Chapter 1

Introduction
"There's plenty of room at the bottom" - Richard Feynman

1.1 Introduction

Cancer is the term used for a disease in which abnormal cells divide without control and are able to invade other tissues. This disease can spread from one part to other parts of the body through the blood and lymph systems (US National Cancer Institute, 2014). Cancer is one of the leading causes of death in India. The WHO reported that the incidence of cancer in India is expected to be about 700,000 in the coming years. About 21.7 million new cases are expected to be diagnosed and 13 million cancer deaths are expected to be recorded in 2030 worldwide. Cancer incidence rate is almost 25% higher in men than in women (www.cancer.org; Estanquerio et al., 2015). It is a terminal disease, many-a-times leading to death. The cancer death rates based on geographic variation and the trends reflect differences, based on various risk factor patterns such as alcohol, smoking, life style, genetics, insufficient physical activity and obesity, as well as distribution of poverty and heath care access (Siegel et al., 2015).

In modern socio-economic systems, cancer represents a stressful problem. The main treatment of cancer are surgery and radiation, recommended for the local treatments. Another option is injection of a cytotoxic drug, which is known as chemotherapy. It has been the traditional system for treating cancer. It has low efficacy for some types of cancer such as lung, pancreatic and ovarian cancer and also creates cytotoxic effects in healthy tissues. The escape of cancer cells from chemotherapy is a major reason for systemic cancer treatment failure. So far, only limited progress has been made in the fight against cancer (Coccia and Wang, 2015; Estanquerio et al., 2015; Santoni et al., 2015; www.cancer.org). However, research efforts worldwide have opened up several promising avenues in our fight to conquer cancer.
In this context, nanomedicine is emerging as a forerunner. Nanomedicine has been investigated for cancer therapy for several decades, with several products in clinical trials or available in the market. Poorly water soluble and low permeable chemotherapeutic drugs are a tough task to overcome, where nanomedicine creates a new path to solve all the defects of conventional cancer medicines. The nanomaterials generally possess high biocompatibility and have a number of distinctive properties that make them promising and efficient drug delivery carriers in biomedical applications (Wang et al., 2015a).

Nanostructured materials are known as “intelligent” or “smart” materials, 1-100nm in size, used for constructing controlled drug release systems and possess the ability to control drug dosing in terms of quantity, location and time for drug delivery system. The controlled drug release systems are responsive to circumstances such as temperature, pH, applied magnetic or electrical field, ultrasound, light or enzymatic action (Shi et al., 2014a). Employing nanotechnology, along with the natural products, such as phenolic-enriched plants and fruits and their derivatives, is expected to provide tremendous potential and a new way to find a path to fight against cancer.

Plants have been a rich source of therapeutic compounds for various traditional medicines throughout the world for thousands of years, due to the presence of active phytocomponents, which help in preventing various oxidative stress-associated diseases (Akhtar et al., 2015). One of the most important groups of such active compounds are phenols. Several researchers have shown interest in working with these compounds because of their therapeutic performance. More than 8000 phenolic phytochemicals have been reported, which exist in various fruits and vegetables (Li et al., 2015). Polyphenols are considered as secondary metabolites of plants, with various chemical structures and activities. Moreover, several studies have reported that polyphenols correlate with the prevention of degenerative diseases such as cardiovascular diseases and cancer. They also possess
anti-inflammatory, anticarcinogenic, antidiabetic and antiulcer properties (Soto et al., 2015).

Most of the phenolic compounds are present in the herbs, which is widely used in traditional medicine to cure various diseases. But the major disadvantages of the phenolic compounds in general are their susceptibility when exposed to adverse environment, low oral bioavailability and poor stability, especially in the environment of gastro intestinal tract (Madureira et al., 2015). The biological activity might vary depending upon the nature of the phenolic compound. A way to tackle the above problem is by conjugation of phenolic compounds with the nanoparticles to improve the bioavailability, absorption and potential biological activity.

Among the various metal nanoparticles synthesized (such as silver, gold, zinc, palladium or platinum), now-a-days, silver nanoparticles (AgNPs) are one of the promising nanoparticles in the nanotechnology field, because of their applications in various disciplines like biomedical, catalysis, energy and materials (Ashokkumar et al., 2015). Right from olden days, humans have been in contact with silver, with brazing or soldering, coins, tableware, jewellery and dental filling (Hadrup and Lam, 2014). Silver nanoparticles were observed as a safe inorganic and non-toxic agents, possessing antibacterial and antifungal effects. Silver, in the nano size, encompasses a wide range of applications in biomedical field.

During the last few years, much research work has been carried out in the development of green synthesis methods for nanoparticles. Using the green synthesis approaches, around 200 plants belonging to different families have been screened for their potential effect and ability in the synthesis of silver, gold, copper, palladium and iron nanoparticles. Various plants enriched with medicinal properties have been used to fabricate silver nanoparticles, and the research is focusing on producing AgNPs with different capping molecules that exhibit different morphologies and size. The role of plant extract is acting as capping layers and to shape the nanoparticles during the growth with the nanosize. The synthesis of
nanoparticles from plant not only facilitates the effective physicochemical properties, but also carries potential biological properties like antimicrobial and anticancer activity (Rajan et al., 2015; Rauwel et al., 2015).

