Green Synthesis, Characterization and Antimicrobial Effect of Silver Nanoparticles Using *Alchornea laxiflora* and *Spondia mombin* Against Microorganisms from Wound and Gastrointestinal Tracts Infections

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Abstract: Silver nanoparticles play a huge roles in creating new anti-microbial properties to tackle a quite number of disease-causing microbes. This study aimed at determining the *in vitro* anti-microbial properties of silver-nanoparticles (AgNPs) green synthesis on multidrug resistance organisms from Nigerian Institute of Medical Research, Lagos State and University college Hospital, Ibadan. The AgNPs was synthesized using extracts of *Spondia mombin* and *Alchornea laxiflora* and characterized using UV-vis spectroscopic and fourier-transform infra-red spectroscopy analysis, scanning electron microscopy and Energy-dispersive X-ray analysis. Phytochemical analysis of the ethanolic extract was determined using standard methods. The synthesized AgNPs was assayed for their antimicrobial efficacy using standard methods against some pathogenic organisms. A colour change in the solution from colourless to dark-brown suggested AgNPs formation and spectra of UV-vis at sharp peak of 380–450 nm ascertained the synthesized AgNPs. The fourier-transform spectrum confirmed the presence of some functional groups like hydroxyl group, ammonium ion, aliphatic iodo-compounds and many more which showed major absorption peaks amongst others. The phytochemical screening of ethanolic extracts showed the presence of phenol, saponins, flavonoids, alkaloids among others. The synthesized nanoparticles showed a significant anti-microbial potentials against the tested strains. The minimum inhibitory concentration and minimum bactericidal concentration values ranged between 25 – 50 mg/ml. The medicinal plant extracts used in this study have varied antibacterial properties and ability to mediate the synthesis of AgNPs. The synthesized AgNP suggested their application as a potential therapeutic agents which can be used to formulate new therapeutic drugs for medical use to combat antimicrobial resistance.

Key word: *Alchornea laxiflora-silver nanoparticles; gastrointestinal tract infections; Spondia mombin extracts; green synthesis; wound Infections*.

INTRODUCTION

The widespread usage (abuse) of antibiotics has resulted into a problem known as antibiotic resistance. Antibiotic resistance develops primarily due to abuse of antibiotics, which turns in to proliferation of bacteria that does not respond to treatment with antibiotics (Mancuso *et al*., 2021). Antibiotic resistance is a growing public health concern not only in Nigeria, but worldwide. Antibiotic resistance threatens human health because it reduces or eliminates the effectiveness of therapeutic agents used in treating infections. Researches has been directed and focused on the use of nano-biotechnology to search for and develop new anti-microbial drugs less toxic with broad-spectrum activity to fight and eliminate the existence of resistance, emergence, and re-emerging pathogens. The applications of the antibacterial properties of nano-technology are growing rapidly in the field of science and the utilization of metals in nanoparticles (NPs) creation as a replacement to antibiotics is high (Adebayo-Tayo *et al*., 2022).

Plant extract-based biosynthesis approaches are gaining popularity as a simple, efficient, cost-effective, and practical alternative to traditional nanoparticle creation methods. By using plants or microorganisms, green synthesis techniques can be employed to create nanoparticles from natural sources

(Solomon *et al*., 2007). Several plants, including different extracts from *Cardiospermum halicacabum* L leaves (Mahipal *et al*., 2013), and *Lantana camara* L. fruits (Edy Parwanto *et al*., 2013), have been employed to manufacture and synthesize Ag nano-particles, and testing their antibacterial activity.

Alchornea laxiflora (*A. laxiflora*), a deciduous shrub in the Euphorbiaceae family wildly cultivated in Nigeria is refers to as Opoto in Yoruba in South-West Nigeria. Traditional medicine regularly uses the plant's leaf infusion as an antimalarial (Adeloye *et al*., 2005). Also, the plants are utilized as preservatives for kola nuts and several perishable vegetables and fruits, while the branched stems are utilized as a traditional tooth brush (chewing sticks) in Nigeria. The leaves' decoction is commonly used to treating infections and inflammatory diseases (Adeloye *et al*., 2005). *Spondias mombin* (*S. mombin*) is popularly called hog-plum in English. The Igbo call it Ijikara, while it is known as Tsardar masar in Hausa, Yoruba calls it Ewe iyeye. According to the literature, the leaves of this plant have anti-microbial and anti-radical effects. It has also been reported that aqueous, methanolic, and ethanolic extracts of *S. mombin* leaves show antihelminthic effects, as well as strong antibacterial capabilities against *Bacillus cereus*, *Streptococcus pyogenes*, and *Mycobacterium fortuitum* (Fasogbon *et al*., 2021).

