CHAPTER5 CONCLUSIONS AND SUMMARY

In the present study following point have been concluded which are inlisted bellow

- A total of 14 endophytic fungal isolates were screened from ***Calotropis procera*** **root.**
- Most efficient fungal isolate was tested against selected bacterial and fungal species and selected for study of production of antimicrobial metabolite production.
- Morphological and molecular identification of fungal isolate was done and it was named as **CPR 5.**
- **MIC** value of crude extract from fungal isolate was evaluated.MIC value of bacterial species were lower than fungal species. The extract obtained from isolate CPR5 had MIC values **of 240µg/ml, 150µg/ml, 200µg/ml, 250µg/ml and 140µg/ml for *E.coli*, *B subtilis, Penicillium chrysogenum, S aureus* and *Xanthomonas oryzae* respectively. MIC value of 750 µg/ml, 600 µg/ml, 400 µg/ml, 450 µg/ml and 550µg/ml were observed for *C. albicans, Penicillium chrysogenum, P exigua*, *Sclerotium rolfsii* and *Sclerotinia scleratiourum*** respectively.
- Production media was optimized for some selected production parameters.
- Among various Carbon sources, **Starch** was observed to support highest level of antimicrobial agent production **(Fig. 11).**
- Various **yeast extract concentrations (0.5 g/l to 4.5 g/l)** were tested to see the effect on growth and secondary metabolite production among the various concentration tested, yeast extract of **3 gm/l** showed better results followed by **3.5 g/l and 2.5 g/l** concentrations **(Fig. 12).**
- Productions level of bioactive antibacterial compounds were observed at different temperatures like 15 ^{0}C, 20 ^{0}C, 25 ^{0}C, 30 ^{0}C, 35 ^{0}C and 40 ^{0}C. Maximum growth and

bioactive metabolite production by isolate **CPR5** was recorded at incubation temperature **25 ^{0}C (±2)** and it was followed by 30 ^{0}C, 35 ^{0}C, respectively **(Fig. 13).**

- When different concentration of **NaCl** was used it was observed that NaCl concentration at **3%, 3.5% and 4%** showed noticeable increase in both cell biomass and bioactive metabolite production and **3.5% of NaCl** showed maximum observable values **(Fig. 14).**
- Initial **pH 5.5** of the medium was observed to be the optimal for growth and bioactive metabolites production by **CPR5**. Although initial pH 7.0 also supported biomass production and bioactive metabolite production but lower yield was observed. No growth was observed at pH 3, 4 and pH 9, 11**(Fig. 15).**
- When incubation period was observed for antimicrobial metabolite production it was observed that maximum cell biomass and antimicrobial metabolite production was observed at **8 days**. Continuous decrease in production of metabolite was observed after 8 days **(Fig. 16)**
- Effect of various Nitrogen sources was also studied. Equimolar concentration (2%, w/v) various nitrogen sources were mixed in the production medium and **yeast extract** was observed as the best nitrogen source for enhanced production of antimicrobial agent followed by **potassium nitrate** by **CPR** 5 (Fig. 17).
- Chromatographic separation of crude extract (2.57 gm) was performed and for each fraction TLC was performed to conform that each fraction has compound of only on type.

- A total of 8 compounds were isolated and ^{1}H and C^{13} NMR, FT-IR and ESI-MS were performed for each compound. Structure of each compound was elucidated on the basis of above mentioned analysis.
- **Compound 1** was observed active against *E.coli, Streptococcus pneumonia, B. subtilis, Staphylococcus hyicus, B sphaericus, Staphylococcus aureus,* and *pseudomonas aeruginosa, Candida albicans, Sclerotium rolfsii, Sclerotinia, scleratiourm, Fusarium species* and *Penicillin sp.*
- **Compound 2** was observed active against *X.oryzae,* E.coli, *Streptococcus pneumonia, B.subtilis, Staphylococcus hyicus, B. sphaericus, Staphylococcus aureus*, and *pseudomonas aeruginosa, Candida albicans, Sclerotium rolfsii,* Phoma exigua, *Sclerotinia, scleratiourm*
- **Compound 3** was observed active against- *E.coli*, *Streptococcus pneumonia, B.subtilis, B. sphaericus, Staphylococcus aureus*, and *pseudomonas aeruginosa, Candida albicans, Sclerotium rolfsii, Phoma exigua*, *Sclerotinia, scleratiourm, scleratiourm*
- **Compound 4** was active against- *E.coli, Streptococcus pneumonia, B.subtilis, Staphylococcus hyicus, B. Sphaericus, Staphylococcus aureus, and pseudomonas aeruginosa Candida albicans, Phoma exigua, Sclerotium rolfsii, Sclerotinia, scleratiourm Fusarium sp. and Penicillin sp.*
- **Compound 5** was observed active against *E.coli, Streptococcus pneumonia, B subtilis, Staphylococcus hyicus, B.sphaericus, Staphylococcus aureus, and pseudomonas aeruginosa, Candida albicans, Sclerotium rolfsii, Sclerotinia, scleratiourm and Penicillin sp*

