5. Summary and Conclusion

The present research work is on phytochemical investigation and validation of herbal potential of two folkloric medicinal plants *Pisonia grandis* R.Br. of plant family Nyctaginaceae and *Andrographis stenophylla* C.B Clarke of plant family Acanthaceae. The prime focus of the study is the isolation and chemical characterisation of their phytoconstituents, which area of work is one major stage towards chemical standardisation of the plants. Herbal standardisation strategies have also been applied to make it a wholesome record of validation of the folkloric use of the chosen plants.

This research study is presented in five chapters.

Chapter I comprises of a brief introduction to the study and a mention of its prime focus.

The main objectives of this research work are:

- To isolate and characterize the chemical constituents of two folkloric medicinal plants *Pisonia grandis* R.Br. and *Andrographis stenophylla* C.B Clarke
- To validate the herbal potential of the chosen plants by standardisation studies

Review of literature pertaining to the research study is presented in Chapter II.

It covers recent reports on:

- Earlier work on the chosen plants *Pisonia grandis* and *Andrographis stenophylla*
- Earlier work on the bio-pharma potential of the bio-actives of the chosen plants

The methodology adopted for the research work comprising of two major stages is presented in Chapter III.

The outline of the strategy adopted for the research work is presented in the following sections:

- Isolation and characterization of chemical constituents from the chosen plants *Pisonia grandis* and *Andrographis stenophylla*
- Validation of the Herbal Potential of the chosen plants

The results pertaining to the research work and the ensuing discussions are presented in Chapter IV.

Two folkloric plants have been extensively investigated in this research study. The constituents of their non-polar and polar extracts have been revealed. Both plants have been validated for their herbal potential by exhaustive standardisation studies.
The Highlights of the results and their significance:

As an outcome of the work on isolation and characterization of chemical constituents from the chosen medicinal plants, potentially active constituents elaborated by both plants have been identified in their extracts.

- Five compounds have been characterised from the dewaxed ethanol extract concentrate of stems of *Pisonia grandis*.
- Three compounds have been characterised from the methanol soluble portion of aqueous extract concentrate of stems of *Pisonia grandis*.
- Five compounds have been characterised from the dewaxed ethanol extract concentrate of aerial parts of *Andrographis stenophylla*.
- The non-polar extracts of both plants are found to contain palmitic acid with a larger percentage of it found in extracts of *Pisonia grandis*. Oleic acid and phytol are the other major constituents of *Pisonia grandis*.

The bioactive molecules allantoin and pinitol identified to be present in the stem and roots of *Pisonia grandis* R.Br. are also significant constituents of its leaves also as revealed by a preliminary lead from our laboratory.

These bioactives could be isolated directly from the concentrated extract. Stem extract has a higher percentage of allantoin (5.3%) and pinitol (6%) compared to leaves and roots. This quantification is based on the direct isolation protocol adopted in the study without resorting to column chromatography. This has been ascertained by analytical quantification studies also.

Both allantoin and pinitol isolated from *Pisonia grandis* possess immense pharmacological potential as seen from the large number of scientific reports on their bio pharma potential. Hence extracts of *Pisonia grandis* of leaves, stems and roots of *Pisonia grandis* are of pharmacological significance owing to the presence of medicinally valuable allantoin and pinitol. A molecular entity comprising of a combination of allantoin and pinitol has also been isolated from the leaf, stem and roots of this plant in the present study. This revelation is of significance since such an entity has not been reported in literature though combination compounds of allantoin with various small molecules and of pinitol with small molecules have been earlier reported.

Allantoin and pinitol have proven anti diabetic potential however it was found that allantoin gets degraded in the gastrointestinal tract and may be lost after oral administration. A combination compound with allantoin and pinitol may be of much pharmacological significance especially in diabetes treatment. This is the first report of the isolation of such an addition complex of allantoin and pinitol. Ascertaining the bio potential of these two compounds in the combined form especially the anti diabetic
potential in future work may lend lead to a valuable anti diabetic herbal formualtion with the active extracts of *Pisonia grandis*.

The plant *Andrographis stenophylla* C.B Clarke is a medicinal plant of the *Acanthaceae* family. It is a rare and little known endemic species of India. Chemical investigation of polar extracts of this plant revealed the presence of flavonoidal constituents. *Andrographolide*, a terpenoidal lactone has been also isolated as a major constituent of these extracts. This molecule is a bio marker of the genus *Andrographis*. *Extracts of Andrographis stenophylla possessing this molecule are also are of pharmacological significance.*

In the present research work focus was on standardization of the chosen folkloric plants and their extracts to validate the herbal potential of the plant for use as a Phytomedicine.

