Introduction

CHAPTER NO 1: INTRODUCTION

1.1 BACKGROUND

One of the most lethal forms of cancer that can affect both men and women is lung cancer (LC) [1]. In addition, poor postoperative healing due to LC is a primary cause of mortality, creating it among the most common tumour types. Primary therapies were administered to patients for an average of 5 years, with nearly half of stage I patients and one third of stage II patients. There are two primary forms of LC, known as small lung carcinoma and non-small lung carcinoma, which are responsible for 15 and 85 percent of all lung cell cancers, respectively [2]. Squamous cell carcinoma, adenocarcinoma, and large cell carcinoma are the subtypes that fall under the umbrella of “Non-Small Cell Lung Cancer” (NSCLC). “Squamous cell carcinoma” is responsible for between 25 and 30 percent of all LC cases. The bronchial tubes in the central region of the epithelial lungs contain early copies of the cells that make up the spinal column. It is estimated that approximately forty percent of all cases of LC are caused by adenocarcinoma, thus the most common kind of LC is adenocarcinoma, and it can occur in both smokers and people who have never smoked. Additionally, adenocarcinoma develops more slowly than the majority of other types of LC, and it is typically discovered before it spreads to other parts of the body. There is no squamous or glandulary maturation in this kind of carcinoma and other possibilities can thus be considered essentially random after exclusion. LC is, by a wide margin, the most notable malignant tumor that can develop in the pulmonary system. Multiple histological subtypes are found in around 30 percent of cases of LC. Direct diagnosis of small cell LC can be accomplished through the use of random reactions to treatment and the relatively low survival rates of neuroendocrine dysfunction [3].

The "Indian National Cancer Registry Program" Initiatives, which gathered data from six geographically disparate parts of the nation revealed varying results in each location [4]. Males in Bombay, Delhi, and Bhopal saw the highest rates of malignancies due to tumor of the larynx, thoracic duct, and lungs. Furthermore, in the Indian cities of Madras, it ranked as the second highly prevalent and Bangalore's third highly prevalent kind of tumor, respectively. Barshi, a remote town, was where its rarity was most apparent. Bombay and Bhopal were the only two cities in India where the illness was in the top 10 most common malignancies, and females were disproportionately affected. It held the seventh place in the ranking. The data collected from

hospitals located in various sections of the country likewise displayed varying trends. Global Cancer Observatory performed a survey on the mortality and incident cases of LC patients at world level [5]. They discovered that out of the 223,930 people who were admitted to hospitals, 863 of them had LC (0.38 %).

