CHAPTER 1

INTRODUCTION

India possesses rich floristic wealth and diversified genetic resources of medicinal plants. It has a widely ranging tropical and the agro climatic conditions which are conducive for introducing and domesticating new and exotic plant varieties (Harshburger, 1895). Current estimates suggest that in many developing countries, a large proportion of the population relies heavily on traditional practitioners and medicinal plants to meet primary health needs. For historical and cultural reasons herbal medicines have often maintained popularity (Ford, 1978).

Heavy metals are well known to be toxic to most organisms when present in excessive concentrations. First observations of the effects of heavy metals on soil microbial processes date back to the beginning of this century. But only when the large adverse effects of emissions of heavy metals from smelters on surrounding ecosystems was observed in the 1960-70’s it was realized how severely soil microorganisms and soil microbial processes can become disrupted by elevated metal concentrations, sometimes resulting in severe ecosystem disturbance.

However, toxic metal pollution of the biosphere has intensified rapidly since the onset of the industrial revolution, posing major environmental and health threats. Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues. Industrial exposure accounts for a common route of exposure for adults.
However in plants the toxic range of elements may be altogether different as plants respond to abiotic stress through secondary metabolite elicitation.

These metals may exert abiotic stress on the plant species that inhabit the industrially polluted areas. These factors or range of pollutants may impose an abiotic stress on medicinal plants too, which may alter metabolic profiles and developmental trajectories and thereby induce the production of various secondary metabolites. This in turn assists the plants to adapt to varied environmental changes (Anthony and Singh, 2006).

WHO recommends that medicinal plants which form the raw material for the finished products should be checked for presence of heavy metals (Ajasa et al., 2004). The World Health Organization estimates that 80% of the people in developing countries rely on traditional medicine for their primary health care and about 85% of traditional medicine involves the use of plant extracts. This means that about 3.5 to 4 billion people in the world rely on plants as sources of drugs. According to World Health Organization medicinal plants would be the best source to obtain variety of drugs. However, such plants should be investigated to better understand their properties, safety and efficiency (Arun, 2009). Knowledge of chemical constituents of plants is desirable because such information will be of value for synthesis of complex chemical substances (Mojab et al., 2003; Parekh and Chanda, 2007).

Due to the presence of toxic heavy metals, many medicinal herbs can present health risks. This can be harmful at various degrees depending on their oxidation states as well as their concentration (Piper, 1950). Of the known elements in the periodic table, relatively a small fraction of elements are known to be biologically important. Natural
abundance limits the availability of the elements for such use. Life evolved utilizing those elements that were abundant and available to it. Those elements that were rare were not utilized by the living system as they were not available, neither the system evolves the mechanism to cope with them. Among all array of heavy metals Cu, Co, Fe, Mo, Ni and Zn are essential micronutrient mineral elements, whereas Cd, Pb, Hg, As etc. have no physiological function in plants and are potential toxins to living organisms.

The plants (*Ocimum sanctum* Linn., *Gomphrena celosioides* Mart-Beitr., *Achyranthes aspera* Linn., *Abutilon indicum* Linn., *Azadirachta indica*) growing profusely in the area selected for the study are known to have curative bioactive compounds for various human ailments. Since the above plants are found abundantly in the mentioned pollutant areas, they are likely to be subjected to abiotic elicitation of phytochemicals by various industrial metal pollutants. The health giving properties of the medicinal plants are due to the presence of various complex chemical substances of different composition which occur as the secondary metabolites (Karthikeyan *et al*., 2009). They are grouped as alkaloids, glycosides, flavonoids etc., They have been found to be effective antimicrobial substances against a wide array of microorganisms (Ekwenye, 2005). Plants containing terpenoids, steroids, phenolic compounds and alkaloids have been reported to have antimicrobial activity (Hostettmann and Nakanishi 1979).

The above mentioned plants, subjected to metal stress, may trigger the secondary metabolites of pharmacological importance which in turn may be beneficial to the mankind. Therefore, accumulation of phytochemicals and therapeutic values are to be studied. It is aimed to find the effect of these pollutants on the pharmaceutically
important biochemicals present in the above mentioned plant species. Increased biochemical yields are critical to the commercialization as the biochemical demand is going up enormously.

The phytochemicals have recently become of great interest owing to their versatile applications. At present, herbal remedies are preferred due to less or no side effects for many diseases and due to financial constraints (Mesorini, 1987). It was further added that the use of plant extracts and phytochemicals with antimicrobial properties may be of importance in therapeutic treatments. Studies have been conducted to prove such efficiencies (Oyi et al., 2007; Bukar et al., 2010). Therefore the present study includes determining the pharmacological properties based on the efficiency of plants with respect to antimicrobial activity and cytotoxicity.

