REVIEW OF LITERATURE
Chapter 2

REVIEW OF LITERATURE

2. INTRODUCTION

2.1. GENUS PREMNA: AN OVERVIEW

2.1.1. TAXONOMIC HISTORY OF THE GENUS PREMNA

2.1.1.1. INDIAN HISTORY OF THE GENUS PREMNA

2.1.1.2. PERPLEXITY IN CLASSIFICATION OF THE GENUS PREMNA

2.1.2. DISTRIBUTION OF THE GENUS PREMNA

2.2. PREMNA SERRATIFOLIA: AN OVERVIEW

2.2.1. TAXONOMIC HISTORY OF PREMNA SERRATIFOLIA

2.2.2. DISTRIBUTION OF PREMNA SERRATIFOLIA

2.2.3. POLYMORPHIC STATUS OF PREMNA SERRATIFOLIA

2.3. PREMNA SERRATIFOLIA: A POTENTIAL MEDICINAL PLANT

2.3.1. TRADITIONAL MEDICINAL USES OF PREMNA SERRATIFOLIA

2.3.2. PREMNA SERRATIFOLIA IN AYURVEDIC FORMULATIONS

2.3.3. DESCRIPTION OF PREMNA SERRATIFOLIA UNDER SANSKRIT NAMES IN TRADITIONAL MANUSCRIPTS

2.3.4. CONTROVERSIAL STATUS OF PREMNA SERRATIFOLIA IN AYURVEDA

2.4. PHARMACOGNOSTIC, PHYTOCHEMICAL AND PHARMACOLOGICAL STUDIES ON PREMNA SERRATIFOLIA

2.4.1. PHARMACOGNOSTIC STUDIES ON PREMNA SERRATIFOLIA

2.4.2. PHYTOCHEMICAL STUDIES ON PREMNA SERRATIFOLIA

2.4.3. PHARMACOLOGICAL STUDIES ON PREMNA SERRATIFOLIA

2.5. CONCLUSION
Chapter 2

REVIEW OF LITERATURE

2. Introduction

The taxonomic status of Premna serratifolia L. 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. In the Indian traditional Ayurvedic system of medicine, the plant Agnimantha (Sanskrit name) is described as a highly valuable ingredient 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, for the preparation of Ayurvedic formulations having Agnimantha as ingredient, Clerodendrum phlomidis is used as the source drug in North India and Premna serratifolia is used in South Indian States. However, in Kerala, various morphotypes of Premna serratifolia are used as the source drug and Clerodendrum species viz., Clerodendrum inerme is used as a substitute drug of Clerodendrum phlomidis. Hence, an attempt is made in the present investigation to resolve the ambiguity regarding the taxonomic as well as Ayurvedic status of Premna serratifolia using the most reliable and sophisticated tools and techniques in pharmacognosy and phytochemistry. In the above context, a brief review of the available literature on the genus Premna and the species Premna serratifolia are presented under appropriate heads.

2.1. Genus Premna: An Overview

An overview of the genus Premna with special reference to its taxonomic history, classification and distribution in the tropical and subtropical regions is presented in this section. The genus, from its first report in 1771, was under the confused category due to its indistinguishable taxonomic characters and diverse morphological features. Presently,
it is one of the largest genera of the family Verbenaceae with two hundred species worldwide which are mainly distributed in tropical and subtropical Asia, Africa, Australia and the Pacific Islands (Harley et al., 2004). As this research work is concerned with Premna serratifolia (type species of the genus Premna), it is relevant to have a bird’s eye view of the genus and its taxonomic history.

2.1.1. Taxonomic History of the Genus Premna

The generic name Premna was derived from the Greek word ‘Premnon’ meaning ‘tree stump’, referring to the short and twisted tree trunks of the type species. Linnaeus (1771) described the genus Premna with two species, viz., Premna serratifolia and Premna integrifolia, the types of which were collected by Paul Hermann from Ceylon and kept in ‘Didynamia Angiosperma.’ This treatment was followed by Murray (1774), Gmelin (1791), Willdenow (1800), Sprengel (1825), Roxburgh (1832), Blanco (1837) and Dietrich (1843). Scopoli (1777) placed the genus in ‘Personatae’, which was later recorded in ‘Centuria Quarta’ by Gaertner (1788), in ‘Vitices’ by Jussieu (1789), in ‘Plasyrgophyta’ by Necker (1790) and under the tribe ‘Verbeneae’ of Labiatae by Reichenbach (1828). In the year 1806, Jussieu referred it to the family, ‘Verbenaceae’ where it has been retained by majority of the botanists (Munir, 1984).

Dumortier (1829) was the first author to assign the genus Premna to a tribe, Viticeae. In 1830, Bartling split Verbenaceae into two sections viz., Viticea and Verbenea, and the genus Premna was kept in Viticea. Endlicher (1841) divided the family into three tribes: Lippieae, Lantaneae and Aegiphileae and the genus Premna was included in the tribe Lantaneae. In 1847, Schauer classified the family Verbenaceae into three tribes: Verbeneae, Viticeae and Avicennieae, with the genus Premna in the tribe Viticeae. The genus Premna was retained in this new tribe by majority of botanists such as Bentham (1870), Bentham & Hooker (1876), Hooker (1885), King and Gamble (1909) and Fletcher (1938). Schauer (1847) subdivided the tribe Viticeae into three subtribes: Symphoremeae, Caryopterideae and Viticeae, with Premna in the subtribe,
Viticeae. He further split the genus *Premna* into two sections, viz., *Guimira* and *Premnos*, based chiefly on their calyx being regularly 4 or 5 toothed and also based on the nature of corolla. Miquel (1858) and Bentham (1876) accepted the above classification proposed by Schauer (Munir, 1984; Rajendran and Daniel, 2002).

Briquet (1895) while reclassifying Verbenaceae elevated the tribe Viticeae to a sub-family Viticoidea with four tribes viz., Callicarpeae, Tectoneae, Vitaceae and Clerodendreae and kept *Premna* in the tribe Viticeae. Authors namely, Dalla Torre and Harms (1904), Lam (1919), Gardner (1931), Junell (1934), Moldenke (1959, 1971) and Melchior (1964) followed the above classification of Briquet. Briquet (1895) in his classification subdivided the genus *Premna* into five sections viz., *Holopremna, Odontopremna, Gumira, Premnos* and *Holochiloma*, chiefly based on the size and number of calyx lobes. The above division of the genus *Premna* was accepted by Dalla Torre and Harms (1904). However, most of the later taxonomists did not recognize any intrageneric divisions in *Premna* (Rajendran and Daniel, 2002).

2.1.1.1. Indian History of the Genus *Premna*

A brief history of the genus *Premna* is given in the taxonomic revision of the Indian Verbenaceae by Rajendran and Daniel (2002). The first report of the genus *Premna* (Linnaeus, 1771) was based on the material (*Premna serratifolia*) collected by König from peninsular India (Nicolson *et al*., 1988). In the beginning of 18th century; Wildenow (1800) described *Premna tomentosa* based on Klein’s collection from India. Rottler (1803) described *Premna corymbosa* based on his collection at Tempakkam near Madras. He also specified about *Premna serratifolia* in his work. Jussieu (1806) described a species, *Premna flavescens* from Madras, which is now treated as a synonym of *Premna tomentosa*. Roth (1821), reported *Premna mollissima*, which is now treated as a variety under *Premna latifolia* Roxb. Roxburgh (1832) described 11 species of *Premna* from India. Graham (1839) reported four species viz., *Premna cordifolia, Premna integrifolia, Premna scandens* and *Premna nimmoniana* from
western India. Walpers (1845) described 11 species of *Premna* from India in his monograph. In another study, Schauer (1847) included 25 species from India of which 9 were new and they were based on Wallich’s numerical list. From peninsular India, Wight (1849) reported *Premna glaberrima* as a new species. Clarke (1885) described *Premna bengalensis, Premna coriacea, Premna khasiana, Premna milleflora* and *Premna villosa* as new species. Parkinson (1922) reported *Premna integrifolia* and *Premna divaricata* from the Andaman Islands. In another study, Rao (1986) reported the occurrence of *Premna coriacea, Premna parasitica, Premna pubescens* and *Premna pyramidatus* in Andaman Islands. Deshpande (1961) reported a new species, *Premna resinosa* to the Indian flora. According to Santapau and Henry (1973), there are 25 species of *Premna* in India. However, according to Moldenke (1980), there are 31 species and 11 varieties of *Premna* in India. In the taxonomic revision of Indian Verbenaceae, Rajendran and Daniel (2002), recognized 31 species and 6 varieties of *Premna*. Of these three species, viz., *Premna balakrishnanii, Premna debiana* and *Premna mundanthuraiensis* are new species reported from India. Recently, Prabhu Kumar *et al.* (2013) reported the discovery of a new species *Premna rajendranii* from Western Ghats (Chinnar and Madukkarai) of Kerala. Apart from this, a research team comprising Robi, Augustin, Sasidharan and Udayan (2013) rediscovered an endemic and rare species of *Premna* viz., *Premna paucinervis* (C. B. Clarke) Gamble from the Vagamon hills along South Western ghats of Kerala after a lapse of 140 years of its original type collection by R.H. Beddome (1872) from Anamalayas, Western Ghats (Tamilnadu).

2.1.1.2. Perplexity in Classification of the Genus *Premna*

Perplexity in taxonomic classification usually occurs due to the usage of vague characters as distinguishing features. In taxonomic history of *Premna*, such a confusing situation occurred by the separation and consequent merging of genus *Pygmaeopremna* with genus *Premna*. In 1910, Merrill described a new genus *Pygmaeopremna*, based on two collections from Luzon, Philippines. The type species was named *Premna humilis*
Merr. and the genus was related to *Premna* and *Vitex*, and have shown more resemblance to the genus *Premna*. The genus *Pygmaeopremna* was distinguished from *Premna* only by its very small size. Lam (1919) recognized *Pygmaeopremna* as a valid genus, but at the end of his generic description noted: “without regard to characteristics of less consequence, the genus differs from *Premna* only by its extraordinary small size; therefore perhaps we had better combine it with the genus.” Further in 1921, Lam & Bakhuizen suggested that “*Pygmaeopremna* could not be distinguished from *Premna* L.” Based on taxonomic investigations, Munir (1984) stated that the flowers of all available *Pygmaeopremna* collections were similar to those of *Premna*. The only differential character that really holds is the dwarf habit of *Pygmeopremna*, and this character is not good enough to recognize the genus. Hence, following Merrill (1910, 1923, 1951), *Pygmaeopremna* is now considered as a synonym of *Premna* (Munir, 1984).

### 2.1.2. Distribution of the Genus *Premna*

The genus *Premna* L. now contains about 200 species worldwide which are mainly distributed in the tropical and subtropical regions of Asia, Africa, Australia and the main distribution extends from India to Japan, Southward to Indochina, Malaysia, and tropical Australia and eastward to Polynesia (Munir, 1984; Kirtikar and Basu, 1992; Harley *et al.*, 2004; The Forest Herbarium, 2001; Kadareit, 2004). A study of the genus in Thailand was first undertaken by Fletcher (1938), enumerating 30 species. Later, Moldenke (1980) and Govaerts *et al.*, (2008) reported the checklists of *Premna* with 39, 19 and 33 taxa, respectively. In another study, Rajendran and Daniel (2002), reported 31 species and 6 varieties of *Premna* from India.

### 2.2. *Premna serratifolia*: An Overview

An overview of *Premna serratifolia* with special reference to its taxonomic history (nomenclature, species complexity & synonyms, polymorphic status and geographic distribution) is described in this section.
2.2.1. Taxonomic History of *Premna serratifolia*

The type species of the genus (generic type) for the genus *Premna* is *Premna serratifolia* L. 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. Of the above mentioned species, the type of *Premna integrifolia* was collected by Paul Hermann in Ceylon. This species was first described by J. Burman (1737) under the name ‘*Sambucus zeylanica odorata aromatica*’ and Linnaeus (1747) renamed it using a single name ‘*Cornutioides*’. Later, N. L. Burman (1768) for the first time renamed this Ceylon material by giving a binomial name, *Cornutia corymbosa*.

