2 Review of Literature

2.1 Ayurveda for cancer

Ayurveda is a natural health care system that originated in India more than 5000 years ago. It emphasizes prevention and health promotion and provides treatment for disease. Western Science has explored only ~5% of the approximately 25,000 species of higher plants for drug leads.

Ayurveda provides a list of herbs known for nootropic activity as well as their multi-dimensional utility in various conditions. A research was conducted to review the updated knowledge on pharmacological properties, major chemical constituents, therapeutic actions, preclinical studies, safety and possible mode of action of the selected herbs from ayurvedic pharmacopoeia. Concurrently, it opened up for further research and standardization on nootropic herbs. (Kulkarni et al., 2012)

Chern et al., (2012) stated that the research was encouraged in Ayurveda, Unani, Siddha and Homeopathic by Government initiatives, but they were all insufficient to promote focused and aggressive evaluation of potential herbs. Particular emphasis should be given to clinical pharmacokinetics, drug interactions and clinical trials in specific cancers for the evaluation of dosage, safety, efficacy and concomitant use with chemotherapy.

*Emblica officinalis* Gaertn. or *Phyllanthus emblica* Linn, commonly known as Indian gooseberry or amla, is arguably the most important medicinal plant in the Indian traditional system of medicine, the Ayurveda. Various parts of the plant are used to treat a range of diseases, but the most important is the fruit. The experimental studies have shown that amla and some of its phytochemicals possess radiomodulatory, chemomodulatory, chemopreventive effects, free radical scavenging, antioxidant, anti-inflammatory, antimutagenic and immunomodulatory activities, properties that are efficacious in the treatment and prevention of cancer. (Baliga and Dsouza, 2011)

For centuries, Ayurveda has recommended the use of bitter melon (*Momordica charantia*) as a functional food that prevent and treat diabetes associated complications. It is noteworthy to mention that bitter melon extract has no-to-low side effects in animals as well as in humans. The anti-tumor activity of bitter melon has recently begun to emerge. Several groups of investigators have reported that treatment of bitter-melon-related products in a
number of cancer cell lines induces cell cycle arrest and apoptosis without affecting normal cell growth (Nerurkar and Ray, 2010).

*Alstonia scholaris* has been used for centuries in Ayurvedic medicine for treatment of various disorders. Jahan *et al.*, (2009) demonstrated the chemopreventive potential of *Alstonia scholaris* bark extract in DMBA-induced skin tumorigenesis in Swiss albino mice.

*Butea monosperma* (Lam.) (Fabaceae) popularly known as 'flame of the forest' has been widely used in the traditional Indian medicinal system of 'Ayurveda' for the treatment of a varieous of ailments including liver disorders. The aqueous extract of *Butea monosperma* flowers were used to impose growth arrest and trigger pro-apoptotic death in cell culture that strongly correlated with its strong chemopreventive effect *in vivo* when given orally (Choedon *et al.*, 2010).

*Swertia chirata* is a plant with bitter taste used since an early date in traditional medicinal systems of our country for treatment of varied human ailments. In Ayurveda, the plant is used as stomachic, febrifuge, antihelminthic, diuretic as well as for treatment of some types of mental disorders. In view of the antioxidative, anti-inflammatory and anticarcinogenic activities reported in recent times, the plant demands a more detailed probe to determine its use in pharmaceutical industry for preparation of drugs for prevention and treatment of chronic human diseases like diabetes, cardiac problems and cancer (Saha and Das, 2010).

Sharma *et al.*, (2007) reported that Ayurveda is a comprehensive natural health care system that originated in India more than 5000 years ago. It is still widely used in India as a system of primary health care, and interest in it is growing worldwide as well. There are encouraging results observed from Ayurveda’s comprehensive treatment than Western allopathic treatment.

Stan *et al.*, (2008) demonstrated that Withaferin from *Withania somnifera* treatment causes G2 and mitotic arrest in human breast cancer cells and suggested that G2-M phase cell cycle arrest may be an important mechanism in antiproliferative effect of WA against human breast cancer cells. Ayurveda have the ability to manage chronic disorders that Western medicine has been unable to and improve the health status of the world's population (Sharma *et al.*, 2007).
2.2 Phytochemicals for cancer

Plant-based foods, containing significant amounts of bioactive phytochemicals, may provide desirable health benefits beyond basic nutrition to reduce the process of cancer. Thymoquinone is an active ingredient isolated from \textit{Nigella sativa} are mediated through different modes of action, including anti-proliferation, apoptosis induction, cell cycle arrest, ROS generation and anti-metastasis/anti-angiogenesis. This quinone was found to exhibit anticancer activity through the modulation of multiple molecular targets, including p53, p73, PTEN, STAT3, PPAR-γ, activation of caspases and generation of ROS. (Poojari \textit{et al}., 2012)

A recent study provided novel insight into the mechanism of apoptosis induction by withaferin A, which is a bioactive constituent of an Ayurvedic medicine plant (\textit{Withania somnifera}). Exposure of MDA-MB-231 and MCF-7 human breast cancer cells to WA treated animals resulted in suppression of XIAP, cIAP-2, and Survivin protein levels. These results indicated important contribution of Survivin suppression in WA-induced apoptosis. (Eun-Ryeong and Shivendra, 2012)

Polyphenol rich fractions derived from the pomegranate fruit have been studied for their potential chemopreventive and/or cancer therapeutic effects in several animal models. Although data from \textit{in vitro} and \textit{in vivo} studies look convincing, well designed clinical trials in humans are needed to ascertain whether pomegranate can become part of our armamentarium against cancer. There is increased appreciation by the scientific community that dietary phytochemicals can be potential weapons in the fight against cancer (Syed \textit{et al}., 2012).

Takata \textit{et al}., (2012) suggested that dietary riboflavin intake may be inversely associated with lung cancer risk among female. Lunasin is a 43-amino acid peptide identified in soybean and other plants whose anti-carcinogenic activity has been demonstrated both in \textit{in vitro} and \textit{in vivo} assays (Hernández \textit{et al}., 2012).

The ability of phytochemicals to induce apoptosis of cancer cells and bacterial cell damage may be, at least partly, due to their prooxidant properties (Paszkiewicz \textit{et al}., 2012). Based on earlier research findings, this is to be suggested that the daily consumption of natural dietary components that are rich in phenolics could be beneficial for the prevention of cancer metastasis (Weng and Yen, 2012).
The chemopreventive potential has been related to the bioactive phytochemicals present in the brain portion of the rice such as ferulic acid, tricin, β-sitosterol, γ-oryzanol, tocotrienols/tocopherols, and phytic acid. Studies have been shown that the anticancer effects of the rice bran-derived bioactive components are mediated through their ability to induce apoptosis, inhibit cell proliferation, and alter cell cycle progression in malignant cells. (Henderson et al., 2012)

Betulinic acid (BetA), a lupine-type penta cyclic triterpenoid saponin from plants, has shown antitumor activity in some cell lines in previous studies. The results of earlier research work showed that BetA effectively suppressed tumor growth in vivo (Wang et al., 2012). Natural agents generally have lower side-effects, are readily available and hence are cost effective. Zerumbone, a cyclic eleven-membered sesquiterpene, isolated from the tropical plant Zingiber zerumbet that has attracted great attention recently for its potent anticancer activities in several tumor models and also on various in vitro and in vivo studies related to the effects of zerumbone on numerous pivotal molecular targets in cancer and its reported chemopreventive/therapeutic effects in different models of cancer. (Prasannan et al., 2012)

The recent study demonstrated that the flavonoid quercetin, naturally present in the diet and belonging to the class of phytochemicals, is able to sensitize several leukemia cell lines and B cells isolated from patients affected by chronic lymphocytic leukemia (B-CLL). Considering the low toxicity of the flavonoids toward normal peripheral blood cells, the experimental results are in favor of a potential use of quercetin in adjuvant chemotherapy in CLL or other types of cancer. (Spagnuolo et al., 2012)

