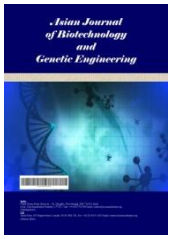

Asian Journal of Biotechnology and Genetic Engineering

5(2): 20-29, 2022; Article no.AJBGE.86042

Antimicrobial Evaluation of Biologically Synthesized Silver Nanoparticles using Aqueous Peel Extracts of Guava (*Psidium guavaja*) and Pumpkin (*cucurbita pepo*)

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MT, TMA and YA designed the Study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ZS and MM managed the analyses of the study. Authors RKM, AS, SSB and ALA managed the literature searches. All authors read and approved the final manuscript.

Article Information

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/86042

Original Research Article

Received 09 February 2022
Accepted 19 April 2022
Published 28 April 2022

ABSTRACT

Introduction: Green nanoparticle synthesis is a new field of nanotechnology that uses ecologically friendly resources such as entire cells, metabolites, agricultural waste such as peel, or extracts from plants and microbes to make metallic nanoparticles. In this study, silver nanoparticles were synthesized from aqueous extracts of *Psidium guavaja* (Guava) and *Cucurbita pepo* (Pumpkin) peels, and their antibacterial properties were evaluated against gram positive and negative bacterial isolates.
Methods: The effect of silver nanoparticles was tested against *Staphylococcus aureus, Proteus mirabilis*, and Gentamycin antibiotic sensitivity disks used as positive control, and the synthesised nanoparticles were analyzed using UV-visible spectroscopy, SEM, and FTIR.
Results: The UV-visible spectra obtained at different peaks between 200nm and 700nm confirmed

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the presence of synthesized silver nanoparticles, while the FTIR revealed the presence of certain functional groups such as C=C stretch, C-H bonding, and Alcohol OH stretch, which represent bioactive compounds such as phenol, amine, and others. The capping and reducing properties of the produced silver nanoparticles are due to these biomolecules. The SEM indicated that synthesized nanoparticles had a spherical, hexagonal, rod, and triangular form. The antibacterial activities of the Nano-particles, such as MIC and MBC, demonstrated their efficiency against the tested bacterial isolate. Antibacterial activity of *guava* and *pumpkin* nanoparticles against *Proteus mirabilis* and *Staphylococcus aureus* were found to be effective.

Conclusion: The studies confirmed that aqueous peel extract of *Psidium guavaja* (Guava) and *Cucurbita pepo* (Pumpkin) are good sources for synthesis of silver nano-particles via green route, the biologically synthesized silver nano-particles were found to have effective broad spectrum of antimicrobial activity against *Staphylococcus aureus* and *Proteus mirabilis.*

Keywords: Silver nano-particles; guava; pumpkin; peel; antimicrobial; UV-VIS; FTIR; SEM.

1. INTRODUCTION

Nanoparticles are small objects that range in size from 1 to 100 nanometers. Copper, zinc, titanium, magnesium, gold, and silver are increasingly being used to create a variety of metallic nanomaterials. Nanoparticles are used in a range of applications, including medicinal treatments, energy storage in a variety of industries, such as solar and oxide fuel batteries, and extensive incorporation into everyday goods like cosmetics and apparel [1]. Electronics, medicine, food processing, environmental applications, and cosmetics are known applications for nanoparticles [2].

Green nanoparticle synthesis is a new field of nanotechnology that uses ecologically friendly resources such as entire cells, metabolites, or extracts from plants and microbes to create metallic nanoparticles. It has several advantages over chemical and physical processes, including the fact that it is safe, easy, cost-effective, and relatively repeatable, as well as the fact that it often produces more stable materials [3]. The integration of green chemistry principles with nanotechnology has emerged as an important subject in nanoscience that has gotten a lot of attention in recent years. Biological approaches are utilized to synthesize metal and metal oxide nanoparticles of desired size and morphology, since they improve nanoparticle properties in a more environmentally friendly way.

