Chapter V

DISCUSSION

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5.1. Qualitative and Quantitative Phytochemical Estimation

The Plant Secondary metabolism produces products that aid in the growth and development of plants but are not required for the plant to survive. The Secondary metabolism facilitates the primary metabolism in plants. The primary metabolism consists of chemical reactions that allow the plant to live. In order stay healthy, secondary metabolism plays a pinnacle role in keeping all the of plants' systems working properly. A common role of secondary metabolites in plants is defence mechanisms. They are used to fight off herbivores, pests, and pathogens. Although researchers know that this trait is common in many plants and it is still difficult to determine the precise role each secondary metabolite. Secondary metabolites are used in anti-feeding activity, toxicity or acting as precursors to physical defence systems.

The Secondary metabolites are organic compounds that are not directly involved in the normal growth, development, or reproduction of an organism. (Fraenkel and Gottfried, 1959). The Secondary metabolites are often restricted to a narrow set of species within a phylogenetic group.Secondary metabolites often play an important role in plant defence against herbivory (Stamp and Nancy, 2003) and other interspecies defences. (Samuni-Blank *et al,* 2012) Humans use secondary metabolites as medicines, flavourings, and recreational drugs.

A steroid is a type of Organic compound that contains a characteristic arrangement of four cycloalkane rings that are joined to each other. The steroids include the dietary lipid cholesterol, bile acids, the sex hormones estradiol and testosterone and the anti-inflammatory drug dexamethasone. Steroids are a class of organic compounds with a chemical structure that contains the core of gonane or a skeleton derived therefrom. Usually, methyl groups are present at the carbons C-10 and C-13 – an alkyl side-chain at carbon C-17 may also be present. Hundreds of distinct steroids are found in plants, animals and fungi. All steroids are made in cells either from the sterols lanosterol (animals and fungi, see below right) or from cycloartenol (plants). Both lanosterol and cycloartenol are derived from the cyclization of thetriterpene squalene.Steroids along with phospholipids function as components of cell membranes. Steroids such as cholesterol decrease membrane fluidity. (Sadava *et al,* 2011) Steroidogenesis is the biological process by which steroids are generated from cholesterol and transformed into other steroids. (Hanukoglu, 1992)

The Sugar, the monosaccharides and disaccharides, which are smaller (lower molecular weight) carbohydrates, are commonly referred to as sugars. Saccharides and their derivatives include many other important biomolecules that play key roles in the immune system, fertilization, preventing pathogenesis, blood clotting, and development. (Rhoades and David, 1979)

Alkaloids are produced by a large variety of organisms, including bacteria, fungi, Plants, and animals, and are part of the group of natural products. Many alkaloids can be purified from crude extracts by acid-base extraction. Many alkaloids are toxic to other organisms. They often have pharmacological effects and are used as medications, as recreational drugs, or in entheogenic rituals. The local anaesthetic and stimulant cocaine, the psychedelic psilocin, the stimulant caffeine, nicotine, the analgesic morphine, the antibacterial barbering, the anticancer compound vincristine, the anti-hypertension agent reserpine, the cholinomimetic galantamine, the spasmolysis agent atropine, the vasodilator vincamine, the anti-arrhythmia compound quinidine, the anti-asthma therapeutic ephedrine, and the antimalarial drug quinine. Although alkaloids act on a diversity of metabolic systems in humans and other animals, they almost uniformly invoke a bitter taste. (Rhoades and David, 1979)

The Flavonoids are one class of secondary plant metabolites that are also known as Vitamin P or citrin. These metabolites are mostly used in plants to produce yellow and other pigments which play a big role in colouring the plants. In addition, the Flavonoids are synthesized by the phenylpropanoid metabolic pathway where the amino acid phenylalanine is used to produce 4-coumaryol-CoA, and this is then combined with malonyl-CoA to produce chalcones which are backbones of Flavonoids (Crozier *et al.* 2006) Chalcones are aromatic ketones with two phenyl rings that are important in many biological compounds. The closure of chalcones causes the formation of the flavonoid structure. Flavonoids are also closely related to flavones which are actually a sub class of flavonoids, and are the yellow pigments in plants. In addition to flavones, 11 other subclasses of Flavonoids including, isoflavones, flavans, flavanones, flavanols, flavanolols, anthocyanidins, catechins (including proanthocyanidins), leukoanthocyanidins, dihydrochalcones, and aurones.

The Phenolic compounds are widely distributed in nature. Their chemical structures may vary greatly, including simple phenols (C6), such as hydrobenzoic acid derivatives and catechols, as well as long chain polymers with high molecular weight, such as catechol melanins (C6)6, lignins (C6-C3) n and condensed tannins (C6-C3-C6) n. Stilbenes (C6-C2-C6) and flavonoids (C6- C3-C6) are phenolic compounds with intermediate molecular weight that inters many pharmacological and biological activities. Flavonoids, including anthocyanins, flavonols (such as quercetin and myricetin), isoflavones (such as daidzein and genistein) and others are formed by multiple biosynthetic branches that originate from chalcone. Phenolic compounds have been widely fractionated in medicinal, aromatic and food plants using CC. Repeated silica gel, sephadex-LH20, RP-18, RP-8, MCI-gel, diaion and toyopearl chromatography columns have been used to fractionate simple phenolics, flavonoids and tannins from kernels and nuts (Zhang *et al,* 2009 and Karamac, 2009), fruits such as apples, Morus nigra, Punica granatum (Lee *et al,* 2010 and Pawlowska *et al,* 2008); olive oil (Khanal *et al*, 2011), tea (Gao *et al,* 2010 and Liu *et al*, 2009), seeds such as lentils (Amarowicz and Karamac 2009), medicinal species, including Ulmus davidiana and Tridax procumbens (Jung *et al,* 2008 and Agrawal, 2011); and aromatic plants, including mint and sage (She *et al*, 2010 and Wang *et al,* 1998).

