Chapter 1:
Introduction, Plant Profile and Literature Review

1.1 Introduction

1.2 Plant Profile and Literature Review

1.3 Objectives

1.4 References

1. Introduction

1.1 General Introduction

In 1820, an ancient herbal cure was transformed into a chemical drugwith the isolation of quinine from Cinchona. Western scientists initiated toreinvent traditional herbal cures by extracting their active principles to create new and profitable drugs.

Ancient literatures of world of medicine suggest that the primitive people of antiquity and those of earlier centuries have been using several kinds of medicinal plants for combating diseases. The herbal medicinal of ancient times practised by the Assyrians (4000 B.C.), Sumerians (3500 B.C.), Indians (3500 B.C.), Chinese (3000 B.C.) and Egyptians (2500 B.C.) was temporarily subdued under the influence of modern medicine, but now has staged a comeback and an `herbal renaissance' is blooming across the world.

Natural product is a chemical compound produced by a living organism which has medicinal value, pharmaceutical use and can be used for drug discovery and development. Nowadays natural product research determines for modern drug development that comes from or derived from natural resources. Phytochemicals of natural product includes terpenoids, steroids, glycosachharides, polyketides, flavonoids, cardiac glycoside, sterols, amino acids, protein, carbohydrates, lipid and fat, nucleic acid bases, ribonucleic acids (RNA), deoxyribonucleic acid (DNA) etc (Spainhour,2005).

Synthetic and combinational chemistry have scientific and technical aspects and advantages too but still natural productshave some unknown novel compounds hidden in them which can be miraculous to some diseases and have enormous contribution to drug discovery still today

(Franswoth et al., 1985; Balandin et al., 1993). The development of novel compounds from natural resources presents obstacles and many difficulties like insufficient resources of samples, poor collection and extraction, inappropriate purificationand isolation of active components from the sample may be faced but these problems are usually not seen in case of synthetic compounds. Chromatographic analysis showsthat more than 60% compound in approved drugs are obtained from natural recourses. In recent years many drugs from these natural resources has gone for first phase trials for many critical diseases. Discovery of efficient traditional medicinal plants with their active components against many diseases has been facilitated by newer bioassay methods. Bioactive components are secondary metabolites in majority. In China the traditional medicine is called traditional Chinese medicines, in Japan it is Campo, in Indonesia it is Jamu, and in India Ayurvedic medicine. Natural products and related drugs are used to treat 87% of all categorized human diseases including bacterial infection, cancer and immunological disorder (Newman et al., 2007). About 25% ofprescribeddrugs in the world originate from plants (Rates, 2001) and over 3000 speciesofplants have been reported to have anticancerproperties (Graham, 2000).

India has a rich and esteemed heritage ofherbal medicines among the South Asian countries. Various species ofmedicinalplants are estimated as growing inIndia and most of the species ofthem are used for the preparationoftraditional medicines. However, the majority of these plantshave not yet undergone chemical, pharmacological and toxicologicalstudies to investigate their bioactive compound(s). Traditionalrecords and ecological diversity indicate that Indianplantsrepresent an exciting resource for possible lead structuresin drug design. However, in order to make these remedies acceptable to modern medicine, there is a need

toscientifically evaluate them to identify the active principles and understand the pharmacological action (Vaidya, 1998).

1.1.1 Medicinal plant and herbal utilization

According to the World Health Organization, "a medicinal plant is the plant which containssubstances that can be used for therapeutic purposes, or which areprecursors for chemopharmaceutical semisynthesis".Medicinal plants are avital source of herbaland synthetic drugs. According to Alves and Rosa (2007), 20,000 plant species are used for medicinal commitments. India and China have been on the front position when one refers to the history of herbal drugs. The traditional systems of medicines viz. Ayurveda, Siddha, Unani, Homeopathy, Western Herbal Medicine and Traditional Chinese Medicine have origins in medicinal herbs. The Napralert database at the University of Illinois establishes ethno medicinal uses for about 9200 of the 33,000 species of monocotyledons, dicotyledons, gymnosperms, lichens, pteridophytes, and bryophytes. Patterns of herbal utilization are depicted in Figure 1.1

