REVIEW OF LITERATURE

Since ancient times, plants are used as a source of medicine for health benefits. It has been estimated that about 80-85% of the world population relies upon conventional medicines for their use in health care needs. It is assumed that majority of long established therapies involve the use of plant extracts and their active principles (Ignacimuthu et al., 2006). Plants promote the host resistance to infections by reestablishing body homeostasis and conditioning the body tissues (Madhuri, 2008; Madhuri, 2009; Agarwal et al., 2001; Panday and Madhuri, 2006), as they produce a variety of active ingredients which are known as secondary compounds. Many secondary metabolites have been utilized by human beings for making medicines and also as preventing agents by the people of Homeopathy, Unani, Ayurvedic medicine producers and practitioners (Altundad and Ozturk, 2011). “Ayurveda” – known as the Indian system of holistic medicine uses mainly the plant derived drugs or formulations to treat different diseases including cancer. The phytochemical examination of plants have a long history of their use in folklore for the cure of cancer (Dhanamani et al., 2011). Along with many recent advances in cancer chemotherapy, natural products are the components of approximately 60 available cancer curing drugs (Gordaliza, 2007). There are four main classes of plant derived anticancer agents viz. vinca alkaloids, epidodophyllotoxin, taxanes and camptothecin derivatives which are used clinically in the United States (Newman and Cragg, 2000).

Medicinal plants are the best option for the treatment of cancer. The chemical mechanism of medicinal plants possesses the antioxidant properties which contribute to their anticancer potential. Bioactive constituents like flavones, isoflavones, flavonoids, anthocyanins, coumarins, lignans, catechins and isocatechins are the major classes which are responsible for the antioxidant action (Nema et al., 2013). The immense potential of plant based compounds is responsible for the treatment and prevention of cancer and is attributed to their benefits like safety, low cost and oral bioavailability. The present expensive conventional therapies for the treatment of cancer like chemotherapy and radiotherapy have a number of side effects such as myelosuppression, neurological, cardiac, pulmonary and renal toxicity which exert
serious harm to the life (Alonso Castro et al., 2011). Therefore, there is a need to develop treatment options that include more effective and less toxic anticancer drugs as compared to existing drugs. Medicinal plants represent an alternative to cancer treatment in many countries of the world and play an important role in the primary health care system. Among rural population, synthetic anticancer treatments are beyond the approach of common man because of the high cost (Gerson Cwilich et al., 2006; Tascilar et al., 2006). The cytotoxic screening of a number of plants has been done to correlate their anticancer activity and expand their scope for drug development (Akter et al., 2014). The potential benefits of plants has increased their use as drugs for the prevention and treatment of cancer across the world (10%-40%) specifically on the Asian continent which execute their therapeutic effect by inhibiting the cancer activating enzymes and hormones, stimulating DNA repair mechanism, promoting assembly of protective enzymes inducing antioxidant action as well as by enhancing immunity (Cassileth and Deng, 2004; Molassiotis et al., 2006).

There are many reports which indicate that phytochemicals such as carotenoids, tocopherols and polyphenols present in fruits and vegetables inhibit the process of carcinogenesis at different phases (Dragsted et al., 1993; Liu, 2003; Naczk and Shahidi, 2006). The phytochemicals especially, the polyphenols show high radical scavenging properties that reduce the risk of cancer as well as cardiovascular diseases (Ames et al., 1993; Alonso et al., 2006; Vita, 2005). The polyphenols are known to act as effective anticancer agents. The complex mechanisms of their action indicate that their cancer preventive properties are due to the combinations of various chemicals present in fruits and vegetables (Liu, 2003; Mertens Talcott and Percival, 2005; Kim et al., 2006).

There are many reports in literature which indicate that the highly reactive oxygen and nitrogen species damage the macromolecules which in turn cause the cell injuries. The damage to cells result into the development of several chronic diseases especially the cancer (Gutteridge and Halliwell, 2000; Halliwell, 1996; Valko et al., 2007). Antioxidants present in plants help to protect the human body from the damaging effect of free radicals and also prevent oxidative stress (Ferreira et al., 2009; Lopez et al., 2007). The plants play a very important role in health care as they are source of many pharmacologically active products (Lopez et al., 2007).
With this in view, an attempt has been made to review briefly the antioxidant and antiproliferative properties of different plants.

