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Green synthesis and antibacterial activities of coconut husk extract-mediated silver nanoparticles

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Abstract

This work reports the synthesis of silver nanoparticles (AgNPs) using coconut husk extract and the evaluation of its antibacterial activity. Antibacterial activity of the biosynthesized AgNPs was evaluated against some selected pathogenic microorganisms. The biosynthesised AgNPs were characterised using UV-Visible, Fourier Transmission Infrared Spectroscope (FTIR), Energy Dispersive X-ray Spectroscope (EDX) and Field Emission Scanning Electron Microscope (FESEM). The absorption spectrum of the synthesized AgNPs showed a maximum spectrum of 448 nm while FTIR analysis showed different functional groups present on the surface of the AgNPs with broad peak between 839 and 3454 cm^{-1}. The FESEM showed a large number of spherically shaped nanoparticles with sizes ranging from 12.96 to 97.04 nm. The coconut husk extract possess biomolecules which aided the bioreduction, formation and stabilization of the AgNPs, which in turn inhibited distinctly the growth of the selected microorganisms with zones ranging from 9-13 mm. This study showed that coconut husk can be used for the synthesis of AgNPs possessing high antibacterial properties.

Keywords: Coconut husk; antibacterial; silver nanoparticles; green synthesis

Introduction

Nanotechnology is considered an emerging technology due to the possibility to advance well-established and to create new products with totally new characteristics and functions with enormous potential in a wide range of applications. (Logothetidis 2014)

While bacteria sizes are about 10^{-6}m, nanotechnology manages structures as little as 10^{-9}m. Disregarding this 10^{-3}m contrast, the improvement of numerous technologies in the 1980s made possible the combination of these two universes. Therefore, nanotechnology advanced to an important branch of nanomedicine making it possible to have numerous applications in health sciences. Recent advancements in biomedical research incorporate a great variety of features and applications in nanobiotechnology and nanomedicine (Amitava *et al*. 2011). Several researchers have reported some of these applications. Lateef *et al.* (2016) reported its usefulness, both in the industry as paint additives and also in medicine as antimicrobial agents. Other applications that have been reported include anticoagulant (Jeyaraj *et al*. 2013), antioxidant (Otunola *et al*. 2017) and larvicidal potentials (Morejon *et al.* 2018).

Various biological materials have been used by several authors in synthesising numerous nanoparticles, the most common being silver nanoparticle (Benakashani *et al.* 2016; Ahmed *et al*, 2016; Ndikau 2017; Vilchis-Nestor *et al*. 2008; Huang *et al*. 2007; Chandran *et al.* 2006) However, studies on the synthesis of AgNPs using coconut husk extract and its biomedical applications have remained unexplored and this study hence focuses on the synthesis of AgNPs from coconut husk extract and the evaluation of its antibacterial activity

Cocos nucifera (belonging to the family Arecaceae) commonly known as coconut, is a versatile plant with a variety of uses. Coconut is unique in terms of their fruit morphology. Fibrous coconut fruit is not just fit for human consumption but also popular for its wide variety of uses. Asides that it is highly nutritious, the plant have also been widely studied for medicinal qualities for kidney, liver and heart disorders. Most recently, Shettigar *et al*. (2014) reported its use in the reduction of HIV viral load. Raj and Magesh (2017) also reported the various medicinal uses of coconut. In another study, Victor and Jeroh (2012) highlighted the anti-diabetic effects of coconut husk. Esquenazi *et al*. (2002) in their study also reported the antiviral and antimicrobial activity of the polyphenolics from coconut husk extract. In a similar study by Al-Adhroey *et al*. (2011), it was reported that the plant's white flesh extract had antimalarial properties. Rinaldi *et al*. (2009) evaluated the Crude extract and fractions obtained from *Cocos nucifera* for antinociceptive and anti-inflammatory activities. Other biomedical applications of coconut that have been evaluated includes anti-inflammatory, antioxidant and antimicrobial activity (Silva *et al*. 2013), antifungal activity (Venkataraman *et al*. 1980), hepatoprotective and antioxidant activity (Loki & Rajamohan 2003) anti-neoplastic activity (Koschek *et al*. 2007), antimalarial activity (Adebayo *et al*. 2013), cytoprotective, antihyperglycemic and phytochemical activity (Renjith *et al*. 2013).

