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*Research Article*

# Efficacy of silver and gold nanoparticles obtained from vermiwash: *In vitro* study on antimicrobial and antidiabetic activities

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**Abstract**
Emerging nanobiotechnology has provided innovative techniques to synthesize nanoparticles through biological methods to explore the potentialities of biological sources like phytoextracts, microbes, animal secretions and excretion. This research studies the potential of vermiwash to synthesize the silver and gold nanoparticles and evaluate its *in vitro* effect of antimicrobial and antidiabetic activities. The characterization of the nanoparticles was analyzed through various techniques. Ultraviolet (UV)-Visible spectroscopy showed the maximum absorption spectrum at 413 nm for silver and 541 nm for gold nanoparticles. Fourier transform infrared spectroscopy (FTIR) revealed the reducing agent involved in nanoparticles synthesis. Scanning electron microscope (SEM) images revealed the size of the silver and gold nanoparticles as 24 nm and 50 nm, respectively. Energy dispersive X-ray (EDAX) analysis revealed the elemental composition of the synthesized nanoparticles. X-ray diffraction (XRD) analysis confirmed the crystalline nature of the nanoparticles that displayed the preferential orientation of the crystals toward the (111) plane. Antimicrobial activity was assessed using the resazurin assay method. A minimum inhibitory concentration (MIC) of less than 7.8 µg was observed in *Staphylococcus aureus* and *Klebsiella pneumoniae*. In the antifungal activity, MIC at 250 µg was noted in *Mucor sp.* and *Candida albicans*. Antidiabetic activity was assessed by α-amylase and α-glucosidase inhibitory assay. $IC_{50}$ of α-amylase and α-glucosidase activity of the silver nanoparticles was noted as 218 and 221 µg/mL, respectively. IC 50 value for the enzymatic assay dose-dependently confirmed the effect. Conclusively biosynthesized nanoparticles from vermiwash showed potential efficiency of antibacterial, antifungal and antidiabetic activities.

**Keywords:** Antimicrobial, Antidiabetic, Minimum inhibitory concentration, Nanobiotechnology, Nanoparticles

## INTRODUCTION

Vermiwash is a liquid extract obtained from vermicomposting beds and is used as an organicfertilizer for crop plants. Generally, it is the wash of earthworms present in the medium collected after the passage of water through the different layers of worm culture unit ( Vadamalaikrishnan and Fathima, 2021). The composition and quality of vermiwash vary depending on the raw organic matter used for vermicomposting. The composition of vermicompost and vermiwash prepared from the same organic matter is essentially the same. It comprises numerous chemicals, viz, hormone, mucous, enzyme, vitamins, proteins, different macro and micronutrients, and microbes that distinguish the two products ( Das *et al*., 2014). It is rich in nutrients, vitamins, growth hormones that act as a disease and pest suppression agent( Machfudz *et al*., 2020).

Besides its role as a biofertilizer to increase crop productivity, it can also be applied in disease suppression and pest control due to the presence of essential antimicrobial and antipest chemicals (Kanchan *et al*., 2013, and Sudeshna Thakur & Sood,2019)]. Earlier studies reported that the effect of vermiwash and mucus extracted from *Eisenia fetida*on *Fusariumgraminearum* greatly inhibited the growth of pathogenic fungi,

which significantly influenced both the quality and production of wheat *Triticum aestivum*(Akinnouye-Adelabu *et al*.,2019). Earthworm vermiwash was found to have potent antimicrobial activity (Govindarajan and Prabakaran, 2012). The advantage of biological systems has unique biocompatibility with functional biomolecules, which improves the reduction of metal ions (Rai and Ingle, 2021). Hence, nanobiotechnology was employed in this study to biosynthesize metallic nanoparticles such as silver and gold using vermiwash through biological methods.

Nanobiotechnology is the combination of biotechnology and nanotechnology that have several applications in biological fields. Biotechnology deals with metabolic and other physiological processes of biological resources, including microorganisms, along with nanotechnology, nanobiotechnology can play a vital role in improving, establishing and implementing many useful tools in the study of life. Nanoparticles range between 1 and 100 nanometers in size, untraceable by the human eye. It attributes different physical and chemical properties to its larger material counterparts**.** Material properties alter as their size reaches the atomic scale due to the increased surface area to volume ratio, resulting in the material's surface atoms influencing material performance. Due to their nanoscale dimension, nanoparticles have a huge surface area to volume ratio compared to bulk material, such as powders, plates, and sheets. This unique feature made nanoparticles to possess specific optical, physical and chemical properties, as they are tiny enough to confine their electrons and produce quantum effects.

Of all nanomaterials, gold and silver nanoparticles are the most explored nanostructures for biomedical applications. The unique properties of GNPs have been exploited for many advanced biomedical applications such as in bio-imaging, gene delivery, contrast enhancement of X-ray computed tomography, targeted drug delivery, diagnostics, plasmonic bio-sensing, colorimetric sensing, tissue engineering, photo-induced therapy, and cancer therapy. Silver nanoparticles are noticeable due to their attractive physicochemical properties and biological functionality, including their high antimicrobial efficiency (destroying or inhibiting the growth of microorganisms and especially pathogenic microorganisms) and relatively nontoxic, a wide spectrum of bactericidal properties, anticancer properties, and other therapeutic abilities, their unique ability to form diverse nanostructures and their relatively low manufacturing cost (Duran *et al*., 2016). Various photo sources, microbial sources like bacteria, fungi and algae are reported to be effective in biological method of nanoparticle synthesis and are effective in therapeutic applications such as antimicrobial, antidiabetic, anticancerous, anti-inflammatory, antioxidative and so on. But limited reports are available in animal source-mediated nanoparticle synthesis, especially at the invertebrate level. Based on this context, the present work was framed i) to synthesis the silver and gold nanoparticles from Vermiwash, ii) to analyze the spectroscopic, morphological, chemical and structural characterization of the synthesized nanoparticles, iii) to evaluate the in vitro antimicrobial activities and iv) ) to study *in vitro* antidiabetic activities.

## MATERIALS AND METHODS

### Chemicals and materials required

Vermiwash was procured from Manidharma Biotech Pvt.Ltd,India contains excretory products and excess secretions of earthworms plus micronutrients from soil organic molecules. It has high quantities of nitrogen, phosphorus, potash, calcium, magnesium and zinc. Silver nitrate ($AgNO_3$ 99.99%), aurochloric acid (H $AuCl_4$, 99.99%) , Whatman filter paper No1, nutrient agar, nutrient broth, potato dextrose agar, potato dextrose broth, 96-well microtiter plate, resazurin tablet ($C_{12}H_6NNaO_4$ ), α –amylase, starch solution (1% w/v), 3,5-dinitrosalicylic acid (DNSA reagent), sodium phosphate buffer, α-glucosidase, acarbose, $Na_2CO_3$ *and p*-nitrophenyl-*α*-D-glucopyranoside (pNPG) were used .