### 2.1. Antioxidative studies

Free radicals are consistently formed as a result of byproducts of aerobic metabolism in the living body (Davies, 2000). They are mainly reactive oxygen or nitrogen species which are present in *in vivo* are mainly superoxide (O$_2^-$), hydroxyl radical (OH$_2^-$), peroxyl radical (RO$_2^-$), nitric oxide (NO) and peroxynitrite (ONOO$^-$). All the above mentioned ROS have been associated with many chronic and degenerative diseases causing diabetes, cancer and overall aging (Diaz *et al.*, 1997; Droge, 2002; Finkel and Holbrook, 2000). The nutritional antioxidants present in diet are believed to be good external sources to neutralize the free radicals in the body (Kaur and Kapoor, 2001). A wide number of methods have been developed to assess the total antioxidant capacity (TAC) of different food samples. Kalaivani and Mathew, (2010) examined that the ethanol leaves extract of *Acacia nilotica* had strong antioxidant activity and was significant in comparison to all the controls used. Ethyl gallate is a known food additive and reported to have antiparasitic (Calderon *et al.*, 2006), anticancer (Yoshioka *et al.*, 2000; Saleem *et al.*, 2002), antimicrobial (Shibata *et al.*, 2005) and radical scavenging activities (Zheng *et al.*, 2009). In addition to biological effects of ethyl gallate, this study also showed antioxidant mechanism like the cytotoxic and hemolytic activities. The results provide important information to the food industry to the use of compound as an antioxidant and a health related medicine.

Pranuthi *et al.* (2014) carried out the qualitative analysis for *Ficus dalhousiae* Miq (Moraceae) which is used in the traditional and Unani medicine for the treatment of liver and skin diseases. It is scarcely found in India. In this methodology, the plant (whole aerial part) was dried and phytochemicals were extracted by the use of solvents of different polarities (hexane, chloroform, acetone, methanol, water). The results showed great variations in the phytochemical composition. The methanol extract was found to have more phyto constituents as others like hexane and chloroform showed the presence of fewer compounds. The acetone and water extract showed moderate range as *F. dalhousiae* has extensive range of chemical constituents and are useful for drug discovery as well as for the development of various new drug formulations.
The plants are used as a source of pharmacologically active products (Barros et al., 2011). Plants like Cytisus multiflorus, Filipendula ulmaria and Sambucus nigra have been commonly used as important medicinal plants in the Iberian Peninsula because of their various benefits to health. These plants were tested for their effect to scavenge the free radicals and also explored for the presence of different phytochemicals. *F. ulmaria* was found to be rich in phenolics, flavonoids, ascorbic acid and vitamin E viz. 228 mg GAE/g DW, 62 mg CE/g DW, 2700 µg/g DW and 497 µg/g DW respectively. The antioxidant activity was found in the order as: *F. ulmaria* > *S. nigra* > *C. multiflorus*. Also, Krishnaiah and co-workers (2009) studied six Malaysian medicinal plants (*Moringa oleifera, Imperata cylindrica, Hibiscus rosa-sinensis Azadirachta indica, Centella asiatica and Emblica officinalis*). Each plant belongs to different family and compared to have various phenolics. The results showed that plants contain phlobatannins, terpenoids, saponins, alkaloids, tannins, cardiac glycosides and flavonoids. The plants were analyzed for the presence or absence of tannins, saponins, flavonoids, terpenoids and alkaloids and it was seen that phlobatannins were present only in *Centella asiatica* and *Moringa oleifera* whereas absent in other plants. *A. indica, C. asiatica* and *I. cylindrica* showed the presence of cardiac glycosides and absent in *Emblica officinalis, Hibiscus rosa-sinensis* and *Moringa oleifera*. The ethanol extract (leaves and shoot) of the fresh *Eichhornia crassipes* was screened for the various phytochemicals. The results showed that the plant contain alkaloids, flavonoids, phenols, sterols, terpenoids, anthoquinones and protein (Lalitha and Jayanthi, 2012).