However, 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. Linnaeus cited under *Premna integrifolia* N.L. Burman’s validly published binomial *Cornutia corymbosa*, thus making the binomial *Premna integrifolia* an illegitimate name (Art67, Int.code Bot.Nom. 1978). Linnaeus cited his invalid single name ‘*Cornutioides Fl.Zeyl.416*’ under the plant material with serrated leaf margin, *Premna serratifolia*. According to Linnaeus, the serrate leaved plant in Hermann’s Ceylon material was different from Burman’s *Cornutia corymbosa*. Since the serrate-leaved segregate of the Ceylon material, on which *Premna serratifolia* was based, did not involve the entire leaved type material of *Cornutia corymbosa* Burm.f., and would seem that *Premna serratifolia* is a legitimate name. According to Lourteig (1966), the type of *Cornutia corymbosa* Burm.f., on which *Premna integrifolia* was based, is preserved at the Institute de France, Paris (Munir, 1984). Based on the above discussions, it can be concluded that taxonomically *Premna integrifolia* and *Premna serratifolia* belongs to the same species, but nomenclaturally they are based on two
different types, segregated from Paul Hermann’s Ceylon material. Schauer (1847) pointed out these two as synonymous and hence united these two species under the name *Premna serratifolia* L.

Rottler and Willdenow in 1803 described one of Rottler’s collections from Madras, India, as a new *Premna* species, *Premna corymbosa*. In the protologue, they mentioned this as *Premna corymbosa* Nob., making no reference to *Cornutia corymbosa* Burm. f. Rottler contrasted *Premna corymbosa* with *Premna integrifolia* L. and in a footnote to his paper, Willdenow contrasted three other species of *Premna* with *Premna corymbosa*, without mentioning Burman’s *Cornutia corymbosa*. Here also, taxonomically *Premna corymbosa* Rottl. & Willd. and *Cornutia corymbosa* Burm. f. are identical, but nomenclaturaly *Premna corymbosa* Rottl. & Willd. was based on a specimen from India while *Cornutia corymbosa* Burm. f. on a specimen from Ceylon respectively. Therefore, the combination of *Premna corymbosa* (Merrill, 1917) based on *Cornutia corymbosa* Burm.f. would be a later homonym and illegitimate. In 1917, Merrill clearly mentioned that *Premna corymbosa* (Burm.f.) Rottl. & Willd. is the correct name for the plant that Linnaeus named *Premna integrifolia*. He further pointed out that ‘all three’ (i.e. *Premna integrifolia*, *Premna serratifolia* and *Premna corymbosa*) “are typified by the same material”. This view was later accepted by Fletcher (1936) and Meeuse (1942). However, Fosberg (1953) disagree with the above argument and pointed out that *Premna corymbosa* Rottl. & Willd. (1803) was described as a new species from India and it was not based on *Cornutia corymbosa* Burm.f., the type of which came from Ceylon. Nevertheless, Fosberg (1953) did not appreciate the fact that Paul Hermann’s Ceylon material, on which *Cornutia corymbosa* Burm.f. was based, had been treated by Linnaeus (1771) as a mixed collection; he considered that both names were based on the type of *Cornutia corymbosa* Burm.f., and were therefore, illegitimate. The fact that *Premna serratifolia* was differently typified and therefore legitimate have been missed by Fosberg (1953) and Moldenke (1971, 1980). They therefore, accepted *Premna obtusifolia* R. Br. (1810) which they considered to be the next available name for this species. As it is evident from the above

Currently, *Premna serratifolia* L. is accepted as the oldest validly published name and all other names viz., *Premna integrifolia* L., *Premna obtusifolia* R.Br. and *Premna corymbosa* (Burm.f.) Rottl. & Willd. are treated as its synonyms (Munir, 1984).

### 2.2.2 Distribution of *Premna serratifolia*

This species is seen distributed in Papua New Guinea, New Britain, New Ireland, East Africa, India, Southern China, Java, Moluccas, Malaysia, Philippines, Samoa, Fiji, Indochina, Australia and Japan. According to Lam (1919) the species is seen distributed from Madagascar, Mauritius, India to Malacca and Thailand, East Bengal, Ceylon, Andaman, Nicobar, Hong Kong, Malaya, Philippines and Polynesia. Apart from these localities Moldenke in 1971 recorded it from Hainan, Taiwan, Ryukyu Archipelago and Melanesia.

In India, it is distributed in Assam, Khasi hills, Goa, Gujarat, Karnataka, Kerala, Lakshadweep, Maharashtra, Orissa, Tamilnadu, West Bengal and coastal forests of Andaman and Nicobar Islands (Kurz, 1974; Hooker, 1985; Rajendran and Daniel, 2002; Khare, 2007). According to Gamble (1967) the species is seen distributed in Deccan forests, Kodur in Cuddapah, Nellore, Chingleput and Madras to the Javadis near the coast. It is reported from Sylhet and Coromandel of Bombay, South Kanara, South Carnatic and Travancore-Cochin, where it grows under saline and mesophytic conditions (Chopra et al., 1956; Somasundaram, 1963; Drury, 1858; Pharmacognosy of Ayurvedic drugs, 1978; Varier, 1995). The plant is common in many parts of India especially towards sea-coasts (Parkinson, 1922; Chopra and Chopra, 1955; Nairne
2.2.3. Polymorphic status of *Premna serratifolia*

The genus *Premna* was considered as an “extremely difficult genus in which flower features are almost as vague and little distinct as those of the extremely variable leaves” (Beer & Lam, 1936). 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…….” Earlier taxonomists like Schauer (1847), Schumann and Hollrung (1889) and H.J. Lam (1919) stressed the importance of merging of related species into a large and very polymorphic one, being called by the name of eldest, probably, *Premna integrifolia*. H.J. Lam (1919) regarded *Premna integrifolia* as “a very polymorphic species” and stated that “for examining a large number of specimens, we found, that several other species were inseparably united with one another by all possible intermediate forms and with *Premna integrifolia*…….” According to Kok (2013), *Premna serratifolia* shows significant morphological variation across a wide geographical distribution, especially in leaf shape, leaf margin, inflorescence size and calyx form. This was recognized and well illustrated by Lam (1919) and Munir (1984). Nevertheless, Lam (1919) accepted that two subspecies could be recognized viz., *Premna integrifolia* sub sp. *truncatolabium* and *Premna integrifolia* sub sp. *dentatolabium* mainly based on the leaf shape. After studying the specimens available, Lam & Bakhuizen van den Brink (1921) decided that these sub species could no longer be recognized, and instead suggested five different types: type I- *integrifolia*, type II- *abbreviate*, type III- *cyclophylla*, type IV- *sambucina* and type V- *foetida*. These subdivisions were suggested based on a series of continuous characters and ecological data (habitat; from seashore to inland; from shrub to tree; lamina becoming bigger and more acuminate and calyx becoming more distinct and toothed) which, according to the authors, overlapped and formed a cline. In 1919, H.J.
Lam expressed the difficulty of subdividing the species exclusively based upon the form of calyx, which, as in some other species, is often inconstant/ variable. He also commended on the wrong and unscientific way to discover the truths using old methods of mere morphological examination and recommended the application of new branches of science like genetics to solve the problems in systematic botany. In spite of such serious attempts to study the wide range of variations in *Premna serratifolia*, no solid evidences have been generated either to recognize or differentiate the different morpho-variants of this taxon. However, when an extensive amount of material is examined the variation is found to be continuous and might have resulted by the process of gradual evolutionary changes.

### 2.3. *Premna serratifolia*: A potential Medicinal plant

*Premna serratifolia* L. is a well-known medicinal plant belonging to the family Verbenaceae. Regardless of its morphological variations, this polymorphic species has a unique status in traditional systems of medicines like *Ayurveda* and folklore medicines. A brief review of literature pertaining to its traditional medicinal uses and traditional medicine preparations are summarized below.

#### 2.3.1. Traditional Medicinal uses of *Premna serratifolia*

The indigenous 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 and Samuel, 2003; Thomas and George, 2005; George *et al.*, 2006; Prajapati *et al.*, 2006). As reported by Kartick (1984) and Sivarajan and Indira (1996), its root, stem/ stem bark and leaves are used for different traditional preparations in medicine. *Premna serratifolia*, also known by the name *Agnimantha*, is acrid, bitter, astringent, cardio tonic, carminative, alterative, laxative, stomachic and tonic. It improves digestive power and is useful in constipation, fever, heart diseases and neurological diseases. It overcomes *kapha* and *vata* disorders,
anemia, piles, oedema, poison, anasarca and abdominal diseases. Traditionally, the drug is highly valued for its anti-inflammatory property (Kolammal, 1979; Kurup et al., 1979). It is pungent and hot in action and used to treat weak digestion and excessive fatty condition of the body (Pharmacognosy of Ayurvedic drugs, 1978). An ethnobotanical study conducted among traditional Ayurvedic vaidyans of Kerala by George (2006), revealed many hitherto unreported indigenous uses of *Premna serratifolia*. According to this study, the leaves have more medicinal applications (55.55%) followed by roots (36.11%) and bark (8.34%). As reported in this study, *Premna serratifolia* is specific to anemia, weak digestion, piles, constipation, amavatam, accumulation of fluid in the tissues or in body cavities and also effective to reduce serum cholesterol level and eliminating poison/ heavy metals from the body parts. *Premna serratifolia* is traditionally used in the treatment for beriberi, vaginal irritation, to relieve headache and as eye lotion (Dassanayake and Fosbergeds, 1980; Padua, 1999).

Traditional healers consider leaf as one of the useful parts for internal as well as external uses. The leaves are galactagogue, and are useful in agalactia, catarrah, rheumatalgia and tumours (Varier, 1995). Leaves are used as an external application to piles and tumours (*Ayurveda*). Leaf decoction of the species is used for bathing infants. Leaves are also used for the treatment of arshas (hemorrhoids) in the form of *Avagaha sweda* (sitz bath) to reduce inflammation and pain. Sugared decoction of *Premna serratifolia* leaves with the juice of calamansi (a citrus fruit) helps to loosen up phlegm and effective for coughs. Decoction of leaves is used as curative medicine for fever, blisters of the lips and stomach aches (Sturtevant, 1972). Leaves when applied over the urinary bladder facilitate urination (Kumar and Jain, 2002). The leaves are eaten by the inhabitants of the Coromandel Coast (Whitelaw, 1984). In Peninsular Malaysia and Indonesia, boiled young leaves are consumed as vegetable. The leaves are also used for the preparation of natural ‘jin’ and ‘sherri’ (Hussain et al., 1992; Steven, 2005). The leaf is diuretic and indicated in dropsy (Agarwal, 1997). The leaf decoction is reported to be good for the treatment of colic, rheumatism and neuralgia (The Wealth of India,
1948). The plant is mentioned as *appel* in Hortus Malabaricus by Rheede (1678-1693), and the decoction of its leaves is suggested for pains and wind in the stomach. According to Burkill (1966), specifically the leaves of *Premna obtusifolia* are used in folklore medicines. George (2006) also reported that, of the four types of *Premna serratifolia*, the leaves of *Neelan munja* are effective for marma treatment, neuralgia and as an analgesic to treat vata disorders. The tender plant is used for neuralgia and rheumatism. Leaves rubbed along with pepper administered in colds and fevers; in the form of a decoction given for flatulence; in form of soup used as stomachic and carminative. Infusion of the leaves (1 in 10) is used in eruptive fevers, colic and flatulence, in doses of 1 to 2 ounces (Nadkarni, 2007). The whole plant is given in decoction for pains in the head and body, as well as, in rheumatism. This plant is an essential ingredient of *Medhahara kwata* which is often prescribed by traditional vaidyans for obesity. According to George (2006), the leaf extract of *Premna serratifolia* can be effectively utilized to evolve an eco-friendly pest-management strategy to control chicken fleas, coconut pest (*Oryctes rhinoceros*) and a variety of vegetable pests.