There are many metals that are of concern because of occupational or residential exposure. Therefore, it is important for us to inform ourselves about the heavy metals and to take protective measures against excessive exposure (John, 2001). Interestingly, small amounts of these elements are common in our environment and diet and are actually necessary for good health, but large amounts of any of them may cause acute or chronic toxicity. The present study concentrates on 25 elements which includes heavy metals, essential, beneficial and non-essential elements. Essential elements perform several functions. They participate in various metabolic processes in the plant cells such as permeability of cell membrane, maintenance of osmotic concentration of cell sap, electron-transport systems, buffering action and enzymatic activity. They also act as major constituents of macromolecules and co-enzymes. There are some beneficial elements which are required by higher plants.
The acceptance of the traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has led researchers to investigate the antimicrobial activity of medicinal plants (Bisignano et al., 1996; Hammer et al., 1999). Higher plant-derived natural products constitute 25% of the total natural products, their derivatives and analogs represent over 50% of all drugs in clinical use (Balandrin et al., 1993). Evidence of the importance of natural products is provided by the fact that almost half of the world’s 25 best selling pharmaceuticals in 1991 were either natural products or their derivatives (Neill, 1993). It has been estimated that 14 - 28% of higher plant species are used medicinally and that 74% of pharmacologically active plant derived components were discovered after following up on ethno medicinal use of the plants (Ncube et al., 2008).

Current estimates suggest that in many developing countries, a large proportion of the population relies heavily on traditional practitioners and medicinal plants to meet primary health needs. For historical and cultural reasons herbal medicines have often maintained popularity (Ford, 1978). Therefore a few medicinal plants used in traditional medicine were collected from Eastern Ghats to study with respect to metal accumulation and their antimicrobial and antitumor activity. Phenolic compounds are known to be major contributors to antimicrobial activity derived from spices and culinary herbs (Kisko & Roller, 2005). Essential oil and their constituents have been used as flavouring agents in the formulation of different pharmaceutical products (Cowan, 1999). Natural products have been used as anticancer agents (Frie, 1982), such as vincristine and vinblastin from Catharanthes roseus (Johnson, 1963). Even vegetables and fruits may help reduce the risk of cancer in humans (Moon et al., 2011; Chen et al., 2006).
Presently study has been undertaken to investigate the anticancer activity of two plants namely *Azadirachta indica* and *Ocimum sanctum*. Limonoids are metabolically altered triterpenes. Of the 300 limonoids known today, about one third also known as meliacins, are obtained from Meliaceae species. Limonoids have wide range of properties permitting both pharmacological and plant protection applications (Isman, 2006; Akhtar et al., 2008). These above mentioned plants are well known, easily available, cheap, identity is non-controversial, free from antitoxicity (Meghna et al., 2008). The goal of screening medicinal plants is to search for excellent anticancer agent avertable to human malignancies. In defiance of astonishing advances in modern science such as surgery, radiotherapy, chemotherapy and hormone therapy, cancer disease remains a worldwide health problem thus endeavoring the search for new alternate approach. Cancer is the second leading cause of death following heart disease, accounting for 23% of all deaths (Malayakkad et al., 2012).

Plants contain almost unlimited capacity to generate compounds that fascinates researchers in the quest for new and novel chemotherapeutics (Reed and Pellecchia, 2005). The search for new anticancer compounds in plant medicines and traditional foods is a realistic and promising strategy for its prevention (Yan-wei et al., 2009). Numerous compounds found in plants with anticancer properties are such as alkaloids, phenols and terpenoids (Kintzios, 2006, Park et al., 2008). Studies have observed the presence of a large number of bioactive compounds in the methanolic extracts of the other plants including tannins, alkaloids, steroids, saponins, terpenoids and flavonoids which exhibit various biological activities (Gulecha & Sivakumar, 2011; Kumar et al., 2011; Kumbhare et al., 2012).
AIMS AND OBJECTIVES

The present study was aimed to identify and collect the plants species showing luxuriant growth. Plant species are collected from two areas of concern, one is located at Visakhapatnam industrial area contaminated with various pollutants. The second area is Chintapalli of Visakhapatnam which is free from industries.

The comparative analysis of elements in the soils of two areas and the uptake of the elements by the selected plants grown in those areas.

Further the research, details the effect of the elements on phytochemicals of those selected plants and their effect on therapeutic action such as antimicrobial and anticancer activity.

The plants included in the study are the following

1) *Ocimum sanctum* Linn., Lamiaceae

2) *Gomphrena celosioides* Mart-Beitr., Amaranthaceae

3) *Achyranthes aspera* Linn., Amaranthaceae

4) *Abutilon indicum* Linn., Malvaceae

5) *Azadirachta indica* A.Juss., Meliaceae

These plants when subjected to metal stress may trigger the secondary metabolites of pharmacological importance which may be boon to the pharmacological industries.

The research is undertaken with the following objectives

- Identification, selection and collection of medicinal plant species that are grown abundantly and luxuriantly in both the areas of the study.
Metal analysis in the soils and selected medicinal plants grown at both the sites.

Qualitative analysis of phytochemicals, estimation of total phenols and flavonoids.

Effect of the extracted phytochemical on antimicrobial and anticancer activity.

The comparative study on the effect of metabolic phytochemicals and their antibacterial activity.

Role of phytochemicals on hepatocarcinoma cells (HepG2 cell lines).