Wounds and inflammations can also be treated with crushed leaf juice or dried leaf powder of *S. mombin*. Gum is used as an expectorant and to remove tapeworms (Sameh *et al*., 2018). A leaf infusion is used as cough medicine, as well as a laxative for fever, constipation and gonorrhea. It is used to treat poliomyelitis and measles as well. The juice collected from young heated leaf is administered to children with stomach problems, while a decoction produced from crushed *S. mombin* leaves is used as an eye salve. The extract exhibits anti-inflammatory effects in wistar rats (Nworu *et al*., 2011). Furthermore, the leaves are believed to possess antibacterial, antiviral and antiseptic properties that can combat various microbial problems such as gonorrhea, colds, cystitis, flu and so forth. There is an increase in awareness of infectious diseases caused by arrays of organisms. So, due to the development and high percentage of antibiotic resistance to common antibiotics, the search for new and efficient therapeutic agents (antibacterial substances) is imperative. Therefore, the need for alternative therapy in combating this issues using green synthesis methods for synthesizing nanoparticles informed this study.

MATERIALS AND METHODS

Collection and preparation of plant samples: *Spondia mombin* and *Alchornea laxiflora* leaves were harvested and collected in Odeomu, Osun State, Nigeria. After-which, it was identified and authenticated at the Department of Botany, University of Ibadan, Oyo State, Nigeria. The leaves were washed under running tap to remove dust and also rinsed with deionized water after which the leaves air-dried under shade for 3-4 weeks until constant mass was obtained. The leaves were chopped into small pieces and then grounded into powder for further use. The extraction process was conducted (Samuggam *et al*., 2021). The extracts was concentrated using rotary evaporation (Rotary Evaporator RE-52A, Union Laboratories, England), and then kept in a refrigerator at 4ºC for further analysis.

Extract sterility test: This was done by introducing a loopful of the extract into nutrient agar plate and incubated at 37ºC for 24 hours (Sule and Agbabiaka, 2008). The absence of growth on the plates after incubation indicated that the extracts are sterile while the presence of growth indicated not sterile.

Synthesis of silver nanoparticles using plant extracts: Solution of 0.4 M silver-nitrate solution was prepared with 50 ml of distilled H_2O. The diluent was mixed for 20 minutes and 5 ml of the leaf extracts each of *S. mombin* and *A. laxiflora*, was mixed to the solution with continuous stirring at 1000 rpm. Also, 0.3 ml of 2.0 M NaOH was added drop-wisely to enhance precipitation and maintaining a pH of 7. There was continuous stirring of the whole mixture for one hour. The precipitate was taking out, washed with ethanol repeatedly twice for the removal of impurities, after which it was dried in the air at 60ºC for six hours to yield a dark powdered silver nanoparticle (AgNP).

Phytochemical screening of the extract: Qualitative phytochemical screening of leaf extract of *Alchornea laxiflora* and *Spondia mombin* was examined to identify and determine some of the active potential phytochemicals such as, saponins, pholobatannins, quinone, flavonoids, terpenoids phenols, alkaloids, anthraquinones, glycosides, steroids and anthocyanin (Thonda *et al*., 2020).

