5: DISCUSSION:

Considerable attention has been focused in recent years on the exploration of phytotherapeutic agents for the treatment of oxidative stress and mutation related disorders. The use of medicinal plants is perhaps the oldest method of coping with illness because they can be easily metabolized inside the body without any harmful effects that leads to the phytochemical based remedies (Sangwan *et al*., 1998; Ma and Kineer, 2002; De Flora and Ferguson, 2005; Anetor *et al*., 2008). Reactive oxygen intermediates like superoxides, hydrogen peroxides and hydroxyl radicals are known to mediate macromolecular damages by reacting with nucleic acids, proteins as well as various membrane components thus act as direct and indirect initiators of mutagenesis and carcinogenesis (Halliwell and Gutteridge, 1985). On the basis of this it has been hypothesized that the involvement of antioxidant might be considered as the safest approach in the prevention of process leading to mutagenesis. The chemistry of free radicals is so complicated that it causes a major limitation in the identification of free radical scavenging activity. To withstand this problem the potential antioxidant substance are tested in *in vitro* model and such approaches expand the scope of antioxidant activity. The mechanism that contribute to the antioxidant capacity of phenols and flavanoids present in medicinal plants include free radical scavenging ability, hydrogen or electron donation ability, chelation of redox active metals ions, modulation of gene expression and interaction with the cell signalling pathways (Arouma, 2003) . Therefore, we have examined the rhizome extracts of *Curcuma caesia* Roxb. for its antioxidant activity by DPPH free radical scavenging assay, ABTS radical cation scavenging assay, superoxide anion radical scavenging assay and total phenol content as well as reducing power assay were also determined**.** The different extracts of the rhizomes of *Curcuma caesia* Roxb showed the presence of different kinds of secondary metabolites as tested through preliminary phytochemical screening (table no.5) and total phenol content was found

to be more in EECC followed by MECC>EaECC>AECC (table no. 6 and 7) and according to it further analysis were performed.

5.1: In Vitro Antioxidant Studies:

5.1.1: DPPH radical scavenging assay, Total phenol contents of the extracts:

The use of DPPH assay provides an easy and a rapid way to evaluate the antioxidants present in plants by spectrophotometer (Huang *et al.,* 2005). The purple color of DPPH reduces to light yellow with the intervention of plant extract; the most probable mechanism of action was because of hydrogen donation by the extracts (Prior and Cao, 1999). Out of the four different extracts of rhizome of CC, DPPH radical scavenging activities follows the order of EECC> MECC > EaECC >AECC with their percentage inhibition ranging from -15.27 (least percentage of inhibition at lowest concentration) to 86.91 percentage (table no.9; fig:18). In fact, the tested extracts are the complex mixtures of several compounds which have diverse chemical structures that determine various properties. The rich source of phenolics, flavanoids and terpenes are of increasing interest nowadays because they retard the oxidative degradation of bio molecules (Wojdylo *et al*., 2007; Sotto *et al*., 2008). The chemical structure of phenolic compounds has hydroxyl group attached to benzene ring in its structure and it enables them the ability to act as free radical scavenger (Clark, 2007). When reactive oxygen species are present at a certain concentration the bond between O and H is broken and the released hydrogen ion is made available to nucleophilic radicals which subsequently quenched the free radicals (Rice *et al.*, 1997). The phenol content of the *Curcuma caesia* Roxb. extracts was found to be 52.11mg/GAE for MECC,68.64 mg/GAE for EECC,38 mg/GAE for EaECC and 4.82 mg/gGAE for AECC in 100g of dry weight of the extract in the present study (table no.7). Sarangthem *et al.,* 2010 also confirms that maximum curcuminoids, oil content, flavonoids, phenolics, different

important amino acids, protein and high alkaloids are contained in the rhizome of this species (*Curcuma caesia* Roxb).

5.1.2: Reducing power assay of the extracts:

Antioxidants present in plants have been reported to act as scavengers of singlet oxygen and free radicals in biological systems (Rice-Evans *et al.,* 1997; Jorgensen *et al.*, 1999). As stated by Oyaizu, 1986, plant extracts has the reducing ability to transform Fe^{3+} to Fe^{+2} and reductones are responsible for it (Duh *et al.,* 1999). The reducing agents of plant extracts have been found to exert antioxidant activity by breaking the free radical chains by donating a hydrogen atom (Gordon, 1990). In the present study, the reducing power of extracts of *Curcuma caesia* Roxb.was found to be remarkable and each extract was found to rise the reducing ability as the concentration of the extracts gradually increases. The reducing power of the extracts follows the order EECC> MECC > EaECC >AECC as shown in the table as well as in the graph (fig: 17, table no. 8).

