1. INTRODUCTION

1.1 General Introduction

Plants are being used as medicine for combating diseases since times immemorial and have fitted the immediate personal need of man as they are accessible and inexpensive. The Indian subcontinent is enriched by a variety of flora - both aromatic and medicinal plant due to diversity of climatic conditions ranging from desert to swamplands. This extensive flora has been greatly utilized as a source of many drugs in the Indian traditional system of medicine. Plants and herbs were used extensively for cures and general well being around the world. But it was only in period of the Ayurvedic Samhitas that there were serious attempts in studying plants scientifically (Agarwal and Paridhavi, 2007).

Plants are the only economic source of number of well-established and important drugs. In addition, they are also the source of chemical intermediates needed for the production of some drugs and it is gratifying to note that the World Health Organization have shown an abiding interest in plant derived medicines, described in folklore of various countries. Additional factors accountable for the continued and extensive use of herbal remedies in India are their effectiveness, easy availability, low cost, comparatively less toxic effects and the shortage of practitioners of allopathic medicine in rural areas. The traditional system of medicine is so engrained in our culture that, even now an estimate 70% of the Indian population depends on this indigenous system for relief. With such a huge section of ever increasing population relying on herbal remedies, it is imperative that the plant products which have been in use be scientifically supported for their efficacy.

Ayurveda is an original holistic system of medicine whose principles of therapeutics are applicable universally. The name Ayurveda is derived from two words: Ayur meaning "life" and Veda meaning “Knowledge or science” i.e., the "Science of Life". Ayurveda system of medicine as mentioned developed over a long period of time with the Rigveda, one of the oldest repositories of human knowledge, mention use of 67 plants for therapeutic use. Ayurvedic knowledge and experiential database can provide new functional leads to reduce time, toxicity and money, the three main hurdles in drug development (W.H.O., 2000).
In this 21st Century, researcher have no doubt that nature is still the preeminent synthetic chemist and that in plants particularly, there are infinite reserve of fascinating chemical constituents with actual and potential effects on human body. As example, importance of plants as a source of useful anti-hypertensive was supported by the isolation of reserpine from Rauwolfia serpentina, by Muller et al. in 1952. Taxol and vincristine have been isolated for treatment of cancer from plant source Taxus baccata linn which is shauneyaka in Ayurveda and plant Vinca rosea known as sadabahar. Natural products research continues to explore a variety of lead structures, which may be used as template for the development of new drugs by the pharmaceutical industry (Harborne J.B., 1998).

In Ayurvedic system of medicine the genus Clerodendrum is a large in demand on account of its value in treatment of many chronic and acute diseases and disorders. No scientific work is yet reported on Clerodendrum splendens regarding its Chemical constituent & anti-asthmatics, anti-inflammatory potential. In present research work Clerodendrum splendens (Family: Verbenaceae) is selected. Leaves, stem and flowers of this plant are used to investigate for pharmacognostic, phytochemical and pharmacological studies.

In the present work scientific study will be done to validate pharmacognostic profile helpful in developing standards for quality, purity, identification along with chemical constituent & anti-asthmatics, anti-inflammatory potential.
1.2. Introduction to plant *Clerodendrum splendens*

1.2.1 **Plant profile:**

*Clerodendrum splendens* (glory tree, flaming glorybower) is a species of genus *Clerodendrum* belonging to family Verbenaceae.

**Common/ vernacular names:**

Sanskrit: Angaravallari, Phanjika, Yashti

English: Flaming glory bower

Hindi: Barangi

Marathi: Bharangi

1.2.2 **Taxonomic classification:** (http://www.gbif.org)

- **Kingdom:** Plantae
- **Family:** Lamiaceae / Verbenaceae
- **Genus:** Clerodendrum
- **Species:** splendens

1.2.3 **Distribution:**

The genus *Clerodendrum* [Family Laminaceae (Verbenaceae)] is widely spread in the tropical and subtropical regions of the world, with most of the species occurring in tropical Africa and Asia. The first description of the genus was given by Linnaeus in 1753, with the identification of *C. infortunatum*. Clerodendrum genus has more than 500 species and compromises from herbs to small tree (Moldenke, 1985; Rueda 1993).

1.2.4 **Botanic Description of Clerodendrum splendens:**

*Clerodendrum splendens* also known as the Flaming glory bower is a woody or semi-woody evergreen vine which grows to about 3.7 m long and climbs by twining. It has ovate to oblong lustrous dark green leaves which are arranged in opposite pairs (Huxley, 1992). The flowers are salverform (tuba shaped) with a slender tube and an abruptly expanded corolla. They are with red flowers, of persistent calyx produced in profusion at the tip of the vine.

