



Cashew Plant-Mediated Biosynthesis of Silver Nanoparticles and Evaluation of their Applications as Antimicrobial Additive for Consumer Care Products

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Received 1 Aug 2020, Revised 30 Sep 2020, Accepted 3 Oct 2020, Published Oct 2020

<https://dx.doi.org/10.4314/tjs.v46i3.17>

Abstract

This study reports the biogenic synthesis of silver nanoparticles (AgNPs) using aqueous extract obtained from *Anacardium occidentale* stem bark and evaluation of their antibacterial activities as well as their potential applications as antimicrobial additives for detergent and toothpaste. The biosynthesized AgNPs were analyzed using UV-Vis spectroscopy, Fourier-transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM). The particles were dark brown in colour with maximum absorbance at wavelength 419 nm. They were mainly spherical in shape with size ranges from 20 to 60 nm. The peaks of FTIR spectrum revealed that their formation was facilitated by protein molecules present in the extract. The AgNPs exhibited strong antibacterial property against pathogenic strains of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Furthermore, the incorporation of the AgNPs into a locally-made liquid detergent and toothpaste led to complete inhibition of bacterial and fungal contaminants such as *S. aureus*, *E. coli*, *P. aeruginosa*, *Aspergillus niger*, and *Aspergillus flavus*. The results obtained herein therefore suggest potential applications of the AgNPs in biomedical area and as antimicrobial additives in the production of consumer care products like detergents and toothpastes. To the best of our knowledge, this is the first reference to the use of biosynthesized AgNPs as antimicrobial additives for detergents and toothpastes.

Keywords: *Anacardium occidentale*; Silver nanoparticles; Biosynthesis; Antimicrobial activity; Antimicrobial additive.

Introduction

Nanotechnology is a multidisciplinary field concerned with developing, manipulating and utilizing materials at the nano scale. It is a branch of science that is growing very rapidly in recent times due to their numerous applications in many aspects of human endeavors (Lateef et al. 2019). Nanoparticles (NPs) are the building blocks of nanotechnology with unique characteristics such as large surface-to-volume ratio, high

reactivity, electrical conductivity and several others. They are synthesized conventionally by a number of physical and chemical methods. However, in recent times, biological approaches which employ environmentally benign and non-toxic bioresources like extracts from plants, animals and microorganisms for their synthesis have been extensively researched due to their simplicity, cheapness, eco-friendliness and biocompatibility over the

conventional methods (Adelere and Lateef 2016, Lateef et al. 2016a,b, Adelere et al. 2017, Elegbede et al. 2018).

Metallic nanoparticles are the most common and well-studied group of nanoparticles because of their versatility for many applications ranging from electronic, cosmetic, biomedical, catalysis and many others (Oladipo et al. 2017, Lateef et al. 2018). Amongst the metallic nanoparticles, AgNPs is the most researched with very distinctive areas of applications owing to their strong antimicrobial efficacy (Gong et al. 2007, Adelere et al. 2017). Authors have exploited very considerable varieties of plants to fabricate functional and effective AgNPs (Adelere et al. 2017, Azeez et al. 2017, Balu et al. 2020). Plant materials including fruit (Kumar et al. 2019a), leaf (Kumar et al. 2019b), bark (Tanase et al. 2020), fruit peel (Skiba and Vorobyova 2019), seed (Adelere et al. 2017) and root (Shaikh et al. 2019) extracts have been successfully utilized for the syntheses of AgNPs through their biomolecules such as flavonoids, saponins, alkaloids, sugar and some other phyto-molecules with reducing property. Furthermore, many authors have reported the synthesis of nanoparticles using extracts/products obtained from various parts of cashew plant such as leaf (Sheny et al. 2011, Sunderam et al. 2019), gum (Araruna et al. 2020, Araújo et al. 2020, Souza et al. 2020), fruit (Kumar et al. 2019a) and nut shell (Velmurugan et al. 2014). However, there exists no report on biosynthesis of nanoparticles using the stem bark extract of cashew plant.

