Materials and Methods

Selection of Plants

*Cassia* sps. (Family: *Ceasalpiniaceae*) 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.
Test for Phenols

Ellagic acid test

The test solution was treated with few drops of 5% (V/v) glacial acetic acid and 5% (W/v) NaNO3 solution. The solution turns muddy yellow, olive brown, niger brown, deep chocolate colours depending on the amount of ellagic acid present.

Phenol test

0.5% of FeCl3 (W/v) solution was added to 2 ml of test solution, formation of an intense colour indicates the presence of phenols.

Test for Sterols/Terpenoids

Libermann-Burchard test

A green colour was formed, when the Libermann-Burchard reagent was added to test solution, indicates the presence of sterols/terpenoids.

Salkowski test

A wine red colour was developed when chloroform and concentrated H2SO4 were added to the test solution, indicate the presence of steroid/terpenoids.

Test for Tannins:

Gelatin test

The test solution was dissolved in gelatin, 1% of gelatin was prepared in 10% sodium chloride, observe the formation of white precipitate.
NaCl test

The extracts were mixed with few drops of NaCl and observe the formation of ppt.

**Test for lignins**

Lobat test

Formation of olive green colour, when the gallic acid is added to the test solution, indicates the presence of lignin.

Lignin test

Formation of red colour when 2% (W/v) furfuraldehyde is added to the test solution indicates the presence of lignin.

**Test for Saponins**

Foam test

0.1g of crude extract was shaken vigorously in 2ml distilled water. Formations of honeycomb like froth persist for a few minutes indicate the presence of saponins.

**Preparation of leaf extract**

Dried leaves were powdered and aqueous extract was prepared with different concentrations (1%, 3%, 5% and 10%) by boiling the powder in distilled water, filtered supernatant was used as reducing agent.
Phytosynthesis of Gold Nanoparticles

Dried leaves of *Cassia auriculata* were powdered and aqueous extract was prepared with different concentrations (1%, 3%, and 10%) by boiling the powder in distilled water, filtered supernatant was used as reducing agent and treated with 1mM Chloroauric chloride solution for the reduction of gold nanoparticles. 10ml of aqueous leaf extract was added to 100ml of $10^{-3}$M HauCl$_4$ solution in a 250 ml conical flask at room temperature. Later 1% of aqueous leaf extract of *Cassia auriculata* was subjected to different concentrations (0.5mM, 1mM, and 2mM) of chloroauric chloride.

Phytosynthesis of Silver Nanoparticles

Dried leaves of *Cassia auriculata* were powdered and aqueous extract was prepared with different concentrations (1%, 3%, and 5%) by boiling the powder in distilled water, filtered supernatant was used as reducing agent and treated with 1mM Silver nitrate solution for the reduction of silver nanoparticles. 10ml of aqueous leaf extract was added to 100ml of $10^{-3}$M AgNO$_3$ solution in a 250 ml conical flask at room temperature. Later 1% of aqueous leaf extract of *Cassia auriculata* was subjected to different concentrations (0.5mM, 1mM, and 2mM) of Silver nitrate.

Synthesis and Characterization of Gold and Silver Nanoparticles by Microwave Irradiation

100 ml aqueous solution of 1mM HauCl$_4$ and AgNO$_3$ taken in separate flasks. 10 ml of 1% *Cassia auriculata* leaf extract was added in a 250 ml conical flask and immediately the whole mixture was put in a domestic microwave oven (Samsung Model GW73BD). The mixture was subjected to several short burst of microwave irradiation at frequency of
2.45 GHz, at power output of about 100W in a cyclic mode (on 15 s, off 15 s) to prevent overheating as well as aggregation of metals. The irradiation process was conducted for a minimum of 1 upto maximum of 23 cycles in silver nanoparticles synthesis and 15cycles for the synthesis of gold nanoparticles. The reduction of Au and Ag ions were monitored by sampling an aliquot (2ml) of the solution after 1,3,5,7,9,15 and 23 cycles and measured the UV-Vis spectra of the solution.

