PART-V

Experimental
PART-V: EXPERIMENTAL

**General:** Melting points were determined by the use of Kofler type electrical melting point apparatus and are uncorrected. All the analytical samples were tested for homogeneity on TLC plates in different solvent systems. TLC plates were prepared in glass plates using a slurry of Silica gel G (Merck, India) in EtOAc/ethanol and the spots of plates were visualized by either exposing the plates in iodine chamber or spraying with 10% H$_2$SO$_4$ in ethanol followed by heating at 110$^\circ$C. Silica gel (mesh 60-120, Merck, India), basic Alumina (Al$_2$O$_3$) (Merck), Diaion HP-20 (Mitsubishi Chemicals, Japan) and Sephadex LH-20 (Pharmacia Fine Chemicals) were used for column chromatography (CC). CC and TLC were performed at room temperature (20-30$^\circ$C). The optical rotation was measured on a Jasco Dip-370 digital polarimeter. UV-vis spectra were recorded on Perkin Elmer Lambda 25 spectrophotometer and were expressed in $\lambda_{\text{max}}$ solvent nm ($\log\epsilon$). IR spectra in KBr disc were recorded on a Shimadzu 8100 FT-IR spectrophotometer and were expressed in $\bar{\nu}_{\text{max}}$ cm$^{-1}$. $^1$H, $^{13}$C and 2D-NMR spectra were recorded on a Varian XL- 400 and 600 MHz NMR / Brucker Advance II 600 NMR spectrometer. Chemical shifts were expressed in $\delta$ (ppm) with tetramethylsilane (TMS) as an internal standard, and the coupling constants were expressed in hertz (Hz). EI-MS, HR-EI-MS were recorded on a Jeol JMS 700 mass spectrometer and FAB-MS and HR-FAB-MS were recorded on a Jeol JMS-HX 110 mass spectrometer. In MS the mass ion peaks were given in $m/z$ values with their relative abundances in % with respects to the base peak in a spectrum. NMR-DEPT experiments were carried out with flip angle $\theta$ of 45$^\circ$, 90$^\circ$ and 135$^\circ$. GC/MS/MS analysis was carried out on a Hewlett-Packard (Palo Alto, CA) 5890 GC interfaced with a Finnigan MAT TSQ 700 triple-quadrupole mass spectrometer using a 30 m $\times$ 0.25 mm DB-5 column and GC injection port temperature, 250$^\circ$C. The helium carrier gas flow rate was 2.0 mL / min. The temperature of the transfer line was 250$^\circ$C. The initial oven temperature was 100$^\circ$C and was held for 1 min. The oven was then heated to 270$^\circ$C at a rate of 10$^\circ$C/min and was held at
that temperature for 10 min. 1µl sample was injected in the splitless mode. Cholesterol was used as internal standard for comparison of sterols. After a GC injection, the SRM scanning mode of mass spectrometer was set to detect cholesterol of mass m/z 386.

**Extraction and Isolation of Compounds:**

1. From *Pouzolzia indica*:

Whole plants of *Pouzolzia indica* were collected from Udaipur, Gomati Tripura in March, 2009 and were identified by Prof. B. K. Datta, Plant Taxonomist, Department of Botany, Tripura University. A voucher specimen (TU/H/1510) has been deposited in the laboratory of B. K. D. Air dried whole plants (2.5 Kg) of *P. indica* were extracted with MeOH (8L × 2, 6d each time) and the MeOH extracts were evaporated in vacuo under reduced pressure using a rotavapour to a residue (250 g). The major portion (240 g) of the residue was suspended in H₂O (~80ml) and extracted successively with CH₂Cl₂, EtOAc and n-BuOH (3 × 100 mL with each solvent). The residue (35 g) from CH₂Cl₂ extract was subjected to silica gel (Si gel) column chromatography and the column was eluted with a solvent mixture of petroleum ether (PE)-CHCl₃ (8 : 2) to afford 1-hentriacontanyl palmitate (1) (40 mg) in white amorphous solid. Elution with PE-CHCl₃ (7 : 3) afforded myricyl alcohol (2) (50 mg) in white amorphous powder. Elution of the column with PE-CHCl₃ (5 : 5) gave 6, 7-dimethoxy coumarin (3) (20 mg); while elution with PE-CHCl₃ (4 : 6) afforded friedelin (4) (150 mg) in colorless needles. Elution of the column with PE-CHCl₃ (3 : 7) afforded trichadonic acid (5) (25 mg) in white amorphous solid. The residue (75 g) of EtOAc extract was also subjected to Si gel column chromatography with a gradient of solvent mixture, PE-CHCl₃ of increasing polarity. Elution of the column with PE-CHCl₃ (3 : 7) afforded trichadonic acid (5) (35 mg) and with PE-CHCl₃ (2 : 8) afforded pouzolzin (6) (120 mg) in white amorpous powder.
Flow sheet 1: Isolation of compounds from *P. indica*

