INTRODUCTION
Cancer is a class of disease characterized by uncontrolled growth, distortion of adjacent tissues and metastasis. More than 20 million people are living with cancer worldwide and estimated that 9 million people will die due to the fatal disease in 2015 (Raj et al., 2012). According to the global burden of cancer study (GLOBOCAN) survey in the year 2012, 19.3 million new cases are estimated to rise by 2025 and the burden of cancer will increase to 23.6 million cases every year by 2030 (Ferlay et al., 2015). In India, 979,786 cancer related death was recorded in the year 2010 and it may increase up to 1,148,757 cases in the year 2020, which indicates that India contributing 7.8% of the global cancer burden and 8.33% of global cancer deaths (Saranath and Khanna, 2014). About 90-95% of the cancers are rising due to modifications in lifestyle, environmental factors and 5-10% due to genetics. It is caused by external factors such as tobacco, chemicals, radiation, infectious organisms and internal factors like an inherited mutation, hormones, immune conditions and metabolic mutation.

Cancer is mainly occurred by DNA alteration in proto-oncogene like ras and tumor suppressor gene like p53, which influence the regulation of essential cellular processes like proliferation, differentiation and apoptosis (Scholzova et al., 2007). Chemical substances such as benzene and nickel have the efficacy to induce cancer by damaging the genetic material (Lin et al., 2009). In some cases, infectious agents like human papilloma virus, epstein-barr virus and Helicobactor pylori also involved in the induction of cancer (Sanchez et al., 2014). The reactive oxygen species (ROS) are one of the accepted free radical which plays an important role in the complex process of carcinogenesis and progression of tumor (Valavanidis et al., 2013). The continuous exposure of irradiation by X-ray and mitochondria-catalyzed electron transport reaction are the source by which ROS are generated in biological system. In living organisms,
the harmful effect of ROS is regulated and detoxified by different cellular enzymes like superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), catalase (CAT) etc. However, over accumulation of ROS leads to the oxidation of DNA by lipid peroxidation which causes DNA damage and finally leads to the cancer development (Valko et al., 2006).

Based on the function and location of cells from which it originates the cancer are broadly categorized by carcinoma, leukemia and central nervous system cancer (NCI, 2009). They include anal, bladder, breast, cervical, colon, endometrial, esophageal, kidney, liver, lung, lymphoma, ovarian, pancreatic, penile, prostate, skin and stomach cancer. Cancer strikes most of the cell types which are present in our body. Among them, lung cancer is the most common cause of cancer-related death and due to lung cancer, about 224,210 new cases and 159,260 deaths are expected as an outcome in 2014 (American Cancer Society, 2014). The continuous division of tumor cells and their metastatic abilities cause cancer to be so difficult to treat very easily.

**Lung cancer**

Lung cancer is one of the troublesome health problems and the leading cause of cancer related death in both men and women. It is estimated that about 2,13,380 new cases are diagnosed and approximately 1,60,390 deaths occurred in the year 2010. The overall death caused by lung cancer is as high as 30% after diagnosis due to poor prognosis. Among the males, lung cancer is the leading fatal disease in India with an estimation of 0.063 million new cases in 2011 and it would reach 1.03 million cases in 2016. Consumption of tobacco is one of the major risk factors for developing lung cancer however, a significant number of patients with lung cancer were never smokers (Ferlay et al., 2010; Ganesh et al., 2011; Dsouza et al., 2013). The disease is often discovered in a last stage, but also in earlier stages, lung cancer patients have worse
outcome than patients with other cancer. The maximum lung cancer incidence rates are found in North America, Northern Europe and Eastern Asia. In India, approximately 63,000 new lung cancer cases are reported every year. The five year survival rate of lung cancer is very poor when compared to other cancers. Approximately, 70% of survival rate was reported with stage-I associated lung cancer, 40%-50% for stage-II and 15-30% for stage-III A (Douillard et al., 2010).

According to the Global data epidemiologist forecast, the number of incidences in Non-Small Cell Lung Cancer (NSCLC) will grow from 1.03 million cases in 2012 to 1.26 million by 2022 at a rate of 2.2% per year. China has the highest number of NSCLC cases growing from 0.55 million in 2012 to 0.69 million by 2022 at the rate of 2.5% per year. India and Japan also have the significant increases of NSCLC cases in the next 10 years due to growing older population and high smoking prevalence (Global Data, New York, 2013).

Histology of lung cancer

Generally, lung is made up of about 90% of air, 10% of solid tissues and other significant components include the bronchi, bronchioles and alveoli. About 90-95% of the lung cancer arises from the epithelial cells, bronchi, bronchioles and surrounding tissues of the lungs. Based on the histological characteristics of the tumor cell, therapy and prognosis, lung cancer can be divided into two types such as small cell lung cancer (SCLC) and NSCLC by World Health Organization (Oguz et al., 2013).

Small cell lung cancer (SCLC)

SCLC comprises of 20-30% of lung cancer and it is characterized by relatively sensitive to chemotherapy and radiotherapy. It is characterized by small cell with scant cytoplasm, ill-defined cell borders, finely granular chromatin and without or
inconspicuous nucleoli. Cigarette smoking is one of the prominent causes of lung cancer, with 5 year survival rate is less than 10% because, the tumors are very aggressive and have a rapid growth rate with distant metastases at diagnosis. It accounts for about 30-40% of primary lung cancer and grow in major bronchi as a stratified or pseudoductal arrangement. It has great potential to divide quickly, from large tumors and spread to lymph nodes and other parts of the body (Zahir and Mirtalebi, 2012). It has high grade neuroendocrine carcinoma with high rate of mitotic activity. The common antigen leucocyte (CD45/LCA) and keratin cocktail are mostly used in the diagnosis of SCLC. The morphological features on microscopic evaluation, a positive keratin and negative CD45/LCA stains support the diagnosis of SCLC. There are two stages of SCLC distinguished in clinical practice developed by the Veteran's Administration Lung Cancer Study Group (VALSG), such as limited disease and extensive stage (LD-ED). Limited disease is confined to one hemithorax and can be encompassed within a single radiation port. Extensive diseases have been spread beyond the hemithroax with metastasis at distant sites (Ganti et al., 2013).

**Non-small cell lung cancer (NSCLC)**

NSCLC comprises approximately 85% of all lung cancer cases and it can be divided into two major types: non-squamous carcinoma (NSC) and squamous cell or epidermoid carcinoma (SCC). The NSC includes adenocarcinoma, large cell carcinoma (LCC) and other types. NSCLC is less sensitive to chemotherapy therefore; surgery is one of the most suitable treatments for this type of cancer. Squamous cell carcinoma (SCC) is characterized by keratin formation and most of them arise from the central or proximal tracheal-bronchial tree in areas of squamous cell metaplasia, dysplasia and carcinoma *in situ*. One third of SCCs is poorly differentiated and it spread, particularly in liver and small intestine.
Adenocarcinoma

Adenocarcinoma is one of the most common types of lung cancer in women and non-smoker and it is originated from the glands of lung that produces mucus. It embraces up to 40% of non small cell lung cancer. It commonly arises in the outer and peripheral regions of the lung and sometimes it spread along the preexisting alveolar walls. Smoking is one of the most prominent causes of adenocarcinoma. It is the most common type of lung cancer, however, the sub-type of this cancer bronchi alveolar adenocarcinoma (BCA) tends to be slow growing and have fewer prospects to metastasize for this reason it has a more favorable prognosis than other forms of NSCLC (Subramanian et al., 2007). Adenocarcinoma is characterized by the definite gland formation or by the presence of mucin production in solid tumors. Generally it originates from peripheral airways and alveoli but traditionally it was thought to originate from scars “scar carcinoma” and large cell carcinoma which is characterized by large cells with large amounts of euchromatin (Boffetta et al., 2002).