### Microorganisms used:

### Bacterial species

*Pseudomonas aeruginosa (P.aeruginosa)* ATCC 27853,*Klebsiella pneumoniae (K. pneumoniae*) ATCC2146, *Salmonella typhi (S. typhi*) ATCC14028, *Staphylococcus aureus ( S. aureus)* ATCC 25923 and *Bacillus.subtilis ( B.subtilis)* ATCC 6633.

### Fungal species

*Aspergillus niger (A. niger)* ATCC6275, *Aspergillus flavus (A. flavus)* ATCC9643, *Aspergillus fumigatus( A. fumigatus)* ATCC 1022, *Mucor sp.* ATCC 56650 and *Candida albicans ( C.albicans)* ATCC 10231.

### Biosynthesis of silver and gold nanoparticles

Vermiwash was procured from Manidharma Biotech Pvt.Ltd and filtered using Whatman filter paper No1. Ten millilitres of the filtrate was added to 90 ml of 1mM silver nitrate solution to synthesize of silver nanoparticles (Jain, 2009). The change in the colour of the solution from light yellow to dark brown indicated the bioreduction of silver nitrate. To synthesize gold nanoparticles, 10-mL filtrate was added to 90 mL of 1mM chloroauric and gold chloride reduction ( Aljabali *et al*.,2018) .The change in the colour of the solution from light yellow to dark purple was observed.

### Characterization of silver and gold nanoparticles

The biosynthesized silver and gold nanoparticles were

monitored using a UV-Visible spectrophotometer. The absorption spectrum band was noted within the range of 200–900 nm. FTIR characterization revealed the functional groups in nanomaterials. SEM and EDAX provided detailed high-resolution images and percentages of the elemental composition of the synthesized nanoparticles. XRD analysis was done to identify and characterize the compounds in the nanoparticles based on the diffraction pattern

***In vitro* antimicrobial activity**

Antimicrobial activity was determined by the resazurin microtiter assay method ( Sarkar *et al*.,2007). Any colour changes from purple to pink or colorless were recorded as positive. The lowest concentration at which colour change occurred was taken as the minimal inhibitory concentration (MIC) value (Gulnaza and Savitha, 2013). Minimum inhibitory concentrations (MICs) are the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation.

***In vitro* antidiabetic activity:**

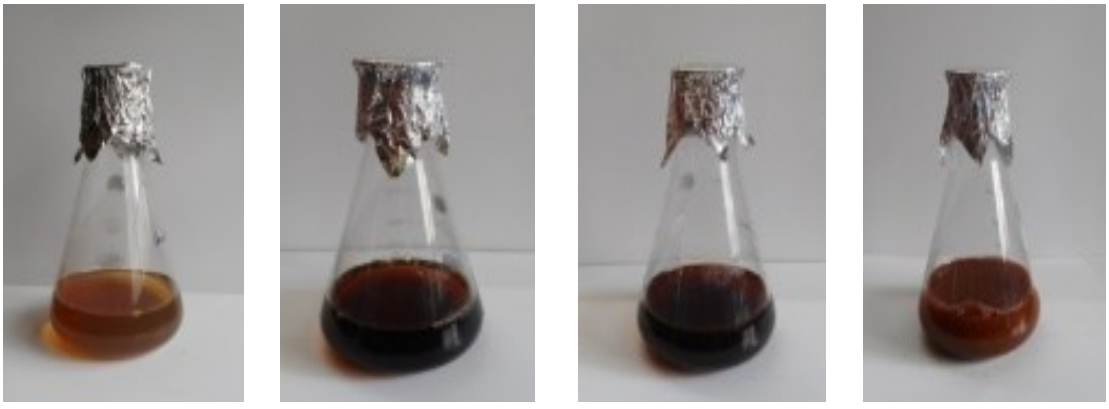
α-amylase inhibition assay was conducted to quantify the amount of maltose liberated during the experimental process( Bhutkar and Bhise 2012). Inhibition of α- glucosidasewas conducted by the method performed by Kim *et al*., 2005. Acarbose was used as a positive control in both assays. Inhibition of the enzyme activity was calculated as-

## RESULTS AND DISCUSSION

**Biosynthesis and characterization 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 , respectively ( Fig 1a −1d) UV-Visible spectroscopy identified the formation of metal nanoparticles in the reaction mixture. The maximum absorbance monitored with the wavelength ranging between 200 and 900 nm was peaked at 413 nm for silver nanoparticles and 541 nm for gold nanoparticles (Fig 2 (a) and (b). A similar absorbance spectrum of about 410 nm was noted in the celomic fluid of *Eudriluseuginae-mediated* silver nanoparticle synthesis (Lekshmi Packia *et al*., 2014). The biosynthesis of gold nanoparticles using Chenopodium album leaf extract showed a similar peak of absorbance at 540 nm (Dwivedi and Gopal, 2010). The characteristic surface plasmon band from 500 to 550 nm indicates the spherical shape of gold nanoparticles. This band appears due to the surface plasmon oscillation modes of conduction electrons, which are coupled through the surface to external electromagnetic fields.

FTIR spectra with the peak value of the infrared region for the synthesized silver and gold nanoparticles are shown in Fig 3a and b. From the peak value, the functional groups and nature of chemical bonds are determined using IR chart. The frequency (cm-1) of characteristic vibration due to the synthesis of silver nanoparticles was observed as 3500–3200 (O-H stretch, H bonded alcohols and phenols), 1650–1580 (N-H bond, Primary amines),1390-1380( C-H bending, aldehyde) whereas 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). In an earlier study of photo source-mediated synthesis of nanoparticles, FTIR revealed a broad peak at 3304.06 corresponded to O-H stretch of phenol, a medium peak obtained at 2916.37 and 2846.93 showed C-H stretch of alkane, medium broad peaks at 1595.13 corresponded to N-H bend of amine, a weak peak at 1381.03 represented the C-H bend of alkane, medium peak at 1029.99 corresponded to C-O stretch of alkane and reported that the aldehyde group has played a role in nanoparticle



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

**Fig. 1.** *Synthesis of Nanoparticles (a) Agcontrol (b) silver nanoparticle synthesis (c) Au control (d) gold nanoparticle synthesis*

