Antimicrobial activity of silver nanoparticles synthesized from fruit epicarp of *Glycosmis pentaphylla*

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Abstract: Our study aims to characterize and assess the antimicrobial effect of silver nanoparticles (AgNPs) synthesized from the fruit epicarp of *Glycosmis pentaphylla* against few crops and human pathogens. Our study suggests a novel method for biosynthesis of silver nanoparticles from the epicarp of *Glycosmis pentaphylla*. The study confirms the ability of the fruit epicarp extract of *Glycosmis pentaphylla* for the biosynthesis of silver nanoparticles grown under in-vitro conditions. The green synthesis of AgNPs from (Ethyl alcohol) EtOH extracts of *Glycosmis pentaphylla* was performed through standard protocols. The synthesized AgNPs were confirmed by colour changes (green to brown) within <10 minutes and characterized by UV-visible spectral, SEM and TGA analysis (Scanning electron microscopy, Thermal gravimetric analysis). Antimicrobial activities of the silver nanoparticles were performed by agar well diffusion method against crops pathogenic fungus and human pathogenic bacteria. The highest antifungal activities of silver nanoparticles were found against *Colletotrichum lindemuthianum* and *Alternaria alternata*. The antibiotic activity was measured through the zone of inhibition against *S. aureus* (18 mm), *S. typhimurium* (17.33 mm), *S. mutans* (17 mm) and *E. coli* (17 mm). The antimicrobial potential of AgNPs was determined by minimum inhibitory concentration (MIC), minimum fungicidal concentration (MFC) and minimum bactericidal concentration (MBC) tested against human and plant pathogens. In addition, AgNPs displayed the significant synergistic antibacterial effect when it combined with Streptomycin and Ciprofloxacin in the ratio of 1:1. This eco-friendly, biocompatible and sustainable phytofabrication approach of bioactive AgNP synthesis is a progressive step towards various applications to control few crops (Chilli, and Tomato) and human pathogens in near future.

Keywords: *Glycosmis pentaphylla*, fruit epicarp, silver nanoparticles, antimicrobial activity, synergistic effect
INTRODUCTION

The worldwide agricultural production gets compromised over the past few years due to crop pathogens. The harmful effects on fruits and vegetables of these pathogens have compromised the quality of the crops along with economical loss globally. The consumption rate of fruits and vegetables has increased up to 40% during the past few years. Every year, the amount of loss is approximately 20% of all fruits and vegetables. The fresh fruits and vegetables are exposed to contamination by microorganisms, especially plant fungi from direct contact with soil, dust, water during harvesting or post-harvesting. Now a day’s the traditional antibacterial treatment against the growing resistance of human pathogenic bacterial strains has become a big challenge. The microbial infection is a serious problem in the agriculture and healthcare sector worldwide. Therefore, it is needed to develop a new antimicrobial agent including different characteristics, such as eco-friendliness, cost-effective, and biocompatibility and good compatibility. In this situation, nanoparticles (NPs) have accepted as alternative to chemical pesticides worldwide, due to their electrostatic attraction between positively charged NPs and negatively charged microbial cells, and a large surface to volume ratio, resulting in improved physicochemical properties and enhanced antimicrobial activities of the NPs. The antibacterial and antifungal properties of NPs have recently been widely reported. The application of metal nanocomposites enhanced antimicrobial activity against multi-drug resistance bacterial infection. The antibiotics could be more effective when combined with metal NPs under specific conditions. The fruit epicarp extract was prepared by taking 50 g of dry dust fruit epicarp in a 500 ml conical flask with 100 ml of different solvents to analyze its photochemical properties. The biochemical analysis was done for various chemicals estimated, such as total phenolics, flavonoids, tannin, saponins, phytate, and oxalate. The dry fruits epicarp were crushed in dust form and extracted in different solvents to analyze its photochemical. The fruit epicarp extract was prepared by taking 50 g of dry dust fruit epicarp in a 500 ml conical flask with 100 ml of 30% ethanol (EtOH) for 24 hrs at 40°C room temperature. The crude extracts were filtered through Whatman’s No.-1 filter paper and stored at 4°C for the synthesis of AgNPs.

