Progress of nanotechnology is becoming rapid in the development of innovative synthesis protocols and characterization techniques (Sharma et al. 2009). Sau and Rogach (2010) stated most of the synthesis methods were limited to synthesis of nanoparticles in small quantities and poor morphology. The earlier methods used for the synthesis were chemical and physical, which often results in synthesis of nanoparticles with poor morphology. Birla et al. (2009) reported these methods were toxic to the environment due to the use of toxic chemicals, higher temperatures for synthesis process. Since then biological synthesis of nanoparticles with controlled morphology captured attention, by using biological means like bacteria (Shahverdi et al. 2009), fungi (Govender et al. 2009), actinomycetes (Ahmad et al. 2003), algae (Chakraborty et al. 2009), etc. Mukherjee et al. (2008) stepped towards a greener approach which was environment friendly, as no toxic chemicals, temperature and pressure conditions used in synthesis. Hence, bio-researchers are focusing towards the synthesis of biogenic nanoparticles compared to the chemically or physically synthesized nanoparticles (Thakkar et al. 2010). The yield of different size and shape of nanoparticles involves use of different types of chemical, physical and biological agents. The most often used method for the chemical synthesis of nanoparticles is the chemical reduction method using chemical like sodium borohydride or sodium citrate as reducing agents (Cao and Hu 2009). Physical methods of synthesis of nanoparticles include thermal decomposition, condensation, laser irradiation, electrolysis, diffusion etc. For example, in thermal decomposition method, the synthesizing process was carried out at very high temperature (Yang and Aoki 2005). Nowadays the biological agents used for the synthesis of nanoparticles include mainly
microbes (Gajbhiye et al. 2009) and plants (Jha et al. 2009, Javad et al. 2014) which results in both extracellular and intracellular synthesis (Shaligram et al. 2009). Physical and chemical synthesis methods requires both strong and weak chemical reducing agents and protective agents (sodium borohydride, sodium citrate and alcohols) which are mostly toxic, flammable, cannot be easily disposed due to environmental issues and also shows low production rate (Bar et al. 2009). For example, in the seeded growth method chemical reducing agents like sodium citrate and sodium borohydride (Jana et al. 2000) were used. Kim et al. (2007) used methanol and ethanol in the polyol synthesizing process. The biological method provides a wide range of resources for the synthesis of nanoparticles. The rate of reduction of metal ions using biological agents found to be much faster at room temperature. The greener synthesizing process is less labor-intensive, low-cost technique and nontoxic. The exact mechanism for the synthesis of nanoparticles using biological agents has not been devised yet as different biological agents react differently with metal ions. Rai et al. (2011) elucidated the mechanism for intra and extracellular synthesis of nanoparticles which is different in various biological agents. The biologically synthesized silver nanoparticles can be utilized in area of electronics, silica coated Ag nanowires and electric circuits (Kvistek and Prucek 2005). Gold bionanoparticles mainly concentrated in medicine due to its biocompatibility and strong interaction with soft bases like thiols which play a major role in the treatment of cancer (Bhattacharya and Mukherjee 2008).

Kumar and Yadav (2009) explained plant based nanoparticle synthesis advantageous over other biological methods (microbial), the reaction rate for the synthesis of nanoparticles
is very high and no need to grow the microbes, which is very cumbersome and also no need to maintain specific conditions.

**Biosynthesis of Gold and Silver Nanoparticles**

Several plants have been reported for their potential in biosynthesis of silver and gold nanoparticle. Shankar *et al.* (2003) explained geranium leaf extract led to rapid formation of highly stable, crystalline silver nanoparticles (16-40nm), which assembled in the reaction medium into quasilinear super structures. Shankar *et al.* (2004) reported the extracellular synthesis of pure metallic silver, gold and bimetallic Au/Ag nanoparticles by neem (*Azadirachta indica*) leaf broth.

Ankamwar *et al.* (2005a) explained bioreduction of HAuCl$_4$ by tamarind leaf extract which resulted in formation of flat and thin single crystalline gold nanotriangles with unique and highly anisotropic planar shapes. These nanoparticles have application in photonics, optoelectronics and optical sensing.