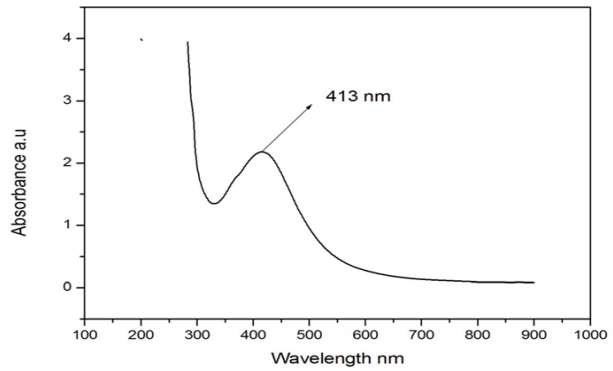



**Fig. 2 (a)**. *UV- Visible spectra of silver nanoparticles*

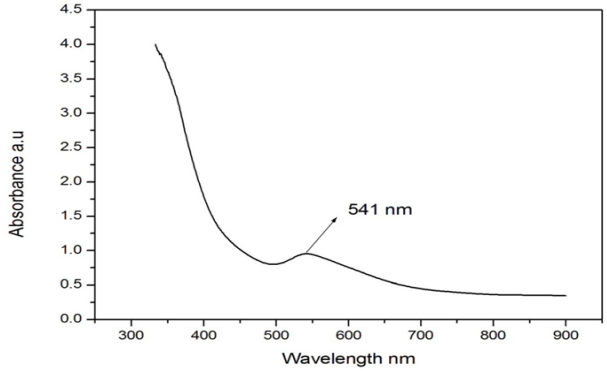



**Fig. 2(b).** *UV- Visible spectra of gold nanoparticles*

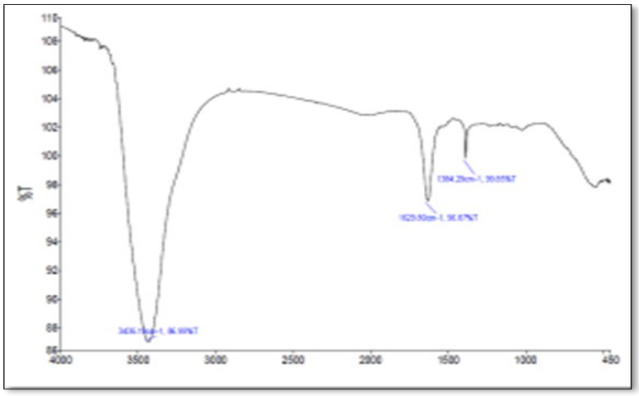



**Fig. 3(a).** *FTIR spectra of silver nanoparticles*

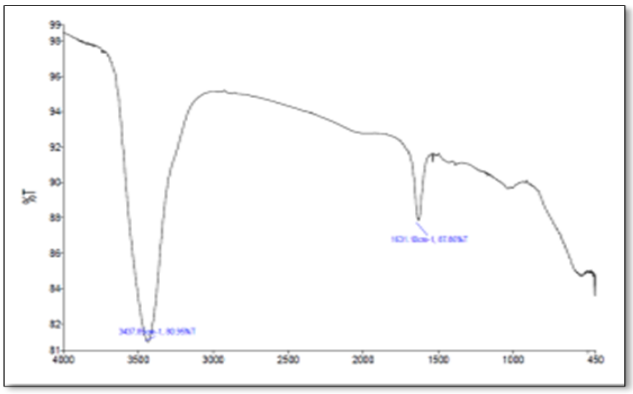
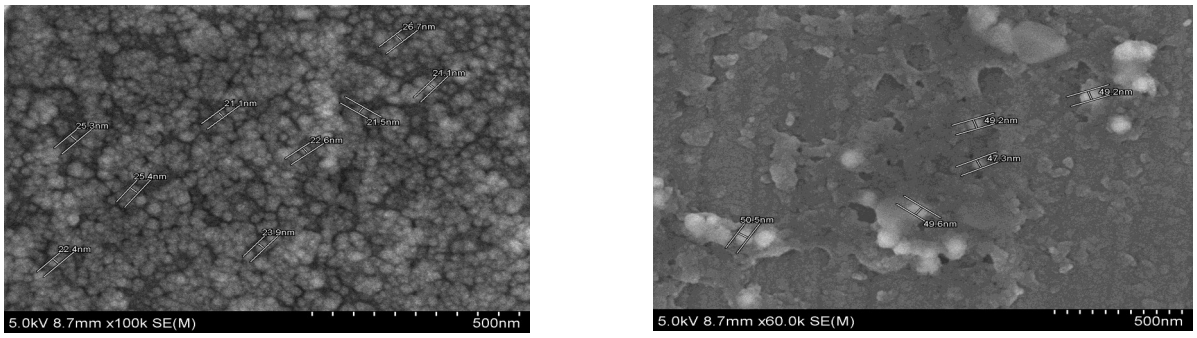



**Fig. 3 (b).** *FTIR spectra gold nanoparticles*




**(a)** **(b)**

**Fig. 4.** *SEM image of (a) silver nanoparticles and (b) gold nanoparticles*

synthesis ( Happy Agarwal *et al*., 2018) which is a supporting reference for this work. An earlier study suggested that various 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 nanoparticle synthesis. (Guo *et al*., 2015). From the obtained fair peaks in our study, surface residue and functional groups like phenol, hydroxyls, and aldehydes, which were attached to the nanoparticles' surface, confirmed their role in synthesis for efficient reduction and stabilization.

The high resolution, three-dimensional images of nanomaterials produced by scanning electron microscopy provided topographical, morphological and compositional information. SEM image revealed the size range of silver nanoparticles as 24 nm due to hydrogen bonds and electrostatic interactions between the bioorganic capping molecules bound to the silver nanoparticles. Similar results were reported in silver nanoparticle synthesis using dried fruit extract of the plant *T. Terrestris* (16 to 28 nm).( Gopinath *et al*., 2012, and Bhau *et al.,* 2015). Basavegowda *et al.* (2013) synthesized gold nanoparticles in size range of 10–50 nm using *Punica granatum* (PuG) extract as a reducing agent, whereas the size of gold nanoparticles synthesized ranges from 47 -51 nm in this work. EDAX spectra of the nanoparticles are given in figures 5(a) and (b). It is an analytical technique, which is used for the identification of compositions of different elements in a specific sample. It relies on an interaction between some sources of X-ray excitation and a sample. It can be used to determine which chemical elements are present in a sample

(qualitative analysis) and can be used to estimate their relative abundance (quantitative analysis). The intensities of peaks provided the elemental concentration of a specific element present in a sample ( Paulkumar *et al*., 2014). EDAX spectra revealed a peak for elemental silver at 3kev and a strong peak of gold at 2kev[9]. In this analysis, the elemental compositions of silver and gold nanoparticles in the sample were found to be 54.85 and 24.53%, respectively confirmed the nanoparticle synthesis.

