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GREEN SYNTHESIS OF COPPER NANOPARTICLES USING ECLIPTA PROSTRATA LEAVES EXTRACT AND THEIR IMPACT ON SEED GERMINATION AND SEEDLING GROWTH OF SORGHUM VULGARE

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ABSTRACT

Nanotechnology is a vital and rapidly evolving breakthrough with a wide range of applications. It entails the mixing and application of materials with one of the measurements ranging from 1 to 100 nm. For the blending of nanoparticles, a variety of physico– synthetic techniques are presently being used (NPs). Nanoparticles' size, orientation, and physical features have been proven to alter the performance of any material. For several years, scientists have been experimenting with various nanoparticle synthesising processes. In contrast to chemical-mediated or green synthesis, the green technique of nanoparticle synthesis is simple, efficient, and environmentally benign. Chemical synthesis involves toxic solvents, high pressure, energy conversion, and high temperature. Microbial synthesis is not industrially feasible due to its laboratory maintenance. Since green synthesis is the best choice for nanoparticle synthesis, the focus of this research is on the cheapest and simplest way to synthesize copper nanoparticles using Eclipta Prostrata leaves extract. The synthesized lead nanoparticles were confirmed by Visible UV spectroscopy, XRD and particle size analyser. The toxicity was tested by studying the size of the 87 nm nanoparticles obtained, and the synthesized nanoparticles were used to study seeds and the effects on growth. The antimicrobial activity of lead nanoparticles was analysed with pathogenic fungi, bacteria and adequate photographic algae. Synthetic nanoparticles showed an efficient antimicrobial activity against bacterial and pathogenic fungi. In the same way, synthetic nanoparticles showed an efficient activity against the aggregate to spirulina culture.

Keywords: green synthesis, microwave, copper nanoparticles, Eclipta prostrata leaf extract, biological application, antibacterial activity and influence on germination.

INTRODUCTION

Metal nanoparticles have a high specific surface area and a high proportion of surface atoms. Due to the unique physical and chemical properties of nanoparticles, including catalytic activity, optical properties, electronic properties, antibacterial properties and magnetic properties, scientists are increasingly interested in their new synthesis methods. In recent years, the synthesis of metal nanoparticles is an important research topic in modern materials science. Nanocrystalline silver particles have huge applications in the fields of high-sensitivity biomolecular detection, diagnosis, antibacterial, therapy and catalysis. The chemical, physical and fungal synthesis of silver and gold nanoparticles has been reported in the literature, but studies on the use of Eclipta Prostrata leaves extract to synthesize copper nanoparticles and their possible applications, as well as not much is given about its positive and negative effects

on germination of seeds. Therefore, this research aims at the green synthesis, characterization and possible applications of copper nanoparticles.

Copper is a highly toxic heavy metal that affects every organ and system in the body. Ingestion of copper-contaminated food or water can cause poisoning. It can also enter the human body through inhalation of leaded paint, leaded gasoline, dirt and dust. Therefore, to understand the physical and chemical properties of nano-lead and its toxicity, please compare with metallic lead. Generally, nanoparticles are designed with surface modifications to meet the needs of the specific application in which they will be used. The enormous diversity of nanoparticles (Figure 1) comes from their wide range of chemical properties, shapes, and morphologies, the medium in which the particles exist and the dispersion state of the particles.

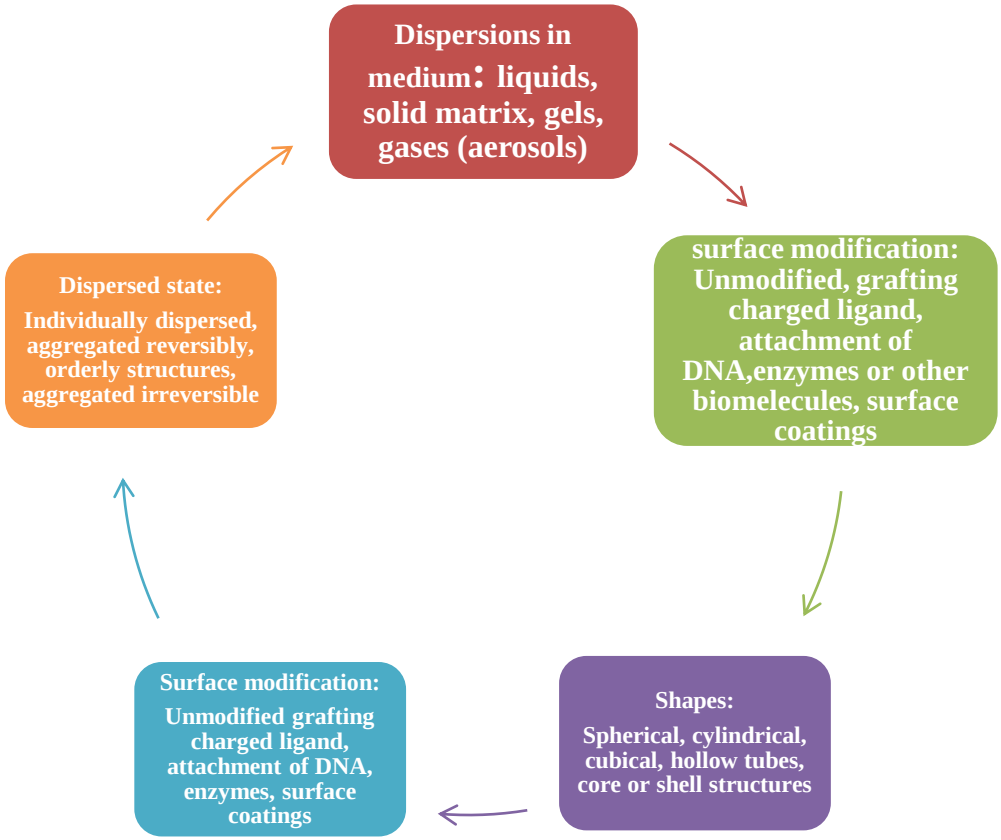

Fig (1): Several features that contribute to the diversity of engineering nanoparticles. The same chemical substance can produce a variety of nanoparticles.

MATERIALS AND METHODS

Preparation of Eclipta Prostrata leaves extract

Eclipta Prostrata leaves were purchased from the local rythu market in Secunderabad, washed several times with water to remove dust particles, then dried to remove residual water and ground to powder. Next, the leaf extract was prepared by mixing 1% of the extract with deionized water in a 250 ml Erlenmeyer flask (Borosil, India). The solution was then cultivated for half an hour. Then centrifuge for half an hour with 4000 revolutions per minute at room temperature. With the help of a vacuum filter, filter paper (what kind of filter paper) is used to separate and filter the supernatant. The solution is then used to reduce the lead ions to copper nanoparticles.

Source of Copper (Cu)

1 litre of 0.001 M copper (Cu) solution was prepared by using Copper acetate, as precursor source for the synthesis of copper nanoparticles.

Preparation of copper nanoparticles

Preparation of an aqueous solution of copper acetate (0.001 M) to synthesize copper nanoparticles was done. Add 10 mg of Eclipta prostrata leaf extract to 90 ml of 0.001M aqueous copper acetate solution to reduce to Cu + 2 ions, and keep in a 700watt microwave oven for 5 minutes. Here, the filtrate acts as a reducing agent and stabilizer for the copper acetate (0.001 M).