*Pouzolzia indica* whole plant (2.5 kg)

1. MeOH, percolation method
2. MeOH extract (250 g)
   - Fractionated
3. *Pouzolzia indica* whole plant (2.5 kg)
   - CH$_2$Cl$_2$ sol. fraction
   - EtOAc sol. fraction
   - n-BuOH sol. fraction
   - Silica gel CC

1-Hentriacontanyl palmitate (1)

Amorphous powder

IR (KBr) $\nu_{\text{max}} \text{ cm}^{-1}$: 1736, 1365, 1081, 826.

FAB-MS $m/z$ (%): 691 [M + H]$^+$ (21), 663 (37), 653 (53), 607 (47), 451 (42), 1.435 (100), 315 (21), 257 (37), 239 (18)

Myricyl alcohol (2)

White amorphous powder, mp 90-92$^\circ$C (lit. 88$^\circ$C[1])

ESI-MS: 439 [M (C$_{30}$H$_{62}$O + H)$^+$ (30%)]

$^1$H-NMR (600 MHz, CDCl$_3$): $\delta$ 0.88 (3H, t, $J = 7.0$ Hz), 1.25 (54H, br s)

1.60 (2H, m), 3.64 (2H, t, $J = 6.8$ Hz)

$\text{CH}_3$ - (CH$_2$)$_{28}$ - CH$_2$OH

2
6,7-Dimethoxy coumarin (3)

Pale yellow needles, mp 145-146°C

UV (MeOH) $\lambda_{max}$ : 344

IR (KBr) $\nu_{max}$ cm$^{-1}$: 1688 (C = O), 1540, 1460, 1388, 1362.

HR-EI-MS $m/z$ : 206.1544 [M]+ (Calcd for C$_{11}$H$_{10}$O$_{4}$ : 206.1576)

$^1$H-NMR (600 MHz, CDCl$_3$) : 6 6.24 (1H, d, $J$ = 8.5 Hz, H-3), 7.62 (1H, d, $J$ = 8.5 Hz, H-4), 6.90 (1H, s, H-5), 6.85 (1H, s, H-8), 3.85 (3H, s, MeO-6), 3.88 (3H, s, MeO-7)

$^{13}$C-NMR (150 MHz, CDCl$_3$) : 161.2 (C-2), 113.7 (C-3), 143.2 (C-4), 111.4 (C-4a), 108.2 (C-5), 150.2 (C-6), 152.8 (C-7), 100.0 (C-8), 146.4 (C-8a), 56.4 (2×OMe) [2, 3]

![Diagram of 3](image)

Friedelin (4)

Colourless needles. mp 262°C (lit 265-266°C [4])

$[\alpha]_D^{24}$ +2.6 (C = 0.2, MeOH)

IR (KBr) $\nu_{max}$ cm$^{-1}$: 1717, 1464, 1389, 1111, 1073.

FAB-MS $m/z$ (rel.int.%) : 427 [M + H]$^+$ (100), 412 (7), 221 (12), 273 (32), 205 (76), 191 (18), 152 (28).

$^1$H and $^{13}$C-NMR (CDCl$_3$) : Table 1.4

Trichadonic acid (5)

White amorphous solid, mp 249-251°C (lit 248-249°C [5])

IR (KBr) $\nu_{max}$ cm$^{-1}$: 3381, 2928, 1714, 1685, 1385, 1081.

