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Green synthesis,Characterization,Anti-bacterial,Anti-diabetic, Anti-Cancerous Activity of Momordica Charantia leaf extract mediated Cu-Ag Nanoalloys

Jasmine jacob[1*], Augustine Arul Prasad .T[2],Scholastica Mary Vithiya .B[3]
[1,2]Department of Chemistry,Dwarakadoss Goverdhan doss Vaishnav College (Autonomous),Chennai-600 106.
[3]Department of Chemistry,Auxilium College (Autonomous),Chennai-632 006.
*Corresponding e mail: jasminejacob215@gmail.com.

ABSTRACT

The present study reports the green synthesis of Cu-Ag bimetallic nano alloy(NA) from *Momordica Charantia* leaf extract in aqueous media.The reduction and stabilization is carried out by the antioxidants present in the *Momordica Charantia* leaf extract. Toxic substances are not used as reducing and stabilizing agents in this synthesis. UV-Visible spectroscopy confirms the formation of Cu-Ag NAs.Transmission electron microscope(TEM) revealed that the particles size is less than 20nm. Scanning electron microscope (SEM-EDAX) gives evidence for the presence of silver-copper nanoparticles.The electrochemical response of the bimetallic nanoparticles is evaluated using Cyclic Voltammogram. The results concluded that Cu-Ag NAs synthesized from *Momordica Charantia* can be effectively used in medicinal field because of its Anti-bacterial, Anti-diabetic and Anti-cancerous activity.

Keywords
Green synthesis,Cu-Ag bimetallic nanoparticles,Anti-bacterial ,Anti-diabetic,Anti-cancerous.

INTRODUCTION

Nanotechnology is the growing field of research interest across the world.It has multiple properties and applied in several areas.Nowadays bio- based processes are alternative to conventional methods for preparing nanoparticles[1] .The main focus on this field is because of their certain characteristics such as size, morphology and distribution. There are lot of new applications emerging day by day[2]. In recent years, due to so many pollutants in environment for the synthesis,the uniqueness of bio synthesis of metal nanoparticles are really necessary to maintain the ecosystem.As nano-sized particles has unique nature it is utilized in biomedical, pharmaceutical, agriculture and so on[3]. Eco-friendly materials like pant extract[4] , microbes[5], enzymes[6] which are utilized for any synthesis reduces toxic chemicals which can be employed in any field without any side effects.As this bio- method reduces harmful effects,it is highly utilized in medicinal field which is inexpensive and can be processed for large scale

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synthesis.The synthesis by microbes and enzymes has limited industrial use due to the requirement of antiseptic conditions , synthesis by plant extract is more valuable.

Natural antioxidants lies in plant phenols and antimicrobial compounds are present in some of them.Anti microbial compounds present in plants act as essential components for microbial infection resistance[7]. Lots of treatment are going on for diabetic mellitus because the percentage of people having this chronic disease is increasing as days going on.Treatment without side effects can be achieved by using herbs/natural products.Plant medicines are greatly preferred for treating this disease through out the world[8]. Investigations have proved that the compounds present as such may be unstable in gastrointestinal tract and this condition cause the compounds to be ineffective.To improve this properties of drugs, nanoparticles come into picture for drug delivery to the target area[9].Cancer is a deadly disease, where the cases rise from 14 million in 2012 to 22 million in the next decades[10]. Therefore various effective drugs are prepared in which the exploitation of natural products are the most successful method.Two different metals combined to form bimetal.When compared to mono-metallic, bimetallic nanoparticles are more useful because they exhibit different magnetic, thermal and optical properties[11]. Nanoalloys play an important role in curing various diseases.Among nanoparticles Ag and Cu are highlighted the most because of their certain properties and wide applications in electrical device, biological etc[12]. On synthesis of Cu-Ag nano alloy very few works are reported[13].In this we have come across the formation of core shell nanoparticle, which shows the reduction of metal ion of “shell” on the “core” which is pre - synthesized.Core-shell Cu-Ag helps us to lower the cost of Ag and to increase the oxidation stability of Cu. In this work, the plant used is Momordica Indica which is a herbal medicine used for treating and preventing many diseases.It is a powerful weapon against AIDS, Cancer, Diabetics and many other infectious diseases. This plant extract itself act as good stabilizing and reducing agent because of phyto- chemicals present in it.This work explains about the synthesis, characterization of Cu-Ag nanoalloy from momordica indica and their antibacterial, anti diabetic and anti cancerous activity has been reported.As per our knowledge Momordica Indica has not been used for synthesis of Cu-Ag nanoalloy.

