Original Research Article

ANTIMICROBIAL EVALUATION OF BIOLOGICALLY SYNTHESIZED SILVER NANOPARTICLES USING *AQUEOUS* PEEL EXTRACTS OF *GUAVA (Psidium guavaja)* AND PUMPKIN *(cucurbita pepo)*.

List of abbreviations

AgNPs -Silver nanoparticles, SEM-Scanning electron microscopy, FTIR-fourier transform infrared, SPR -surface Plasmon resonance, MIC-minimum inhibitory concentration, GP-AgNPs-Guava peel silver nanoparticles, PP-AgNPs-Pumpkin peel nanoparticles.

ABSTRACT

Introduction: The green synthesis of nanoparticles is an emerging branch of nanotechnology in which environmentally benign materials in the form of whole cells, metabolites, agricultural waste like peel etc, or extracts from plants and microorganisms are used for the synthesis of metallic nanoparticles. In this research a single step, fast, low cost and environment friendly green synthesis procedures was used to synthesize and characterized silver nanoparticles from aqueous extract of *Psidium guavaja* (Guava) and *Cucurbita pepo* (Pumpkin) peel and antimicrobial property against gram positive and negative bacterial isolates was also evaluated.

Methods: Silver nanoparticles were synthesized by treating silver nitrate solution with the aqueous extracts that served as reducing agents, the synthesized nanoparticles were characterized using UV-visible spectroscopy, SEM and FTIR, and their effect was tested against *Staphylococcus aureus*, and *Proteus mirabilis* and *Gentamycin* antibiotic sensitivity disk was used as positive control.

Results: The result showed that UV-visible spectra obtained between 200nm to 700nm at different peaks confirm the presence of synthesized silver nanoparticles, while the FTIR showed the presence of certain functional groups such as C=C stretch, C-H bonding and Alcohol OH stretch which represent the bioactive compounds such as phenol, amine etc this biomolecules are responsible for the capping and reducing property of the synthesized silver nanoparticles.While the SEM revealed a spherical , hexagonal , rod and trianagular shape depicted by synthesized nanoparticles. The antimicrobial activities such as MIC and MBC showed the efficacy of the Nano-particles against the tested bacterial isolate. Both guava and pumpkin nanoparticles shows an effective antibacterial activity against *Proteus mirabilis* and *Staphylococcus aureus*

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

1.0 Introduction

Nano-particles can be defined as an objects ranging in size from 1-100 nm. Presently, different metallic nanomaterials are being produced using copper, zinc, titanium, magnesium, gold and silver. Nanoparticles are being used for diverse purposes, for medical treatments, using various

branches of industry production such as solar and oxide fuel batteries for energy storage, to wide incorporation into diverse materials of everyday use such as cosmetics or clothes [1]. Nanoparticles cover a large area of interest including electronics, medicine, food industry, environmental applications and cosmetics [2].

The green synthesis of nanoparticles is an emerging branch of nanotechnology in which environmentally benign materials in the form of whole cells, metabolites, or extracts from plants and microorganisms are used for the synthesis of metallic nanoparticles. It is advantageous over chemical and physical methods as it is safe, simple, cost-effective, relatively reproducible, and often results in more stable materials [3]. The integration of the principles of green chemistry with nanotechnology has become a key area in nanoscience and has received great attention in recent years. Biological methods are used in the synthesis of metal and metal oxide nanoparticles of desirable size and morphology as they enhance the properties of nanoparticles in greener route.

Silver nanoparticles (AgNPs) have been successfully synthesized using plant extracts, bacteria, and fungi. Biosynthesis of AgNPs is a bottom-up approach that mostly involves reduction/oxidation reactions [4]. In this way, microbial enzymes and phytochemicals with reducing potentials have been found responsible for the capping and stabilization of nanoparticles. This is achieved in an eco-friendly manner, which does not lead to the use of toxic chemicals. AgNPs have been used in different ways; such as in the treatment of burns, as dental materials, in textile fabrics, for water treatment, and as sunscreen lotions [5]. Also have applications in the production of antimicrobial paint [6], non-linear optics, by selectively coating for solar energy absorption and intercalation materials for electrical batteries, optical receptors, catalysis in chemical reactions, bio-labeling and antibacterial agents [7-8].

