EXPERIMENTAL
PART-IV: 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) 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°C. The spots were also visualized by spraying Dragendorff reagent in case of alkaloids. Silica gel (mesh 60-120, Merck), 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°C). The optical rotation was measured on a JASCO DIP-370 digital polarimeter. UV-vis spectra were recorded on a Perkin Elmer Lambda 25 spectrophotometer and were expressed in $\lambda_{\text{max}}$ solvent nm (log $\varepsilon$). IR spectra in KBr disc were recorded on a Shimadzu 8100 FT-IR spectrophotometer and were expressed in $v_{\text{max}}$ cm$^{-1}$. $^1$H, $^{13}$C and 2D-NMR spectra were recorded on a Varian XL-300, 400 and 600 NMR / Bruker Avance II 600 NMR spectrometer. Chemical shifts were expressed in $\delta$ (ppm) with tetramethylsilane (TMS) as an internal standard, and the coupling constants were in hertz (Hz). EI-MS, HR-EI-MS were taken using 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°, 90° and 135°. 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 30m x 0.25nm DB-5 column and GC injection port temperature, 250°C . The helium carrier gas flow rate was 2.0 mL/min. The temperature of the transfer line was 250°C. The initial oven temperature was 100°C and was held for 1 min. The oven was then heated to 270°C at a rate of 10°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 phytochemicals from Sida glutinosa*

**Plant material:** The aerial parts of *Sida glutinosa* were collected from Kalsi (Jolaibari), South Tripura in March 2008. The plant was identified by Prof. B. K. Datta, taxonomist, Department of Botany, Tripura University. A Voucher specimen (#BD/01/08) has been deposited in the Central National Herbarium, Botanical Survey of India, Govt. of India, Botanic Garden, Howrah-711103, West Bengal.

**Extraction of aerial parts of S. glutinosa:** Air-dried powdered aerial parts of *S. glutinosa* (3.3 kg) were extracted three times with MeOH (6 L × 3) at room temperature for 48 hours. The MeOH extract was concentrated under reduced pressure in vacuo to a gummy mass (106 g). The residue (90 g) was suspended in water (100 ml) and extracted three times with CH$_2$Cl$_2$, CHCl$_3$ and n-BuOH successively.

One part of n-BuOH soluble fraction was subjected to Diaion HP-20 column chromatography (CC) and eluted with a stepwise gradient of H$_2$O/MeOH (100:0, 75:25, 50:50, 25:75, 0:100) and 250 ml fraction of each were collected. The residues obtained from H$_2$O/MeOH 75:25 and 50:50 were mixed together and was subjected to Silica gel CC using CHCl$_3$ and CHCl$_3$/MeOH mixtures of different ratios as eluents. The fractions eluted with CHCl$_3$ and CHCl$_3$/MeOH was further subjected to basic Alumina CC and the column was eluted with EtOAc and EtOAc/MeOH (95:5, 90:10, 80:20, 60:40, 50:50), 250 ml each fraction were collected. The eluates of the column were monitored by TLC in different solvent systems. EtOAc/MeOH (90:10) fraction a solid, which on repeated crystallization from CHCl$_3$/MeOH (80:20) mixture gave 24(28)-dehydromakisterone A (45 mg). EtOAc/MeOH (50:50) fraction on crystallization from MeOH gave glutinoside (35 mg).
The CHCl₃ fraction was subjected to Silica gel CC using Petroleum ether (PE)/CHCl₃ mixture (9:1, 6:1, 3:1). The PE/CHCl₃ (6:1) eluate gave a residue, which on crystallization from CHCl₃/MeOH (90:10) gave chrysin (60 mg) in pale yellow needles.

Another part of n-BuOH soluble fraction was churned with 5% aqueous citric acid for 6 h and the aqueous filtrate was basified with dilute NH₄OH (~2 N) and extracted with n-BuOH. The n-BuOH soluble part was applied to Silica gel CC and eluted with CHCl₃ and CHCl₃/MeOH (different ratios). CHCl₃/MeOH (70:30) eluate gave alkaloid (25 mg).

The CH₂Cl₂ soluble fraction was subjected to column chromatography through Silica gel to get pure phytochemicals. Petroleum ether (PE)/EtOAc (15:1) gave the sterol mixture (50 mg), PE/EtOAc (12:1) gave 1-triacontanol (25 mg) and PE/EtOAc (9:1) gave docosanoic acid (15 mg).

The isolation of phytochemicals from S. glutinosa are shown in Flow-Sheet-I.
**Flow-Sheet-I: Isolation of phytochemicals from S. glutinosa.**