Plant extracts, bacteria, and fungi have all been used to make silver nanoparticles (AgNPs). The biosynthesis of AgNPs is a bottom-up process involving primarily reduction/oxidation processes [4]. Microbial enzymes and phytochemicals with reducing potentials have been discovered to be responsible for nanoparticle capping and stability in this fashion. This is done in an environmentally responsible way that avoids the use of hazardous chemicals. AgNPs have been used in a variety of applications, including burn therapy, dental materials, textile textiles, water purification, and sunscreen lotions [5]. They can also be used to make antimicrobial paint [6], non-linear optics, and solar energy absorption by selectively coating surfaces and intercalation materials for electrical batteries, optical receptors, catalysis in chemical reactions, bio-labeling and antibacterial agents [7-8].

Guava is a tropical American tree and shrub belonging to the genus *Psidium* (family *Myrtaceae*). The word "guava" is said to come from the Arawak word guayabo, which means "guava tree," and is derived from the Spanish word *guayaba* [9]. It has been adapted in a similar manner in many European and Asian languages. Apple guava, yellow fruited cherry guava, strawberry guava, and red apple guava are all common guava varieties. It is typically consumed raw (ripe or semi-ripe) or in the form of juice, jams, and jellies [10-11]. The fruit of the common guava has a yellow skin and white, yellow, or pink flesh [12]. Guavas are well-known for their sweet, tart flavor and numerous applications, but there's a lot more to this fruit than meets the eye. Many consider it a “magical” fruit because of its array of nutrients and medicinal uses [13].

P. guajava has a long and illustrious ethno-medical history. Many portions of the plant are employed in various indigenous medical systems, particularly to treat gastrointestinal ailments. Crushing the leaves and using the liquids that come out of them to wounds, cuts, ulcers, boils, skin and soft tissue infection sites, and rheumatic locations are some of the ethno-medicinal uses

[14]. Pumpkin is a vegetable that belongs to the *Cucurbitaceae* family and is grown all over the world. Pumpkin gets its name from the Greek word Pepon, which means huge melon. In the stages of development, the American colonists replaced the ion with kin giving rise to pumpkin [5].Pumpkin is cultivated from northern Mexico to Argentina and Chile and has spread to Europe (France and Portugal, for example), Asia (India and China), African countries and Western America. Pumpkin is an annual vine or trailing plant and can be cultivated from sea level to high altitudes. It is famous for its edible seeds, fruit and green [16].

2. MATERIALS AND METHODS

2.1 Samples Collection

The fresh Guava (*psideum guavaja*), and Pumpkin (*cucurbita pepo*) was collected from Naibawa 'yan lemo market Kano State, Nigeria. And as authenticated at biology department Kano University of science and Technology Wudil, Kano with identification NO KUSTBIO-0022 and KUSTBIO-0024for guava and pumpkin respectively. And were washed with fresh clean water, and the peel were removed carefully, follows by shade dried for 14 days at room temperature and milled into powder with the aid of Mortar and pestle.

2.2 Preparation of the Extracts

The procedure is based on Perez and Bazerque's [17] approach with minor modifications: 10g of milled peel was weighed and suspended in 100 ml of distilled water. The resulting mixtures were heated for 1 hour in a water bath at 60°C, cooled, and filtered using Whatman No.1 filter paper before centrifugation at 4000 rpm for 20 minutes. Each sample's supernatants were collected and used for further research [17].

2.3 Synthesis of Silver Nanoparticles (AgNPs) Using Guava and Pumpkin Peel

The extracts from the Guava and Pumpkin peels were used to synthesised AgNPs according to the process described by Lateef et al., [18]; 1 ml of the extracts was put to a reaction vessel containing 40 ml of 2mM silver nitrate (AgNO3) solution for silver ion reduction. The reaction was carried out at room temperature (302°C) for 2 hours in a static state. The generation of AgNPs was monitored using a UV-visible spectrophotometer to detect the absorbance spectrum of the reaction mixture and ocular observation of color change.

2.4 Characterization of the Synthesized Silver Nano-particles

The synthesized silver nano-particles were characterized by UV-Vis spectroscopy, Scanning electron microscopy (SEM) and fourier transform infrared spectroscopy (FTIR).