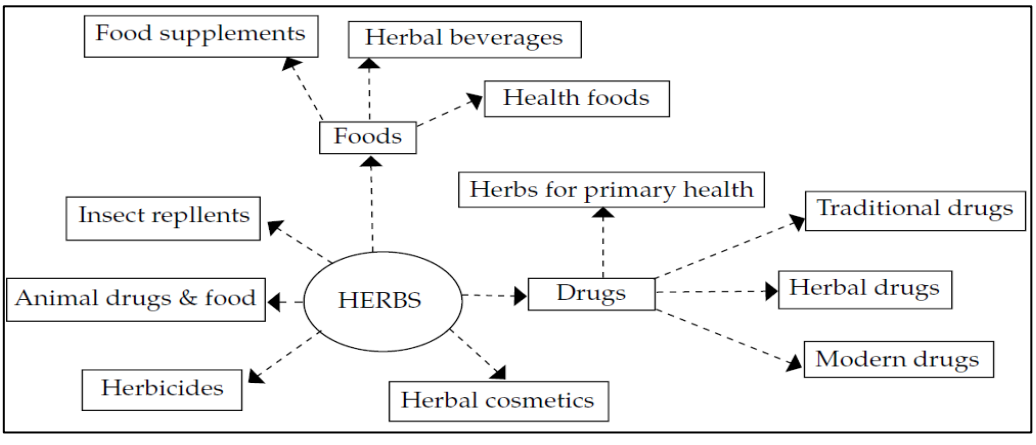

Figure 1.1 Patterns of herbal utilization.

1.1.2 Antioxidant agents from medicinal plants

As antioxidant is a molecule capable of preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electron from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radicals intermediates, and inhibit other oxidation agents such as thiols, ascorbic acid or polyphenols (Sies, 1996). Although oxidation reactions are crucial for life, hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and peroxidase. Low levels of antioxidants, or inhibition of the antioxidants enzymes, cause oxidative stress and may damage cells.

First line defense

These are enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GTX), glutathione reductase and some minerals like Se, Mn, Cu etc. SOD mainly acts byquenching of superoxide (O_2), catalase by catalyzing the decomposition of hydrogenperoxide (H_2O_2) to water and oxygen. GTX catalyses the reduction of H_2O_2 and

lipid peroxide generated during lipid peroxidation to water using reduced glutathione as substrate.

Second line defense

These are glutathione, Vit. C, uric acid, albumin, Vit. E, carotenoids, flavonoid etc. Vit. C interacts directly with radicals likeO_2, $OH^{\cdot}$**.** GSH is a good scavenger of many free radicals like O_2, $OH^{\cdot}$and various lipid hydroperoxides and may help to detoxify many inhaled oxidizing air pollutants like ozone

Third line defense

These are a complex group ofenzymes for repair of damaged DNA, protein,oxidized lipids and also to stopchain circulation of peroxyl lipid radical. Theseenzymes repair the damaged biomolecules andreconstitute the damaged cell membrane (Gupta et al.,2006).

The *in vitro* antioxidant activity of fixed oil isolated from seed of *L. cylindrical* showed DPPH radical, nitric oxide radical, hydroxyl and peroxide radical scavenging activities (Yoganandam et al., 2010). The methanolic extract and aqueous extract of *Benincasa hispida* (Rana and Suttee, 2012), various solvent extracts of *Sonchus asper* (L.) (Khan et al., 2012), stem bark extracts of *Moringa oleifera* (*M. oleifera)* (Kumbhare et al.,2012),ethanol extract on the aerial parts of *Cocculus hirsutus* (Panda et al.,2011), methanol extracts of *Momordica charantia* (Leelaprakash et al.,2011), *Sphaeranthus indicus* Linn (Shirwaikar and Prabhu,2006), *Asparagus racemosus* (Velavan et al.,2007), *Annona squamosa*(Kaleen et al.,2006), eucalyptus oil (Kokate and Purohit,2004), *and Triphala* is a traditional ayurvedic

herbal formulation (Yamanara and Mochizuki ,1988; Boddows and Kelly,2005 ; Saini et al.,2007) exhibited free radical scavenging activity as antioxidants.

1.1.3. Anti-inflammatory agents from medicinal plants

Plants as natural anti-inflammatory agents

Inflammation has very central role to protect an organism against local injury and infections, however inflammation may turn to chronically damaging or painful state which needed pharmacological treatment (Gao et al., 1996; Laupattarakasem, 2003). Inflammatory diseases like asthma, rheumatoid arthritis, colitis and hepatitis are most leading diseases which cause death and disability (Jiang and Ames, 2003). Chronic inflammation also leads to develop more dangerous diseases like cancer, neurodegenerative and cardiovascular disorders (Jiang and Ames, 2003).

Presently much interest have been given in the searching of medicinal plants with anti-inflammatory activity which may lead to the discovery of new therapeutic agent that is not only used to suppress the inflammation but also used in diverse disease conditions where the inflammation response in intensifying the disease process. The bark extract of *Albizia lebbeck* , hydroalcoholic extract of *Argyreia speciosa* root, hydroalcoholic extract of *Sphearanthus indicus*, *B.prionitis* roots, petroleum ether and methanolic extract of *Benincasa hispida* fruit, ethyl alcohol extract of *Calendula officinalis* flower, methanol

extract of leaves of *Clerodendron infortunatum* Linn, aqueous extract of *Cynodon dactylon*, petroleum ether, benzene, chloroform, ethanol and aqueous extracts of *Desmostachya bipinnata* Stapf, aqueous and methanolic extract of leaves of *E. heterophylla*, acetone extract of fruit of *Garcinia mangostana*, methanolic extract of *Hibiscus rosa- sinensis* leaves, ethanol extract of leaves *Indigofera tinctoria* , aqueous and ethanolic extract of the stem bark of *Moringa oliefera*, alcoholic extract of seeds of trigonella-foenum-graceum (Fenugreek), showed prominent anti-inflammatory activity (Kumar et al.,2013).

