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Chapter 1

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Chapter 1

INTRODUCTION

1.1. Context of the study

Plants have been used as drugs for centuries and the accumulated traditional knowledge over the years lead to the discovery of many novel drugs derived from plant based biologically active principles. Even today, vast majority of people in this world rely on indigenous medicinal plants and plant derived products for their every day health care needs. As Cunningham (1988) rightly pointed out a large proportion of the population in developing countries uses traditional medicines, either as a result of the high cost of western pharmaceuticals and health care or because the traditional medicines are more acceptable from cultural and spiritual perspective. According to the reports of WHO, 70% - 95% of citizens in majority of the developing countries rely on traditional medicine as their primary source of medication (Robinson and Zhang, 2011). Nowadays, herbal drugs represent as a major component not only in traditional systems of medicine and health care but also in nutraceuticals and cosmetics industry. As reported by Gurib-Fakim (2006), one quarter of the medical prescriptions are formulations based on substances derived from plants or plant-derived synthetic analogs. Above all, it is believed that drugs derived from medicinal plants have added advantage of being simple, effective and offering a broad spectrum of activity with an emphasis on the preventive action (Mills and Kerry, 2000; Gupta, 2003). There are more than 120 distinct chemical substances derived from plants that are considered as important drugs currently in use in one or more countries in the world. Many of these drugs were either copies of the naturally obtained substances or prepared by processing of the actual plant materials or synthesized by simple synthetic modifications.

In the present global scenario, natural medicines are gaining prominence, since they are economical, easily available and relatively free from side effects. In most of the developing countries, traditional medical practices are regarded as an integral part of
their culture. As the world population is increasing, three fourth of the population cannot afford the modern synthetic drugs and they have to depend upon the use of traditional medicines mainly derived from plants. WHO has emphasized the importance of better utilization of the indigenous system of medicine, ‘save plants to save lives’ was the call given by WHO a few years ago to stress the role of medicinal plants in achieving the goal of ‘health for all’ (Singh and Govil, 2009). The renewed interest in herbal medicines is likely to continue globally because of increasing population, better affordability and paradigm shift in attitude towards alternative or complementary medicines (Madge, 1987; Sacks, 1992). However, traditional system of medicine cannot remain confined to the use of conventional conservative norms of medication. It has to accept the new challenges and be prepared to answer the queries of the modern man who would have a right to know how the drug administered to him are collected, processed, preserved and used. To meet this new thrust of inquisitiveness, standardization of drugs in traditional systems of medicine is mandatory. Hence, with ever growing commercialization of herbal drugs, there is an urgent need to develop appropriate standards for quality control of the raw materials and plant based products. Moreover, the present trend of commercialization in the preparation and marketing of herbal medicines has necessitated the need to develop appropriate methods and standards to ensure quality products and proof of their efficacy for the universal acceptance of the plant based drugs.

Many countries are registering their standardized herbal drugs with proven clinical efficacy & safety and as dietary supplements. But India is unable to exploit the world market due to unsatisfactory system of quality control which necessitates the establishment of quality standards for herbal single drugs and compound formulations. The medicinal plants conventionally used in many Ayurvedic formulations have not been subjected for detailed studies to screen their bioactive principles. Most of the studies so far reported are based on the use of plant extracts which do not seem to provide adequate and accurate analyses with respect to the bioactive compounds responsible for the observed effects. As pointed out by Taylor et al. (2001) and Holmstedt and Bruhn (1995), it is imperative that the identification of biologically
active principles in such cases needs to be interpreted in the light of their traditional uses and indigenous drug preparations. Hence, prioritizing research to develop acceptable quality standards for the herbal medicines ensuring their consistency, safety and efficacy with good manufacturing practices is the need of the hour. The present study was an attempt in the above context and it aims to develop pharmacognostic and phytochemical standards for the medicinal plant, *Premna serratifolia* L. in Kerala.

### 1.2. Need and Significance of the study

*Premna serratifolia* L. is an important medicinal plant belonging to the family Verbenaceae. This medicinal plant has great demand in pharmaceutical industry, since it is an essential component of a large number of *Ayurvedic* preparations like *Arishtam, Avaleham, Kwatham, Ghritham* and *Thailam*. The medicinal properties of *Premna serratifolia* have been reported by many Indian researchers (Chopra *et al*., 1956; Chopra, 1969; Nadkarni, 1976; Rathore *et al*., 1977; Kartick, 1984; Rastogi and Mehrotra, 1991; Warrier *et al*., 1994; Lalithamma, 1996; Yoganarasimhan, 2000; George, 2006). A preliminary survey conducted by George (2006), revealed that the roots of different morphotypes of *Premna serratifolia* are used as the source drug ‘Agnimantha’ in different parts of Kerala.