Anacardium occidentale Linn. (cashew) is a hardy drought-resistant tropical evergreen tree that belongs to family Anacardiaceae. The family comprises of over 70 genera and 400 to 600 species (Lim 2012). It is indigenous to Brazil and now extensively cultivated throughout the tropics particularly in many parts of Asia and Africa because of its high economic importance. *A. occidentale* is commonly known as cashew in English,

“atiya” in Twi, “kashu” in Hausa and “kaju” in Yoruba. It produces very juicy, fibrous and edible fruit. The plant is a cash crop with high medicinal values most especially its stem bark and leaves (Akinpelu and Ojewole 2001). Its leaves and stem bark extracts possess antidiabetic, anti-inflammatory, antiulcerogenic and antibacterial properties (Okpashi et al. 2014). Cashew apple is used to produce varieties of products like jams, syrups and beverages (Trevisan et al. 2006). They are also used as traditional medicines to treat gastrointestinal disorders, throat infections, mouth ulcers, rheumatism and hypertension (Nugroho et al. 2013, Chotphruethipong and Benjakul 2019). Though cashew plant has very numerous applications, however, its importance has not been fully exploited in nanotechnology. Hence, this study reports the green synthesis of antibacterial AgNPs using the stem bark aqueous extract of *Anacardium occidentale*. The study also evaluates the potential applications of the biosynthesized nanoparticles as antimicrobial additives for detergents and toothpastes.

Materials and Methods

Sample collection

The stem barks of *Anacardium occidentale* (Figure 1) were harvested on 17th June 2019 from a field in Bosso area of Minna, Niger State, Nigeria and identified in the Department of Plant Biology, Federal University of Technology, Minna, Nigeria. The bark was washed thoroughly with tap water until no impurities remained. It was dried at room temperature ($30 \pm 2^\circ\text{C}$) for 10 days, pulverized into powder form by grinding machine and stored at the same temperature for further use.



Figure 1: Dried stem bark of *Anacardium occidentale*.

Preparation of cashew stem bark aqueous extract

Approximately 10 g of the cashew stem bark powder was dispersed in 100 ml of deionized water and was heated in water bath at 60 °C for 2 h. Thereafter, the mixture was centrifuged at 4000 rpm for 15 minutes while the supernatant collected was filtered using Whatman No. 1 filter paper. The filtrate collected served as the aqueous extract of cashew stem bark and was stored under ambient conditions until further use (Lateef et al. 2016).

Biosynthesis and characterization of AgNPs

The aqueous cashew stem bark extract was used to synthesize AgNPs as described by Adelere et al. (2017). Exactly 1 ml of the extract was transferred into a reaction vessel containing 10 ml of 1 mM silver nitrate (AgNO₃) solution. The reaction was held under bright sunlight for almost 60 min and was monitored through visual observations for possible colour change. Absorbance in the range of 270-800 nm was measured via UV-visible spectrophotometric analysis. The control experiment consisted of only silver nitrate solution and was held under the same conditions.

The biomolecules responsible for the AgNPs formation were investigated using Fourier transform infrared (FTIR) spectroscopy (BUCK M530 Spectrophotometer, Buck, USA) according to the methods of Bhat et al. (2011). The colloidal AgNPs were centrifuged at 10,000 rpm for 20 min. The solid residue was dried at room temperature and the powder obtained was mixed with potassium bromide (KBr) pellets for the FTIR measurements. The morphology and sizes of the particles were studied using scanning electron microscopy (SEM). Dried sample of AgNPs was mounted on specimen stub coated with copper and micrograph was obtained using scanning electron microscope (JEOL, Model 6390).

Evaluation of antibacterial properties of biosynthesized AgNPs

Antibacterial properties of the biosynthesized AgNPs were investigated using broth dilution and agar diffusion methods. Clinical isolates of *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* obtained from the General Hospital Minna were used as test organisms. The AgNPs were graded into different concentrations (5, 25, 50, 100, and 150 µg/ml) by diluting the stock concentration with deionize water. The minimum inhibitory concentration (MIC) was determined using broth dilution method. In this test, 1 mL of each graded concentration was added to a tube containing 10 mL of nutrient broth and each tube was then inoculated with a standardized number of test organisms. Tubes were incubated at 37 °C for 24 h and the optical density was thereafter measured (OD₆₀₀) to evaluate microbial growth in each tube. The MIC was defined as the minimum concentration of the biosynthesized AgNPs that prevented visible bacterial growth. In the agar diffusion test, each of the test isolates was grown overnight in peptone water for a period of 18 h before seeded on Mueller-Hinton agar plates. The MIC of the AgNPs was dispensed into wells of 7 mm diameter created on the inoculated plates using a sterile cork borer. The plates were then held at 37 °C and zones of inhibition were measured by metric rule after 24 h of incubation (Lateef et al. 2015).

