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BIOSYNTHESIS OF COPPER NANOPARTICLES USING AQUEOUS GUAVA EXTRACT –CHARACTERISATION AND STUDY OF ANTIBACTERIAL EFFECTS

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ABSTRACT

Vegetable mediated synthesis of nanoparticles is a green chemistry approach that connects nanotechnology and biotechnology. In the present investigation, we have used a fast, convenient and environment friendly method for the synthesis of copper nanoparticles by biologically reducing copper nanoparticles with aqueous extract of psidium guajava L (guava) under optimum conditions (pH 10). The formation of copper nanoparticles was indicated by the colour change from colourless to brown. Biosynthesized nanoparticles were characterized by UV-VIS, FT-IR, XRD, SEM, TEM and EDAX analysis. These biologically synthesized copper nanoparticles were tested for antibacterical activity against two human pathogens viz, Escheria coli and staphylococcus aures. The reducing property of aqueous extract due to the presence of antioxidant viz, ascorbic acid, polyphenols which is confirmed by quantitative assay.

KEY WORDS

Psidium guajava L, FT-IR, XRD, SEM, TEM and EDAX.

1. INTRODUCTION

Nanoscience (science at 1- 100 nanoscale) is the most promising technology that can be applied almost in all spheres of life, ranging from electronics, pharmaceutical, defence, transformations, heat transfer to sports and aesthetics. Metallic nanoparticles are great interest due to their excellent physical and chemical properties, such as high surface-to-volume ratio and heat transfer (thermal conductivity) [1].

The nanoparticles can be synthesized by physical, chemical and biological methods. The physical methods are laser ablation method, arc discharged method, High energy ball billing method and the chemical vapour deposition method the chemical methods are co-precipitation method, sol-gel method, micro emulsion method, hydrothermal method, sono chemical method, microwave method [2]. The biosynthesis of nanoparticles is also considered to be a bottom up technique, where the oxidation or reduction is the main reaction that occurs during the production of nanoparticles. Metal compounds usually reduce into their respective nanoparticles because of microbial enzymes or the plant phytochemicals with antioxidant or reducing properties.

Although biosynthesis of copper nanoparticles by plants viz, ginger (zingiber officinal)[3], Aspergillus species [4], plant leaf extract of magnolia[5], leaf extract of tridax procumbens [6], pseudomonas stutzeri isolated from soil [7], lantana camara[8]. Copper nanoparticles due to their excellent physical and chemical properties and low cost of preparation have been of great interest. Copper nanoparticles have wide applications as heat transfer system, antimicrobial materials[9,10], super strong materials[11-12], sensors[13-15] and catalyst[16-

18]. Copper nanoparticles can easily oxidize to form copper oxide. To protect copper nanoparticles from oxidation, they are usually encapsulated in organic and inorganic coating such as carbon and silica [19, 20]. Cuprous oxide is p-type semiconductor with a direct band gap of 2.17eV [21, 22].

Psidium guajava L , the Indian guava from Spanish guayaba is a deciduous tree of the Myrtales family. It is known for its edible fruit. Although these fruits are reputed to contain high amounts of ascorbic acid (vitamin C), 479mg/100g, it also contains polyphenols like flavonoids, ellagic acid and gallic acid. Guavas are rich in dietary fiber, vitamins A and C, folic acid, and the dietary minerals, potassium, copper and manganese. Having a generally broad, low-calorie profile of essential nutrients, a single common guava (*P. guajava*) fruit contains about four times the amount of vitamin C as an orange [23].

Nanoparticles have been intensively studied over the last decade due to its characteristic physical, chemical, electronic, electrical, mechanical, magnetic, thermal, dielectric, optical and biological properties [24]. The oxides of transition metals are an important class of semiconductors, which have applications in magnetic storage media, solar energy transformation, electronics, gas sensors, and catalysis [25]. Micro emulsion method [26] arc submerged nanoparticles synthesis system [27] flame based aero sol methods [28] sonochemical [29] hydrothermal [30] and solid state techniques [31].

2. MATERIALS AND METHODS

2.1. Material: Guava sample for the biosynthesis of copper nanoparticles was produced from the local supermarket. Copper sulphate, sodium hydroxide, PEG 6000 and other reagents used in the study were a analytical grade. The bacterial strains employed in this work were produced from microbial type culture collection center (MTCCC) technology, Chandigarh, India (E-coli and staphylococcus).

2.2. Preparation of sample extract

50gm of guava fruit (psidium guajava L) was accurately weighed, thoroughly washed under running tap water followed by washing it with double deionised water to remove surface impurities. They were crushed using a blender and finely macerated. After homogenisation 100ml of double deionised water was added and heated over a water bath maintained at 80^0C for 15 minutes. The extract obtained was filtered through muslin cloth and then through whatmann No.1 filter paper (pore size 25 µm) and used immediately for the biosynthesis of copper nanoparticles.

2.3. Pharmocognostic evaluation of aqueous extract

Fresh extract of the fruit of guava was used for phytochemical screening-Qualitative analysis.

2.3.1. Phytochemical Screening – Qualitative Analysis

Preliminary phytochemical screening was carried out for the identification of carbohydrates, flavonoids, steroids, tannins, alkaloids, glycosides, saponins, triterpenoids and phenol using standard phytochemical methods.[32].

2.4. Synthesis of copper nanoparticles

The biosynthesis of copper nanoparticles involves four stages Copper sulphate (0.02M) was prepared in deionised water and a blue solution was obtained. Polyethylene glycol 6000 (0.01M) was dissolved in water and added to the aqueous solution containing the copper salt with vigorous stirring. In this step, the colour of the solution changed from blue to white. In the third step, guava extract was added to the copper sulphate solution containing PEG. The colour of the solution remains the same. Finally, 0.1M

sodium hydroxide was added in drops to the solution under continuous rapid stirring. The colour of the aqueous phase changed from white to green. The appearance of this colour indicates that the reduction has started. The formation of copper nanoparticles is confirmed by the colour change from green to brown when it is kept on the water bath under 80^0C. The formation of copper nanoparticles is inferred by visual observation followed b UV-Visible spectrum, FTIR, SEM, XRD and EDAX studies [15].

2.5. Fixation of parameters for biosynthesis of copper nanoparticles

2.5.1. Biosynthesis of copper nanoparticles using different ratios

The biosynthesis of copper nanoparticles was carried out at different ratio of extract and copper sulphate (1:1, 1:2, 1:3, 1:4, 1:5) at pH10. Time taken for the colour change in the reaction mixture as well as the formation of nanoparticle was monitored by visual inspection and also by UV-Visible spectrophotometer.