The root has an agreeable aromatic odour (Dey, 1980). The root forms an ingredient of *dasamula*, a preparation often prescribed by the native physicians in obstinate fevers (Kirtikar and Basu, 1992). The root is pungent, useful in chyluria, and swellings. It is thermogenic, anodyne, alexeteric, expectorant, depurative, febrifuge and antibacterial. The roots are useful in cardiac disorders, hepatopathy, cough, asthma, bronchitis, leprosy, migraine, jaundice, malaria, skin diseases, dyspepsia, inguinal hernia, kidney stones, rhinitis, rheumatoid arthritis, abscess haemorrhoids and is also indicated in *shotha* (inflammation), *vatavyadhi* (neurological disorders), *prameha* (diabetes mellitus), *medoroga* (obesity) and *agnimandya* (loss of appetite). In *Unani*, the root is suggested good for liver complaints. The species is known to have various folklore usages (Nadkarni, 1985; *The Wealth of India*, 2005; Fairchild, 1943; USDA, 2007). This root is prescribed in decoction, as a gentle cordial and stomachic in fevers. For fevers, a quantity of half a tea-cupful, twice daily is prescribed. Decoction of root is
good for liver complaint. Decoction of the root (1 in 10) or about 4 ounces in a pint of water and boiled for 15 minutes, is given in doses of 2 to 4 ounces twice daily as a stomachic and a tonic, and also in gonorrhea and during convalescence from fevers. Leaves are also used for the same purpose. Root forms an ingredient of Dasamula and thus used in a variety of afflictions. Root rubbed into a paste with water is recommended to be taken with clarified butter in urticaria and roseola for a week (Nadkarni, 2007). The root boiled in salt water is used in gout, which is externally used (Drury, 1858). Traditionally, it is used in bone fractures and also to reduce pains in bones and rheumatic aches. In various parts of Indonesia, an infusion of the leaves and roots are used against fevers and shortness of breath and to promote breast-milk production in women. In Indo-China, the leaves and roots are used in traditional medicine as a diuretic, stomachic and febrifuge (Hussain et al., 1992).

Decoction of stem bark decreases force of contraction of heart and produces dilation of the pupils (Chopra et al., 1956). The alkaloids in the species have a sympathomimetic action (Chopra and Chopra, 1955). On Guam, in the Pacific Ocean, a tea made from the boiled bark is used to treat neuralgia. It is very effective for the treatment of kidney disease, liver problems, and constipation (Hussain et al., 1992).

2.3.2. *Premna serratifolia* in Ayurvedic formulations

*Premna serratifolia* is one of the drugs that constitute the drug groups (gana) such as Dasamula, Brahatsphotamula, Viratarvadi, Varunadi gana (Sushruta); Sothahara, Sitaprasamana and Anuvasantopaga (Charaka) (Pandey, 2002). In Ayurveda various drug formulations like arishtam, rasayanam, kwatham, ghritham, and thailam are used as a part of its treatment strategy. In Arishtams (naturally fermented herbal decoctions) such as Amritharishtam, Dandyarishtam, Dasamoolarishtam and Balarishtam, *Premna serratifolia* is used as a major constituent. The major rasayanams (nutritional juices of medicinal plants) having *Premna serratifolia* as an essential constituent are Agasthyarasayana, Chyavanaprasam, Dasamoolarasayanam and
Brahmarasayanam. In Ayurvedic medicines, kwatham is an unfermented form of herbal decoction prepared by adding water in specified proportion to the coarsely powdered herbal material followed by further boiling and filtering processes. The main kwathams prepared from the powdered roots of Premna serratifolia are Indukanthakwatham, Dhanwantharakwatham, Varanadikwatham, Sapthasarakwatham, Medhahara kwatham, Maharasnadi kwatham and Luhuna Kolla kwatham. Ghritham are ghee preparations of plant extracts used for internal consumption. Inducantha ghritham, Sukumara ghritham, Dhanwanthara ghritham, Dasamoola shatpala ghritham and Medhahara ghritham are some of the major ghrithams containing Premna serratifolia as major ingredient. Thailams are mainly the oil preparations having herbal extracts which is usually used for external applications. Dhanwanthara thailam, Prabhanjana thailam, Vimordhana thailam, Narayana tailam and Sahacharadi thailam are prepared with poly herbal extracts containing Premna serratifolia as a key constituent (API, 2001). Premna serratifolia is also used in preparations like Dasamoola hareetaki, Agnimantha-mulkalka and Agnimantha-kasaya (Dey, 1980).

2.3.3. Description of Premna serratifolia under Sanskrit names in Traditional Manuscripts

Premna serratifolia is described in the traditional Ayurvedic manuscripts under many Sanskrit and vernacular names depicting its morphological and diverse medicinal/therapeutic uses. The most commonly used Sanskrit vernacular name attributed to this medicinal plant is Agnimantha. The name Agnimantha is derived from its use during Vedic period, where its stem/sticks were used to produce fire (The Wealth of India, 1972). Other Sanskrit names of Agnimantha viz., Vahnimantha and Havirmantha also indicates, that the tree was used to produce fire in the sacrificial ceremonies by rubbing the sticks together (Sivarajan and Indira, 1996).

The different uses of Agnimantha were mentioned in Rig-Veda and Atharva-Veda. In Astanga Sangraha, the plant is described by the name Arani which is the synonym of Agnimantha. The different medicinal properties of Agnimantha were
described in *Laghutrayis*. In *Nighantukaras*, besides medicinal properties, its morphological characters were explained in detail. The major manuscripts having the description of this species are: *Bhavaprakasa, Saligrama Nighantu, Dhanwanthari Nighantu, Madanapala Nighantu, Nighantu Ratnakara, Abhidhanamanjari*, and *Raja Nighantu* (Pharmacognosy of Ayurvedic drugs, 1978). In these traditional Sanskrit texts, many synonyms such as *Agnibijaka, Agnimantharkari, Ananta, Arani, Aranika, Araniketu, Ganakasika, Ganikaarikaa, Ganikasika, Gankarika, Girikarnika, Harimantha, Havirmantha, Jaya, Jayanthi, Jayee, Jyotishka, Kanika, Karnika, Kethu, Kshudragnimantha, Manthanam, Mathana, Nadeyi, Nadija, Nathy, Pavaka, Pavakarini, Pittamata, Rakthangam, Shriparna, Shriparni, Tanutvaka, Tarkari, Tejomantha, Vaataghni, Vahnimantha, Vaijayantika, Vanhimula, Vijaya, Vijayantika and Vyganthika* were given to *Agnimantha* to describe either its medicinal attributes/properties or its morphological features.

*Premna serratifolia* is also known by different names in different states of India. Its common names in different regional languages are as follows.

Hindi : *Arni, Agetha, Arani, Ganiari*

Tamil (Tamilnadu) : *Munnai, Munnay, Munney, Munni, Munni-vayz, Pasumunnai, Peyminnay.*

Telugu (A.P.) : *Gabbunelli, Ghebunelli, Kanika, Karnika, Nagura, Nelli, Nelli chettu, Padmaka, Pinnanelli, Tukkadu.*

Malayalam (Kerala) : *Munja, Munna, Appa, Appel, Kozhychedy.*

Kanada (Karnataka) : *Agnimandha, Naravalu, Takkila.*

**2.3.4. Controversial status of *Premna serratifolia* in Ayurveda**

In the *Ayurvedic* system, there is considerable disagreement regarding the identity of genuine medicinal plants since many of the drug plants are not described with scientific precision in classic *Ayurvedic* texts. Hence, many unauthentic plants or
plant parts are being used for the preparation of standard classic Ayurvedic medicine in different places and sometimes even in the same locality. Premna serratifolia, is one such medicinal plant coming under controversial drug plants of India. The review of related literature revealed that there is much disagreement among the commentators of modern Nighantus regarding the identity of genuine drug, Agnimantha. When a few scholars did not refer to different source plants for Agnimantha, majority of the Ayurvedic scholars (Purandare, 1896; Sthana, 1916; Acharya, 1950; Nadkarni et al., 1954; Vaidya, 1965; Krishnamurthy et al., 1972; Meulenbeld, 1974; Chaturvedi et al., 1983; Jain, 1986; Handa and Kaul, 1996; Sharma, 1996; Meulenbeld and Wujastyk, 2001; Puri, 2002; Bishnupriya et al., 2003; Nair, 2004) raised doubts regarding the identity of genuine source drug of Agnimantha.

A very serious limitation of the Ayurvedic system is the difficulty in ascertaining the identity of the genuine medicinal plants prescribed by the founders of the system. In the original texts, the descriptions of the medicinal plants were given in poetic language and hence very often, lack scientific precision. Hence, the interpretation of the description in Sanskrit is largely influenced by the views of the interpreter. A short review of the views of different authors is compiled below.

Two types of Agnimantha viz., Laghu (small) and Brihat (big) having somewhat similar properties were mentioned in Nighantus. Charaka and Sushrutha have mentioned them separately as Agnimantha and Tarkari respectively. In Sushrutha Samhita, Agnimantha is mentioned as one among Brihat panchamula and it is equated to Premna integrifolia by many commentators. Sushrutha while explaining Varunadi gana, Tarkari and Agnimantha were mentioned separately, indicating these two as separate drugs. In Charaka Samhitha also has mentioned about two varieties viz., Agnimantha and Tarkari and are described as separate trees. However, Charaka described these two, Agnimantha and Tarkari, together in a similar context. Later authors have equated Clerodendrum phlomidis to Laghu and Premna corymbosa
(Premna serratifolia, Premna integrifolia) to Brihat as the respective sources of the two varieties of Agnimantha (Bapalal Vaidya, 1982; Chunekar, 1982).

The texts and lexicons of Ayurveda mentioned about two varieties of Agnimantha, namely Brihat Agnimantha (big variety) and Laghu Agnimantha or Ksudragnimantha (small variety) which have been correlated to Premna integrifolia and Clerodendrum phlomidis respectively by many scholars. While writing vimarsha for the sloka, ‘Tarkari dwayam’ in Ashtanga samgraha, the commentator explains that Agnimantha is of two varieties Tarkari and Agnimantha; Tarkari is also called as Laghu Arani and it is botanically equated to Premna integrifolia, while Agnimantha is called as Vruddha Arani and is botanically equated to Clerodendrum phlomidis. Among the Nighantu, Amarakosha considered Agnimantha and Tarkari as different plants. Sodhala (1994) mentioned Tarkari and Agnimantha (Arani) as two different varieties (Clerodendrum phlomidis and Premna integrifolia as the botanical sources of Tarkari and Agnimantha respectively). Some of the modern time scholars of Dravyaguna consider Tarkari as Laghu Agnimantha. Bhavamishra mentioned only one variety i.e., Agnimantha for which Tarkari is the synonym. In Nighantu Ratnakara, Agnimantha is described as two types viz., Laghu (smaller) and Brihat (bigger). This text explains that Laghu variety has better sothahara property than the Brihat variety. Kaiyyadeva Nighantu also quoted Agnimantha as a better sothahara and vatahara drug. In Dhanwanthari Nighantu two varieties of Agnimantha are mentioned, Agnimantha and Kshudragnimantha or Kshuragnimantha. Commentator considered Clerodendrum phlomidis as Agnimantha and Premna integrifolia as Kshudragnimantha. In Priya Nighantu, Arani and Agnimantha are used synonymously. The commentator considered Premna integrifolia as Agnimantha. In Raja Nighantu and Saligrama Nighantu, two varieties are mentioned: Agnimantha and Kshudragnimantha. Both these are mentioned under Prabhadradiyarga in Raja Nighantu. The synonyms of Agnimantha are also given for Jayanti, included under Satahavadiyarga. For these the commentators cite the Latin name Clerodendrum phlomidis, though Tarkari is regarded as a synonym of Agnimantha. According to Pandey (1998), Agnimantha is of two types viz., Brihat
Agnimantha and Kshudragnimantha and are equated to Clerodendrum phlomidis and Premna integrifolia respectively.

