2:REVIEW OF LITERATURE:

Considerable attention has been focused in recent years on the exploration of phytotherapeutic agents for the treatment of oxidative stress and mutation related disorders. Oxidative stress and mutation related disorders arise from the production of reactive oxygen species or reactive nitrogen species. ROS consists of stable free radicals such as hydroxyl (OH^-), superoxide(O_2^-), Nitric oxide(NO), peroxyl (RO_2^-), lipid peroxyl radicals(LOO^-), and non free radicals such as hydrogen peroxide(H_2O_2), singlet oxygen(O_2^-), ozone(O_3), lipid peroxide (LOOH) and they are different activated forms of oxygen (Halliwell *et al*., 1999; Yildirim *et al*., 2000 and Gulcin *et al*., 2002). ROS are not only the end products of normal metabolic process of aerobic organisms but are also produce from harmful exogenous agents (Harman, 1956 and 1961; Briviba and Sies, 1994, Devasagayam *et al*., 2005; Rizzo *et al*., 2010). The interaction of this free radicals with polyunsaturated fatty acids , nucleotides and disulphide bonds (Machlin and Bendich, 1987, Grigorov, 2012; Shobha *et al*., 2013, Dizdaroglu *et al*., 2002; Hollstein *et al.,* 1991; Cooke *et al*., 2003) has been implicated as the major factor to cause the oxidation of the biological compounds that eventually leads to mutations (Devasagayam *et al.,* 2005, Lobo *et al*., 2010) ultimately causing mutagenesis and carcinogenesis (Lobo *et al*., 2010; Keith *et al*., 1999). Therefore the recent investigations explored the protective properties of plants against the free radicals generated diseases because of their chemical compositions. The phytochemicals present in plants do not only enable the plant to withstand the harsh conditions but also help in life expectancy improvement strategies (Nobili *et al*., 2009). The remedies, based on the use of herbs and their bioactive principles are currently being developed as a part of protective mechanism to provide the organism more resistant to mutagens and oxidative stress.

2.1: Source of oxidative stress and its implications:

Oxidative stress results from the lost of balance between the ROS/RNS and counteracting antioxidant mechanisms (Domej *et al*., 2014). ROS/RNS are formed as a natural metabolic byproduct of oxygen and has important role in signaling and homeostasis, moreover they are generated by tight regulations of endogenous enzymes such as nitric oxide synthase (NOS) and NAD (P) H oxidase isoforms, xanthine oxidase and xanthine dehydrogenase respectively (Devasagayam *et al.,* 2005; Valko *et al*., 2007, Sharma *et al*., 2012; Weidinger and Kozlov, 2015; Leimburg-Apers *et al*., 2015). The presence of primary species such as superoxide radical, nitric oxide do not appear to have deleterious effect even at their high concentrations, the only prerequisite to be a deleterious effect is the formation of secondary species such as hydroxyl radical, peroxinitrite etc. upon reaction of primary species with secondary species or with any transition metal (Weidinger and Kozlov, 2015). Oxidative stress is believed to contribute to the development of a number of diseases significantly, particularly age related diseases. Environmental stress also aid in increasing the number of secondary species dramatically resulting in the lost of redox homeostasis (Devasagayam *et al*., 2005; Domej *et al*., 2014; Ridnour *et al*., 2004; Valko *et al*., 2001, 2006). Scavenging or detoxification of these excess ROS/RNS is suggested to be achieved by an efficient antioxidative system comprising of both enzymatic as well as non enzymatic antioxidants such as superoxide dismutase, catalase, glutathione peroxidase (enzymatic antioxidants) and vitamin E (tocotrienols, tocopherols), glutathione, vitamin C (non enzymatic antioxidants) (Noctor and Foyer, 1998; Valluru., 2014). Superoxide dismutase are manganese dependent (MnSOD or SOD2) which are localized in the mitochondrial matrix and it rapidly converts superoxide radical anion into peroxide with the help of superoxide dismutase (copper and zinc dependent) located in the cytosol, nucleus and mitochondrial

intermembrane space (Murphy, 2009; Tyler, 1975; Weisiger and Fridovich, 1973). In turn, hydrogen peroxide is converted into water and oxygen by the catalase enzymes which are mainly located in the peroxisomes as well as mitochondria (Salvi *et al*., 2007). However within mitochondria, hydrogen peroxide is mainly removed by the action of glutathione peroxidae-1(Gpx1; Cox *et al*., 2010; Esposito *et al*., 2000; Esworthy *et al*., 1997), peroxiredoxins 3 and 5 (Prx3 and Prx5) and the thioredoxine-2 (Trx2) system (Chae *et al*., 1999; Chang *et al.,* 2004; Cox *et al*., 2010) with the help of glutathione(GSH). Oxidised GSH (GSSG) is regenerated to GSH with the help of glutathione reductase (Leimburg-Apers *et al*., 2015). The overloading of free radicals produced from exogenous and endogenous agents however limits the function of these endogenous antioxidants resulting in the accumulation of ROS/RNS causing irreversible damage to all kinds of biomolecules such as damage to DNA, oxidation of polyunsaturated fatty acids (lipid peroxidation), oxidation of amino acids in proteins, deactivate specific enzymes by oxidation of co-factors thereby leading to oxidative stress related diseases such as cancer, autoimmune disorders, aging, cataract, rheumatoid arthritis, cardiovascular and neurodegenerative diseases (Pham *et al*., 2008; Brooker and Robert, 2011; Liemburg-Apers *et al*., 2015; Weidinger and Kozlov, 2015).