Figure-2: Eclipta prostrata leaves and extract

Eclipta prostrata leaf extract weighing was thoroughly washed and dried into 100ml distilled water

↓

Filtered through whatmann filter paper followed by filtration through 0.22µ*m* sized filter paper

↓

0.001M copper acetate was prepared in 1000ml distilled water

↓

10mg of Eclipta prostrata leaf extract was added into 90ml of aqueous solution of 0.001M copper acetate

↓

Cultivation in microwave oven at 750watts for five minutes

↓

Copper nanopartices formation

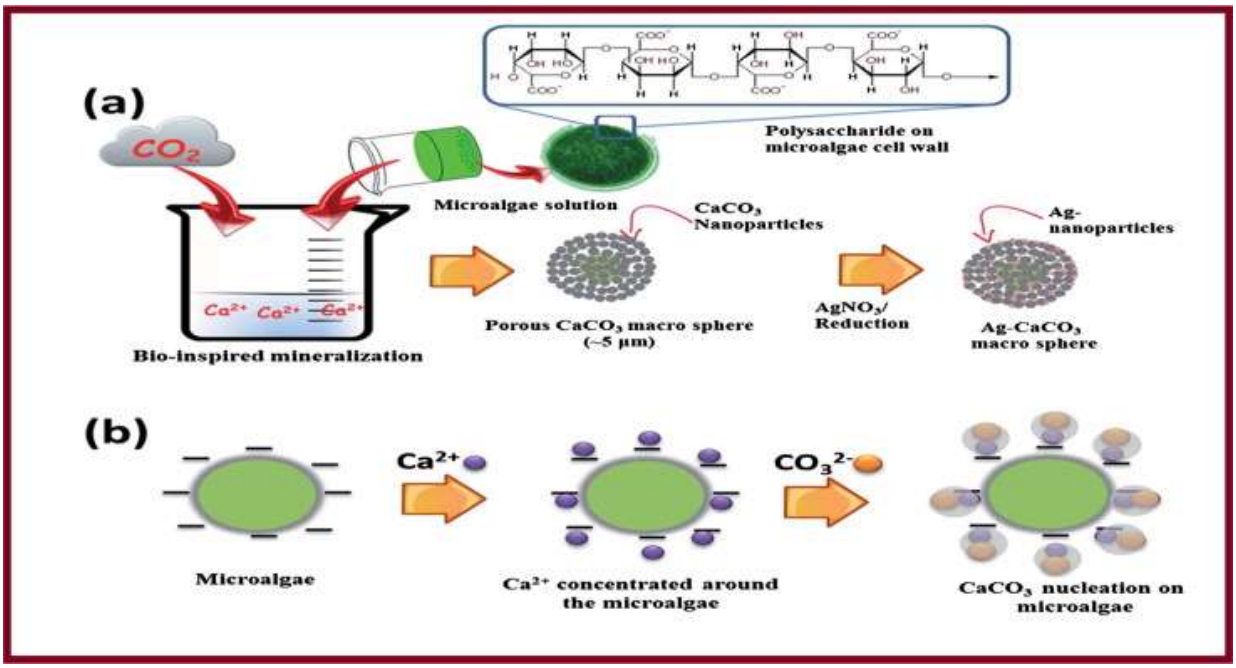

Figure-3: Method of Biological Synthesis of copper Nanoparticles and detailed Mechanism

Figure 4: Green synthesis of copper nanoparticles

Advantages of using Plant leaf extracts

The use of hybrid procedures to synthesize nanoparticles has always created pressure among tree hugs because they have an adverse effect on their science. From now on, due to their healthy properties on the planet, the use of plant extracts for nanoparticle game projects is favoured. In fact, even in business, it can significantly reduce the generation of hazardous waste. The plant supplements the diminishing host and compensator of nanoparticles. Generally speaking, these nanoparticles should be included in various programs remotely.

By standing out from the factory system, the monetary gain from the engineering method is less because the cost of aid is fundamentally less and the exchange of waste requires less effort among various factors. This method is surprisingly superior to any method using conventional techniques, because the entire plant system is far less useful than a culture that needs to handle a large number of tiny, miraculous life forms. Subsequent inspection showed that the regulating effect of plants, of which nanoparticles can be decomposed, can be saturated on the particles in

a similar way. In this way, the carrier of the treatment material can be beautified to reach the site. action and also eliminate errors. Need to develop specific drugs for pain.

CHARACTERIZATION OF COPPER NANOPARTICLES

UV-Vis Analysis

The optical properties of copper nanoparticles were measured by an ultraviolet-visible spectrophotometer (Lambda 35, Germany). After adding copper acetate to the seed extract, the spectra were collected between 350 nm and 500 nm at different time intervals, namely 1 to 5 minutes.

FTIR analysis

The FTIR spectrometer (Perkin Elmer LS55 luminescence spectrometer) was used to study the chemical composition of the synthesized copper nanoparticles. The solution was dried at 75 ° C and the dry powder was characterized by the KBr particle method in the 4000-400 cm 1 range.

Particle Size Distribution

A MicrotracModel S3500 particle size analyzer (IICT, Hyderabad, Telangana) was used to further characterize the particle size distribution of colloidal copper nanoparticles synthesized with Eclipta prostrata leaf extract. The size distribution is determined based on the dynamic scattering of a red laser with a wavelength of 750 nm. Due to the Brownian motion of the colloidal copper nanoparticles, light is scattered. In the total percentage of the particle size distribution, the D50 value of 50% of the particle size distribution is considered.

XRD Analysis

The phase type and grain size of the synthesized copper nanoparticles were determined by X-ray diffraction spectroscopy (Philips PAN analysis). CUKα radiation was used to study the synthesized copper nanoparticles under a voltage of 30 kV and a current of 20 mA at a scanning speed of 0.030 / s. The different phases present in the synthesized sample are determined by the X'pert high score software with search and comparison functions. The particle size of the prepared sample is determined using the Scherrer equation as follows

$$D \approx 0.9\lambda / \beta \cos\theta$$

where D is the size of the crystal, λ is the wavelength of the X-rays, θ is the Bragg angle in radians, and B is the full width at half the maximum of the peak in radians.

Anti-Bacterial & Anti-Fungal Activity of Copper Nanoparticles

All pure cultures of experimental bacteria and fungi come from the Microbial Type Culture Collection and Gene Bank (MTCC) of the Institute of Microbiology Technology (IMTECH) in Chandigarh. Pure bacterial cultures are maintained on nutrient agar medium and fungal cultures are maintained on Potato Dextrose Agar (PDA) medium. Each bacterial and fungal culture was further maintained by regular subculture in the same medium and stored at 4 ° C before being used in experiments.

Media Preparation & Its Sterilization

For the agar hole diffusion method, antibacterial sensitivity was tested on a solid medium (agar) in a Petri dish. For bacterial assays, Nutrient Agar (NA) (40 g / L) and fungal PDA (39 g / L) are used to culture colony growth on the surface. The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum bactericidal concentration (MFC) were determined by the E-test. In suspension culture, Lauria broth at 2% (w / v) was used for the growth of bacterial cells, and PDB (potato dextrose broth) at 2.4% (w / v) was used for the evaluation of the growth of fungal cells. Then, autoclave all prepared media at (121 ° C) for 20 minutes.

