Green Synthesis of Antimicrobial Silver Nanoparticles using Fruit Extract of *Glycosmis Pentaphylla* and its Theoretical Explanations.

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Research

Keywords: *Glycosmis pentaphylla* fruit, Silver nanoparticles, Antibacterial, Antifungal, Synergistic effect, Bavistin

Posted Date: February 9th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-200301/v1>

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Version of Record: A version of this preprint was published at Journal of Molecular Structure on January 1st, 2022. See the published version at <https://doi.org/10.1016/j.molstruc.2021.131361>.

Abstract

The present study reports a novel, one-pot, cost-effective, green synthesis route of silver nanoparticles (AgNPs) from the fruit extract of *Glycosmis pentaphylla* (FGP). The UV–vis spectroscopy (UV-Vis), dynamic light scattering (DLS) and transmission electron microscopy (TEM) studies confirmed that the synthesis produces stable, monodispersed AgNPs with an average size of 17 nm. Theoretical simulation using density functional theory (DFT) established that among the different compounds of FGP, arborine is mainly responsible for the stabilization of AgNPs with a binding energy of 58.45 kJ/mol. Synthesized AgNPs showed strong antifungal and antibacterial activity. The synergistic study of AgNPs with fungicide Bavistin and antibiotic Streptomycin produced remarkable morphological abnormalities of *A. alternata* as observed under the light microscope. Hence, the AgNPs synthesis approach is a progressive step towards various applications to soon control crop and human pathogens.

1. Introduction

Globally, fruit and crop production is under the risk of several biotic and abiotic factors resulting in a downfall in the targeted amount than expected. Disease caused by various pathogenic fungi is one of the main reasons for reducing agricultural production (Katan 2017). Thus, the quality of fruits and vegetables is decreasing along with economic loss due to these pathogens. In the last couple of years, the amount of loss of all fruits and vegetables is approximately 20%, whereas the consumption rate of fruits and vegetables is increased by 40% (Droby 2005). Therefore, minimizing the gap between produce and consumption is the greatest challenge to agricultural scientists. The fresh vegetables and fruits get exposed to several microorganisms (mainly plant fungi) during harvesting or post-harvesting storage (Angela Obaigeli Eni 2010). The most common fungal pathogens are *Alternaria alternata*, *Colletotrichum lindemuthianum*, *Fusarium moniliforme*, etc. (Gabriel et al. 2016; Ito et al. 2004; Martins et al. 2019; Möller et al. 1999; Volova et al. 2018). The utilization of pesticides is the most common practice to increase food and crop production, but pesticides interfere with the food chain (Hayat et al. 2019). Chemical pesticides are very much unsafe when they are used beyond the prescribed limit. Moreover, these chemicals create environmental hazards, and pathogens also develop resistance against these chemical fungicides. Hence, agricultural scientists are trying to find out some eco-friendly, inexpensive, innocuous and highly effective pesticides to trim down or eliminate chemical fungicides. On the other hand, candidiasis is another infectious disease caused by *Candida* sp. (Spanakis et al. 2010). These pathogens are also resistant to many anti-candida drugs due to their high biofilm-forming ability (Basavegowda and Lee 2013). The traditional antibacterial treatment for human beings against human pathogenic bacterial strains' growing resistance becomes a big challenge (Rodríguez and Sanchez 2010). Above 70% of bacterial infections show resistance to traditionally used antibiotics (Diekema et al. 2004). Thus, the upheaval of multidrug resistance bacteria became a global problem (Sondi and Salopek-Sondi 2004). These problems direct the whole scientific community to develop new antimicrobial agents with eco-friendly, low toxicity, antimicrobial potency, and excellent compatibility properties. In this situation, nanoparticles (NPs) can be an alternative to chemical pesticides worldwide due to its

electrostatic attraction towards microbial cells and a large surface to volume ratio(Jogaiah et al. 2019; Kavyashree et al. 2015). The antibacterial and antifungal properties of NPs have recently been widely reported (Dutta et al. 2020a; Dutta et al. 2019; Dutta et al. 2020d; Dutta et al. 2020j).

In recent years, the green synthesis of metal NPs gained significant attention due to its eco-friendly nature and massive application in the biomedical field(Barman et al. 2020; Chinnappan et al. 2018; Chowdhury et al. 2021). Among these NPs, silver nanoparticles (AgNPs) are gaining importance due to its significant biological applications like antibacterial(Govarthanan et al. 2014; Shao et al. 2019), antifungal(Dutta et al. 2020d), antidiabetic (Shao et al. 2019), anti-inflammatory(Ahmad et al. 2015), antiviral(Ragaseema et al. 2012), anti-angiogenesis(Vicente et al. 2011), antiplatelet(Kowshik et al. 2002) and anticancer(Dutta et al. 2020j) activities. The biological activity of AgNPs depends on its synthesis process. The synthesis of stable, eco-friendly, monodispersed, biocompatible AgNPs is a tremendous challenge to the researchers. Over the last decades, researchers are trying to find out different AgNPs synthesis routes to increase its biocompatibility, stability and versatile utility. Traditional methods of AgNPs synthesis like electrochemical, sonochemical, photochemical, etc. utilizes synthetic chemicals, and as a result, synthesized AgNPs are hardly biocompatible and eco-friendly(Chinnappan et al. 2018). In contrast, green synthesis of AgNPs through biological methods from plants and microbes are found cost-effective and environmentally friendly(Vicente et al. 2011). Thus, several biological systems like fungi, bacteria (He et al. 2013; Veerasamy et al. 2011; Vilchis-Nestor et al. 2008) and plant parts (Ijaz et al. 2020; Rafique et al. 2017; Rauwel et al. 2015) are being exploited to synthesize AgNPs.