Ankamwar *et al.* (2005b) reported that *Emblca officinalis* fruit extract leads to rapid reduction of the silver and chloroaurate ions to highly stable silver and gold nanoparticles. TEM images confirmed the formation of silver (10-20nm) and gold (15-25nm) nanoparticles.

However, Chandran *et al.* (2006) obtained control over the shape and size of gold and silver nanoparticles with the use of *Aloe vera* leaf extract as reducing agent. The extract volume used for the synthesis of nanoparticles and temperature during the reaction had a great impact on the synthesis of characteristic nanoparticles.

Ghule *et al.* (2006) achieved the bioreduction of aqueous Au (III) ions directing the formation of triangular gold prisms. Proteins and biomolecules from bengal gram beans
(Cicer arietinum) mediated the synthesis and controlled the morphology of gold particles by varying compositions of gram bean seed extract and aqueous Au (III) solution.

Huang et al. (2007) described the use of Sun dried biomass of Cinnamomum camphora leaf and incubated with aqueous silver or gold precursors for the production of both silver and gold nanoparticles (55-80nm). The marked difference in shape of gold and silver nanoparticles could be due to protective and reductive biomolecules from leaf extracts. They explained polyol and water soluble heterocyclic components responsible for the reduction of silver ions or chloroaaurate ions.

Armendariz et al. (2004) has also shown pH-dependent synthesis of various shaped gold nanoparticles with oat (Avena sativa) biomass. The biomass and solution of Au(III) was reacted for a period of 1 hr at pH 2-6. The Au (III) ions binding to biomass have been found to be pH dependent and the highest adsorption (~80%) occurred at pH 3. Smaller nanoparticles, in fair amounts, were observed at pH 3 and 4, whereas larger nanoparticles were observed at pH 2.

Efforts have been made towards identifying the molecules involved in synthesis of nanoparticles. Egorova et al. (2000) observed the plant metabolite quercetin (3,5,7,3,4-pentahydroxyflavon, C_{15}H_{10}O_{7}O_{2}H_{2}O) was involved in very quick, simple and highly stable nanoparticle synthesis.

Li et al. (2007) identified the responsible biomolecules involved in the synthesis and 3 nm protein moieties capped the silver nanoparticles. Furthermore, amine groups containing proteins were found to cause the reduction of silver ions, leading to silver nanoparticle synthesis in the solutions.
Udayasoorian et al. (2011) have reported AgNPs synthesis from leaf extract of *Cassia auriculata* complete reduction of silver ions was observed after 48 hr of reaction at 30°C under shaking condition resulted in spherical shaped, polydispersed particles size ranging from 20 to 40 nm.

Ganesh Kumar et al. (2011) synthesized gold nanoparticles (AuNPs) using *Cassia auriculata* aqueous leaf extract and found stable at a wide range of pH (3.4-10.2).

Velavan et al. (2012) showed silver nanoparticles from *Cassia auriculata* flower extract were polydispersed, size ranging from 10-40 nm, and further reported nanoparticles exhibited strong antioxidant activity.

Amaladhas et al. (2012) reported water soluble components from the leaves, probably the sennosides, served as both reducing and capping agents in the synthesis AgNPs from *Cassia angustifolia* leaf extract.

Daisy and Saipriya (2012) reported that *Cassia fistula* mediated stable gold nanoparticles with different morphologies have promising antidiabetic properties.

Dhayananthaprabhu et al. (2013) reported the bio reduction of gold ions by *Cassia auriculata* flower extract with spherical, hexagonal and triangular shaped nanoparticles size ranging from 10 to 55nm.

Gaddam et al. (2014) reported biofabrication and antimicrobial activity of silver nanoparticles from *Cassia alata* leaf extract.

In addition to the individual synthesis of either silver or gold nanoparticles, there are number of reports available in the literature on the extracellular biosynthesis of Ag and Au nanoparticles using several plants with their possible biomolecules (Table-1).
Table-1. Biosynthesis of AuNPs and AgNPs using plants with possible biomolecules.

