



INTRODUCTION

Medicinal plants are important natural products for a large population, especially because of their use as herbal medicines resulting from traditional knowledge arising from direct contact with nature (Abdala and Carlos, 2020). The use and reliance on plants as medicines by man has been in existence since the time of human evolution and man continues to look for plants as drugs for a specific disease within his reach. Medicinal plants continue to perform an important role in the healthcare system for a considerable proportion of the world's population, especially in developing nations where traditional medicine has a long history of use. With growing awareness of the health risks and toxicity linked with the random use of synthetic medications and antibiotics, attention in the use of plants and plant-based drugs has been rejuvenated all over the world. The medicinal plants are used as drugs or formulations to treat various human diseases because of the presence of several components of therapeutic significance (Singh *et al.*, 2016).

Plants are considered as potent resources of herbal remedies and these plants form the basis for the production of modern medicines (Dar *et al.*, 2017). The broad structural diversity and outspread pharmacological activities of natural products from plant origin has made its wide acceptance in the pharmaceutical industry (Cragg and Newman, 2013). The therapeutic potential of these plants resides in the presence of active phytochemical components that produce precise physiological effects on the human body. A wide range of bioactive chemicals including alkaloids, flavonoids, polyphenols, saponins, terpenoids, carotenoids, tannins, curcumins, and phytosterols have been documented from medicinal plants (Dhivya and Kalaichelvi, 2017). These active metabolites from natural origin are good candidates for the development of drugs, because being elaborated within the living systems, they are believed to show more similarities to drugs and exhibit more biological friendliness than the synthetic ones (Dias *et al.*, 2012). These natural compounds formed the foundation of modern drugs as we use today.

There is a forceful dependence on medicinal plants for a herbal remedy against the deadly and infectious diseases due to the irreversible effects of modern therapies and increasing drug resistance (Shrivastava and Dwivedi, 2015). Typically, the therapeutic efficacy of plant extracts is attributable to the synergistic and combined action of many chemicals. Current drug discovery strategies and modern medicine aid the identification and isolation of many of the active principles from plants in pure form and these components along with the synthetic compounds are the drugs now almost exclusively used in the management of diseases (Thomford *et al.*, 2018). Pure compounds or standardized extracts from medicinal plants have been found to contribute in indefinite ways for new drug leads because of the unrivaled accessibility and chemical diversity of bioactive components from the plant kingdom (Sasidharan *et al.*, 2011). The discovery of active constituents in natural sources is the primary stage in the development of novel drugs and thus searching for plant extracts is a novel method for identifying bioactive curative components in a variety of plant species (Olivia *et al.*, 2021).

The discovery of ethnomedicines with substantial antioxidant effects but moderate cytotoxicity has received a lot of attention recently. Plants synthesize secondary metabolites with biological activity, namely an antioxidant, which on the whole are phenolic compounds serving in plant defense mechanisms to counteract free radicals (Azhid *et al.*, 2016). Free radicals are tiny diffusible molecules which include reactive oxygen and nitrogen species and they are produced by numerous metabolic routes in the body. Unfettered production of free radicals in the cells degrade biological molecules like membrane lipids, proteins, and DNA results in oxidative stress and eventually leads to cell death. Many degenerative diseases, including diabetes, neurological disorders, atherosclerosis, cancer, Alzheimer's disease, ageing, and inflammatory diseases, are caused by these excess free radicals when they are not neutralised by the antioxidants present in the human body (Ifeanyi, 2018).

Antioxidant defense systems balance the reactive species production and halt the chain reaction mediated by free radicals even before the vital molecules are impaired thereby inhibiting cellular damage. When the defense system in the cell is imbalanced, defense against free radicals can be improved by taking adequate quantities of exogenous antioxidants (Pande and Sanda, 2020). There is a greater attention towards antioxidants present in natural phytocompounds, and they are now being used therapeutically for quenching reactive species, thereby attenuating oxidative stress-

mediated diseases. The antioxidant effect of plant products has been determined to be primarily due to the radical scavenging ability of phenols and flavonoids (Unuofin and Lebelo, 2020).

Cancer is one of the most dreadful global diseases caused due to extreme free radical detriment, which eventually causes damage to the DNA, lipids and proteins thereby transforming normal cells into a cancer cell (Jambunathan, 2014). Carcinogenesis is a multifaceted and multi-stage process comprising mutations in the gene and epigenetic alterations that change major cellular progressions and pathways including propagation, differentiation, and invasion in successive transformative events. A characteristic feature of cancer is massive deregulation of all aspects of the epigenetic machinery. Normal gene regulation is disrupted, and normal cellular activities such as cell cycle, DNA repair, cell proliferation, differentiation, and death are hampered by these modifications (Schnekenburger *et al.*, 2014).