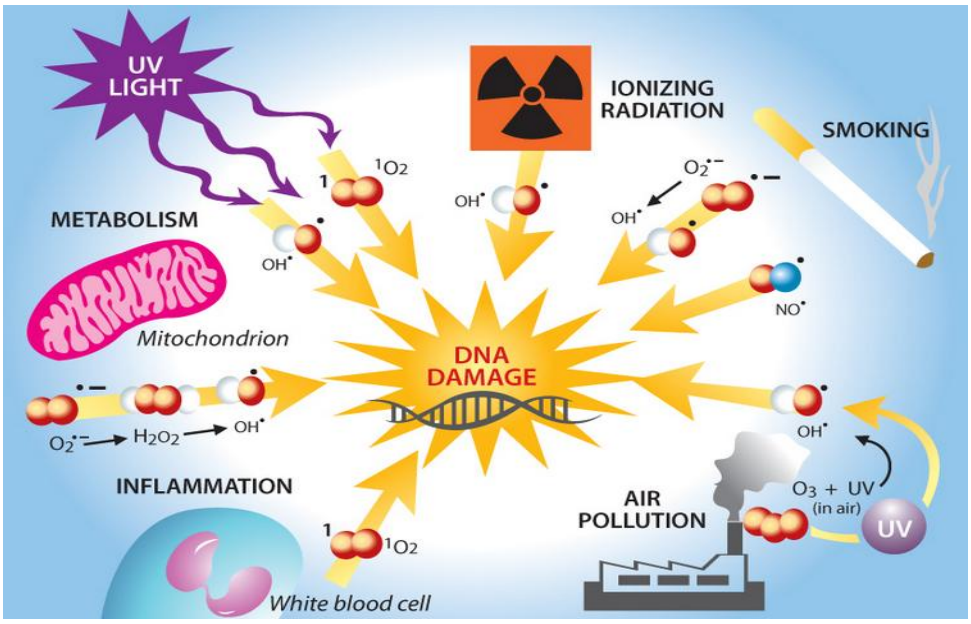

Fig.1: Common source of ROS/RNS (Cadenas and Davies, 2000).

2.2: Lifestyle pattern as risk factors:

Changing lifestyle play an important role in the initiation, promotion and proliferation of cancer and its risk factors include consumption of alcohol, cigarette smoke, exposure to sun, inclusion of fried foods and red meat in diet, nuclear emissions, obesity, stress, infections and physical inactivities (Anand *et al*., 2008; Jermal *et al*., 2011; Stewart and Wild, 2014). Tobacco smoking accounts for about 25-30% of cancer and volatile organic compounds form tobacco smoke are associated with increased risk of cancer, cardiovascular, respiratory diseases, heart attacks,, birth defects, chronic obstructive pulmonary diseases (Helen *et al*., 2014; Anand *et al*., 2008; Tobacco Fact Sheet, 2014). Many pesticides which are commonly used commercially, agriculture, home and garden are also proved to be associated with increased risk of cancer and IARC, 2015 has classified five of the commonly used organophosphate pesticides (glyphosate, malathion, diazinon, tetrachlorvinphos and parathion) to be highly carcinogenic to humans.

10% of both ionizing and non ionizing radiations are known to induce cancer and carcinogenesis (Anand *et al.,* 2008). Non ionizing radiations such as ultraviolet radiations, radio frequencies radiation imparted from mobile phones, electric power transmissions serve as a risk factor for different forms of cancer, such as non melanoma skin cancer (Tornaletti and Pfeifer, 1996; Cade *et al*., 2005, IARC, 2011). Ionizing radiations such as X-rays have potential to induce lung and breast carcinoma, while radioactive nuclei have been reported to develop gastric cancer in rats (Cohen, 2002; Ron, 2003; Kleinerman, 2007). The risk factors caused by both ionizing as well as non ionizing radiations depend upon the time of exposure, physiological state and age etc (Anand *et al*., 2008; Little, 2009).

2.3: Implications of oxidative stress:

2.3.1:Oxidative DNA damage:

Overproduction of ROS/RNS results in the structural alterations in DNA, causing oxidation in guanine nucleotide readily as compare to cytosine, thymine and adenine as the oxidation potential is high in guanine (DNA oxidation; Wikepedia). Increase level of oxidized guanine nucleotide serves as a biomarker of oxidative stress and found to be associated with carcinogenesis and many diseases by two mechanisms such as first mechanism involves the modulation of gene expression and second mechanism involves the induction of mutations (Evans *et al*., 2003; Valavanidis *et al*., 2013).

2.3.2: Oxidative protein damage:

Excessive production of ROS/RNS not only damage DNA but also damage proteins as a result of their abundance in living cells (Daveis, 2005). It has been reported that side chains and backbone of amino acids in the protein are more prone to oxidative damage and amongst all the amino acids methionine easily gets oxidized to methionine sulfoxides which is somewhat repairable while other protein damage are almost non repairable (Daveis, 2005).

2.3.3: Oxidative lipid damage:

Lipid peroxidation of membranes is also another major cause of ROS/RNS**.** The end product of lipid peroxidationare reactive aldehydes such as MDA and 4-hydroxynonenal (HNE) (second messenger of free radicals and major active marker of lipid peroxidation) and if they are not terminated fast enough they may be mutagenic and carcinogenic (Marnett, 1999). The end product, MDA reacts with deoxyadenosine and deoxyguanosine of DNA and form DNA adducts primarily (Marnett, 1999).

2.4: Mechanism of cancer Development and cancer progression:

Different forms of cancer develop due to the documentation of cascades of mutation and finally lead to carcinogenesis. Carcinogenesis is generally recognized as a complex and multistep process in which oxidative stress and inflammation plays a crucial role in altering distinct molecular and cellular structures (Fanco *et al*., 2008; Kryston *et al*., 2011). Free radicals not only cause the mutations but also in turn trigger the initiation stage of carcinogenesis (Johnson, 2007). Many mutagens act through the generation of reactive oxygen species (ROS); therefore the removal of ROS in the process of antimutagenesis becomes a mandatory field. There is also increasing evidence that compounds with antioxidant properties can remove ROS before the reaction of mutagens and DNA that can result in mutation (Lee *et al.*, 2011).

2.5: Chemoprevention by synthetic antioxidants and its side effects:

Chemoprevention is defined as the introduction of synthetic, natural/biological agents for the purpose of reducing cancer (Steward and Brown, 2013; Tsao *et al*., 2004; Sanner and Dybing, 1996). The preference to natural antioxidants obtained from natural sources has been increased recently as compared to the synthetic antioxidants such as BHA; BHT etc since BHA, BHT are linked to cause stomach as well as liver tumors and many diseases, (Kahl and Kappus, 1993; Williams *et*

al., 1999; Lobo *et al*., 2010) so they have been restricted by many regulatory agencies to include in human foods. Thus the search for non toxic, effective natural compounds has been intensified to cope with the diseases related to oxidative stress.

