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*Research Article*

# Synthesis and Characterization of Gold Nanobiocomposite of Asparaginase using *Aspergillus terreus*

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## Abstract

Nanotechnology has made it possible to coat therapeutic agents on the nanoparticles. Nanoparticles are used as drug carrier to deliver anticancer therapeutic agents to cancer cells for increased bioavailability. Fungal L-asparaginase is a potential anticancer agent with lesser side effect. In the present study, L-asparaginase synthesized by *Aspergillus terreus* was conjugated with gold nanoparticles to form gold nanobiocomposite of asparaginase. The formation of gold nanobiocomposite was confirmed by absorption peak at 540 nm in UV-Visible spectrometer. The crystalline nature of synthesized gold nanobiocomposite was confirmed using XRD analysis. Both spherical shaped needle shaped gold nanobiocomposites were observed in SEM analysis with the size ranges between 150-250 nm and 60-80 nm respectively. The MTT assay showed that decrease in viability of MCF-7 cancer cell line. The cell viability was decreased to 36.74% when MCF-7 cancer cells treated with gold nanobiocomposite of asparaginase. Thus the synthesized gold nanoparticles conjugated with asparaginase can be used as bi-functional nanomaterials for cancer diagnosis and treatment.

**Keywords**: Gold nanoparticles; Nanobiomaterials; Characterization; L-Asparaginase; Anticancer activity.

## Introduction

Nanotechnology is the field of synthesizing, characterization and applications of nanomaterials for various applications. Nanobiotechnology is the field of developing nanobiomaterials for biomedical and other industrial applications. Various nanomaterials have been used to bind biological drug molecules, used as a drug carrier for improved bioavailability [1,2]. Gold nanoparticles have been reported for various medical applications for its better imaging, diagnostics, and therapeutic value. Gold nanoparticles are biocompatible and can easily conjugate with biological molecules for labeling, imaging, optical and electrochemical sensing, diagnostics and therapy [1,3,4].

Nature has provided us with a number of biological resources such as plants and plant products, algae, fungi, yeast, bacteria, and viruses for synthesis of nanoparticles [5,6]. Both extracellular and intracellular biosynthesis of gold nanoparticles have been reported using bacteria such as *Pseudomonas aeruginosa* [7] *and Pseudomonas fluorescens* [8] and fungi such as *Penicillium brevicompactum* [9], *Epicoccum nigrum* [10], *Fusarium oxysporum* [11] and *Sclerotium rolfsii* [12]. Marine sponge and several plants also reported for biosynthesis of gold nanoparticles. *Acanthella elongate* a marine sponge [13], *Crocus sativus* extract [14] and *M. uniflorum* (dry land legume crop) [15] were reported biosynthesis of gold was reported for reported the green synthesis of gold nanoparticles.

Treating the tumour cells with L-asparaginase decreases the available L-asparagine content in the surrounding. Exposure of normal cells to L-asparaginase also reduces the L-asparagine content in the surrounding environment but the L-asparagine produced using asparagine synthetase is enough for cell survival and maintaining its viability. Hence this makes L-asparaginase a potent anti-cancerous agent [16]. Fungal asparaginase has been reported in literature for better anticancer activity with lesser side effect than bacterial asparaginase [11,17].

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Metalloproteins are nothing but metal nanoparticles coated with proteins on its surface which may be therapeutic value. Metalloproteins have many advantages over the metal nanoparticles in medicine field [18-20]. Thus the present work was focused on synthesis of multifunctional therapeutic metalloproteins of gold nanoparticles using asparaginase from *Aspergillus terreus* and for in cancer therapy. The gold nanoparticles synthesized by asparaginase was characterized and tested for its anticancer activity against MCF-7 cancer cells.

## Materials and methods

### *Fungi growth conditions for inoculum*

*Aspergillus terreus* used for the production of asparaginase was obtained from IMTECH, Microbial Type Culture Collection (MTCC), Chandigarh, India. The fungus was sub-cultured in modified Czapek agar slants for 96 hrs at 32°C and refrigerated at 4°C for further use.

