Australian Journal of Science and Technology

ISSN Number (2208-6404)
Volume 5; Issue 1; March 2021

Original Article

Antimicrobial effect of green synthesized silver nanoparticles using aqueous extract of *Peperomia pellucida*

David Timi[1]*, Subramaniyam Gopalakrishnan[1], Francis Hombhanje[2] and Macquin Maino[3]

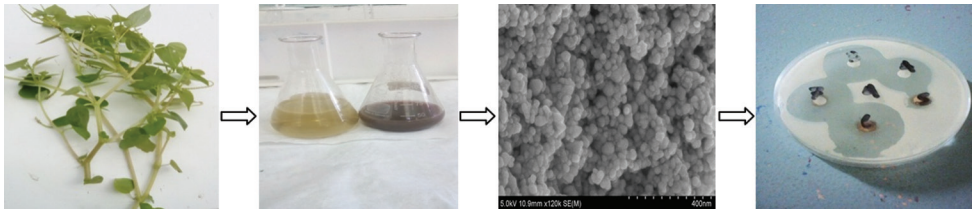
[1]Department of Applied Sciences, Papua New Guinea University of Technology, PMB, Lae, Papua New Guinea, [2]Centre for Health Research and Diagnostics, Divine Word University, Madang, Papua New Guinea, [3]Department of Agriculture, Papua New Guinea University of Technology, PMB, Lae, Papua New Guinea

ABSTRACT

Plant-mediated biosynthesis of silver nanoparticles (AgNPs) and the microbiological activity on selected pathogenic microorganisms is discussed. In this study, aqueous extracts of the aerial parts of a medicinal herb, *Peperomia pellucida,* were utilized to construct AgNPs. The synthesized AgNPs were characterized by ultraviolet–visible, Fourier-transform infrared (FT-IR), and X-ray diffraction spectrometer and scanning electron microscopy analysis. The biosynthesized AgNPs exhibited comparable antimicrobial activity with gentamycin and chloramphenicol reference antibiotics against five indicator microorganisms including two Gram-positive (G+) bacteria, two Gram-negative (G−) bacteria, and a protozoa (Pz). The organisms are, namely, *S. subtilis* (G+), *Staphylococcus aureus* (G+), *Escherichia coli* (G-), *Streptococcus pneumoniae* (G-), and *Trichomonas vaginalis* (Pz). Assessment of the efficacy of the biosynthesized AgNPs on the microorganisms was carried out through the disc diffusion technique.

Keywords: Antimicrobial effects, green synthesis, *Peperomia pellucida*, silver nanoparticles

Submitted: 26-02-2021, **Accepted:** 01-03-2021, **Published:** 30-03-2021



BACKGROUND

Nanotechnology is a new and rapidly emerging area of research that has got application in all areas of sciences and engineering disciplines including medical sciences.[1] Nanotechnology is based on the manipulation of atoms and molecules at nanoscale level (1–100 nm) because of the unique physical, chemical, and biological properties demonstrated by certain nanomaterials.[2] Silver nanoparticle (AgNP) is one such nanomaterial that has drawn attention for its application in nanomedicine due primarily to its outstanding chemical and biological properties.[3] Among all metal nanoparticles, silver is more biocompatible and has demonstrated excellent antimicrobial activity.[3]

Several procedures are followed in the construction of nanoparticles including AgNPs and these are physical, chemical, and biological (green) methods.[4] From these methods, green synthesis is preferred as it is more eco-friendly in that it involves the use of benign biomolecules derived from biological sources unlike physical and chemical processes. Among the green methods of synthesis, phytosynthesis is inexpensive and a more convenient method.[5] Thus, plant-

Address for correspondence: David Timi, Department of Applied Sciences, PNG University of Technology, PMB, Lae, PNG.
E-mail: david.timi@pnguot.ac.pg

mediated green synthesis of nanoparticles has gained more reputation and consequently in the recent past, extracts from a good number of plants have facilitated the synthesis of AgNPs and their chemical and biological properties assessed.[6] Some of these reported cases include bioassay conducted on the efficacy of AgNPs on antibiotic-resistant strains of bacteria.[7]