Plant extract mediated synthesis of AgNPs has some extra advantages than other synthesis methods as it eliminates the several steps of the synthesis methods and can be scaled up for large scale synthesis in a non-aseptic environment(He et al. 2013). Plants secrete the functional molecules (terpenoids, flavonoids, polyphenols, etc.), acting as both reducing and capping agents and compatible with the green chemistry principle(He et al. 2013). Few plant parts have long been used from ancient times due to their medicinal properties. One such kind of plant *Glycosmis pentaphylla* (Retz.) DC. (Family Rutaceae) has a protracted history of utilization in traditional medicine against different types of ailments around the globe. This plant has been used against jaundice, fever, cough, anaemia, rheumatism, vermifuge, etc. in ayurvedic and other medicinal practices(Sreejith et al. 2012). Different phytochemicals (flavonoids, alkaloids, terpenes, etc.) present in these plant parts with critical pharmaceutical applications like antiseptic, anti-ulcer, and antiviral antioxidant, anti-tumour, and hepatoprotective properties(Sreejith et al. 2012). On the other hand, a computational simulation is an excellent tool for understanding the interaction between stabilizing compounds and AgNPs(Dutta et al. 2020j). This data is crucial to identify the exact compound responsible for the stability of AgNPs which leads other researchers to isolate that compound from the mixture of the compounds of plant parts extract for more controlled synthesis of AgNPs.

The present study reports on the synthesis of AgNPs from the fruit extract of *G. pentaphylla* without using any hazardous chemicals. The compound, majorly responsible for stabilizing AgNPs, among the mixture of the compounds of FGP, was identified by the computational simulation using DFT. The

synthesized AgNPs were assessed for antifungal, antibacterial, and synergistic activities and its effects on fungal cell morphology under a light microscope.

2. Materials And Methods

2.1. Experimental microorganisms: The referred microbial strains of fungus and bacteria were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. Reference fungal strains included *Alternaria alternata* (MTCC-8459), *Colletotrichum lindemuthianum* (MTCC-8474), *Fusarium moniliforme* (MTCC-2015) and *Candida glabrata* (MCC 1445). The bacterial strains included two Gram-positive *Bacillus subtilis* (MTCC 121), *Streptococcus mutans* (MTCC 497) and two Gram-negative bacteria *Escherichia coli* (MTCC 723) and *Salmonella enterica* serovar Typhimurium (MTCC 98).

2.2. Plant sample collection and extract preparation: The ripening fruits of *G. pentaphylla* (FGP) were collected from Bidhan Chandra Krishi Viswavidyalaya, Haringhata campus, West Bengal. The fleshy epicarp separated from the fruit and dried in hot air oven at 40°C and crushed in dust form. 50 g of dry dust fruit epicarps was extracted with 100 ml of 30% ethanol (EtOH) (Laboratory-grade, Fischer Scientific) for 24 h at 30°C room temperature. The crude extracts were filtered through Whatman's No.1 filter paper and stored at four °C for the synthesis of AgNPs.

2.3. Synthesis of Silver nanoparticles (AgNPs): The aqueous solution of 1 mM silver nitrate (AgNO_3) (Sigma Aldrich, 99.9%) was prepared and used for the synthesis of AgNPs. Into 50 ml of the aqueous solution of 1 mM AgNO_3 , 5 ml fruit extract was added with continued stirring (250 rpm) on a magnetic stirrer. The brown colour of the solution indicated the generation of AgNPs. Synthesized AgNPs were isolated by centrifugation (6000 rpm up to 25 minutes), repeated washing and drying at 65°C for further characterization. The overall synthesis process is represented by Fig. 1

2.4. Characterization techniques: UV–vis spectroscopic analysis of AgNPs was recorded in the range of 200 to 600 nm (Jasco V550 spectrophotometer), where fruits extract of *G. pentaphylla* in Ethyl alcohol acts as a blank. The DLS study was done in a Malvern instrument (model No. ZS-90). The TEM study sample was prepared by placing a drop of a very dilute solution of AgNPs on a carbon-coated copper grid and then drying at room temperature for 24 h before examined. TEM analysis was done by JEOL electron microscope (JEOL 200 FX-II).

2.5. Computational study: At first, ground state geometry optimization of ten major compounds (Acutifolin, Arborine, Arborinine, 3-(3',3'-dimethylallyl)-4,8- dimethoxy-N-methylquinolin-2-one, γ - fagarine, Glycocitlone, Glycopentaphyllone, 1-hydroxy-3,4-dimethoxy-10 methylacridan-9-one, Skimmianine) of FGP were carried out by Quantum Espresso *ab initio* simulation package (Abid et al. 2002). Generalized gradient approximation (GGA) was employed with the Perdew-Burke- Ernzerh (PBE) (Perdew et al. 1996; Perdew et al. 1992) function and ultra-soft pseudopotential (Vanderbilt 1990). Interaction of AgNPs with these compounds was studied with the cut off energy of the plane-wave basis set of 40 Ry. The force on

each atom was reduced below 0.01 eV/Å during geometry optimization, and the final structures were used to analyze adsorption energies and different geometric parameters. A vacuum region of above 40 Å was used to confirm the decoupling between neighbouring systems. xCrySDen package was utilized for visualization (Kokalj 1999). One silver atom was considered a model of AgNPs to minimize the computational cost, and this type of simplification captured the basic character of AgNPs without changing the projected result (Dutta et al. 2020a; Dutta et al. 2020d). To identify the nature of the interaction, the silver atom was placed near different electrophilic positions of the target compounds.