Agar well diffusion method

Antibacterial activity was determined by the Kirby-Bauer disk diffusion method. Clean the nutrient agar (NA) and potato dextrose agar (PDA) plates with the culture broth of the respective bacteria and fungi for 4 hours (sterile cotton swabs). Use a sterile cork drill to make holes in each of these plates (10mm in diameter and approximately 2cm each). A stock solution of plant extracts and copper acetate was prepared at a concentration of 1 mg / ml. Use a sterile syringe to add approximately 10 80 µl of copper nPs of different concentrations into the holes and allow them to diffuse for 2 hours at 20-22°c.

Incubate the two wells with 100 µl of plant extract and copper acetate solution to understand the best activity of the precursor reagents used in the preparation of copper nPs. A control experiment was set up containing an inoculum without plant extracts and copper nPs. For bacterial pathogens, incubate the plate at 37 ° C for 18-24 hours and fungal pathogens at 28 ° C for 48 hours. Determine the diameter (mm) of the zone of inhibition and calculate the activity index. Hold three repetitions, the experiment is repeated three times, for each repetition, read the readings in three different fixed directions and record the average value.

Minimum Inhibitory concentration

The minimum inhibitory concentration is defined as the lowest concentration that can inhibit the growth of any visible bacteria on the culture plate. This is determined by the readings on the culture plate after incubation. The most commonly used methods are slide method and Kirby-Bauer disk diffusion. Prepare serial dilutions of the product in bacterial and fungal growth media. The test organism is then added to the product dilution, incubated, and the growth is scored. This procedure is a standard antimicrobial test. In diagnostic laboratories, the minimum inhibitory concentration is important to confirm the resistance of microorganisms to antimicrobial agents and to monitor the activity of new antimicrobial agents. MIC is generally considered to be the most basic laboratory measurement of the activity of antimicrobial agents on organisms.

Preparation of Inoculum

Test for antibacterial activity

The antibacterial test is carried out by the microdilution method to determine the antibacterial activity of the tested compound against pathogenic bacteria. The bacterial suspension was

adjusted with sterile saline at a concentration of 1.0 X 107 CFU / ml. Prepare the inoculum and store at 4 ° C until use. The inoculum dilution is grown on solid medium to verify that there is no contamination and to check the efficacy of the inoculum. All experiments were repeated 3 times.

Test for Antifungal Activity

To study the antifungal activity of the extract, a modified microdilution technique was used. Wash the fungal spores from the surface of the agar plate with sterile 0.85% saline containing 0.2% (v / v) Tween 80. The spore suspension is adjusted with sterile saline to a concentration of approximately 1.0-107, and the final volume is 100 µl / well. The inoculum is stored at 4 ° C to determine the MIC for later use. Incubate a dilution of the inoculum on solid potato dextrose agar to verify that there is no contamination and to check the efficacy of the inoculum.

The minimum inhibitory concentrations (MIC)

MBC and MFC are performed by serial dilution technique using 96-well microtiter plates. Take different concentrations of CuNP, use luria broth to serially dilute the extract for bacterial culture and fungus, and the corresponding inoculum in potato dextrose broth medium. The microplates were incubated at 28°C for 72 hours. The lowest concentration with no visible growth (under a binocular microscope) is defined as the MIC.

Determination of MBC

MBC is determined by subculturing 2 µl serially into a microtiter plate containing 100 µl broth per well and incubating for a further 72 hours. The lowest concentration with no visible growth is defined as MBC, indicating that the destruction rate of the original inoculum is 99.5%. The optical density of each well was measured by a microplate reader (Perlong, ENM8602) at a wavelength of 655 nm and compared with the bacterial standard ampicillin (Himedia Laboratories, India) as a positive control and the experiments were repeated thrice

The fungicidal concentration (MFC) was determined by serially passing 2 µl to a microtiter plate containing 100 µl broth per well and incubating for a further 72 hours at 28°C. The lowest concentration with no visible growth was defined as MFC, indicating that the killing rate of the original inoculum was 99.5%. All experiments were repeated 3 times.

Determination of MFC

The fungicidal concentration (MFC) was determined by serially passing 2 µl to a microtiter plate containing 100 µl broth per well and incubating for a further 72 hours at 28°C. The lowest concentration with no visible growth was defined as MFC, indicating that the killing rate of the original inoculum was 99.5% and the experiments were repeated 3 times.

Anti - Algal Activity of Copper Nanoparticles

Metals with small attachments are essential for algae cells to perform cellular functions. They act as part of photosynthetic electron transfer proteins (Cu, Fe) and photosynthetic water oxidant focal point (Mn), or as a component of vitamins (Co). In addition, they serve as catalysts

of interest for the fixation of CO2 (zinc in carbonic anhydrase), the interpretation of DNA (zinc in RNA polymerase) and the acquisition of phosphorus (zinc in alkaline phosphatase) N2 penetration (Mo, Fe, V in nitrogenase) and reduction of nitrate (Mo in nitrate and Fe in nitrite reductase). Nevertheless, the high degree of convergence of these metals and other large amounts of excess metals (Hg, As, Cd, Pb, Cr) can cause negative effects (weakened photosynthesis mechanism, hindered cell division, restricted catalytic effect) in microalgae cells.

Metals also affect the morphology of algae cells. In this study, the anti-algae potential of transgenic CuNPs against cyanobacteria that form toxic blooms was evaluated. Spirulina was used to study the growth inhibitory effect of the use of engineered lead nanoparticles. Cell density, fresh biomass weight change, dry biomass weight, and growth inhibition percentage were used to determine the toxic effects of CuNPs. The media used for the growth of Spirulina is listed in the table below:

Table-1: Chemical Components used in Media Preparation for Algal Growth

S.NO.	INGREDIENT	AMOUNT (g/L)
1.	$NaHCO_3$	16.8
2.	$NaNO_3$	2.5
3.	NaCl	1.0
4.	K_2SO_4	1.0
5.	K_2HPO_4	0.5
6.	$MgSO_4.7H_2O$	0.2
7.	$FeSO_4.7H_2O$	0.01
8.	$CaCl_2.2H_2O$	0.04
9.	EDTA	0.08
10.	H_3BO_3	0.00286
11.	$MnCl_2.4H_2O$	0.00181
12.	ZnO	0.00022
13.	MoO_3	0.00001
14.	$CuSO_4.5H_2O$	0.00008
15.	Distilled Water	1000

All the experiments in this study were performed three times. Transfer 50 ml of the prepared synthetic medium to a series of sterile Erlenmeyer flasks and inoculate 0.2 g of dried spirulina culture. Erlenmeyer flasks are represented by the numbers 1, 2, 3, 4, 5, 6, and contain copper nanoparticles of 1 µg/ml to 3 µg/ml, respectively. These inoculated flasks were kept in a BOD incubator, and the toxicity of the nanoparticles in Spirulina was observed at fixed time intervals of 10, 15, and 20 days. CuNPs toxicity is expressed as the percentage of cyanobacteria growth inhibition, calculated using the following formula:

$$\text{cyanobacteria growth inhibition} = \frac{\text{Control} - \text{treatment}}{Control} \text{ X } 100$$

Impact of CuNPs on Seed Germination of Sorghum vulgare

Due to the positive impact on many economic sectors, including agriculture, the use of man-made nanomaterials has increased. Silver nanoparticles (AgNPs) are now used to promote seed

germination, plant growth, and photosynthetic quantum efficiency. In this study, we examined the effect of CuNPs dose on sorghum seed germination to examine the toxicity levels of the metals Cu and CuNPs.