2.5 Antibacterial Activities of the Synthesized Silver Nano-particles (AgNps)

The antibacterial activity of the synthesized AgNPs was determined by agar well diffusion method as described by Perez *at al*.,[19]. The test bacteria such as gram positive (*Staphylococcus aureus*) and gram negative (*Proteus mirabilis*) were obtained from the Department of Microbiology laboratory, Kano University of Science and Technology, Wudil. Each bacterium was grown overnight in nutrient broth medium and the turbidity of the 24hrs old cultures of the test bacteria was adjusted to 0.5MacFarland turbidity standard and then inoculated on the surface of the muller hinton agar plates by streaking method using sterilized cotton swab and then allow to dry for 15minutes. The plates were bored using a cork borer (7 mm) to create wells. The wells were loaded with graded concentrations of silver nanoparticles (AgNPs). Gentamycin antibiotic sensitivity disk were used as a positive control. The plates was incubated at 37^{o}C for 24 hrs. At the end of incubation, the plates were examined for diameter zones of inhibition using standard meter rule.

2.6 Minimum Inhibitory Concentration (MIC) of Silver Nano-particles (AgNPs)

The MIC of the produced nanoparticles was determined using the broth dilution method. Each test tube was labeled, and 5 mL of nutrient broth was poured to each before inoculating with 0.5 mL of bacterial suspension. The extract was then placed in sterile nutrient broth test tubes and incubated at 37oC for 24 hrs. Two control test tubes was used, the first control contained nutrient broth and test bacteria while the second

control contained nutrient broth and nano-particles extract. After incubation, growth of the test bacteria was checked by comparing the turbidity of the three sets of test tubes. The MIC was calculated using the lowest concentration of produced nanoparticles required to preclude observable growth [20].

2.7 Minimum Bactericidal Concentration (MBC) of Silver Nano-particles (AgNPs)

Standard techniques were used to determine the MBC of the produced nanoparticles. The test tubes that exhibited no obvious growth were sub-cultured onto fresh nutrient agar plates and incubated for 24 hours at 37 degrees Celsius. The minimum bactericidal concentration was defined as the lowest concentration at which the organism did not grow [21].

3. RESULTS AND DISCUSSION

3.1 Synthesis of Silver Nano-particle using Guava and Pumpkin Peel Aqueous Extracts

The guava and pumpkin peel aqueous extracts were used to synthesized silver nanoparticles (AgNPs) by using 2mM silver nitrate reagent, the color changed after 1 hr from yellowish to reddish brown and became dark brown after 24 hours, the Change indicate the presence of AgNPs which was normally brown in color. The biosynthesis of silver nanoparticles was confirmed by a change in color of colloidal suspension from yellowish to reddish [22] (See Figs. 1a and 1b).

3.2 Characterization of Synthesized Silver Nano-Particles Agnps of Guava and Pumpkin Peel Aqueous Extract

3.2.1 UV -visible spectrophotometer analysis of biosynthesized AgNPs

The surface Plasmon resonance (SPR) of the different reaction mixtures was investigated using UV-visible absorption spectroscopy. The UV-Vis spectra of the biosynthesized nanoparticles at 2mM revealed different peaks in the visible region ranging from 200 to 700nm, as shown in Fig. 2a and 2b for the two extracts of Guava and Pumpkin peel, indicating that silver nanoparticles were formed (AgNPs). Organic biomolecules in guava and pumpkin peel extracts served as effective reducing and capping agents for nanoparticle synthesis. The reacting mixture changed from yellowish color to light brown solution after 15 minutes of extract addition and incubation in a water bath at 60oC, indicating the formation of colloidal silver nanoparticles in the mixture. After 2 hours of incubation in a oven at 30±2°C, the color increased to dark brown. After two hours of incubation, the generation of silver nanoparticles was measured by scanning the mixture under UV-Vis spectrometer. The maximum absorbance was reported between 200nm and 450nm, which might correlate to colloidal silver nanoparticles' surface Plasmon resonance [23].