The taxonomic status of *Premna serratifolia* L., belonging to the family Verbenaceae, has been a topic of heated discussion among taxonomists from the early period of Linnaeus due to extremely polymorphic features exhibited by this species reported from different geographic regions of the world. The nomenclature and identity of this widespread and very polymorphic species has been subjected for much heated discussions and negotiations among taxonomists (Merrill, 1917; Fletcher, 1936; Meeuse, 1942; Fosberg, 1953). Many authors have given different names viz., *Premna integrifolia* L., *Premna serratifolia* L., *Premna corymbosa* (Burm.f.) Merr., *Cornutia corymbosa* Burm.f., for this species complex. According to Beer & Lam (1936), the genus *Premna* is considered as an “extremely difficult genus in which flower features are almost as vague and little distinct as those of the extremely variable leaves”. As Bentham (1870) rightly pointed out “there are a number of forms including *Premna*
integrifolia and Premna serratifolia of Linnaeus which seem to pass into each other by numerous intermediates, and it would require a much more detailed study of good specimens from different localities…” Linnaeus (1771) considering the complexity of the Ceylon material being a mixture of more than one taxon, divided this into two species viz., *Premna integrifolia* and *Premna serratifolia* based on the morphology of their leaf margin being entire and serrate respectively. The above situation necessitates detailed studies based on different morphotypes of *Premna serratifolia* in Kerala.

From the *Ayurvedic* point of view, there is considerable disagreement among the commentators of modern *Nighantus* regarding the identity of *Premna serratifolia* as a genuine drug. In the *Ayurvedic* system, it is treated under ‘controversial drug plants’ of India. In this context, it is a real challenge to resolve the ‘controversial drug status’ of this medicinal plant species since the real species to be used as a specific drug could be singled out and the controversy should be resolved by laying down ‘pharmacopoeial standards’ for its proper identification and authentication (Dash and Kashyap, 1980; Dhar *et al*., 2002; API, 2011). A thorough scientific approach based on the systematic identification of the single drugs of controversial nature from botanical, pharmacognostical, chemical and pharmacological point of view may be an answer to solve the prevailing controversies and difficulties regarding the identity of *Premna serratifolia* in Kerala. There are mainly four aspects for ensuring, quality control and standardization of plant drugs (Dhar *et al*., 2002; Roy and Chourasia, 2002). These include: confirmation of botanical identity of raw materials, pharmacognostical examination of raw material, phytochemical analysis of raw materials and biological assay of raw materials. Hence, in the present study we aim to resolve the ambiguity of *Premna serratifolia* by subjecting the raw drugs of its six morphotypes for the above mentioned quality control and standardization processes.

In the Indian traditional *Ayurvedic* system of medicine, the drug plant *Agnimantha* (Sanskrit name) is described as an essential constituent of *Dashamoolam*. Two different species viz., *Premna serratifolia* L. and *Clerodendrum phlomidis* L.f. (*Clerodendron phlomidis*) are equated to the source plant *Agnimantha*, providing a controversial drug
status to these two medicinal plants. Consequently, Clerodendrum phlomidis is widely used as the source drug of Aganimtha in most of the North Indian states. However, Premna serratifolia L. is used instead of Clerodendrum phlomidis as the genuine drug of Aganimtha throughout Kerala and in neighboring South Indian states for the preparation of Ayurvedic formulations. In Kerala, various morphotypes of Premna serratifolia are used by traditional practitioners attributing differential therapeutic properties to these morphotypes and Clerodendrum species viz., Clerodendrum inerme is rarely used as a substitute drug of Clerodendrum phlomidis. The present study gains significance in the light of above mentioned aspects. A perusal of literature revealed that not many studies were done on the pharmacognostic and phytochemical aspects of the different morphotypes of Premna serratifolia L. in Kerala. George (2006), in his preliminary study entitled ‘Ethnobotanical, Phytochemical and Pharmacognostic studies on Premna serratifolia L.’ reported many indigenous uses of this medicinal plant and stressed the importance of further studies based on different morphotypes of Premna serratifolia L. in Kerala. The present research work is an extension of his previous work and it aims to develop quality standards with special reference to six morphotypes of Premna serratifolia L. in Kerala. An earnest attempt is made in the present investigation to resolve the ambiguity regarding the taxonomic as well as Ayurvedic status of Premna serratifolia L. using the most reliable and sophisticated tools and techniques in biological sciences. The study is entitled: “PHARMACOGNOSTIC AND PHYTOCHEMICAL STUDIES ON PREMNA SERRATIFOLIA L. IN KERALA.”

1.3. Hypothesis of the study

Present study was designed to test the following hypothesis:

- The ambiguity regarding the Taxonomic and Ayurvedic status of Premna serratifolia L. can be resolved by subjecting the different morphotypes of this species for detailed pharmacognostic and phytochemical investigations.

- The different morphotypes of P. serratifolia L., in spite of distinct morphological variations, may have certain unifying features in terms of
anatomical/ pharmacognostic and phytochemical characteristics showing their origin from a common ancestor.