Selection of Seeds

Select sorghum seeds to study the germination effect of CuNPs. 20 sorghum seeds are counted and screened by flotation method. Use standard methods to perform physical and chemical characterization of the soil before and after germination. The microwave digestion method was used to mix different concentrations of CuNPs with 500 g of soil.

RESULTS & DISCUSSION

Physical observation of the formation of CuNPs

The formation of CuNPs was first concluded by the colour change of the copper acetate solution when mixed with the fennel seed extract. Different ratios of Eclipta prostrata leaf extract showed different changes in colour changes, as shown in Figure 5.

Figure 5: Different ratios of Eclipta prostrata leaf extract showed different changes in colour

UV- Visible Spectroscopy

Ultraviolet visible Absorption is key tools to determine structure and optical properties of metal nanoparticles because the absorption band is related to the precise diameter and aspect ratio of metal nanoparticles. Colloidal CuNPs have a unique yellow-orange solution. At the nanometre size, the surface electron cloud can vibrate and absorb electromagnetic radiation of specific energy. CuNP samples are prepared by chemical methods, and the changes in the UV-visible spectrum of the resulting solution are observed to analyse the effect of the size of metal nanoparticles on surface plasmon resonance (SPR). The figure shows the absorption spectrum of a reaction mixture containing copper acetate (0.001 mM) and an aqueous solution of Eclipta prostrata leaf extract. The obtained absorption spectra show that when mixed with plant extracts, CuNP is produced within 5 minutes under microwave irradiation (Figures 4 and 5). The above plant extracts were added to the Copper acetate solution, and the solution changed from brown to yellow-brown. As time goes by, the final colour will darken and eventually become dark brown. It was found that the intensity of absorbance increased as the reaction proceeded.

CuNPs, which are dark brown in water, come from surface plasmons. This is due to the dipole oscillation generated when the electromagnetic field in the visible range is coupled with the collective oscillation of conductive electrons. The established fact is that metal nanoparticles with a size of 210 nm show a strong and broad surface plasmon peak. The light absorption spectrum of metal nanoparticles controlled by surface plasmon resonance (SPR) shifts to elongated wavelengths as the particle size increases. The position of the absorption band is also highly dependent on the dielectric constant of the medium and the substance adsorbed on the surface.

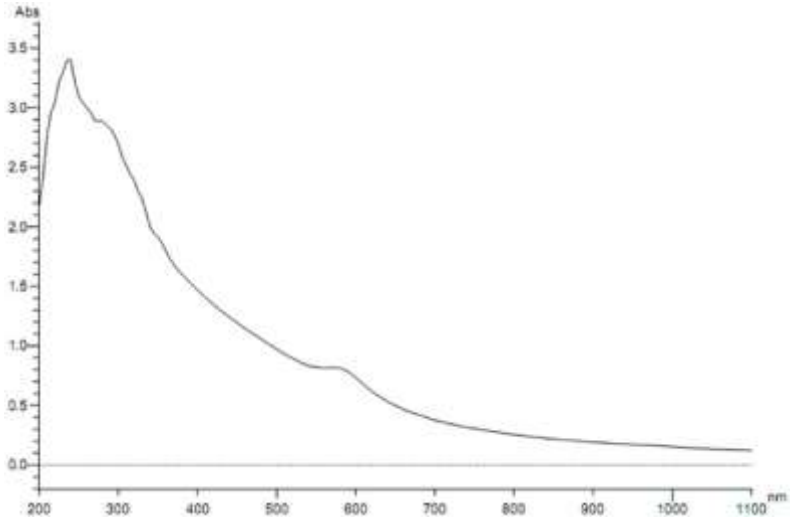

Figure-6: UV- Visible Spectra of Copper Nanoparticles

Particle Size Analysis of CuNPs

Use dynamic light scattering measurement technology to determine the particle size of the synthesized lead nanoparticles. Dynamic Light Scattering (DLS) is a technique to characterize the size of colloidal scattering, which uses the illumination of a particle or molecular suspension. An autocorrelator is used to analyse the fluctuations in the intensity of the scattered light that occurs over time, which determines the autocorrelation function of the signal. The size distribution of the synthesized CuNPs is shown in Figure 6. It can be seen from the figure that the obtained particles are polydisperse mixtures in the range of 40 to 150 nm. The mean size of the silver nanoparticles synthesized with berry extract is about 87 nm. The size and shape of metal nanoparticles are affected by many factors, including pH, precursor concentration, reducing agent concentration and incubation time. The zeta potential of the synthesized CuNPs was measured in water as a dispersant. The zeta potential is found to be -68.07 mV. The high value confirms the repulsive force between the particles, thus increasing the stability of the formulation. The zeta potential value can be positive or negative; the negative potential value shown by the CuNPs may be due to the fact that the bioorganic components of the extract may be blocked.