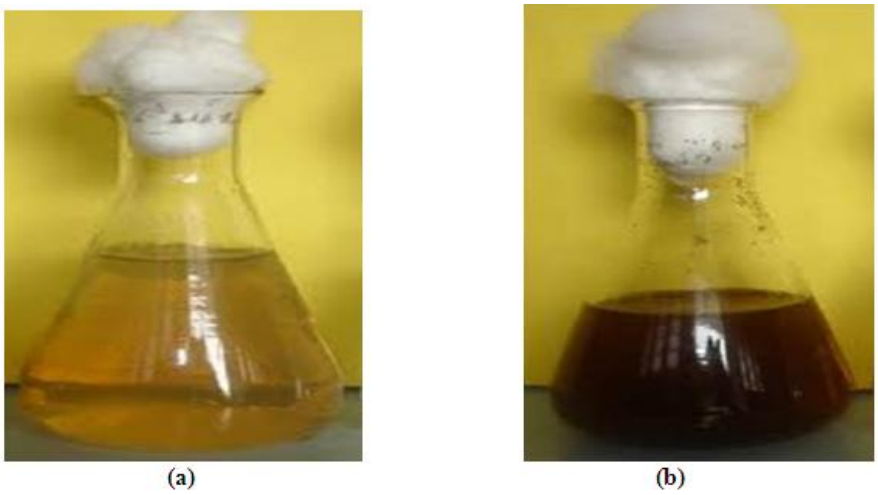

Fig. 1a and 1b. color confirmation of the synthesized silver nanoparticle guava and pumpkin peel

There are also minor differences in absorbance values between these two extracts, indicating that the differences are attributable to particle size or shape [24]. The effect of reaction time on the synthesis of silver nanoparticles was studied using UV-Vis absorption spectra, which revealed that as reaction time increased, the peaks became much stronger; this increase in intensity is associated with an increase in nano particle formation due to the continued reduction of silver nitrate by the biomolecular component in the aqueous extracts. The Plasmon resonance became constant after two days, suggesting that the process had achieved equilibrium. However, when the reactions continued for another two weeks in both guava and pumpkin extracts, the observed color began to fade, and no significant changes in color were noticed at this point. When 2 mM silver nitrate solution, ordinary guava, and pumpkin peel extract were utilized, there was no evidence of peaks] [25].

3.3 FT-IR Analysis of Guava and Pumpkin Synthesized Silver Nanoparticles

The FT-IR results for both guava and pumpkin silver nano particles were shown in table1, it displays some functional groups related to biochemical constituent of the nanoparticle that can be act as stabilizing and capping ability of the biologically synthesized AgNPs. These functional groups are within a distinctive frequency. Figure 3 shows the recorded FTIR spectrum of the produced silver nanoparticles. Alkyl amine is linked to the bands in the 1020 - 1220 cm-1 range[26]. Whereas bands at 858-733 cm^{-1} regions is attributed to C-H bonding[26]. Bands between 1535 to 1640 cm^{-1} can be attributed to keto group of diketones [26] , those between 2100 - 2260 cm^{-1} are considered to be for C=C of alkene [27] and the band between 3200 - 3550 are attributed to hydroxy group of alcohol and phenols[28]. It is thought that bioactive functional molecules found in the extracts of guava and pumpkin, such as phenols, amines, alkenes, and others, are responsible for the reduction of silver ions and the stabilization of colloidal particles during reactions.

3.4 Scanning Electron Microscopy (SEM) of the synthesized silver Nanoparticles using guava and pumpkin aqueous peel extract

The scanning electron microscopy was used to examine the morphology of silver nanoparticles made from guava and pumpkin peels (SEM). Figure 4a and 4b shows SEM images of synthesized silver nanoparticles. The particles of guava peel nanoparticles (GP-AgNPs) and pumpkin nanoparticles (PP-AgNPs) were discovered to be variable in form and size, with some being spherical, oval, and irregular in shape, which is a defining trait of nanoparticles [29]. Guava nanoparticles varied in size from 10–70 nm, while pumpkin nanoparticles ranged from 5-80 nm.