**Characterization of Nanoparticles and Associated Molecules**

Kumar and Yadav (2009) explained characterization techniques of nanoparticles. The formation of nanoparticles from different salts gives characteristic peaks at different absorptions that can be monitored by UV-Vis spectroscopy. Silver nanoparticles show an absorption peak around 450nm, while gold nanoparticles show absorption peak around 550nm. The progressive increase in the characteristic peak with increase in reaction time
and concentration of plant extracts with salt ions is a clear indicator of nanoparticle formation. UV-Vis absorption spectra shows characteristic peaks of the surface plasmon resonance of nanosized particles.

The X-ray diffraction (XRD) technique is used to establish the metallic nature of particles. The wavelength of X-rays is comparable to the size of atoms, they are ideally suited for probing the structural arrangement of atoms and molecules in a wide range of materials. The energetic X-rays can penetrate deep into the materials and provide information about the bulk structure (Putnam et al. 2007). For very small crystallite sizes, signals in XRD are broadened, a phenomenon described by the Scherrer equation (materials and methods).

Fourier transform infrared (FTIR) spectroscopy is a chemical analytical technique, which measures infrared intensity versus wavelength (wavenumber) of light. It is used to determine the nature of associated molecules of plants or their extracts with nanoparticles. A FTIR spectrometer obtains infrared spectra by first collecting an interferogram of a sample signal with an interferometer, which measures all of the infrared frequencies simultaneously. A FTIR spectrometer acquires and digitizes the interferogram, performs the FT function and outputs the spectrum. This technique has been used in the characterization of nanoparticles and their associated biomolecules from plant extracts in various studies.

Microscopic techniques such as scanning electron microscopy (SEM), transmission electron microscopy (TEM) and atomic force microscopy are mainly used for morphological studies of nanoparticles.
Characterization of Associated Biomolecules

According to Iravani et al. (2011) the bioreduction of metal NPs occurs by the combinations of biomolecules present in the plant extracts such as enzymes, proteins, amino acids, vitamins, polysaccharides, typically obtained by contact of a broth of plant leaves with metal salts. In a biological medium, NPs may interact with biomolecules such as proteins, nucleic acids and biological metabolites due to their nanosize and large surface-to-mass ratio. The adsorption of proteins on the nanoparticle surface results in nanoparticle protein complexes (NP-PC). These proteins functionalize the nanoparticles making them biocompatible. The NP-PC can influence the biological reactivity of the NP (Casals 2010). Kannan (2010) reported the involvement of cell wall proteins, carbohydrates and biomembranes in microbial bioreduction.

Xie et al. (2007) explained active biomolecules played an important role in controlling bio-chemical composition, size and shape distributions in the biosynthesis of nanoparticles.

Chandran et al. (2006) isolated 28 kDa protein from green alga provided the dual function of the gold ions reduction and size and shape controlled agent. Zhang et al. (2011) identified three fungal proteins associated with gold nanoparticles related to the energy metabolism of live fungal cells.

Kaur et al. (2009) described that proteins might have major role in controlling the characteristics of NPs from biological or chemical origin. In most cases, plants have broad variety of metabolites that can aid in the reduction of ions, and are quicker than microbes in the synthesis. The main mechanism considered for the reduction process is
due to plant biomolecules which plays dual function in synthesis and stabilization of nanoparticles.

**Immobilation of nanoparticles within polymer**

The macroscopic gels have become more promising as templates/nanoreactors for *in situ* synthesis and immobilization of smaller size nanoparticles and this approach became a new concept in hybrid or composite structures in green chemistry, biomedicine and engineering science (Takahito *et al*. 2009). Mitamura *et al*. (2008) developed nanocomposite by incorporation of prepared gold nanorods. Preparation of Au (Pal *et al*. 2005) and Au-Ag (Pal *et al*. 2007) nanoparticles in aqueous sodium alginate also reported. Tokarev *et al*. (2010) developed biosensors based on thin hydrogel films loaded with noble metal nanoparticles with enzyme reactions.

