Chapter 5

Summary and conclusions
5. SUMMARY & CONCLUSIONS

Medicinal plants are moving from fringe to mainstream use with a greater number of people seeking remedies and health approaches free from side effects caused by synthetic drugs. Recently, considerable attention has been paid to utilize eco-friendly and bio-friendly plant-based products for the prevention and cure of different human diseases. Considering the adverse effects of synthetic drugs, the Western population is looking for natural remedies which are safe and effective. It is documented that 80% of the world population has faith in traditional medicine, particularly plant drugs for their primary healthcare (Dubey et al., 2004).

The plant based indigenous knowledge was passed down from generation to generation in various parts of the world throughout its history and has significantly contributed to the development of different traditional systems of medicine. The use of plants as medicine has involved the isolation of active compounds, beginning with the isolation of morphine from opium in the early 19th century and subsequently led to the isolation of early drugs such as cocaine, codeine, digitoxin and quinine, of which some are still in use. Isolation and characterization of pharmacologically active compounds from medicinal plants is in practice till today.

More recently, drug discovery techniques have been applied to the standardization of herbal medicines, to elucidate analytical marker compounds. It is estimated that around 2,50,000 flowering plant species are reported to occur globally. Approximately half of these species are found in the tropical forests. They continue to provide natural products to chemists with invaluable compounds for development of new drugs. The potential for finding new compounds is enormous as till date only about 1% of tropical species have been studied for their pharmaceutical potential. The
success of drug discovery from plants resulted principally in the development of anti-
inflammatory agents.

Plants and their parts are used as crude drugs in many medicinal preparations from centuries. Proper identification of drugs and their evaluation on a scientific basis is therefore of prime significance. The present investigation is on the three ethno-
pharmacologically important plants *Anisomeles malabarica* R.Br., *Clerodendrum serratum* L. and *Atalantia monophylla* DC for antioxidant, anti-inflammatory and immunomodulatory activities has lead us to explore these plants thoroughly to get novel lead molecules for various inflammatory conditions.

In our investigation, an attempt has been made to outline the most important aspects of the empirical approach to find new lead compounds from these plants. The different parts of the three ethno-pharmacologically important plants *Anisomeles malabarica* R.Br., *Clerodendrum serratum* L. and *Atalantia monophylla* DC. have been studied for their phytochemical properties, antioxidant potentiality, immunomodulatory and anti-inflammatory activities.

Initially the aerial parts of the plants *Anisomeles malabarica* R.Br. and *Clerodendrum serratum* L. and leaves of *Atalantia monophylla* DC were subjected to preliminary phytochemical analysis using solvents like petroleum ether, chloroform, ethyl acetate, methanol, ethanol and water.

The aerial parts of *Anisomeles malabarica* showed the presence of carbohydrates and alkaloids in all the extracts except ethyl acetate. Sterols were present in petroleum ether and ethanol; tannins in petroleum ether and aqueous extracts; saponins in ethanol and aqueous extracts; di and triterpenes in ethyl acetate, ethanol and aqueous extracts; glycosides were present only in ethyl acetate extract;
whereas flavanoids were present in all the extracts. Phenols, oils and fats and proteins and amino acids were totally absent in all the extracts.

The aerial parts of Clerodendrum serratum showed the presence of carbohydrates in all the extracts except ethyl acetate and alkaloids except petroleum ether and ethyl acetate. Steroids were present in ethanol extract only, phenols in chloroform and ethanol only and diterpenes in aqueous extract only. Tannins were present in petroleum ether and aqueous and glycosides in ethyl acetate, ethanol and aqueous extracts. Flavanoids were present in all the extracts except petroleum ether and ethanol. Proteins, saponins and oils and fats were totally absent in all the extracts tested.

The petroleum ether extract of leaves of Atalantia monophylla showed the presence of tannins, oils and fats, proteins and amino acids and triterpenes. The chloroform extract of leaves of Atalantia monophylla showed the presence of carbohydrates and phenols only. The ethyl acetate extract exhibited the presence of carbohydrates, phenols, proteins and amino acids, triterpenes and flavanoids. The methanolic extract confirmed the presence of carbohydrates, alkaloids, steroids, phenols, oils and fats, diterpenes and glycosides, whereas the aqueous extract showed the presence of carbohydrates, alkaloids, steroids, tannins, phenols, saponins and triterpenes.

