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ORIGINAL ARTICLE

Biogenic synthesis and characterization of Silver nanoparticles from seed extract of Spondia mombins and screening of its antibacterial activity

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Abstract

The seed extract of *Spondia mombins* was used in the biofabrication of silver nanoparticles (AgNPs) in this study. Biosynthesized AgNPs was characterised using UV-Vis Spectroscopy, FTIR, FESEM, TEM, XRD and EDX. The antimicrobial efficacy of the synthesized AgNPs was evaluated against certain pathogens. The absorption spectrum peaked at 425 nm when the UV analysis was carried out, with a large peak of 3000 to 3800 cm−1 indicating different functional groups on the AgNPs surface when the FTIR analysis was conducted. An examination of FESEM and TEM showed a number of spherical particle structures varying from 10 to 50 nm. The XRD analysis also confirmed this size range. The synthesized AgNPs inhibited growth of the microorganisms used in this study. This study demonstrates that *Spondia mombins*'s seed extract has biomolecules that helped to bio reduce and stabilize the synthesized AgNPs, hence confirming the possibility of using *Spondia mombins* seeds in AgNPs synthesis.

Keywords: *Antibacterial; Extraction; Green Synthesis; Silver Nanoparticles; Spondia Mombins.*

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INTRODUCTION

Nanotechnology is an emerging science field that deals with the synthesis and creation of different nanomaterials. Nanoparticles are structures of 1-100 nm in size that can vary in size from bulk material. Currently, various nanomaterials are made of silver, gold, copper, zinc, titanium, magnesium and alginate [1]. Nanotechnology has found wide uses in biology and modern medicine, using dispersed nanoparticles for the treatment of medicine, tumour detection and destruction [2], pathogens bio-detection [3], protein detection [4], probing of DNA structure [5], tissue engineering, bio-diagnostic separation and purification [6]; Nanoparticles are smaller in size, with a large surface-to-volume ratio, which is critical for catalytic reactivity and even antibacterial activity. Nanoparticles can adsorb or encapsulate a drug to protect it from enzyme degradation [7].

The most widely marketed nanoparticles are silver nanoparticles, which attract significant attention for chemical visualization, the delivery of pharmaceuticals, photonics, microelectronics, biomedicine and biosensors [8]. There is a broad

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variety of physical and chemical methods for synthesizing AgNPs that use a wide range of chemical reduction methods, as AgNPs can be synthesised in mild conditions. Recently, the main focus was the synthesis of environmentally friendly nanoparticles, using herbal extracts [9], bacteria [10] and fungi [11]. Green synthesis is, therefore, a reliable process with great stability and sufficient dimensions, because it has a number of advantages over other chemical and physical methods [12]. Green nanoparticle synthesis is advantageous because it allows nanoparticles to be generated on a large scale, is cost-effective and eco-friendly [13].

Spondias mombin (Hog plum) grows in the tropical and subtropical regions of the world. It is commonly used in traditional medicine for the treatment of many ailments e.g ulcer, arthritis, hypertension, etc. Pharmacological studies of various *Spondias* species have shown that they exhibit cytotoxic [14], antioxidant [15], anti-ulcers [16], immune-boosting [17], anti-inflammative, anti-arthritis, anti-pyretic, analgesic, thrombolytic, hypoglycaemic, antifertility, antimicrobial and anthelmintic activities [18] as a result of a broad variety of phytoconstituents found in this genus [18]. Several biomolecules such as tannins, flavonoids, sterols, triterpenes, saponins, essential oils, amino acids and polysaccharides, have been reportedly present in plants belonging to the genus *Spondias* [19].

The aim of this research is to investigate the characterization and antibacterial activity of synthesized AgNPs from the seed extract of *Spondias mombin*. The synthesized AgNPs were characterized by UV-Vis, FTIR, XRD, SEM and TEM. The antibacterial activity of the AgNPs was evaluated against selected microorganisms

MATERIALS AND METHODS

Sample Collection and Preparation

Mature, fresh and healthy fruits of *Spondias mombin* was purchased from Iperu market, Ogun State, Nigeria. The samples were placed in a sterile container and transported to the Microbiology Laboratory, Babcock University, Ilishan-Remo, Ogun State. The fruit of *Spondias mombin* washed thoroughly with distilled water and the pulp was separated from the seed. Seed and pulp samples were oven dried separately at 40 ^{o}C for 7 days. Dry samples were pulverized using a laboratory blender (Lexus MG-2053 Optima) and stored in air-tight transparent containers at room temperature for further analysis.