$^1$H and $^{13}$C-NMR (CDCl$_3$) : Table 1.5

FAB-MS $m/z$ (%) : 457 [M + H]$^+$ (100), 442 (24), 303 (70), 235 (27) and 191 (64).
Pouzolzin (6)

White amorphous powder, m.p. 150-153°C
$[\alpha]_D^{24} + 5.32$ (C 0.2, MeOH)

IR $\nu_{\text{max}}$ (KBr) cm$^{-1}$: 3434, 2923, 2851, 1735, 1715, 1562, 1465, 1432, 1385, 1077.

$^1$H and $^{13}$C-NMR (CDCl$_3$) : Table 1.3

FAB-MS $m/z$ (%) : 663 [M + Na]$^+$ (13), 648 [M Na - Me]$^+$ (7), 473 (4), 427 (6), 296 (7), 221 (6), 203 (12), 167 (15), 149 (53), 57 (100).

Alkaline hydrolysis of compound 1

Compound 6 (50 mg) was dissolved in 5% KOH in EtOH-H$_2$O (1:1) (10 mL) and the solution was refluxed at 80°C for 2h. The resultant solution was cooled, acidified with dil. H$_2$SO$_4$ and extracted with EtOAc. The EtOAc extract was dried with anhy. Na$_2$SO$_4$, concentrated and column purified to get lauryl alcohol 6b (11mg) and 7β-hydroxy-3-oxo-friedelan-28-oic acid (6a) (20mg)

7β-Hydroxy-3-oxo-friedelan-28-oic acid (6a)

Amorphous powder, m.p. 225-228°C

IR (KBr) $\nu_{\text{max}}$ cm$^{-1}$: 3440, 2925, 1712, 1690, 1450, 1385, 1370, 780.

FAB-MS $m/z$ (%) : 495 [M (C$_{30}$H$_{48}$O$_4$) + Na]$^+$ (30), 480 (25), 289 (27), 221 (21), 203 (29), 95 (100).

Lauryl alcohol (6b)

Semisolid mass,

IR (KBr) $\nu_{\text{max}}$ cm$^{-1}$: 3436, 2965, 1385, 620.

FAB-MS $m/z$ : 209 [M + Na]$^+$.

It was identical with authentic sample of lauryl alcohol in co-TLC.
2. From *Oroxylum indicum*

Stem-barks of *Oroxylum indicum* were collected from Agartala, Tripura in January 2010. It was identified by Prof. B. K. Datta, Plant Taxonomist, Department of Botany, Tripura University.

Air dried powdered stem-bark of *O. indicum* was extracted with MeOH (5L × 3). The MeOH extract was evaporated under reduced pressure to a thick condensate (140 mg) and dissolved in 150 mL of H₂O. The aqueous solution was extracted three times each with hexane, EtOAc and n-BuOH (100 mL each time for each solvent). The hexane extract (20 g) was subjected to column chromatography over silica gel (60 mg) using a gradient of hexane-CH₂Cl₂ (1 : 1) to afford chrysin (70 mg) and elution with hexane-CH₂Cl₂ (1 : 2) to afford baicalein (130 mg). EtOAc extract (50 mg) was column chromatographed (CC) over silica gel using solvent gradient of CH₂Cl₂-EtOAc. Elution of the column with CH₂Cl₂-EtOAc (95 : 5) afforded Oroxylin A (65 mg). Elution with CH₂Cl₂-EtOAc (80 : 20) afforded a mixture of two compounds, which on repeated CC gave scutellarein (40 mg) and scutellarein 4'-O-methyl ether (30 mg). Elution of the column with CH₂Cl₂-EtOAc (70 : 30) afforded pectolinarigenin (25 mg). The BuOH extract (40 g) was also subjected to CC over silica gel. Elution of the column with EtOAc-MeOH (90 : 10) afforded dihydrobaicalein 7-O-(6"-benzoylglucoside), OI-20 (25 mg) and elution with EtOAc-MeOH (70 : 30) afforded β-sitosterol glucoside (38 mg).
Flow sheet 2: Isolation of compounds from *O. indicum*

*Oroxyllum indicum* Stem bark (2.0 kg)

MeOH, percolation

MeOH Extract, 140 g

Merck

Hexane sol. fraction (20 g)