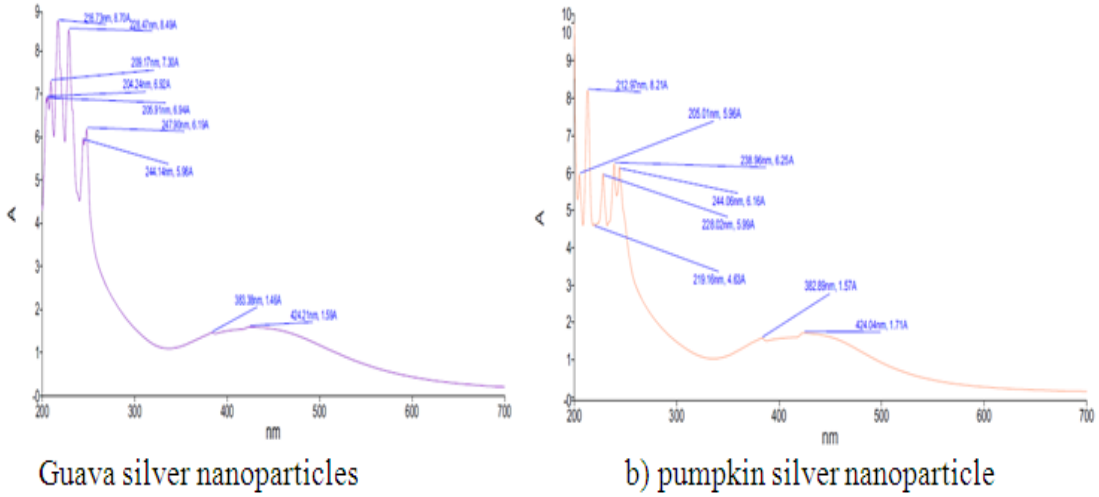

Fig. 2. The UV-vis absorption spectrum of the biosynthesized AgNPs for 2mM silver nitrate solution of guava and Pumpkin peel aqueous extract

Table 1. FTIR of Synthesized guava and pumpkin silver nanoparticles

Concentrations	Frequencies	Functional group	Intensity	Assignment
2mM Guava AgNPs				
	3260	Alcohol OH stretch	Strong	Alcohol
	2140	C=C stretch	Variable	Alkenes
	1637	Diketones	Variable	Ketones
	1060	Alkyl amine	Variable	Amines
	881	C-H bonding	Strong	Alkanes
	750	C-H bonding	Variable	Alkanes
2mM Pumkin AgNPs				
	3335	Alcohol OH Stretch	Strong	Alcohol
	2121	C=C stretch	Variable	Alkenes
	1640	Diketones	Variable	Ketones

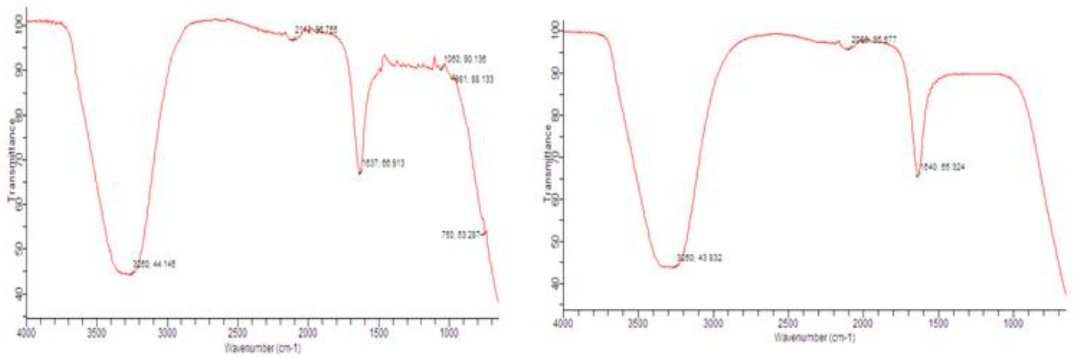

Fig. 3 . The FT-IR of biosynthesized AgNPS for 2mM silver nitrate solution for guava and pumpkin aqueous extract

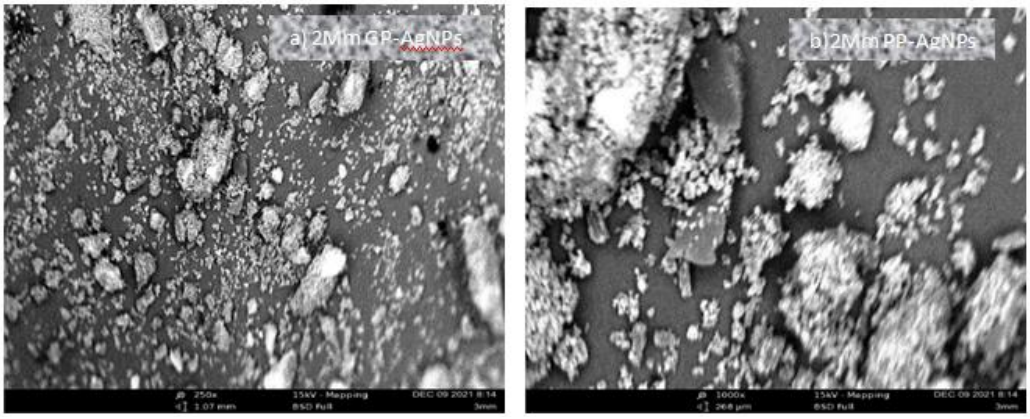

Fig. 4a and 4b. The SEM micrograph obtained for the Synthesized Silver Nanoparticles using guava and pumpkin aqueous peel extract

