ANTIMICROBIAL ACTIVITIES AND EVALUATION OF BIOGENIC SILVER NANOPARTICLES AS ANTIMICROBIAL ADDITIVE IN PAINT

ADELERE, I. A., BABAYI, H., ABOYEJI, D. O., ADABARA, N. U., JAGABA, A., & SUNDAY, H. Z.
Department of Microbiology,
Federal University of Technology, PMB 65, Minna, Niger State, Nigeria
Email: isiaka.ade@futminna.edu.ng **Phone No:** +234-806-218-7217

Abstract
In the present study, aqueous seed extract of bitter kola (Garcinia kola) was utilized for biological synthesis of silver nanoparticles (AgNPs) under the influence of solar irradiation. Antimicrobial activities and potential application of the biogenic AgNPs as antimicrobial additive in emulsion paint were examined. The AgNPs were characterized by UV-vis spectroscopy, fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM). The particles displayed brown coloration with maximum absorbance at wavelength 437nm. The FTIR spectrum showed distinct peaks at 3267 and 1635 cm^{-1} indicating the role of biomolecules in the capping and stabilization of the biogenic AgNPs. The particles were spherical in shape with sizes of 20-60 nm. Furthermore, the biogenic AgNPs exhibited moderate antibacterial activity against clinical isolates of Staphylococcus aureus and Salmonella Typhi while a very remarkable antifungal efficacy was also demonstrated against strains of Aspergillus niger and Aspergillus fumigatus. The AgNPs when incorporated as an additive into white emulsion paint completely inhibited the growth of Staphylococcus aureus, Bacillus subtilis, Aspergillus niger and Aspergillus flavus. Hence, the results obtained suggest that the biogenic AgNPs have promising biomedical application and could also serve as an essential ingredient in the manufacture of novel nanopaint.

Keywords: Aqueous extract, antimicrobial property, biological synthesis, *Garcinia kola*, and silver nanoparticles

Introduction

The introduction of green chemistry principles to the field of nanotechnology has greatly revolutionized the area and has attracted remarkable attentions in recent years (Adelere & Lateef, 2016). The method has been extensively researched and has successfully led to the synthesis of very effective and stable metallic and non-metallic nanoparticles. It shows advancement over the conventional chemical and physical methods due to its cost effectiveness, simplicity, eco-friendliness, biocompatibility, and diverse areas of applications (Adelere & Lateef, 2016; Elegbede *et al.,* 2018; Adelere *et al*., 2020). Biosynthesized nanoparticles especially silver nanoparticles have found applications in so many areas including agriculture, environmental management, biomedical field, catalysis, electronics, in the manufacture of personal care products among others (Keat *et al*., 2015). Silver nanoparticles had received unprecedented attentions compare to other metallic nanoparticles due to its strong antimicrobial efficacy.

Various kinds of biomolecules from microorganisms and plants have been utilized for the synthesis of metallic nanoparticles, but synthesis involving plant materials is more advantageous due to the complexity of microbial culture maintenance (Singaravelu *et al*., 2007). The rich biodiversity of plants and their secondary metabolites has prompted their utilization in the fabrications of varieties of nanoparticles (Madhumitha *et al*., 2013). Plant extracts contain abundant biomolecules like steroids, flavonoids, saponins, tannins, alkaloids and others. They act as reducing and stabilizing agents in the green synthesis of metallic nanoparticles (Kuppusamy *et al.,* 2016). Researchers have documented several reports on

the synthesis of stable and very effective silver nanoparticles mediated by phytochemicals obtained from varieties of medicinal and non-medicinal plants (Lateef *et al*., 2015b; 2016a, b; Devadiga *et al*., 2015; Bhakya *et al.,* 2016; Adelere *et al*., 2017; Adelere *et al*., 2020).
Bitter kola (*Garcinia kola*) is a perennial crop that belongs to family *Guttiferae*. It is widely distributed in the wild forest of West and Central Africa with an average height of 12m (Iwu, 1993). It has nutritional and medicinal importance (Okwu & Morah, 2006); the root and stem are used to produce chewing sticks in some parts of West Africa (Okwu & Ekeke, 2003). The seed is characterized with bitter taste and is chewed extensively as a masticatory and is often used to entertain visitors especially in the Eastern part of Nigeria (Uko *et al.,* 2001). *G. kola* seeds (Fig. 1) has both seeds coat dormancy and physiological dormancy probably imposed by the chemicals in the seed (Oboho & Urughu 2010). They contain phytochemicals such as phenolic compound, tannin, and flavonoids. The flavonoids and phenolic compounds are responsible for their antimicrobial, anti-inflammatory, anti-oxidant, anti-tumour, and anti-hepatotoxic properties. Traditionally, the plant has been involved in the treatment of colic disorders, cirrhosis, hepatitis and cough (Okwu & Morah, 2006).