Evaluation of antimicrobial properties of AgNPs as additives for detergents

The potential applications of the AgNPs as antimicrobial additives for detergents were investigated by incorporating them into a locally-made liquid detergent purchased from a retailer outlet in Bosso Market, Minna. Detergent of 19 ml was dispensed into McCartney bottles, autoclaved at 121 °C for 15 min and thereafter incorporated with 1 ml (150 µg/ml) of the biosynthesized AgNPs. After cooling, 0.5 ml of 18 h bacterial cultures of *E. coli*, *S. aureus* *P. aeruginosa*

and 0.5 ml of 48 h fungal cultures of *Aspergillus niger* and *Aspergillus flavus* were inoculated separately into each detergent bottle. The control experiments consisted of detergent and test organisms only. The bottles were held under ambient conditions for 5 days. Thereafter, 1 ml was drawn from the contents of each bottle, inoculated appropriately into freshly prepared nutrient agar and potato dextrose agar plates using pour plate technique and then incubated at 37 °C and 30 °C, respectively for 72 h.

Evaluation of antimicrobial properties of AgNPs as additives for toothpaste

The biosynthesized AgNPs were further investigated for their potential applications as antimicrobial additives for toothpaste. One gram of toothpaste purchased from a retailer outlet in Bosso Market, Minna was dislodged in 19 ml of sterile distilled water inside McCartney bottles and then incorporated with 1 ml (150 µg/ml) of the biosynthesized AgNPs. Each of the bottles was inoculated with 0.5 ml of 18 h culture of *E. coli* and 48 h culture of *Candida albicans*. The control experiment consisted of the toothpaste and test organisms only. The bottles were held under ambient conditions for 5 days. Thereafter, 1 ml was drawn from the contents of each bottle, inoculated appropriately into freshly prepared nutrient agar and potato dextrose agar plates using pour plate technique and then incubated at 37 °C and 30 °C, respectively for 72 h.

Results and Discussion

Biosynthesis and characterization of AgNPs

The aqueous stem bark extract of *A. occidentale* mediated the synthesis of dark brown AgNPs under ambient conditions. Mixture of the extract and AgNO₃ solution earlier produced light yellow colouration after about 10 minutes of reaction; it later developed gradually to stable dark brown after 30 min of incubation (Figure 2). The colour change was due to excitation of

surface plasmon resonance induced by reduction of silver ion by biomolecules present in the stem bark extract. Authors have reported different colours of biologically synthesized AgNP such as light yellow, yellow brown and dark brown (Netala et al. 2016, Adelere et al. 2017, Oladipo et al. 2017, Aritonang et al. 2019, Aina et al. 2019, Balu et al. 2020). The colour variations have been attributed to differences in the biomolecule compositions.



Figure 2: A, AgNO₃ as control; B, biosynthesized AgNPs after 30 minutes of bioreduction.

The biosynthesized AgNPs showed maximum UV Vis absorbance at wavelength of 419.5 nm (Figure 3), which corresponds with AgNPs absorbance characteristics previously reported (Priyadarshini et al. 2013, Lateef et al. 2016a, Oladipo et al. 2017, Adelere et al. 2017, Balu et al. 2020).

The FTIR absorption spectrum (Figure 4) showed peaks at 3268.9, 2109.7 and 1636.3 cm⁻¹. The distinctive bands at 3268 and 1636 cm⁻¹ correspond to the bond vibrations of amine and amide groups, respectively. Thus, indicating that the biosynthesized AgNPs were capped and stabilized by the proteins present in the aqueous stem bark extract of *A. occidentale*. The SEM images revealed that the biosynthesized AgNPs were dispersed, spherical in shape with sizes range of 20-60 nm (Figure 5). This is in agreement with the results obtained in the previous studies (Zaki et al. 2011, Kannan et al. 2013, Lateef et al. 2016a, Adelere et al. 2017). In a related study, Aiswarya et al. (2011) reported the synthesis of polydispersed and spherical shape AgNPs from cashew apple extract. Similarly, the synthesis of spherical AgNPs with a size range of 4-33 nm was also

reported by Oladipo et al. (2017). Nanoparticles are gaining importance in recent times due to their fascinating

morphologies, sizes and chemical compositions which had made them suitable for many applications.

