APPENDICES

APPENDIX - I

Bioaccumulation studies

Preparation of phosphate buffer, pH 6

0.25M potassium phosphate, monobasic (KH_2PO_4) in distilled water was prepared. The initial pH of 4.8 was confirmed by using a pH meter. The pH is adjusted upward to 6 with 0.25M sodium phosphate, dibasic (Na_2HPO_4) to get phosphate buffer of pH 6.

Preparation of inhibitors

5g of powdered leaves of CFL, BSL and the flowers of MJF taken in R.B.flask, added 80ml of distilled water refluxed for 1h, cooled, filtered and made up to 100ml. This 5% extracts were used for the experiments.

Partition co-efficient of *cassia fistula* leaves, *Bougainvillea spectabilis* leaves and *Mirabilis jalapa* flowers in octanol and water system was determined by measuring the absorbance in two steps.

1. Absorbance (B) of octanol layer separated from (octanol+inhibitor) mixture.

2. To the (inhibitor+octanol) mixture from step 1 aqueous phosphate buffer of pH 6 was added. Shaken vigorously for 30 minutes, allowed to stand overnight, octanol layer separated and absorbance A was measured.

APPENDIX - II

The presence of phytochemical constituents in the acid extracts of *Cassia fistula* leaves, *Bougainvillea spectabilis* leaves and *Mirabilis jalapa* flowers was confirmed by the positive results of the test.

Analysis of the acid extracts of *Cassia fistula* leaves

S.No	Experiment	Observation	Inference
1.	**Wagner's Test** Extract+Wagner's reagent	Reddish brown precipitate	Presence of Alkaloids
2.	**NaOH test** Extract + aq. NaOH+HCl	Yellow orange colour	Presence of Flavanoids
3.	**Lead acetate test** Extract +lead acetate	White precipitate	Presence of Flavanoids
4.	Extract+2,4 dinitrophenyl hydrazine solution	Yellow solution	Presence of Carbonyl group

Analysis of the acid extracts of *Bougainvillea spectabilis* leaves

S.No	Experiment	Observation	Inference
1.	**Wagner's Test** Extract+Wagner's reagent	Reddish brown precipitate	Presence of Alkaloids
2.	**NaOH test** Extract + aq. NaOH+HCl	Yellow orange colour	Presence of Flavanoids
3.	**Lead acetate test** Extract +lead acetate	White precipitate	Presence of Flavanoids
4.	Extract+2,4 dinitrophenyl hydrazine solution	Yellow solution	Presence of Carbonyl group
5.	Extract + 10% alcoholic $FeCl_3$ solution	Green colour	Presence of Tannins

Analysis of the acid extracts of *Mirabilis jalapa* flowers

S.No	Experiment	Observation	Inference
1.	**Wagner's Test** Extract+Wagner's reagent	Reddish brown precipitate	Presence of Alkaloids
2.	**NaOH test** Extract + aq. NaOH+HCl	Yellow orange colour	Presence of Flavanoids
3.	**Lead acetate test** Extract +lead acetate	White precipitate	Presence of Flavanoids
4.	Extract+2,4 dinitrophenyl hydrazine solution	Yellow solution	Presence of Carbonyl group