Figure 1: Bitter kola seeds

In this study, we synthesized silver nanoparticles using aqueous seed extract of bitter kola as a reducing, stabilizing and capping agent. The antimicrobial activities of the synthesized silver nanoparticles were tested on some clinical bacterial isolates and also evaluated for their potential application as an antimicrobial additive in emulsion paint.

Materials and Methods

Preparation of Bitter kola Seed Extract

Bitter kola seeds were purchased from Bosso central market of Minna, Niger State, Nigeria. They were thoroughly washed in the laboratory to remove dirts and other extraneous materials. The seeds were chopped into smaller pieces and dried at room temperature (30 ± 2℃) for 2 weeks. The dried seeds were milled into powdery form using an electric blender. About 1 g of the powder was added into 100 ml of deionized water and heated in water bath at 60 ℃ for 1 h. The extract was then filtered using Whatman No. 1 filter paper and stored at 4℃ for further studies.

Biosynthesis and Characterization of Silver Nanoparticles

The biosynthesis of silver nanoparticles was carried out using the procedure described by Lateef *et al*. (2015a, b; 2016a, b). In this method, 1 ml of aqueous extract of bitter kola seeds was mixed with 10 ml of 1 mM silver nitrate ($AgNO_3$) and allowed to stand under bright sun light for 1 h. The reaction was visually monitored to observe color development while the preliminary characterization to determine the absorption spectrum of the resulting solution was carried out using UV-vis spectrophotometric analysis.

The AgNPs solution was freeze dried and mixed with KBr pellet. The resulting mixture was used for fourier transform infrared (FTIR) spectroscopy analysis using BUCK M530 Spectrophotometer according to Bhat *et al*. (2011). For scanning electron microscopy (SEM)

analysis, dried sample of the AgNPs was mounted on specimen stub coated with copper and the image was taken under scanning electron microscope (JEOL, Model 6390). The particle size was calculated using the magnification size in micrometer to determine the scale bar and was converted to nanometer.

Antimicrobial Activities of the Biogenic AgNPs

The agar disc diffusion method was used to investigate the antibacterial activity of the biogenic AgNPs using method of Ahmed and Ikram (2015). The test organisms were clinical isolates of *Staphylococcus aureus* and *Salmonella* Typhi obtained from General Hospital, Minna. The 6 mm diameter paper discs were prepared from Whatman No.1 filter paper and sterilized at 60℃ in hot air oven for 1 h. The isolates were grown in peptone water for 18 h, each was used to seed the plates of Mueller-Hinton agar. The paper discs were impregnated with graded concentrations of AgNPs (50µg ml^{-1}, 100µg ml^{-1} and 150µg ml^{-1}) prepared by dilution with sterile distilled water. The AgNPs impregnated discs were gently placed on surface of those seeded plates of Mueller-Hinton agar and thereafter incubated at 37℃ for 24 h. After incubation, the plates were examined for the zones of inhibition and were measured using metric rule.

The method of Khatami *et al.* (2015) was adopted for the evaluation of antifungal activity of the biogenic AgNPs. In this method, the AgNPs (150 µg/ml) was used to treat about 45℃ cooled saboraud dextrose agar (SDA) (1:10, v/v), wells of 6 mm diameter were thereafter created on the plates of treated SDA using sterile cork borer, the wells were inoculated with mycelial agar plug (6 mm) of 48-h old cultures of fungal strains including *Aspergillus niger* and *Aspergillus fumigatus.* In the control experiments, fungal plugs were inoculated on SDA plates without the AgNPs incorporation. All the plates were incubated under ambient conditions (30 ± 2 ℃) for 72 h. The diameters of fungal growth in all plates were measured and used to determine the percentage of fungal growth inhibitions as follows:

$\frac{D_{control} - D_{test}}{D_{control}} \times 100\ \%$ where D is the diameter of fungal growth on the SDA plates.

Evaluation of the BiogenicAgNPs as Antimicrobial Additive in Paint

In this test, antimicrobial efficacy of the biogenic AgNPs was evaluated in white emulsion paint as described by Lateef *et al.* (2016b). White emulsion paint purchased from a reputable retailer outlet was prepared in the laboratory according to manufacturer's instructions. The prepared paint was dispensed as 19 mL into McCartney bottles, autoclaved at 121 ℃ for 15 minand thereafter mixed with 1 ml of 150 µg/ml of biosynthesized AgNPs. After cooling, 1mL of 18 h old cultures of *Escherichia coli* and *Bacillus subtilis* were inoculated into separate bottles. In case of *Aspergillus niger* and *Aspergillus fumigatus*, 1 ml of 48 h old cultures was used as inoculum. The control experiments consisted of the paint and test organisms only. All bottles were incubated at 30 ± 2℃ for 48 h. After incubation, 1 mL of the contents of each bottle was drawn and inoculated into fresh plates of nutrient agar and potato dextrose agar for bacterial and fungal cultures respectively using pore plate technique. The plates were then incubated at ambient temperature for 48 h and thereafter observed for microbial growth.