2. MATERIALS AND METHODS

2.1. Plant sample collection and extract preparation

The ripened fruits of Glycosmis pentaphylla were collected from the college ground of Sreegopal Banerjee College, Bagati, Mogra, Howrah, India. Identification and authentication of Glycosmis pentaphylla was done by Dr. Monoranjan Chowdhury, Associate Professor, Taxonomy of Angiosperm and Biosystematic Laboratory, Botany Department, University of North Bengal, India, with the voucher number SBC/132/2017. The fleshy epicarp separates from the fruit and dries in hot air oven at 40°C. The dry fruits epicarp were crushed in dust form and extracted in different solvents to analyze its photochemical. The fruit epicarp extract was prepared by taking 50 g of dry dust fruit epicarp in a 500 ml conical flask with 100 ml of 30% ethanol (EtOH) for 24 hrs at 40°C room temperature. The crude extracts were filtered through Whatman’s No.-1 filter paper and stored at 4°C for the synthesis of AgNPs.

2.2. Phytochemical analysis of the crude extract

The fruit epicarp extract was dipper in different solvents for extraction of different secondary chemicals. The biochemical analysis was done for various chemicals estimated, such as total phenolics, flavonoids, tannin, saponins, and alkaloids. Determination of each biochemical analysis was repeated three times and expressed in a percent dry weight basis.

2.3. Synthesis of Silver Nanoparticles (AgNPs)

The aqueous solution of 1 mL silver nitrate (AgNO₃) [analytical grade (AR), purchased from E. Merck (India)] was prepared and used for the synthesis of AgNPs. The fruit epicarp extract was added to the 1 mL silver nitrate solution, and the mixture was kept for 24 hrs at room temperature with occasional stirring. The brownish color of the mixture indicated the successful synthesis of AgNPs.
epicarp extract (5 ml) was added into 50 ml of an aqueous solution of 1 mM AgNO₃ for reduction of Ag⁺ to Ag⁰. The reaction mixture was incubated (15 minutes) at room temperature till the turn up of green to brown colour. The particles were isolated by centrifugation (6,000 rpm up to 15 minutes), repeated washing and drying at 75°C for further characterisation.

2.5. Characterization of synthesized AgNPs using UV-visible spectrophotometer

The reduction of Ag⁺ to Ag⁰ was monitored by measuring the UV-Vis spectrum of each reaction mixture at different time intervals (10, 20, 30, 40, 50, 60, 120, 180, 240, 300 minutes) within the range of 370-500 nm in the UV-Vis spectrophotometer (Shimadzu UV-Vis Spectrophotometer, Japan). MBC of aqueous solution of AgNO₃ and green synthesized AgNPs solution exhibited λₘₓ at about 220 nm and 430 nm, respectively.

2.6. Characterization of Synthesized AgNPs by Scanning Electron Microscopy

Morphological characterization of AgNPs was done by Scanning electron microscopy. For SEM analysis, the EtOH is used as a blank reference. The isolated dried and powdered AgNPs were used for SEM study. A thin film of each sample was prepared separately on a small glass cover slip (2×3 mm), and set on a copper stub for electron microscopy using Hitachi made Scanning Electron Microscope (SEM) (Model: S530 with IB2 ion coater, Japan).

2.7. Characterization of synthesized AgNPs using Thermal Gravimetric Analysis (TGA)

TGA analysis of the synthesized AgNPs was also observed with an increasing temperature range of 160–550°C.