In nanotechnology, the some parts of the coconut fruit have equally been utilized in the biosynthesis of nanoparticles. In 2014, Elumalai *et al*. synthesized AgNPs using the coconut water as reducing agent. Bello *et al*. (2015), employing the top down approach, also utilized coconut shell powders in the synthesis of coconut shell nanoparticles

The above highlighted medical potentials of coconut have inspired this current study to explore the nanobiotechnological potential of this plant. In this study, the biosynthesis of AgNPs using coconut husk extract and the exploitation of its antimicrobial properties were reported.

Materials and methods

Sample collection

The coconut fruit was purchased from Bere Market, Ibadan, Nigeria. It was brought down to the Microbiology laboratory, Babcock University for further processing. The husk was decorticated and blended into powdery form before storage at ambient temperature in air-tight container.

Collection of clinical isolates

Four clinical bacterial isolates were obtained from LAUTECH Teaching Hospital, Ogbomoso, Nigeria. They included 3 Gram negative organisms (*Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) and 1 Gram positive organism (*Staphylococcus aureus*).

Preparation of extract

The aqueous extract of coconut husk extract used for this study was prepared according to the methods of Lateef *et al*. (2018). To a 100ml of distilled water, 1g of the powdered coconut husk was suspended. Subsequently, this was followed by heating for 1h at 60^{o} C in the water bath. It was further filtered and the resulting solution was subject to centrifugation for 20 min at 4000 rpm. The supernatant that resulted was used for further experiment.

Synthesis of the nanoparticles

The biogenic synthesis of the AgNPs was carried out by drawing 1 ml of the already prepared coconut husk extract and introduced into 40 ml of 1 mM $AgNO_3$ and the observation for colour changed followed suit. The whole synthesis which took approximately 2 h was carried out at room temperature (Lateef *et al*., 2018)

Characterisation

The biosynthesis of the AgNPs was monitored via visual observations for the colour change in the reaction vessel. After stabilization, the absorption spectra was monitored at 190-900nm using the UV-Visible spectrophotometer (Cecil, USA). The vibrational frequencies of the nanoparticle were gotten from FT-IR transmission spectra by KBr method using FTIR model 8400S (Shimadzu, Japan). The grinded KBr powder was pressed with SHIMADZU MHP-1 mini hand press to form homogenized pellet for background measurement. A 5% dilution of AgNPs with KBr was pulverized with agate mortar to have a homogenized mixture. Suitable pellets were used for the scan in the percent transmittance mode in the mid IR region of 400–4000 cm^{-1}. The presence and ratio of the elements in the particles was analysed using the EDX Silicon-drift detector (X-MaxN, Oxford Instruments, UK). For the size and morphological properties of the synthesized nanoparticle, the synthesized nanoparticle was analysed using the FESEM. Powder samples were sonicated in ethanol medium in ultrasonic probe sonicator to have maximum deagglomeration of the particles before imaging. The surface morphology of the synthesized particle was observed using a FESEM (Lyra 3, Tescan, Czech Republic), accelerating voltage of 20 up to 30kV.

Antibacterial activity

The antibacterial efficacy of the nanoparticle was evaluated in accordance with the methods of Perez *et al*. (1990). To assess the efficacy of the biosynthesized AgNPs against some selected isolates of clinical importance, the agar well diffusion was the method employed. An 18 h-old culture of each of the organisms prepared overnight in peptone broth was used to inoculate an already prepared plate of Mueller Hinton agar. Holes were bored on the inoculated plate using a sterile 6 mm cork borer and labelled 10, 20, 40, 60, 80 and 100 µg/ml respectively. Varying concentrations of 100 µl of the synthesized AgNPs was then introduced into the corresponding holes. The plates were incubated for 18 h at 37 ^oC after which the zone of inhibition was computed. Control experiments were set up with CDE, silver nitrate (positive control) and distilled water (negative control). The agar well diffusion test was performed in triplicate