Eugenol (4-allyl-2-methoxyphenol), which is the active component of Syzigium aromaticum (clove s) which has a wide range of applications like perfumeries, flavorings, essential oils and in medicine as a local antiseptic and anesthetic. Increasing volumes of literature showed eugenol possesses antioxidant, antimutagenic, antigenotoxic, anti-inflammatory and anticancer properties. Molecular mechanism of eugenol-induced apoptosis in melanoma, skin tumors, osteosarcoma, leukemia, gastric and mast cells has been well documented. This abstract also highlighted the antiproliferative activity and molecular mechanism of the eugenol induced apoptosis against the cancer cells and animal models. (Jaganathan and Supriyanto, 2012)

Phytoestrogens are phytochemicals are widely distributed in diet and herbs and have shown anti-cancer activity via mechanisms including estrogen receptor modulation,
aromatase inhibition, and anti-angiogenesis. Genistein, daidzein and resveratrol are some of the most studied PE examples. Quality control in product manufacturing and clinical study design is a critical issue in developing them as clinically effective chemopreventive agents for breast cancer (Liu et al., 2012). Several epidemiological studies have indicated that abundant consumption of foods from plant origin is associated with a reduced risk of developing several types of cancers (Lamy et al., 2012).

Researchers found data on grapes, berries, tea, cocoa, coffee, myrtle, chamomile, honey/propolis, aloe extracts and the three main groups of polyphenols (stilbenes, flavonoids and proanthocyanidins). Their effects on caries, gingivitis, periodontal disease, candidiasis, oral aphtae, oral mucositis, oral lichen planus, leukoplakia and oral cancer were investigated. The data have shown interesting activities of polyphenols against the most common oral diseases as well as in oral cancer prevention. (Varoni et al., 2012)

Phytoestrogens are plant-derived, non-steroidal phytochemicals with anticarcinogenic potential. The human lignans enterodiol and enterolactone were more biologically active than their precursors secoisolariciresinol and matairesinol, and might be defined as the real drugs in cancer prevention. (Abarzua et al., 2012)

Soy isoflavones, linked to reduced cancer risk in Asian epidemiology, may suppress cox-2 induction by activating ERbeta. This is suggested that a comprehensive lifestyle strategy targeting cox-2 expression and bioactivity may have tremendous potential for cancer prevention (McCarty, 2012). Quercetin can be considered the prototype of a naturally-occurring chemopreventive agent because of its key roles in triggering the "hallmarks of cancer". The design of specific clinical trials was extremely warranted to depict possible applications of quercetin in adjuvant cancer therapy (Russo et al., 2012).

Curcumin, the principal polyphenolic curcuminoid, obtained from the turmeric rhizome Curcuma longa has long been used to cure several chronic ailments, such as neoplastic and neurodegenerative diseases. A recent study suggested that curcumin may have antitumor, antioxidant, and anti-inflammatory properties (Darvesh et al., 2012).

Xiao et al., (2011) showed that inadequate intake of fruit and vegetable makes a significant contribution to the cancer burden. Increasing consumption of fruit and vegetable could prevent many cancer deaths and save many lives. Promoting the consumption of fruit and vegetable is an important component in diet-based strategies for preventing cancer.
Epidemiological studies confirmed that, among many flavonoids, apigenin, epigallocatechin gallate, delphinidin and genistein appear to be beneficial compounds in various stages of carcinogenesis (Clere et al., 2011).

*Phyllanthus niruri* is a well known medicinal plant which has been used in Ayurvedic medicine as hepatoprotective, antiviral, antibacterial, analgesic, antispasmodic and antidiabetic. The results of this work suggested that *P. niruri* extract exhibits significant anti-tumor activity, which supports the traditional medicinal utilization of this plant. (Sharma et al., 2009).

### 2.3 Free Radicals and Antioxidants

Free radicals are known to play a definite role in a wide variety of pathological manifestations. Overproduction of free radicals can cause oxidative damage to biomolecules, eventually leading to many chronic diseases, such as atherosclerosis, cancer, diabetes, aging and other degenerative diseases in human. Antioxidants fight against these free radicals and protect us from various diseases either by scavenging the reactive oxygen species (ROS) or protecting the antioxidant defense mechanisms (U mamaheswari and Chatterjee, 2008).

The basic principle underlying the ABTS decolorization assay is that ABTS on reaction with $\text{K}_2\text{S}_2\text{O}_8$ forms a greenish blue radical cation. Standard and sample antioxidants that are able to transfer an electron to ABTS radical scavenge the color of the solution proportionate to their amount. The extent of scavenging depends both upon the concentration of antioxidant and time duration for the reaction. ABTS assay is an excellent tool for determining the antioxidant activity of hydrogen-donating antioxidants and of chain-breaking antioxidants (Muhammad Nadeem Asghar et al., 2008).

Hochestein and Atallah (1988) stated that the hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells. This radical has the capacity to join nucleotides in DNA and cause strand breakage which contributes to carcinogenesis, mutagenesis and cytotoxicity related to its antioxidant activity. The resulting radical can undergo further reactions, such as reacting with oxygen to give peroxylradicals, or decomposing to phenoxy1-type radicals by water elimination. The effectiveness of the plant extracts to quench hydroxyl radicals seems to insinuate that they are good scavengers of oxygen species. The ability of the above...
mentioned fractions to quench hydroxyl radicals seems to be directly related to the prevention of propagation of the process of lipid peroxidation and seems to be good scavenger of active oxygen species, thus reducing the rate of the chain reaction (Manian et al., 2008).

2.4 Elaeagnaceae for cancer

*Elaeagnus glabra* (Thunb.), an evergreen shrub with alternate leaves, has been used as a medicinal plant in Korea. Li et al., investigated the effect of the methanol extract of *E. glabra* on tumor cell invasion. The invasiveness of HT1080 human fibrosarcoma cells were reduced in a dose-dependent manner following 24h treatment of up to 200µg/ml of the *E. glabra* extract, at which concentration no cytotoxicity occurred. Furthermore, gelatinolytic activities, and the protein and mRNA levels of MMP-2 and MMP-9 were also suppressed with increasing concentrations of the extract. (Li et al., 2009)

A 70% ethanol extract of the branches of *Hippophae rhamnoides* exhibited remarkable antitumor activity in an *in vivo* two-stage carcinogenesis test in mice using 7,12-dimethylbenz[a]anthracene as an initiator and 12-O-tetradecanoylphorbol-13-acetate (TPA) as a promoter. From the active fraction of the 70% ethanol extract, three phenolic compounds, (+)-catechin (1), (+)-gallocatechin (2), and (-)-epigallocatechin (3) and a triterpenoid, ursolic acid (4) were isolated and identified. These compounds were evaluated for their inhibitory effects on TPA-induced inflammation (1µg/ear) in mice. Within the tested compounds, 3 and 4 showed marked anti-inflammatory effects, with a 50% inhibitory dose of 1.7 and 0.2µM/ear. (Yasukawa et al., 2009)

The autumn olive (*Elaeagnus umbellata* Thunb.) was evaluated for antioxidant capacity and anti-cancer properties. Pretreatment of JG6 P+ mouse epidermal cells with autumn olive extracts inhibited the activation of activator protein-1 (AP-1) and nuclear factor-kappaB (NF-kappaB) induced by either 12-O-tetradecanoylphorbol 13-acetate (TPA) or ultraviolet-B (UVB). Extracts of all autumn olive genotypes inhibited proliferation of human leukemia HL-60 cancer cells and human lung epithelial cancer A549 cells and induced apoptosis of HL-60 cells. (Wang et al., 2007)

Hippophae (Sea buckthorn) is a deciduous species, widely distributed throughout the world. It contains different kinds of nutrients and bioactive substances such as vitamins, carotenoids, flavonoids, polyunsaturated fatty acids, free amino acids and elemental components. The clinical trials and scientific studies during the 20th century confirm
medicinal and nutritional value of sea buckthorn, and the most important of them is its anti-
carcinogenic properties. This mini-review is focused on the anti-carcinogenic potential of
lipids from this plant, in order to open up a clear understanding for further detailed study in
this regard. (Zeb, 2006)

2.5 Green Synthesis of Silver Nanoparticles

Kharissova et al., (2013) suggested that greener routes synthesis of nanoparticles of
zerovalent metals, metal oxides, and salts with an emphasis on recent developments. Products
from nature or those derived from natural products, such as extracts of various plants or parts
of plants, tea, coffee, banana, simple amino acids, as well as wine, table sugar and glucose,
have been used as reductants and as capping agents during synthesis. Polyphenols found in
plant material often play a key role in these processes. The techniques involved are simple,
environmentally friendly, and generally one-pot processes. Tea extracts with high polyphenol
content act as both chelating/reducing and capping agents for nanoparticles.