2.5.2. Biosynthesis of copper nanoparticles at different temperatures

The biosynthesis of copper nanoparticles for the fixed composition was done at different temperatures namely; room temperature and by heating in the waterbath at 60^0C,and 80^0C . The time taken for visual colour change from green to brown was recorded followed by recording UV-Visible spectrum.

2.5.3. Biosynthesis of copper nanoparticles at different pH

The biosynthesis of copper nanoparticles for 1:3 ratios of extract and copper sulphate was carried out at different pH viz. 6, 8, and 10. The time taken for colour change as well as the UV- Visible spectrum for the reaction mixture was monitored.

2.5.4. Biosynthesis of copper nanoparticles at different intervals of time

The synthesis was carried out at pH10 in the ratio 1:3(extract:$CuSO_4$) and the time taken for the formation was noted at an noted at an every 10 minutes and completion of the reaction was monitored by the colour change as well as the UV- Visible spectrum.

2.5.5. Biosyntheis of copper nanoparticles in the presence of PEG

Biosynthesis of copper nanoparticles was carried at pH 10 in the ratio 1:3 with and without PEG 6000 and completion of the reaction was monitored by the colour change as well as by recording the UV-Visible spectrum.

2.5.6. Stability of copper nanoparticles

The stability of the colloidal aqueous solution of copper nanoparticles was determined at room temperature at an interval of 24 hrs for 15days.

2.6. Characterisation of biosynthesized copper nanoparticle

2.6.1. Visual inspection

The bio-reduction of the aqueous solution of copper sulphate using guava extract was monitored and the appearance of brown colour indicates the formation of Copper nanoparticles.

2.6.2. pH analysis

The pH of the extract, precursor as well as the resulting mixture after addition of PEG 6000 and NaOH was determined using digital pH meter.

2.6.3. UV spectroscopy

The reduction of copper sulphate to copper was monitored by recording UV-Visible spectrum of the reaction mixture after diluting a small aliquot of the sample with deionised water. The measurements are recorded on Shimadzu dual beam spectrometer (model uv-1650pc) operated at resolution of 1nm.

2.6.4. FT-IR analysis of bio-mass before and after bio-reduction

FT-IR measurement was carried out for both the extract and copper nanoparticles to identify

the possible bioactive molecules responsible for the reduction of the copper ions and the capping of the copper nanoparticles by the guava extract using KBr pellet and the spectrum was recorded in the wavelength interval 4000 to 400cm$^{-1.}$ The FT-IR spectrum was also recorded for the solid copper nanoparticles isolated after centrifugation.

2.6.5. X-Ray diffraction studies

X-ray diffraction (XRD) measurement of the guava reduced copper nanoparticles was carried out using powder x-ray diffractometer instrument (SEIFERT JSO DEBYEFLEX-2002) in the angle range of 10^{0}-70^{0} operated at a voltage of 40kV and a current of 30mA with CuKα radiation in a θ-2θ configuration. The crystallite domain size was calculated by using Debye- Scherrer formula.

2.6.6. Scanning electron microscopy (SEM)

The sample was prepared by placing a drop of colloidal solution of copper sulphate on carbon coated copper grid and subsequently drying in air, before transferring it to the microscope operated at an accelerated voltage of 130kV (Hitachi-S 3400N).

2.6.7. Energy dispersive x-ray spectroscopy (EDAX)

The presence of elemental copper was confirmed through EDS. Energy dispersive analysis x-ray spectrometer takes advantage of the photon nature of the light. In the x-ray range the energy of a single photon is just sufficient to produce a measurable pulse x-ray. A semiconductor material is used to detect the x-ray along with processing electronics to analysis the spectrum. The EDS observations were carried out by instrument coupled with SEM.

2.6.8. Transmission electron microscopy (TEM)

TEM techniques was employed to visualise the size and shape of copper nanoparticles. The 200kV high resolution transmission electron microscope (FEITECNAI F -20)was added. TEM grid was prepared by placing a drop of the particle solution and drying under a IR lamp.

2.7. **Pharmocognostic evaluation of biosynthesized copper nanoparticles**

2.7.1 Determination of antibacterial activity

Antibacterial activity of the extract was determined on Muller and Hinton Agar (Hi-Media Pvt. Ltd .Mumbai) using Kirby-Bauer disk diffusion method [33]. Test pathogens were spread on the test plates- Muller Hinton Agar (MHA) for bacterial using sterile swabs. Sterile wells are made with the help of a sterile cork borer at aseptic conditions. Samples (1500µg and 2000µg) were added to the wells at aseptic conditions. Stock solutions of the extract were prepared using DMSO. The test plates were incubated for 24hrs. The zone of inhibition (in mm diameter) were read and taken as the activity of the extract against the test organisms.

3. RESULTS AND DISCUSSION

3.1. Qualitative Pharmocognostic evaluation of extract

The results of qualitative phytochemical analysis of the guava extract are shown in table-1 which indicates the presence of secondary metabolites such as carbohydrates, flavonaids, alkaloids, steroids, glycosides, tannins, saponins, phenols and triterpenoids.

Table 1: Qualitative phytochemical screening of fresh guava extract

1	Carbohydrates	+
2	Flavonaids	+
3	Alkaloid	+
4	Steroids	+
5	Glycosides	+
6	Tannins	_
7	Saponins	_
8	Triterpenoids	+
9	Phenon	+

Indication of sign (+) present and (-) absent

The presence of ascorbic acid, polyphenols and other phytonutrients present in aqueous guava extract is mainly responsible for the bio-reduction process [16]. From the literature it has been found that the amount of ascorbic acid (natural vitamin C) present in guava extract was found to be 479mg of ascorbic acid/100gm of fruit. Polyphenolic compounds are very important plant constituents because of the scavenging ability of their –OH groups. The antioxidant property of polyphenolic compounds is mainly due to the redox property which allows them to act as reducing agents [23].

3.2. Visual characterisation

The preparation of copper nanoparticles from guava extract involves a four stage process. When the pH of the solution was increased to 10 by the addition of 0.1M sodium hydroxide, the colour change of the solution changed from colourless to green and finally brown on heating in a water bath. The colour change to brown indicates the reduction of copper sulphate and formation of copper nanoparticles. Fig 1 indicates the formation of nanoparticles.