EtOAc sol. fraction (50 g)

BuOH sol. fraction (40 g)

Silica gel CC

Silica gel CC

Silica gel CC

Baicalein, Chrysin

Oxyxlin A, Scutellarein

Scutellarein 4’ methylether

Pectolinarigenin

Oxryxlin A

Scutellarein

Scutellarein 4’ methylether

Pectolinarigenin

Chrysin (7)

Yellow needles, mp 274° C

C₁₅H₁₀O₄ (M⁺ 254)

UV (MeOH) \( \lambda_{max} (\log \epsilon) \) nm: 247 sh (4.58), 268 (4.90) and 314 (4.48)

IR (KBr) \( \bar{\nu}_{max} \) cm⁻¹: 3427, 1653, 1611, 1578, 1506 and 806

\(^{1}\)H-NMR (600 MHz, DMSO-\( d_6 \)) : \( \delta_H \) 6.92 (1H, s, H-3), 6.50 (1H, d, \( J = 2.0 \) Hz, H-8), 6.19 (1H, d, \( J = 2.0 \) Hz, H-6), 8.04 (2H, dd, \( J = 8.0 \) and 2.0 Hz, H-2’, 6’), 7.54 (2H, t-like, \( J = 8.0 \) Hz, H-3’, 5’), 7.59 (1H, m, H-4’), 12.80 (1H, br s, HO-5) and 10.89 (1H, br s, HO-7) [6]
$^{13}$C-NMR (100 MHz, DMSO-$d_6$) : δC 163.1 (C-2), 105.1 (C-3), 181.8 (C-4), 103.9 (C-4a), 157.4 (C-5), 103.9 (C-6), 164.0 (C-7), 94.0 (C-8), 157.4 (C-8a), 130.7 (C-1'), 126.4 (C-2', 6'), 129.1 (C-3', 5') and 132.0 (C-4').

EI-MS m/z (%) : 254 [M]$^+$ (100), 253 [M-1]$^+$ (21), 226 (48), 124 (24), 102 (8) and 77 (11) [6]

Baccalein (8)

Light brown needles, mp 262.0°C

C$_{15}$H$_{10}$O$_5$ (M$^+$ 270)

UV (MeOH) $\lambda_{max}$ (log $\epsilon$) nm : 247 (4.83), 273 (5.03) and 323 (4.72)

IR (KBr) $\tilde{\nu}_{max}$ cm$^{-1}$: 3412, 1655, 1611, 1585 and 827

$^1$H-NMR (400 MHz, DMSO-$d_6$) : δH 6.91 (1H, s, H-3), 6.61 (1H, s, H-8), 8.04 (2H, dd, $J = 8.0$ and 1.5 Hz, H- 2', 6'), 7.55 (2H, t-like, , $J = 8.0$ Hz, H- 3', 5'), 7.58 (1H, m, H- 4'), 12.64 (1H, br s, HO-5), 10.57 (1H, s, HO-7) and 8.81 (1H, br s, HO-6).

$^{13}$C-NMR (100 MHz, DMSO-$d_6$) : δC 162.1 (C-2), 104.5 (C-3), 180.1 (C-4), 104.3 (C-4a), 153.6 (C-5), 129.3 (C-6), 147.0 (C-7), 94.0 (C-8), 149.8 (C-8a), 130.9 (C-1'), 126.3 (C-2', 6'), 129.1 (C-3', 5') and 131.8 (C-4').[6]

EI-MS m/z (%) : 270 [M]$^+$ (100), 242 (45), 168 (35), 140 (20) and 105 (45) [6]
**Oroxylin A (9)**

Light yellow needles, mp 204°C

C_{16}H_{12}O_{5} (M^+ 284)

UV (MeOH) $\lambda_{\text{max}}$ nm: 218, 271 and 320

IR (KBr) $\tilde{\nu}_{\text{max}}$ cm\(^{-1}\): 3412, 1655, 1618, 1580 and 830

$^1$H-NMR (400 MHz, DMSO-$d_6$) : $\delta_{\text{H}}$ 6.94 (1H, s, H-3), 6.61 (1H, s, H-8), 8.04 (2H, dd, $J = 8.5$ and 1.5 Hz, H-2', 6'), 7.57 (3H, m, , H-3', 4', 5'), 3.74 (3H, s, MeO-6), 12.90 (1H, s, HO-5), 10.80 (1H, br s, HO-7).