Varaprasad *et al*. (2010) stated that silver nanoparticles (AgNPs) within hydrophilic, biocompatible polymers can be new antimicrobial materials. Monteiro *et al*. (2009) reported that polymeric nanocomposites were effective in biocompatibility with diversity of forms and structures for biomedical applications such as antimicrobial coatings, wound dressings and potential tissue implants.

Sepulveda *et al*. (2009) described the formation of colloidal gold within alginate gels which received a great deal of attention due to its multiple properties, especially for optical sensing. Rakel *et al*. (1998) found calcium alginate transparent film to be the best dressing in terms of patient comfort, infection rate and healing quality.

Alginate is a naturally occurring biocompatible polymer having numerous applications in the nanobiotechnology as non-toxic material with biodegradability (Madhusudana Rao
et al. 2013). According to Xia et al. (2003) the mechanical properties of hydrogel nanocomposites were superior to those of traditional hydrogels and becoming popular.

APPLICATIONS

Antimicrobial activity

It is well known fact, that silver ions and nanoparticles are highly toxic and hazardous to microorganisms. The silver nanoparticles have many inhibitory and bactericidal effects as it was an excellent antibacterial agent used from history. Stohs et al. (1995) reported that metal ions induce generation of intracellular reactive oxygen species (ROS) in bacterial cells. Feng et al. (2000) explained the inhibitory effect of silver ions is higher in gram negative bacteria, and less in gram positive bacteria due to the cell wall thickness of the peptidoglycan layer which prevent the action of the silver ions. Matsumura et al. (2003) suggested that silver ions (particularly Ag+) released from silver nanoparticles can interact with phosphorus moieties in DNA, resulting in inactivation of DNA replication, or can react with sulfur containing proteins, leading to the inhibition of enzyme functions. Morones et al. (2005) explained Ag nanoparticle less than 20nm diameter get attached to the sulfur containing proteins of bacterial cell membranes leading to greater permeability of the membrane, which causes the death of the bacteria. The antibacterial activity also depends on particle size. Panacek et al. (2006) investigated the antimicrobial activity of the colloidal silver particles with variable sizes (44, 50, 35 and 25nm) synthesized by the reduction of (Ag(NH3)2)+ complexes with carbohydrates.

Pal et al. (2007) determined the AgNPs also exhibit a shape dependent interaction with the bacterial cells. The truncated triangular silver nanoplates displayed the strongest biocidal action against E. coli compared to the spherical and rod shaped nanoparticles.
Kim et al. (2007) showed yeast and E. coli was inhibited at a low concentration of AgNPs, the study of mechanisms revealed that free radicals and oxidative stress was responsible for the antibacterial activities. Several mechanisms have been proposed to explain the inhibitory effect of silver nanoparticles on bacteria. It is assumed that the high affinity of silver towards sulfur and phosphorus as the key element of the antimicrobial effect. Due to the abundance of sulfur containing proteins on the bacterial cell membrane, AgNPs can react with sulfur containing amino acids inside or outside the cell membrane, which in turn affects bacterial cell viability.

Shrivastava et al. (2007) studied the dose dependent effect of AgNPs (size ranges from 10-15nm) on gram negative and gram positive microorganisms. Jung et al. (2008) studied the effect of silver nanoparticles on the cell morphology of Escherichia coli and Staphylococcus aureus using TEM, SEM and X-ray microanalyses. It was revealed that treatment with the silver ions results in similar morphological changes in both the gram positive and gram negative bacteria. The cytoplasmic membrane detaches from cell walls and an electron light region containing condensed deoxyribonucleic acid molecules appears in the center of the cell. Geoprincy et al. (2011) compared the zone of inhibition produced by various antibiotics with the inhibitory zone produced by the silver nanoparticles.