Free radicals are the primary cause of oxidative damage to biological molecules in the human body and are related to many diseases such as cancer, aging, heart diseases etc (Kim *et al*., 2008). ROS oxidizes lipids, proteins, and nucleic acids leading to the cell death or transformation (Gawlik-Dziki *et al*., 2013). However such ROS, which act as secondary signalling pathways in the development of cancer, are interfered by plant secondary metabolites (Dai and Mumper, 2010) which are usually termed as chemoprevention. Chemoprevention is a novel approach for controlling cancer that involves the use of specific natural products from fruits, vegetables, teas, spices and traditional medicinal herbs and they can act as potent anti-inflammatory, antioxidant or anticancer agents (Sumalatha, 2013).

5.1.3: ABTS$^+$ radical scavenging activity:

To validate the antioxidant activity of plant extracts against the free radicals we further extend the evaluation of antioxidant assays against ABTS$^+$ and SOD free radicals generated in the laboratory besides DPPH assay to determine the antioxidant activity of *Curcuma caesia* Roxb, rhizome extracts. ABTS$^+$ assay was based on the ability of different substance to scavenge ABTS$^+$ radical cation. Each extract of the rhizome of *C.caesia* Roxb. at different concentrations (5-100µg/ml) were tested for their scavenging activity against ABTS$^+$ radical cation. The values were compared to that of the standard compound Gallic acid and it was observed that EaECC scavenged the ABTS$^+$ radical cation by 41.15% at its lowest concentration (5µg/ml of EaECC) and 77.93% at the highest concentration (100µg/ml of EaECC) as shown in table no. 10. The inhibitory percentage at its highest concentration is comparable to that of the inhibitory activity of Gallic acid which lies in between 70.57% (60 µg/ml) and 92.78% (80µg/ml) as shown in table no.10, fig: 19. EECC, MECC and AECC have also shown same pattern of ABTS$^+$ scavenging activity. The IC50 value of ethyl acetate extract, ethanolic extract, methanolic extract and aqueous extracts of *C.caesia* are 14.44µg/ml, 43.63µg/ml, 51.994µg/ml and 76.992µg/ml respectively while that of Gallic acid standard is 4.37µg/ml. The order of scavenging activity of *C.caesia* extracts against ABTS$^+$ in terms of IC50 value is EaECC> EECC>MECC>AECC. DPPH and ABTS$^+$ assays share some common features of extracting electrons/hydrogen from the antioxidants present in plants causing the changes in their color with the acceptance of electrons/hydrogens. However comparing the different activities of the extract EECC showed highest antioxidant activity against DPPH and EaECC showed highest antioxidant activity against ABTS$^+$. EECC has highest phenolic content while EaECC has low phenolic content however EaECC showed highest antioxidant activity against ABTS$^+$,

may be because the position and number of phenols present does not affect the scavenging activity of $ABTS^+$ (Nenadis *et al*., 2004).

5.1.4: Superoxide Anion Radical Scavenging Assay:

Superoxide anion radicals are the important class of host defense system originated in electron transport chain with the reduction of electron from molecular oxygen with the help of many enzymes. Although they act as second messengers in host defense mechanisms, their presence beyond the limit may induce cytotoxicity to normal cells by reacting with membrane lipids and induce tissue damage that leads to carcinogenesis (Halliwell and Gutteridge, 1989; Valko *et al*., 2007; Aruoma., 1996; Pulido *et al*., 2000; Evan and Vousden., 2001). In the present study, O_2^- was generated non enzymatically from the dissolved oxygen in the presence of PMS-NADH system that further reduces the NBT to diformazan which is indicated by the presence of blue color and it was measured spectrophotometrically at 560nm (Nishikimi *et al*., 1972). It was found that all the tested extracts at different concentrations (20-200µg/ml) (table no.12 and 13) showed dose dependent scavenging activity of superoxide anion radicals generated non- enzymatically. Amongst all the tested extracts, ethyl acetate extract of *Curcuma caesia* Roxb was found to be most effective followed by other extracts.