$^{13}$C-NMR (100 MHz, DMSO-$d_6$) : $\delta_{\text{C}}$ 163.3 (C-2), 104.7 (C-3), 182.3 (C-4), 104.4 (C-4a), 152.6 (C-5), 131.6 (C-6), 157.7 (C-7), 94.5 (C-8), 152.8 (C-8a), 130.8 (C-1'), 126.5 (C-2', 6'), 129.2 (C-3', 5') and 132.1 (C-4') 60.0 (MeO-6). [6]
Scutellarein (10)

Orange needles, mp >300°C

C_{15}H_{10}O_{6} (M^+ 286)

UV (MeOH) $\lambda_{\text{max}}$ nm (log ε): 286 (4.94), 336 (5.00).

IR (KBr) $\bar{\nu}_{\text{max}}$ cm$^{-1}$: 3427, 2931, 2631, 1653, 1610, 1578, 1555, 1499, 1356, 1313, 1169, 806.

$^1$H-NMR and $^{13}$C-NMR (DMSO-$d_6$): Table 2.4

EI-MS $m/z$ (%): 286 (100), 285 (5), 258 (4), 168 (35) and 140 (7), 121 (11), 93 (4). [6]

Scutellarein 4’-O-methyl ether (11)

Yellow amorphous powder, mp 187-189°C

C_{16}H_{12}O_{6} (M^+ 300)

UV (MeOH) $\lambda_{\text{max}}$ nm: 282 and 332

IR (KBr) $\bar{\nu}_{\text{max}}$ cm$^{-1}$: 3428, 2930, 2840, 1654, 1610, 1580 and 1360.

$^1$H-NMR (400 MHz, DMSO-$d_6$): $\delta$H 6.50 (1H, s, H-3), 6.76 (1H, s, H-8), 7.98 (2H, d, $J = 8.5$ Hz, H- 2’, 6’), 7.12 (2H, d, $J = 8.5$ Hz, H- 3’, 5’), 3.86 (3H, s, MeO-4’), 12.80 (1H, s, HO-5), 10.35 (1H, br s, HO-7) and 8.68 (1H, br s, HO-6).

EI-MS $m/z$ (%): 300 [M]$^+$ (100), 285 [M – Me] (30), 168 (38), 140 (13), 132 (15), 107 (20), 105 (50) and 91 (33)
**Pectolinarigenin (12)**

Yellow needles, mp 209-210°C (lit. 210-211°C [9])

C_{17}H_{14}O_{6} (M^+ 314)

UV (MeOH) \( \lambda_{max} \) nm : 276 and 332

IR (KBr) \( \bar{\nu}_{max} \) cm\(^{-1} \) : 3457, 1643, 1610, 1561, 1177.

\(^1\)H- and \(^{13}\)C-NMR (DMSO-\(d_6\)) : Table 2.5

FAB-MS \textit{m/z} (%) : 315 [M + H]^+ (100), 314 [M]^+ (72), 299 (25), 286 (8), 271 (6), 219 (11), 183 (11), 167 (19), 107 (35)

**Dihydrobaicalein 7-O-(6''-benzoylglucoside), OI-20 (13)**

Pale yellow amorphous powder, mp 176-178°C

C_{28}H_{26}O_{11} (M^+ 538)

UV (MeOH) \( \lambda_{max} \) nm : 242sh, 285 and 348

CD (MeOH) : [\( \theta \)]_{338} + 7890, [\( \theta \)]_{290} - 33532, [\( \theta \)]_{214} + 88772

IR (KBr) \( \bar{\nu}_{max} \) cm\(^{-1} \) : 3437, 1653, 1611, 1578, 1499, 1448, 1356, 1169, 1032 and 806.