1.2.5. **Ethnomedicinal claims:**

The leaves, roots and stem extracts of *Clerodendrum splendens* are used extensively in traditional medicine for treating many diseases in African folklores. *Clerodendrum*
splendens is used for hemorrhoids, sinus disease, menstrual troubles, diarrhea, healing scars, and as a febrifuge and vermifuge agent. The leaves and stem are also used to treat scrofulous infection. Decoction in water (boiling) is the most common mode of preparation. Many plant species in the genus Clerodendrum have been used majorly in the treatment of disorders like asthma and inflammation.

1.2.5.1. Leaves:
The leaves decoction consumed; part of the decoction was mixed in shea butter and applied locally to hemorrhoids. Leaves vapour bath inhale once a day for 3 days for sinus disease. Leaves decoction is consumed 2 times a day for 2 months for menstrual troubles in women. Leaves decoction consumed 3 times a day for 3 days for relief in diarrhea. Pounded fresh leaves rubbed on the scars for healing skin infection. Leaves decoction is used as Febrifuge and vermifuge consumed 2 times a day for 1 week (Kouakou et al., 2013).

1.2.5.2. Stem:
The pounded or crashed fresh leaves and stem were applied or rubbed on skin infection (Kouakou et al., 2013).

1.2.5.3. Root:
The roots and leaves decoction are used for the treatment of skin diseases, cancer, syphilis, gonorrhea, ulcers, and various inflammatory diseases (Okwu et al., 2009).
Figure 1: Various parts of *Clerodendrum splendens* plant. 
A - Flower, B – Stem, C – Plant, D – leaf
1.3 Review of Literature

1.3.1 Phytochemistry review of genus Clerodendrum

The genus Clerodendrum [Family Verbenaceae)] is widely spread in the tropical and subtropical regions of the world, with most of the species occurring in tropical Africa and Asia. The first description of the genus was given by Linnaeus in 1753, with the identification of C. infortunatum. Clerodendrum genus has more than 500 species and compromises from herbs to small tree. Clerodendrum species are extensively used in various indigenous systems of medicine for the treatment of many diseases around the world. Various literature reviews for biological activities of these species are associated with chemical constituents present in species (Shrivastava and Patel, 2007). Clerodendrum genus are rich source of phenolic, flavonoid, diterpenoid and steroidal compounds hence plant extracts are traditionally reported to be used for remedial purpose in asthma, inflammatory, cancer, diabetes and malaria.

1.3.1.1. Flavonoids

Flavonoids are one of the major groups present in Clerodendrum genus possessing promising biological activities. Kaempferol [1] has been isolated from the leaves of C. fragrans (Gao et al., 2003). Apigenin [2] has been isolated from the stem and leaves of C. inerme (El-Shamy et al., 1996), from whole plant of C. trichotomum (Min et al., 2005), from the flowers of C. infortunatum (Sinha et al., 1981). It has also been isolated from the flowers of C. phlomidis (Seth et al., 1982) and also from the stem of C. serratum (Jaya et al., 1997). Scutellarein [3] has been isolated from the stem of C. serratum (Jaya et al., 1997) and from the flower of C. indicum (Gunasegaran et al., 1993). The flavone cirsimaritin [4] from the root bark of C. mandarinorum (Zhu et al., 1996). Hispidulin [5] has been isolated from the flowers of C. phlomidis (Roy and Pandey, 1994) and from the flowers of C. indicum (Gunasegaran et al., 1993). Luteolin [6], a major flavonoid has been isolated from the flowers of C. phlomoidis (Roy and Pandey, 1994) and from the roots and leaves of C. serratum (Nair, et al., 1976). Salvigenin [7] has been isolated from the leaves of C. inerme (Raha and Das, 1989). 7-Hydroxyflavanone [8] has been isolated from the leaves and flowers of C. phlomidis (Roy and Pandey, 1994). Quercetin-3-methyl ether and quercetin [9] has been isolated from roots and flower of C.
mandorinorum (Zhu et al., 1996) and *C. infortunatum* (Singh et al., 1980). Pectolinarigenin [10] has been isolated from flowers of *C. tomentosum* (Pal et al., 1989).
**CHAPTER 1**

**INTRODUCTION**

Pharmacognostic, Phytochemical and Pharmacological studies on *Clerodendrum splenden*.

*Family* - Verbenaceae.