Mittal et al., (2013) stated that biomolecules present in plant extracts can be used to
reduce metal ions to nanoparticles in a single-step green synthesis process. The reducing
agents involved include the various water soluble plant metabolites (e.g. alkaloids, phenolic
compounds, terpenoids) and co-enzymes. Silver (Ag) and gold (Au) nanoparticles have been
the particular focus of plant-based syntheses. Extracts of a diverse range of plant species have
been successfully used in making nanoparticles. In addition to plant extracts, live plants can
be used for the synthesis.

Dubey et al., (2013) furnished a simple protocol for preparation of various plant
leaves extract and their application to green synthesis of the metallic nanoparticles. Five plant
leaves extract showed mild reduction and stabilization ability for silver and gold
nanoparticles (AgNPs and AuNPs) at room temperature. The particle size range varied from
25 to 42nm and 21 to 47nm for AgNPs and AuNPs, respectively. Plant leaves extract-
mediated nanoparticles were characterized to confirm the shape, size, crystallinity, and
content using different spectroscopic investigations. Differences in stability of nanoparticles
at different pH were also measured by zeta potential.

Silver nanoparticles were quickly synthesized from aqueous silver nitrate through a
simple method using leaf extract of a plant-Cynodon dactylon which served as reducing
agent, while sunlight acted as a catalyst. The formation of AgNPs was indicated by gradual

Antitumour Potentials of Silver Nanoparticles from Elaeagnus indica Servett.
change in colour and pH and confirmed by ultraviolet-visible spectroscopy. The Ag-NPs showed a surface plasmon resonance at 451nm. Based on the decrease in pH, a possible mechanism of the synthesis of Ag-NPs involving hydroxyl (OH(-)) ions of polyphenols of the leaf extract is postulated. Ag-NPs having and crystal lattices were confirmed by X-ray diffraction. Scanning electron microscopy revealed the spherical nature of the Ag-NPs, while transmission electron microscopy showed that the nanoparticles were polydispersed with a size range of 8-10nm. The synthesized Ag-NPs also demonstrated their antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella typhimurium*. (Sahu et al., 2012)

Park *et al.*, (2012) reported a green synthesis of silver nanoparticles that uses extracts from the aerial part of *Artemisia capillaris*. Both water and 70% ethanol extracts successfully generated silver nanoparticles. The formation of silver nanoparticles was confirmed by surface plasmon resonance bands, Fourier transform-infrared spectra, high resolution-transmission electron and atomic force microscopic images. Various shapes of silver nanoparticles were generated with an average diameter of 29.71nm with water extract and 29.62nm with 70% ethanol extract. An improvement in antibacterial activity was observed against total of twenty different strains of Gram-negative and Gram-positive bacteria. The results of this study suggested that plant extracts have the potential to be used as powerful reducing agents for the production of biocompatible silver nanoparticles possessing enhanced antibacterial activities.

Aqueous extract of fresh leaves of *Prosopis juliflora* was used for the synthesis of silver (Ag) nanoparticles. UV-Vis spectroscopy studies were carried out to asses silver nanoparticles formation within 5m, scanning electron microscopic was used to characterize shape of the Ag nanoparticles, X-ray diffraction analysis confirms the nanoparticles as crystalline silver and face-centered cubic type and Fourier transform infra-red assessed that shows biomolecule compounds which are responsible for reduction and capping material of silver nanoparticles. The anti microbial activity of silver nanoparticle was performed using sewage. The approach of plant-mediated synthesis appears to be cost efficient, eco-friendly and easy methods. (Raja *et al.*, 2012)

The use of plants in the green synthesis of nanoparticles emerges as a cost effective and eco-friendly approach. The green biosynthesis of silver nanoparticles using *Callicarpa maingayi* stem bark extract has been reported here. Characterizations of nanoparticles were
done using different methods, which include; ultraviolet-visible spectroscopy (UV-Vis), powder X-ray diffraction (XRD), transmission electron microscopy (TEM), scanning electron microscopy (SEM), energy dispersive X-ray fluorescence (EDXF) spectrometry, zeta potential measurements and Fourier transform infrared (FT-IR) spectroscopy. UV-visible spectrum of the aqueous medium containing silver nanoparticles showed absorption peak at around 456nm. The TEM study showed that mean diameter and standard deviation for the formation of silver nanoparticles were 12.40 ± 3.27nm. The XRD study showed that the particles are crystalline in nature, with a face centered cubic (fcc) structure. The most needed outcome of research will be the development of value added products from *Callicarpa maingayi* for biomedical and nanotechnology based industries. (Shameli *et al*., 2012)

The recent study dealt with the synthesis, characterization of silver nanoparticles using *Iresine herbstii* and evaluation of their antibacterial, antioxidant and cytotoxic activity. The reaction mixture turned to brownish gray color after 7 days of incubation and exhibits an absorbance peak around 460nm characteristic of Ag nanoparticle. Scanning electron microscopy (SEM) and EDX analysis showed silver nanoparticles were pure and polydispersed and the size were ranging from 44 to 64nm. X-ray diffraction (XRD) studies revealed that most of the nanoparticles were cubic and face centered cubic in shape. Fourier transform infrared spectroscopy (FTIR) showed nanoparticles were capped with plant compounds. Biosynthesized silver nanoparticles showed potent antibacterial activity against human pathogenic bacteria. Phytosynthesized nanoparticles exhibited strong antioxidant activity as well as cytotoxicity against HeLa cervical cell lines. The approach of green synthesis seems to be cost efficient, eco-friendly and easy alternative to conventional methods of silver nanoparticles synthesis. The powerful bioactivity demonstrated by the synthesized silver nanoparticles leads towards the clinical use as antibacterial, antioxidant as well as cytotoxic agent. (Dipankar *et al*., 2012)

The plant, *Tribulus terrestris* L. fruit bodies are used in this study, where the dried fruit body extract was mixed with silver nitrate in order to synthesis of silver nanoparticles. The active phytochemicals present in the plant were responsible for the quick reduction of silver ion (Ag⁺) to metallic silver nanoparticles (Ag⁰). The reduced silver nanoparticles were characterized by Transmission Electron Microscope (TEM), Atomic Force Microscope (AFM), XRD, FTIR, UV-vis spectroscopy. The spherical shaped silver nanoparticles were observed and it was found to be 16-28nm range of sizes. The diffraction pattern also
confirmed that the higher percentage of silver with fine particles size. The antibacterial property of synthesized nanoparticles was observed by Kirby-Bauer method with clinically isolated multi-drug resistant bacteria such as *Streptococcus pyogens*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus*. The plant materials mediated synthesis of silver nanoparticles have comparatively rapid and less expensive and wide application to antibacterial therapy in modern medicine. (Gopinath *et al.*, 2012)

