CHAPTER 10

SUMMARY AND CONCLUSION

The thesis entitled Pharmacognostical, Phytochemical and Pharmacological evaluation of the leaves of *Symplocos cochinchinensis* (Lour.) S.Moore ssp. *laurina* (Retz.) Nooteb. (*Symplocaceae*) deals with pharmacognostical, phytochemical and pharmacological investigation of traditionally used medicinal plant Kamblivettai (in Tamil). A perusal of the literature revealed that only fragmentary information was available on this plant species regarding pharmacognosy, phytochemistry and pharmacological activity by any other researchers. This study was designed first time for the identification, screening of pharmacological activities and isolation of unexplored compound in order to establish its folklore claims.

Plants are becoming potential source for phytoconstituents with varied pharmacological activities. Identification of such plants of potential use in medicine is of significance and as a prelude to this, it becomes necessary to examine the various pharmacognostical characters of the plant before further investigation.

In pharmacognostical studies the macroscopy, microscopy, histochemical studies, physico-chemical constants and inorganic mineral analysis were carried out. Pharmacognostical standards obtained during the observation are valuable tools for the identification of the plant material.
Morphological study had provided a characteristic identity of leaf which was dark green in colour, sweet taste and tea like odour are the diagnostic feature of the leaves.

The various distinguishing features of the plant observed through anatomical studies were

i) Transverse section of the leaves showed a fairly prominent midrib, lateral veins and dorsiventral lamina. The vascular strand is omega shaped with an abaxial arc and two lateral out curved wings.

ii) The lamina has smooth and even surface with two layers of palisade cells and the marginal part of the lamina is curved down and bluntly conical.

iii) The petiole consists of a thin layer of epidermis which consists of small, thick walled squarish cells, homogenous ground tissue and a deep bowl shaped vascular strand with two lateral smaller accessory strands.

iv) The stem is circular and has thin continuous epidermal layer, a wide cortex with homogenous layers of parenchyma cells and vascular cylinder consists of a thin, discontinuous layer of sclerenchyma abutting in phloem and a wider zone of xylem elements.

v) Bark has narrow periderm, fairly wide cortex and wider granular secondary phloem. Secondary phloem is wider part of the bark. It is differentiated into outer zone of wide collapsed phloem (crushed phloem) and inner zone of non collapsed or intact phloem.

The microscopical examination of the powder showed numerous uniseriate multicellular covering trichomes (1-3 celled), which are slightly
curved, paracytic stomata made up of rectangular or polygonal epidermal cells.

Histochemistry is mainly used to localize the chemical compounds within the cells and tissues using some chemical reagents have been done and it showed the presence of lignin, proteins, strach, mucilages, fatty acids and flavanoids.

Various physico-chemical parameters such as ash values, extractive values, loss on drying, crude fibre content and foaming index were found to substantiate its standard values. Any significant deviation in the percentage of any parameters reported in this work may indicate adulteration or substitution in the drug. Presence of calcium and iron added up its nutritional value, fluorescence analysis is also a part of diagnostic tool for the presence of chromophore in the particular species.

The pharmacognostical details evolved from the present study would help to fix up the standards for *Symplocos cochinchinensis* (Lour.) in relation to its identification, authentication and differentiation from other related species and adultrants. This is first report on the pharmacognostical standardisation on the leaf of *Symplocos cochinchinensis* (Lour.).

In Phytochemical evaluation various extracts were prepared and studied for qualitative chemical analysis, TLC and HPTLC finger print analysis.

The qualitative preliminary phytochemical analysis was performed to detect the nature of the phyto-constituent and their presence in powder and various extracts. Hexane and chloroform extracts showed the presence of steroids. Ethyl acetate extract was found to contain flavanoids, glycosides, proteins, saponins, alkaloids and carbohydrates. Methanolic extract showed
the presence of carbohydrates, flavanoids, phenols, saponins, proteins, glycosides and alkaloids.

Qualitative chromatographic analysis (TLC) was performed for the identification of different components in the extracts qualitatively.

The HPTLC finger print of various extracts were also studied. HPTLC was scanned at 280 nm with the best solvent to detect the maximum number of components and peak abundance qualitatively. HPTLC fingerprint is one of the versatile tool for qualitative and quantitative analysis of active constituents. It is also a diagnostic method to find out the adulterants and to check the purity.

The therapeutically active extract to carry out the pharmacological studies in animals was selected on the basis of in-vitro studies like in-vitro cytotoxic study against human cancer cell lines, anti-inflammatory activity by HRBC membrane stabilization method and anti-snake venom activity by prevention of HRBC membrane lysis method. Among the four extract the methanolic extract showed significant activity. It was selected for the screening of in-vivo pharmacological studies.

The chapter pharmacological activity dealt with the screening of acute toxicity study, hepatoprotective, anti-inflammatory, anti-snake venom and anti-cancer activities of methanolic extract of the leaves of *Symlocos cochinchinensis* (Lour.).

The methanolic extract was found to be very safe up to 2000mg/kg body weight by acute toxicity model study as per the OECD guidelines 423.
The methanolic extract showed promising hepatoprotective, anti-inflammatory, anti-snake venom and anti-cancer activities in a dose dependent manner.

The various pharmacological activities established by the test extract can be investigated further in future to get their meaningful extension in clinical use. The therapeutical activities were carried out scientifically and reported for the first time in this plant.

The most effective methanolic extract was selected for column chromatographic separation in an attempt to isolate the compounds responsible for the therapeutic activity. Three compounds were isolated from the extract and the structure of the compounds were confirmed by IR, $^1$H NMR, $^{13}$C NMR and Mass spectral data. The compunds were found to be Glucuronic acid, Rhamnetin-3-O-β-D-Galactosyl-4- O-β-D-galactopyranoside and oleanolic acid derivative (3-O-[α-l-Arabinopyranosyl-(1,4)-O-β-D-glucuronopyranosyl]) -31-O-(β-D-glucopyranosyl) oleanolic acid).

The oleanolic acid derivative was screened for the in-vivo anti-cancer activity using DAL cancer cell lines and it showed significant anti-cancer activity.

From the above mentioned studies it can be concluded that the pharmacognostical standards generated will be useful for the proper identification of a plant. These kinds of microstructures have been recognized as tools to measure the phylogenetic relationship under light microscope to resolve the taxonomic controversies. The study also helps to establish the botanical identity for this plant that could be made use of, those who deal with the species and also in the quality assurance of the plant species. With the support of phytochemical studies and in-vitro pharmacological activities the methanolic extract was selected and subjected to in-vivo pharmacological
studies. The methanolic extract at the dose level of 400mg/kg showed significant hepatoprotective, anti-snake venom, anti-inflammatory and anti-cancer activities. The compound III oleanolic acid derivative isolated from the methanolic extract also showed promising anti-cancer activity by in-vivo model.

Further studies were focused on structural activity relationship of phytoconstituents isolated from the methanolic extract. This scientific study revealed the efficacy of the drug and it would definitely have wide scope in future. Hence, the leaf can be recommended therapeutically for the investigated medicinal claims. These observations will stimulate further research in the field of phytochemistry and also in the clinical application of phytochemical constituents of *Symlocos cochinchinensis* (Lour.) S.Moore ssp. *laurina* (Retz.) Nooteb.