XRD patterns of the biosynthesized silver and gold nanoparticles are shown in Fig. 6 (a) and (b). The prominent peaks at the 2 theta value that correspond to the Miller indices of the synthesized silver and gold nanoparticles are shown in Table 1.XRD analysis showed that the most intense signal of crystalline silver and gold nanoparticles displayed the preferential orientation of the crystals toward the (111) plane at the 2 theta value of 63.26°. This specific plane proved the structure of the face center cubic structure of metallic nanoparticles (fcc), the crystalline nature of the synthesized nanoparticles. (Garibo *et al*.,2020). The scattering of X-rays from atoms produces a diffraction pattern, which contains information about the atomic arrangement within the crystal. It computes the molecular form factor by accounting for the scattering factors of all atoms in the molecule and their relative positions ( Thanh-truc *et al*., 2019). The XRD pattern indicated that biosynthesized nanoparticles are crystalline.

### *In vitro* antimicrobial and antidiabetic activity

The resazurin microtiter assay was conducted to study the antimicrobial activity of silver and gold nanoparticles at various concentrations ranging from about 1000,

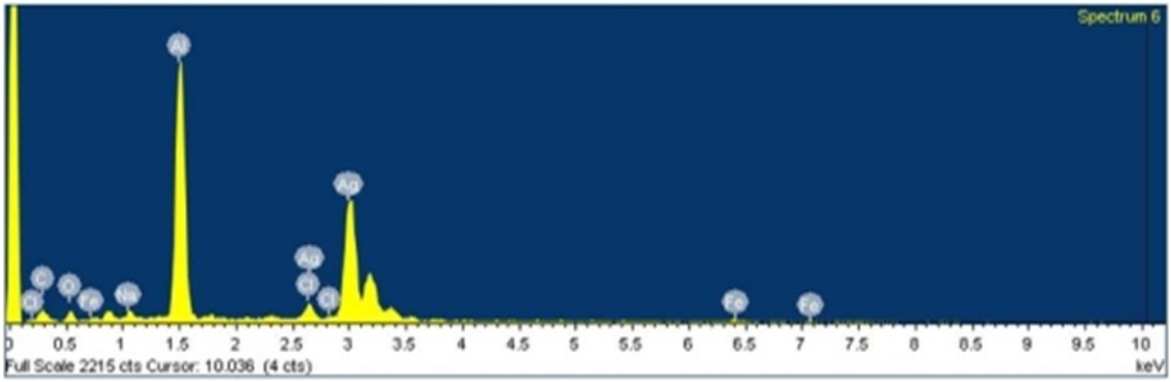



**Fig. 5(a).** *EDAX spectrum of silver nanoparticles*

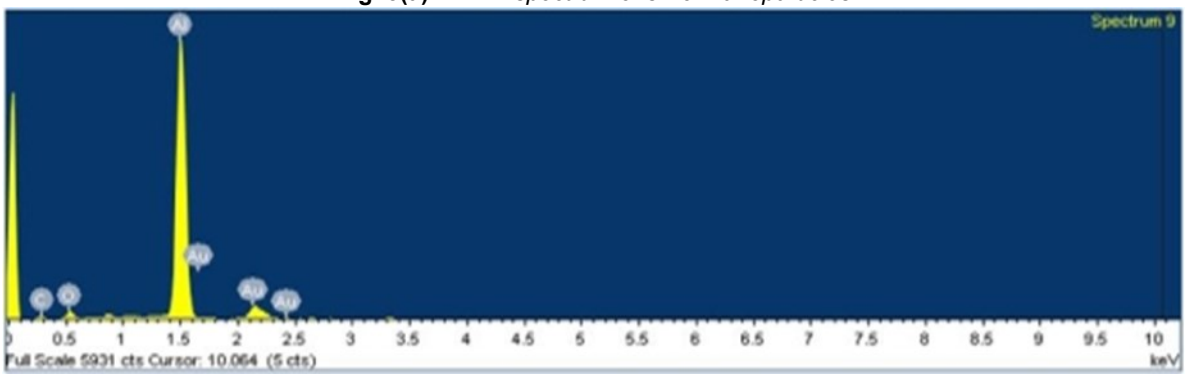



**Fig. 5(b).** *EDAX spectrum of goldnanoparticles*

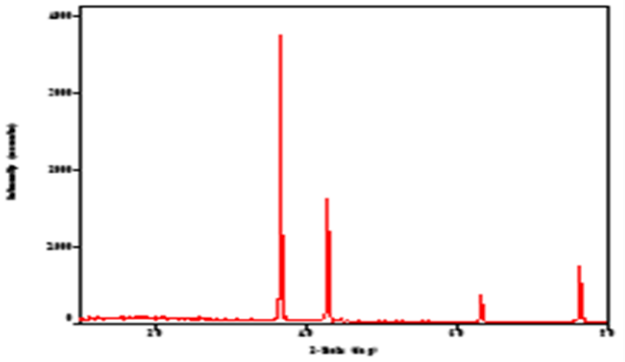


**Fig. 6 (a).** *XRD pattern of silver nanoparticles*

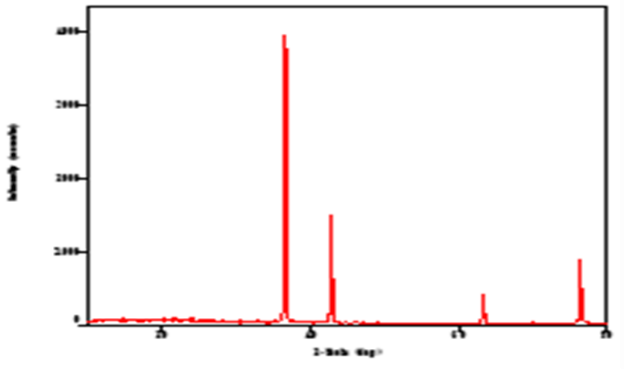


**Fig. 6(b).** *XRD pattern of gold nanoparticles*

**Table. 1.** Peak list analysis of XRD patterns of nanoparticles

| Nanoparticles | 2θ | d(Ang) | FWHM (deg) | h | k | l | Crystallite size (nm) |
|---|---|---|---|---|---|---|---|
| Silver | 36.69 | 2.44 | 0.15(2) | 1 | 0 | 0 | 57.16 |
| | 42.91 | 2.11 | 0.19(3) | 1 | 1 | 0 | 46.70 |
| | 63.26 | 1.47 | 0.17(7) | 1 | 1 | 1 | 56.69 |
| | 76.41 | 1.25 | 0.18(4) | 2 | 1 | 0 | 56.20 |
| Gold | 32.85 | 2.72 | 0.10(3) | 1 | 0 | 0 | 86.54 |
| | 36.69 | 2.44 | 0.16(2) | 1 | 0 | 0 | 53.33 |
| | 42.87 | 2.11 | 0.17(5) | 1 | 1 | 0 | 53.40 |
| | 63.26 | 1.47 | 0.18(7) | 1 | 1 | 1 | 53.28 |
| | 76.41 | 1.25 | 0.16(4) | 2 | 1 | 0 | 67.29 |