Leaf extract of *Morinda citrifolia* L. was assessed for the synthesis of silver nanoscale particles under different temperature and reaction time. Synthesized nanoscale (MCAgNPs) particles were confirmed by analysing the excitation of surface plasmon resonance (SPR) using UV-visible spectrophotometer at 420nm. Further SEM, HRTEM analysis confirmed the range of particle size between 10 and 60nm and SAED pattern authorizes the face centered cubic (fcc) crystalline nature of the MCAgNPs. Fourier transform infrared spectrum (FTIR) of synthesized MCAgNPs confirms the presence of high amount of phenolic compounds in the plant extract which may possibly influence the reduction process and stabilization of nanoparticles. Further, inhibitory activity of MCAgNPs and plant extract were tested against human pathogens like *Eschericia coli*, *Pseudomonas aeroginosa*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Bacillus cereus* and *Enterococci* sp. The results indicated that the MCAgNPs showed moderate inhibitory actions against human pathogens than crude plant extract, demonstrating its antimicrobial value against pathogenic diseases. (Sathishkumar *et al.*, 2012)

The shape-directing role of cetyltrimethylammonium bromide, CTAB, is reported to the green synthesis of Ag-nanoparticles for the first time using Neem (*Azadirachta indica*) leaf extract. UV-vis spectroscopy, transmission electron microscopy (TEM), and selected area electron diffraction (SAED) patterns were used to monitor the growth kinetics, morphology and crystalline nature of Ag-nanoparticles, respectively. It was observed that the growths of Ag-nanoparticles are stopped within 40m of reaction time. The Ag-nanoparticles are polydispersed spherical and exhibiting an interesting triangle, flat, plate-like hexagonal and some irregular morphology in presence of different. Hexagonal particles aggregated in a systematic manor, leads to produce a fine tiles-like arrangement of Ag-nanoparticles with dimensions between 10 and 37nm. The nature of reaction-time curves to the reduction of
Ag(+) ions by Neem leaf extract are much different than those observed by us in our earlier studies using different bio-reductants. (Khan et al., 2012)

Green synthesis of nanoparticles is one of the crucial requirements in today's climate change scenario all over the world. In view of this, leaf extract (LE) of Bauhinia variegata L. possessing strong antidiabetic and antibacterial properties has been used to synthesise silver nanoparticles (SNP) in a controlled manner. The synthesised SNPs were found stable in double distilled water, BSA and phosphate buffer (pH 7.4). On the contrary, incubation of SNP with NaCl induced aggregation. This suggests the safe use of SNP for various in vivo applications. (Kumar and Yadav, 2012)

A green rapid biogenic synthesis of silver nanoparticles (Ag NPs) using Terminalia chebula (T. chebula) aqueous extract was demonstrated. The formation of silver nanoparticles was confirmed by Surface Plasmon Resonance (SPR) at 452nm using UV-visible spectrophotometer. The reduction of silver ions to silver nanoparticles by T. chebula extract was completed within 20m which was evidenced potentiometrically. Synthesised nanoparticles were characterised using UV-vis spectroscopy, Fourier transformed infrared spectroscopy (FT-IR), powder X-ray diffraction (XRD), transmission electron microscopy (TEM) and atomic force microscopy (AFM). The hydrolysable tannins such as di/tri-galloyl-glucose present in the extract were hydrolyzed to gallic acid and glucose that served as reductant while oxidised polyphenols acted as stabilizers. In addition, it showed good antimicrobial activity towards both Gram-positive bacteria (S. aureus ATCC 25923) and Gram-negative bacteria (E. coli ATCC 25922). Industrially it may be a smart option for the preparation of silver nanoparticles. (Mohan Kumar et al., 2012)

A novel biosynthesis route for silver nanoparticles (Ag-NPs) was attempted in this present investigation using aqueous extracts of Trachyspermum ammi and Papaver somniferum. The main constituents in T. ammi are thymol, p-cymene and γ-terpinene, while P. somniferum consists of morphine and codeine. The essential oil in T. ammi was found to be a good reducing agent than the alkaloids present in P. somniferum for the formation of biocompatible Ag-NPs. The effectiveness of both the extracts was investigated by using same dosage of extract in the synthesis of silver nanoparticle. The results showed that for the same dosage of extracts the T. ammi synthesized various size triangular shaped nanoparticles measuring from 87nm, to a fewer nanoparticles having a size of 998nm diagonally. P.
somniferum resulted in almost spherical shaped particle ranging in size between 3.2 and 7.6μm diagonally. (Vijayaraghavan et al., 2012)

Silver nanoparticles were successfully synthesized from aqueous AgNO₃ through a simple green route using the leaf extract of Coccinia grandis as a reducing as well as capping agent. The results obtained from UV-vis spectrum, X-ray diffraction (XRD), scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS) and Fourier-transform infra red spectroscopy (FTIR) and high-resolution transmission electron microscopy (HRTEM) revealed that the biosynthesis of silver nanoparticles were in the size range of 20-30nm and is crystallized in face centered cubic symmetry. Further, the thermal stability of nanoparticles was studied using thermo gravimetric analyzer (TGA) and differential scanning calorimeter (DSC). Photocatalytic property of the Ag nanoparticles was investigated by degradation of Coomassie Brilliant Blue G-250 under UV light. The results showed that Ag nanoparticles have suitable activity for the degradation of Coomassie Brilliant Blue G-250 (Arunachalam et al., 2012).

Development of reliable and eco-friendly process for the synthesis of metallic nanoparticles is an important step in the field of application of nanotechnology. Kumar et al., have developed modern method by using agriculture waste to synthesize silver nanoparticles by employing an aqueous peel extract of Annona squamosa in AgNO₃. Controlled growth of silver nanoparticles was formed in 4h at room temperature (25°C) and 60°C. AgNPs were irregular spherical in shape and the average particle size was about 35±5nm and it is consistent with particle size obtained by XRD Scherer equation. (Kumar et al., 2012)

Development of an environmentally benign process for the synthesis of silver nanomaterials is an important aspect of current nanotechnology research. Among the 600 species of the genus Dioscorea, Dioscorea bulbifera has profound therapeutic applications due to its unique phytochemistry. Ghosh et al., report on the rapid synthesis of silver nanoparticles by reduction of aqueous Ag(+) ions using D. bulbifera tuber extract. This was the first report on the synthesis of silver nanoparticles using D. bulbifera tuber extract followed by an estimation of its synergistic potential for enhancement of the antibacterial activity of broad spectrum antimicrobial agents. (Ghosh et al., 2012)

A simple, green method was described for the synthesis of Gold (Au) and Silver (Ag) nanoparticles (NPs) from the stem extract of Breynia rhamnoides. Unlike other biological methods for NP synthesis, the uniqueness of our method lies in its fast synthesis rates (~7m

Antitumour Potentials of Silver Nanoparticles from Elaeagnus indica Servett.
for AuNPs) and the ability to tune the nanoparticle size (and subsequently their catalytic activity) via the extract concentration used in the experiment. The phenolic glycosides and reducing sugars present in the extract were largely responsible for the rapid reduction rates of Au(3+) ions to AuNPs. Efficient reduction of 4-nitrophenol (4-NP) to 4-aminophenol (4-AP) in the presence of AuNPs (or AgNPs) and NaBH₄ was observed and was found to depend upon the nanoparticle size or the stem extract concentration used for synthesis. (Gangula et al., 2011)

Vidhu et al., (2011) reported the green synthesis of silver nanoparticles using aqueous seed extract of Macrotyloma uniflorum. The as prepared samples are characterized using XRD, TEM, UV-Visible and FTIR techniques. The formation of silver nanoparticles was evidenced by the appearance of signatory brown colour of the solution and UV-vis spectra. The XRD analysis showed that the silver nanoparticles were of face centered cubic structure. Well-dispersed silver nanoparticles with anisotropic morphology had size ~12nm were seen in TEM images. FTIR spectrum indicated the presence of different functional groups in capping the nanoparticles.