2.6: Antimutagens:

Antimutagens are those agents that interfere the mutagenicity of substance that may either prevents the transformation of mutagenic compound into mutagen or inactivates the mutagen-DNA reaction. The antimutagenic agents can be of natural and synthetic compounds and they are classified based on their mechanism of action such as compounds with antioxidant activity and compounds that inhibit the action of mutagens (Sloczynska *et al*., 2014). It has been reported that several antitumor agents act through antimutagenic mechanism (Sloczynska *et al*., 2014; Tsai *et al.,* 1996; Dion *et al*., 1997; Ikken *et al*., 1999), hence in recent years; it has been focused on the publications of screening of both natural and synthetic compounds. Therefore searching for antimutagenic compounds has become a rapidly expanding field for cancer research (El-Sayed and Hussain, 2013; El-Sayed *et al*., 2013) and it has been suggested that plants which have high antioxidant potential could show antimutagenic and can counteract the effect of mutagens and carcinogens (Cardador-Martinez *et al*., 2006, El-Sayed and Hussain., 2013). Such bioactivities of medicinal plants can only be studied through extraction of plant secondary metabolites using different suitable solvents.

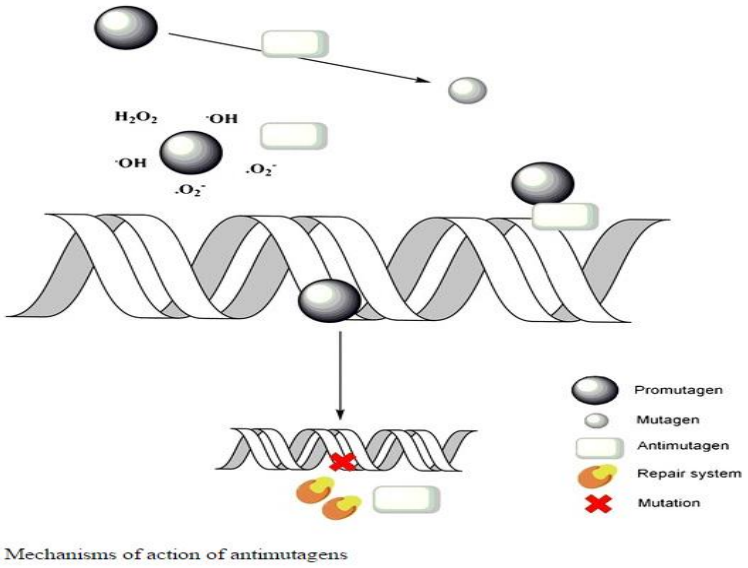

Fig.2: Mechanisms of action of antimutagens (Sloczynska *et al*., 2014).

2.7: Preparation of plant samples for extraction:

The principle methods of extraction is to separate all the soluble secondary metabolites from the insoluble residues where the extractives contains mixture of plant metabolites and can be further used as medicinal agents in the form of fluid or may need further processing (Azwanida, 2015). Both the fresh and dried samples can be used for extraction but dried samples are most preferred because fresh samples are more fragile and tend to deteriorate faster than dried samples (Sulaiman *et al*., 2011). The preservation of plant biomolecules present in the medicinal plants is an important task prior to extraction and it can be done by extraction from either fresh or dried plant materials, besides grinding and drying aid in the preservation of phytochemicals present in the final extracts. For efficient extraction to occur grinding of plant materials is important to reduce the size of particles so that it can easily come in contact with the solvents to ease the extraction procedure (Methods optimization in Accelerated Solvent extraction, 2013).

2.8: Extraction and choice of solvents:

To successfully determine the biological activities of the compounds present in the plants, the first and foremost method is to extract the plant bioactive compounds using different solvents through standard procedures (Handa *et al*., 2008). The general techniques for the extraction of medicinal plants include maceration, infusion, percolation, decoction, hot continuous extraction (Soxhlet extraction) etc. and any part of the medicinal plant can be used for the extraction of its constituents (Pandey and Tripathi 2014). The commonly used extraction technique is by using Soxhlet or hot continuous extraction. Soxhlet uses finely ground sample placed in a porous bag or filter paper in the Soxhlet chamber where the extraction solvents will be heated and allowed to condense the sample in the Soxhlet chamber with the vapours. As soon as the liquid content reaches the siphon arm of the Soxhlet, the liquid contents again emptied back into the bottom flask again and process continues.

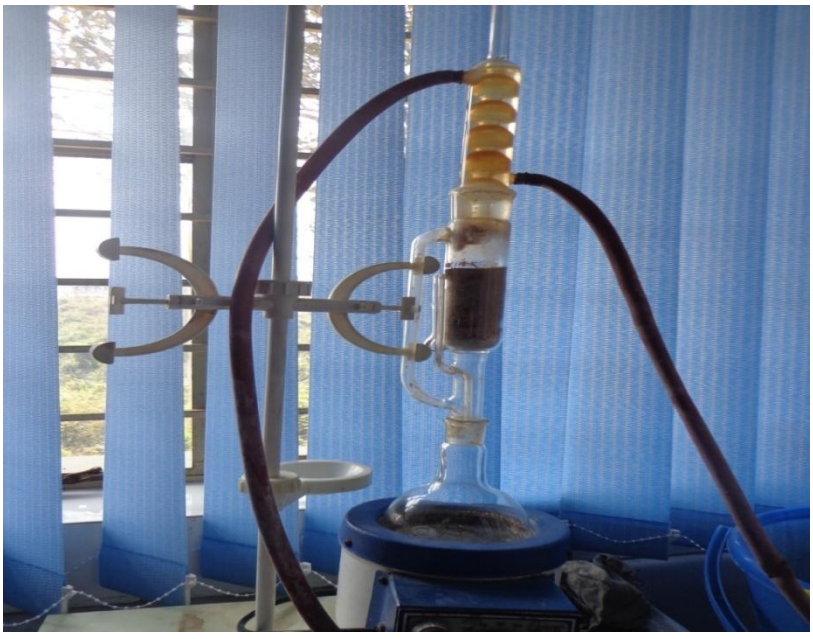

Fig:3: Extraction with Soxhlet apparatus.