Unscientific nomenclature of medicinal plants followed in classical traditional Sanskrit texts is a serious defect of Ayurvedic system. In Ayurveda, unlike modern botany, there is no precise and uniform system of nomenclature. Dozens of names may be found given to one and the same plant, each name indicative of one minor attribute or diagnostic property of the plant. The loose, intuitive and unscientific way by which ancient authors have named plants is the source of much confusion today, because the qualitative names are very often applicable to more than one plant. Similarly, there are also cases of different species of plants (taxonomically related or unrelated species) having common medicinal properties, owing to the presence of same organic compounds. In the case of Agnimanta, the two species suggested are Premna serratifolia and Clerodendrum phlomidis, which are botanically closely related species belonging to the same family Verbenaceae (Singh et al., 1972). In South India, particularly in the state of Kerala, Premna serratifolia has been used as the genuine drug. However, in North India Clerodendrum phlomidis is used instead of Premna serratifolia. The properties attributed to these two species are considered to be the same. The property of Laghu Agnimanta is same as Brihat Agnimanta but Laghu is better for lepa and poultice in swelling.

Most of the regional names of Agnimanta such as Gineri, Agethu, Tekara or Tankali are actually distorted forms of the original Sanskrit names- Ganikarika, Agnivadhu and Tarkari. There is not much difference in the tree sizes of the two kinds of Agnimanta and thus any attempt to differentiate them as Vrhat (large) and Ksudra (small) kinds appears untenable. Their separation on the basis of plant size may, however, be limited to different species of Premna only. It is, however, reasonable and useful for the sake of field identification to name the Premna species as Ksuragnimantha (thorny Agnimanta) and Clerodendrum species as Aksuragnimantha (without thorns). Arani and Agnimanta may be treated as common names for both the
species but *Tarkari* may be accepted as a permanent name for *Clerodendrum* species. The justification for accepting the other names as synonyms for *Agnimantha* and its varieties has been discussed based on their properties and uses. It may be noted that the differences in uses and properties of the two varieties have not been pointed out either in the *Nighantus* or in the preparations (*Yogas*) of the texts. Instances are not inadequate where *Agnimantha* and *Tarkari* have been treated as equivalents in the identical *Yogas* of different texts.

Several authors have correlated *Agnimantha* to other species of the genus *Premna*. Kolammal (1979) and Nair (2004) correlate *Agnimantha* to *Premna serratifolia* L. and *Premna latifolia* Roxb. as a substitute. Warrier et al. (1994) and Sivarajan and Indira (1996) correlate *Agnimantha* to *Premna corymbosa* Rottl. Sharma (2006) mentions two varieties: *Agnimantha* (bigger variety) correlated to *Premna mucronata* Roxb. and *Tarkari* (smaller variety) equated to *Clerodendrum phlomidis*. *Premna serratifolia* L. and *Premna spinosa* Roxb. are the other species correlated to *Agnimantha*. *Premna latifolia* Roxb. var. *mucronata* C.B.Clarke (a botanical synonym of *Premna mucronata* Roxb.) has also been considered by some authors as *Agnimantha*.

In most of the traditional texts of Kerala except *Bhavaprakasham* and *Madanapala Nighantu*, two types of *Munna* are mentioned and their synonyms are often used interchangeably. The two types of *Munna* mentioned in *Madanapala Nighantu* are *Munna* and *Kattumunna*. P.V. Sharma (1998) in *Dravyagunavinjan* has considered *Agnimantha* to be *Valiya munna* (*Premna mucronata*) and *Tarkari* to be *Ceriya munna* (*Clerodendrum phlomidis*). However, throughout Kerala *Premna* species is used for *Agnimantha* and *Tarkari* (Varier, 1995). In *Ayurveda Vishwa Vijnan Kosham* three types of *Munja* viz., *Puzhamunja*, *Munja* and *Cherumunja* were described.

There are various views regarding the genuine source plant and substitute of *Agnimantha*. According to ‘Data base on medicinal plants used in Ayurveda’,
Clerodendrum phlomidis is considered as the genuine drug and Premna serratifolia as the substitute since these two plants have similar medicinal properties (i.e., in ‘Guna karma’ they are treated identical). Chunekar (1982) in his commentary on Bhavaprakasa Nighantu also opined these two as substitutes for each other, since they have similar medicinal properties. A few authors like Nadkarni (1976), Kamat and Mahajan (1972) pointed out Clerodendrum inerme (L.) Gaertn. as the source plant of Agnimantha. However, majority of the authors like Kirtikar and Basu (1918), Vaidya (1936), Kurup et al. (1979), Dey (1980) and Mooss (1980) equate the drug with Premna serratifolia L.

In the first edition (Part-I) of Ayurvedic Formulary of India Clerodendrum phlomidis Linn.f. is mentioned as the authentic botanical source and Premna integrifolia Linn. and Premna mucronata Roxb. as a substitute (AFI, 1978). In its second edition (Part-I), Premna integrifolia has been mentioned as the authentic plant source and Clerodendrum phlomidis and Premna mucronata as the substitutes (AFI, 2000). In the first edition (Part-II) of Ayurvedic Formulary of India also Clerodendrum phlomidis. Linn.f. has been listed as the authentic Agnimantha and instead of Premna integrifolia Linn., Premna obtusifolia R. Br. and Premna mucronata Roxb. are listed as the substitutes (AFI, 2000). As Aparna et al. (2012), rightly pointed out, the basis for this variation in listing of botanical sources for Agnimantha are not provided and hence not clear.

As it is evident from the above discussions, it is very difficult to resolve the controversy regarding the identity of Agnimantha as the available evidences are inadequate to equate this drug plant to either Clerodendrum phlomidis L./ Clerodendrum. inerme (L.) Gaertn. or Premna serratifolia L. Based on the available evidences listed in classical texts and Nighantas, it is not easy to judge the authenticity and exact identity of the source drug Agnimantha. Based on comparative morphological characters described in classical texts, it is quite likely that both plants (Premna serratifolia and Clerodendrum phlomidis/ Clerodendrum inerme might have identical
therapeutic properties as evidenced from their time tested ethno medical uses. Marked discrepancy in pharmacognostical parameters of these plants were reported by different authors. However, it is expected that in depth studies based on modern tools and techniques in pharmacognosy and phytochemistry will resolve the existing ambiguity and may provide additional evidences for establishing the identity and purity of the real source plant.

2.4. Pharmacognostic, Phytochemical and Pharmacological studies on *Premna serratifolia*

A brief report of the studies done with respect to Pharmacognostic, Phytochemical and Pharmacological studies of *Premna serratifolia* L. is presented below under appropriate heads.

2.4.1. Pharmacognostic studies on *Premna serratifolia*

Pharmacognosy is one of the indispensable tools in distinguishing medicinally important plants from its substitute or adulterant counter parts. In botanical pharmacognosy prime importance is given to the botanical aspects of the plant and its growing habitat, harvesting and processing conditions rather than for its chemistry for ensuring the plant’s medicinal efficiency. The interest in traditional herbal medicines is reemerging worldwide, precisely because many modern drugs, whether synthetic or derived from nature, are failing to serve the health care needs of majority of the world population. The classical botanical pharmacognosy mainly deals with and concentrates in maintaining the quality of the plant, the environment in which it grew, and its myriad compounds and actions that are of importance and most appropriate in the development of traditional herbal medicines that people worldwide rely upon in self healing and traditional healing systems. The pharmacognostic studies carried out with special reference to *Premna serratifolia* are summarized below.

Studies on pharmacognostic, ethnobotanical and phytochemical aspects of different ecotypes of *Premna serratifolia* in Kerala were initiated under the leadership
of George in the year 2003, in Department of Botany, C.M.S. College, Kottayam. He conducted an extensive survey among the traditional Ayurvedic practitioners of Kerala. He collected many morpho-variants of *Premna serratifolia* from different regions and the germplasm of these morpho-variants were maintained in C.M.S. College herbal garden and also in his experimental home garden at Karunyna lane, Kalathilpady, Vadavathoor P. O., Kottayam - 686010. A project (funded by UGC) entitled ‘Ethnobotanical, Phytochemical and Pharmacognostic studies on *Premna serratifolia* L.’ was successfully completed and submitted to University Grants Commission (George, 2006). In another study entitled ‘Pharmacognostic studies on Agnimantha’, George *et al.* (2006) identified four different ecotypes of Agnimantha viz., Kozhimunna (Ecotype-1), Chemparathimunna (Ecotype-2), Cherumunna (Ecotype-3) and Neelanmunna (Ecotype-4) from different parts of Kerala. The study also revealed the existence of different ecads for Kozhimunna and Chemparathimunna. As revealed in this study, the existence of different ecotypes and ecads of *Premna serratifolia* in the different geographical areas of Kerala shows the high degree of plasticity possessed by Agnimantha.

The macro and microscopical characters of the roots of *Premna integrifolia* and *Clerodendrum phlomidis* were studied by Gokani *et al.* (2008). Morphologically the roots of both species resemble each other except for their colour and size. Microscopically they can be differentiated by noting the presence of rhytidoma in roots of *Premna integrifolia*. Starch grains were found distributed only in the xylem parenchyma and xylem rays in *Clerodendrum phlomidis*, whereas in *Premna integrifolia* starch grains were detected in all tissues except cork. In another study, Rajendran and Susheela (2010) conducted a preliminary investigation to standardize certain pharmacognostical parameters viz., physico-chemical, phytochemical and fluorescence analysis of stem bark and stem wood of *Premna serratifolia* collected from Tamilnadu State. The anatomical features of the root and stem of *Premna serratifolia* L. were reported by earlier researchers (Pharmacognosy of Ayurvedic drugs, 1978; Lalithamma, 1996; George, 2006).
Thirumalai et al. (2013) studied the morphological and microscopical characters of *Premna herbacea*, a related species of *Premna serratifolia*. In this study, the roots and root powder of *Premna herbacea* were subjected to morphological (colour, odour, taste, shape and texture) and microscopic structural evaluation. Coarse powder samples of the plant roots were used to perform physiochemical studies such as total ash, acid insoluble ash, water soluble ash, extractable matter, loss on drying, foaming index and swelling index. Root powder samples were treated with different reagents and observed for fluorescence under visible light and under UV light of short and long wavelength. They exhibited fluorescence. The physiochemical parameters of the plant were within the limits. Phytochemical analysis of the root extracts *Premna herbacea* in different solvents (ethanol, chloroform, petroleum ether and water) revealed the presence of triterpenoids and alkaloids with trace amounts of carbohydrates and flavonoids. TLC and HPTLC analysis of the various extracts also yielded satisfactory results.

### 2.4.2. Phytochemical studies on *Premna serratifolia*

Plants serve as the primary sources of medicine from ancient period and the search for novel plant derived lead molecules of therapeutic importance has gained momentum in recent years. A brief description of the phytochemical compounds reported from different parts of *Premna serratifolia* along with different approaches adopted for identification, screening, isolation, characterization and *in vitro* production of active compounds are summarized in this section.