1.1.4 Anticancer agents from medicinal plants

Chemoprevention is recognized as an importantapproach to control malignancy. Recentstudies have focused on the search for desirablechemopreventive agents. Natural products,particularly dietary substances, have played animportant role in creating new chemopreventive agents (Surh, 2003). Interesting patterns of differentialcytotoxicity have been associated with knownclasses of compounds, such as cardenolides,lignans or quassinoids (Cardellina et al., 1993). In any cancer drugdiscovery program, a paradigm based onethnobotanical and ethnopharmacological datawould be more economical and beneficial inidentifying potential anticancer molecules thanmass screening of plant species(Nair et al.,2009). Naturalproducts have been regarded as important sourcesof potential chemotherapeutic agents and manyanticancer drugs have originated from naturalsources (Tan et al., 2006).

Ayurveda, a traditional Indian medical practiceusing plant drugs has been successful from veryearly times in using these natural drugs andpreventing or suppressing various tumours withdifferent lines of treatment (Balachandran and Govindarajan, 2005).

The National Cancer Institute collected about35, 000 plant samples from 20 countries and hasscreened around 114,000 extracts for anticanceractivity (Mohammad, 2006). Over 3000 species of plants withantitumour properties have been reported (Hartwell, 1982).Cancer is one of the most prominent diseases inhumans and currently there is considerablescientific and commercial interest in thecontinuing discovery of new anticancer agentsfrom natural product sources (Kinghorn, 2003).

Anticancer properties of many naturalcompounds isolated from different Indian plantextracts have been reported. Research is beingcarried out throughout the world to find out a leadcompound which can block the development ofcancer in humans. Nature has always been a greatcontributor towards this goal. Plant-derivednatural products such as flavonoids, terpenoidsand steroids have received considerable attentiondue to their diverse pharmacological properties,which include cytotoxic and chemopreventiveeffects (Abdullaev,2001). The isolation of the vinca alkaloids,vinblastine and vincristine from the *Catharanthus roseus* introduced anew era in the use of plant material as anticanceragents. They were the first agents to advance intoclinical use for the treatment of cancer (Cragg and Newman, 2005).The medicinal plants contain many antioxidants such as vitamins (A, C, E, K), carotenoids,flavonoids (flavones, isoflavones, flavonones, anthocyanins, catenchins, isocatechins), polyphenols (ellagic acid, gallic acid, tannins),saponins, enzymes and minerals (selenium,copper, manganese, zinc, chromium, iodine,etc).

In this review, 50 anticancer medicinal plants ofIndian origin belonging to 35 families arereported along with detailed information regarding part used, extract used, type of themodel used, types of tested cancer cell lines, etc.Some medicinal plants have been studied invarious *in vivo* and *in vitro* experimental modelsof cancer and have shown significant inhibitionof cancer cell proliferation. *Abrus precatorius* in Yoshida's sarcoma, carcinomaand Dalton's lymphoma ascites cancer (Sivakumar and Alagesaboopathi, 2008), *lstonia scholaris* in Ehrlich ascitescarcinoma (Jagetia and Baliga, 2006; Kulkarni and Juvekar,2008),*Cymbopogon flexuosus* inEhrlich ascites carcinoma, leukemia and sarcoma-180(Sharma et al.,2009), *Phellinus rimosus* in lymphoma andcarcinoma (Ajith and Janardhanan,2002), *Allium sativum* insarcoma 180(Ejaz et al.,2003 and Balasenthila et al.,2001),*Tinospora cordifolia* in Ehrlich'sascites carcinoma (Prince and Menon, 1999),*Viscum album in* Ehrlich's carcinoma (Cebovic et al.,2008), *Andrographis paniculata* in lymphoma andcarcinoma (Geethangili et al.,2008), *Tiliacora racemosa* inleukaemia and carcinoma (Chakroborty et al.,2004) were reported to have anticancer activity.

1.1.5Apoptosis

The process of programmed cell death, or apoptosis, is generally characterized by distinct morphological characteristics and energy-dependent biochemical mechanisms. Apoptosis is considered a vital component of various processes including normal cell turnover, proper development and functioning of the immune system, hormone-dependent atrophy, embryonic development and chemical-induced cell death. Inappropriate apoptosis is a factor in many human conditions including neurodegenerative diseases, ischemic damage, autoimmune

disorders and many types of cancer. The ability to modulate the life or death of a cell is recognized for its immense therapeutic potential. Therefore, research continues to focus on the elucidation and analysis of the cell cycle machinery and signaling pathways that control cell cycle arrest and apoptosis. To that end, the field of apoptosis research has been moving forward at an alarmingly rapid rate. Although many of the key apoptotic proteins have been identified, the molecular mechanisms of action or inaction of these proteins remain to be elucidated.