Parashar et al., (2011) described a novel green synthesis of silver nanoparticles using Guava (Psidium guajava) leaf extract. Fourier transform infrared (FTIR) spectroscopic analysis of the used extract and as-synthesized silver nanoparticles suggested the possible reduction of Ag(+) by the water-soluble ingredients of the guava leaf like tannins, eugenol and flavonoids. The possible reaction mechanism for the reduction of Ag(+) has been proposed and discussed. The time-dependent electron micrographs and the simulation studies indicated that a physical interaction between the silver nanoparticles and the bacterial cell membrane may be responsible for this effect. Based on the findings, it seemed very reasonable to believe that this greener way of synthesizing silver nanoparticles was not just an environmentally viable technique but it also opened up scope to improve their antibacterial properties.

Philip (2009) reported on extracellular synthesis method for the preparation of Au, Ag and Au-Ag nanoparticles in water, using the extract of Volvariella volvacea, as reducing and protecting agents. Gold nanoparticles of different sizes (20-150nm) and shaped from triangular nanoprisms to nearly spherical and hexagonal were obtained by this novel method. The size and shape of gold nanoparticles were also found to depend on temperature of the extract. The silver nanoparticles were spherical with size approximately 15nm. There was
increased productivity of nanoparticles as shown by sharp and intense surface plasmon resonance bands for the nanoparticles prepared using an excess of the extract. The Au-Ag nanoparticles prepared by co-reduction has only one plasmon band due to alloying of the constituents. All the synthesized nanoparticles were found to be photoluminescent and were highly crystalline as shown by SAED and XRD patterns with fcc phase oriented along the plane. FTIR measurements were carried out to identify the possible biomolecules responsible for capping and efficient stabilization of the nanoparticles. It was found that Au nanoparticles were bound to proteins through free amino groups and silver nanoparticles through the carboxylate group of the amino acid residues. The position and intensity of the emission band was found to depend on composition of the nanoparticles indicating the possible use in therapeutic applications.

Based on a green chemistry mechanism, small silver clusters were obtained by using biosynthesis with alfalfa (*medicago sativa*), controlling the size of the nanoparticles base don different pH conditions. The analysis of the samples was made with help of transmission electron microscopy methods, mainly with high angle annular dark field and high resolution transmission electron microscopy. The optimal conditions were identified when the sample was obtained at pH10, which allowed obtaining an average size of 4.09nm and a standard deviation of 1.59, mainly based on cubic like structures. (Tavera-Davila *et al.*, 2009)

A green, low-cost and reproducible Eclipta leaves negotiated synthesis of silver nanoparticles is reported. X-ray and transmission electron microscopy analyses are performed to ascertain the formation of Ag nanoparticles. Nanoparticles almost spherical in shape having a size of 2-6nm are found. UV-visible study revealed the surface plasmon resonance at 419nm. The lattice strain is estimated to be 0.0045 using Williamson-Hall approach. The use of Eclipta for the synthesis of silver nanoparticles offers the benefit of ecofriendliness and amenability for large scale production through scaling up. (Jha *et al.*, 2009)

Mukherjee *et al.*, (2008) synthesized silver nanoparticles at first time from a transparent solution of the cell-free filtrate of *Trichoderma asperellum* containing 1mM AgNO$\text{\textsubscript{3}}$ which turns progressively dark brown within 5d of incubation at 25 °C. An intense surface plasmon resonance band at $\sim$410nm in the UV-vis spectrum clearly reveals the formation of silver nanoparticles. The size of the silver particles using TEM and XRD studies is found to be in the range 13-18nm. These nanoparticles are found to be highly stable and even after prolonged storage for over 6 months they do not show significant aggregation. A
plausible mechanism behind the formation of silver nanoparticles and their stabilization via capping has been investigated using FTIR and surface-enhanced resonance Raman spectroscopy.

2.6 Green synthesized Silver Nanoparticles for Cancer

*Albizia adianthifolia* (AA) is a plant of the Fabaceae family that is rich in saponins. The biological properties of a novel AgNP, synthesized from an aqueous leaf extract of AA (AAAgNP), were investigated on A549 lung cells. Cell viability was determined by the MTT assay. The result of this study suggests that AAAgNP induces cell death in the A549 lung cells via the mt mediated intrinsic apoptotic program. (Govender *et al.*, 2013)

A recent investigation of AgNPs presented the green synthesis of AgNPs using leaf extract of *Podophyllum hexandrum* Royle and optimized with various parameters such as pH, temperature, reaction time, volume of extract and metal ion concentration for synthesis of AgNPs. TEM, XRD and FTIR were adopted for characterization. The synthesized nanoparticles were found to be spherical shaped with average size of 14nm. Effects of AgNPs were analyzed against human cervical carcinoma cells by MTT Assay, quantification of ROS, RT-PCR and western blotting techniques. The overall result indicated that AgNPs can selectively inhibit the cellular mechanism of HeLa by DNA damage and caspase mediated cell death. This biological procedure for synthesis of AgNPs and selective inhibition of cancerous cells gives an alternative avenue to treat human cancer effectively. (Jeyaraj *et al.*, 2013)

The capability of crude ethanolic extracts of certain medicinal plants like *Phytolacca decandra*, *Gelsemium sempervirens*, *Hydrastis canadensis* and *Thuja occidentalis* used as homeopathic mother tinctures in precipitating silver nanoparticles from aqueous solution of silver nitrate has been explored. Nanoparticles thus precipitated were characterized by spectroscopic, dynamic light scattering, X-ray diffraction, atomic force and transmission electron microscopic analyses. Silver nanoparticles showed differences in their level of anticancer and anti-bacterial potentials. The nanoparticles of different origin interacted differently with CT-DNA, showing differences in their binding capacities. Particle size differences of the nanoparticles could be attributed for causing differences in their cellular entry and biological action. (Das *et al.*, 2012)
The earlier was aimed to synthesize silver nanoparticles by using aqueous extract of *Coleus amboinicus* lour. For the synthesis of silver nanoparticles, 50ml extract filtrate was mixed with equal volume of 1mM silver nitrate [AgNO₃ (1mM)] and agitated at room temperature in dark. The synthesis of silver nanoparticles was investigated by UV-Vis spectroscopy, X-ray diffraction, and scanning electron microscopy (SEM) with Energy dispersive X-ray (EDX). Results indicated the synthesis of silver nanoparticles in the reaction mixture. Obtained silver nanoparticle showed the antioxidant activity and cytotoxicity against the Ehrlich’s ascite carcinoma (EAC) cell line. (Vadivel and Suja, 2012)

Cost effective and eco-friendly technique for green synthesis of silver nanoparticles from 1mM AgNO(3) solution using the extract of *Piper longum* leaf as reducing as well as capping agent was described by Jacob et al.,. Nanoparticles were characterized using UV-Vis absorption spectroscopy, FTIR, and SEM. SEM analysis showed the spherical nanoparticles with 17.6-41nm in size. These biologically synthesized nanoparticles were also exhibiting excellent cytotoxic effect on HEp-2 cell lines. (Jacob et al., 2012)

A recent study also demonstrated the efficacy of biologically synthesized silver nanoparticles (AgNPs) as an antitumor agent using Dalton's lymphoma ascites (DLA) cell lines *in vitro* and *in vivo*. Acute toxicity, ie, convulsions, hyperactivity and chronic toxicity such as increased body weight and abnormal hematologic parameters did not occur. AgNPs significantly increased the survival time in the tumor mouse model by about 50% in comparison with tumor controls. AgNPs also decreased the volume of ascitic fluid in tumor-bearing mice by 65%, thereby returning body weight to normal. Elevated white blood cell and platelet counts in ascitic fluid from the tumor-bearing mice were brought to near-normal range. Histopathologic analysis of ascitic fluid showed a reduction in DLA cell count in tumor-bearing mice treated with AgNPs. These findings confirm the antitumor properties of AgNPs, and suggested that they may be a cost-effective alternative in the treatment of cancer and angiogenesis-related disorders. (Sriram et al., 2010)

Safaepour et al., (2009) have been investigated the synthesis of silver nanoparticles using geraniol. They reported that they successfully synthesized uniformly dispersed silver nanoparticles with a uniform size and shape in the range of 1 to 10nm with an average size of 6nm. Also the cytotoxicity of the prepared silver nanoparticles was investigated using a cancer cell line (Fibrosarcoma-Wehi 164). The cytotoxicity analysis of the sample shows a direct dose-response relationship; cytotoxicity increased at higher concentrations. At
concentration of 1µg/ml, silver nanoparticles was able to inhibit the cell line's growth by less than 30%. Conversely, the presence of 5µg/ml of silver nanoparticles significantly inhibited the cell line's growth (>60%). The concentration necessary to produce 50% cell death was 2.6µg/ml for this silver nanoparticles prepared with geraniol.