5.2: Antimutagenicty Assay:

The properties of antioxidant present in phytochemicals are connected to their ability to forage the free radicals generated from either endogenous or exogenous agents. These preventive agents can inhibit the mutation and cancer initiation process by modulating phase I and phase II enzymes, blocks the reactive species either by scavenging, electron donation or through chelation and finally maintains the DNA structure. The inhibition of mutagenesis by antioxidants are grouped into two namely desmutagens and bioantimutagens and it has been hypothesized

that bioantimutagens act as second stage inhibitors that blocks the mutagen before they could attack the DNA (Ramel *et al*., 1986) and phytochemicals which has bioantimutagenic effect is determined in co incubation method (Hung *et al*., 2009). The different extracts of *Curcuma caesia* Roxb.have shown the following order of antimutagenicity EaECC>EECC>MECC>AECC; against indirect acting mutagen Cyclophosphamide (500µg/plate) (table 14). The results were based on the number of induced revertant colonies detected. According to Ames *et al.,* 1975, a compound is classified as a mutagen if it is able to increase at least twice the number of revertants as compared to spontaneous revertants. Earlier Morffi*et al.,* 2012 have reported the antimutagenic activity of *Magnifera indica* against CP in the strain TA100. Higher mutagenicity was found when CP was activated with S9 but inhibition of this microsomal activity was observed in the presence of rhizome extract. The present results showed the antimutagenic activity in Ames test and it may be attributed in part to powerful radical scavenger associated with the extract. According to Negi *et al.,* 2003, a compound is found to possess its less antimutagenic activity if its percentage of inhibition is less than 25%, a moderate activity if the percentage inhibition values lie between 25% and 40% and a strong antimutagenicity effect if it is more than 40%. Ethyl acetate extract reduces the mutagenicity caused by CP by 93.07% in TA 98 (5000µg/ml) and by 72.55% in TA100 (5000µg/ml) in the presence of S9 which shows strong antimutagenic activity (table no.14). Ethanolic extract follows the reduction of mutagenicity caused by indirect acting mutagen cyclophosphamide by 90.95 % in TA 98 and 72.32% in TA100 at their highest concentration (5000 µg/plate) in the presence of S9 which is again followed by MECC and AECC respectively in the strains TA 98 and TA100 (table no. 14).

5.3: Acute toxicity studies:

Supporting the role of antimutagenic activity we further evaluate the genotoxicity and antigenotoxicity of the rhizome extracts of*Curcuma*

caesia Roxb against cyclophosphamide. Genotoxicity is defined as the property of chemical agents that affects either somatic cells or germ cells causing DNA mutations which may lead to cancer and passed to future generations (Kolle and Susane, 2012). It is therefore an imperative task to identify the molecules which are toxic to cells with predetermined genetic background by cell based assays (Dunstan *et al*, 2002; Siddique and Afzal 2005; Mathur and Bhatnagar, 2007) and needs to be addressed earlier for safety assessment and it has been regarded as an important regulatory program worldwide (Jena *et al*., 2002). The genotoxic studies of naturally occurring substances or plant products are also of great importance because of their widespread used in various forms (Umar *et al*., 2004). The genetic damages can be manifested in the form of gene mutation and chromosomal aberrations and the major cause of chromosomal damage is indicated by the appearance of micronuclei at the end of mitosis and is an indication of genotoxic insult in the nuclei (Salam *et al*., 1993).

Before studying further experiments we briefly examined about the acute toxicity of plant extracts. The definition of acute toxicity lies behind the fact that a substance results in undesirable effects either from single or multiple exposure, usually within 24 and adverse effects occur within fourteen of administration of the substance (The MSDS Hyper Glossary: Acute Toxicity, 2006 and IUPAC, Compendium of Chemical Terminology, 2006). It has been suggested that acute toxicity studies should have to be performed for a substance to select the dose at or above human suggested doses for chronic and sub chronic studies (Rhiouani *et al*., 2008). In the present investigation *C.caesia* Roxb rhizome extract was subjected to acute toxicity as per the guidelines no 420. Accordingly, the study was planned with stepwise treatment of fixed doses to observe any gross behavioral changes during the study period. The results have revealed that *C.caesia* Roxb rhizome extract

was well tolerated upto 2000mg/kg.b.wt with no adverse effects observed (table no. 15, 16 and 17).