2.8. In vitro antifungal activity of green synthesized AgNPs

The antifungal activity of bio-synthesized AgNPs was tested against various crop pathogens according to Loo et al., 2018, agar well diffusion method. The test sample was serially diluted up to 5.26 µg/ml and 30% ethanol was serving as control. The spore suspension of test fungus was prepared by scraping the spores from 7-day-old PDA slant culture. 10 µl spore suspension was picked up from slant through micropipette, checked the CFU and poured into each fresh Potato dextrose agar plate. 10 µl of the test samples from each concentration were loaded into the 5 mm diameter well in five test fungus plates and incubated at 28°C for 48 hrs. The MIC end-point criterion was defined as the lowest AgNPs concentration showing no visible growth after 48 hrs incubation. MIC values were calculated by comparing the germination of spores in PDA plates containing different concentrations of AgNPs. The MFC was determined from the concentration of the compound in which no fungal growth was found. To determine MFC, 2, 4, 8, and 10 times higher concentrations of MIC were taken and the colony-forming units (CFU) were counted after 24 hrs of incubation at 37°C to observe complete growth inhibition of the fungal organisms.

2.9. Determination of Minimal Inhibitory Concentration (MIC) and Minimal Fungicidal Concentration (MFC)

The MIC values of synthesized AgNPs against four Bacterial pathogens were determined using the standard protocol. The stock AgNPs sample were serially diluted up to 5 µg/ml and MIC values were measured using NA media. 10 µl of the test sample was loaded into the well of pre-inoculated nutrient agar plate of target bacteria, incubated for 24 hrs at 37°C and observed for zone of growth inhibition. The MBC was determined by checking the viability of the bacterial cells after treating with 2 × concentration of MIC of AgNPs and dilution plating on a nutrient agar plate. In brief, the actively growing bacterial strains (log phase growth) were treated with test samples (different AgNPs samples) at higher concentration of MIC and incubated for 1 hr. The treated culture was then plated on a nutrient agar plate at a dilution of 10⁻⁵ to 10⁻¹ in triplicates and incubated in similar conditions for observation for any viable colony formation. MBC is noted as the concentration where no viable cells were noticed.

2.10. In Vitro antibacterial activity of green synthesized AgNPs

2.10.1. Antibacterial activity by the agar well diffusion method

Assessment of antibacterial activity of AgNPs sample against two Gram-positive bacteria (Bacillus subtilis, Staphylococcus mutans) and two Gram-negative bacteria (E. coli and Salmonella typhimurium) was measured by the agar well diffusion method. 8 mm wells were cut in each fresh inoculated bacterial plate and 10 µl of different concentration of the test sample was loaded into the 5 mm diameter well seeded with test bacteria and incubated for 24 hrs at 37°C. The potency was compared by measuring zone diameter of growth inhibition with standard antibiotics, Streptomycin (10 µg/ml).

2.11. Determination of Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC)

The MIC values of synthesized AgNPs against four Bacterial pathogens were determined using the standard protocol. The stock AgNPs sample were serially diluted up to 5 µg/ml and MIC values were measured using NA media. 10 µl of the test sample was loaded into the well of pre-inoculated nutrient agar plate of target bacteria, incubated for 24 hrs at 37°C and observed for zone of growth inhibition. The MBC was determined by checking the viability of the bacterial cells after treating with 2 × concentration of MIC of AgNPs and dilution plating on a nutrient agar plate. In brief, the actively growing bacterial strains (log phase growth) were treated with test samples (different AgNPs samples) at higher concentration of MIC and incubated for 1 hr. The treated culture was then plated on a nutrient agar plate at a dilution of 10⁻⁵ to 10⁻¹ in triplicates and incubated in similar conditions for observation for any viable colony formation. MBC is noted as the concentration where no viable cells were noticed.

2.12. Synergistic activity with antibiotics

To performed this experiment, 6 mm diameter sterile Whatman No.-1 filter paper discs were soaked with the AgNPs sample at MIC values (5.5 µg/ml) and filter sterilized antibiotic solutions at MIC values (i.e. Streptomycin, 0.5 µg/ml and Ciprofloxacin, 0.5 µg/ml) and placed at the centre of the each culture plate seeded with target bacterium and incubated at 37°C for 24 hrs and were observed growth inhibition. The synergistic potential was determined by comparing the magnitude of antibacterial activity of AgNPs and antibiotics with the antibiotics alone using the following formula: FI-Fold Increase (FI) = [(b−a)/a] × 100; where, 'b' stands for...
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3. STATISTICAL ANALYSIS

All the data of phytochemical regime and antimicrobial activity were analyzed using one way ANOVA, Tukey HSD and Pearson correlation. All the statistical analysis was performed using the statistical program SPSS v. 13.0 (SPSS, 2004).