**Sida glutinosa aerial part (3.3 kg)**

- MeOH (6L X 3), 48 h, Percolation
- MeOH extract (106 g)
  - Divided into two parts
  - MeOH extract-A (70 gm)
    - Fractionated
      - CHCl₃ Fraction
        - CHrysin (250) (60 mg)
        - CHCl₃ Fraction
          - CC through Silica gel
            - MeOH Part (3.5 g)
              - Divided into two parts
                - Part-I (2 g)
                  - Repeatedly CC through Silica gel & basic Al₂O₃
                    - Glutinoside (247) (35 mg)
                - Part-II (1.5 g)
                  - CC through Silica gel and basic Al₂O₃
                    - 24(28)-dehydromakisterone A (248) (45 mg)
  - MeOH extract-B (35 gm)
    - i) Churned with 5% aq. citric acid (300 ml) for 6 h. & filtered.
      - ii) Filtrate was basified with dil. NH₄OH (~2N, PH 8-9)
      - iii) Fractionated
    - CH₂Cl₂ Fraction
      - MeOH Part (3.5 g)
        - Divided into two parts
          - Part-I (2 g)
            - Repeatedly CC through Silica gel & basic Al₂O₃
              - Glutinoside (247) (35 mg)
          - Part-II (1.5 g)
            - CC through Silica gel and basic Al₂O₃
              - 24(28)-dehydromakisterone A (248) (45 mg)
      - CHCl₃ Fraction
        - CC through Diaion HP-20
          - MeOH Part (3.5 g)
            - Divided into two parts
              - Part-I (2 g)
                - Repeatedly CC through Silica gel & basic Al₂O₃
                  - Glutinoside (247) (35 mg)
              - Part-II (1.5 g)
                - CC through Silica gel and basic Al₂O₃
                  - 24(28)-dehydromakisterone A (248) (45 mg)
      - n-BuOH Fraction
        - CC through Silica gel
          - Alkaloid (249) (25 mg)
Glutinoside (247) \(= \text{Kaempferol-5-O-}\beta-d-(6''-O-trans-coumaroyl)\) glucopyranoside:

Yellow crystals, mp 225°C. UV (MeOH) \(\lambda_{\text{max}}\) (nm): 255 (4.08), 322 sh (3.68), 372 (4.18). UV (+NaOAc) \(\lambda_{\text{max}}\) (nm): 248, 321, 353 nm; IR (KBr) \(\nu_{\text{max}}\) (cm\(^{-1}\)): 3460, 3252, 1695, 1684, 1655, 1628, 1607, 1589, 1501, 1360, 1294, 1182, 1067, 827; \(^1\)H and \(^{13}\)C NMR spectral data are given in Table 1.4. FAB-MS \(m/z\) (relative intensity): 617 [M+Na]\(^+\) (38), 595 [M+H]\(^+\) (13), 287 [aglycone+H]\(^+\) (100), 315 (9), 299 (14), 286 [aglycone]\(^-\) (72), 259 (16), 165 (28), 147 (75), 107 (50), 77 (59), 65 (22). HR-FAB-MS \(m/z\) (positive ion): 617.1256 [M+Na]\(^+\) (calcd for C\(_{30}\)H\(_{26}\)O\(_{13}\)Na : 617.1270).

24(28)-Dehydromakisterone A (248):

A light yellow crystals, mp 220°C (dec). UV (MeOH) \(\lambda_{\text{max}}\) (nm): 248 (3.61); IR (KBr) \(\nu_{\text{max}}\) (cm\(^{-1}\)): 3389, 1658, 1642, 1464, 1445, 1381, 1150, 1059, 874; \(^1\)H and \(^{13}\)C NMR spectral data are given in Table 1.5. FAB-MS \(m/z\) (relative intensity): 515 [M+Na]\(^+\) (60), 493 [M+H]\(^+\) (53), 475 (60), 457 (100), 439 (40), 363 (33), 345 (35), 327 (13), 301 (33), 191 (27), 173 (47), 165 (47), 147 (33), 129 (40). HR-FAB-MS \(m/z\) (positive ion): 515.2998 [M+Na]\(^+\) (calcd for C\(_{28}\)H\(_{44}\)O\(_7\)Na : 515.2984).

Alkaloid (249) \(= 1, 2, 3, 9\)-Tetrahydropyrrolo \([2,1-b]\)-quinazolin-3-amine:

Amorphous powder, mp 188-190°C (dec.) \([\alpha]_D^{20} \approx -115.2^\circ\) (c = 0.15, MeOH). UV (MeOH) \(\lambda_{\text{max}}\) (nm): 226.11 (3.99) and 299.28 (3.88). IR (KBr) \(\nu_{\text{max}}\) (cm\(^{-1}\)): 3431-3381 br, 1634, 1597, 1570, 1501, 1483, 1458, 1333, 1306, 1102, 1080, 1059, 874, 760; \(^1\)H and \(^{13}\)C NMR spectral data are given in Table 1.6. EI-MS \(m/z\) (relative intensity): 188 [M+H]\(^+\) (16), 187 [M]\(^+\) (100), 170 [M-NH\(_3\)]\(^+\) (75), 159 [M-C\(_2\)H\(_4\)]\(^+\) (90), 131 (92), 104 (77), 89 (74), 77 (80), 51 (31). HR-EI-MS \(m/z\) (positive ion): 188.1172 [M+H]\(^+\) (calcd for C\(_{11}\)H\(_{14}\)N\(_3\) : 188.1188).