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METHODS AND MATERIALS

Materials

Silver nitrate, Copper nitrate used in this synthesis is purchased from Sigma Aldrich with high purity.The leaves of Momordica Charantia is collected from Thrissur district in Kerala, India.Deionized water is utilized through out the process.

Extract Preparation

Freshhealthy leaves of Momordica Charantia are used for bio-synthesis of Cu-Ag bimetallic Nano Alloys(NA).Leaves are dried completely without any moisture content and 10gms of leaves are ground into fine powder in a 500mL beaker with 100mL of de-ionized water.The pure extraction of leaves obtained by heating it for 80^0c and non-turbid filtrate is stored at 4^0cfor further studies.

Ag-Cu NAs formation

In this work the formation of Cu-Ag is carried out using silver nitrate($AgNO_3$),cupric nitrate($Cu(NO_3)_2$ and plant extract.10mL of plant extract is added drop by drop to the equal concentration of prepared 1mM $AgNO_3$ and 1mM $Cu(NO_3)_2$ with constant stirring by conventional heating method for about 3hrs at 80^0C.After that the mixture is kept shaking at room temperature for excellent mixing of extract with solution.The NAs formed is turbid greenish in colour [14] and is taken for characterization.

Characterization studies of NAs

It is very important to carry out characterization studies to confirm the synthesis of Cu-Ag NAs and its applications.Cu-Ag NAs formed is confirmed using UV-Visible spectra in the range between 300-800nm using Hitachi U-2900 Double beam spectrometer, Scanning Electron Microscopy (SEM) by FEI-Quanta FEG 200F, Transmission Electron Microscopy (TEM) by FEI-Tecnai G2 20 Twin, Cyclic voltametry by CHI 604E.

In-vitro Anti-bacterial Activity

Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonous aurgenosa are the test bacterial strain from IMTECH, Chandigarh.Well diffusion method[15] is used for this antibacterial study.Petri plate with 10 mL molten Mueller Hinton agar is kept for 18hrs growth in which 100 µl of the pathogenic bacteria is added and made culture lawn with sterile L-rod spreader.Sterile cork borer is used to make 5mm well on agar. 25 µl/well, 50 µl/well, 75 µl/well,

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100 µl/well are the different concentrations of samples taken in well and 24hrs of incubation is done 37^0c. Azithromycin (30µl/well) is the positive control By antibiotic zone scale, the antibacterial activity is measured by zone of inhibition around the well.

Anti-diabetic Activity

Yeast α-glucosidase inhibitory activity

The enzyme and substrate used are Yeast α-glucosidase (0.7 U) dissolved in 100 mM phosphate buffer (pH 7.0) containing 2 g/l bovine serum albumin, and 0.2 g/l NaN3, and 5 mM pnitrophenyl- α-D-glucopyranoside in the same buffer (pH 7.0) .Absorbance with 405nm measured with UV spectrometer for the enzyme solution (1000 µl) and 100 µl of the test sample at various concentrations were mixed.After 5 min of incubation , 50 µl of the substrate solution were added and again incubated. ThePercentage of the blank control gives inhibitory activity.

By using this formula % inhibition is calculated

$$\text{Inhibition (\%)} = \frac{\text{Abs 405 (control)} - \text{Abs 405 (extract)}}{\text{Abs 405(control)}} \times 100$$