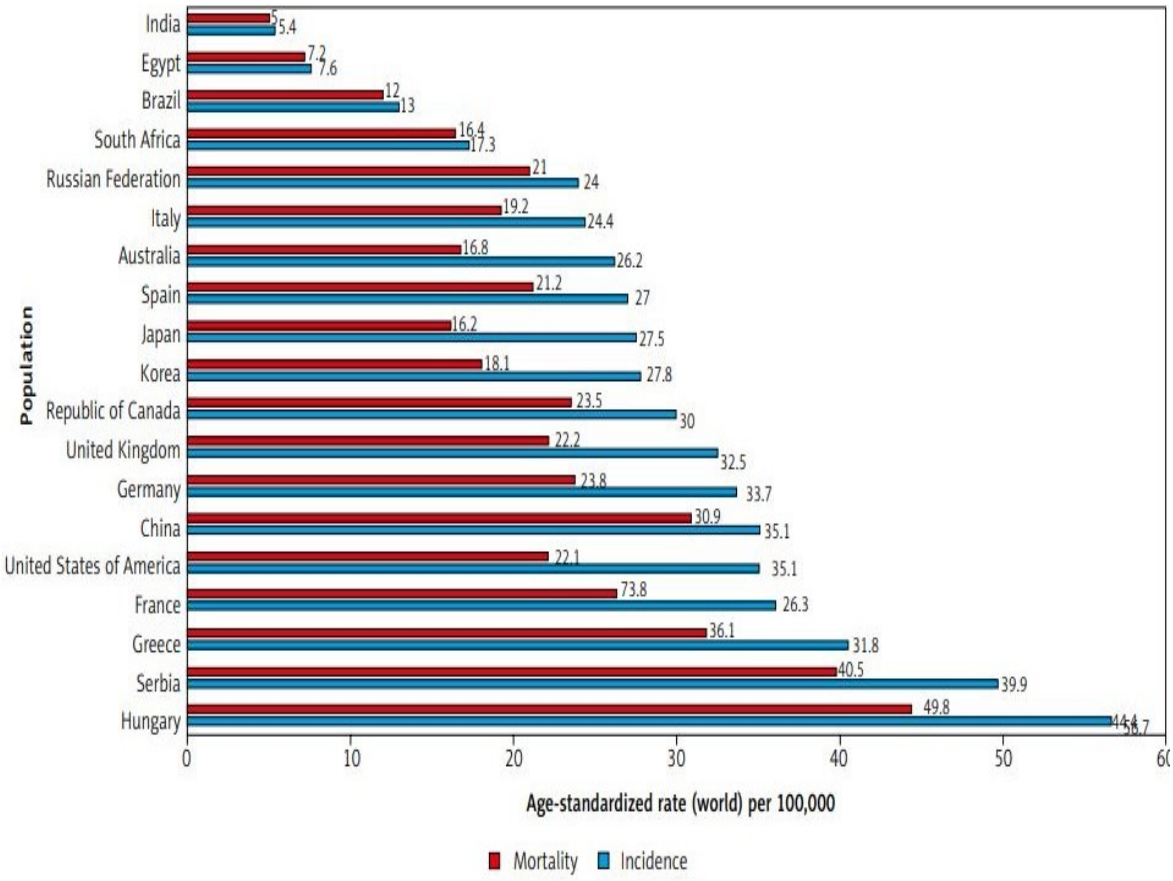

Figure 1.1: Approximately age-adjusted global incidence and death rates for 2018 from the 2018 edition of Globocan [5].

Lung tumor growth is the most unsafe and far-reaching tumor growth on the planet as per phase of revelation of the diseased cells in the lungs, so the procedure of early identification of the illness plays a vital and fundamental job to keep away from genuine propelled stages to reduce its level of dissemination. The point of this exploration was to identify features for precise pictures examination as pixels rate and tumor cell marking.

1.2 LUNG CANCER TREATMENT AND TREATMENT CHOICES

Generally, the treatment for adenocarcinoma is given dependent on the area on the human body it develops [6]. Be that as it may, for the most part, the treatment methodology includes:

- **Surgery:**

If the NSCLC has not migrated beyond the lungs, the tumours may be surgically removed at stage I or, in rare cases, stage II. Lobectomy, wedge resection and pneumonectomy are all popular surgical procedures for the removal of lung malignant cells. However, it is not indicated when the tumour is big, when it is near a key organ, or when there are distant metastases. Though anywhere from 10–35% of cancers may be successfully treated, the damaged cells are always going to be there. Some patients have a recurrence or return following surgery. Nonetheless, individuals with severe heart or lung illnesses are not usually candidates for surgery due to the high risk of death associated with the procedure. SCLCs are less amenable to surgery because the tumours are less likely to be contained to a single region. Surgery has additional drawbacks as well. There is a risk that post-operative patients may have respiratory complications, discomfort, and tiredness along with bleeding, infection, and other problems are possible [7].

- **Radiation treatment:**

SCLCs and NSCLCs may be treated with radiation therapy. X-rays or ionising radiation destroy cancer cells. Even as a solitary treatment, this sort of therapy reduces a tumour by destroying DNA or creating ROS [7]. It is a popular therapy if a patient opposes surgery, if tumours have spread irregularly, or if surgery poses a life-threatening risk. Radiation treatment focuses radiation from all angles on a tumour. It has two forms: (a) “teletherapy”, when the individual is “irradiated” from an external source and it’s risk-free and (b) “brachytherapy”, where “radioactive pellets” are inserted near the tumour [5]. However, using this tharapy with surgery may improve outcomes. Before surgery, it shrinks tumours or kills lingering dangerous cells [8].