**Figure 2:** Structures of flavonoids reported from *Clerodendrum* plants.

1.3.1.2. Terpenes

Many terpenoids have been reported from this genus and includes: monoterpenes, diterpenes, triterpenes, iridoids and sesquiterpenes. The triterpene α-amyrin [11], has been isolated from the roots and stem of *C. fragans* (Singh and Singhi, 1981) and from the leaves and stem of *C. inerme* (Singh and Prakash, 1983). β-amyrin [12], another triterpene has also been isolated from the roots of *C. colebrookianum* (Joshi et al., 1979), from the leaves and stem of *C. inerme* (Singh and Prakash, 1983) and from the roots of *C. paniculatum* (Joshi et al., 1979). Betulin [13] and oleanolic acid [14] have been isolated from the leaves and stem of *C. inerme* (Singh and Prakash 1983). Friedelin [15] has been isolated from the stem of *C. cryophyllum* and *C. inerme* (Tian et al., 1993; Rao et al., 1993). Lupeol [16] added triterpene has been isolated from the stem bark and roots of *C. neriifolium* (Ganapaty and Rao, 1985) and (Zhua et al., 1996). The diterpene...
Clerodendrin A [17] has been isolated from the roots of *C. phlomoidis* (Joshi et al., 1979). Clerodendrin B and C [18] have as well been isolated from the leaves of *C. inerme* (Rao et al., 1993) & Clerodendrins (A-H) is been isolated from whole plant of *C. trichotomum* (Kawai et al., 1998). The diterpenes clerodinin A, B and C [19] have also been isolated from the leaves of *C. brachyanthum* (Lin et al., 1989). Clerodin [20] which is a diterpene has been isolated from the flowers of *C. infortunatum*, from the leaves of *C. brachyanthum* (Lin et al., 1989) and from the roots of *C. phlomoidis* (Joshi et al., 1979). Two new diterpenes, bungone A and B, together with three known compounds, uncinatone [21], teuvincenone F and sugiol have been isolated from the stem of *C. bungei* (Fan et al., 1999). The iridoid monoterpene, harpagide [22] has been isolated from the leaves of *C. tomentosum* (Jacke and Rimpler, 1983). Ajugoside [23] has as well been isolated from the leaves of *C. thomsonae* (Lammel and Rimpler, 1981). Mi-saponin A [24] is been reported as isolated from roots of *C. wildi* (Toyota, 1990).
Pharmacognostic, Phytochemical and Pharmacological studies on
*Clerodendrum splendens*. Family- Verbenaceae.
Figure 3: Structures of terpenoids reported from Clerodendrum plants.
1.3.1.3. Steroids

*Clerodendrum* genus contains steroids as major class of chemical constituents. Clerosterol [25] has been isolated from the aerial part of *C. fragrans* (Singh and Singhi, 1981), also been reported in aerial parts of *C. infortunatum* (Thakur et al., 1988), *C. inerme* (Rehman et al., 1997) and *C. nutans*, (Joshi et al., 1985), isolated from stem of *C. cryptophyllum* (Tian et al., 1993) and leaves and stem of *C. inerme* (Akihisa et al., 1989), the root bark of *C. mandarinorum* (Zhu et al., 1996) and the roots of *C. phlomidis* (Joshi et al., 1979). β-sitosterol [26] has been reported to be present in the roots of *C. serratum* (Jaya et al., 1997), *C. paniculatum* (Joshi et al., 1979) and *C. fragrans* (Singh and Singh, 1981). β-sitosterol has also been reported in the leaves of *C. inerme* (Singh and Prakash, 1983), *C. infortunatum* (Joshi et al., 1978) and *C. neriifolium* (Ganapaty and Rao, 1989), and in the leaves and aerial parts of *C. colebrookianum* (Goswami et al., 1996). β-sitosterol has as well been reported in the aerial parts of *C. nutans* (Joshi et al., 1985) and in the stems of *C. fragrans* (Singh and Singh, 1981) and *C. indicum* (Prakash and Garg, 1981). Stigmasterol [27] was reported from aerial part of *C. cryptophyllum* (Wu, 1980) and *C. serratum* (Banerjee et al., 1969). Colebrin A [28] and B [29] have been isolated from the aerial parts of *C. colebrookianum* (Yang et al., 2000). Cholesterol [30] and 22-Dehydroclerosterol [31] have been isolated from the leaves and stem of *C. fragrans* (Akihisa et al., 1988). Campesterol [32] has been reported to be present in the leaves of *C. neriifolium* (Ganapaty and Rao, 1989), the leaves and stem of *C. scandens* (Akihisa et al., 1990) and *C. inerme* (Akihisa et al., 1989). Steriods such as taraxerol, glochidone, glochidonol, glochidiol were isolated from *C. bungei* (Gao et al., 2003).
Figure 4: Structures of steroids reported from Clerodendrum plants.
1.3.2. Phytoconstituents from Clerodendrum splendens