Squamous cell carcinoma (SCC)

SCC commonly arises in the central part of bronchi in a stratified or pseudoductal arrangement through squamous metaplasia and characterized by keratinization and presence of intercellular bridges between adjacent cells are most common features. It is also known as epidermal carcinoma, which embraces 30-40% of primary lung cancer. SCC can spread to other parts such as bones, adrenal glands, liver, small intestines and brain. It is most common type of NSCLC, highly associated with cigarette smoking (Travis et al., 2011; Uddin, 2002).

Large cell carcinoma (LCC)
This is least common type of NSCLC which is large and heterogeneous group of indifferent malignant neoplasm arise from epithelial cells in lung. It is commonly differentiated from SCLC by the large size of the anaplastic cells. It embraces about 10% of all lung cancer and it can be differentiated from SCC by the larger size of the anaplastic cells with higher cytoplasmic content. It occurs in any part of lung and the prognosis is generally less than other type of NSCLC (Blachhall et al., 2007).

Causes of lung cancer

Cigarette smoking is one of the most predominant causes of lung cancer, which accounts for over 90% of lung cancer case (Bray et al., 2004). Workplace agents such as asbestos, arsenic, chromium, nickel and environmental factors like passive smoking, indoor radon and air pollution also contributing to the onset of lung cancer (Alberg et al., 2005). An accurate staging system of lung cancer is more important for appropriate treatment of lung cancer and determining the prognosis of lung cancer. The International system for staging lung cancer consists of T-tumor-N-regional lymph node-M-metastasis (TNM) staging system is accurate and widely used system in the management of NSCLCs. Based on the tumor size, the presence of malignant cells in regional lymph-nodes and the absence of metastasis stages, lung cancer is classified into four major stages; stage-I (I, IIA), stage-II, stage-III (IIIA to IIIB) and stage IV. The stage-I to IIA represent localized tumor in the absence of metastasis, stage IIB to IIIB represent localized tumors with metastasis in lymph-nodes and stage IV is an advanced stage with distant metastasis. More than three forth of NSCLC patients have locally advance or widespread metastatic stages (stage IIIB and IV) at diagnosis (Mountain et al., 1997).
Table 1. Stages of lung cancer and their features of tumor regional lymph node metastasis (TNM) (Mountain et al., 1997).

<table>
<thead>
<tr>
<th>Stages of Lung cancer</th>
<th>TNM features</th>
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<tbody>
<tr>
<td>Stage 0</td>
<td>TIS</td>
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<tr>
<td>Stage IA</td>
<td>T1, N0, M0</td>
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<tr>
<td>Stage IB</td>
<td>T2, N0, M0</td>
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<td>Stage IIA</td>
<td>T1, N1, M0</td>
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<tr>
<td>Stage IIB</td>
<td>T2, N1, M0</td>
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<tr>
<td>Stage IIIA</td>
<td>T1-3, N2, M0, T3, N1, M0</td>
</tr>
<tr>
<td>Stage IIIB</td>
<td>T4, any N and M0, any T, N3, M0</td>
</tr>
<tr>
<td>Stage IV</td>
<td>Any T, any N, M0</td>
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</tbody>
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Symptoms and diagnosis

Due to the lack of clinical symptoms, lung cancer is more difficult to predict in early stage. It can grow to large size in an asymptomatic patient usually and a mass is not discovered until it invades some other parts like blood vessels, a cough receptor and pleural pain receptor. Cough, dyspnoea, hemoptysis and post obstructive pneumonia are common symptoms associated with lung cancer, although in some patients lung cancer can present without symptoms. There are several methods such as Computerized Tomography (CT), Magnetic Resonance Imaging (MRI) and Positron Emission Tomography (PET) scans are used to diagnose the early stage of lung cancer. These scanning results are used for three dimensional treatment planning system for more
precise radiation therapy treatments. Tissue diagnosis is one of the reliable methods for diagnosing lung cancer, which include, sputum cytology, a thoracentesis, excisional biopsy of an accessible node, flexible bronchoscopy, transthoracic needle aspiration and video-assisted thoracotomy. Sputum cytology is a simple, reliable, cost effective and non-invasive method in which a sample of sputum is examined under microscope. This test is more helpful to find cancer, such as most squamous cell lung cancer, but, it is not reliable for another type of non small-cell cancer and its use is limited to tumors that extend into the airways and the yield is lower than other techniques such as bronchoscopy and needle aspiration. The sensitivity of this technique is 77% for central tumors and 50% of peripheral tumors. In some cases, the pleural effusion is found between the lung and the chest cavity, in such cases thoracentesis can be performed. Bronchoscopy is used to find out tumor or blockage in the large airways of the lungs and the value of this method depends on the location of the tumor. It also examines the transbronchial anatomy, transbronchial biopsy and staging of lymph nodes by transbronchial needle aspiration (Firmin et al., 1982).

Table 2. Common symptoms of lung cancer (Thanossioris, 2000).

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>Cough</td>
<td>60%</td>
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<tr>
<td>Loss of weight</td>
<td>56%</td>
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<tr>
<td>Dyspnea</td>
<td>46%</td>
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<tr>
<td>Chest pain</td>
<td>34%</td>
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<tr>
<td>Hemoptysis</td>
<td>27%</td>
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</table>
Treatment for lung cancer

The aim of the cancer treatment is to remove or destroy the cancer cells while sparing normal cells. Lung cancer treatment mainly depends on the histological type of SCLC and NSCLC, the stages of disease, and the performance status. SCLC is usually metastatic in nature, consequently, chemotherapy represents the mainstay treatment for the lung cancer and therefore, surgery is rarely applied to lung cancer. Commonly, the combination of cisplatin and etoposide were used as a potential drug molecule to treat the SCLC with chemotherapy (Murray et al., 2006; Johnson et al., 2004).

Radiation therapy and chemotherapy can be applied as an initial treatment for lung cancer to shrink the tumor for complete resection of borderline respectable tumors (stage III). It can also be used after surgery for complete eradication of micometastasis. Tumor resection is the best treatment of NSCLC, which gives the best survival rate in patients who have pathologically confirmed stage-I and it progressively, decreases the effect of cancer depending on the stages of lung cancer (Arriagada et al., 2004).

Spiral Computed tomography (CT) and auto-fluorescence bronchoscopy are used as a screening method to investigate the effectiveness of lung cancer. The test is used to measure the auto-antibodies (Ab) against six tumor-associated antigens like p53, SOX2, CAGE, NY-ESO-1, GBU4-4 and Annexin 1 which are produced in tumor cells and act as markers of an immune response within a host (Talbot-Smith et al., 2003). In chemotherapy, prior to the development of cisplatin, the survival rate of NSCLC rarely exceeded 20%, but later during the use of the combination of cisplatin with other compounds like vinca alkaloid or cisplatin with etoposide increases the survival rates of 20-40%. Recently, new anti-cancer drugs such as paclitaxel,
vinorelbine, docetaxel and CPT-11 are introduced in the treatment of lung cancer, which increases the survival rates to as high as 50-60% (Bunn, 2000).

**Lung cancer surgery**

Commonly, three types of surgery are used to remove the lung cancer that includes wedge resection (segment resection), lobectomy and pneumonectomy. In wedge resection method, a small portion of the lung with tumor and some surrounding tissues are removed and it is the widely used method for the treatment of very early stage of lung cancer. Lobectomy is another type of surgery in which lobe of the lungs is removed, whereas in pneumonectomy an entire lung is removed (Zhang et al., 2013).

Surgery is one of the best treatments for NSCLC, however approximately 75% of the non-small cell lung cancer is inoperable at the stage of diagnosis. In this case, chemotherapy is used as standard treatment to help patients live longer (Burdett et al., 2007). For both NSCLC and SCLC patients, often given by a small dose of radiation with chemotherapeutic drugs used to control the symptoms of lung cancer. This type of treatment is used to rectify the blockage of a large airway lope and difficult to operate tumor affected area. Chemotherapy was used as a neo-adjuvant therapy before the surgery or radiotherapy to shrink the tumor size and improve the outcome after surgery to improve the survival rate in lung cancer patients (Celebioglu et al., 2002).

