Chapter 1

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1.1. Cancer

WHO has assumed cancer as the single important cause of death with an estimation of 12 million deaths worldwide by 2030. Cancer is one of the killer diseases and major public health burden causing 12 % of all deaths globally. Every year about 8,50,000 new cancer cases are being diagnosed of which about 5,80,000 deaths result from cancer in India. In male oral, lung, stomach cancers and in female cervical, breast and oral cancers are the three main causes of cancer related illnesses and death (Dhanamani *et al*., 2011). Cancer is the rapid and uncontrolled growth of normal cells, possessing unique characteristics such as immortality, continuous growth invading nearby tissues forming tumors and metastasizing to distant parts of body, which when untreated causes death. Cancer results from many factors such as environmental, toxic chemicals, viruses, life style, epi-genetic and genetic (Ramalakshmi and Muthuchelian, 2011). In genomic level cancer is caused by genetic level changes at oncogenes, tumor-suppressor gene and microRNA genes. Of the 50,000 to 1,00,000 genes in our genome, about 100 have been identified as proto-oncogenes and about a dozen as tumor suppressor genes (Spandidos and Siminovitch, 1978).

1.2. Anticancer Drugs

The main forms of treatment for cancer in humans are surgery, radiation and drugs. Cancer chemotherapeutic drugs such as 5-fluorouracil derivatives, cisplatin, mitomycin and adriamycin have been used extensively for the treatment of different types of cancer but they provide only temporary relief of symptoms, prolong life, and cures rarely. Problems associated with these synthetic drugs include short life, aqueous solubility, non-selectivity, severe gastrointestinal toxicity, diarrhoea, mucosis and haematological toxicity, with leucopoenia and immune suppression. In recent years, a lot of effort has been applied for the synthesis of potential anticancer drugs with minor side effects.

An ideal anticancer drug should kill cancer cells without causing excessive damage to normal cells (www.cancergov.com). Medicinal plants have served as a source of drugs for the treatment of a number of diseases including cancer. Since ancient times, medicinal plants are the important source of drugs for diseases including cancer as they are easily available, cheaper and possess no toxicity as compared to the modern (allopathic) drugs. Various anticancer herbs used in the herbal formulations are designed in such a way that they do not attack the normal cells but target and kill cancerous cells in the body (Saxe, 1987). The active compounds of plant origin are said to possess extremely complex and novel structures. Plant natural product chemistry is an exciting field having a significant role in discovery of drug candidate compounds from plant sources, where 50 % of drugs entering commercial markets are from natural sources. Several semi-synthetic and synthetic derivatives of plant-derived anticancer drug compounds are synthesized and tested at various pre-clinical and clinical stages.

The various anticancer drugs from plant sources include vinblastine, vincristine, campothecin (derivatives such as topotecan and irinotecan), epipodophyllotoxin (derivatives such as etoposide and teniposide), paclitaxel, homoharringtonine, betulinic acid, silvestrol, lapachol and β-lapachone (Cragg and Newmann, 2005). A lot of new promising anticancer drugs are in various stages of preclinical and clinical studies such as flavopiridol and combretastin A4 phosphate, 10-hydroxycamptothecin, (-)-sophocarpine, monocrotaline, d-tetrandrine, lycobetaine, indirubin, colchicinamide, curcumol, curdione and gossypol (Lee *et al*., 1998; Wang and Lee, 1998). According to the consultancy firm Frost and Sullivan, the anti-cancer drug market touched Rs.2, 000 crore in the year 2013 and is projected to grow to Rs.3, 831 crore by 2017 in India (www.frost.com).

1.3. Medicinal Plants

India is said to be a rich source of all plant species and has been selected as one of the 12 mega biodiversity countries in world. Before the advancements in medicine field, traditional knowledge on medicinal plants has helped 75 % of world's population to cure a variety of diseases (Duke, 1985). Herbs are taken regularly by people in their

daily diet to protect the body from cancer as they detoxify body due to the active phytochemicals present.

Due to rise in population, economy, inadequate supply of drugs and prohibitive cost of treatments in developing countries, people living with less income depend on usage of plant drugs as they can't afford to buy modern medicines. Due to the emergence of new infectious diseases and resistance among the existing pathogens, even modern people go for medicinal plants and herbs for treatment of wide variety of human ailments.

1.4. Phytochemicals

Plant metabolites are phytochemical substances that can be produced in plants due to primary or secondary metabolic activities, where the secondary metabolites are most active playing an important role in plant defense system. The secondary metabolites such as terpenoids, quinines, tannins and capsaicin are responsible for plant odor, pigment production, characteristic flavor (Cowan, 1999).