- **Major Phytochemical compounds in *Premna serratifolia***

Many phytochemical compounds were reported from *Premna serratifolia* by earlier researchers. Preliminary screening of phytochemical compounds in *Premna serratifolia* revealed the presence of sterols and triterpenes (Debelmas *et al*., 1973), resin, alkaloids [premnine (Basu and Dandiya, 1947), ganikarine (Basu and Joneja, 1949), premnazole (Barik *et al*., 1992) and ganiarine], Flavonoid-luteolin (Dasgupta *et al*., 1984), glycosides, tannins, phenolic compounds, carbohydrates, amino acids and some
unsaturated aromatic hydrocarbons (Chopra and Chopra, 1955; Chopra et al., 1956; Nadkarni, 2007; Mali and Bhadane, 2010). According to Kartick (1984), this drug plant contains a volatile alkaloid to the extent of 0.05% and some resins soluble in alcohol. The alkaloid premnmine decreases the force of contraction of heart and produces dilation of the pupils (Dey, 1980). Yuasa et al. (1993) isolated a new phenylethanoid, and other phenolic compounds from its stem. As reported by Rastogi and Mehrotra (1990), a compound with mp 155° was isolated from root bark of Premna integrifolia and it was active against gram-positive organisms. Rastogi and Mehrotra (1991) also reported β-sitosterol isolated from the leaves and stem bark and betulin from the stem bark of Premna integrifolia. From the leaves, a verbascoside-iridoid glycoside conjugate was isolated along with premnafolioside (Otsuka et al., 1993). According to Chunekar (2005), this plant mainly contains p-methoxy cinnamic acid and linalol, linoleic acid, β-sitosterol and flavone luteolin. As reported by Rajendran et al. (2008) and Rao et al. (1985), iridoid glycoside is one of the major active principles of Premna serratifolia. In an attempt to standardize Dasamula containing formulations, Alam et al. (1993) reported large quantities of alkaloids from Premna serratifolia. Researchers like Ky et al. (2005), Caldecott and Tierra (2006), Daniel (2006), Bagchi et al. (2008) and Hang et al. (2008) also reported the presence of premnine, ganiarine, ganikarine, premnazole, aphelandrine, pentacyclic terpene betulin, caryophellene, premnenol, premnaspirodiene and clerodendrin-A from this medicinal plant. The aqueous extracts of this plant showed a powerful action on the uterus and gout of the experimental animals (Khare, 2007). According to this study, the leaves contain an isoxazole premnazole, which was found to reduce granuloma formation in rats (34.62%) and its activity was comparable to phenylbutazone (35-36%). The study suggested that premnazole acts probably by controlling the activity of the adreno-corticotropic hormone.

- **Phytochemical methods adopted for the isolation and characterization of novel compounds**

  Earlier researchers have adopted different methods to identify, screen and isolate
phytochemical compounds from the roots, stem and leaves of *Premna serratifolia*. The preliminary phytochemical screening followed by evaluation of biological properties were reported by researchers like Rajendran *et al.* (2008), Rajendran *et al.* (2009), Rajendran *et al.* (2009), Rajendran (2010), Rajendran and Krishnakumar (2010), Rajendran and Saleem (2010), Thirumalai *et al.* (2011), Kumar *et al.* (2013a, 2013b) and Muthukumaran *et al.* (2013). Some researchers have adopted successive extraction of the plant materials in different organic solvents like petroleum ether/ hexane, chloroform, ethyl acetate, methanol/ ethanol and water or combination of different solvent systems for the isolation of different classes of compounds (Mali and Bhadane, 2010; Rajendran, 2010). Isolation of major compounds (alkaloids, flavonoid glycosides, steroids) by TLC/ HPTLC profiling were also reported (Gokani *et al.*, 2008; Rajendran *et al.*, 2008; Gokani and Shah, 2009; Yadav *et al.*, 2011; Yadav and Gupta, 2013).

As a part of developing chemical markers for ensuring the quality standards in *Ayurvedic* formulations, Yadav *et al.* (2010) isolated three novel diterpenoids from the root bark of *Premna integrifolia* and their structure were identified from their 1D and 2D NMR data. Further, these diterpenoids were also evaluated for antibacterial activity (Yadav *et al.*, 2010). A sensitive, selective and robust densitometric High Performance Thin Layer Chromatographic method was developed and validated for the determination of diterpenoid compounds. Diterpenoids 1β,3α,8β-trihydroxy-pimara-15-ene(A), 6α,11,12,16-tetrahydroxy-7-oxo-abieta-8,11,13-triene (B) and 2α,19-dihydroxy-pimara-7,15-diene (C) were used as chemical markers for the standardization of *Premna integrifolia* plant extracts. The separation was performed on silica gel 60F (254) High Performance Thin Layer Chromatography plates using hexane: acetone: ethylacetate (60:20:20 v/v) as mobile phase. The quantification of diterpenoids was carried out using densitometric reflection/ absorption mode at 475 nm after post-chromatographic derivatization using vanillin-sulfuric acid reagent. A precise and accurate quantification was performed for compounds A, B and C in the linear working concentration range of 1-10 μg/spot with good correlations (r(2))=0.9985, 0.9996 and
0.9992, respectively. The method was validated for peak purity, precision, robustness, limit of detection (LOD) and quantification (LOQ) as per the International Conference on Harmonization (ICH) guidelines. Specificity of quantification was confirmed using retention factor (Rᵣ) and spectra correlation of markers in standard and sample tracks (Yadav et al., 2011).

Yadav et al. (2013) also reported two new furofuran lignans premnadimer (1) and 40-hydroxyasararinin 10 glucopyranoside (2) along with 9 known compounds isolated from the stem bark of *Premna integrifolia*. Their chemical structures were elucidated using detailed spectroscopic studies. Their relative configurations were established using analysis of NOESY correlations and coupling constants observed in 1H NMR. Compounds 1 and 2 together with four known iridoid glycosides were evaluated for radical scavenging and ferric reducing antioxidant power. Radical scavenging activity was found maximum in 4-hdroxy-E-globularinin followed by 19-0-trans-p-coumaroylcatalpol and the new dimer. In FRAP assay, premnosidic acid, 10-0-trans-p-coumaroy1-6-0-α-9-rhamnopyranosyl catalpol showed maximum ferric reducing ability supported by high reducing power.

As a continuation of their previous studies, Yadav and Gupta (2013), attempted to develop a High Performance Thin Layer Chromatography (HPTLC) method for quantitative estimation of iridoid glycosides [10-O-trans-p-coumaroyletalpol; 4''-hydroxy-E-globularinin; and premnosidic acid] from the stem bark of *Premna integrifolia*. Separation was performed on silica gel 60 F254 HPTLC plates. The solvent system consisted of ethyl acetate- methanol- H₂O- acetic acid (80:12:6:2 v/v). Densitometric analysis of iridoids was carried out in the absorbance mode at 510 nm after post-chromatographic derivatization using vanillin-sulphuric acid reagent. The method was validated as per the International Conference on Harmonization (ICH) guidelines. This HPTLC method was found to be reproducible, accurate, and can detect iridoids at a nanogram level. Hence, this HPTLC method can be employed as an important tool in the quality control method for polyherbal formulations.
Gokani and Shah (2009) isolated and quantified a chemical marker compound viz., Clerodendrin-A from the roots of Clerodendrum phlomidis by simple and precise method of HPTLC using n-hexane: ethyl formate (7:3) as mobile phase, pre-coated TLC plates (silica gel 60 F254) as stationary phase and H2SO4 as derivatizing agent. The method was validated in terms of linearity, precision, repeatability and accuracy. The limit of detection and limit of quantification was determined on the basis of signal to noise ratio. Content of clerodendrin-A was found to be 0.073 and 0.04% w/w in Clerodendrum phlomidis and Premna integrifolia respectively.

Different methods viz., steam distillation, vacuum distillation from a hexane concentrate and fractionation by silica gel chromatography, GC and GC/MS were employed for the analysis and quanification of volatile compounds. Teai et al. (1998) identified ninety-four compounds from Premna serratifolia representing about 81% of the distillate. The major components identified are: 1-octen-3-ol (16.9%), (Z)-3-hexenol (10.2%), 2-phenylethylalcohol (8.9%), (E, Z)-2,4-nonadienal(6.2%), (E,Z)-2,6-nonadienal (5.0%) and linalool (4.4%). In another study Rahman et al. (2011) identified twenty-nine compounds representing 94.81% of the total leaves oil from Premna serratifolia. The major compound identified are: Cyclohexane (1.03%), Hexan-1-ol (0.65%), α-Pinene (0.86 %),1-Octen-3-ol (8.21%), β-Pinene (1.11%), 3-Octanol (0.97%), 1,8-Cineole (0.93%), cis-2-Octenal (1.13%), Phenylethyl alcohol (5.81%), Indole (0.91%), Decanal (0.87%), Dodecane (0.49%), Damascenone (0.18%), Eugenol (6.69%), Azulene (0.61%), Isoeugenol (1.83%), β-Caryophyllene (0.92%), Benzofuranone (0.98%), α-Humulene (14.21%), Spathulenol (12.12%), Caryophyllene oxide (2.60%), Cubenol (1.67%), Tetradecanal (0.32%), Tumerone (0.83%), Pentadecanol (0.64%), Pentadecanoic acid (0.23%), Hexadecanoic acid (1.06%), Eicosane (0.62%) and Phytol (27.25%).

Singh et al. (2011) also made an attempt to study the chemical constituents of the leaves and roots of Premna serratifolia. The alcoholic extract of Premna serratifolia leaves were analysed and reported many compounds from ethanolic extract. The
Review of Literature | Chapter 2

Compounds reported are: Glycerin (2.79%), 2,5-Furandione, 3-methyl- (9.27%), Benzofuran, 2,3-dihydro- (29.94%), 2-Hydroxy-3-methylbenzaldehyde (6.39%), Dodecanoic acid (7.88%), 2-Propenoic acid, 3-(4-methoxyphenyl)- (13.84%), Phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy- (1.54%), 2-Propenoic acid, 3-(4-methoxyphenyl), ethyl ester (1.35%), 1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester (2.50%), n-Hexadecanoic acid (13.94%), Phytol (6.78%), Octadecanoic acid ethylester (1.68%) and Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl (2.11%). The alcoholic extract of *Premna serratifolia* roots were analysed to have Glycerin (1.14%), 2,5-Furandione, 3-methyl- (2.89%), 2-Furancarboxaldehyde, 5-(hydroxymethyl)- (2.44%), Benzofuran, 2,3-dihydro- (9.86%), 2-Hydroxy-3-methylbenzaldehyde (34.58%), Seychellene (2.30%), Dodecanoic acid (0.71%), 1H-Cycloprop[e]azulen-7-ol, decahydro- 1, 1, 7- trimethyl-4- methylene-, [1ar-(1aà,4aà,7á,7aá,7bà)]- (2.98%), 2-Propenoic acid, 3-(4-methoxyphenyl)- (13.99%), 2s,6s-2,6,8,8-Tetramethyltricyclo[5.2.2.0(1,6)]undecan-2-ol (6.35%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (1.34%), n-Hexadecanoic acid (4.87%), Phytol (1.90%), Octadecanoic acid, ethyl ester (0.59%) and 2-Phenanthrenol, 4b, 5, 6, 7, 8, 8a, 9, 10-octahydro-4b, 8, 8-trimethyl-1-(1-methylethyl)-, (4bS-trans)- (4.77%).

**Production of secondary metabolites by Tissue culture**

Production of secondary metabolites by tissue culture method is a viable option for the mass production of useful secondary metabolites produced from the roots and leaves of medicinal plants without destroying their natural habitat. Advances in the area of cell cultures for the production of medicinal compounds has made possible the production of a wide variety of pharmaceuticals like alkaloids, terpenoids, steroids, saponins, phenolics, flavonoids and aminoacids. In this respect, a study was undertaken by Singh (2011) to evaluate the effect of leaf and root callus extract of *Premna serratifolia* against selected human pathogens. The study revealed an increase in inhibitory activity of root derived callus compared to the root and other extracts. The order of antimicrobial activity of *Premna serratifolia* extracts was reported as: root callus extract
natural root extract > leaf extract > natural leaf extract. In a similar investigation, Singh and his co researchers studied the efficacy of callus extracts from the root and leaf callus of *Premna serratifolia* for luteolin production and tested its anti-inflammatory activity against carrageenan induced paw edema (Singh et al., 2012).