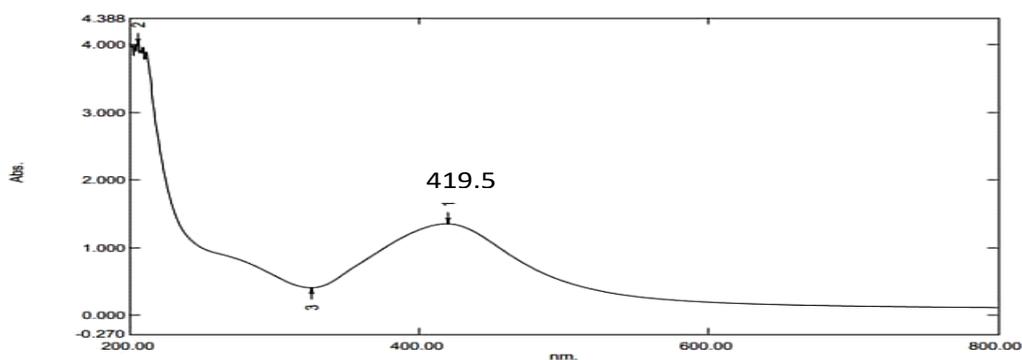


Figure 3: UV-Vis absorption spectrum of the biosynthesized AgNPs using aqueous stem bark extract of *A. occidentale*.

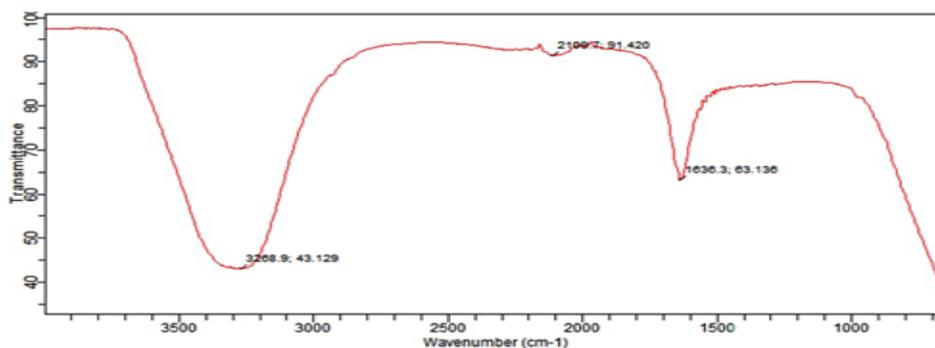


Figure 4: FTIR spectrum of the biosynthesized AgNPs using aqueous stem bark extract of *A. occidentale*.

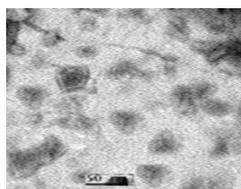


Figure 5: SEM image of AgNPs biosynthesized using aqueous stem bark extract of *A. occidentale*.

Antibacterial properties of biosynthesized AgNPs

The MIC of the AgNPs against clinical isolates of *P. aeruginosa*, *S. aureus* and *E. coli* was found to be 50 µg/ml. The agar

diffusion test revealed that the particles at concentrations of 50-150 µg/ml remarkably inhibited the growth of *P. aeruginosa*, *S. aureus* and *E. coli* strains (Figure 6) with zones of inhibition ranging from 12 to 27 mm (Table 1). Antibacterial properties of the AgNPs obtained in this study corroborate those reported in the previous studies (Priyadarshini et al. 2013, Oladipo et al. 2017, Adelere et al. 2017, Lateef et al. 2018). Balu et al. (2020) recently reported the zones of inhibition of 11-19 mm induced by biosynthesized AgNPs from the culture free extract of *Saccharum spontaneum* against some pathogenic bacterial strains. Similarly,

Kumar et al. (2019a) reported zones of inhibition of 9 mm and 10 mm produced against *Klebsiella pneumoniae* and *Bacillus subtilis* by cashew fruit extract-mediated AgNPs. AgNPs are strong antimicrobial agents with multiple modes of action against microorganisms even at very low concentrations. Though, the actual mechanism of antibacterial activities of AgNPs is not yet known. However, it has been hypothesized that AgNPs usually bind to bacterial cell wall to disrupt its structure and lipopolysaccharides membrane before penetrating into the cell to denature DNA molecule, inactivate enzyme activities and interfere with electron transport chain which eventually result to bacterial death (Jasuja et al. 2014). They are the most extensively researched types of nanoparticles and have found applications in many areas of human endeavours owing to their potent antimicrobial properties (Elegbede and Lateef 2019).