- **Chemotherapy:**

Since it has been shown that late-stage LC patients do not benefit from surgical procedures, more extreme treatments are used for these individuals. After surgical removal of malignant tissue, chemotherapy consists of injecting medications into the patient to kill any remaining cancer cells or to stop them from dividing. Potentially alleviates cancer pain and discomfort. Chemotherapy is an option for treating both SCLCs and NSCLCs. Patients with SCLCs had their survival times increased by a factor of four to five thanks to treatment. Boosted tumour

penetration and retention is crucial to the chemotherapy process. Increased expression of "vascular endothelial growth factors" (VEGFs), "nitric oxide" (NO), "bradykinin peptides," "prostaglandins," "collagenases," etc., contributes to the hyperpermeability of tumours. Accordingly, macromolecules that target tumours may be able to readily target and diffuse through the porous vasculature of tumours. Conversely, cancers have a remarkable capacity to hold on to whatever foreign compounds they encounter. Tumors differ from typical tissues in that the invading macromolecules tend to accumulate in the interstitial spaces due to the poor lymphatic drainage of cellular components due to excessive interstitial pressure and a lack of lymphatic vessels [9].

- **Photodynamic Therapy (PDT)**

PDT involves injecting a photosensitive biological chemical into the individual, which is quickly taken up by cancer cells notwithstanding the tumour's stage. In both its early and late stages, LC is often treated with porfimer sodium. When a sufficient number of photosensitizers have accumulated at the affected sites, they undergo excitation from the ground state into excited singlet and finally triplet states in the influence of an ambient source of light of a specified spectrum. Tissues contain a plethora of molecular oxygen species, all of which remain in their ground triplet states. Energy is transferred from the excited photosensitizers to the molecular oxygen species, causing the oxygen molecules to enter an excited singlet state. Highly reactive singlet oxygen species are responsible for the targeted destruction of cancer cells. Non-specificity is overcome by the treatment's potency, making it a viable option for inoperable LCs. However, individuals undergoing this sort of treatment may have inflammation and trouble breathing or swallowing. Since PDT has shown to be an efficient anticancer therapy method, several PDT drugs have been developed. In contrast, most PDT agents have poor solubility in water. Therefore, it is difficult to transport PDT chemicals to tumours, and an efficient carrier medium is necessary for their accumulation at a desired site [10, 11].

1.3 THE CHALLENGES AND CURRENT LIMITATIONS

Treatment options for metastatic disease, such as LC, include systemic (“chemotherapy”, “biological therapy”, “targeted therapy”, and “hormonal therapy”) and localized (“surgery”, “radiation”) approaches, as well as combinations of the two. Treatment options are determined by a number of factors, including the original cancer's characteristics (such as volume, area, and

the number of tumours), the patient's general fitness, oldness, and the cancer stage. Treatment options include those that are curative and those that are palliative. Chemotherapy and radiation are two forms of postoperative treatment used to eradicate any leftover cancer cells following surgical excision of a tumour [10]. Following are some major limitations found in majority of the literatures and the present work deal to eliminate or minimize all these limitaions of LC treatment.

- LC treatment is very costly.
- The treatments of LC have many adverse effects on patient's body.
- LC treatment is out of reach treatment to the common person due to its unavailability in villages and even in majority of metropolitan cities.

1.4 MEDICINAL PLANTS

Mankind has long searched the surroundings for plants that might meet all of their essential requirements, including those for food, habitat, fuel, and wellness. As a consequence, many different plants have been used; in notably, significant cultivation of food plant species has produced crops with great yields. Such breeding has mainly not occurred in the case of therapeutic plants since nature could offer an adequate supply. There are between 40,000 and 70,000 medicinal plants worldwide. This suggests that roughly 25% of all plant species are used medicinally in some capacity. It has led to the separation and synthesis of pure active chemicals (such as morphine, atropine, and digoxin) as well as the subsequent invention of innovative synthetic compounds based on this knowledge. Our predecessors left us a medical heritage that has developed further in modern European treatment. Some of these artificial substances based on natural substances have had great success, such as acetylsalicylate, which was created as a result of the usage of Salix bark as an analgesic [12]. The completely distinct healthcare systems that conventional medicines are enmeshed in, such as various illness classification systems, tailored medications, and complicated chemical combinations, provide one of the challenges in analysing them. High Throughput Screening (HTS) may find some promising compounds with activity using current drug development methods [13]. In reality, a sincere approach to researching the activity of medicinal plants is required by the holistic concepts of conventional healthcare systems. Because of the lengthy history of usage and continued popularity of these drugs, acute toxicity is very improbable; however, long-term toxicity would necessitate additional investigation. Medical investigation is used in systems biology to examine an

organism in a variety of contexts, but no assumption is formed beforehand. Instead, one attempts to measure as many factors as feasible, analyse all the data using multivariate analysis or other relevant statistical methods, and then make inferences about the hypothesis. This information could comprise chemical and physiological characteristics. Such a comprehensive strategy may lead to the discovery of pro-drugs and synergy. This method may also disclose new forms of action. The many medical systems may share knowledge with one another, making significant advancements and generating new ideas and concepts in the process. Combining the finest methods will greatly improve everyone's access to healthcare [13].

