

Isolation of bioactive compounds from *Calophyllum inophyllum* was carried out in this study. From the fresh leaves collected, Column chromatography was carried out. Extract that was obtained was spotted on commercially available silica gel TLC plates. Alike samples were further pooled and preparative TLC was carried out. Separated compounds were matched with the Standards by comparing their R_f values. By this procedure, we were able to isolate compounds like Inophyllum C and Calophylloide.

Plant Tissue Culture of the seeds and leaf of *Calophyllum inophyllum* was carried out. The main objective behind this was to determine the % of callus induction in both the explants. Woody Plant Medium, (WPM) Mc.Cown BH & Lloyds G (1981) was used as the basal medium. It was supplemented with auxin- IBA and cytokinin- BAP. After 30 days of incubation, callus induced in seed explants The Callus was initially white, friable, profuse which turned dark brown on subsequent sub-culturing/transferring, while callus induced from leaf explant was nodular, some were irregular, creamish colored and turned little brown on sub-culturing. Various hormone concentrations were tried, but 2ppm IBA was found to be most suitable. Addition of BAP reduced the % of callus induction in explants. Moreover the % contamination in leaf was high as compared to seed. From the above studies, it can be inferred that seed explants are better suited for the callus production as compared with the leaf.

The callus obtained from the Plant Tissue Culture was used for determining the concentration of bioactive compounds in them. HPLC technique was used for this qualitative and quantitative study. Ethyl acetate and Petroleum ether was used as the mobile phase. The compounds got separated at their respective Retention time. Later, the Retention time of the sample was compared with the Retention time of authentic peaks of the standard compounds.

The presence of bioactive compounds was confirmed by Column chromatography and quantitatively by HPLC. From the above studies carried out, it can be deduced that the concentration of compounds like Inophyllum C, Inophyllum P and Calophylloide was found to be more in seeds as compared to the leaf. Thus seed should be chosen as the explant for callus production in *Calophyllum inophyllum*.

The *Calophyllum inophyllum* species produces callus that contains secondary metabolites such as **Calanoloid** and **Inophyllum**. In the above work we tried to optimize the parameters that were suitable for the callus production. Calanoloid A, Calanoloid B, InophyllumB, InophyllumP and InophyllumG were reported to act as Non nucleoside Reverse Transcriptase Inhibitor of HIV- 1. If the compounds give positive results in human trials, it might become a remedy for the treatment of AIDS. Optimization of callus production using hormones like IBA and BAP was done. Still studies can be carried with other auxins and cytokinins.

Cell Suspension Culture can also be carried out. It is one of the best method to raise the concentration of secondary metabolites. The compounds can be qualitatively and quantitatively estimated by HPLC. Callus production can carry out by using this study. However further studies on effect of hormones on callus induction is still awaited.

CHAPTER-V

BIBLIOGRAPHY