Ethanol and Methanol extracts of the samples were prepared by cold maceration using the modified method of Usunobun *et al*. [20]. One hundred grams (100 g) of the crude pulverized samples were macerated into 1000 mL of absolute ethanol and methanol in separate conical flasks at room temperature for 72 hrs. The suspensions were filtered using the Whatman No.1 filter paper. The extracts were then concentrated under a reduced temperature using a rotary evaporator.

Phytochemical Analysis

Phytochemical screening of ethanol and methanol extracts was carried out using the standard methods described by Sofowora [21] for qualitative and quantitative analysis of bioactive metabolites such as alkaloids, saponins, tannins, flavonoids, polyphenol, glycosides, reducing sugar, phlobatannin, steroids and anthraquinones.

Silver Nanoparticles Synthesis and Characterization

The hot water extraction method of Azeez *et al*. [22] was used to obtain *Spondias mombins* seed extract (SMSE) for use in the AgNPs synthesis. One gram (1 g) of the pulverized samples was suspended in 100ml of distilled water**.** It was heated in hot water bath at 60^{o} C for 1hr to obtain the extracts**.** Centrifugation is carried out at 4000 rpm for 20 min. The supernatant was collected for further analyses.

The AgNPs were biogenically synthesized by adding 1 ml of the prepared extract to 40 ml of 1 mM $AgNO_3$ and a change of colour was observed. The whole reaction was carried out at room temperature and lasted about 2 hours [23].

Spondias mombin seed extract-silver nanoparticles (SMSE-AgNPs) were synthesized from a reaction mixture containing 20 ml of 1 mM silver nitrate ($AgNO_3$) and 1 ml of SMSE at room temperature, as the stability of plant metabolites requires working at ambient temperature(30 ± 2°C) as described by Azeez *et al*. [22]. It has been reported that an optimal concentration of Ag^{+} ions is required as a deficiency of ions at a lower concentration and excessive ions at a higher concentration result in the formation of improper and/or complex intermediates that ultimately bring about poor conversion efficiencies [23], hence the choice of using 1mM $AgNO_3$ and the mixture ratio. Synthesis was confirmed by a

Table 1. PHYTOCHEMICAL PROPERTIES OF Spondias mombin SEED EXTRACTS.

	Alkaloid	Polyphenol	Saponin	Tannin	Flavonoid	Glycoside	Anthraquinones	Reducing sugar	Sterols	Phlobatannins
Ethanolic Extract	-	+++	+	+++	++	+	++	+	-	++
Methanolic Extract	+	+++	+	+	+	+++	-	+++	-	+++

+ Present; - Absent

change in colour from transparent yellow to brown which was stabilized after 10 min using visual detection, and the formation of SMSE-AgNPs were confirmed by measuring its absorbance spectrum using UV/Visible spectroscopy at 200-900 nm as described by Lateef *et al*. [24]. The identity of the biomolecules that took part in the green synthesis was determined by Fourier transform infrared (FTIR) spectroscopy, while the size, morphology and elemental compositions of SMSE-AgNPs was unravelled by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) as described by Azeez *et al.* [23] and Deepak & Madhurika [25]. The X-ray diffraction (XRD) analysis was investigated using PANalyticalX'pert PRO with CuKa radiation at 40 kV and 30 mA.

Antibacterial Activity of Synthesized AgNPs

Antibacterial activity of the biosynthesized silver nanoparticles was studied using the agar diffusion method of Azeez *et al*. [22] and Bauer *et al.* [26]. It was evaluated against bacterial isolates such as *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*, *Klebsiella, Salmonella, Enterobacter, Acinetobacter, Proteus vulgaris, Bacillus subtilis, Streptococcus pyrogenes.* The bacterial isolates were grown in a nutrient broth for 24 hrs and then inoculated on Mueller Hinton agar plates using spread plate method. Thereafter, holes were made on the plates using sterilized cork borer (5 mm) and loaded with silver nanoparticles at different concentrations (10 µL, 20 µL, 40 µL, 60 µL, 80 µL and 100 µL) under aseptic conditions and incubated at 37°C for 24 hr. After 24 hr of incubation, the formation of zone of inhibition was observed and measured. The agar well diffusion test was performed in a triplicate.