Plants contain many phytochemicals that are useful sources of natural antioxidants such as alkaloids, polyphenols, flavonoids, terpenoids, carotenoids and chlorophyll (Lee *et al*., 2004). The presence of active phytochemical phenols reduce the lipid peroxidation of food and its products which boost the quality and nutritive value of food (Mathew and Abraham, 2006), so it is now common to incorporate crude extracts of important medicinal plants, herbs, vegetables and fruits. The toxicity of phenols on microbes takes place due to the activity of functional and non functional groups such as hydroxyl present in them interacting with enzymes and proteins in microbes (Mason and Wasserman, 1987). Other phytochemicals such as flavonoids and carotenoids are natural, brightly colored pigments synthesized by plants for defense process to fight against microbes through oxidative deterioration and to compete with other plants (Dixon *et al*., 1983; Akoh and Min, 1997). These active compounds possess strong antioxidant, antimicrobial, anticancer (Surh and Chun, 2007; Mou, 2011) and other biological activities due to their difficult, unusual structures.

1.5. Antioxidants

Free radicals are generated through interaction of human with hazardous chemicals, environmental pollutants, exhausts from industries, automobiles, air pollutants, pesticides and radiation. In human body the dynamic balance between the amount of free radicals produced by various reactions and antioxidants and enzyme systems work in order to prevent the oxidative stress and damage caused to cell, when there is no stability it leads to severe diseases (Nose, 2000). The main role of antioxidants in cell is to prevent damage caused by the action of reactive oxygen species (ROS), which are unstable and highly reactive, causing damage by chain reactions, such as lipid peroxidation or free radical damage to lipids, proteins, flavonoids and nucleic acids (Choi and Lee, 2009). Synthetic antioxidants butylated hydroxyl anisole (BHA), *tert*-butyl hydroxyl quinine (TBHQ) and butylated hydroxy toluenes (BHT) are available but their use was restricted by legislative rules because of the genotoxicity and carcinogenicity at higher concentrations (Gutteridge and Halliwell, 2010).

Natural foods are rich sources of antioxidants such as vitamins C and E, phenolic compounds, terpenoids, carotenoids and phytonutrients. Plants and fruits have advanced level of antioxidant defense, by synthesising different phytochemicals and enzymes as to maintain growth and metabolism system (Pandhair and Sekhon, 2006). Thus considerable interest by the industry, food and health scientists, people's preference for natural antioxidants over synthetic compounds, has given more impetus to explore natural source of antioxidants. Nowadays more attention has been directed towards the development of ethnomedicines with strong antioxidant properties but low cytotoxicities.

1.6. Antimicrobials from Plants

Though each day variety of antibiotics and drugs come to market, infectious diseases are said to be the foremost cause of deaths worldwide in tropical countries. Each antimicrobial though have wide spectrum of action on microbes to cure diseases, they cause side effects. To minimize the side effects caused by synthetic compounds, the huge therapeutic potential of medicinal plants are explored as they are effective and

gentle. The phytomedicines are still proving its potential by development of new antimicrobials by meeting the requirements of common people and drug industries.

1.7. Brine Shrimp Cytotoxicity Assay

Brine shrimp cytotoxicity assay is a simple bench-top assay used to measure cytotoxicity of plant extracts as well as synthetic compounds. This assay indicates not only cytotoxicity but also a wide variety of pharmacological activities (anticancer, antiviral, insecticidal and pesticidal potential) (Mayer *et al*., 1982; McLaughlin, 1990; Colegate and Molyneux, 2007). Brine shrimp eggs are available commercially, and being used as fish food.

1.8. Phytotoxicity Assay

Weed species grow stronger, competing neighbouring crops for light, water and nutrients, affecting the crop yields. During competition between the exotic and native species a large amount of toxins named as allelochemicals are created from leaves, flowers, seeds, stems and roots to repel other species (Indergit and Dakshini, 1998). These allelochemicals are produced by plant metabolic pathways which may be of secondary origin not used by plants which include alkaloids, phenols and terpenoids. Of the allelochemicals studied, phenols are identified as profuse substances secreted under field conditions affecting seed germination and growth of other species (Lodhi, 1976). This allelopathic potential study gives a scheme to identify and develop ecofriendly, cheap and effective green growth promoters (Oudhia *et al*., 1998).