Anti-Cancerous Activity

HeLa cells Dulbecco's Modified Eagle's Medium (DMEM) with 10%FCS is poured into 25mL flask. Passage of cells is suspended by pipette and cells are homogenized.Various concentration of test sample(0-100µg/mL) is collected in 24 well culture plate with 1mL homogenized cell suspension and 37^0C incubation is done in 5% CO_2 in humidified CO_2 incubator for about 48hrs.Cytotoxicity is studied with 80% confluence of cells.The assay was carried out using (3-(4, 5-dimethyl thiazol-2yl)-2, 5- diphenyltetrazolium bromide (MTT). Purple product formazan is formed when MTT is cleaved by mitochondrial Succinate dehydrogenase and reductase of viable cells. This formazan production is directly proportional to the viable cell number and inversely proportional to the degree of cytotoxicity.MTT is added to the wells and incubated for 48hrs and left in room temperature for 3 hrs. 100µl SDS in DMSO were added to dissolve the formazan crystals and wells were removed the content using pipette.At 540nm, absorbancewere read in Lark LIPR- 9608 micro plate reader [16].

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RESULTS AND DISSCUSSION

UV-Visible Spectral Analysis

UV- Visible spectrum is taken for the formed bimetallic Cu-Ag NAs shown in fig1.In the fabrication of Cu-Ag NAs it was observed that the NAs were formed with the average size of 20nm.The formation of Ag shell nanoparticle over the surface of the Cu core nanoparticle has been confirmed by the broad adsorption peak of Cu-Ag core shell structure at ~430nm[17].This observation indicates that the Na has been formed with Cu as it core and completely coated by the Ag shell.This also gives us the strong evidence for Cu-Ag NAs formed from leaf extract of Momordica Charantia itself as reducing and stabilizing agents.

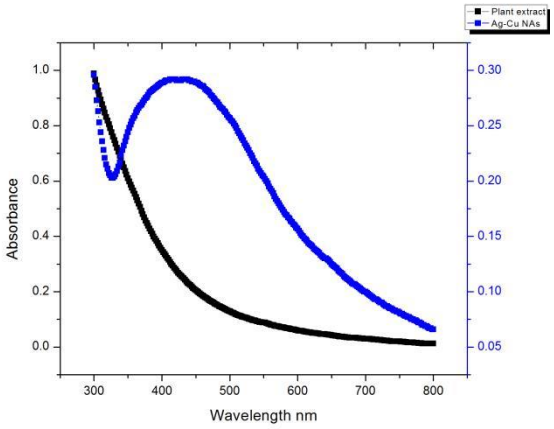

Figure 1:UV-Visible spectrum of plant extract of Momordica Charantia and formed bimetallic Cu-Ag NAs from the plant extract.

Scanning Electron Microscopy(SEM)

To determine the structure (or) shape of the compound, SEM analysis is employed.It is carried out within accelerating voltage of 30Kv at 6000x magnification.The presence of Ag-Cu NAs is confirmed by EDAX in which other elements present are from the plant extract which also proves that the bio - molecules from plant extract act as stabilizer and reducing agent in this synthesis process.The SEM studies reveals the fact that Cu-Ag core shell structures as been formed with rod shaped surface morphology and it is shown in Fig 2(a) and 2(b).

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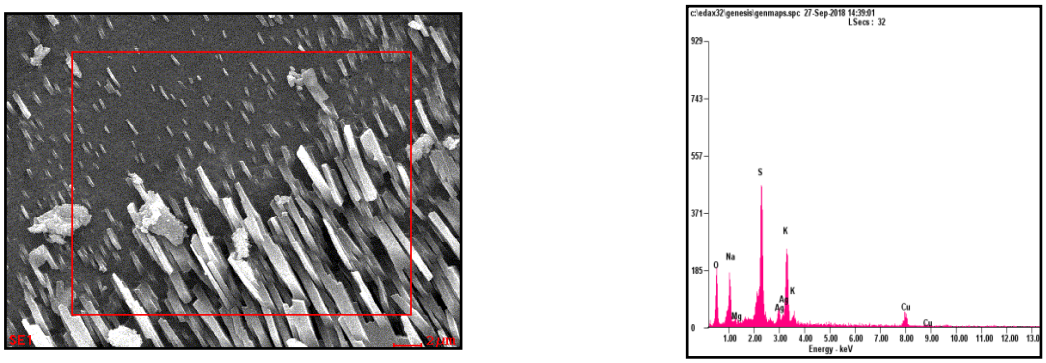

(a) (b)