Synthesis and Characterization of Gold and Silver Nanoparticles by Sunlight Irradiation.

100 ml aqueous solution of 1mM HAuCl₄ and 1mM AgNO₃ taken in separate flasks and mixed 10 ml of 1% Cassia auriculata leaf extract in a 250 ml conic flask and immediately the whole mixture was exposed to bright sunlight, the change of color takes place within few minutes from colorless to ruby red in gold nanoparticles and colorless to brown color in silver nanoparticles. The experiment was performed in midnoon and the temperature was recorded 45° C in the month of April. The reduction was confirmed by optical property and UV-Vis spectra were recorded at different intervals of time.

Characterization

UV-Vis spectroscopy

Small aliquot of gold and silver nanoparticles solution was used for UV-Vis spectroscopy. The measurement was carried out on a JASCO dual-beam spectrophotometer (model V-570) operated at a resolution of 1 nm.
Fourier Transform Infrared (FTIR) spectroscopy

After complete reduction of gold and silver ions by the *Cassia auriculata* leaf broth and the solutions of gold and silver nanoparticles were centrifuged at 5000 rpm for 20 minutes to isolate the gold and silver nanoparticles from free proteins or other compounds present in the solution. The nanoparticle pellets obtained after centrifugation were redispersed in water and centrifuged 3 times to get nanoparticles free from traces of free proteins or other compounds present in the solution. FTIR measurements were carried out on a Perkin-Elmer (Model-783) in the diffuse reflectance mode operating at a resolution of 4 cm\(^{-1}\).

X-ray diffraction (XRD)

XRD measurements of the bioreduced nanoparticles drop coated on glass were done on a powder X-ray diffractometer instrument (PXRD-6000 SCHIMADZU) in the angle range of 10°C-80°C at 2θ, scan axis: 2:1 sym. The crystallite domain size was calculated from the width of the XRD peaks using the Scherrer formula.

\[
\langle D \rangle = \frac{0.9 \lambda}{\beta \cos \theta} \quad \text{…………… (Equation.1)}
\]

where \(\langle D \rangle\) is average crystallite size, \(\beta\) will indicate the line broadening value of the full width at half maximum (FWHM) of peak, \(\lambda\) is wavelength of irradiated X-rays, \(\theta\) is maximum peak position value.
Transmission Electron Measurement (TEM)

TEM samples of the gold and silver nanoparticles synthesized by the biological reduction were prepared by placing a drop over carbon coated copper grids and allowing the solvent to evaporate. TEM measurements were performed on a JEOL model 1200EX instrument operated at an accelerating voltage at 80 kV.

Biochemical Studies

Estimation of Protein by Lowry’s Method

Estimation of protein associated with gold and silver nanoparticles was done by Lowry’s method along with the aqueous leaf extract of Cassia auriculata.

Preparation of Reagents

Reagent A

2 g of sodium carbonate dissolved in 100 ml of 0.1N sodium hydroxide.

Reagent B

500 mg of copper sulphate incorporated into 100 ml of 1% potassium sodium sulphate.

Reagent C

This reagent was prepared fresh just before use by mixing 49 ml of Reagent A and 1ml of Reagent B.

Reagent D (Folin-Ciocalteau reagent)

Folin-Ciocalteau reagent was diluted with water, just before use’ in the ratio of 1:1.
**Protein solution (stock solution):**

50 mg of BSA was dissolved in distilled water and the volume made up to 50ml adding distilled water.

**Working standard**

In a standard flask, 10 ml of the stock solution was diluted to 50 ml with distilled water. 1 ml of this solution contained 200µg protein.

**20% Trichloroacetic acid**

20 g of Trichloroacetic acid dissolved in 100 ml of distilled water.

**1M Tris HCL buffer**

6.50 g of tris base dissolved in 250 ml of distilled water and pH was adjusted to 8.5.

