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# Biogenic nanoparticles from excretory pellets of silkworm Bombyx mori .L and its bioactive properties

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***Abstract:*** **- Increasing need for the strategies to encounter the agrowaste management is a current challenge, as there is more number of agro based cottage industry blooming to meet out the need of the rural economy. Silkworm excrement from the Lepidopteran larvae *Bombyx mori* is one of the organic biowastes. As novel study silkworm excreta was used to synthesize silver and gold nanoparticles and explored the efficiency of this organic waste in the nanotechnology. Synthesized silver nanoparticles showed maximum absorbance at 418 nm and gold nanoparticles at 555 nm scale. FTIR showed peaks at 34564.49cm$^{-1}$ &1626.93cm$^{-1}$ for silver nanoparticles and 3468.85cm$^{-1}$ & 1636.04cm$^{-1}$ for gold nanoparticles. Scanning electron microscopy revealed the average size of biosynthesized silver and gold nanoparticles were about 42 nm& 45 nm respectively. EDAX value of biosynthesised silver and gold nanoparticles showed 33.08% and 14.77% respectively. XRD show diffraction pattern of about 5 peaks for both silver and gold nanoparticles. Antimicrobial activity was assessed using the resazurin assay method. MIC values obtained for the tested organisms revealed antimicrobial activities.*K. pneumoniae*, showed MIC at 62.5 µg for silver nanoparticles and *A. niger, A.flavus and C.albicans* showed MIC at 125 µg for gold nanoparticles. IC $_{50}$ values of α- amylase activity was found to be 574 and 1075 µg/ml for the silver and gold nanoparticles, respectively. IC$_{50}$ values are about 3521 and 415.97 µg/ml for the silver and gold nanoparticles, respectively, in α-glucosidase activity. Conclusively silver and gold nanoparticles synthesized from the silkworm excreta revealed antibacterial, antifungal and antidiabetic activities**

**Keywords: Nanotechnology Nanoparticles, MIC, Antidiabetic properties**

## INTRODUCTION

Sericulture is a rural agro based industry which was in vogue from second century BC which depends on farm and non –farm activities. It pays crucial part in socio economic development of the country through silk production. The waste generated from the silk industry in tons due to the established silk-based industrialization around the world, huge amount of silkworm excrements (faeces) are expected, causing severe issues in the community. The chemical compounds of silkworm excreta that have basically been reported are chlorophyll and chlorophyll derivatives, xanthophyll, carotenoid, and flavonoids[1] . It is a rich source of vitamin E AND K, polysaccharides, amino acids such as lysine and alpha-glucosidase inhibitor. Silkworm excreta or pellets are excreted from silkworm (Bombyx mori) and considered as a major waste product of sericulture industry . Traditional source reports that silkworm excrement has pharmaceutical and food commercial uses. In customized Asian medicine excreta have been used as a therapeutic agent to treat infectious diseases, headache and abdominal pain, as well as lower LDLcholesterol and blood pressure [2,3] . However, few data are available on the bioactive compound profile of silkworm feces, and their application so far. The breeding waste of silkworm and their excrements were utilized in biogas production[4].

The silver nanoparticle hasextensive applications due to the huge degree of commercialization. Silver (Ag) is an captivative material for its distinctive properties, such as good conductivity, chemical stability,catalytic activity, and antimicrobial activity [5] . Gold nanoparticles (AuNPs), as a kind of unique and non toxicmaterial have noticed widespread interest for therapeutic applications in treating disease for humans[6].Gold nanoparticles (AuNPs), have become highly workable materials for treating malignant tumors due to their unique quenchingabilities, biocompatibility and significant surface modifiability [7]. It was reported that Astra Zeneca accompanying with

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Cytimmune, focused on AuNPs-based nanomedicine in cancer treatmet[8]. Employing the techniques of nanobiotechnology excretory pellets of B mori .L is utilised for the synthesis of silver and gold nanoparticles for first time. The objective of this study are (i) to synthesis the silver and gold nanoparticles from excretory pellets of B mori .L (ii) to analyze the spectroscopic, morphological, chemical and structural characterization of the synthesized nanoparticles and (iii) to evaluate their in vitro antibacterial and antifungal activities.( iv) ) to evaluate their in vitro antibacterial, antifungal and antidiabetic activities.

