CHAPTER 2

LITERATURE REVIEW

India possesses rich floristic wealth and diversified genetic resources of medicinal plants. The Eastern Ghats or Purva Ghat is a discontinuous range of mountains along India’s eastern coast. The Eastern Ghats run from West Bengal state in the north, through Odisha and Andhra Pradesh to Tamil Nadu in the south passing some parts of Karnataka. The mountain ranges run parallel to the Bay of Bengal. These ranges are not as high as the Western Ghats. Most of the medicinal plants belong to the higher flowering plants. Analysis of medicinal plants indicates that they are distributed across diverse habitats. Around 70% of India’s medicinal plants are found in tropical areas mostly in various forest types spread in Western and Eastern Ghats. Less than 30% are found in temperate and alpine areas and higher altitudes.

The effect of pollution on flora and fauna is a burning global issue. Eastern Ghats located at industrial area of Visakhapatnam is one of the rich districts of Andhra Pradesh with rich biodiversity and good forests (Padal et al., 2010). Therefore the soil and several plant specimens used in traditional medicine have been collected based on ethno medical significance to study the effect of industrial metal pollution.

To compare the data, the soil and the medicinal plants, flourishing in Chintapalli division of Visakhapatnam district, at the higher altitude in the hilly tracts of Eastern Ghats of Andhra Pradesh are collected. This area has second highest tribal population.
The site at which the plants have been collected at Chintapalli is considered to be free from industries, the biosphere in this zone is less polluted and therefore has been selected as a control site.

Upon studying one of the contaminated sites of Eastern Ghats with respect to plants exhibiting luxuriant growth, few plants have been selected that are known to have curative action on respiratory ailments along with other general medicinal properties. The study site is contaminated with heavy metals that have mainly originated due to industrial activity in Gajuwaka area of Visakhapatnam.

In the developing countries heavy metal pollution becomes serious due to mining, mineral, smelting and tannery industry. Medicinal plants are the most important source of life saving drugs for the majority of the world’s population. Medicinal, aromatic and spice plants grown in different regions of Austria were monitored as to their Cd, Cu, Fe, Mn, Pb, Zn and As contents. Arsenic (As) is a naturally occurring toxic element in the environment (Rahimi et al., 2012).

Although synthetic drugs and antibiotics are essential for current medical practice, plants provide a major contribution to the pharmaceutical industry (Flower, 1983; Sahoo et al., 1997). Unfortunately, rapid industrialization and urbanization has led to overexploitation and loss of valuable natural resources, including medicinally important herbaceous plants (Soumen et al., 2010). As study area, two zones of Eastern Ghats have been selected from Visakhapatnam district. One is large residential area at lower altitude of Eastern Ghats in Visakhapatnam, where there are more than fourteen major hazardous industries, among which seven are present in Malkapuram cluster. The soil and medicinal plants thriving in Malkapuram cluster are chosen as source of industrially polluted zone.
However toxic metal pollution of the biosphere has intensified rapidly since the onset of industrial revolution, posing major environmental and health threat (Chandra and Srivastava, 2003). Several years ago the World Health Organization made an attempt to identify all medicinal plants that exist in the world. More than 20,000 species were included on that list.

### 2.1 MEDICINAL PLANTS

Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of metabolites that perform various biological functions, and to defend from predators such as insects, fungi and herbivorous mammals (Lai and Roy, 2004). Chemical compounds in plants mediate their effects on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs. Thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to be effective as conventional medicines.

During the past decade, traditional systems of medicine have become increasingly important in view of their safety. Current estimates suggest that in many developing countries, a large proportion of population relies heavily on traditional practitioners and medicinal plants to meet primary health needs. Although modern medicine may be available in these countries, herbal medicines have often maintained popularity for historical and cultural reasons (Farnsworth and Soejarto, 1991). The use of the plants, plant extracts and pure compounds isolated from natural sources provided the foundation to modern pharmaceutical compounds. The well known Indian systems of Medicine,
namely Ayurveda, Siddha and Unani use predominantly plant based raw materials. Most of these traditional preparations and formulations have been found to be a reservoir of pharmaceuticals (Arora et al., 2003). Medicinal plants of about one hundred and eighteen were collected from Eastern Ghats and screened by the brine shrimp lethality assay and eleven out of the one hundred and eighteen showed significant toxicity of the brine shrimp (Alluri et al., 2006). Of the above mentioned plants species, three plant specimens namely Abutilon, Ocimum and Azadirachta species are included in the present study.