**Apoptosis**

Programmed cell death is a major phenomenon in normal development of unicellular and multicellular organisms which maintain homeostasis and elimination of damaged cells. Apoptosis, necrosis, autophagy, paraptosis and slow cell death are the different type of cell death. Among them, apoptosis and necrosis are the two fundamental types of cell death in cancer (Darzynkiewicz et al., 1997). The term
Introduction

Apoptosis is derived from Greek word “dropping off” and refers to the falling of leaves from trees in autumn. It is a widely investigated process in biology as described by Kerr et al. (1970). Apoptosis can occur when a cell is damaged beyond repair, infected with a virus, or undergoing stress conditions such as starvation. Apoptosis occurs mainly in intrinsic pathways (mitochondria dependent) and generated by signals within the cell. It involves the activation of mitochondria to release activating proteins like caspases. Numbers of morphological changes of apoptosis have been observed under light and electron microscope including cell shrinkage and chromosome condensation (Kamba et al., 2014). The main apoptotic features are cytoplasmic membrane blebbing and formation of apoptotic bodies. Failure of apoptotic mechanisms are considered as an important determinant of fetal abnormalities, if a cell is unable to undergo apoptosis, due to mutation or biochemical imbalance and it can continue to divide and develop into a tumor (Ronco et al., 1998).

The different patterns of apoptosis such as early and delayed apoptosis, homo-phase, homo-cycle and post-mitotic apoptosis have been identified (Darzynkiewicz, 1997). According to Darzynkiewicz, when the cells are exposed to a fairly high concentration of toxic agents, cells are rapidly undergoing apoptosis. Early apoptosis was occurring, when the injury was brought on the cell in the same cell cycle or the same phase. Homo-phase apoptosis is the term used to define the apoptosis, which take place in the same phase of cell cycle wherein the cells are originally exposed to the apoptosis inducing agent. In this stage, cells stay arrested and die without continuing into the next phase of the cell cycle. Homo-cycle apoptosis take place when the cells are exposed to the noxious agent (initially), excluding the specifying cell phase, so the cells die before or during the first mitosis following the induction of the injury. The phrase post-mitotic apoptosis occurred in the cell cycle is originally exposed to the
Introduction

harmful agent and it also indicative of a delayed apoptosis, which frequently taking place in the cells is pulse-exposed to a fairly low concentration of noxious agent. It occurs due to damage to the genes which are vital for survival.

Morphological changes of apoptosis

Nuclear chromatin condensation and nuclear fragmentation are an important morphological features of apoptosis, which are accompanied by rounding up of the cell, reduction in cellular volume (pyknosis) and retraction of pseudopodes (Kroemer et al., 2005). In nuclear membrane, the nuclear condensation forming a crescent or ring like structure further condenses until it breaks up inside a cell with an intact membrane, a feature known as karyorrhexis (Manjo and Joris, 1995). In addition to nucleus, other morphological features such as membrane blebbing, ultrastuctural modification of cytoplasmic organelles and a loss of membrane integrity were observed in plasma membrane at a later stage of apoptosis (Kroemer et al., 2005).

There are two different pathways initiates apoptosis in mammals; they are an extrinsic pathway or cytoplasmic pathway and intrinsic pathway or mitochondrial pathway.

Extrinsic pathway

Extrinsic pathway triggered by caspase-8 protein which initiates the binding of a ligand like Fas, tumor necrosis factor (TNF) to a cell-surface receptor (Zapata et al., 2001). Fas are a cell surface protein, which belongs to the tumor necrosis factor receptor super family. Fas/Fasl system plays an important role in cell mediated cytotoxicity against lung cell carcinomas. The over expression of Fas/Apo-1 is effectively induced apoptosis in lung cancer (Fulda, 2006). Tumor necrosis factor related apoptosis inducing ligand (TRAIL) also act as a potential anticancer agent. This
ligand binding region activates the pro-apoptotic death receptors DR4 and DR5. The activation of death receptors activates procaspase 8 and 10 which turn from the death-inducing signaling complex (DISC) through Fas associated death domain (FADD) and consequently activates caspase-8 (Nagata, 1999). Caspase-8 cleaves procaspase (3 & 7) into caspase (3 & 7) which trigger apoptosis. Caspase-8 provides a link between the extrinsic and intrinsic pathways of apoptosis, through an alternative mechanism whereby it cleaves and activates the pro-apoptotic Bcl-2 family protein, Bid. Then, the pro-apoptotic Bid protein interact with anti-apoptotic Bcl-2 family protein on the mitochondrial outer membrane to promote mitochondrial-mediated apoptosis (Billen et al., 2008). TNF-alpha receptor is a well known cytokine which share similar cystein rich extracellular domain which plays a crucial role in various cellular activities including, transmitting the cell signal, differentiation and apoptosis (Wang et al., 1998).

**Intrinsic pathway**

The intrinsic pathway activates death signal by the release of cytochrome-c from mitochondria which in turn activates caspase-9 and effector proteins such as caspase-3 & 9. Thus, the activated caspases kill the cells by proteolytic activity of key cellular substrates. In this pathway, mitochondria play an important role in the regulation of both cell death and cell survival through the regulation of Bcl family (Wang et al., 2009). It is divided into two subgroups such as pro-apoptotic members and anti-apoptotic members. The pro-apoptotic members such as Bak and Bax are responsible for the release of cytochrome-c. Bcl-2/Bcl-xL is an anti-apoptotic member which inhibits the initiation of apoptosis by inferring with the interaction of activated Bak/Bax proteins thus preventing the release of cytochrome-c (Rehm et al., 2009).

Malfunction of apoptosis plays a crucial role in pathogenesis of various types of tumors. It induces the cell survival by inactivation of pro-apoptotic signaling or
activation of anti-apoptotic pathways (Devarajan et al., 2002). Genetic changes are one of the major phenomenon during which the normal cell is transformed into a malignant tumor while evasion of cell death and cause this malignant transformation (Hanahan and Weinberg, 2000). Generally, two mechanisms are effectively involved in reduction of apoptosis or apoptosis resistance, which includes disturbed balance of pro-apoptotic protein and anti-apoptotic protein, reduced caspase function and impaired death receptor signaling.

But in case of cancer cells, the process of apoptosis is uncontrolled. Because, the cancer gain the ability of escaping apoptosis by mutating tumor suppressor gene like p53, and over expression of apoptotis regulatory oncogenes like Bcl-2, c-myc and inhibiting pro-apoptotic proteins like Bax and cytochrome-c. The accumulation of mutation by impaired repair of DNA alteration leads to malignant cell transformation (Hanahan and Weinberg, 2000). In multicellular organisms, they have developed certain defense mechanism to protect them against the development of cancer. Tumor suppressor gene, p53 plays a key role in cancer protection. The protein p53 regulates the expression of multiple genes by which it modulate the apoptotic machinery. It also regulates the activation of Bcl-2 family members. Losses of p53 gene alter the expression of Bax and causes accelerated tumor growth (Schmitt et al., 2002).