\(^1\)H- and \(^{13}\)C-NMR (DMSO-\(d_6\)) : Table 2.3
**Natural Products Chemistry**

FAB-MS $m/z$ (%) : 539 [M + H]$^+$ (100), 538 [M]$^+$ (59), 435 (60), 273 (26), 167 (10)

**β-Sitosterol glucoside (14)**

White amorphous powder, mp 275-278°C

$C_{35}H_{60}O_6$ (M$^+$ 576)

IR (KBr) $\nu_{\text{max}}$ cm$^{-1}$: 3391, 2957, 2870, 1651, 1445, 1379, 1076, 1028 and 972.

$^1$H- and $^{13}$C-NMR (C$_5$D$_5$N) : Table 2.6

FAB-MS $m/z$ (%) : 599 [M + Na]$^+$ (29), 413 [M - glucosyl]$^+$ (43), 397 (100), 329 (71), 273 (71), 255 (36) and 219 (86).

**Alkaline hydrolysis of OI-20 (13)**

Compound 13 (10 mg) was dissolved in 0.1M aq-methanolic NaOH (8 ml) and the solution was stirred at 40°C for 4 h. The resultant solution was concentrated, diluted with water, acidified with dil. H$_2$SO$_4$ and extracted with EtOAc. The EtOAc extract was concentrated and column purified over silica gel to get benzoic acid (2 mg).

**Acid hydrolysis of OI-20 (13)**

Compound 13 (12 mg) was dissolved in 2M aq-methanolic HCl (10 ml) and the solution was refluxed for 2 h. The resultant solution was concentrated, diluted with water, and extracted with EtOAc. The EtOAc extract was concentrated and column purified over silica gel to get benzoic acid (1.5 mg) and dihydrobaicalein (3.5 mg). The aqueous layer was concentrated. The presence of D-glucose in the concentrated aq. solution was detected by co-TLC with an authentic sample of D-glucose. The aq. solution showed sp. rotation of +52.5°.
**Acid hydrolysis of β-Sitosterol glucoside (14)**

Compound 14 (15 mg) was dissolved in 2M aq-methanolic HCl and the solution was refluxed for 2 h. The work up of resultant solution (as per above) afforded D-glucose and β-sitosterol (4 mg).

3. From *Justicia gendarussa*:

Whole plants of *Justicia gendarussa* were collected from Madhupur, near railway station, Agartala, Tripura in March, 2009 and were identified by Prof. B. K. Datta, Plant Taxonomist, Department of Botany, Tripura University. A voucher specimen (TU/H/1512) has been deposited in the laboratory of B. K. D.

Air dried powdered whole plants (2.0 Kg) of *Justicia gendarussa* were extracted with MeOH (5L × 2, 6d each time) and the MeOH extracts were concentrated under reduced pressure to a residue (120 g). The major portion of the residue (110 g) was suspended in H₂O (60 ml) and extracted successively with CH₂Cl₂, EtOAc and n-BuOH (3 × 100 mL with each solvent). The residue (30 g) from CH₂Cl₂ extract was subjected to silica gel column chromatography. Elution of the column with PE-CH₂Cl₂ (9 : 1) afforded α-amyrin (20 mg) and elution with PE-CH₂Cl₂ (8 : 2) afforded a residue of the mixture of two sterols, which on GC MS analysis indicated β-sitosterol (70%) and stigmasterol (30%). Elution of the column with PE-CH₂Cl₂ (6 : 4) afforded lupeol (35 mg). The residue (45 g) of the EtOAc extract was subjected to silica gel column chromatography using a gradient of PE-EtOAc and EtOAc-MeOH mixture of increasing polarity. Elution of the column with EtOAc-MeOH (9 : 1) afforded ursolic acid (45 mg). The residue of n-BuOH extract (15g) was subjected to silica gel chromatography. Elution of column with EtOAc-MeOH (9 : 1) gave ursolic acid (30) mg.
Flow sheet 3: Isolation of compounds from *J. gendarussa*

**Justicia gendarussa** whole plant (2.0 kg)

- MeOH, percolation
  - MeOH extract
    - Fractionated
      - CH$_2$Cl$_2$ sol. fraction
        - Silica gel CC
          - α-Amyrin
      - EtOAc sol. fraction
        - Silica gel CC
          - β-Sitosterol
      - n-BuOH sol. fraction
        - Silica gel CC
          - Stigmasterol
          - Ursolic acid
          - Lupeol

- MeOH extract
  - Fractionated
  - Merck

**α-Amyrin (15)**

Colourless needles, mp 182-183$^\circ$C (lit. 184-186$^\circ$C)

C$_{30}$H$_{50}$O ($M^+$ 426)

UV (MeOH) $\lambda_{max}$ nm: 218, 271 and 320

IR (KBr) $\tilde{\nu}_{max}$ cm$^{-1}$: 3460, 2895 and 1470.