2.2 METAL ANALYSIS

Environmental pollution is one of the burning issues of the world. Heavy metal pollution of soil is a significant environmental problem and has its negative impact on human health and agriculture. (De Jing and Yang, 2007). Most heavy metals are transition elements with incompletely filled orbitals. Fortunately, not all the 53 heavy metals have a good or bad biological function (Neis, 1999).

Ba, Pb, Rb, Sr, Zr, Y have no known biological functions. Fe, Mo, Mn are important trace elements with low toxicity. Zn, Ni, Cu, V, Co, Cr are toxic elements with high to moderate importance as trace elements. As, Ag, Sb, Cd, U have limited beneficial function. Se, Si, Na are beneficial. Al and Ti are non essential. K, Ca, Mg are representative elements. The heavy metal which is of some biological importance is simply based on the solubility function under physiological conditions and the toxicity, which involves its affinity to sulphur plus interaction with macrobioelements (Weast, 1984). Some heavy metals with no biological function such as Cd, Pb, As, Sb, U are toxic to living organisms. These non-biodegradable insidious pollutants have potential
ecological risk. The heavy metals are toxic for human biosystems, even at very low levels of intake and they are usually present in plants because of the increasing industrialization and associated pollution of the biosphere (Heintz, 2003; Chen, 1992).

Since the dawn of civilization, metal pollutants have been part of human history. However, toxic metal pollution of the biosphere has intensified rapidly since the onset of industrial revolution, posing major threat to environment and health. Lipman and Burgess (1914) have reported that heavy metal toxicity to microorganisms in soil and first observations of the effects of heavy metals on microbial processes that date back to the beginning of this century. World Health Organisation (WHO, 1998) mentions maximum permissible limits in raw materials only for As, Cd, Pb. A World Health Organisation survey indicated that about 70–80% of the world populations rely on non-conventional medicine mainly of herbal sources for their primary healthcare. Plant materials are used in developed and developing countries as home remedies and raw materials for the pharmaceutical industry. The World Health Organization (WHO) recommends that medicinal plants which form the raw materials for the finished products may be checked for the presence of heavy metals, pesticides, bacterial or fungal contamination (Khan et al., 2007). The permissible limits set by FAO/WHO (1984) in edible plants for iron (Fe) and zinc (Zn) were 27.4 ppm and 3.00 ppm respectively. The permissible limits for Cu set by China and Singapore for medicinal plants were 20 ppm and 150 ppm respectively (WHO, 2005). According to Jabeen et al. (2010) the range of Cu in agricultural products should be between 4 to 15 ppm. The range of Cu contents in the 50 medicinally important leafy material growing in India were 17.6 ppm to 57.3 ppm.
The determination of metal in environment samples such as soil and plants is very necessary for monitoring environmental pollution (Zhou et al., 1997, Tuzen, 2003, Al-Khashman, 2007).

2.3 ANALYSIS OF METAL CONTENT IN PLANTS

The use of aromatic medicinal herbs to relieve and treat human disease has been increased worldwide because of their mild features and low side effects (Abu-Darwish, 2009). Soil to plant transfer is one of the key processes of human exposure to heavy metals through the food chain. Heavy metal uptake via the roots from contaminated soils and direct deposition of contaminants from the atmosphere onto plant surface can lead to plant contamination by heavy metals (Zhuang et al., 2009). A soil test is the analysis of a soil sample to determine nutrient and contaminated content, composition and other characteristics such as the acidity or pH level. A soil test can determine fertility or the expected growth potential of the soil which indicates nutrient deficiencies, potential toxicities. The test is used to mimic the function of roots to assimilate minerals (Summer, 2000).

The level of essential elements in plants is conditional, the content being affected by geochemical characteristics of the soil and by the ability of plants to selectively accumulate some these elements (Chan 2003). There is a common concept among people that herbal medicines have no side effects and that “being natural in origin, herbs are safe”. The assimilation of heavy metals in plants is obvious because of widespread heavy metals in the soil due to geo-climatic conditions (Khan et Al., 2007).
Standards for herbal medicine that clearly stipulate the maximum allowable value of heavy metals in herbal medicine have been enacted and put into effect by many countries (Chen & Jia, 2005). Accumulation of heavy metals may have impacts on medicinal plants that are different from their impacts on farm crops, therefore, it is necessary to improve quality standards of phytochemicals or herbal medicines by examining and revising the maximum allowable values of heavy metals in medicinal plants, using research based on medicinal plants (Cao et al., 2009).