Figure 2:(a) and (b) are the Scanning Electron Microscopy and EDAX of Cu-Ag bimetallic NAs from Momordica Charantia leaf extract respectively

High Resolution -Transmission Electron Microscopy(HR-TEM)

To measure the size of the particle the most simple and useful method to apply is HR-TEM analysis. It not only provide the size but also the crystalline nature of particles.The average size of the particle is found to be 20nm and is shown in Fig 3.HR-TEM image of Cu-Ag NAs depicts that the particles are spherical in shape and are dispersed substantially with no evidence of accolomeration .The SAED diffraction pattern also provides the possibility of understanding that Ag is deposited on the surface of Cu particle with the uniform shell structure.EDAX spectrum of the Cu-Ag NA confirms that the main component of the core shell was Cu with the high intensity peak for elemental Cu.

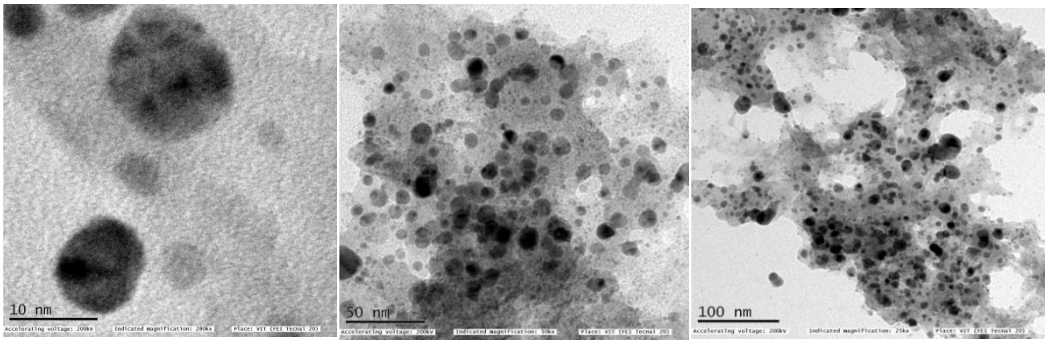

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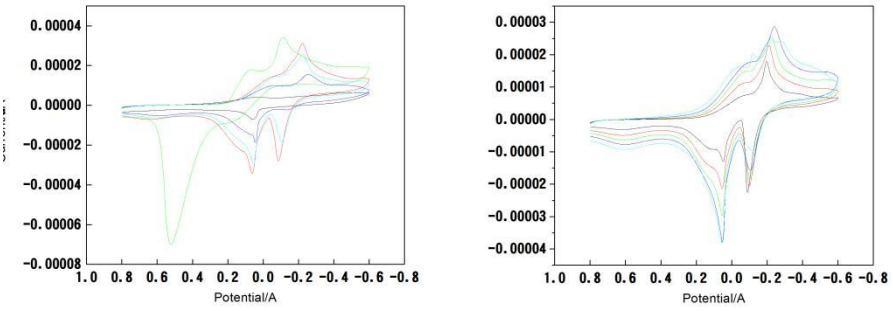
Fig 3: Transmission Electron Microscopy & EDAX of Ag-Cu bimetallic NAs from Momordica Charantia leaf extract.

Cyclic Voltammetric Analysis(CV)

Cyclic Voltametric technique is scanned here with different concentration 0.001M,0.005M,0.008M,0.01M,0.02M at various scan rates from 20mV/s to 100mV/s of bimetallic NAs with colloidal media(plant extract).CV curve shows strong anoidic and cathodic peaks.The anoidic peak is because of the oxidation of Ag^0 to Ag^+cathodic peaks says the reduction of two components ,Cu^{2+} to Cu^+ and Ag^+ to Ag^0 respectively.Quasi- reversible electron transfer process is revealed by CV curves and seen in Fig 4(a) and 4(b).



(a) (b)

Fig 4(a): Cyclic Voltammogram of bimetallic nanoparticle of colloidal media with different concentration 0.001M,0.005M,0.008M,0.01M,0.02M at scan rate of 60mV/s.

Fig 4(b) :Cyclic Voltammogram of bimetallic nanoparticle of colloidal media with different scan rate 20mV/s to 100mV/s at concentration of 0.008M.

