



CHAPTER – 4

Aim and Objectives

4.1. AIM:

To isolate and characterize medicinally important compounds from traditional plants- *Ammania baccifera* Linn. and *Bergenia ciliata* (HAW) Sternb. by activity-directed isolation and characterization approach.

4.2. OBJECTIVES**a) Collection and authentication of traditional medicinal plants under study.**

Ammania baccifera Linn. whole plant and *Bergenia ciliata* (HAW) Sternb. leaves shall be collected from surrounding areas of Hyderabad and local forests of Chota Shimla respectively, with the help of Botany experts and shall be authenticated by organoleptic evaluation and by comparing gross morphological features with the literature with guidance from botanists. Microscopical examination shall also be carried out for *Bergenia ciliata* leaves.

b) Phytochemical investigations of prepared extracts.

Extracts shall be prepared from the powdered whole plant of *Ammania baccifera* and *Bergenia ciliata* leaves by continuous hot extraction method at 50⁰C in soxhlet extraction apparatus using organic solvents in the increasing order of polarity. Each organic solvent extract shall be tested for the presence of different class of phytoconstituents by qualitative chemical tests, and shall be subjected to TLC studies.

c) Biological evaluation of extracts for acute toxicity, antilithiatic, antioxidant, antityphoid, antitubercular activities (*Ammania baccifera* whole plant) and antilithiatic, antioxidant, antibacterial activities (*Bergenia ciliata* leaves).

Prepared extracts shall be evaluated for acute toxicity (The Up and Down or 'Staircase' model), antilithiatic (Effect on experimentally prepared kidney stones of calcium oxalate and phosphate *in vitro* model; Ethylene glycol-induced hyperoxaluria in albino rats *in vivo* model), antioxidant (Reaction with 1-1 – Diphenyl-2-Picryl-Hydrazyl (DPPH), Scavenging of Hydroxyl Radicals-Thiobarbituric Acid Reactive Substances (TBARS) Assay, Rapid Screening for Scavenging Compounds -Dot-blot test on TLC silica layers *in vitro models*, and CCl₄-induced hepatotoxicity in rats & Isoproterenol-induced myocardial infarction in rats – *in vivo* models), antityphoid (Agar well diffusion *in vitro* method), antitubercular (Microplate Alamar Blue Assay) activities (*Ammania baccifera* whole plant) and antilithiatic, antioxidant, antibacterial (Agar well diffusion *in vitro* method) activities (*Bergenia ciliata* leaves) .

d) Isolation and purification of bioactives from promising extracts.

Extracts which show promising actions in test models, shall be subjected to column chromatography technique for the separation of compounds (judged by the TLC studies) using suitable eluents. Isolated compounds shall be purified by re- column chromatography technique and recrystallization process.

e) Biological evaluation of purified constituents for the said activities.

Isolated and purified compounds shall be evaluated for antilithiatic, antioxidant, antityphoid, antitubercular and antibacterial activities by suitable *in vitro* & *in vivo* models (wherever applicable) .

f) Characterization of potential bioactive compounds.

Isolated and purified compounds which demonstrate significant biological actions (*in vitro* & *in vivo* evaluation), shall be characterized by following physical, chemical, chromatographic and spectral parameters.

1. Primary physical tests (appearance, colour, odour, solubility, melting point)
2. Chemical tests (Basic chemical tests)
3. Chromatography (TLC & HPLC)
4. Spectral (UV, FTIR, MASS, NMR).

5. Collection and Authentication of plants under study.

6. Organoleptic and Morphological characters study.

Microscopical characteristics study (*Bergenia ciliata* leaf).

7. Phytochemical studies:

a) Preparation of extracts

b) Detection of phytoconstituents class by primary qualitative chemical tests

c) Preliminary TLC studies.

8. Evaluation for biological activities:

a) Pharmacological studies - 1. Acute toxicity studies

2. Evaluation for antiurolithiatic activity

3. Evaluation for antioxidant activity

b) Microbiological studies – 1. Evaluation for antibacterial activity

2. Evaluation for antitubercular activity

3. Evaluation for antityphoid activity

9. Isolation of compounds from biologically promising extracts

9. Purification of isolated promising compounds

10. Characterization of biologically promising isolated compounds.

Scheme of procedure followed for isolation and characterization of biologically active compounds from plants under study.



