CHAPTER-6

SUMMARY AND CONCLUSION

Aim of the present investigation was to study the genetic variability and bioactive molecules production by *Ganoderma* species. The findings or salient features of the present study are summarized below:

During the mycofloristic survey of different regions of Himachal Pradesh and Punjab, it was found that *Ganoderma* is present in both hot and cold places but more frequently in hot regions. Both laccate and non-laccate *Ganoderma* are found in NW Himalaya. A great variation was recorded in microscopic and macroscopic traits of *Ganoderma* isolates in the present study. 37.07% hosts were not identifiable as those were highly degraded. Among identifiable hosts most common were *Dalbergia sissoo* (Sheesham), *Acacia* (khair) and *Albizia chinensis* (Oyi) (13.04%) followed by *Cedrus deodara* (Deodar) and *Acacia nilotica* (Babul), both accounted for 8.69% of the total hosts.

Best suitable medium and optimum culture conditions such as temperature, pH and light were determined for vegetative growth of *Ganoderma* isolates. Various liquid media routinely used for cultivation of fungi were used to screen best suitable medium and malt extract broth was found to be the best medium for majority of isolates except isolate GL2, GL9 and GL12, which produced maximum biomass in potato dextrose broth. During the evaluation of different temperatures on mycelial growth it was observed that all the isolates produced maximum biomass at 30 °C. pH 5.5 was found optimum for the mycelial growth of 85% of isolates except isolate GL6, GL8 and GL9, which showed maximum mycelial growth at pH 6.0. As light is considered to be an essential factor for reproductive growth of *Ganoderma*, the effect of light and darkness on mycelial growth was also determined and it was found that all isolates produced maximum biomass under darkness; although there was no significant difference in biomass yield under these two conditions.
Genetic variability among the 31 *Ganoderma* isolates was determined by RAPD analysis. Similarity values varied from 25-60% among these tested isolates, which revealed high genetic diversity in test population. RAPD data suggested that the genetic variation may depend upon the geographic origin of the mushroom, whereas, no relation was found according to the hosts of the isolates.

Antimicrobial activities of twenty selected *Ganoderma* isolates was evaluated, it was observed that GL1, GL2, GL3, GL4 and GL5 (collected from Bilaspur) showed higher inhibitory activities against all isolates in comparison to others. Specifically GL4 (collected from Bilaspur) showed very good inhibition against all tested pathogens.

Polysaccharide content varied from 2.47%- 8.67% in the twenty isolates. Highest content was recovered from isolate GL4 (collected from Bilaspur). The identity of this isolate (GL4) with good antimicrobial activities and highest polysaccharide content was confirmed as *Ganoderma lucidum* by amplifying and sequencing the Internal Transcribed Spacer (ITS) DNA. Further, this *G. lucidum* isolate was taken for cultivation trials and it was found that forestry wastes i.e., mango, sheesham and poplar sawdusts gave high yield and biological efficiency in comparison to agricultural wastes. Overall mango sawdust was found the best substrate as it showed maximum yield and biological efficiency, and shortest spawn run period as well as shortest primordium initiation period.

While determining optimum spawn level on forestry wastes it was found that 3% of spawn level showed highest yield on all substrates. Overall mango sawdust inoculated at 3% spawn level gave maximum yield and biological efficiency, and shortest spawn run period as well as shortest primordium initiation period. Further, during evaluation of effect of supplementation at different concentrations, it was found that 20% of all supplements gave high yield irrespective of the substrate but overall highest yield was obtained when mango sawdust was supplemented with 20% wheat bran.

Polysaccharides extracted from *G. lucidum* fruit bodies showed the presence of many sugars of medical relevance such as Rhamnose, Fucose, Galactose, Mannose and Glucose on paper chromatography. The antimicrobial activities and antioxidant activities of this polysaccharide fraction was compared with fruit body and mycelial extract and
polysaccharide fraction was found to be better antimicrobial and antioxidant agent as compared to fruit body and mycelial extracts.

Major findings of the current study provide scientific support to many claims of the ancient herbalists that *Ganoderma* can be used to combat many diseases as it proved an efficient antimicrobial and antioxidant agent and showed the presence of high amount of polysaccharides with sugars of medical importance. Thus, *G. lucidum* polysaccharides can be used to make carbohydrate-based vaccines with therapeutic efficacy for infectious diseases and cancer. Genetic diversity in *Ganoderma* reflects much of the genetic universe within which the tools of selection, breeding and genetic engineering can be applied in future research to provide strains adapted to a wider range of substrates, environments and cultivation methods. On the other hand, *Ganoderma* cultivation offers a unique opportunity to bio-convert agro-forestry pollutants into economically valuable medicinal products, which in turn minimize health hazards in the environment and serve to generate incentives to Indian economy.