Guava is of numerous trees and shrubs of the genus *Psidium* (family *Myrtaceae*) native to tropical America. The term “guava” appears to derive from Arawak guayabo “guava tree”, via the Spanish guayaba [9]. It has been adapted in many European and Asian languages, having a similar form. The common types of guava include apple guava, yellow fruited cherry guava, strawberry guava, and red apple guava. It is mostly eaten raw (ripe or semi-ripe) or consumed in the form of juice, jams, and jellies [10-11]. The common guava has a fruit with a yellow skin and white, yellow, or pink flesh [12] Guavas are known for their sweet and tangy flavor and many uses, but there’s much 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 rich ethno-medicinal history. Different parts of the plant are used in various indigenous systems of medicine, primarily for the treatment of gastrointestinal disorders. Some of the ethno-medicinal uses includes the crushing of the leaves and the application of the liquids coming out from them on wounds, cuts, ulcers, boils, skin and soft tissue infectious site, rheumatic places [14]. Pumpkin belongs to the family *Cucurbitaceae* and is a widely grown vegetable all over the world. The name pumpkin originated from a Greek word Pepon which means large melon. French converted the Pepon to Pompon and English adapted the word Pompion. 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].

2.0 Materials and Methods

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.

Preparation of the Extracts

The preparation is based on the method adopted by Perez and Bazerque [17] with slight modification; 10g of the milled peel was weighed each and suspended in 100 ml of distilled water. The mixtures obtained were heated in water bath at 60°C for 1hr, cooled and filtered using Whatman No.1 filter paper and then centrifuged at 4000 rpm for 20 min. The supernatants of each samples were collected and used for further study [17].

Synthesis of Silver Nanoparticles (AgNPs) Using guava and pumpkin peel

The supernatant of Guava and Pumpkin peel obtained were used to synthesized AgNPs using procedure describe by Lateef *at al*., [18]; 1 ml of the extracts was added to the reaction vessel containing 40 ml of 2mM silver nitrate ($AgNO_3$) solution for the reduction of silver ion. The reaction was carried out in static condition at room temperature (30±2°C) for 2 hrs. The formation of AgNPs was monitored through visual observation of the change of color and measurement of the absorbance spectrum of the reaction mixture using UV-visible spectrophotometer.

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).

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

The antibacterial activity of the synthesized AgNPs was determined using agar well diffusion method described by Perez *at al*.,[19]. The test bacteria used in this study was obtained from the Department of Microbiology laboratory, Kano University of science and technology, Wudil. They include gram positive (*Staphylococcus aureus)* and gram negative *(Proteus mirabilis) bacteria*. 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 then 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

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Minimum inhibitory concentration (MIC) of Silver Nano-particles (AgNPs)

The MIC of the synthesized nanoparticles was determined using broth dilution method. Different test tubes were labeled and 5ml of nutrient broth was introduced into each test tubes, 0.5 ml of bacterial suspension was inoculated. This was followed by the addition of the extract to the sterile nutrient broth test tubes and incubated at 37^{o}C 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 determined by the lowest concentration of the synthesized nanoparticles that prevent visible growth [20].