2.7 Ehrlich Ascites Carcinoma for Cancer Study

Kumar et al., (2012) investigated the antitumour and antioxidant activities of the ethanol extract of Scutia myrtina (EESM) against Ehrlich's ascites carcinoma (EAC) in mice. Twenty-four hours after tumour inoculation, EESM was administered at doses 100, 200 and 300 mg kg\(^{-1}\) bodyweight/mice/day for 21 days. EESM caused a significant (p < 0.01) decrease in ascites volume, packed cell volume and viable cell count, and also prolonged the life span of EAC tumour-bearing mice. Haematological profiles reverted to near-normal levels in extract-treated mice (p < 0.01). EESM also produced protective effects by significantly decreasing the activity of serum enzymes and bilirubin and increasing the protein and uric acid levels (p < 0.05). EESM significantly (p < 0.05) decreased the levels of lipid peroxidation, while it significantly (p < 0.05) increased the levels of enzymatic and non-enzymatic antioxidants.

Habib et al., (2012) explored the anticancer activity of di-(2-ethylhexyl) phthalate (DEHP) isolated from Calotropis gigantea flower against Ehrlich ascites carcinoma cells (EAC) in Swiss albino mice. The activity of DEHP was evaluated at doses of 10, 20 and 40mg kg\(^{-1}\) body mass applied intraperitoneally. DEHP showed a significant decrease in viable cell count (p < 0.05), mass gain (due to tumour burden) and elevated the life span of EAC cell bearing mice. Altered hematological profiles such as RBC, hemoglobin, WBC and differential count were reverted to normal levels in DEHP-treated mice. DEHP also brought back altered biochemical parameters (glucose, cholesterol, triglycerides, blood urea, SALP and SGOT) to normal level. Results of this study indicated that DEHP showed potent dose dependent antitumour activity against EAC in vivo.

The effect of oral supplementation of α-lipoic acid (LA) on growth of Ehrlich ascites carcinoma cells (EACs) and hepatic antioxidant state in mice was investigated and it was concluded that LA acted as a potential therapeutic complement in the treatment or prevention of different pathologies that may be related to an imbalance of the cellular oxidoreductive status associated with malignancy. (Al Abdan et al., 2012)
Sreelatha et al., (2012) investigated that the antioxidant capacity and the possible protective effects of *Amaranthus paniculatus* leaves on the antioxidant defense system in Ehrlich's ascites carcinoma (EAC) -treated mice. Oral administration of the leaf extract at different doses caused a significant decrease in tumor volume, viable cell count and tumor weight and elevated the life span of EAC bearing mice. It also showed an improved antioxidant potential as evidenced by a significant increase in the cellular antioxidant defense system such as catalase, superoxide dismutase and reduced glutathione and also significantly reduced the levels of TBARS. The levels of RBC, hemoglobin and lymphocyte count were altered in EAC bearing mice and were reverted back to near normal levels after the treatment with the leaf extracts.

Kar et al., (2012) stated that *Mimusops elengi* (MEME) showed significant (p < 0.001) decrease in tumor volume, packed cell volume, and viable cell count, and increased the life span of EAC bearing mice. Hematological, biochemical profile, and in vivo antioxidant parameters were significantly restored toward normal levels in MEME-treated mice as compared to EAC control. MEME also showed direct cytotoxicity on EAC cell line in a dose-dependent manner. The results of recent study showed that *Anthocephalus cadamba* (MEAC) has direct cytotoxicity on EAC cell line in a dose dependant manner. MEAC exhibited significant (P<0.01) decrease in the tumor volume, viable cell count, tumor weight and elevated the life span of EAC tumor bearing mice. The hematological profile, biochemical estimations and tissue antioxidant assay were reverted to normal level in MEAC treated mice. (Dolai et al., 2012)

Sundaram et al., (2012) evaluated antitumor activity of *Gracilaria edulis* in Swiss albino mice with Ehrlich ascites carcinoma (EAC). Tumors were induced in mice by intraperitoneal injection of EAC cells. Ethanol extract of *Gracilaria edulis* (EEGE) was administered to the experimental animals in different doses after 24h of tumor inoculation. The antitumor effect of the EEGE was evaluated by assessing in vitro cytotoxicity, survival time, biochemical parameters and hepatic enzyme levels. EEGE increased the life span of EAC-bearing mice compared with that of the model control mice (P<0.05 or P<0.01). EEGE treatment also converted the changes of biochemical parameters and hepatic enzyme levels in the EAC-bearing mice (P<0.05 or P<0.01). EEGE induced inhibition of tumor formation in EAC-bearing mice compared with that of the model control group (P<0.05 or P<0.01).
Bhattacharya and Haldar (2011) assessed the influence of treatment of hydroalcoholic extract of *Trichosanthes dioica* root (TDA) on Ehrlich ascites carcinoma (EAC) in Swiss albino mice with effects on antioxidant systems. Twenty-four hours after intraperitoneal inoculation of tumor (EAC) cells in mice, TDA was administered at 25 and 50mg/kg for 8 consecutive days. On the 9(th) day, half of the mice were sacrificed for estimation of tumor proliferation, hematological, and hepatic antioxidative parameters. The rest were kept for assessment of survival parameters. TDA exhibited dose dependent and significant increase in tumor weight, tumor volume, packed cell volume and viable cells and reduced non-viable cells and life span of EAC bearing animals. Hematological parameters were significantly worsened in TDA-treated mice. TDA treatment significantly aggravated the hepatic antioxidative parameters. This study demonstrated that *T. dioica* root possessed tumor promoting activity in EAC bearing albino mice, plausibly mediated by attenuation of endogenous antioxidant systems.

Chromatographic investigation of fruits obtained from Citrullus colocynthis, growing in Saudi Arabia, led to isolation of two compounds; Cucurbitacin E glucoside (Cu E, 1), and Cucurbitacin I glucoside (Cu I, 2). The chemical structures of 1 and 2, were elucidated by spectroscopic analyses include; 1D ((1)H and (13)C) and 2D (COSY, HMQC and HMBC) NMR and ESI-MS spectroscopy. The *in vitro* cytotoxic activity against hepatoma cell line (HepG2) and mice-bearing tumor of Ehrlich's ascites carcinoma (EAC) of the compounds were estimated. Both compounds had potent inhibitory activity on HepG2 with IC(50) 3.5 and 2.8nmol/mL, respectively. In addition to these activities, the *in vivo* study employing EAC, showed the capability of both compounds to prolong the survival time, life span and normalize the biochemical parameters of the infected mice with EAC. (Ayyad *et al.*, 2012)

Hossain *et al.*, (2012) evaluated the *in vitro* and *in vivo* antitumor activity of the methanol extract of *Dregea volubilis* leaves (MEDV) and elucidated its possible mechanism of action. *In vitro* antitumor activity of MEDV was evaluated against Ehrlich ascites carcinoma (EAC) cell-line. *In vivo* antitumor and antioxidant activity of MEDV at three dose levels (50, 100, and 200 mg/kg) were determined against EAC tumor-bearing mice. After 24 h of EAC inoculation, the extract was administered for 9 consecutive days. After the administration of the last dose on the 9th day followed by 18 h fasting, mice from all groups were sacrificed to determine antitumor activity and hematological profiles along with liver related biochemical parameters like lipid peroxidation, antioxidant enzymatic activity, etc.
For \textit{in vitro} antitumor activity, IC(50) value of MEDV for EAC tumor cells was 85.51 ± 4.07\,\mu g/ml. The MEDV showed a decrease in tumor volume, packed cell volume and viable cell count and an increase in the non-viable cell count of the EAC tumor-bearing mice (p < 0.001). Hematological profile reverted near to normal level in extract treated mice. MEDV decreased the hepatic lipid peroxidation level and enhanced superoxide dismutase and catalase level in tumor-bearing mice (p < 0.001). They concluded that MEDV exhibited \textit{in vitro} and \textit{in vivo} antitumor activity in EAC tumor-bearing mice mediated through augmenting antioxidant defense system.