Karthick et al. (2011) determined the concentrations of 20µg, 40µg, 60µg and 80µg of the nanoparticle where Staphylococcus aureus exhibited characteristic inhibitory zones 14mm, 16mm, 18mm and 20mm diameter, whereas Enterococcus faecalis exhibited 11mm, 13mm, 14mm and 17mm diameter of zone of inhibiton respectively. Srivastava et al. (2011) explained that biological effectiveness of nanoparticles depends on increased
surface area and surface energy of the nanoparticles. Smaller nanoparticles with larger surface area to volume ratio provide more effective antibacterial activity even at lower concentration. Ramya and Sylvia (2012) reported nanosilver as much effective and fast acting fungicide against broad spectrum of common fungi including *Aspergillus*, *Candida* and *Saccharomyces*. Aditi *et al.* (2011) used standard well diffusion method to assay the antibacterial activity against human pathogenic bacteria such as *Escherichia coli*, *Pseudomonas aeroginosa*, *Bacillus subtilis* and *Klebsiella pneumonia*. Geoprincy *et al.* (2013) explains the silver nanoparticles have relatively higher antibacterial activity against gram negative bacteria than gram positive bacteria, which may be due to the thinner peptidoglycan layer and presence of beta barrel proteins called porins. Awwad *et al.* (2013) reported the AgNPs as an effective antibacterial agent toward *Escherichia coli*.

**Seed Germination Studies**

**Effect of Nanoparticles on seed germination and seedlings growth**

Nanotechnology has the potential to revolutionize the agriculture at molecular level in living cells and Nano agriculture involves the employment of nanoparticles in agriculture with the ambition that these particles impart some beneficial effects on the crops (Kotegooda *et al.* 2011). The important genetic engineering technique like gene gun or particle bombardment is one of the popular tools to deliver DNA into intact plant cells (Deng *et al.* 2001). Particles used for bombardment are typically made of gold since they readily adsorb DNA and are nontoxic to cells. The use of nanoparticles in growth of plants and control of plant diseases is a recent practice (Shah *et al.* 2009).
The transition metals such as silver, gold and cobalt (Gomez 2002), and non transition elements like aluminum (Ghanati et al. 2005) proved to have a stimulatory effect on plant growth. Nel et al. (2006) explained nanoparticles of size below 100nm generate both positive and negative biological effects in living cell. Torney et al. (2007) reported the mesoporous silica nanoparticle (MSN) coating triggers delivering DNA in the plants without any toxic effects in tobacco and corn plants. An et al. (2008) have reported an increase in ascorbate and chlorophyll contents in leaves of Asparagus treated with silver nanoparticles. Monica et al. (2009) suggested nanotechnology can be applied to plant science research to analyze plant genomics and gene function as well as improvement of crop species. According to Perea-de-Lugue et al. (2009) nanocapsules can enable effective penetration of herbicides through cuticles and tissues, allowing slow and constant release of the active substances. Gonzales-Melendi et al. (2008) reported that the nanoparticles act as smart treatment delivery systems in plants.

Nair et al. (2010) explained nanoparticles can serve as ‘magic bullets’, containing herbicides, chemicals(fungicides, insecticides, etc.) or genes, which target particular plant parts to release their content for improved plant disease resistance, efficient nutrient utilization and enhanced growth. Roghavyeh et al. (2010) have also reported an increase in pod weight, leaf and pod dry weight and yield of soybean, on treatment with nano-iron. Lee et al. (2010) observed positive influence of nano-Al₂O₃ on root elongation of Arabidopsis thaliana. Shah et al. (2009) reported application of metal NPs such are silica, palladium, gold and copper nanoparticles significantly influenced the growth of lettuce plants by increased in the shoot/root ratio.
Sandeep Arora et al. (2012) suggested use of nanotechnology in agriculture offers a viable alternative to GM crops, for enhancing growth and crop productivity. Prasad TNVKV et al. (2012) reported nanoscale ZnO improves the pea nut germination, root growth, shoot growth dry weight and pod yield of the treated seeds. Vineet Kumar (2013) found a considerable correlation between expression of key plant regulatory molecules, microRNAs (miRs) and seed germination, growth and antioxidant potential of *A. thaliana* on GNP exposure. Lu et al. (2002) reported the beneficial effects of nanoparticles on seed germination and growth. On the contrary, Yang and Watts (2005), Lin and Xing (2007) reported the phytotoxic nature of various nanoparticles through the inhibition of seed germination and root growth. Brumfield et al. (2003) stated concern over the potential harmful effects of such nanoparticles have stimulated the advent of nanotoxicology as a unique and significant research discipline. Prasad et al. (2004) recognized the heavy metals inhibits seed germination, growth, and development of plants, and disturb their biochemical and physiological processes.