Chrysin (250):

Yellow needles, mp 270°C, UV (MeOH) \(\lambda_{\text{max}}\) nm (log\(\varepsilon\)) : 247 sh (4.58), 268 (4.90), 314 (4.48). IR (KBr) \(\nu_{\text{max}}\) cm\(^{-1}\): 3427, 1653, 1611, 1578, 1555, 1499,
1H-NMR (400 MHz, DMSO-d$_6$) $\delta$: 6.93 (1H, s, H-3), 12.80 (1H, s, HO-5), 6.19 (1H, d, $J = 2.0$ Hz, H-6), 10.89 (1H, s, HO-7), 6.50 (1H, d, $J = 2.0$ Hz, H-8), 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'); $^{13}$C-NMR (100 MHz, DMSO-d$_6$): 163.12 (s, C-2), 105.14 (d, C-3), 181.83 (s, C-4), 161.43 (s, C-5), 98.98 (d, C-6), 164.40 (s, C-7), 94.04 (d, C-8), 157.42 (s, C-9), 103.93 (s, C-10), 130.68 (s, C-1'), 126.37 (d, C-2', 6'), 129.09 (d, C-3', 5'), 131.96 (d, C-4').

EI-MS $m/z$ (%): 254 (M$^+$, 100), 253 (21), 226 (M-CO, 48), 152 (40), 124 (26), 113 (21), 105 (6), 102 (8), 77 (11); HR-EI-MS $m/z$ (%): 254.0579 (100%) Calcd. for C$_{15}$H$_{10}$O$_4$: 254.0579.

Sterol mixture:
White solid, mp 110-113°C; The composition of the sterol mixture was determined by GC and GC/MS analysis: DB-5 column, using He gas and the following temp conditions: isothermal 250°C, injector and separator temps 270°C; cholesterol was used as internal standard. The major component was $\beta$-sitosterol (68.2%) which was accompanied with campesterol (16.6%) and stigmasterol (15.2%). IR $\nu_{\text{max}}$ cm$^{-1}$ (KBr): 3404, 2933, 2870, 1693, 1462, 1379, 1271, 1049. On GC analysis the sterol mixture showed three peaks corresponding to campesterol ($R_R$ : 1.28, content : 16.6%), stigmasterol ($R_R$ : 1.37, content : 15.2%), and $\beta$-sitosterol ($R_R$ : 1.63, content : 68.2%), which were identified by comparison of $R_R$s relative to cholesterol ($R_t$ : 1.00) as well as co-GC with authentic samples under identical conditions. (i) EI-MS of campesterol $m/z$ (rel. int.): 400 [M]$^+$ (46), 385 (15), 382 (26), 367 (20), 315 (28), 289 (32). (ii) EI-MS of stigmasterol $m/z$ (rel. int.): 412[M]$^+$ (85), 394 (28), 327 (15), 314 (12), 300 (20), 273 (38), 271 (42), 255 (100), 213 (30). (iii) EI-MS of $\beta$-sitosterol $m/z$ (rel. int.): 414[M]$^+$ (100), 399 (16), 396 (35), 381 (25), 329 (18), 303 (50), 273 (35), 255 (35), 231 (50), 229 (18), 213 (60).

1-Triacontanol:
Amorphous solid, mp 86°C. IR $\nu_{\text{max}}$ cm$^{-1}$ (KBr): 3304, 2931, 2849, 1747, 1462, 1061. EI-MS $m/z$ (rel. int.): 438 [M]$^+$ (0.5), 420 [M-H$_2$O]$^+$ (16), 392 [M-46]$^+$ (57), 364 (33), 336 (10), 85 (98), 83 (100). $^1$H-NMR (CDCl$_3$, 400 MHz): $d$ 0.88 (3H,
t, \( J = 6.8 \text{ Hz}, \ H_3-30 \), 1.26–1.34 (br s, \ CH_2 \text{ grs.}), 1.58 (m, \ CH_2 \text{ gr.}), 3.64 (2H, t, \ J = 6.8 \text{ Hz}, \ H_2-1).

![1-Triacontanol](image)

**Docosanoic acid:**

Amorphous solid, mp 82°C. IR \( \nu_{\text{max}} \text{ cm}^{-1}(\text{KBr}) \): 2635, 2916, 2848, 1710, 1474, 1462. EIMS \( m/z \) (rel. int.): 340 [M]+ (100), 323 (26), 312 (31), 284 (12), 256 (9), 60 (8), 57 (10). \(^1\text{H-NMR} (\text{CDCl}_3, 400 \text{ MHz}): \delta 0.88 (3H, t, \ J = 6.8 \text{ Hz}, \ H_3-22), 1.26–1.32 (br s, \ CH_2 \text{ grs.}), 1.63 (2H, quint, \ H_2-3), 2.35 (2H, t, \ J = 6.8 \text{ Hz}, \ H_2-3).

![Docosanoic acid](image)

**• Extraction and isolation of phytochemicals from Neanotis wightiana**

**Plant material:** The whole aerial parts of *Neanotis wightiana* were collected from Kalsi (Jolaibari), South Tripura in March 2008 and identified by Prof. B. K. Datta, taxonomist, Department of Botany, Tripura University. A Voucher specimen (#BD/02/08) has been deposited in the National Herbarium, Botanical Survey of India, Botanical Garden, Howrah 711 103.