Fig.1 Formation of copper nanoparticles

Different parameters were optimized for the biosynthesis of copper nanoparticles viz.,

- Volume ratio of extract and copper sulphate,
- Temperature,
- In the presence and absence of PEG 6000 (capping agent),
- Different pH,
- Effect of time.

3.3. Ratio of volume of extract: $CuSO_4$: The formation of Copper nanoparticle depends on the volume of extract to $CuSO_4$ ratio are $_{4:}$ The time taken isgiven in (Table-2).

Table 2: Time taken for the formation of copper nanoparticles using different ratio of volume of fresh aqueous guava extract and aqueous 0.02M $CuSO_4$ at 80^0C in the presence of PEG at pH 10.

S.No	Ratio (extract: $CuSO_4$)	Time taken for the formation of nanoparticles	λ_{max}
1.	1:1	2 Hours	324
2.	1:2	1 Hour	268
3.	1:3	30 Min	294

The UV-Visible spectrum was recorded for the shift in SPR peaks position with variation in the amount of precursor salt to extract as shown in the Fig 2.

A blue shift in the wavelength from 324 to 268nm was observed with the increase in amount of precursor salt. This shift can be explained on the basis of increased nucleation rate due to greater amount of cu^{2+} ions and generation of smaller nanoparticle in the solution. However with further increase in the precursor ion from 1:2 to 1:3, a red shift was observed in SPR from 268 to 294nm. This may be due to collision between smaller nanoparticle which leads to particle growth [4]. The inset digital photograph in fig 2 clearly shows the formation of copper nanoparticle. Brown color was noted for the optimum amount of precursor

and extract producing greatest number of copper nanoparticle in aqueous medium.

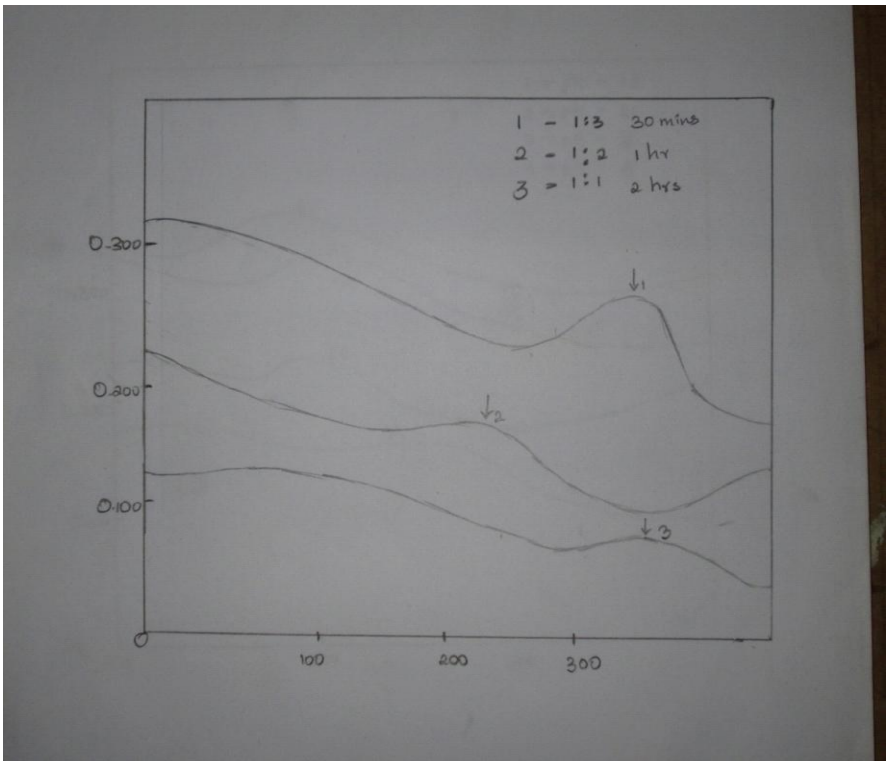


Fig.2 UV- Visible spectrum of biosynthesized Copper nanoparticle at different ratios

From the table it is also seen that the time taken for the formation of Copper nanoparticle was found to be less for 25ml of the extract and 75ml of 0.02M $CuSO_4$ solution (1:3). This ratio was found to be ideal as the biosynthesized nanoparticles showed maximum absorption at 294nm which is in agreement with the values reported in the literature.

3.4. Effect of temperature on biosynthesis of copper nanoparticles

The effect of temperature on the rate of formation of Copper nanoparticle was studied for the 1:3 composition of the extract and $CuSO_4$ solution. The CuNp were formed within 3o mins at 80^0C. However, at room temperature and 60^0C the formation of CuNp were formed after 1 day and 2 hours respectively and above 80^0C under boiling condition the solution becomes charred and no particle formation is seen. Hence, the reaction at 80^0C favours the biosynthesis of CuNP using aqueous guava extract.

3.5. Effect of PEG

An important feature in the production of the metal nanoparticles is to prevent agglomeration and oxidation process. The stabilization is commonly achieved by using surfactants which avoid the aggregration by binding to the nanoparticle surface. PEG 6000 is frequently used as the stabilizer or capping agent of metal colloids because of its availability, low cost and non toxicity. The stabilization of metal colloids and the shape of nano material depend strongly on PEG. In the present work PEG 6000 was used which works as size controller and polymeric capping agent because it hinders the nuclei from aggregation through the polar group which are strongly adsorbed at the surface of the CuNp with the co-ordination bonds. Fig- 4 shows UV-Visible absorbance spectra of CuNp synthesized

under identical condition in absence and presence of PEG. The spectrum shows a blue shift in the position of SPR from 326-294nm in the presence of PEG. This indicates that the PEG molecule was adsorbed on the CuNp surface keeping them from excessive growth and leading to the generation of smaller nanoparticles [34]. Formation of CuNp in the presence and absence of PEG 6000 is shown in the Fig 3.