Lihu *et al.* (2005) studied the changes in phenolic compounds in fresh tea (shoots) grown in different seasons in Australia by using HPLC method. In this, epigallocatechin gallate (EGCG), epicatechin gallate (ECG) and epigallocatechin (EGC) along with total catechins (Cs), total catechin gallates (CGs), total flavanols (Fla) and total polyphenols (PPs) were estimated and compared for their presence during the commercial harvest seasons. The results indicated the highest levels of EGCG, ECG and CGs in the fresh tea shoots in the warm months and lower levels during cooler months. In contrast, the levels of EGC and total catechins were high and consistent in the cooler months and low in the warmer months. Likewise, Kumar *et al.* (2008) showed the presence of six polyphenols viz. rutin, quercetin, kaempferol, gallic acid,
quercitrin and myricetin in all the extracts of fresh flowers of *Rosa bourboniana* and *R. brunonii* and marc (left after industrial distillation of rose oil) of *Rosa damascena* by HPLC method.

Bernal *et al.* (2013) studied the variations in the content of phenolic compounds under the influence of UV-B changes of the environment. Firstly, the seasonal and altitudinal changes in the content of phenolic compounds in the leaves as well as cuticles of *Buxus sempervirens* L. were estimated and then related to the natural fluctuations in UV-B levels. Secondly, the specific phenolic compounds was also studied and used as the biomarkers of ambient UV-B levels. In this study, the plant samples were collected after every three months during one year from different sites i.e. at the lowest and the highest altitudes. In addition to this, UV-exclusion studies were also done to know whether the observed changes are due to the natural variations in UV-B radiations. A critical analysis of the results showed that the total phenolic content of leaves was found to be lower in June than other months which suggested no role of UV-B radiations on the leaf content. With respect to the elevational gradient, it was observed that the overall amount of phenolic acids and neolignan in entire leaves increased with altitude whereas the total amount of flavonoids in leaf cuticles decreased. However, in the absence of a significant effect of UV-exclusion treatment on the content of plant lead to the conclusion that the observed variations with the altitudinal gradient would respond to other factors rather than to UV-B.

Ambasta, (1994) had also reported that *Acacia nilotica* is a multipurpose tree (Fabaceae) used for the curing of various ailments. In Chhattisgarh, this tree is used for the treatment of cancer of mouth, bone and skin. In West Africa also the bark and gum is used against ear, eye, or testicles cancer and roots are used for tuberculosis, wood for smallpox and the leaves for the treatment of ulcers. Ali *et al.* (2012) found that *Acacia nilotica* have inspiring range of medicinal uses including antioxidant activities due to the presence of a wide range of secondary metabolites viz. alkaloids, volatile essential oils, phenols, terpenes, tannins, phenolic glycosides, resins, oleosins and steroids. It mainly consists of condensed tannins. The different parts such as seeds, roots, fruits, bark, flowers, gum, leaves and immature pods also exhibit different bioactivities.
Agrawal and coworkers, (2010) reported the free radical scavenging potential of methanol extract of *Acacia nilotica* (AN) and *Berberis chitria* (BC) in different *in vitro* techniques viz. ABTS, DPPH, nitric oxide, hydroxyl radical and hydrogen peroxide. The results indicated that both the extracts possess significant antioxidant properties. Among, *Acacia nilotica* and *Berberis chitria*, it was observed that the extracts of *A. nilotica* had higher potential to scavenge the free radicals than *B. chitria*. The total content of phenols in *Acacia nilotica* and *Berberis chitria* extract was observed to be 9.88 and 2.73 μg/ml respectively. The results of above study suggested that *Acacia nilotica* is rich in polyphenolic compounds therefore it can be good candidate to be used for the treatment of diseases that are caused by free radicals.

Rasool *et al.* (2013) reported the antioxidant and cytotoxic activities of extracts (roots) of *Acacia nilotica*. The antioxidant activity was calculated by using different methods viz. reducing power, DPPH radical scavenging and linoleic acid oxidation. In addition, TPC and TFC were also estimated. The results showed total phenolic content (1.47-6.61 GAE mg/g of dry matter), total flavonoid content (2.31-6.42 CE mg/g of dry matter), DPPH radical scavenging activity (IC<sub>50</sub> 10.53-70.23) and linoleic acid oxidation (33.8-86.61%). The antioxidant potential of extract/fractions was also evaluated by using corn oil as the oxidation substrate. The oxidative alterations were also evaluated by studying the conjugated dienes (CD), conjugated trienes (CT), p-anisidine, free fatty acid (FFA) and peroxide (PO) values. The results showed that the plant roots can be used as a potential source of antioxidant agents.