2.6. In vitro antifungal activity AgNPs: The antifungal activity of synthesized AgNPs were tested against various fungal pathogens [*A. alternata* (MTCC-8459), *C. lindemuthianum* (MTCC-8474), *F. moniliforme* (MTCC-2015) and *C. glabrata* (MCC 1445)] by the standard agar well diffusion method (Dutta et al. 2020d). The spore suspension of test fungus was prepared by scrapping the spores from the 7-day-old PDA slant culture. 10 µl spore suspension was picked up from slant through micropipette, checks the CFU and poured into each fresh Potato dextrose agar plates. The antifungal activity of synthesized AgNPs was done by agar well diffusion assay in a Potato Dextrose agar plates. 30 µl of each fungal spore's suspension containing 1×10^6 colony-forming units (CFU) per ml was inoculated on the Petri plates by a sterile glass rod, and 5 mm cup was cut with the help of a sterile cork borer in each inoculated plates. The well was filled with 10 µl synthesized AgNPs (40 µg/ml) solution and incubated at 28°C for five days. The process was repeated for different concentration (35 µg/ml, 30 µg/ml, 25 µg/ml and 20 µg/ml) of AgNPs. Control (ethanolic FGP extract) was used under the same conditions.

2.7. In vitro antibacterial activity AgNPs: Assessment of antibacterial activity of AgNPs sample against two Gram-positive bacteria (*B. subtilis* MTCC 121, *Strep. mutans* MTCC 497) and two Gram-negative bacteria (*E. coli* MTCC 723 and *Sal. enterica* serovar Typhimurium MTCC 98) was measured by standard the agar-well diffusion method (Dutta et al. 2020d). 5 mm wells were cut in each fresh inoculated bacterial plates and 10 µl of different concentrations (40 µg/ml, 35 µg/ml, 30 µg/ml, 25 µg/ml and 20 µg/ml) of AgNPs was loaded into the 5 mm diameter well seeded with test bacteria and incubated for 24 h. at 37°C. Ethanolic FGP extract was used as a control solution.

2.8. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) and Minimum Bacterial Concentration (MBC) of AgNPs: The MIC values of synthesized AgNPs against the same fungi and bacteria were determined by the standard protocol (Dutta et al. 2020d). AgNPs, starting from 30 µg/ml concentration, were serially diluted using pure ethanol to check MIC values against different fungi. 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 lowest concentration considered as MIC resulted in inhibition of spores germination compared to the germination in control well. The MFC means the lowest compound concentration at which no visible growth of fungi was observed. Evaluation set up for MFC were prepared as same as MIC experimental set up for fungi. The MFC and MBC values of AgNPs was determined; a

higher concentration of their corresponding MIC values was used. The same concentrations of AgNPs were used as made previously to check the MIC values against bacteria. 10 µl of the test sample of different concentrations was loaded into the well of the pre-inoculated nutrient agar plate of target bacteria, incubated for 24 hrs at 37°C and observed for the zone of growth inhibition. The MBC was determined by checking the bacterial cells' viability after treating higher concentrations than their corresponding MIC values of AgNPs.

2.9. Synergistic effect of AgNPs: Synergistic effect was checked with a fungicide Bavistin and an antibiotic Streptomycin. *A. alternata* fungus and *B. subtilis* bacteria were chosen for synergistic activity by the agar well diffusion method, as discussed earlier. For comparison purposes, we have used the concentration of 40 µg/ml for every case. The degree of increasing inhibition zone diameter was measured after 48 hrs of incubation (28°C) in a BOD incubator by the equation: Fold Increase (FI) = $[(b - a)/a] \times 100$; where 'b' stands for 'inhibition zone diameter (mm) for fungicide or antibiotics + AgNPs'; 'a' stands for 'inhibition zone diameter (mm) for fungicide or antibiotics alone'.

2.10. Effect of AgNPs on cell morphology: For cell morphology study on *A. alternata*, MIC concentration of AgNPs was applied on *A. alternata* as per the agar-well diffusion method as discussed earlier. After 1–3 days of incubation at 28°C in a BOD incubator, the mycelia from the inhibited zones were checked under a light microscope (Phase-contrast microscope DM750, Leica, Germany) to check either there are any cellular abnormalities or not.

3. Results And Discussion

3.1. UV-VIS Spectroscopic analysis of AgNPs: The colour change of the reaction mixture (Fig. 1) was the first indication of AgNPs synthesis. The UV-Vis spectrum (Fig. 2) of the amber colour AgNPs showed a surface plasmon absorbance band at 417 nm, an indication of the presence of smaller size spherical AgNPs(Datta et al. 2017). According to the principle of UV-Vis spectroscopy, the shape and position of plasmon absorption depend on particle size, shape and morphology. Previous studies suggest that the plasmon absorption peak at 417 nm in UV-Vis spectroscopy due to the presence of AgNPs below 30 nm(Hildebrandt and Stockburger 1984). Mie theory is also able to predict this kind of phenomenon(Link and El-Sayed 1999). The sharpness of the UV-Vis peak reflects the monodispersed particles(Dutta et al. 2020a). The stability of the synthesized AgNPs is an essential parameter for its biomedical application. Up to 120 days from the synthesis of AgNPs, we have the same UV-Vis curve for the synthesized AgNPs.