Further analysis of various parts of the plants (Anisomeles malabarica and Clerodendrum serratum) was carried out by using methanol.

The methanolic extracts of leaves of A.malabarica showed the presence of carbohydrates, phenolics and tannins. The extracts of roots of A.malabarica showed the presence of carbohydrates, saponins, flavanoids, phytosterols and triterpenoids.
The extracts of aerial parts *Anisomeles malabarica* showed the presence of carbohydrates, phytosterols and triterpenoids. The extracts of roots of *C.serratum* showed the presence of glycosides, alkaloids, phenolics, tannins, phytosterols and triterpenoids. The extracts of aerial parts of *C.serratum* showed the presence of glycosides, alkaloids, flavanoids. The extracts of leaves of *Atalantia monophylla* showed the presence of carbohydrates, alkaloids, phenols and tannins.

The maximum DPPH radical scavenging activity was exhibited by CsA and CsR with a very high IC$_{50}$ value, followed by moderate to good activity by the AmA and AmL extracts. AmR showed poor antioxidant activity and AmoL exhibited the least antioxidant activity when compared to all the extracts tested. Except for CsA all the remaining five extracts exhibited poor nitric oxide (NO) radical scavenging activity when compared with the standard. These findings establish the antioxidant potential of the various parts of these plants.

The in vitro anti-inflammatory activity was undertaken to study the anti-rheumatic and immunomodulatory role of aerial parts, leaves and roots in lipopolysaccharide (LPS) mediated signaling in mouse macrophage and mouse connective tissue cell lines. Among the six extracts tested, AmA and CsR showed better activity with by reducing the LPS induced TNF-α production when compared to the standard drug Dexamethasone. Taken together, these findings from the present in-vitro studies suggest the anti-rheumatic and immunomodulatory properties of the extracts of *A.malabarica* and *C.serratum* when compared to the standard immunomodulator.

On the basis of the results obtained from the in vitro anti-inflammatory activities, the best among the six plant extracts were subjected to anti-inflammatory
activity using the carrageenan-induced paw oedema in rats. The results suggested that the extracts of aerial parts of *A. malabarica* (AmA) and roots of *C. serratum* (CsR) possess potent anti-inflammatory activities by reducing the inflammation in the hind paw of rats. These activities were related to the dose and these results corroborate the potential traditional use of these plants in folk medicine. Further investigations were carried to isolate and characterize the active components from these two plant parts.

Isolation and characterization of phytoconstituents from the extract of aerial parts of *Anisomeles malabarica* R.Br. (AmA) and roots of *Clerodendrum serratum* L. (CsR) was carried out by subjecting the plant extracts to $^1$H-NMR, $^{13}$C-NMR, IR and LC-ESI-MS spectral studies. Data obtained from the above spectral studies revealed the presence of Pentadecanoic acid ethyl ester from the extracts of aerial parts of *Anisomeles malabarica* and $\beta$-sitesterol from the extracts of roots of *Clerodendrum serratum*. The identity of these two pure compounds was confirmed from the previously published literature.

The two pure isolated compounds namely Pentadecanoic acid ethyl ester and $\beta$-sitesterol from the two plants were further subjected to *in vitro* anti-inflammatory and immunomodulatory studies using RAW-32 and L-929 cell lines. Of these two pure compounds, $\beta$-sitosterol exhibited potent anti-inflammatory activity with by reducing the LPS induced TNF-$\alpha$ production when compared to the standard drug dexamethasone, whereas, pentadecanoic acid ethyl ester exhibited moderate TNF-$\alpha$ modulatory effect.

Thus, the present findings suggest that the plants *Anisomeles malabarica* R.Br. and *Clerodendrum serratum* L. could be exploited for the therapeutic management of various inflammatory conditions in human beings including rheumatism.