1.5 PLANTS IN CANCER CHEMOPREVENTION

Currently used in cancer therapy are chemicals originating from plants. The periwinkle Catharanthus roseus, native to the rain forests of Madagascar, is a good example of a plant that produces a substantial family of alkaloids called vinca alkaloids. Etoposide, another drug obtained from a natural source, has shown promising results in the treatment of testicular cancer when combined with bleomycin and cisplatin. Mandrake (*Podophyllum peltatum*) and wild chervil (*Podophyllum emodi*) are both sources for the epipodophyllotoxin known as etoposide. It has shown substantial action against small-cell LC [14, 15].

Since they may help stop diseases before they even start, biologically active chemicals found in plants have concerned a lot of attention. Vegetable and organic food consumption, which is rich in polyphenols, has been shown to lessen the risk of certain types of cancer. Over 40,000 secondary plant metabolites, including polyphenols, help plants develop several plant-ecological relationships [16] and offer chemical defence mechanisms against pathogens and environmental stress. Polyphenols, including phenolic acids, flavonoids, stilbenes, and lignans, may be found in abundance in plant-based diets eaten all over the globe. Polyphenols come in many different forms, but they always share the presence of an aromatic ring and one or more hydroxyl groups [8, 9]. Flavonoids are characterised by a structure of two aromatic rings (A and B rings) connected by three carbon atoms and contained in an oxygenated heterocycle ring (C ring). Flavonoids are divided into subgroups called flavonols, flavones, catechins/condensed tannins, anthocyanidins, and isoflavones based on structural changes in the C ring.

Paclitaxel and docetaxel two taxanes exhibit substantial antitumor activity in clinical studies. Irinotecan and topotecan, camptothecin compounds, are effective against colorectal and

ovarian cancer [17]. Bark of Nyssacea *Camptotheca accuminata* was used to extract the compounds [18]. Taxanes and camptothecins have been approved for human usage. Flavopiridol, a synthetic flavone made from the plant alkaloid rohitukine, may cure colorectal, prostate, renal cell, and NSCLC, as well as non-lymphoma Hodgkin's and chronic lymphocytic leukaemia [19, 20]. Many more plant-derived compounds are being researched than those in Table 1.1.

Table 1.1: Anticancer agents based on plant extract available in market [15]

Compound	**Plant source**	**Type of cancer**
Vincristine	*Catharanthus roseus*	Leukemia, lung, pediatric solid cancers
Topotecan	*Camptotheca acuminata*	Ovarian, lung and pediatric cancer
Irinotecan	*Camptotheca acuminata*	Colorectal and LC
Acronyciline	*Acronychia baueri*	Under Preclinical stage, LC
Bruceantine	*Brucea antidysenterica*	Under Preclinical stage, pediatric cancer, lung
Thalicarpin	*Camellia sinensis*	Under Preclinical stage, Leukemia, LC

Table 1.2 provides a comprehensive catalogue of anticancer drugs that have been identified from plants.

Table 1.2: Anticancer agents extracted fractions from various plants

Name	**Clinical status**	**Target**	**Source**	**Reference**
Beta Lapachone	Phase II	topoisomerase I&II	*Tabebuia avellanedae*	[21, 22]
Diadzein &Genistein	Phase II	inhibits 3A 4-mediated metabolism	Lupinus species	[23, 24]
Ellipticine	Lead molecule	topoisomerase II inhibitor	*Ochrosia borbonia*	[25, 26]
Kanglaite	Lead molecule	Inhibits mitosis of tumor cells during G2/M phase	*Coix lachryma-jobi*	[27]
Phenoxodiol	Phase I	inhibits plasma membrane electron transport & cell proliferation	Plant isoflavone genistein	[28]
Pervilleine A	Lead molecule	inhibition of P-glycoprotein	*Erthroxylum porvillei*	[29]
Silvestrol	Lead molecule	trigers mitochondrial pathway of apoptosis	*Aglaia foveolata*	[30]
Berbamine	Lead molecule	caspase-3-dependent apoptosis	*Berberis amarensis*	[31, 32]