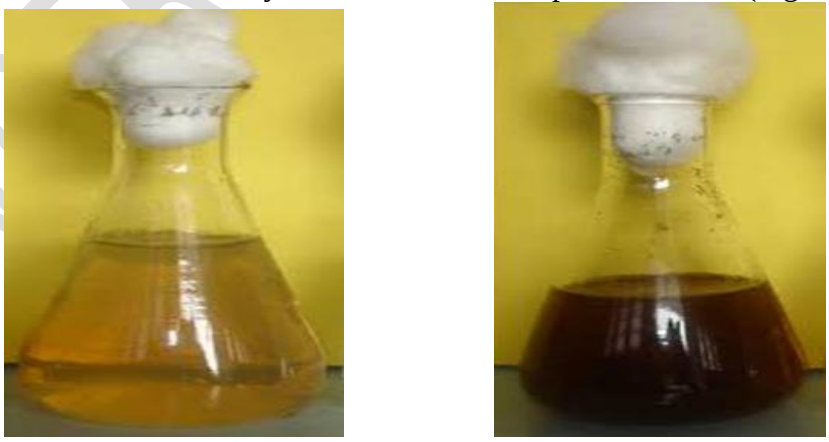
Minimum bactericidal concentration (MBC) of Silver Nano-particles (AgNPs)

The MBC of the synthesized nano-particles was determined according to standard procedures. The test tubes that showed no visible growth was sub-cultured onto fresh nutrient agar plates and then incubated at 37^{o}C for 24 hrs.The least concentration at which the organism did not grow was considered as the minimum bactericidal concentration [21].

3.0 Results and Discussion

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. A change in color from yellowish to reddish of colloidal suspension confirmed the biosynthesis of silver nanoparticles [22]. (Figure 1a and 1b).



(a) **(b)**

Figure 1a and 1b: color confirmation of the synthesized silver nanoparticle guava and pumpkin peel

Characterization of Synthesized Silver Nano-particles AgNPs of Guava and Pumpkin Peel Aqueous Extract

UV -Visible Spectrophotometer Analysis of Biosynthesized AgNPs

UV-visible absorption spectroscopy was used to investigate the surface Plasmon resonance (SPR) of the various reaction mixtures. The UV-Vis spectra of the biosynthesized nanoparticles at 2mM showed different peaks in the range of 200 to 700nm of the visible region, as shown in Fig. 2a and 2b for the two extracts of Guava and Pumpkin peel which confirm the formation of silver nanoparticles (AgNPs).

The guava and pumpkin peel extract contains organic biomolecules which served as effective reducing and capping agents for synthesis of nanoparticles. After 15min of extract addition and incubation in water bath at 60^{o}C, the reacting mixture turned from yellowish color to light brown solution, which indicates the formation of colloidal silver nanoparticles in the mixture. The color intensified to dark brown after 2 hrs of incubation periods in an oven at (30±2°C). The formation of silver nano-particles was monitored gradually by scanning the mixture under UV-Vis spectrometer after two hrsof incubation. The peak absorbance was observed maximally between 200nm-450nm which may correspond to the surface Plasmon resonance of colloidal silver nanoparticles [23]. There are some little variations in the values of absorbance between these two extracts which signifies that the changes are due the particle size or shape [24]. Effect of reaction time on the synthesis of silver nanoparticles was monitored with UV-Vis absorption spectra, and it indicated that an increase in time, the peaks become well strong, the increase in intensity is associated with the increases in the formation of nano particles due to continue reduction of silver nitrate by the biomolecular component in the aqueous extracts. The Plasmon resonance become constant after 2days which showed that the reaction is at equilibrium. But start diminishing as the reactions left further for two weeks in both guava and pumpkin extracts and at this stage no any significant changes of the observed color. There was no any indication of peaks when 2 mM silver nitrate solution, plain guava and pumpkin peel extract were used] [25].