Tannins, Maximilian Nierenstein studied natural phenols and tannins (Drabble and Nierenstein. 1907). Found in different plant species. Working with Arthur George Perkin, he prepared ellagic acid from algarobilla and certain other fruits in 1905. (Perkin and Nierenstein. 1905). He suggested its formation from galloyl-glycine by *Penicillium* in 1915. (Nierenstein. 1915). Tannase is an enzyme that Nierenstein used to produce m-digallic acid from gallotannins. (Nierenstein. 1932). He proved the presence of catechins in cocoa beans in 1931. (Adam *et al.* 1931) He showed in 1945 that luteic acid, a molecule presents in the myrobalanitannin, tannin found in the fruit of *Terminaliachebula*, is an intermediary compound in the synthesis of ellagic acid.(Nierenstein and Potter, 1945). At present molecule formulas were determined through combustion analysis. The discovery in 1943 by Martin and Synge of paper chromatography provided for the first time the means of surveying the phenolic constituents of plants and for their separation and identification.

5.2. GCMS Analysis

Correa and Alcantara (2010) reported that ethanolic extract of *Mussaenda frondosa* has been subjected to GC-MS analysis. Twenty chemical constituents have been identified. The major chemical constituents are (-)-Quinic acid (32.87%), 4- (IE)-3-Hydroxy-1-Propenyl)-2-methoxy phenol (8.30%), Naphthalene, decahydro-2-methoxy-(7.20%), 1, 2, 3- Benzenetriol (7.70%) (Ahmed John and Koperuncholan, 2012) The plant leaves were carefully removed and washed with sterile distilled water, separately. Thereafter, the dried soil and plant samples were crushed by agate mortar and pestle. The samples were treated with aqua-regia mixture (i.e. HCl:HNO3= 3:1) in Teflon bomb and were incubated at 140 °C for 2-3 days after dried and sieved samples.(Ahmed John andAnnadurai, 2015). The bioactive compounds of *Polygonum glabrum* have been evaluated using-MS (Ramesh *et al,* 2014). The chemical compositions of the whole plant ethanol extract of *P. glabrum* were investigated using Perkin-

Elmer Gas Chromatography-Mass Spectrometry, while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library (Koperuncholan and Ahmed John 2011). GC-MS analysis of *P. glabrum* whole plant ethanol extract revealed the existence of the ether compound –Propane 1,1-diethoxy- (64.86%), alkane compound -2-Heptane, 5-ethyl-2,4-dimethyl- (13.51%), sulphur compound –Tiophene-2-Carboxamide, N-(2-furfuryl)- (8.11%), alcoholic compound - 1,14-Tetradecanediol (5.41%), and plasticizer compounds -1,2-Benzenedicarboxylic acid, isodecyloctyl ester5.41%) and 1,2,3-Benzenetriol (2.79%). The results of this study offer a base of using *P.glabrum* as herbal alternative for the synthesis of antimicrobial agents (Cho *et al,* 2010).

5.3. Heavy Metals Studies

5.3.1. Heavy Metals *vs* Plants

Heavy metals are significant environmental pollutants, and their toxicity is a problem of increasing significance for ecological, evolutionary, nutritional and environmental reasons. The term ‘‘heavy metals’’ refers to any metallic element that has a relatively high density and is toxic or poisonous even at low concentration (Lenntech Water Treatment and Air Purification, 2004). ‘‘Heavy metals’’ in a general collective term, which applies to the group of metals and metalloids with atomic density greater than 4 g/cm^3, or 5 times or more, greater than water (Hawkes, 1997). The chemical properties of the heavy metals are the most influencing factors compared to their density. Heavy metals include lead (Pb), cadmium (Cd), nickel (Ni), cobalt (Co), iron (Fe), zinc (Zn), chromium (Cr), iron (Fe), arsenic (As), silver (Ag) and the platinum group elements. The

Environment is defined as totally circumstances surrounding an organism or group of organisms especially, the combination of external physical conditions that affect and influence the growth, development and survival of organisms. (Annadurai and Ahmed John, 2015).

The Heavy metal toxicity in plants varies with plant species, specific metal, concentration, chemical form and soil composition and pH, as many heavy metals are considered to be essential for plant growth. Some of these heavy metals like Cu and Zn either serve as cofactor and activators of enzyme reactions e.g., informing enzymes/substrate metal complex (Mildvan, 1970) or exert a catalytic property such as prosthetic group in metalloproteins. These essential trace metal nutrients take part in redox reactions, electron transfer and structural functions in nucleic acid metabolism. Some of the heavy metal such as Cd, Hg and as is strongly poisonous to metal-sensitive enzymes, resulting in growth inhibition and death of organisms.