Table: 1: Solvents used in the extraction of active Compounds:

Solvents	Compounds	References
Methanol, Ethanol, Acetone, Propanol, Ethyl Acetate	Extraction of Phenolics	Durling *et al*., 2007; Alothman *et al*., 2009; Xu *et al*., 2007; Shi *et al*., 2005.
Methanol, Ethanol, Ethyl Acetate	Extraction of hydrophilic compounds	Sasidharan *et al*., 2011
Dichloromethane, Dichloromethane+ Methanol(1:1 ratio)	Extraction of lipophillic compounds	Sasidharan *et al*., 2011
Hexane	Chlorophyll	Cosa *et al*., 2006
Methanol/ ethanol	Anthocyanin rich phenolic extracts	Dai and Mumper, 2010
Methanol	Low molecular weight polyphenols	Metivier *et al*., 1980; Prior *et al*., 2001; Guyot *et al*., 2001.
Methanol, ethanol, acetone, water	Extraction of Flavanoids	Naczk and Shahidi, 2004; Zhu *et al*., 2010; Biesaga, 2011.
Aqueous acetone	High molecular weight flavones	Metivier *et al*., 1980; Prior *et al*., 2001; Guyot *et al*., 2001.

Table: 2: Solvents used for active component extraction

(Prashant *et al*., 2011):

Acetone	Ether	Chloroform	Methanol	Ethanol	Water
Phenol	Alkaloids	Terpenoids	Anthocyanins	Tannins	Anthocyanins
Flavanoids	Terpenoids	Flavanoids	Terpenoids	Polyphenols	Starches
	Coumarins		Saponins	Polyacetylenes	Tannins
	Fatty Acids		Tannins	Flavanol	Saponins
			Xanthoxyllines	Terpenoids	Terpenoids
			Totarol	Sterols	Polyphenols
			Quassinoids	Alkaloids	Lectins
			Lactones		
			Flavones		
			Phenones		
			Polyphenols		

2.9: Antioxidant and Antimutagenic activity of plant extracts:

Chou *et al*., 2012, demonstrated that hot water extract of *Glechoma hederaceae* has antioxidant and antimutagenic activity. The antioxidant activity of the plant extract which was tested against DPPH, $ABTS^{+}$, Suproxide anions, lipid peroxides as well as reducing ability and was found to be effective. The scavenging activity of plant extract was found to be higher even than the standard compounds tested such as Trolox and Vitamin C. The mutagenic, antimutagenic ability of the plant extract was tested employing Ames test against 2- Aminofluorine, 4-nitroquinoline-N-oxide (4-NQO), t- Butyl Hydroperoxide (t-BHP), Sodium Azide (AzNa), 2- Anthramine (2-AA) using the strains TA97, TA 98, TA 100, TA 102 and TA 1535 in the presence and absence of metabolic activator S9. There was marked inhibition of the extracts against the mutagenicities caused by mutagens. The experiment concluded that the herbal plants are rich source of antioxidants and plants rich in antioxidants are antimutagenic and anticarcinogenic.

Issazadeh and Aliabadi, 2012, have screened the antimutagenic properties of aqueous extract of Olive leaf employing Ames test. Olive leaf aqueous extract was tested against sodium azide and 2-nitroflurorine using *Salmonella typhimurium* strains TA100 in the presence and absence of rat microsomal liver enzyme S9. The mutagenicity induced by the mutagens was observed to be increased in the positive control groups; however, it was found that the extract inhibits the mutagenic potential of sodium azide by 54.21% and 2-nitrofluorine by 51.62%.

Moriff *et al*., 2012, also evaluated the antimutagenic properties of *Magnifera indica* L.stem bark extract against ten chemical mutagens including cyclophosphamide using the strain TA100. Amongst the ten chemicals tested, cyclophosphamide was one of them and CP (500µg/ plate) was found to be more mutagenic in the presence of metabolic activator S9 in Ames test, however the stem extract of the plant was found to inhibit the mutagenicity caused by cyclophophamide.

Abdillahi *et al*., 2012, have demonstrated that leaf and stem extracts of Podocarpus species are not mutagenic and antimutagenic against the tested chemical mutagens. The antimutagenicity test was based on plate incorporation method and neural red uptake (NRU) assay using five Salmonella strains TA98, TA 100, TA 102, TA 1535 and TA 1537. The extracts were demonstrated to have strong antimutagenic activity against 4-nitroquinoline-N-oxide (4-NQO) induced mutagenesis in a dose dependent manner. The extracts which have antimutagenic activity were also tested for their cytotoxic activity against human hepatocellular liver carcinoma cell line (HepG2). Petroleum ether extracts of Podocarpus species were found to be more cytotoxic than ethanolic extracts of Podocarpus species.

2.10: In vitro models for studying Antigenotoxicity activity:

The genotoxic testing is to determine whether a substance will induce genetic defect or may lead to cancer in which it can be tested by using bacterias, yeast or mammalian cells (Furman and Grace 2008). Using the knowledge of genotoxic testing, we can control diseases related to genotoxic substances (Kolle and Susane, 2012). The genotoxic substances produces many aberrations like chromatid and chromosome breaks, chromatid deletions, fragmentation, translocation, complex rearrangement and many more (Furman and Grace, 2008) which can be observed in the micronucleus test and chromosomal aberration test conducted on mammalian cells (Kolle and Susane, 2012). Treatment of such genotoxic effects are done with the help of genotoxic drugs which is usually termed as genotoxic chemotherapy. The rapidly dividing cells are sensitive to genotoxic drugs but affects the normal cells too that may also be mutagenic and cytotoxic, raising the risk of secondary cancers (Walsh and Declan, 2013). So to reduce the side effects of chemotherapeutic drugs, scientist has been using the plants extractives or compounds as an alternative means for the treatment of clastogenic/genotoxic effects.