- As part of the standardisation studies a survey was conducted on the folkloric use of the chosen plants. The survey led to the documentation of the folkloric use of the plants. It was found that the plants chosen for the present investigation have been extensively used by locals and tribals as anti-diabetic and anti-inflammatory agent. Mostly leaves of the chosen plants have been more prioritized among the people to treat various ailments.

- Morphological data of both plants has been documented in literature and this has mention in the present work since documentation of morphological data of medicinal plants is an essential part of the validation and this will help in selection of the correct species of the plant for formulation.

- Physicochemical characteristics of both plants have been recorded and the results reveal that both plants are suitable for use as an Ayurvedic medicine.

- Heavy metal analysis of leaves stem and roots of *Pisonia grandis* and aerial parts of *Andrographis stenophylla* revealed that the metal contaminations are within the WHO permissible limits and thus the extracts of the chosen medicinal plant parts are safe for use.

- Chemical fingerprinting of the plants was done by phytochemical analysis HPTLC, HPLC, NMR and GC-MS finger printing of extracts and quantification of bio actives.

- The NMR finger prints of the polar extracts of *Pisonia grandis* showed that the dewaxed ethanol extracts possess fewer constituents than its non polar pet-ether extracts. The NMR resonances expressed by the polar extracts correlate with the
active constituents isolated from the same by direct fractionation and by column chromatography and the NMR finger prints reveal these constituents as the major constituents.

- The chemical shift ranges exhibited by the extracts in $^1$H NMR and $^{13}$CNMR represent the metabolite finger prints of the polar extracts
- GC-MS analysis of pet-ether concentrates of leaves, stems and roots of *Pisonia grandis* (PGSP, PGRP, and PGLP) revealed that the most prevailing phytoconstituents are palmitic acid, oleic acid and phytol. The GC-MS chromatograms represent the fingerprints.
- The GC-MS analysis of pet-ether extract of *Andrographis stenophylla* (ASAP) revealed the presence of chondrillasterol, stigmasterol, palmitic acid, oleic acid, dotriacontane, nonadecane and n-pentadecanol. The GC-MS chromatograms represent the fingerprints.
- The bioactives isolated from both plants were quantified in the extracts by HPLC method. This led to a revelation on the relative percentage of the bioactives in the various extracts. The relevance of this study lies in the validation of the extracts for its bioactive content which is of principal significance in the development of any herbal formulation.
- Stem and roots of *Pisonia grandis* contain a higher percentage of pinitol whereas leaves and stems possess a higher percentage of allantoin.
- The ethanol extracts of *Andrographis stenophylla* (ASAE and dASAE) have a higher percentage of the biomarker molecule andrographolide ($32 \mu g$ and $39 \mu g$ per $100 \mu g$ of extract respectively) compared to the non-polar extract (ASAP)
- **Biological Standardization:** The various extract concentrate of the plants *Pisonia grandis* and *Andrographis stenophylla* were standardized by biological activity studies. The biological activity studies may be considered as biological fingerprints of the extracts of these plants.
- The efficacy of the isolated constituents also has been tested and compared with that of the extract concentrates of *Pisonia grandis* and *Andrographis stenophylla*.
- At a concentration of $50 \mu g/mL$ the dewaxed stem ethanol concentrate DPSE showed highest DPPH Radical Scavenging potential compared to the other extracts. At $90 \mu g/mL$ DPGSE showed 80% inhibition.
In the analysis of the anti angiogenic potential of the extracts it is worthy of mention that the leaf petroleum ether extract of *P.grandis* shows slightly higher percentage of blood vessel formation (50% increase) than the leaf ethanol extract (44%) whereas the dewaxed ethanol extract of leaves of *P.grandis* express an equivalent decrease (44%). It may be proposed that the non-polar constituents of the leaf petroleum ether extract of *P.grandis* might play a greater role in stimulating angiogenesis than the constituents of the dewaxed leaf ethanol extract; being identified to contain the bio actives allantoin and pinitol.

The biological standardisation study forms part of the validation of the herbal potential of the chosen plants.

**Conclusion**

*In the present research work, two folkloric medicinal plants have been investigated extensively for their chemical constituents. Medically valuable molecules have been identified and characterized from the non-polar and polar extracts of leaves, stem and roots of *Pisonia grandis* and aerial parts of *Andrographis stenophylla*. All feasible herbal standardisation strategies have been applied to make it a wholesome record of validation of the folkloric use of both the plants. This work may lend a major lead to the development of herbal formulations particularly anti-diabetic, wound healing and anti-arthritis formulations.*