An alternative classification of metals based on their coordination chemistry, categorizes heavy metals as class B metals that come under non-essential trace elements, which are highly toxic elements such as Hg, Ag, Pb, Ni (Nieboer and Richardson, 1980). Some of these heavy metals are bioaccumulative, and they neither break down in the environment nor easily metabolized. Such metals accumulate in ecological food chain through uptake at primary producer level and then through consumption at consumer levels. Plants are stationary, and roots of a plant are the primary contact site for heavy metal ions. In aquatic systems, whole plant body is exposed to these ions. Heavy metals are also absorbed directly to the leaves due to particles deposited on the foliar surfaces.

5.3.2. Effects of Heavy Metals on Plants

Like all living organisms, plants are often sensitive both to the deficiency and to the excess availability of some heavy metal ions as essential micronutrient, while the same at higher concentrations and even more ions such as Cd, Hg, as are strongly poisonous to the metabolic activities. Researchers have been conducted throughout the world to determine the effects of toxic heavy metals on plants (Reeves and Baker, 2000). Contamination of agricultural soil by heavy metals has become a critical environmental concern due to their potential adverse ecological effects. Such toxic elements are considered as soil pollutants due to their widespread occurrence and their acute and chronic toxic effect on plants grown of such soils.

5.3.3. Effects of Zinc on Plants

Zinc (Zn) is an essential micronutrient that affects several metabolic processes of plants (Cakmak and Marshner, 1993) and has a long biological half-life. The phytotoxicity of Zn and Cd is indicated by decrease in growth and development, metabolism and an induction of oxidative damage in various plant species such as *Phaseolus vulgaris*and *Brassica juncea*. Cd and Zn have reported to cause alternation in catalytic efficiency of enzymes in *Phaseolus vulgaris* and pea plants. The Concentrations of Zn found in contaminated soils frequently exceed to those required as nutrients and may cause phytotoxicity. The Zn concentrations in the range of 150–300 mg/kg have been measured in polluted soils. High levels of Zn in soil inhibit many plant metabolic functions, in retarded growth and cause senescence. Zinc toxicity in plants limited the growth of both root and shoot. Zinc toxicity also causes chlorosis in the younger leaves, which can extend to older leaves after prolonged exposure to high soil Zn levels. The chlorosis may arise partly from an induced iron (Fe) deficiency as hydrated Zn^2 and Fe^2 ions have similar radii (Marschner, 1986). Excess Zn can also give rise to manganese (Mn) and copper (Cu) deficiencies in plant shoots. Such deficiencies have been ascribed to a hindered transfer of these micronutrients from root to shoot. This hindrance is based on the fact that the Fe and Mn concentrations in plants grown in Zn-rich media are greater in the root than in the shoot. Another typical effect of Zn toxicity is the appear-ance of a purplish-red colour in leaves, which is ascribed to phosphorus (P) deficiency (Lee *et al.* 1996).

5.3.4. Effects of Cadmium on Plants

The regulatory limit of cadmium (Cd) in agricultural soil is 100 mg/kg soil (Salt *et al,* 1995). Plants grown in soil containing high levels of Cd show visible symptoms of injury reflected in terms of chlorosis, growth inhibition, browning of root tips and finally death (Guo *et al,* 2008). The inhibition of root Fe (III) reductase induced by Cd led to Fe (II) deficiency, and it seriously affected photosynthesis. In general, Cd has been shown to interfere with the uptake, transport and use of several elements (Ca, Mg, P and K) and water by plants. Cd also reduced the absorption of nitrate and its transport from roots to shoots, by inhibiting the nitrate reductase activity in the shoots. Appreciable inhibition of the nitrate reductase activity was also found in plants of *Silenecucubalus* (Mathys, 1975). Nitrogen fixation and primary ammonia assimilation decreased in nodules of soybean plants during Cd treatments (Balestrasse *et al,* 2003). Metal toxicity can affect the plasma membrane permeability, causing a reduction in water content; in particular, Cd has been reported to interact with the water balance. Cadmium treatments have been shown to reduce ATPase activity of the plasma membrane fraction of wheat and sunflower roots. Cadmiumproduces alterations in the functionality of membranes by inducing lipid peroxidation and dis-turbances in chloroplast metabolism by inhibiting chloro-phyll biosynthesis and reducing the activity of enzymes involved in CO_2 fixation (De Filippis and Ziegler, 1993).

5.3.5. Effects of Copper on Plants

Copper (Cu) is considered as a micronutrient for plants (Thomas *et al*, 1998) and plays important role in CO_2 assimilation and ATP synthesis. Cu is also an essential component of various proteins like plastocyanin of photo-synthetic system and cytochrome oxidase of respiratory electron transport chain. Oxidative stress causes disturbance of metabolic pathways and damage to macromolecules. Copper toxicity affected the growth of Alyssum montanum and Cd of cucumber and *Brassica juncea*. The Copper and Cd in com-bination have affected adversely the germination, seedling length and number of lateral roots in *Solanummelongena* (Neelima and Reddy, 2002).

