CHAPTER1 INTRODUCTION

1.1 Medicinal plants

Medicinal plants are remained the major source of both conventional and traditional medicine worldwide. A variety of medicinal plants has been used in the remedy of various diseases from ancient times. According to a report given by world health organization, 70 percent of population depends for their primary health care on traditional Medicinal plants (Alves and Rosa 2007). People in developing country mostly use these plants as remedial medicines often have negligible side effects. Another possible reason, relatively high cost of synthetic medicine in developing country make them to be dependent on Medicinal plants. In recent years a lots of efforts have been made to identify and characterize the molecules from natural sources which exhibited a great range of clinical and pharmacological properties in different disease (bacterial and fungal infection) conditions. This feature thus made an urgent view for an extensive research on the molecules extracted from plants and their associated microorganisms. *Calotropis procera* belongs to family *Asclepiadaceae* with 80 genera and 2200 species distributed mainly in tropical and subtropical region of the world. Only two species *Calotropis procera* and *Calotropis gigantea* are of economic importance. These two species resemble to each other and find similar medicinal uses.

The plants are generally known as milk weeds because of the latex they produce. *Calotropis species* are mostly considered as common weeds in some parts of the world. The flowers are fragrant and are often used in making floral tassels in some main land Southeast Asian cultures. Fibers of these plants are called madar. *Calotropis* species are usually found in abandoned farmland.

Calotropis procera - Scientific classification

Kingdom –Plantae- plants

Subkingdom-Tracheobionta – Vascular plants

Superdivision -Spermatophyta Seed plants

Division-Magnoliophyta – Flowering plants

Class-Magnoliopsida – Dicotyledons

Subclass-Asteridae

Order-Gentianales

FamilyAsclepiadaceae – Milkweed family

Genus *Calotropis*

Species *procera*

1.2 General Description

Worldwide the plant is known by several names as sodom apple, calotrope, French cotton, small crown flower (English), algodón de seda, bomba (Spanish), cotton-france, arbre de soie, and bois canon (French) (Howard 1989, Liogier 1995, Neal 1965, Parrotta 2001). But, mostly it is known as Giant milkweed. This plant is a soft-wooded, evergreen, perennial shrub. It has one or a few stems, few branches, and relatively few leaves, mostly concentrated near the growing tip. The bark is corky, wrinkled, and light gray in colour. All parts of plant contain white viscus milky sap which flows whenever any part (root, leaf, and stem) of plant is cut or broken. Giant milkweed has a very deep, stout taproot having only few or no lateral roots near-surface. Roots were found to have few branches and reach of 1.7

to 3.0 m in Indian sandy desert soils (Sharma 1968). The opposite leaves are oblong obovate to nearly orbicular, short-pointed to blunt at the apex and have very short petioles below an early clasping, and heart-shaped base. The length of the leaf blade ranges from 7 to 18 cm long and 5 to 13 cm broad, slightly leathery, and have a fine coat of soft hairs that rub off. The colour of leaf blades are light to dark green and veins are mostly white. The flower clusters are umbelliform cymes in arrangement that grow at or near the ends of twigs. The flowers are superficially campanulate with five sepals that are 4 to 5 mm long, fleshy and variable in color which varies from white to pink, often spotted or tinged with purple. The fruits are inflated, obliquely ovoid follicles that split and invert when mature to release flat, brown seeds with a tuft of white hairs at one end (Howard 1989, Liogier 1995, little and others 1974).

Habitat—Giant milkweed is native to West Africa as far south as Angola, North and East Africa, Madagascar, the Arabian Peninsula, southern Asia, and Indochina to Malaysia (Rahman and Wilcock 1991). The species is now naturalized in Australia, many Pacific islands, Mexico, Central and South America, and the Caribbean islands. Ecology.—Giant milkweed favors open habitat with little competition. This condition is most completely met in overgrazed pastures and rangeland. Other common habitats are beach front dunes, roadsides and disturbed urban lots. The species grows in dry habitat (150 to 1000 mm precipitation) and sometimes in excessively drained soils in areas with as much as 2000 mm of annual precipitation. Giant milkweed may be found in areas up to 1,000 m in elevation in India (Parrotta 2001). It roots very deeply and rarely grows in soils that are shallow over unfractured rock. Soils of all textures and derived from most parent materials are tolerated, as well as soils with high sodium saturation. Beachfront salt spray is not detrimental.

Competition with tall weeds, brush and especially grass weakens existing plants, and being overtopped and shaded by trees soon eliminates them. During droughts in Puerto Rico, giant milkweed is attacked by the orange aphid, Aphis nerii Boyer de Fonscolombe, which causes defoliation, death of branches, and aborted fruits (Little and others 1974).

1.3 Medicinal uses

All the parts, viz. root, stem, leaf and flowers of *Calotropis* are in common use in indigenous system of medicine Giant milkweed tissues, especially the root bark, are used to treat a variety of illness including leprosy, fever, menorrhagia, malaria, and snake bite (Parrotta 2001). The leaves of *Calotropis procera* are said to be valuable as an antidote for snake bite, sinus, fistula, rheumatism, mumps, burn injuries, and body pain. The leaves of *Calotropis procera* are also used to treat jaundice.

