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Comparative Evaluation Of Antibacterial, Antifungal And Antioxidant Activity Of Silver Nanoparticles Synthesized Using Cassia Tora L Leaf Extract And Glucose

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Abstract. **In this study, we compared the antibacterial, antifungal and antioxidant efficacy of silver nanoparticles (AgNPs) synthesised using Cassia tora L leaf extract (biological method) and Glucose (chemical method). The synthesized nanoparticles were characterised using various spectroscopic methods such as UV–visible absorption spectrophotometry (UV–vis), Dynamic Laser-Light Scattering Study (DLS), Energy Dispersive Spectroscopy and Transmission electron microscopy (TEM). The Biologically synthesized silver nanoparticles (EAgNPs) showed plasmon resonance band at 420 nm and has size particle sizes of 19–22 nm. The chemically synthesized silver nanoparticles (GAgNPs) showed a plasmon resonance band at 425 nm with particle sizes of 33–42 nm. The comparative antibacterial, antifungal, and antioxidant studies of biological synthesised and chemical AgNPs were evaluated using disk diffusion assay, poison food assay, and DPPH assay respectively. The biological synthesised AgNPs showed superior antibacterial, antifungal and antioxidant potential in comparison to chemically synthesised AgNPs.**

I. INTRODUCTION

In recent decade metallic nanoparticles due to their unique catalytic, optical, magnetic and electrical properties have found wide range of applications in the fields of catalysis, photonics, optoelectronics, pharmaceutical, biological tagging, environmental pollution control, drug delivery systems [1-5]. Among various metal-based nanoparticles, silver nanoparticles (AgNPs) due their antibacterial and antifungal potential has found wide application in wide range of products such as clothing, catheters, electric home appliances, and biomedical implants [6-7]. The silver nanoparticles are also gaining importance in agricultural sciences due to their ability to control many phytopathogenic fungi which become resistant to chemical fungicides [8].

Generally, metal nanoparticles are synthesised using physical and chemical methodology. The physical method is an energy intensive method and yield are the low quantity of nanoparticles, while chemical method uses harsh conditions, toxic chemicals and solvents for the synthesis of nanoparticles which are also not environment-friendly [9-12]. Therefore, there is urgent need to develop a simple, rapid, cost-effective and environment-friendly method for synthesis of nanoparticles. Recently biological method using microorganism and plant extract has emerged as a viable alternative to chemical and physical method [13-18].

In this present work, we have demonstrated synthesis of AgNPs by reduction of aqueous silver nitrate (AgNO3) using methanolic Cassia tora L leaf extract. The synthesised nanoparticles were characterized using various spectroscopic methods such as UV–visible absorption spectrophotometry (UV–vis), Dynamic Laser-Light Scattering Study, Energy Dispersive Spectroscopy and Transmission electron microscopy (TEM). The antibacterial, and antifungal were determined using different pathogens. Further, we also determined antioxidant potential of synthesised nanoparticle using DPPHassay. We further compared the antibacterial, antifungal and antioxidant potential of biological and chemical synthesised silver nanoparticles.

II. MATERIALS AND METHODS

Materials

All the reagents used were of analytical grade and obtained from Sigma-Aldrich and used without any further purifications. Silver nitrate was obtained from Qualigens Fine Chemicals,Mumbai, India.The Bacterial culture E.coli (MTCC 443), Staphylococcus aureus (MTCC 3160), Pseudomonas aeruginosa (MTCC 2581) and Klebsiella pneumoniae (MTCC 7028) were procured from the microbial type culture collection and gene bank, Institute of Microbial Technology, Chandigarh. The fungal culture Fungal culture of R. solani (ITCC 4502), S. Rolfsii (ITCC 6263), Fusarium oxysporum and M. phaseolina (ITCC 6267) and R. solani were obtained from the Indian type culture collection, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute (IARI), New Delhi.