5.3.6. Effects of Chromium on Plants

Chromium (Cr) compounds are highly toxic to plants and are detrimental to their growth and development. Although some crops are not affected by low Cr (3.8 9 10^{-4}lM) concentrations, Cr is toxic to higher plants at 100 l kg^{-1} dry weight (Davies *et al,* 2002). Since seed germination is the first physiological process affected by Cr, the ability of a seed to germinate in a medium containing Cr would be indicative of its level of tolerance to this metal. Seed germination of the weed Echinochloacolona was reduced to 25% with 200lM Cr. High levels (500 ppm) of hexavalent Cr in soil reduced germination up to 48% in the bush bean *Phaseolus vulgaris* (Parr and Taylor, 1982). (Peralta *et al*, 2001) found that 40 ppm of Cr (VI) reduced by 23% the ability of seeds of Lucerne (Medicago sativa cv. Malone) to germinate and grow in the contaminated medium. Reductions of 32–57% in sugarcane bud germination were observed with 20 and 80 ppm Cr, respectively. The reduced germination of seeds under Cr stress could be a depressive effect of Cr on the activity of amylases and on the sub-sequent transport of sugars to the embryo axes. Protease activity, on the other hand, increases with the Cr treatment, which could also contribute to the reduction in germination of Cr-treated seeds. Decrease in root growth is a well-documented effect due to heavy metals in trees and crops (Tang *et al,* 2001). (Prasad *et al*, 2001) reported that the order of metal toxicity to new root chromium stress is one of the important factors that affect photosynthesis in terms of CO_2 fixation, electron transport, and photophosphorylation and enzyme activities. In higher plants and trees, the effect of Cr on photosynthesis is well documented. it is not well understood to what extent Cr-induced inhibition of photo-synthesis is due to disorganization of chloroplasts' ultra-structure, inhibition of electron transport or the influence of Cr on the enzymes of the Calvin cycle. Chromate is used as a Hill reagent by isolated chloroplast. The more pronounced effect of Cr(VI) on PS I than on PS II activity in isolated chloroplasts has been reported by (Bishnoi *et al,* 1993) in peas. In whole plants, both the photosystems were affected. Chromium stress can induce three possible types of metabolic modification in plants: (i) alteration in the production of pigments, which are involved in the life sustenance of plants (e.g., chlorophyll, anthocyanin) (ii) increased production of metabolites (e.g., glutathione, ascorbic acid) as a direct response to Cr stress, which may cause damage to the plants and (iii) alterations in the metabolic pool to channelise the production of new biochemically related metabolites, which may confer resistance or tolerance to Cr stress (e.g., phytochelatins, histidine) (Schmfger, 2001). Induction and activation of superoxide dismutase (SOD) and of antioxidant catalase are some of the major metal detoxification mechanisms in plants. (Gwozdz *et al,* 1997) found that at lower heavy metal concentrations, the activity of anti-oxidant enzymes increased, whereas at higher concentrations, the SOD activity did not increase further and catalase activity decreased.

5.3.7. Effects of Lead on Plants

Lead (Pb) is one of the ubiquitously distributed most abundant toxic elements in the soil. It exerts adverse effect on morphology, growth and photosynthetic processes of plants. Lead is known to inhibit seed germination of *Spartianaalterni flora*, *Pinushelipensis* (Nakos 1979). Inhibition of germination may result from the interference of lead with important enzymes. (Mukherji and Maitra, 1976) observed that at 60 lM lead acetate inhibited protease and amylase by about 50% in rice endosperm. Early seedling growth was also inhibited by lead in soya bean, rice, maize, barley, tomato, eggplant and certain legumes. Lead also inhibited root and stem elongation and leaf expansion in Allium species, barley and *Raphanussativas*. The degree to which root elongation is inhibited depends upon the concentration of lead and ionic composition and pH of the medium. The Concentration-dependent inhibition of root growth has been observed in *Sesamumindicum*. A high lead level in soil induces abnormal morphology in many plant species. Irregular radial thickening in pea roots, cell walls of the endodermis and lignification of cortical parenchyma. Lead also induces proliferation effects on the repair process of vascular plants. Lead administrated to potted sugar beet plants at rates of 100–200 ppm caused chlorosis and growth reduction. In contrast, there were no visual symptoms of lead toxicity in alfaalfa plants exposed to 100 mg/mL. Low amounts of lead (0.005 ppm) caused significant reduction in growth of lettuce and carrot roots. Inhibitory effects of Pb^2 on growth and biomass production may possibly derive from effects on metabolic plant processes (Van Assche and Clijsters, 1990). The primary cause of cell growth inhibition arises from a lead-induced simulation of indol-3 acetic acid (IAA) oxidation. Lead is also known to affect photosynthesis by inhibiting activity of carboxylating enzymes. The High level of Pb also causes inhibition of enzyme activities, water imbalance, alterations in membrane permeability and disturbs mineral nutrition. Pb inhibits the activity of enzymes at cellular level by reacting with their sulfhydril groups. High Pb concentration also induces oxidative stress by increasing the production of ROS in plants (Reddy *et al,* 2005).