**Extraction of whole aerial parts of N. wightiana:**

Fresh air-dried aerial parts of *N. wightiana* were dried in shaded floor and crushed into coarse powder. Dried coarse powders (3.0 kg) were extracted three times with MeOH (10 L × 3) at room temperature for 1 week each time. The MeOH extract was concentrated under reduced pressure in *vacuo* to a semi solid mass (400 g). The residue (350 g) was suspended in 125 mL water and extracted three times with hexane, chloroform, ethyl acetate and \( n-\text{BuOH} \) (each 200 mL), successively.
The hexane (10.2 gm), chloroform (5 gm) and ethyl acetate (20.2 g) soluble fraction of the crude MeOH extract were separately column chromatographed through Silica gel repeatedly to get the pure compounds. The n-BuOH soluble fraction of MeOH extract was subjected to column chromatography through Diaion ion HP-20 and Silica gel successively. The isolation of phytochemicals from different soluble fractions of methanol extract of N. wightiana are shown in Flow-Sheet-II.

Hexane soluble fraction was subjected to CC over Silica gel using stepwise solvent gradient of PE/CHCl₃ (100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 20:80; each 500 ml). The residue obtained from PE/CHCl₃ (70:30) eluate gave hexacosanoic acid (50 mg). The residue obtained from PE/CHCl₃ (20:80) eluate on repeated CC on Silica gel gave β-sitosterol (22 mg) and stigmasterol (28.2 mg). The CHCl₃ soluble fraction was subjected to CC on Silica gel using solvent gradient PE/CHCl₃ (50:50, 20:80, 10:90 and 0:100) and CHCl₃/EtOAc (9:1, 6:1, 3:1). The CHCl₃ eluate gave oleanolic acid (48 mg). CHCl₃/EtOAc (9:1) eluate gave ursolic acid (36 mg). CHCl₃/EtOAc (6:1) eluate gave NW-2 (45 mg). CHCl₃/EtOAc (3:1) eluate gave a residue which on repeated CC over Silica gel using CHCl₃/EtOAc mixture of different ratios gave stigmasterol glucoside (18.4 mg) and β-sitosterol glucoside (12.5 mg).

n-BuOH fraction was subjected to Diaion HP-20 CC and eluted stepwise with H₂O/MeOH (100:0, 75:25, 50:50, 25:75, 0:100; each 125 ml). The residue from MeOH eluate was subjected to Silica gel CC with a stepwise gradient of solvent, EtOAc/MeOH (100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 0:100; each 100 ml). The fraction eluted with EtOAc/MeOH (90:10) gave a residue, which on repeated crystallization from MeOH afforded neanoside A (65 mg) in colourless needles.
**Flow-Sheet-II:** Isolation of phytochemicals from *N. wightiana*.

*M. wightiana* aerial part (3.0 kg)

- MeOH (10 L X 3), 1 week Percolation

  - MeOH extract (400 g)
  - Suspended in H₂O & Fractionated

  - Hexane Fraction (10.2 g)
  - CHCl₃ Fraction (5 g)
  - Ethyl acetate Fraction (20.2 g)
  - n-BuOH Fraction (90 g)

  - CC through Silica gel
  - β-sitosterol glucoside (12.5 mg)
  - Stigmasterol glucoside (18.4 mg)
  - Ursolic acid (36 mg)
  - Oleanolic acid (48 mg)
  - NW-2 (45 mg)
  - Repeatedly CC through Silica gel

  - Hexacosanoic acid (50 mg)
  - β-sitosterol (22 mg)
  - Stigmasterol (28.2 mg)

  - Repeatedly CC through Silica gel

  - Neanoside A (65 mg)

  - NW-10 (25 mg)

**Neanoside A (97):**

White needles from MeOH; mp 220⁰C (dec); [α]_D⁰²⁶ + 12.4⁰ (c 0.75, MeOH). IR (KBr) max cm⁻¹: 3434, 2943, 1694, 1633, 1459, 1385, 1081, 1046, 624; H and C NMR spectral data are given in Table 2.3. HR-FAB-MS (positive ion...
Acidic hydrolysis of neanoside A (97):

Compound 97 (25 mg) was refluxed in 5 mL of 2 M HCl (MeOH-H2O, 1:1) for 2 h in a water bath. After removal of MeOH, the solution was extracted with EtOAc (10 L X 3). The extractant (EtOAc extract) was washed with H2O and concentrated to get bayogenin (98, 6 mg), identified by co-TLC with authentic sample.

Alkaline hydrolysis of neanoside A (97):

Compound 97 (6 mg) was refluxed with 5 mL of 2 M KOH (MeOH-H2O, 1:1) for 4 h. After cooling, the reaction mixture was neutralized with 2 M HCl and then extracted with n-BuOH (5 mL X 2). The BuOH layer was evaporated to dryness under vacuo and the residue was subjected to TLC. It was identical with parent compound 97 in co-TLC.

3α-Hydroxyolean-12-en-27-oic acid (99):

Amorphous solid, mp 248–250°C, HR-FAB-MS: m/z 457.3682. Other peaks of FAB-MS are 439 [MH–18], 411, 248, 207 and 163 (base peak)1H and 13C-NMR are given in Table 2.4.