Muneeb *et al*., 2012, evaluated the antigenotoxicity and antioxidant efficacy of the naturally occurring plant polyphenol, Ellagic acid against the antineoplastic agent cyclophosphamide. The study was conducted on some groups of Swiss albino mice dividing into positive, negative and treatment groups. It was observed that the treatment of CP only induced genotoxicity in the cells of mice which were marked by the increase number of micronuclei when assayed through micronucleus test. Besides that CP was observed to induce oxidative stress in the kidney of mice causing disturbances in the renal infiltration as well as renal architectures were found to change which was indicated by swelling and fatty changes. Endogenous antioxidants such as glutathione, glutathione peroxidase, glutathione S-transferase were found to be reduced in the CP treated groups on the other hand lipid peroxidation was found to be increased as compared to the normal groups. Moreover the pre-treatment of some groups of mice with the Ellagic acid improved the changed morphology of the kidney and other changes brought about by cyclophosphamide as well reduces the genotoxicity caused by CP.

Alkan *et al*., 2012, evaluated that the extract of *Salvia officinalis* protect against cyclophosphamide-induced genotoxicity and oxidative stress in rats. Different doses of *Salvia officinalis* extract (50, 100, 150mg/kg.b.wt) were prepared and pretreated some groups of animals with the extracts before the treatment of CP (40mg/kg.b.wt). Then the biochemical activities were determined in the liver, kidney and heart tissues and the number of micronuclei was also determined using micronucleus assay with mice bone marrow cells. The pretreatment of extracts at different concentrations was observed to revert the changes done by cyclophosphamide. The results concluded that the protective activity of the plant extract was related to its high antioxidant potential.

Mishra *et al*., 2013, extracted the dried powdered leaves of *Murraya koenigii* (Curry leaf) with two solvents aqueous and ethanol using hot continuous extraction method, Soxhlet. Extracts were further

processed and used for preliminary phytochemical testing as well as some bioactivities such as genotoxicity and antigenotoxicity assay. One group of mice was injected intraperitoneally with CP (50mg/kg.b.wt) and other groups of mice excluding negative control groups were administered orally with the extracts of *Murraya koenigii* (Curry leaf) with two different doses of 100mg and 200mg per kg body wt of mice for seven consecutive days and sacrificed for the evaluation of micronucleated polychromatic erythrocytes (MnPCEs) The values of micronucleated polychromatic erythrocytes (MnPCEs) were counted and compared with the positive control groups. Higher doses (200mg/kg.b.wt) of both the extracts were found to reduce the aberrations induced by cyclophosphamide thereby concluding that both the extracts have the antimutagenic potential that prevents the mutagenic effect of various cytotoxic drugs.

Shokrzadek *et al*., 2013, used the extracts of the plant *Citullis colocynthis* (L) fruit (CCT) and evaluated its antioxidant and genoprotective effects. Different concentrations of CCT fruit extracts (0.5-3mg/ml) were tested against DPPH and maximum inhibitory effects was found to be obtained in 3mg/ml of the extract with the percentage inhibition of 95.862% (IC 50 value of 1.564mg/ml). Further the CCT extract was evaluated for micronucleus assay against the mutagen cyclophosphamide using mice bone marrow cells. The frequency of micronuclei was observed to be increased significantly ($p<0.0001$) in CP treated groups as compared to the normal control groups. However, the frequency of MnPCEs was found to be reduced significantly in CCT+ CP treated groups of mice by a factor of 5.9 and 6.67 for the doses 100mg and 200mg per kg b. wt as compared to the CP treated groups. It indicates the suppressing action of CCT against the clastogenic effects of CP. Treatment of CP showed pronounced cytotoxic effect in the bone marrow cells of mice as well as decreases the mitotic activity (% PCE) which was observed to be altered by the treatment of CCT fruit extracts. Increased lipid peroxidation level induced by CP was also found to be reduced by

the treatment of CCT fruit extracts. The GSH/GSSG ratio was observed to be increased significantly ($p<0.001$) as compared to the CP treated groups. The study concludes from the results obtained that CCT fruit extract can be used as a supplement in the patients undergoing chemotherapy as it has profound bioactivities against the clastogenic agents.

Fahmi *et al*., 2013, have evaluated that the extracts of *Vitis vinifera* cultivars was found to posses high antioxidant and antimutagenic activity against DPPH and Endoxan(CP). Grape cultivars were found to exhibit high concentration of total phenolic content ranging from 115-960 mg/L Gallic Acid Equivalent (GAE). HPLC analysis of each cultivar was found to possessed significant difference of phenolic compounds such as Cyanidine chloride, Myricetin, Chrysin, Quercetin, Delphinidine chloride, Malvidine chloride, Naringenin, Galangin and Caffeic acid.

Different grape cultivar extracts was found to scavenge DPPH by 47-60% and all the clastogenic effects such as chromosomal aberrations induced by Endoxan was found to be reduced significantly with the treatment of grape cultivar extracts in mice. Mitotic index decreased by the Endoxan was also enhanced with the treatment of the grape cultivar extracts. The study concluded that the grape cultivar extracts are the good source of phenolic compounds and because of that the bioactivities shown by it was more effective towards the exogenous harmful agents.

Sheetla *et al.,* 2013, studied changes in biochemical parameters brought about by cyclophosphamide in adult male *Rattus norvegicus*. Cyclophosphamide at the rate of 0.4ml/100g.b.wt was administered in two groups of mice once in a week for a period of 7 and 35 days. After such treatment the levels of protein and creatinine were found to be reduced which indicates that exposure to cyclophosphamide impairs the functional activities of liver and kidney of male *Rattus norvegicus* by interfering enzymatic metabolic activities and protein synthesis.

Tripathi *et al*., 2013, investigated that essential oils of *Foeniculum vulagre* (FEO)was found to possess antimutagenic activity against

cyclophosphamide induced genotoxicity and oxidative stress in mice. Two groups of mice were administered with two different doses of FEO (1, 2 mL/kg body weight of mice) and another group was administered with cyclophosphamide continuously for 3 days and the result showed that there was significant increase in the formation of micronuclei in the positive control group as well as the activities of superoxide dismutase, catalase, glutathione was found to be reduced markedly in the CP treated group of mice. On the other hand CP intoxication increases MDA (end product of lipid peroxidation) content. However, pretreatment of FEO inhibits the micronuclei formation and antagonized the CP induced reduction of SOD, CAT and GSH, also inhibits the increased MDA content in the liver.

