INTRODUCTION
1. INTRODUCTION

1.1 General Introduction and Need for the Study

India is a country endowed with a unique wealth of biotic and abiotic factors. The biotic factor includes a large number of medicinal and aromatic plants. People of this country are also found to be expertise in ethnic diversity and traditional knowledge about the medicinal plants and their uses. Generally, according to scientists, medicinal plants are considered to be the treasure house of potential drugs. Most of these medicinal plants are edible and contain different amounts of vitamins, protein and carbohydrates which help the body to replace worn out cells or tissues, to digest food and combat ailments among other health related problems (Nwachukwu et al., 2011).

Plants have an inert ability to combat with the diseases and to maintain good health, since it provides the body with vital nutrients. The traditional knowledge about medicinal plants is a wealth of knowledge and it has been passed on orally from generation to generation without any written document. Hence, documenting this indigenous knowledge through ethno botanical studies is important for the conservation and utilization of biological resources. A recent report states that, 80% of the total global population depends on the traditional medicines and therefore use of medicinal plants is now becoming popular in the modern World (Shyma and Devi, 2012). The reason is that modern allopathic drugs in the recent years have many side effects and hence people are attracted towards herbal medicines and their consumption. Another major reason reported by World Health Organization (WHO) for the use of herbal medicine is that 80% of the world’s population in developing countries, lack the access to modern medicine due to poverty and hence they mainly depend on plants for their primary healthcare (Joao, 2005).
In developing countries like India, medicinal plants provide a real alternative for primary health care system and it has been estimated that 70,000 of plant species are used in folk medicine Worldwide. Generally, plant based drugs are easily available, less expensive, safer to use and moreover the drug is highly efficient and it rarely have side effects. Those plants which have a historical record of medicinal value over thousand years constitute the most obvious choice for a new therapeutically effective drug in the case of life threatening disease conditions such as ulcer, cancer, inflammation and microbial infections (Faizul et al., 2011).

Medicinal plants contain some organic compounds which provide a definitive physiological action on the human body and these compounds are termed as bioactive compounds and include bioactive substances like tannins, alkaloids, terpenoids, steroids and flavonoids. These compounds are synthesized in plants by secondary metabolism. These secondary metabolites are chemically and taxonomically have extreme diverse structures with wide range of functions which are used in the human therapy, veterinary medicine, agriculture and other scientific researches. For example, the phenolic and terpene compounds are the major constituents of many plant’s essential oil and it is used in the treatment of various human ailments (Yadav and Munin, 2011).

Essential oils are odorous and volatile aromatic liquid extracts obtained only in 10% of the plant kingdom and found in special secretary structure like glands, secretory hairs, secretory ducts, secretory cavities and resin ducts. Generally, a plant essential oil contains 20-60 individual compounds but, in some cases it may contain even more than 100 different compounds. At the same time the total amount of essential oil present in a plant is generally very low and rarely exceeds as 1%. But, in some of the plants like nutmeg (Myristica fragrans) and clove (Syzygium aromaticum) it reaches more than 10% (Ciccarelli et al., 2008). Majority of essential oils fall into the category in which the compounds contain molecules of carbon and hydrogen and are classified into
different categories such as Hydrocarbons (e.g., Pinene, Limonene), Alcohols (e.g., Linalol, Santalol), Acids (e.g., Benzoic acid, Geranic acid), Aldehydes (e.g., Citral), Cyclic aldehydes (e.g., Cuminal), Ketones (e.g., Camphor), Lactones (e.g., Bergaptene), Phenols (e.g., Eugenol), Phenolic ethers (e.g., Anethole), Oxides (e.g., Cineole) and Esters (Geranyl acetate). All these compounds may be classified into two main categories such as Terpenoids (Terpenes) and Phenylpropanoids. These terpenes are further classified into monoterpenes ($C_{10}$), sesquiterpenes ($C_{15}$) and di-terpenes ($C_{20}$) (Andrade et al., 2011).