Contaminated soils with heavy metals can potentially lead to the uptake and accumulation of these metals in the edible plant parts causing health risk. (Jagrati et al., 2012). Medicinal plants are genuinely useful for primary health care. World Health Organisation (1978) has advocated traditional medicine as safe remedies for ailments of both microbial and non-microbial origin. Various studies have been conducted on the phytotoxic effects of heavy metals. The phytotoxic effect of heavy metals such as Cd, Cu, Ni, Pb and Zn on the growth was detected under artificial as well as natural conditions. To test under artificial conditions a greenhouse experiment was conducted. The seed germination, root and shoot growth were found significantly affected by these metals at higher concentration of 40 and 50 ppm. However, the lower concentration of heavy metals ranging from 5 to 20 ppm doses were observed to be stimulating the root and shoot length and increase biomass of the sunflower plant (Chhotu et. al., 2008).

In order to ascertain accumulation of heavy metals including Fe, Co, Ni, Cu, Cr, Mn, Cd and Pb in *Euphorbia helioscopia* L. found in polluted and unpolluted (natural) sites of Peshawar city, investigations were performed by using atomic absorption spectrometry. The results showed heavy metals accumulation in plants, procured from
polluted sites as compared to unpolluted sites (Khan et. al, 2007). Eleven elements (K, Ca, Zn, Cu, Fe, Mn, Cr, Ni, Br, Rb and Zr) were determined by using Energy Dispersive X-ray Fluorescence (EDXRF). The highest concentration of Ca (8107 ppm), Br (33 ppm), Rb (19.9 ppm), Zn (18.11 ppm), Cu (15.9 ppm), K (15451 ppm), Fe (1426 ppm), Cr (13.10 ppm), Ni (7.6 ppm), Mn (10.6 ppm) and Zr (8.2 ppm) were found in various medicinal plants (Shad Ali khan et al., 2008).

The plants grown in contaminated areas have high risk of having heavy metal concentrations beyond the permissible limit for each of them as compared to the less contaminated areas. (Khan et. al., 2007). Heavy metals are among the most important sorts of contaminant in the environment. Several methods already used to clean up the environment from these kinds of contaminants, but most of them are costly and difficult to get optimum results. Currently, phytoremediation is an effective and affordable technological solution used to extract or remove inactive metals and metal pollutants from contaminated soil and water. This technology is environmental friendly and potentially cost effective (Tangahu et. al., 2011). Heavy metals cannot be chemically degraded and need to be physically removed or be transformed into nontoxic compounds (Gaur and Adholeya, 2004).

Plants with exceptional metal-accumulating capacity are known as hyperaccumulator plants. Phytoremediation takes the advantage of the unique and selective uptake capabilities of plant root systems, together with the translocation, bioaccumulation, and contaminant degradation abilities of the entire plant body (Cho-Ruk et. al, 2006; Hinchman et. al., 1995). The most common heavy metal contaminants are Cd, Cr, Cu, Hg, Pb, and Zn. Metals are natural components in soil (Lasat, 2000).
Some of these metals are micronutrients necessary for plant growth, such as Zn, Cu, Mn, Ni, and Co, while others have unknown biological function, such as Cd, Pb, and Hg (Gaur and Adholeya, 2004).

Heavy metal cations play an important role as “trace elements” in sophisticated biochemical reactions. At higher concentrations, however, heavy metal cations form unspecific complex compounds in the cell, which leads to toxic effects. Some heavy metal cations like Hg$^{2+}$, Cd$^{2+}$, Ag$^{+}$ form strong complexes which makes them too dangerous for any physiological function. Even Zn$^{2+}$, Ni$^{2+}$ and especially Cu$^{2+}$ are toxic at high concentrations (Weast, 1984; Nies, 1999). Plants generally do not accumulate trace elements beyond near-term metabolic needs and these requirements are small ranging from 10 to 15ppm. The exceptions are “hyper accumulator” plants, which can take up toxic metal ions at levels in the thousands of ppm (Tu et al., 2004).

