2.1 Introduction to extraction:

In present days there is an increasing interest in the study of the mechanism of action of natural products, especially as part of drug discovery programs, as they represent a formidable reservoir of potentially useful leads for new medicines. The natural products of interest are organic molecules, which are also frequently called secondary metabolites and are produced by various living organisms, e.g., plants, microbes, marine organisms, insects and amphibians (Newman et al., 2007). Plants produce complex mixtures of natural products and the selection of the best protocol for an efficient extraction of these substances is not a simple task.

Plants produce a range of secondary metabolites with different functional groups and polarities. Although water is used as an extractant in many traditional protocols, organic solvents of varying polarities are generally selected in modern methods of extraction (Ravishanankara M N et al., 2001). Solvent extraction procedures applied to plant metabolites include maceration, ultrasound assisted solvent extraction, percolation, Soxhlet extraction, pressurized solvent extraction, extraction under reflux, steam distillation and acid-base extraction. The selection of a method is done on trial and error basis.

2.2 Materials and Methods:

2.2.1 Sample Collection:

The voucher specimen of Saraca indica was deposited in the herbarium of Botany Department, Osmania University, Hyderabad, Andhra Pradesh and also planted Andhra Pradesh forest department nursery, erragadda, Hyderabad, India. The plant
authentication was kindly carried out by Prof. Ram reddy, Department of Botany, Osmania University. The bark of *Saraca indica* was collected from Public garden, Nampally, Hyderabad, Andhra Pradesh, India.

500g of bark of *Saraca indica* plant was dried under shadow for 120 hours (5 days) to remove the moisture and later chopped into small pieces weighing 250g which was further dried for another 120 hours in the shadow. Then in it was kept in hot air oven at 60°C for overnight to make it 100% moisture free and ground it to fine powder weighing 230g as shown in figure.2.1.

### 2.2.2 Extraction:

50 g of bark powder of the *Saraca indica* was placed in a thimble-holder and 400 ml of hexane added to the solvent flask. The temperature was raised to 45°C for 16 hours (using heating mantle) and cooled to room temperature, separated the pet ether layer. Then added 400 ml of ethyl acetate and ran the soxhlet at 55°C for 16 hours. At the end, the ethyl acetate layer was separated. To the above residuary gum 400 ml of methanol was added and repeated the above procedure. Finally the extracted samples were concentrated under reduced pressure with rotary evaporator (Heidolph) to get the residues.

The above solvent extractions were checked in different mobile phases like 2%, 4%, 6%, 10% and 15% ethyl acetate: pet ether. It was observed that 4% ethyl acetate: pet ether was the best eluent to separate the components in hexane extraction and other extractions didn’t contain any compound. Pet ether extracted brown gum was checked by TLC, where three bands were observed under UV and Iodine. The above (pet ether
extracted) gum was subjected to column chromatography, eluting with ethyl acetate: pet ether and 300 fractions each 20 ml were collected and examined with thin layer chromatography (TLC). We observed three major spots (bands) on TLC under UV as well as in Iodine vapors as shown in figure 2.2.

Chart. 2.1: schematic presentation of *Saraca indica* extraction methodology
2.3 Results:

The column packed with hexane extracted gum (containing three spots) is eluted with the solvents whose polarity gradually increased from 1% to 2% to 3% and finally 4% of ethyl acetate: pet ether mixture. The first spot was obtained in fractions 100 to 120 at 2% ethyl acetate: pet ether with an yield of 0.5g, and second spot was eluted in the fractions 170 to 200 (3% ethyl acetate: pet ether) with an yield of 1.0g and finally third spot was eluted in the fractions 250 to 290 (4% ethyl acetate: pet ether) with an yield of 1.5g.

Figure.2.1: a) Stem of the Saraca indica b) Bark of the Saraca indica c) Powdered bark.
TLC images are shown below before and after column chromatography.

Figure 2.2: a). TLC profile at 2% EA: PE. Figure 2.2: b). TLC profile at 3% EA: PE.

Note: All the above photographs are taken under UV light.

Figure 2.2: c). TLC profile at 4% EA: PE. Figure 2.2: d) TLC profile of all the extractions (2%, 3%, 4% EA: PE) under Iodine vapors.