(a) (b)

Fig.3 Formation copper nanoparticle a) In the absence of PEG 6000 for the composition (1:3) and b) In the presence of PEG 6000 for the composition (1:3) (extract: $CuSO_4$)

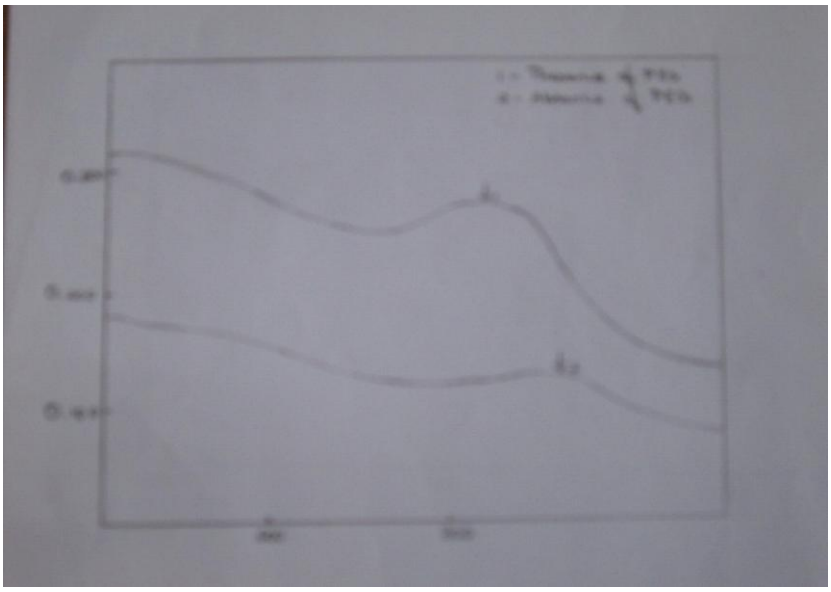


Fig. 4 Biosynthesized copper nanoparticle in the presence and absence of PEG.

3.6. Effect of pH

The present work shows that the pH of the solution has an influence on the progress of bio-reduction of Copper sulphate solution. The pH of the guava extract, $CuSO_4$ and PEG on mixing was found to be 5.4. The probable kinetic enhancement could also be conducive to a reduction in crystallite size because of the enhancement of the nucleation rate [34]. The ascorbic acid present in the extract induces a reduction in the solution pH which was adjusted back in the range from 6 to 12 with addition of 0.1M NaOH solution. Fig-7 shows UV-Visible absorption spectra for the pH ranging from 6 to 12. The surface Plasmon absorbance of copper colloids was obtained for all pH except at PH 6. This probably indicates very small particles at such low pH. The Plasmon resonance is clearly

visible for pH 8 to 10 at 308 and 294nm respectively. At pH 12, the peak is still detectable but much weaker when compared with other pHs.

The maximum blue shift in SPR peak around the maximum value at pH 10 could be attribute to the decrease in the particle size [35], but the exact position of the Plasmon absorption may depend on several factors (including particle size, shape, solvent type and capping agent) and in this case, there might be some variation in the arrangement of the capping molecules around the copper particles as a consequence of the variation in pH. Thus pH is found to be ideal due to the appearance of brown color within 30 mins because biosynthesized CuNp showed maximum absorption at 294nm which is in agreement with reported values in the literature [4]. Fig-5 shows the formation of CuNp at different pH.

Fig.5 Formation of copper nanoparticle at different pH for the ratio1:3 (extract: $CuSO_4$).

Table 3: Time taken for formation of CuNp at different pH for ratio 1:3(extract: $CuSO_4$) in the presence of PEG 6000.

S.No	pH	Time taken for the formation of Copper Nanoparticles	Absorbance λ_{max}(nm)
1.	6	1 day	350
2.	8	4 hours	308
3.	10	30 mins	294
4.	12	45 mins	272

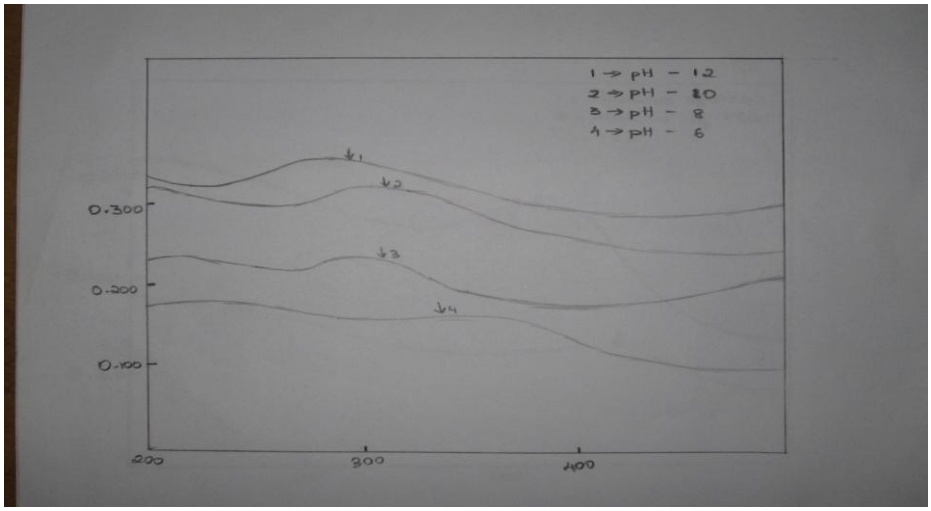


Fig.6 Formation copper nanoparticles at different pH for the ratio (1:3)

3.7. Effect of reaction time

Time is a very important parameter in nanoparticle synthesis. As an empirical rule the availability of a larger number of nuclei at a given time induces a decrease in the nanoparticle size because smaller metal nuclei grow and consume metal ions at the same time. Copper nanoparticle synthesis was carried out under optimum condition. In order to clarify the reaction process the UV-Visible absorption spectra was recorded for every 10 mins. The evaluation of the UV-Visible spectrum is shown in Fig 8. Initially there was no characteristic absorption peak.

Table 4: Formation of Copper nanoparticle at different intervals of time.

S.No	Reaction Time of Solution (mins)	Colour	λ_{max} (nm)
1.	Immediately after the addition of NaOH	Greenish yellow	-
2.	10	Dark green	-
3.	20	Pale brown	350
4.	30	Brown	294
5.	40	Brown	277

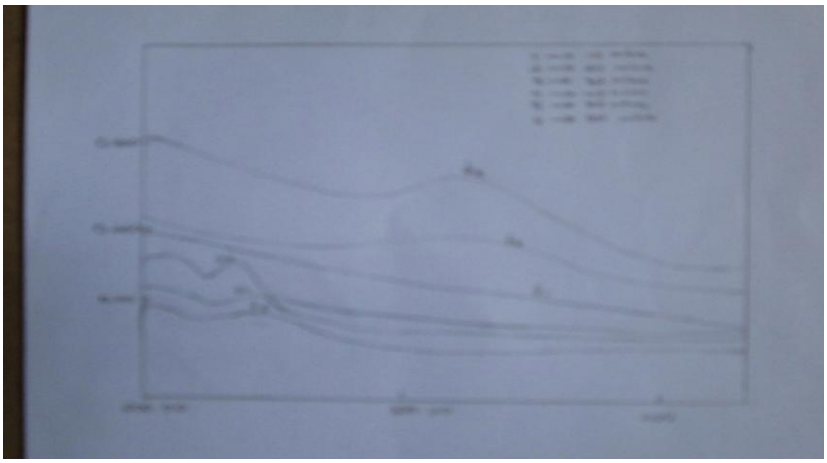


Fig.7 UV-Visible spectrum of biosynthesized copper nanoparticles at different intervals of time.