**Procedure**

1. 0.2, 0.4, 0.6, 0.8, and 1 ml of the working standard was taken in series of test tubes.
2. 1mg of gold and silver nanoparticles powder was taken in two test tubes.
3. The volume was made up to 1 ml in all the test tubes by adding distilled water. A tube with 1 ml of water served as blank.
4. To this 5 ml of alkaline copper reagent was added including blank. The tube were mixed well and allowed to stand for 10 minutes.
5. Then 0.5 ml Folin-Ciocalteau reagent was added, mixed well and incubate at room temperature in the dark for 30 min until blue color developed.
6. The optical density was noted at 660 nm.

7. A standard graph was drawn and the amount of protein in the sample was calculated with the help of graph.

**Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis**

SDS PAGE analysis of gold and silver nanoparticles associated protein, plant extract was done by using Genei SDS PAGE Kit (cat no: 626103000011730) provided with molecular weight marker. The molecular weight determines ‘pure’ protein.

**Principle**

SDS PAGE is most widely used method for qualitatively analyzing any protein mixture, monitoring protein purity and to determine their molecular weight. It is based on the separation of proteins according to their size and then locating them by binding to a dye. SDS or sodium dodecyl sulphate is an anionic detergent that binds strongly to proteins, causing their denaturation. In the presence of excess SDS, about 1.4g of the detergent binds to each gram of protein, giving the protein a constant negative charge per unit mass. As a result, protein-SDS complex move towards the anode during electrophoresis and owing to molecular sewing properties of the polyacrylamide gel, get separated based on their molecular weight. Since, the principle of this technique is separation proteins based on size differences, by running standard proteins of known molecular weight s on the same gel as unknown proteins, molecular weight of the unknown protein can be determined. Mobility of protein in SDS gel electrophoresis is expressed as relative mobility (Rf) with respect to the tracking dye, bromophenol blue.
\[ R_f = \frac{\text{Distance migrated by protein}}{\text{Distance migrated by tracking dye}} \]  

\[ \ldots \ldots \text{(Equation.2)} \]

\( R_f \) values of protein marker of known size was used to generate a standard curve by plotting the molecular weights against the \( R_f \) value on a semi-log graph. The molecular weight of the unknown protein can then be extrapolated from its \( R_f \) value.

**Procedure**

The PAGE slab was prepared and fixed to vertical electrophoresis apparatus as the procedure given in the kit. The nanoparticles were separated from the solution by centrifugation. Protein samples (50\( \mu \)g) were denatured by boiling in sample loading buffer containing Tris buffer of pH 6.8, SDS, \( \beta \)-Mercaptoethanol (reduces disulphide bonds), sucrose and bromophenol blue (as tracking dye) for 5 minutes in hot water bath and loaded into the wells and proteins were resolved on 12% polyacrylamide gel.

**Immobilization of Gold and Silver Nanoparticles**

**Metal Solutions**

Silver nitrate and Chloroauric chloride (1mM, 3mM and 5mM) were prepared by diluting stock solutions containing chloroauric acid (HAuCl\(_4\)) and silver nitrate (AgNO\(_3\)) using deionized water.

**Preparation of Gold and Silver nanocomposites**

The sodium alginate solution was agitated in an orbital shaker at 100 rpm for 24h at room temperature to promote better uniformity. Sodium alginate solution was prepared at a concentration of 1\%, 3\% and 5\%, Calcium chloride (CaCl\(_2\)) solutions of 0.3M and 0.5 M
were prepared by dissolving the chemicals in double distilled water. Calcium alginate films were produced by cross linking with Ca\(^{2+}\) using CaCl\(_2\). Later CaCl\(_2\) was replaced by Ca(OH)\(_2\) in gels used for silver sorption to avoid silver precipitation from contact with chloride ions. After trying several preparations we standardized 3% sodium alginate, 0.5M Ca(OH)\(_2\) and 3mM metal solutions. 10ml of 3% sodium alginate was spreaded in separate petriplates (85mm diameter), slowly 20ml of 0.5M of Ca(OH)\(_2\) was added and left for 4hrs to complete polymerization of calcium and alginate forming calcium alginate hydrogels. Hydrogel was about 1mm thickness and cut into 10mm square from all sides to make a film. Rinsed thrice with double distilled water and immersed in 3mM choloroauric acid (HAuCl\(_4\)) and silver nitrate (AgNO\(_3\)) solutions for 5hrs. 1% and 5% of plant extract was prepared by adding 1gm and 5gm of leaf powder in 100ml of double distilled water used as reducing and stabilizing agent. The leaf extract then immediately added to petri plates containing alginate films for complete reduction and immobilization of the gold and silver nanoparticles inside the films.