The antibacterial susceptibility test was carried out using a panel of commercial broad-spectrum antibiotics on Mueller Hinton agar plates containing bacterial isolates using a disc diffusion method defined by Lateef *et al*. [24]. Plates were incubated at 37 °C for 24 hr and the zone of inhibition was measured. Gram positive bacteria was screened against antibiotics containing (µg) ceftazidime (CAZ), 30; cefuroxime (CRX), 30; gentamicin (GEN), 10; ceftriaxone (CTR), 30; erythromycin (ERY), 5; cloxacin (CXC), 5; Ofloxacin (OFL), 5; and Augmentin (AUG), 30. While Gram negative bacteria was tested against antibiotics containing (µg): septrin (SXT), 30; chloramphenicol (CH), 30; sparfloxacin (SP), 10; ciprofloxacin (CPX), 10; gentamicin (CN), 10; augmentin (AU), 30; amoxicillin (AM), 30; pefloxacin (PEF), 30; tarivid (OFX), 10; streptomycin (S), 30. The test was carried out in triplicate

RESULTS AND DISCUSSIONS

Preliminary phytochemical screening of LMCA revealed the presence of different kind of chemical groups that are summarized in Table 1. The presence of a color change in the reaction preliminarily confirmed by the biosynthesized silver nanoparticle. The solution, initially colorless formed a stable dark brown color after stability within 10 min (see Fig. 1). The stabilized dark brown colored solution was used for the purposes of this study. After stabilization, no particle aggregate was found at the bottom of the flask, which took about 30 min. This color change is due to the excitation of the surface plasmon resonance (SPR), as previously reported [27-29]. The *Spondia mombins* seed extract was used as a reduction and capping molecules, as shown in Fig. 1, leading to the formation of silver nanoparticles. The UV-Vis spectrum of the synthesized AgNPs peaked at 425 nm as shown in Fig. 2. This greatly correlates with previous findings from various researchers. Aina *et al*., [30] have reported similar reduction of the Ag^+ to Ag resulting in SRP of the UV-visible with maximum absorbance at 425 nm. Generally, the SPR peaks' Gaussian shape of the spectrum between 410 and 460 is that of silver nanoparticles [31, 32]. The single, strong and

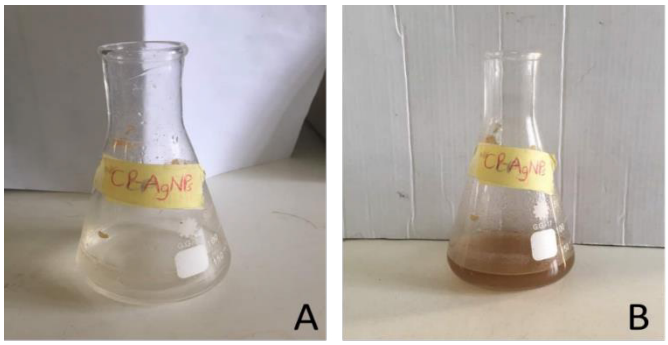

Fig. 1. Synthesis of the AgNPs (a) 0 min after the addition of the plant extract to the silver nitrate (b) Formation of deep brown colouration after 30 min.