### *Synthesis of gold nanoparticles using Aspergillus terreus*

*A. terreus* was inoculated in 500 ml Erlenmeyer flasks containing 200 ml modified Czapek-Dox liquid media containing (g/100ml): 3.0 glucose, 2.0 sodium nitrate, 0.052 pottasium chloride, 0.15 Di-pottasium hydrogen sulphate, 0.001 zinc sulphate, 0.001 copper sulphate, 0.001 ferrous sulphate and 0.052 magnesium sulphate at pH 6.2. The fungal cultures were grown aerobically by agitating in an orbital shaker at 160 rpm at 32°C for 4 days. After the inocubation time the fungal cultures were filtered under vacuum through Whatman#2 filter paper [21]. The filtrate obtained was mixed with $HAuCl_4$ (1 mM) in 500 ml Erlenmeyer flasks and kept in a shaking incubator at 150 rpm at 32°C for 24 hrs. Change in the colour of filtrate in the flask from yellow to dark green colour confirmed the formation of gold nanoparticles. The suspended particles in the three flasks were separated by centrifugation at 10,000 rpm for 10 min and lyophilized for characterization purpose.

### *Synthesis of gold nanobiocomposite of asparaginase using Aspergillus terreus*

*A. terreus* was used to produce metalloproteins as it has the ability to produce both L-asparaginase and nanoparticles. *A. terreus* was inoculated in three different 500 ml Erlenmeyer flask containing modified Czapek Dox liquid medium containing (g/100ml): 2.0 L-Proline, 1.0 L-Asparagine, 0.2 glucose, 1.0 sodium nitrate, 0.052 pottasium chloride, 0.152 Di-pottasium hydrogen sulphate, 0.001 zinc sulphate, 0.001 copper sulphate, 0.001 ferrous sulphate and 0.052 magnesium sulphate, maintained at pH 6.2. The fungus was grown aerobically by agitating in an orbital shaker at 160 Rpm at 32°C for 4 days. After the incubation period the culture was filtered under vacuum through Whatman #2 filter paper which is rich with both L-Asparaginase and proteins required for reducing the metal ions to nanoparticles. The filtrate obtained was mixed with $FeCl_3$ (1 mM) in 500 ml Erlenmeyer flasks and kept in a shaking incubator at 150 rpm at 32°C for 24 hrs [21]. Change in the colour of filtrate in the flask from yellow to dark green colour confirmed the formation of gold nanoparticles. The suspended particles in the flasks were separated by centrifugation at 10,000 rpm for 10 min and lyophilized for characterization.

### *Characterization of gold nanoparticles and gold nanobiocomposite*

#### *UV spectrum analysis of gold nanoparticles and gold nanobiocomposite*

Optical properties of the synthesized gold nanoparticles and gold nanobiocomposite were analyzed using UV-Visible spectroscopy. The samples collected after the transformation process was analyzed using SYSTRONICS Double Beam UV-Visible spectrophotometer 2201. The spectrum values were obtained between the wavelength range 200 to 900 nm. The characteristic functional groups present in the synthesized gold nanoparticles and gold nanobiocomposite were analyzed using Fourier Transform-Infra Red (FT-IR) spectroscopy. FT-IR spectroscopy was measured on BRUKER α-T FT-IR Spectrometer. The samples were mixed with KBr (binding agent) and were made into discs at high pressure using hydraulic press. These discs were scanned in the range of 500 to 4000 $cm^{-1}$ to obtain FT-IR spectra.

#### *HR-SEM and EDS analysis of gold nanobiocomposite*

The surface morphology and size of the lyophilized gold nanobiocomposite was analyzed by SEM on a QUANTA 200 with magnification

 

range 35 to 30,000, equipped with Energy Dispersive Spectroscopy (EDS).

*X-Ray Diffraction analysis of gold nanobiocomposite*

The structure of the synthesized gold nanobiocomposite was studied using XRD analysis. XRD patterns were recorded on a XPERT-PRO diffractometer. This diffractometer uses Cu-K as an anode, acts as a X-Ray source (wavelength = 1.54060 Å), operating with Cu-tube radiation at 40 KV and 30 mA. The scan step for 2θ was 0.0170° with a scan step time of 38.1s. Approximately 1 g of the lyophilized sample was taken and XRD analysis was performed.

*Assay of L-asparaginase activity*

L-Asparaginase catalyzes L-asparagine to form aspartic acid and ammonia at 37°C. The concentration of ammonia was determined by using Nesslers reagent method. 0.1 ml of the cell free filtrate was taken. 900 µl of 0.1 M phosphate buffer pH 8.5 and 1ml of 0.04 M L-Asparagine solution was added to it and incubated at 37°C for 10 min. The reaction was stopped by the addition of 500 µl of 15% Trichloro acetic acid. Centrifugation was done at 6000 rpm for 15 min. Then 0.1 ml of the supernatant was taken and diluted to 8 ml with distilled water. 1ml of 2M NaOH and 1ml of Nessler's reagent was added. After 10 min the absorbance value was taken at 480 nm [22].

