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Evaluation of Cu-Ag Bimetallic Nanoalloys as Antibacterial, Antidiabetic, Anticancerous Drug Biosynthesized from *Curcuma aromatica*

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Drug resistant strains are formed due to unsuitable uses of antibiotics and insufficient check of infections. In present years, due to the extensive antimicrobial properties, metallic nanoparticles and metallo-pharmaceutics are highly proposed. Therefore, the synthesis of bimetallic nanoparticles are exploring towards the evolution of more productive amalgamative antimicrobials composed of combined metals. In this study, the green synthesis of Cu-Ag bimetallic nano-alloys using aqueous extract from the leaves of *Curcuma aromatica* is carried out. Synthesized Cu-Ag nano-alloys were characterized by UV-visible spectroscopy, scanning electron microscope (FE-SEM), transmission electron microscope (TEM-EDAX), cyclic voltammogram (CV). The characterization studies reveals that the biosynthesis produced core-shell Cu-Ag nano-alloys with spherical shape and average diameter size of 15 nm. The synthesized Cu-Ag nanoalloys exhibited antibacterial activity against both Gram-positive and Gram-negative bacteria. The antidiabetic potential of Cu-Ag nanoalloys shows an effective inhibition against α-glucosidase. Anticancerous activity of Cu-Ag nanoalloys indicates its greater efficacy in destroying cancer cells. The biosynthesis of Cu-Ag nanoalloys can be employed in medical and industrial fields on a large scale with cost reductive method.

Keywords: Bimetallic nanoparticles, *Curcuma aromatica*, Antibacterial activity, Antidiabetic activity, Anticancerous activity.

INTRODUCTION

Drug-resistant infectious diseases are fast spread and causes a global health threat in this era. If this continues, then after 50 years around 10 million people die every year due to these drug-resistant infections [1]. As antimicrobial agents, metal nanoparticles are utilized. Tiny particles with different properties and applications are nanoparticles [2]. Noble metal nanoparticles, such as silver, gold, copper, *etc*. exhibits strong antibacterial action against wide range of microorganisms; thus it is used in cosmetics, medical devices, food preservatives, dental resin composites and implants [3]. Bimetallic nanoparticles (combination of two metal elements), show more advantages when compared to mono-metallic nanoparticles and are highly focused due to their distinct optical, electronic, catalytic properties [4]. Biosynthesis of metal nanoparticles provide a new scope due to its simple, stable, eco-friendly, inexpensive methods. In bimetallic nanoparticles synthesis, environment friendly reducing agent, plant extract is utilized. To reduce metal ions into metal nanoparticles, plant phytochemicals with antioxidant or reducing properties are used [5]. In nanoparticles synthesis, the composition of plant leaf extract is also an important factor *i.e*.) each one have different concentration level of phytochemicals [6]. Flavones, terpenoids, ketones, aldehydes, sugars, carboxylic acids and amides are the main phytochemicals in plants, responsible for bioreduction [7]. In theoretical point of view metal ions with stronger reduction potential are reduced faster. Thus in Au-Ag bimetallic nanoparticles which is a well-known system, Au ions are reduced first while Ag ions are reduced later, forming core-shell structure [8]. *Curcuma aromatica*, a traditional medicinal herb, belong to Zingeberaceae family, used for treating biliary disorder, anorexia, cough, diabetic wounds, hepatitis disorders, rheumatism, sinusitis [9]. Around 235 compounds are found in *C. aromatica*, which is categorized under phenolic compounds and terpenoids. *C. aromatica* has 22 diaryl heptanoids, 8 phenyl-

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propene, other phenolic compounds, 68 monoterpenes, 109 sesquiterpenes, 5 diterpenes, 4 sterols, 3 triterpenoids, 2 alkaloids and 14 other compounds [10]. The main component in *Curcuma aromatica* is Curcumin. It has antioxidant, anticancer, anti-inflammatory, antiarthritic, antimicrobial, neuroprotective, cardioprotective and radioprotective activities [11]. It is safe with minimum toxicity and proved to have potential for preparation of green synthesis nanoparticles such as Ag, Au, Cu, Fe, Ni, *etc*. [12,13]. Bimetallic nanoparticles are oriented in different structures such as cluster-in-cluster, core shell, alloy with intermetallic compound, which cannot be seen in monometallic nanoparticles [14].