From the table-4and fig-8 it is seen that the CuNp were formed within 30 mins exhibited plasmon resonance at 294nm. For 50 and 60 mins no clear Plasmon resonance was obtained despite a clear absorption in the lower range of wavelength. It suggests a homogenization mechanism, which provides a larger number of nuclei with time. This could indicate a even smaller particle size. At this moment the mechanism associate with this phenomenon is not clearly understood. Ascorbic acid present in guava extract is well known to scavenge free radicals thus provides anti-oxidant action during copper nuclei formation. This provides the right condition for subsequent rapid reduction by phytonutrients, polyphenols along with ascorbic acid and hence copper nanoparticle formation [34].

3.8. Stability

The stability of nanoparticles dispersion is a key factor in itsapplication. In order to prevent agglomeration of nanoparticles , several capping agent is added in this media. In this work, ascorbic acid present in the guava extract is used as both reducing and capping agent along with the protecting agent viz PEG 6000. The ascorbic acid and PEG stabilized copper nanoparticles dispersion was centrifuged at 8000 rpm for 15 mins. It is seen the residue get settled at the bottom of the centrifuge tube. The supernant liquid was decanted under ambient conditions and no sign of sedimentation was observed even after 15 days of storage. The UV-Visible spectrum for the biosynthesized CuNp using 25ml of the extract and 75ml of $CuSO_4$ solution was recorded over a period of time for 15 days. There was no change in UV spectrum.

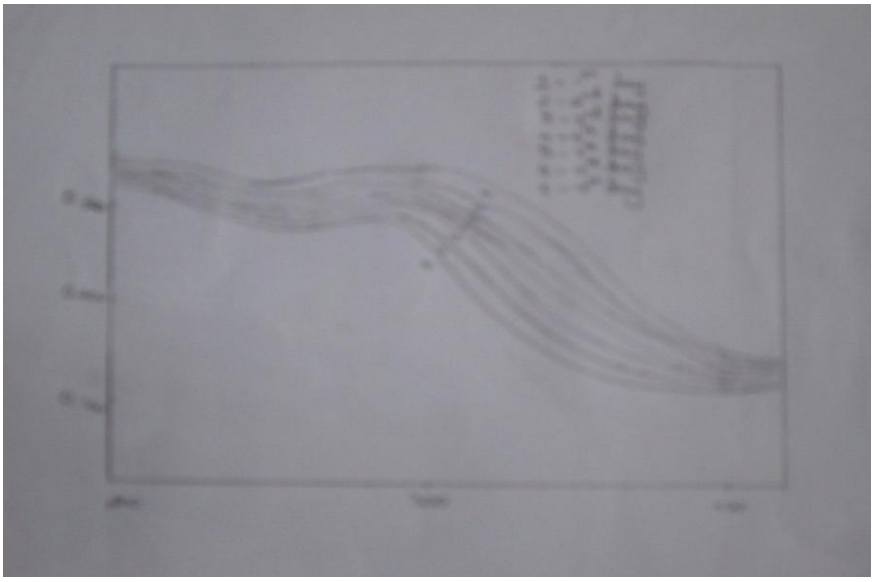


Fig.8 UV-Visible spectra showing stability of biosynthesized copper nanoparticle

There is no change in the SPR and the λ_{max} was found at 294nm without any change. This indicates that the ascorbic acid, PEG 6000 stabilized copper nanoparticle are highly stable due to the extreme capping effect of both the ascorbic acid, PEG 6000.

This stability against oxidation is likely attributable to the presence of ascorbic acid and PEG which forms a capping layer at the surface of the particle. From the observation (fig 8), it is understood that ascorbic acid plays a key dual role as reducing agent and capping agent. During particle synthesis Cu ions can coordinately bond

with carbon and oxygen present in PEG, so that the synthesized CuNp are covered by an adsorbed layer of PEG [35].

3.9. Characterisation of biosynthesised nanoparticle by spectral methods

3.9.1 UV-Visible studies on copper nanoparticles

Nanosized particles exhibit unique optical properties having an exponential-decay Mie scattering profile with deceasing photon energy [36]. UV-Visible absorbance spectroscopy has proved to be a very useful technique for studying metal nanoparticles because the peak positions and shapes are sensitive to particle size. The effect of ascorbic concentration in the extract on the UV-Visible absorbance spectroscopy of the synthesized CuNp showed single peak at around 294nm. The surface Plasmon peak of CuNp has been reported to appear at around 570nm. However, when the particle size is less than 4nm, the distinctive Plasmon peak is known to be broadened and replaced by a featureless absorbance, which increases monotonically towards higher energies [37-39].

In our work, the resulting Cu dispersion did not show a plasmon peak at around 570nm, but displayed a broadened peak at a short wavelength, indicating the presence of very small separated CuNp . In fig 9 showed the UV-Visible spectrum for CuNp for the ratio 1:3 [35].