The leaves of *Coccinia grandis* are a potential source of natural antioxidants. The studies conducted by Umamaheshwari and Chatterjee, (2008) in different *in vitro* assays on the hydromethanolic extract of *Coccinia grandis* L. Voigt. (Cucurbitaceae) showed its good potential which was more than the known antioxidants. It was seen that all the fractions come out to be effective hydrogen donor, reducing power, free radical scavengers, metal chelators ability as well as inhibitors of β-carotene bleaching. The activity was found to be dose dependent. The free radical scavenging activities may be attributed to the presence of phenolics and flavonoids present in the fractions.
Re et al. (1999) reported a decolorization assay which was used for the screening of antioxidant activity. This method is applicable to flavonoids, hydroxycinnamates, carotenoids, lipophilic, hydrophilic and plasma antioxidants. The generation of pre-formed radical monocation of ABTS⁺ i.e. 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) is formed by oxidation of ABTS with potassium persulfate and reduced in the presence of hydrogen donating antioxidants. The influence of both the concentrations of antioxidant and duration of reaction on the inhibition of the radical cation absorption is taken into account when determining the antioxidant activity. ABTS assay has many advantages. Firstly, the chemistry involves the immediate generation of the ABTS radical monocation with no involvement of an intermediary radical. Secondly, it is a decolorization assay, so the radical cation is pre-formed prior to addition of antioxidant test systems, rather than the generation of the radical taking place continually in the presence of the antioxidant. Thirdly, it is appropriately used for both aqueous and lipophilic systems.

Osman et al. (2009) examined the antioxidant activity of plant extracts (fresh and dried) of Paederia foetida and Syzygium aqueu. The study was done by using β-carotene bleaching and ABTS radical cation assays. The antioxidant activity in terms of percentage for all the extract samples in both the assays was in the range of 58-80%. The results showed that the fresh samples of both the plants had higher antioxidant activity as compared to the dried samples. The results of the β-carotene bleaching assay were correlated with the results of ABTS. Kalaivani and Mathew, (2009) studied the free radical scavenging activity of the different extracts of Oroxylum indicum by applying assays viz. total antioxidant and β-carotene bleaching assays. In addition, qualitatively the phytochemistry of the plant was also analyzed by using various methods. The stem and bark powder of the plant was extracted with different solvents by using sequential extraction method in the order of increasing polarity. The results of scavenging activity for ethanol and chloroform extracts showed highest effectiveness as compared to the other extracts as the ethanol extract exhibited maximum antioxidant potential in β-carotene bleaching assay. Further, the chloroform extract showed maximum reducing power in total antioxidant activity.
Kaur and Arora, (2011) explored the antioxidant potential of bark and leaves extracts (hexane, chloroform, ethyl acetate, methanol, water) of Cassia siamea and Cassia javanica by applying superoxide anion radical scavenging assay. The results showed the inhibition of superoxide radicals in a dose dependant manner. The methanol extract showed the inhibition of 60.5% (800 µg/ml) among all the bark extracts of C. siamea and the water extract showed strong antioxidant potential of 51.3% (1000 µg/ml). The water extract of C. javanica (bark) showed strong antioxidant potential (55%) at 1000 µg/ml concentration. The various leaf extracts of C. siamea showed good antioxidant potential (25-50%) at 1000 µg/ml. Likewise, the methanol leaf extract of C. javanica showed strong antioxidant potential of 50.4% at 300 µg/ml concentration.

Apak et al. (2008) studied the application of CUPRAC (cupric reducing antioxidant capacity) assay which is used for the dietary polyphenols, vitamin C, vitamin E. It utilises the Cu(II)-Nc (copper(II)-neocuproine) reagent as the chromogenic oxidant. This method involved the mixing of antioxidant solution with CuCl₂, neocuproine and ammonium acetate (pH 7). The absorbance was measured at 450 nm. The CUPRAC antioxidant capacities of polyphenolics were reported and compared to ABTS/persulfate and Folin assays. The trolox equivalent capacities (TEC) of the antioxidants were linearly correlated (r=0.8) with ABTS. The results showed highest antioxidant capacities in the CUPRAC method for epicatechin gallate, epigallocatechin gallate, quercetin, fisetin, epigallocatechin, catechin, caffeic acid, epicatechin, gallic acid, rutin and chlorogenic acid in accordance with theoretical expectations.