Swarnlata *et al.,* 2014, evaluated the antioxidant and hepatoprotective effect of aqueous extract of *Phyllanthus fraternus* (AEPF) against chemotherapeutic drug cyclophosphamide. CP administered intraperitonially at the rate of 200mg/kg.b.wt induced liver damage in mice featuring substantial increase in serum glutamic oxaloacetic transaminase, serum glutamate pyruvate transaminase. CP caused strong oxidative stress which was indicated by increased lipid peroxidation. However, treatment with AEPF showed significant inhibition of oxidative stress and augmented endogenous antioxidants. The tissue injury caused by CP in liver was also found to be reverted to its normal morphological structure of liver with the treatment of AEPF. The study also concluded that AEPF is the promising medicinal herb in complementary chemotherapeutic modalities.

2.11: Plant secondary metabolites and its relationship to cytotoxic activities:

Cytotoxic agents i.e chemotherapy in the treatment of cancer usually targets rapidly dividing cells (Chemotherapy Principles, 2014) in which the cells may either undergo necrosis (the cells will lost their membrane integrity and die as a result of lysis) or apoptosis (cells may

undergo active genetic program of control cell death) where the cells undergoing necrosis do not have appropriate time to show the apoptotic markers (Promega Corporation, 2015) but the cells undergoing apoptosis have well defined cytological and molecular events like shrinkage of cytoplasm and nuclear condensation as well as cleavage of DNA into regularly sized fragments (Promega Corporation, 2015). The cells which are under culture will undergo secondary necrosis besides undergoing apoptosis (Promega Corporation, 2015; Riss *et al*., 2004) which can be measured using various methods and one amongst them is the measuring of cytotoxicty using the 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT). MTT assay measures the reducing ability of the viable cells using a color reagent called formazan. The treatment of cancer cells with cytotoxic drugs causes various serious side effects in normal cells (Saeed *et al*., 2015) so finding an alternative way for the treatment of cancer has been an increasing interest. Pharmacological screening of plants is an important task for the production of new, effective drugs therefore medicinal plants has been screening worldwide for their cytotoxic activity towards the rapidly dividing cancer cells that do not harm the normal cells. Around 60% of anticancer drugs which has been approved today by the United States of America are derived from the natural origin (Stevigney *et al*., 2005; Newman and Cragg, 2007). So many researchers from various disciplines have been gaining interest on the screening of medicinal plants for their cytotoxicity activity through their antioxidant activities.

Doudach *et al.*, 2013, evaluated the antioxidant and cytotoxic properties of various extracts of *Corrigiola telephifolia* Pourr using DPPH and MTT assay. Several cyclohexane, dichloromethane and methanol extracts of the plant were tested for their cytotoxicity activities against WM-266 and CT-26 cell lines by MTT assay. The cytotoxicity activity of cyclohexane extract showed dependent cytotoxic against both the cancer cell lines with the IC 50 value of 70µg/ml in CT-26 and IC 50 value of

120µg/ml in WM-266 while the cytototoxic activity of dichloromethane in WM-266 (80± 4.56 µg/ml) was more active than CT-26 (IC50 value= 70± 6.1 µg/ml). The cytotoxic activity of the methanolic extract of the plant was also active in dose dependent manner with the IC 50 value of 70± 6.1 µg/ml in CT-26 and IC 50value of 160± 4.56 µg/ml. Besides the cytotoxic activity, free radicals scavenging activity of the plant extracts was also tested against DPPH and all the extracts were observed to scavenge the free radicals in dose dependent manner and the extracts showed moderate inhibitory as compared to the standard compound Trolox. The study concluded that the plant extract showed average inhibitory and cytotoxic activity which gives an idea of developing anticancer or pharmaceutical products to treat cancer.

Mariswamy *et al*., 2013, demonstrated that methanolic extract of root, flower, stem and leaves of *Aerva lanata* L. is a potent therapeutic agent. Number of compounds were found to present in the extracts of *A. lanata* when analysed through GC-MS. Major components found in the analysis displayed various bioactivities such as antineurotoxic, anticarcinogenic, antialcoholic, antiviral, anti-inflammatory, antiprotozoal, antiparasitic, antimutagenic, antineoplastic, cytoprotectant etc.

Ghali *et al.,* 2013, have demonstrated the cyto-protective, antiproliferative and antioxidant potential of hydro-alcoholic extract of *Jatropa podagrica.* Total phenolic and flavanoid content of the seeds of the plant extract was found to be higher as compared to that of the leave extracts. Two tumor cell lines such as A549 and PC12 were used for testing the antiproliferative activity of the plant extract through MTT assay. Low concentration of *Jatropa podagrica* extract exhibits protection against hydroxyl free radicals generated in Fenton's reaction causing DNA damages, protein carbonylation and lipid peroxidation. On the other hand, the extract of *Jatropa podagrica* only does not show the generation of hydroxyl free radicals and DNA fragmentation in vitro. The results concluded that the extracts of *Jatropa podagrica* exhibits antioxidant as

well as antiproliferative activity against A549 and PC12 that deserves further research for chemoprevention and treatment of cancer.

Propolis, a natural resinous hive product collected by Bees from plant exudates were analysed for its chemical constituents through GC-MS and all the major peaks in the chromatogram were compared with NIST data library. 44 compounds were found to present in the Indian Propolis of different sources. Following that four propolis extracts were evaluated for their cytotoxic activity at different concentrations (5-320µg/ml) against cell lines; human breast cancer (MCF 7), colon cancer (HCT 116) and prostate cancer (PC 3). The results concluded that all the propolis showed significant cytotoxic activity against all the cancer cell lines despite of the different sources of propolis therefore propolis extracts can be used as a chemopreventive agent (Shubharani *et al*., 2014).