Pro-apoptotic proteins like BH3, Noxa and PUMA also regulated in a p53 dependent manner. PUMA involved in DNA damage induced neuronal death assisted by the activation of DNA damaging agent and endoplasmic reticulum-stress, but the PUMA induced apoptosis was abolished in p53-knockout mice (Villunger et al., 2003). Additionally, the Apaf-1 gene plays a key role in the activation of DNA-damage induced apoptosis in p53 dependent manner. Thus, p53 regulates apoptosis outside the nucleus and outer mitochondrial membrane by the activation of Bax and cytochrome-c.
The special interest of p53 gene due to its functions in many pathways associated with growth regulation and DNA repair, and also its capability to arrest the cell division, even though with multiple genetic defects. It also regulates a cell-cycle checkpoint in response to DNA damage, and determines the common and radio-sensitivity of cancer cells and it trigger apoptosis by up-regulating other anti-apoptotic protein such as Bax and Fas/APO1, and down regulating apoptosis regulating protein such as Bcl-2 (Hussain and Harris, 1998; El-Deiry et al., 1998). However, the mutated p53 gene can regulate the tumor suppression function in a negative fashion because it can bind and inactivate the wild-type protein (Vogelstein and Kinzler, 1992). The most frequent mutations have been detected in almost 90% of SCLC and 50% of NSCLC. In lung cancer, the negative regulator of p53, the MDM2 binds to p53 and facilitate the transfer of p53 to the nucleus and simulates its degradation (Rodin and Rodin, 2005).
There are three different independent pathways of p53 activation have been identified. The pathway-1 is triggered by DNA damage caused by anthracyclines and ionizing radiation, this pathway is activated by two dependent protein kinase namely, ATM (Ataxia Telangiectasia Mutated) and Chk2 (Checkpoint kinase 2). The pathway-2 is induced by ultraviolet light and protein kinase inhibitors and it is not dependent on intact ATM or Chk2, instead of these two protein kinases ATR (Ataxia Telangiectasia Related kinase) is involved in this pathway for the activation of p53. The pathway-3 is triggered by aberrant growth signals. These oncogenes stimulate the transcription of p14 which binds to MDM2 and inhibits its activity, by which it preventing the p53 binding and degradation (Sherr and Weber, 2000; Vogelstein et al., 2000).

In normal cells, DNA damage is triggered by anthracyclines and ionizing radiation is detected by the protein product of the ATM, transduced towards cell cycle arrest and apoptosis through p53 pathway.

**Figure 1.** The intrinsic and extrinsic pathway of apoptosis (Wang, 2011)

**Figure 2.** p53 apoptotic pathway followed by DNA damage. ATM activates Chk2 by
phosphorylation, which activates the p53 phosphorylation. Arrows indicate the activation and bud end arrow indicates the inhibition.

DNA damage, activates the production of ATM and the ATM activates Chk2 by phosphorylation of amino acid, Thr68 (Matsuoka et al., 2000). It also activates the phosphorylation of p53 at Ser15, a site close to the interaction of a region of MDM2. It prevents the binding of MDM2 and increase the stability of p53 (Delia et al., 2000). ATM can also activate the phosphorylation of MDM2 at Ser395 to decrease its affinity to p53 (Maya et al., 1999).

The member of Bcl-2 family proteins plays a crucial role in the regulation of apoptosis. The effects of Bcl-2, Bcl-xL and Bax protein have been investigated on the regulation of apoptosis in cancer cell induced by chemotherapy. Over expression of Bax protein sensitizes cancer cell to several chemotherapeutic agents (Chou et al., 2003). Kaliberov et al. (2002), reported the expression of Bax gene in cancer cell using a single adenoviral vector and reported that the over expression of Bax induces apoptosis in lung cancer cell line, but not in normal cell line and the loss of Bax have been associated with an accelerated tumor growth which resulted from the loss of p53 in a brain mode.

**Free radicals**

Free radicals are chemically unstable molecules possessing one or more unpaired electrons and are highly reactive. It is naturally generated as by-products in a living system when the cells use oxygen to produce energy and plays an essential role in anaerobic life and metabolism. ROS can be produced by both enzymatic and non enzymatic reactions involving xanthine oxidase (XO) and NADPH oxidase, respectively. It can also produce by exogenous sources such as xenobiotics, chlorinated
compounds, environmental agents, metals (redox and non-redox), ions and radiation (Valko et al., 2006). There are two types of ROS such as free radical and non-radical species. The high concentration of ROS production can damage the cell structures, nucleic acids, lipids and proteins. Superoxide radical (\(\text{O}_2^{\cdot}\)) effectively involved in lipid peroxidation and also has the capability to decrease the antioxidant defense mechanism of CAT and GPx enzymes. Thus, it causes damage to the ribonucleotide consequently induce the DNA damage. The human body evolved an antioxidant system itself to protect against free radicals that include endogenous antioxidants and exogenous antioxidants (Lee and Lee, 2006). The most important oxygen-containing free radicals such as superoxide radicals (\(\text{O}_2^{\cdot}\)), hydroxyl radicals (\(\text{OH}^{\cdot}\)), hydrogen peroxide (\(\text{H}_2\text{O}_2\)), peroxyl radical (\(\text{ROO}^{\cdot}\)), nitric oxide radical (\(\text{NO}^{\cdot}\)) and singlet oxygen (\(\text{1O}_2^{\cdot}\)) are inevitable intermediates from oxygen to water reduction in human body. Most of the organisms have a defense mechanism to prevent the formation of free radicals through scavenging and stabilizing free radicals by producing chain-breaking antioxidants. Free radicals and other reactive oxygen species can be derived from either normal essential metabolic process in human body or from external sources like exposure to X-rays, ozone, cigarette smoking, air pollutants and industrial chemicals (Rahmann et al., 2007).

Superoxide radical (\(\text{O}_2^{\cdot}\)) is one of the strongest reactive oxygen species among the free radicals which generates either directly or indirectly through enzyme or metal-catalyzed processes. Superoxide radical anion has a long half-life and reacts with NO to generate peroxynitrite radicals (\(\text{ONOO}^{\cdot}\)). In both plant and animal cells, the extracellular superoxide is converted into hydrogen peroxide (\(\text{H}_2\text{O}_2\)) by oxidative enzyme such as superoxide dismutase. Hydrogen peroxide has the capability to cross the cell membrane and can inactivate the enzymes by oxidizing their thiol group. When
superoxide (O$_2^-$) radicals react with hydrogen peroxide (H$_2$O$_2$) in metal catalyzed reaction (Fenton reaction: iron catalyzed Haber Weiss reaction) it produces highly reactive oxygen centered hydroxy radical (OH*) which has the capability to damage all molecules which leads to membrane destruction and genetic mutation (Beckman and Ames, 1999).  

Hydroxyl radical is one of the most reactive free radicals and effectively reacts with the first molecule it encounters. It can be formed from superoxide anion and hydrogen peroxide in the presence of metal ions such as copper or iron. The formation of hydroxyl radical causes the oxidation of lipids, proteins and nucleic acids, base modification and DNA cross linking have also been observed (Mandal et al., 2009).

Nitric oxide plays an important role in mediating many aspects of diseases. It is produced by phagocytes and endothelial cells, to yield more reactive species which decomposed to form OH* radicals (Aiyegoro and Okoh, 2010).

**Antioxidants**

An antioxidant is a molecule which prevents oxidation of other molecules. The aerobic organisms developed antioxidant defense mechanisms by itself to reduce the free radical production in two different ways either by preventing the generation of free radical or by intercepting the formation of any radicals. An ideal antioxidant should be readily absorbed and quench free radicals and chelate redox metals at physiologically relevant levels. Antioxidant help to minimize oxidative damage and also prevent the age related diseases (Raghu et al., 2010). Antioxidants are vital substance which has the potential to deactiv ate the free radicals and inhibit the effect of oxidants by donating a hydrogen atom or by chelating metals (Bursal and Gulcin, 2011).

Based on the mode of action, the antioxidant can be divided into two main
types; primary antioxidants or chain-breaking antioxidant, which can inhibit or scavenging free radicals by donating hydrogen and convert them to more stable and secondary antioxidant or preventive antioxidant, which have the efficacy to bind the metal ion, scavenge oxygen, and absorb UV radiation (Maisuthisakul et al., 2007). The antioxidant systems are widely classified into two major groups, such as enzymatic and non enzymatic antioxidant.

**Enzymatic antioxidants**

The enzymatic antioxidant system includes SOD, GPx and CAT and these endogenous defence mechanisms to help protect against free radical-induced cell damage. The antioxidant enzymes like SOD, GPx and CAT metabolize oxidative toxic intermediates. These enzymes also require co-factors such as selenium, iron, copper, zinc, and manganese for optimum catalytic activity. It has been suggested that an inadequate dietary intake of these trace minerals may compromise the effectiveness of these antioxidant defense mechanisms. The consumption and absorption of these important trace minerals may decrease with aging (Khalid Rahman, 2007; Lobo et al., 2010).

