Chapter 4

Experimental Section

4.1 General Experimental Remarks

1. IR spectra were recorded on a Perkin Elmer L 120 – 000 A grating IR spectrophotometer, using KBr pellets.

2. $^{1}{ }^H$ NMR (400 MHz) spectra were recorded on a BRUCKER- ACF 300 spectrometer with Tetramethylsilane (TMS) ($\delta$ 0.00) as the internal standard in CDC1$_3$ (as solvent) at room temperature. All chemical shifts ($\delta$) are quoted in ppm.

3. Solvents like hexane, petroleum ether, ethyl acetate, methanol etc. (MERCK, Qualigen) and commercially available reagents (MERCK) were used as received without further purification unless otherwise specified and whenever needed, were purified by standard protocols prior to use. Anhydrous solvents were prepared by standard method and were freshly distilled before use.

4. Visualization of spots on TLC plates was achieved by development with iodine vapour.

4.2 Collection of the plant materials

The plants were collected from the local market of Fatakazar in Silchar, Rangirkhari and Meherpur located in south of Cachar District and Shyama Prasad Road, Lala town, Hailakandi in the state of Assam, India during April to August 2002.
The plants were identified by Dr. M. Dutta Choudhury, Department of Life Science and Bioinformatics, Assam University, Silchar

4.3 Chemicals Required

All the chemicals used were of analytical grade except ethyl acetate, acetone, carbon tetrachloride and petroleum ether which were of laboratory grade. Silica gel (60-120 mesh) for column chromatography was obtained from S.D. Fine Chemicals Ltd., Boisar. The solvents acetone, methanol, benzene, petroleum ether and hexane were supplied by Qualigens Fine Chemicals, Mumbai, India. Silica gel G used for TLC and ethyl acetate were purchased from E. Merck (India) Ltd. Ethanol and Diethyl ether was purchased from Bengal Chemicals & Pharmaceuticals Ltd.

The solvents used for extraction and chromatographic separation were distilled at their respected boiling points before use.

4.4 Phytochemical tests

1. Alkaloid Test: For detecting alkaloids in phytochemical screening, two types of reagents were used i.e., alkaloidal precipitants and spray or dip reagents.

a) Mayers – reagent test: The residue was treated with Mayers reagent, formation of precipitation or turbidity indicated the presence of alkaloid.

b) Dragendroff’s – reagent test: The residue was treated with Dragendroff’s reagent, formation of precipitation or turbidity indicated the presence of alkaloid.
2. Screening of Steroids and Terpenoids

a) Liebermann – Burchard Reaction: After spraying the TLC plates with LB-reagent and heating at 100 °C for 5 minutes, terpenes gave yellow to violet colour while sterols gave pink colour when observed under UV light.

b) Salkawaski Reaction: When the chloroform solution of the compound was treated with 2-3 drops of conc. H₂SO₄, if brown red colour appeared in the chloroform layer, the compound was a steroid.

c) Teschugajew Reaction: Chloroform solution of the compound was mixed with acetyl chloride in excess and a little of zinc chloride, and boiled. A colour with characteristic fluorescence was developed, a green colour for terpenes (i.e. triterpenes) and yellow to pink colour for steroids.

d) Winogradon reaction: Solution of the compound in chloroform was mixed with 90% trichloroacetic acid (in HCl) and mixture was boiled, a yellow colour appeared for triterpenes and pinkish yellow to blue for sterols.

e) Phosphoric acid: To 20% ethanolic solution of phosphoric acid was added to chloroform solution of the compound, the colour appeared more intense on heating. Blue grey colour was developed by keto steroids, and alcoholic steroids produced brown to violet red color.

Test for Saponins: A small portion of the residue, obtained after evaporating the chloroform extract, was dissolved in distilled water and shaken vigorously. A honey comb froth persisting for 15 minutes indicated the presence of saponins. A few drops of conc. H₂SO₄ and 1 ml of acetic anhydride were added to the filtrate. Appearance of
a blue, bluish green or reddish brown colour, after accompanied by the formation of a pink ring, showed the presence of saponins.

**Test for Flavonoids:** A few drops of conc. HCl and magnesium turnings were added to 1 ml of the chloroform extract. The presence of flavonoids was indicated by the development of pink or magneta-red-colour.

**Test for Glycoside**

*Liebermann – Burchard Reaction:* After spraying the TLC plates with LB–reagent and heating at 100 °C for 5 minutes, **Glycosides** gave pink colour.

**4.5 General Extraction and Purification of the compounds obtained from the plants**

The dried powdered shells, leaves and seeds of *Basella rubra* Linn. and the dried powdered stems and leaves of *Basella alba* Linn. were defatted with hexane and subsequently extracted with acetone. The extracts, on removal of the solvent under reduced pressure, were separated and purified on silica gel column. The isolated fractions were subjected to preparative TLC (Silica Gel 60; chloroform-ethyl acetate | 9.5 : 0.5). One compound (3) from the shell, three compounds (4, 5, and 6) from the seeds and four compounds (5, 7, 8, and 9) were isolated from the leaves of *Basella rubra* Linn.; and two compounds (10 and 11) from the stems and two compounds (12 and 13) from the leaves of *Basella alba* Linn. were also isolated.