Stigmasterol glucoside (100):

Colourless needles, mp 270°C, HR-FAB-MS: m/z 597.4134 [M+Na]+ (calcd for C55H88O6Na: 597.4131) in positive mode. Other peaks of FAB-MS are 411 [M–glucosyl]+, 395 [M–glucose+H]+, 271 and 255. 1H and 13C-NMR are given in Table 2.5.

Oleanolic acid:

White amorphous solid, mp 276°C; C30H48O3 (M+ 456); IR (KBr) νmax cm−1: 3428 (OH), 1705 (–COOH); 1H-NMR (600 MHz, CD3OD) δ 0.81 (3H, s, H3-
26), 0.86 (3H, s, H₃-24), 0.95 (3H, s, H₃-29), 0.97 (3H, s, H₃-25), 1.03 (3H, s, H₃-30), 1.09 (3H, s, H₃-23), 1.23 (3H, s, H₃-27), 2.96 (1H, dd, J = 12.0, 5.0 Hz, H-18), 3.22 (1H, dd, J = 11.5, 4.0 Hz, H-3), 5.38 (1H, t, J = 3.5 Hz, H-12); ¹³C-NMR (150 MHz, CD₃OD) δ: 39.9 (C-1), 26.6 (C-2), 78.9 (C-3), 38.9 (C-4), 55.6 (C-5), 19.2 (C-6), 33.4 (C-7), 40.1 (C-8), 48.6 (C-9), 37.6 (C-10), 24.6 (C-11), 124.3 (C-12), 144.2 (C-13), 42.2 (C-14), 28.2 (C-15), 23.5 (C-16), 46.9 (C-17), 42.8 (C-18), 46.6 (C-19), 30.7 (C-20), 34.5 (C-21), 34.0 (C-22), 28.2 (C-23), 16.4 (C-24), 15.8 (C-25), 18.0 (C-26), 26.2 (C-27), 177.8 (C-28), 33.3 (C-29), 23.6 (C-30) [3, 4].

Oleanolic acid

**Ursolic acid:**

White powder, mp 265–67°C; C₃₀H₄₆O₉ (M⁺ 456); IR (KBr) ν_max cm⁻¹: 3420 (OH), 1705 (–COOH); ¹H-NMR (600 MHz, CD₃OD) δ: 0.76 (3H, s, H₃-25), 0.83 (3H, d, J = 6.5 Hz, H₃-30), 0.87 (3H, d, J = 6.3 Hz, H₃-29), 0.93 (3H, s, H₃-24), 0.95 (3H, s, H₃-26), 0.96 (3H, s, H₃-27), 1.09 (3H, s, H₃-23), 2.20 (1H, d, J = 11.5 Hz, H-18), 5.22 (1H, m, H-12); ¹³C-NMR (150 MHz, CD₃OD) δ: 39.8 (C-1), 27.7 (C-2), 79.7 (C-3), 39.9 (C-4), 55.9 (C-5), 19.4 (C-6), 34.2 (C-7), 40.8 (C-8), 47.6 (C-9), 38.2 (C-10), 24.3 (C-11), 126.7 (C-12), 139.8 (C-13), 42.8 (C-14), 29.1 (C-15), 25.3 (C-16), 47.8 (C-17), 54.2 (C-18), 40.2 (C-19), 40.4 (C-20), 31.8 (C-21), 38.2 (C-22), 28.8 (C-23), 15.9 (C-24), 16.4 (C-25), 17.6 (C-26), 24.2 (C-27), 181.6 (C-28), 17.7 (C-29), 21.4 (C-30), EL-MS m/z (%): 456 (M⁺, 20), 438 (15), 248 (100), 207 (30), 203 (48), 189 (20) [5].

**Natural Products Chemistry**

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\[ \text{Sitosterol glucoside:} \]

Colourless crystals, mp 283–285°C; C\textsubscript{35}H\textsubscript{60}O\textsubscript{6} (M\textsuperscript{+} 576); FAB-MS (Na-\textsuperscript{m}-nitro benzyalcohol) m/z (%): 599 [M+Na\textsuperscript{+}] (35); IR (KBr) \( \nu_{\text{max}} \text{ cm}^{-1} \): 3434 (OH), 1640 (C=C), 1095 (glycosidic); \(^1\)H-NMR (300 MHz, C\textsubscript{5}D\textsubscript{5}N) \( \delta \) 0.67 (3H, s, H\textsubscript{3}-18), 0.88 (3H, d, \( J = 6.5 \) Hz, H\textsubscript{3}-26), 0.90 (3H, d, \( J = 6.5 \) Hz, H\textsubscript{3}-27), 0.95 (3H, s, H\textsubscript{3}-19), 1.02 (3H, d, \( J = 6.0 \) Hz, H\textsubscript{3}-21), 3.98 (1H, m, H-3), 5.36 (1H, brd, \( J = 4.5 \) Hz, H-6); \(^{13}\)C-NMR (75 MHz, C\textsubscript{5}D\textsubscript{5}N) \( \delta \): 38.5 (C-1), 31.1 (C-2), 79.2 (C-3), 40.2 (C-4), 141.6 (C-5), 122.8 (C-6), 33.1 (C-7), 32.9 (C-8), 51.0 (C-9), 37.8 (C-10), 22.1 (C-11), 40.6 (C-12), 43.6 (C-13), 57.6 (C-14), 24.2 (C-15), 29.4 (C-16), 57.0 (C-17), 12.9 (C-18), 20.2 (C-19), 37.2 (C-20), 19.8 (C-21), 35.1 (C-22), 27.1 (C-23), 46.8 (C-24), 30.4 (C-25), 20.1 (C-26), 20.8 (C-27), 25.4 (C-28), 12.9 (C-29), 103.4 (C-1\textprime{}), 76.2 (C-2\textprime{}), 79.4 (C-3\textprime{}), 72.4 (C-4\textprime{}), 79.2 (C-5\textprime{}), 63.4 (C-6\textprime{}) [6].