**Fresh and Dry weight/ Moisture content**

After complete reduction and immobilization of gold and silver nanoparticles, the alginate films were blotted off excess water weighed immediately for fresh weight in triplicates. The films were dried for 1hr under oven to determine the dry weight and moisture content by using the equation:

\[
\text{Moisture Content} = \left(\frac{W_1}{W_2}\right) \times 100
\]

[Equation.3]

Where, \(W_1\)- Dry weight of film, \(W_2\)- Fresh weight of film.
Swelling and degrading studies

The films after complete reduction and immobilization of nanoparticles were immersed in phosphate buffer (pH-7.0) and double distilled water to determine the swelling time and degradation of films.

Characterization

UV-Vis spectroscopy

After the dissolution of films in phosphate buffer, UV vis spectra was recorded on a JASCO dual-beam spectrophotometer (model V-570) operated at a resolution of 1 nm.

Fourier Transform Infrared (FTIR) spectroscopy.

After complete reduction and immobilization of gold and silver ions on alginate films by the Cassia auriculata leaf broth, the films were powdered for FTIR measurement. FTIR measurements were carried out on a Perkin-Elmer (Model-783) in the diffuse reflectance mode operating at a resolution of 4 cm\(^{-1}\).

Transmission Electron Measurement (TEM)

TEM samples of the gold and silver nanocomposites were prepared by placing a drop over carbon coated copper grids and allowing the solvent to evaporate. TEM measurements were performed on a JEOL model 1200EX instrument operated at an accelerating voltage at 80 kV.
Thermogravimetry Analysis

Thermogravimetric analysis (TGA) curves of the Au-BNC and Ag-BNC were recorded using a sequential thermal analyzer Mettler STAR® SW-8.10 in the temperature range of 50°C to 1000°C in nitrogen atmosphere with the heating rate of 10°C ml/min.

Measurement of width of dry films

The width of gold and silver embedded dry films were measured by using micrometry scale and was found to be 0.8µm of gold film and 1.3µm for silver film. Formula used was

\[ W = \frac{Y \times 10\mu m}{X} \]

............... (Equation. 4)

X- Eyepiece line (Ocular), Y-Slide line (Stage).

Application studies

Antibacterial activity

Antimicrobial assay of synthesized nanoparticles was performed on *Escherichia coli*, *Bacillus subtilis* and fungi *Aspergillus niger*, *Aspergillus flavus* by disc diffusion method. The cultures were obtained from Department of Microbiology, Gulbarga University, Gulbarga. Sterile 6mm diameter of whatman no.1 filter paper disks were prepared by applying 30µg/ml 1%, 3% and 5% of synthesized AgNP’s and cefotaxime as standard for bacteria and 50µg/ml of AgNP’s and Clotrimazole as standard for fungi. The test organisms were subculture in nutrient broth media (bacteria) and Czapekdox broth media
(fungi). These cultures were used for antimicrobial assay. The nutrient agar media/Czapekdox agar media was poured in sterile petriplates under sterile conditions and left to solidify. About 0.5ml of bacterial/fungal suspension was uniformly spread on media and the prepared disks were placed. The culture plates were incubated at 38º C and zone of inhibition was measured after 24hrs and 48hrs. The experiment was performed in triplicate and average diameter of zone of inhibition was recorded by analyzing the data using SPSS (Mean values ± S.E).