Preparation of Cassia tora L leaf extract

The dry leafs of Cassia tora L were purchased locally. The leaf powder (200 gm) was Soxhlet extracted with methanol for 8 hours. The extraction was repeated thrice. Then the extracted crudes were combined and concentrated under reduced pressure. The concentrated crude extract was weighed and stored at 0°C till further study.

Synthesis of silver nanoparticles

Biological synthesis of silver nanoparticles

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To synthesise silver nanoparticle 25 ml of 10% crude extract in methanol was added to 50 mL of 1 mM AgNO3 and mixed on a magnetic stirrer. The reaction mixture was stirred for 1h at room temperature to ensure thorough mixing. A control setup was also maintained without crude extract. The change in reaction mixture color to yellow after 1h of stirring confirmed the formation of silver nanoparticles. No color change was observed in control setup indicating that plant extract is working as reducing agent. The synthesized AgNPs were separated from the reaction mixture by centrifuging them at 10,000 rpm for 15 min and washed for three times with deionized water. The free flowing powder of the silver nanoparticles was obtained by freeze drying.

Chemical synthesis of silver nanoparticles using glucose as reducing agent

To synthesise silver nanoparticle we added 5ml of 0.1M glucose solution to 45 ml 1 mM AgNO3 and reaction mix was allowed to stir at 90 oC for 15 min. The change in reaction mixture color to yellow confirmed the formation of silver nanoparticles. The synthesized AgNPs were separated from the reaction mixture by centrifuging them at 10,000 rpm for 15 min and washed for three times with deionized water. The free flowing powder of the silver nanoparticles was obtained by freeze drying.

Characterization of silver nanoparticle

The synthesised AgNPs were characterized using different spectroscopy techniques. The nanoparticles obtained were scanned after the color change using a UV–vis spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) within a working wavelength range of 300 to 700 nm using a dual beam operated at 1 nm resolution. The High-resolution transmission electron TEM measurements were performed with a Philips Technai G230 transmission electron microscope was then used to examine the surface morphologies and sizes of the biologically synthesized AgNPs and chemically synthesised AgNPs. The size distribution of the dispersed particles was measured using a Zetasizer Nano ZS90 (Malvern 153 Instruments Ltd., UK).

In vitro antibacterial activity:

The antimicrobial potential of synthesized metal complexes was tested using a disk diffusion assay. Briefly, the nutrient agar medium (25 mL) was poured into Petri dishes (90 cm in diameter) under aseptic conditions in a laminar flow hood. The plates were kept in the laminar flow chamber for solidification of the media. After solidification 100 µL of fresh culture (log phase) was spread on the surface of the solidified medium with the help of a spreader. The plates were then kept in laminar flow for drying. Once dried five plain sterile disks were placed on the plate and 5 µL of test solution of different concentration (200-12.5 ppm) was loaded on each disk. In control plate commercially procured ampicillin (10 µg/disk) was used. Plates were then kept at 37 ºC. After 24 hours plates were taken out from the incubator and zone of inhibition (in mm) was recorded for all the compounds tested and commercial antibiotic. All experiments were in triplicate for each treatment against each bacteria.

In vitro antifungal activity:

The antifungal assay was carried out by a poisoned food technique using potato-dextrose-agar (4% PDA) medium against four phytopathogenic fungi Rhizoctonia solani, Sclerotium rolfsii, fusarium oxysporum and Macrophamina phaseolina at different concentrations (200-12.5ppm). 1 mL of stock solution was added to 50 mL of PDA medium in to obtain desired concentrations. The medium was then poured into two Petri plates under aseptic conditions in a laminar flow chamber. Similarly, other concentrations were prepared by serial dilution process. 1 mL of methanol was used as a control.