4. RESULTS AND DISCUSSION

4.1. Phytochemical Regime

The biochemical analyses of fruit epicarp extract represent variation in secondary (Phenols, flavonoids, tannin, saponins, alkaloids, phytate and oxalate) metabolites which are very much similar to the Roy and Barik, 2010 experiment. The phytochemical regime of the plant is presented in Fig.1. All Secondary chemicals were higher than other plant parts.

Fig. 1: Phytochemical variations of *Glycosmis pentaphylla* fruit epicarp extract (Mean ±5 observations).

4.2. UV-VIS spectroscopy characteristics of AgNPs

During the green synthesis of AgNPs through the fruit epicarp extract, changes colour from green to brown as previously reported by researchers (Fig. 2) Parvekar et al, 2020. The brown colour due to the reduction of Ag$^+$ confirms the formation of AgNPs and was characterized by UV-Vis Spectroscopy as in Roy and Barik, 2010. The reduction of pure silver ions (Ag$^+$) was estimated by UV-Vis spectral analysis in the frequency range of 270 to 500 nm at room temperature and which was represented the peak at around 420-430 nm for a long time interval (10-300 minutes) specific for the synthesis of AgNPs with longer stability (Fig. 3). The band at 420 - 430 nm can be attributed to the property surface plasmon resonance (SPR) due to the oscillation of electrons (Mie scattering) for the strong interaction of light with the AgNPs. In both cases EtOH act as blank. The $\lambda_{max}$ of AgNPs was observed at around 430 nm whereas in EtOH extract it was at around 380 nm, respectively within the time span of 10-300 minutes. The UV-visible spectra showed absorption bands in the 350 to 355 nm region which confirms the formation of AgNPs.

Fig. 2: The colour changes from green (A) to brown (B and C) during the reaction of Ag$^+$ into AgNPs due to the photochemical present in fruit epicarp extract of *Glycosmis pentaphylla.*
Fig. 3: UV-Vis absorption spectra recorded at different time intervals (10 min, 30 min, 1h, 24h, 48h, 72h) of AgNPs synthesized from fruit epicarp extract *Glycosmis pentaphylla* (Mean of 3 observations).

4.3. SEM and TGA characteristics of AgNPs

Microscopic surface features including morphology and particle size of synthesized AgNPs was assessed by SEM analysis. The SEM image provided roughly spherical topography of AgNPs was about 75±3 nm in size (Fig. 3). The SEM image also confirms that the synthesized nanoparticles are well separated with no aggregation (Fig. 4). TGA data of the synthesized AgNPs showed steady weight loss due to desorption of its bioorganic compounds with an increasing temperature range of 160–550˚C. Previously reported SEM images of AgNPs from different extracts showed spherical particles, aggregated spherical particles, irregularly shaped particles, and cubic particles. The moderate particles sizes observed were 40, 41, 42, 43, 44, and 50 nm for AgNPs synthesized by different biological extracts using water as a solvent. This observation aligns well with the previously reported particle sizes 40, 41, 42, 44, 47. In an article comparing the advantages and drawbacks of these methods and the applications of nanoparticles in various domains, a synthesis of chemical, physical, and biological methods for obtaining AgNPs of different shapes and dimensions (from 2 to 300 nm) was described 48.

Fig. 4: The SEM images of AgNPs synthesized from fruit epicarp extract *Glycosmis pentaphylla* at 25.0 kV × 1 k.
Fig. 5: TGA of the synthesized AgNPs showed steady weight loss within the temperature range of 160–550°C.