Esterbauer and co-workers (1991) defined lipid peroxidation as an established mechanism of cellular injury in both the plants and animals. This is used as an indicator of oxidative stress in cells and tissues. Lipid peroxides are unstable and decompose to form a complex series of compounds including reactive carbonyl compounds. On decomposition, polyunsaturated fatty acid peroxides generate malondialdehyde (MDA) and 4-hydroxyalkenals (HAE). The measurement of malondialdehyde and 4-hydroxyalkenals has been used as an indicator of lipid peroxidation. The oxidation of lipids is the subject of great importance from the analysis of the mechanisms, dynamics, product analysis, involvement in diseases, inhibition and biological signaling (Niki et
al., 2005). In general, there are three distinct mechanisms known for oxidation of lipids which are oxidation by enzymes, free radical mediated oxidation and non-radical oxidation. Each of the mechanisms yields specific products. The relative response of lipids to oxidation depends on the reaction background and also on their inbuilt structure. Among all the by-products, lipid hydroperoxides are formed as the major primary products because there are substrates for different enzymes and also undergo various secondary reactions. Phospholipid hydroperoxides are reduced to the corresponding hydroxides by seleno proteins in vivo. Various kinds of antioxidants with different functions restrain lipid peroxidation and the deleterious effects caused by the lipid peroxidation products.

Singh and Arora, (2009) confirmed the peroxyl radical scavenging activity of aqueous bark extract/fractions of *Acacia nilotica* (L.) Willd. Ex Del. in lipid peroxidation assay. The results were compared with a known antioxidant viz. BHT. The extraction of bark powder with different solvents of increasing and decreasing polarity was done. Further, the water extract was partitioned with ethyl acetate. The scavenging activity of extract was found to increase on fractionating the extract.

Fukuzawa et al. (2000) examined a number of variables which determined the efficiency of lipid peroxidation in egg yolk phosphatidylcholine liposomes and microsomes. They are exposed to enzymatically generated superoxide radicals. The initiation of the peroxidation process requires the presence of preformed lipid peroxides and a chelated metal catalyst. The comparison of the effectiveness of four iron chelating agents showed that the chelate must be fixed to the membrane by coulombic attraction in between the charged membrane and a chelate which carry the opposite net charge. Among the chelates tested, only the carcinogenic ferric nitriloacetate (Fe$^{3+}$-NTA) was an effective catalyst for the oxidation of all the membranes whether carrying a net charge or not. The unique catalytic capacity of the ferric nitriloacetate (Fe$^{3+}$-NTA) was explained by its existence in two forms at neutral pH, each binds to oppositely charged membranes and initiate their peroxidation and gives the complex a unique ability to bind to any membrane which may be a factor in its carcinogenicity.
2.2. Antiproliferative studies

The global burden of cancer is continuously increasing, largely because of the factors like aging and human population growth rate (Jemal et al., 2011). Furthermore, the changing lifestyle and smoking in the developing countries has also an additional factor. The fast food, colas and habits like drinking, paan chewing, stressful lifestyle, toxic medicines and environmental pollution cause the building up of toxins that lowers the immunity and thus cause cancer. The study based on the GLOBOCAN in 2008 estimated that about 12.7 million cancer cases as well as 7.6 million cancer deaths have occurred in 2008 and out of these, 56% of cases and 64% of deaths occurred in the economically developing world. The most frequently diagnosed cancer is breast cancer which is the main cause of cancer death amongst the females which accounts for 23% of the total cancer cases and 14% of the cancer deaths. Likewise, lung cancer is the main cancer in males which comprise 17% of the total new cancer cases and 23% of the total cancer deaths.

The major cause of death of women is due to cervical cancer (Kma, 2013). As it is a major health problem among women therefore, early detection and affordable drugs with clinical efficacy have to go hand-in-hand in order to comprehensibly address this serious health challenge. Plant based drugs with potent anticancer effects and no side effects are required at cheaper rate. The plants and their products are known to exhibit antitumorigenic potential in human cervical carcinoma cells. A number of alkaloids are isolated from Cynanchum vincetoxicum and Tylophora tanakae, naucleorals A and B from the roots of Nauclea orientalis, (6aR)-normecambroline from the bark of Neolitsea dealbata that showed excellent effects in cervical carcinoma cell lines with IC₅₀ values of 4.0-8 μg/mL. The rhinacanthone and neolignans isolated from different plants kill cervical cancer cells at very low concentrations. Among plant based anticancer drugs, noni, from the plant Morinda citrifolia perhaps is the best candidate. It was found that cisplatin in combination with noni showed significantly better effect against the different human cervical carcinoma cells.