Synthesis of silver nanoparticles is achieved by electrochemical [15], photochemical [16], chemical reduction [17], sonochemical method [18,19], pulsed laser method [20], *etc*. The cost of silver materials had made its use to minimize [21]. Copper is used to replace silver because of its low cost and has high electrical conductivity. The main drawback on copper is that it is easily oxidized [22]. To overcome this, we have adopted the Ag-shell on Cu core formation. This reduces the cost of silver and oxidation stability of copper [23]. The keen interest on this alloy is because of its high conductivity and use in lead free alloy preparation. Metallic alloy has many applications but there are many difficulties in preparation with simple low cost when it comes to large scale synthesis [24]. So the preparation of metallic core-shell alloys with low cost simple way is of great importance.

Biosynthesis of Au-Ag, Ag-Pd, Ag-Fe bimetallic nanoparticles and their applications are reported [25-27]. While, copper-silver (Cu-Ag) bimetallic nanoparticle biosynthesis, characterization and application are less reported. Therefore, we reported about the synthesis of Cu-Ag bimetallic nanoalloys using the leaf extract of *Curcuma aromatica*, their spectroscopic studies and studied their antibacterial, antidiabetic and anticancerous activities. The results and findings in this work contribute to the advanced knowledge for synthesis and antimicrobial properties of Cu-Ag bimetallic nanocomposites. Moreover, this study pave way for the development of more effective in the treatment of infections caused by both sensitive and drug-resistant clinically relevant pathogens in the future.

EXPERIMENTAL

Silver nitrate, copper nitrate with high purity provided by Sigma-Aldrich. The leaves of *Curcuma aromatica* is obtained from Alappuzha district in Kerala state, India. Throughout the process, deionized water was used.

The samples UV-VIS absorption spectra was obtained with the use of Hitachi U-2900 Double beam spectrometer in the range of 300-800 nm. Scanning electron microscopy (SEM) is carried out by FEI-Quanta FEG 200F. Transmission electron microscopy (TEM) analysis is taken using FEI-Tecnai G2 20 Twin. Cyclic voltametric studies were preformed using CHI 604E.

Extract preparation: Biosynthesis of Cu-Ag bimetallic nanoalloys were prepared from fresh healthy leaves of *Curcuma aromatica*. Without any moisture content leaves were dried completely and fine powder of 10 g of leave was taken in 500 mL beaker with 100 mL of deionized water. At 70 ºC, the pure extraction of leaf extract was obtained and then stored at 4 ºC for studies.

Cu-Ag bimetalic nanoalloys formation: In present work, Cu-Ag was formed using silver nitrate, cupric nitrate and plant extract. Into equal concentration of prepared 1 mM $AgNO_3$ and 1 mM $Cu(NO_3)_2$, 10 mL of plant extract were added dropwise with constant stirring at 80 ºC for 3 h with conventional heating. Then this mixture was kept for shaking for excellent mixing at room temperature. Turbid greenish colour [28], nanoalloys formed was preserved for further characterization studies.

***in vitro* antibacterial activity:** *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonous aurgenosa* were the test bacterial strains and procured from IMTECH, Chandigarh, India. Antibacterial study was carried out by well diffusion method [29]. Molten Mueller Hinton agar (10 mL) in petri plate kept for 18 h growth, 100 µL of pathogenic bacteria added and made culture lawn with sterile L-rod spreader. Sterile cork borer was used to make 5 mm well on agar. Different concentrations of samples as 25, 50, 75, 100 µL/well were incubated for 24 h in 37 ºC. Positive control used was azithromycin (30 µL/well). The diameter of zone of inhibition around the well by antibiotic zone scale determines the antibacterial activity.

