ABSTRACT

It has been established that in developing countries like India, lung cancer is the main reason of many deaths. There has been worldwide concern about the rising mortality toll from lung cancer. While several methods, including surgery and radiation, are employed to combat the disease, Lung cancer remains a formidable foe. Unfortunately, in countries like India, where a large percentage of the population lives in rural areas, access to these therapies is limited for a number of reasons, including (a) a general lack of knowledge about cancer and its treatment options, (b) a low cancer hospital-to-cancer patient ratio, and (c) the high cost of treatment and available medication. However, even among those who are able to receive treatment, the success rate is low. This may be due todifferentelements sd following: (1) delayed identification of the illnesses; (2) those few who approach the health center at an initial stage of the disease can be efficiently cured; however, whose who approach the health center at an advanced cancer stage remain saddening to back of the life; (3) in certain instances, surgical extraction remains ineffective for the overall extraction of the tumor tissue, which leads to death. In addition, there are many other things including smoking, occupational hazzards, having a family history of the illness, etc.

Accordingly, the scientific community has proclaimed a global war against lung cancer, as seen by the rising number of articles covering topics from simple detection to effective treatment. However, researchers are struggling to find drugs that are both affordable and suitable, with few negative effects. As a result, there is a greater demand for research into medications that are not only biocompatible but also affordable enough to reach the vast populations of developing countries like India. The natives have been using plants as medicine to cure a wide range of illnesses for a very long time. This is due to the abundance of bioactive compounds that provide a formidable barrier to the spread of illness. This has piqued the scientific community's interest in the anti-cancer properties of phytochemicals. Many plants have been shown to have anticancer effect against lung cancer in in-vitro and in-vivo studies, and these findings arevery prominent. Therefore, primarygoal of this research is to uncover the untapped “Anticancer Potential” (AP) of ethanomedicinal plants against lung cancer. There are many different indigenous peoples living in the eight states that make up Northeast India. These states comprise of “Assam”, “Arunachal Pradesh”, “Manipur”, “Meghalaya”, “Mizoram”, “Sikkim”,

“Nagaland”, and “Tripura”. Tribal peoples have relied on the region's abundant wildlife for centuries, as shown by their traditional understanding of the importance of the area's diverse plant and animal life for survival. From this context, we chose *Etlingera linguiformis* (CAN) of the Zingiberaceae family and *Smilax ovalifolia* (LEX) of the Smilacaceae family, both of which include bioactive chemicals whose theoretical and practical applications are yet largely unknown.

To investigate the anticancer effectiveness of the hydro-alcoholic extract, we first screened it for cytotoxic activity employing an in-vitro cell culture system with cancerous cells, including A549, NCI-H522, and NCI-H23, all of which are associated with lung cancer. Compared to controls, the findings demonstrated a substantial “dose and time” relying reduction in cancer “cell viability”. However, PBMCs, which served as a healthy control cell, showed no signs of diminished viability. We have decided to focus on *Etlingera linguiformis* and *Smilax ovalifolia* because of their promising antioxidant and cytotoxic properties. In contrast to the control group, Balb/C, animals given this plant extract showed no significant differences in the aforementioned indices of important organ function (liver, kidney, and blood) even at the highest dosage tested. For improved comprehend the function of phytochemicals in cancer prevention, the present study's findings prompted the evaluation of the anticancer potential of extracts from the selected CAN free phenolic fraction, CAN bound phenolic fraction, LEX free fraction, and LEX bound phenolic fraction against induced lung cancer.

We assessed the antitumorgenicity efficacy of the selected plants to identify the methodology of anticancer potency in the CAN free phenolic fraction and LEX free fraction of the investigated plant leaf isolate on A549 triggered cancer in athymic nude mice. The effectiveness of the plant extract was measured using a tumour burden; tumour volume; tumour incidence.

Using solvent extraction, we separated the bound phenolic and free phenolic of CAN and LEX to determine their anticancer potential. Subsequently, an MTT test was used to evaluate the cytotoxicity of CAN bound phenolic, LEX bound phenolic, CAN free phenolic, and LEX free phenolic on A-549, NCI-H522, and NCI-H23 cell lines. Cell viability was shown to decrease considerably for those exposed to free and bound phenolic of CAN and LEX compared to the control group, although the IC_{50} values for these fractions were lower. Because of their

promising anticancer properties, the CAN free phenolic fraction, the CAN bound phenolic fraction, the LEX free fraction, and the LEX bound phenolic fraction were subjected for further study. The “cell cycle” phases and “apoptosis” rates were determined by “flow cytometry” and AO, respectively. ROS generation was measured using flow cytometry; Rhodamine-123 fluorescence was measured in an MMP analysis; flow cytometry was used to measure autophagy; fluorescent microscopy images displayed autophagy; and clonogenicity and cell migration were evaluated using cell culture system.

Inhibition of clonogenicity and cell migration was observed at increasing doses of CAN free phenolic fraction, CAN bound phenolic fraction, LEX free fraction, and LEX bound phenolic fraction, as well as a reduction in cell viability, cell cycle at G1/G0/S-phase, induction of autophagy, apoptosis, DNA damage, decreased MMP, increased ROS production in a dose-dependent mode, and decreased MMP. In contrast, the free phenolic fraction of LEX was shown to be more effective as an anticancer medication than the bound phenolic fraction of LEX, the free fraction of CAN, or the bound phenolic fraction of CAN. As a result, “High Resolution Liquid Chromatography Mass Spectrometry” (HR-LCMS) became necessary to discover new fragments that potentially have high anticancer potential. Analytical techniques like HR LCMS may reveal which biomolecules are present in a given sample. Thus, HR-LCMS was used to separate bioactive molecules from the parent fraction.

We have found a fraction containing a combination of recognised anticancer phytochemicals and some new molecular peaks that may have worked across numerous biochemical and molecular pathways, resulting in increased anticancer potentials against lung cancer. Based on the results of this study, it is safe to say that *Etlingera linguiformis* (CAN) and *Smilax ovalifolia* (LEX) tested to have the utmost anti-proliferative and pro-apoptotic potentials. The molecular mechanism behind their anticancer potential requires more, in-depth research, which may be conducted using a gene knockout model of cancer. NMR analysis may also be used to identify previously undiscovered compounds. This might lead to the identification of a novel chemical/compound with therapeutic potential against lung cancer cells.