### 2.4.3. Pharmacological studies on *Premna serratifolia*

Today, plant derived drugs have paramount importance for the treatment and prevention of diseases. Worldwide, there has been an upsurge of interests in adopting and studying traditional systems and exploring their potential applications in developing novel healthcare systems. In the traditional system *Premna serratifolia* is used for *Vatavyadhi* and *Amavatham* (rheumatism and arthritis), nerve/ neuralgic complaints; urinary complaints, *prameha* (diabetes), digestive disorders (appetizer, deranged digestion, dyspepsia, diarrhea, and also as laxative), liver diseases, cardiac disorders, chest pain, obesity, piles, glandular enlargement, muscular pain (body pain, head ache, back ache), fever, skin diseases, gonorrhea, asthma, respiratory problems [acting as *kaphagna* removing obstruction of respiratory tract] (George, 2006). Many pharmacological studies have been undertaken during the last decade to evaluate the indigenous biological properties of *Premna serratifolia* as described in traditional manuscripts and folklore literature. A brief review of the works reported with special reference to major biological activities is presented below.

- **Antiarthritic and anti-inflammatory activity**

  Rheumatic disease is one of the oldest diseases of mankind affecting the people in the active period of their life and no substantial progress has been made in achieving a permanent cure for this disease. The inflammatory process is a series of events that can be elicited by numerous stimuli such as infectious agents, ischaemia, antigen-antibody interactions and thermal or physical injury through years of ingenious synthesis and structural modifications. The present review on antiarthritic and anti-inflammatory property of *Premna serratifolia* is attempted in the above context. Few
studies were reported to validate the ethno-pharmacological claim regarding the anti-arthritic and anti-inflammatory properties of *Premna serratifolia*. Karthikeyan and Deepa (2010\(^b\), 2011) evaluated the acute toxicity and anti-arthritic activity of *Premna corymbosa* (syn. *Premna serratifolia*) ethanolic leaf extract (PCEE) in experimental animals. In the acute toxicity study, a single dose of PCEE, 2000 mg kg (-1) body weight, was administered. The animals observed for 48h showed no clinical signs, no mortality and the extract was found to be safe. To evaluate the anti-arthritic activity, PCEE in Complete Freund's Adjuvant (CFA)- induced arthritis in rats were conducted. The results indicated that the long-term treatment significantly (p<0.01) suppressed the development of chronic arthritis induced by CFA. The study thus established the anti-arthritic activity of *Premna corymbosa* (*Premna serratifolia*) leaves. In a similar study, Rajendran and Krishnakumar (2010) monitored the anti-arthritic activity of ethanolic extract of the wood of *Premna serratifolia* by Freund's adjuvant induced arthritis model in albino rats. The results of the investigation revealed significant anti-arthritic activity against adjuvant induced arthritis thereby justifying its therapeutic role in arthritic condition. Rathore *et al.* (1977) reported the anti-inflammatory property of *Premna serratifolia*. Jantan *et al.* (1996) reported significant PAF receptor binding activity for ethanolic extract of *Premna integrifolia* when Malaysian medicinal plants were subjected to their inhibitory activity. This finding provides additional evidence for the anti-inflammatory property of *Premna serratifolia*. In another study, Kumari *et al.* (2011) conducted a comparative experimental evaluation of anti-inflammatory activity of the leaves of *Premna obtusifolia* L. and *Premna latifolia* Roxb. in Charles foster rats. As revealed in this study, both species of *Premna* are having significant anti-inflammatory activity and among the two species, *Premna latifolia* was found to have better activity. According to Barik *et al.* (1992), premnazole, an isoxazole alkaloid isolated from *Premna integrifolia* L. and *Gmelina arborea* L. (Verbenaceae) demonstrated significant anti-inflammatory activity in reducing cotton pellet-induced granuloma formation in rats. As reported by Barik *et al.* (1992), the anti-inflammatory activity was comparable to that of phenylbutazone. Studies conducted by Singh *et al.*
(2012) revealed better luteolin production and good anti-inflammatory activity of the root callus extract of *Premna serratifolia* than its normal root extract against carrageenan induced paw edema.

The anti-inflammatory and antioxidant activities of *Premna integrifolia* was also studied by Gokani *et al.* (2011). As revealed in this study, pre-treatment with a single dose of methanolic extract of *Premna integrifolia* (PIM) (300 mg/kg b.w.) produced significant inhibition on carrageenan-induced rat hind paw edema, histamine induced wheal formation and acetic acid-induced mouse vascular permeation. In a 7-day study, daily administration of PIM suppressed formalin induced paw edema and cotton pellet-induced rat granuloma formation. The extract also showed significant inhibition of cyclo-oxygenase (COX-I) activity on rat uterus and plasma membrane stabilization. The study also revealed the antioxidant activity (*in vitro*) of the plant extract in terms of anti radical, superoxide scavenging, erythrocyte membrane stability, anti lipid peroxidation, hydroxyl radical scavenging, nitric oxide scavenging and reducing power (ferric thiocynate method and β-carotene bleaching test) assays. The results demonstrated the anti-inflammatory activity of *Premna integrifolia* roots in various experimental models through their antihistaminic, antikinin, COX-inhibitory and antioxidant action, justifying its traditional use.

• **Antimicrobial activity**

The root bark (Kurup, 1964; Kapoor, 2001), essential oil (Rahman *et al.*, 2011), stem wood and stem bark (Rajendran, 2010; Rajendran and Saleem, 2010) of *Premna serratifolia* were subjected to antimicrobial screening.

The antimicrobial activity of the alcoholic extract of the root bark from fresh roots of *Premna integrifolia* was studied against gram-positive organisms by Kurup (1964). The anti-bactericidal activity of the extract (in [μg/cm³]) were noted in: *Staphylococcus aureus* 0.3; *Bacillus subtilis* [long dash] 0.3 and *Streptococcus haemolyticus* [long dash] 0.25. However, the extract was not active against *E.coli,*
Salmonella typhosa and Shigella dysenteriae. Kapoor (2001) investigated the antibacterial activity of the phenolic substances from the root-bark of Premna serratifolia against Staphylococcus aureus, Bacillus subtilis and Streptococcus haemolytics. In another investigation, Rahman et al. (2011) examined the chemical composition of the essential oil of Premna integrifolia L. and tested the efficacy of the oil and various organic extracts as an antibacterial potential. The chemical compositions of the essential oil were analyzed by GC-MS. Twenty-nine compounds representing 94.81% of the total leaves oil were identified. The oil (15 μL disk⁻¹) and extracts (300 μg disk⁻¹) of Premna integrifolia displayed a great potential of antibacterial activity against Sarcina lutea (IFO 3232), Bacillus subtilis (IFO 3026), Escherichia coli (IFO 3007), Pseudomonas sp. (ATCC 13867), Klebsiella pneumoniae (ATCC 10031) and Xanthomonas campestris (IAM 1671) with their respective zones of inhibition of 12.0 ±1.2 to 22.1 ±1.2 mm and MIC values of 62.5-250 μg mL⁻¹. Rajendran (2010) studied the antimicrobial activity of stem wood and bark of Premna serratifolia against bacteria (Staphylococcus, Escherichia coli, Klebsiella pneumonia, Salmonella typhi, Salmonella paratyphi A, Salmonella paratyphi B, Pseudomonas aeruginosa, Vibrio cholera) and fungus (Aspergillus flavus, Aspergillus niger, Penicillium notatum, Candida albicans). As revealed in this study, ethyl acetate, ethanol and aqueous extracts exhibited significant antibacterial activity; n-hexane and chloroform extracts exhibited moderate antibacterial activity and these five extracts exhibited significant antifungal activity. Premna serratifolia L., was also screened to evaluate in vitro antimicrobial activity against the selected human pathogenic organisms including gram +ve and gram -ve bacterial organisms. The study suggests that Premna serratifolia L. is a promising medicinal plant for the treatment of various pathogenic diseases which in future can be developed as a potential antimicrobial agent with reduced toxicity and adverse effects when compared with the synthetic chemotherapeutic agents (Rajendran and Saleem, 2010). In another study, Singh (2011), evaluated the antimicrobial activity of natural leaves, roots of Premna serratifolia L. and its respective callus induced with help of various plant growth regulators against the selected human pathogens (Bacillus sp.,
Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Non-haemolytic Streptococci, Streptococcus epidermidis, Pseudomonas aeruginosa, Salmonella typhimurium). As reported in this study, increased inhibitory activities of callus extracts were found better than the natural plant material extracts (Singh, 2011). The results of the above mentioned studies suggest that the natural products derived from the roots, leaves and callus of Premna serratifolia can be used as potential preservatives or antimicrobial agents in food, pharmaceutical and/or agro industries.

• **Antidiabetic activity**

To date, over 250 million people worldwide are known to suffer from diabetes. The disease is now reported to be increasing in emerging and many developing countries at epidemic proportions. India is among the highest affected emerging economy country with 41 million diabetes cases reported in 2006 and a projected rise to 70 million by the year 2025 (WHO, 2012). In the above context, the pharmacological studies on antidiabetic property of Agnimantha remain relevant. Studies conducted by Kar et al. (2003) revealed the hypoglycaemic activity of Premna integrifolia along with 24 known medicinal plants. Dash et al. (2005), studied the antihyperglycemic activity of the roots of Premna corymbosa which was conducted on both normoglycemic and hyperglycemic rats at dose levels 200-400mg/kg. As revealed in this study, the extract produced marked reduction in blood glucose concentration at tested dose levels in a dose dependent manner. However in normoglycemic animals the extract at 400mg/kg dose level produced significant reduction of blood glucose at the 8th hour of administration. Thiruvenkata and Jayakar (2010) studied the effect of extracts of Premna corymbosa on blood glucose levels and serum lipid profile (total cholesterol, triglycerides, phospholipids, low density, very low density and high density lipoprotein) in the diabetic and non diabetic rats. The study revealed significant reduction in Total cholesterol, LDL cholesterol, VLDL cholesterol and improvement in HDL cholesterol in diabetic rats, there by proving the antidiabetic and antihyperlipidaemic activity of this medicinal plant in HDL cholesterol in diabetic rats (Thiruvenkata and Jayakar, 2010).
Majumder *et al.* (2014), investigated antidiabetic activity along with *in vitro* antioxidant activity of the methanolic bark extract of *Premna integrifolia* (MEPI). Oral glucose tolerance test (OGTT), normoglycemic test and alloxan induced diabetic test were conducted for the evaluation of antidiabetic activity. In addition, total phenolic content, total antioxidant activity, scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical as well as reducing power assessment were used to evaluate antioxidant potentiality of MEPI. The continuous post treatment for 120 min with the MEPI showed potential hypoglycemic activity in OGTT and normoglycemic rats. At a dose of 300 mg/kg the extract, considerable drop in elevated blood glucose level was observed in the alloxan induced diabetic (p<0.05) rat after 7 days. The study also revealed radical scavenging properties in the DPPH assay (IC = 8.61 ± 0.16µm) (Majumder *et al.*, 2014). The results of the above mentioned studies provide additional evidences to validate the traditional claim of the roots of *Premna serratifolia* for treating diabetes.