Antidiabetic activity

Yeast α-glucosidase inhibitory activity: 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 NaN_3 and 5 mM pnitrophenyl-α-D-glucopyranoside in the same buffer (pH 7.0) were employed as enzyme and substrate solution. Absorbance with 405 nm measured with UV spectrometer for the enzyme solution (1000 µL) and 100 µL of the test sample at various concentrations were mixed, measured as 405 nm absorbance with UV spectrometer. After 5 min of incubation, 50 µL of the substrate solution were added and again incubated for 5 min [30]. From time zero, an increased absorbance was measured and the percentage of the blank control gives the inhibitory activity. The % inhibition is calculated as follows:

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

Anticancerous activity: After discarding the culture medium, HeLa cells in Dulbecco's modified Eagle's Medium (DMEM) was tryspinized separately and DMEM with 10% FCS was poured into 25 mL flask. The cells was suspended in the medium by gentle passage with the pipette and the cells homogenized.

Various concentration of test sample (0-100 µg/mL) was collected in 24 well culture plate with 1 mL homogenized cell suspension and 37 ºC incubation was done in 5% CO_2 in humidified CO_2 incubator for about 48 h. Cytotoxicity was 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 was formed when MTT cleaved by the mitochondrial succinate dehydrogenase and reductase of viable cells. MTT was added to the wells and incubated for

48 h and left in room temperature for 3 h. The SDS (100 µL) in DMSO was added to dissolve the formazan crystals and the wells were removed the content using pipette. At 570 nm, absorbance were read in Lark LIPR-9608 micro plate reader [31,32].

RESULTS AND DISCUSSION

UV-visible studies: UV-visible absorption spectrum for Cu-Ag bimetallic nanoparticles is shown in Fig. 1. The position and intensity of localized surface plasmon resonance (LSPR) gives the particles formation confirmation. Navas & Soni [33] revealed that the absorption band position for core-shell structures in Ag/Cu particles relays on the thickness of shell. The Cu LSPR peak in this Cu-Ag was weak and appears in higher wavelength region. The broad absorption peaks at 420 nm and 520 nm were observed, describes that bimetallic particles with Cu rich (core) - Ag rich (shell) were formed. Trinh *et al.* [34] also reported the peaks at 410 nm and second one from 525 nm to 580 nm were proved for Cu/Ag core-shell nanoalloys.

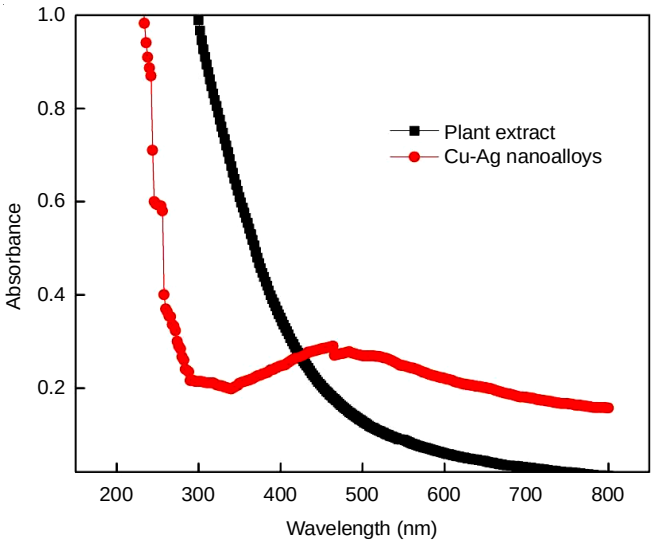

Fig. 1. UV-Visible spectrum of *Curcuma aromatica* plant extract and formed bimetallic Cu-Ag nanoalloys from the plant extract