***Anticancer activity of gold nanobiocomposite of asparaginase***

*MCF-7 cell culturing*

The MCF-7 cells were cultured to carryout *in vitro* anti-cancerous studies. The cells were grown in folate-free Dulbecco Modified Eagles Medium (FFDMEM) containing 10% fetal bovine serum (FBS), 100 units/mL penicillin and100 µg/mL streptomycin and 4 mM L-glutamine, incubated at 37 °C in a 5%CO2/95% air humidified atmosphere.

*Cytotoxicity by MTT Assay*

The cytotoxicity of the prepared samples were measured using MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide, a yellow tetrazole) assay. The cells were grown in 96 wells tissue culture plates at a density of 0.2 X $10^6$ cells/well. After 24 h the old medium from each well was discarded and replaced with increasing concentration of gold nanobiocomposite of asparaginase. After 24 hr incubation, 10 µl of MTT solution was added at a final concentration of 0.5 mg/mL in phenol-red-free Roswell Park Memorial Institute (RPMI) medium containing 10% heat inactivated fetal bovine serum (FBS) in the dark at 37 °C. The cells were incubated for 4 h and the supernatant from each well were carefully removed without disturbing blue formazan crystals produced by the viable cells. The purple crystals were then dissolved completely using 100 µl of solubilising buffer (1:1dimethyl formamide/20% sodium dodecyl sulfate). Colorimetric measurements were performed at 595 nm with a scanning multi-well spectrometer (Thermo, USA). The untreated cells absorbance was used as control reference. The cell viability was calculated using the formulae given in equation 1.

% of cell viability = OD540 (sample)/OD540 (control) X 100 ……. (1)

where O.D. of the sample represents gold nanobiocomposite of asparaginase treated MCF-7 cells and O.D. of control refer to untreated MCF-7 cells [20,23].

**Results and discussion**

The synthesis of gold nanoparticles by fungus *Aspergillus terreus* filtrate was confirmed by change in colour of the culture filtrate from pale yellow to pale green solution after incubation. This colour change might be is due to the excitation of surface plasmon resonance, indicates the reduction of auric chloride ions by the proteins present in fungal culture filtrate which resulted in the formation of nanoparticle.

***UV spectrum analysis of gold nanoparticles and gold nanobiocomposite of asparaginase***

Optical properties of the gold nanoparticles were studied by UV spectrum analysis. Fig. 1 shows the absorption peaks obtained for gold nanoparticles and gold nanobiocomposite. An absorption peak at 540 nm in both the samples confirms the presence of gold nanoparticles in the green solution. Formation of absorption peak at 540 nm is due to the excitation of surface plasmon resonance. Peaks observed at 220 and 270 nm (Fig. 1) might be due to the presence of proteins/asparaginase along with the gold

 

nanoparticles which is of great importance in cancer treatment.

### *FT-IR spectrum analysis of gold nanobiocomposite of asparaginase*

The synthesized gold nanoparticles were subjected to FT-IR analysis to detect the various characteristic functional group associated. The peaks indicate the characteristics functional group present in the synthesized gold nanoparticles. It is inferred from Fig. 2 that the samples have absorption peaks in the range of 1645.56 $cm^{-1}$, 1537.61 $cm^{-1}$, 1380.16 $cm^{-1}$, 1011.26 $cm^{-1}$ and 474.43 $cm^{-1}$. The absorption peak at 474.43 $cm^{-1}$ may corresponds to vibrational mode of polysulfides or gold metal. The peak at 1011.26 $cm^{-1}$ is ascribed to cyclohexane and aliphatic fluoro compounds. The peak at 1380.16 $cm^{-1}$and 1537.61 $cm^{-1}$ are ascribed to gem-dimethyl or "iso", phenol or tertiary O-H group. The peak at 1645.56 $cm^{-1}$ is ascribed to the vibrational modes of secondary amine N-H bend.