5.4: Antigenotoxicity Assay:

In continuation of acute toxicity, the present study also concerns about the genotoxic potential of the rhizome extracts of *Curcuma caesia* Roxb and antigenotoxic potential of the extracts against cyclophophamide using mice bone marrow micronucleus assay. Bone marrow are the rich source of vascularised rapidly cycling cells which are vulnerable to aberrations by clastogens through the inhibition of DNA repair processes or may also involve the generation of free radicals in mice, which further binds to the DNA bases i.e purines and pyrimidines leading to base substitutions and breakage of DNA and ultimately induces mutation (Sang and Li, 2004). Micronuclei originate from the chromosomal breakage done by clastogenic event and are unable to migrate with the rest of the chromosome during anaphase cell division. Therefore micronucleus assay is regarded as an assay for chromosomal aberration. Micronucleus test has now been regarded as one of the most successful and reliable test system for genotoxic carcinogens causing DNA damage (OECD guidelines, 2007) and can be detected using DNA dyes and their frequency can be quantified microscopically (Nusse *et al*., 1994). The in vivo micronucleus assay is normally conducted by examining polychromatic erythrocytes in bone marrow of mice for the presence of micronuclei (Krishna and Hayashi, 2000). In the present study, cyclophosphamide; a clastogenic agent that can induce micronuclei in the mice bone marrow cells is administered intraperitonially to determine the increase number of micronuclei formation.

The chemotherapeutic agent cyclophosphamide has been used for the treatment of various types of cancer however IARC 1987 has reported that cyclophosphamide induces mutation and is highly carcinogenic for both animals and humans. Reactive metabolites of CP

crosslink purine bases of DNA affecting DNA replication, transcription, translation processes leading to decrease in the activity of endogenous antioxidants (Ray *et al*., 2010) as well as damages DNA, resulting in the formation of micronuclei (MN) and death of normal cells (Morre *et al*., 1994; Murata *et al*., 2004). Micronuclei are the acentric form of chromatid and chromosome and detection of it has been regarded as the potential effect of clastogens (Hosseinimehr *et al.,* 2008). In the present study, number of micronuclei in CP (50mg/kg. b. wt) treated group (PC group) (table no. 18, 19, 20 and fig: 26 and 27) was observed to be increased significantly ($p< 0.002$) as compared to the negative control group. However, the pre treatment of all extracts of C. *caesia* Roxb. at different concentrations (100mg, 250mg, 500mg/kg.b.wt) showed dose dependent inhibition of micronuclei significantly ($p<0.01$ and $p<0.05$). The obtained result was in agreement with the previous studies on the pre treatment of plant extracts of *Solanum lycopersicum* (Devi *et al*., 2014) and *Nasturtium officinale* (Cassanova *et al*., 2013) which has shown the inhibition of micronuclei formation. These results suggest that the plant extracts can prevent the interaction of DNA and reactive metabolites of CP. C.*caesia* Roxb.is a good source of antioxidant and antimutagenic activity (Devi *et al*., 2015) and it is said that plants possessing antimutagenic activity help in the prevention of cancer and other diseases (Berhow *et al*., 2000; Nishino, 1998; Surh and Ferguson, 2003). Many natural antioxidants are also found to protect against oxidative damage including DNA damage, risk of cancer and other degenerative diseases caused by reactive oxygen species (Vilela *et al*., 2008). The present study showed no sign of toxicity for all the extracts tested i.e there was no sign of genotoxicity caused by extracts at the tested concentrations as the value of micronuclei formation was not significantly different from the negative groups (table no.18, 19, 20 and fig: 27).

5.5: Biochemical Analysis:

5.5.1: SGOT and SGPT Analysis:

It is said that administration of CP leads to the production of free radicals: Phosphoramide and Acrolein, acrolein induces nephrotoxicity (Singh *et al,* 2014) and hepatotoxicity (Chakraborty *et al*, 2009). Phosphoramides alkylates (Singh *et al*, 2014) cellular proteins and other macromolecules with a continuous attack on the membrane lipids such as poly unsaturated fatty acids in the presence of oxygen to produce lipid peroxides as a result of which the cytosolic enzymes SGOT and SGPT leak out leading to their raised levels in the blood, ultimately leading to depression in protein synthesis, and many other cytosolic enzymes (Senthilkumar *et al*, 2006). Therefore it is an important task to measure the activities of various enzymes in the tissues and body fluids that plays a significant role in disease investigation and diagnosis (Malomo 2000) that bother the organs/tissues such as liver, kidney, bone marrow etc and to a reasonable extent, the toxicity of the drug (Yakubu *et al*, 2003).