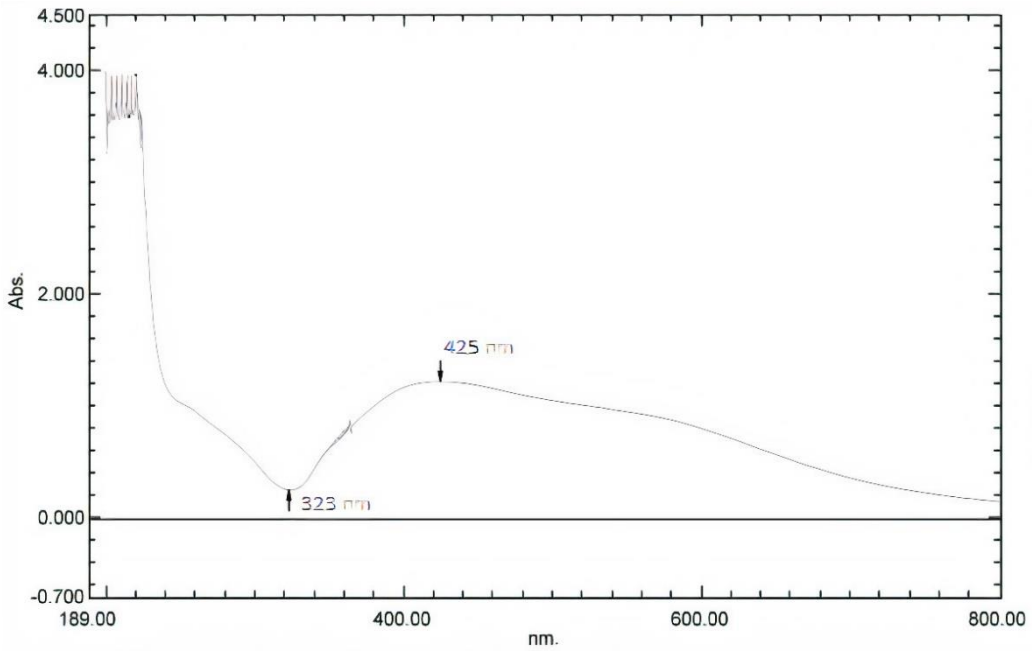

Fig. 2. The UV-Visible spectrum of the biosynthesized SMSE-AgNPs.

broad SPR observed at 425 nm is an indication of nanoparticles formation.

The use of plant extracts in the synthesis of nanoparticles is important for the functionalization of NPs. The extracts capped the NPs and introduced certain functional groups on the surface of the NPs, which are easily confirmed with the use of FTIR spectroscopy. A characteristic peak at 3431 cm^{-1} was observed in the FTIR of the present NP (Fig. 3); this is assigned to stretching vibration of $–NH_2$ (amide I) and/or –OH of phenolic compounds. The infrared bands at 2924, and 2361 cm^{-1} have been linked to the atmospheric CO_2 absorption [30, 33]. This CO_2 adsorption indicated the potential use of nanoparticle to extract ambient carbon dioxide. The 1773 cm^{-1} peak is presumed to be that of C–H bonding (weak overtone) of aromatic compounds, while the 1385 cm^{-1} peak is a well-known in plane bending common in alkenes and aromatics. The 1628 cm^{-1} peak is that of –N–H bend of amides and the 1217, 1184, 1055 cm^{-1} peaks are typical of C – N stretching aliphatic amine, The NPs surface vibrational mode of carbonate is at 779 cm^{-1} and Ag-O stretching are observed at 679 and 467 cm^{-1} [34]. The FTIR of the SMSE-AgNPs shows that NP is capped by biomolecules of functional groups of carbonyls, amide and hydroxyls. Negatively charged groups presented in the plant extract