$^1$H-NMR (400 MHz, CDCl$_3$) : $\delta_{H}$ 3.18 (1H, dd, $J = 5.0$ and 11.0 Hz, H-3), 5.08 (1H, t, $J = 3.5$ Hz, H-12), 0.73 (3H, d, $J = 6.8$ Hz, H$_3$-30), 0.84 (3H, d, $J = 6.8$ Hz, H$_3$-29), 0.74 (6H, s, H$_3$-24, 25), 0.89 (3H, s, H$_3$-26), 0.94 (6H, s, H$_3$-23, 28) and 1.01 (3H, s, H$_3$-27). [11]

$^{13}$C-NMR (100 MHz, CDCl$_3$) : $\delta_C$ 38.7 (C-1), 28.7 (C-2), 79.7 (C-3), 38.6 (C-4), 55.0 (C-5), 18.4 (C-6), 32.1 (C-7), 40.6 (C-8), 47.8 (C-9), 36.6 (C-10), 23.2 (C-11), 124.2 (C-12), 139.4 (C-13), 42.0 (C-14), 27.1 (C-15), 26.6 (C-16), 33.6 (C-17), 59.0 (C-18), 39.5 (C-19), 39.7 (C-20), 31.2 (C-21), 41.4 (C-22), 28.1 (C-23), 15.6 (C-24), 15.5 (C-25), 16.8 (C-26), 23.1 (C-27), 28.0 (C-28), 17.3 (C-29) and 21.4 (C-30). [11]
FAB-MS $m/z$ (%) : 427 $[M + H]^+$ (100%)

$\beta$-Sitosterol (16)

C$_{29}$H$_{50}$O (M$^+$ 414)

EI-MS $m/z$ (%) : 414 (100), 399 (30), 396 (40), 381 (25), 329 (50), 303 (55), 273 (35), 255 (50), 231 (50), 229 (20) and 213 (78). [12]
Stigmasterol (17)

C$_{29}$H$_{48}$O (M$^+$ 412)

EI-MS $m/z$ (%): 412 [M$^+$] (30), 394 (5), 379 (30), 314 (30), 300 (40)

273 (42), 271 (65), 255 (100), 231 (50), 229 (30) and

213(94). [12]

Lupeol (18)

Colourless needles, mp. 214-215$^0$C

C$_{30}$H$_{50}$O (M$^+$ 426)

IR (KBr) $\nu_{\text{max}}$ cm$^{-1}$: 3433, 2934, 2849, 1640, 1464, 1381 and 1063.

$^1$H- and $^{13}$C-NMR (CDCl$_3$) : Table 3.2

FAB-MS $m/z$ (%): 426 [M$^+$] (57), 411 (15), 315 (15), 257 (11), 234 (17)

218 (75), 207 (100) and 189 (92).

Ursolic acid (19)

Amorphous solid, mp 265-267$^0$C

C$_{30}$H$_{48}$O$_3$ (M$^+$ 456)

IR (KBr) $\nu_{\text{max}}$ cm$^{-1}$: 3433, 2934, 1709, 1639, 1464, 1381, 1063 and 970.
1H- and 13C-NMR (CD3OD) : **Table 3.3**

FAB-MS m/z (%): 457 [M + H]+ (57), 439 (100), 411 (96), 248 (65), 203 (91) and 189 (90).

4. From *Vitex peduncularis*:

   Fresh leaves of *Vitex peduncularis* were collected from Belonia, South Tripura in March, 2010 and were identified by Prof. B. K. Datta, Plant Taxonomist, Department of Botany, Tripura University.