Results and Discussion

Biosynthesis and Characterization of the Biogenic AgNPs

The aqueous extract of bitter kola reduced silver ion to dark brown AgNPs within 10 min of reaction under bright sun light (Fig. 2) while the control silver nitrate solution without bitter kola extract showed no color change. The result obtained corroborates previous studies where authors reported closely related colours of biologically synthesized AgNPs such as

light yellow, deep yellow, yellow brown and dark brown (Lateef *et al.,* 2016a, Adelere *et al.,* 2017, Aina *et al.,* 2019; Adelere *et al.,* 2020).There had been variations in the colors of the previously reported biosynthesized AgNPs owing to complexity of reducing biomolecules employed. The color formation in the AgNPs synthesis is mainly due to excitation of surface plasmon resonance (SPR) in the metallic nanoparticles (Selvi & Sivakumar, 2012).

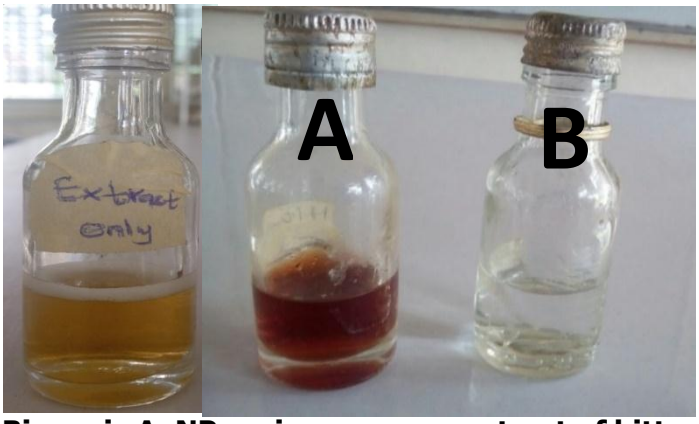

Figure 2: Biogenic AgNPs using aqueous extract of bitter kola seed: A, synthesized AgNPs within 10 min; B, control silver nitrate solution

The SPR band displayed by the biogenic AgNPs was 420 nm (Fig. 3) which is within the range of 391-460 nm AgNPs absorbance characteristics earlier reported (Thirumurugan *et al.,* 2011; Zaki *et al.,* 2011; Kannan *et al.,* 2013; Priyadarshini *et al.,* 2013; Lateef *et al*., 2015a, b; 2016a, b; Adelere *et al.,* 2017; Adelere *et al.,* 2020). The UV-vis absorbance characteristic of AgNPs is also the function of their surface plasmon resonance (Creighton *et al*., 1979). The FTIR absorption spectrum showed distinct peaks at 3267 and 1635 cm^{-1} (Fig. 4). These bands correspond to N-H bond of amines, and C=C stretch of alkenes or C=O stretch of amides, respectively (Shankar *et al.,* 2014). Kummara *et al.* (2016) reported that most studies recognized phenolic compounds as the active reducing compounds among other biomolecules in the synthesis. Also, proteins are involved in the stabilization and capping of nanoparticles by binding either through their free amine groups or cysteine residues (Gole *et al.,* 2001; Mandal *et al*., 2005). Hence, it can be inferred that the phytochemicals present in the bitter kola seed extract are responsible for the reduction of silver ion.

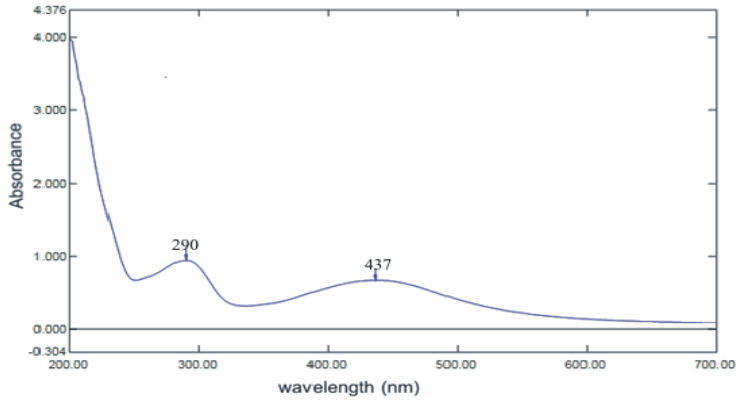

Figure 3: UV-vis absorption spectrum of the biogenic AgNPs using aqueous extract of bitter kola seeds