Concentration of phytochemicals or qualitative and quantitative analysis of phytochemicals when the economically important plants are exposed to metal pollutants has been studied by various people in several plants. Effect of heavy metals on crop plants, vegetables, medicinal plants has been studied. Time to time several studies have been made by many researchers to determine heavy metal levels in spices (Farooq et al., 2008), vegetables (Kumar et al., 2007; Yargholi et al., 2008; Kala and Khan, 2009), weeds as well as medicinal plants (Hussain and Khan, 2010; Ijeoma et al., 2011). The level of heavy metals in plants is conditional depending upon geochemical characteristics of the soil and the ability to selectively accumulate some of these elements (Tolonen, 1990). Bioavailability of the elements depend upon the form of their bond with the constituents of the soil. Plants easily assimilate such elements through the roots, which
dissolve in water and occur in ionic forms. It is believed that the great majority of heavy metals act as key components of essential enzyme systems or other proteins, e.g. the haemoprotein, haemoglobin which perform vital biochemical functions. As, Cd, Hg, Pb etc are highly toxic for the human bio system even at very low levels of intake and they are usually present in plants because of the increasing industrialization and associated pollution of the biosphere, taken up from the soil, water, fertilizers, pesticides treatment and anthropogenic operations (Hunt, 2003; Chen, 1992).

Hyper accumulators achieve a shoot-to-root metal-concentration ratio greater than one. Nonaccumulating plants typically have a shoot-to-root ratio considerably less than one. Ideally, hyperaccumulators should thrive in toxic environments, require little maintenance and produce high biomass, although few plants perfectly fulfill these requirements. Metal accumulating plant species can concentrate heavy metals like Cd, Zn, Co, Mn, Ni, and Pb up to 100 or 1000 times than those taken up by nonaccumulator (excluder) plants (Mwegoha, 2008). Although, a lot of toxic/trace elements were recorded in the plants, these levels or concentrations were far below the World’s Permissible Levels (Bowen, 1979; Pendias and Pendias, 1984).

Again, the high levels of iron (Fe) in the plant organs may probably make it a very powerful tonic to boost anaemic conditions in humans. It is worth mentioning that almost all the essential elements required as the natural remedies for diabetes are present in all the three plant species even though in lower quantities compared to the permissible levels. zinc, which lowers blood sugar; chromium, improves glucose tolerance and magnesium, that leads to improved insulin production in elderly people and reduces eye damage (Ameyaw et al., 2012).
However, it has been established that over dose or prolonged ingestion of medicinal plants leads to the chronic accumulation of different elements which causes various health problems. This is because these essential metals can also produce toxic effects when the metal intake is in high concentrations, whereas non-essential metals are toxic even in very low concentrations for human health (Sharma et al., 2009). Elemental contents of the medicinal plants are therefore very important and need to be screened for their quality control (Liang et al., 2004; Arceusz et al., 2010).

2.4. PHYTOCHEMICAL AND ANTIMICROBIAL ANALYSIS OF MEDICINAL PLANTS

Use of plant based drugs and chemicals for curing various ailments and personal adornment is as old as human civilization. Plants and plant-based medicaments are the basis of many of the modern pharmaceuticals we use today for our various ailments.

Drug resistance is a serious global problem and spread of resistance poses additional challenges for clinicians and the pharmaceutical industry. Use of herbal medicines in the developed world continue to rise because they are rich source of novel drugs and their bioactive principles form the basis in medicine, nutraceuticals, pharmaceutical intermediates and lead compounds in synthetic drugs (De N et al., 2002 and Ncube et al., 2008). Screening medicinal plants for biologically active compounds offers clues to develop newer antimicrobial agents. These compounds after possible chemical manipulation provide new and improved drugs to treat the infectious diseases
Plant based products are cheaper alternatives to the development of synthetic drugs. (Reddy et al., 2013)

The acceptance of the traditional medicine as an alternative form of health care and the 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). Plants containing terpenoids, steroids, phenolic compounds and alkaloids have been reported to have antimicrobial activity (Hostettmann and Nakanishi, 1979). 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 et al., 1963). Even vegetables and fruits may help reduce the risk of cancer in humans (Moon et al., 2011; Chen et al., 2006). The plants used in the present study have the following phytochemicals and antimicrobial history.

