CHAPTER – 6

SUMMARY & CONCLUSION
6.1. Summary

Medicinal plants have played an important role in drug discovery, with many pharmaceutical products originating from plants. Isolation and characterization of antifungal compounds is still urgent today because of continuing development of resistance of pathogenic fungi to antifungal drugs. Therapy with herbal drugs is an old tradition and plants have been used over the years for the treatment of numerous health problems including infectious and non-infectious skin disorders. This dependency on medicinal plants for such a long time has raised questions of efficacy and safety of traditional medicine. On the other hand, it has provided the opportunity for researchers and scientists to evaluate the biological activity and safety of medicinal plants to promote their usage, and for the development of new pharmaceuticals which may be less toxic and more potent than currently used antifungal and antibacterial drugs. Plants produce a vast number of secondary constituents some of which may act to protect the plants against invading pathogens. These constituents are important for human and animal lives because they can be used in the production of new pharmaceuticals or feed additives. The extraction of medicinal plants with appropriate solvents can result in extracts rich in potential antimicrobially active constituents.

The aim of the study was to evaluate the antifungal activity of extracts prepared from nine tree species (Aegle marmelos (CBT 003), Capparis aphylla (CBT 009), Callistemon lanceolatus (CBT 008), Commelina bengalensis (CBT 013), Justicia adhatoda (CBT 017), Argemona mexicana (CBT 004), Achyranthes aspera (CBT 002), Catharanthus roseus (CBT 012), and Syzygium cumini (CBT 022)) selected based on their use in respiratory and other disorders in traditional systems of medicine. Three opportunistic pathogenic fungi; Aspergillus fumigatus (ITCC 4517), A. flavus (ITCC 5192) and A. niger (ITCC 5405), were used as test organisms. A serial microplate dilution method was used to determine the minimal inhibitory concentration (MIC) and Gas Chromatography-Mass Spectroscopy (GC-MS) used to determine the number of antifungal compounds in the extract and their Retention factor values. Extracts of all the plant species were active with average MIC values ranging from 0.156 to 6.0 mg/ml against the three pathogenic strains of fungi. In disc diffusion assay, nine out of forty five leaf extracts had activity with clear zones of inhibition against all three pathogenic Aspergillus species.

MIC values as low as 156.0-312.0 µg/ml were obtained with J. adhatoda and C. bengalensis extracts against Aspergillus species. The toxicity (in vitro and in vivo) of various
fractions of *J. adhatoda* and *C. bengalensis* extracts were evaluated, in which the extracts isolated from *J. adhatoda* were found to be non-toxic. Therefore, *J. adhatoda* was chosen for further investigation because (a) it had good antifungal activity against three pathogenic strain of fungi with MIC value as low as 156.0-312.0 µg/ml, (b) there were several active compounds against all the tested fungi based on GC-MS, (c) it is common and easily available in Haryana state of India, and (d) as far as our literature survey could ascertain there was no published information on the antifungal activity of this plant species.

The bulk powdered leaves of *J. adhatoda* were extracted with into five fractions (Petroleum ether, chloroform, Acetone, Methanol and Water) using the Soxhlet’s extraction, to group the phytochemicals based on their polarity. Petroleum ether, chloroform and acetone fractions were the most active with MIC values as low as 156.0-312.0 µg/ml for the *Aspergillus* species. One antifungal compound was isolated from the Petroleum ether fraction using column chromatography with silica gel as the stationary phase and petroleum ether: ethyl acetate gradient as the mobile phase from low to high polarity. The isolated compound was identified as acyclic diterpene alcohol named 3, 7, 11, 15 tetramethyl-3-hexadec-en-1-ol by using NMR, FTIR, GC-MS and mass spectrometry. Phytol (3, 7 11, 15 tetramethyl-2-hexadecen-1-ol) had the best activity with low MIC values of 93.0 µg/ml against the three pathogenic strains of fungi and negligible toxicity experimented by haemolytic and comet assay.

Moreover, the present study reveals that highest antibacterial potential with aqueous extract fraction of *C. roseus* was found against all pathogenic microbes. Furthermore, our study clearly evidenced that the extracts of *A. marmelos*, *C. aphylla*, *J. adhatoda* and *A. aspera* are rich in phenolics and may be responsible for the observed antioxidant capacities of different extracts. These results imply that the extracts or the derived phytochemical (2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl) from *J. adhatoda* have great potential to prevent diseases caused by the overproduction of radicals, and they may be suitable for the treatment of degenerative diseases. Consequently, this (*J. adhatoda*) plant contains various compounds which can be useful in modern medicine. Though, the present research on the natural resources encouraged the scientific community for explore the bioactive molecules and funding should be made available for the development and production of new pharmaceuticals or herbal extracts of therapeutic value.
6.2. Conclusion

The present study proved *J. adhatoda* as the most promising species for further work based on the presence of best antifungal (*A. fumigatus*, *A. flavus* and *A. niger*) activity and the low average MIC value of petroleum ether extract as 0.156 mg/ml. Column chromatography and GC-MS led to the isolation and identification of three constituents [2-Hexadecen-1-ol, 3, 7, 11, 15-tetramethyl-, (64.20%),1-Hexadecyne (28.55%) and 1-Octadecyne (7.24%)] present in most bioactive sub-fraction of the *J. adhatoda* leaves extract (petroleum ether). Finally, the most bioactive purified compound was identified as acyclic diterpene alcohol named 3, 7, 11, 15 tetramethyl-3-hexadec-en-1-ol by using NMR, FTIR, GC-MS and mass spectrometry. The findings of this study suggest that *J. adhatoda* may be considered as useful natural alternative therapy for patients with aspergillosis disorders either alone or in combination with other suitable antifungal/antimicrobial agents. Thus, further in-depth investigations on such herbal drugs with the aim of isolating the active compound(s) and optimizing the anti-aspergillosis formulation for current delivery are justified.

Furthermore, our study also proved that *J. adhatoda* synthesizes potent antioxidant metabolites with extremely low toxicity to human cells. Expectantly, our study could contribute to the development of newer and highly active antimicrobial and antioxidant drugs which would be rapidly progress to various stages of formulation development and enter the pharmaceutical market, further contributing to a reduction in the incidence of infections and degenerative diseases.