Physiologic apoptosis

The role of apoptosis in normal physiology is as significant as that of its counterpart, mitosis. It demonstrates a complementary but opposite role to mitosis and cell proliferation in the regulation of various cell populations. It is estimated that to maintain homeostasis in the adult human body, around 10 billion cells are made each day just to balance those dying by apoptosis (Renehan et al., 2001).

Apoptosis is critically important during various developmental processes. Both the nervous system and the immune system arise through overproduction of cells. This initial overproduction is then followed by the death of those cells that fail to establish functional synaptic connections or productive antigen specificities, respectively (Nijhawan et al., 2000; Opferman and Korsmeyer, 2003).

Apoptosis is also necessary to rid the body of pathogen-invaded cells and is a vital component of wound healing in that it is involved in the removal of inflammatory cells and

the evolution of granulation tissue into scar tissue (Greenhalgh, 1998). Dysregulation of apoptosis during wound healing can lead to pathologic forms of healing such as excessive scarring and fibrosis. Apoptosis is also needed to eliminate activated immune cells either during maturation in the central lymphoid organs or in peripheral tissues (Osborne, 1996).

Furthermore, as organisms grow older, some cells begin to deteriorate at a faster rate and are eliminated via apoptosis. One theory is that oxidative stress plays a primary role in the pathophysiology of age-induced apoptosis via accumulated free-radical damage to mitochondrial DNA (Harman, 1992; Ozawa, 1995).

Pathologic apoptosis

Abnormalities in cell death regulation can be a significant component of diseases such as cancer, autoimmune lymphoproliferative syndrome, AIDS, ischemia, and neurode-generative diseases such as Parkinson's disease, Alzheimer's disease, Huntington's disease, and Amyotrophic Lateral Sclerosis. Some conditions feature insufficient apoptosis whereas others feature excessive apoptosis.

Cancer and apoptosis

Cancer is an example where the normal mechanisms of cell cycle regulation are dysfunctional, with either an overproliferation of cells (King and Cidlowski, 1998). In fact, suppression of apoptosis during carcinogenesis is found to play a central role in the development and progression of some cancers (Kerr et al., 1994). There are a variety of molecular mechanisms that tumor cells use to suppress apoptosis.

Tumor cells can acquire resistance to apoptosis by the expression of anti-apoptotic proteins such as Bcl-2 or by the down-regulation or mutation of pro-apoptotic proteins such as Bax. The expression of both Bcl-2 and Bax is regulated by the p53 tumor suppressor gene (Miyashita, 1994). Certain forms of human B cell lymphoma have overexpression of Bcl-2, and this is one of the first and strongest lines of evidence that failure of cell death contributes to cancer (Vaux et al., 1988). Alterations of various cell signaling pathways can result in dysregulation of apoptosis and lead to cancer. The *p53* tumor suppressor gene is a transcription factor that regulates the cell cycle and is the most widely mutated gene in human tumorigenesis (Wang and Harris, 1997). The critical role of *p53* is evident by the fact that it is mutated in over 50% of all human cancers. *p53* can activate DNA repair proteins when DNA has sustained damage, can hold the cell cycle at the G_1/S regulation point on DNA damage recognition, and can initiate apoptosis if the DNA damage proves to be irreparable (Pientenpol and Stewart, 2002). Tumorigenesis can occur if this system goes twisted. If the *p53* gene is damaged, then tumor suppression is severely reducedOther cell signaling pathways can also be involved in tumor development. For example, upregulation of the phosphatidylinositol 3-kinase/AKT pathway in tumor cells renders them independent of survival signals. In addition to regulation of apoptosis, this pathway regulates other cellular processes, such as proliferation, growth, and cytoskeletal rearrangement (Vivanco and Sawyers, 2002).