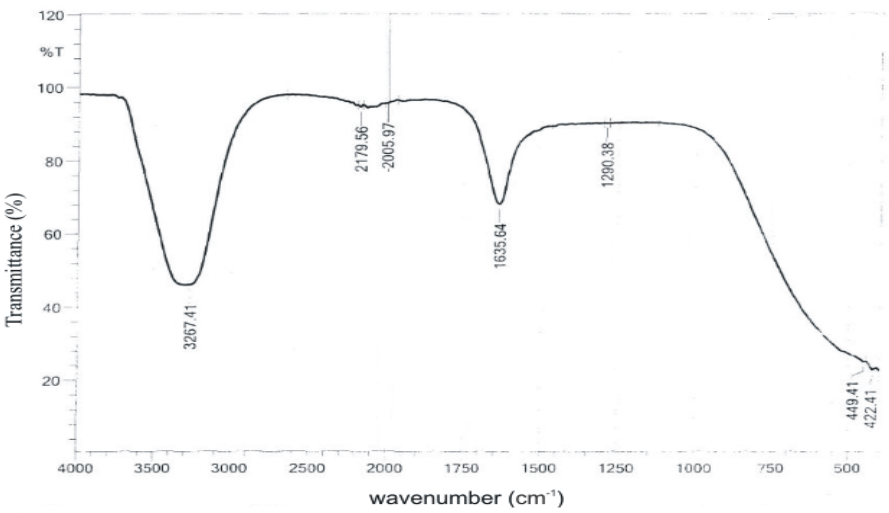

Figure 4: FTIR spectrum of the biogenic AgNPs using aqueous extract of bitter kola seeds

The characterization of size and shape of the biogenic AgNPs by scanning electron microscopy revealed that the particles are spherical in shape with the size ranging from 20-60 nm (Fig. 5). Zaki *et al*. (2011) and Kannan *et al*. (2013) had reported spherical shape of biosynthesized AgNPs while Lateef *et al*. (2015a) reported the synthesis of spherical shaped AgNPs in the size range of 5-30 nm.

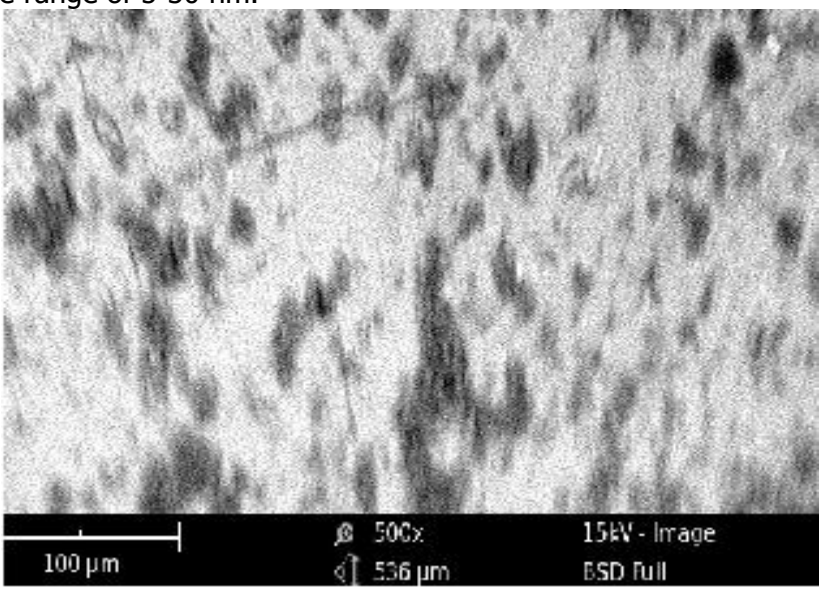

Figure 5: SEM image of AgNPs synthesized using aqueous extract of bitter kola seeds

The AgNPs exhibited considerable antibacterial activities against the clinical isolates of *Staphylococcus aureus* and *Salmonella* Typhi (Fig. 6). The particles at concentrations of 50-150 µg/ml moderately inhibited the growth of tested bacterial strains by inducing inhibition zones of 11-13 mm (Table 1).There are several reports on antibacterial efficacy of AgNPs against various bacterial species, however, these findings agree with the results of Nanda and Saravanan (2009) and Saravanan *et al.* (2011), on the antibacterial activities of biosynthesized AgNPs against *Staphylococcus aureus* and *Salmonella* Typhi, respectively.

Furthermore, the biosynthesized AgNPs demonstrated tremendous antifungal effects against the strains of *Aspergillus niger* and *Aspergillus fumigatus* (Fig. 7) by producing over 65 % fungal growth inhibition against the tested fungal strains (Table 2). Literatures have reported several mechanisms of antimicrobial activity of AgNPs. For instance, Morones *et al.* (2005) ascribed the efficient antimicrobial activity of AgNPs to their minute sizes, as well as their large surface area to volume ratio which facilitates their interaction with microbial cells.

Similarly, studies have suggested that size, morphology, and chemical compositions play an active role in the antimicrobial activity of nanoparticles. Moreover, AgNPs is considered one of the most potent antimicrobial type of nanoparticles, as it attack and disrupt cell wall and cytoplasmic membrane through electrostatic attraction (Raffi *et al.,* 2008). Silver ions release by AgNPs disrupt microbial cells by denaturing cell membrane, interfering with DNA replication and electron transport chain (Feng *et al*., 2000; Sondi and Salopek-Sondi, 2004; Morones *et al*., 2005; Song *et al*., 2006). Microorganisms are unable to develop resistance against AgNPs compared to conventional antibiotics owing to the fact that AgNPs are capable of attacking many microbial organelles and molecules.