Other compounds have been found to have bactericidal and vermicidal properties. The latex contains a proteolytic enzyme called caloptropaine. An infusion of bark powder is used in the treatment and cure of leprosy and elephantiasis. It is inadvisable to use bark that has been kept for more than a year. The root bark is an emetic, the flower a digestive, and a tonic is used for asthma and cataract. Bark and wood stimulate lactation in cattle. Roots (extremely poisonous) are applied for snake bite. The milky sap is used as a purgative agent. The latex is used for treating ringworm, guinea worm blisters, scorpion stings, venereal sores and ophthalmic disorders; also used as a laxative. Its use in India in the treatment of skin diseases has caused severe bullous. *Calotropis procera* a popular traditional medicinal plant that have ethnobotanical history for traditional medicinal plant was selected for studies for endophytic fungal community. There are some earlier reported works which claim that the biochemicals

produced from endophytic fungal species originally possess properties like host plants which might be related genetic recombination of endophytic fungus with host plant occurred during evolutionary time (Zao et al., 2001).

1.4 Calotropis procera: Chemical constituents

Phytochemical studies on *Calotropis procera* have revealed several types of compounds such as, triterpinoids, alkaloids, resins, anthocyanins and proteolytic enzymes in latex, flavonoids, tannins, sterol, saponins, and cardiac glycosides. latex contains caoutchouc, calotropin, calotoxin 0.15%, calactin 0.15%, uscharin 0.45%, trypsin, voruscharin, uzarigenin, syriogenin and proceroside (Atef et al., 1999). The flower contains the flavonoids, queretin-3- ratinoside, sterol, calactin, calotoxin, calotropagenin, calotropin, polysaccharides cardenolide, gigantin, giganteol, isogiganteol, voruscharin a-calotropeol, 3-epimoretenol, α-lactuceryl acetate and α-lactuceryl isovalerate, flowers contain terpenes, multiflorenol, and cyclisadol he latex contains caoutchouc, calotropin (Sharma et. al., 2011). Root bark of *Calotropis procera* contains triterpenes. A new norditerpenyl ester, named Calotropterpenyl ester, and two unknown pentacyclic triterpinoids, namely calotropursenyl acetate and calotropfriedelenyl acetate, akundarol isovalerate, mundarol isovalerate and quercetin -3-rutinoside.

1.5 Why to focus research on endophytic fungi in Calotropis procera?

Endophytes are microorganisms that live in the intercellular spaces of various parts of plants viz; stems, petioles, roots and leaves without showing any disease symptoms due to their presence and have typically gone unnoticed. The symbiotic relationship between plant and endophytes have been well established between the two namely, the former protects and feeds the latter which produces 'in return' bioactive (plant growth regulatory, antibacterial,

antifungal, antiviral, insecticidal, etc.) substances to enhance the growth and competitiveness of the host in nature.

Endophytic fungi are important component of biodiversity and only a little study have been done therefore more research work should be carried out to explore in detail their chemical and biochemical aspects because the plant has long been used as a medicinal plant. There are many aspect of endophytic fungi that could be investigated some important aspect of current research with endophytic fungi are following and concerned with study of-

1. antimicrobial metabolites
2. anticancer metabolites
3. insecticidal compounds
4. pesticide compounds
5. immune modulator compounds
6. antioxidant metabolites
7. Isolation of plant growth enhancer metabolites
8. Study of plant disease through endophytic fungi

1.6 Aims and objectives of study

As for as our work was mainly concerned with the study of properties of bioactive metabolite, the aim of study was to isolate and identify endophytic fungi from medicinal plant *Calotropis procera* and to isolate and characterize bioactive metabolites extracted from selected endophytic fungi which might have some pharmacological potential.

This fact is based on the rational that endophytic fungi protect their host plants in various adverse conditions to bring about the above mentioned investigation *Calotropis procera* was selected, a number of fungal isolates were cultured at small scale and their metabolites

extracted using ethyle acetate and N-hexane solvents and these extracts were then used to study biological activity.

The research work was completed with the under given objectives-

- Isolation and taxonomic characterization of fungal species exibiting higher potential for bioactive metabolite production.
- Study of antibacterial property of bioactive metabolite with special reference to pathogenic bacteria.
- Study of antifungal property of novel bioactive metabolite with special reference to pathogenic fungi.
- Characterization of bioactive metabolite for molecular structure study using analytical tools (NMR, FT-IR, and ESI-HRMS).

  After that the most active fugal species was selected for production of bioactive metabolites, crude metabolites were fractionated, purified, and again tested again for antimicrobial properties. Finally structure of each compound was elucidated based on the information obtained from NMR, FT-IR and ESI-HRMS.

Therefore, the most active fungal species was selected for production of bioactive metabolites. Structural elucidation of yielded secondary metabolites with the aid NMR (^{1}H, ^{13}CNMR) and mass (EI MS, ESI MS) were explored.