## MATERIALS AND METHODS

Chemicals and Materials Required:

Excretory pellets of silkworm, silver nitrate(AgNO3 99.99%) , aurochloricacid (H AuCl4, 99.99%) , Whatman filter paper No1, nutrient agar, nutrient broth, potato dextrose agar, potato dextrose broth, 96-well microtiter plate, resazurin tablet (C12H6NNaO4 ), α –amylase, starch solution (1% w/v), 3,5-dinitrosalicylic acid (DNSA reagent), sodium phosphate buffer, α-glucosidase, acarbose, Na2CO3, p-nitrophenyl-α-D-glucopyranoside (pNPG).

Microorganisms Used :

Bacterial species: Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella typhi, Staphylococcus aureus and Bacillus. subtilis. Fungal species: Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Mucor spp. and Candida albicans.

Biogenesis of silver and gold nanoparticles from excretopry pellets of B.mori.L:

The extract of excretory pellets was filtered using Whatman filter paper No1. Ten milliliters of filtrate was added to 90 ml of 1mM silver nitrate solution for the synthesis of silver nanoparticles. The bioreduction of silver ion was noted by the change of colour of the solution from light yellow to dark brown. To synthesize gold nanoparticles, 10-mL filtrate was added to 90 mL of 1mM chloroauric and gold chloride reduction was observed by the change of the colour of the solution from light yellow to dark purple.

Characterization of silver and gold nanoparticles synthesized from excretory pellets of B.mori.L:

Biosynthesized nanoparticles were characterized by UV-Visible spectrophotometer, Fourier transformed infrared spectroscopy (FTIR), X-ray diffraction (XRD) ,Scanning electron microscopy and EDAX to understand their structural, morphological and chemical characterization.

In vitro antimicrobial activity:

Antimicrobial activity was assessed by resazurin microtiter assay method [9]. The colour changes from purple to pink or colorless were recorded as positive. The lMinimum concentration at which colour change occurred was taken as the minimal inhibitory concentration (MIC) value[10] .

In vitro antidiabetic activity: α-amylase and α- glucosidase inhibitory assays:

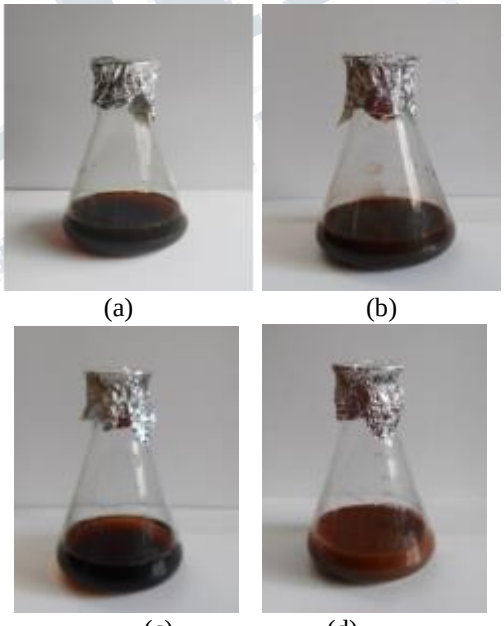
In α-amylase inhibition assay the amount of maltose liberated was quantified using the method adopted by Bhutkar and Bhis[11].Inhibition of α- glucosidase was assessed by the method carried out by Kim.et al.,[12]. Acarbose was used as a positive control in both assays. Inhibition of Enzyme activity was calculated as

$$\% \text{ of Inhibition} = \frac{\text{Abs control} - \text{Abssample}}{\text{Abs contrl}} \times 100$$

## RESULTS AND DISCUSSION

Biosynthesis of silver and gold nanoparticles:

The change in the colour within 24hr from light yellow to dark brown and light yellow to purple, confirmed the bioreduction of silver and gold nanoparticles respectively( Figures 1a −1d) .