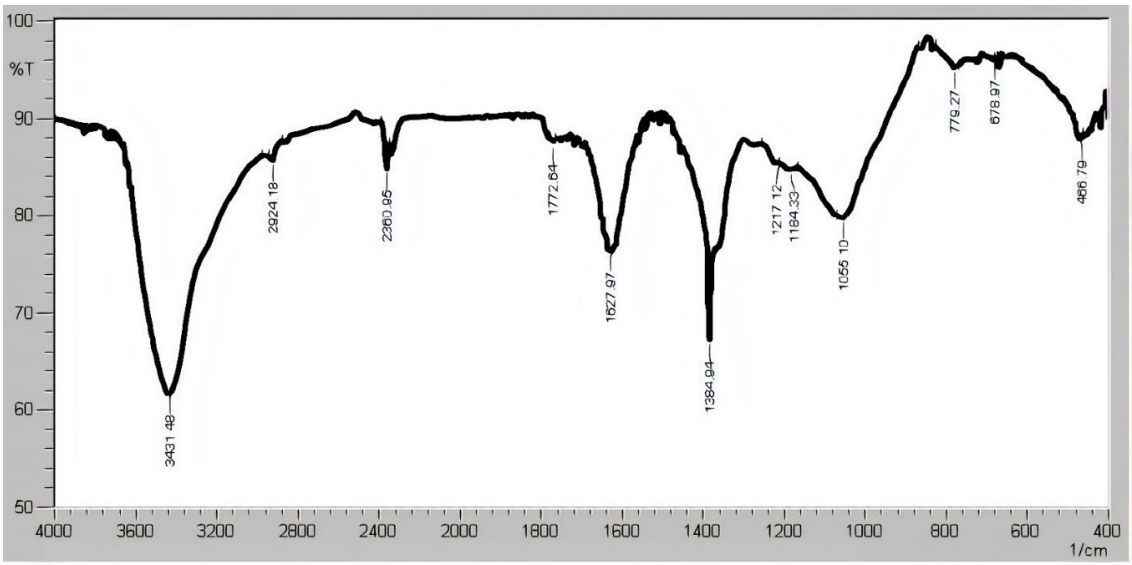

Fig. 3. FTIR SPECTRA OF the biosynthesized SMSE-AgNPs.

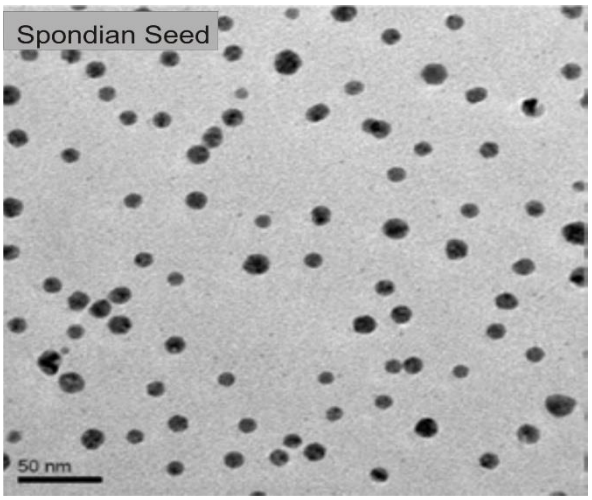

Fig. 4. TEM ANALYSIS of the biosynthesized SMSE-AgNPs.

and polar groups such as OH and CO have a high tendency to attach on the Ag^{+} surface. These groups also contribute to both the reduction and stabilization of Ag ions [35]. It can be concluded that plant metabolites containing OH, CO and especially COO^{-} such as carnosic acid, flavonoids and proteins have a pivotal role in the possible mechanism for the bioreduction of silver ions. Characterization of the size and shape of silver nanoparticles synthesized by *Spondias mombin* aqueous extract with Transmission electron microscopy (TEM) revealed that the particles were spherical in shape, distributed randomly, polydisperse and varying from 10 to 50 nm (Fig. 4). Similar results were obtained by Karwa *et al*., [36] when they used medicinal mushroom for silver nanoparticles synthesis. They also reported the polydisperse nature of their nanoparticles, however their sizes ranged from 10 to 70 nm. Lateef *et al*. [24] reported the synthesis of spherical AgNPs in the size range of 3-50 nm. The TEM morphological analysis in most of the studies revealed that the synthesized AgNPs were spherical in shape [24, 36], whereas others reported irregular, triangular, hexagonal, isotropic, polyhedral, flake, flower, pentagonal, anisotropic

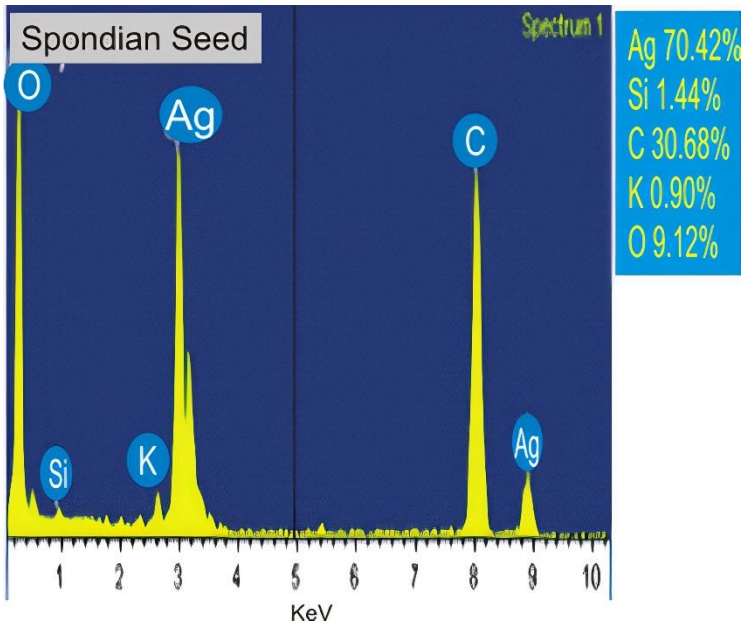

Fig. 5. EDX SPECTRA of the biosynthesized SMSE-AgNPs.