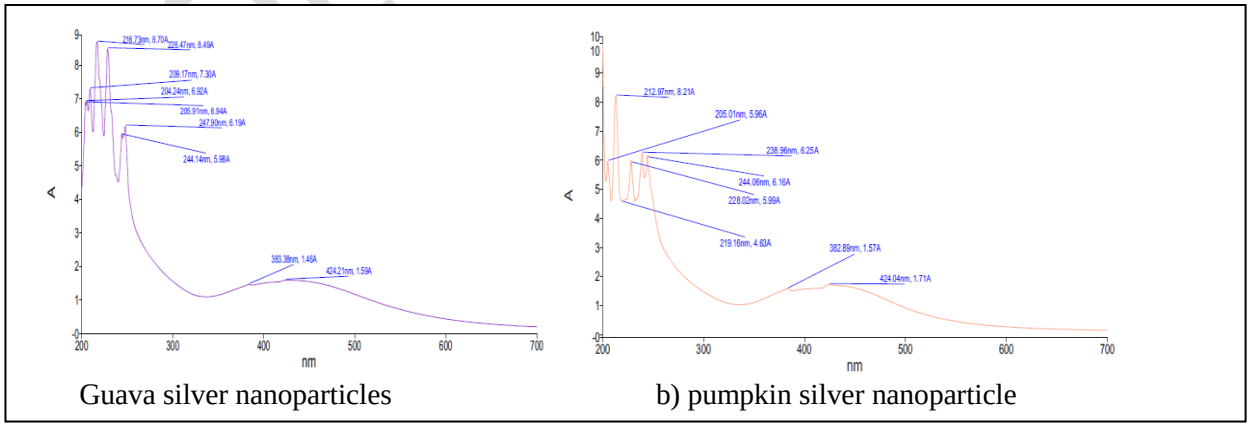


Figure 2. The UV-vis absorption spectrum of the biosynthesized AgNPs for 2mM silver nitrate solution of guava and Pumpkin peel aqueous extract**.**

Fourier Transform Infrared Spectroscopy (FTIR) of Guava and Pumpkin Silver nanoparticles

FTIR 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. The recorded FTIR spectrum of the synthesized silver nanoparticles were shown in Figure 3.The bands at region between 1020 - 1220 cm^{-1} is associated with alkyl amine[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]. Its believed that the bioactive functional molecules like phenols, amines, alkenes and others, were found to be present in the extracts of guava and pumpkin and are responsible for the reduction of silver ions and stabilize the colloidal particles during interaction.

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

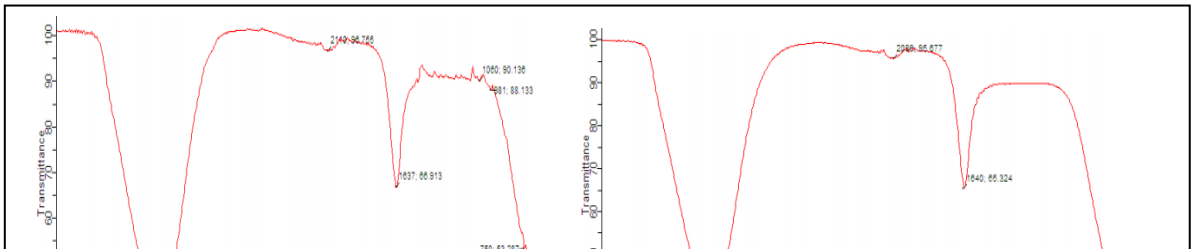


Figure 3 . The FT-IR of biosynthesized AgNPS for 2mM silver nitrate solution for guava and pumpkin aqueous extract

Scanning Electron Microscopy (SEM) of the Synthesized Silver Nanoparticles using guava and pumpkin aqueous peel extract.

The morphological studies of guava and pumpkin peel silver nanoparticles were performed using scanning electron microscopy (SEM). SEM images of synthesized silver nanoparticles were shown in Figure (4a and 4b). The guava peel nano particle (GP-AgNPs) and pumpkin nanoparticle (PP-AgNPs) showed that the particles were varied in shape and size, some were found to be spherical, oval and irregular in shape which is the characteristic property of nano particles [29]. The nanoparticles for guava were in size ranged from 10–70 nm while that of pumpkin were in ranged of 5-80nm.