A 5 mm thick disc of fungus was put at the center of the medium in the test Petri plate and the plates were kept in BOD incubator at 28±1°C till the fungal growth in the control dishes was completed (6-10 days). The mycelial growth (cm) in both treated (T) and control (C) Petri-plates use measured diametrically in three different directions. From the mean growth of above readings, percentage inhibition of growth (I) was calculated by using the following equation:

$$\text{Percent growth inhibition I (\%)} = [(T - C)/ C] \times 100$$

In vitro antioxidant activity:

The in vitro antioxidant activity was determined using DPPH assay.The working solutions of test extracts and standards were prepared in methanol. Quercetin and Gallic acid solution were used as standard and DPPH solution (0.1mM, 1 ml) as blank. Different concentrations (200-6.25 µg/mL) of test compounds were pipetted into the test tubes and volume adjusted to 3 mL with methanol. 1 ml of DPPH (0.1mM) solution was mixed with 1 ml of sample and standard solution separately. The samples were vortexed, incubated in dark at room temperature for 30 min. and the absorbance measured at 517 nm against blank samples in a spectrophotometer. The absorbance was recorded and radical scavenging activity was expressed as percentage inhibition of DPPH radical and was calculated by the following equation:

% Inhibition = (Absorbance of control - Absorbance of sample

/Absorbance of control) x 100

I. RESULTS AND DISCUSSION

Characterization of Biological synthesised Silver nanoparticle

The methanolic leaf extract Cassia tora L is rich in various plant secondary metabolites such as a flavonoid, anthraquinones, and alkaloids which can act as reducing and capping agent for synthesis of nanoparticles. Batra and coworkers have reported the significant presence of emodin and Quercetin in leaf extract of Cassia tora L [19]. Based on this observation proposed prospective mechanism reduction of Ag (II) to Ag (0) for the synthesis of silver nanoparticle is shown in scheme 1.

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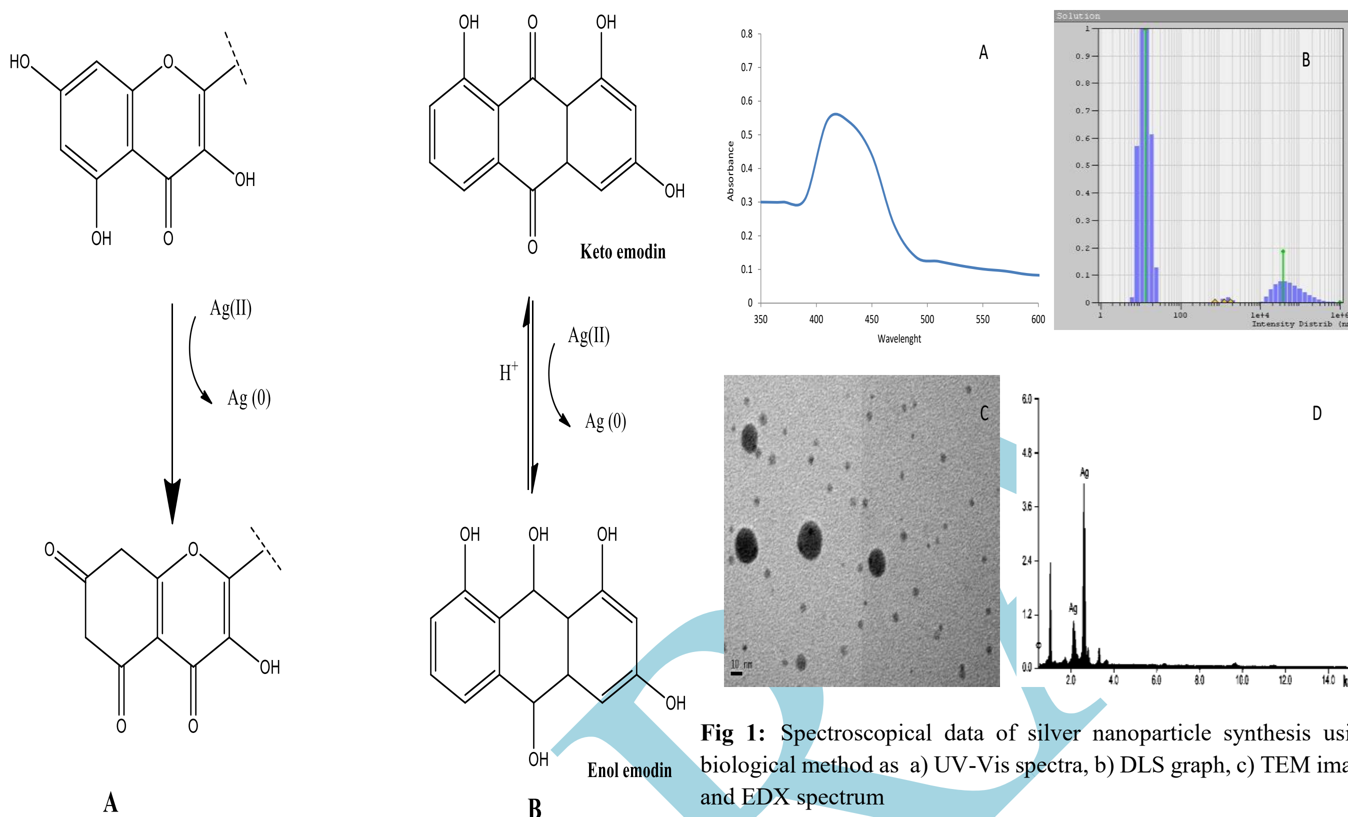