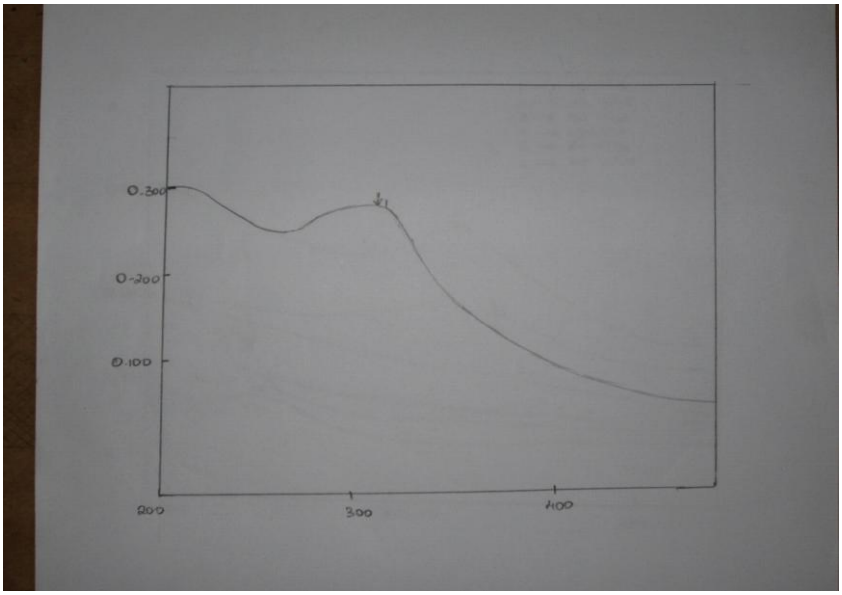


Fig.9 UV-Visible spectrum for the formation of Copper Nanoparticle under optimum conditions.

3.9.2. Fourier Transform-Infrared (FT-IR) Characterization

FT-IR spectroscopy was used to investigate the interactions between different species and changes in chemical compositions of the mixtures. FT-IR measurements of both the aqueous guava extract and the synthesized dried copper nanoparticles were carried out to identify the possible bio-molecules responsible for the reduction, capping of and efficient stabilization of the bio-reduced CuNp.

The FT-IR spectra of the guava extract and the synthesized CuNp are shown in Fig 10 a and b. The guava extract displays a number of adsorption peaks, reflecting its complex nature. Terpenoids from the Psidium guajaya L (guava) can be identified by the strongest peaks of hydroxyl at 3419 cm^{-1}, α, β- unsaturated ketone band at 1710 cm^{-1}, olefinic band at 1610 cm^{-1},

primary and secondary alcohols functionalities bands at 1043 cm^{-1} as well as the peaks around 3000 and 1400 cm^{-1} attribute to aliphatic C-H stretching and bending modes [40].

By comparing the spectrum of copper nanoparticles with that of the guava extract, we can conclude that the two spectra are similar in their spectral features. There is no question, therefore, that the compound on the surface of copper nanoparticles has a very close chemical composition to the guava extract of not identical. It was found that many peaks obtained by the guava extract have been repeated in the FT-IR spectrum of copper nanoparticles with changes in the position as well as the intensity of absorption.

The absorption peaks at 3419, 1604, 1107 and 1053 cm^{-1} corresponding to OH, C=C and C-O observed in the plants extract get narrower and shifted to higher frequently regions, while those at around 3000 and 1400 cm^{-1} attributable to aliphatic C-H stretching and bending modes deceased in intensity and shifted to low frequency regions. In addition the disappearances of $\gamma_{C=O}$ stretching vibration of the α, β- unsaturated ketone at 1710 cm^{-1} confirm that the reduction and the stabilization of copper nanoparticles proceed via these groups which confirm that water soluble compounds such as terpenoids are present in guava extract has the ability to perform dual functions of reduction and stabilization of copper nanoparticles. A similar observation has been reported by several works [41].

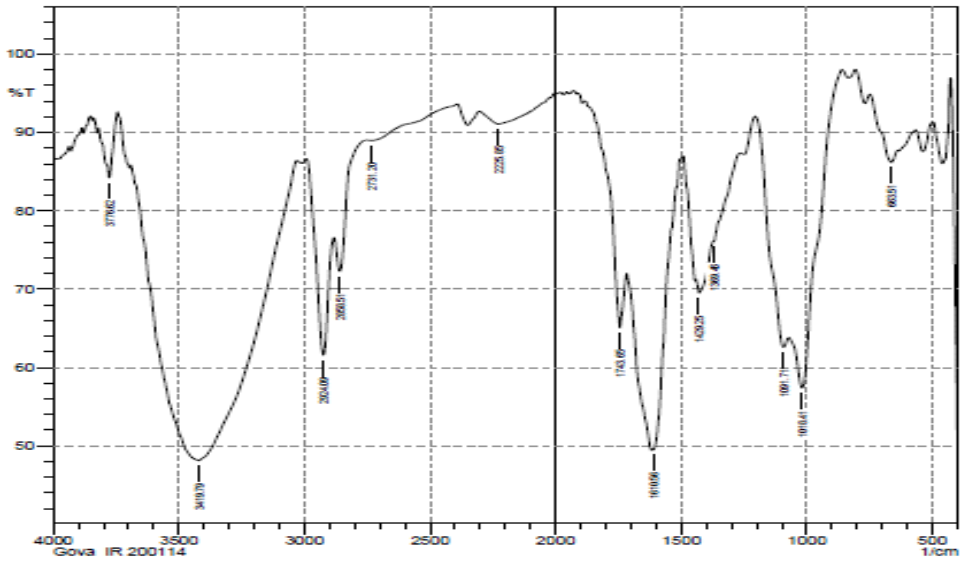


Fig 10 (a) IR spectrum of aqueous guava

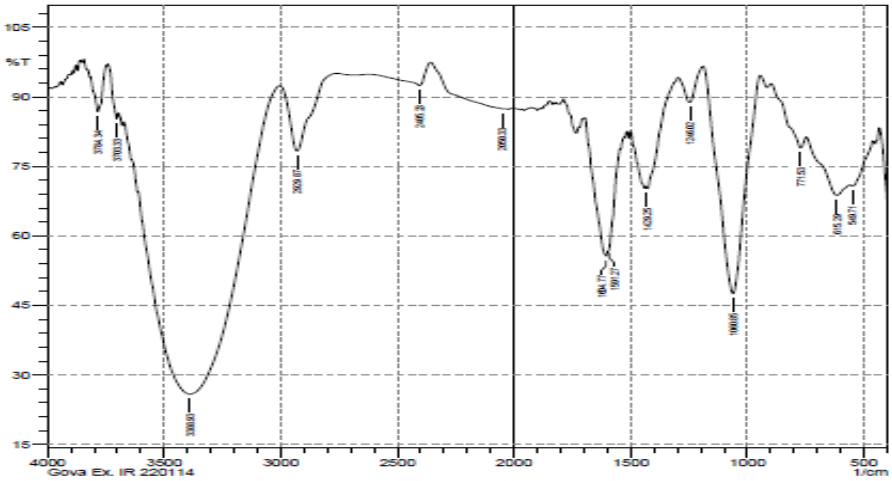


Fig 10(b) IR spectrum of copper nanoparticles

3.9.3. XRD

XRD patterns taken using powder X-ray diffractometer instrument (SEIFERT JSO DEBYEFLEX 2002) in the angle range 10-70^0 of the copper nanoparticles at 2θ, scan axis 2:1 sys is shown in fig 11. A number of Bragg reflections corresponding to (111), (200) and (220) sets of lattice planes are observed, which can be indexed to face-centered cubic copper [42]. The peaks match with the Joint Committee of powder Diffraction Standards (File No. 089-2838), which further proves the formation of crystals of copper nanoparticles. Furthermore, the average diameter of the copper nanoparticles is calculated in the range 15-30nm by Scherrer formula using FWHM obtained from the diffraction peaks:

$D = 0.89\lambda/\beta\cos\theta$

Where D is the mean grain size, λ is the wavelength of copper target, β is the FWHM of the diffraction peaks and θ is the diffraction angle. Thus XRD is commonly used to determine the chemical composition and crystal structure of a material.

XRD pattern was taken for both copper nanoparticle in the presence and the absence of PEG 6000 (capping or protecting agent). The fig 11 a and b shows copper nanoparticle formation in the presence and absence of PEG 6000. In the absence of PEG 6000 XRD showed that the Cu_2O has formed whereas in the presence of capping or protecting agent ie,PEG 6000 it showed that Cu has formed. This clearly proves that PEG 6000 plays vital role in the formation of copper nanoparticles and protects them from oxidation.

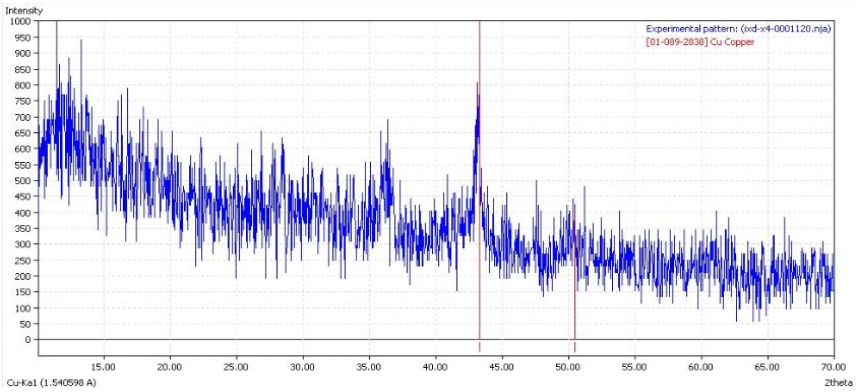


Fig.11 (a) XRD spectrum of biosynthesized copper nanoparticle in the presence of PEG 6000

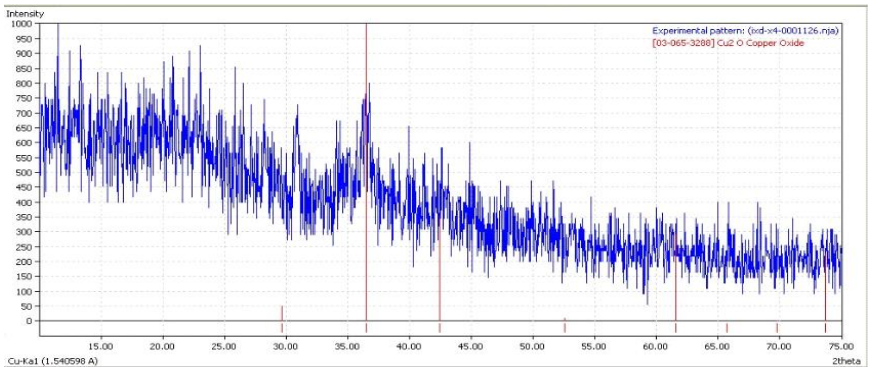


Fig.11 (b) XRD spectrum of Copper nanoparticle in the absence of PEG 6000

3.9.4. SEM (Scanning electron microscopy)

Scanning Electron Microscopy provided further insight into the morphology and size details of the copper nanoparticles. The experimental result showed that the diameter of the prepared nanoparticle was about 15-30nm and the shape is found like flakes as shown in the Fig 12 a and b. Similar phenomenon was reported [43].

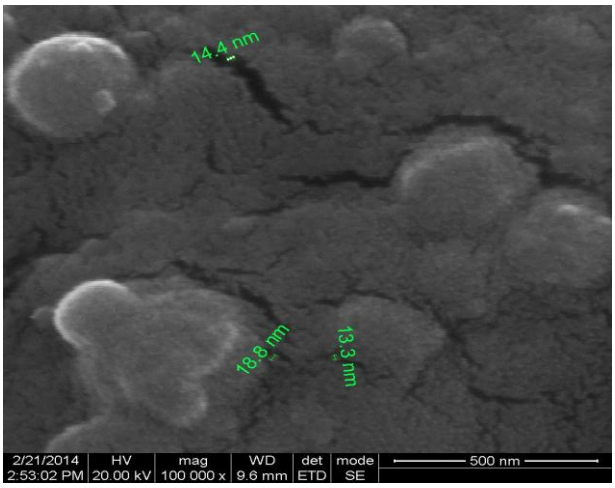


Fig 12: SEM image of copper nanoparticles

3.9.5 EDAX

The EDAX pattern clearly shows that copper nanoparticle formed by the reduction of copper ions using fresh aqueous guava extract are crystalline in nature (fig 13). The EDAX spectrum was recorded in the spot-profile mode. The optical absorption peak is observed at 8keV, which is typical for the absorption metallic copper nanoparticles. Strong signals from the copper atoms are observed, while weaker signals for C, O, Si, Mg and K atoms were also recorded. From the EDS signals, it is clear that copper nanoparticles reduced by aqueous guava extract have the weight percentage of elemental copper as 75%.