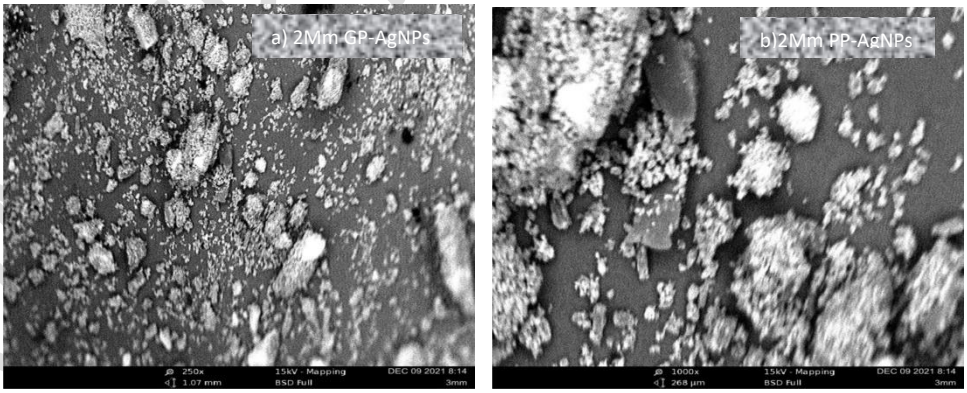


Figure 4: The SEM micrograph obtained for the Synthesized Silver Nanoparticles using guava and pumpkin aqueous peel extract.

Antimicrobial Activity of synthesized Silver Nanoparticles of Gauva and Pumpkin

The antibacterial activity of the synthesized silver nanoparticles of the aqueous peel extracts of Guava and Pumpkin was determined against *Staphylococcus aureus* and *Proteus mirabilis*. Measurement of the diameter zone of inhibition, minimum inhibitory concentration and minimum bactericidal concentration were evaluated to determine the antimicrobial efficacy of the synthesized guava and pumpkin silver nanoparticles. However, gentamycin antibiotics was used as a positive control (Figure 5a,5b,6 a and 6b).

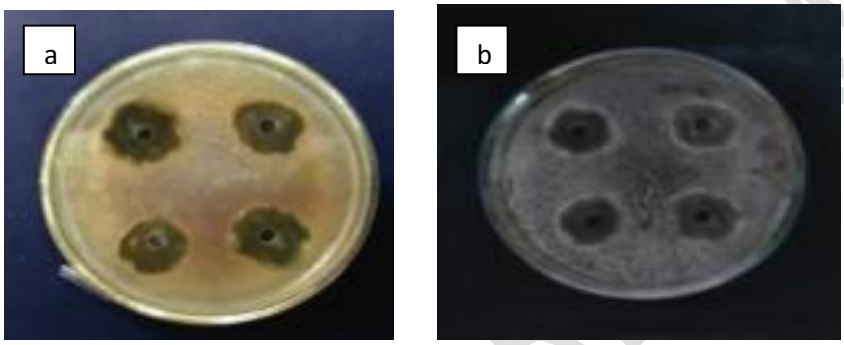


Figure 5 a and 5b: Antibacterial activity of silver nanoparticles synthesized using (a) guava peel, (b) pumpkin peel, extract against *Staphylococcus aureus.*

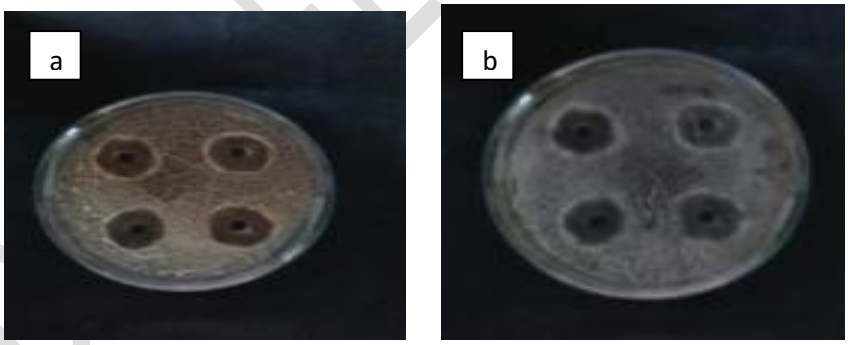


Figure 6a and 6b : Antibacterial activity of silver nano-particles synthesized using (a) guava peel, (b) pumpkin peel, extract against *Proteus mirabilis*

