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INTRODUCTION

The most significant and essential element for life in aerobic organisms is molecular oxygen, because it aids the formation of Reactive Oxygen Species (ROS). It plays a pivotal role in various physiological and biochemical processes when present in small quantities and highly toxic at higher concen*trations. When cells are exposed to pollutants, UV-rays, xenobiotics, smoke* and other endogenous metabolites of the redox and respiratory chain during the transfer of electrons, ROS is generated constantly (Al-Amiery *et al.*, 2017). The highly reactive molecule, ROS, induces stress in living organisms through DNA damage and results in various disorders such as cardiovascular, neurodegenerative, cancer and ageing. An organism that functions normally can counterbalance the highly formed radical species, but in most cases, these disorders result in the imbalance between free radicals and scavenging of molecules thereby results in oxidative stress (Aldosari *et al*., 2018).

Free radicals are atoms, molecules, or ions with unpaired electron that are highly unstable and active towards chemical reactions with other molecules. Endogenous (mitochondria, peroxisomes, and endoplasmic reticulum) and exogenous (electromagnetic radiation, cosmic radiation, ultraviolet radiation, ozone, smoking, environmental pollutants, drugs, pesticides, and industrial solvents) sources of free radicals can adversely affect various biomolecules, thus changing the normal redox condition and thereby increases the oxidative stress (Umamaheswari *et al*., 2015 and Zishan *et al*., 2017). Reactive Oxygen Species shield a widespread array of chemical components enclosing superoxide anion, hydrogen peroxide, hydroxyl radicals, nitric oxide and peroxynitrite (Bagchi *et al*., 2000). Though the innate defense in the human body may not profuse for rigorous or persistent oxidative stress, the exogenous antioxidants are constantly requisite to sustain a adequate level of antioxidants to balance the ROS (Karuna *et al*., 2018). The assemblage of free radicals is one of the main factor accountable for

cell damage which revitalizes diseases such as diabetes mellitus, cancer, cardiovascular and liver problems (Boligon *et al*., 2014). Antioxidants have the capability to prevent the oxidative damage caused by the free radicals and present a conflict against oxidative stress by preventing from them (Pavithra and Vadivukkarsi, 2015). The free radicals cause an inconsistency in homeostatic phenomena between the oxidants and antioxidants in the body and the disparity leads to ageing and various degenerative diseases (Tiwari, 2001).

Cancer is a major public health concern and the world's second largest cause of death. Several factors such as sedentary lifestyle, fast foods, hygiene and exposure to UV, ionizing and non-ionizing radiations causing mutations are likely to cause cancer (Anjali and Gayatri, 2015). As per the statistical survey, there would be 17 million fatalities and 26 million new cases per year by 2030 (Graidist *et al*., 2015). This rise is because of the lifestyle changes, increasing life expectancy, urbanization, industrialization and overpopulation.

Lung cancer has become one of the most common malignant tumours globally and is also the foremost root of cancer-associated death due to high incidence, rapid progression and poor prognosis (Sikdar *et al*., 2014 and Thun *et al*., 2010). It is the prime cancer killer and public health problem with an altogether five-year survival rate of <15%. A total of 2,20,000 people have been diagnosed with lung cancer, with 85 percent of cases being categorised as non-small cell lung carcinoma (NSCLC) and the remainder instances being classified as small cell lung carcinoma (SCLC). The A549 cell line is a type of non-small cell lung cancer cell that is used to test cytotoxicity under *in vitro* conditions. The most critical etiological risk factors for lung cancer development are tobacco smoking, industrial exposure, genetic polymorphism, overexpression of EGFR protein and a family history of p53 mutation (Kanwal *et al*., 2017). The exploitation of cigarette is accountable for elevated prevalence of lung cancer, where long-term smoking causes a 10-fold increase compared with non-smokers (Mccarthy *et al*., 2012).