3.5 Antimicrobial Activity of Gauva and Pumpkin Silver Nanoparticles

The antibacterial activity of produced silver nanoparticles from aqueous peel extracts of Guava and Pumpkin against Staphylococcus aureus and Proteus mirabilis was determined. The antibacterial efficiency of the produced guava and pumpkin silver nanoparticles was determined by measuring the diameter zone of inhibition, minimum inhibitory concentration, and minimum bactericidal concentration. Antibiotics gentamycin, on the other hand, were utilized as a positive control (Fig. 5a,5b,6 a and 6b).

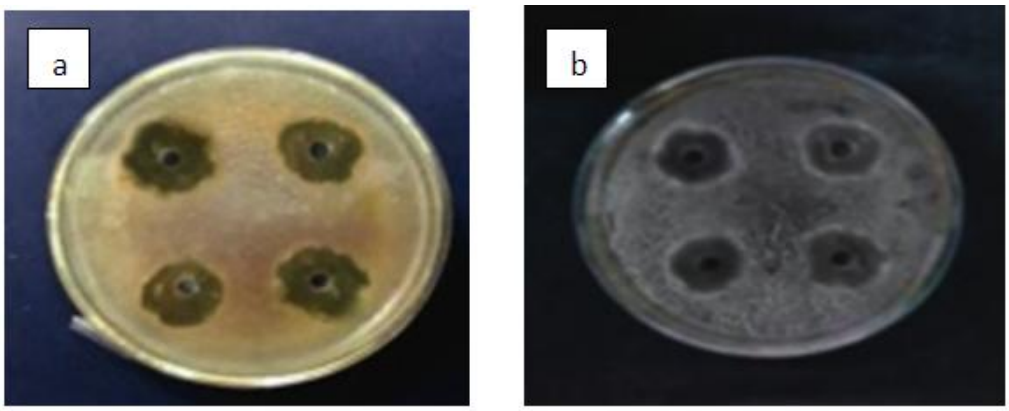

Fig. 5 a and 5b. Antibacterial activity of silver nanoparticles synthesized using (a) guava peel, (b) pumpkin peel, extract against *Staphylococcus aureus*

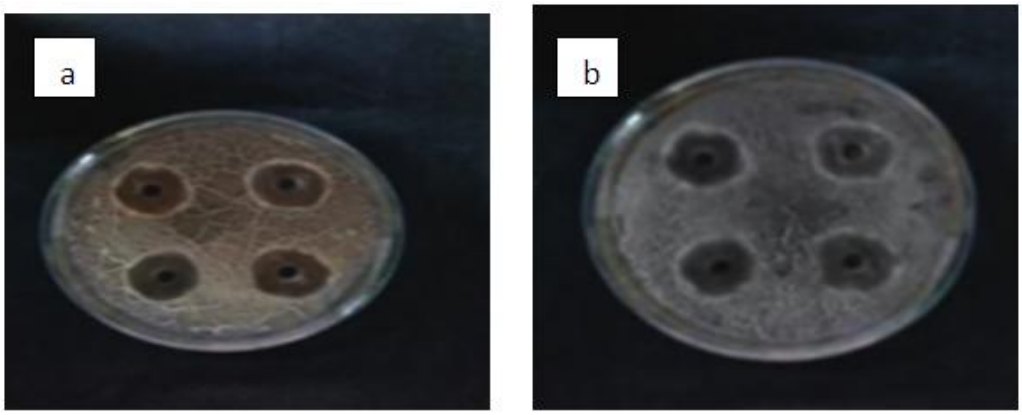

Fig. 6a and 6b. Antibacterial activity of silver nano-particles synthesized using (a) guava peel, (b) pumpkin peel, extract against *Proteus mirabilis*

