CHAPTER – 9

Isolution and Purification

ISOLATION OF COMPOUNDS FROM PROMISING EXTRACTS

Extracts which showed promising action (s), in *in vitro* biological evaluation studies, were selected for isolation of compounds by column chromatography technique using suitable eluents. [31-34]

Biologically promising extracts:

⇒ Ethyl acetate extracts of *Ammania baccifera* and *Bergenia ciliata* demonstrated promising antilithiatic and antioxidant activities hence, designated as promising extracts for isolation of compounds.

⇒ Ethyl acetate extract of *Bergenia ciliata* exhibited promising antibacterial activity, hence designated as promising extract for isolation of compounds.

⇒ *Ammania baccifera* chloroform extract showed encouraging antitubercular action hence, selected for isolation of compounds.

⇒ Ethyl acetate extract of *Ammania baccifera* exhibited promising antityphoid activity hence, designated as promising extract for isolation of compounds.

Isolation of compounds from *B. ciliata* ethyl acetate extract by column chromatography:

- Adsorbent: Silica gel activated for column chromatography
- Dimensions of the column: L -45cm ,Diameter—outer -3 cm, inner -2.8 cm
- Length of adsorbent packed: 35cm
- Rate of elution: 8-10 drops per minute
- Volume of elute collected: 5ml fraction
- Type of elution: Constant elution.

Preparation of sample

10g of ethyl acetate extract was dissolved in 15ml of methanol and mixed with 30gm of silica gel (60-120 mesh size) and dried in vacuum oven at 45^{O}C .The adsorbed material obtained was transferred to the column.

Eluent

Ethyl Acetate: Glacial acetic acid: Formic acid: Distilled water (126.8: 5: 5: 12.2)

Column Packing

300g of silica gel was activated in hot air oven at 110^{0} C for 1 hour. A small amount of glass wool was fixed at the bottom. The slurry of activated silica made in the mobile phase, was charged in to the column in small portions by keeping the knob open and with gentle taping (to ensure uniform packing & to avoid the air bubble formation which otherwise can interfere in separation) after each addition, in order to ensure the uniform packing. A small volume of solvent was allowed to remain at the top of the column (about 4cms) in order to avoid the drying or cracking of the column. The column was run fast for some time with the solvent system to remove any impurities. Prepared sample was then charged into the column and was allowed to settle. Then it was eluted with the mobile phase to collect fractions of 5 ml each. Several 5ml fractions were collected & each fraction was evaluated by TLC. These fractions were grouped according their homogeneity, judged from TLC analysis.

TLC of Column Elutes [31-32]

Adsorbent : Silica gel G.

Solvent system: Glacial acetic acid: Formic acid: Distilled water (126.8: 5: 5: 12.2)

Visualizing agent : 5%w/v Ferric chloride in Alcohol.

Table No.7

Consolidated details of fractions eluted by the column chromatography

Sl.no	Fraction Number	Number of spots	R_f values
1	1-18	No spot	---
2	19-28	One	0.92
3	29-31	Three	0.92,0.86,0.78
4	32-41	Three	0.86,0.78,0.72
5	42-47	One	0.56
6	48-51	Two	0.56,0.52
7	52-55	One	0.52
8	56-60	No spot	---
9	61-65	No spot	---

Fractions showing same number of compounds and R_f values were combined, concentrated and evaporated to dryness, weighed and named as **P-1, P-2** and **P-3.**

Isolation of compounds from *A. baccifera* ethyl acetate extract by column chromatography:

Preparation of sample- 10g of ethyl acetate extract was dissolved in 15 ml of methanol and mixed with 30gm of silica gel (60-120 mesh size) and dried in vacuum oven at $45^{O}C$ .The adsorbed material obtained was transferred to the column.

Eluent- Ethyl Acetate: Glacial acetic acid: Formic acid: Dist. water (126.8: 5: 5: 12.2)

Column Packing-Column was packed and eluted with the mobile phase in the same manner as described above.

TLC of Column Elutes [31-32]

Adsorbent : Silica gel G.

Solvent system: Glacial acetic acid: Formic acid: Distilled water (126.8: 5: 5: 12.2)

Visualizing agent : 5%w/v Ferric chloride in Alcohol.

Table No.8

Consolidated details of fractions eluted by the column chromatography

Sl.No	Fraction Number	Number of spots	R_f values
1	1-18	No spot	---
2	19-28	One	0.50
3	29-31	Two	0.43, 0.50
4	32-41	Two	0.43, 0.50
5	42-47	One	0.50
6	48-51	No spot	---
7	52-55	No spot	---
8	56-58	No spot	---

Fractions showing same number of compounds and R_f values were combined, concentrated, evaporated to dryness, weighed and named as **A-1 & A-2.**

Isolation of compounds from *A. baccifera* chloroform extract by column chromatography:

Preparation of sample- 10g of chloroform extract was dissolved in 15 ml of methanol and mixed with 30gm of silica gel (60-120 mesh size) and dried in vacuum oven at 45^{O}C .The adsorbed material obtained was transferred to the column.

Eluent- Ethyl Acetate: chloroform (60:40)

Column packing-Column was packed and eluted with the mobile phase in the same manner as described earlier.

TLC of Column Elutes [31-32]

Adsorbent: Silica gel G. Solvent system: Chloroform: Ethyl Acetate (60:40)

Visualizing agent: 5%w/v Ferric chloride in Alcohol.

Table No.9

Consolidated details of fractions eluted by the column chromatography

Sl.No	Fraction Number	Number of spots	R_f values
1	1-18	No spot	---
2	19-28	No spot	---
3	29-31	No spot	---
4	32-41	One	0.56
5	42-47	Two	0.56,0.42
6	48-51	Two	0.56,0.42
7	52-55	One	0.42
8	56-58	No spot	----
9	59-65	No spot	----

Fractions showing same number of compounds and R_f values were combined, concentrated, evaporated to dryness, weighed and named as **C-1 & C-2.**

PURIFICATION OF ISOLATED COMPOUNDS

Re-column chromatography:

Isolated compounds P-1, P-2, P-3, A-1, A-2, C-1 and C-2 were purified by re-column chromatography using the same eluent which was used for their separation from respective extracts.

Re-crystallization:

Re-column chromatographed compounds were dissolved in pure methanol, at times by warming, and then evaporated it on hot water bath to the extent of 85-90%, allowed to cool at room temperature. Compounds precipitated, dried naturally and weighed.

BIOLOGICAL EVALUATION OF PURIFIED COMPOUNDS

Purified compounds P-1, P-2, P-3 (from *Bergenia ciliata*), A-1, A-2, C-1 and C-2 (from Ammania *baccifera*) were evaluated for following activities by respective *in vitro*, *in vivo* & *ex vivo* evaluation models as described earlier.

- Purified compounds P-1& P-2 for antiurolithiatic, antioxidant and antibacterial actions (*In vitro*, *in vivo* & *ex vivo* evaluation).
- Purified compounds A-1 & A-2 for antiurolithiatic & antioxidant actions (*In vitro*, *in vivo* & *ex vivo* evaluation).
- Purified compounds A-1, A-2, C-1 & C-2 for antitubercular and antityphoid actions (*In vitro* evaluation).