Fig 1. Scheme 1: The prospective mechanism for the synthesis of the silver nanoparticle using Cassia tora L leaf extract A) Quercetin and B) Emodin.

Fig 1: Spectroscopical data of silver nanoparticle synthesis using biological method as a) UV-Vis spectra, b) DLS graph, c) TEM image and EDX spectrum

Due to surface plasmon resonance (SPR) phenomenon exhibited by metallic nanoparticles UV–vis spectroscopy is used as a first technique in the characterization of metallic nanoparticles. The biologically synthesised nanoparticles showed the strong absorption peak centering at 420 nm (fig 1a) due to its surface plasmon which is a characteristic band for silver nanoparticles and in accordance with a previous report [20].

The DLS size distribution histogram (fig 1b) shows that the size of biological synthesis silver nanoparticles is 22 nm. We also observe some distribution at the lower range of particle size indicates that the synthesized particles are polydisperse in nature not monodisperse.

The surface morphology of synthesised particles was observed using TEM microscopy. The TEM micrograph (fig 1c) shows synthesise particles are circular in nature with smooth edges. The TEM micrograph also showed particles are well dispersed with considerable stability.

The Energy Dispersive X-ray Spectroscopy (EDAX) analysis of the silver nanoparticles was conducted on a Technai G230 TEM instrument as it is equipped with high energy electron beam which could provide the dispersive pattern of a characteristic atom in the form of its elemental composition. The EDAX analysis (fig 1d) showed a characteristic peak of elemental silver with a weight percentage of about 48.9%. In addition, other peaks such as Cu, C, and O which could be mainly attributed to the presence of carbon coated copper grids were also observed.

Characterization of chemical synthesised Silver nanoparticle

In the case of chemical synthesised silver nanoparticles, we observed strong absorption peak centered at 425 nm in UV-Vis spectra (fig 2a) indicating towards the successful formation of silver nanoparticles. The single peak in UV-Vis spectra indicates synthesised particles are of uniform shape and size which is in accordance with a previous report [21]. The TEM spectroscopy was used to observe surface morphology of synthesised particles. The DLS histogram (fig 2b) shows that the average size of chemically synthesised silver nanoparticles is larger than biologically synthesised particles. The average size of chemically synthesis nanoparticle was 33 nm. Also, we observe that some distribution at the higher range of particle size indicates that the synthesized particles are polydisperse in nature not monodisperse. The TEM micrograph (fig 2c) showed

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that chemically synthesised particles are different from biologically synthesised particles, they spherical in nature with smooth edges in comparison to circular shaped particles synthesised biologically.