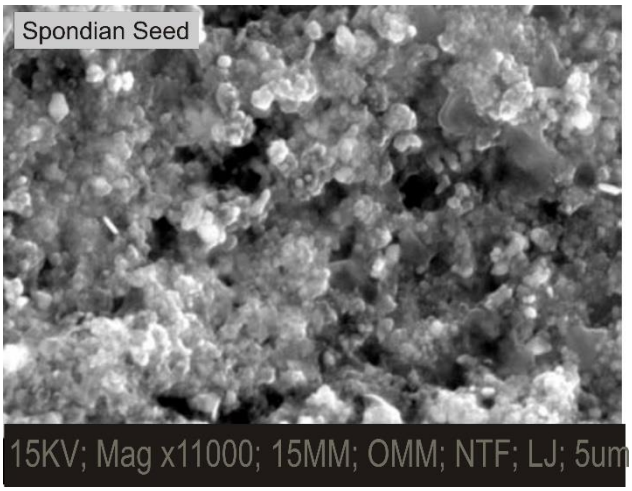

Fig. 6. FESEM ANALYSIS of the biosynthesized SMSE-AgNPs.

and rod like structures [37]. It is well known that the physiochemical properties of AgNPs contribute immensely to their versatility.

Energy Dispersive X-ray (EDX) analysis confirmed the presence of elemental silver in the synthesized AgNPs. A strong silver signal peak was observed at 2.7 keV, while weak silver signal peaks were observed at 2.9, 3.0, and 3.2 keV (Fig. 5). Rao and Tang [38] recorded strong silver signal peak at 3.0 keV in their silver nanoparticles synthesized with the aqueous extract *Eriobotrya japonica* leaf. Jagtap and Bapat [39] reported an optical absorption limit of approximately 2.983 keV. Additional peaks were also observed, including Na, Si, Mg, K, C, O and Cl. C and O are possibly from the extract while the rest are impurities.

A large number of spherical-shape nanoparticles of sizes ranging from 9.53 to 52.38 nm were found in the Field Emission Scanning Electron Microscope (FESEM), which revealed the morphology and scale of the synthesized silver nanoparticles (Fig. 6). The interactions of nanoparticles with stabilizers and inductors around them have been known to influence

Table 2. ANTIBACTERIAL ACTIVITIES OF Spondias mombin SEED EXTRACTS MEDIATED AgNPs.

	Mean Zone of Inhibition (mm) ± standard deviation (SD)							
Isolates	10µg/ml	20µg/ml	40µg/ml	60µg/ml	80µg/ml	100µg/ml	CPE	Distilled H_20
Klebsiella sp.	-	-	-	-	-	8±0.2	-	-
Pseudomonas (ATCC 27435)	-	-	-	-	-	8±0.2	-	-
Staphylococcus aureus (25382)	-	-	-	8±0.2	10±0.2	12±0.2	-	-
Salmonella (11357 b/d)	-	-	8±0.2	10±0.2	12±0.2	14±0.2	-	-
Candida albicans (25333)	-	-	-	-	12±0.1	15±0.2	-	-
Escherichia coli (254)	-	-	8±0.2	10±0.1	12±0.2	14±0.1	-	-
Enterobacter (14821)	-	-	-	-	10±0.1	12±0.2	-	-
Acinetobacter (15841 b/d)	7.1±0.1	8±0.2	9±0.2	10±0.2	11±0.2	13±0.1	-	-
Staphylococcus aureus (25425)	-	-	6±0.1	7±0.2	9±0.2	12±0.2	-	-
Proteus vulgaris	7±0.2	9±0.2	11±0.2	11±0.2	12±0.2	12±0.2	-	-
Staphylococcus aureus (ear)	-	-	-	-	-	-	-	-
Klebsiella pneumoniae (urine)	-	-	-	-	8±0.2	12±0.2	-	-
Escherichia coli (ATCC25922)	9.3±0.2	10±0.1	11±0.2	12±0.2	13±0.1	14±0.1	-	-
Bacillus subtilis (ATCC6633)	-	-	-	-	11±0.2	12±0.1	-	-
Escherichia coli (stool)	8±0.1	9±0.2	10±0.2	12±0.2	13±0.2	14±0.2	-	-
Staphylococcus aureus (ATCC27853)	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa (25923)	-	-	-	-	-	8±0.1	-	-
Pseudomonas aeruginosa (25923)	-	-	-	-	-	11±0.2	-	-