Table 2. Antibacterial activity of silver nanoparticles of Guava and Pumpkin feels against bacterial isolates

Test Organisms	Gentamycin (mm)	2mM GP-AgNPs (mm)	2mM PP-AgNPs (mm)
Staphylococcus aureus.	14.84±0.24	13.33±1.55	11.43±2.15
Proteus M.	13.68±0.47	12.78±0.14	12.98±1.16

Key: Result were presented in triplicate as mean ± standard deviation, GP-AgNPs=Guava peel silver nanoparticles, PP-AgNPs= Pumpkin peel nanoparticles

Table 3. Minimum Inhibitory Concentration (MIC)

Test Organism	gentamycin (mm)	2mM GP-AgNPs (mm)	2mM PP-AgNPs (mm)
Staphylococcus aureus	-	-	-
Proteus mirabilis.	-	-	-

Key: negative = absence of growths, positive= presence of growths

Table4. Minimum bactericidal concentration

Test Organism	gentamycin Control (mm)	2mM GP-AgNPs (mm)	2mM PP-AgNPs (mm)
Staphylococcus aureus	-	-	-
Proteus mirabilis	-	-	-

Key: negative = absence of growths, positive = presence of growths

These synthesized silver nanoparticles were tested for antibacterial activity against *Staphylococcus aureus* and *Proteus mirabilis*. Table 2 shows the results for the diameter zones of inhibition. Synthesized silver nanoparticles of guava and pumpkin peels have showed remarkable antibacterial activity against *Staphylococcus aureus* and *Proteus mirabilis*, according to the findings of this study. The results of this investigation matched those of a previous study by Roy et al.,[27]. Pumpkin nanoparticles against *Proteus mirabilis* had the largest diameter zones of inhibition, whereas Pumpkin nanoparticles against Staphylococcus aureus had the smallest diameter zones of inhibition. The phytochemical contents of the extracts and the release of silver ions (Ag+) from silver nanoparticles into bacterial cells are responsible for the antibacterial actions of silver nanoparticles from Guava and Pumpkin peels [30]. Although the mechanisms of action of silver nanoparticles are unknown, the biochemical nature of a bacterial cell's cytoplasmic membrane can allow silver ions (Ag+) to enter the cell due to electrostatic forces of attraction [31]. As a result, the cytoplasmic composition may change rapidly, influencing cell permeability. This deteriorates cellular transport even further, resulting in cell death [32].

To observe the growth of bacteria in the nutrient broth, the MIC was carried out using the broth dilution method. Table 3 shows that silver nanoparticles suppressed the growth of Staphylococcus aureus and Proteus mirabilis at a concentration of 2Mm, whereas table 4 shows that the lowest bactericidal concentration revealed that nanoparticles of Guava and Pumpkin peels were bactericidal at a concentration of 2Mm. This study's findings are consistent with those of Yahya et al.,[28] who conducted another recent investigation.

4. CONCLUSION

Using aqueous peel extracts of guava and pumpkin, ecofriendly, easy, and nontoxic biological processes were used to produce silver nanoparticles in this study. Silver nanoparticles produced were shown to be effective capping and reducing agents. UV-visible spectroscopy, FT-IR spectroscopy, and SEM were used to analyze the guava and pumpkin silver nanoparticles. Silver nanoparticles synthesized were discovered to be 200-700nm in size, with a variety of forms including spherical, hexagonal, rod, and triangular-shapes, as well as diverse functional groups associated to biological molecules that provide the nanoparticles their capping capacity. Antibacterial activity was found in both guava and pumpkin nanoparticles against gram-negative (*Proteus mirabilis*) and gram-positive (*Staphylococcus aureus*) pathogens. The researchers discovered that silver nanoparticles made from guava and pumpkin peel extracts can be utilized to treat bacterial infections caused by *Staphylococcus aureus* and *Proteus mirabilis*.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge.

ACKNOWLEDGEMENT

The authors wish to acknowledged the support of Tertiary Education Trust fund (TETFUND) Nigeria and Kano University of Science and Technology Wudil, Kano, Nigeria for sponsoring the Research under Institutional Based Research Grant (TETFUND/DR&D/UNI/WUDIL/RG/2018/VOL.I)

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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