Growing antibiotic drug resistance has been a serious medical concern in recent years. Since the discovery of the first antibiotic in 1928, all bacterial infections were well contained by the existing antibacterial drugs.[8] However, this situation has changed over the last few years as a result of the emergence of multidrug resistance bacteria known as superbugs.[9,10] The problem developed as a result of both the overuse and misuse of the antibiotics over the years. When a bacterium no longer responds to a treatment directed to eliminate the associated infection, then it has developed resistance to the drug.[11] Some of the reported multidrug-resistant strains of bacteria are *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa*.[9] Without the availability of alternative antibiotics, this has become a major global health concern because it is now difficult to effectively treat common diseases caused by bacteria such as pneumonia, TB, blood poisoning, gonorrhea, and food-borne diseases.[9,10] There have already been many reported cases of death in developed western countries as a result of antibiotic resistant bacteria.[11] To contain this emerging situation, associated research activities are being conducted to find alternative drugs. *In vitro* antibacterial study conducted on nanomaterials such as AgNPs is one such research effort.

In this study, aqueous extracts of a medicinal herb, *Peperomia pellucida* (Piperaceae), were utilized to synthesized AgNPs and the antimicrobial property of these nanoparticles was examined by *in vitro* assay against five selected pathogenic microorganisms followed by the determination of minimum inhibition concentration (MIC) of the AgNP. MIC is the lowest concentration of an antimicrobial agent or a drug that will inhibit the visible growth of a microorganism after overnight (18–24 h) incubation.[12] It is used to assess the *in vitro* activity of a new antimicrobial agent and often the information leads to the establishment of MIC breakpoints.[13] In addition, breakpoint is a chosen concentration of an antibiotic (mg/L or µg/ml) that determines whether a species of pathogenic microorganism is susceptible or resistant to an antibiotic. If the MIC against a microorganism is less than or equal to the susceptibility breakpoint, the organism is considered susceptible to the antibiotic.[13]

P. pellucida is a shallow-rooted annual herb that normally grows up to 15–45 cm long. It flourishes well in loose humid soils and is found all over Asia and the other tropical and subtropical regions.[14] The whole aerial part of the plant is edible and can be eaten either raw or cooked. Although in PNG, there is no known recorded medicinal use of the plant, in other countries, the plant is reported to be used for the treatment of variety of ailments including abdominal pain, abscesses, acne, boils, colic, fatigue, gout, headache, renal disorders, rheumatic joint pain, fever, wound dressing, and to stop hemorrhages.[14,15] *P. pellucida* is further reported to have demonstrated a broad antibacterial activity in addition to its antifungal property.[14,16] The major chemical constituents of the plant are found to be alkaloids, cardenolides, saponins, tannins, and carbohydrates.[17]

METHODOLOGY

Preparation of AgNPs

Preparation of silver nitrate ($AgNO_3$) solution

On an analytical balance, 9.5 g of $AgNO_3$ was weighed and transferred into a 500 ml volumetric flask, dissolved, and made up to volume with distilled water giving a concentration of 0.1177 moles/L. From this, 2.5 ml was transferred to a 100 ml volumetric flask and diluted to mark with distilled water, giving a final concentration of 0.003 moles/L (3 millimoles).

Sampling and preparation of plant sample

Young matured diseases free aerial parts of *P. pellucida* were collected, botanically identified and 10 g of these were weighed and washed thoroughly with tap water and then rinsed with distilled water.

Aqueous extract of plant sample

Ten grams of the plant were chopped up to smaller pieces with a kitchen knife and placed into 250 ml volumetric flask. Two hundred milliliters of distilled water were added and heated on a Bunsen burner to the first sign of boiling, then removed and left to cool to room temperature. This was then filtered with a Whatman No. 1 filter paper and the filtrate was used for the synthesis of AgNPs.

Synthesis of AgNPs

With a measuring cylinder, 50 ml of aqueous plant extract was measured and transferred into 250 ml volumetric flasks and to this 100 ml of 3.0 millimoles of silver nitrate was added, gently shaken and placed under direct sunlight. Color changed from yellow to dark brown of the mixture indicated the formation of AgNPs followed by agglomeration of the particles. After about 2 h, this was removed and placed in a room for agglomerated colloidal AgNPs to settle overnight before they were centrifuged at 2500 rpm. The supernatants were discarded and the AgNPs were washed with about 50 ml of distilled water and centrifuged again. The same washing process was repeated 3 times and the AgNPs for bioassay were stored as aqueous suspension (30 ml) in a vial. Those for characterization were stored as dried samples (~250 mg) and sent away for spectral analysis.