500, 250, 125, 62.5, 31.2 µg,15.6 and 7.8 µg are shown in Fig. 7(a) and (b) and Fig. (8a) and (b). Among the bacterial species, silver nanoparticles showed low MIC of< 7.8 µg for *S.aureus* and *K.pneumoniae,* and 7.8 µg against *P.aeruginosa, S.typhi* and *B.subtilis*, which was similar to work in Procyanidin Capped Silver Nanoparticles Where *P. aeruginosa* with MIC of 31.25 µg/mL and even as low as 15.63 µg/mL for *S. aureus*. ( Badeggiet al., 2020) and for gold nanoparticles *P.aeruginosa ,S aureus and B. subtilis* exhibited MIC at 125 µg, whereas *K. pneumoniae* and *S. typhi* exhibited MIC at 250 µg.( Table 2 and Fig. 9). Earlier studies reported antibacterial activities of polychaete extract in disc diffusion wherein the maximum antibacterial activity was observed against *S. aureus* that showed 13 mm zone of inhibition followed by *E. coli* (10 mm), *P.aeruginosa* (10 mm), and *S. typhi* (9 mm) (Singh *et al*., 2014). Of the different methods adopted, the nanoparticles exhibited the antibacterial property identical to the results obtained in the present study. Among fungal species, silver nanoparticles showed MIC at 250 µg in *Muco*r sp and *C.albicans* and 500 µg of MIC was noted in *A.niger, A.flavus, and A.fumigatus* ( Table 3 and Fig. 10). Similar antifungal activity against C. *albicans* (18mm zone of inhibition) was noted in *Abelmoschus* mediated nanoparticle synthesis (Chidambaram Jayaseelan *et al*., 2013), which is effective in our study and the coelomic fluid of *Eudrilus euginae* was reported to possess antifungal activities against *A. flavus* ( Nadana *et al*., 2020) as also evidenced in our study. Hence, it is evident from the present study, silver and gold nanoparticles biosynthesized from vermiwash possessed antimicrobial efficacy.

### *In vitro* antidiabetic activity of silver and gold nanoparticles

The antidiabetic activity was analyzed through α-amylase and α-glucosidase assay in this study. It revealed that the silver and gold nanoparticles dose-

**Table. 2.** MIC values of silver and gold nanoparticles for bacterial species

| Bacterial species | MIC Value (µg) AgNPs | MIC value (µg) AuNps |
|---|---|---|
| P. aeruginosa | 7.8 | 125 |
| K. pneumoniae | Less than 7.8 | 250 |
| S. typhi | 7.8 | 250 |
| S. aureus | Less than 7.8 | 125 |
| B. subtilis | 7.8 | 125 |

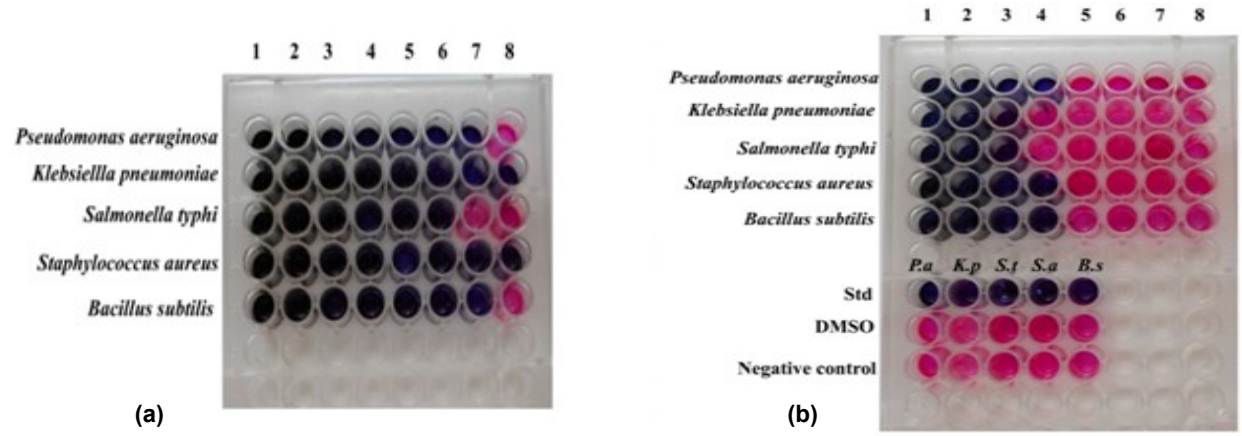



**Fig. 7**. *MIC of (a) silver and (b) gold nanoparticles in bacterial species at different concentrations. 1-1000 µg; 2-500 µg; 3-250 µg; 4-125 µg; 5-62.5 µg; 6-31.2 µg; 7-15.6 µg;8- 7.8 µg. Std-streptomycin (Positive control), DMSO-Vehicle control*