So far researches showed that most of the plant’s essential oils are found to have an enormous therapeutic potency in curing various animal and human ailments. They are valuable natural products and hence they are used as a raw material in many fields, including perfumes, cosmetics, aromatherapy, phytotherapy, spices and nutrition. The essential oils of different plants have been reported that efficient anti-bacterial, anti-viral and anti-fungal activity against many pathogenic strains (Hulin et al., 1998). It is also found that essential oils and their individual aroma components showed cancer suppressive activity when tested on number of human cell lines. It is shown that that the essential oils are rapidly absorbed when it is used via oral, intra dermal, intraperitoneal and pulmonary administration and they cross the blood-brain barrier and interact with the receptors in the central nervous system (Ahmadi et al., 2002). Therefore, in the present study we need to evaluate the therapeutic potential of the selected medicinal plant’s essential oil such as anti-microbial, anti-inflammatory, anti-oxidant and anti-cancer activity by using different experiment models.

In general, infectious diseases are one of the major reasons for the higher proportion of health related problems in the developing countries like India. Since, microorganisms have developed resistance to many antibiotics there prevails an immense clinical problems in the treatment of infectious diseases. Even though,
pharmaceutical industries have produced a number of new antibiotics for the past three decades, resistance to these drugs by micro-organisms has increased and being increasing in a drastic way (Sunitha and Mahendra, 2008). Many research reports have shown that the plants containing large number of phytochemicals have been well documented to have inhibitory effects on all types of micro-organisms in-vitro. The use of plant compounds (plant extract and essential oil) for pharmaceutical purpose has gradually increased in both the developed as well as developing countries due to their greater significance in therapeutic treatments. According to biologist, active compounds found in plants appear to be more adaptable, acceptable and safer than synthetic compounds and display a wealthy source of potential anti-microbial agents. On the other hand, research reports also supports the above mentioned statement that the plants extracts and essential oils are effective against fungal and bacterial pathogens (Gislene et al., 2000).

Generally, if the body is injured it calls upon its defenses to protect it by a process called inflammation. Similarly, when an infectious agent bacteria or viruses attack the body and thereby it can cause injury by harming its various tissues. Treatment for infection and inflammation includes managing both the pathogen and its resulting symptoms. For example, modern antibiotics are commonly used to deal with an infection process. But recently, natural products such as plants extracts and essential oils have been used for treating inflammation caused by infection. In particular, essential oils have two prominent features such as low toxicity and low risk of resistance development by pathogenic microorganisms (Amini et al., 2012). Further formulation and elaborate in-vitro and in-vivo experiments are necessary to achieve these targets.

Inflammation is a complex reaction to injurious agents and other insults. The insult may be physical, mechanical, chemical and even by microorganisms. Usually,
inflammation is classified according to its time course as acute and chronic inflammation (Joao et al., 2003). Acute inflammation is the initial and often transient series of tissue reactions to injury and it may last from a few hours to few days. During, the earlier stages of acute inflammation odema, fibrin and polymorph neutrophils accumulate in the extracellular spaces of the damaged tissue. The presence of these above mentioned cellular components is essential for the diagnosis of acute inflammation by histopathological analysis. Since, these cells begin to appear in the wound rapidly after damage has occurred, usually achieving their maximum population within 48 hours. But, neutrophils have a very short life span and begin to decline after 72 hours particularly if there is no infection (Sharma et al., 2010).

On the other hand, chronic inflammation is the subsequent and often prolonged tissue reactions following the initial response i.e., if the agent causing acute inflammation has not removed, the acute inflammation may progress to chronic inflammation. According to earlier research reports chronic inflammation may be defined as an inflammatory process in which lymphocytes, plasma cells and macrophage predominate and is usually accompanied by the formation of granulation tissue resulting in fibrosis. It is also commonly known among medical experts that chronic inflammation is usually primary, but does occasionally follow acute inflammation. Many types of inflammation based diseases are known which includes rheumatic fever, rheumatoid arthritis, spondylitis, systemic lupus erythematosus and osteoarthritis (Gupta et al., 2011). Treatments that are currently available use modern anti-inflammatory drugs, but these drugs are highly associated with severe side effects and hence the identification of new anti-inflammatory drugs with fewer side effects is needed. Plants have been used by man as drugs to cure various types of inflammatory disorders. Moreover, plant based herbal drugs are used widely because scientists considered them to be safer, efficient in their activity and mainly they are cost effective and easily available for use (Mohini et al., 2012).
Other treatment for these inflammatory disorders is available in traditional medicines using various herbal preparations. In order to reduce the side effects produced in modern medicine, vast studies are carried out to discover a suitable herbal drug. So far, available modern medicines are derived originally from traditional herbal sources. With this background knowledge about traditional herbal medicine scientists have evolved to produce conventional medicine, which uses both synthetic drugs and isolated natural compounds (Agnihotri et al., 2010). We intend to study the effect of the study plant’s essential oils on the chemically induced inflammatory condition.