3.2. DLS study: Prediction of the hydrodynamic size of the synthesized AgNPs from the DLS study (Fig.3) is an integral part of the characterization of AgNPs. It reflects an average of 90 nm of hydrodynamic size with a PDI value of 0.289, indicating the monodispersity of the AgNPs(Hackley and Clogston 2011). The sharpness of the DLS peak also indicates of monodispersed AgNPs.

3.3. TEM study: As per TEM study, our synthesis route produces spherical shape AgNPs (Fig. 4a). The particle size distribution graph (Fig. 4b) of the TEM image reveals that the AgNPs is 17 nm. Previous studies also support this type of observation(Dutta et al. 2020j).

3.4. Compound analysis: As per the compound analysis of previous studies, we have selected ten major compounds (Table 1), present in the fruits of *G. pentaphylla* for computational study.

Table 1: Major compounds present in the fruits of *G. pentaphylla*

SLNo.	Name of the Compounds	Molecular Formula	Structure of compounds	References
1.	Acutifolin	C ₁₇ H ₂₁ NO ₄		(Aye et al. 2019; Sripisut et al. 2012)
2.	Arborine	C ₁₀ H ₁₂ N ₂ O
3.	Arborinine	C ₁₀ H ₁₂ NO ₄
4.	Dictamine	C ₁₂ H ₈ NO ₂
5.	3-(3',3'-dimethylallyl)-4,8-dimethoxy-N-methylquinolin-2-one	C ₁₇ H ₂₁ NO ₃
6.	γ-fagarine	C ₁₃ H ₁₁ NO ₃

7.	Glycocitlone C	C ₁₇ H ₂₁ NO ₄
8.	Glycopentaphyllone	C ₁₇ H ₂₁ NO ₃
9.	1-hydroxy-3,4-dimethoxy-10-methylacridan-9-one	C ₁₈ H ₁₅ NO ₄
10.	Skimmianine	C ₁₄ H ₁₃ NO ₄

3.5. Computational Study: The DFT study shows the interaction energies of AgNP with the principle compounds of the fruit of *G. pentaphylla*. This study gives us a clear picture of the interaction between AgNP and the selected compounds. Figure 4 represents the optimized geometries of the ten compounds that bind with AgNP. Minimum energy structures of these complexes were confirmed by frequency analysis. The binding energies (E_b) between AgNP and the target molecules were carried out by the equation " $E_b = E_{\text{Ag-Compound}} - (E_{\text{Ag}} + E_{\text{Compound}})$ ", where $E_{\text{Ag-Compound}}$ represents the energy of AgNP binds with the target molecule, E_{Ag} and E_{Compound} represent the energy of AgNP and the target compound respectively. Binding energy calculations (Table S1) clearly show that AgNP is stabilized with the highest binding energy of 58.45 kJ/mol (Fig. 5) by arborine.

The moderate values of all the compounds' binding energy reflect the interaction between AgNP and the selected compounds. The lowest bond distance between the AgNP and active carbonyl functional group of arborine concerning other compounds also reveals that arborine is majorly responsible for the stability of AgNP.

3.6. Antifungal and antibacterial activities of synthesized AgNPs: The synthesized AgNPs (Fig. 6) shows a broad-spectrum of antimicrobial activities against different fungi and bacteria (Table S2 & S3). So, synthesized AgNPs can inhibit fungi and bacteria's growth, whereas the control solution does not show any growth inhibition zone. AgNPs showed the highest activity against *F. moniliforme* fungus and *S. enterica* serovar Typhimurium bacteria.

3.7. Determination of MIC, MFC and MBC of AgNPs: The MIC, MFC and MBC values give us a clear idea about the potency of AgNPs against different pathogenic microorganisms. The synthesized AgNPs showed MIC values against fungi and bacteria in the range of 9.5–15.5 µg/ml and 6.5-9 µg/ml, respectively (Table S4; Fig. 7). On the other hand, MFC and MBC values of AgNPs vary in the range of 12–19 µg/ml and 11.5–15 µg/ml, respectively (Fig. 7). Hence, synthesized AgNPs are more active against bacteria than fungi. This study also suggests that AgNPs show the highest antifungal activity against *F. moniliforme* and the highest antibacterial activity against *S. enterica* serovar Typhimurium. The previous study revealed that green synthesized AgNPs could inhibit *Candida* sp. and *E. coli* with MIC values of 21.4 µg/ml(Lateef et al. 2016) and 43.2 µg/ml(Perni et al. 2014), respectively which are much higher than the MIC values reported herein. Hence, AgNPs, synthesized by the route mentioned above, show higher antimicrobial activity than previously synthesized AgNPs. As per the previous study, the penetration of AgNPs through the bacterial cell wall is the reason behind its antibacterial effect(Alsammarrarie et al. 2018). If this fact is only responsible for the antibacterial activity, AgNPs always show higher antibacterial activity against Gram-negative bacteria than Gram-positive bacteria as Gram-positive bacteria have a thick peptidoglycan layer outside the plasma membrane(Griffith et al. 2015; Shrivastava et al. 2007). However, in our study, no particular trend of antibacterial activity is observed by the synthesized AgNPs. So, the antibacterial efficacy of AgNPs is not only manifested by the penetration of the AgNP through the bacterial cell wall. Releasing of Ag⁺ from AgNP may be another reason(Sotiriou and Pratsinis 2010). The high affinity of Ag⁺ ion towards the protein thiol group also causes bacterial death(Sotiriou and Pratsinis 2010).