Colchicine	Lead molecule	anti-mitotic	*Colchium automole*	[33]
Dimethyl xanthene-9-on4-acetic acid	Phase I/II	TNF-á induction	Flavone-8-acetic acid analog	[34, 35]
4-Ipomeanol	Lead molecule	DNA binding	*Ipomoea batatas*	[36]
Meisoindigo	CD terminated	Induces apoptosis by locking Stat 3 signaling	*Indigofera tinctoria*	[37]
Perillyl alcohol	Phase I	Activates capase 3 apoptosis	*Limonene analogue*	[38, 39]
Roscovitine (CYC 202)	Phase II	CDK inhibitor	Olomucine (*Raphanus sativus*)	[40, 41]
Santonin	Lead molecule	Inhibition of NF-Kb	*Artemisia maritima*	[42]
Berberine	Lead molecule	Inhibition of NF-Kb	*Hvdrastis canadensis*	[43]

Using plant compounds to combat illness is vital and India's diversified geography makes us rich in medicinal plants. Many Indian medicinal plants have similar effects to medicines. They're widely available throughout Indian flora. Finding innovative bioactive chemicals with minimal side effects should be a priority. To manufacture effective, affordable drug, its chemical make-up, mechanism of action, and therapeutic efficacy must be determined.

The primary focus of this investigation is to establish the anticancer potential of selected plants from northeast India, the Zingiberaceae family plant *Etlingera linguiformis* and the Smilacaceae family plant *Smilax ovalifolia*.

1.6 REVIEW OF SELECTED PLANTS

- ***Etlingera linguiformis* of Zingiberaceae family**

Description: Native to northeast India, the *Etlingera linguiformis* of Zingiberaceae is a valuable medicinal and fragrant plant that thrives in warm regions and loamy soil rich in humus. While the plant's essential oil is utilised in fragrance, the rhizome is used to cure cancer, sore throats, stomachaches, rheumatism, and respiratory ailments. *Etlingera linguiformis* is a perennial plant that may grow up to 2 metres in height and has a thick, abundantly runner-bearing rhizome that gives off a pleasant odour. The hairless, oblong, lance-shaped leaves may grow to be 30–45 centimetres in length. Red spikes with a length of approximately 8 centimetres, that are

elongated and tapered at both ends, few-flowered, and generally buried in the earth, bear the flowers. The flower's lip is 5 centimetres in length and oblong in shape. It is coloured brilliant red and yellow and is deflexed, or folded below the centre. The Eastern Himalayas, from Northeastern India to Myanmar, are home to the elongated Torch Ginger [44].

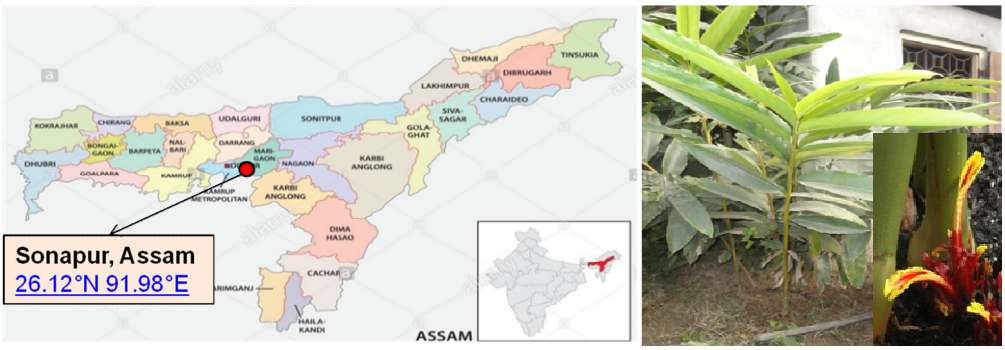

Figure 1.2: *Etlingera linguiformis*

Medicinal uses: Sore throats may be alleviated by chewing on pieces of rhizome wrapped in betel leaf. For stomach pain, try taking 5 millilitres of fresh juice made from the plant's rhizomes twice day. Rheumatism may be treated by taking pea-sized tablets produced from the plant's rhizomes three times daily for 15 days and applying fomentation to the affected area. The rhizome of the plant is used to alleviate coughs and other respiratory problems [44].