Steroidal compounds like 24β-ethylcholesta-5, 22, 25-triene-3-β-ol [33], clerosterol [25] and cycloartenol [34] have been isolated from leaves of *C. splendens* (Pinto et al., 1985). β-Amyrin [11] and Clerodolone [35] have been isolated from the aerial parts of the plant (Joshi et al., 1985). The flavonoids apigenin [2] and hispudilin [5] including its glycoside have been isolated from the leaf extract of the plant (Shrivastava and Patel, 2007). Four clerodane diterpenes, 2α-acetoxy-3β-(2’,3’-diacetoxy-2’-methyl)-butanoyloxy-14-hydro-15 hydroxyclerodin [36], 2α-15-dihydroxy-14-hydro-clerodin [37], 2α,15-dihydroxy-3β-(2’-hydroxy-2’-methyl-3’-acetoxy)-butanoyloxy-6α,18-diacetoxy-4α,17-epoxy-clerodan 11,16-lactone [38], and 3β,14S,15-trihydroxy-6α,18-diacetoxy-4α,17-epoxy-clerodan-11,16-lactone [39]. One new phenylpropanoid β-(3,4-dihydroxyphenyl)ethyl-O-β-D-xylopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-6-O-caffeoyl-β-D-glucopyranoside [41], along with nine known compounds, 14,15-dihydro-15-hydroxy-3-epicarioptin [40], phlinoside B, verbascoside, isoacteoside, hispidulin-7-O-β-D-glucopyranoside, hispidulin-7-O-neohesperoside, luteolin-7-O-neohesperoside, rosmarinic acid and 2β-angeloyloxy-5β-hydroxy-7α,10β-methyl-eudesm-3-ene-1-one were isolated from aerial part of plant (Laura Faiella et al., 2013).
Pharmacognostic, Phytochemical and Pharmacological studies on *Clerodendrum splendens*. Family- Verbenaceae.
Pharmacognostic, Phytochemical and Pharmacological studies on Clerodendrum splendens. Family- Verbenaceae.

CHAPTER 1

INTRODUCTION

Pharmacognostic, Phytochemical and Pharmacological studies on Clerodendrum splendens.

Family - Verbenaceae.

1.3.3. Pharmacological review of genus Clerodendrum

Following pharmacological activities were reported by researcher in published literature.

1.3.3.1. Anti-histaminic, anti-allergic and anti-asthmatic Activity

Several scientists have highlighted anti-allergic and anti-asthmatic potential of Clerodendrum serratum (Gupta, 1968; Gupta, 1971; Gupta and Tripathi, 1973; Hazekamp and Verpoorte, 2001) in the bronchial hyper-reactivity study on aqueous extract of root and stem at high (180 mg/kg) and low (90 mg/kg) doses using milk induced leucocytosis in mice and egg albumin induced asthma in guinea pigs. These studies signified the use of C. serratum roots (180 mg/kg) for anti-allergic and anti-inflammatory diseases like asthma (Bhangare et al., 2012). Alcoholic extract (100 and 200 mg/kg) of C. serratum roots showed potent anti-asthmatic activity in ovalbumin induced experimental mice model (Thalla et al., 2012). Other investigators have also confirmed the anti-asthmatic potential of C. serratum roots through several in-vivo and in-vitro screening models in guinea pig and mice (Bhujbal et al., 2009, 2010).

1.3.3.2. Anti-inflammatory Activity

Many species of the genus Clerodendrum showed potent anti-inflammatory activity. Narayanan et al., 1999 reported alcoholic extract of roots of C. serratum showed significant anti-inflammatory activity in carrageenan and also in the cotton pellet model in experimental mice, rats and rabbits. C. phlomidis was reported for significantly

Figure 5: Structures of compounds reported from Clerodendrum splendens plant.

\[
\beta-(3,4\text{-dihydroxyphenyl})\text{ethyl}-\beta-D\text{-xylopyranosyl}\text{-}(1\rightarrow2)-\alpha-L\text{-rhamnopyranosyl}(1\rightarrow3)-6\text{-O-tcaffeyl}\beta-D\text{-glucopyranoside [41]}
\]
decreasing paw edemas induced by carrageenan in rats at a dose of 1g/kg (Surendrakumar, 1988). *C. petasites* plant extract was reported to show moderate anti-inflammatory activity by inhibiting prostaglandin synthesis in acute phase of inflammation in rats (Panthong et al., 2003).

