This chapter deals with identification of compound/compounds from the most active extract.

MATERIALS AND METHODS

Flavonoid extracts of all the selected plant parts were observed to have inhibitory activity (‘*in vitro’*) against salivary alpha amylase enzyme. Hence, flavonoid extracts were selected for the identification of compound/compounds through TLC, PTLC, MP, IR spectral and GCMS studies.

Thin Layer Chromatography (TLC)

Flavonoid extracts of selected plant parts were dissolved in ethyl acetate and applied on Silica gel coated (0.2-0.3 mm) and activated glass plates (20 x 20 cm). Plates were developed in selected organic solvent systems viz., Benzene: Acetic acid: Water (125:72:3) and n-Butanol: acetic acid: water (4:1:5) in air tight chambers.

Developed glass plates were air dried and sprayed with 5% ethanolic ferric chloride solution; heated in an oven at 100^0C for 5 minutes. Reagent positive spots were observed in plates developed in the selected solvent

system and then Rf values were calculated.

Preparative thin layer chromatography (PTLC)

Free flavonoid extracts of the plant parts were selected for PTLC as were found to be the most active extracts. About 300 silica gel coated and activated glass plates (20 x 20 cm) were used for preparative thin layer chromatography. Solvent used was Benzene: Acetic acid: Water in the ratio of 125:72:3 and Butanol: Acetic acid: Water in the ratio of 4:1:5 . Spots coinciding with standards (Apigenin, Quercetin and Kaempferol and Leuteolin obtained in diffrenet extracts) were eluted separately. Elutes were co-chromatographed with standards to test the purity of compounds.

Melting point (MP) and Infra Red (IR)

Compounds isolated from PTLC were crystallized, weighed and subjected to MP (melting point) and IR spectral studies on Perkin Elmer model-555 spectrophotometer in KBr pellets. Comparable MP and superimposed spectra of the compounds isolated and standards, further confirmed the nature of compound.

GC-MS analysis

Gas chromatography-mass spectrometry (GC-MS) analysis of the most active flavonoid extract from stem bark of *Mangifera indica* Linn. was carried out by GC-MS Shimadzu Model QP-2010 mass spectrometer under the following conditions: DB-Polyethylene glycol coated fuse silica capillary column (30 m length × 0.25 mm ID × 0.25 μm film thickness): Helium carrier gas (1.34 ml/min); 250^0C injector temperature; 240^0C interface temperature; and 200^0C on source temperature. Column temperature programmed at 60^0C with 10^0C/min rise to 230^0C. for GC-MS detection ionization energy of 70ev was used. The components were identified based on National Institute of Standards Technology (NIST) Library.

RESULTS

TLC of active extracts of flavonoids from different plants was carried out using the solvent system Benzene: Acetic acid: Water (125:72:3). Following results were obtained for different selected plants.

In flavonoid extract of *Aloe vera* L. three Rf values as 0.56, 0.80 and 0.93 were observed in solvent system Benzene: acetic acid: water (125:72:3) and three Rf values as (0.78, 0.64 and 0.83) were recorded in solvent system Butanol: acetic acid: water (4:1:5) which were coinciding

with the standered compounds. Which were coinciding with the standered compounds Leuteolin, Quercetin and Kaempferol respectively.

In flavonoid extract of *Allium cepa* L. two Rf values as 0.56 and 0.93 in solvent Benzene: acetic acid: water and 0.78 and 0.83 in solvent n-Butanol: acetic acid: water were observed. those were coninciding with standered compounds Leuteolin and Kaempferol respectively.

In flavonoid extract of *Allium sativum* L. two Rf values as 0.56 and 0.80 in solvent system Benzene; acetic acid: water and 0.78 and 0.64 in solvent system n-butanol: acetic acid: water were observed which were coinciding with standered compounds Leuteolin and Quercetin respectively.

In flavonoid extract of *Azadiracta indica* A Juss. two Rf values as 0.64 and 0.80 in Benzene: acetic acid: water and 0.89 and 0.64 were observed which were coinciding with satndered compounds Apigenin and Quercetin respectively.

In flavonoid extract of *Mangifera indica* L. two Rf values as 0.64 and 0.93 in solvent system Benzene: acetic acid: water and 0.89 and 0.83 in n-butanol: acetic acid: water were observed which were coinciding with Apigenin and kaempferol respectively.

In day light, the identified spot of Apigenin and Leuteolin were yellow whereas Quercetin and Kaempferol were grayish yellow in colour. In **UV light,** the four spots showed different colours viz: fluorescent yellowish green, fluorescent dull yellow, fluorescent yellowish brown and fluorescent yellowish blue, respectively.

Exposure to ammonia fumes made the identified apigenin and leuteolin spost yellowish green and that of Quercetin and Kaempferol spot dull yellow in colour. On exposure to **iodine vapors** spots became prominent and yellowish brown in colour. **Spraying with 5% ethanolic $FeCl_3$ solution** converted the spots in three different colours viz: brown, brown, blackish gray and brown, respectively whereas spraying with **5% ethanolic $AlCl_3$ solution** resulted the spots of yellow, dull yellow and yellow colour respectively (Table 4.1).

Melting points of isolated samples were found similar to those of standard Leuteolin, Apigenin, Quercetin and Kaempferol (330°C, 340°C, 309-311°C and 271-273°C respectively).

IR spectra of all the four identified samples superimposed with the authentic standard compounds (Leuteolin, Apigenin, Quercetin and Kaempferol) which further confirmed the presence of Leuteolin,

Apigenin, Quercetin and Kaempferol in the samples tested (Fig. 4.1-4.4).

Since flavonoid extract from stem bark of *Mangifera indica* L. showed maximum inhibitory activity on salivary alpha amylase with minimum IC_{50} value, the extract was explored for chemical constituents by GC-MS analysis. The spectral data of GC-MS analysis of the extract are shown in Fig. 4.5. In all, 50 compounds were identified in the sample. The retention time, name, molecular weight and the structure of the components of the test extract were ascertained (Table 4.2).