3.8. Synergistic effect of AgNPs: The combined effect of AgNPs and fungicides or antibiotics against different pathogenic microorganisms is shown in Table-2. Bavistin shows an increase in the inhibition zone of 25% against *A. alternata* combined with AgNPs. On the other hand, AgNPs also show a synergistic effect with an increase of 33.3% inhibition zone diameter against *B. subtilis* when combined with Streptomycin. Increased antimicrobial activity of antibiotics or fungicide is due to bonding between AgNP and active functional groups (hydroxyl, amide, etc.) of antibiotics or fungicide(Batarseh 2004). In this way cost of antibiotics or fungicide can be reduced as a lower concentration is required to get the same activity and also fungicide or antibiotic resistance microbes can be handled by this process.

Table 2
Synergistic effect of AgNPs with Bavistin and Streptomycin

Name of the Fungi	Inhibition zone diameter (mm) for AgNPs	Inhibition zone diameter (mm)		
		Bavistin		
		Only Bavistin (a)	Bavistin + AgNPs (b)	FI % [(b - a)/a × 100]
<i>A. alternata</i>	11 ± 0.33	16 ± 0.66	20 ± 0.57	25
Name of the Bacteria	Inhibition zone diameter (mm) for AgNPs	Inhibition zone diameter (mm)		
		Streptomycin		
		Only Streptomycin (a)	Streptomycin + AgNPs (b)	FI %
<i>B. subtilis</i>	15.5 ± 0.57	18 ± 0.94	24 ± 0.35	33.3

3.9. Effect of AgNPs on cell morphology: The effect of AgNPs on the cell morphology of *A. alternata* was studied. As it is the least affected fungus by AgNPs, we have chosen this fungus for microscopic observation. Several morphological abnormalities (like hyphal swelling) have been observed (Fig. 8) when normal healthy mycelia were treated with AgNPs. This type of unusual swelling of fungus hyphae was also reported by the previous scientists (Chitarra et al. 2003; Li et al. 2007).

4. Conclusion

In summary, a low cost, one-pot, less time consuming, green synthesis method has been developed to prepare AgNPs using the fruit extract of *G. pentaphylla*. The phytochemicals have provided both the reducing and capping agents to produce a monodispersed, stable AgNPs of an average size of 17 nm. DFT calculation indicates that arborine of fruit extract of *G. pentaphylla* stabilizes the AgNPs with a binding energy of 58.45 kJ/mol. Synthesized AgNPs have both antifungal and antibacterial activity. According to MIC, MFC, and MBC study, AgNPs exhibit the highest activity against *F. moniliforme* in case of fungi and *S. enterica* serovar Typhimurium in case of bacteria. Synthesized AgNPs also show synergistic activity with the fungicide Bavistin and antibiotic Streptomycin by increasing their activity by 19% and 33.3%, respectively. The microscopic study indicates morphological abnormalities of *A. alternata* as an effect of AgNPs. Therefore, these green synthesized AgNPs could provide an effective alternative of fungicide and antibiotic to the pharmaceutical industries.

Declarations

5.1. Ethics approval: Not applicable

5.2. Consent to participate: Not applicable

5.3. Consent for publication: Not applicable

5.4. Availability of data and material: The data has been submitted as a supplementary compressed file along with this article.

5.5. Conflicts of interest/Competing interests: The authors declare no conflict of interest.

5.6. Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

5.7. Authors' contributions:

Conceptualization and design of experimentation: Vivekananda Mandal, Asoke P. Chattopadhyay and Mahuya Das

Methodology: Tanmoy Dutta, and Swapan Kumar Chowdhury did the experimentations; Tanmoy Dutta, and Narendra Nath Ghosh did the computational analysis.

Formal analysis and investigation: Mahuya Das, Asoke P. Chattopadhyay, and Vivekananda Mandal

Writing - original draft preparation: Tanmoy Dutta, Swapan Kumar Chowdhury and Narendra Nath Ghosh. Tanmoy Dutta wrote the first draft of the manuscript and all the authors commented on the manuscript. They drafted the work or revised it critically for important intellectual content. All authors read and approved the final manuscript. All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Writing - review and editing: Vivekananda Mandal

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5.8. Acknowledgements: The authors would like to thank JIS College of Engineering, Kalyani and Department of Botany, University of Gour Banga for Infrastructural support.

References

Abid J-P, Wark A, Brevet P-F, Girault H (2002) Preparation of silver nanoparticles in solution from a silver salt by laser irradiation Chemical Communications:792-793 doi:<https://doi.org/10.1039/B200272H>

Ahmad N, Bhatnagar S, Ali SS, Dutta R (2015) Phytofabrication of bioinduced silver nanoparticles for biomedical applications International journal of nanomedicine 10:7019 doi:<https://doi.org/10.2147/IJN.S94479>

Alsammarraie FK, Wang W, Zhou P, Mustapha A, Lin M (2018) Green synthesis of silver nanoparticles using turmeric extracts and investigation of their antibacterial activities Colloids and Surfaces B:

Biointerfaces 171:398-405 doi:<https://doi.org/10.1016/j.colsurfb.2018.07.059>

Angela Obaigeli Eni IAO, Oranusi U. Solomon (2010) Microbial quality of fruits and vegetables sold in Sango Ota, Nigeria African Journal of Food Science 4:291-296
doi:<https://doi.org/10.5897/AJFS.9000186>