Apoptosis is a highly organized cell death event involving a variety of biochemical and genetic pathways that contribute to the elimination of unwanted and undesired cells in order to retain a healthy balance between cell existence and cell death in normal tissues and is characterized by membrane blebbing, DNA fragmentation, reduction in cell size, chromatin condensation, and apoptotic bodies. Inadequate apoptosis has been linked to cancer and autoimmune diseases, while excessive cell death has been observed in acute and chronic degenerative illnesses, immunodeficiency, and infertility. Under many stressful conditions, such as precancerous lesions, DNA damage control point pathways may operate to eliminate harmful DNA-damaged cells by inducing apoptotic cell death to prevent cancer. Therefore, the apoptotic indications serve to protect the integrity of the genome whereas the apoptotic pathway dysregulation not only stimulates carcinogenesis but also makes the cancer cell resilient to anticancer drugs. Hence, the evasion of apoptosis is a noticeable characteristic of cancer resulting in impaired apoptotic signaling, which facilitates tumor development and metastasis (Hassan *et al.*, 2014). Many anticancer drugs work by stopping the cell cycle at a certain checkpoint, triggering apoptosis, or a combination of both cyclic block and apoptosis (Sumathi *et al.*, 2013).

Lung cancer, bronchogenic malignant tumors stemming from airway epithelioma, is the primary cause of cancer-associated deaths among men and women worldwide (Wadowska *et al.*, 2020). The major causes of lung cancer are smoking, hereditary

factors, certain poisonous gases, heavy metals, air pollution, and radon gas. Small-cell lung carcinoma (SCLC) and non-small-cell lung carcinoma (NSCLC) are the two most common types of lung cancer, the most prevalent form of lung cancer is non-small-cell lung cancer, which accounts for 85 to 90% of cases. Chemotherapy, immunotherapy, surgery and radiotherapy are the commonly used treatment possibilities for lung cancer. Despite the fact that chemotherapy is the most widely used treatment, it has many disadvantages, including toxicity, reduced effectiveness, and drug resistance (Samarakoon *et al.*, 2017).

Over the last decade therapeutic interventions such as surgery, radiotherapy and chemotherapy proved to be toxic to normal cells along with cancer cells because of non-selectivity and often cause serious side effects (El-Nabi *et al.*, 2016). Furthermore, despite the high cost of development, newer synthetic chemotherapeutic medicines now in use have failed to meet expectations, necessitating the creation of new, effective, and affordable anticancer drugs.

Cancer is the world's second leading cause of death, despite recent improvements in the discovery and development of new cancer medicines. Chemicals derived from plants have dominated as a substantial source of anticancer drugs for many years, owing to their availability and low toxicity when compared to chemotherapy, and have performed a crucial role in the prevention and treatment of cancer. More than 3000 plant species have been identified with the potential to treat cancer and about thirty plant-derived chemicals have been extracted and studied in cancer clinical studies. The majority of them are well known to trigger apoptosis which can be executed through intrinsic or extrinsic pathways, and caspase and/or p53-dependent or independent mechanisms (Solowey *et al.*, 2014).

The use of medicinal plants for cancer treatment has been increasing due to its availability, affordability and relatively less adverse effects when compared to the commercially available chemotherapeutic agents (Sharmila and Padma, 2013). Therefore, plant extracts are considered as chemical reservoirs of structurally different compounds of therapeutic potential and hence their investigation constitute a prominent approach in drug discovery. The potential biocompatible anticancer agents are bioactive phytochemicals that demonstrate the ability to inhibit cancer cytogenesis by preventing the initiation, promotion and progression of the tumour. (El-Nabi *et al.*, 2016).

To maximise the patient compliance and avoid recurrent administration, phytotherapeutics need a scientific approach to distribute the components in a persistent way. This may be accomplished by developing Novel Drug Delivery Systems (NDDS), which not only diminish the need for periodic administration to overcome noncompliance, but also help to improve curative efficacy of the drugs by lowering toxicity and boosting bioavailability. Herbal nano-drug delivery systems offer the likelihood to improve the activity and overcome the issues associated with plant medications, which are important in the management of chronic diseases such as asthma, diabetes, and cancer. Nanotechnology is extensively used to deliver targeted medication therapy, diagnostics, tissue regeneration, cell culture and biosensors (Aruna *et al.*, 2014). The adequacy, specificity, durability and therapeutic index of analogous drugs were declared to be enhanced by nanomedicines. Nanoparticles (NPs) are found to have vast applications including their usage in highly responsive diagnostic studies, drug and gene delivery, thermal ablation, radiotherapy improvement, antimicrobial activity, and mechanical, electronic and catalytic applications. Advances in nanoscience are promptly enabling the evolution of nanoparticles with distinct properties which address the limitations of traditional disease diagnostic and therapeutic agents (Rajendran *et al.*, 2015).