\[ \text{\( \beta \)-Sitosterol glucoside} \]
**β-Sitosterol:**

Colourless needles, mp 135–137°C; C_{29}H_{50}O \text{ (M}^{+} 414); IR (KBr) \nu_{\text{max}} cm^{-1}: 3400 (OH), 1625 (C=C), 1460, 1440; \text{ }^1\text{H-NMR (300 MHz, CDCl}_3) \delta: 0.68 (3H, s, H_{3-18}), 0.80 (3H, d, \ J = 6.5 \text{ Hz, } H_{3-27}), 0.83 (3H, d, \ J = 6.5 \text{ Hz, } H_{3-26}), 0.92 (3H, d, \ J = 6.5 \text{ Hz, } H_{3-21}), 1.02 (3H, s, H_{3-19}), 3.50 (1H, m, H-3), 5.34 (1H, brd, \ J = 5.0 \text{ Hz, } H-6); \text{ }^{13}\text{C-NMR (75 MHz, CDCl}_3) \delta: 37.3 (C-1), 31.7 (C-2), 72.0 (C-3), 42.4 (C-4), 140.8 (C-5), 121.7 (C-6), 32.0 (C-7), 32.1 (C-8), 50.2 (C-9), 36.6 (C-10), 21.2 (C-11), 39.8 (C-12), 42.3 (C-13), 56.8 (C-14), 24.2 (C-15), 28.2 (C-16), 56.1 (C-17), 11.9 (C-18), 19.3 (C-19), 36.2 (C-20), 19.1 (C-21), 34.0 (C-22), 29.3 (C-23), 50.2 (C-24), 26.2 (C-25), 18.8 (C-26), 19.7 (C-27), 23.2 (C-28), 11.9 (C-29) [7, 8].

![β-sitosterol](image)

**Stigmasterol:**

Colourless crystals, mp 152°C; C_{29}H_{48}O \text{ (M}^{+} 412); IR (KBr) \nu_{\text{max}} cm^{-1}: 3380 (OH), 1640, 875 (C=C); \text{ }^1\text{H-NMR (600 MHz, CDCl}_3) \delta: 0.69 (3H, s, H_{3-18}), 0.80 (3H, d, \ J = 6.5 \text{ Hz, } H_{3-27}), 0.84 (3H, d, \ J = 6.5 \text{ Hz, } H_{3-26}), 0.92 (3H, d, \ J = 6.6 \text{ Hz, } H_{3-21}), 1.02 (3H, s, H_{3-19}), 3.52 (1H, m, H-3), 5.01 (1H, dd, \ J = 14.5, 8.0 \text{ Hz, } H-22), 5.16 (1H, dd, \ J = 14.5, 9.0 \text{ Hz, } H-23), 5.35 (1H, brd, H-6); \text{ }^{13}\text{C-NMR (150 MHz, CDCl}_3) \delta: 37.2 (C-1), 31.6 (C-2), 71.8 (C-3), 42.3 (C-4), 140.8 (C-5), 121.7 (C-6), 31.7 (C-7), 31.9 (C-8), 50.2 (C-9), 36.5 (C-10), 21.2 (C-11), 39.8 (C-12), 42.2 (C-13), 56.9 (C-14), 24.4 (C-15), 28.9 (C-16), 56.0 (C-17), 12.2 (C-18), 19.4 (C-19), 40.5 (C-20), 21.1 (C-21), 138.3 (C-22), 129.3 (C-23), 51.2 (C-24), 31.9 (C-25), 19.0 (C-26), 21.2 (C-27), 25.4 (C-28), 12.0 (C-29); EI-MS \text{ } m/z (%) : 412

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Hexacosanoic acid:

White powder, mp 88–89°C; C\textsubscript{26}H\textsubscript{52}O\textsubscript{2} (M\textsuperscript{+} 396); FAB-MS m/z: 397 [M+H]\textsuperscript{+}; IR (KBr) \nu_{\text{max}} \text{ cm}^{-1}: 1710 (COOH); \textsuperscript{1}H-NMR (600 MHz, CDCl\textsubscript{3}) \delta 0.87 (3H, t, J = 7.2 Hz, H\textsubscript{3}-26), 1.53 (2H, quint., J = 6.6 Hz, H\textsubscript{2}-3), 2.36 (2H, t, J = 7.2 Hz, H\textsubscript{2}-2); \textsuperscript{13}C-NMR (150 MHz, CDCl\textsubscript{3}) \delta: 179.0 (C-1), 33.8 (C-2), 24.6 (C-3), 29.0–29.7 (C-4–23), 31.9 (C-24), 22.7 (C-25), 14.1 (C-26) [11].