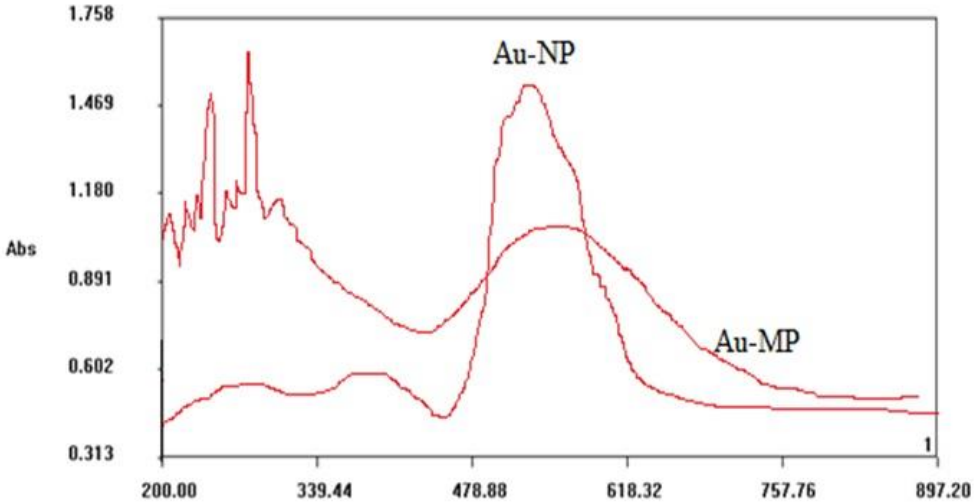


Fig. 1. UV Spectrum of gold nanoparticles (Au-NP) and gold nanobiocomposite of asparaginase (Au-MP)

The synthesized gold nanobiocomposite was subjected to FT-IR analysis to detect the various characteristic functional group associated. The peaks indicate the characteristics functional group present in the synthesized gold nanobiocomposite. It is inferred from Fig. 3 that the samples have absorption peaks in the range of 2923.61 $cm^{-1}$, 1629.78$cm^{-1}$, 1400.11 $cm^{-1}$, 1366.90 $cm^{-1}$, 1007.90 $cm^{-1}$and 568.61 $cm^{-1}$. The absorption peak at 568.61 $cm^{-1}$ may correspond to metal (gold stretching vibrations) vibrational mode. The peak at 1007.26 $cm^{-1}$ is ascribed to cyclohexane and aliphatic phosphate compounds. The peak at 1366.90 $cm^{-1}$and 1400.11 $cm^{-1}$ are ascribed to tri-methyl or tert-butyl group and phenol or tertiary alcohol O-H bend. The peak at 1629.78 cm-1 is ascribed to alkenyl C=C stretch, primary amine N-H bends, organic nitrates and open chain imino group. The peak at 2923.61 $cm^{-1}$ methylene C-H asym./sym. stretch. Thus different absorption peaks for nanoparticles and nanobiocomposite were observed in FT-IR analysis. Presence of different functional groups in gold nanobiocomposite might be due to proteins conjugated to the surface of gold nanoparticles.

### *XRD analysis of gold nanobiocomposite of asparaginase*

The X-ray diffraction patterns of gold nanoparticles can be observed in the Fig. 4. Sharper and stronger diffraction peaks were observed at 38.06°, 44.24° and 64.74°. These peaks were assigned to the planes 111, 200 and 220 of the cubic centered gold. This data matches with the data's in the database of joint committee on powder diffraction standards. The average crystallite size was calculated by the Debye Sherrer formula $D = K \lambda/\beta_{1/2}\cos\theta$ where K is the Sherrer constant (K=0.9 for spherical particle), $\lambda$ is the X-ray wavelength ($\lambda$=1.54060 Å), $\beta_{1/2}$is the full width of the XRD peak at half height; $\theta$ is the Bragg diffraction angle. Substituting these values D = 0.9(1.54060)/ (0.53)(0.96)=2.76 nm. The XRD pattern thus clearly shows that the gold nanoparticles formed by the reduction of Au+ ions by asparaginase enzyme present in the culture filtrate are crystalline in nature.

 

***Structural characterization of gold nanobiocomposite of asparaginase***

Scanning Electron Microscope was used to deduce the particle size and morphology of the synthesized gold nanobiocomposite. The localization of gold nanobiocomposite was done in SEM using a secondary electron detector. As the metal particles are good conductors, they are observed as such without any prior carbon coating at a magnification of 5000Xin a voltage of 8.0 kV. It can be clearly seen from the Fig. 5 that the gold nanobiocomposite in the samples were needle and round shaped. The size range of the needle shaped particles was in between 150-250 nm. The size range of spherical particles was in between 60-80 nm.