- ***Smilax ovalifolia* of Smilacaceae family**

Description: *Smilax ovalifolia* of Smilacaceae family is native Indian medicine of the northeastern part of India. Leathery shiny ovate to elliptic (7-15 x 4-11 cm) with a rounded to briefly wedge-shaped base of 3 to 5 nerves. The leaf stalk is 1.5 centimetres long, sheathing at the base, and ends in tendrils. White flowers clustered in umbels at the end of each leaf stalk. Opaque bracts Mature blooms have a recurved perianth, with the outer three segments measuring 4 mm in length and shaped like thin oblongs. This flower is harvested between the months of January and April, and you may find it growing everywhere from the northern Himalayas to Peninsular India, at elevations of 200 to 1500 m.

Medicinal uses: Diseases such as dysentery, urinary problems, and rheumatic swellings all benefit from its use [45].

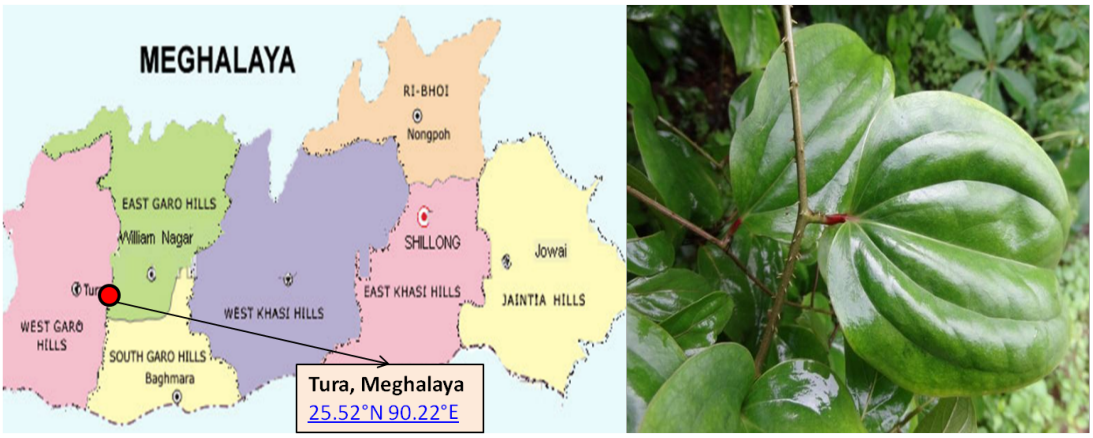

Figure 1.3: *Smilax ovalifolia*

1.7 MOTIVATION FOR THE PRESENT STUDY

Lung cancer growth problem is one of the feared diseases in the advanced world. Lung tumor growth is one of the primary sources of death among tumor maladies in the world. Viability of the treatment and survival rate of the patient's straight forwardly related to the early analysis, direction, treatment and guiding is limited. In a large portion of the cases, the tumor is analyzed uniquely at the propelled stage, with the outcome, the life span of the patients reduced. Following are a portion of the reasons owing to the late finding of the tumor.

- Patients' non-awareness about the reason and seriousness of the tumor.
- Similarities of the signs of LC with other illnesses like "Chronic Obstructive Pulmonary Disease" (COPD), Tuberculosis.
- Majority of the common people are not capable of bearing the cost of the expense to experience the screening procedure.
- Lack of cutting edge screening centers in the provincial territories.
- Lack of direction and guiding at the ideal time and right place.

1.8 SCOPE OF THE PRESENT STUDY

Cancer is a major cause of death and suffering across the world. That's why ample studies aim to find better ways to treat people, so that they may live longer. There are many different types of phytochemicals, and they are all stored in plants. As a result of tumour recurrence and other treatment-related adverse effects, the effectiveness of most anticancer medicines has been diminished for the treatment of LC has been evaluated in present study. The discovery of effective new anticancer drugs relies heavily on the use of phenolic extracts from plants. The

pharmaceutical industry can also use of phenolic extracts for the treatment of LC, in addition to their many medicinal applications. When used in conjunction with other medicinal treatments, various extracts from plants have the ability to improved cancer therapy outcomes across the board.

1.9 OBJECTIVES OF THE PRESENT STUDY:

The objectives of the present studies are as follows:

- Selection and identification of medicinal plant(s) based on traditional knowledge of northeastern India and preparation of extract of from selected parts.
- Fractionation and characterization of plant extract for identification of active molecule/fraction(s).
- Study of molecular events in response to the fractionated extract/active molecule to understand the anticancer action using *in vitro* cell culture system and *in vivo* animal model.