CHAPTER I

INTRODUCTION

CHAPTER 1

1.1 Medicinal plants (*Adhatoda Beddomei*)

There are about 3 lakh different plant species on our planet. It is estimated that there are over one million microorganisms are associated with many plants, exist in nature. Many of these are medicinal plants and globally these plants have been the main source of both unconventional and traditional medicine. All these medicinal plants have long been used in the treatment of various diseases. According to a report by the World Health Organization, 70 percent of the population relies on traditional medicinal plants for their main health concern. (Aly et al., 2010).

People in developing countries, mostly use these drugs because traditional medicines often leave negligible side effects. Another possible reason is that the relatively high cost of synthetic medicine in a developing country makes them dependent on medicinal plants. In recent years a great deal of effort has been made to identify and characterize molecules from natural sources, used in various diseases (bacterial and fungal infections) conditions, and exhibits a large range of pharmacological properties Are, thus created an essential approach to extensive research on molecules derived from plants and their associated microorganisms. One of the medicinal plants is *Adhatoda Beddomei* (Adusa). This has its many medicinal properties. *Adhatoda beddomei,* generally known as Malabar nut, adulsa, Adhatoda, vasa, or vasaka, is a medicinal plant native to Asia, widely used in Siddha Medicine, Ayurvedic, homeopathy and Unani Chain of medicine. Its roots go to a very low depth in the ground and it does not grow in a place with deep soil. All the parts, root, bark, stem bark, leaf, and flowers of Adhatoda beddomei are mainly used in the native system of medicine. Root bark used in leprosy, fever, menorrhagia, malaria, and snake bite. Leaves used for snakebite, sinus fistula, rheumatism, mumps, burn Injuries Jaundice and body pain. Chemical constituents of *Adhatoda beddomei* are Cardenolide, triterpenoids,

alkaloids, resins, anthocyanins and proteolytic. *Adhatoda* is a shrub-like plant with lance-shaped leaves 10 to 15 cm wide. They are inversely arranged, ornate-edged and originate on small petioles (Arnold et al., 2000). When they dry, they begin to look light brownish-green, they are very bitter in taste, probably because of their chemical composition. When a leaf is cleaned with chloral hydrate and viewed under a microscope, the stoma appears oval (Arnold et al., 2001).

The flowers of *Adhatoda* are usually white and the inflorescence is of medium size, with dense, axillary spikes. Fruits are pubescent and with club-shaped capsules.

1.2 *Adhatoda beddomei* - Scientific classification.

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision-	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Scrophulariales
Family	Acanthaceae
Genus	Adhatoda
Species	*Adhatoda beddomei*

This plant is softwood, evergreens, perennial shrub. They have few branches and one or more stems and relatively few leaves are mostly concentrated near the growing tip. Leaves are shorter, less broad, more linear, alternate, simple, elliptic or elliptic-lanceolate and acuminate. Flowers are white, in dense spikes, bracts ovate or obovate, calyx deeply five-lobed, stamens glabrous and Bites the ovum or ovary, it is stifling. Which causes a very bad feeling when inhaled? This species grows in dry habitats (150 to 1000 mm rainfall) and sometimes in areas with more than 2000 mm annual rainfall. This plant is found in every part of India.

1.3 ***Adhatoda beddomei*****: Chemical constituent's** Photochemical studies on *Adhatoda beddomei* reveal many types of compounds such as anthocyanins, aminophylline, alkaloids, cardiac glycosides, isoprenaline, triterpenoids, resins vasicinone, vasicine, and proteolytic enzymes in latex, flavonoids, tannins, sterol, saponins, and Latex contains caoutchouc, calotropin, calotoxin 0.15%, calactin 0.15%, uscharin 0.45%, trypsin, voruscharin, uzarigenin, syriogenin and proceroside (Asford, 1997). The Root bark of *Adhatoda beddomei* 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.

1.4 Medicinal uses: *Adhatoda beddomei* is a popular traditional medicinal plant that an ethnographic history was selected for study from the endophytic bacterial community. All parts of beddomei, that is, root, stem, leaf, and flower are mainly used in indigenous medical treatment, giant spongy tissue, especially root bark, is used in many types including leprosy, fever, bleeding. Used to treat illnesses such as malaria, and snakebite. The leaves of Adhatoda

beddomei are used as an antidote for snakebite, sinus fistula, and arthritis. It is used for mumps, burns and body aches

There is some earlier reported work which claims that the biochemical's produced from endophytic bacteria species originally possess properties like host plants which might be related to genetic recombination of endophytic bacteria with host plant occurred during evolutionary time.

1.5 Endophytes: In the natural environment, internal tissues of plants contain many different types of microorganisms, called endophytes. The term endophyte was first used by Day-Barry in 1866. Endophytes were discovered in Germany in 1904 by Freeman, who confirmed the occurrence of an endophytic fungus in Persian darlin (annual grass). Endophytes are microbes that are found inside plant cells. The first actinobacterial endophyte was isolated by Frankia an N2-fixing actinobacterium that formed actinorhizae with 8 families of angiosperms. The term 'endophyte' is a ubiquitous topographic term that includes all these organisms that, during a variable period of their lives, symptomatically colonize the living internal tissues of their hosts (Wang et al., 2007).