**1.4. Objectives of the study**

The major objectives of the present study are:

1. To compare the morphological and anatomical characters of the morphotypes of *Premna serratifolia* L. in Kerala.
2. To develop pharmacognostic standards for the morphotypes of *P. serratifolia* L.
3. To identify major classes of secondary metabolites in the morphotypes of *P. serratifolia* L.
4. To develop a common chemical marker for the roots of different morphotypes of *P. serratifolia* L. in Kerala.
5. To detect and screen selected bioactive compounds in different morphotypes of *P. serratifolia* L.
6. To study the antioxidant property of the root extract with special reference to pure compound isolated from the root wood of *P. serratifolia* L.

**1.5. Materials and Methods in brief**

Six morphotypes of *Premna serratifolia* L. (M1, M2, M3, M4, M5, and M6) were collected from different locations of Kerala state, authenticated and acclimatized in the experimental garden at Kottayam. The roots/leaves of the acclimatized morphotypes alone were used for further pharmacognostic and phytochemical analysis. The six morphotypes were subjected to morphological, anatomical and standard pharmacognostic evaluations including studies on micro foliar features, physical evaluation, fluorescence analysis and heavy metal analysis. The plant morphotypes were then subjected to phytochemical analysis viz., qualitative detection, HPTLC screening, chemical marker development, detection and characterization of bioactive compounds and screening of volatile fractions in roots and leaves using GC-MS. The antioxidant property of root wood extract with reference to pure compound isolated
from the reference sample was also carried out. The phytochemical studies were done with the aid of chromatographic techniques such as HPTLC, HPLC, LC-MS, GC-MS and Column chromatography followed by spectroscopic methods such as UV, FTIR and NMR.

1.6. Scope of the study

A thorough understanding of the pharmacognostic, phytochemical properties of the different morphotypes of *Premna serratifolia* in Kerala would be a rewarding endeavor to bring forth its morphe-anatomical & phytochemical diversity, biological significance and chemical affinity with respect to major classes of secondary metabolites. The pharmacognostic and phytochemical standards developed with respect to six morphotypes of *Premna serratifolia* can be used as reference standards for ensuring the quality standards of the raw drugs as well as finished products with *Premna serratifolia* as a constituent. The study thus provides a means to identify the genuine drug from its adulterant or substitute species. Further, the study will provide a baseline data to identify useful bioactive compounds which in turn helps to conduct further studies on bio-activity based research. It is expected that the study provides a means for bioprospecting of the species *Premna serratifolia* L. in Kerala with special reference to its anti-arthritic, anti-inflammatory, antimicrobial, anti-diabetic, anti-obesity, cardio-protective, gastro-protective, hepato-protective and antioxidant activities. The study also highlights the importance of stringent measures to conserve the different morphotypes of *Premna serratifolia* in Kerala.

1.7. Format of the report

The report is presented in 5 chapters and the details are given as follows:

**Chapter 1: Introduction**

The introductory chapter as noted above contains all appropriate sections of an introductory chapter, such as a brief account of context of the study, need and significance of the present study, objectives of the study, hypothesis of the study, a
short note on the materials and methods used for the study, scope of the study and format of the report.

**Chapter 2: Review of Literature**

This chapter deals with the major conclusions that have emerged from the review and appraisal of the literature pertaining to taxonomic history of the genus *Premna* and species *Premna serratifolia*, medicinal potential and controversial *Ayurvedic* status of *Premna serratifolia* followed by an in-depth review on recent studies with special reference to pharmacognostic, phytochemical and pharmacological activities of this medicinal plant.

**Chapter 3: Materials and Methods**

This chapter deals with detailed description of materials and methods employed in pharmacognostic and phytochemical studies. The schematic representations of the experiments are also included under this title.

**Chapter 4: Results and Discussion**

This chapter gives major results which emerged from the pharmacognostic and phytochemical studies on six morphotypes of *Premna serratifolia* in Kerala. The results emerged from morpho-taxonomical, anatomical, organoleptic & fluorescence analysis, evaluation of physical constants, extractive values and heavy metal analysis are discussed under pharmacognostic studies. Preliminary detection of phytochemicals by qualitative analysis, screening of major secondary metabolites by HPTLC, identification, separation and characterization of marker compound, selected alkaloids, novel cardiac glycoside and analysis of the volatile fractions from the roots and leaves of six morphotypes of *Premna serratifolia* are dealt under phytochemical studies. The evolutionary relationship among the six morphotypes of *Premna serratifolia* with respect to major secondary metabolites (essential oils, flavonoids, phenolic compounds, cardiac glycosides, saponins and iridoid glycosides) are also discussed. The antioxidant
property of the root wood extract with special reference to the active antioxidant principle, acteoside, is also discussed in this chapter.

**Chapter 5: Summary and Conclusion**

This chapter summarizes the present study in retrospect. The major findings, scope and implications of the study are also discussed in this chapter.