CHAPTER 5

SUMMARY AND CONCLUSION

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Fungi are regarded as the second-largest group of eukaryotic organisms on earth, with an estimation of 1.5 to 5.1 million species. They are morphologically, metabolically, ecologically and phylogenetically diverse. They are notorious to produce various bioactive molecules, which makes them valuable for researchers to pursue the discovery of novel chemical diversity for industrial, pharmaceutical, agricultural and biotechnological applications. Despite their importance in basic (taxonomy, ecology) and applied (bioprospecting, genomics) research, taxonomic identification of fungi to species level remains a paramount task for researchers. Identification of fungi and their taxonomy based on morphology is essential but can be misleading due to several factors including cryptic speciation, hybridization and convergent evolution. As a consequence, DNA-based approaches over morphology-based approaches have emerged for differentiating between species among several phyla. Recently, DNA barcoding and DNA taxonomy, a new epoch for the molecular identification of fungi, that make use of the DNA sequences generated from the small subunit (nrSSU-18S), large subunit (nrLSU-26S or 28S) and the 5.8S rRNA gene (Internal Transcribed Spacer region, ITS1 and ITS2). DNA barcoding utilizes a short-standardized region (between 400 and 800 base pairs) to discriminate between species together with the D1/D2 domain of the Large Subunit (LSU, 26S) nuclear DNA (nrDNA) sequences.

Gujarat state is well-known for its varying climatic conditions and possess different types of forests such as moist deciduous forest in south Gujarat, dry deciduous to scrub forest in central and north Gujarat with some part of Saurashtra while Rann of Kachchh is known for its typical desert conditions. Despite of all such climatic conditions, the fungal diversity of the state is poorly investigated till 2015. However, no special efforts have been made in the state for the documentation of the fungal diversity. Previous studies from our laboratory have collected and identified several

fungal species as a part of the Gujarat Biodiversity Board activity for the documentation of fungal biodiversity in Gujarat state. During this period several interesting and important fungal taxon have been collected from different forest regions of the state on which no in-depth information is available. Hence, there is an urgent need to identify and explore such important and uncommon fungal species for their therapeutic evaluation.

Fruiting bodies of some unique fungi were collected from different forest regions of Gujarat State. These include Vansda National Park, Vansda; Zand Hanuman, Panchmahal; Wilson hills, Dharampur; Community Science Centre, Vadodara; Bhavnath, Junagadh; Sagai, Narmada; Panjraghat, Narmada; Panas, Valsad; Kheralu, Mehsana; Ratanmahal, Dahod; Jambughoda, Narmada and Gir Forest National Park, Junagadh. In the present study, molecular identification of unique fungi was done as a preliminary study. Based on ITS rDNA sequences, fungi were identified in addition to their morphological characterization. Identification was done by 99% base pair match of the sequence obtained to the closest available reference sequences. The selected fungi were identified as *Clathrus delicatus*, *Trichaleurina javanica*, *Itajahya galericulata*, *Cyathus stercoreus*, *Geastrum triplex*, *Geastrum saccatum*, *Geastrum rufescens*, *Disciseda candida*, *Scleroderma bovista*, *Pisolithus albus*, *Pisolithus tinctorius*, *Dictyophora indusiata* and *Dictyophora multicolor*. The occurrence of *C. delicatus* and *C. stercoreus* were reported for the first time in Gujarat state. *T. javanica* and *D. candida* were reported from India for the first time whereas *I. galericulata* was the first report of the species for India and Asia. The present study reported three species of *Geastrum* as a new distributional record for the Gujarat state.

Fungi are a biotechnologically important yet understudied group of microorganisms. Nowadays, there is an increased interest in fungi not only as a high protein food but also as a source of bioactive compounds having therapeutic potential like anticancer, immunopotentiating, hepatoprotective, antiviral etc. To discover the innovative therapeutic possibilities, more research is required into the mechanisms underlying the multiple health benefits of the fungus to humans. The screening of the secondary metabolite is almost entirely motivated by the discovery of novel bioactive compounds in therapeutics since many years. Metabolite profiling is a tool that may be used to find diverse applications in all aspects of understanding, utilization and discovery, making it a key point in studies of fungal physiology and taxonomy. In a view of this background, the study was focused on the therapeutic application of bioactive metabolites extracted from fungi and their identification by HR-LC/MS.

Out of the several fungal strains screened, seven species *viz*. *P. albus*, *P. tinctorius*, *G. triplex*, *G. saccatum*, *D. indusiata*, *I. galariculata* and *C. stercoreus* were used to check their therapeutic potential. Ethanolic extracts were prepared from the dried fruiting body of the selected fungus for the metabolic profiling of bioactive metabolites and the evaluation of their therapeutic effects. The ethanolic extracts of all the fungi in a concentration range of 50-200 µg/ml were tested for antioxidant activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydrogen peroxide (H_2O_2). The results revealed that ethanolic extracts of all the selected fungi possess a concentration-dependent scavenging effect. The scavenging potentials against DPPH were *D. indusiata* > *I. galariculata* > *P. tinctorius* > *P. albus* > *G.triplex* > *G. saccatum* > *C. stercoreus*. The scavenging potentials against H_2O_2 were *D. indusiata* > *I. galariculata* > *P. tinctorius* > *P. albus* > *G.triplex* > *G. saccatum* > *C. stercoreus*.

Around the globe, cancer-related deaths are increasing as the incidence continues to rise. Fungi are regarded as a potential repository of novel chemical compounds for cancer research. Therefore, the anticancer potentials of crude extract of seven selected species of fungi that are medicinally important were evaluated against three different cancer cell lines viz MDA-MB-231, HCT-116 and A-549 by MTT assay. Significant inhibition of colorectal cancer cells HCT-116 was found than lung cancer cells A-549 and breast cancer cells MDA-MB-231. Dose-dependent anticancer potentials of fungal extracts were observed i.e. increase in concentration (50, 75, 100, 150 and 200 µg/ml), escalates the potentiality of selected fungus. The crude extract of *D. indusiata* showed the highest inhibition activity in all the three tested cell lines.

Fungi are well known for their unique ability of bioactive potentials, including antiviral capability. Therefore, crude extracts of five selected fungi viz. *P. albus*, *G. triplex*, *D. indusiata*, *I. galariculata* and *C. stercoreus* were exploited for profiling their bioactive compounds using HR-LC/MS. With the detailed mass spectrum data, absorbance spectra and retention times were compared with the available literature and found that all the fungi possess different bioactive compounds which belong to various classes and have different therapeutic potential. Based on medicinal uses and binding energy, active sites were covered after molecular docking analysis; three potent compounds (bergenin, quercitrin and dihydroartemisinin) were selected for in-depth *in-silico* analysis as potential inhibitors against SARS-CoV-2 M^{pro}. The docking analysis, ADMET predictions and medicinal properties indicated that bergenin, quercitrin and dihydroartemisinin are the most significant compounds as potential inhibitors of SARS-CoV-2 M^{pro}, which could be explored further.

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In conclusion, the present study was successful in reporting the occurrence, distribution, morpho-taxonomic and molecular identification of some unique fungi from the Gujarat state, and in evaluating their therapeutic properties like antioxidant, anticancer and antiviral potential.