CHAPTER 5

SUMMARY AND CONCLUSIONS
CHAPTER 5

Summary and Conclusions

The present study focussed on isolation of endophytic fungi from medicinal plants such as Tulsi, Bittergourd and Menthya. Ethyl acetate extracts of isolated endophytic fungi were screened for alpha amylase, alpha glucosidase and aldose reductase inhibitors. The active compounds from two endophytic fungi responsible for enzyme inhibition and anticancer activity were identified.

India has been declared as the diabetes capital of the world and diabetes ranked as the third important cause for global mortality after cancer and cardiovascular diseases. Therefore there was a need for development of novel drugs. With this context, alpha amylase and aldose reductase inhibitors were chosen as main targets for the development of antidiabetic drugs in this study. Literature suggests there are evidences on medicinal plants producing this type of inhibitors. But these plant drugs lack efficacy and also found to be unstable. Therefore, there is a huge demand for antidiabetic compounds which are safe, stable and efficient. There are also evidences that endophytic fungi can produce same or similar compounds as that of their host plant. Earlier studies on plant extract of Tulsi, bittergourd and menthya for the alpha amylase and aldose reductase inhibitors was not promising. But there are indications of antidiabetic properties by these plants extracts. Hence, this study was carried out to explore the possibility of endophytic fungi for the production of inhibitors of enzymes related to diabetes.

Endophytes are the microorganisms that include fungi or bacteria that live within the plants without causing any injury to the plants and they are symbiotically associated.
The host plant protects endophytes and provides nutrients and in turn these endophytes help in plant growth and also produce secondary metabolites which help the plant to fight against plant pathogens. Endophytic fungi have the capability to produce bioactive compounds such as alkaloids, terpenoids, steroids, quinones, lignans, phenols and lactones. In this study 84 endophytic fungi were isolated from *Ocimum sanctum* (Tulsi) plants, 11 endophytic fungi from Bittergourd and 11 from Menthya leaves. The endophytic fungal isolates were transferred into new agar plates regularly to maintain live culture. These endophytic fungal cultures were also preserved for long at 4°C in agar slants and sterile Milli-Q water.

Endophytic fungi from three medicinal plants were screened for porcine pancreatic α-amylase inhibitor, α-glucosidase inhibitor and aldose reductase inhibitors. Ethyl acetate extracts of seven endophytic fungal isolates from Tulsi, three from Bittergourd and seven from Menthya inhibited both pancreatic α-amylase and α-glucosidase enzyme. Ethyl acetate extracts of five fungal isolates from Tulsi, three from Bittergourd and three from Menthya inhibited aldose reductase enzyme. The current antidiabetic drugs acarbose available in the market was used as standard for the pancreatic α-amylase and α-glucosidase inhibition estimation. Quercetin was used as standard for the estimation of aldose reductase enzyme inhibition.

The ethyl acetate extract of endophytic fungi *Trichoderma* sp have shown very good aldose reductase enzyme inhibition activity with IC\(_{50}\) value very much similar to the standard quercetin. The enzyme inhibition activity of ethyl acetate extracts of endophytic fungi (*Alternaria tenuissima, Alternaria carthami, Colletotrichum gloeosporioides, Diaporthe* sps. and *Trichoderma* sp.) isolated from *Ocimum sanctum* proved to be the most promising due to their low IC\(_{50}\) value which is very close to acarbose and quercetin standard. The ethyl acetate extract of endophytic fungi isolated
Summary and Conclusions

from bittergourd have shown the best aldose reductase inhibition activity. The highest aldose reductase inhibition was by endophytic fungi *Trichoderma atroviride* isolated from bittergourd.

Secondary metabolites extracted from endophytic fungi showing anti-diabetic properties independently without plants components in it. This proved that endophytic fungi can produce anti-diabetic compounds independently in the culture. In this way one can produce antidiabetic compounds from fungi and indirectly save endangered medicinal plants which otherwise used for diabetic treatments. Many of synthetic drugs and plant extracts are used for the treatment of diabetes but they are not satisfactory. Therefore, production of anti-diabetic compounds from endophytic fungi would be a better approach for the treatment of diabetes due to their fast growth rate, lesser area for the culture growth as well as easy extraction method. There is every possibility that purified compounds might emerge as an efficient natural compound which can control diabetes.