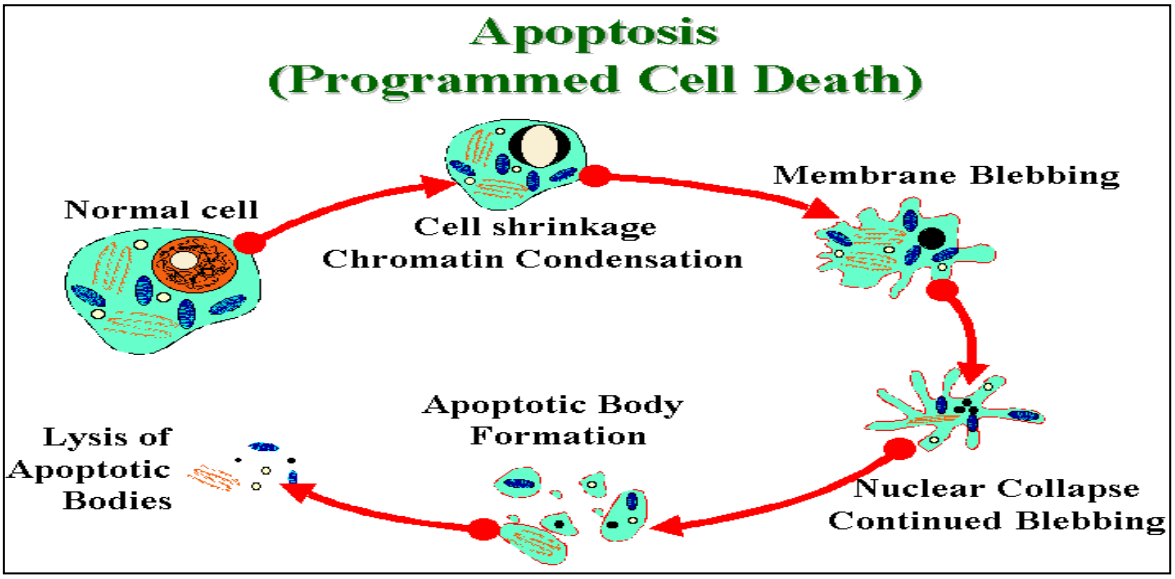

Figure 1.2 Apoptosis (Programmed cell death)

1.2 Plant Profile of *Calotropis gigantea* Linn. and literature review

1.2.1 Geographical distribution and description of the plant

Calotropis gigantea (C. gigantea) (Crown flower) is a species of *Calotropis*, native to Indonesia, Malaysia, Philippines, Thailand, Sri Lanka, India and China. *Calotropis gigantea*, commonly known as milkweed or swallow-wort, is a common wasteland weed (Singh et al., 1996). *Calotropis* belongs to Asclepiadaceae family which includes 280 genera and 2,000 species of world-wide distribution but most abundant in the sub-tropics and tropics, and rare in cold countries. *Calotropis* grows throughout the country (Sastryet al., 1990) on a variety of soils in different climates, sometimes where nothing else grows.

It is a largeshrub growing to 4 m tall. Its stems are erect, up to 20 cm in diameter.The plant consists of oval, light green leaveswith the size of 9-20 cm x 6-12.5 cm but sub sessile.and

milky stem. It has clusters of white or lavender coloured waxy flower having five pointed petals and a small, elegant "crown" rising from the centre thatholds the stamens. The scentless flowers are about 1 cm across and longdurable and the seeds spread by wind. The inflorescence stalk is 5-12 cmlong; the stalk of an individual flower is 2.5-4 cm long. Sepal lobes are largelyegg-shaped with a size of 4-6 mm x 2-3 mm. Petal is 2.5-4 cm in diameter. The petal lobes measuring 10-15 mm x 5-8 mm,arebroadly triangular. The egg-shaped or boat-shaped fruits are mostly in pairs,inflated, 6.5-10 cm x 3-5 cm.

Figure 1.3 Mature *Calotropis gigantea* plant.

1.2.2 Taxonomy

Kingdom: *Planatae*, Subkingdom: *Tracheobionta,* Superdivision: *Spermatophyta,* Division:*Magnoliophyta*, Class: *Dicotyledones*, Sub class: *Asteridae,* Series: *Bicarpellatae*, Order: *Gentianales*, Family: *Apocynaceae*, Subfamily: *Asclepidiaceae*, Genus: *Calotropis,* Species: *gigantea*, Binomial name :*Calotropis gigantea* (Linn.)(Singh et al., 1996).

1.2.3 Vernacular Names

a. Common names: Giant Milkweed, Crown Flower, Swallow Wort.
b. English: Crown flower, giant Indian milkweed. Bowstring hemp, crownplant, madar(Singh et al., 1996).

1.2.4 Traditional use of *Calotropis gigantea*

Calotropis is used as a traditional medicinal plant (Rastogi et al., 1991) which has unique properties (Oudhia and Tripathi 1998).

In Siddha:

Traditionally *Calotropis* is used alone or with other medicinal (Caius, 1986) to treat common disease such as fevers, rheumatism, indigestion, cough, cold, eczema, asthma, elephantiasis, nausea, vomiting, diarrhoea (Das et al, 1996). The roots and leaves of *Calotropis gigantea* are used traditionally for the treatment of abdominal tumours, boils, syphilis, leprosy, skin diseases, piles, wounds, insect-bites, ulceration and elephantiasis (Ghani A, 2003). The

leaves of *C. gigantea* are used for the treatment of poisonous snake bites, periodic fever, intestinal worms and ulcers. Latex of this plant is used to cure dental problems, rat bite, swellings, gonococcal arthritis and other rheumatic complaints.