Holsapple et al. (2005) reported positive and negative effects of nanoparticles on higher plants depends on its variable shape and size, however it is difficult to predict the positive or negative effect and its mode of action in the environment and within living systems. Nel et al. (2006) stated that small size nanoparticles can modify the physiochemical properties of the nanomaterials, which can lead to adverse biological effect on living cells with the main mechanism of oxidative stress through NPs. Lin and Xing (2007) investigated the effects of five types of nanoparticles (multi-walled carbon nanotube, aluminum, alumina, zinc and zinc oxide) on seed germination and root growth of six higher plant species (radish, rape, ryegrass, lettuce, corn, and cucumber) and stated that,
except nano-zinc on rye grass and nano-zinc oxide on corn, at 2,000 mg L$^{-1}$, germination of the plants tested did not get affected.

Di Salvatore et al. (2008) investigated the phytotoxicity of NPs via seed germination and root elongation tests which evaluate the acute effects of NPs on plant physiologies. Gubbins et al. (2011) reported toxic effects of AgNP on plants at concentrations as low as 5 mg/L. Jiang et al. (2012) reported the impact of AgNPs have detrimental effects on plant growth in vascular plants. Luca Marchiol et al. (2014) observed the in vivo formation of silver nanoparticles (AgNPs) in Brassica juncea, Festuca rubra and Medicago sativa.

**Anticancer Studies of Nanoparticles**

Green synthesis of gold and silver nanoparticles using leaves extract and cytotoxic effects on different cell lines are well documented.

Xia et al. (2006) has demonstrated that ROS generation and oxidative stress are the primary method of cytotoxicity, used as a paradigm to assess NP toxicity. Kim et al. (2007) demonstrated cytotoxicity induced by AgNPs in human hepatoma HepG2 cells and observed that AgNPs agglomerated in the cytoplasm and nuclei of treated cells, induced intracellular oxidative stress. Nan et al. (2008) stated that cancer cells are more resilient towards nanoparticle toxicity than normal cells due to an increased rate of proliferation and metabolic activity. Carlson et al. (2008) reported AgNPs induced apoptosis due ROS generation. Hsin et al. (2008) provided evidence for the molecular mechanism of cytotoxicity of AgNPs. They showed that AgNPs acted through ROS and JNK to induce apoptosis via the mitochondrial pathway in fibroblast cells. However
Asharani et al. (2009a) demonstrated the uptake of AgNPs occurs mainly through clathrin mediated endocytosis and macropino-cytosis and predicted AgNPs have multiples cellular targets that vary among different cell types. Asharani et al. (2009b) also reported that production of reactive oxygen species (ROS) implicates DNA damage caused by AgNPs. Though the exact mechanism by which AgNPs exert in vivo toxicity is not yet clear and believed that AgNPs cause oxidative stress (i.e., by releasing reactive oxygen species (ROS)) and DNA damage in human cell lines.

Kim et al. (2011) suggested that silver ions (particularly Ag⁺) released from silver nanoparticles could interact with phosphorus moieties in DNA, resulting in inactivation of DNA replication, or react with sulfur containing proteins, leading to the inhibition of enzyme functions, which results in loss of cell viability and eventual cell death. Satyavani et al. (2011) investigated anticancer activity of silver nanoparticles synthesized from calli extract of Citrullus colocynthis on human epidermoid larynx carcinoma cell line. Raghunandan et al. (2011) reported in vitro anticancer efficacy against human colorectal adenocarcinoma, human kidney, human chronic myelogenous, leukemia, bone marrow, and human cervix from biofunctionalized gold and silver nanoparticles synthesized using different plant extracts of guava and clove. Prabhu et al. (2013) investigated synthesized AgNPs from leaf extract of Vitex negundo has shown antitumor activity against human colon cancer cell line.