Electron microscopy analysis: To know the surface morphology of Cu-Ag nanoalloys, FESEM micrographs were taken. From the image (Fig. 2), it is understood that almost all particles are in spherical shape . Fig. 3a-b are the HR-TEM images, which give the size and shape of the formed nanoalloys. The HR-TEM image gives the information of spherical shape and average size of particles are 15-20 nm. SAED diffraction pattern (Fig. 3c) gives clear idea that Ag deposits on the Cu particles. In Fig. 3d, EDAX spectrum gives evidence for the formation and presence of Cu-Ag core shell nanoalloys.

Ag-Cu nanocomposites electrochemical response: Reducing nature of the extract can be supported by cyclic voltammetric (CV) studies. Here, CV technique was scanned with different concentration 0.001, 0.005, 0.008, 0.01 and 0.02 M at different scan rates from 20 to 100 mV/s of Cu-Ag nanoalloys with plant extract. Strong anoidic and cathodic peaks are shown by CV curve. The oxidation of Ag^0 to Ag^+ as anoidic peak, reduction of two components Cu^{2+} to Cu^+ and Ag^+ to Ag^0 as cathodic peaks. The CV curves revealed the quasi-reversible electron transfer process which is shown in Fig. 4a-b.

Antibacterial assay: The antibacterial activity of biosynthesized Cu-Ag nanoalloys from *Curcuma aromatica* showed good activity against Gram-positive *Staphylococcus aureus*, *Bacillus subtilis* and Gram-negative *Escherichia coli*, *Pseudomonous aeruginosa* bacteria. Present study shows zone of inhibition highest 28 mm against *Escherichia coli* and lowest 19 mm for *Bacillus subtilis*. Increase in concentration increases antibacterial activity against control inhibition value of 30 mm azithromycin. Results disclosed that antibacterial activity of Gram-negative bacteria (*E. coli* and *P. aeruginosa*) were more compared to Gram-positive (*S. aureus* and *B. subtilis*) (Table-1).

Antidiabetic assay: At α-glucosidase concentration 0, 10, 20, 30, 40, 50 µL/mL, the % inhibition by Cu-Ag NAs from *Curcuma aromatica* was increased in a concentration dependent manner (Fig. 5).

Anticancerous assay: Biosynthesized Cu-Ag nanoalloys showed HeLa cell lines were proned to be cytotoxic. For cytotoxicity on HeLa cell lines, the sample was kept for 48 h, which