Furthermore, inflammation has long been associated with cancer. There is a strong association between chronic inflammation in the body and the occurrence of cancer. Cancer is a major public health problem in both developed as well as developing countries like India. Occurrence of one in 4 deaths is due to cancer. Cancer begins when cells in a part of the body start to grow out of control and forms malignant tumors and invade the nearby parts of the body. There are many types of cancer of which the major types include breast, skin, lung, colon, prostate and lymphoma and the symptoms of cancers are mainly based on its types. Primarily cancers are an environmental disease with 90-95% of cases attributed to environmental factors and only 5-10% is due to genetic factors (Oleg, 2009).

Development of cancer involves two main stages namely an initial and a final stage. In the initial stages of cancer it will start with just one or a few cells in the body that undergo a change and become malignant or cancerous and the later stages of cancer starts in a process called metastasis where some of the cancer cells split off and go into the lymph channels or blood stream and to other parts of the body (Idowu and Powers, 2010). Among all the known cancers, lung cancer is one of the most common and malignant diseases to be known Worldwide. It is the leading cause of death with an
estimate of more than 1.3 million new cases each year. There are two major types of lung cancer namely small cell-lung cancer and non-small cell-lung cancer. About, 85-90% of lung cancer is non-small cell lung cancer (NSCLC) and there are three main sub-types of NSCLC. The cells of these sub-types differ in their shape, size and chemical make-up. But they are grouped together because the approach of treatment and prognosis are very similar. On the other hand, about 10-15% of lung cancers are small cell-lung cancer and the name small cell-lung cancer is due to their size and appearance when viewed under microscope (Sasho, 2005).

Treatment for lung cancer is also different but the most common undergoing treatments include surgery, chemotherapy and radiation therapy. Surgery is often the first treatment option. On the other hand, both radiation and chemotherapy might be used to shrink the tumor before or after the surgery. The term chemotherapy refers to the use of any anti-cancer drugs to kill cancer cells. Usually, these anti-cancer drugs are given intravenously or taken orally. Whereas, radiation therapy is carried out using high energy rays such as X-rays as external radiation outside the body to kill cancer cells or shrink tumors (Akira and Nagahiro, 2003).

In general, according to researchers, any anti-cancer drug should have the capacity to suppress the activity of angiogenesis and metastasis and thereby it has to induce apoptosis also. Angiogenesis is the formation of new blood vessels from a pre-existing blood vessel and is a process involving the proliferation and migration of endothelial cells (ECs). The process of angiogenesis occurs even during normal wound healing process and it is also an essential component of the metastatic pathway because, the new vessel formation process paves the principal route by which tumor cells exit the primary tumor site and enter the circulation (Melo et al., 2010). Earlier reports and research data has shown that there exists a correlation between angiogenesis and tumor metastasis in which a deceased vascularity in the primary tumor is virtually always
associated with decreased formation of metastatic colonies. One of the major limitations of the conventional cancer therapeutic drugs is that they are often limited in their activity if the tumor has undergone distant or widespread metastasis (Jean et al., 2010).

On the other hand, the major hallmark that has to be induced by any anti-cancer drug is apoptosis. Apoptosis or programmed cell death is a normal component involved in the development and health of multi-cellular organisms. Chemotherapeutic agents have also been reported to induce apoptosis which includes the alkylating agents (Cyclophosphamide), topoisomerases II inhibitors (Daunorubicin) Cisplatin, Microtubule disrupters (Vinblastine, Vincristine and Taxol) (Scott and Athena, 2000). Similarly, p53 is the first tumor suppressor gene linked directly to apoptosis. p53 mutation occurs in the majority of human tumors and is often associated with advanced tumor stage. Hence, the role of p53 in apoptosis is indirectly linked to DNA damage and could be stimulus and tissue specific. On the other hand, Caspases are a family of proteins that are one of the main executers of the apoptotic process. Induction of apoptosis via death receptors typically results in the activation of an initiator Caspase such as Caspase 8 or Caspase 10. These caspases are responsible for the cleavage of the key cellular proteins, such as cytoskeleton proteins that leads to the typical morphological changes observed in cells undergoing apoptosis (Kam and Frech, 2000).