Revathi, *et al*., 2014, screened the phytochemicals present in the leaf extract of *Bruguira cylindrica (B. cylindrica*) and analyzed the bioactive compounds present in it through GC-MS analysis. It was found to contain carbohydrates, Protein, Amino Acids, Lipids, Fatty acids, Fibre, Alkaloids, Flavanoids, Tannin, Tri-terpenoids, Saponins, Phenols/ Gallic acid equivalent, Glycosides, Cardiac Glycosides, Lignin, Volatile Oil and steroids in the phytochemical screening of sequential extracts of *B. cylindrical*.44 bioactive compounds were found to present in the GC-MS analysis out of which, the compounds such as 2-Cyclopenten-1-one, 2-hydroxy (Diterpene: anticancer, anti-inflammatory); 2-Coumaranone (Coumaran compound: antioxidant); 4H-Pyran-4-one, 2, 2-dihydroxy-6-methyl (Flavanoid: anproliferative, antimicrobial); Benzofuran, 2,3-dihydro (Coumaran compound: Antimicrobial, anti-inflammatory); Tetradecanoic acid (Myristic acid: antioxidant, cancer preventive); 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (terpene alcohol: antimicrobial); n- Hexadecanoic acid (Palmitic acid: antioxidant); 3-O-Methyl-d-Glucose (sugar moity: preservative activity); Phytol (Diterpene: antioxidant, anticancer); 9, 12- Octadecanoic acid, methyl ester (Linoleic acid ester:

Cancer preventive, Hepatoprotective); 9-Octadecenal (Z) (Fatty acid: antitumor); 2- Cyclopenten-1-one, 2-Hydroxy (Diterpene: anticancer); Phenol (cytotoxic activity, antimutagenic, anticarcinogenic, antioxidant) etc. present in the ethanolic leaf extract of *B.cylidrica* are found to have strong biological activities.

Bugarin *et al*., 2014, identified that 31 compounds were found to present in the n-hexane extract of *Eucalyptus Gunnii* Hook (EO) when analyzed through GC-MS analysis of *Eucalyptus Gunnii* Hook. The compounds are dominated by oxygenated monoterpenes such as 1, 8, cineole, α-pinene, linalool, α-thujene, 2-β-pinene, β-myrcene etc. The compounds were matched with NIST Library. The n-Hexane extract of *Eucalyptus Gunnii* Hook was tested for its antioxidant activity against DPPH at different concentrations (1.25-15µl/ml) and its scavenging activity was found to be slight and lower than the standard antioxidant compounds BHA, BHT and PG. The EO extract was tested for its antimutagenic activity against spontaneous and induced t-BOOH mutagenesis in *Escherichia coli* IC202 oxyR mutant strain deficient in removing reactive oxygen species (ROS). Antimutagenic activity of EO extract towards the spontaneous mutagenesis was observed to be slight however the extract showed significant reduction towards oxidative mutagen in dose dependent manner. The study concluded that the bioactivities shown by the plant is because of the chemical compositions present in the plant extract.

2.12: Terpenes and antioxidant activity:

Recently terpenes are found to have antimutagenic activity in bacterial reverse assay (Sotto *et al*., 2008). Terpenes and terpenoidsare large and diverse groups of plant secondary metabolites and are the important constituents of essential oils of many plants and flowers. Terpenes and terpenoids are widely used as fragrances in perfumery, flavors in food additives and as alternative medicines in aromatherapy. Terpenes are derived from the isoprene subunits which has the molecular

formula C_5H_8. Isoprene subunits link together from head to tail to form linear chains or rings. As chains of isoprene subunits are built up, resulting classification of terpenes are based on their size such as monoterpene (consist of two isoprene subunit; example: limonene; $C_{10}H_{16}$); hemiterpenes (consist of one isoprene subunit); sesquiterpenes (three isoprene subunits; $C_{15}H_{24}$; three isoprene subunits; example, humulene, farnesol); diterpenes (four isoprene subunits; $C_{20}H_{32}$; example: retinol, retinal, phytal) etc.

Monoterpenes with two isoprene subunits may form linear or cyclic rings and biochemical modifications and oxidation produce the related compounds monoterpenoids. Limonene is one of the examples of monoterpens formed by cyclisation of isoprene subunits.

α-terpinol, linalool, eucalyptol(cineole) and α-pinene are the terpenes from the essential oils and found to have antimicrobial against gram positive and gram negative bacteria and antioxidant activity against DPPH (Zengin and Baysal, 2014).

Methanolic fraction of the roots of the plant *Centaurea centaurium* L. (Asteraceae) showed scavenging activity against DPPH with the IC 50 value of 57µg/ml and the n-hexane fraction of the the root of the plant also showed highest inhibitory action against the α-amylase with the IC 50 value of 150 and 5 µg/ml.n-hexane fractions of the plant contains the fatty acids such as 11,14-eicosadienoic acid methyl ester, 9-octadecanoic acid methyl ester, and 9-octadecanoic acid as well as terpenes such as cypirene, α-zingiberene, β-farnesene, β-santalene, β-bisabolene, β- himachalene and azulene were found to present (Conforti *et al*., 2008). Saroglu *et al*., 2007, investigated the leaves of the plant *Teucrium royleanum* through GC-MS analysisand was observe to contain about 44 compounds out of which 42% of the compounds are sequiterpenes in which β-Santalene and cis-α-bisbolene are main components. The plant extract reacts with stable free radical DPPH slightly in dose dependent manner, concluding that the plant has antioxidant activities. Also Modzelewska *et al*., 2005 reported that

sesquiterpenes, the natural products are the potential anticancer agents that help in the prevention of progression of cancer. Besides all these properties, β-Santalene isolated from *Lavandula angustifolia* Miller (Lamiaceae) has anti-inflammatory and perfumery (Duke, 2008).

Various types of phytochemicals were found to present in the plant *Calendulla officinalis* Linn (Asteraceae) such as terpenoids, flavanoids, coumarins, quinones, volatile oils, carotenoids and others. The plant was observed to exhibit various pharmacological activities such as anti-HIV, anti-cancer (dual activity), anti-inflammatory, hepatoprotective, spasmodic concluding that it is an essential potentially important medicinal plant of human use (Muley *et al*., 2009).