5.3.8. Effects of Nickel on Plants

Nickel (Ni) is a transition metal and found in natural soils at trace concentrations except in ultramafic or serpentinic soils. However, Ni^2 concentration is increasing in certain areas by human activities such as mining works, emission of smelters, burning of coal and oil, sewage, phosphate fertilizers and pesticides. Ni^2 concentration in polluted soil may range from 20-

to 30-fold (200–26,000 mg/kg) higher than the overall range (10–1,000 mg/kg) found in natural soil (Izosimova, 2005). Excess of Ni^2 in soil causes various physiological alter-actions and diverse toxicity symptoms such as chlorosis and necrosis in different plant species, including rice. Plants grown in High-Ni-containing soil showed impairment of nutrient balance and resulted in disorder of cell membrane functions. Thus, Ni^2 affected the lipid composition and H-ATPase activity of the plasma membrane as reported in Oryza sativa shoots. Exposure of wheat to high level of Ni^2 enhanced MDA concentration (Pandolfini *et al,* 1992). Moreover, (Gonnelli *et al,* 2001) found that the increase in MDA concentration of Ni^2-sensitive plants compared to a Ni^2-tolerant saline. Such changes might disturb membrane functionality and ion balance in the cytoplasm, par-ticularly of K, the most mobile ion across plant cell membrane. Other symptoms observed in Ni^2-treated plants were related with changes in water balance. High uptake of Ni^2 induced a decline in water content of dicot and monocot plant species. The decrease in water uptake is used as an indicator of the progression of Ni^2 toxicity in plants (Gajewska *et al,* 2006).

5.3.9. Effects of Iron on Plants

Iron as an essential element for all plants has many important biological roles in the processes as diverse as photosynthesis, chloroplast development and chlorophyll biosynthesis. Iron is a major constituent of the cell redox systems such as heme proteins including cytochromes, catalase, peroxidase and leghemoglobin and iron sulfur proteins including ferredoxin, acontiase and superoxide disumutase (SOD) (Marschner, 1995). Although most mineral soils are rich in iron, the expression of iron toxicity symptoms in leaf tissues occurs only under flooded conditions, which involves the microbial reduction of insoluble Fe^3 insoluble Fe^2. The appearance of iron toxicity in plants is related to high Fe^2 uptake by roots and its transportation to leaves and via transpiration stream. The Fe^2 excess causes free radical production that impairs cellular structure irreversibly and damages membranes, DNA and proteins (de Dorlodot *et al,* 2005). Iron toxicity in tobacco, canola, soybean and *Hydrillaverticillata* are accompanied with reduction of plant photo synthesis and yield and the increase in oxidative stress and ascorbate peroxidise activity (Sinha *et al,* 1997).

5.3.10. Plant – Heavy Metal

As one of the consequences of heavy metal pollution in soil, water and air in some cases in India, plants were also seen to be polluted by heavy metals (Zhang *et al,1996),* which consequently threatens the health of animals and human beings via the food chain (Wang *et al,* 2001). It is urgently necessary to clean and remediate heavy metals from areas, where crops, vegetables, fruits and pasturages have been grown, in order to protect the health of animals and human beings. The Plants play an important role in solar energy transport to bio-energy and can clean the environment in an environmentally friendly manner; they would also play an important role in heavy metal remediation. To understand the effects of heavy metals on plants and resistance mechanisms would be helpful for using plants to clean and remediate heavy metal pollution.

5.3.11. Effects of Heavy Metals on the Growth of Plants

Heavy metals such as Cd and Pb are non-essential elements for plants. If plentiful amounts are accumulated in the plants, heavy metals will adversely affect the absorption and transport of essential elements, disturb the metabolism, and have an impact on growth and reproduction (Xu and Shi, 2000). The germinating ratio and growth rate of barley declined, for instance when polluted by Cd and the decline was related to the dosage and duration. The germinating ratio was lower than 45 % and the growth of roots were stagnant under10 -2 mol/L Cd treatment. The seedlings of bean became brown and died under Cd stress. The roots were one of the target organs of Cd pollution, so that the root growth of crops such as wheat, maize, pumpkin, cucumber, and garlic *(Allium sativum* L) were inhibited (Liu *et al,* 2000).

The Seedlings represent a more easily damaged stage of the lifecycle. In crops such as rice and cotton (Qin *et al*, 2000), and vegetables such as spinach *(Spinaciaoleracea*Linn.)*, Brassica chinensis* L.and *Braseniaschreberi* L., seedlings were easily injured and inhibited by the heavy metal pollution in hydroponically exposure. The growth of vegetables such as cabbage, carrots, broccoli and cucumbers were inhibited under exposure to 10 mg/L Cd solution (Liu *et al,* 1995*).* (Yang *et al*, 1999) investigated that the effects of Cr 6+ on *Hydrochairsdubia (B.l)* and showed that Cr prevented it from absorbing water. The degree of damage was positively relative to the cultural concentration and time. At a high concentration of Cr 6+ (16-32 mg/kg), the edge of the leaves began to dry and the root tips rotted in a short period of time. But allow concentration of Hg (10 -s mol/L) stimulated the growth of wheat seedlings. The reason for this may be that low concentrations of Hg increased the activities of amylase, proteinase and lipase, sped up the decomposition of endosperm and the respiration rate, so that the germination was more rapid. Root vitality is reduced under heavy metal stress. (Shu *et al,* 1997) observed the root vitality of

*Stylosanthes guianensis*in mine tailings, it was reduced by heavy metals (Pb, Zn, Cuand Cd), and the absorption of inorganic nutrients was prevented and led to evident chlorosis, which significantly affected the growth.