Aye MM, Aung HT, Sein MM, Armijos C (2019) A review on the phytochemistry, medicinal properties and pharmacological activities of 15 selected Myanmar medicinal plants Molecules 24:293
doi:<https://doi.org/10.3390/molecules24020293>

Barman A, Dutta T, Khamrui A, Basu A (2020) Review on Green Synthesis of ZnO Nano Particles and their Applications Available at SSRN 3541374 doi:<http://dx.doi.org/10.2139/ssrn.3541374>

Basavegowda N, Lee YR (2013) Synthesis of silver nanoparticles using Satsuma mandarin (Citrus unshiu) peel extract: a novel approach towards waste utilization Materials letters 109:31-33
doi:<https://doi.org/10.1016/j.matlet.2013.07.039>

Batarseh KI (2004) Anomaly and correlation of killing in the therapeutic properties of silver (I) chelation with glutamic and tartaric acids Journal of Antimicrobial Chemotherapy 54:546-548
doi:<https://doi.org/10.1093/jac/dkh349>

Chinnappan S, Kandasamy S, Arumugam S, Seralathan K-K, Thangaswamy S, Muthusamy G (2018) Biomimetic synthesis of silver nanoparticles using flower extract of Bauhinia purpurea and its antibacterial activity against clinical pathogens Environmental Science and Pollution Research 25:963-969 doi:<https://doi.org/10.1007/s11356-017-0841-1>

Chitarra G, Breeuwer P, Nout M, Van Aelst A, Rombouts F, Abee T (2003) An antifungal compound produced by Bacillus subtilis YM 10–20 inhibits germination of Penicillium roqueforti conidiospores Journal of Applied Microbiology 94:159-166 doi:<https://doi.org/10.1046/j.1365-2672.2003.01819.x>

Chowdhury SK, Dutta T, Chattopadhyay AP, Ghosh NN, Chowdhury S, Mandal V (2021) Isolation of antimicrobial Tridecanoic acid from Bacillus sp. LBF-01 and its potentialization through silver nanoparticles synthesis: a combined experimental and theoretical studies Journal of Nanostructure in Chemistry:1-15 doi:<https://doi.org/10.1007/s40097-020-00385-3>

Datta LP, Chatterjee A, Acharya K, De P, Das M (2017) Enzyme responsive nucleotide functionalized silver nanoparticles with effective antimicrobial and anticancer activity New Journal of Chemistry 41:1538-1548

Diekema DJ, Dodgson KJ, Sigurdardottir B, Pfaller MA (2004) Rapid detection of antimicrobial-resistant organism carriage: an unmet clinical need Journal of clinical microbiology 42:2879-2883
doi:<https://doi.org/10.1128/JCM.42.7.2879-2883.2004>

Droby S Improving quality and safety of fresh fruits and vegetables after harvest by the use of biocontrol agents and natural materials. In: I International Symposium on Natural Preservatives in Food Systems 709, 2005. pp 45-52. doi:<https://doi.org/10.17660/ActaHortic.2006.709.5>

Dutta T, Chattopadhyay AP, Mandal M, Ghosh NN, Mandal V, Das M (2020a) Facile green synthesis of silver bionanocomposite with size dependent antibacterial and synergistic effects: a combined experimental and theoretical studies Journal of Inorganic and Organometallic Polymers and Materials 30:1839-1851 doi:<https://doi.org/10.1007/s10904-019-01332-8>

Dutta T, Ghosh NN, Chattopadhyay AP, Das M (2019) Chitosan encapsulated water-soluble silver bionanocomposite for size-dependent antibacterial activity Nano-Structures & Nano-Objects 20:100393 doi:<https://doi.org/10.1016/j.nanoso.2019.100393>

Dutta T, Ghosh NN, Das M, Adhikary R, Mandal V, Chattopadhyay AP (2020d) Green synthesis of antibacterial and antifungal silver nanoparticles using Citrus limetta peel extract: Experimental and Theoretical studies Journal of Environmental Chemical Engineering 8:104019 doi:<https://doi.org/10.1016/j.jece.2020.104019>

Dutta T, Ghosh NN, Das M, Adhikary R, Mandal V, Chattopadhyay APJJoECE (2020j) Green synthesis of antibacterial and antifungal silver nanoparticles using Citrus limetta peel extract: Experimental and Theoretical studies:104019

Gabriel MF, Postigo I, Tomaz CT, Martínez J (2016) *Alternaria alternata* allergens: markers of exposure, phylogeny and risk of fungi-induced respiratory allergy Environment international 89:71-80 doi:<https://doi.org/10.1016/j.envint.2016.01.003>

Govarthanan M et al. (2014) Biosynthesis and characterization of silver nanoparticles using panchakavya, an Indian traditional farming formulating agent International journal of nanomedicine 9:1593 doi:<https://doi.org/10.2147/IJN.S58932>

Griffith M, Udekwu KI, Gkotzis S, Mah T-F, Alarcon EI (2015) Anti-microbiological and anti-infective activities of silver. In: Silver Nanoparticle Applications. Springer, pp 127-146

Hackley VA, Clogston JD (2011) Measuring the hydrodynamic size of nanoparticles in aqueous media using batch-mode dynamic light scattering. In: Characterization of Nanoparticles Intended for Drug Delivery. Springer, pp 35-52

Hayat K et al. (2019) Insecticide toxic effects and blood biochemical alterations in occupationally exposed individuals in Punjab, Pakistan Science of The Total Environment 655:102-111 doi:<https://doi.org/10.1016/j.scitotenv.2018.11.175>

