -methoxy – 8 - ethylflavonyl- (6→8a)-5a,7a,2′a,3′a,4′a-pentahydroxyflavone. This is a new biflavone molecule isolated from a natural or synthetic source for the first time.

Compound 6, designated as pinnatabichalconyl myristate, exhibited UV absorption maxima at 238 and 350 nm distinctive to aurones [7,10,13] and IR absorption bands for hydroxyl group (3436 $cm^{-1}$), ester function (1721 $cm^{-1}$), carbonyl groups (1708 $cm^{-1}$) and a long aliphatic chain (744 $cm^{-1}$)). Its molecular ion peak was established at *m/z* 716 on the basis of mass and $^{13}C$ NMR spectra corresponding to a bichalconyl ester, $C_{46}H_{52}O_7$. The ion peaks arising at *m/z* 701 $[M – Me]^+$, and 506 $[M – 210, CH_3(CH_2)_{12}CO]^+$ indicated that a $C_{14}$ fatty acid was esterified with the bichalcone. The $^1H$ NMR spectrum of **6** showed two one-proton doublets at δ 7.88 (*J* = 2.6 Hz) and 7.61 (*J* = 2.6 Hz) assigned to *meta*-coupled aromatic H-2′ and H-2 protons, respectively. Two one-proton doublets at δ 7.93 (*J*=7.6 Hz) and 7.56 (*J* = 7.9 Hz) were attributed to *ortho*-coupled aromatic H-5 and H-5′ protons, respectively. Four one-proton doublets at δ 7.78 (1H, *J* = 8.8 Hz), 7.72 (1H, *J* = 8.8 Hz), 7.35 (1H, *J* = 8.8 Hz) and 7.26 (1H, *J* = 8.6 Hz) were accounted correspondingly to *cis*-oriented vinylic H-8, H-8′, H-7 and H-7′ protons. The remaining aromatic protons appeared as one-proton multiplets at δ 7.53 (H-6), 7.50 (H-6′), 7.01 (H-15), 6.89 (H-15′) and as two-proton multiplets at δ 7.47 (H-11, H-11′), 7.42 (H-12, H-12′) and 7.12 (H-14, H-14′). The methoxy protons resonated as three-proton broad singlets at δ 4.14 and 4.11. A two-proton triplet at δ 2.12 (*J* = 6.8 Hz) was accounted to methylene $H_2$-2″ proton adjacent to the ester group. A two-proton multiplet at δ 1.91, a two-proton broad singlet at δ 1.45 and an eighteen-proton broad signal at δ 1.20 were associated with the remaining methylene protons of the ester chain. A three-proton triplet at δ 0.78 (*J* = 6.4 Hz) was due to primary C-14″methyl protons. The $^{13}C$ NMR spectrum of **6** displayed signals for carbonyl carbons at δ 186.07 (C-9) and 184.04 (C-9′), ester carbon at δ 172.35 (C-1″), vinylic carbons at δ 105.96 (C-7), 129.82 (C-8), 105.92 (C-7′) and 131.16 (C-8′), aromatic carbons between δ 106.72-158.77, methoxy carbons at δ 61.02 and 54.36, methylene carbon between δ 34.19-22.60 and methyl carbon at δ 14.21 (C-14″). The absence of carbon signals near δ 163 in the $^{13}C$ NMR suggested attachment of chalcone units through (13→13′) linkage. Alkaline hydrolysis of 6 yielded a bichalcone, 506 $[M]^+$ ($C_{32}H_{26}O_6$). Its $^1H$ NMR spectrum showed aromatic and vinylic protons between δ 7.91 – 6.85 and methoxy protons as three-proton broad singlets at δ 4.08 and 4.03. The fatty acid was characterized as myristic acid, $[M]^+$ at *m/z* 228 ($C_{14}H_{28}O_2$); co-TLC comparable. On the basis of these evidences the structure of 6 has been formulated as *cis*-,*cis*-13-(3′-methoxy-4′-hydroxychalconyl-(13→13′)-3-*n*-tetradecanyloxy-4-methoxychalcone. This is a new bichalcone ester.

The compounds 4 and 5 were the known phytoconstituents characterized as lanost-5-en-3β-ol 3-β-D-glucopyranoside and glyceryl 1-octadec-9′-enoate 2-tetradecanoate 3-phosphate, respectively [14, 15].