4.4. *In vitro* antifungal potentiality of AgNPs

The antimicrobial activities of AgNPs against various crop pathogenic fungi were investigated as shown in Fig. 6. The biosynthesized AgNPs inhibits the growth of *Fusarium oxysporum*, *Fusarium moniliforme*, *Alternaria alternata*, *Colletotrichum lindemuthianum* and *Colletotrichum gloeosporioides*. Thus, AgNPs could be considered as excellent broad-spectrum antifungal agents for sustainable crop production and also could potentially be used widely in clinical applications against human pathogenic fungi. The various research reports of AgNPs synthesized from plants and microbes had broad range antifungal activity against *Fusarium oxysporum*, *Fusarium moniliforme*, *Fusarium tricinctum*, and *Alternaria* sp. by agar well diffusion method. The antifungal potentiality in other reports of AgNPs showed against crop pathogens such as *Aspergillus niger*, *Rhizoctonia solani*, *Curvularia lunata*, *Colletotrichum* sp. and *Fusarium* sp.

Fig. 6: Antifungal activity of AgNPs in PDA media by agar well diffusion method; (A) *Colletotrichum lindemuthianum*; (B) *Alternaria alternata*; (C) *Colletotrichum gloeosporioides*; (D) *Fusarium moniliforme*

4.5. Determination of MIC and MFC of AgNPs

The minimum inhibitory concentration and minimum fungicidal concentration of AgNPs for five different fungal strains as shown in Table 1. The results suggest that the plants synthesized AgNPs are capable of inhibiting crop fungi like *Fusarium oxysporum*, *Fusarium moniliforme* *Alternaria alternata*, *Colletotrichum lindemuthianum* and *Colletotrichum gloeosporioides*. The highest MIC values shown in *Alternaria alternata*, *Fusarium oxysporum*, *Fusarium moniliforme* was 6.2 µg/ml and the highest MFC values was 7.14 µg/ml. Green synthesized silver nanoparticles had antimicrobial effects against *A. flavus*, *F. oxysporum* and *P. digitatum* on PDA in vitro. Inhibition (97.3%) was obtained against *A. flavus* treated with a 10 µg/ml concentration of silver nanoparticles and the minimal level of inhibition was found against human pathogenic fungi.
against P. digitatum and F. oxysporum with 2 µg/ml concentrations of AgNPs. This could be possible to adhere AgNPs to fungal hyphae and deactivate plant pathogenic fungi. DNA loses its ability to replicate upon treatment with Ag+ resulting in inactivated expression of ribosomal subunits proteins, as well as certain other cellular proteins and enzymes essential to ATP production.

### Table 1: MIC and MFC values of synthesized AgNPs against different fungal pathogens

<table>
<thead>
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<th>Name of the crop pathogens</th>
<th>Concentration of AgNPs (µg/ml)</th>
<th>MIC</th>
<th>MFC</th>
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<td></td>
<td>50 µg/ml</td>
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<tr>
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<td>16.67 µg/ml</td>
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<td>Colletotrichum gloeosporioides</td>
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<td>Fusarium moniliiforme</td>
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<td>Fusarium moniliiforme</td>
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<tr>
<td>Fusarium oxysporum</td>
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Here ‘+’ indicated the positive inhibition zone and ‘-’ indicated the absence of inhibition zone.

### 4.6. Antimicrobial Activity of AgNPs

The application of AgNPs against human pathogenic bacteria showed significant growth as shown in Fig. 7. The antibacterial activity was measured through the zone of inhibition against B. subtilis (18 mm), S. typhimurium (17.33 mm), S. mutans (17 mm) and E. coli (17 mm) which was shown in Fig. 8. It was observed that the AgNPs was a more potent antibacterial compound than other. The comparison of a single application of AgNPs, Streptomycin, Gentamicin and Ciprofloxacin against the Gram-positive and Gram-negative bacterial strains which was shown in Fig. 9. The results showed that antibiotics and AgNPs have more or less parallel potency by means of formation of inhibition zone (mm) by the application of the same volume (10 µl) and same concentration (6 µg/ml). The antimicrobial activity of silver nanoparticles was previously reported to penetrate the cell wall of bacteria and kill them. Due to the presence of a thin peptidoglycan layer in cells of Gram-negative bacteria show potent higher than Gram positive. Anyhow, our study, carryout the new features of antibacterial.