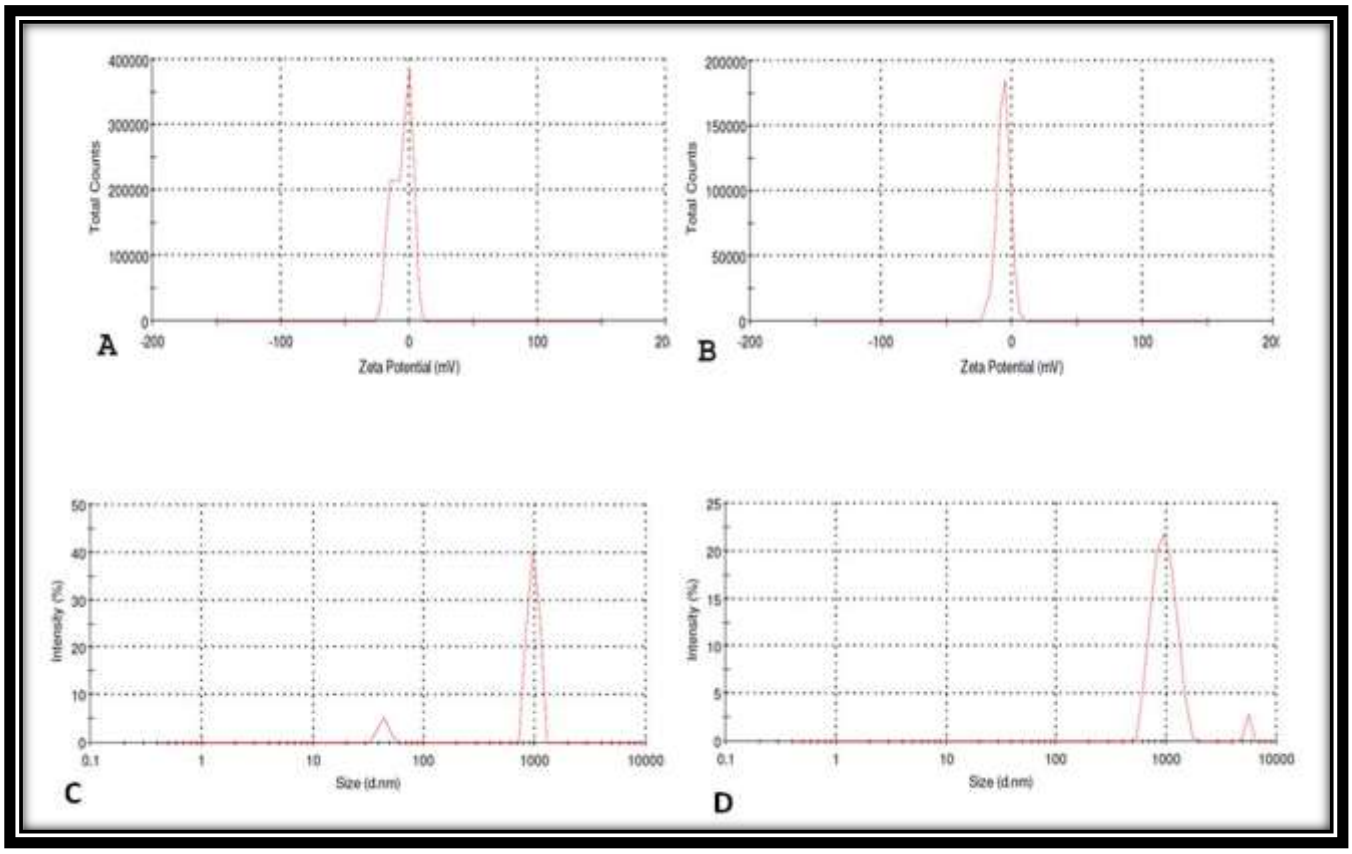

Figure 7: Particle Size Analysis of Green Synthesized Copper Nanoparticles

FTIR Spectral Analysis

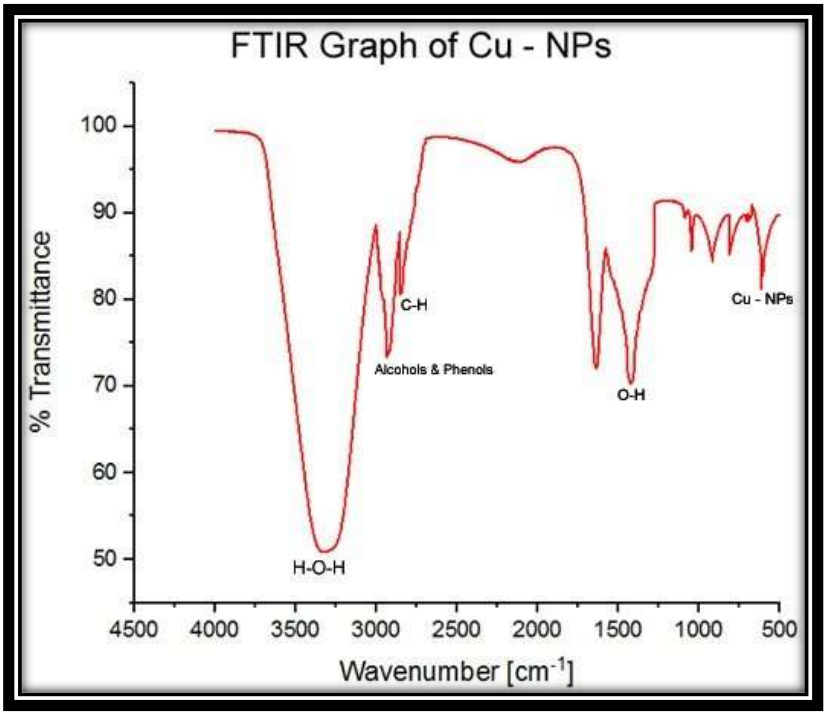

Figure-8: FTIR spectrum of copper nanoparticles

X-Ray diffraction studies of copper Nano particles:

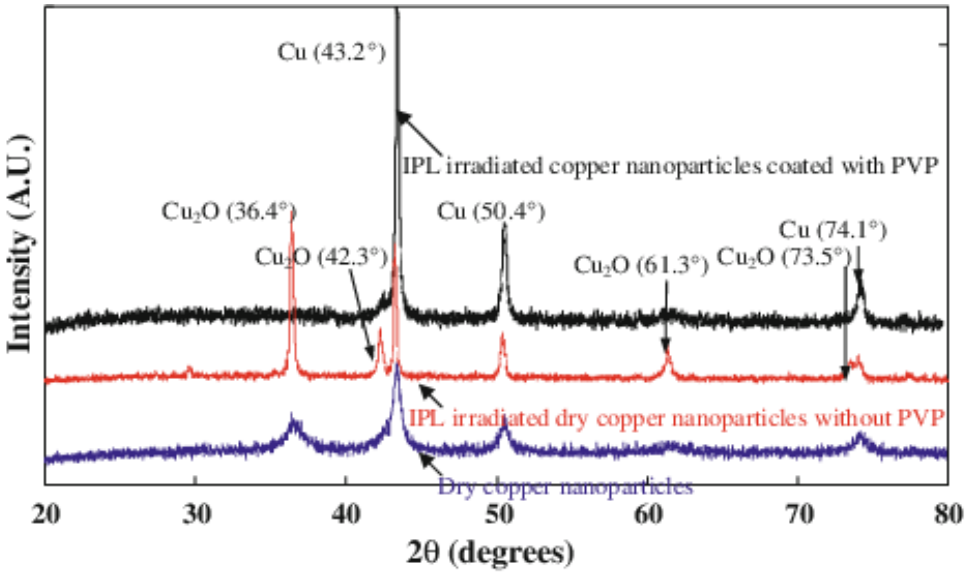

Figure 9: XRD Spectrum of Copper nanoparticles

X-ray powder diffraction studies can provide information on translational symmetry, size, and unit cell shape of any compound. It is an important tool used in solid size chemistry and materials science.

Provides information on perfect particle deviations and defects extending from the shape and width of the peak.

Peak Indexing:

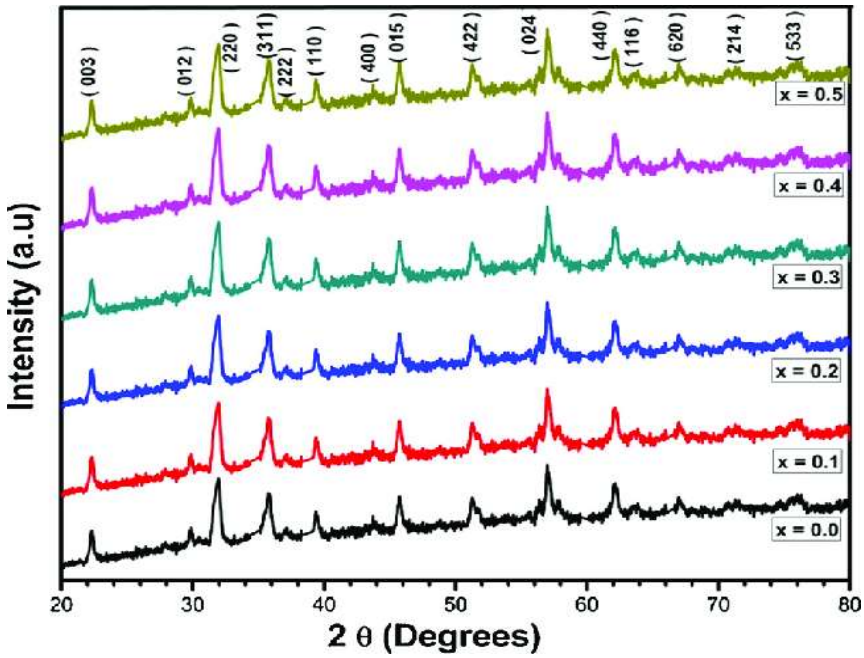

Figure 10: XRD Spectrum, peak indexing and Reflections

Anti - bacterial activity

Maintaining control (10 µl of CuNps, 10 µl of copper acetate and 10 µl of Eclipta prostrata leaf extract), the agar well diffusion method was used to detect the effects of CuNP synthesized from the extract of Cuminum cyminum seed on E. coli and Gram-negative bacteria The antibacterial activity of copper acetate The antibacterial effect of copper acetate is determined according to the inhibition circle (mm) in the figure. From the figure it can be concluded that the Eclipta prostrata leaf extract did not show antibacterial activity and the CuNPs showed a higher zone of inhibition. Aqueous solution of copper acetate and streptomycin antibiotic standards. Escherichia coli is a gram-negative bacterium with a maximum inhibition zone of 18 mm for CuNPs.

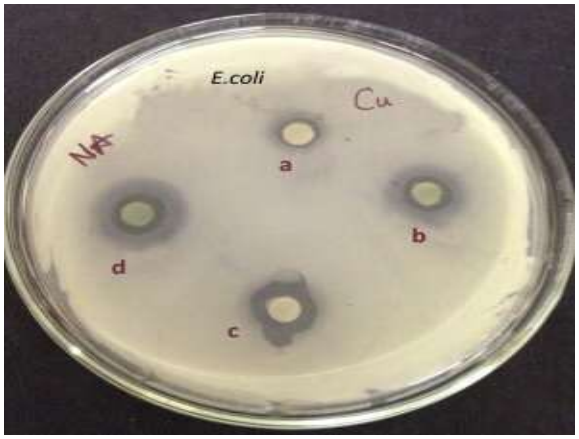

Figure 11: Anti-Bacterial Activity of Copper Nanoparticles against E.Coli

Anti – fungal activity

The antifungal activity of CuNPs synthesized from Eclipta prostrata leaf extracts against Candida albicans was examined using a controlled agar well diffusion method (10 µl CuNps, 10 µl copper acetate, and 10 µl Eclipta prostrata leaf extract. The effect of copper acetate is determined according to the circle of inhibition (mm) in the figure. It can be concluded from Figure 11 that the Eclipta prostrata leaf extract has no antibacterial activity, and CuNPs show a higher zone of inhibition. Candida albicans and CuNPs show a maximum inhibition zone of 22 mm.

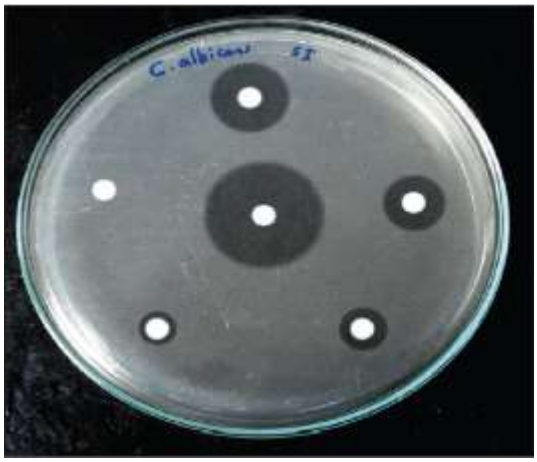

Figure-12: Determination of Anti - Fungal Activity of Copper Nanoparticles against Candida albicans

Anti – Algal activity

Spirulina is known as the most common cyanobacterium (CB) and exists in a eutrophic water environment. The use of nanoparticles to control algae growth is a promising new technology for water restoration. In this study, the use of CuNP to control the growth of CB was studied. To study the effect of CuNPs on spirulina growth, cells were exposed to increasing concentrations of CuNP from 1 to 3 mg / 500 ml per 0.2 g of spirulina. The growth of the control and CuNP treatments was estimated at 0, 15, 20 and 25 days of exposure. The biomass weight (dry and fresh biomass) of CB cells cultured at different concentrations of nanoparticles is plotted on the graph. As shown in the graph, it can be concluded from the graph that the cell growth rate decreases as the CuNP concentration increases, up to 15 days. When the contact time was increased, the spirulina cell concentration decreased due to increased toxicity and a decrease in cell growth was observed. On day 25, the cell growth rate reached equilibrium compared to the control. The reason behind this is that as the incubation period increases, the biomass increases and the concentration of CuNP used in this study is not sufficient to achieve this growth rate and there will be no change. Low concentration of nano metal can stimulate the growth and production of target compounds, but excessive metal can have harmful and fatal effects on algae culture. Therefore, the cultivation of algae in waste water contaminated by nano-metals must be designed in such a way that the interaction between cells and metals is limited, so that the metal concentration only has a beneficial effect on the growth of algae and the biosynthesis of products. The response of algae cells to the presence of nanometals depends on many factors, such as culture conditions, availability of nutrients, and the presence of organic compounds.

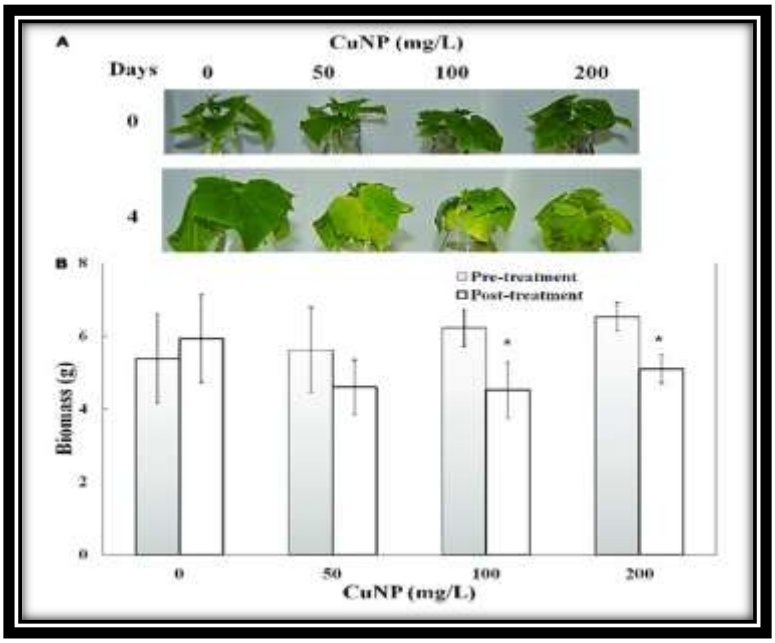

Figure 13: Effect on Biomass development of Spirullina under various concentrations of Copper Nanoparticles (Dry weight).