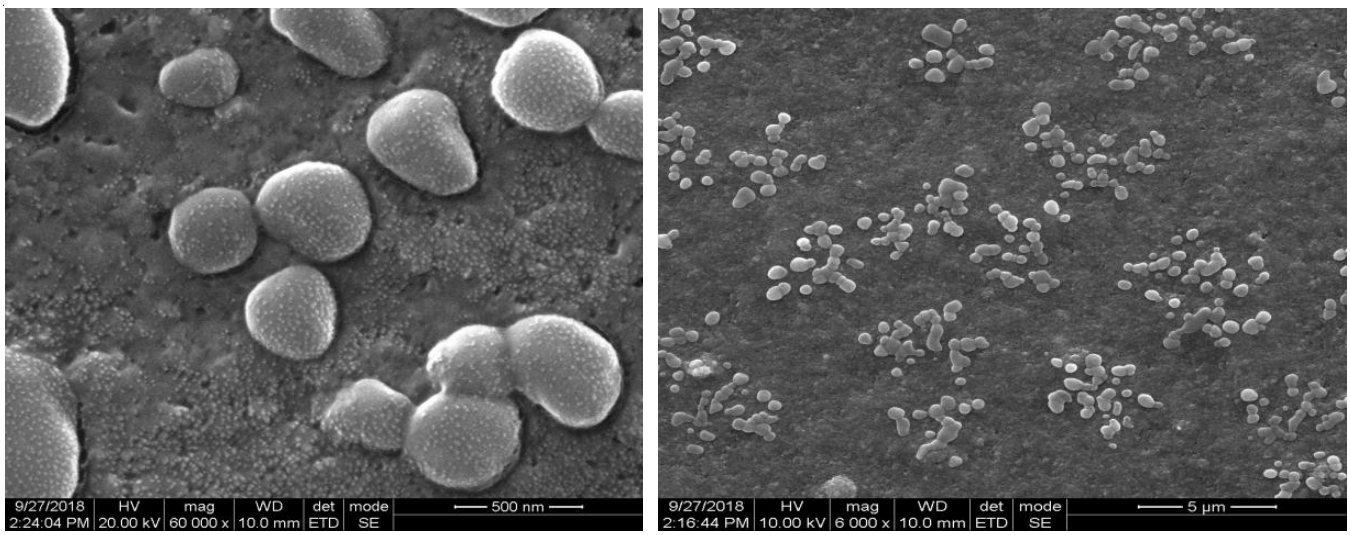

Fig. 2. FESEM images of Cu-Ag nanoalloy prepared at 80 °C from the plant extract

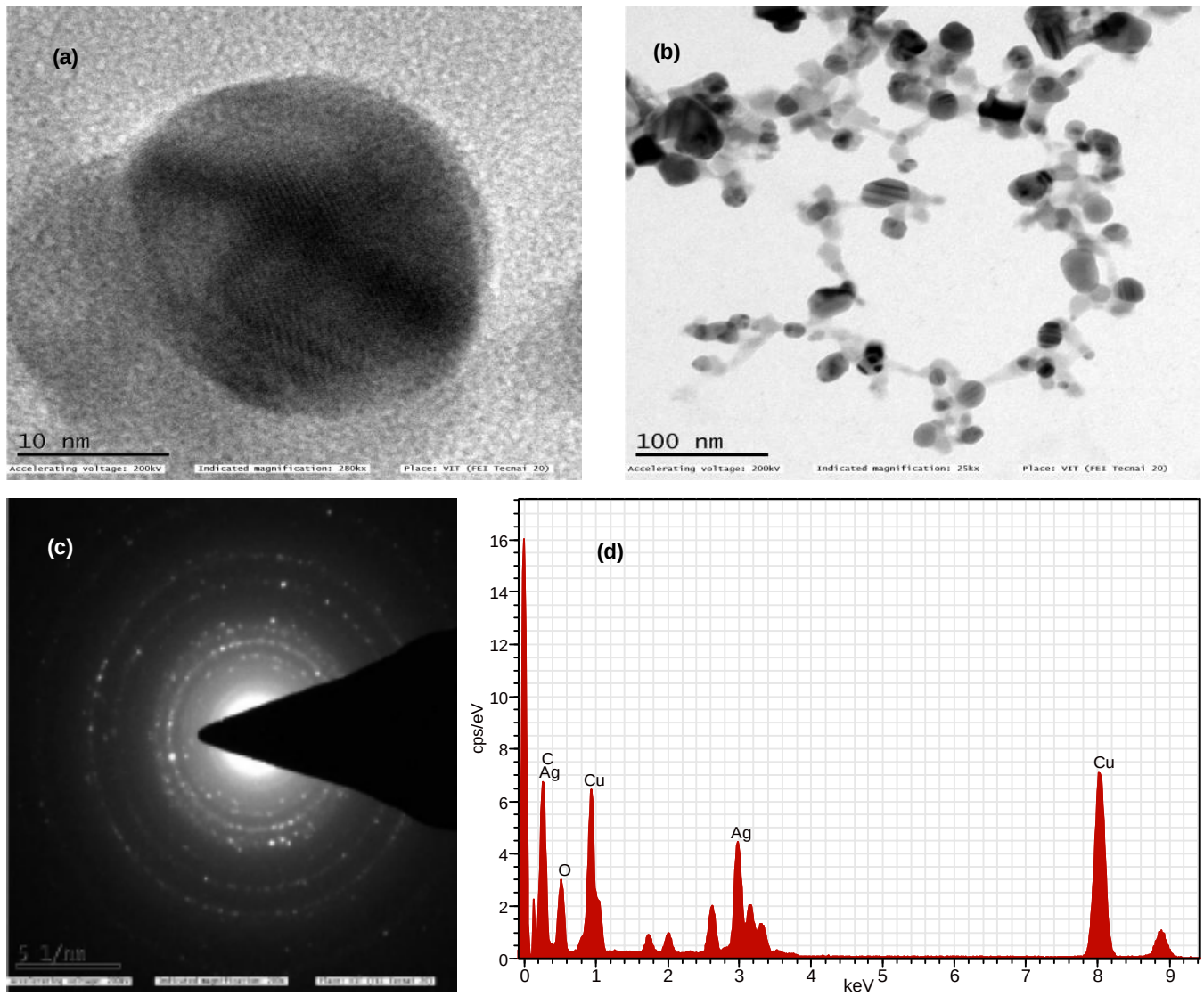

Fig. 3. (a&b) are the HR-TEM image of synthesized Cu-Ag NAs, (c) is the SAED diffration pattern, (d) EDAX spectrum

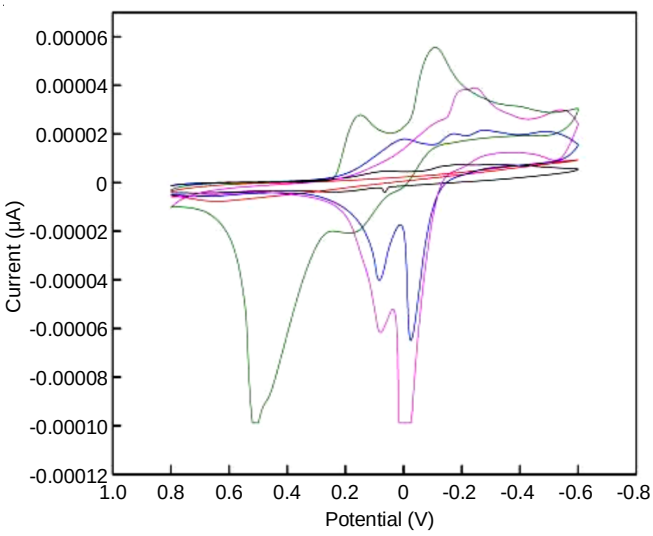