(a) (b) (c) (d)

**Figure 1- (a)Ag control; (b)- silver nanoparticle synthesis;(c)Au control;(d)-gold nanoparticle synthesis**

Characterization of silver and gold nanoparticles :

UV-Visible Spectroscopy

Uv –Visible spectroscopy is useful to identify the formation of metal nanoparticles in the reaction mixture.The maximum absorbance was visualised with the wavelength that was peaked at 418 nm for silver nanoparticles and 555 nm for gold nanoparticles.(Figures 2(a) &(b).Earlies studies

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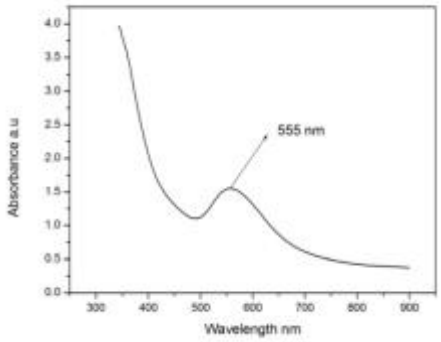
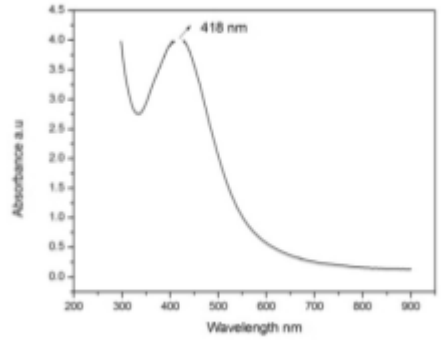
conducted by coriander leaf extract showed similar peak of absorbance gold nanoparticles[13]. Generally, the characteristic surface plasmon band from 500 to 550 nm indicates the spherical shape of gold nanoparticles[14] andrey. The surface plasmon oscillation modes of conduction electrons, which are coupled through the surface to external electromagnetic fields caused this band appearance.




(a)

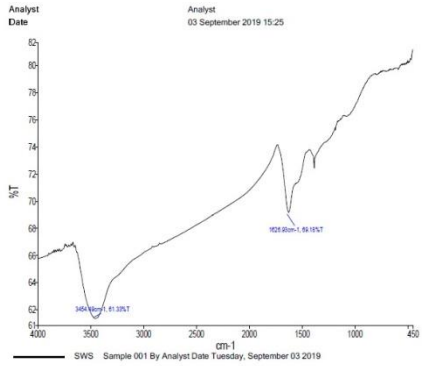



(b)

**Figure 2: UV- visible spectra of (a) silver and (b) gold nanoparticles**

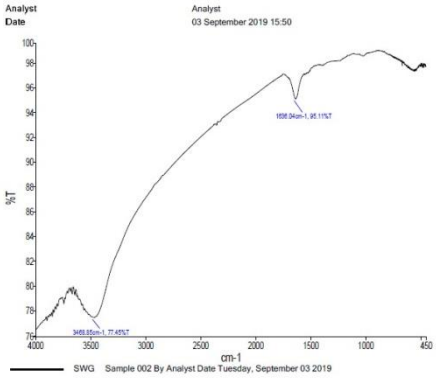
Fourier Transform Infra Red Spectroscopy(FTIR)

FTIR spectra evidenced with the peak value of infrared region for the silver and gold nanoparticles are shown in figures 3a& 3b.The frequency (cm-1)of characteristics vibration due to synthesis of silver nanopaticles were observed as 3500-3200 ( O-H stretch, H bonded alcohols& phenols and1650-1580( N-H bond, Primary amines) [15,16]. Gold nanoparticles showed characteristic vibration with two peaks of about 3500-3200 (O-H stretch, H bonded alcohols and phenols)and 1650-1580( N-H bond, Primary amines), different functional groups like O-H stretching of phenol and alcohol, the group of N-H, O-H, and C-H bend aldehyde are said to be involved in the process of nanoparticles synthesis [17]. FTIR spectroscopy exhibits residues in the surface and functional groups like phenols,flavonoid and hydroxyls, which attaches to the surface of nanoparticles and responsible for reduction and stabilization during nanoparticle synthesis [18].