1.9. Genotoxicity Studies of Plants- Cytokinesis-Blocked Micronucleus (CBMN) Assay

The break through of the cytokinesis blocked method by Fenech and Morley (1985) using cytochalasin – B derived from a mould metabolite isolated from *Helminthosporium dematioideum* made this method a golden standard to evaluate genotoxic agents. A micronucleus is a small structured (1/5 to 1/20 the size of the nucleus) extranuclear body resulting from chromosome breaks or whole chromosomes lagging behind during anaphase. In the field of mutagenicity testing, the human *in vitro* micronucleus (MN) test has turned out to be a fast and reliable assay for scientists.

1.10. Cytotoxicity Studies Using MTT Assay

Now-a-days cell-based assays have become increasingly important for the pharmaceutical industry as well as high throughput screening of active compounds as drugs. MTT assay is a colorimetric assay for measuring the respiratory ability of the mitochondrial succinate-tetrazolium reductase system that reduces yellow MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide, a tetrazole) to purple color formazan product. Thus MTT system serves as quantitative, sensitive and convenient method for evaluating cell population's response to new compounds. In this method, the growth or death rate of cells can be calculated directly due to the linear relationship between cell activity and absorbance.

1.11. Nanomedicine

Nanotechnology involves the synthesis, characterization and application of nanomaterials in the nanometer scale or one billionth of a meter for application in diagnosis, imaging, therapy or in combinations (Jain, 2005). Nano devices of small size (10^{-9}) can easily penetrate and transport to most cells and even blood vessels interacting with the target, delivering the drug material without altering the behaviour and biochemical property of those molecules.

Recent researches in nanotechnology is involved in development of devices with small molecules, such as a contrast agent or a multicomponent diagnostic system capable of assaying a cell's metabolic state, resulting in high sensitivity in detecting cancer at the earliest stages (Kubik and Sugisaka, 2002). The modern trend in nanomedicine devices are used for both drug delivery ('therapy') as well as 'diagnosis', where the term 'theranostics' is employed. Thus nanotechnology enables in the delivery of anticancer drugs to the target tumor tissues by crossing various barriers and protects the surrounding normal tissues from toxic effects. The development of nanoparticles based drugs provides less side effects and targeted action on cancer cells as they are targeted selective and specific towards tumors resulting in better treatment (Ali *et al*., 2011).

1.12. Silver Nanoparticles

Silver nanoparticles (AgNP) in their 'nano' range possess increased large surface to volume ratio, easily crossing the obstacles in cell and have a direct effect on microbes. Silver nanoparticles are famous for their antimicrobial effect on variety of microbes (Zhao and Stevens, 1998). The various applications of AgNP include their use as catalysts, as optical sensors, integrated circuits, filters, antimicrobial deodorant fibres, cell electrodes, textile engineering, electronics and optics (Iravani, 2011) due to their antimicrobial properties.The medical application of AgNP in form of silver ion includes the use in the formulation of dental resin composites and in coatings of medical devices. Prabhu and Paoulose (2012) make obvious that nano form of silver have been applied in intravenous catheters, endotracheal tubes, wound dressings, bone cements, disinfectants and dental fillings.

In cancer diagnosis, AgNP based Surface-Enhanced Raman Spectroscopy (SERS) in non-invasive detection of cancer is said to be a remarkable research by Lin *et al*. (2011), involving novel blood plasma analysis with membrane electrophoresis is a highly promising technique. Kwan *et al*. (2011) have developed wound dressing made of AgNP and found to have better healing rate due to better collagen alignment after healing, giving better mechanical strength. In targeted action, folated silver-dendrimer composite nanodevices upon exposure of light energy destroy cancer cells effectively, where these AgNPs uptake light energy and result in microbubbles (Tse *et al*., 2011).

1.13. Research Objectives

The present study was made to screen these two plants for their basic activities as well as anticancer activity with the following objectives:

- To successively extract bioactive compounds from *Mallotus tetracoccus* and *Tabebuia rosea* using polar and non-polar solvents.
- To analyse qualitatively and quantitatively for phytochemical compounds such as phenols, flavonoids and carotenoids.
- To identify volatile profile of plant extract by GC-MS analysis.

- To screen the various fractions of *T. rosea* and *M. tetracoccus* for antioxidant activity and antimicrobial activity against gram positive and negative organisms.
- To screen for brine shrimp lethality, phytotoxicity and genotoxicity studies of all fractions.
- To select the proven herbal extract and prepare bionanoparticles and characterize them.
- To compare cytotoxicity of prepared nanoparticles and plant extract using MTT assay.

Chapter 2

Review of Literature