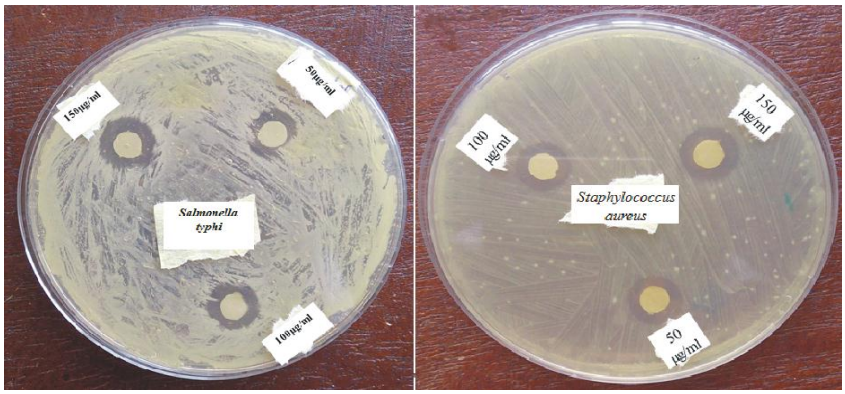

Figure 6: Antibacterial activities of the biogenic AgNPs using aqueous extract of bitter kola seeds against clinical strains of *Staphylococcus aureus* (right) and *Salmonella* Typhi (left)

Table 1: Antibacterial Activity of Biogenic AgNPs and zones of Inhibition (mm)

Concentration (µg/ml)	Zone of inhibition (mm)	
	S. aureus	*S.* Typhi
50	12	11 ± 0.02
100	13	12 ± 0.01
150	15	15 ± 0.04

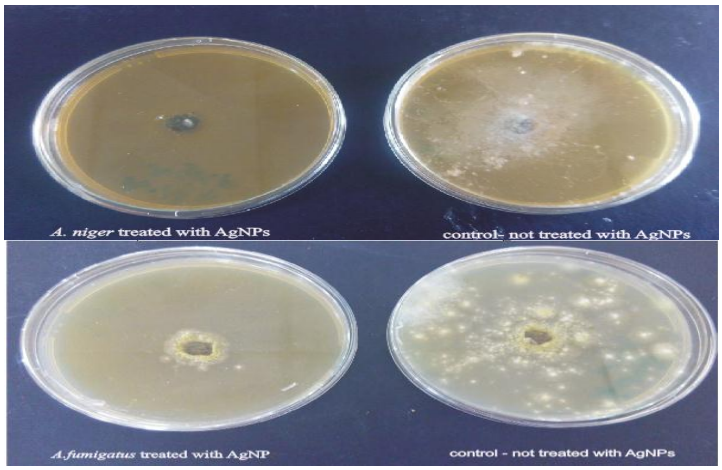

Figure 7: Antifungal activities of the biogenic AgNPs against strains of *A. niger* and *A. fumigatus*

Table 2: Antifungal activities of biogenic AgNPs on fungal isolates

Organisms	PDA plate + AgNPs	PDA plate only	% of mycelial inhibition
. *Niger*	20 ± 0.01	60 ± 0.02	66.7
. *Fumigatus*	20 ± 0.00	80 ± 0.01	75

The particles displayed excellent antimicrobial effects against the activities of tested isolates of *Bacillus subtilis, E. coli, Aspergillus niger,* and *Aspergillus flavus* in the AgNPs-treated paint unlike heavy growth observed in the control plates (Fig. 8). Kumar *et al.* (2008) had earlier proposed the application of AgNPs as an additive to make eco-friendly antimicrobial nanopaint. Thereafter, many authors reported successful application of AgNPs as an antimicrobial additive in paint (Rajarathinam *et al.,* 2014; Lateef *et al.,* 2015). In view of this, the biogenic AgNPs could therefore be incorporated in the manufacture of emulsion paint to safeguard microbial attack and other forms of biodegradation.