SOD is a metallo protein and is the first enzyme involved in the antioxidant defense. In oxidative stress condition the SODs catalyze the dismutaiton of superoxide into oxygen and hydrogen peroxide (Sharma et al., 2012)

\[
2O_2^- + 2H^+ \xrightarrow{\text{SOD}} O_2 + H_2O_2
\]

Catalase is an enzymatic antioxidant widely distributed in all animal tissues and the highest activity is found in the red blood cells and in the liver. It is composed of four identical subunits present in peroxisomes which protect the cells from toxic effect of hydrogen peroxide by conversion of H\textsubscript{2}O\textsubscript{2} into molecular oxygen and water without
the production of free radicals (Putnam et al., 2000).

\[ 2\text{H}_2\text{O}_2 \xrightarrow{\text{CAT}} 2\text{H}_2\text{O} + \text{O}_2 \]

Gpx is a selenoenzyme (selenium-dependent enzyme) which protect the cell membrane and other cellular components from oxidative stress by reduction of variety of H$_2$O$_2$ into water.

\[ \text{H}_2\text{O}_2 + \text{TrxS}_2 \xrightarrow{\text{GPx}} \text{Trx(SH)}_2 + \text{H}_2\text{O} \]

Four different isoforms of Gpx are present in mammalian cell, include cytosolic Gpx (cGpx), plasma Gpx (PGpx), phospholipid-hydroperoxide Gpx (PHGpx) and gastrointestinal Gpx (GI-Gpx). All forms of Gpx required glutathione (GSH) as cofactor for the reduction of hydrogen peroxide (Mates, 2000).

**Non-enzymatic antioxidants**

Non-enzymatic antioxidants are widely present in the cell and most of them are low molecular weight molecules providing secondary defense against ROS production. It can be classified into two groups: endogenous and exogenous antioxidants. The major endogenous antioxidant includes cerulopsalmin, transferrin, hepatoglobin and albumin. They are found in human plasma and it can easily bind with transition metal by which it controls the production of metal-catalyzing free radicals. The endogenous antioxidants include lipoic acid, uric acid, bilirubin, ubiquinone and glutathione which effectively inhibit the oxidation reaction by scavenging the free radicals. However, the body obtains the rest of anitoxidants from external sources, primarily the diet. These exogenous antioxidants are commonly called dietary antioxidant. The natural antioxidants have the ability to protect the human body from harmful free radicals by which it can arrest the progress of chronic disorders. Some dietary plants, including grains, fruits, vegetables, tea etc, possess antioxidants. Vitamins-E, ascorbic acid,
carotenoids (beta carotene), polyphenols, flavonoids and phenolic acids are common exogeneous antioxidants which have potent antioxidant activity by acting as hydrogen donors or chelating with metal ions.

**Medicinal plants**

Nature has been a wide source of therapeutic agent from ancient period and an impressive number of currently used drugs have been isolated from natural sources. The plant based traditional medicine system play an important role in health care. According to World Health Organization, about 80% of the world population used medicinal plants for their primary health care through a traditional medicine system (Owolabi et al., 2007). In India, ayurveda, siddha, unani and folk medicine are the major systems of indigenous medicine. Among these, ayurveda is one of the most developed and widely practiced systems. It provides a route to the prevention and treatments of various diseases by employing a large number of pharmaceutical lead molecules preparations. Herbs and spices are important sources of antioxidants and it is also considered to have a great potential as food preservatives. Natural bioactive compounds are derived either from whole plant or from different parts, like leaves, stem, bark, root, flower, seed etc. Some compounds may derive from excretory plant products such as resin, gum and latex. Medicinal plants offer a valuable source of potent bioactive compounds with wide variety of biological activities and novel structure which lead to novel drug discovery (Vuorela et al., 2004).

Nature has created an antioxidant defense mechanism composed of a group of compounds to protect against the destructive action of free radicals which have the capacity to remove free radicals before they cause cell damage. Plants are a good source of biologically active principles known as phytochemicals which act as potent antioxidants by scavenging free radicals (Molan et al., 2012). The major class of
phytochemicals such as flavonoids, phenolic acid, tannins, alkaloids, carotenoids, tocopherols and its derivatives play an essential role in antioxidant properties. Medicinal plants have high amounts of antioxidants such as polyphenols, which play an important role in absorbing and neutralizing free radicals and decomposing peroxides. Nowadays medicinal plant extracts could be used to treat various diseases by virtue of synergistic effects of one or more components which are present in the medicinal plants (Velioglu et al., 1998). These compounds act directly on the different biological system or through altering the metabolism of animals and human. Therefore, the isolation, purification and characterization of bioactive compounds from medicinal plant extract by various analytical methods are important aspects in the revolutionary world. The medicinal properties of a plant could be based on the therapeutic effects of the phytochemicals (Adesokan et al., 2008).

In 19th century, scientists began to isolate and purify the bioactive compounds from the active fractions of medicinal plants. Salicyclic acid was identified as the active ingredient in a number of plants which has good pain-relieving properties and was first synthesized in 1853 and led to the development of the most widely used drug aspirin (Figueroa et al., 2005). The medicinal properties of plant species or groups are unique because of the combination of secondary products in a particular plant are often taxonomically distinct (Wink, 1999).

**Isolation of bioactive compound**

Generally, the plant extract is a complex mixture which contains more than thousands of compounds. The isolation and identification of active components from medicinal plants are important to evaluate their pharmacological, pharmacokinetic and toxicological mechanisms. Approximately, 90% of the extracts of plant material are insoluble cellular matrix as well as artifacts like chlorophylls, cellulose, starch, salts
and glycoproteins. About, 10% of plant extract are secondary metabolites which are organic compounds synthesized by plants. Based on the biosynthesis pathway, the secondary metabolites are classified into three large families include phenols, terpenes and alkaloids (Mokoka et al., 2000). Therefore, the isolation and purification of active constituents extremely difficult and need a powerful extraction method. It should be simple, rapid and give good yields of analytes with minimum impurities. New techniques like high performance liquid chromatography (HPLC), solid phase extraction (SPC) with spectroscopic analysis like nuclear magnetic resonance (NMR) and mass spectrometry (MS) are used to shorten the time duration to isolate active components from natural sources (Harborne, 1999).

**Bioassay guided isolation**

To evaluate the potential efficacy of compounds from crude extracts, some kind of test must be employed to identify the compounds which possess the type of activity which was present in the crude extract. Bioassay guided fractionation is the usual method for isolation of active compounds. Several bioassays are available to evaluate the different type of compounds which have different type bioactivities and the assay can be chosen based on the nature and type of activity that is desired to isolate the active components (Rahman, et al., 2001). Bioassay is a biological system to evaluate the properties of crude extract, chromatographic fraction, mixture or pure compounds. It included anticancer, antibacterial, anti-HIV and antifungal assays (Sarker et al., 2006). The cytotoxic assay is one of the best methods to detect anticancer properties of the extract which measure the ability of drugs to kill the cancer cells. These assays are based on parameters, such as metabolic activity and cell morphology.

**Chromatographic techniques**
Chromatography is a separation technique which was first discovered by Tsweet in 1906 for separation of chlorophylls on paper. Nowadays chromatography is an extremely versatile technique used to separate the mixture of compounds. It separates the analytes in a mixture based on their physical properties like molecular weight, charge, solubility and how they are interacting with two phases, the mobile and stationary phases (Ahuja et al., 2003). Silica alumina or c-18 powder and sephadex are commonly used as the stationary phase while the mobile phase can be liquid or gas. The mobile phases carry the analytes across the stationary phase. Open column chromatography, paper chromatography, thin layer chromatography have widely used techniques in the separation process. However, the two major type chromatography namely, gas chromatography and liquid chromatography was used for separation of bioactive compounds (Villas-Boas et al., 2007).