Moustafa et al. (2014) examined two hundred wild and cultivated plants collected from different localities which were grown in Egypt. The study showed the
significant cytotoxic effect of methanol extracts of plants against MCF-7, HCT-116, HepG2 and A549 cell lines. The selectivity index (SI) was also estimated for the botanicals by using normal skin cell line of human (BJ-1). The plants that exhibited maximum cytotoxic activities were *Dovyalis caffra*, *Gingko biloba*, *Ipomoea carnea* and *Lonchocarpus speciosus*. On the basis of SI values, the branch extract of *Dovyalis caffra* showed relatively high selectivity to the lung tumor cell line. The methanol extracts of leaves of *Gingko biloba* and *Ipomoea carnea* as well as the bark extract of *Lonchocarpus speciosus* exhibited markedly high SI values for colon cancer.

The roots of *Ichnocarpus frutescens*, *Cissampelos pareira*, *Bauhinia vahlii* and *Ardisia solanacea* were processed and given the combination orally for the treatment of stomach cancer to the tribes of Chotanagpur and Santhal parganas of Bihar. The results of above study were confirmed by Singh and Singh, (2014) by evaluating the *in vitro* anticancer activity of methanolic roots extracts of *I. frutescens* (MIF) as well as isolated triterpenes by 3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay in MCF-7, BEL-7402, SPC-A-1 and SGC-7901 cell lines.

Chronic degenerative diseases are “non-communicable, a heterogeneous group of conditions that contribute to mortality by a small number of outcomes (diabetes, cancer and cardiovascular diseases)”- Cordova, (2008). Many studies showed therapeutic effectiveness for certain types of cancer as Ngeh et al. (2012) reported *Vernonia guineensis* as antiprostate cancer agent. Chung, (2008) found that *Ganoderma tsugae* is effective against anticolorectal cancer. Plastina, (2012) reported that *Ziziphus* is effective against breast cancer. Yu, (2012) reported that *Echinacea purpurea* and *Garcinia mangostana* has anticancer, antioxidant and apoptosis inducing properties (Moongkarndi, 2004). *Azadirachta indica* eliminates prostate cancer cells (Suresh, 2006) and *Viscum cruciatum* showed high anticancer and antimicrobial potential (Assaf, 2013). Bandana, (2012) reported that *Tomentosa roem* and *Schult wrightia (Apocynaceae)* is a potent agent for treating the breast cancer.

The study for the phytochemical analysis of methanolic extract of *Datura metel* leaves showed the presence of alkaloids, terpenoid, steroids, flavonoids, phenolic compounds, saponins, tannins as well as glycosides (Banu et al., 2014). Further, the
results showed that the ethanol extract of leaves had highest anticancerous activity as compared to stem extract. Both the leaves extracts were studied on Vero and MCF-7 cell lines and results showed the lowest IC$_{50}$ values. Withanolide, a steroidal lactones present in the plant, isolated from *Datura metel* have been reported to have high anticancer activity against colorecto carcinoma (HCT-116) cell line (Glotter, 1991; Nash *et al.*, 1993). This plant has significant anticancer potential as confirmed by other reports given by Donatus and Ephraim, (2009); Akharaiyi (2011); John and Herin, (2011); Arshad *et al.* (2008); Ma *et al.* (2006). Also, Paul and Manjula, (2012) studied six medicinal plants (*Pavonia odorata*, *Gardenia latifolia*, *Canthium dicoccum*, *Limonia monophylla*, *Bridelia roxburghiana* and *Wrightia tinctoria*) for their cytotoxicity and antiproliferating activities. In this study, crude methanol extract of leaves was obtained by using soxhlet apparatus. An amount of 10 mg/ml of each plant extract was used for MTT assay against Calu-6, Colo-205 and HL-60 cell lines. On the basis of results obtained by MTT assay, *Pavonia odorata* and *Wrightia tinctoria* were further studied for the cell cycle analysis by the use of Calu-6 cells and results showed the cytotoxicity and antiproliferating activities of the leaves extracts.