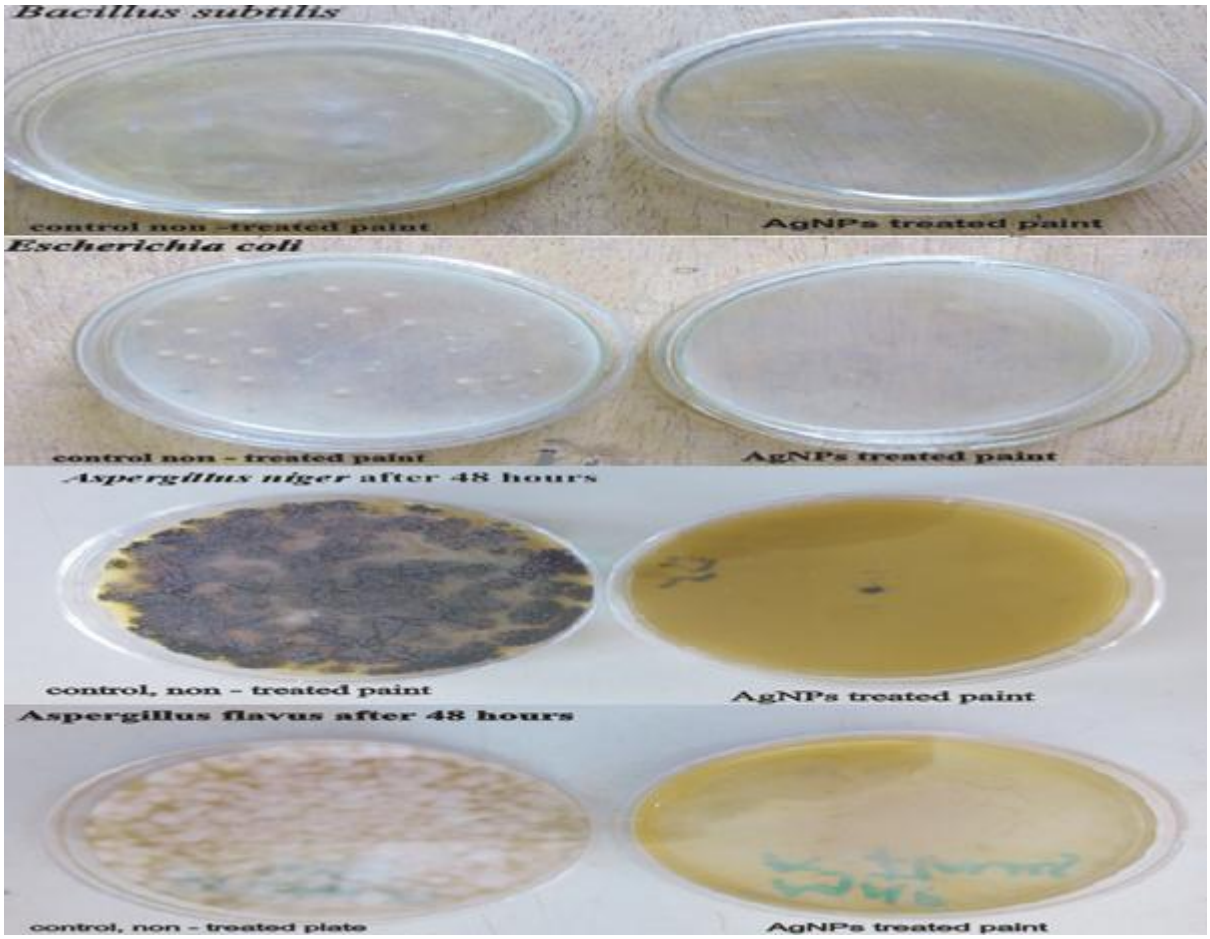

Figure 8: Antimicrobial activities of biogenic AgNPs on fungal and bacterial strains inoculated into emulsion paint

Conclusion

This study has led to the synthesis of AgNPs using aqueous extract of bitter kola through a simple, cheap and environmentally benign method. The phytochemicals present in the extract played a vital role in the reduction of Ag from +1 to 0 oxidation state. The particles are spherical in shape with size of 20-60 nm. They showed remarkable antimicrobial effects against the clinical bacterial isolates of *Staphylococcus aureus* and *Salmonella* Typhi as well as fungal strains of *Aspergillus niger* and *Aspergillus fumigatus*. Furthermore, the AgNPs produced tremendous antimicrobial effects against the activities of *Staphylococcus aureus*, *Bacillus subtilis*, *Aspergillus niger* and *Aspergillus flavus* in emulsion paint. This study has therefore shown that the bitter kola-mediated biogenic AgNPs have promising applications in the development of broad spectrum antimicrobial agents and also in the manufacture of novel antimicrobial nanopaint.

Acknowledgement

AIA thanked authority of FUT, Minna for providing facilities to undertake this study.

References

Adelere, I., Aboyeji, D., Akindurodoye, F., Adabara, N., & Babayi, H. (2020). Cashew plant-mediated biosynthesis of silver nanoparticles and evaluation of their applications as antimicrobial additive for consumer care products. *Tanzania Journal of Science*, 46(3), 768-778.

Adelere, I. A., Lateef, A., Aboyeji, D. O., Abdulsalam, R., Adabara, N. U., & Bala, J. D. (2017). Biosynthesis *of silver nanoparticles using aqueous extract of buchholzia coriacea (wonderful kola) seeds and their antimicrobial activities*. *Annals. Food Science and Technology*, 18(4), 671-679.

Adelere, I. A., & Lateef, A. (2016). Novel approach to the green synthesis of metallic nanoparticles: The use of agro-wastes, enzymes and pigments. *Nanotechnology Reviews,* DOI: 10.1515/ntrev-2016-0024.

Ahmed, S., & Ikram, S. (2015). Silver nanoparticles: One pot green synthesis using *Terminalia arjuna* extract for biological application. *Journal of Nanomedicine and Nanotechnology,* 6, 4.