Impact on Seed germination

The role of nanoparticles in plants varies from plant to plant and species to species. Nanomaterials like nano-silica, carbon nanotubes, and nano-titanium dioxide have a significant impact on the germination of various crops. These findings are useful and important because increasing germination parameters has a major impact on increasing yield and sustainable agricultural production. Non-toxicity is receiving increasing attention. These small nanoparticles can change the physicochemical properties of materials, causing adverse biological effects in living cells. Lead nanoparticles will not negatively affect the survival process of the seeds, but improve the process compared to the control. This improvement may be due to the creation of nanopores in the seed coat, which must have led to improved germination conditions and the slow release of lead ions. This may be one of the reasons why copper nanoparticles do not have a significant effect on seed germination. This study showed that the nanoparticles did not change the germination of the seeds much. Lower concentrations of copper nanoparticles have no adverse effects on plants, but higher concentrations of nanoparticles will have adverse effects on plant species. Compared with control and seed germination, the germination rate of bicolour sorghum is very low and the growth of germinated seeds is not obvious.

Figure – 14: Impact of Copper Nanoparticles on Seed Germination of Sorghum vulgare

It can be seen in the experiment that the soil treated with CuNP germinated in 5 days, but the untreated sample (control) germinated in 48 hours and the nanoparticles acted as stimulants for the germination of two-coloured sorghum. Generally, even at lower concentrations, copper solutions will show high toxic effects. By applying lead nanoparticles to agricultural and food systems, reducing the toxicity of copper nanoparticles can benefit society. It is very important to focus on the impact of nanoparticles on the soil microbial system. More research is needed because the harmful effects of nanoparticles on plant growth can be positive or negative,

depending on the dose of the nanoparticles, the treatment time of the plant species, and the stage of development. The physiological and visual toxicological effects of plants may not be sensitive indicators that require toxicity studies at the proteomics.

Possible mechanism for the formation of copper nanoparticles

The use of plants and plant extracts in nanoparticle fusion is considered more beneficial than microbe-based structures because it reduces unpredictable methods of maintaining cellular society. By changing the mixing conditions, such as pH, reducing agent concentration, temperature, mixing degree of reagents, etc., the improvement of particle evaluation can be similarly controlled. Extracts of plant origin must be extracellular or intracellular. Intracellular extracts occur within the plant, but extracellular binding occurs in vitro. Inspections have shown that extracellular extracts using plant isolates are considered best when not combined in cells. The biosynthesis of CuNPs eliminated by plants, for example, ginger [35], coconut and coriander has been represented. So far, some articles describing the framework and parts of the dynamic biomolecules in the extract have been misappropriated. These controls indicate that the proximity of phytochemicals in plant isolates is a key part of the reduction and shift of copper particles. The phytochemicals responsible for the reduction are terpenoids, flavonoids, ketones, aldehydes, amides, and carboxylic acids. Water soluble metabolites such as flavonoids, normal acids and quinones are only responsible for the biological reduction of particles. The same phenomenon applies to the formation of copper nanoparticles. Fourier infrared transmission Spectroscopy (FTIR) The spectrum of biosynthetic CuNP has been used to show that the biomolecules present in the extract are responsible for the mixing of nanoparticles. One of the biological molecules that they fundamentally share is terpenoids. Terpenoids are commonly called isoprene, a common mixture that often occurs in plants and contains five isoprene carbon units. Some authorities have investigated the presence of terpenoids in cumin seed extracts, which are important participants in the biosynthesis of CuNP. The result of the comparison was the discovery of Cinnamomum zeylanicum (cinnamon) feces containing eugenol, which may be the reason for the reduction of silver nitrate in AgNP. In view of the FTIR spectral data, it has been proposed that the deprotonation of the eugenol hydroxyl molecule triggers the resonance structure change process, which can also be reduced to a nanometric width by reducing the metallic particles. Another important class of plant metabolites are flavonoids. Flavonoids are a collection of polyphenol mixtures that contain 15 carbon particles and are soluble in water. Flavonoids can be combined into isoflavones, bioflavonoids, and neoflavonoids, which can act as chelating agents and reducing metal particles. The common-sense social events introduced in the flavonoids are solely responsible for the nanoparticle game project. The difference in flavonoids from enols to ketones will result in the reduction of metal particles to characterize nanoparticles.

CONCLUSIONS

The synthesis of nanoparticles by using Eclipta prostrata leaf extract has a significant potential over traditional methods of synthesis. The green synthesis of nanoparticles technology has to be scaled up to check the cost effectiveness. The process of synthesis is eco-friendly, rapid,

followed green approach mechanism. The synthesised nanoparticles showed efficient anti - microbial activities against bacteria and pathogenic fungi. Similarly, the synthesised nanoparticles showed efficient anti algal activity against Spirulina culture. Many studies confirmed the plants can absorb lead from environment and it gets accumulated in roots and higher concentration of copper higher is the toxicity. It reduces the germination also to great extent due to presence of lead but this effect is not observed in the case of lead nanoparticles in where the toxicity is reduced. This is due to creation of nano holes on the seed coats. The crystalline nature of nanoparticles is evident from the sharp peaks in the XRD spectrum and average particle size is 87 nm. Therefore, there is a need for more studies to evaluate and understand the actual plant-dependant mechanism. Similarly, the germination studies have enhanced the work of toxicity checkups but it has further studied with respect to genomic proteomic and metabolic levels.

REFERENCES

1) Catauro M, R.M., De Gaaetano FD, Marotta A, "Sol–gel processing of drug delivery materials and release kinetics.". J Mater Sci Mater Med, 16(3)261-265, 2005.
2) Crabtree JH, B.R., Siddiqi Ra, Huen IT, Handott LL, Fishman A, "The efficacy of silver-ion implanted catheters in reducing peritoneal dialysis-related infections.". Perit Dial Int, 23(4)368-374, 2003.
3) Krolikowska A, K.A., Michota A, Bukowska J, "SERS studies on the structure of thioglycolic acid monolayers on silver and gold.". Surf Sci, 532, 227-232, 2003.
4) Zhao G, S.J., "Multiple parameters for the comprehensive evaluation of the susceptibility of Escherichia coli to the silver ion.". Biometals, 11, 27-34, 1998.
5) Brayner R., 2008. The toxicological impact of nanopartcicles, Nano Today. 3, 48 – 55.
6) Fresta M., G. Puglisi, G. Giammona, G. Cavallaro, N. Micali., Pefloxacine mesilate-loaded polyethyl cyano acrylate nanoparticles characterization of the colloidal drug carrier formulation. Journal of Phram. Sci. 84; 895-902, 1995.
7) James G.V., Water treatment 4th Edition, Cleveland. CRC pres. 1971. P. 30.
8) Stoimenov P.K., Klinger R.L., Marchin G.L., Klabunde K.J., Metal oxide nanoparticles as bactericidal agents. Langmuir, 18; 6679 – 6686, 2002.
9) Sondi I., Sondi B.S., Silver nanoparticles as antimicrobial agents; a case study on E.coli as a model for gram negative bacteria. J. Colloid. Interf. Sci. 275; 177-182, 2004.
10) Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken HR Manual of Clinical Microbiology, 6th Ed. ASM Press, Washington DC, 15-18, 1995.
11) Olurinola PF. A laboratory manual of pharmaceutical microbiology, Idu, Abuja, Nigeria, 69-105, 1996.
12) Andersen, R.A. Algal Culturing Techniques; Elsevier Academic Press: San Diego, CA, USA, 2005.
13) Moroney, J.V.; Bartlett, S.G.; Samuelsson, G. Carbonic anhydrases in plants and algae. Plant Cell Environ. 24, 141–153, 2001.
14) Sunda, W.G. Feedback interactions between trace metal nutrients and phytoplankton in the ocean. Front. Microbiol. 2012.
15) Bothe, H.; Schmitz, O.; Yates, M.G.; Newton, W.E. Nitrogen fixation and hydrogen metabolism in cyanobacteria. Microbiol. Mol. Biol. Rev. 74, 529–551, 2010.