Perveen \textit{et al.}, (2012) explored the cytotoxic activity of ethanol extract of \textit{Alpinia calcarata} Rosc (EEAC) rhizome against Ehrlich ascites carcinoma (EAC) tumor bearing Swiss Albino mice. It was found that EEAC at dose 8mg/kg/day (i.p.) significantly decreased tumor weight (62.0\%; P <0.01), increased life span (70.25\%; P <0.01) and reduced tumor cell growth rate (85.7\%; P <0.01) in comparison to those of EAC bearing mice. The plant extract also improved the depleted haematological parameters like RBC, WBC, Hb\%, differential counts (e.g. lymphocytes, neutrophils, monocytes etc) of EAC bearing mice towards normal. The host toxic effects were not very high and recovered gradually towards normal within a few days after treatment. EEAC exhibits potent \textit{in vivo} cytotoxic activity against EAC tumor bearing Swiss Albino mice. So, the plant can be considered as a probable new source of antitumor agents.

Biswa \textit{et al.}, (2012) evaluated the methanol extract of \textit{Terminalia arjuna} Roxb. (Combretaceae) leaf (META) for antitumour activity against Ehrlich ascites carcinoma (EAC) in Swiss albino mice. Twenty-four hours after intraperitonial inoculation of tumour (EAC) cells in mice, META was administered at 100 and 200 mg kg(-1) body weights for 9 consecutive days. On day 10, half of the mice were sacrificed and the rest kept alive for an assessment of the increase in life span. The antitumour effect of META was assessed by evaluating tumour volume, tumour weight, viable and non-viable tumour cell counts, median survival time and increase in life span of EAC-bearing hosts. Haematological profiles were estimated. META showed a significant (p<0.001) decrease in tumour volume, tumour weight and viable cell count, and also increased the life span of EAC-bearing mice. Haematological profiles were significantly (p<0.001) restored to normal levels in META-treated mice compared to the EAC control. Therefore, from this study, it can be concluded that \textit{T. arjuna} leaf exhibited remarkable antitumour activity against EAC in Swiss mice.
Anticancer potential of seed extract of *Ziziphus mauritiana* *in vitro* against different cell lines (HL-60, Molt-4, HeLa, and normal cell line HGF) by MTT assay as well as *in vivo* against Ehrlich ascites carcinoma bearing Swiss albino mice was investigated. The extract was found to markedly inhibit the proliferation of HL-60 cells. Annexin and PI binding of treated HL-60 cells indicated apoptosis induction by extract in a dose-dependent manner. The cell cycle analysis revealed a prominent increase in sub Go population at concentration of 20 μg/ml and above. Agarose gel electrophoresis confirmed DNA fragmentation in HL-60 cells after 3h incubation with extract. The extract also exhibited potent anticancer potential *in vivo*. Treatment of Ehrlich ascites carcinoma bearing Swiss albino mice with varied doses (100-800 mg/kg b.wt.) of plant extract significantly reduced tumor volume and viable tumor cell count and improved haemoglobin content, RBC count, mean survival time, tumor inhibition, and percentage life span. The enhanced antioxidant status in extract-treated animals was evident from decline in levels of lipid peroxidation and increased levels of glutathione, catalase, and superoxide dismutase. (Mishra *et al*., 2011)

Sreelatha *et al*., (2011) explored the anticancer activity of the ethanol extract of *Sesbania grandiflora* against Ehrlich ascites carcinoma (EAC)-bearing Swiss albino mice. Anticancer activity of ethanol extract of *Sesbania grandiflora* (EESG) of both leaves and flowers were evaluated in Swiss albino mice against Ehrlich Ascites Carcinoma (EAC) cell line at the doses of 100 and 200 mg/kg body weight intraperitoneally. The extracts were administered for 14 consecutive days. Twenty-four hours of last dose and 18h of fasting, the mice were sacrificed and the anticancer effect of EESG was assessed by evaluating tumor volume, viable and nonviable tumor cell count, tumor weight, hematological parameters and biochemical parameters of EAC bearing host. The results of this study showed that *Sesbania grandiflora* extracts showed significant decrease in (p<0.01) tumor volume, viable cell count, tumor weight and elevated the life span of EAC bearing mice. Hematological profile such as RBC, hemoglobin and lymphocyte count reverted to normal level in EESG treated mice. The extracts significantly (p<0.05) decreased the levels of lipid peroxidation and significantly (p<0.05) increased the levels of GSH, SOD and CAT. From this results, it was observed that the ethanol extract of *Sesbania grandiflora* was effective in inhibiting the tumor growth in ascitic models and that is comparable to 5-Fluorouracil.

The antitumour activity of the hydroalcoholic extract of the leaf (PCL) and stem bark (PCB) of *Prosopis cineraria* (L.) in Swiss albino mice was evaluated against an Ehrlich
ascites carcinoma (EAC) tumour model. The activity was assessed using survival time, peritoneal cells, haematological studies, lipid peroxidation, antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione S-transferase, solid tumour mass and in vitro cytotoxicity. PCL and PCB were found to be potent and possessed significant cytotoxicity towards EAC tumour cells. (Robertson et al., 2011)

Kundusen et al., (2011) evaluated the methanol extract of Citrus maxima leaves for its antitumor activity against Ehrlich's Ascites Carcinoma cell in Swiss albino mice. Oral administration of the extract at the doses of 200 and 400 mg/kg significantly decreased tumor parameters such as tumor volume, viable tumor cell count and increased body weight, hematological parameters and life span in respect of the EAC control mice. Experimental design exhibits significant antitumor activity of the extract (MECM) in a dose dependant manner.

Luteoskyrin, a hydroxyanthraquinone has been proved to be a potent inhibitor against Ehrlich ascites tumor cells. The comparative antitumor activity and antioxidant status of MT81 and its structural analogue [Acetic acid-MT81 (Aa-MT81)] having polyhydroxyanthraquinone structure were assessed against Ehrlich ascites carcinoma (EAC) tumor in mice. The in vitro cytotoxicity was measured by the viability of EAC cells after direct treatment of the said compounds. In in vivo study, MT81 and its structural analogue were administered (i.p.) at the two different doses (5, 7mg MT81; 8.93, 11.48mg Aa-MT81/kg body weight) for 7 days after 24h of tumor inoculation. The activities were assessed using mean survival time (MST), increased life span (ILS), tumor volume, viable tumor cell count, peritoneal cell count, protein percentage and hematological parameters. Antioxidant status was determined by malondialdehyde (MDA) and reduced glutathione (GSH) content, and by the activity of superoxide dismutase (SOD) and catalase (CAT). MT81 and its structural analogues increased the mean survival time, normal peritoneal cell count. They decreased the tumor volume, viable tumor cell count, hemoglobin percentage and packed cell volume. Differential counts of WBC, total counts of RBC & WBC that altered by EAC inoculation, were restored in a dose-dependent manner. Increased MDA and decreased GSH content and reduced activity of SOD, and catalase in EAC bearing mice were returned towards normal after the treatment of MT81 and its structural analogue. Being less toxic than parent toxin MT81, the structural analogue showed more prominent antineoplastic
activities against EAC cells compared to MT81. At the same time, both compounds exhibit to some extent antioxidant potential for the EAC-bearing mice. (Choudhury et al., 2010)