Results and discussion

Synthesis and characterisation of the synthesized silver nanoparticles

The preliminary confirmation of the biosynthesized silver nanoparticle was done by the presence of colour change in the reaction vessel. An initially colourless solution, after stability, became deep brown (Figure 1). As previously reported by various researchers (Vanaja *et al*. 2013; Krishnaj *et al*. 2010; Bar *et al*. 2009), this colour change resulted from the excitation of the surface plasmon resonance. As observed in figure 1, the coconut husk extract served as both the capping and reducing molecule, leading to the formation of the silver nanoparticle.

Figure 1: Synthesis of the Coconut husk extract mediated silver nanoparticles (a) immediately after the addition of the coconut husk extract to the silver nitrate; (b) Formation of deep brown colouration after 30 min.

As indicated in Fig 2, the UV- Vis spectra analysis of the synthesised silver nanoparticles displayed a broad absorption peak at 448nm. This further confirms the formation of silver nanoparticles as reported by other researchers to be within ranges of 390-470nm (Sinha & Paul 2015) Kumar *et al*. (2016) also reported a broad absorption peak at 434nm when *Adansonia digitata* L. fruit pulp extract was utilized in biosynthesising silver nanoparticles. The broad band was said to be attributed to the wide size of the synthesised nanoparticle (Link & El-Sayed 1999).

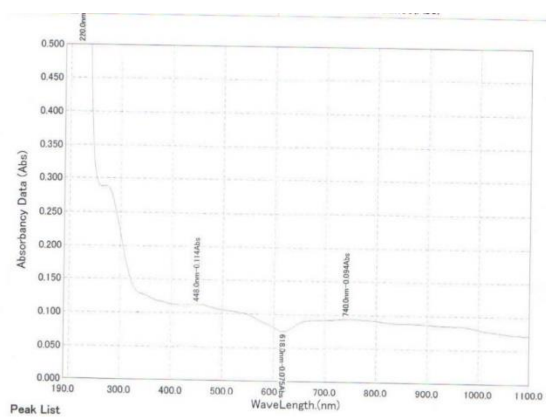

Figure 2: UV-Vis spectra of silver nanoparticles synthesized from Coconut husk extract

Figure 3 represents the FT-IR spectrum of the AgNPs, which shows prominent absorption bands at 3454, 2000, 2359, 1384, 1047, and 839 cm^{-1}. The band at 1635 cm^{-1} is characteristic of –N-H bending vibrations of amine functional group (Sun *et al.* 2004; Lin *et al.* 2005), while the band at 3454 is either that of O-H, N-H stretching vibrations of amide, alkanes and/or alcohol (Ofudje *et al.* 2017). The peak at 1384 cm^{-1} represents to in-plane C-H bend of alkenes or aromatic (Rahmaniyan *et al.* 2015) while its stretching vibration is at 2359 cm^{-1}. The C-N stretching of aliphatic amines is reported at 1047 cm^{-1} and The Ag-O stretching modes are observed at 669 cm-1 and 420 cm^{-1} (Taghavi Fardood *et al*. 2017). Summarily, the FTIR study showed that the AgNPs have phytochemicals as capping agents on their surfaces.