This study also concludes that *Stemphylium globuliferum* isolated from menthya was one of the best endophytic fungi having both α-amylase inhibition and α-glucosidase inhibition activity and the first report from India. The ethyl acetate extract having the α-amylase inhibition activity better than the present available drug acarbose was very interesting and promising. The α-glucosidase inhibition activity of the same fungal species was comparable to the standard acarbose activity. This indicates there is better scope for optimization of secondary metabolite production and further purification of compounds for the pharmaceutical applications. It was the first study on *Stemphylium* sp isolated from menthya and proved to have a potent antidiabetic agent.

*Alternaria carthami* isolated from *Ocimum sanctum* L., and *Trichoderma atroviride* L. from *Momordica charantia* L was chosen for detailed study. Ethyl acetate extract
of these two endophytic fungi were purified and characterized for active components responsible for alpha amylase and aldose reductase inhibition. From the literature it was evident that *Trichoderma atroviride* produces a wide range of bioactive secondary metabolites. In this study also we have found that column purified fractions of ethyl acetate extracts from *T. atroviride* inhibits α-amylase and aldose reductase significantly and their IC₅₀ values are less the IC₅₀ of standard drug acarbose and quercetin respectively.

There are no studies available on production of aldose reductase or α-amylase inhibitors from *T.atroviride*. Therefore, this endophytic fungus might emerge as a major producer of strong antidiabetic compound in near future. Pyrrolo (1, 2-a) pyrazine 1, 4-dione, hexahydro was identified as the major compound present in column purified fraction of *T.atroviride*. There are no studies on antidiabetic activity of Pyrrolo (1, 2-a) pyrazine 1, 4-dione, hexahydro and *Trichoderma atroviride*. Therefore these findings have explored wide advantages of *Trichoderma atroviride* and its secondary metabolite Pyrrolo (1,2-a) pyrazine 1,4-dione, hexahydro as antidiabetic and anticancer agent.

Secondary metabolites characterised from column purified fraction of *A.carthami* was dihydronorwogonin, cerebroside C, alternariol, tenuazonic acid and stearamide possessing antidiabetic and anticancer activity. Secondary metabolites isolated from *Alternaria sp* has been studied extensively for anticancer activity and very few studies have been carried out on alpha amylase and aldose reductase inhibition activity. Ethyl acetate extracts of *A.carthami* showed effective RLAR activity with an IC₅₀ value of 15 µg/mL which proved to efficient when compared to quercetin. Cerebroside C characterized in the study may also be responsible for RLAR activity.
Hence study of enzyme inhibition activity of *A. carthami* metabolite may lead to exploration of these fungi as an alternative source for development of natural drug. Column purified fraction of *Alternaria carthami* also proved to be the most effective anticancer agent against C6 and MCF-7 cell lines. Alternariol have been widely studied for its cytotoxic activity by many groups. In this study alternariol present in the column purified fraction of *A. carthami* may be responsible for anticancer activity. In the present study dihydronorwogonin from *A. carthami* (Tulsi) and Pyrrolo 1, 2 a pyrazine hexahydro from *T. atroviride* (Bittergourd) are found to be the most probable alpha amylase and aldose reductase inhibitors.

Both active compounds have anticancer activity. Presence of dihydronorwogonin has been reported for the first time in endophytic fungi. Dihydronorwogonin and Pyrrolo 1, 2 a pyrazine hexahydro reported for the first time as antidiabetic agents. Docking studies on binding of inhibitors to alpha amylase and aldose reductase enzymes clearly proved that dihydronorwogonin was the best fitting molecule for both enzymes. The binding affinity for dihydronorwogonin was better than quercetin indicates that further detailed study on this would bring out a novel antidiabetic compound.