*Escherichia. coli*: It is a gram negative bacterium. These organisms found in the intestinal tract or partially in all vertebrates. These organisms go rapid multiplication and cause diseases in animals and human beings under favorable conditions and cause septicemia meningitis, appendicitis peritonitis, diarrhea, cystitis pyelites, urinary tract infections, food poisoning, and neonatal meningitis.

*Bacillus subtilis*: It is a gram positive rod shaped bacilli found in soil cause food poisoning.

*Aspergillus niger*: It is a saprophytic, septate mycelia ascomycetous fungus. The nephrotoxins produced by *A.niger*, cause kidney and liver damage, convulsions, hemorrhages of lung and brain. It also causes aspergillosis in ducks and chickens.

*Aspergillus flavus*: It is a mold fungus, pathogenic associated with aspergillosis cause corneal and nasoorbital infections. *A.flavus* is the second most common isolate of aspergillosis, the first being *A.fumigates* infects arteries of lungs or brain and cause infection.
Media Preparation

Preparation of Nutrient Agar Media (1000ml)

Peptone - 5gms
Beef extract - 3gms
Agar - 20gms
Distilled water - 1000ml

5 gms peptone and 3 gms of beef extract was dissolved in 1000 ml of distill water in a conical flask. Shaken well till completely dissolved. The pH adjusted to 7, then 20 gms agar was added and autoclaved the flask at 15 lbs pressure for 20 minutes for sterilization.

Preparation of Nutrient Broth Media (500ml)

Peptone - 2.5gms
Beef extract - 1.5gms
Distilled water - 500ml

2.5gms of peptone and 1.5gms of beef extract was dissolved in 500 ml of distill water in a conical flask. Shaken well till completely dissolved. The pH was adjusted to 7.0, then the flask was sterilized by autoclaving at 15 lbs pressure for 20 minutes. The strains were obtained from the lab stock, on the day before testing, the organisms were subculture into sterile broth media and the suspension was used as inoculum for the test.
Preparation of Czepek dox Agar medium

Sodium nitrate - 2.0gm
Dipotassium hydrogen phosphate - 1.0gm
Magnesium sulphate - 0.5gm
Potassium sulphate - 0.5gm
Ferrous sulphate - 0.01gm
Sucrose - 30gm
Agar - 20gm
Distilled water - 1000ml
pH - 6-6.5

All the above chemicals were dissolved in 1000 ml of distill water in a conical flask. The pH adjusted to 6-6.5, then 20gm agar was added and autoclaved the flask at 15 lbs pressure for 20 minutes for sterilization.

Preparation of Fungal suspension for Bioassay

The fungal cultures *Aspergillus niger* and *Aspergillus flavus* were maintained on Czepek dox broth media incubated at room temperature for 4 days.
Seed Germination Studies

Preparation of nanoparticle dispersion

The AuNPS and AgNPs were suspended in double distilled water and were dispersed using ultrasonic vibrations (100W, 30kHz) for 30 minutes to produce required concentrations for the experiment. The organic meioties coated on biologically synthesized nanoparticles stabilize the nanoparticles and prevent from aggregation in the medium.

Germination experiment

Pearl millet (*Pennisetum glaucum*) seeds were collected from Agricultural research station Gulbarga, Karnataka. The seeds were first soaked in liquid soap solution and bavistne (fungicide) for 3 minutes and then rinsed twice with deionized water (dH₂O) to remove surface adherents. Then seeds were placed in dH₂O (control) and 20µg/ml, 50µg/ml of AuNPS and AgNPs solutions and shaken gently for two hours (Lin and Xin). All the treated seeds were subsequently transferred into 15 mm × 100 mm petri dishes containing one piece of filter paper (90 mm in diameter, Whatman No.1). 10-15 seeds were evenly spaced on top of the filter paper in each petri dish and 5ml of 20mg/L, 50mg/L nanoparticle suspensions and control (distilled water) were added. Finally, the dishes were covered and allowed to seed germination, set on a lab bench at room temperature (30±5°C). The experiment was performed in triplicate. Germination percentage were determined by comparing the number of seeds that developed a primary root of at least 1 mm to the total number of seeds in each dish. The number of germinated
seeds in each dish was counted after 5 days and germination percentage was calculated using Equation (6).

\[
\text{Germination \%} = \frac{\text{No. of Germinated seeds}}{\text{No. of Inoculated seeds}} \times 100 
\] ……(Equation.6 )

Statistical Analysis

Each treatment was conducted with three replicates and total mean length of primary root, shoot and seedlings were measured after 5 days of inoculation and analyzed the data using SPSS (Mean ± S.E). Each experimental value was compared to its corresponding control.