The Geno toxicity of heavy metals in plants influences the synthesis, and the duplication of DNA and chromosomes both directly or indirectly, as well as inducing chromosomal aberration. The effects relate positively to heavy metal dosage. Hang, (1997) treated the barley by Cd and showed that Cd combined with nuclear acid and damaged the structure of the nucleolus, as well as causing chromosome fragmentation, aberration, conglutination and liquefaction. Exposed to Cd, Pb, Hg, the chromosomes of beans, garlic and onions were injured and revealed polyploidy, C-karyokinesis, chromosomal bridges, chromosomal rings, and chromosome fragmentation, chromosome fusion, micro-nuclei and nuclear decomposition. The higher concentration of heavy metals in medium, in which plants could not grow normally, affected the SCE (sister chromatid exchange) frequency in root tip cells of *Hordeumvulgare.*Cr_2O_3 or $CdC1_2$ detected by See tests even if thereis a lower dose level (Yi and Zhang, 1997).

The grana cascade of chloroplast mitochondria decrease, disappear or swell, and polypeptide compositions of the thylakoid membrane are degraded under heavy metal stress. Peng and Wang, (1991) noticed that the effects of Cd on the cell ultrastructure of maize and showed that the grana cascade of chloroplast mitochondria decreased and/or disappeared under low concentrations of Cd stress. the chloroplast cascade became more extensive and the membranes began to decompose, the mitochondria also became tumorous and decomposed under high concentrations of Cd stress. The damage to the chloroplasts was related to the attachment of Cd to the thylakoid and combined with the protein in the membrane to destroy the enzymatic system of the chloroplasts and to block the synthesis of chlorophyll. *Hydro charisdubia* L. demonstrated that the chromatin condensed, and the thylakoids of chloroplast and lumen of mitochondria swelled in the early stages when the leaves suffered poisoning due to Hg (Hao *et al,* 2001). The polypeptide compositions of the thylakoid membrane of *B.schreberi* were degraded under the stress of Hg and Cd (Chen *et al,* 1999). The changes in the mitochondria resulted from the penetration of K § and H_2O from the lumen to the outside and the disturbance of Cd on the activities of ATE Yang, (1991) also reported the effects of heavy metals on the structure and function of photosynthetic membranes of higher plants and showed that the sub-microstructure of chloroplast were changed. The grana also decomposed and some plasmids were formed. In intact tobacco *(Nicotianatabacure),* the photosynthetic membranes were damaged by Treatment, which might be the main reason for the decreasing of photosynthetic intensity (Jiang, 1995).

Sun and Wang, (1985), reported that the Chlorophyll a/b ratio of macrophytes decreased as the concentration of Cd increased and that Fe 3§could abate the increasing trend (Li *et al,* 1992). Heavy metals affected the function of PSI and PS II, and it was stronger with the latter (Yang *et al,* 1989). The chlorophyll proteins, which took protons for photosynthesis in PSII, were decomposed and decreased under Cd stress. The sub-microstructure of chloroplast was changed and the membrane system was destroyed. Therefore, the capacity of taking protons declined and the photosynthesis function was influenced (Peng and Wang, 1991). The photosynthesis foody plants decreased under pollution with Cd from the atmosphere before the observed symptoms had occurred (Huang and Zhang, 1986). Thus, the photosynthetic yield would be one of the indicators.

5.3.12. Recommendations

To avoid of the negative effects of heavy metals on plants, the resistance mechanisms should be studied further, especially the molecule mechanisms and the role of glutathione (GSH) on the detoxification of heavy metals. This would help to benefit the selection and cultivation of plants for heavy metal phytoremediation. More highly resistant plants can be used to remediate the pollution area, which is the basic idea of green and environmentally-friendly phytoremediation techniques. The bioavailability of heavy metals to plants is dependent on two different aspects, too. There are two different strategies to manipulate the bioavailability of heavy metals.

5.4. Silver Nanoparticles Characterization

When the plant extract was mixed at 0.1% concentration of the respective silver nitrate ($AgNO_3$) aqueous solutions the solutions changed their colour from white to brown for silver nanoparticles. The change in colour is due to the excitation of surface plasmon vibration, which is indicated by the formation of silver nanoparticles at different time intervals.

Once materials are prepared in the form of very small particles, they change significantly their physical and chemical properties. In fact, in nano-dimension, percentage of surface molecule compare to bulk molecule is high and this enhances the activity of the particle in nano dimension and therefore, the normal properties of the particle like heat treatment, mass transfer, catalytic activity, etc are all increases. The non-metal nanoparticles, metal nanoparticles have more industrial application. Nanoparticles offer many new developments in the field of biosensors, biomedicine and bio nanotechnology-specifically in the areas Drug delivery, as medical diagnostic tools, as a cancer treatment agent (Gold nanoparticles).