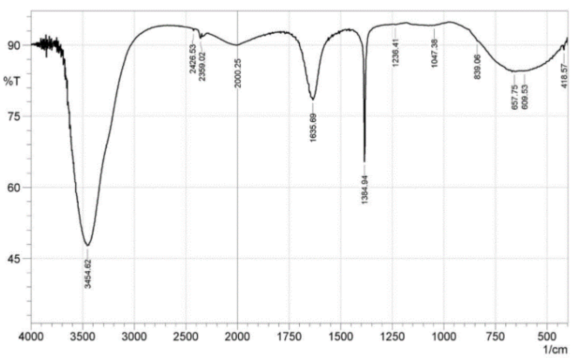

Figure 3: The FTIR spectra of the silver nanoparticles synthesized from Coconut husk extract

The chemical analysis of the Coconut husk mediated silver nanoparticle was shown (Figure 4) by the EDX spectroscopy. Strong elemental signal was observed around the Ag atom at 0.9keV. Other elements present include sodium, oxygen, carbon, magnesium and nitrogen). Similar observations have been reported by other researchers (Islam *et al*. 2017; Puchalski *et al*. 2007) with strong silver signals at the range of 1.5 to 5.0 keV

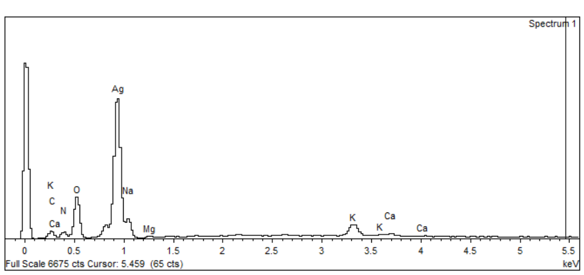

Figure 4: Energy dispersive spectra of the synthesized AgNPs

The size and morphology of the biosynthesized nanoparticle were captured by (Figure 5) FESEM. The particles which ranged from 12.96 to 97.04 nm were spherical in shape. This size range falls within the ranges reported when other plants were used in the biosynthesis of silver nanoparticle (Singha & Paul, 2015; Kumar *et al*. 2016)

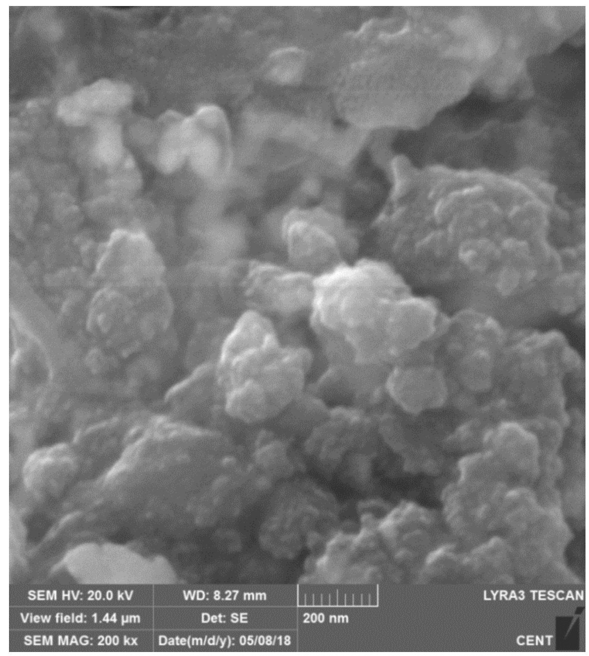

Figure 5: Field Emission Scanning Electron Micrograph of the Synthesized AgNPs

Evaluation of the antibacterial activity of the synthesised Nanoparticles

The biosynthesised silver nanoparticle exhibited its property as an antimicrobial agent when evaluated against some clinical isolates (Figure 6). The highest antibacterial was displayed against *E. coli* (13mm) while the lowest zone was recorded for *S. aureus* with a zone of 9mm. This is particularly similar to what was reported by Sinha & Paul (2014). Several other researchers in their study reported a similar result in relation to the antimicrobial efficacy of green synthesised silver nanoparticles (Sinha *et al*. 2014; Sinha & Paul 2015; Kumar *et al*. 2016; Yuvarajan *et al*. 2014).