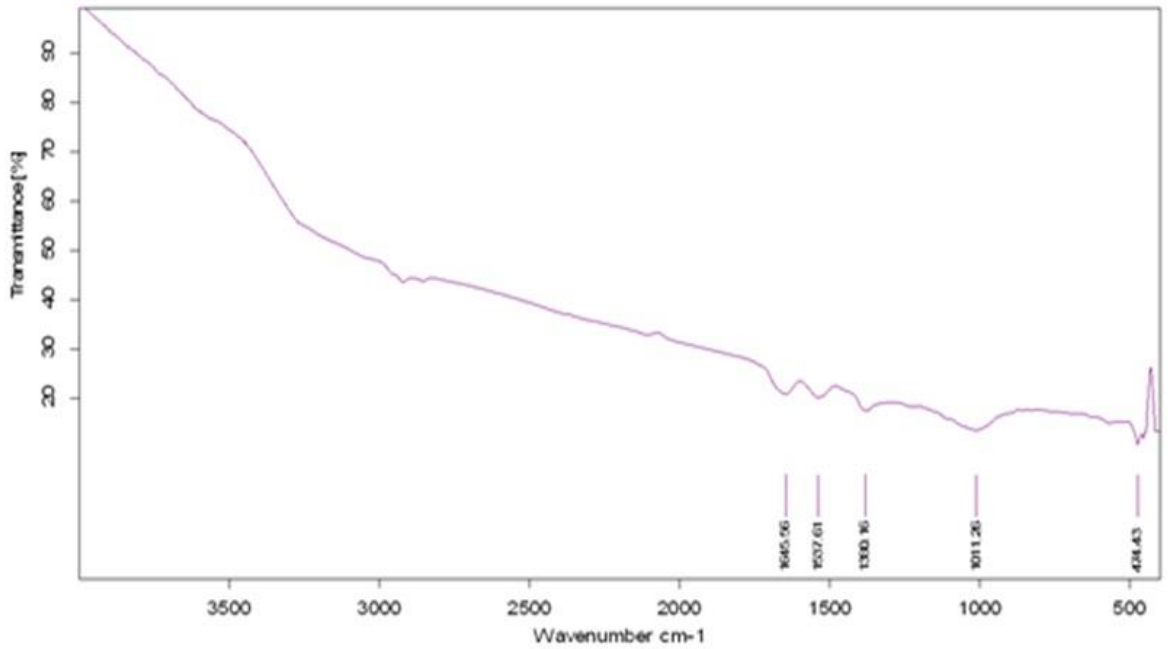


Fig. 2. The smoothed FT-IR spectrum of synthesized gold nanoparticles

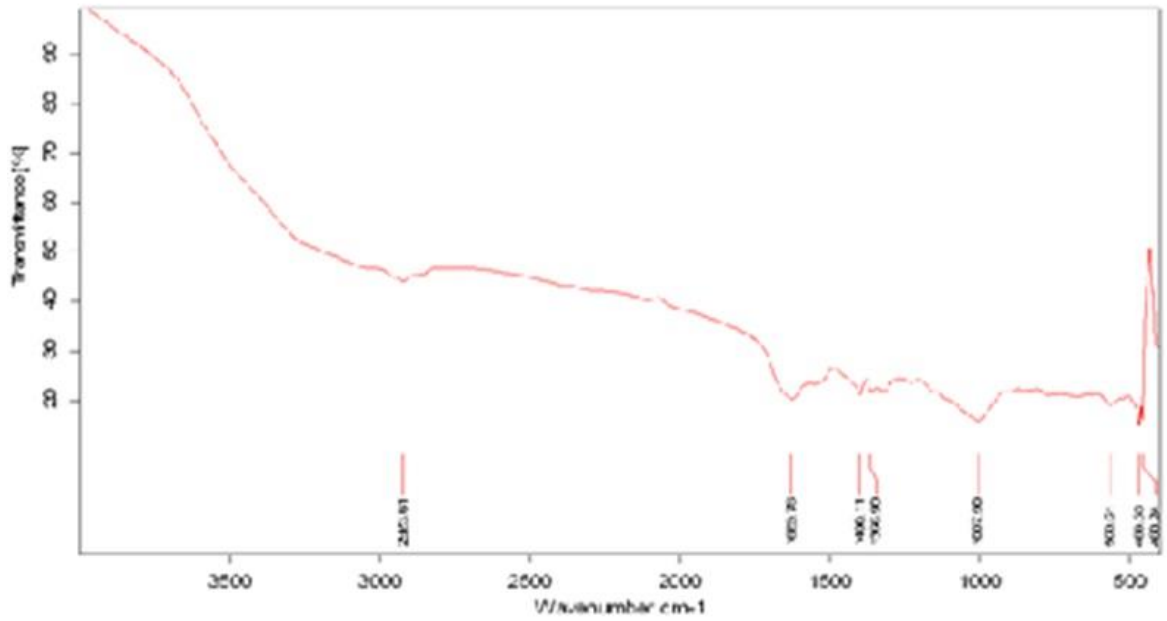


Fig. 3. The smoothed FT-IR spectrum of gold nanobiocomposite of asparaginase

***EDX analysis of gold nanobiocomposite of asparaginase***

The energy dispersive X-ray analysis gold nanobiocomposite was done to find the amount of gold nanoparticles present. EDX analysis of the gold nanobiocomposite has shown strong signals for gold atoms along with weak signals for oxygen and potassium (Fig. 6). These weak signals could have arisen from macromolecules like proteins/enzymes and residual salts used for the growth of fungal biomass. The image shows the presence of glittering spots which are the gold nanoparticles.

 