The UV-Vis absorption spectroscopy is one of the main techniques followed to examine size and shape of the nanoparticles in the aqueous suspensions (Wiley *et al,* 2006). Optical response was recorded under UV-Vis spectroscopy in relation to increase in time duration. The observation of brown and red colours is a characteristic feature for the surface plasmon resonance (SPR) band due to the formation of different sizes of silver nanoparticles in the respective solutions. The observation of reduction of silver ions present in the aqueous solution of silver complex during reaction with the ingredients of the plant extract may be correlated by the formation of silver nanoparticles in the solution under UV-Vis Spectroscopy. This observation could be attributed to the excitation of surface plasmon vibrations and it has resulted in the formation of silver nanoparticles.

Huang *et al,* (2007) reported that the formation of silver nanoparticles when constant aqueous $AgNo_3$ at 50 ml, 1 mM with 0.1 g bio-mass produced silver nanoparticles as indicated by sharp absorbance at around 440 nm in *Cinnamomum camphora*.

The FTIR mechanisms involved in the uptake of metal ions may be intracellular accumulation and surface adsorption. The former one is an active process because the plant must be active to carry out. In the case of surface adsorption, it is a passive process because the chemical groups attached to the cell walls of the plant can bind with metal ions even though when the plant is dead. It is considered as an advantage in phytoremediation technologies by which metal contaminants are removed. If the chemical groups attached to the cell walls are the binding sites then these groups can be adsorbed as metal ions. Therefore, there may be a possibility to use the plant tissues to filter such ions out of the aqueous solutions. This technology is called phytofiltration.

Alfalfa biomass using SEM micrographs and a corresponding elemental composition of Ag L as well as traces of C K, Si K analysis by EDS. They reported the highest being Ag, Si, C with 71.59%, 1.34%, 27.06% respectively and the absence of Fe. As EDS equipment works at low vacuum (1-270 pa) it allows to observe non-conducting samples without the need to cover them with a thin conductive film, and consequently no evidence of noise by the coating material (Huang and Yang, 2004).

5.5. Antimicrobial Studies

Antimicrobial activity can be regarded as a very important study, particularly at this specific period of human history due to bacterial resistance that is constantly imposing new scientific challenges. In spite of great development in drug therapy, infectious diseases remain as the most common reasons for mortality in many developing countries. Resistance to antimicrobial agents has become an increasingly important and pressing global problem. To counter the emergence of resistant microorganisms, considerable resources have been invested in the search for new antimicrobials.

It is well known that plants synthesize a diverse array of secondary metabolites, which are involved in defence mechanisms, and in the last few years it is recognized that some of these molecules have health beneficial effects including antimicrobial properties (Borges *et al*,2013).*Cleome gynandra* is used as a medicinal plant and can be found in all over world .It grows as a weed in paddy fields and also in road sides and in open grass lands (Annadurai and Ahmed John, 2014).According to the WHO, plants are a source of compounds that have the ability to combat disease. *Rauvolfia tetraphylla* is a well-known medicinal plant in the Indian traditional system of medicine. Due to their various biological activities, the plant and its compounds are continuously under investigation. This evidence opens possibilities to the fact that the extract contains compounds that may act by synergism or additive effect which in turn is responsible for its pharmacological activity (Pa and Mathew, 2012). Among the phytocompounds, polyphenols or phenolic compounds are a group of secondary metabolites that are adequately found in medicinal plants with more than 8000 identified compounds (Ebrahimi and Schluesener, 2012), and have been tested in clinical and experimental studies as antimicrobials (Albuquerque *et al*, 1995). The Quality control which is an important challenge in present scenario can be addressed with reliable and sensitive quantization of important biological active metabolite in the sample (Shirolkar *et al,* 2013).

High Performance Thin Layer Chromatography is one of the modern sophisticated techniques than can be used for evaluating the potency, authenticity, quality and purity of crude drugs (Mamatha, 2014). HPTLC offers better resolution, and estimation of active constituents can be done with reasonable accuracy in a shorter time (Syed *et al,* 2011). Polyphenols found in medicinal plants have been extensively investigated against a wide range of microorganisms, and among them tannins and flavanols received more attention due to its broad spectrum and the fact that most of them are able to suppress microbial virulence factors (Daglia, 2012).

Flavonoids like quercetin and catechin are becoming the subject of medical research. Flavonoids interfere with specific intracellular or surface enzymes and many bacterial virulence factors such as toxins, enzymes and signal receptors (Cushnie and Lamb, 2011). *Xanthomonas phaseoli* showed a similar antimicrobial activity confirming that flavonoids may have been responsible for its activity (Aeri and Singh, 2013). Rauvolfia extract was the highest among the tested compounds. *Camellia sinensis* showed profound antibacterial activity against *Shigella*, *Vibrio* and *S. mutans* (Cowan, 1999).