$cm^{-1}$

(a)




$cm^{-1}$

(b)

**Figure 3 : FTIR spectra of (a) silver and (b) gold nanoparticles**

SEM and EDAX analysis:

SEM analysis of the synthesized silver and gold nanoparticles is given in Fig 4 (a) & (b) respectively The average size of the silver and gold nanoparticles size were noted as 40nm and 45nm respectively (Figure 4a &4b)[19]. Energy dispersive X-ray analysis (Edax) spectra of the nanoparticles are given in figures 5(a) & (b). EDAX spectra revealed peak for elemental silver at 3kev and the presence of strong peak of gold at 2kev[16].The elemental proportions of silver and gold nanoparticles were found to be 33.08 and 14.77% respectively. (Figure 5 (a) &(b).

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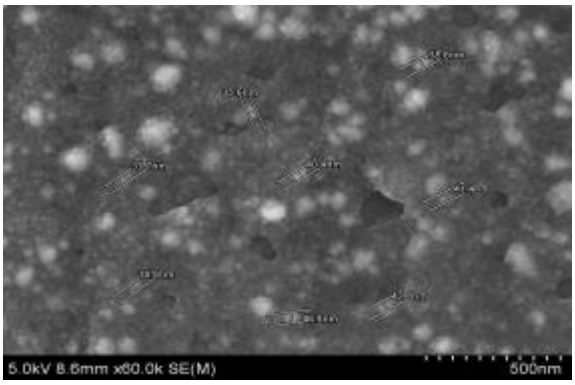
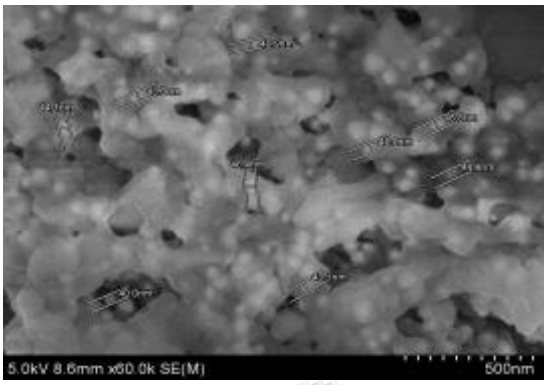



(a)




(b)