Fig. 4a. Cyclovoltammetric curves of bimetallic nanoparticle of colloidal media with different concentration 0.001 M, 0.005 M, 0.008 M, 0.01 M, 0.02 M at scan rate of 60 mV/s

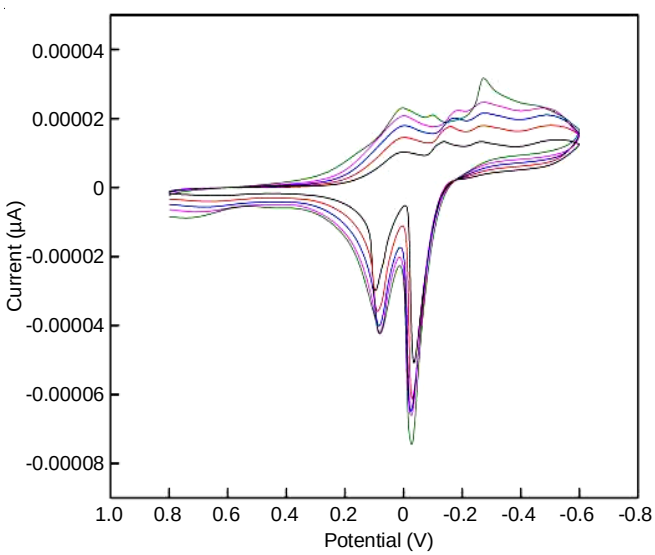

Fig. 4b. Cyclovoltammetric curves of bimetallic nanoparticle of colloidal media with different scan rate 20 to 100 mV/s at concentration of 0.008 M