The extracts of *Jatropha ribifolia* found after hydrodistillation were subjected to GC-MS equipped with flame ionization detection (FID) and 49 compounds were found to present in the extract out of which 39.5% represents monoterpenes, 43% of sesquiterpenes and 8.5% of polypropanoids. The main compounds present in the essential oils were β-pinene (9.2%), isoeugenol methyl ester (8.5%), vatirenene (8.4%), α-gurjunene (7.0%), α-pinene(6.4%) and p-menth-1-ene-8-ol(5.2%) of the total 91.4% of oil isolated from the roots of the plant *Jatropha ribifolia.* Nine human cancer lines were taken and tested for the antiproliferative activity of the root extract of the plant *Jatropha ribifolia* using sulphorodamine B assay. The Jatropa oil was found to exhibit the antiproliferative activity on the growth of the cells NCI-H 460(drug reseistant ovarian) with the GI_{50} value of 6.2µg/ml and OVCAR-3 (ovarian) with the GI_{50} value of 8 µg/ml. The fractions were also shown to exhibit antiproliferative activities against the prostate cancer cell line (PC-3) with the GI50 value of less than 0.25 µg/ml. the study thereby concludes that the fractions of the plant extract hasmore antiproliferating activity against the tested cancre cell lines than crude oil (Silva *et al.,* 2015).

34 constituents of essential oils were identified through GC-MS analysis of the leaves of the plant *Zanthoxylum armatum* out of which β-linalool (53.05%), Bergamot mint oil (12.73%), α-Limonene-diepoxide (11.39%), α-pinene (4.08%), β-myrcene (3.69%), D-Limonene(3.10%) etc are the major compounds. The plant extract was tested for its cytotoxic activity using Brine Shrimp Assay and the extract showed 100% mortality rate of cancer cells at its highest concentration (1000µg/ml) with the IC 50 value of 15.90 µg/ml(Barkatullah *et al*., 2013).

Essential oils and oleoresin of the seed of *Carum nigrum* extracted through hydrodistillation and Soxhlet extraction using acetone were subjected to GC-MS ananlysis and was observed to identify the major components such as dillapiole (29.9%), germacrene B (21.4%), β-caryophyllin (7.8%), β-selinine (7.1%) and nothoapiole (5.8%) and many other minor components in the essential oil of the seed of *Carum nigrum*. The components in the oleoresin of the seed of *Carum nigrum* acetone extract was observed to contain major components such as dillapiole (30.7%), thymol(19.1%), nothoapiole(15.23%) and γ-elemene (8.0%) etc. Both the oleoresin and essential oil of the seed of *Carum nigrum* acetone extracts were tested for their antioxidant activity against mustard oil and it monitored peroxidation, thiobarbituric acid, total carbonyl and p-anisidine values of the oil substrate and was observe to reduce the oxidation rate in accelerated conditions as compared to the synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene at 0.02%. Complete zone of inhibition was also observed against *Bacillus cereus* and*Pseudomona aeruginosa* at 2000 and 3000 ppm by the seed extracts of *Carum nigrum* when employed through agar diffusion method. The fruit essential oil also showed 100% mycelia zone inhibition against *Penicillium purpurogenum* and *Acrophialophora fusispora* at 3000ppm in the poison food method. Thereby the study concluded that both the essential oil and oleoresin of the seed of the plant *Carum nigrum* can be

used as an additive in food and pharmaceutical preparations after screening (Singh *et al*., 2006).

32 connstituents were observed to be identified through GC-MS analysis of the leaves of the plant *Solanum Spirale* in which 73.36% of the constituents are oil components. The major components present are E-phytol (48.10%), n-Hexadecanoicacid (7.34%), β-selinene(3.67%), α-selinene(2.74%), octadecanoic acid (2.12%), hexahydrofarnesylacetone(2.00%). The extract was tested for its antioxidant activity against DPPH and it showed weak scavenging activity however it showed significant antibacterial activity against both the gram positive Staphylococcus aureus gram negative Escherichia coli with Minimum Inhibitory Concentration (MIC) values of 43.0µg/ml and 21.5 µg/ml respectively. Cytotoxic activity of the plant extracts were also tested against oral cancer(KB), breast cancer (MCF-7) and small cell lung cancer(NCI-H187) with the IC 50 values of 26.42, 19.69 and 24.02 µg/ml and it was significant (Keawsa-ard *et al*., 2012) .

2.13: Chemical constituents of *Curcuma caesia* Roxb:

The phytochemical studies of *Curcuma caesia* revealed the presence of multiple phytoconstituents like essential oils with camphor, ar-turmerone,(Z) ocemene, ar-curcumene,1,8-cineole, elemene, borneol, bornyl acetate, curcumene etc (Panday and Chowdhary, 2003).

Ghosh *et al*., 2013, isolated a novel terpenoid compound known as (2Z, 2'Z)- 2,2'-(3aR,10aS)- 1,3,5,8,9,9- hexamethyl- 1,2,3,3a-tetrahydrobenzo (f) azulene-4, 10 (5H, 8H, 9H, 10aH)- diylidene) diacetaldehyde using UV, FTIR (IR), HRMS, NMR etc.

Sahu and Saxena, 2014, subjected the crude methanolic extracts of the rhizomes of *Curcuma caesia* Roxb in UV Visible spectroscopy in the wavelength ranging from 200-800nm which showed the peak values at 200nm, 288nm and 300nm. FTIR spectrum profile of the methanolic extract of *Curcuma caesia* Roxb showed the presence of functional groups

such as phenol, cycloalkane, alkene and aromatics and identified the extract to be present as pyrocatechol derivatives.

2.14: Biological activities of *Curcuma caesia* Roxb:

*Curcuma caesia*Roxb; also known as black turmeric is a perennial herb with bluish black rhizomes and it is famous for its medicinal properties. It is recognised as a medicinal herb to possess with various properties such as anti-fungal activity by Banerjee and Nigam, 1976,smooth muscle relaxant and anti-asthmatic activitybyArulmozhi *et al.,* 2006, bronchodilating activity by Paliwal *et al.*, 2011, antioxidant activity by Mangla *et al.* , 2010, anxiolytic and CNS depressant activity, locomotor depressant, anti-convulsant by Karmakar *et al.*, 2011,anthelmintic activity by Gill *et al.*, 2011,anti-bacterial activityby Rajamma *et al*., 2012,anti-ulcer activityby Das *et al*., 2012.