The potential of *Mangifera pajang* kernel extract was studied as a persuasive cytotoxic agent against breast cancer cell line (MCF-7) (Bakar *et al.*, 2010). The growth of cells was found to be inhibited with IC$_{50}$ value of 23 and 30.5μg/ml in MCF-7 and MDA-MB-231 cells respectively. Overall, the extracts caused the cell arrest at sub-G1 phase in time dependent manner. Some of the cell lines showed the cell arrest at G2-M phase which showed substantial subG1 arrest after 48 and 72 hours of incubation. The cell death was observed to be due to apoptosis which was confirmed by exposing the cells to Annexin V-FITC and propidium iodide. The apoptosis appeared to be dependent on caspase-2 and 3 in MCF-7 cells and caspase-2, 3 and 9 in MDA-MB-231 cells.

Sehar *et al.* (2010) studied the anticancer effect of ethanolic (CSL-E) and n-hexane fraction (CSL-F) of *Cannabis sativa* leaves. The extracts were evaluated against a number of human cancer cell lines viz. colon carcinoma (Colo-205), prostate adenocarcinoma (PC-3), lung carcinoma (A-549) and promyelocytic leukemia (HL-60). The results of CSL-E and CSL-F extracts inhibited the growth of cell lines in a dose
dependent manner (IC\textsubscript{50} 32.51-89.28 and 25.71-56.26 μg/ml) respectively. Additionally, CSL-F induced concentration dependent apoptosis in HL-60 cells as calculated by annexin V binding, DNA fragmentation, apoptotic bodies formation and an increase in hypo diploid sub G\textsubscript{o} DNA content. The translocation of Bax to mitochondria leads to loss of mitochondrial membrane potential and release of cytochrome c into the cytosol. These all were associated with significant activation of caspase-3, 6 and 9 which further led to Poly (ADP-ribose) polymerase cleavage. The results demonstrated that CSL-F inhibits the proliferation of cancer cells through induction of apoptosis and CSL-F is a contender for anticancer therapy.

Blanc \textit{et al.} (2000) defined the role of caspases in inducing the cell death via apoptosis. He conducted the study on breast cancer cell line (MCF-7) deficient in caspase-3 and observed that cells showed defective response to the treatment of cisplatin. The reconstitution of MCF-7 cells by stable transfection of \textit{CASP-3} cDNA restores all these defects and results in an extensive apoptosis after cisplatin treatment. The extracts from caspase-3- deficient MCF-7 cells, procaspase-9 processing is strongly impaired after stimulation with either cytochrome \textit{c} or recombinant caspase-8. The reconstitution of MCF-7 cell extracts with procaspase-3 corrects this defect, resulting in an efficient and complete processing of procaspase-9.

The isolated compound of ethanol extract of \textit{Carmona retusa} (Vahl.) Masam, was quercetin (flavonoid) which was studied for its anticancer activities. The studies were carried on HepG2 cell lines by using MTT and Caspase-3 colorimetric assays (Chandrappa \textit{et al.}, 2014). The results showed that quercetin had significant and concentration dependent anticancer activities at different concentrations i.e. 100 μg/ml and 80 μg/ml doses after the treatment for 24 and 48 hours in MTT assay. A significant cell apoptosis have been seen at 53μg/ml concentration of quercetin as well as significant activation of caspase-3 was observed at 100 μg/ml concentration after its exposure for 24 and 48 hours. The results obtained showed that quercetin could be used as a promising anticancer agent.

The apoptosis inducing effect of the alcoholic extract from \textit{Erythrina suberosa} stem bark (ESB) in human promyelocytic leukemia HL60 cells was studied by Agrawal
et al. (2011). The results showed that the ESB inhibited cell growth in a dose as well as time dependent manner. The scanning electron microscopy by the gold standard showed formation of apoptotic bodies as well as formation of blebs. A significant increase in Sub G0 population of cells along with increase in intracellular ROS production up to six fold was observed in ESB treated HL60 cells. In addition to this, the dissipation of mitochondrial membrane potential of intact cells accompanied by increase in cytosolic cytochrome c was observed which was followed by activation of caspase-9 and 3 but not caspase-8. The study showed that ESB induced mitochondria mediated intrinsic apoptotic pathway in HL60 cells.