The antibacterial activity of these synthesized silver nanoparticles was studied against S*taphylococcus* a*ureus* and *Proteus mirabilis. The* results for the diameter zones of inhibition is shown in table 2. According to the findings of this study, synthesized silver nanoparticles of Guava and Pumpkin peels have shown promising antimicrobial activities against S*taphylococcus* a*ureus* and *Proteus mirabilis.* The findings of this study were consistent with another study conducted by Roy *et al*.,[27].The highest diameter zones of inhibition was observed in the synthesized nanoparticles of Pumpkin against *Proteus mirabilis* while the lowest was similarly

observed in the synthesized nanoparticles of Pumpkin against *Staphylococcus aureus.* The antibacterial activities of silver nanoparticles of Guava and Pumpkin peels can be attributed to the phytochemical constituents of the extracts and the release of silver ions (Ag^+) from silver nano-particles in to the bacterial cells [30].The mechanisms of action of the silver nanoparticles is not well understood, however, biochemical nature of cytoplasmic membrane of bacterial cell can allow silver ions (Ag^+) to enter the cell due to the electrostatic force of attractions [31]. As a result, the composition of the cytoplasm may vary rapidly affecting the cell permeability. This further degrades cellular transport and subsequently causes death of the cells [32].

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

Test Organisms	**Gentamycin (mm)**	**2mM (mm)**	**GP-AgNPs**	**2mM (mm)**	**PP-AgNPs**
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

The MIC was conducted using broth dilution method to observe the growth of bacteria in the nutrient broth. The silver nanoparticles have inhibited the growth of *Staphylococcus aureus* and *Proteus mirabilis* at the concentration of 2Mm as shown in table 3 and the minimum bactericidal concentration revealed that nanoparticles of Guava and Pumpkin peels was bactericidal at concentration of 2Mm as shown in table 4 .The results of this study is in line with another recent study conducted by Yahya *et al*.,[28]

Table 3 Minimum Inhibitory Concentration (MIC)

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

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

Table4. Minimum bactericidal concentration

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

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

5.0 Conclusion

In this study, an ecofriendly, simple and non toxic biological procedures was used to synthesized silver nanoparticles using aqueous peel extract of guava and pumpkin. The synthesized silver nanoparticles were found to be effective capping and reducing agent. The guava and pumpkin silver nanoparticles were characterized using UV-visible spectroscopy, FT-IR spectroscopy, and SEM. Synthesized silver nanoparticles were found to be in ranged from 200-700nm with different shapes such as spherical , hexagonal, rod and trianagular-shape and posses different functional groups related to biological molecules which give the nanoparticles its capping ability. Both guava and pumpkin nanoparticles showed an effective antibacterial activity against gram-negative (*Proteus mirabilis*) and gram-positive (*Staphylococcus aureus*) bacteria. The study revealed that the synthesized silver nanoparticles using aqueous peel extract of guava and pumpkin can be used to treat various bacterial infections associated with *Staphylococcus aureus* and *Proteus mirabilis.*

ETHICAL APPROVAL

This study do not involved human or animal subjects.

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COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. 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.

REFERENCES

[1]. Dubchak S., Mieteiski J.W. and Turnau K. Influence of silver and tianium nanoparticles on *arbuscularmycorhiza* colonization and accumulation of *radiocaesium* in *helianthus anus, span. J. Agric. Res*., 2010: 8(1), 103-108.

[2]. Jitendra Mittal, Amla Batra, Abhijeet Singh and Madan Mohan Sharma,. Phytofabrication of nanoparticles through plant as nanofactories. *Adv. Natural Sci. Nanosci. Nanotechnol*., 2014:5: 10.