There are several reports about the potential antifungal activity of polyphenols for treating oral candidiasis. Gel-entrapped catechins (GEC) inhibited the growth of *Candida* strains suggesting that hydrogen peroxide may be involved in the antimicrobial activity of CA (Tamura *et al,* 2011). The Black tea extract containing CA completely inhibited the growth of *T. mentagrophytes* (Okubo *et al,* 1991). Surprisingly, the *C. albicans* was unaffected with heat treatment of tea CAs (Kim *et al,* 2004).

The ethanolic extracts, which certainly indicates that higher concentration of active antimicrobial agents. But also this could be attributable to the polarity nature of active antimicrobial agents. These may include alkaloids, sugar, tannins, which are all found in more abundant amount in *Rauvolfia sp*. Suresh *et al,* (2008) reported that the best antimicrobial activity of *Rauvolfia tetraphylla*, which showed maximum activity against *E. coli* and *Enterobacter aerogenes*, and various tested fungi such as *A. niger* and *Penicillium sp*, were found to be more sensitive to crude extract when compared to others. Several phytoconstituents such as terpenoids (Scortichini and Rossi, 1991), flavonoids (Tsuchiya *et al,* 1966) and tannins are effective against certain microorganisms. The results of the present investigation clearly demonstrate the antibacterial and antifungal activities of the ethanol extracts of the leaves.

Zeng, (2007) investigated that the antimicrobial studies of silver nanoparticles have a great bactericidal effect against several bacteria including multi resistant strains; can be considered as potential antifungal agent. (Anitha *et al,* 2011) found that the Ag nanoparticles have exhibited considerable activity against some human pathogens. The antimicrobial property of silver is found to be the best among different metals in the following order Ag >Hg > Cu >Cd>Cr>Pb>Co>Au>Zn>Fe>Mn>Mo>Sn (Petica, 2008). It is disc known that Ag ions and Ag-based compounds have strong antimicrobial effects (Furno, 2004), and many investigators are interested in using other inorganic nanoparticles as antibacterial agents (Hamouda, 2000) studied the increasing use of silver based products as antimicrobial agents and he concluded that the silver materials are an efficient alternative to antibiotics for the treatment. This nanoparticles release silver ions in the bacterial cells, which enhance their bactericidal activity (Sondi, 2004 and Morones, 2005). Recently it was shown that highly concentrated and nonhazardous nanosized silver particles can easily be prepared in a cost-effective manner and tested as a new type of bactericidal nanomaterial. The Morones, 2005 reported that the silver nanoparticles not only interact at the surface of cell membrane, but also inside the bacteria (Morones, 2005). Hence there is a possibility that the silver nanoparticles may also interact inside the bacteria, which may also cause damage of the cells by interacting with phosphorus and sulfur containing DNA. The mechanism of inhibitory action of silver ions on microorganisms is partially known. It is believed that DNA loses its replication ability and cellular proteins become inactivated on Ag^+ treatment.

Hence, the antimicrobial activity may be due to the presence of catechin and gallic acid in the extract which have been previously reported for their antimicrobial property. It is therefore conceivable that this extract could be used against infections caused by the microbes against which it has shown pronounced effects. The results showed a good correlation between the reported uses of *C. gynandra* derived silver nanoparticles medicine against infectious diseases. The preset fineling may not be adequate to suggest potential antibiotic agent considering the MIC values and the zones of inhibition. However, this approach could be considered as preliminary step to find out promising lead molecules and the possible mechanism of action by which it inhibits microorganisms.

5.6. *In vitro Cytotoxicity*

The cytotoxicity effect was compared with the standard anticancer drug 5-FU against osteosarcoma cell lines cells and their LC_{50} value was observed (Samy et al, 2008). A large number of in vitro studies indicate that AgNPs are toxic to the mammalian cells. Interestingly, some studies have shown that AgNPs has the potential to intervene genes associated with cell cycle progression, also induce DNA damage and apoptosis in cancer cells. Indeed, the results of present study provide conclusive evidence for cytotoxic effect of AgNPs on cancer cell lines rather than normal cell lines.

The greatest service, which can be rendered any country, is to add a useful plant to its culture. Plants have forever been a catalyst for our healing. In order to halt the trend of increased emerging and resistant infectious disease, it will require a multi-pronged approach that includes the development of new drug. Using plants as the inspiration for new drugs provides an infusion of novel compounds or substances for healing disease. Evaluating plants from the traditional Indian system of medicine provides us with clues as to how these plants can be used in the treatment of disease. Many of the plants presented here show very promising activity in the area of antimicrobial agents.

The marked difference in the shape of gold and silver nanoparticles may be attributed to the comparative advantage of protective biomolecules and reductive biomolecules, the environmental-friendly and low-cost candidate as a reductant for synthesizing either silver or gold nanoparticles. This procedure can be extended to the synthesis of other nanoparticles from different chemical compositions.

The Synthesis of Nanoparticles has many advantages by scale up of each process because of its economic viability, possibility of covering large surface area easily by suitable growth of the filaments, etc. Equally the synthesis of metal ion reduction or reaction process in cellular metabolism explaining whether the nanoparticles formed as byproducts of the process has role to play in a cellular activity.