The symbiosis of endophytes bacteria with plants is probably found in different parts of vascular plants. (Arunpanichlert et al., 2010). As suggested by Carroll, phytopathogens are always related to certain endophytes in the environment. (Romero et al., 2001). In some environments, many microorganisms actively manifest themselves through cell openings or lesions to enter plant tissue, as well as using hydrolytic enzymes such as cellulases and pectinases. Some endophytes bacteria originate from the plant's rhizosphere or falloplan microflora, which pass through the root hair to the cell's xylem and phloem. Endophytes are

microorganisms producing allelochemicals that promote plant growth and, as a result, have a beneficial effect on the host plant.

1.6 Secondary bioactive metabolites: These anti-property bioactive metabolites are organic compounds produced by bacterial, fungi or plants, with no direct evidence of growth and production.

Some specific types of secondary metabolites have been limited to an accumulated group of species within the phylogenetic group. It has often been observed that secondary metabolites play an important role in protecting the plant against external invasive elements, herbicides and other interspersion motions. For many centuries' humans have been using secondary metabolites as various drugs, flavor's, dyes, and recreational drugs. Secondary metabolites have always been supportive of their host. They also play an important role in important functions such as protection competition and species interactions. But it has often been observed that hosts never use secondary bioactive metabolites to survive. A special type of specificity is found in each of the metabolites which shows its characteristics. Typically, each of the metabolites is specific to an individual species of microbes. (Pichersky and Gang, 2000). Research also shows that different species are affected in different ways by secondary metabolites. In a similar forest, four separate species of arboreal marsupial folivores reacted differently to a secondary metabolite in eucalyptus (Jensen et al., 2014). Several types of research have shown that bioactive metabolites also have a profound effect on various types of food consumed by humans. According to some research, some volatile bioactive metabolites may be responsible for human food diversity, which are based on various nutritional foods. (Goff and Klee, 2006). It has often been observed that various bioactive

metabolites help the plant to obtain important nutrients, as examples such as nitrogen. As an example, the scientist observed that legumes use flavonoids to indicate a symbiotic relationship with nitrogen-fixing bacteria (Rhizobium) to increase their nitrogen (Croteau et al., 2012). Therefore, many plants that use secondary metabolites are very high in nutrients and are highly beneficial for human consumption.

1.7 Why to focus research on endophytic bacteria in *Adhatoda beddomei.*

Endophytes are a type of microorganism that spend their entire lives in the intracellular parts of plants; Their presence in stems, petioles, roots, and leaves is shown without any symptoms of disease in them. A good relationship has been established between the two. Namely, endophytic and symbiotic.

A variety of bioactive metabolites that produce various bioactive (plant growth regulators, antibacterial, antifungal, antiviral, insecticidal, etc) substances in exchange for substances to increase host growth in nature and one type of competition to an extent.

Endophytic bacteria are an important component of biodiversity and have been little studied, so more research should be done to explore their chemical and biochemical aspects in detail, as it has long been known as a medicinal plant is known. It has been used in many aspects on which endophytic bacteria can be examined, some important aspects of research related to the investigation and study as

- antimicrobial metabolites
- anticancer metabolites
- insecticidal compounds
- pesticide compounds

- immune modulator compounds
- antioxidant metabolites
- Isolation of plant growth enhancer metabolites
- Study of plant disease through endophytic bacteria and fungus.

1.8 Aims and objectives of the studies: As our research were primarily concerned with the studies of the properties of bioactive metabolites, For the purpose of isolating and identifying endophytic bacteria of the medicinal plant *Adhatoda beddomei* and selected for studies to characterize extracted bioactive metabolites that may have some pharmacological potential. This fact is based on the argument that the endophytic bacteria protect their host plants under various adverse conditions to bring about the above investigation, for which *Adhatoda beddomei* was selected, Some bacterial isolates were cultured on a small scale and their metabolites were extracted using ethyl acetate and n-hexane solvents and these extracts were used to study differential biological activity.

1.9 Various research work was carried out with the given objectives-

- ❖ First, we selected the right medicinal plant to obtain the right endophytes that producing bioactive metabolites.
- ❖ Isolation and taxonomic characterization of endophyte bacterial species exhibiting a higher potential for bioactive metabolite production.
- ❖ Study of antibacterial property of different bioactive metabolites with special reference to human pathogenic and non-pathogenic bacteria.

- ❖ Study of the antifungal property of different bioactive metabolites with special reference to human pathogenic fungi.
- ❖ We optimized the production of bioactive metabolites at different temperatures, pH and base on different carbon, nitrogen source.
- ❖ To confirm optimization of production of bioactive metabolites, we have done RSM (Response Surface methodology).
- ❖ Study of molecular structure using analytical instruments (NMR, FT-IR, ESI-MS) to characterize bioactive metabolites.

Thereafter, we selected the most active bacterial species for the production of bioactive metabolites. After that we fractioned the crude metabolites, purified, and again tested again for antimicrobial properties. Finally, the composition of each compound is described as based on information obtained from NMR, FT-IR, and ESI-MS. Therefore, In the summarized way in the present study, the taxonomical characterization of the bacterial strain together with the antimicrobial and structural elucidation of yields secondary metabolites with the aid NMR (1H, 13CNMR) and mass (ESI –MS) was explored.