Liver is the organ for detoxification while kidney helps in the excretion of toxic substance and they are very susceptible to toxic agents (Kumar *et al*., 2005). SGOT and SGPT are useful biomarkers for liver injury (Johnston 1999;McClatchey and Kenneth; 2002,Mengel *et al.,* 2005) and the increase level of SGOT and SGPT in the serum is an indication of liver damage induced by cyclophosphamide. Present study reveals the increase level of SGOT and SGPT in the serum of cyclophosphamide treated mice significantly ($p<0.01$ for SGOT; $p<0.0001$ for SGPT) as compared to negative control groups (table no.21). However pretreatment of all extracts prior to administration of CP at the dose of 50mg/kg.b.wt reduces the leakage of SGOT and SGPT from the liver as indicated in the table no.21. Besides these effects, some other biochemical analysis were also evaluated to observe the adverse effects cause by the reactive metabolites of CP and the protective effects of *C.caesia* Roxb against it.

5.5.2: Lipid Peroxidation Assay in both liver and kidney of mice:

Lipid oxidation is a chain reaction that leads to the damage of structure and function of membrane phospholipids and forms unstable hydroperoxides. The further degradation of hydroperoxides forms various secondary products under the influence of peroxyl, alkoxyl radicals. The attack of peroxyl and alkoxyl radicals on the membrane phospholipids forms an end product MDA that disrupts the cell membrane, thereby increasing permeability to ions (Devasagayam*et al*., 2003; Anoopkumar *et al.,* 2001). MDA on reaction with TBA produces TBARS which is indicated by the presence of pink color indicating the toxicity to the membrane phospholipids which can be measured spectrophotometrically at 532nm (Halliwell and Gutteridge, 1989). The intoxication of mice with CP induced oxidative stress and liver damage has been already reported by Swarnlata *et al*., 2014; Habibi *et al*., 2014 and was also observed in the kidney of CP treated mice (Monika *et al*., 2014; Muneeb *et al*., 2012), since the physiological, anatomical and biochemical features of kidney makes it more sensitive to xenobiotics toxicants including drugs (Muneeb *et al*., 2012). In the present study lipid peroxidation was observed to be more in both the liver and kidney of only cyclophopsphamide treated mice as compared to the negative control group significantly (table no.22,24 and fig: 29, 33) but there was no sign of toxicity in the liver and kidney with the treatment of extracts only. Pretreatment of all extracts at different concentrations prior to the administration of CP reduces the peroxidation level caused by CP in both the liver and kidney (table no.22, 24 and fig: 29, 33).

5.5.3: GSH, GR and Protein Assay in both liver and kidney of mice:

Enzymatic antioxidants also decayed with the treatment of CP (Manda and Bhatia, 2003) and measurement of their activities has been regarded as one of the most important factor to investigate the toxicity of the drug to organ and tissues (Malomo, 2000;Yakubu *et al*., 2003). The antioxidant GSH has the ability to resist oxidative stress by donating its

electrons/H^+ to unstable molecules (Pompella, 2003; Narciso *et al*., 2013) and maintains a reduced environment in healthy cells and tissues (Deponte, 2013; Meister, 1988; Mannervik, 1987). Reactive metabolites of CP (acrolein) conjugate with GSH resulting in the formation of glutathionylpropionaldehyde which induces oxidative stress that leads to the depletion of GSH (Anna *et al*., 2001). GSH, as an antioxidant can detoxify toxic substances by conjugation (Peklak *et al*., 2005) however, the direct coupling with CP and its metabolites leads to the depletion of GSH (Yuan *et al*., 1991). The present study also confirms the decrease level of GSH in the positive control group of both kidney and liver significantly ($p<0.0001$ in liver and $p< 0.05$ in the kidney) (table no.22 and 24) as compared to the negative control group (fig: 30 and 34). It was also observed that the activity of GR and protein in the CP intoxicated mice was observed to be decreased significantly in both the tissues (table no.23 and 25; fig: 31, 32, 35 and 36). But, the pre treatment of all extracts increases the level of GR significantly in both liver and kidney similar to the findings of Muneeb *et al*., 2012. A large number of plants also have been reported to induce GSH (Sharma *et al*., 1999; 2000; Singh *et al*., 2000). The animals which are pre treated with the *Curcuma caesia* Roxb extracts also showed induction of GSH, GR and protein increasing their levels in both liver and kidney.