![Figure 7: Agar well diffusion assay of the AgNPs.](image)

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**Figures/Tables Foot notes**

- **Figures/graphs:** Kindly ensure that all the figures should have respective figure number with caption and suitable foot notes (explaining the figures/graph).

In this example: Here ‘+’ indicated the positive inhibition zone and ‘-’ indicated the absence of inhibition zone.

**Other examples**

![Figure 6: Effect of various extracts on urine volume in 24 h. versus normal control.](image)

**Table 1. Level of Neuropeptide NE, DA and 5-Hydroxytryptophan fresh water fish C. gariepinus exposed to 2**

<table>
<thead>
<tr>
<th>Control</th>
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<tr>
<td>DA</td>
<td>0.98</td>
<td>1.06</td>
<td>1.12</td>
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<tr>
<td>5-HT</td>
<td>0.89</td>
<td>0.97</td>
<td>1.03</td>
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The data are displayed as mean ± standard error. Bar with the same letters indicate no significant differences according to Tukey (HSD) test (P < 0.05). Here alphabets a, b, c, d, e, f, g, h indicates bar with standard error.

Fig 8: Antibacterial assessment of the AgNPs. Here, the data were the average diameter of inhibition zone of triplicate trials.

Fig 9: Potency of antimicrobial activity of AgNPs, Streptomycin, Gentamycin and Ciprofloxacin against the Gram-positive and Gram-negative bacterial strains.

4.7. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The results of Table 3 showed that the MIC values varied from 6~5 µg/ml. The MIC values for B. subtilis and E. coli was 5 µg/ml and 5.26 µg/ml MIC values for S. mutans and S. typhimurium respectively. Similarly, the MBC value for B. subtilis and E. coli were 5.26 µg/ml and for S. mutans and S. typhimurium was 5.55 µg/ml. From the above observations, it is very much clear that AgNPs have the highest antibacterial effect which causes the highest interaction with the bacterial cell wall. Similar observation on antibacterial activity was also observed by the previous studies. We found no particular trend of antibacterial effect for four pathogenic bacteria. Antibacterial activity of AgNPs was observed highest in Gram-negative bacteria than Gram-positive due to the presence of a thick peptidoglycan layer.

Phytobiocatalysed AgNPs with antimicrobial properties have also been investigated against different microbes which actually depend on size, shape, environmental conditions (pH, ionic strength) and capping agent. Recently, efficient antimicrobial activity of green AgNPs was observed against multi drug resistant (MDR) and highly pathogenic bacteria (P. aeruginosa, S. aureus, S. typhi, S. epidermidis and E. coli) and fungi (C. gloeosporioides). Our study shows that S. mutans (Gram positive) and S. typhimurium (Gram negative) were most sensitive which indicates the mode of action was not only affected by cell wall thickness of the bacteria. Ag⁺ release from AgNP is another reason for antibacterial activity. As the smaller AgNPs have the higher surface areas associated with the faster release of Ag⁺ and exert higher toxicity. The high affinity of Ag⁺ towards protein thiol groups of respiratory enzymes inactivated enzymes even died out. AgNPs also plays an important role in biocompatibility as it controls the interaction of AgNP with a living organism.
The application of Streptomycin with AgNPs showed the highest increasing fold 38.24% against B. subtilis. Similarly, the combined application of Ciprofloxacin showed the highest fold increase 20% against B. subtilis followed by 24.64%, 15.95% and 5.67% against S. mutans and S. typhimurium. When bacteria acquire antibacterial resistance, synergistic effect plays an important role as AgNPs and antibiotics kill bacteria in different mechanisms. Our study reveals that, synthesized AgNPs is able to decrease the concentration of Streptomycin and Ciprofloxacin against S. mutans & S. typhimurium with lowering the side effects and cost-effectiveness of antibiotics.