their shape [40], which in turn influences their reaction rate [41]. The presence of compounds such as polyphenols, tannins, saponins etc. in the plant extract resulted in the formation of spherical-shaped nanoparticles. Similar studies conducted on the biological synthesis of spherical-shaped silver nanoparticles also reported similar compounds present on the plant extract used as both stabilizing and reducing agents [42-44]. The existence of Ag nanocubes 90 min after the initial reduction process with cube sizes between 50 and 200 nm was reported in the previous paper on the green biosynthesis of silver nanocubes from leaf extracts from *Eucalyptus macrocarpa* [45].

The XRD pattern of the synthesized AgNPs indicates the presence of peaks corresponding to (111), (200), (220) and (311) silver planes at 2hr = 38.23 °C, 44.42 °C, 64.44 °C and 77.39 °C, respectively (Fig. 6). The AgNPs are polycrystalline and have been shown to be consistent with the substantial peak intensities of the published studies [46, 47]. The presence of individual peaks at various angles of diffraction indicates that the particles are homogeneous and impure from the spectrum. The Scherrer's formula was used to calculate the crystallite size (L) of the nanoparticles.

The antibacterial activity of silver nanoparticles synthesized from *Spondias mombin* seed extract at a concentration of 10-100 µg / ml was observed and computed as the diameter of the millimeter (mm) inhibition zone as shown in Table 2. The seed mediated particles displayed impressive antibacterial effects on the different bacterial strains by inducing an inhibition zone ranging 7-15 mm against the test species. These findings are consistent with the antibacterial activities of AgNPs reported in earlier studies [48-51]. Adelere *et al*. [37] reported that aqueous extract of wonderful kola AgNPs demonstrated a 10–20 mm zone of inhibition against the test species, and Lateef *et al*. [24] reported that cocoa pod husk extract-mediated AgNPs induced a 10-14 mm zone of inhibition against clinical bacterial isolates, while Salem *et al*. [52] documented a 7-19 mm zone of inhibition for biosynthesized AgNPs using leaf isolates. However, the exact mechanism of the AgNP antibacterial activity is not yet known, but researchers have suggested several potential successful mechanisms. For example, AgNPs' antibacterial activity was thought to be related to its sizes [53], the smaller the size, the greater the surface-area-to-volume ratio. This property makes contact with microbial cells simpler. Likewise, Pal *et al*. [54] stated that the AgNPs antibacterial activity is also dependent on structure. AgNPs attack bacterial cells by releasing silver ions into cells that cause antibacterial effects such as cell membrane denaturation, interference with DNA replication and the respiratory chain that eventually leads to death [55, 56]. The inhibition zone clearly displayed the inhibitory effects of silver nanoparticles. While it has been found that the effects of silver nanoparticles on Gram negative bacteria are more comparable with Gram positive bacteria.

Table 3. ANTIBACTERIAL SUSCEPTIBILITY TEST OF GRAM NEGATIVE BACTERIA.