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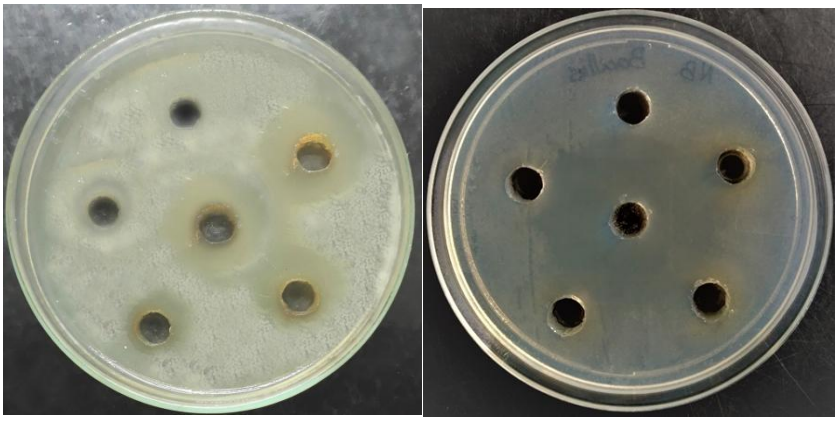
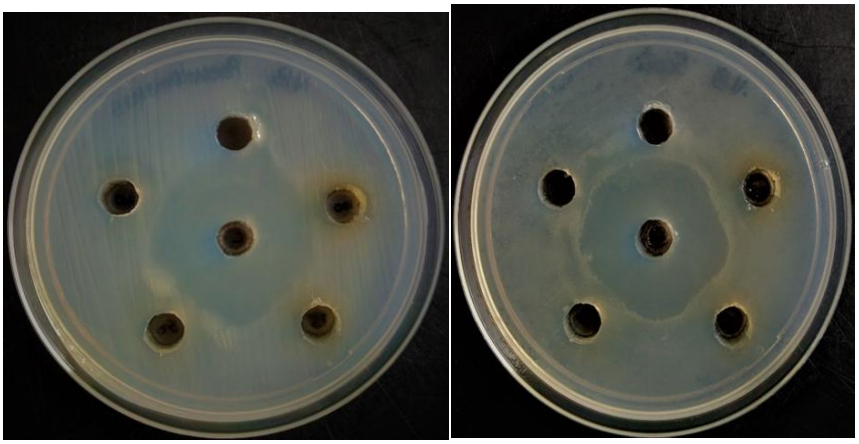
Anti - bacterial assay

Cu-Ag NAs synthesized from Momordica Charantia displayed good anti-bacterial activity against both gram positive Staphlyococcus aureus [Fig 5(a)] , Bacillus subtilis [Fig 5(b)] and gram negative Escherichia coli [Fig 5(c)], Pseudomonas aeruginosa [Fig 5(d)] from Table 1.Zone of inhibition is measured in mm. It is observed that in lower concentration the effect is minimal.Increasing concentration Cu-Ag NAs increases the antibacterial activity .Result showed that the antibacterial activity of Staphlyococcus aureus(gram positive) ,Pseudomonas aeruginosa(gram negative) is more compared to Bacillus subtilis (gram positive), Escherichia coli(gram negative) .



(a) (b)



(c) (d)

Fig 5:(a),(b),(c),(d) are the antibacterial activity of synthesized Cu-Ag NAs against Staphylococcus aureus(+),Bacillus subtilis(+),Escherichia coli(-) and Pseudomonous aeruginosa(-) respectively

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Concentrations (µl/well)	**Zone of inhibition (mm) Staphylococcus aureus**	**Zone of inhibition (mm) Baccilus Subtilis**	**Zone of inhibition (mm) Pseudomonous aeruginosa**	**Zone of inhibition (mm) Escherichia coli**
25	8	-	9	-
50	11	7	12	9
75	19	9	16	12
100	22	12	20	15

TABLE 1:Effect of synthesized Cu-Ag NAs against various pathogenic bacterial strains

Anti-diabetic assay

To breakdown starch, carbohydrate the main enzyme involved is the Alpha -amylase which releases sugar into blood and cause increase in blood sugar level which lead to diabetes.The therapeutic effect on diabetes can be proved from the inhibitory effect of the enzyme.The synthesized Cu-Ag NAs has good anti-diabetic activity which is showed in figure 6. This bimetallic NAs can be used as anti -diabetic drug candidate.