4.8. Synergistic effect of AgNPs

The combined effect of antibiotics and AgNPs against different human pathogenic bacteria was observed in Table 3. The highest inhibition zone was observed in dual (AgNPs + antibiotic) application than single (AgNPs). The combined effect against bacteria increased the diameter of the inhibition zone that may be possible due to binding between antibiotics and AgNPs as the antibiotics generally contain active groups like hydroxy or amino, which bind AgNPs by chelation. The application of Streptomycin with AgNPs showed the highest increasing fold 38.24% against E. coli followed by 31.89, 25.20%

Table 3: Synergistic effect of two antibiotics with AgNPs against B. subtilis, S. mutans and E. coli, S. typhimurium; Fl-fold increase F1% = [(b - a)/a] × 100

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Name of the Pathogens</th>
<th>Inhibition zone diameter (mm) for AgNPs</th>
<th>Inhibition zone diameter (mm) for Streptomycin (AB)</th>
<th>Inhibition zone diameter (mm) for Ciprofloxacin (AB)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Only AB (a)</td>
<td>AgNPs (b)</td>
<td>Only AB (a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhibition zone diameter (mm)</td>
<td>FI %</td>
<td>Inhibition zone diameter (mm)</td>
</tr>
<tr>
<td>1</td>
<td>B. subtilis</td>
<td>21.000 ± 0.577</td>
<td>20.000 ± 0.577</td>
<td>25.000 ± 0.577</td>
</tr>
<tr>
<td>2</td>
<td>S. mutans</td>
<td>20.000 ± 0.577</td>
<td>23.333 ± 0.577</td>
<td>31.000 ± 0.577</td>
</tr>
<tr>
<td>3</td>
<td>E. coli</td>
<td>20.000 ± 0.577</td>
<td>22.667 ± 0.577</td>
<td>31.000 ± 0.577</td>
</tr>
<tr>
<td>4</td>
<td>S. typhimurium</td>
<td>19.000 ± 0.577</td>
<td>23.333 ± 0.577</td>
<td>32.000 ± 0.577</td>
</tr>
</tbody>
</table>

The data are displayed as mean ± standard error according to Tukey (HSD) test (P <0.05)

5. CONCLUSION

The biosynthesis of silver nanoparticles from different parts of plant is low cost, safe, environmentally friendly, less time consuming, and it provides effective satisfactory results without any hazardous chemicals involved. In the present study, AgNPs were successfully synthesized through the green technique at normal room temperature. The SEM studies confirmed that the concentration of the fruit epicarp extract is highly efficient in controlling the shape and size of AgNPs structures. TGA was detecting the steady weight loss due to desorption of its biogenic compounds with increasing temperature. The synthesized AgNPs are lysis the cell wall integrity against pathogenic bacteria and few crop fungi. The applications of AgNPs and antibiotics together improved its efficiency to reduce the dose and also its cost. These results not only provide a new approach for integrative control of plant pathogens but also reduce or avoid the use of various drugs. From the application point of view, these AgNPs could be used as biofungicide for sustainable agriculture and biomedical use against human pathogenic bacteria in future studies.

6. FUNDING ACKNOWLEDGMENT

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7. AUTHORS CONTRIBUTION STATEMENT

Swapan Kumar Chowdhury designed the whole study including sample collection, antibacterial assay, antifungal assay, synergistic effect at Department of Botany, Sreegopal Banerjee College and prepared the manuscript. Nayan Roy conducted statistical analysis, synthesis of AgNPs, Characterization and prepared contribution part of manuscript. Indrani Mukherjee prepared the part of the manuscript. All the authors read and approved the final version of the manuscript.