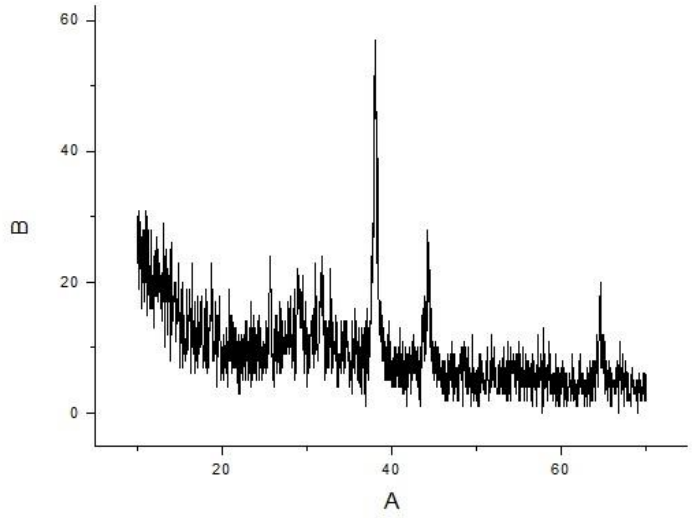


Fig. 4. XRD pattern of gold nanobiocomposite of asparaginase

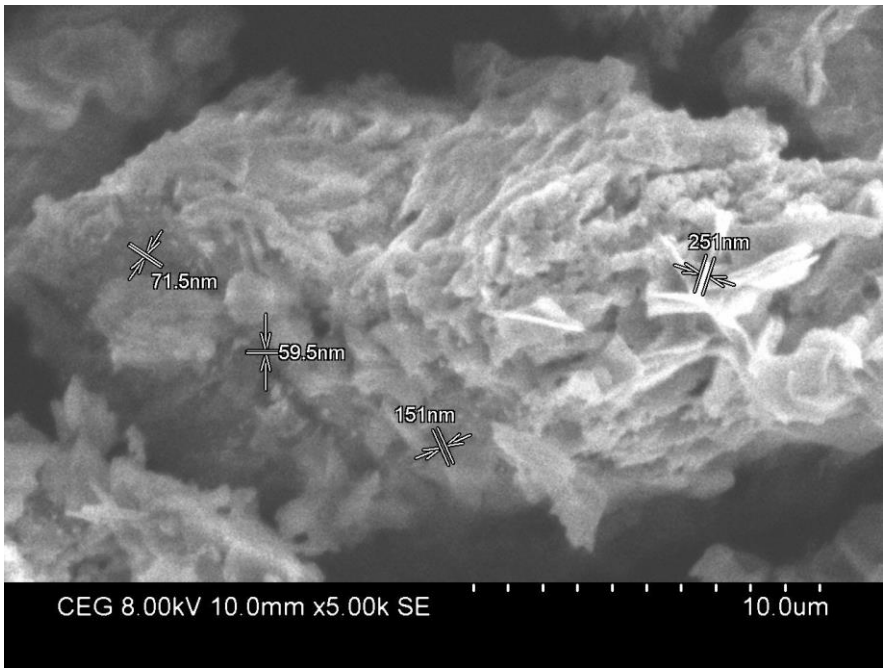


Fig. 5. SEM image of gold nanobiocomposite of asparaginase

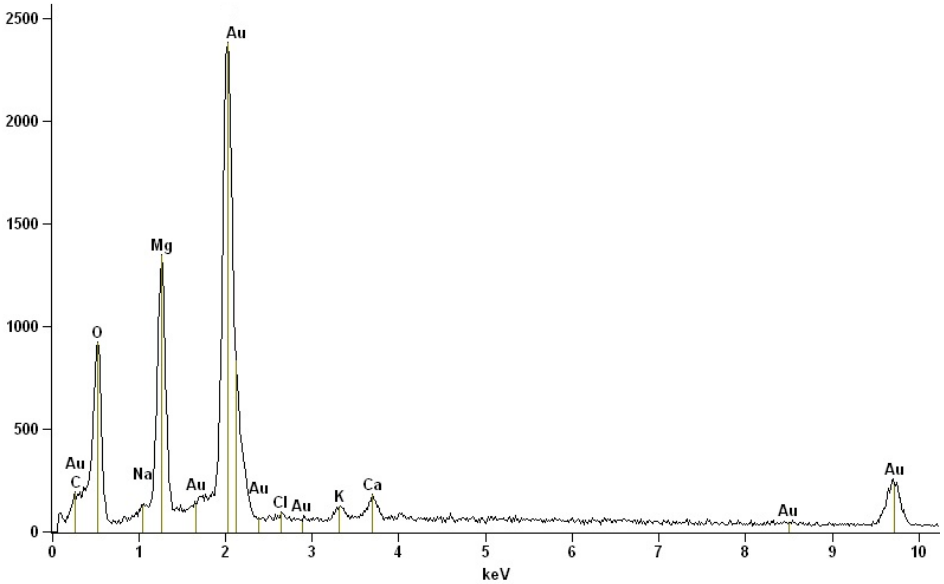


Fig. 6. EDX pattern of gold nanobiocomposite of asparaginase

 

### *Anticancer activity of gold nanobiocomposite of asparaginase*

After culturing MCF-7 cancer cells, the anticancer activity of gold nanobiocomposite of asparaginase was studied through MTT assay. MCF-7 cells were grown in 96 wells tissue culture plates. The cell viability based on the optical value from the incubated control well was calculated. The viability of MCF-7 cancer cells was found decreased with increase in concentration of gold nanobiocomposite of asparaginase. The MCF-7 cells treated with crude asparaginase enzyme reduced the cell viability to 33%. Cell viability of MCF-7 cancer cells treated with gold nanobiocomposite of asparaginase was decreased to 36.74% (Fig. 7). Thus the synthesized gold nanobiocomposite of asparaginase proved to be potential drug for anticancer applications.

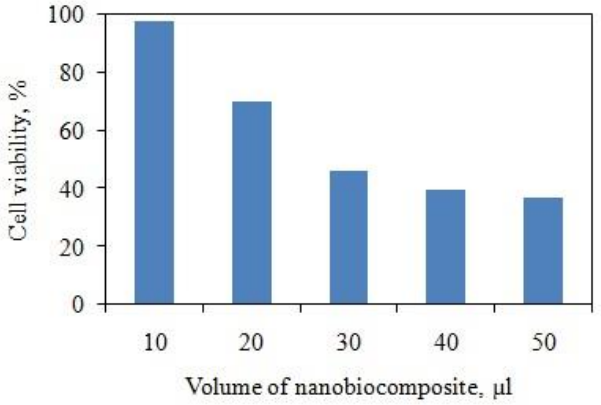


Fig. 7. Anticancer activity of gold nanobiocomposite of asparaginase