## 4. Conclusion

Phytochemical investigation of a methanolic extract of the fruits of *P. pinnata* yielded two bichalcones, one each of furano flavonol, biflavone, lanost-5-en-3β-ol 3-β-D-glucoside and fatty acid glyceride. These compounds may be used as chromatographic markers for standardization of the karanja fruits.

## 5. Acknowledgements

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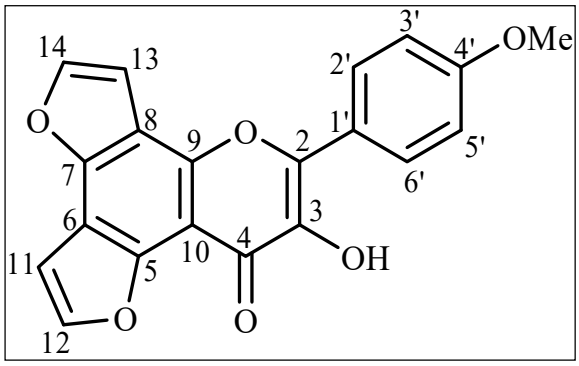
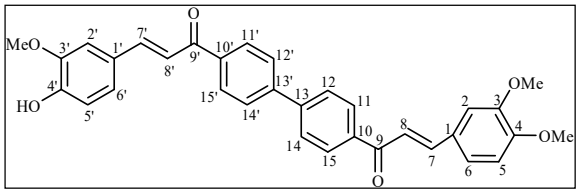
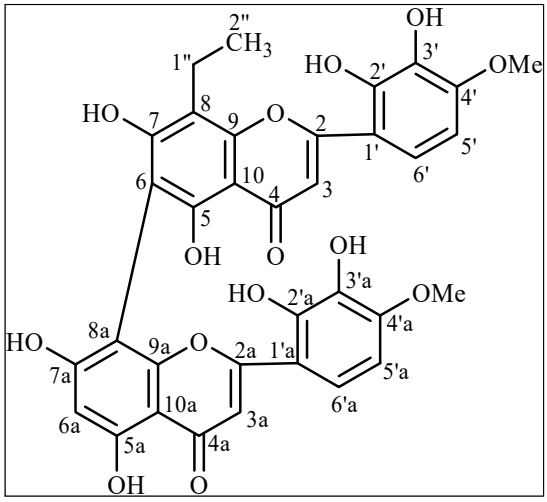


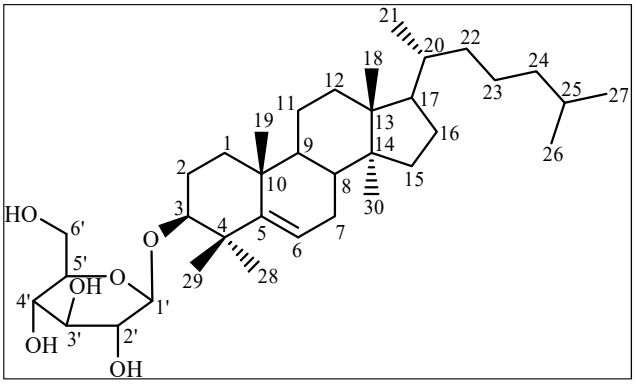
(1) Pinnataflavonol



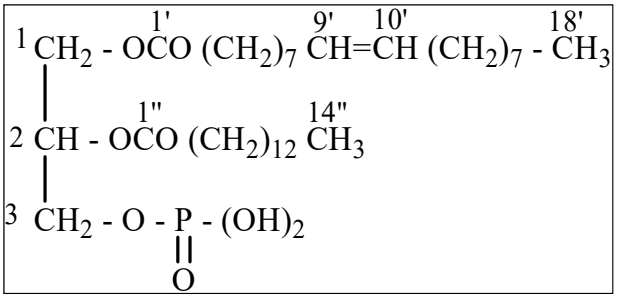
(2) Pinnatabichalcone



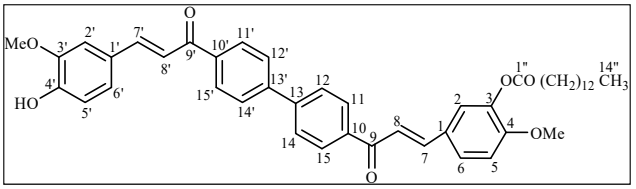
(3) Pinnatabiflavone



(4) Lanosteryl glycoside



(5) Glyceryl 1-oleiyl 2-myristyl 3- phosphate



(**6**) Pinnatachalconyl myristate

**Fig 1:** Strucural formulae of compounds 1 - **6**.

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