8. CONFLICT OF INTEREST

Conflict of interest declared none.
REFERENCES


Saxty M, Mayya KS, Bandyopadhyay K. PH dependent changes in the optical properties of carboxylic acid derivatized silver colloidal particles. Colloids Surf A Biotechnology


Biotechnology
OTHER COMMENTS

1. **CONTENT IN THE SUB HEADING**
   - No subheadings should have very less content. At least ensure that all the subheadings should at least have 60 words.

2. **FOR PLANT OR PHYTO CHEMICAL STUDY OR ANY RELEVANT STUDY**
   - For plant or phyto chemical study or any relevant study, plant material should be authenticated by suitable botanist or Pharmacognosist or phyto chemistry
   - Authentication done by whom ?? (For Plant)
   - Example

   ![Plant Material Authentication Example](https://example.com/Plant_Material_Example)

3. **For studies on specific animals**
   - For studies on specific animals such as fishes, nematodes, insects etc, kindly provide authentication by zoologist for their zoological name.

4. **Ethical Committee Approval for Animals**
   - Kindly ensure that you include ethical committee approval for your animal study with registration or reference number. See the following examples.
   - Examples:

   ![Ethical Committee Approval Example](https://example.com/Ethical_Committee_Approval_Example)

5. **Ethical committee approval for the Patient/human testing**
   - If your paper is related to patients or human testing, kindly include the Institutional permission statement and / or Human Ethical approval committee reference number for your study in the materials and methods. Mention which protocol was followed (Helmsky declaration or any other) for conduction of the study. Also ensure and include appropriate sentence for getting a written patients consent for this study. See some of the following examples.
   - Examples
6. **INCLUSION AND EXCLUSION CRITERIA**

- For patients or treatments using humans you need to provide INCLUSION CRITERIA and EXCLUSION CRITERIA.

- The following link will give some idea about inclusion and exclusion criteria,
  
  
  
  - [https://libguides.city.ac.uk/postgraduate_research/criteria](https://libguides.city.ac.uk/postgraduate_research/criteria)
  

- **Example**

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age 18 years and older</td>
<td>Unable to breastfeed due to illness or delivery complications</td>
</tr>
<tr>
<td>African American (qualitative only)</td>
<td>Taking breastfeeding-contraindicated medications or substances</td>
</tr>
<tr>
<td>38 weeks’ gestation and older</td>
<td>Diagnosis of human immunodeficiency virus</td>
</tr>
<tr>
<td></td>
<td>Department of Social Services involvement</td>
</tr>
<tr>
<td></td>
<td>Non-English speaking</td>
</tr>
<tr>
<td></td>
<td>Admitted to the neonatal intensive care unit</td>
</tr>
<tr>
<td></td>
<td>Congenital abnormalities that prevented breastfeeding</td>
</tr>
<tr>
<td></td>
<td>Died</td>
</tr>
</tbody>
</table>
7. **Placement of Tables/Figures/graphs at appropriate places**

- All figures should be clear (not less than 300 dpi)
- Place all your tables/figures/graphs at or nearby the places were you are explaining or mentioning them.

8. **Number of References**

- There should be minimum 25 references and atleast 5 references should be of recent references
9. Discussion:

- Each and every sentences mentioning any earlier studies for discussing for your results should have respective reference number citation. Results should be discussed in support of citing references. Try to cite many references in support of your result interpretations from your result. Ensure that you have atleast 15 references cited in the discussion. See the following example.

Zeta potential is an important physicochemical parameter that influences the physical stability of colloidal systems. Generally, a colloidal system with zeta potential above +30 mV or below −30 mV is considered to be stable. In our study, zeta potential of the prepared VPT loaded liquid microsomes was determined to be −21.5 mV. The negative charge of liquid microsomes could be due to the trace amount of free oleic acid existed in commercial GMO. However, after surface modification with CS and crosslinking by glutaraldehyde, the reconstituted chito-cubesomes reversed to positive charge with a zeta potential of +35.9 mV. Such charge should be ascribed to the protonation of positive charged CS.

For any query or help or assistance kindly contact us.