Apart from the above mentioned therapies and treatment remedies obtained with the help of medicinal plants constitute a promising avenue for the development of new anti-cancer drugs with fewer side effects. With cancer being a widespread threat to humanity, plants play an important role in cancer prevention, as well as in therapy. Search for anti-cancer agents from plant sources commenced in the 19th century with the discovery and development of Vinca alkaloids, Vincristine, topotecan, irinotecan, etoposide, taxol, paclitaxel and Vinblastine in the year 1950s (Mouli et al., 2009). National Cancer Institute (NCI) have also collected about 35,000 plant samples from 20
countries and has screened around 1,14,000 extracts and essential oils for anti-cancer activity. Out of 119 anti-cancer drugs available all over the World about 64% are from natural origin which includes Plants, Marine and Micro-organisms (Mohammad, 2006).

At the same time, the rapid progress made in cell and molecular biology over the last three decades played a major role in understanding the functioning of cancer cells better. Due to this more specific treatments have been developed and it has become possible to target more precisely the processes involved in the development, proliferation and survival of tumor cells. At present Worldwide, interest aroused in using natural resources, especially medicinal plants which is used to fight disease, maintain health and it is an important ingredient in the health sector and pharmaceutical industry (Rajesh and Howard, 2003).

In the field of modern medicine, particularly in cancer research the use of herbs is increasingly used due to the excessive use of synthetic drugs which is found to have excess toxicity and side effects, which favors the reconsideration of the medicinal plant drugs. Several studies have led to the discovery and development of new active ingredients from natural molecules and these active compounds are used even today in clinical practice. Hence the use of naturally occurring molecules in the treatment of cancer has greatly contributed to the improvement of the therapeutic efficiency of drugs used today in chemotherapy (Elson, 1995). The diverse therapeutic potential of essential oil has turned the attention of the researchers to test them for anti-cancer activity due to the advantage of the fact that the mechanism and mode of action of essential oil is non-toxic. Earlier research reports have put forth that the essential oil component, especially monoterpenes have multiple pharmacological effects on mevalonate metabolism which is responsible for the terpene-tumor suppressive activity. Thus essential oils and their constituents can hopefully be considered in the future for more clinical evaluations and possible applications in cancer treatment. Moreover, it
can be used as an adjuvant to current medications (Ghada, 2013). We have also focused our study to find out the efficacy of the essential oils of study plants on the prevention of lung cancer metastasis and growth *in-vivo*.

The other major cause of cancer is excessive free radical damage in cells that harms DNA and results in some cells mutating into cancerous cells. These free radicals can be defined as atoms or molecules containing one or more unpaired electrons in their orbitals. Formation of free radicals occurs continuously in the cells as a consequence of both enzymatic as well as non-enzymatic reactions. In a recent research it has been estimated that an average person has around 10,000 to 20,000 free radicals attacking each cell of body each day. Increased or uncontrolled free radical activity might combine with other factors to cause some diseases or disorders such as neurodegenerative diseases, diabetes, pre-mature aging, cardiac arrest and cancer. Several reports have highlighted the increased level of free radicals and decreased level of antioxidants in cancer conditions such as cancer patients as well as induced *in-vivo* cancer models. Hence, there needs a balance between the production of free radicals and these antioxidant defenses in the body has important health related benefits (Funda and Hakan, 2008).

The major connective link between antioxidant and free radicals is that antioxidants generally help to counteract the effects of free radicals and they are substances that are typically found in nature such as vitamins and minerals in foods for example Vitamin A and C, beta-carotene, selenium. Most fruits and vegetables that generally we consume in our routine life are considered to be high in anti-oxidants (Valko *et al.*, 2006). In nature there are a wide variety of naturally occurring antioxidants which are found to be different in their composition, physical and chemical properties, mechanisms and site of action. Past few decades search for the novel natural sources of antioxidants was found to be very important and during that time medicinal
plants have been extensively studied for their antioxidant activity. The ingestion of fresh fruits, vegetables and tea rich in natural antioxidants has been associated with the prevention of cancer, cardiovascular diseases and lowers the risk of mortality from these diseases. Among plant based natural antioxidants, polyphenols are the most significant compounds and their activity is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, metal chelators and reductors of ferryl hemoglobin (Mariagrace, 2010).