Lung cancer is triggered by polycyclic aromatic hydrocarbons, particularly Benzo(a)pyrene [B(a)P] released from cigarette smoke (Moorthy *et al*., 2015 and

Yu *et al*., 2015). Moreover, B(a)P is used as a tumour-inducing agent in mouse models since the tumour has molecular and morphological resemblances comparable to human beings. However, B(a)P toxicity is due to its intermediate metabolites and oxidative stress caused by ROS, both of which play a role in B(a)P induced lung carcinogenesis (Wang *et al*., 2013). Thus, lung cancer could be a highly aggressive challenging disease worldwide that leads to high mortality. The counterpart to any disease is the planning of effective treatment strategies. In the long run, surgical resection, chemo and radiotherapy were extensively used in lung cancer treatment. Among them, chemotherapy is the most preferred treatment, which causes non-specific effects with normal cells and also causes severe side effects such as trauma, toxicity and also reduces the survival rate of the patients (Li *et al*., 2020). Advanced approaches for identifying molecular determinants of carcinogenesis, such as genetic mutations in oncogenes (Kras, cMyc, EGFR, ALK, etc.) and tumour-suppressor genes like p53, RASSF1, RB and FHIT have recently been developed (Pfeiffer *et al*., 2012). But, given a responsibility to the scientific community to replace the above, the treatment of various cancers have been streamlined with the use of bioactive molecules from the medicinal plants, which are target specific, less toxic and in turn trigger the immune stability of the system to balance the homeostasis of the patient (Broker and Giaccone, 2002).

Critical analysis based on the plethora of review reports suggested that there has been a shift to novel / nutritive or non-nutritive phytoconstituents in recent years and the prospective of natural product molecules has been documented and traditionally implemented for the lung cancer treatment. Their efficacy has been proven in pre-clinical testing both *in vitro* and *in vivo*, as well as in clinical trials. One of the compelling reasons to believe that bioactive compounds are superior to synthetic drugs is that these natural product molecules are not only potent anticancer agents for lung cancer treatment, but they also play an important role in regulating multi-molecular targets while causing minimal side effects to normal cells surrounding the tumour site.

As a result, cancer chemoprevention is a safe way to reduce the incidence and mortality of lung cancer by consuming phyto or pharmaceutical products that block cancer induction, prevent DNA damage, stop tumour progression in premalignant cells, and reduce metastasis without causing harmful side effects (Bodduluru *et al*., 2014).

Despite the numerous advantages of natural products, there are still significant challenges in standardizing their usage in preventing and treating lung cancer. They include the crude character of the medications, which are most usually used as powders but have low purity and the trouble of consuming them due to their non-palatability and repulsiveness of taste and odour. Finally, non-conformal dosage estimation and dose compensation must be standardized for any bioactive molecule to be considered potentially useful against cancer and other diseases. Thus in considering the downside of the natural products, it is imperative that researchers devise unique method to resolve the aforementioned challenges and verify the credibility of potential herbs.

Phenolic compounds are one of the important plant-derived chemotherapeutic agents, which are highly concentrated by scientists because of their dynamic role in therapeutic applications. They are ubiquitous in plants and possess antioxidant properties mostly due to their redox potential, which empowers them to act as reducing agents, hydrogen atoms or electrons donors and singlet oxygen quenchers or metal chelators (Gini and Jothi, 2018). One such phytophenol renowned for assorted health benefits is syringic acid (4- hydroxyl-3-5 dimethoxy benzoic acid), synthesized via the shikimic acid pathway and possess a wide range of therapeutic applications in the prevention of diseases, especially cancer, diabetes, cerebral ischemia, etc. It has outstanding properties such as free radical scavenging, antioxidant, antimicrobial, anti-inflammatory, anti-endotoxic and hepatoprotective activities. The therapeutic property of syringic acid is due to the existence of methoxy groups onto the aromatic ring at third and fifth positions. Further syringic acid has the capability to amend the protein dynamics, enzyme activity and assorted transcription factors

involved in oxidative stress, inflammation, cancer and angiogenesis (Srinivasulu *et al*., 2018).