5.6: Histopathological Analysis:

5.6.1: Histopathological Analysis of liver:

The protective effect of EaECC, EECC, and MECC was further supported by the histopathological analysis of both liver and kidney (fig: 38-43). It has been suggested that alterations in histoarchitechture in the major organs like liver and kidney are the most consistent result of toxic manifestations. The diseases related to the liver arise due to the proliferation of fibrosis as well as liver damage resulting in the distortion of normal structure as well as impairment of liver functions. Enzymes that can metabolize the drugs are located in the liver microsomes which

help in transforming drugs and xenobiotics that can lead to either detoxification of the reactive metabolites produced by the drug or may increase the toxicity (Athar, *et al*., 1997; Plaa, 1991). Thus liver acts as main organ targeted by toxic chemicals and drugs. Many toxic chemicals and drugs have been reported to cause severe damages in the liver of human beings which are difficult to manage with medical therapies. The reactive metabolites of CP are so fatal that it induces hepatic damage in humans as well as experimental animals (Chakraborty *et al*., 2009; Stankiewicz *et al*., 2002; Swarnlata *et al*., 2014). CP treated liver showed degeneration of hepatocytes in the present study, however pretreatment of *C.caesia* Roxb extracts at different concentrations (100, 250, 500mg/kg b.wt) almost normalized the toxic effects in dose dependent manner (fig: 38, 39 and 40) .

5.6.2: Histopathological Analysis of kidney:

Kidney also contains variety of metabolizing enzymes that can act as xenobiotic transporters, so they are also the target organs for xenobiotic toxicants including drugs (Muneeb *et al*., 2012). Half life of cyclophosphamide is about 6.5hrs, so very small amount of it is excreted from the kidney since its absorption is high (Milsted *et al*., 1982). Acrolein, the metabolic product of CP is highly reactive and found to damage kidney cells (Muneeb *et al*., 2012, Kennedy *et al*., 2013) inducing nephrotoxicity (Singh *et al*., 2014) resulting in urine sediment abnormalities, electrolyte imbalances and most commonly decline in glomerular fiitration rate (Nolin and Himmelfarb,2010) . Cyclophosphamide treated rats showed glomerular nephritis, interstitial edema, cortical tubular vacuolization (Muneeb *et al*., 2012; Kennedy *et al.,* 2013; Singh *et al.,* 2014). In the present study treatment of mice with CP only shows tubular necrosis, desquamation of epithelial cells, and invasion of inflammatory cells in the cortex and medullary regions of kidney. The key histological finding of this study is that pretreatment of EaECC, EECC, MECC influenced the recovery of kidney architecture

induced by CP (fig 41-43). Kidney section of positive control group showed loss of normal renal architecture, causing inflammatory cell infiltration and fatty changes with swelling of cells (fig 27-29B) and the liver section of CP treated group showed inflammation in the central vein, decreasing sinusoidal spaces (fig 24-26B). However the pre-treatment of EECC at different concentrations (100, 250 and 500mg/kg. b. wt of mice) showed the improvement of kidney and liver morphology (fig 24-26). The results obtained are similar to the works of (Muneeb *et al*., 2012; Lata *et al*., 2014).