Sansevieria roxburghiana Schult. & Schult. f. (Agavaceae) is a herbaceous perennial plant traditionally used for coughs, rheumatism; as an expectorant, febrifuge, purgative, and tonic. Haldar et al., (2010) evaluated the hydroalcoholic extract of S. roxburghiana rhizome (HASR) for antitumor activity against Ehrlich ascites carcinoma (EAC) in Swiss albino mice. Twenty-Four hours after intraperitoneal inoculation of tumor (EAC) cells in mice, HASR was administered at 50 and 100 mg/kg body weight for nine consecutive days. On day 10 half of the mice were sacrificed and rest were kept alive for assessment of increase in life-span. The antitumor effect of HASR was assessed by evaluating tumor volume, packed cell count, viable and non-viable tumor cell count, median survival time and increase in life-span of EAC bearing hosts. Hematological profiles and serum biochemical parameters were estimated. Further, antioxidant properties were assessed by estimating lipid peroxidation, reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT). They observed that HASR showed a significant (p < 0.001) decrease in tumor volume, packed cell volume and viable cell count and increased the life span of EAC bearing mice. Hematological and serum biochemical profiles were restored to normal levels in HASR treated mice as compared to EAC control. HASR treatment significantly (p <0.001) decreased lipid peroxidation and recovered GSH, SOD and CAT towards normal as compared to EAC control. The present study demonstrated that S. roxburghiana rhizome exhibited remarkable antitumor activity in Swiss mice that was plausibly attributable to its augmenting endogenous antioxidant mechanisms.

Chemoprotective effect of diphenylmethyl selenocyanate against cyclophosphamide (CP) induced cellular toxicity and antitumor efficacy was evaluated in mice bearing Ehrlich ascites carcinoma. Diphenylmethyl selenocyanate (3mg/kg.b.w.) was administered orally and CP was given intraperitoneally (25mg/kg.b.w). The effects were observed on the level of lipid peroxidation, antioxidant enzymes status, serum transaminase (ALT, AST) activity, hematological profile, transplantable murine tumor growth, apoptosis induction in tumor cells, and life span of tumor bearing hosts. The selenium compound restored the levels of antioxidant enzymes system, decreased the level of lipid peroxidation and serum transaminase activity. Hematological profile reverted to near normal level after selenium compound treatment. Treatment with the selenium compound also resulted in significant
tumor growth regression along with significant upregulation of apoptosis, increased in mean survival time and life span of tumor bearing host. Results clearly indicated that diphenylmethyl selenocyanate has the potential to reduce the cellular toxicity of CP at the same time improving its antitumor efficacy. (Chakraborty et al., 2009)

The methanol extract of *Careya arborea* bark (MECA) was tested for antioxidant and hepatoprotective activity in Ehrlich ascites carcinoma (EAC) tumor-bearing mice. Tumor control animals inoculated with EAC showed a significant alteration in the levels of antioxidant and hepatoprotective parameters. The extract treatment at 50, 100 and 200mg/kg body weight doses given orally caused a significant reversal of these biochemical changes towards the normal in serum, liver and kidney when compared to tumor control animals indicating the potent antioxidant and hepatoprotective nature of the standardized extract. (Senthilkumar et al., 2008)

Rajkaptor et al., (2007) evaluated the antitumor and cytotoxic activity of methanol extract of *Phyllanthus polyphyllus* (MPP) in mice and human cancer cell lines, the antitumor activity of MPP was evaluated against an Ehrlich ascites carcinoma (EAC) tumor model. The activity was assessed using survival time, hematological studies, lipid peroxidation (LPO), antioxidant enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), glutathione S-transferase (GST), solid tumor mass, and short-term *in vitro* cytotoxicity. The cytotoxic activity of MPP was evaluated using human breast cancer (MCF7), colon cancer (HT29), and liver cancer (HepG2) cell lines. Oral administration of MPP (200 and 300mg/kg) increased the survival time and significantly reduced the solid tumor volume in a dose-dependent manner. Hematological parameters, protein, and packed cellular volume (PCV), which were altered by tumor inoculation, were restored. MPP significantly decreased the levels of LPO, GPx, GST, and significantly increased the levels of SOD and CAT. In a cytotoxicity study against human cancer cell lines, MPP was found to have IC$_{50}$ values of 27, 42 and 38µg/ml on MCF-7, HT-29, and HepG2 cells respectively. MPP possessed significant antitumor and cytotoxic activity on EAC and human cancer cell lines.

The recent study was carried out to assess the effect of *Pterios volitans* venom (mixture of peptides) on Ehrlich's ascites carcinoma (EAC) and its influence on antioxidant status in the liver. Among six groups of albino mice, three were treated with sublethal doses of venom, along with the standard drug, 5-fluorouracil. In EAC-bearing mice, mean life span
and antioxidants were significantly decreased, whereas, body weight, tumor volume, viable tumor cell count, lipid peroxidation and expression of proliferating cell nuclear antigen were significantly increased. The findings were further confirmed by histopathological observations. (Sri Balasubashini et al., 2006)

Diospyrin, a bisnaphthoquinonoid plant product, showed inhibitory activity against murine tumour in vivo and human cancer cell lines in vitro. Hazra et al., (2005) had made the efforts to obtain synthetic derivatives of diospyrin with the objective of improved therapeutic effects. With the goal to reduce the toxicity towards normal cells and enhance the efficacy to tumour cells, diospyrin was encapsulated in liposomal vesicle and its antitumour potential was observed on the growth of Ehrlich ascites tumour in Swiss mice. It was found that the longevity of the tumour-bearing mice was significantly enhanced by treatment with liposomal diospyrin as compared with the free drug. Biochemical assay of liver function enzymes, viz. LDH, AP, GOT and GPT in blood serum of the tumour-bearing mice showed substantial alterations in the activity of these enzymes. These parameters were restored to near normal level when the drug treatment was given encapsulated in a liposome. Histopathological studies on the liver tissues indicated a near normal pathological status in the treated animals despite being challenged by tumour cells.

Bhosle et al., (2005) also observed that combined treatment of tumor with ellagic acid and radiation enhanced oxidative stress and cytotoxicity in tumor cells. Ellagic acid protected normal cells against radiation damage. Similarly, Gupta et al., (2004) has studied the antitumor effect and antioxidant role of Bauhinia racemosa. They evaluated the methanol extract of Bauhinia racemosa stem bark (50, 100, and 200mg/kg) against Ehrlich ascites carcinoma (EAC) tumor in mice. They concluded that the methanol extract of Bauhinia racemosa stem bark exhibited antitumor effect by modulating lipid peroxidation and augmenting antioxidant defense system in EAC bearing mice. Similarly,
Mukhopadhyay et al., (2003) noted that chronic ingestion (for 22-30 consecutive days) of caffeine (20mg/kg/day, p.o.) increased the activities of the hepatic enzymes- catalase (CAT) and superoxide dismutase (SOD) and decreased its lipid peroxidation (LP) in mice. Development of Ehrlich ascites carcinoma (EAC) cell decreased the activities of hepatic CAT and SOD and increased LP. But pretreatment of caffeine for 12 consecutive days and continuation of its treatment during the course of development of EAC cells restored the EAC cell-induced changes in liver CAT, SOD and LP to their corresponding control values. The results of this experiment confirmed the results of others previously published, suggested that caffeine is an antioxidant and might act as an anticarcinogen.