Summary

Bioactive metabolites isolated from endophytic fungi from different plants have a new hope in medicinal chemistry. In this scenario, several other medicinal plants were targeted in search for endophytic fungi for screening of bioactive potential from Western Ghats. The investigation of endophytes exposed a varied assemblage of taxa, including several species. Salacia species is explored in Ayurveda as a medicinal plant for several disorders like antibacterial, anti-diabetic and anti-cancer agent. But there is a gap in research which endophytes present in this Salacia species were less exploited. The present work is targeted on the screening and profiling of endophytic extracts for their bioactivity viz., anti-diabetic, anti-tumor, antimicrobial properties, Genomic mining for TAXOL-Producing endophytic fungi and phytochemical analysis followed by purification and characterization.

In the present study, diversity of the fungal endophytes was investigated the fungal diversity of the endophytic isolates in the stem portions of Salacia species at different seasons of the year. 474 endophytic isolates were categorised as 21 species viz., Alternaria alternata, Aspergillus niger, Aspergillus terreus, Botryosphaeria rhodina, Cladosporium herbarum, Colletotrichum species, Coriolopsis caperata, Curvularia species, Diaporthe perjuncta, Drechslera species, Fusarium oxysporum, Gliocladium roseum, Lasiodiplodia theobromae, Myrothecium verrucaria, Penicillium notatum, Pestalotiopsis species, Phoma species, Sterile species, Trichoderma longibrachiatum, Trichophyton mentagrophyte and Xylaria species. In the rainy and winter seasons, the species richness was comparatively lower than in the summer season. The colonization frequency was highest during the winter season for Penicillium notatum, Pestalotiopsis species and Phoma species. Fungal endophytes such as Trichoderma longibrachiatum, Aspergillus species, Coriolopsis caperata, Curvularia species, Diaporthe perjuncta, Drechslera species, Fusarium oxysporum, Gliocladium roseum, Lasiodiplodia theobromae species were isolated from the winter and summer seasons. Their isolation could be due to the
ability of their spores to survive harsher conditions and grow at low humidity levels.

The endophytes isolated from *Salacia macroperma*, *Salacia fruticosa*, *Salacia oblonga* and *Salacia chinensis* were screened for α-amylase inhibitory activity as preliminary screening for anti-diabetic activity. In total 172 endophytic fungi isolates from *Salacia chinensis* among which 32 isolates showed inhibition more than 25% in crude extracts and highest inhibition was shown by the isolate SCS5 (*Phoma* sp.) of activity 39.4%. Among the 124 endophytic fungal isolates from *Salacia oblonga*, 15 isolates showed inhibition more than 25% in crude extracts and highest inhibition was shown by the isolate SOM15 *Colletotrichum* species of activity 39.38. Among the 128 endophytic fungal isolates from *Salacia fruticosa*, 15 isolates showed inhibition more than 25% in crude extracts and the highest inhibition was shown by the isolate SFM17 *Trichoderma longibrachiatum* (activity of 33.60%). Whereas, in *Salacia macroperma*, out of 50 isolates, 06 isolates showed inhibition more than 25% in crude extracts and the highest inhibition was shown by the isolate SMW8 *Phoma* species 33.00%. The antimicrobial activity of isolated endophytic fungi was tested against representative Gram positive and Gram negative bacteria by Well Diffusion Assay. In the present study, the ethyl acetate extract of endophytic fungi has shown effective Zone of inhibition of antibacterial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Listeria monocytogenes*, *Bacillus subtilis* and *Klebsiella pneumoniae*. Similarly, *E. coli* and *Salmonella typhi* showed zones of inhibition. It has been observed that *Salacia* sp. also contains antibacterial activity and the endophytes obtained from all four sp. of *Salacia* also exhibit antibacterial activity.

Chemical analysis was carried out on the isolated endophytic fungal extracts to determine the presence of chemical components as a prospective source for medicinal and industrial uses. The active metabolites contain chemical groups such as Steroids, Tannins, Sugars, Proteins, Flavonoids, Saponins, Terpenoids and Glycosides. In this study, phytochemical analysis
of ethyl acetate extracts of 39 isolates out of 68 isolates showed the presence of Steroids, 21 of Tannins, 45 of Sugars, 33 of Proteins, 26 of Flavonoids, 33 of Saponins, 25 of Terpenoids and 32 of Glycosides. The samples were screened for the potential to produce Taxol or taxanes, employing PCR. The resulted data have been sequenced to confirm the presence of the two genes implicated in Taxol biosynthesis, 10-deacetylbaccatin III-10-O-acetyl transferase (DBAT) and C-13 phenylpropanoid side chain-CoA acyltransferase (BAPT). Seven samples showed the amplicons of DBAT gene and one showed the amplicons of BAPT gene. Sequencing of these products was carried out, of which one sample has revealed sequence homology to the original DBAT gene from Taxus sps. The present work confirms and substantiates the potential of genomic mining approach to discover novel Taxol-producing endophytic fungi.