Aina, D. A., Owolo, O., Lateef, A., Aina, F. O., Hakeem, A. S., Adeoye-Isijola, M., Okon, V., Asafa, T. B., Elegbede, J. A., Olukanni, O. D., & Adediji, I. (2019). Biomedical applications of *Chasmanthera dependens* stem extract mediated silver nanoparticles as antimicrobial, antioxidant, anticoagulant, thrombolytic, and larvicidal agents. *Karbala Int. J. Modern Sci.,* 5(2), 2.

Bhakya, S., Muthukrishnan, S., Sukumaran, M., Muthukumar, M., Senthil Kumar, T., & Rao, M. V. (2015). Catalytic degradation of organic dyes using synthesized silver nanoparticles: A green approach. *Journal of Bioremediation & Biodegradation*, 6, 5.

Bhat, R., Deshpande, R., Ganachari, S. V., Huh, D. O., & Venkataraman, A. (2011). Photo-irradiated biosynthesis of silver nanoparticles using edible mushroom *Pleurotus florida* and their antibacterial activity studies. *Bioinorganic Chemistry and Applications,* (2011), 1-7.

Creighton, J. A., Blatchford, C. G. & Albrecht, M. G. (1979). Plasma resonance enhancement of Raman scattering by pyridine adsorbed on silver or gold sol particles of size comparable to the excitation wavelength. *Journal of the Chemical Society, Faraday Transactions,* (2)75, 790-798.

Devadiga, A., Shetty, K. V. & Saidutta, M. B. (2015) Timber industry wasteteak (*Tectonagrandis* Linn.) leaf extract mediated synthesis of antibacterial silver nanoparticles. *International Nano Letter,* 5, 205–214.

Elegbede, J. A., Lateef, A., Azeez, M. A., Asafa, T. B., Yekeen, T. A., Oladipo, I. C., Adebayo, E. A., Beukes, L. S., & Gueguim-Kana, E. B. (2018). Fungal xylanases-mediated synthesis of silver nanoparticles for catalytic and biomedical applications. *IET Nanobiotechnol.,* 12(6), 857-863.

Feng, Q. L., Wu, J., Chen, G. Q., Cui, F. Z., Kim, T. N., & Kim, J. O. (2000). A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus. Journal of Biomedical Materials,* 52(4), 662-668.

Gole, A., Dash, C., Ramachandran, V., Sainkar, S. R., Mandale, A. B. & Rao, M. (2001). Pepsin-gold colloid conjugates: preparation, characterization, and enzymatic activity. *Langmuir,* 17, 1674-1679

Iwu, M. M. (1993). Handbook of African Medicinal Plant. CRC Press, 2nd Edition, Arbon London. Tokyo, pp. 83-184

Kannan, R. R. R., Arumugam, R., Ramya, D., Manivannan, K. & Anantharaman, P. (2013). Green synthesis of silver nanoparticles using marine macroalga *Chaetomorphalinum*. *Applied Nanoscience,* 3, 229–233.

Keat, C. L., Aziz, A., Eid, A. M., & Elmarzugi, N. A. (2015). Biosynthesis of nanoparticles and silver nanoparticles. *Bioresour. Bioprocess.,* 2, 47.

Khatami, M., Pourseyedi, S., Khatami, M., Hamidi, H., Zaeifi, M., & Soltani, L. (2015). Synthesis of silver nanoparticles using seed exudates of *Sinapisarvensis* as a novel bioresourse, and evaluation of their antifungal activity. *Bioresources and Bioprocesses,* 2, 19.

Kumar, A., Vemula, P. K., Ajayan, P. M., & John, G. (2008). Silver-nanoparticle-embedded antimicrobial paints based on vegetable oil. *Nature Materials,* 7(3), 236-241.

Kuppusamy, P., Yusoff, M. M., Maniam, G. P., & Govinda, N. (2016). Biosynthesis of metallic nanoparticles using plant derivatives and their new avenues in pharmacological applications – an updated report. *Saudi Pharmaceutical Journal,* 24, 473–484

Lateef, A. Adelere, I. A., Gueguim-Kana, E. B., Asafa, T. B. & Beukes, L. S. (2015a). Green synthesis of silver nanoparticles using keratinase obtained from a strain of *Bacillus safensis*LAU 13. *International Nano Letter,* 5, 29-35.

Lateef, A., Azeez, M. A., Asafa, T. B., Yekeen, T. A., Akinboro, A., Oladipo, I. C., Ajetomobi, F. E., Gueguim-Kana, E. B., & Beukes, L. S. (2015b). *Cola nitida*-mediated biogenic synthesis of silver nanoparticles using seed and seed shell extracts and evaluation of antibacterial activities. *BioNanoSci*ence, 5(4), 196-205.

Lateef, A., Azeez, M. A., Asafa, T. B., Yekeen, T. A., Akinboro, A., Oladipo, I. C., Azeez, L., Ojo, S. A., Gueguim-Kana, E. B., & Beukes, L. S. (2016a). Cocoa pod husk extract-mediated biosynthesis of silver nanoparticles: its antimicrobial, antioxidant and larvicidal activities. *Journal of Nanostructure and Chemistry,* 6, 159–169.