Many numbers of medicinal plants, spices and their purified constituents like plant extracts and essential oils have shown beneficial therapeutic potentials as antioxidants. These antioxidant activity exerted by the medicinal plants, is due to the presence of flavones, iso-flavones, flavonoids, anthocyanin, coumarin lignans, catechins and iso-catechins. Some of the examples of antioxidant sources from plants are green and black tea leaves due to the presence of polyphenols, which includes flavonols, flavandiols, flavonoids, phenolic and gallic acids. On the other hand, essential oils of spices like *piper longum* (Black Pepper) and *Elettaria cardomomum L* (Cardomomum) have been reported to have significant Superoxide dismutase and Catalase antioxidant activity using *in-vivo* animal models (Nooman *et al.*, 2008). Dietary phenolics have been recently reported by many researchers that they exhibit pro-oxidant and cytotoxic properties under certain conditions. The antioxidant and pro-oxidant activity of phyto-phenolics is due to their metal-reducing potential, chelating behavior, pH and solubility characteristics. It is also reported that the plants which produce essential oils rich in monoterpenes such as β-myrcene, α-pinene, β-phellandrene, β-pinene, β-ocimene, Carvocrol, Thymol, Phellandrene, cymene, caryophyllene, D-limonene and γ-terpinenes possess many important pharmacological activities (Ren *et al.*, 2010).

Recent articles have stated that if the combinations of limonene and perillyl alcohol, carvocrol and thymol present in a plant’s essential oil it may have
chemopreventive activity against cancer since these compounds belong to monoterpenes (Lucindo et al., 2013). Very recent study have shown that, the monoterpenes isolated from medicinal plants have been described as redox-active molecules, since they have the ability to scavenge specific reactive species such as hydroxyl radicals, nitric oxide and prevents the oxidation of biomolecules. It is well known that the reactive species and oxidative stress are linked to a wide array of pathological conditions. The evaluation of the redox action of monoterpenes with potential pharmacological activity may indicate new pharmacological agent for free radical’s related diseases like cancer (Lucindo et al., 2013). Hence, in the future, the specific compounds with high antioxidant capacities should be isolated, purified and identified especially from medicinal plants to develop and discover new novel natural antioxidants in order to reduce the free radicals related disease and disorders. Most of the in-vivo cancer models developed by chemicals as well as cell lines have shown a marked oxidative stress associated with cancer development. The B16F-10 cell line used for our study on lung cancer also has been reported for the development of oxidative stress and generation of free radicals (Shukla and Gude, 2003). Hence, we intend to study the antioxidant property of the study plants essential oil on such oxidative stress which may be the cause of cancer preventive action.

In the current study, we have selected three plants based on their traditional uses as background that includes Plectranthus amboinicus (Lour), Wedelia chinensis (Osbeck) and Tridax procumbens L. The plant Plectranthus amboinicus (Lour) belongs to the family Lamiaceae and is a perennial herb found throughout India, Ceylon and Moluccas. The plant Tridax procumbens L is an annual herb belonging to the family Asteraceae, native to tropical America and rapidly spread in many parts of tropical, subtropical and mild temperate regions Worldwide. Whereas, Wedelia chinensis (Osbeck) belongs to the family Asteraceae and it consists of approximately 65 species and is distributed in tropical and warm temperature regions like India, China and Japan.
These plants have been studied for their chemical constituents and have shown various biological functions.

Recent researchers have studied on different extracts of these plants for their significant activity in curing wounds and found to have anti-fungal, anti-inflammatory as well as anti-tumor activity. Reports are available for the extracts of these plants for lowering blood pressure, blood glucose level and also are documented for their significant hepatoprotective activity in rats. All these in-vivo studies and traditional use did not reveal any deleterious effects in rats and higher animals including man. In particular the leaf extract of *Tridax procumbens L* is being used widely as traditional medicine for healing open wounds and for conjunctivitis due to its greater anti-inflammatory effect. Therefore, it is very clear from the above mentioned research reports and literature data that medicinal plants occurring in nature in particular those plants which are rich in essential oil have enormous therapeutic potential within them. Since, our study plants also come under essential oil containing aromatic plants; it is worthwhile to study the therapeutic potential of these plant’s essential oils. Moreover, previous studies on the different solvent extracts of the selected three plants were found to have significant therapeutic value in the treatment of many diseases. Hence, we need to evaluate the therapeutic potency of these plant’s essential oils using different experimental models.