In the present work, we have attempted towards isolation, purification and characterization of bioactive compounds and their application towards antioxidant, anti-inflammatory, anti-diabetic, anti-tumor, antimicrobial properties of purified products. From the preliminary screening of four plants, S. chinensis, S. oblonga, S. macrosperma and S. fruticosa we have selected Colletotrichum sps.- SOM15 from S. oblonga which showed a promising results towards antioxidant, anti-diabetic and antimicrobial properties. Initially, SOM15, isolated from Salacia oblonga in the Monsoon season which showed highest bioactive potential and further was selected and subjected to fermentation. Then the culture filtrates were extracted with ethyl acetate followed by evaporation of the solvent using Rotary flash evaporator. The ethyl acetate extract of SOM15 was fractionated by column chromatography. Thin layer chromatograms were developed by using ethyl acetate extract using optimized solvent system, petroleum ether and ethyl acetate in the ratio 1:1 which facilitates good resolution of metabolites based on polarity on pre-coated TLC silica plates. Similar results were found in fungal endophytes like Colletotrichum gloiospoides and Fusarium oxysporum found in the spikes of Pinus roxburghii when extracted and analyzed indicating the presence of
phytoconstituents like phenols, flavonoids, terpenes, saponins, alkaloids and tannins by fermentation followed by solvent extraction and also contributed towards various bioactivities.

In the present work, to study anti-bacterial activity, the chromatograms were transferred to sterile petri plates and overlaid with Mueller Hinton soft agar Brain heart infusion medium incorporated with 2, 3, 5-triphenyltetrazolium chloride and inoculated with 1% standardized axenic microbial inocula. After incubation, the upper agar was flooded with 10ml of microbiological agar of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) 5 mg/ml which was converted into a formazan dye by the active dehydrogenase enzyme of the test microbe. Inhibition zones were observed as clear spots against red on chromatogram corresponding to the antimicrobial metabolites resolved. The bioactive fractions numbered from 80-101 with Rf value of 0.52 were collected and confirmed by Bioautography for anti-bacterial activity.

Antioxidant potential of the natural products is generally attributed to the presence of a wide array of secondary metabolites that include phenolics, flavonoids, tannins and terpenes. The endophytic extracts associated with medicinal plants have been tested for their antioxidant capacity through various assays. In vitro chemical-based DPPH and ABTS antioxidant assay compounds exhibited good activity with standards 5.72 and 3.56 µg of IC\textsubscript{50} values and 7.56 and 6.1µg of SOM15 respectively. The ethyl acetate extract of Colletotrichum sp. (SOM15) has shown effective zone of inhibition of antibacterial activity against Staphylococcus aureus of 12mm, Staphylococcus epidermidis of 10mm, Listeria monocytogenes of 7mm, Bacillus subtilis of 8mm, and Klebsiella pneumoniae of 8mm at 50µg. Similarly in 100µg, the zone of inhibition is 22mm, 18mm, 15mm, 12mm, 14mm, whereas for E. coli and Salmonella typhi, 20mm and 19mm was the zones of inhibition. The minimum inhibitory concentrations of isolated endophytic fungi tested against Klebsiella pneumonia has shown the lowest concentration at 6.25µg, Bacillus subtilis at

The potency of the inhibition was determined experimentally, and the data were expressed using IC50 values. These results have shown that the ethyl extract of SOM15 is a potent inhibitor of α-amylase and α-glucosidase enzymes. α-amylase inhibitory assay is based on the breakdown of starch to maltose while α-glucosidase inhibitory assay is based on the breakdown of maltose to glucose. SOM15 extract showed a dose-dependent inhibition of both the enzymes, but in different concentrations at 106.11 µg/ml concentration, and showed 124.62 µg/ml 50% of inhibition for α-amylase and α-glucosidase respectively. Bioanalytical analysis like LC, MS, FTIR and NMR spectroscopy are the most powerful analytical methods for identification and structural elucidation of compounds. Compared to FTIR, the NMR analysis and MS spectroscopy studies show relatively high sensitivity and measurements. The metabolite elucidated from the endophytic isolate SOM15 was identified by LC-MS analysis and also was further supported by spectroscopic analyses such as IR and 1H-NMR data which provides prominent signals for the important functional groups and protons which are necessary for the formation of the phenolic compound 3-(4-Hydroxyphenyl) propionionic acid. The Homonuclear correlation spectroscopy (COSY) sequence, employed to identify spins which are coupled to each other at 4.6 ppm and 3.0 ppm and Heteronuclear Multiple Bond Coherence (HMBC) showed 2-dimensional inverse of H, C correlations which enabled to justify the determination of carbon to hydrogen connectivity.