**Figure 4: SEM image of (a) silver and (b) gold nanoparticles**

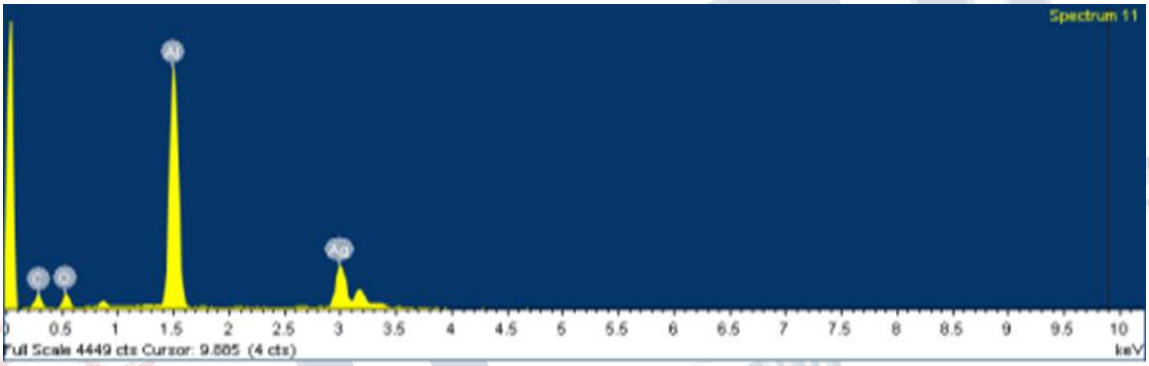



Figure 5(a): spectrum of silver nanoparticles

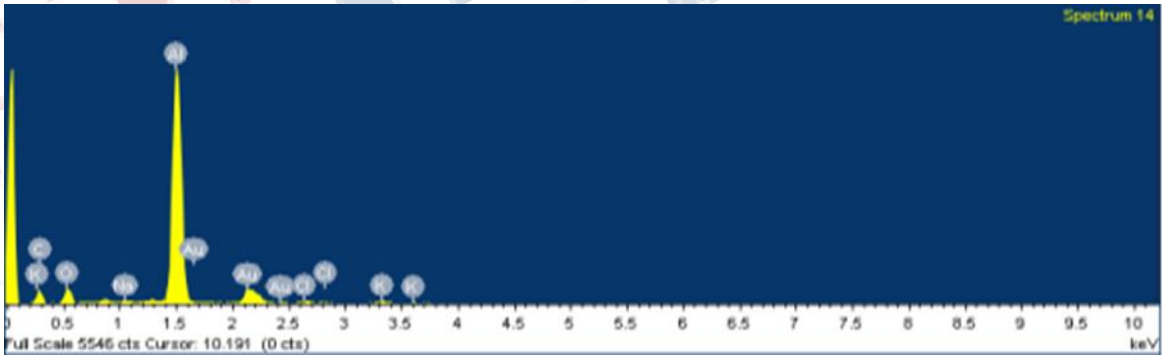



Figure 5(b): spectrum of gold nanoparticles

**X-Ray diffraction pattern**

X-ray diffraction pattern of the biosynthesized silver and gold nanoparticles are shown in figures 6 (a) & (b)). The prominent peaks at the 2 theta value of silver nanoparticles are 32.89,36.69, 42.89,63.26 and 76.39 which correspond to the Miller indices{ 100,100,110,111,210}. Likewise for gold nanoparticles 32.89, 36.73, 42.95, 63.30 and 76.42 that correspond to Miller indices {100,100,110,111,210}.XRD analysis showed that the most intense signal of crystalline silver nanoparticles displayed the preferential orientation of the crystals toward the (111) plane[20].This confirms that the particles in(111) plane are fcc crystalline in natute [21]. A similar results are evidenced in silver and gold silver mediated nanoparticle synthesis [22].

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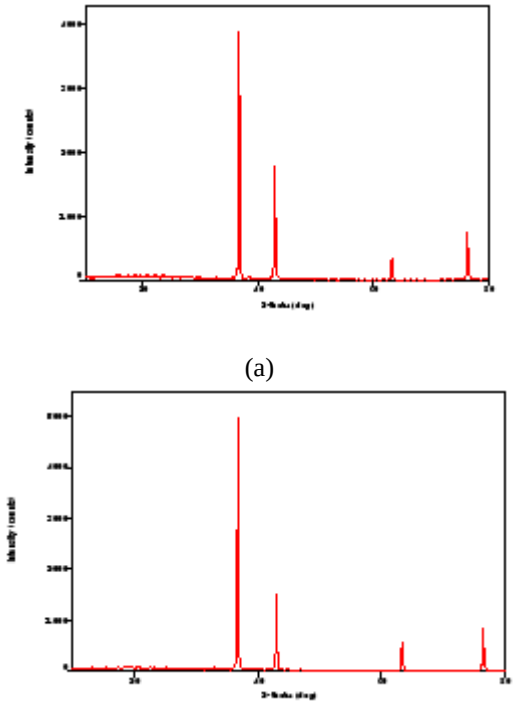


(a)

(b)

**Figure 6:XRD pattern of (a)- Silver and (b) gold nanoparticles**

In vitro antimicrobial activity

The antibacterial activity of the siver and gold nanoparticles assessed using resasurin assay method is depicted in figure 7&8. The MIC values of silver and gold nanoparticles exhibited against bacterial and fungal species are shown in figure &. Among the bacterial species, silver nanoparticles showed MIC at 62.5 µg against K.pneumoniae,and 125 which was similar to work in Procyanidin Capped Silver Nanoparticles [23] and for gold nanoparticles P.aeruginosa ,K.pneumoniae and B subtilis exhibited MIC at 125 µg (Figure 9). Among fungal species, silver nanoparticles showed MIC 125 µg against the tested Aspergillus sp and 250 µg of MIC was noted in C.albicans (Figure 10). Earlier work on Abelmoschus mediated nanoparticles synthesis revealed similar antifungal activity against C.albicans [24].Hence, bioactive potentialities of excretory pellets of B.mori.L are enhanced through metal nanoparticles proved to be antimicrobial in this study.

