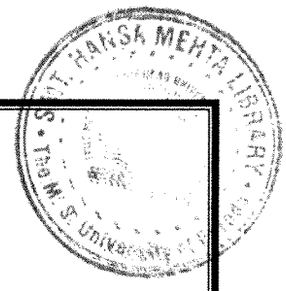

Chapter 4

Summary And Conclusion

4. SUMMARY AND CONCLUSION

In India numbers of traditional systems of medicine like Ayurveda, Siddha and Unani are practiced since centuries. Though these systems possess rich heritage as health care, these are confined to Vaidyas, Hakeems, Dais and massagers etc. Due to lack of documentation of scientific facts regarding usage of herbs as medicament, manufacturing at industrial level was not possible.

Many of the important drugs of modern medicine are derived from plants as their constituents or those which are the modified form of original constituents like digoxine, vincristine, quinine, taxol derivatives etc. Investigations of plants for their biological utility have become important task on scientific basis. The lead for these investigations originates from the literature available. There is a remarkable contribution of folklore practitioners in sharing the knowledge regarding herbal drugs. This provides a basis for screening of natural products using modern techniques. Though modern system of medicine is well equipped for combating various disorders, certain diseases cannot be treated symptomatically. Therefore there is a need to evaluate the agents from alternative system of medicine.

Some plants are believed to promote positive health and maintain resistance against infection by establishing body equilibrium. The concept of adaptogens relates to nonspecific activation of the function and efficiency of macrophages, granulocytes, complement natural killer cells and lymphocytes and also to the production of various effector molecules generated by activated cells. It is expected that theses nonspecific effects give protection against different pathogens including bacteria, viruses, fungi etc. and constitute an alternative to conventional chemotherapy.

There is a difference in the concept of resistance of the body to disease in Ayurveda and modern system of medicine. According to Ayurvedic theory harmonious balance between three humors of body viz Vayu, Pitta and Kaf is needed for positive health. A significant part of Ayurvedic therapeutics is preventive in nature. Many plants have been identified as Rasayana in Ayurvedic system of medicine. These substances have been described to possess pharmacological properties like immunostimulant, tonic, antiaging, antistress, antirheumatic, adaptogenic anticancer, antibacterial etc.

During the literature search, it was observed that there are many plants not included in the Rasayana category but reported to have rejuvenative properties. The idea of present study was evolved due to the use of several drugs as rejuvenators other

than Rasayana. These drugs are used in many Ayurvedic formulations along with Rasayana drugs. The selected plants *P. integerrima* and *H. spicatum* though not included in the Rasayana category possess the rejuvenative properties. Both the plants are used in Chyavanprash which is a well known Ayurvedic formulation used as rejuvenator. Chyavanprash contains Rasayana drugs like *Pueraria tuberosa, Withania somnifera, Asparagus racemosus, Tinospora cordifolia etc. P. integerrima* is used in formulations like Kumari Asav, Chyavanprash Avaleha. *H. spicatum* is used in Piptonil tablets, Brainokan and Cardiokan which are used as tonic. The present study aims to investigate these plants for their phytochemical profile. Based on their phytochemical profile, the plants were screened for the biological activity to confirm the claims made on them. There is a need to evaluate the plants on the scientific basis including their chemical nature and biological activity. In our present study, the efforts have been taken to evaluate the selected plants for their phytochemical profile and different activities which include assessment of rejuvenating potential of the plant, antioxidant and hepatoprotective activity.

The selected drugs were identified carefully by macroscopic and microscopic evaluation. The crude drugs were evaluated for different parameters like ash value, water soluble and alcohol soluble extractive value. Preliminary phytochemical screening of successive extracts of the crude drugs was made for qualitative evaluation of phytoconstituents and their presence was confirmed by TLC. On the basis of preliminary phytochemical screening, the extracts were prepared for biological screening. Since the selected drugs were reported to contain phytoconstituents with variation in their chemical nature, the methods selected for the preparation of extracts were different.

The present studies were planned to assess the adaptogenic activity in animals using different models like *E. coli* induced abdominal sepsis, cyclophosphamide induced myelosuppression, carbon clearance test, forced swim test and anoxia tolerance test. The studies were conducted on extracts and activity guided fractions. The studies on the selected plants were carried out as per WHO guidelines for quality control methods for medicinal plant materials.

The selected plant drugs were subjected to pharmacognostic evaluation to identify the diagnostic features, by studying morphological and microscopic characters. Morphological features of both the plant drugs were compared with the standard text

for confirming their identity. Microscopy of entire plant drug and powder characteristics helped in identification and evaluation of the plant drugs.

The plant drugs were then subjected to proximate analysis in order to develop standards such as extractive value, ash value, foreign organic matter, loss on drying, foaming index, swelling index, heavy metals, bitterness value, volatile oil content and microbial content. One of the drugs *P. integerrima* which was found to be rich in phenolic and flavonoid content was subjected to determination of total phenolic and flavonoid content.