## Conclusions

*Aspergillus terreus* was used to produce bi-functional gold nanobiocomposite of asparaginase which can be used for diagnosis and treatment cancer. The formation of gold nanobiocomposite of asparaginase was confirmed by absorption peak at 540 nm in UV-Visible spectrometer. The crystalline nature of synthesized gold nanobiocomposite was confirmed using XRD analysis. Both spherical shaped needle shaped gold nanobiocomposites were observed in SEM analysis with the size ranges between 150-250 nm and 60-80 nm respectively. Decrease in the cell viability of MCF-7 cell lines on treatment with gold nanobiocomposite of asparaginase proved its anticancer activity. The anticancer activity can be further augmented when purified asparaginase is directly conjugated to gold nanoparticles.

## Conflict of interest

Authors declare there are no conflicts of interest.

## References

[1] Kaittanis C, Shaffer TM, Daniel LJ, Thorek DLJ, Grimm J. Dawn of Advanced Molecular Medicine: Nanotechnological Advancements in Cancer Imaging and Therapy. *Crit Rev Oncog.* 2014;19:143-176.

[2] Astruc D. Introduction to Nanomedicine. *Molecules*. 2016;21(1):4.

[3] Boisselier E, Astruc D. Gold nanoparticles in nanomedicine: preparation, imaging, diagnostics, therapies and toxicity. *Chem Soc Rev.* 2009;38:1759-1782.