Several studies have reported that silver nanoparticles have potent antimicrobial effect. The nanoparticles are known to attach to the surface of the cell membrane or bind to the enzymes and proteins within the bacteria, which severely damages the cell and its major functions such as permeability, regulation of enzymatic signaling activity, cellular oxidation and respiratory processes, resulting in the bacterial death (Lim et al., 2015). They also penetrate into the cell easily due their small size and larger surface area, which unique character helps to kill the microorganisms (Abdel-Aziz et al., 2014). Reithofer et al. (2014) have reported that silver nanoparticles with the size range from 20-25nm were the most effective in suppressing the growth of clinically relevant bacteria with moderate to high antibiotic resistance. Many researchers have demonstrated the cytotoxicity of silver nanoparticles in cancer cells, which affects the membrane integrity and induces various apoptotic signaling genes, leading to programmed cell death (Butler et al., 2015).

Earlier studies conducted in our laboratory proved Piper betle leaf extract to be inherently rich in phenols, and to have antioxidant and hepatoprotective activity (Saraswathi, 2006). The leaves have also been shown to be antimutagenic and anticarcinogenic (Padma et al., 1989a; Padma et al., 1989b). Grapes is also rich in polyphenols, which neutralize free radicals and have applications in the treatment of cancer and neurodegenerative diseases (Aizpurua-Olaizola et al., 2016). Another study conducted in our research group using grape skin extract against oxidative stress-induced apoptosis in Saccharomyces cerevisiae cells, showed that the extract exhibited protective effect (Merlyn, 2014). Based on this background, we selected both the Piper betle and Vitis vinifera as the candidate plants for the present study.
Betel leaves are rich in eugenol (Punuri et al., 2012) and grapes are known to be rich in resveratrol (Carter et al., 2014). Eugenol and resveratrol are natural compounds that possess tremendous antimicrobial and anticancer activity (Hwang and Lim, 2015; Pramod et al., 2015). Eugenol causes anticancer effect by inducing apoptosis through the caspase-3 activation pathway and cell cycle arrest with mitochondrial membrane loss (Yi et al., 2015). Various literatures have suggested that resveratrol acts through multiple mechanisms like proapoptotic, anti-proliferative, anti-inflammatory and anti-angiogenesis in cancer management, which could be exploited as a chemotherapeutic agent (Singh et al., 2015).

The target of the present study was to optimize the appropriate method for the synthesis of silver nanobioconjugates from betel (Piper betle) leaves (Athur variety), grape (Vitis vinifera) seeds and their active phenolic components, eugenol and resveratrol respectively.

1.1 Hypothesis

Based on the earlier studies conducted in our laboratory, the study focused on the synthesis of silver nanobioconjugates and their anticancer effect from the Piper betle extract, Vitis vinifera extract and their active compounds, eugenol and resveratrol respectively.

The null hypothesis (H₀) formulated for our study was:

The silver nanobioconjugates synthesised from Piper betle extract, Vitis vinifera extract and their active compounds, eugenol and resveratrol, do not possess anticancer effect.

The alternate hypothesis (Hₐ) drawn was:

The silver nanobioconjugates synthesised from Piper betle extract, Vitis vinifera extract and their active compounds, eugenol and resveratrol, exhibit good anticancer effect.
1.2 Objectives

To test these hypotheses, the study was laid out with the following objectives:

- To synthesize and characterize silver nanobioconjugates from anticancer phenolic phytochemicals (eugenol and resveratrol) and their major plant sources, namely the extracts of *Piper betle* and *Vitis vinifera* respectively, and to study their bioactivity.

- To compare the efficacy of the silver nanobioconjugates of eugenol and resveratrol with those prepared with the extracts of *Piper betle* and *Vitis vinifera* respectively.

- To study the anticancer effect of the prepared nanodrugs using cancer cell lines.

1.3 Layout of the thesis

The thesis includes seven chapters. Chapter 1 introduces the reader to the field of nanotechnology and the recent developments in establishing nanoparticles as drugs, especially in cancer. A brief review of literature pertaining to the present study is presented in chapter 2. Chapters 3 to 7 deal with the aspects of the present study. Among these, chapter 3 deals with the preparation of the bioconjugates in the nanoscale, using silver as the component metal, and the phytoextracts and the individual major component phenols. This chapter also presents the aspects related to the optimization of the conditions for the green synthesis of the AgNPs and testing their bioactivity. The fourth chapter deals with the detailed characterization of the prepared nanobioconjugates. In chapter 5, the properties associated with the druggability of the nanobioconjugates prepared were studied. This encompassed the recording of the drug release kinetics and testing human biocompatibility of the AgNPs to ensure that there was no toxicity of the bioconjugates towards human cells. Chapter 6 presents the anticancer activity of the silver nanobioconjugates prepared.
using the phytocomponents, namely *Piper betel* leaf extract, its component phenolic eugenol, *Vitis vinifera* seed extract and its component phenol resveratrol. This activity was tested and reported in two cancer types namely oral carcinoma (KB cell line) and lung adenocarcinoma (A549 cell line). The non-toxicity of the conjugates were further affirmed in human buccal cells and human peripheral lymphocytes, which were used as the non-cancerous control cells. Chapter 7 summarizes all the results described in chapters 3 to 6 and presents the major conclusions drawn from the outcome of our study. References that are quoted in all the chapters are listed at the end of the thesis.