In Ayurveda:

According to Ayurveda, dried whole plant is a good tonic, expectorant, depurative, and antihelminthic. The dried root bark is a substitute for *ipecacuanha.* The root bark is febrifuge, antihelminthic, depurative, expectorant, and laxative. This root bark is also used in cutaneous infections, intestinal worms, helminthic infections, cough and ascites. The powdered root used in asthma, bronchitis, and dyspepsia and it promotes gastric secretions. The leaves are useful for the treatment of paralysis, arthralgia, swellings, and intermittent fevers. The flowers are bitter, digestive, astringent, stomachic, antihelminthic, and tonic (Agharkar, 1991). *Calotropis* is also a reputed homoeopathic drug (Ghosh, 1988).

1.2.5 Medicinal properties of *Calotropis gigantea*

1.2.5.1 Analgesic activity

The alcoholic extract of the flowers of *C. gigantea*was reported for analgesic activity inchemical and thermal models in mice (Pathak et al., 2007).

1.2.5.2 Antimicrobial activity

Methanol, ethanol, aqueous, and petroleum ether extracts of the leaves of C. gigantea were reported to possess anti-candida activity against clinical isolates of *Candida albicans*, *C.parapsilosis*, *C.tropicalis*and *C. Krusei* (Kumar et al., 2010a). *C. gigantea* latex also

possesses potent fungicidal activity (Subramanian et al., 2010).Leaves and latex exhibited anti-bacterial activity (Kumar et al., 2010b), (Kumar et al.,2010c).The ethanol latexextractwas active only against *Staphylococcus aureus*, *Shigella dysenteriae*and did not show any significant antibacterial properties (Sarkar et al., 2013).

1.2.5.3 Wound healing activity

Root bark extract of *C. gigantea*given orallyaccelerated wound healing at 100, 200 and 400 mg/kg dosein Wistar rats (Deshmukh et al., 2009). At a dose of 200 mg/kg/day *C. gigantean* latex showed the significant wound healing activity (Nalwaya et al., 2009).

1.2.5.4 Anti-diarrhoeal activity

The hydroalcoholic (50:50) extract of aerial part of *C. gigantean* exhibited significant anti-diarrhoeal activity against castor oil-induced-diarrhoea model in rats (Chitme et al., 2004).

1.2.5.5 Anti-pyretic activity

Chitme et al., (2005) reported the anti-pyretic activity of the water: ethanol (50:50) extract of *C. gigantean* roots by using yeast and TAB (Typhoid) vaccine induced pyrexia in albino Swiss rats and rabbits. At the dose of 200 and 400 mg/kg body weight (intraperitoneal injection), extract significantly reduced the fever and body temperature.

1.2.5.6 Pregnancy interceptive properties

Different organic solvents of *C.gigantea* roots were reported to exhibit pregnancy interceptive activity in rats (Srivastava et al., 2007).

1.2.5.7 Procoagulant activity

The latex of *C. gigantean* is reported to carry procoagulant activity. Proteins present in the latex of *C. gigantea* are strongly proteolytic and responsible for procoagulant activity of *C. gigantea* (Rajeshet al., 2005).

1.2.5.8 Hepatoprotective effects

Ethanol extract of stems of *C. gigantea* was reported for hepatoprotective activity in male Wistar rats against carbon tetrachloride induced liver damage.

1.2.5.9 Insecticidal activity

Methanol extract of *C. gigantea* root bark and its chloroform and petroleum ether fractions showed high insecticidal activity against *T. castaneum* followed by petroleum ether fraction and chloroform fraction. None of the sample showed fumigant toxicity (Alam et al., 2009).

1.2.5.10 Cytotoxic activity

The cardenolide glycosides collected from the root *C. gigantea* were reported to carry cytotoxic activity against several human and mouse cell lines. Calotropin, frugoside and 4'–O-β-Dglucopyransylfrugoside was found as the active principles (Kiuchi et al., 1998). Ethanol extract of the roots of *C.gigantea* were reported to display inhibitory effects towards chronic myelogenous leukemia K562 and human gastric cancer SGC-7901 cell lines (Wang et al.,2008)

1.2.5.11 Antioxidant activity

Hydroalcohlic extract of *C. gigantea* leaves were reported to carry antioxidant activity by showing DPPH radical, nitric oxide scavenging activity (Kumar et al., 2010).

1.2.6. Phytochemistry

C. gigantea was reported to possess alkaloids, cyanogenic, glycosides, phenolics, tannins (Mahajan et al., 2010), cardenolides, (Seeka et al., 2010), flavonoids (Sen et al., 1992) , terpene (Gupta et al., 2000 and Anjaneyulu et al.,1968) sterols , Proteinases (Abraham et al., 1979) and nonprotein amino acid as major phytochemical groups.