5.7: Cytotoxicty Assay of the extracts against cancer cell lines:

Nowadays the antioxidant activity of the tested material has been verified with the measurement of antiproliferating activity against various carcinoma cells (Ghali *et al.,* 2013; Dela Rosa *et al*., 2014; Loizzo *et al*., 2014). Most recent studies on medicinal plant extracts have revealed that plants of various parts are the good source of antioxidants and has antiproliferating activity (Baharum *et al*., 2014; Kokosa *et al*., 2015; Lim *et al*., 2004) and they have been reported to have long history in the treatment of cancer (Hartwell, 1971; Conforti *et al*., 2008; Jain and Jain, 2011). MTT assay is a widely used to evaluate the safety of plant extracts in the laboratory (Fatemeh and Khorso, 2013). MTT is a yellow water soluble salt and the succinate dehydrogenase exnzymes of metabolically active cells convert yellow color of MTT to an insoluble purple colored product, formazan by reductive cleavage of tetrazolium ring. The cells are solubilised with an organic solvent (isopropanol/ DMSO) and then releasesformazan and formazan serves as an estimate of the number of mitochondria and the number of living cells in the sample (Meerloo *et al.,* 2011; Aziz, 2006; Shakya *et al*., 2014). The goal of the present study was to determine whether the plant extracts tested exerted an inhibitory effect on the cancer cell proliferation in the studied cancer cell lines (table no. 26 and 27). According to Atjanasuppat *et al.,* 2009*,* a plant extract is regarded to have high antiproliferative active if

their IC50 value is ≤ 20 μg/ml which is reagarded as an active form, IC50 > 20-100 μg/ml, which is denoted as moderately active, IC 50> 100- 1000 μg/ml is regarded as weakly active. However United States National Cancer Institute plant screening program stated that a crude extract will be generally considered to have cytotoxicty activity if its IC 50 value is < 30-40 μg/ml (Oskoueian *et al*., 2011). The results of our study suggest that EaECC has highest antiproliferating activity against the tested cell lines with the IC 50 value of 35.89 μg/ml in MDAMB cells and 38.405 μg/ml in Calu6 cells. The moderate antiproliferating activity was shown by other extracts EECC, MECC with the IC 50 value of 82.12 μg/ml, 89.33 μg/ml in MDAMB cells respectively and the IC 50 value of EECC, MECC in Calu 6 were 93.24 μg/ml and 83.84 μg/ml respectively.

5.8: GC-MS and UV-Visible Spectral Analysis of EaECC and EECC.

Natural products play an important prominent role in the development of drugs which have found important applications in the field of treatment and prevention of various diseases (Sandosh *et al*., 2013; Pico and Cozmutza, 2007; Zer *et al*., 2005). A large number of medicinal plants have been shown to have therapeutic potentials by many researchers from different fields. Therefore in order to promote the use of the medicinal plant it is an important task to investigate the compositions present in it and further validate its uses (Nair and Chanda 2006). The in vitro screening of phytoconstituents present in plants are mainly resoluted by often laborious and expensive techniques such as GC/LC-MS or GCMS and GC-MS can identify pure compounds present at less than 1gm (Eisenhauer *et al*., 2009; Liebler *et al*., 1996). However simple cost effective, rapid test such as UV-Visible methods can also be used in this sense as well as conventional methods (Sandhosh *et al*., 2013; Sahaya *et al*., 2012) and UV-Visible spectroscopy has been proved

as a reliable and sensitive method for the detection of biomolecular components (Sandosh *et al.,* 2013; Sahaya *et al*., 2012).

In recent years spectroscopic methods have become as a key factor for profiling secondary metabolites present in both plant and non plant species (Fernie *et al*., 2004; Robertson, 2005). The results of GC-MS analysis in the tested solvent extracts of rhizomes of *Curcuma caesia* Roxb reveals the existence of various chemical functional groups (table 28 and 29; figure: 46 and 47) and UV-Visible spectrum of EaECC showed the characteristic absorption peak at 300 and 380nm while the absorption peak of EECC showed at 200, 300nm (table no. 30 and 31 and fig: 48 and 49). According to Sahu and Saxena 2013, absorption bands of flavanoids are devided in two regions in UV- Visible Spectrum, region: 230-290nm is regarded as band I for flavanoid absorption maxima and region 300-350nm is regarded as band II absorption maxima for flavanoids. Adopting these values, the present study observes that EaECC lies in the absorption region II at 300nm with the absorption of 1.196 while EECC lies in both regions at 200nm and 300nm with the absorption values of 0.748 and 1.067. The relative precise positions and intensities of maxima give valuable information on the nature of the flavanoids. May be because of the presence of various compounds in the rhizome of *Curcuma caesia* Roxb, the bioactivities shown by the plant is so effective. Therefore the results of the present study may help in the development of novel medicines for using in treatment of cancer and other diseases.