[4] Llevot A, Astruc D. Application of gold nanoparticles to the diagnostic and therapy of cancer. *Chem Soc Rev*. 2012;41:242-257.

[5] Li X, Xu H, Chen ZS, Chen G. Biosynthesis of nanoparticles by microorganisms and their applications. *Journal of Nanomaterials*. 2011:ID 270974.

[6] Patra JM, Panda SS, Dhal NK. A review on green synthesis of gold nanoparticles. *Int J Pharm Bio Sci.* 62015;6:251-261.

[7] Husseiny IM, El-Aziz AM, Badr Y, Mahmoud AM. Biosynthesis of gold nanoparticles using *Pseudomonas aeruginosa*. *Spectrochimica Acta Part A.* 2007;67:1003-1006.

[8] Radhika Rajasree SR, Suman TY. Extracellular biosynthesis of gold nanoparticles using a gram negative bacterium *Pseudomonas fluorescens*. *Asian Pacific Journal of Tropical Disease*. 2012;S:795-799.

[9] Mishra A, Tripathy SK, Wahab R, Jeong SH, Hwang I, Yang YB, Kim YS, Shin HS, Yun S. Microbial synthesis of gold nanoparticles using the fungus *Penicillium brevicompactum* and their cytotoxic effects against mouse Mayo blast cancer $C_2C_{12}$ cells. *Appl Microbial Biotechnol.* 2011;92:617-630.

[10] Sheikhloo Z, Salouti M, Katiraee F. Biological Synthesis of Gold Nanoparticles by Fungus *Epicoccum nigrum*. *J Clust Sci*. 2011;22:661-665.

[11] Anitha TS, Palanivelu P. Synthesis and Structural Characterization of Polydisperse

 

Silver and Multishaped Gold Nanoparticles Using *Fusarium Oxysporum* MTCC 284" *Digest Journal of Nanomaterials and Biostructures.* 2011;6(4):1587-1595

[12] Narayanan KB, Sakthivel N. Facile green synthesis of gold nanostructures by NADPH-dependent enzyme from the extract of *Sclerotium rolfsii*. *Spectrochimica Acta Part A.* 2011;380:156–161.

[13] Inbakandana D, Venkatesan R, Ajmal Khan S. Biosynthesis of gold nanoparticles utilizing marine sponge *Acanthella elongate*. *Colloids and Surfaces B: Biointerfaces*. 2010;81:634-639

[14] Vijayakumar R, Devi V, Adavallan K, Saranya D. Green synthesis and characterization of gold nanoparticles using extract of anti-tumor potent *Crocus sativus*. *Physica E.* 2011;44:665-671.

[15] Aswathy Aromal S, Vidhu VK, Philip D. Green synthesis of well-dispersed gold nanoparticles using *Macrotyloma uniflorum*. *Spectrochimica Acta Part A.* 2012;85:99-104.

[16] Broome JD. L-Asparaginase: Discovery and development as a tumor-inhibitory agent. *Cancer treatment reports*. 1981;65(S4):111–114.

[17] MIM Sarquis, Oliveira EMM, Santos AS, da Costa GL. Production of L-asparaginase by filamentous fungi. *Mem Inst Oswaldo Cruz*. 2004; 99:489-492

[18] Pal T, Pal A, Panigrahi S. Nanotechnology in Biology and Medicine: Methods, Devices, and Applications (Ed. Tuan Vo-Dinh), CRC Press; Boca Raton, FL: 2006.

[19] Pathak P, Katiyar VK. Multi-Functional Nanoparticles and Their Role in Cancer Drug Delivery – A Review, *AZojomo (Journal of Material online)*. 2007:DOI: 10.2240/azojono0114.

[20] Baskar G, Chandhuru J, Fahad KS, Praveen AS, Chamundeeswari M. Anticancer activity of fungal L-asparaginase conjugated with zinc oxide nanoparticles. *Journal of Materials Science: Materials in Medicine*. 2015;26:43.doi:10.1007/s10856-015-5380-z.

[21] Baskar G, Renganathan S. Production of L-asparaginase from natural substrates by *Aspergillus terreus* MTCC 1782: Optimization of carbon source and operating conditions. *Int. J. of Chem. Reactor Engineering*. 2011;9(1): DOI: 10.1515/1542-6580.2479.

[22] Wriston JC Jr, Yellin TO. L-asparaginase: a review. *Adv Enzymol Relat Areas Mol Biol*. 1973;39:185-248.

[23] Mossman T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J Immunol Methods.* 1983;65:55-63.

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