**Antiobesity activity**

Over the last few decades, there have been dramatic changes in the environment, lifestyle, life expectancy, dietary habits and behavior of people at global level. These changes have resulted in escalating obesity rates, deteriorating the quality of life and making shorter life expectancy in developed and developing countries of the world. In the above context, researchers like Ghosh and Sukumar (2009), Mali *et al.* (2013) and Patel and Patel (2012) have made pharmacological studies to evaluate the therapeutic efficacy of *Agnimantha* as an antiobesity agent. Ghosh and Sukumar (2009) studied the antiobesity property of *Premna obtusifolia* R. Br. (syn. *Premna serratifolia* L.) in 26 subjects with severe form of obesity and the results were compared to age and sex matched controlled subjects. Subjects of treatment showed remarkable decrease in body mass index (BMI), triglyceride, cholesterol-HDL ratio, uric acid, LDL-HDL ratio and midtriceps skin-fold thickness (Ghosh and Sukumar, 2009). In another investigation Mali *et al.* (2013) evaluated the anti-obesity activity of chloroform: methanol extract of *Premna integrifolia* (CMPI) in mice fed with cafeteria diet. Female Swiss Albino mice
were divided into six groups, which received normal and cafeteria diet, standard drug simvastatin (10 mg/kg) and CMPI (50, 100 and 200 mg/kg) daily for 40 days. Parameters such as body weight, body mass index (BMI), Lee index of obesity (LIO), food consumption, locomotor behavior, serum glucose, triglyceride, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), atherogenic index, organ weight and organ fat pad weight were studied for evaluating the anti-obesity activity of *Premna integrifolia*. The result of this study corroborates the result of Ghosh and Sukumar (2009). In another study, Patel and Patel (2012) evaluated the antihyperlipidaemic activity of *Premna integrifolia* on nicotine induced hyperlipidaemia in male albino rats. For the experiment, male albino rats weighing 200-250 gm were divided into 4 groups viz. Normal control; Nicotine control (NC); Nicotine control (NC) and *Premna integrifolia* (Agnimantha) (500 mg/kg) treated; Nicotine control (NC) and Atorvastatin treated (standard control). Blood samples were collected after 7 days, for lipid estimation. As noted in this study, Nicotine caused significant increase in the serum Cholesterol, Triglyceride, VLDL, LDL & significant reduction in HDL level. *Premna integrifolia* and Atorvastatin treatment showed significant prevention in increased serum Cholesterol, Triglyceride, LDL as compared to Nicotine control (NC) group. While HDL level was significantly increased in treated and standard group as compared to Nicotine control (NC) group.

- **Cardioprotective activity**

Heart failure is an extremely severe cardiac state associated with high mortality rate. In traditional system of medicine, *Premna serratifolia* is considered as an important plant having the potential to tone up the functioning of heart and circulation of blood. Rajendran and Saleem (2008) tested the cardioprotective effect of ethanol extract of *Premna serratifolia* Linn. on Isoproterenol administered experimental myocardial infarction in rats. The cardioprotective effect of ethanol extract of *Premna serratifolia* L. on Isoproterenol induced myocardial infarction in rats was confirmed by ECG study, electrophoresis analysis of serum protein, serum A/G ratio, biochemical
studies such as heart tissue proteins, glycogen, nucleic acids and blood glucose. The ethanol and aqueous extracts of stem bark and stem wood of *Premna serratifolia* were subjected to screening for their cardiac stimulant activity using isolated frog heart perfusion technique and biochemical parameters in heart tissue and serum of albino rats after administrating the extracts for 7 days (Rajendran *et al.*, 2008). The preliminary phytochemical detection and HPTLC profiling were monitored along. The ethanol extract produced significant positive ionotropic and negative chronotropic actions similar to that of digoxin on frog heart while aqueous extract produced positive ionotropic and chronotropic effects similar to that of adrenalin. A significant decrease in membrane Na+ K+ ATPase and Mg2+ ATPase and an increase in Ca2+ATPase further confirmed the cardiotonic activity. The results suggest that the ethanol extract produced cardiotonic effect and the aqueous extract produced β-adrenergic effect. Ethanol extract showed positive reaction for alkaloids, glycosides, flavonoids and steroids whereas the aqueous extract showed positive reaction for alkaloids, glycosides and phenolic compounds. HPTLC profile of ethanol extract showed 10 peaks in the solvent system of n-hexane: ethylacetate (3:1) at 260 nm and aqueous extract showed 7 peaks in the solvent system of chloroform: methanol: water (7:2.6:0.4) at 260 nm. It is believed that phytoconstituents like iridoid glycosides, alkaloids, flavonoids and phenolic compounds in *Premna serratifolia* have protective myocardial property. Anticoagulation activity of the flavonoids in *Premna integrifolia* was reported in earlier study by Gopal and Purushothaman (1984).

- **Gastro protective potential**

  Many medicinal plants and dietary nutrients have been shown to possess gastro-protective activity. In clinical practice, peptic ulcer is one of the most prevalent gastrointestinal disorders in developed countries. Treatments available for peptic ulcer is generally non specific and is usually aimed at reducing the production of gastric acid and re-enforcing gastric mucosal protection such as regular food, adequate rest and avoidance of ulcer generating soft drinks, coffee, alcohol and tobacco. In traditional
medicine, the drug *Agnimantha* is one of the specific drugs prescribed in such conditions to enhance digestive power and also to eliminate the ill effects of *kapha* and *vata*. In this context, Jothi *et al.* (2010) studied the gastro protective potential of *Premna serratifolia* L. leaves against Aspirin induced ulcer in albino rats. As revealed in this investigation, the ethanolic extract of *Premna serratifolia* has significant antiulcer and antisecretory activity when compared to the drug, Ranitidine. In a similar investigation, Rajathi and Indumathi (2013) investigated the anticulcer activity of methanolic bark extract of *Premna serratifolia* against Aspirin induced gastric ulcer models of male Wistar rats. In Aspirin induced pylorus ligation model, various parameters viz. volume and pH of gastric juice, total acidity, free acidity, ulcer score, ulcer index and percentage protection were studied. Ranitidine (50 mg/kg p.o.) was used as the standard drug. Pre-treatment with the extracts (200 & 400 mg/kg p.o.) showed significant protection against ulcer model. In Aspirin induced pylorus ligated model, the methanolic bark extract of *Premna serratifolia* showed significant decrease in the volume of gastric juice, free and total acidity, ulcer score, ulcer index and increase in pH of gastric juice as compared to the toxicant control group. In short, the antiulcer assay of the leaf and bark extract of *Premna serratifolia* revealed a decrease in gastric acid secretion thereby reducing the prevalence of causing peptic ulcer. Further, as revealed in these studies, *Premna serratifolia* possess significant antiulcer and cytoprotective effect. The activity may be due to the presence of phytoconstituents like alkaloids, iridoid glycosides and flavonoids in *Premna serratifolia*.

- **Hepatoprotective activity**

Liver is one of the most vital organs in our body that functions as the centre of metabolism of nutrients such as carbohydrates, proteins, lipids and also helps in the excretion of waste metabolites. By handling the metabolism and excretion of drugs and other xenobiotics from the body, liver provides protection against foreign substances by detoxifying and eliminating them. Liver disease is one of the most common causes of mortality for both men and women and the trend for liver disease is steadily increasing
in developed and developing countries. The key risk factors that cause individuals to develop liver disease are: excessive alcohol consumption, infection with hepatitis B and C virus, obesity and related metabolic syndrome. Today, natural products and their active principles as sources for developing novel hepatic drugs have attracted attention since these drugs have less side effects compared to synthetic drugs.

In the above context, George (2006) evaluated the hepatoprotective activity of the leaf and root extract of *Premna serratifolia* L. (500 mg/kg) by analyzing the hepatic enzyme parameters viz., alkaline phosphatase (ALP), acid phosphatase (ACP), serum glutamate oxalate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) in the blood serum and liver of albino Wistar rats after a period of 21 days treatment. The hepatoprotective activity was studied by comparing the levels of the hepatic enzymes of normal group Wistar rats with that of the treatment groups (CCl4 group & CCl4 + *Premna serratifolia* leaf/ bark extract groups). The study revealed that the leaves and roots of *Premna serratifolia* are very effective to tone up the functioning of liver by nullifying the ill effects caused by CCl4. Besides, an attempt was made to compare the hepatoprotective activity of *Premna serratifolia* with that of a proven hepatoprotective drug plant, *Eclipta alba* (500mg/kg). In another study, Vadivu et al. (2009) evaluated the hepatoprotective and in vitro cytotoxic activity of alcoholic extract of leaves of *Premna serratifolia* L. Hepatoprotective activity was studied by carbon tetrachloride induced hepatotoxicity in rats and the in vitro cytotoxic activity was carried out by tryphane blue exclusion method using EAC cell lines. The degree of protection in hepatoprotective activity has been measured by using biochemical parameters such as SGOT, SGPT, ALP, bilirubin and total protein. The results suggested that the alcoholic extract at the dose level of 250mg/kg had produced significant (p < 0.001) hepatoprotection by decreasing the activity of serum enzymes, bilirubin, and lipid peroxidation which was comparable to that of standard drug Silymarin. The alcoholic extract also does exhibit the IC50 value of 75µg/ml which indicated the significant in vitro cytotoxic activity of the extract. As it is evident from the above mentioned studies, the alcoholic extract of leaves/ roots of *Premna*
serratifolia L. is not only an effective hepatoprotective agent, but also possesses significant antitumor activity. Muthukumaran and Pattabiraman (2010) studied the hepatoprotective activity of the aqueous extract of Premna serratifolia L. against carbon tetrachloride- and paracetamol- induced hepatotoxicity in rats. As revealed in this study, Premna serratifolia exhibited significant hepatoprotective activity by reducing carbon tetrachloride- and paracetamol- induced change in bio-chemical parameters that was evident by enzymatic examination. The plant extract was interfered with free-radical formation, providing hepatic protection. Acute toxicity studies revealed that the LD sub. 50 value is more than the dose of 4 g/kg body wt. (Muthukumaran and Pattabiraman, 2010).

Studies conducted by Singh et al. (2011)\textsuperscript{a} using the ethanolic extract of root and root derived callus extracts of Premna serratifolia L. against paracetamol induced oxidative stress and hepatotoxicity in blood and liver of male albino rats provide additional evidence to supplement the results generated by earlier researchers. According to Singh et al. (2011)\textsuperscript{a}, the effects of root callus extracts were comparable to that of standard drug-Silymarine. Histopathological findings also suggest that the root callus extracts of Premna serratifolia L. is effective to prevent the development of chronic damage. It was concluded that the ethanolic extract of root callus is not only an effective hepatoprotective agent but also possess significant antioxidant activity that can be attributed to the flavanoid- luteolin and other alkaloids like premnine, ganikarin and ganiarin which helps to overcome the paracetamol-induced toxicity.