**1.3.3.3. Antimicrobial Activities**

The antibacterial potential of *Clerodendrum* species was evaluated for gram positive (*Staphylococcus aureus* and *Staphylococcus haemolyticus*) and gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria. George et. al., (1949) reported antibacterial activity with alcoholic extract of leaves and flower of *C. inerme*. Misra et al. (1995) reported that hexane extract of the leaves of *C. colebrookianum* at concentrations of 1000 and 2000 ppm also showed strong antibacterial activities. Two flavonoids from roots of *C. infortunatum*, cabruvin showed activity against *Alternaria carthami* and *Helminthosporin oryzae* and quercetin showed activity against *Alternaria alternate* and *Fusarium lini* (Roy et al., 1996). Mi-saponin-A, a triterpenoid saponin isolated from the roots of *C. wildii*, showed potent antifungal activity against *Cladosporium cucumerinum* (Toyoto et al., 1990). The leaf extracts of *C. inerme* in ethyl acetate and hexane, at 1mg/ml exhibited activity against both animal and plant fungi, *Epidermophyton floccosum*, *Trichophyton mentagrophytes*, *Trichophyton tonsurans*, *Aspergillus flavus* and *Aspergillus niger* (Anitha and Kannan, 2006).

**1.3.3.4. Antimalarial Activities**

The alcoholic extract of *C. phlomidis* showed antimalarial activity against *Plasmodium falciparum* with an IC50 value of 48 μg/ml (Simonsen et al., 2001). Another Indian species, *C. inerme* also inhibited the growth of larvae of *Aedes aegypti*, *Culex quinquefasciatus* and *Culex pipiens* at 80 and 100 ppm concentration of petroleum ether and ether extracts (Gayar and Shazll, 1968; Kalyanasundaram and Das, 1985). *C. myricoides* a species from Southern Africa was also tested positive for its antimalarial activity against both sensitive and resistant strains of *P. falciparum* with IC50 < 30 μg/ml (Muregi et al., 2004), it also showed 31.7% suppression in parasitaemia against cloroquine tolerant strain of *Plasmodium berghei* NK65 (Muregi et al., 2007). These plants may be useful as a source for novel anti-plasmodial drugs/compounds from natural origin (Shrivastava et al., 2007).
1.3.3.5. Antioxidant Activities

*C. inerme* have been reported as antioxidant drugs in various indigenous systems of medicines (Masuda et al., 1999). Organic and aqueous extracts of *C. colebrookianum* showed significant inhibition of lipid peroxidation in vitro and in vivo induced by FeSO4-ascorbate in rats (Rajlakshmi et al., 2003).

1.3.3.6. Other Activities

Antidiarrheal activity from leaves extract of *C. multiflorum* was reported by Rani et al. (1999) and 0.1% leaf juice showed anthelmentic activity against *Ascaris lumbricoides*, *Phreitima posthuma* and *Taenia solium* (Garg and Sidique, 1992). Ethanolic extract (2.25-9.0 mg/ml) of *C. petasites* evaluated for spasmolytic activity in guinea-pigs showed spasmolysis on tracheal smooth muscles; it also relaxed the smooth muscle which was contracted by exposure to histamine (Hazekamp, 2001). The hexane and methanolic extracts of the whole plant of *C. phlomidis* at concentrations of 100, 300 and 500 mg/kg body weight were established to reduce yeast-induced pyrexia in rats (Ilango et al., 2009).

*C. pholmidis* Methanolic leaf extract showed antispasmodic activity in mice (Murugesan et al., 2011).

1.3.4. Pharmacological activities of *Clerodendrum splendens*

Kouakou et al. (2013) have reported on immunomodulatory activity of polysaccharide isolated from leaves of *C. splendens*. Gbedema et al., (2010) have reported on the antimicrobial and wound healing activities of the leaves of *C. splendens*. A methanolic extract exhibited MIC ranging from 64 to 256 μg/ml against the wild type organisms. The plant extract also significantly promoted in vivo wound healing and wound contraction (69.2%) in 7 days as compared to the control (46.2%) and nitrofurazone (67.5%) when 100 mg of a 33.3% w/w ointment of *C. splendens* was applied 500 mm² area to an excision wound on Male Sprague- Dawley rats.