   Air dried powdered leaves (1.5 kg) of *Vitex peduncularis* were extracted with MeOH (4L × 2) and the MeOH extracts were concentrated to a gummy residue (78 g). The major part of the residue (70 g) was suspended in water and extracted successively with CH2Cl2, EtOAc and n-BuOH. The EtOAc extract (35 g) was subjected to silica gel column chromatography. Elution of the column with PE-EtOAc (7 : 3) afforded vitipecin (48 mg). While elution of the column with PE-EtOAc (6 : 4) afforded vitexin (25 mg). The residue (20 g) of the n-BuOH extract was subjected to column chromatography over silica gel. Elution of the column with EtOAc-MeOH (9 : 1) afforded ursolic acid (30 mg).

**Flow sheet 4: Isolation of compounds from *V. peduncularis***

```
V. peduncularis leaves (1.5 kg)  
MeOH, percolation  
  
MeOH extract  
  | Fractionation  
  |  
  | MeOH extract  
  |  
  | CH2Cl2 sol. fraction  
  |  
  | EtOAc sol. fraction  
  |  
  | Silica gel CC  
  |  
  | Vitecin  
  |  
  | Vitexin  
  |  
  | Merck  
  |  
  | n-BuOH sol. fraction  
  |  
  | Silica gel CC  
  |  
  | Ursolic acid  
```

Natural Products Chemistry 17
Vitecin (20)

Brown needles, mp 232\(^0\)C

\(C_{18}H_{16}O_8 (M^+ 360)\)

UV (MeOH) \(\lambda_{max} \text{ nm} : 252, 263\text{sh} \text{ and } 360\)

IR (KBr) \(\nu_{max} \text{ cm}^{-1} : 3472, 2872, 1643, 1474 \text{ and } 1089.\)

\(^1\)H- and \(^{13}\)C-NMR (DMSO-\(d_6\)) : Table 4.3

FAB-MS \(m/z \) (%) : 361 [M + H]\(^+\) (100), 360 (40), 359 (18), 345 (18), 317 (8), 299 (6) and 167 (14).

Vitexin (21)

Yellow amorphous powder,

\(C_{21}H_{20}O_{10} (M^+ 432)\)

UV (MeOH) \(\lambda_{max} \text{ nm (log } e) : 268 \text{ (3.88), 333 (3.90).}\)

\(^1\)H-NMR (600 MHz, DMSO-\(d_6\)) : \(\delta_H 6.64 \text{ (1H, s, H-3), 6.28 \text{ (1H, s, H-6), 7.98 \text{ (2H, d, } J = 8.5 \text{ Hz, H-2', 6'\text{'}, 6.90 \text{ (2H, d, } J = 8.5 \text{ Hz, H-3', 5'\text{'}, 4.62 \text{ (1H, d, } J = 9.0 \text{ Hz, H-1'\text{'}, 4.68 \text{ (1H, br d, } J = 10.5 \text{ Hz, H-6'\text{'}, 4.98 \text{ (1H, br d, } J = 10.5 \text{ Hz, H-6'\text{'}, 13.17 \text{ (1H, s, HO-5), 10.84 \text{ (1H, br s, HO-7), 10.35 \text{ (1H, br s, HO-4'\text{'}. \[13\]}

\(^{13}\)C-NMR (100 MHz, DMSO-\(d_6\)) : \(\delta_C 164.0 \text{ (C-2), 102.3 \text{ (C-3), 182.1 \text{ (C-4), 104.1 \text{ (C-4a), 161.2 \text{ (C-5), 98.2 \text{ (C-6), 162.6 \text{ (C-7), 104.6 \text{ (C-8), 156.0 \text{ (C-8a), 121.6 \text{ (C-1'\text{'), 129.0 \text{ (C-2'\text{'}, 6'\text{'), 115.8 \text{ (C-3'\text{'}, 5'\text{'), 160.4 \text{ (C-4'\text{'}, 73.4 \text{ (C-1'\text{'), 70.9 \text{ (C-2'\text{'}, 78.7 \text{ (C-3'\text{'}, 70.6 \text{ (C-4'\text{'), 81.9 \text{ (C-5'\text{'}, 61.3 \text{ (C-6'\text{'}. \[14, 15\]}

FAB-MS \(m/z \) (%) : 433 [M + H]\(^+\) (100%).
References