- **Antioxidant activity**

The stem bark, stem wood, roots and leaves of Premna serratifolia were screened for their *in vitro* antioxidant property by researchers like Rajendran et al. (2009)\textsuperscript{a}, Selvam et al. (2010), Shilpa et al. (2012), Jain et al. (2013), Muthukumaran et al. (2013) and Mali (2014). The stem bark and stem-wood extracted with 95% ethanol and double distilled water were screened for their *in vitro* antioxidant potential.
Inhibitions of oxygen-derived free radicals, viz., assays for free radical scavenging by DPPH, reducing power ability and nitric oxide scavenging were performed. All the antioxidant activities were compared with standard antioxidant-ascorbic acid. Both the extracts of this plant showed effective free radical scavenging activity, reducing power and nitric oxide scavenging activity. All these antioxidant properties were concentration dependent. The highest antioxidant activity was observed with ethanol extracts. Besides, the antioxidant activities of the ethanol extract of stem-bark & stem-wood were also determined by high fat diet (HFD) induced oxidative stress in rabbits (Rajendran et al., 2009). The study revealed significant antioxidant activity by lowering the enzyme levels of thiobarbituric acid reactive substance (TBARS) and by increasing the enzyme levels of catalase (CAT), glutathione (GSH) and super oxide dismutase (SOD), which was comparable with the standard Atorvastatin in a dose dependent manner and also demonstrated remarkable activities to scavenge reactive oxygen species (ROS), which may be attributed to the high amount of hydrophilic phenolic compounds in Premna serratfolia (Rajendran et al., 2009). The antioxidant activity of methanolic extract of Premna serratfolia leaf in paracetamol intoxicated Wistar albino rats was studied by Selvam et al. (2010). The experiment comprised of five groups such as healthy control group, disease control (Paracetamol treated), positive control group (paracetamol + Silymarin), treatment groups (test drug + Paracetamol) lower dose (100mg/kg b.wt.) and higher dose (200mg/kg b.wt.), having six animals in each group. The animals were maintained in the standard laboratory conditions and the test extract was administered p.o. The antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase were evaluated in the blood samples and as well as in the isolated tissue samples of liver, kidney and heart. The disease control group showed decreased status of antioxidant enzymes in blood and tissue samples. But the elevated levels of SOD, catalase, glutathione were observed significantly (p<0.05, p<0.01) in liver, kidney and heart tissue samples and blood samples of the extract administered groups. The activity was found to be dose dependent. The overall efficacy of the extract was comparable with the standard drug.
silymarin. The study thus confirmed the potential antioxidant activity of the methanolic extract of *Premna serratifolia* in the animal model system. In another study, Shilpa *et al.* (2012) investigated the *in vivo* antioxidant activity of ethanolic extract of *Premna corymbosa* (Rottl.) root against streptozotocin induced oxidative stress in different organs (liver, kidney, brain, heart and pancreas) of rats. Ethanolic extract of *Premna corymbosa* (Rottl.) root was administered orally (200 mg/kg body weight) and the effect of extract on enzymatic antioxidants like SOD, CAT, glutathione peroxidase (GPx), glutathione-S-transferase (GST) and polyphenol oxidase (PPO), non enzymatic like vitamin C, vitamin E and glutathione were determined. Lipid peroxidation like basal, ascorbate and peroxide induced lipid peroxidation were also estimated. Glibenclamide was used as standard reference drug. A significant increase in the activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase and reduced glutathione were observed in different organs of diabetic rats on treatment with 200 mg/kg body weight of *Premna corymbosa* (Rottl.) root extract and glibenclamide for 30 days treatment. Both the treated groups showed significant decrease in lipid peroxidation, suggesting its role in protection against lipid peroxidation induced membrane damage.

Jain *et al.* (2013) studied the *in vitro* antioxidant activity of aqueous and methanolic extracts of *Premna integrifolia* roots with special reference to DPPH, DMSO, ABTS, Nitric oxide and Iron chelation assay. Ascorbic acid was used as the standard. In all the models studied, the aqueous extract showed IC 50 values of 111.009, 101.369, 99.976, 109.827, 105.239μg/ml and the methanolic extract showed IC 50 values of 98.252, 96.559, 88.163, 100.631, 95.005μg/ml for DPPH, DMSO, ABTS, Nitric oxide and Iron chelation assay, respectively.

Muthukumaran *et al.* (2013) in another study, investigated the antioxidant effect of the wood extract *Premna serratifolia* by various antioxidant assays (1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2’-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and hydrogen peroxide scavenging method). The antioxidant activities were compared
to standard antioxidant- ascorbic acid. The wood extract showed a significant antioxidant activity in DPPH, ABTS and H2O2 scavenging methods. In a recent investigation, Mali (2014) evaluated the beneficial effect of extracts of *Premna integrifolia* root on human leucocytes and erythrocytes against hydrogen peroxide (H2O2) induced oxidative damage. Chloroform: methanol (1:1) extract of *Premna integrifolia* (CMEPI) and aqueous extract of *Premna integrifolia* roots were used to screen CAT, SOD, GPx, GSH and LPO levels in H2O2 induced oxidative damage. The study revealed that, there was an increase in the CAT, SOD, GPx and reduction of the GSH and LPO levels in H2O2 group compared with the control. *Premna integrifolia* root extract treated groups showed the reduction of CAT, SOD, GPx and increased in the GSH and LPO levels as compared with H2O2 group. CMEPI was found to be more effective than aqueous. The study suggests that, extracts of *Premna integrifolia* root possess beneficial effect on human leucocytes and erythrocytes against H2O2 induced oxidative damage, which has substantiated their use in ethnomedicine as an antioxidant. Observed effect was attributed due to the flavonoid and phenol contents in the plant.

The results obtained from the above mentioned studies indicated that entire parts viz., leaves, stem and roots of *Premna serratifolia* are potential source of natural antioxidants. The *in vitro* antioxidant activity of this plant shows the presence of antioxidant principles present in its roots, stem and leaves. These findings points to the great interest of the *Premna serratifolia* whose phytochemistry and phytopharmacology should be subjected to detailed investigation so as to bring forth all possible phytotherapeutic uses of the species, *Premna serratifolia* and its related species in different parts of the world.

- **Immunomodulatory, Antinociceptive, Analgesic, Neuropharmacological, Anticonvulsant, Tumor cell suppression and Anti-parasitic activities**

Studies on various pharmacological aspects such as immunomodulatory, antinociceptive, analgesic, neuropharmacological, anticonvulsant, tumor cell
suppression and anti-parasitic activities of *Premna serratifolia* L. were subjected to
detailed investigations by researchers in recent years. The results emerged from these
studies are summarized below.

**Immunomodulatory potential:** Immunomodulatory potential of *Agnimantha*
drugs with special reference to the roots *Clerodendrum phlomidis* and *Premna*
integrifolia were investigated by Gokani *et al.* (2007). Methanol root extracts of the
above mentioned plants (300 mg kg\(^{-1}\) x 7 days) were administered orally to mice prior
to immunization with Sheep Red Blood Cells (SRBC) and it resulted in a significant
increase in haemagglutinating antibody titre, plaque forming cell assay and delayed type
hypersensitivity to SRBC. *Clerodendrum phlomidis* and *Premna integrifolia* enhanced
the non specific immune response in carbon clearance test and showed significant
immunoprophylactic effect, when tested on *E. coli* induced abdominal sepsis. In the
study *Clerodendrum phlomidis* showed higher response to specific immune activity as
compared to *Premna integrifolia*, where as in case of non specific immune activity both
the roots showed almost equal response.

**Antinociceptive activity:** Karthikeyan and Deepa (2010)\(^a\) evaluated the acute
toxicity and antinociceptive activity of an ethanolic extract of *Premna corymbosa* in
animal models. In the acute toxicity study, the ethanolic extract showed no clinical
signs and mortality of the animals and was found to be safe. In the acetic acid-induced
writhing model, the ethanolic extract at a dose of 200 or 400 mg kg\(^{-1}\) body weight
significantly (p<0.01) inhibited the writhing response by 42.57\% and 54.67\%,
respectively. In the hot plate test, the extract produced a significant (p<0.01) increase in
latency with 34.50\% and 51.08\% of protection in a dose-related manner. The study
established the analgesic properties of *Premna corymbosa*. It is speculated that \(\beta\)-
sitosterol or luteolin in the ethanolic extract might have contributed to the
antinociceptive activity of *Premna corymbosa*. 

**Analgesic, Neuropharmacological and Anticonvulsant property:** In traditional medicine, *Premna serratifolia* is recommended as an analgesic to relieve body pain, back ache and head ache. In this backdrop, Karmakar *et al.* (2011) assessed analgesic activity of the ethanolic extract of the leaves of *Premna integrifolia* using acetic acid induced writhing model in mice. In analgesic activity, the extract produced 52.17% (p<0.01) acetic acid induced writhing inhibition in mice at the dose of 500 mg/kg body weight, which was comparable to diclofenac sodium 65.21% (p<0.01) at the dose of 25 mg/kg body weight.

In a recent investigation, Khatun *et al.* (2014) studied **neuropharmacological, analgesic, and anti-inflammatory activities** of the methanolic bark extract of *Premna integrifolia* (MEPI). Neuropharmacology study was done by open field and hole-cross test whereas acetic acid writhing test and formalin induced pain was done for analgesic activity of MEPI. Carrageenan induced inflammatory model was considered for anti-inflammatory activity evaluation. A statistically significant (p<0.05) decrease in locomotor activity was observed at all doses in the open-field and hole-cross tests. The extract significantly (p<0.05) and dose dependently reduced the writhing reflex in the acetic acid-induced writhing test as well as licking response in the formalin induced inflammatory pain. At 200 mg/kg body weight dose, MEPI showed 71.16% inhibition in carrageenan induced anti-inflammatory activity.

Baby *et al.* (2011) evaluated the **anticonvulsant** effect of the extract of *Premna corymbosa* against Pentylenetetrazole and maximal electroshock induced convulsions in mice.

**Tumor cell suppression potential:** Selvam *et al.* (2012) evaluated the free radical scavenging activity and tumor cell suppression potential of the methanolic leaf extract of *Premna serratifolia* in various in vitro model systems. The superoxide radical scavenging activity, nitric oxide radical, hydroxyl radical, DPPH radical and ABTS radical scavenging activity and lipid peroxidation were determined. The tumor cell
suppression cell potential was determined in three different cancer cell lines MCF7 (breast cancer), HepG2 (liver cancer) and A549 (lung cancer) by SRB assay. As noted in this study, the methanolic extract of Premna serratifolia has free radical scavenging activity against superoxide radical, nitric oxide radical, hydroxyl radical, DPPH radical, ABTS radical and also has the potential to inhibit lipid peroxidation. The efficacy was dose dependent as it is very clear from the IC 50 value. The test extract showed cytotoxic activity against MCF7, HepG2 and A549 cell lines. The GI50, TGI and LC50 values were determined against each cell line and compared with standard drug Adriamycin. Thus the study proved the free radical scavenging activity and tumor cell suppression potential of Premna serratifolia leaf in the selective in vitro model systems.

**Anti-parasitic activity:** Desrivot et al. (2007) reported antiparasitic activity for Premna serratifolia against Leishmania donovanim with IC 50 values between 0.5-5µg/ml.

In short, the pharmacological evaluation studies on Premna serratifolia L. so far reported with special reference to antiarthritic, anti-inflammatory, antimicrobial, antidiabetic, antiobesity, cardioprotective, gastro protective, hepatoprotective and antioxidant activities have shown promising results for bioprospecting Premna serratifolia L. as a ‘Neutraceutical Panacea’.

2.5. Conclusion

The taxonomic review of the genus Premna and the species Premna serratifolia L. in particular revealed much perplexity in delimiting the different species of the genus Premna with special reference to Premna integrifolia, Premna serratifolia, Premna corymbosa and Premna obtusifolia and also with respect to different morpho-variants of Premna serratifolia. Even though many studies have been undertaken by earlier researchers based on the morphology and taxonomy of related species of Premna serratifolia, the confusion was not fully resolved due to high polymorphism among different morphotypes of this species. In the Ayurvedic/ traditional systems of medicine,
Premna serratifolia has a significant position. However, this plant has a controversial drug status in Ayurveda since the source drug Agnimantha is equated to many species like Clerodendrum phlomidis L., Clerodendrum inerme (L.) Gaertn., Premna serratifolia L., Premna latifolia Roxb. and Premna obtusifolia L. in different parts of India. The major source of the drug in Eastern and Central parts of India is Premna obtusifolia and that in the North and Western regions is Premna latifolia. Clerodendrum phlomidis is considered as the source plant in certain North Indian states and in Gujarat state while Clerodendrum inerme is the accepted source plant of Kshudragnimantha in certain parts of South Indian states especially Kerala state. This controversial drug status of Premna serratifolia with other substitute medicinal plant species viz., Clerodendrum phlomidis and Clerodendrum inerme necessitates further studies based on phytochemical and pharmacognostic characteristics of these plant species. The earlier pharmacognostic and phytochemical studies reported were based on either limited samples or morphotypes of Premna serratifolia. A perusal of literature revealed that no research studies have so far been done based on the comparison of pharmacognostic and phytochemical characteristics of the different morphotypes of Premna serratifolia L. in Kerala. The present investigation is an attempt in this context and it aims to study and compare the different morphotypes of Premna serratifolia in Kerala using relevant tools and techniques in Phytochemistry and Pharmacognosy. It is expected that the results emerged from this study will be helpful to resolve the controversial status of Premna serratifolia in taxonomy and Ayurvedic system of medicine.