Evaluation of AgNPs as antimicrobial additives in detergents and toothpastes

The AgNPs demonstrated excellent inhibitory effects against strains of *E. coli*, *S. aureus*, *P. aeruginosa*, *A. niger*, and *A. flavus* in the detergent. Profuse growth was found in each of the control plates consisted of only the detergent and test organism, while the plates of AgNPs-treated detergent showed no sign of microbial growth (Figure 7). Furthermore, investigation on applications of the AgNPs as

antimicrobial additives in toothpaste was also considered. The biosynthesized AgNPs displayed potent antimicrobial activities against strains of *E. coli* and *C. albicans* in the toothpaste. Dense growth was observed on the control plates devoid of AgNPs, while no growth was recorded on the AgNPs-treated toothpaste plate (Figure 8). Though there are very little or no information on applications of AgNPs as antimicrobial additives in detergents and toothpastes, but there exist studies on enhancements of their antimicrobial qualities using natural plant extracts (Verkaik et al. 2011, Rhoades et al. 2013, Rossi et al. 2014). However, most recently, Ahmed et al. (2019) reported a high antibacterial efficacy induced by nanosilver-containing toothpaste against a strain of *Streptococcus mutans*. Antimicrobial effects displayed by the biosynthesized AgNPs in this study could be ascribed to gradual release of silver ion by AgNPs to pose serious threats to microorganisms present in the detergent and toothpaste. AgNPs in detergent would add antimicrobial effects to its cleansing property such that as it removes dirt from a material it also destroys germs that may be present. More so, incorporation of AgNPs into toothpaste would assist in removing some oral pathogens to safeguard against dental plaque. Therefore, the results obtained in this study suggest that the biosynthesized AgNPs could be utilized as antibacterial and antifungal additives to enhance the quality of some consumer care products.

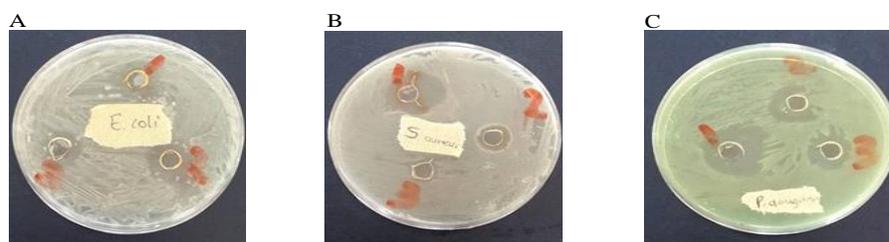


Figure 6: Antibacterial activities of the AgNPs (50-150 $\mu\text{g/ml}$) against clinical bacterial isolates: A, *Escherichia coli*; B, *Staphylococcus aureus*; C, *Pseudomonas aeruginosa*. The AgNPs graded concentrations 150, 100, and 50 $\mu\text{g/ml}$ were represented by 1, 2, and 3, respectively.

Table 1: Inhibition of growth of clinical bacterial isolates by the biosynthesized AgNPs

Organisms	Concentration of AgNPs ($\mu\text{g/ml}$)	Zone of inhibition (mm)
<i>P. aeruginosa</i>	150	27
	100	19
	50	15
<i>E. coli</i>	150	18
	100	15
	50	13
<i>S. aureus</i>	150	20
	100	15
	50	12