Characterization techniques

Four spectroscopic techniques were employed to characterize the biosynthesized AgNPs. They included ultraviolet–visible (UV–vis) spectroscopy, Fourier-transform infrared (FT-IR) spectroscopy, X-ray diffraction (XRD) spectroscopy, and scanning electron microscopy (SEM).

The UV–vis analysis of the AgNPs was carried out using a Varian Cary 50 Bio UV–vis spectrophotometer. The absorption maxima (λ_{max}) of the colored solution of AgNPs in a cuvette (1 cm path length) were taken from a wavelength range set at 200–700 nm and slit width at 1 nm. FT-IR analysis was carried out in the range of 450–4000 cm^{-1}. XRD pattern was recorded using Cu Kα radiation (λ = 1.54060 Å) with nickel monochromator in the range of 2θ from 10° to 70°. The morphology and size range of the synthesized AgNPs were examined by SEM.

Bioassay

Preparation of test microorganisms

With a pre-flamed inoculating loop, five test microbials (four bacteria and a protozoa) were obtained from mother cultures, transferred to sterilized nutrient broth (Oxoid) in McCartney bottles and all five organisms were subcultured overnight at 37°C. Nutrient broth (13 g) was dissolved in a liter of distilled water and sterilized in an autoclave at 121°C for 15 min.

Preparation of assay plates and paper discs

Twenty-eight grams of nutrient agar (Oxoid) were dissolved in 1 L of distilled water and sterilized in an autoclave for 15 min at 121°C. After sterilization, 15–20 ml of nutrient agar were dispensed into sterile plastic Petri discs having internal diameter of 9 cm. After the agar solidified, the plates were stored in a refrigerator at 4°C awaiting bioassay.

Table 1: Antimicrobial activity of AgNPs synthesized using leaves of *P. pellucida*

Name of microorganisms	Zone of inhibition in mm			
	AgNPs	Gen.	Chl.	Blank
B. subtilis (G+)	+++	++	++	0
S. aureus (G+)	+++	+++	+++	0
E. coli (G−)	++	+++	++	0
S. pneumoniae (G−)	++	++	+	0
T. vaginalis (Pz)	+++	+++	++	0

G+: Gram-positive bacteria, G−: Gram-negative bacteria, Pz: Protozoa, Gen.: Gentamicin reference drug, Chl.: Chloramphenicol reference drug, +: 6–9 mm zone of growth inhibitions, ++: 10–19 mm zone of growth inhibitions, +++: 20–30 mm zone of growth inhibitions. AgNPs: Silver nanoparticles. *B. subtilis: Bacillus subtilis, S. aureus: Staphylococcus aureus, E. coli: Escherichia coli, T. vaginalis: Trichomonas vaginalis, P. pellucida*: *Peperomia pellucida*

Paper discs (6 mm) were cut out from Whatman Millipore filter paper (1 mm thick) with a paper hole puncher and were sterilized at temperature of 120°C in an oven for about 24 h.

In vitro antimicrobial test

To the nutrient agar plates, 100 µL of subcultured microorganisms were transferred with a sterile micropipette and streaked evenly with a glass hockey rod under sterile condition. To the seeded media, paper discs impregnated with 30 µL suspension of AgNPs were gently pressed down to ensure complete contact and were incubated at 37°C for 18–24 h. After incubation, zones of inhibitions were measured (mm) and tabulated [Table 1].

Determination of minimum inhibitory concentration (MIC)

Five microorganisms were included in the bioassay using disc diffusion technique to observe MIC (see section 3.0 for definition) of the phytosynthesized AgNPs. The organisms are *B. subtilis*, *S. aureus*, *E. coli*, *Streptococcus pneumoniae,* and *Trichomonas vaginalis.*

From the aqueous suspension of AgNPs, 2.0 µL were removed with a micropipette and transferred to glass vials. These were then diluted by adding appropriate volume of distilled water using a 1000 µL micropipette. Several dilutions were prepared, ranging from 5× to 100× dilution. Applying the bioassay method described in Section 2.2.3 above, the dilution that exhibited minimal growth around the edge of the 6 mm paper disc was taken as the minimum inhibitory concentration and the results are shown in Table 2.