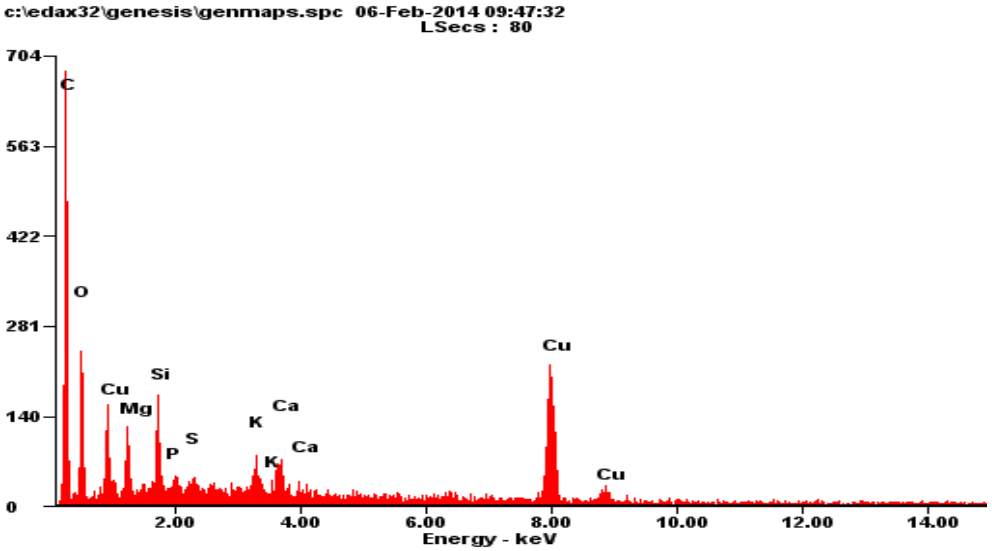


Fig.13 EDAX of biosynthesized copper nanoparticles

3.9.6 Transmission electron microscopy (TEM)

TEM analysis reveals that the Copper nanoparticles are predominantly Spherical (Fig.14a). The overall morphology of the copper nanoparticles produced by reduction of cu^{2+} ions with 2Mm $CUSO_4$ is composed of almost uniform nanoparticles.

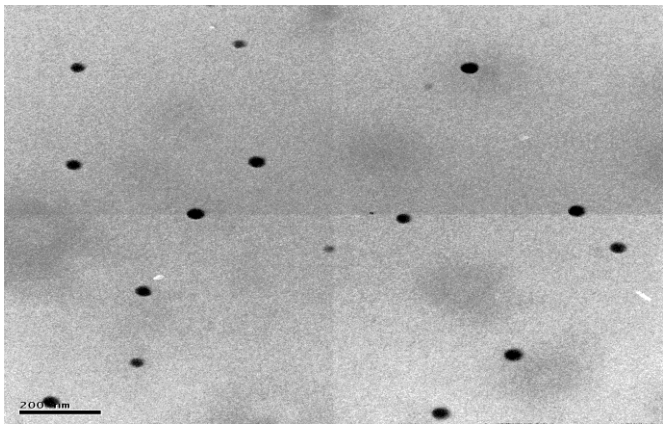


Fig.14a TEM images of biosynthesised copper nano particle

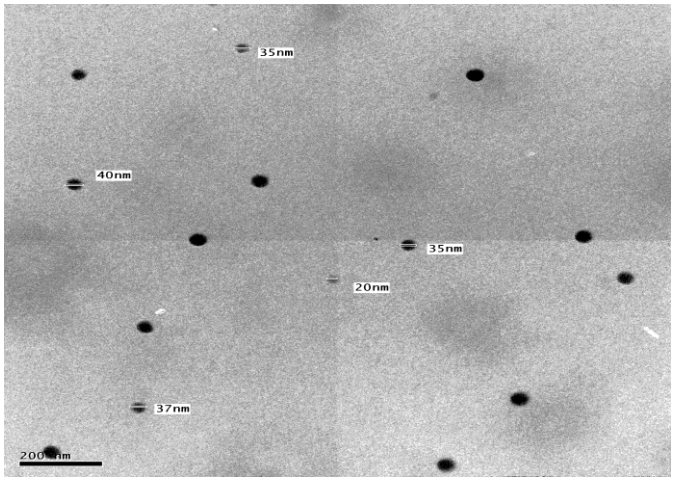


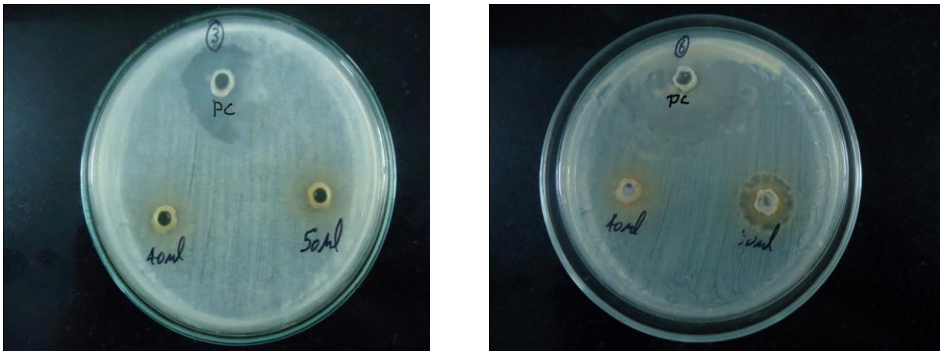
Fig.14b Biosynthesised Nanoparticles showing capping ability of aqueous guava extract.

Further the capping ability of guava nanoparticles was observed (Fig. 14b).Tem image shows selected area electron diffraction pattern (SAED) of the copper nanoparticles. The cu particles are amorphous as can be seen from the selected area diffraction pattern recorded from one of the nanoparticles in the aggregate (Fig 14c).

Fig. 14 c selected area electron diffraction pattern (SAED)

3.10 Antimicrobial activity of copper nanoparticles

Antimicrobial activity of biosynthesized copper nanoparticles was examined out on two pathogens, such as E.coli (gram –ve) and staphylococcus aureus (gram +ve). Biosynthesized copper nanoparticle showed clear zone of inhibition as indicated in the table-5 against E.coli and staphylococcus aures. It is reported that Cu nanoparticles attach to the surface of the cell membrane, disturbs its function and penetrates directly with the bacterial outer membrane and release Cu ions. Ciprofloxacin 25µg/ml was used as +ve control.



(a) (b)

Fig.15 Zone of inhibition of green synthesis of CuNps against (a) E-Coli and (b) Staphylococcus aures.

Table 5: showing zone of inhibition against bacterial pathogens

Culture name	Copper nanoparticle 1500µg	2000µg	Pc
E. coli	10	14	24
Staphylococcus aureus	-	-	22

4. CONCLUSION:

The present investigation revealed that the fresh guava extract is capable of producing copper nanoparticles that are quiet stable for 15 days at room temperature without any sign of precipitation.

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