Chromatography technique such as gravity column chromatography, thin layer chromatography, and HPLC are used for the isolation, purification or separation of compounds from crude extract. Spectroscopic method like Fourier transform infrared spectroscopy (FTIR), MS and NMR are used for structure elucidation of purified compounds.

**Medicinal plants as a source of anticancer drugs**

There are several methods have been used in drug discovery, including isolation of compounds from natural sources, synthetic chemistry, combinatorial chemistry and molecular modeling (Lombardino and Lowe, 2004). Nowadays, pharmaceutical companies and funding organization were focusing their interest on natural products, particularly from medicinal plants which plays an important source of new drugs, new drug leads and new chemical entities (Newman et al., 2000). Today, about half of the approved currently available drugs were obtained from natural sources. In 1982,
Hartwell published more than 3000 plants that are used in the treatment of cancer. The vinca alkaloids such as vinblastine and vincristine were isolated in minute quantities from *Catharanthus roseus* (L) G. Don (Apocynaceae). Both vinblastine and vincristine are widely used to treat lymphoma type of cancer. It is effectively involved in disassembly of the microtubules by which it stops the process of cell division at the metaphase stage. It also prevents the polymerization of tubulin, which segregate chromosomes into daughter cells at mitosis, their disruption results in blocked mitosis (Warber, 1999). Vinorelbine or navelbeine is a semi-synthetic compound of vinca alkoloid used as an anti-mitotic chemotherapy drug for the treatment of NSCLC and metastatic breast cancer. Microtuble inhibitor vinflunine analogues of vinca alkoids has significant antitumor activity on human xenografts in mice which effectively inhibit the tumor growth and increase the survival prolongation (Unnati et al., 2013).

The plant *Podophyllum peltatum* was initially used as a purgative and in the treatment of venereal warts (Kaplan, 1942). The anticancer properties of podophyllotoxin obtained from *P. peltatum* were reported to inhibit the cell growth by binding irreversibly to tubulin (Wilson et al., 1974). The modified analogs of podophyllotoxin such as etoposide and teniposide also have the efficacy to cause cell death by inhibition of topoisomerase II, thereby preventing the cleavage of the enzyme-DNA complex and arresting the cell growth (Srivastava et al., 2005).

The anticancer property of *Taxus brevifolia* and the active phytoconsituent Paclitaxel (Taxol) was discovered and reported (Wani, 1971). The mechanism of action of the paclitaxel was established by Horwitz, (1979). Paclitaxel inhibit cell proliferation and causes programmed cell death by irreversibly binds to the beta tubulin, thus promoting microtubule stabilization like previously reported anticancer drugs such as vinblastin, vincristine and podophyllotoxin (Wilson et al., 1995). Tubulin microtubule
equilibration is an essential activity for cell multiplication and tubulin stabilization which lead to cell death. Paclitaxel was first discovered compound which promotes microtubule formation and it has been used in the treatment of various cancers, but most commonly used to treat NSCLC, ovarian and breast (Kinghorn et al., 1996).

Homoharringtonine, a potential anticancer drug was isolated from the root and rhizome of the Chinese herbal plant *Cephalotaxus harringtonia* (*C. harringtonia*) have high chemotherapeutic efficacy on human acute agranulocyte leukemia and acute myelocitic leukemia. Ipomeanol, a pneumotoxic furan derivative was isolated from sweet potatoes, *Ipomoea batatas*, has potential anticancer property against lung cancer (Kinghorn et al., 1993). A pentacyclic triterpene betulinic acid from the outer bark of white birch tree *Betula alba* which have efficacy to induce apoptosis in human melanoma and neuroblastoma cells (Fulda et al., 1998). Camptothecin is one of the anticancer agents which derived from the deciduous tree *Camptotheca acuminate* and its derivatives are effectively involved in the inhibition of topoisomerase and cause cell death by inducing DNA damage. It also binds to ribosomes in a cell by which it leads to the inhibition of protein synthesis (Hsiang et al., 1985).

Combretastatins is a potent anticancer compound isolated from the bark of the South African “bush willow” tree *Combretum caffrum*. It was used to treat solid tumors in clinical development due to their anti-angiogenic property by which it causes vascular shutdowns in cancer cells and resulting in tumor necrosis (Cragg and Newmann, 2005; Srivastava et al., 2005). The anticancer activity of a water soluble analogue, combretastatin A-4 phosphate (CA₄) has also been reported and it is in early clinical trials. There are number of CA₄ mimics being developed and among them, three are in clinical trials and eleven are in preclinical development. Two novel alkaloids, such as schischkinnin and montamine have been isolated from the seeds of
Centaurea schischkinii and Centaurea montamine, respectively. Both of the isolated compounds exhibited significant cytotoxicity against human colon cancer cell lines (Shoeb et al., 2005). The cardenolides such as 2''-oxovoruscharin, uscharin and voruscarin from Calotropis procera which exhibits antiproliferative effects against lung cancer cell line A549 (Van Quaquebeke et al., 2005; Sawadogo et al., 2012).

Medicinal plant used in the treatment of lung cancer

Medicinal plants and their products have been recognized as one of the attractive approaches for lung cancer treatments. The plant derived drugs have proven to be useful and effectively sensitizing the conventional agents, prolonging survival time, preventing side effects of chemotherapy and improving the life span in lung cancer patients. Herbal medicines and phytochemicals act as a potent anticancer agent for lung cancer treatment by regulating multi-molecular targets involved in angiogenesis, metastasis (Cragg and Newman, 2013; Jeong et al., 2011). Several medicinal plants such as Acalypha wilkesiana, Annona senegalensis, Balanites aegyptiaca, Calotropis procera and Holarrhena floribunda (Sawadogo et al., 2012), Asparagus cochinchinensis, Hedyotis diffusa and Pseudostellaria heterophylla (Cho, 2010), Abutilon indicum (Kaladhar et al., 2014), were reported to represent excellent anticancer activities against human lung cancer cells.

In this direction, the Indian traditional plant, Luffa acutangula belongs to the cucurbitaceae family was selected for the present study to evaluate the anticancer activity of the active principle(s).

Cucurbitaceae

The cucurbitaceae family comprises 121 genera and over 750 species, arranged into 8 tribes and 2 sub-families. The plants belong to this family are rich in oxidatively
modified triterpenes called cucurbitacin. Nearly, 36 genera and 100 species of cucurbitaceae family are found in India (Heiser et al., 1988). It is a major family, which has economically important domesticated plant species. Among them, Luffa species has a long history of cultivation in the tropical and subtropical countries of Asia and Africa.

**Luffa acutangula (L.) Roxb**

*Luffa acutangula* is a large annual climber, 3-6 m long or more. The leaves are smaller and orbicular in outline with 15-20 cm long and prominent veins and veinlets. Flowers are smaller and yellow in color, both male and female flower present in the same plant. The male flower is auxiliary with 12-20 flowered racemes and female are solitary peduncle and furrowed. It has a tomentoes type of ovary. Fruits are conical at the both ends (15-30 cm long, 3-4 cm thick) and outer surface being covered with 8-10 longitudinal ribs, bitter taste, transverse section of fruit shows a single layer of thick cuticle followed by 4-6 layers of parenchymatous cells. Seeds are smaller and brown in color which is enclosed in a thin fibrous network. It is mostly grown in Southern India and Bengal. The nutritive proportion of 100 grams of fruit is 54% of protein, 0.1 g of carbohydrate, 0.2 g of fiber and 3.3 g of organic acid as major ingredients and also has minerals like calcium, potassium magnesium, zinc, thiamin, riboflavin and niacin in the proportion of 14 mg, 160 g, 14 mg, 0.2 mg 0.05 mg, 0.01 mg and 0.02 mg respectively (Dandge et al., 2012).