Characterization of the synthesized AgNPs: To trace the development of synthesised *Alchornea laxiflora* (AgNP-AL) and *Spondia mombin* silver nanoparticles (AgNP-SM), the UV-vis spectra of the reaction fluids were measured using a V-630 spectrophotometer double beam at wavelengths ranging from 200 to 800 nm (Varadavenkatesan *et al*., 2021). The functional group of AgNP was characterized using FT-IR spectroscopy (FTIR-84000S, IR Prestige-21, IR affinity-1) following the method of Joselin *et al*., (2014) and Fagbemi *et al*. (2024), scanning electron microscopy (SEM) imaging was conducted to observe and evaluate the morphology and sizes of the silver nanoparticles being synthesized, while EDX was used to determine the Ag concentration and other elements present in the nanoparticles (Oyewole *et al*., 2023). Energy Dispersive Analysis presents the elements quantitative and qualitative status which are responsible for the formation of the AgNPs (Oyewole *et al*., 2023)

Collection and sources of test organisms: The isolates used are pathogenic and multidrug resistance microorganisms obtained from Nigerian Institute of Medical Research (NIMR), Yaba, Lagos State, Nigeria and University College Hospital (UCH), Ibadan, Nigeria. The organisms' sources are gastrointestinal tract infections (GIT) and wound infections. The test organisms used were: *Klebsiella* spp, *Proteus mirabilis*, *Escherichia coli,* while the fungal isolates used was *Candida albicans*. The test organisms were subcultured on selective media for colonial, morphology and biochemical characterization for the confirmation of the test microorganisms (Thonda et al., 2020).

Antimicrobial activity of the synthesized nanoparticles (AgNP-SM and AgNP-AL): The antimicrobial activities of AgNP-SM and AgNP-AL was established using the method of Kirby-Bauer disk diffusion susceptibility test as described by Oyewole *et al*. (2023). Briefly, the test microorganisms were introduced in nutrient broth for 18-24 hrs and turbidity of the culture was adjusted to 0.5 McFarland's standard. Upon dipping sterile swab stick into the bacterial inoculum, it was aseptically spread on the Mueller-Hinton agar. Thereafter, wells of about 6 mm in diameter were aseptically created on the agar plates using a sterile cork-borer and aliquots of the synthesized AgNPs of different concentrations (100, 50 and 25 mg/mL) and controls were dispensed into the wells. Incubation of the plates was done at 37ºC for 24 hrs. At the end of incubation, the diameter of the zones of inhibition was measured in triplicates (mm) and recorded. The susceptibility and resistance of the test organism to each antibiotics tested was compared to the Clinical Laboratory Standards Institute standard (CLSI, 2024).

Anticandidal activity of the synthesized nanoparticles (AgNP-SM and AgNP-AL): The anticandidal potentials of the AgNP-SM and AgNP-AL were tested against *Candida albicans* (*C. albicans*) using the disk diffusion method (Dzoyem *et al*., 2022), while ketoconazole was used as standard antifungal agent, The *C. albicans* in broth media were spread uniformly on potato-dextrose-agar media using a sterile swab-stick. Six millimeter diameter wells were bored onto the agar plate and filled with AgNP-AL and AgNP-SM of different concentrations (100, 50 and 25 mg/mL). These were grown at 28°C for 48 hrs. The anticandidal activities of the AgNP was determined by measuring the diameters of zones of inhibition and the results were recorded.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the synthesized nanoparticles: The MIC of AgNP-AL and AgNP-SM was determined using the method described by Akinpelu *et al*. (2015). The NPs were the diluted to obtain 50, 25, 12.5, 6.25 and 3.125 mg/ml concentrations. The lowest concentration that inhibited bacterial growth was defined as the MIC, while MBC of the nanoparticles was determined to be the lowest concentration of AgNP that did not exhibit any growth on fresh plates.

Statistical analysis of data obtained: The experiments were repeated in three replicates, and data were presented as means ± standard deviation (SD) and were statistically analyzed using ANOVA at 5% significant level.

RESULTS

Qualitative phytochemicals constituents of *Spondia mombin* and *Alchornea laxiflora* extracts

The phytochemicals present in the extracts of *Alchornea laxiflora* and *Spondia mombin* are depicted in Table 1. Findings revealed that extracts contained some phytochemical components such as phenol, saponins, flavonoids and alkaloid.

Visual observation of the synthesized AgNPs

In this study, the biological reduction of the Ag^{+} into the AgNPs was performed successfully by the plant extracts used as a bioreducing agents. The formation of AgNPs was confirmed by a change in colour observed from colourless $AgNO_3$ solution to dark-brown colour as shown in Figure 1a.

