

# Aim 10

## To Investigate the Presence of Phytochemical Constituents in the Plant sample

### Introduction

Various human diseases can be cured using medicinal plants having bioactive compounds. Phytochemicals have two categories i.e. Primary and secondary constituents.

Chlorophyll, proteins, sugar and amino acids are primary constituents while terpenoids and alkaloids are secondary constituents. Medicinal plants have antifungal, antibacterial and anti-inflammation activities. For curing various diseases, new drugs can be prepared by phytochemical analysis of the plants. Preliminary screening of phytochemical is an important step for the detection of bioactive principles present in medicinal plants and may lead to novel environment friendly bioherbicides and drug discovery.

Standard methods are used for the screening of the plants and resulted in the detection of presence of tannins, flavonoids, phenolics, steroids, cardiac glycosides and alkaloids.

### Requirements

Beakers, test tubes, measuring cylinder, pipettes, centrifuge, Whatman filter paper no. 1, methanol, 10% ammonia, Conc. HCl, Zinc metal powder, 5% ferric chloride, glacial acetic acid, chloroform, Conc.  $H_2SO_4$ , centrifuge, Whatman filter paper no. 1, shaker and plant sample.

### Procedure

#### 1. Phytochemical investigation of plants:

- a. Collect the leaf of selected plant species.

- b. Wash with distilled water and dry them.
- c. Crush the plant material using electric blender.
- d. Use powder sample for qualitative analysis using chemical treatment method.

### **2. Test for Anthraquinone:**

2 ml of the extract is taken in a test tube, add 5 ml of chloroform and shake well for 5 min. filter the extract. Stir the filtrate and add an equal volume of 10% ammonia solution. The presence of anthraquinone is confirmed by the appearance of pink or violet colour in an ammonical layer.

### **3. Test for Flavonoids:**

20 g of powdered leaf sample is taken and add 100 ml methanol to it. Place it in a shaker for 24 hours and use Whatman filter paper no. 1 for filtration. Titrate 5 ml methanolic extract with 0.5 g Zinc metal powder and add 3 drops of Conc. HCl. There are two possibilities that may occur:

- a. A deep red to magenta colouration indicates the presence of flavonols.
- b. A weak pink to magenta colouration indicates the presence of flavonol and flavonoids.

### **4. Test of Saponins:**

20 g of powdered leaf sample is taken and add 100 ml methanol. Put it in a shaker for 24 hours. Filter it through Whatman filter paper no.1. 5 ml of methanolic extract is taken and add 5 ml of distilled water and stir, shake it well. On shaking, If froth formation occur, indicates the presence of saponins.

### **5. Test for Tannins:**

50 g of the dried powdered sample is taken and boil it for 10 min in 200 ml distilled water. Then, filter it and add 3 drops of 5 % (w/v) ferric chloride in the filtrate. Brownish-green or blue-black coloration indicates the presence of tannins.

### **6. Test for Cardiac Glycosides:**

20 g of the powdered sample is taken and add 100 ml methanol. Put it in a shaker at 200 rpm for 24 hours. Use Whatman filter paper no.1 to filter the extract. 5 ml of the methanolic extract is taken and add 2 ml of glacial acetic acid containing one drop of 5% ferric chloride solution to it. Now carefully transfer the solution to the surface of 1 ml Conc.  $H_2SO_4$  which further leads to formation of reddish brown ring at the junction of two liquids. This ring formation indicates the presence of deoxysugar. The formed layer becomes bluish green which darkens on standing indicates the presence of cardiac glycosides.

### **Conclusion**

Nature generally produces many secondary metabolites which are important for the development of new environmental friendly pesticides, herbicides and many pharmaceutical drugs. Plants have been a source of medicinal agents for thousands of years.