**Taxonomic position of Luffa acutangula**

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>: Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phylum</td>
<td>: Mangnoliophyto</td>
</tr>
<tr>
<td>Class</td>
<td>: Mangnoliopsida</td>
</tr>
<tr>
<td>Order</td>
<td>: Violales</td>
</tr>
<tr>
<td>Family</td>
<td>: Cucurbitaceae</td>
</tr>
</tbody>
</table>
Genus : *Luffa*
Species : *Luffa acutangula*

**Figure. 3. Luffa acutangula (Jadhav, 2012)**

**Vernacular names of Luffa acutangula**

- **Assam** : Jhing, jika
- **Bengali** : Sataputi, Titotorai, Titodhundul
- **Hindi** : Gudari, Sataputitorai, Torai, Tori
- **Kannada** : Eere Kaayi, Heere kaayi Balli, Kadupadagila
- **Malayalam** : Cheru-peeram, Peechhakam, Puttalpiram
- **Manipuri** : Mayang-shebot
- **Marthi** : Dodka, Ghosavala Shiralla, Gomsali
- **Sankrit** : Sushodhani, Sutikta, Tikta
- **Tamil** : Patarkay, Peerakai, Peerkankai, Peerkku
- **Telugu** : Beera, Beerakaaya, Urkai
- **Urdu** : Bhol

**Properties and uses**

*L. acutangula* possesses laxative and purgative property. It is tonic to the intestines, and used to cure vata, anemia, leucoderma, piles, bronchitis, uterine and vaginal tumors. Fruits are useful in the treatment of urinary discharge, fever,
leucoderma, bronchitis, asthma and biliousness. The fruit pulp is given with water to cure dog bite and also other kinds of bite. The juice of young fruit was used to cure headache. In Ayurveda system of medicine, the ripe seed are employed as an emetic, cathartic and errhine and it possess great control over dysentery. It is used as an antidote for snake poisoning and also used as a bitter tonic and diuretic, recommended in splenic enlargement (Kirtikar and Basu, 1991).

**Chemical constituents**

Fresh fruits contain 91.77% moisture, and completely dried fruits contain 0.87% albuminoids (containing nitrogen-0.14), 73.47% soluble carbohydrates, 16.56% woody fiber, and 6.12% ash. Dried seeds contain a principle allied to colocynthin, and a gelatinous bitter compound luffin. The trypsin inhibitors such as LA-1 and LA-2 have been isolated from seeds of *L. acutangula*. The trypsin inhibitors LA-1 and LA-2 consist of 28 and 29 amino acid residues, respectively. But they lack threonine, alanine, valine and tryptophan residues and both are effectively inhibited the trypsin by forming enzyme inhibitor complex (Haldar *et al*., 1996). Ribosomal inactivating peptide, luffangulin have been isolated from seeds with 5.6 kDa, which inhibited cell-free translation with an \( IC_{50} \) of 3.5 nM (Wang *et al*., 2002). A chito-oligosaccharide, lectins have been isolated from the fruits of *L. acutangula* (Anantharam *et al*., 1986). It has a molecular weight of 48 kDa and stoke radius of 2.9 nm which has 31% alpha helix and no beta sheet. The bioactive compounds like bitter taste cucurbitacin-B, oleanolic acid and sapogenin have been isolated from the seeds of *L. acutangula* (Pal *et al*., 1968). The seed oil contains nutritional components like oleic and linoleic acid, which constitutes 68% of the total kernel oil. It is a good source of certain amino acids, phosphorus, iron and magnesium (Kamel *et al*., 1982). Nagao *et al.* (1991) have reported the structure of seven oleanane-type triterpene
Introduction

Saponins, acutosides A, B, C, D, E and F. Totally, 19 fatty acids were detected in fruit of *L. acutangula* which include major monosaturated fatty acid like pentadecanoic acid (33.40%), palmitic acid (20.10%), linoleic acid (17.10%) (Jadhav *et al.*, 2012).

Pharmacological activity of *Luffa acutangula*

Misar *et al.* (2004) reported that the dose dependant CNS-depressant activity of ethanol extract of the fruits of *L. acutangula* in mice. It also enhanced pentobarbitone sodium-induced hypnosis in single dose treated groups. According to Gill *et al.* (2011), it is quite apparent that the ethanolic seed extract of *L. acutangula* possesses significant antioxidant activity, antiinflammatory and analgesic activity. The antilarvicidal activity of *L. acutangula* was tested against the late third larval stage of *Culex quinquefasciatus*. It shows significant mortality with the LC$_{50}$ value of 839.81 ppm (Prabakar *et al.*, 2004). Hydroalcoholic extract of *L. acutangula* shows significant hepatoprotective activity against carbon tetrachloride (CCl$_4$) and rifampicin induced hepatotoxicity in rats. It significantly decreased the levels of serum marker enzymes like aspartate transaminase (AST), antichymotrypsin (ACT) and lactate dehydrogenase (LDH) and increased activity of non-enzymatic intracellular antioxidant like CAT and SOD (Jadhav *et al.*, 2010). Antidiabetic activity of fruit extract of *L. acutangula* has reported that more significant reduction ($p<0.01$) in blood glucose level in alloxan induced wistar rats (Priyanka *et al.*, 2010).

The ethanol extract of the fruits of *L. acutangula* showed remarkable anticancer activity against human lung cancer adenocarcinoma epithelial cell line A-549 (Reddy *et al.*, 2009). It also significantly inhibits the expression of vascular endothelial growth factor (VEGF), matrix metallo proteinases-2 (MMP) and MMP-9 (Reddy *et al.*, 2009). Nehha *et al.* (2010) isolated mucilage from *L. acutangula* fruits and it showed small
retardation in drug release from tablet in high concentration and due to its binding properties it can be used as a tablet binder in pharmaceutical formulations. Kalyani et al. (2011) reported the antioxidant activity of ethanol extract of aerial parts and seed oil of *L. acutangula*, respectively. Pimple et al. (2011) reported that the methanol and aqueous extract of *L. acutangula* fruit may have beneficial effects in the treatment of type-II diabetes mellitus. It acts as a good source of natural alpha glucosidase inhibitors for suppression of post prandial hyperglycemia in the management of type II diabetes mellitus.

The antioxygenic activity of pulp and peel powder extract of *L. acutangula* was evaluated by using different methods, including linoleic acid peroxidation, beta carotene-linoleic acid bleaching and 2, 2-diphenyl-1-picryl hydrazyl (DPPH). An ethanol/water extract of the pulp and peel showed highest antioxidant activity due to the presence of phenols and flavonoids which have been reported as a potential antioxidant (Padmashree et al., 2012). The ameliorative effect of *L. acutangula* was studied on doxorubicin (DXR) induced cardio and nephrotoxicity in mice by evaluating different serum biomarkers, antioxidants and histoarchitecture alteration. Administration of hydro-alcoholic extract of *L. acutangula* moderately reduced the DXR induced cardio and nephrotoxicity in mice and improved the histoarchitecture of both heart and kidney. It significantly reduces the oxidative stress by scavenging free radicals, enhancing enzymatic and non-enzymatic antioxidant and stabilization of membrane (Jadhav et al., 2013).

**Computational studies**

Nowadays, computational approaches have been considerably used in drug discovery and development. It is used in understanding the molecular interactions and drug activity, energy calculations and geometric considerations in an effort to narrow
down the search of potent pharmaceutical drugs with high specificity, potency, efficacy and favorable pharmacokinetics using computer-aided drug design (CADD). Depending on the structural, functional and other information of the target protein/receptor and the ligand, there are two major strategies for CADD currently used in the drug designing process such as direct and indirect approaches. In direct approach, the three dimensional feature of the targets are directly considered for the drug design while in case of indirect approach, the drug molecule are designed based on the comparative analysis of the structural features of known active and inactive compounds (Ooms, 2000).