Researchers have given attention to metal nanoparticles because of their vast range of unique features compared to bulk metals, and they have a wide range of uses in diagnostics, cell labelling, antimicrobial agents, drug administration, and cancer therapy (Krishnan *et al.*, 2015). Amongst many metal nanoparticles, Silver nanoparticles (AgNPs) have acquired a lot of attention because of their unique physicochemical properties, such as chemical stability and electrical conductivity, as well as biological properties like antibacterial, antifungal, anti-inflammatory, antiviral, antiangiogenesis, anticancer, and antiplatelet activities (Al-Sheddi *et al.*, 2018). AgNPs have provoked researchers' attention in cancer research due to their ease of surface modification and production, robustly augmentable and adaptable visual features, and improved biocompatibility (Pei *et al.*, 2019).

AgNPs are least toxic to human cells compared to other metal nanoparticles, and their tiny size allows them to easily pass through the cell membrane, making them a potential antibacterial agent. Due to their extensive use in areas including catalysis, optics, antimicrobials, and biomaterial processing, AgNPs have become the subject of intensive research. Depending on their size, morphology, and distribution, silver

nanoparticles reveal new or enhanced properties (Dawad, 2021). The biological activity of silver nanoparticles are influenced by several factors including surface chemistry, size, size distribution, shape, morphology of the nano particle, composition of the particle, coating/capping, agglomeration, and dissolution rate, particle reactivity in solution, ability of ion release and type of the cell. One of the most important factors in determining cytotoxicity is the kind of reducing agents utilised in AgNP production (Zhang *et al.*, 2016).

Substantially, nanoparticles are prepared by a range of chemical and physical methods which are rather costly and possibly lethal to the environment which involves the use of poisonous and perilous chemicals (Ahmed, 2015). The use of plant products for nanoparticle production provides a new dimension to modern nanobio-technology by reducing drug degradation and undesirable side effects while also increasing drug bioavailability and the fraction of the drug deposition in the desired zone (Aruna *et al.*, 2014). Bio-based outlook towards the synthesis of nanoparticles are promptly gaining importance due to their ease of synthesis, eco-friendliness and formation of stable and biocompatible nanoparticles, notably bacteria, fungi, plants and seaweeds being ideal sources utilized for the synthesis of nanoparticles. The green synthesis with plant extracts with reported medicinal and/or anticancer properties are simpler and advantageous over other biological processes as they are safe to handle and they negotiate nano synthetic cues while the unused metabolites enhance therapeutic efficacy (Jha and Prasad , 2014).

Tabebuia roseo-alba (Ridl.) Sand, commonly known as White Trumpet tree of the family Bignoniaceae, is a deciduous tree with straight, pyramidal crown; it can grow up to 7 - 16 meters long (Lorenzi, 2002) and is considered as an economically important species due to its ethnopharmacological components.

With this framework the present study was formulated to explore the antiproliferative and apoptotic activity of the ethanolic extract of *Tabebuia roseo-alba* (*T. roseo-alba*) leaves and their silver nanoparticles under *in vitro* conditions.

Hypothesis of the study

The present study was planned to assess the following hypothesis:

Null hypothesis (H₀): Ethanolic extract of *T. roseo-alba* leaves do not possess antiproliferative and apoptotic activity in cells grown under *in vitro* conditions.

Alternate hypothesis (H_A): Ethanolic extract of *T. roseo-alba* leaves possess antiproliferative and apoptotic activity in cells grown under *in vitro* conditions.

Therefore, the present study was designed with the following objectives

- Phytochemical Characterization and Assessment of Free radical scavenging potential of various extracts of *T. roseo-alba*
- Synthesis and Characterization of silver nanoparticles using *T. roseo-alba*
- Evaluation of *in vitro* cytotoxic, antiproliferative activity and apoptotic inducing ability of crude extract and AgNPs of *T. roseo-alba* using A549 Lung cancer cell lines
- Assessment of *in silico* interactions between selected phytoconstituents of *T. roseo-alba* and targets from cancer pathways

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