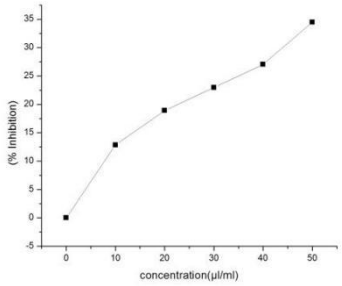

Fig 6: Anti-diabetic assay of synthesized Cu-Ag bimetallic NAs from Momordica Charantia

Anti-Cancerous assay

When tested with green synthesized Cu-Ag bimetallic NAs showed HeLa cell lines are prone to be cytotoxic. For about 48 hrs the samples are treated shows the cytotoxicity effect on HeLa cell lines and and as concentration of the showed drug increases there is increase in the cytotoxicity. It was evident that the samples tested at high as 200 µg/ml showed cytotoxicity activity as nearly 90%

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against HeLa cell lines and showed in Table 2 and Fig 7,8. IC50 value calculated for the bimetallic Cu-Ag NAs is found to be 67.82µg/ml.

Sample concentration(ug/mL)	HeLa Cell viability(%)
0	100
1.5625	88.28
3.125	81.19
6.25	70.46
12.25	62.95
25	49.89
50	36.77
100	13.75

Table 2: In vitro toxicity effect of Cu-Ag bimetallic nanoparticles against HeLa cell lines

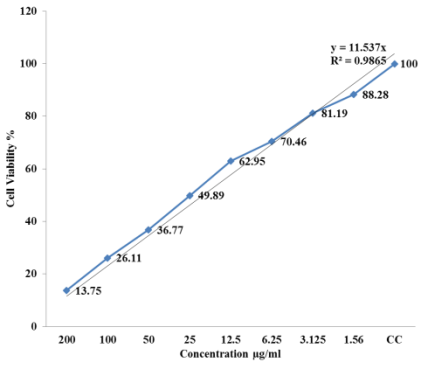

Fig 7:Anticancerous activity of Cu-Ag bimetallic nanoparticles against HeLa cell lines.

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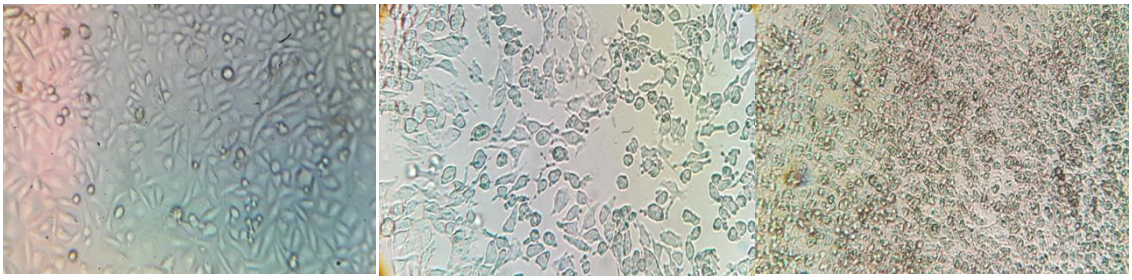

(a) (b) (c)

Fig 8: (a),(b),(c) are the control,con(100µg/mL),con(200µg/mL) of Cu-Ag bimetallic nanoparticles against HeLa cell lines

CONCLUSION

In this study , a green and effective synthetic method for the synthesis of Cu-Ag core shell NAs has been evaluated using Momordica Charantia.The Cu-Ag core shell has been synthesized by a single step bio-genic reduction method.Cu-Ag NAs size distribution has been found to be 20±5nm.It is also observed that Ag shell coated over Cu core protects it from oxidation which further enhances the stability of the NAs. The formed NA were further analyzed for anti-bacterial,anti-diabetic and anti-cancerous assay which gives a very goog promising effective results.This cost effective method can be widely utilized for the synthesis of such Cu-Ag core shell NA and can be used for various biological purposes.

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