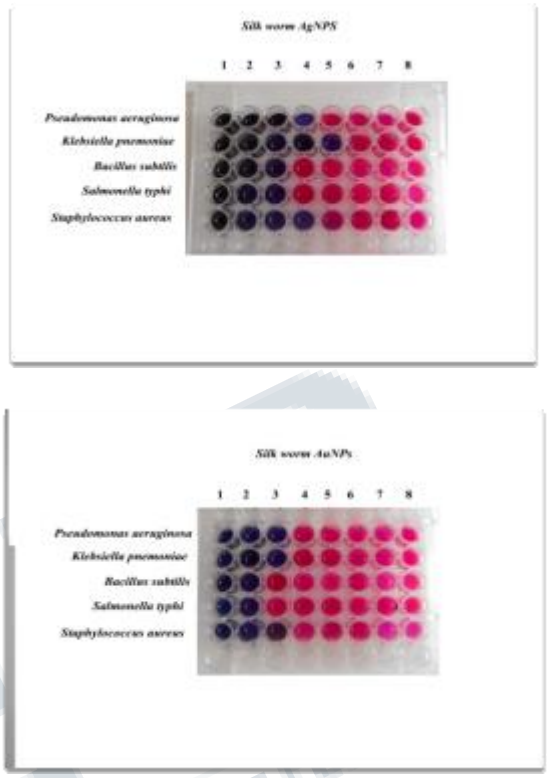
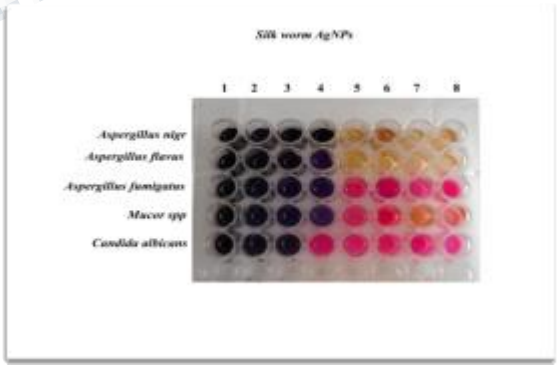



**Figure 7: MIC of siver nanoparticles at different concentrations. 1-1000 µg; 2-500 µg; 3-250 µg; 4-125 µg; 5-62.5 µg; 6-31.2 µg; 7-15.6 µg;8- 7.8 µg.**



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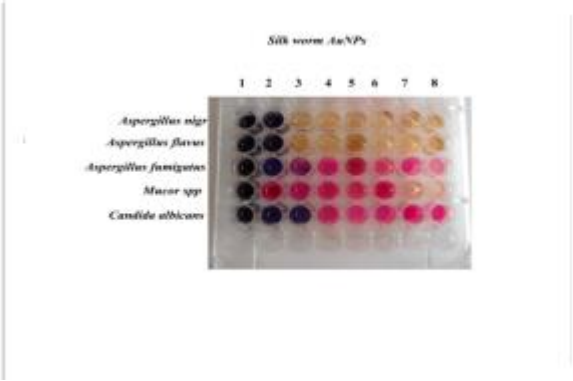

**Figure 8: MIC of gold nanoparticles at different concentrations. 1-1000 µg; 2-500 µg; 3-250 µg; 4-125 µg; 5-62.5 µg; 6-31.2 µg; 7-15.6 µg;8- 7.8 µg.**