Jeyaraj et al. (2013) reported invitro cytotoxicity of AgNPs synthesized by Sesbania grandi flora leaf extract on human breast cancer (MCF-7). Govender et al. (2013) studied cytotoxic activity of Albizia adianthifolia mediated silver nanoparticles and showed the activation of AgNPs in the intrinsic apoptotic pathway in A549 lung carcinoma cells.
Lokina and Narayanan (2013) reported good anticancer activity of gold nanoparticles synthesized from grapes extract against HeLa cell lines. Ghassan et al. (2013) reported biomedical potential of silver nanoparticles synthesized from extract of *Rosmarinus officinalis* reduces viability of the HL-60 cells in a dose-dependent manner by MTT assay. Firdhouse & Lalitha (2013) described green synthesis of silver nanoparticles *Alternanthera sessilis*. The cytotoxic activity of synthesized nanosilver was carried out against prostate cancer cells (PC3) by MTT assay and found to show significant activity. Ravi et al. (2013) reported biological synthesized gold nanoparticles induced apoptosis and DNA damage in HL-60 cells.
Selection of Plants

*Cassia* sps. (Family: *Ceasalpiniaeae*) have been used as traditional medicine for centuries. The whole plants have been employed in herbal medicine around the world (Burkill 1995). The selected plants were: 1. *Cassia auriculata*, 2. *Cassia fistula*, 3. *Cassia occidentalis*, 4. *Cassia sophera*, 5. *Cassia Tora*.

Collection and Screening of Plants for potent Gold and Silver Nanoparticle Synthesis

The plants were collected from the botanical garden, Gulbarga University and by visiting local places. The leaves of the collected plants were gently washed with soap solution and bavistine to remove the dust and any other contamination then shade dried at room temperature for about 10-15 days. All these plants were preliminary screened by using 1% of leaf extracts, treated with 1mM of AgNO$_3$ and HAuCl$_4$ and based on good results, *Cassia auriculata* was selected to carry out further work.

Phytochemicals Screening

Qualitative analysis:

The details of qualitative analysis given below:

**Test for Alkaloids**

Iodine test

1 ml of KI in Iodine solution was added to the 2 ml of test solution. A brown precipitate formation indicated the presence of alkaloids.

Dragendroff’s reagent

2 ml of Dragendroff’s reagent and 2 ml of diluted HCl were added to 2 ml of test solution. The formation of reddish brown precipitate indicates the presence of alkaloids.

Mayers test

To a little of test solution add few drops of Mayers reagent. While precipitate formed indicates the presence of alkaloids. Some alkaloids are soluble in excess of the reagent. If no precipitate occurs with the addition of few drops more reagent is to be added.

**Test for Flavonoids**

Pew’s Test (Zn/HCl)

A pinch of zinc powder and about 5 drops of 5N HCl were added to the 2 ml of test solution. It results in deep purple red or cherry red colour.

Shinoda Test (Mg/HCl)
A pinch of magnesium and 5N HCl were added to the test solution and a deep red or magenta colour is formed.

NaOH test

1 ml of 1N NaOH solution was added to the test solution formation of yellow colour indicates the presence of flavonoids.

Test for Glycosides

Keller-Killiani test

1 ml of glacial acetic acid was carefully added to 2 ml of test solution of the extract and mixed well. 2 drops of ferric chloride solution was added after cooling. These contents were transferred carefully to the test tube containing 2 ml of concentrated H2SO4. A reddish brown ring was observed at the junction of two layers.

Concentrated H₂SO₄ test

1 ml of Concentrated H2SO4 was added to 1 ml of test solution and is allowed to stand for two minutes. The formation of reddish colour indicates the presence of glycosides.

Molicsh test

A mixture of Molischs reagent and concentrated H2SO4 (1:1) was added to the test solution. Formation of reddish violet ring at the junction of two liquids shows the presence of glycosides.