CHAPTER-2

2.1 PLANT MATERIAL

The plant *Nyctanthes arbor-tristis* Linn. was collected from the local areas of Muzaffarnagar (UP) and identified by the courtesy of Dr. Veena Chandra, Scientist “F” Incharge of Systematic Botany, Forest Research Institute, Dehradun. The dried and crushed leaves, stem and flower parts have been investigated for the study.

2.2 CHEMICALS AND REAGENTS

All organic solvents (petroleum ether, chloroform, ethylacetate, ethanol, butanol etc.) and other chemicals (FeCl$_3$, CH$_3$COOH, NaOH, NaHCO$_3$ etc.) used were of Qualigens AR grade. Column chromatography was carried out using Perfit glass column (100 cm length, 44 mm outer diameter) and Qualigens Silica gel (60-120 mesh). Thin layer chromatography was done using Merck TLC (8”×8”) plates.

2.3 METHODS OF SCREENING FOR PHYTOCHEMICAL CONSTITUENTS

Plant extract obtained after the extraction is a complex mixture of many phytoconstituents such as alkaloids, terpenoids, carbohydrates, glycosides, saponins, sterols etc. To analyse their presence, extract of various parts of NAT was subjected to different phytochemical investigation as per the standard methods [1, 2].

**Test for Flavonoids :**

(a) NaOH Test-A small amount of extract was treated with aqueous NaOH and dilute HCl. Formation of red colour shows the presence of flavonoids.

(b) H$_2$SO$_4$ Test-A fraction of extract was treated with concentrated H$_2$SO$_4$. Formation of white precipitate indicates the presence of flavonoids.
Test for Carbohydrates:
(a) Molisch’s Test- Few drops of Molisch’s reagent (10% solution of α-naphthol) was added to 1 mL extract diluted with distilled water. This was followed by the addition of 1 mL of conc. H₂SO₄ by the side of the test tube, allowed to stand for two minutes and then diluted with 5 mL of distilled water. Formation of red colour at the interphase of the two layers shows the presence of carbohydrates.
(b) Fehling’s Test- About 0.5 mL of extract was dissolved in the distilled water and filtered. The filtrate was heated with 5 mL of equal volumes of Fehling solution A (copper sulphate solution) and B (alkaline sodium potassium tartarate). Formation of red coloured cuprous oxide ppt indicates the presence of reducing sugar.

Test for Terpenoids:
(a) Liebermann-Burchard Test- 1 mL of extract was treated successively with chloroform, acetic anhydride and few drops of H₂SO₄ were added. Formation of dark green colour shows the presence of terpenoids.
(b) Salkowski’s Test- 1mL of extract was treated with chloroform and filtered. The filtrate was treated with few drops of concentrated H₂SO₄, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenoid.
(c) Noller’s Test- 1 mL of extract was treated with tin and 1 mL of thionyl chloride, heated in water bath. Appearance of purple colour indicates the presence of triterpenoid.
(d) TNM Test-1 mL of the extract was dissolved in 2 mL of chloroform and 2-3 drops of tetra nitro methane (TNM) were added. Generation of golden yellow colour indicates the presence of unsaturation in the terpenoid compound.
(e) Vanillin Reaction Test- A spot of the extract was over imposed on paper chromatography, run in BuOH/AcOH/H₂O (63:10:27) and sprayed with
vanillin reagent (4 gm of vanillin, 100 mL of methanol and 4 mL of conc. HCl). The paper was heated at 100-110°C for a few minutes in an oven. The violet coloured spot indicates the presence of iridoids [3].

**Test for Phytosterols:**
(a) Liebermann-Burchard Test- 1 mL of extract was treated with chloroform and acetic anhydride and 3-4 drops of H$_2$SO$_4$ were added. Formation of dark pink colour indicates the presence of phytosterol.
(b) Salkowski Test-1 mL of extract was dissolved in chloroform and concentrated H$_2$SO$_4$. Formation of red colour indicates the presence of phytosterol.

**Test for Alcohol:**
Few drops of the extract were dissolved in 0.5 mL of dioxane. This solution was added to 0.5 mL of ceric ammonium nitrate reagent (4 gm of ceric ammonium nitrate was dissolved in 10 mL of 2N HNO$_3$ on mild heating) and shaken well. Formation of red colour indicates the presence of alcoholic hydroxyl group.

**Test for Phenols:**
(a) Ferric chloride Test- The fraction of extract was treated with 5% neutral ferric chloride. Formation of deep blue, purple or green colour shows the presence of phenols.
(b) Liebermann’s Test- The extract was heated with sodium nitrite and 2-3 drops of H$_2$SO$_4$ were added, diluted with water and treated with excess of dilute NaOH. Formation of deep red or green or blue colour shows the presence of phenols.

**2.4 INSTRUMENTATION**
The C and H contents of the sample were determined with the help of Elemenlar Vario EL III Elemental Analyser. Melting points were obtained using Perfit (India) melting point apparatus. IR spectra were recorded on Thermo Nikolet Nexus FT-IR Spectrometer, using 16 scans and were
reported in cm$^{-1}$. UV absorption spectra were recorded using methylalcohol as solvent on Thermo Scientific UV-visible Spectrophotometer. A matched pair of quartz cell of path length 1 cm was used. Mass spectra were obtained with Bruker qTOF time of flight Spectrometer. $^1$H NMR and $^{13}$C NMR spectra were recorded on Bruker AVANCE. Spectrometer at 300 MHz and 100 MHz respectively. Chemical shifts were presented in δ values relative to internal standard Me$_4$Si (TMS) for all residual protium in the deuterated solvents (DMSO-d$_6$, CDCl$_3$, MeOH-d$_4$, CD$_3$COCD$_3$).
REFERENCES