**Molecular docking**

Computer-aided molecular docking is one of the important tools for understanding the binding interaction of receptors (protein) with small molecules (ligand). Nowadays, prediction of binding interactions of small molecules to known targets is an important component in drug discovery process (Seeliger and Groot, 2010). Molecular docking is used to identify and optimize the small molecules by examining the binding interaction of ligand with protein molecule. In this aspects, many software packages are used for prediction of protein-ligand complex using molecular docking simulations such as DOCK (Shoichet and Kuntz, 1993), AutoDock (Goodsell et al., 1996), FlexX (Rarey et al., 1996), GOLD (Jones et al., 1997) and Gilde (Halgren et al., 2004). Glide (grid-based ligand docking with energetic) is the most popular and widely used docking tool to predict the potent small (lead) molecules against wide variety of targets. In docking process, the ligand conformations and orientation are generated and the most appropriate conformation is selected as best ligand molecule. Scoring functions are applied to the estimation of binding energy between the ligand and protein complex (Kapetanovic, 2008).
Molecular dynamics (MD) simulation

MD simulation is one of the theoretical methods for analyzing the relationship between structure and function of biomolecules. It can serve as a computational microscope to study the motion of atoms within a molecular system which are difficult to observe experimentally. It is also used to obtain the information of kinetic and thermodynamics nature of macromolecules and it can provide fine details concerning the motions of individual atoms as a function of time (Straatsma and McCammon, 1992). MD simulation is widely used method for X-ray structure refinement (Brunger et al., 1987), internal motions in biomolecular functions (opening and closing of active site) fluctuations (Colonna-Cesari et al., 1986; Case and Karplus, 1979) and role of conformational entropy in proteins and nucleic acids (Brooks and Karplus, 1983).

According to the model chosen for representation of physical system, the MD simulation is classified into two main families such as classical mechanics and quantum mechanics approaches. In classical approach, the molecules are treated as classical objects which resembling the ball and stick model. Atoms are represented by soft ball and the bonds are represented by elastic stick. The quantum mechanics MD simulation provide the information about quantum nature of the chemical bond, electron density function for the valence electrons using quantum equations. AMBER (Cornell et al., 1995), CHARMM (Brooks et al., 1983) and GROMOS (van Gunsteren et al., 1996) are available force field to study the kinetic and thermodynamic properties of proteins and nucleic acids (Meller, 2001). NAMD (Nanoscale Molecular dynamics), AMBER, GROMACS and Desmond are widely used software package for MD simulation. Desmond is a newly developed software package (D.E. Shaw Research, DESRES), which significantly accelerates parallel MD simulations. A reasonable representation of
a protein environment is an important component for characterizing the properties of proteins throughout simulation. An aqueous solvent is selected as the environment for the vast majority simulations. There are wide range of water models used in molecular dynamic simulation, includes TIP3P, TIP4P (Jorgensen et al., 1983) TIP5P (Mahoney et al., 2000) SPC and SPC/E (Berendsen et al., 1987).

**Similarity search (2D fingerprint method)**

Shape similarity searching is an important concept for molecular recognition and this method represent the molecular shape and to quantify the shape similarity between the molecules. The prediction of activity of very large libraries of molecules to identify the most promising lead molecules for further experimental studies with the help of computers is known as virtual screening. Due to the size of the chemical database the similarity based screening needs efficient methods for screening a potent molecular representations for molecules from database which are sufficiently similar to the query molecule (Finn and Morris, 2013; Kristensen et al., 2013). Fingerprints (FPs) or SMILES strings are popular descriptors for chemical similarity searching for the identification of novel active compounds on the basis of known reference molecules (Heikamp and Bajorath, 2012). They are designed to encode various 2D or 3D molecular features and descriptors, mostly in a binary format and also as count FPs which monitor the presence of features in a molecule and their frequency occurrence (Stumpfe and Bajorath, 2011).

**Binding free energy calculation**

The accurate binding free energy calculation of protein-ligand complex is an important strategy to study the macromolecule recognition and computer-aided drug design (McCammon, 1998; Jorgensen, 2004). Predicting the binding free energy of
ligands to the receptors can have great practical values for searching of novel small molecules that can act as potential therapeutic drugs (Wlodawer, 2002). The absolute binding affinity may be calculated on the basis of a continuum solvent approximation assuming quadratic fluctuations around a unique configuration (Luo and Sharp, 2002; Roux and Simonson, 1999). Two popular approaches are widely used for estimation of the binding free energy such as Molecular Mechanics/Poisson-Boltzmann and Surface area (MM-PBSA) and Molecular mechanics/generalized-Born and Surface area (MM-GBSA). MM-PBSA relies on a mixed scheme combining configurations sampled from molecular dynamics (MD) simulations with explicit solvent, together with free energy estimators based on an implicit continuum solvent model (Massova and Kollman, 2000).

**Molecular Electrostatic Potential (MEP)**

MEP is a classical tool which has been used for over 30 years for studying molecular interaction and molecular properties and can be used in drug design (Bonnacorsi et al., 1970). The electrostatic potential created in the surrounding space around a molecule by its nuclei and electrons of a molecule for analyzing and predicting the molecular reactive behavior. An important feature of MEP is that it can be determined experimentally by diffraction method, as well as computational method. A variety of methods for calculating MEP is available, at different levels of rigor. Density functional theory (DFT) is increasingly used technique for studying MEP of a molecule (Politzer et al., 1985; Labanowski and Andzelm, 1991). It commonly applied in the form of the local density approximation in which the functional relationship between exchange/correlation energy and approximate is expressed in terms of the uniform electron gas model and it does take account of electron correlation (Parr and Yang, 1989).
Absorption, distribution, metabolism and excretion (ADME) properties

ADME are important molecular properties which are crucial for drug design. Therefore, the importance of optimizing ADME properties for potential drug molecules have been widely recognized (Segall et al., 2009). After administration the drug molecule should pass through the cell membrane through passive diffusion, carrier-mediated uptake, or active transport. The potent drug must reach the target at sufficient concentration with specific time to achieve the required pharmacological effect and also must be safe at therapeutic concentration without much side effects (Gola et al., 2006). Several screening paradigms, including absorption, have been employed to enhance the probability of success through the drug development stage. There are several methods used alone or combination to assess absorption which include in situ, in vivo, in vitro and in silico models (Lin et al., 2003; Fujikawa et al., 2005). Recently, in silico modeling of ADME properties have been used in drug design using different approaches such as quantitative structure-activity relationship (QSAR), similarity searches, 3-dimensional (3D) QSAR, ligand-protein docking and pharmacophore modeling (Yamashita and Hashida, 2004).

SCOPE OF THE STUDY

Lung cancer is the one of the major health problem in human and it is the second leading cause of death worldwide. It also associated with significant amount of poor prognosis in lung cancer patients. Recent years, herbal medicine is being considerably used as a therapeutic source for cancer treatment with diminutive side effect. The use of many plants derived compounds alone or in combination, is still studied by in vitro studies to understand their mechanism of action against lung cancer. The Indian traditional plant, Luffa acutangula possesses various pharmacological activities including antioxidant, anticancer activity. The fruit ethanol extract of
*L. acutangula* have the ability to inhibit cell proliferation on lung cancer cell line. However, there is no detailed study on the anticancer activity of aerial parts of *L. acutangula* and its secondary constituents. Also, there is no report on the bioassay-guided fractionation and isolation of active principle responsible for anticancer effects. Therefore, the present study is carried out to evaluate the anticancer potential of *L. acutangula* through *in vitro* and *in silico* approaches.

**OBJECTIVES OF THE PRESENT STUDY**

- To evaluate the preliminary phytochemical screening and antioxidant activity of crude extract of *Luffa acutangula* by various *in vitro* antioxidant assays.

- To determine the *in vitro* anticancer activity of crude extract of *L. acutangula* against human non-small-cell lung cancer cell line.

- To isolate and identify the potent anticancer compound through bioassay-guided fractionation by MTT.

- To determine antiproliferative and anti-apoptotic activity of the isolated bioactive compound of *L. acutangula* on lung cancer cell line through MTT, MMP, ROS, AO-EtBr, RT-PCR and western blotting.

- To evaluate the interaction of bioactive compound with lung cancer targets through computational studies.