2.4.1 Abutilon indicum Linn.

Abutilon indicum Linn. (Malvaceae) commonly called “Country Mallow” is a perennial plant up to 3m in height. It is abundantly found as weed in sub-Himalayan tract, hotter parts of India, adjoining countries, Malaya, Philippine islands and China. The plant is used in traditional medicine in India, Pakistan, China and Philippines for treatment of several diseases like bronchitis, body ache, toothache, jaundice, diabetes, fever, piles, leprosy, ulcers, cystitis, gonorrhea and diarrhea (Kirtikar and Basu, 1987; Sivarajan and
Balachandran, 1994; Bakshi et al., 1999; Nicholson, 1991). *Abutilon indicum* Linn. is reported to have hepatoprotective (Porchezhian and Ansari, 2005), hypoglycemic (Lakshmayya et al., 2003), antimicrobial (Mehta et al., 1997), male contraceptive (Shah et al., 1997) and antidiarrhoeal (Chandrashekhar et al., 2004) activities. A large number of phytoconstituents have been isolated from different parts of *Abutilon indicum* Linn. viz. carbohydrates, essential oil, flavonoids, sesquiterpenes, fatty acids, amino acids and sterols (Ahmed et al., 1993; Gaind and Chopra, 1976; Matlawska and Sikorsba, 2002).

The pharmacognostical profile of root of *Abutilon indicum* Linn. which covers preliminary phytochemical screening, morphology, histology, powder analysis, ash values, extractive values, loss on drying and paper partition chromatography of carbohydrates and amino acids. It is the first report for *Abutilon indicum* (Sharma and Goyal, 2010).

### 2.4.2 Achyranthes aspera Linn.

Available literature on *Achyranthus aspera* indicates that the antibacterial activity is due to different chemical agents present in the extract including essential oils especially thymol, flavonoids and triterpenoids and other natural phenolic compounds or free hydroxyl groups. These are classified as active antimicrobial compounds (Hasan et al., 1994). Flavonoids have a number of nutritional functions and have been described as biological response modifiers; most act as an anti-oxidant and some have anti-inflammatory properties. Flavonoids have been shown to prevent or slows the development of some cancers (Narayana et al., 2001). Several investigators have reported that the methanolic extracts of the leaves of *Achyranthes aspera* have significant
antimicrobial activity against the Gram-Positive (S. aureus, Bacillus subtilis) Gram-negative bacteria (K. pneumoniae, E. coli) and fungal species (Aspergillus niger, C. albicans) (Londonkar et al., 2011; Sharma et al., 2011).

*Achyranthes aspera* belonging to the family Amaranthaceae is one of the important medicinal plants. It is used as traditional medicaments in the treatment of fever, especially malaria fever, dysentery, asthma, hypertension and diabetes (Girach and Khan 1992; Tang and Eisenbrand, 1992). The chloroform and ethanol root extracts of the *A. aspera* are reported to have anti-implantation and abortifacient activity (Bhom et al., 1992; Vasudeva and Sharma, 2006). The ethanol extract of the root possess spermicidal activity (Paul et al., 2006). The literature survey indicates that aqueous and methanolic extracts of the whole plant have hypoglycaemic effect (Mohammad and Javed, 1991). Roots are used as astringents to wounds in abdominal tumor and stomach pains (Ghani, 2003). The stem shows abortifacient activity in the rat. Leaf extracts were reported to possess thyroid stimulating and antiperoxidative properties (Tahiliani and Kar 2000; Chandrakant et al., 2012).

### 2.4.3 *Gomphrena celosioides* Mart-Beitr

As of now, there is no literature available on phytochemical, antimicrobial and metal analysis on *Gomphrena celosioides*. 

22
2.4.4 *Azadirachta indica* A. Juss.