RESULTS AND DISCUSSION

Color Change

The formation of AgNPs was evident from the change of color of the reaction mixture from pale yellow to a dark brown solution within 20 min of exposure to direct sunlight.

Characterization

The UV–vis spectrum of the AgNPs generated from the aqueous extract of *P. pellucida* exhibited an absorption maxima (λ_{max}) at 425 nm that is characteristic of the UV–vis signal of the surface plasmon resonance of AgNPs.[18] FT-IR frequencies of the biomolecules involved in the reduction of silver ion show peaks at 3387 (N-H), 1625 (C=C), and 1379 (C-H bending) cm^{-1}.[19] The XRD pattern of the AgNPs [Figure 5] shows three distinct diffraction patterns 2θ peak values at about 38°, 44°, and 64° that are characteristic of the planes (111), (200), and (220) for the face-centered cubic silver.[18,20] Image from the SEM [Figure 6] shows the shape of the AgNPs to be mainly spherical and their sizes ranged between 25 and 50 nm.

Table 2: Dilution factors (1/x) of aqueous suspension of AgNPs that produced minimum growth inhibition of 6 mm

	Dilutions (1/x)				
	Microorganisms				
	B. subtilis	***S. aureus***	***E. coli***	***S. pneumoniae***	***T. vaginalis***
Dilutions	20×	5×	20×	20×	70×

AgNPs: Silver nanoparticles. *Bacillus subtilis: Bacillus subtilis, S. aureus: Staphylococcus aureus, E. coli: Escherichia coli*

Bioassay

Antimicrobial property of the green synthesized AgNPs was assessed by performing bioassay against five indicator organisms including two Gram-positive bacteria (*B. subtilis* and *S. aureus*), two Gram-negative bacteria (*E. coli* and *S. pneumoniae*), and one protozoa (*T. vaginalis*). Table 1 highlights different levels of the efficacy of phytosynthesized AgNPs on the five microorganisms. The activities were demonstrated by the clear zones of growth inhibition of the microbes. Under this experimental conditions, the synthesized AgNPs (30 µL) have shown to be comparable with the two reference drugs, chloramphenicol (10 µg) and gentamycin (10 µg).

Experimental results of the evaluation on the minimum inhibitory concentration (MIC) of the synthesized AgNPs are given in Table 2. Due to the thicker cell wall of the two Gram-positive bacteria, they proved less susceptible while the two Gram-negative bacteria with thinner cell walls together with *T. vaginalis* were more susceptible to the toxic effect of AgNPs. This observation is consistent with reports of past similar studies.[20] Gram-positive bacteria have thicker cell walls and so are less prone to the toxic effects of AgNPs unlike Gram-negative bacteria that have thinner cell walls.[20]

The activity mechanism of phytosynthesized AgNPs leading to bacterial death is still not clearly known. However, there have been suggestions of several hypothetical attacking sites by nanosilver. AgNPs can lodge in between cell walls and causes plasmolysis, leading to death of bacteria.[18,20] If nanosilver get pass the cell wall and get into the cytoplasm, Ag^+ ions are released as a result of electron transfer and in doing so can interact with sulfur and phosphorus containing compounds, thus interfering with protein synthesis and DNA replications.[18,20] Ag^+ ions can also interact with thiol groups in enzymes such as NADH dehydrogenase and disrupt the bacterial respiratory mechanism. Another possible explanation to bacterial cell death could be linked to the formation of free radicals by AgNPs that induce oxidative stress.[18,20]

CONCLUSION

In the present study, plant-mediated green synthesized AgNPs were assayed to examine the microbiological activity against five pathogenic microorganisms. These AgNP suspensions at 30 µL have shown antimicrobial activity comparable to two reference antibiotics, gentamycin (10 µg) and chloramphenicol (10 µg). The investigations have also shown the protozoan, *T. vaginalis* to be more susceptible to the AgNPs followed by the two Gram-negative bacteria and the less susceptible organisms to be the two Gram-positive bacteria. Overall, this finding supports various literature reports on the broad range antimicrobial activity of AgNPs.[20] In addition, biologically active and stable AgNPs for possible applications in consumer products can be constructed from the aqueous extract of *P. pellucida*. Plant species of *Peperomia* could be a good source of biomolecules required for the fabrication of stable bioactive nanosilver. These nanomaterials like others need further investigation for possible formulation as an alternative antimicrobial agent to be applied against antibiotic-resistant bacteria.[18] This study has also highlighted the extended ethnobotanical use of the plant, *P. pellucida*.[14,15]

ACKNOWLEDGMENT

We are grateful to the Research Committee of PNG University of Technology for the funding assistance toward this research. In addition, we wish to acknowledge the Department of Applied Sciences, PNG University of Technology for the use of the microbiology laboratory for doing the bioassay. Artis Vinas of the Forestry Department, PNG University of Technology assisted in plant identification.