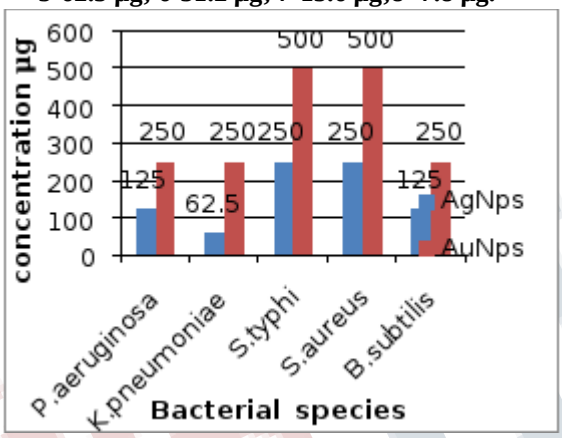


**Figure 9: In vitro antibacterial activity**

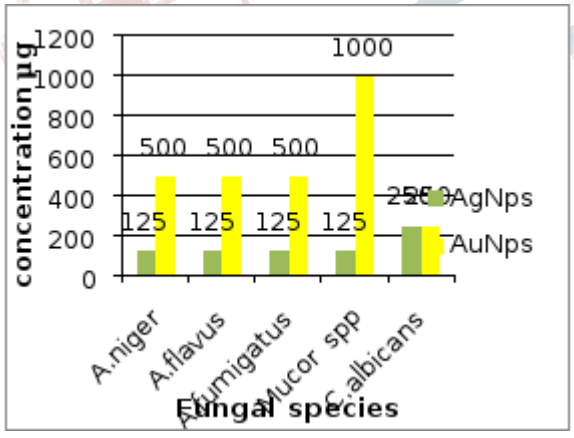


**Figure 10: In vitro antifungal activity**

In vitro antidiabetic activity

Using nanoparticles of trace elements for treating diabetes mellitus is considerable because of their nanoscale properties that enable for delivery to target cells are loaded with therapeutic agents [25].The silver and gold nanoparticles showed inhibitory effect on the enzymes in a dose-dependent manner ( Figures: 11 & 12).IC 50 values of α- amylase activity were found to be574 and 1075 µg/ml for the silver and gold nanoparticles respectively [26] and for α-glucosidase, the IC50 values of about 3521 and 415.97 µg/ml for the silver and gold nanoparticles respectively [27] . The results revealed that synthesized nanoparticles possess antidiabetic properties with the significant IC 50 value in both α- amylase assay and α- glucosidase assay.

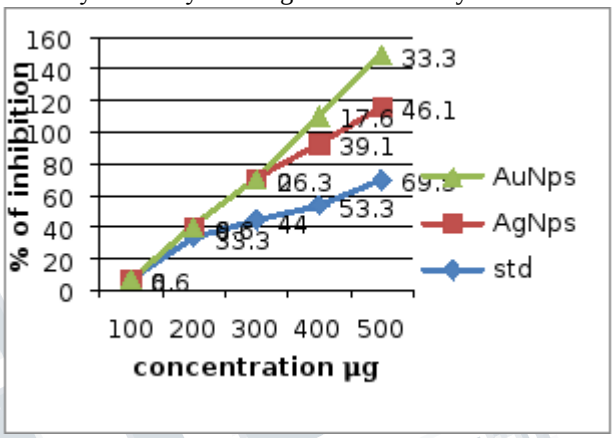


Figure 11:Inhibitory activity of α-amylase

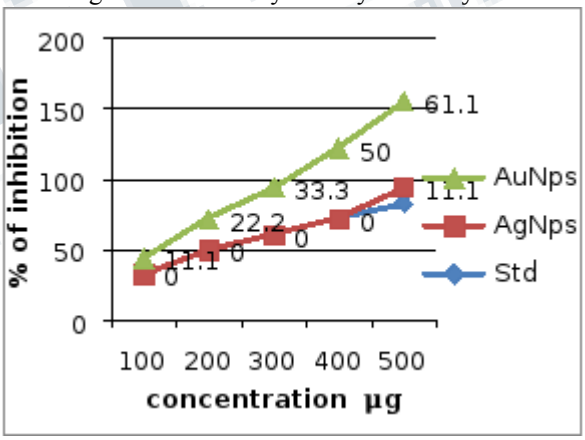


Figure 12:Inhibitory activity of α-glucosidase

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**CONFLICT OF INTEREST:**

The authors declare no conflict of interest

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