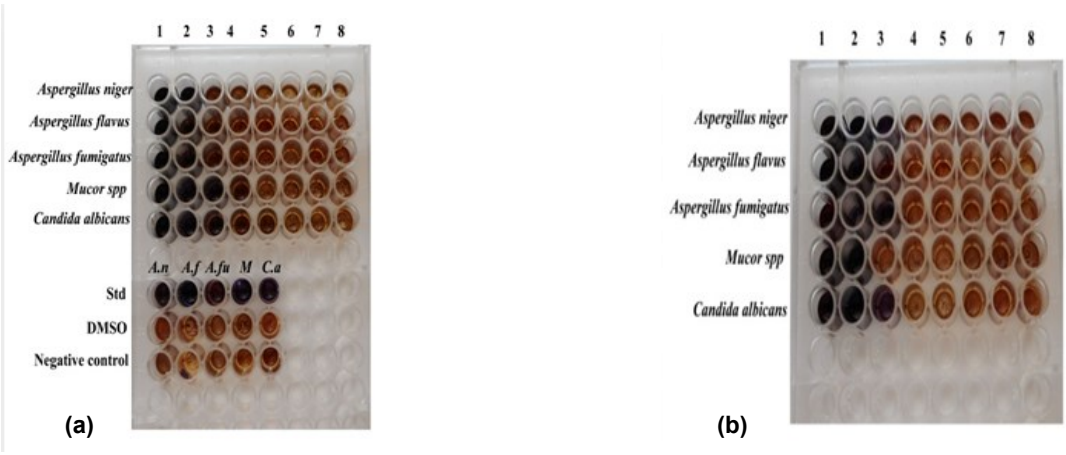



**Fig. 8.** *MIC of (a) silver and (b) gold nanoparticles in fungal species at different concentrations. 1-1000 µg; 2-500 µg; 3-250 µg;4-125 µg; 5-62.5 µg; 6-31.2 µg; 7-15.6 µg;8 7.8 µg. Std-streptomycin (Positive control), DMSO-Vehicle control*

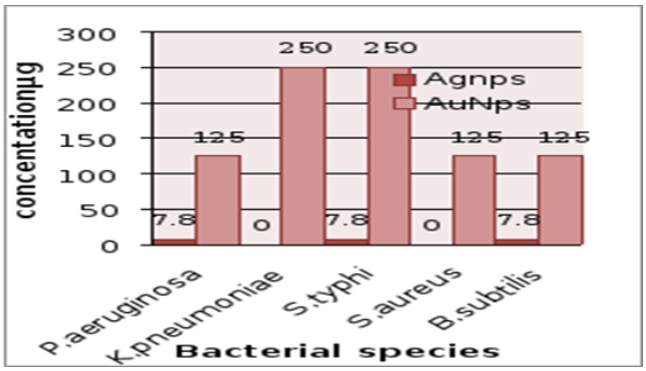



**Fig. 9.** *In vitro antibacterial activity*

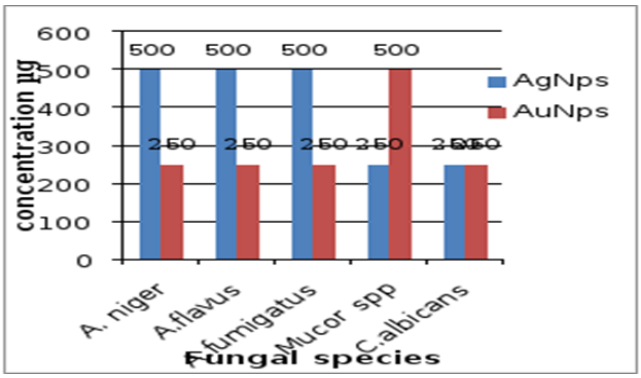



**Fig.10**. *In vitro antifungal activity*

dependently showed an inhibitory effect on the enzymes (Fig. 11 and 12). IC 50 values of α- amylase activity was found to be 218 and 221 µg/ml for the silver and gold nanoparticles, respectively and for α- glucosidase, the IC50 values of about 384 and 290 µg/ml for the silver and gold nanoparticles, respectively, are exhibited in our study, which is comparatively found to be less than the IC50 values of the metallic nanoparticles like silver and gold synthesized through phytoextracts and microbial sources. For example, IC50 values of silver nanoparticles in the α- amylase activity (280.39 and 273.48 µg/m)l reported in phytoextracts (Vishnu Kiran and Murugesan, 2013). Likewise, C*olpomeniasinuosa* (Marine algae) exhibited α-amylase (IC50-490 ± 0.02 mg/ml) and α- glucosidase (IC50-385 ± 0.02 mg/ml) activity, which is similar to the reports of the inhibitory assay performed in biosynthesized nanoparticles (Manam and Murugesan, 2014). α-amylase and α-glucosidase are carbohydrate digestion enzymes that convert them stepwise to simple sugars and are suitable for absorption. Inhibition of these enzymes is needed in non-insulin diabetics to slow down the release of glucose into blood (Thatoi *et al*., 2016). The inhibitory efficiency of AgNPs for the amylase activity shall play an important role in controlling hyperglycemia and, therefore, will be able to cope with diabetes ( Debnath *et al*.,2020). Comparatively synthesized nanoparticles showed a minimal IC 50 value in α- amylase assay than α- glucosidase assay. Thus the antidiabetic potential of silver and gold nanoparticles synthesized from biological resources like vermiwash is significant.

## Conclusion

The present study gives perceptivity to the antimicrobial and antidiabetic potential of the vermiwash combined with silver and gold nanoparticles. The combined approach of nanobiotechnology using vermiwash was confirmed and characterized using various techniques. Compared to gold nanoparticles, silver nanoparticles

**Table 3.** MIC values of silver and gold nanoparticles for fungal species

| Fungal Species | MIC Value (µg) AgNPs | MIC value (µg) AuNps |
|---|---|---|
| A.niger | 500 | 250 |
| A. flavus | 500 | 250 |
| A.fumigatus | 500 | 250 |
| Mucor sp. | 250 | 500 |
| C.albicans | 250 | 250 |