**Anticancer Studies**

**Materials**

The cancer cell lines such as A549 (Human lung carcinoma), MDA-MB (Human adenocarcinoma mammary gland), LNCap-FGC (Human carcinoma prostate) were purchased from National Center for Cell Sciences (NCCS, Pune India), and was maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic solution.

**In vitro cytotoxic activity**

The effect of gold and silver nanoparticles on the viability of A549, LNCap-FGC, MDA-MB was determined by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay, the reduction of yellow tetrazolium salt by mitochondrial dehydrogenase of metabolically active viable cells forms blue-purple formazan that could be measured
spectrophotometrically. Hence, the intensity of the color in the solution is directly proportional to cell viability. Cells were cultured in Dubelco’s modified essential medium (DMEM) with 10% foetal bovine serum containing pencillin (100 units/ml) and streptomycin (100μg/ml). The cells were maintained at 37°C in a humidified 5% CO₂ incubator. A549, LNCap-FGC, MDA-MB cells in the concentration of 1×10⁶ cells/ml were taken into 96 well plates. Then the cells were treated with different concentrations of AuNPs and AgNPs (10, 20 and 30 µg/ml) and incubated for 4h in the presence of 5% CO₂ and 95% humidity at 37°C by adding MTT (100µl). The formazan crystals were dissolved in 100µl of DMSO and the absorbance of wells containing cells and blank was measured at 490nm. The absorbance values of the test (treated) and control (untreated) cells were used for the determination of the percentage cell viability. Cell survival in control cells was assumed to be 100%. The percentage cell viability was calculated by formula:

\[
\text{% Cell viability} = \frac{\text{O.D. of test} \times 100}{\text{O.D. of control}} \quad \text{(Equation.7)}
\]

DNA Fragmentation Assay

A549, LNCap-FGC, MDA-MB (10⁶ cells ml) were seeded in 6well microplates and treated with 10, 20 and 30µg/ml of AuNPs and AgNPs. After 24h of treatment, the culture medium was removed, and the cells were harvested by scraping with 1ml of PBS and lysed with 500µL of lysis buffer (20mM Tris-HCl (pH 8.0), 5mM EDTA, 400mM NaCl, 1% SDS, and 10mg/ml proteinase K) for 1 h at 55°C. Fragmented DNA was extracted with phenol/chloroform/isoamyl alcohol (25 : 24 : 1 v/v/v), precipitated with
ethanol, and resuspended in Tris-EDTA buffer (TE, pH 8.0) containing 20μg/ml RNase-A. For quantitative analyses of DNA content, an equal amount of DNA was loaded and run on a 1.0% agarose gel containing 1μg/ml ethidium bromide at 70V, the DNA fragments were visualized by exposing the gel to ultraviolet light followed by photography.

**Antibacterial Activity of Gold and Silver Nanocomposites**

Antibacterial activity of prepared nanocomposites was determined against gram negative *E. coli* and gram positive bacteria *B. subtilis*. The test organisms were subcultured in nutrient broth media for 12hrs. The nutrient agar media was poured in sterile petriplates under sterile conditions and left to solidify. About 0.5ml of bacterial suspension was uniformly spread on media, the gold and silver nanocomposites/films and control film of pure alginate was placed. The culture plates were incubated at 38º C and zone of inhibition was measured after 24hrs. The experiment was performed in triplicate and average diameter of zone of inhibition was recorded by analyzing the data using SPSS (Mean values ± S.E).