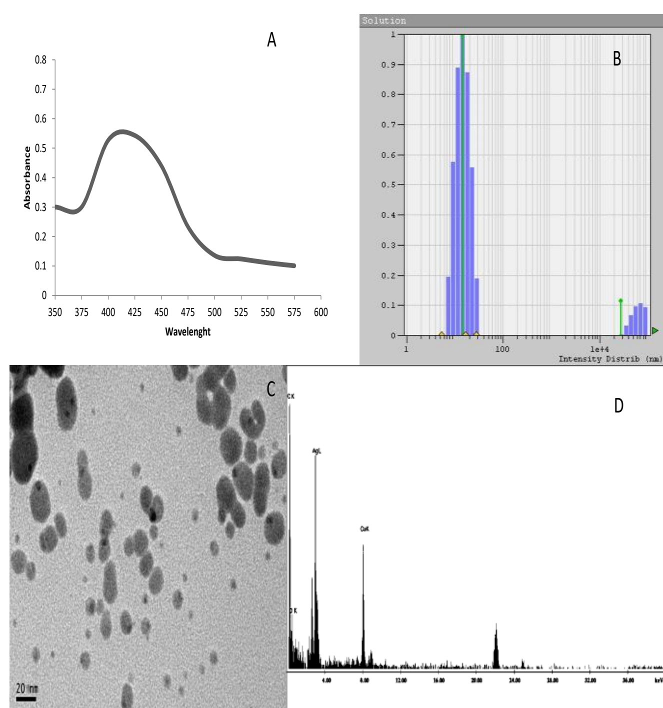

Fig 2. spectroscopical data of silver nanoparticle synthesis using glucose as reducing agent a) UV-Vis spectra, b) DLS graph, c) TEM image and EDX spectrum.

The TEM micrograph also shows that chemical synthesised particles were aggregated in comparison to well disperse particle in the case of biologically synthesise particles. This aggregation of nanoparticles can be attributed to lack of capping agent in chemical synthesised particles, while in the case of biologically synthesise particle reducing agent act as capping agent which prevent aggregation. The EDAX analysis (fig 2d) of chemical synthesis showed a characteristic peak of elemental silver with a weight percentage of about 46.7%. In addition, other peaks such as Cu, C, and O which could be mainly attributed to the presence of carbon coated copper grids were also observed.

3.2 Antibacterial studies

The antibacterial activity of biologically and chemically synthesised AgNPs (fig 3) were tested against Gram-negative bacteria E. coli, P.aeruginosa, K.pneumoniae and Gram-positive bacteria S. aureus. The silver in bulk form is used as an antibacterial agent for a long time [22]. Generally, silver is used in its nitrate form for preparing antibacterial formulations, but due to the large size of bulk silver molecules, the effectiveness of formulation are limited. In contrast to this silver nanoparticles show better antibacterial potential due to their small size and high surface area. The exact mechanism how silver nanoparticle kill bacteria is not fully understood but it is generally accepted that silver nanoparticles penetrate the cells causing intracellular loss leading to cell death and this mechanism depends on the concentration of the AgNPs [23].

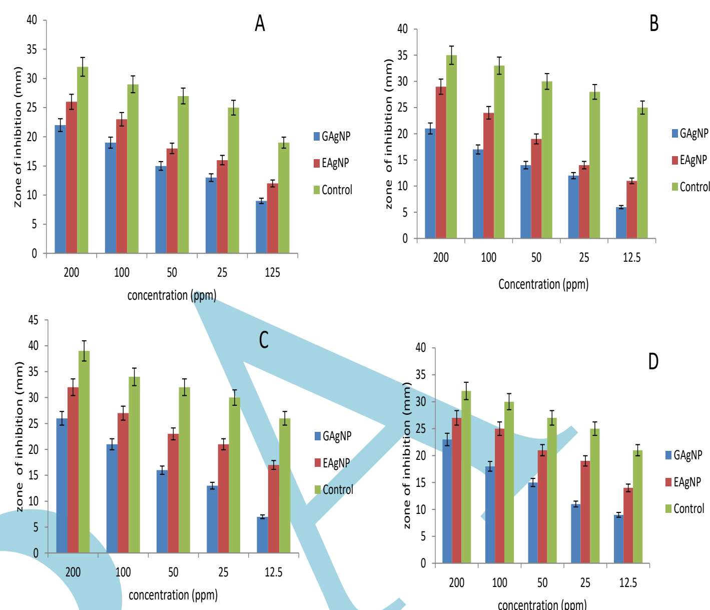

Fig 3: Antibacterial activity of chemically and biologically synthesized AgNPs against A) E. coli, B) S. aureus, C) P.aeruginosa, and D) K.pneumoniae.