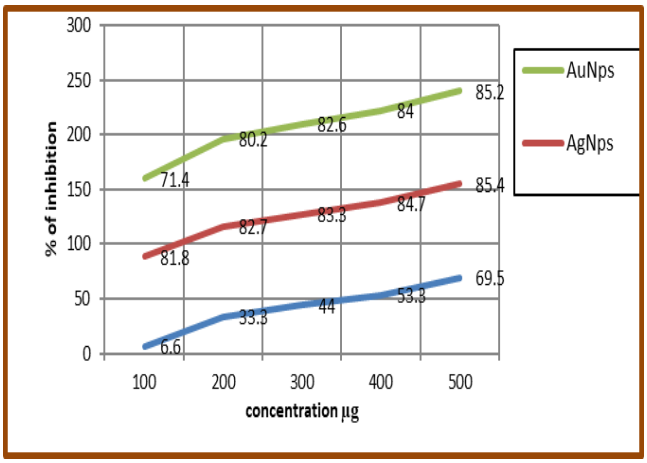



**Fig.11.** *Inhibitory activity of α-amylase*

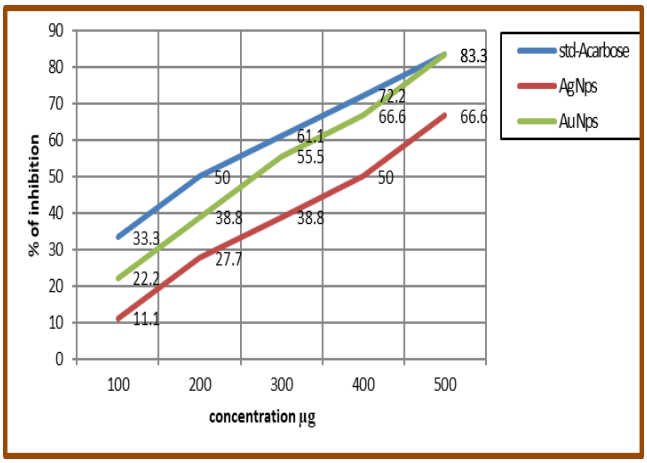



**Fig. 12.** *Inhibitory activity of α-glucosidase*

were found to be effective in the *in vitro* antimicrobial and antidiabetic activity. Vermiwash, as the excretion of earthworm, is known for its use as a biofertilizer in plant growth promotion, pest control and disease suppression agent. Present findings explored its antimicrobial and antidiabetic properties, and these biological applications need to be further explored using *in vivo* experimental models to substantiate the results obtained in this study. Identifying compounds with therapeutic applications will be helpful for encountering various issues like microbial drug resistance, diabetic management, etc. This finding would benefit future research and turn the focus on animal sources to exploit their potentialities in developing therapeutic and pharmaceutical products.

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### Conflict of interest

The authors declare that they have no conflict of interest.

## REFERENCES

1. Vadamalaikrishnan, K. & Fathima (2021). M. Effect of vermiwash and plant growth regulators on growth and stem anatomy of *Hibiscus cannabinus* L.*International Journal of Pharmaceutical Sciences* and *Research,* 12(2), 1190-95. doi: 10.13040/IJPSR.0975-8232.12(2).1190-9
2. Das, S.K., Avasthe, R.K. & Gopi, R.(2014). Vermiwash: use in organic agriculture for improved crop production. *Popular Kheti,* 2 (4), 45-46 www. popularkheti. info
3. MacHfudz, M., Basit, A. & Handoko, R.N.S. (2020).Effectiveness of vermicompost with additives of various botanical pesticides in controlling *Plutellax ylostella* and their effects on the yield of cabbage (*Brassica oleracea L. var. Capitata*). *Asian Journal* of *Agricu lture* and *Biology*, 8 (3), 223-232.
4. Kanchan, M., Keshav, S. & Tripathi, C.P.M. (2013).Management of pod borer (*Helicoverpa armigera*) infestation and productivity enhancement of gram crop (*Cicer aritenium*) through vermiwash with biopesticides.*World Journal of Agricultural Sciences ,* 9(5), 401-408 . DOI: 10.5829/idosi.wjas.2013.9.5.1749.
5. Sudeshna Thaku & Sood, A.K.(2019). Lethal and inhibitory activities of natural products and biopesticide formulations against Tetranychusurticae Koch (Acarina: Tetranychidae). *International Journal of Acarology*, 45 (6-7), 381-390, DOI: 10.1080/01647954.2019.1666920.
6. Agarwal, H., Venkat Kumar. S. & Rajesh Kumar, S. (2018). Antidiabetic effect of silver nanoparticles synthesized using lemongrass (Cymbopogon citratus) through conventional heating and microwave irradiation approach. *Journal of Microbiology, Biotechnology and Food Sciences*,7(4), 371-376.
7. Akinnuoye-Adelabu, D. B., Hatting, J, C., De Villiers, Terefe, T. & Bredenhand E. (2019). Effect of redworm extracts against fusarium root rot during wheat seedling. *Agronomy Journal*, 111 (5), 2610-2618. https:// doi.org/ 10.2134/ agronj2018 .11.0743.
8. Govindarajan, B. & Prabakaran, V. (2012). Antibacterial activity of vermiwash of *Eisenia fetida* (Earthworm). *International Journal* of *Biological Technology*, 3(3), 15-16.
9. Rai, M. & Ingle, A. (2021).Role of nanotechnology in agriculture with special reference to management of insect pests. *Applied Microbiology and Biotechnology,* 94(2), 287 –293. https://doi.org/10.1007/s00253-012-3969-4.
10. Duran, N., Durán, M., de Jesus, M.B., Seabra, A. B., Favaro, W. J. & Nakazato, G.(2016) Silver nanoparticles: A new view on mechanistic aspects on antimicrobial activity. *Nanomedicine*,12(3), 789-799. doi: 10.1016/j.nano.2015.11.016.
11. Jain, Devendra, Daima, Hemant, Kachhwala, S. & Kothari, Shanker. (2009). Synthesis of Plant-Mediated Silver Nanoparticles using Papaya Fruit Extract and Evaluation of their Anti Microbial Activities. *Digest Journal of Nanomaterials and Biostructures,* 4, 557-563.