TABLE-1
EFFECT OF SYNTHESIZED Cu-Ag NANOALLOYS AGAINST VARIOUS PATHOGENIC BACTERIAL STRAINS

Conc. (µL/well)	Zone of inhibition (mm)			
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*
25	10	8	15	12
50	12	10	20	15
75	18	14	25	20
100	20	19	28	24

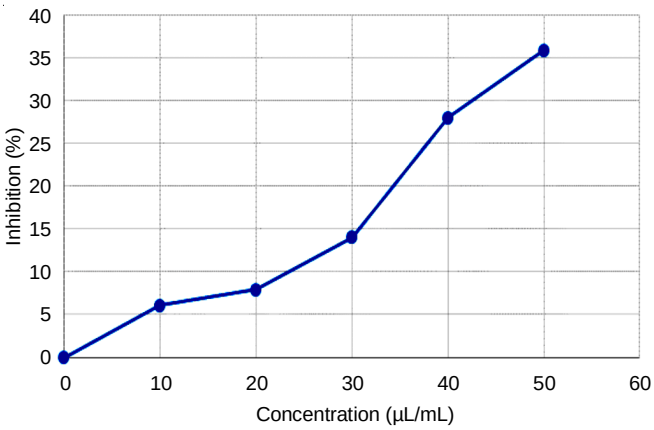

Fig. 5. Antidiabetic assay of synthesized Cu-Ag bimetallic nanoalloys from *Curcuma aromatica*

showed an increase in the cytotoxity as the concentration of biosynthesized drug increases. The anticancerous activity of Cu-Ag nanoalloys is showed in Fig. 6. The calculated IC_{50} value was found to be 79.24 µg/mL for the synthesized Cu-Ag nanoalloys.

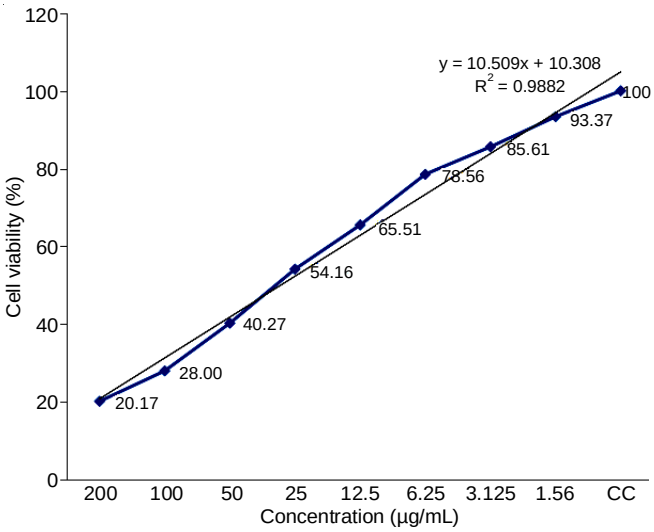

Fig. 6. Anticancerous activity of Cu-Ag bimetallic nanoalloys against HeLa cell lines

Conclusion

In present study, Cu-Ag bimetallic nanocomposites were prepared from the leaf extract of *Curcuma aromatica*, which is used as both reducing agent and stabilizer. The characterization studies confirmed the presence of Cu-Ag nanoalloys and redox behaviour of the formed nanoparticles. The Cu core was protected by thin silver shell from oxidation and thus increased the stability of Cu-Ag nanoalloys. The antibacterial, antidiabetic, anticancerous evaluation shows the ability of green synthesis process are more effective to treat drug-resistant infections.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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