Plant part	**Chemical nature**	**Chemical constituents**	**Reference**
Root	Cardiac glycoside	Calotropogenin	Tiwari et al., 1978
Root	Cardiac glycoside	Calotropin	Tiwari et al., 1978
Root	Cardiac glycoside	Uscharin	Tiwari et al., 1978
Root	Cardiac glycoside	Calotoxin	Tiwari et al., 1978
Root	Cardiac glycoside	Calactin	Tiwari et al., 1978
Root	Cardenolide glycoside	Coroglaucigenin	Kiuchi et al., 1998.
Root	Cardenolide glycoside	Frugoside	Kiuchi et al., 1998
Root barks	Cardenolide glycoside	4-0 Beta-D-glucopyranosylfrugoside	Kiuchi et al., 1998
Root	Terpene	Calotropnapthalene	Gupta et al., 2000.
Root	Terpene	Calotropisesquiterpenol	Gupta et al., 2000
Root	Terpene	Calotropises juiterpenol	Gupta et al., 2000
Root	Terpene	Calotropisesterterpenol	Gupta et al., 2000
Root	Aromatic product	Calotropbenzofuranone	Gupta et al., 2000
Root	Calotropone	12β-*O*-benzoyl-3β,14β,17β-trihydroxypregnane-20-one	Wang et al., 2008

Root bark	Sterols	Stigmasterol	Habibet al., 2007
Root bark	Sterols	β-sitosterol	Habibet al., 2007
Leaves	Cardenolides	19-Nor-and18,20-Epoxycardenolides	Lhinhatrakool et al., 2006
Leaves	Cardenolides	16alpha-hydroxycalactinic acid methyl ester	Seeka et al., 2010
Leaves	Cardenolides	15beta-hydroxycardenolides	Seeka et al., 2010
Arial parts	Flavonol	Isorhamnetin-3-*O*-rutinoside	Sen et al., 199
Arial parts	Flavonol	Isorhamnetin-3-*O*-Glucopyranoside	Sen et al., 1992.
Arial parts	Flavonol	Taraxasteryl acetate	Sen et al., 1992
Latex	Proteinases	Calotropain-F1	Abraham et al., 1979
Latex	Proteinases	Calotropain-FII	Abrahamet al., 1979
Latex	Proteinases	Calotropins DI	Pal et al., 1980.
Latex	Proteinases	Calotropins DII	Pal et al., 1980
Latex	3′-methylbutanoates of α-amyrin	triterpene esters	Thakuret al., 1984
Latex	ψ-taraxasterol	triterpene esters	Thakuret al., 1984
Flowers	Triterpenoids	Di-(2-ethylhexyl) Phthalate	Habibet al., 2009
Flowers	Triterpenoids	Anhydrosophoradiol-3-acetate	Habibet al., 2009

Table 1.2 Compound isolated from different parts of *Calotropis gigantea.*

Latex part of *Calotropis gigantea*

The latex of *Calotropis gigantea* contains resinols as esters of steamvolatile fatty acids (acetic and isovaleric), similar to uscharin and calcium oxalate. The resinol portion consists mainly of two new alcohols, α-calotropeol and β-calotropeol in almost equal quantities and minor amounts of β-amyrin. The latex contains calotropin, calotoxin, uscharin, voruscharin, uschridin, uzarigenin, syriogenin, calotonic acid and proceroside, calotropin DI and DII (Sengupta et al., 1984; Pal et al., 1980), and cysteine proteinases calotropain FI and FII (Abraham et al., 1979).

Several proteinases (Abraham et al., 1979) such as two carbohydrate-containing proteinases, calotropain FI and FII, the former being very similar in its properties to chymopapain, the latter more closely resembleedpapain. Pal et al. (Pal et al., 1980) also described the isolation, crystallization, structure and properties of two other papain-like proteinases, calotropins DI and DII that do not contain carbohydrate. Two proteinases contained two carbohydrates, namely calotropain-FI and calotropain-FII, purified from *C. gigantea* latex by CM-Sephadex C-50 chromatography. Both calotropain-FI and FII were found to be homogeneous by rechromatography on CM-Sephadex C-50, gel filtration on Sephadex G-100, electrophoresis on polyacrylamide gel and by N-terminal amino acid analysis. Some properties of these enzymes are reported by Abraham et al., 1979.

The physicochemical properties of both calotropins were similar to those of other plant cysteine proteases. As in the case of papain, their sedimentation coefficients were independent of protein concentration. The average molecular weights calculated from

sedimentation and diffusion coefficient values, Archibald experiment, amino acid composition, and sodium dodecyl sulfate-gel electrophoresis were 23,800 for calotropin DI and 24,200 for calotropin DII. Their amino acid compositions were very similar with minor differences in individual amino acids. Like papain they were devoid of carbohydrate moieties. In the enzymic properties both calotropins were found to be indistinguishable. Hydrolysis of azoalbumin by each enzyme was optimal in the pH range 7.5–8.0 at 55 °C. Their caseinolytic activities were about one-third as active as papain or ficin. They, unlike papain and ficin, showed no measurable activity toward synthetic substrates such as benzoyl-l-arginine ethyl ester, p-tosyl-l-arginine methyl ester, carbobenzoxy glycine p-nitrophenyl ester, benzoyl-dl-arginine p-nitroanilide, and benzoyl-l-arginine amide.