[3]. Kannan, R.R.R., Arumugam, R., Ramya, D., Manivannan, K., Anantharaman, P. (2013). Green synthesis of silver nanoparticles using marine macroalga Chaetomorpha linum. *Appl. Nanosci*. 2013: 229–233

[4]. Lateef A., Ojo S.A., Akinwale A.S., Azeez L., Gueguim-Kana E.B., Beukes L.S. Biogenic synthesis of silver nanoparticles using cell-free extract of Bacillus safensis LAU 13: antimicrobial, free radical scavenging and larvicidal activities. *Biologia* , 2015: 70, 1295–1306.

[5]. Kumar, A., Vemula, P.K., Ajayan, P.M., John, G. (2008). Silver-nanoparticle-embedded antimicrobial paints based on vegetable oil. *Nat. Mater.* 7(3), 236–241

[6]. Nelson, D., Priscyla, D.M., Oswaldo, L.A., Gabriel, I.H., DeSouza, E.E. Mechanistic aspects of biosynthesis of silver nanoparticles by several *Fusarium oxysporum* strains. *J. Nanobiotechnol*. 2005:3, 1–7

[7]. Adamu A.U, Abdulmumin Y., Mustapha R.K. Green Synthesis, Characterization and Phytochemicals Analysis of Silver Nano-Particles Using Aqueous Peel Extract of *Cucumis sativus. Journal of Materials and Environmental Science*. 2022: 12(12), pp. 1627-1636

[8]. Izquierdo, L. & Sendra, J.M. Citrus fruits: Composition and characterization. In B. Caballero, L. Trugo & P. Finglas (Eds.). *Encyclopedia of food sciences and nutrition*, 2003:(Vol. 2, pp. 1335-1341). Oxford, UK: Academic Press11

[9]. Diplock, A.T. (1994). Antioxidants and disease prevention. *Molecular Aspects of Medicine*. 1994: 15, 293-376

[10]. Childs, L.N. Marketing issues for functional foods and nutraceuticals. In R.E.C. Wildman (Ed.), Handbook of nutraceuticals and functional foods.2001: (pp. 517-528). Boca Raton, FL: CRC Press Inc.11

[11]. Lockwood, B. Nutraceuticals , 2007:(pp. 1-18). London, UK: Pharmaceutical Press.11

[12]. Begum S, Hassan SI, Siddiqui BS. Two new triterpenoids from the fresh leaves of Psidium guajava. *Planta Med*. 2002; 68:1149-1152.

[13]. Aliyu BS. Some ethno-medicinal plants of the Savannah Regions of West Africa Description and phytochemicals. Triumph publishing company. 2006; 1:135-152.11

[14]. Anju K Dhiman, Sharma KD. , Surekha Attri " Functional constituents and processing of pumpkin: A review" *J Food Sci Technol* 2009, 46(5), 411-41711

[15]. Stovel D.D. Pumpkin: A Super Food for All 12 Months of the Year. North Adams, MA: 2005: Storey Publishing, LLC.11

[16]. Song JY, Jang HK, Kim BS. " Biological synthesis of gold nanoparticles using *Magnolia kobus* and *Diopyros kaki* leaf extract".Process Biochem ; 2009: 44:1133–8.11

[17]. Perez C., paul M. Bazerque p. Antibiotics assay by agar well diffusion method. *Acta Biol. Med. Exp*. 1990: 15: 113-115

[18]. Lateef A., Ojo S.A., Akinwale A.S., Azeez L., Gueguim-Kana E.B., Beukes L.S. Biogenic synthesis of silver nanoparticles using cell-free extract of Bacillus safensis LAU 13: antimicrobial, free radical scavenging and larvicidal activities. *Biologia* , 2015: 70, 1295–1306.