The AgNPs are more effective in controlling Gram-negative bacteria in comparison to Gram-positive [24]. This difference in activity is due to the difference in the membrane of gram positive and gram negative bacteria. The gram-positive bacteria membrane consist rigid peptidoglycan layer which is more stable and did not allow negatively charged AgNPs sufficient binding site to enter the cell. While AgNPs can bind to Gram-negative cell wall better and thus show more efficiency in controlling them [25]. In our study, we observed that both chemically and biologically synthesised particles were more effective against gram negative bacteria than gram positive. Also, we observed the biologically synthesised nanoparticle show enhanced antibacterial potential in comparison to the chemically synthesised nanoparticles. This enhancement can be attributed to the smaller size of nanoparticles synthesised using the biological method. These smaller particles have a larger surface area for interaction and will have a stronger bactericidal effect than larger particles synthesised using the chemical method.

3.3 Antifungal Studies

The antifungal potential of biological and chemical synthesised nanoparticles was tested against four phytopathogenic fungus S.rolfsii, R.solani, F.oxysporum, and A.niger. The exact mechanism mode action of silver nanoparticles is still under investigation but it is widely accepted that silver nanoparticle attached to fungal membrane and penetrate the cell and then either it attaches to respiratory enzymes and blocks them leading to cell death or after entering the cell silver

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nanoparticles releases silver ions which disrupt DNA replication pathways and lead to cell death [26]. Both chemically and biologically synthesised silver nanoparticle shows moderate to good antifungal activity.

The biologically synthesised silver nanoparticle show enhanced antifungal activity in comparison to chemical synthesised silver nanoparticles. This superior antifungal activity of biologically synthesised nanoparticle is attributed to their lower size which enhances their

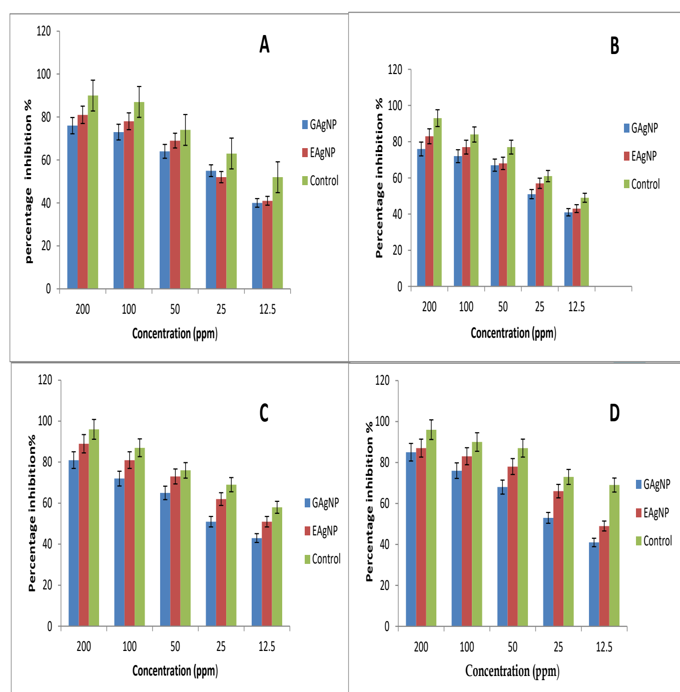

Fig 4. Antifungal activity of chemically and biologically synthesized AgNPs against A) S.rolfsii, B) R.solani, C) F.oxysporum and D) A.niger

penetration to fungal cells and leads to enhanced antifungal activity. The biologically synthesised silver nanoparticle was most active against F.oxysporum and least active against S.rolfsii, while chemically synthesised silver nanoparticles were most active against S.rolfsii and least against A.niger. In both the cases, we observed dose-dependent activity, the antifungal potential decreases with because in the concentration of silver nanoparticles.