Even though its presence is high in many plants, there are meager reports on its existence in *Plectranthus amboinicus*, a phenolic rich flora. Hence, we probed to isolate the syringic acid fraction from the *P. amboinicus* and assessed its efficacy in treating lung cancer using A549 cell lines and B(a)P induced Swiss albino mice.

Plectranthus amboinicus, commonly known as Country borage or Indian borage (Karpuravalli / Omavalli in Tamil) is a dicotyledonous plant belonging to the family Lamiaceae (Warrier, 1994). It is a large succulent, aromatic perennial herb, highly branched, fleshy with distinctive aromatic pubescent smelling leaves. Based on literature evidence on traditional usage, *P. amboinicus* is proved to possess remarkable therapeutic properties due to the existence of aromatic oils (Farshori *et al*., 2013). The active principles present possess urolithiasis, antiepileptic, antitumorigenic, antimutagenic, antiviral, antifungal and neuropharmacological properties (daCosta *et al*., 2010). Besides the comprehensive pharmacological activities of *P. amboinicus*, the leaf is exclusively used to treat inflammations, skin allergies, chronic cough, asthma, arthritis and malarial fever (Venkateshappa and Sreenath, 2013).

In the recent past, tailoring green technology with the high-throughput chemistry-based synthesis in drug development has emerged and led to the expansion of the plant pharmaceutical industries. With the development of molecular dynamics simulation studies in the field of biology, computational research and bioinformatics have considerably increased in comparison with the past decade. Molecular docking is a widely used tool to identify a target, design a drug, and find the best binding molecule present in the phytocompounds (Rathinavel *et al.,* 2017 and Meng *et al.,* 2011). *In silico* approaches such as pharmacokinetic (ADMET) studies, drug-likeness, molecular docking, and DFT analysis of the phytocompounds of *P. amboinicus* address the molecular mechanism of action, binding affinity and interactions of the molecules with the proteins.

Activation of tumour suppressor genes leads to the process of uncontrolled cell growth. The tissue-specific cell-to-cell attachment requires deactivation of apoptotic mechanism and downregulation of cell adhesion receptors, while upregulation of cell adhesion receptors improves cell motility. Hence, research on cancer studies using the entire genome has gained more attention among scientific communities. Research on cancer studies using the entire genome has gained more attention among scientific communities. The genes responsible for the cancer-associated pathways such as cell cycle regulation, damaged/ mutated DNA repair, attachment or detachment of cells and transduction of cellular signals have been considered to evaluate their expression and recognize its role in tumorigenesis (Wheeler and Wang, 2019).

In light of this, the present study was investigated to explore the antilung cancer potential of syringic acid fraction extracted from *P. amboinicus* in comparison with the commercial syringic acid and standard drug, paclitaxel under *in vitro* and *in vivo* approaches.

Hypothesis set for the present study

The purpose of this study was intended to examine the following hypotheses:

Null Hypothesis (H_0): Syringic acid fraction does not possess antilung cancer activity

Alternate hypothesis (H_A): Syringic acid fraction possesses antilung cancer activity

Hence the present study was formulated with the following objectives to test the above mentioned null and alternate hypotheses.

- To screen the phytochemicals in the leaf extracts of *Plectranthus amboinicus*
- To evaluate the free radical scavenging activity of the methanolic extract of *Plectranthus amboinicus*
- To understand the anticancer activity of the phytocompounds of *Plectranthus amboinicus* using *in silico* studies

- To identify the bioactive principles in the *Plectranthus amboinicus*
- To assess the anticancer activity of syringic acid fraction against A549 lung cancer cells through *in vitro* approach
- To evaluate the therapeutic efficacy of syringic acid fraction against B(a)P induced lung cancer tumorigenesis in mouse model

A brief review relevant to the present study is presented in the next chapter.