[19]. Andrews J.M. Determination of minmum inhibitory concentration. *J . Antimicrob. Chemother.* 2001: 48(1):5-16.

[20]. French G.L . Bactericidal agent in the treatment of MRSA infection--the potential role of *daptomycin. J. Antimicrob. Chemother. 2006:*58(6):1107-17.

[21]. Ahmad N., Sharma S., Singh V.N. .Shamsi S.F, Fatma A. and Mehta B.R. Biosynthesis of silver nanoparticles from Desmodium Triflorum a novel approach towards weed utilization, *Biotechnol. Res. Int.* 2011: 454090(1-8).

[22]. Prabhu, S., and Poulose, E.K. Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. *Int. Nano Lett. 2012:* 2, 3210.

[23]. Deepashri C. Lingegowda, J. Komal Kumar, A.G. Devi Prasad, Mahsa Zarei, Shubha Gopal. "FTIR spectroscopic studies on cleome gynandra – comparative analysis of functional group before and after extraction" romanian j. Biophys., 2012: Vol. 22, Nos 3–4, P. 137–143,

[24]. Gong, P., Li, H., He, X., Wang, K., Hu, J., Tan, W : Preparation and antibacterial activity of Fe3O4 and Ag nanoparticles. *Nanotechnology* 2007: 18, 604–611

[25]. Gasaymeh, S.S., Radiman, S., Heng, L.Y., Saion, E., Saeed, H.M . Synthesis and characterization of silver/polyvinilpirrolidone (AG/PVP) nanoparticles using Gamma irradiation techniques. Am. *J. Appl. Sci*. 2010: 7(7), 892–901

[26]. Sunday A.O., Lateef A., Musibau A.A., Suliat M.O., Abiola S.A., Tesleem B.A., Taofeek A.Y, Akeem A., Iyabo C.O., Evariste B.G., and Lorika S.B.: Biomedical and Catalytic Applications of Gold and Silver-Gold Alloy Nanoparticles Biosynthesized Using Cell-Free Extract of Bacillus Safensis LAU 13: Antifungal, Dye Degradation, Anti-Coagulant and Thrombolytic Activities. *Ieee transactions on nanobioscience,2016:* vol. 15, no. 5

[27]. Roy k., Sarkar C.K., Ghosh C.K.: Single-step novel biosynthesis of silver nanoparticles using cucumis sativus fruit extract and study of its photcatalytic and antibacterial activity. *Digest Journal of Nanomaterials and Biostructures* , 2015: Vol. 10, No. 1

[28]. Yahya S. M., Abdulmumin Y.,. Abdulmumin T. M, Sagagi B. S., Murtala M, Salau A. K. and Hassan S. A.: Biological Synthesis, Characterization and Antimicrobial effect of Silver Nanoparticles (Ag-NPs) using Aqueous Extract of Mango Pulp (*Mangifera indica*): *Journal of Complementary and Alternative Medical Research* : 2021: *13(4): 39-50,*

[29]. Selvi, K.V., and Sivakumar, T.: Isolation and characterization of silver nanoparticles from Fusarium oxysporium. *Int. J. Curr. Microbiol. Appl. Sci*. 2012:1(1), 56–62

[30]. Jung W.K., Koo H.C., Kim K.W., S. Shin, S.H. Kim and Y.H. Park. Antibacterial activity and mechanism of action of the silver ion in *Staphylococcus aureus* and *Escherichia coli. Appl. Environ. Microbiol*., 2008: 74(7), 2171

[31]. Zhang X.Q., Xu X., Bertrand N., Pridgen E., Swami A., and Farokhzad O.C.: Interactions of nanomaterials and biological systems: Implications to personalized nanomedicine. *Adv. Drug Deliv. Rev*. 2012: 64, 1363

[32]. Sahoo S. K., Parveen S., and Panda J.J. (2007). The present and future of nanotechnology in human health care. *Nanomedicine: Nanotechnology, Biology and Medicine. 2007:* 3 (1), 20-31