3.4 DPPH free Radical Cavenging assay

The result of DPPH assay is by chemical and biological synthesise silver nanoparticles were shown in fig 5. The DPPH was a stable radical compound and accepts hydrogen or electrons from silver nanoparticles [27].The DPPH activity of the both biological and chemical synthesised AgNPs showed a dose-dependent trend. The antioxidant activity increases with increase in silver nanoparticle concentration in both cases. We observed that T biological synthesised silver nanoparticle show better antioxidant activity than the chemical synthesised silver nanoparticles. This enhancement in antioxidant potential of biological synthesised AgNPs could be attributed to functional groups adhered to them which were originated from the leaf extract. In comparison to biological synthesis AgNPs, the chemical synthesised AgNPs show poor antioxidant activity with 60% inhibition at 100 ppm. Both biological and chemical synthesis nanoparticle show lower antioxidant activity in comparison to standard compounds.

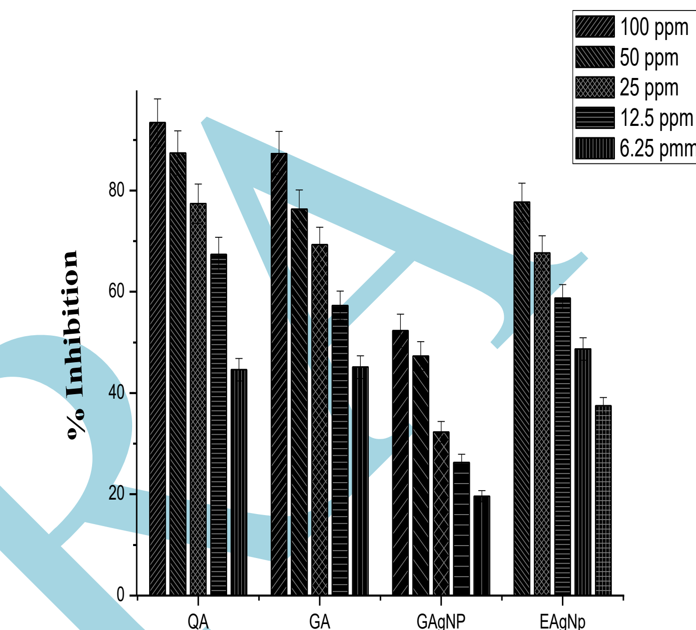

Fig. 5. Antioxidant activity of biological and chemical synthesised AgNPs at various concentrations.

II. CONCLUSION

We have synthesis silver nanoparticle using leaf extract of Cassia tora L and its antifungal, antibacterial and antioxidant potential was evaluated and compared with chemically synthesised silver nanoparticles. We observe that biologically synthesised AgNPs displayed superior antibacterial,antifungal and antioxidant activity compared to Chemically AgNPs. This work shows that biological method can replace chemical method as a cost-effective, green and rapid method for synthesis of silver nanoparticles. In addition, this work showed that biologically synthesised nanoparticles possess superior biological activity in comparison to chemical synthesised nanoparticles. This work opens the future possibility to develop biological synthesis silver nanoparticle for therapeutic purpose.