	SXT	CH	SP	CPX	AM	AUG	GEN	PEF	OFX	S
Klebsiella sp.	-	15±0.2	13±0.1	15±0.1	-	-	-	17±0.1	-	-
Pseudomonas (ATCC 27435)	-	-	16±0.1	15±0.1	-	-	-	15±0.1	14±0.2	16±0.2
Salmonella (11357 b/d)	18±0.1	16±0.1	15±0.2	16±0.2	16±0.1	-	-	16±0.1	15±0.1	17±0.2
Escherichia coli (254)	19±0.1	17±0.1	11±0.1	15±0.2	15±0.1	17±0.1	15±0.1	14±0.1	16±0.2	17±0.1
Enterobacter (14821)	14±0.1	15±0.1	17±0.1	11±0.1	-	-	-	-	12±0.1	-
Acinetobacter (15841 b/d)	17±0.2	16±0.2	19±0.1	15±0.1	-	14±0.1	15±0.1	16±0.1	16±0.1	15±0.1
Proteus vulgaris	-	15±0.1	14±0.1	13±0.2	13±0.1	-	-	13±0.2	14±0.2	15±0.1
Klebsiella pneumoniae (urine)	-	16±0.2	-	16±0.1	-	-	-	-	13±0.2	16±0.2
Escherichia coli (ATCC25922)	-	15±0.1	-	14±0.2	-	-	-	-	13	-
Escherichia coli (stool)		16±0.1	-	13±0.2	-	-	-	11	14	14
Pseudomonas aeruginosa (25923)		15±0.1	14±0.1	15±0.2	12±0.1	-	-	13	13	16

Table 4. ANTIBACTERIAL SUSCEPTIBILITY TEST OF GRAM POSITIVE BACTERIA.

	CAZ	CRX	GEN	CTR	ERY	CXC	OFL	AUG
Staphylococcus aureus (25382)	-	-	9±0.1	-	15±0.1	-	9±0.1	-
Staphylococcus aureus (25425)	9±0.1	10±0.1	-	-	-	-	12±0.2	-
Candida albicans (25333)	-	-	20±0.1	-	-	-	24±0.2	-
Staphylococcus aureus (ear)	-	-	17±0.2	-	-	-	15±0.1	-
Bacillus subtilis (ATCC6633)	-	-	15±0.2	-	-	-	15±0.1	-
Staphylococcus aureus (ATCC27853)	-	-	19±0.1	-	-	-	17±0.1	-
Pseudomonas aeruginosa (25923)	-	-	17±0.1	-	-	-	15±0.2	-

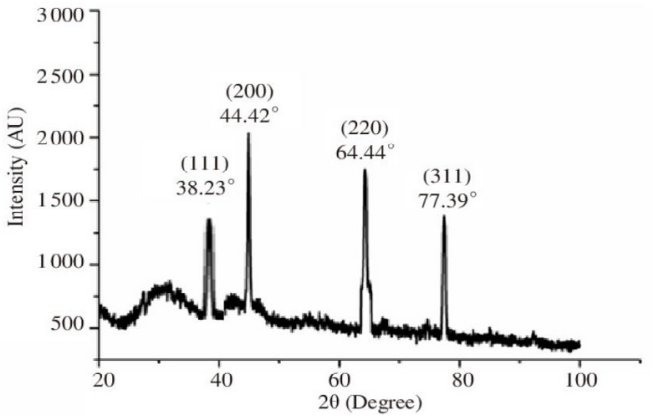

Fig. 7. XRD SPECTRA of the biosynthesized SMSE-AgNPs.

The explanation behind this may be attributed to the thickness of the peptidoglycan layer of the Gram-positive bacteria. An essential function of the peptidoglycan layer is to protect against antibacterial agents such as antibiotics, toxins, chemicals, and enzymes [57]. Singh *et al*. [58] had also conducted a similar research.

After evaluating the antibacterial activity of the SMSE-AgNPs, the antibacterial activity of some classical commercial antibiotics against the selected microorganisms has also been evaluated (Tables 3 and 4). Some of the bacteria displayed resistance to these commercial antibiotics. Nanoparticles are now considered a feasible alternative to antibiotics and seem to have high potential to solve the problem of multidrug resistance arising from bacteria. Recently, AgNPs are considered especially desirable for the development of a new class of antimicrobials that opens up a whole new way of combating a wide range of bacterial pathogens. Although the antibacterial activity of AgNPs has been widely defined, its mechanism of action still needs to be completely understood [59]

CONCLUSION

AgNPs were successfully synthesized in this study using aqueous extract of *Spondia mombin*,. It can be seen that the seed extract of *Spondias mombin* can be used as a reducing and superior long-term stabilizing agent for AgNPs. The particles were spherical in shape and ranged from 10 to 50 nm in size. The AgNPs synthesized demonstrated high antibacterial efficacy against clinical isolates of the bacteria. Green synthesized AgNPs can also be used to produce new antibacterial agents for medical, pharmaceutical and biotechnological applications.

CONFLICT OF INTEREST

Authors have no conflict of interest.

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