He Y et al. (2013) Green synthesis of silver nanoparticles by *Chrysanthemum morifolium* Ramat. extract and their application in clinical ultrasound gel International Journal of Nanomedicine 8:1809

doi:<https://doi.org/10.2147/IJN.S43289>

Hildebrandt P, Stockburger M (1984) Surface-enhanced resonance Raman spectroscopy of Rhodamine 6G adsorbed on colloidal silver The Journal of Physical Chemistry 88:5935-5944

doi:<https://doi.org/10.1021/j150668a038>

Ijaz M, Zafar M, Iqbal T (2020) Green synthesis of silver nanoparticles by using various extracts: a review Inorganic and Nano-Metal Chemistry:1-12 doi:<https://doi.org/10.1080/24701556.2020.1808680>

Ito K, Tanaka T, Hatta R, Yamamoto M, Akimitsu K, Tsuge T (2004) Dissection of the host range of the fungal plant pathogen *Alternaria alternata* by modification of secondary metabolism Molecular microbiology 52:399-411 doi:<https://doi.org/10.1111/j.1365-2958.2004.04004.x>

Jogaiah S, Kurjogi M, Abdelrahman M, Hanumanthappa N, Tran L-SP (2019) *Ganoderma applanatum*-mediated green synthesis of silver nanoparticles: Structural characterization, and in vitro and in vivo biomedical and agrochemical properties Arabian Journal of Chemistry 12:1108-1120

doi:<https://doi.org/10.1016/j.arabjc.2017.12.002>

Katan J (2017) Diseases caused by soilborne pathogens: biology, management and challenges Journal of Plant Pathology 99:305-315 doi:<http://dx.doi.org/10.4454/jpp.v99i2.3862>

Kavyashree D et al. (2015) ZnO superstructures as an antifungal for effective control of *malassezia* furfur, dermatologically prevalent yeast: prepared by Aloe vera assisted combustion method ACS Sustainable Chemistry & Engineering 3:1066-1080 doi:<https://doi.org/10.1021/sc500784p>

Kokalj A (1999) XCRYSDen—a new program for displaying crystalline structures and electron densities Journal of Molecular Graphics and Modelling 17:176-179 doi:[https://doi.org/10.1016/S1093-3263\(99\)00028-5](https://doi.org/10.1016/S1093-3263(99)00028-5)

Kowshik M, Ashtaputre S, Kharrazi S, Vogel W, Urban J, Kulkarni SK, Paknikar K (2002) Extracellular synthesis of silver nanoparticles by a silver-tolerant yeast strain MKY3 Nanotechnology 14:95 doi:<https://doi.org/10.1088/0957-4484/14/1/321>

Lateef A, Ojo SA, Oladejo SM (2016) Anti-candida, anti-coagulant and thrombolytic activities of biosynthesized silver nanoparticles using cell-free extract of *Bacillus safensis* LAU 13 Process Biochemistry 51:1406-1412 doi:<https://doi.org/10.1016/j.procbio.2016.06.027>

Li L, Mo M, Qu Q, Luo H, Zhang K (2007) Compounds inhibitory to nematophagous fungi produced by *Bacillus* sp. strain H6 isolated from fungistatic soil European journal of plant pathology 117:329-340 doi:<https://doi.org/10.1007/s10658-007-9101-4>

Link S, El-Sayed MA (1999) Spectral properties and relaxation dynamics of surface plasmon electronic oscillations in gold and silver nanodots and nanorods. ACS Publications,

- Martins SJ, Faria AF, Pedroso MP, Cunha MG, Rocha MR, Medeiros FHV (2019) Microbial volatiles organic compounds control anthracnose (*Colletotrichum lindemuthianum*) in common bean (*Phaseolus vulgaris* L.) *Biological Control* 131:36-42 doi:<https://doi.org/10.1016/j.biocontrol.2019.01.003>
- Möller E, Chetkowski J, Geiger H (1999) Species-specific PCR assays for the fungal pathogens *Fusarium moniliforme* and *Fusarium subglutinans* and their application to diagnose maize ear rot disease *Journal of Phytopathology* 147:497-508 doi:<https://doi.org/10.1046/j.1439-0434.1999.00380.x>
- Perdew JP, Burke K, Ernzerhof M (1996) Generalized gradient approximation made simple *Physical review letters* 77:3865 doi:<https://doi.org/10.1103/PhysRevLett.77.3865>
- Perdew JP, Chevary JA, Vosko SH, Jackson KA, Pederson MR, Singh DJ, Fiolhais C (1992) Atoms, molecules, solids, and surfaces: Applications of the generalized gradient approximation for exchange and correlation *Physical review B* 46:6671 doi:<https://doi.org/10.1103/PhysRevB.46.6671>
- Perni S, Hakala V, Prokopovich P (2014) Biogenic synthesis of antimicrobial silver nanoparticles capped with L-cysteine *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 460:219-224 doi:<https://doi.org/10.1016/j.colsurfa.2013.09.034>
- Rafique M, Sadaf I, Rafique MS, Tahir MB (2017) A review on green synthesis of silver nanoparticles and their applications *Artificial cells, nanomedicine, and biotechnology* 45:1272-1291 doi:<https://doi.org/10.1080/21691401.2016.1241792>
- Ragaseema V, Unnikrishnan S, Krishnan VK, Krishnan LK (2012) The antithrombotic and antimicrobial properties of PEG-protected silver nanoparticle coated surfaces *Biomaterials* 33:3083-3092 doi:<https://doi.org/10.1016/j.biomaterials.2012.01.005>
- Rauwel P, Küünal S, Ferdov S, Rauwel E (2015) A review on the green synthesis of silver nanoparticles and their morphologies studied via TEM *Advances in Materials Science and Engineering* 2015 doi:<https://doi.org/10.1155/2015/682749>
- Rodríguez PE, Sanchez MS (2010) Maternal exposure to triclosan impairs thyroid homeostasis and female pubertal development in Wistar rat offspring *Journal of Toxicology and Environmental Health, Part A* 73:1678-1688 doi:<https://doi.org/10.1080/15287394.2010.516241>
- Shao J, Wang B, Li J, Jansen JA, Walboomers XF, Yang F (2019) Antibacterial effect and wound healing ability of silver nanoparticles incorporation into chitosan-based nanofibrous membranes *Materials Science and Engineering: C* 98:1053-1063 doi:<https://doi.org/10.1016/j.msec.2019.01.073>
- Shrivastava S, Bera T, Roy A, Singh G, Ramachandrarao P, Dash D (2007) Characterization of enhanced antibacterial effects of novel silver nanoparticles *Nanotechnology* 18:225103 doi:<https://doi.org/10.1088/0957-4484/18/22/225103>