The presence of different chemical constituents in the selected plant drugs were detected by subjecting them to successive solvent extraction using solvents in the order of increasing polarity. The successive extracts so obtained were subjected to qualitative tests for various chemical constituents. Leaf galls of *P. integerrima* showed presence of volatile oil, carbohydrates, phenolics and flavonoids whereas rhizomes of *H. spicatum* showed presence of volatile oil, terpenoids, steroids, carbohydrates and flavonoids. These successive extracts were further subjected to TLC to confirm the presence of various phytoconstituents. Based on the phytoprofiles obtained from qualitative tests and TLC, the aqueous extract of *P. integerrima* was fractionated into n- Butanol, Ethyl acetate and Methanol extract. The Methanol extract was fractionated into Chloroform, Ethyl acetate, Methanol extract and residual Methanol extract. Methanol extract of *H. spicatum* was fractionated into Chloroform, Ethyl acetate extract and residual Methanol extract. All the fractions were subjected to qualitative tests and TLC for confirming the presence of different phytoconstituents. All the successive extracts and fractions were subjected to HPTLC fingerprinting using different solvent systems and at different wavelengths.

The fractions were selected for isolation of compounds on the basis of the phytoconstituents present. Ethyl acetate fraction of Methanol extract of *P. integerrima* was rich in phenolic and flavonoid content. Therefore it was subjected to column chromatography and preparative TLC to isolate flavonoids and phenolics. PI 1 and PI 2 were of flavonoid nature and PI 3 was phenolic in nature. These compounds were subjected to physicochemical characteristics, TLC, elemental analysis and spectral studies. The structures were predicted from the above data. Methanol extract of *H. spicatum* was subjected to column chromatography and preparative TLC. Only one compound HS was isolated from Methanol extract of *H. spicatum* which was found to be of diterpenoid in nature. The diterpenoid nature of the compound was confirmed

by chemical test and TLC. The IR and NMR spectra were compared with the standard data. The structures of the compounds were predicted from elemental analysis, IR, NMR and Mass. The purity of these compounds was assessed by HPLC method using Acetonitrile: Methanol: water (80:15:5) as mobile phase. The compounds PI 1, PI 2 and PI 3 were scanned at 254nm and compound HS was scanned at 308 nm. Compound PI1 was found to be 83.65% pure. Purity of PI2 was 96.5%, that of PI3 was 97.5% and HS were found to have purity 97.7%. The compounds PI1 and PI2 were quantified by HPTLC. From the physicochemical characteristics and spectral studies the probable structures of the compounds were determine viz PI 1 as Quercetin, PI 2 as Leutiolin, PI3 as Gallic acid and HS as a hedychnone like furanoditerpene. Quercetin and Gallic acid were identified in Methanol extract of *P. integerrima* and Kaempferol was identified in *H. spicatum*. Therefore, these compounds were quantified. Quercetin was found to be 4.67% w/w, Gallic acid was found to be 5.63% w/w and Kaemperol was found to be 0.8% w/w.

The Aqueous and Methanol extracts of *P. integerrima* and *H. spicatum* were subjected to acute toxicity studies as per OECD guidelines no. 423. Female albino rats were used for the acute toxicity studies as per mentioned in the guidelines. The groups of animals were treated with the extracts of both the drugs up to dose of 2000mg/kg. None of the extracts showed toxicity at 2000mg/kg and therefore considered to be safe. The Methanol and Aqueous extract of *P. integerrima* and Methanol extract of *H. spicatum* were found to be effective, theses extracts were further fractionated and subjected to biological activity. The extracts and fractions were screened for adaptogenic activity using different models and different parameters were evaluated.

In *E. coli* induced abdominal sepsis, the WBC and neutrophils were determined. % mortality due to sepsis was another criterion. % mortality observed in the control group was 100 % while in the treatment group it was reduced to 33%.The animals were treated with extracts at dose levels 100, 200 and 500mg/kg. Methanol extract of *H. spicatum* was found to be significant as compared to Aqueous extract. Both Aqueous and Methanol extract of *P. integerrima* were found to be effective. Chloroform fraction of methanol extract was not found to be effective. Acetone fraction of methanol extract was effective at 150mg/kg. Ethyl acetate fraction showed significant activity ($p<0.001$).In case of fractions of Aqueous extract, n Butanol fraction, remaining Methanol fraction and Ethyl acetate fractions were significant at

150mg/kg ($p<0.001$). In case of *H. spicatum* Aqueous and Methanol extract showed dose dependent activity. Ethyl acetate fraction and remaining Methanol fraction of Methanol extract were found to be effective in groups treated with 150mg/kg. The plant drugs were found to be effective as immunoprophylactic.