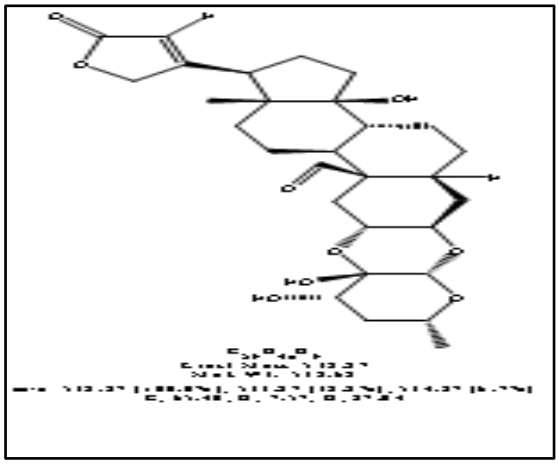

Figure 1.4 Chemical structure of Calotropin.

The hexane and methanol soluble extract of the latex coagulum of *Calotropis gigantea* afforded two new triterpene esters, viz. 3′-methylbutanoates of α-amyrin and ψ-taraxasterol, besides the known 3′-methylbutanoates of three triterpene alcohols. The

compositions of the alkane fraction, total triterpene alcohol fraction, and free, acetyl and 3′-methylbutanoyl triterpene alcohol fractions of the extract were determined(Thakur et al., 1984).

Lupeol, a pentacyclic triterpenoid was extracted for the first time from the latex of *Calotropis gigantea* and characterized by spectral studies. Triterpenoid, lupeol (3˘-hydroxylup-20(29)-ene), are immense bioactive compound present in different medicinal plants (Sturm et al., 1996; Fernández et al.,2001). This compound was reported to be antiangiogenic, antioxidative and anti-inflammatory in nature (Sudhahar et al., 2008a). It inhibits early responses of tumor growth induced by benzoyl peroxide (Saleem et al., 2008). It also plays very important role in normalization of lipid profile (Sudhahar et al., 2007), wound healing activity (Harish et al.,2008), protective effect in hypercholesterolemia associated with renal damage (Sudhahar et al., 2008b)and suppression of immune factors(Vasconcelos et al.,2008; Gallo et al., 2009).

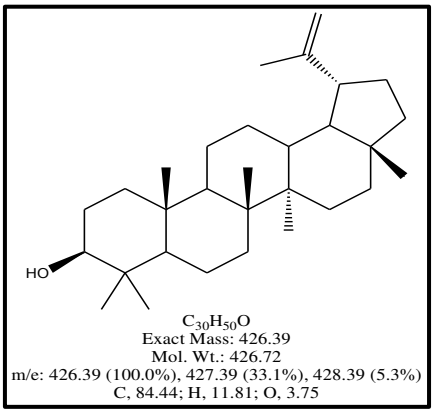

Figure 1.5Chemical structure of Lupeol

1.3 Objectives

- Collection, identification, extraction, characterization and chemical screening of active constituents of*Calotropis gigantean* latex extract.
- Investigation of the acute and sub-acute toxic effects of the ethanol (EECGL) and water (WECGL) extract of *Calotropis gigantea* latexin brine shrimp and zebra fish embryo, human lymphocytesas well as in Swiss albino mice.
- Evaluation of *in-vitro*antioxidant activity of *Calotropis gigantea* latex extracts (EECGL and WECGL).
- Study on the*in-vitro* and *in-vivo*anti-inflammatory effect of the ethanol (EECGL) and water (WECGL) extract of *Calotropis gigantea* latex.
- Investigation of the *in-vitro* cytotoxic activity of ethanol (EECGL) and water (WECGL) extracts *Calotropis gigantea* Linn. latex on human acute T cell leukemia producing Jurkat cells and also to investigate the antimitotic potential of the extracts in *Allium cepa* root tip cells.
- Exploration of the anticancer activity of *Calotropis gigantea* latex extracts (EECGL and WECGL)against Dalton's Ascitic Lymphoma (DLA) cells in *in vitro* and *in vivo* experimental conditions.
- Assessment of the antineoplastic and as well as cytotoxic, antioxidant and apoptotic potential of ethanol (EECGL) and water (WECGL) extract of *Calotropis gigantea* latex against Ehrlich ascites carcinoma (EAC) cells in *in vitro* and *in vivo* experimental set up.

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