REFERENCES

1. Prasad R, Kumar V, Prasad KS. Nanotechnology in sustainable agriculture: Present concerns and future aspects. Afr J Biotechnol 2014;13:705-13.
2. Bandyopadhyay AK. Nano Materials. New Delhi: New Age International Publishers; 2007.
3. Rauwel P, Rauwel E, Ferdov S, Singh MP. Silver nanoparticles: Synthesis, properties, and applications. Adv Mater Sci Eng 2015;2015:624394.
4. Tran QH, Nguyen VQ, Le AT. Silver nanoparticles: Synthesis, properties, toxicology, applications and perspectives. Adv in Nat Sci: Nanosci and Nanotechnol 2013;4:85-99.
5. Ahmed S, Ahmad M, Swami BL, Ikram S. A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise. J Adv Res 2016;7:17-28.
6. Ahamed M, Alsalhi MS, Siddiqui MK. Silver nanoparticle

applications and human health. Clin Chim Acta 2010;411:1841-8.
7. Feng QL, Wu J, Chen GQ, Cui FZ, Kim TN, Kim JO. A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. J Biomed Mater Res 2000;52:662-8.
8. Surwade P, Ghildyal C, Weikel C, Luxton T, Peloquin D, Fan X, *et al*. Augmented antibacterial activity of ampicillin with silver nanoparticles against *Methicillin-resistant Staphylococcus aureus* (MRSA). J Antibiot (Tokyo) 2019;72:50-3.
9. Ventola CL. The antibiotic resistance crisis: Part 1: Causes and threats. P T 2015;40:277-83.
10. Antimicrobial Resistance, World Health Organization. Available from: http://www.who.int/mediacentre/factsheets/fs194/en. [Last accessed on 2018 Apr 03].
11. Cox R. Bacterial Infections 101: Types, Symptoms, and Treatments-on Health; 2016. Available from: https://www.onhealth.com/content/1/bacterial_infections. [Last accessed on 2018 Aug 28].
12. Andrews JM. Determination of minimum inhibitory concentration. J Antimicrob Chemother 2001;48:5-16.
13. Turnidge J, Paterson DL. Setting and revising antibacterial susceptibility breakpoints. Clin Microbiol Rev 2007;20:391-408.
14. *Peperomia pellucida.* Available from: https://www.en.wikipedia.org/wiki/peperomia_pellucida. [Last accessed on 2018 Nov 05].
15. Khan MR, Omoloso AD. Antibacterial activity of *Hygrophila stricta* and *Peperomia pellucida*. Fitoterapia 2002;73:251-4.
16. Bojo AC, Albano-Garcia E, Pocsidio GN. The antibacterial activity of *Peperomia pellucida* (L.) HBK (Piperaceae). Asia Life Sci 1994;3:35-44.
17. Egwuche RU, Odetola AA, Erukainure OL. Preliminary investigation into the chemical properties of *Peperomia pellusida* L. Res J Phytochem 2011;5:48-53.
18. Gopinath K, Gowri S, Arumugam AJ. Phytosynthesis of silver nanoparticles using *Pterocarpus santalinus* leaf extract and their antibacterial properties. J Nanostruct Chem 2013;3:68-85.
19. Roberts RM, Gilbert JC, Rodewald LB, Wingrove AS. Modern Experimental Organic Chemistry. 4th ed. Philadelphia, PA: Saunders College Publishing; 1985.
20. Rather MY, Pandian KJ, Sundarapandian SM, Yogamoorthi A. Biosynthesis and characterization of silver nanoparticles using leaf extract of *Wedelia urticifolia* (Blume) DC and evaluation of antibacterial efficacy. IOSR J Pharm Biol Sci 2017;12:14-23.