Lateef, A., Azeez, M. A., Asafa, T. B., Yekeen, T. A., Akinboro, A., Oladipo, I. C., Azeez, L., Ajibade, S. E., Ojo, S. A., Gueguim-Kana, E. B., & Beukes, L. S. (2016b). Biogenic synthesis of silver nanoparticles using pod extract of *Cola nitida*: Antibacterial, antioxidant activities and application as additive paint. *Journal of Taibah University for Science,* 10(4), 551-562.

Madhumitha G. & Roopan S. M. (2013). Devastated crops: Multifunctional efficacy for the production of nanoparticles. *Journal of Nanomaterials,*DOI:10.1155/2013/951858.

Mandal, S., Phadtare, S., & Sastry, M. (2005). Interfacing biology with nanoparticles. *Current Applied Physics,* 5, 118-127.

Morones, J. R., Elechiguerra, J. L., Camacho, A., & Ramirez, J. T. (2005). The bactericidal effect of silver nanoparticles. *Nanotechnology*, 16, 2346-2353.

Nanda, A., & Saravanan, M. (2009). Biosynthesis of silver nanoparticles from *Staphylococcus aureus* and its antimicrobial activity against MRSA and MRSE*. Nanomedicine,* 5, 452-456.

Oboho, E. G., & Nrughu, J. A. (2010). Effects of per-germination treatment on the germination and early growth of Garcia Kola (Heckel). *African Journal, General Agriculture,* 6 (4), 211-218.

Okwu, D. E., & Morah, F. N. I. (2006). The potentials of Garcinia kola seed as a source for nutraceuticals. *Journal of Medicinal and Aromatic Plant Sciences,* 28, 605-611.

Okwu, D. E., & Ekeke, O. (2003). Phytochemical screening and mineral composition of chewing sticks in South Eastern Nigeria. *Global Journal of Pure and Applied Science,* 9, 235-238.

Priyadarshini, S., Gopinath, V., Priyadharsshini, N. M., Ali, D. M., & Velusamy, P. (2013). Synthesis of anisotropic silver nanoparticles using novel strain, *Bacillus flexus*and its application. *Colloids and Surfaces B: Biointerfaces,* 102, 232-237.

Raffi, M., Hussain, F., Bhatti, T. M., Akhter, J. I., Hameed, A. & Hasan, M. M. (2008). Antibacterial characterization of silver nanoparticles against *E. coli* ATCC-15224. *Journal of Materials Science and Technology,* 24(2), 192-196.

Rajarathinam, M., Dhanapal, D., Morukattu, G., Joseph, S. & Thangavelu, K. P. (2014). Imparting potential antibacterial and antifungal activities to water based interior paint using nanoparticles of silver as an additive-an ecofriendly approach. *Advanced Science, Engineering and Medicine,* 6(6), 676-682.

Saravanan, M., Venu, A. K., & Barik, S. K. (2011). Rapid biosynthesis of silver nanoparticles from *Bacillus megaterium* (Ncim 2326) and their antibacterial activity on multi drug resistant clinical pathogens. *Colloids Surface B.,* 88, 325-331.

Selvi, K. V., & Sivakumar, T. (2012). Isolation and characterization of silver nanoparticles from *Fusarium oxysporium. International Journal of Current Microbiology and Applied Science,* 1(1), 56-62.

Shankar, S., Jaiswal, L., Aparna, R. S. L., & Prasad, V. (2014). Synthesis, characterization, in vitro biocompatibility, and antimicrobial activity of gold, silver and gold silver alloy nanoparticles prepared from *Lansium domesticum* fruit peel extract. *Material Letters,* 137, 75-78.

Singaravelu, G., Arockiamary, J. S., Kumar, V. G., & Govindaraju, K. (2007). A novel extracellular synthesis of monodisperse gold nanoparticles using marine alga, *Sargassum wightii* Greville. *Colloids and Surfaces B.*, 57(1), 97–101.

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 Interface*, 275, 177-182.

Song, H. Y., Ko, K. K., Oh, L. H. & Lee, B. T. (2006). Fabrication of silver nanoparticles and their antimicrobial mechanisms. *European Cells and Materials*, 11, 58.

Thirumurugan, A., Tomy, N. A., Kumar, H. P., & Prakash, P. (2011). Biological synthesis of silver nanoparticles by *Lantana camara* leaf extracts. *International Journal of Nanomaterials and Biostructures,* 1(2), 22–24.

Uko, O.J., Usman, A. & Ataja. A. M. (2001). Some biological activities of *Garcinia kola* in growing rats. *VeterinarskiArhiv,* 71(5), 287–297.

Zaki, S., El-Kady, M. F. & Abd-El-Haleem, D. (2011). Biosynthesis and structural characterization of silver nanoparticles from bacterial isolates. *Materials Research Bulletin,* 46, 1571–1576.