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References

[1]. S. Ahmed, M. Ahmad, S. Ikram. J. Appl. Chem., (2014), 3(2), 493.
[2]. S. Ahmed, M. Ahmad, S. Ikram. J. Adv. Res. (2015). http://dx.doi.org/10.1016/j.jare.2015.02.007.
[3]. S. Ashokkumar, S Ravi, S. Velmurugan. Spectrochim. Acta Mol. Biomol. Spectrosc, (2013), 115, 388.
[4]. P. Banerjee, M. Satapathy, A. Mukhopahayay, P. Das, Bioresources and Bioprocessing, (2014), 1(3), 1.
[5]. M. R. Bindhu, M. Umadevi. Spectrochim. Acta Mol. Biomol. Spectrosc, (2015),135, 373.
[6]. L. S. Devi, S. R. Joshi. J. Micro. Ultra., (2015) 3, 29.
[7]. G. A. El-Chaghaby, A. F. Ahmad. Orien. J. Chem., (2011), 27, 929.
[8]. C. Krishnaraj, E.G. Jagan, S. Rajasekar, P. Selvakumar, P.T. Kalaichelvan, N. Mohan, Colloids Surf. B. (2010), 76, 50.
[9]. P. Gopinath, S.K. Gogoi, P. Sanpui, A. Paul, A. Chattopadhyay, S.S. Ghosh, Colloids Surf. B, (2010),77240.
[10]. M.R. Das, R.K. Sarma, R. Saikia, V.S. Kale, M.V. Shelke, P. Sengupta, Colloids Surf.B. (2011), 83, 16.
[11]. A.J. Kora, R.B. Sashidhar, J. Arunachalam, Carbohydr. Polym. (2010), 82, 670.
[12]. S. Pal, Y.K. Tak, J.M. Song, Appl. Environ. Microbiol. (2007), 73,1712.
[13]. A.R. Shahverdi, A. Fakhimi, H.R. Shahverdi, S. Minaian, Nanomed. Nanotechnol.Biol. Med. (2007), 3, 168–171.
[14]. G.A. Martínez-Casta˜nón, N. Ni˜no-Martínez, F. Martínez-Gutierrez, J.R.Martínez-Mendoza, F. Ruiz, J. Nanopart. Res. (2008), 10 1343–1348.
[15]. M. Chandrasekaran, K. Kannathasan, V. Venkatesalu, K. Prabhakar, World J. Microbiol. Biotechnol. (2009), 25, 155.
[16]. D.G. Yu, Colloids Surf. B. (2007), 59, 171–178.
[17]. J.S. Kim, E. Kuk, K.N. Yu, J.H. Kim, S.J. Park, H.J. Lee, S.H. Kim, Y.K. Park, Y.H. Park,C. Hwang, Y. Kim, Y. Lee, D.H. Jeong, M. Cho, Nanomed. Nanotechnol. Biol. Med. (2007), 3, 95.
[18]. S.S. Shankar, A. Rai, B. Ankamwar, A. Singh, A. Ahmad, M. Sastry, Nat. Mater. (2004), 3, 482.
[19]. Durgesh Nandani, Roop Narayan Verma and Amla Batra. Ann. Phytomed. (2013), 2(2), 96,
[20]. K. Shameli, M.B. Ahmad, P. Shabanzadeh, E.A.J. Al-Mulla, A. Zamanian, Y. Abdullahi,S.D. Jazayeri, M. Eili, F. Azizi Jalilian, R.Z. Haroun, Res. Chem. Intermed. (2013) 40, 1313.
[21]. Ajeet Kumar, Swati Aerry, Dan V. Goia. J. Colloid Interface Sc, (2016), 470, 196.
[22]. J.S. Kim, E. Kuk, K.N. Yu, J.H. Kim, S.J. Park, H.J. Lee. Nanomedicine, (2007), 3, 95.
[23]. S. Gurunathan, J. W. Han, D. Kwon, J.Kim. Nanoscale Res Lett. (2014), 9(1),373.
[24]. W. K. Jung, H. C. Koo, K. W. Kim, S. Shin, S. H. Kim and Y. H. Park Appl. Environ. Microbiol. (2008), 74,2171.
[25]. B.Ramalingam, T. Parandhaman, S.K. Das. ACS Appl. Mater. Interfaces, (2016), 8 (7), 4963.
[26]. K. B. Narayanan, H. H. Park. Eur. J. Plant Path. (2014), 140, 185.
[27]. S. Bhakya, S. Muthukrishnan, M. Sukumaran, M. Muthukumar.Applied Nanoscience, (2016), 6, 755.

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