12. Aljabali, A.A.A., Akkam, Y.A.I.,Zoubi, M.S., Al-Batayneh, K.M., Al-Trad. B., Abo Alrob, O., Alkilany, A.M., Benamara, M. & Evans,D.J.(2018). Synthesis of gold nanoparticles using leaf extract of *Ziziphus zizyphus* and their antimicrobial activity. *Nanomaterials (Basel)*, 8(3), 174. doi: 10.3390/nano8030174.
13. Sarker, S.D., Nahar, L. & Kumarasam, Y.(2007). Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. *Methods*, 42(4), 321-4. DOI: 10.1016/j.ymeth.2007.01.006.
14. Gulnaza, A. & Savitha, G. (2013) .Evaluation of antimicrobial activity of leaf and stem extracts of sidda medicinal plant *Sidacordata. International Journal of Medicine and Pharmaceutical Sciences (IJMPS)*, 3(3), 39-50.
15. Bhutkar, M. A. & Bhise, S. B. (2012). In vitro assay of alpha amylase inhibitory activity of some indigenous plants. *International Journal of Chemical sciences*,10(1), 457–462. DOI: 10.31031/MAPP.2018.01.000518.
16. Kim, Y.M., Jeong, Y.K., Wang, M.H., Lee, W.Y. & Rhee, H.I. (2005). Inhibitory effect of pine extract on alpha-glucosidase activity and postprandial hyperglycemia. *Nutrition*, 21(6), 756-61. doi: 10.1016/j.nut.2004.10.014. PMI D: 15925302.
17. Lekshmi, Packia., Sahila, R., Bharath, S.& Dinesh Kumar, P.A. (2014). Synthesis of nanofibreans silver nanoparticles from coelomic fluid of earthworm eudriluseuginae and Pontoscolexcorethrurus and its antimicrobial potency. *Asian Journal of Pharmaceutical and Clinical Research*, 7 (1), 177-182.
18. Dwivedi, Amarendra Dhar & Gopal, Krishna.(2010). Biosynthesis of silver and gold nanoparticles using Chenopodium album leaf extract,*Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 369(1–3), 27-33 https://doi.org/10.1016/j.colsurfa.2010.07.020.
19. Guo, M., Li, W., Yang, F. & Liu, H. (2015). Controllable biosynthesis of gold nanoparticles from a Eucommia ulmoides bark aqueous extract. *Spectrochimica acta. Part A, Molecular and Biomolecular Spectroscopy*,142, 73-79. DOI: 10.1016/j.saa.2015.01.109.
20. Gopinath, V., MubarakAli, D., Priyadarshini, S., Priyadharsshini, N.M., Thajuddin, N. & Velusamy, P. (2012).Biosynthesis of silver nanoparticles from *Tribulus terrestris* and its antimicrobial activity. *A novel biological approach. Colloids and Surfaces B: Biointerfaces*,96, 69-74. DOI: 10.1016/j.colsurfb.2012.03.023.
21. Bhau, B.S., Sneha Ghosh., Sangeeta Puri., Borah, B.,Sarmah, D.K. & Raju Khan. (2015).Green synthesis Of gold nanoparticles from the leaf extract of nepenthes khasiana and antimicrobial assay. *Advanced Materials Letters*, 6(6), 55-58.
22. Basavegowda, Nagaraj & Sobczak-Kupiec, Agnieszka & Fenn, Rebecca & Dinakar, Salman. (2013). Bioreduction of chloroaurate ions using fruit extract Punica granatum (Pomegranate) for synthesis of highly stable gold nanoparticles and assessment of its antibacterial activity. *Micro & Nano Letters, IET*. 8,: 400-404. 10.1049/mnl.2013.0137.
23. Paulkumar, K., Gnanajobitha, G., Vanaja, M., Rajeshkumar, S., Malarkodi, C., Pandian, K. & Annadurai, G. (2014). Piper nigrum leaf and stem assisted green synthesis of silver nanoparticles and evaluation of its antibacterial activity against agricultural plant pathogens. *The Scientific World Journal*., (7), 829-894 https:// doi.org/10.1155/201 4/829894.
24. Garibo, D., Borbón-Nuñez, H.A., de León, J.N.D. *et al.* (2020).Green synthesis of silver nanoparticles using *Lysilomaacapulcensis* exhibit high-antimicrobial activity. *Sci Rep,* 10(1),12805 , https://doi.org/10.1038/s41598-020-69606-7.
25. Thanh-Truc Vo., Thi Thanh-Ngan Nguyen., Thi Thanh-Tam Huynh., Thi Thuy-Trang Vo., Thi Thuy-Nhung Nguyen., Dinh-Truong Nguyen., Van-Su Dang., Chi-Hien Dan & Thanh-Danh Nguyen.(2019). Biosynthesis of silver and gold nanoparticles using aqueous extract from *Crinum latifolium* leaf and tTheir applications forward antibacterial effect and wastewater treatment. *Journal of Nanomaterials,* (4):1-14 https://doi.org/10.1155/2019/8385935.
26. Badeggi, U.M., Badmus, J.A., Botha, S.S., Ismail, E., Marnewick, J.L., Africa, C.W.J. & Hussein,A.A.(2020) Biosynthesis, Characterization, and biological activities of procyanidin capped silver nanoparticles. *Journal of Functional Biomaterials*, 11(3), 66. https://doi.org/10.3390/ jfb11030066.
27. Singh, R., Sahu, S. &Thangaraj,M.(2014). Biosynthesis of Silver Nanoparticles by Marine Invertebrate (Polychaete) and Assessment of Its Efficacy against Human Pathogens. *Journal of Nanoparticles*, (2), 1-7. http: // dx. doi. org/ 10.1155/2014/ 718240.
28. Chidambaram Jayaseelan., Rajendiran Ramkumar., Abdul AbdulRahuman, & Pachiappan Perumal. (2013). Green synthesis of gol nanoparticles using seed aqueous extract of Abelmoschus esculentus and its antifungal activity. *Industrial Crops and Products,* 45, 423–429. Doi:10.10 16/j.indcrop.2012.12.019.
29. Nadana, G. Raja Vadivu., Rajesh, C., Kavitha, A., Sivakumar, P., Sridevi, G., &Palanichelvam, K. (2020). Induction of growth and defense mechanism in rice plants towards fungal pathogen by eco-friendly coelomic fluid of earthworm. *Environmental Technology & Innovation, 19*, 101011.doi: 10.1016/j.eti.2020.101011 .
30. Thatoi,P., Kerry, R.G., Gouda, S., Das, G., Pramanik, K., Thatoi, H. & Patra, J.K. (2016). Photo-mediated green synthesis of silver and zinc oxide nanoparticles using aqueous extracts of two mangrove plant species, Heritiera fomes and *Sonneratia apetala* and investigation of their biomedical applications .*Journal of Photochemistry and Photobiology B: Biology*,163,311-8. doi: 10.1016/j.jphoto biol.2016.07.029.
31. Vishnu Kiran, M.& Murugesan.S.(2013). Biogenic silver nanoparticles by Halymeniaporyphyroides and its in vitro anti-diabetic efficacy. *J. Chem. Pharm. Res*, 5(12),10018.
32. Manam, D.V.K.& Murugesan, S .(2014).Biological synthesis of silver nanoparticles from marine alga *Colpomeniasinuosa* and its in vitro anti-diabetic activity.*American Journal of Bio-pharmacology Biochemistry and Life Sciences (AJBBL)*, 3(1), 1-7.
33. Debnath, G., Das, P.&Saha, A.K.(2020). Characterization, Antimicrobial and α-Amylase Inhibitory Activity of Silver Nanoparticles Synthesized by using Mushroom Extract of *Lentinus tuber-regium*.Proceedings of the National Academy of sciences*. Sect. B Biological Sciences*, 90(1), 37–45. https://doi.org/10.1007/s40011-019-010 76.