16) Vega, J.M.; Herrera, J.; Aparicio, P.J.; Paneque, A.; Losada, M. Role of molybdenum in nitrate reduction by Chlorella. Plant Physiol., 48, 294–299, 1971.
17) Monteiro, C.M.; Castro, P.M.L.; Malcata, F.X. Metal uptake by microalgae: Underlying mechanisms and practical applications. Biotechnol. Prog. 28, 299–311, 2012.
18) Prathap, S. C., Chaudhary, M., Pasricha, R., Ahmad A., Sastry M. Synthesis of gold nanotriangles and silver nanoparticles using Aloe vera plant extract. Biotechnol. Prog. 22, 577-586, 2006.
19) Xia, Y.; Halas, N. J. Shape-controlled synthesis and surface plasmonic properties of metallic nanostructures. Mrs. Bull., 30, 338, 2005.
20) Kaszuba M., McKnight D., Connah M.T., McNeil-Watson F.K., Nobbmann U., J. Measuring sub nanometer sizes using dynamic light scattering.Nanopart. Res. 10 823-829, 2008.
21) Rao Y.S., Kotakadi V.S., Prasad T.N.V.K.V., Reddy A.V., Sai Gopal D. V.R. Green synthesis and spectral characterization of silver nanoparticles from lakshmi tulasi (Ocimum sanctum) leaf extract. Spectrochim. Acta Part A: Mol. Biomol. Spec. 103 156–159, 2013.
22) Edison T.J.I., Sethuraman M.G., Instant Green Synthesis of Silver Nanoparticles Using Terminalia chebula Fruit Extract and Evaluation of Their Catalytic Activity on Reduction of Methylene Blue. Process Biochem. 47, 1351 – 1357, 2012.
23) Parameshwaran R., Kalaiselvam S., Jayavel R., Green synthesis of silver nanoparticles using Beta vulgaris: role of process conditions on size distribution and surface structure. Mater. Chem. Phys. 140, 135 – 147,2013.
24) Alagar M., Theivasanthi T., Kubera raj A. Chemical synthesis of nano-sized particles of lead oxide and their charecterization studies. Journal of Applied Sciences, 12;398-401, 2012.
25) Nouran HA., Haram MH., Colloidal Silver as a new antimicrobial agent. International Journal of microbiological research. 1(1);33-36,2010.
26) Shrivastava, S; jyung, woo; lungue, M; Characterization of Enhanced Antibacterial Effects of Nano Silver Nano Particles, J Nanotechnology, 25(12):103– 125, 2010.
27) Hu T.L., Jin Z.H., W.F. Chang, J.J. Wu. Anti-bacterial study using nanosilver droped high density polyethylene pipe. Sustain. Environ. Res. 22(3), 153-158, 2012.
28) Dutta, R.K, "Studies on Antibacterial Activity of ZnO Nanoparticles by ROS Induced Lipid Peroxidation", Colloids Surf.B Biointerfaces. 94:143-50, 2012.
29) Selvam, S, Rajiv Gandhi R, Suresh J, Gowri S, Ravi kumar S, Sundrarajan M. "Antibacterial Effect of Novel Synthesized Sulfated Beta-Cyclodextrin Crosslinked Cotton Fabric and Its Improved Antibacterial Activities with ZnO, TiO2 and Ag Nanoparticles Coating." Int.J.Pharm. 434.1-2: 366-374, 2012.
30) Matthews L, Rupinder KK, Shufong Z, Vasu P, Jagat R.K. Applications of Nanomedicine in antibacterial, medical therapeutics and diagnostics. The open tropical medicine journal. 3, 1-9, 2010.
31) Lara, H.H., Ayala-Núnez, N.V., Ixtepan Turrent, L.C., Rodríguez Padilla, C., Bactericidal effect of silver nanoparticles against multidrug-resistant bacteria. World Journal of Microbiology and Biotechnology, 26(4):615-21, 2010.
32) Guangyin, Lei;Synthesis of Nano-Silver Colloids and Their Anti-Microbial Effects Master of Science In Materials Science & Engineering. Blacksburg, Virginia, November 8(2007).
33) Nabipour Y.S., Rostamzad A. Comparing the antimicrobial effects of silver and copper nanoparticles against pathogenic and resistant bacteria Klebsiella pneumonia, Pseudomonas aeruginosa and Staphylococcus aureus. Cumhuriyt University Faculty of Science Journal, 36(3) 2540-2546, 2015.
34) Makarov V.V., Love A.J., Sinitsyna O.V., Makarova S.S., Yaminsky I.V. "Green" nanotechnologies: Synthesis of metal nanoparticles using plants. Acta naturae 6, pp.35-43, 2014.

35) Delma B.T., Vijila B., Jaya Rajan M. Green Synthesis of copper & lead nanoparticles using zingiber officinale stem extract. International journal of scientific and research publication. 6(11), 134-137, 2016.
36) Elango G., Roopom S.M. Green synthesis, spectroscopic investigation & photocatalytic activity of lead nanoparticles. Spectrochimca acta part-A. Molecular & Biomolecular spectroscopy.139. 367-373, 2015.
37) Devendra K., Adya J., Neelam. Green chemistry approach, synthesis, charecterisation and thermal studies of nanocomposites of lead oxide nanoparticles. Journal of Chemical & pharmaceutical research, 8(10), 86-93, 2016.
38) Parashar U.K., Kumar V., Bera T., Saxena P.S., Nath G. Study of mechanism of enhanced antibacterial activity by green synthesis of silver nanoparticles. Nanotechnol 22, pp. 1-13, 2011
39) Shankar S.S., Ahmad A., Sastry M. Geranium leaf assisted biosynthesis of silver nanoparticles. Biotechnol Prog 19, pp.1627-1631, 2003.
40) Sathishkumar M., Sneha K., Won S.W., Cho C.W., Kim S. Cinnamon zeylanicum bark extract and powder mediated green synthesis of nanocrystalline silver particles and its bactericidal activity. Colloids Surf B Biointerfaces 73, pp.332-338, 2009.
41) Ahmad N., Sharma S., Alam M.K., Singh V.N., Shamsi S.F., Rapid synthesis of silver nanoparticles using dried medicinal plant of basil. Colloids Surf B Biointerfaces 81, pp. 81-86, 2010
42) Si S., Mandal T.K. Tryptophan-based peptides to synthesize gold and silver nanoparticles: a mechanistic and kinetic study. Chem Eur J 13, pp.3168.