\[
\text{H}_3\text{C}\begin{array}{c}
\text{COOH}
\end{array}
\]

Biochemical study of 97, 99 and 100:

Serum was separated from human blood sample and the activity of compounds 97, 99 and 100 against human liposomal serum enzymes SGOT, SGPT, ALP and Triglyceride at different concentrations was evaluated colorimetrically.
• **Extraction and isolation of phytochemicals from *Ichnocarpus frutescens***

**Plant material:** The fresh roots of *I. frutescens* were collected from Kalsi (Jolaibari), South Tripura in March 2008. The plant was identified by Prof. B.K. Datta, Taxonomist, Department of Botany, Tripura University. A voucher specimen (#BD/06/08) has deposited in the laboratory of Prof. B.K. Datta, Taxonomist, Department of Botany, Tripura University.

**Extraction of *I. frutescens* roots:**

Fresh air-dried roots of *I. frutescens* were dried in shaded floor and crushed into coarse powder. Dried coarse powder (1.5 kg) was extracted with MeOH (4L × 3) at room temperature for 72 h. The MeOH extract was concentrated under reduced pressure in vacuo to a gummy mass (90 g). The residue (110 g) was suspended in H$_2$O (100 mL) and fractionated into hexane (5 g), petroleum ether (8 g), chloroform (15 g), ethyl acetate (18 g) and *n*-butanol (25 g) soluble fractions by partition between water and hexane, water and petroleum ether, water and chloroform, water and ethyl acetate, water and *n*-butanol, successively.

The Petroleum ether fraction was subjected to Silica gel CC. The elution of the column with petroleum ether-ethyl acetate (9:1) afforded a solid, which on crystallization from petroleum ether-CHCl$_3$ mixture gave 2-hydroxy tricosanoic acid.

The CHCl$_3$ fraction was subjected to Silica gel CC. Elution of the column with PE-CHCl$_3$ (6:1) afforded a solid, which on repeated CC gave β-sitosterol and stigmasterol.

The EtOAc fraction was subjected to CC over Silica gel. The elution of the column with EtOAc-MeOH (9:1) gave stigmasterol glucoside. Elution with EtOAc-MeOH (7:1) gave β-sitosterol glucoside.

The *n*-BuOH fraction was CC over Diaion HP-20 using water, water-methanol (50:50, 25:75) and methanol.

Isolation of all these phytochemicals from different soluble fractions of methanol extract of *I. frutescens* are shown in **Flow-Sheet-III.**
Flow-Sheet-III: Isolation of phytochemicals from *I. frutescens*.

**Ichnopetes frutescens roots (1.5 kg)**

MeOH (5 L X 3), 72 h Percolation

- MeOH extract (90 g) suspended in H₂O & Fractionated
  - Hexane Fraction (5 g)
  - Petroleum ether (8 g)
  - CC through silica gel

- CHCl₃ Fraction (15 g)
  - CC through silica gel
  - β-sitosterol glucoside (22 mg)
  - Stigmasterol glucoside (21 mg)

- Ethyl acetate Fraction (18 g)
  - CC through silica gel
  - β-sitosterol (25 mg)
  - Stigmasterol (26 mg)

- n-BuOH Fraction (25 g)
  - CC through Diaion HP-20
  - 2-hydroxy tricosanoic acid (60 mg)
  - BF-1 (2.5 g)
  - BF-2 (2.5 g)
  - BF-3 (2.0 g)
  - BF-4 (1.5 g)

2-Hydroxy tricosanoic acid (33):

White amorphous powder, mp 76–77°C; C₂₃H₄₆O₃ (M⁺ 370), FAB-MS m/z : 393 [M+Na]⁺, 371 [M+H]⁺, 325 [M–45]⁺, 353 [M–18]⁺, 295, 85, 71 and 57. IR (KBr) νmax cm⁻¹: 3435 (OH), 1705 (COOH); ¹H and ¹³C-NMR spectral data are given in isolation and structure elucidation part.
Stigmasterol:
Colourless crystals, mp 152°C; C_{29}H_{48}O (M^+ 412); IR (KBr) \nu_{\text{max}} \text{ cm}^{-1}: 3380 (OH), 1640, 875 (C=C); ^1H-NMR (600 MHz, CDCl\textsubscript{3}) \delta 0.69 (3H, s, H\textsubscript{3}-18), 0.80 (3H, d, J = 6.5 Hz, H\textsubscript{3}-27), 0.84 (3H, d, J = 6.5 Hz, H\textsubscript{3}-26), 0.92 (3H, d, J = 6.6 Hz, H\textsubscript{3}-21), 1.02 (3H, s, H\textsubscript{3}-19), 3.52 (1H, m, H\textsubscript{3}), 5.01 (1H, dd, J = 14.5, 8.0 Hz, H-22), 5.16 (1H, dd, J = 14.5, 9.0 Hz, H-23), 5.35 (1H, brd, H-6); ^13C-NMR (150 MHz, CDCl\textsubscript{3}) \delta: 37.2 (C-1), 31.6 (C-2), 71.8 (C-3), 42.3 (C-4), 140.8 (C-5), 121.7 (C-6), 31.7 (C-7), 31.9 (C-8), 50.2 (C-9), 36.5 (C-10), 21.2 (C-11), 39.8 (C-12), 42.2 (C-13), 56.9 (C-14), 24.4 (C-15), 28.9 (C-16), 56.0 (C-17), 12.2 (C-18), 19.4 (C-19), 40.5 (C-20), 21.1 (C-21), 138.3 (C-22), 129.3 (C-23), 51.2 (C-24), 31.9 (C-25), 19.0 (C-26), 21.2 (C-27), 25.4 (C-28), 12.0 (C-29); EI-MS \textit{m/z} (%) : 412 (M^+, 45), 394 (8), 379 (30), 314 (35), 300 (35), 273 (40), 271 (60), 255 (95), 231 (50), 229 (35), 213 (100) [9, 10].