In carbon clearance model, significant phagocytic activity was observed at 200 and 500 mg/kg dose of aqueous and methanol extract of *P. integerrima* ($p<0.001$). Acetone fraction, ethyl acetate fraction and remaining methanol fraction of methanol extract showed significant activity. Ethyl acetate fraction and Methanol fraction of aqueous extract of *P. integerrima* were found to be more effective. Methanol extract of *H. spicatum* at 200 and 500mg/kg showed dose dependant activity and its ethyl acetate fraction was significant. The increased phagocytic activity confirms the immunostimulant nature of the extracts.

The extracts were found to offer protection against bone marrow suppression when evaluated in cyclophosphamide induced myelosuppression. Evaluation of the activity was done on the basis of increase in the WBC as compared to the cyclophosphamide treated group. The selected extracts and the fractions of the extracts were found to offer protection against myelosuppression. Other parameters such as HB, HCT, MCV and RBC were not altered so the extracts can be use for long term therapy.

Tolerance to anoxic stress was increased after second and third week. The time of first convulsion was delayed at the end of second week which was still prolonged at the end of third week In forced swim model different parameters like swim time, effect on blood count, organ weight and changes in biochemical parameters were considered. It was observed that there was increase in the swim time duration in the treatment groups when compared with stress control group. No change in the organ weight was observed. There was decrease in the blood count as compared to stress control group. The plant drugs were found to be immunoprotective.

Quercetin, Leutiolin and Gallic acid were isolated from *P. integerrima*. These compounds are known to possess antioxidant activity. Therefore, Aqueous and Methanol extract of *P. integerrima*, their fractions and the isolated compounds were subjected to in vitro antioxidant activity using DPPH scavenging activity, reducing power assay, hydrogen peroxide scavenging activity and hydroxyl radical scavenging activity and found to possess significant antioxidant potential.

It is mentioned in the Ayurvedic text that both the selected plants are used in liver disorders. To ensure the hepatoprotective activity endowed upon them, the isolated

flavonoid and phenolic fractions of *P. integerrima* and diterpene fraction from *H. spicatum* as well as isolated compounds viz PI 1 (Quercetin), PI 2 (Leuteolin), PI 3 (Gallic acid) and HS (Furanoditerprene) from the fractions were subjected to in vitro hepatoprotective activity. In vitro hepatoprotective activity was carried out using paracetamol as toxicant on primary culture rat hepatocytes. These isolated hepatocytes were subjected to primary culturing using culture medium RPMI 1640 supplemented with calf serum (10%), HEPES and gentamycin (1μg/ml). These cells were 97-98% viable as confirmed by trypan blue exclusion method. The assessment of hepatoprotective activity was carried out by measuring an increase in the percentage of viable cells compared with toxicant and control groups. The flavonoid and phenolic fraction from *P. integerrima* and terpenoid fraction from *H. spicatum* were subjected to hepatoprotective activity in the dose level 100, 500 and 1000 mg/kg. Maximum protection was observed with fractions at 1000 mg/kg dose ($p<0.01$). After confirming the activity of the fractions, isolated compounds were subjected at dose level 10, 50 and 100mg/kg. The compounds isolated from *P. integerrima* were of phenolic in nature and compound isolated from *H. spicatum* was a furanoditerpene. All these compounds are reported to possess hepatoprotective activity. In the present study, these compounds showed significant activity against paracetamol induced hepatotoxicity in isolated rat hepatocytes.

Thus from the present study, it can be concluded that the selected plant drugs were found to possess adaptogenic activity through various arms. The extracts were found to possess immunoprophylactic activity in *E. coli* induced abdominal sepsis. The extracts were effective in offering protection against myelosuppression by cyclophosphamide. Immunoprotective activity was observed by inducing stress by different methods and immunoprotective effect via Phagocytosis. The selected fractions and isolated compounds from both the selected drugs were also found to be hepatoprotective in isolated rat hepatocytes. The phytochemical screening revealed presence of phenolics and flavonoids in *P. integerrima. H. spicatum* was found to be rich in terpenoids, diteprnes and steroids. Both types of components are reported to exhibit adaptogenic activity in various experimental models. Potent adaptogenic activity may be observed due to presence of phenolics, flavonoids and diterpenes.

The fractions and isolated compounds were found to show significant hepatoprotective activity in drug induced hepatotoxicity in isolated hepatocytes. The hepatoprotective activity at the higher dose was comparable with that of Sylimarine.

P. integerrima was found to be rich in phenolic and flavonoid content whereas *H. spicatum* showed presence of terpenoids particularly furanodiperpenes. Activity of *P. integerrima* may be due to phenolics and flavonoids. Activity of *H. spicatum* may be attributed to diterpenes present which was similar to that of andrographaloid, a diterpene responsible for hepatoprotective activity. Andrographaloid is also known to possess both specific and nonspecific immune response. The results of our findings were comparable to that of the reported Rasayana drugs.

On the whole, these findings offer justification for the usage of these drugs in the alternative system of medicine as adaptogens.