- Sondi I, Salopek-Sondi B (2004) Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria *Journal of colloid and interface science* 275:177-182
doi:<https://doi.org/10.1016/j.jcis.2004.02.012>
- Sotiriou GA, Pratsinis SE (2010) Antibacterial activity of nanosilver ions and particles *Environmental science & technology* 44:5649-5654 doi:<https://doi.org/10.1021/es101072s>
- Spanakis EK, Kourkoumpetis TK, Livanis G, Peleg AY, Mylonakis E Statin therapy and decreased incidence of positive *Candida* cultures among patients with type 2 diabetes mellitus undergoing gastrointestinal surgery. In: *Mayo Clinic Proceedings*, 2010. vol 12. Elsevier, pp 1073-1079
- Sreejith P, Praseeja R, VV A (2012) A review on the pharmacology and phytochemistry of traditional medicinal plant, *Glycosmis pentaphylla* (Retz.) Correa *Journal of Pharmacy Research* 5:2723-2728
doi:<http://rgcb.sciencecentral.in/id/eprint/232>
- Sripisut T et al. (2012) Glycopentaphyllone: The first isolation of hydroperoxyquinolone from the fruits of *Glycosmis pentaphylla* *Phytochemistry Letters* 5:379-381
doi:<https://doi.org/10.1016/j.phytol.2012.03.007>
- Vanderbilt D (1990) Soft self-consistent pseudopotentials in a generalized eigenvalue formalism *Physical review B* 41:7892 doi:<https://doi.org/10.1103/PhysRevB.41.7892>
- Veerasamy R, Xin TZ, Gunasagaran S, Xiang TFW, Yang EFC, Jeyakumar N, Dhanaraj SA (2011) Biosynthesis of silver nanoparticles using mangosteen leaf extract and evaluation of their antimicrobial activities *Journal of saudi chemical society* 15:113-120 doi:<https://doi.org/10.1016/j.jscs.2010.06.004>
- Vicente T, Mota JP, Peixoto C, Alves PM, Carrondo MJ (2011) Rational design and optimization of downstream processes of virus particles for biopharmaceutical applications: current advances *Biotechnology advances* 29:869-878 doi:<https://doi.org/10.1016/j.biotechadv.2011.07.004>
- Vilchis-Nestor AR, Sánchez-Mendieta V, Camacho-López MA, Gómez-Espinosa RM, Camacho-López MA, Arenas-Alatorre JA (2008) Solventless synthesis and optical properties of Au and Ag nanoparticles using *Camellia sinensis* extract *Materials letters* 62:3103-3105
doi:<https://doi.org/10.1016/j.matlet.2008.01.138>
- Volova TG, Prudnikova SV, Zhila NO (2018) Fungicidal activity of slow-release P (3HB)/TEB formulations in wheat plant communities infected by *Fusarium moniliforme* *Environmental Science and Pollution Research* 25:552-561 doi:<https://doi.org/10.1007/s11356-017-0466-4>

Figures

Figure 1

Synthesis of AgNPs

Figure 2

UV-Vis spectrum of synthesized AgNPs

Figure 3

DLS study of the synthesized AgNPs

Figure 4

(a) The TEM analysis of synthesized AgNPs; and (b) particle size distributions of AgNPs

Figure 5

Optimized geometries of the selected compound-AgNP complex

Figure 6

Binding energies of the selected compounds bond with AgNP

Figure 7

Antimicrobial assessment of the different concentration of AgNPs. (a-c) Inhibition zone diameter of (a) Antifungal assessment; (b) Antibacterial assessment; (c-e) Disc image of (c) antifungal assay against *A. alternata*; (d) antifungal assay against *C. glabrata*; and (e) antibacterial assay against *B. subtilis*

Figure 8

Antimicrobial potency of the AgNPs against the pathogenic microbes. (a) MIC and MFC values of AgNPs against different fungi; and (b) MIC and MBC values of AgNPs against different bacteria.

Figure 9

Microscopic view of *A. alternata* (a) normal mycelia (b) mycelia treated with AgNPs. The morphological abnormalities have been marked with arrows.

Supplementary Files

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