Stigmasterol glucoside:
Colourless needles, mp 270°C, HR-FAB-MS: \textit{m/z} 597.4134 [M+Na]^+ (calcd for C_{35}H_{58}O_6Na: 597.4131) in positive mode. Other peaks of FAB-MS are 411 [M–glucosyl]^+, 395 [M–glucose+H]^+, 271 and 255. ^1H and ^13C-NMR spectral data are similar with respect to Table 2.5.

\beta-Sitosterol:
Colourless needles, mp 135–137°C; C_{20}H_{50}O (M^+ 414); IR (KBr) \nu_{\text{max}} \text{ cm}^{-1}: 3400 (OH), 1625 (C=C), 1460, 1440; ^1H-NMR (300 MHz, CDCl\textsubscript{3}) \delta 0.68 (3H, s, H\textsubscript{3}-18), 0.80 (3H, d, J = 6.5 Hz, H\textsubscript{3}-27), 0.83 (3H, d, J = 6.5 Hz, H\textsubscript{3}-26), 0.92 (3H, d, J = 6.6 Hz, H\textsubscript{3}-21), 1.02 (3H, s, H\textsubscript{3}-19), 3.50 (1H, m, H-3), 5.34 (1H, brd, J = 5.0 Hz, H-6); ^13C-NMR (75 MHz, CDCl\textsubscript{3}) \delta: 37.3 (C-1), 31.7 (C-2), 72.0 (C-3), 42.4 (C-4), 140.8 (C-5), 121.7 (C-6), 32.0 (C-7), 32.1 (C-8), 50.2 (C-9), 36.6 (C-10), 21.2 (C-11), 39.8 (C-12), 42.3 (C-13), 56.8 (C-14), 24.2 (C-15), 28.2 (C-16), 56.1 (C-17), 11.9 (C-18), 19.3 (C-19), 36.2 (C-20), 19.1 (C-21), 34.0 (C-22), 29.3 (C-23), 50.2 (C-24), 26.2 (C-25), 18.8 (C-26), 19.7 (C-27), 23.2 (C-28), 11.9 (C-29) [7, 8].

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**β-Sitosterol glucoside:**

Colourless crystals, mp 283–285°C; C\textsubscript{35}H\textsubscript{60}O\textsubscript{6} (M\textsuperscript{+} 576); FAB-MS (Na–m-nitro benzylalcohol) m/z (%): 599 [M+Na]\textsuperscript{+} (35); IR (KBr) \(v_{\text{max}}\) cm\(^{-1}\): 3434 (OH), 1640 (C=C), 1095 (glycosidic); \(^1\)H-NMR (300 MHz, C\textsubscript{5}D\textsubscript{5}N) \(\delta\) 0.67 (3H, s, H\textsubscript{3}-18), 0.88 (3H, d, \(J = 6.5\) Hz, H\textsubscript{3}-26), 0.90 (3H, d, \(J = 6.5\) Hz, H\textsubscript{3}-27), 0.95 (3H, s, H\textsubscript{3}-19), 1.02 (3H, d, \(J = 6.0\) Hz, H\textsubscript{3}-21), 3.98 (1H, m, H-3), 5.36 (1H, brd, \(J = 4.5\) Hz, H-6); \(^{13}\)C-NMR (75 MHz, C\textsubscript{5}D\textsubscript{5}N) \(\delta\): 38.5 (C-1), 31.1 (C-2), 79.2 (C-3), 40.2 (C-4), 141.6 (C-5), 122.8 (C-6), 33.1 (C-7), 32.9 (C-8), 51.0 (C-9), 37.8 (C-10), 22.1 (C-11), 40.6 (C-12), 43.6 (C-13), 57.6 (C-14), 24.2 (C-15), 29.4 (C-16), 57.0 (C-17), 12.9 (C-18), 20.2 (C-19), 37.2 (C-20), 19.8 (C-21), 35.1 (C-22), 27.1 (C-23), 46.8 (C-24), 30.4 (C-25), 20.1 (C-26), 20.8 (C-27), 25.4 (C-28), 12.9 (C-29), 103.4 (C-1\textsuperscript{'}), 76.2 (C-2\textsuperscript{'}), 79.4 (C-3\textsuperscript{'}), 72.4 (C-4\textsuperscript{'}), 79.2 (C-5\textsuperscript{'}), 63.4 (C-6\textsuperscript{'}).[6]

**References**