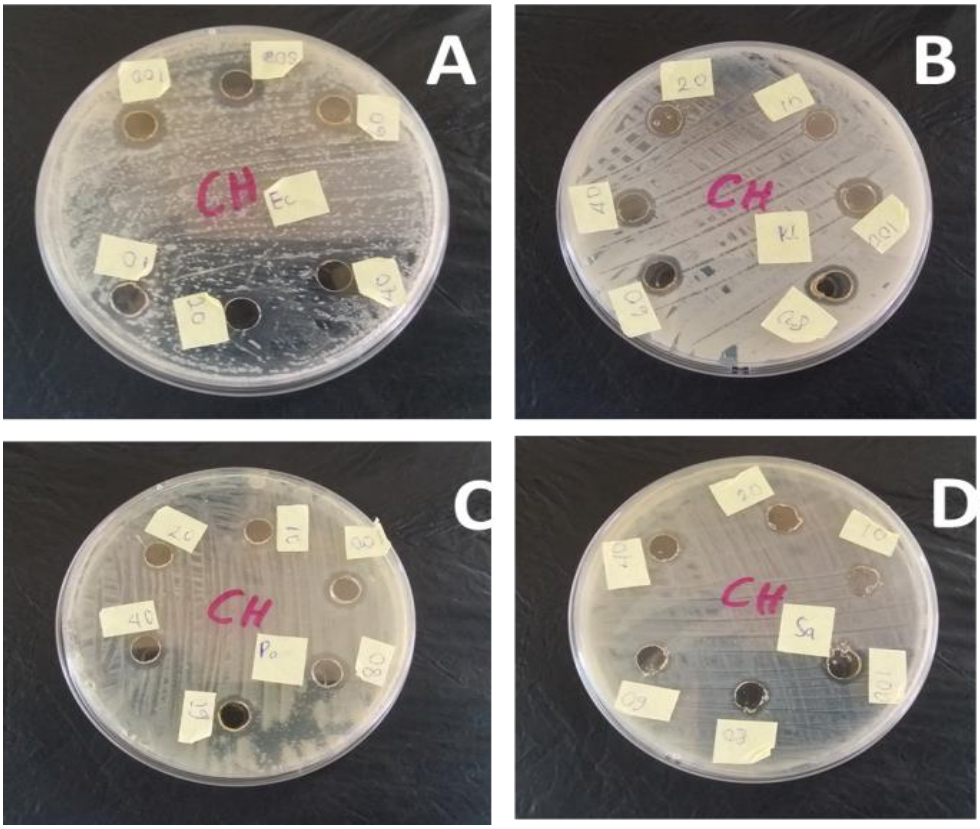

Figure 6: The antibacterial activity of the synthesized AgNPs against some clinical bacterial isolates (a) against *Escherichia coli* (b) against *Klebsiella pneumoniae* (c) against *Pseudomonas aeruginosa* (d) against *Staphylococcus aureus*

TABLES

Table 1: Zone of inhibition of the synthesized AgNPs against some selected pathogens

	Mean Zone of Inhibition (mm) ± standard deviation (SD)								
	AgNPs						$AgNO_3$	CDE	Distilled H_2O
	10µg/ml	20µg/ml	40µg/ml	60µg/ml	80µg/ml	100µg/ml			
Isolate									
Staphylococcus aureus	NZ	NZ	NZ	NZ	NZ	8.0±0.1	6.2±0.2	NZ	NZ
Klebsiella pneumoniae	NZ	NZ	9.0±0.1	10.0±0.1	10.0±0.2	11.0±0.1	7.4±0.2	NZ	NZ
Pseudomonas aeruginosa	NZ	NZ	9.0±0.2	NZ	12.0±0.1	11.0±0.2	7.1±0.1	NZ	NZ
Escherichia coli	NZ	NZ	9.0±0.1	12.0±0.2	11.0±0.1	13.0±0.1	6.9±0.1	NZ	NZ

Conclusion

In this work, coconut husk extract was used to synthesize nanoparticles. The synthesised AgNPs was analysed using UV Spectrophotometer, FTIR, FESEM and EDX. The biosynthesised silver nanoparticle had antibacterial activities against selected clinically important bacteria. Thus Coconut husk extract mediated-AgNPs could have various pharmaceutical applications.

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Conflict of interest

The authors declare that they have no conflict of interest.

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