*Azadirachta indica* (Meliaceae) commonly known as neem is native of India and naturalized in most of tropical and subtropical countries is of great medicinal value and distributed widespread in the world. The Chemical constituents contain many biologically active compounds that can be extracted from neem, including alkaloids, flavonoids, triterpenoids, phenolic compounds, carotenoids, steroids and ketones. Azadirachtin is actually a mixture of seven isomeric compounds labeled as azadirachtin A-G and azadirachtin E is more effective (Verkerk and Wright, 1993). Other compounds that have a biological activity are salannin, volatile oils, meliantriol and nimbin (Jacobson, 1990; National Research Council, 1992). Neem leaf is effective in treating eczema, ringworm, acne, has anti-inflammatory, antihyperglycemic properties and it is used to heal chronic wounds, diabetic foot and gangrene developing conditions. It is believed to remove toxins from the body, neutralize free radicals and purify the blood. It is used as anticancer agent and it has hepato-renal protective activity and hypolipidemic effects (Aditi *et al*., 2011).

Almost every part of a neem tree, *Azadirachta indica* (Meliaceae), is known for its therapeutic values and has been in use as traditional medicine to treat a wide range of human disorders since ancient times. It is an evergreen tree indigenous to south Asia and in most parts of Indian subcontinent (Govindachari, 1993). Antimicrobial activities of neem have widely been recognized. The neem leaf, bark extracts and neem oil are known to suppress several pathogenic bacterial species and its antiviral activities against vaccinia, chikungunya, measles virus and antifungal activities against several human
fungi have also been established (Biswas et al., 2002). Antimicrobial properties of neem can be attributed to several bioactive compounds found in different parts of this tree, which are categorized into two major classes, isoprenoids and non-isoprenoids. Very few compounds, however, could be studied for their specific bioactivities (Chaturvedi et al., 2011).

2.4.5 Ocimum Sanctum Linn.

A preliminary phytochemical analysis was done in Ocimum sanctum by Koche et al., (2010). The qualitative phytochemical analysis of these plants confirms the presence of various phytochemicals like alkaloids, flavonoids, tannins, terpenoid, saponin, steroid and cardiac glycosides in their aqueous leaf extracts (Britto et al., 2012). 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).

Plants containing terpenoids, steroids, phenolic compounds and alkaloids have been reported to have antimicrobial activity (Hostetttmann and Nakanishi, 1979). Further study is undertaken to study anticancer activity of two plants namely Azadirachta indica and Ocimum sanctum. Limonoids are metabolically altered triterpenoids. 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).

Another herb is Ocimum sanctum. It is well known, easily available, cheap, identity is noncontroversial, free from antitoxicity (Adhvaryu et al., 2007). The goal of
screening medicinal plants is to search for excellent medicinal anticancer agent averted to human malignancies. In defiance of astonishing advances in modern science such as surgery, radiotherapy, chemotherapy and hormone therapy, cancer disease remains a world wide health problem, thus endeavouring the search for new alternate approach.

The nature as a huge valuable contributor of potential source for chemotherapeutic agents has recently been reviewed (Newman & Cragg, 2007). Newman and Cragg (2007) reported that out of 974 small molecules possessed by drugs till 2006, 66% were new chemical entities which were classified synthetic, 17% correspond to synthetic molecule containing pharmacophores derived directly from natural products and 12% are modeled on a natural product inhibitor of the molecular target of interest or mimic the endogenous substrate of the active site such as ATP. These facts are in favour with the new call for medical plant identification namely, local plants, in conjunction with anticancer properties. Resistance of microorganisms to antibiotics is a serious global problem. The phytochemical analysis of the methanol, chloroform and hexane extracts are carried out to study the antimicrobial activity of the selected medicinal plants.

*Ocimum* species are widely used for the treatment of various ailments including colds, coughs, abdominal pains, measles and diarrhea (Obeng – Ofori *et al*., 1998). They are also considered a source of aromatic compounds and essential oils containing biologically active constituents that act as insect repellents, particularly against mosquitoes and storage pests (Hassanali *et al*., 1990; Jembere *et al*., 1995; Bekele *et al*., 1996; Obeng-Ofori and Reichmuth, 1997; Kweka *et al*., 2008), antibacterial (Prasad *et al*., 1986) and antioxidant activity (Hakkim *et al*.,2008). Ethnobotanical surveys report
the traditional use of basil, especially camphor-containing basil, as a veterinary medicinal as well (Baerts and Lehmann, 1991).