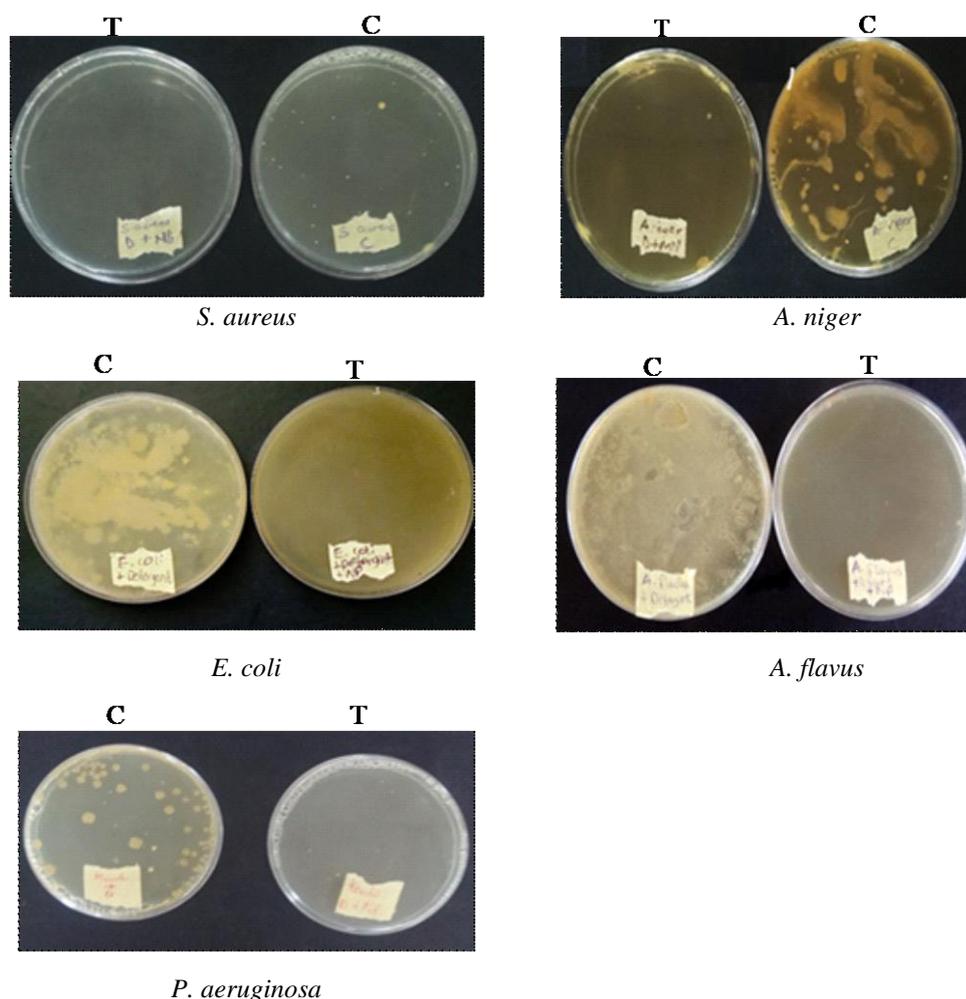


Figure 7: Evaluation of AgNPs as antimicrobial additives for detergent: C is the control (test organism and detergent only); T is the test (AgNPs-treated detergent and test organism).

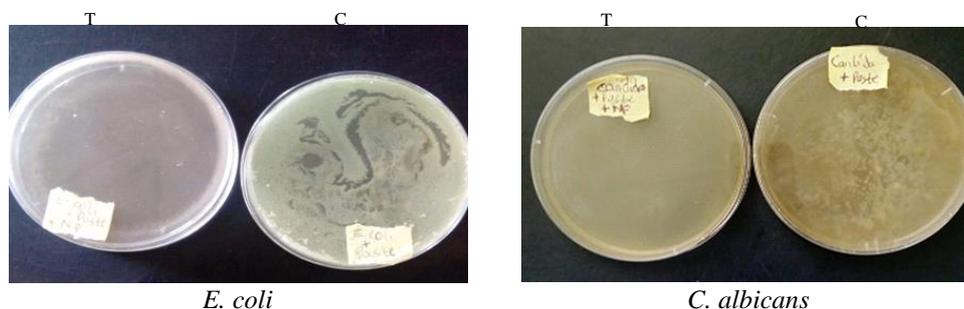


Figure 8: Evaluation of AgNPs as antimicrobial additive for toothpaste: C is the control (test organism and toothpaste only); T is the test (AgNPs-treated toothpaste and test organism).

Conclusion

This study established the biosynthesis of AgNPs from aqueous stem bark extract of *A. occidentale* in a simple, rapid, cost-effective and eco-friendly manner. The biosynthesized AgNPs were mainly spherical with sizes in the range of 20-60 nm. The particles displayed very potent antibacterial activities against selected clinical bacterial isolates and completely inhibited the growth of bacterial and fungal contaminants in toothpaste and liquid detergent. The strong antimicrobial efficacy demonstrated by the AgNPs therefore suggests their feasibility for biomedical applications and also they could be essential ingredients in the formulation of some consumer care products such as detergents and toothpastes. To the best of our knowledge, this study reports the first evaluation of biosynthesized AgNPs as antimicrobial additives in detergent and toothpaste.

Acknowledgement: AIA thanks the authority of FUT, Minna for providing facilities to undertake this study.

Conflict of Interest: The authors declare no conflict of interest as regards this publication.

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