- **Compound 6** was active against *X.oryzae, E.coli,Streptococcus pneumonia, B.subtilis, Staphylococcus hyicus, B. sphaericus, Staphylococcus aureus, and pseudomonas aeruginosa, Candida albicans, Sclerotium rolfsii, Sclerotinia, scleratiourm,Phoma exigua.*
- **Compound 7** was found active against *E.coli, Streptococcus pneumonia, B subtilis, Staphylococcus hyicus, B sphaericus, Staphylococcus aureus, pseudomonas aeruginosa and Xanthomonas oryzae, Candida albicans, Sclerotium rolfsii, Sclerotinia, scleratiourm and Penicillin sp. Phoma exigua.*
- **Compound 8** was found active against- *X.oryzae, E.coli, Streptococcus pneumonia, B.subtilis, Staphylococcus hyicus, B sphaericus, Staphylococcus aureus, and pseudomonas aeruginosa, Candida albicans, Sclerotium rolfsii, Sclerotinia, Phoma exigua, scleratiourm, Fusarium sp.*
- Reference for each structure was given which has partial similarity with structure of our compounds.
- The study supports the growing evidence that bioactive substances which are produced by endophytes fungi may not only be involved in the host-endophyte relationship, but also have wide range applicability in pharmaceuticals, agriculture and industry (Strobel, 2002b). They are proven sources of secondary metabolites with pharmaceutical importance. The study of fungal endophytes is expected to become an important component of fungal biology (Maheshwari, 2006). Although it is well known that despite competition from other drug discovery methods, natural products are still providing fair information of emerging new clinical lead molecules (Butler, 2004). Further, still there are some problems needed to be solved before the strains

can be used for production of bioactive compounds by fungal fermentation. Further studies are now needed to identify the active compounds produced using analytical chemistry methods.

- Strain degeneration is one of the major problems in this research field. After storage in the refrigerator for a period of time, it has experimentally been observe that the ability of bioactive compounds production of the strains usually decreases by several folds.
- In many literature it have been reported that yields are generally very low (Wagenaar M, et al., 2000). These is usually from µg/l to less than mg/l levels, and are therefore not yet suitable for fermentative production on an industrial scale (Zhao et al., 2008). Although some studies have been reported which explained some information with this reference (Strobel and Daisy, 2003; Schulz and Boyle, 2005; Yuan et al., 2008; Suryanarayanan et al., 2009; Yuan, 2006).

Summary-

Screening of endophytic fungus from *Calotropis procera* plant was done by fermented broth using disk diffusion method. A total of fourteen Endophytic fungi were screened (coded as CPR1- CPR14) for production of antimicrobial metabolites from root of *Calotropis procera*. Among these fourteen isolates, one isolate CPR 5 was found to show maximum antimicrobial activity, in compare to other isolates, against gram positive, gram negative bacteria and plant and human pathogenic fungi. endophytic isolate *Aspergillus niger* CPR 5 showed high antibacterial antifungal activity against *Escherichia coli, Streptococcus pneumonia, Bacillus subtilis, Staphylococcus hyicus, B. sphaericus, Staphylococcus aureus*, *pseudomonas aeruginosa,* plant pathogenic bacteria; *Xanthomonas oryzae* and human pathogenic fungus,