2.5 ANTICANCER ACTIVITY

Cancer is the second leading cause of death following heart disease, accounting for 23% of all death (Malyakkad et al., 2012). Plants contain almost unlimited capacity to generate compounds that fascinate 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, phenylpropanoids and terpenoids (Kintizios, 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 and Sivakumar, 2011; Kumar et al., 2011; Kumbhare et al., 2012). The methanol fraction as most polar fraction is expected to contain foresaid active components more than others. Studies have observed the presence of a large number of bioactive compounds in the methanolic extracts of these plants.

Natural products have been used as anticancer agents (Frie, 1982), such as vincristine and vinblastine from *Catharanthus roseus* (Johnson et al., 1963), taxol from *Taxus brevifolia* (Wani et al., 1971) and campothecins from *Camptotheca acuminata*
(Wall et al., 1966). Even vegetables and fruits may help reduce the risk of cancer in humans (Moon et al., 2011; Chen et al., 2006).

Six Thai plants were selected, namely Glochidion daltonii, Cladogynos orientalis, Catimbium speciosum, Acorus tatarinowii, Amomum villosum and Pinus kesiya which are also native to China. The cytotoxicity of these six plants against the human hepatocarcinoma (HepG2) cell line were compared to the normal African green monkey kidney epithelial (Vero) cell line. The extract of Pinus kesiya showed the highest selectivity and cytotoxicity in the HepG2 cell line with an IC50 value of 52.0ug/ml. Extract of Catimbium speciosum too exerted cytotoxicity with an IC50 value of 55.7ug/ml. Crude extracts from Glochidion daltonii, Cladogynos orientalis, Acorus tatarinowii and Amomum villosum exhibited cytotoxicity with IC50 values ranging from 100 to 500ug/ml (Sasipawan et al., 2011).

Cytotoxicity of six phenolics of Terminalia and tannic acid were assessed. It was assumed that cell treated with phenolic compounds serve as an example of their activity. Due to the presence of tannins and related compounds as the major phenolics of T. chebula extract, they could be the molecules responsible for the strong antioxidant antigrowth activities. Chebulinic acid was most cytotoxic (IC50=53.2um) while ellagic acid showed slightly weaker activity (IC50=78.5um) (Ashour, 2008). The structural elucidation data were reported by Saleem et al., (2002).

Tannic acid of Terminalia has the ability to show chemoprotective, skin antitumour (Nepka et al., 1999; Perchellet et al., 1992) and growth suppressor activity against HepG2 cell lines (Kamei et al., 1999). Tannic acid was analysed and was found to contain gallic acid and di-, tri, tetra, and pentagalloyl glucoses besides many minor
unidentified compounds. The antioxidants are known to play key role in reducing cancer cell proliferation (Chinery et al., 1998) and tannins are known as strong lipid peroxidation inhibitors (Okuda et al., 1983).

### 2.5.1 CYTOTOXICITY OF NEEM

The cytotoxic activity of crude extracts of *Azadirachta indica* leaves, pulps and seeds were assayed against HepG-2 cell lines. Crude extracts showed remarkable anti-proliferative activity (Jafari et al., 2013). Neem is similarly known worldwide as commercial natural insecticide, pesticide and agrochemical (Carpinella et al., 2006) and is abundant in cytotoxic limonoids (Seema, 2004).

### 2.5.2 CYTOTOXICITY OF *OCIMUM*

Apoptosis inducing effect of essential oils from aerial parts of *Ocimum viride* in human colorectal adenocarcinoma cells was studied. The results revealed that essential oil has apoptosis inducing effect against these cells *invitro* and is a promising candidate for further anticancer activity (Sharma et al., 2009). Essential oil of *Ocimum gratissimum* was investigated for its percent composition and *invitro* toxicity. The findings support the assumption that the cytotoxic activities of the tested essential oil was basically due to its high level content in thymol (Koba et al., 2009).

*Ocimum gratissium* is used as a traditional folk medicine in many countries. Antioxidant activity and cytoprotective activity of an aqueous *O. gratissium* extract was studied against hydrogen peroxide induced toxicity in human HepG2 cells. The findings
indicated that the aqueous extract of *O. gratissium* has an antioxidant capacity and protective effect on oxidative stress in HepG2 cells. This makes this plant extract a promising therapeutic agent in liver diseases. (Yung-Wei chui *et al.*, 2013). Basil contains the procarcinogen estragole that can be metabolized by cytochrome enzymes (Suzanne *et al.*, 2008).