Characterization of the synthesized AgNPs

In order to demonstrate the environmentally friendly synthesis of AgNPs, surface plasmon resonance (SPR) absorption peak was evaluated using UV-visible spectroscopy. The resultant reaction mixtures of UV-vis spectra of AgNPs at 50 mg/ml concentration showed a strong, broad peak at wavelengths between 380 and 450 nm. *Alchornea laxiflora* and *S. mombin* had different peak values of 400 and 450 nm, respectively which indicated that the nanoparticles are within the range of the nano size because of SPR (Figure 1b). The SEM and EDX spectra of silver nanoparticles are shown in Figure 1c and 1d. The SEM images showed that the silver particles is present in nanosize. The SEM micrograph of the silver nanoparticles for *S. mombim* and *A. laxiflora* revealed the particles to be irregular shape with an average diameter of 34 nm–60 nm. The EDX results of *S. mombin* revealed the present of the following elements; silver (52.29 wt.%), iodine (33.05 wt%), nitrogen (1.44 wt%), ceasium (12.83 wt%) and carbon (0.39 wt%), while the element present in *A. laxiflora* are silver (80.27 wt.%), chlorine (4.33 wt.%) and oxygen (15.40 wt.%) (Figure 1c and 1d). The percentage weight of silver (80.37 wt.%) among all element was high in both AgNP-SM and AgNP-AL nanoparticles as revealed by the EDX.

FTIR spectrum of the synthesized nanoparticles

Fourier transform infrared spectroscopy spectrum of AgNP-SM and AgNP-AL is shown in Figure 5. It showed that both the nanoparticles have some peaks range that coincides, thus have the same functional group as expressed in Table 2 as (2357 to 2362 cm^{-1}) which shows O=C=O stretch carbon-dioxide and 611.45-615.31 shows S-S stretch disulfides (Figure 2).

Functional groups and FTIR peak values of AgNPs

The FTIR spectra was employed in identifying and detecting the peaks and functional-groups of the active components in the AgNP, based on the peak value in the region of infrared radiation as depicted in Figures 2. The FTIR spectrum of AgNP-AL indicated the absorption peaks at 457.14, 530.44, 611.45, 700.18, 916.22, 987.59, 1087.89, 1124.54, 1265.35, 1325.14, 1404.22, 1589.40, 1624.12, 2115.98, 2362.88, 2852.81, 2962.76 and 3441.12 cm^{-1} corresponding to the functional groups namely aryl disulfides (S-S stretch), aliphatic-iodo compounds (C-I stretch), aromatic primary-amine (N-H stretch), cyclic-ethers (C-O stretch), aromatic primary amine (CN stretch), secondary amine (CN stretch), hydroxyl group, OH bend, alkenyl (C=C stretch), terminal alkyne (mono-substituted) (C≡C), carbon-dioxide (O=C=O stretch), phenol or tertiary alcohol, H-bonded (O-H stretch), vinyl C-H out-of-plane bend (Table 2). The absorption peaks occurring at 513.08, 615.31, 713.69, 763.84, 974.06, 1051.24, 1180.47, 1224.84, 1274.99, 1396.51, 1616.40, 1749.49, 2090.91, 2357.09, 2943.47, 3232.80 and 3425.69 cm^{-1} for AgNP-SM indicated the presence of aliphatic-iodo compounds (C-I stretch), aliphatic chloro-compounds (C-Cl stretch), disulfides (S-S stretch), C-H mono-substitution (phenyl), aromatic phosphates (P-O-C stretch), primary alcohol (C-O stretch), organic sulfates, aliphatic ether (C-O stretch), organic nitrates, organic sulfates, aromatic ring (C=C-C stretch), alkyl carbonate, cyanide ion, thiocyanate ion, carbondioxide (O=C=O stretch), methyl asym. (C-H Stretch), hydroxyl group, H-bonded (O-H stretch) and aromatic primary-amine (N-H stretch) respectively.

Antimicrobial activity of synthesized-silver nanoparticles against test organisms

The AgNP-SM and AgNP-AL (synthesized AgNP) showed appreciable activities at all the concentrations used against the test microorganisms. The antibacterial activities are shown by a zone of inhibition. Majority of the organisms are resistant to the ampiclox used as positive control. The AgNP-AL had the highest zones at 100 mg/ml against all organisms as depicted in Figure 4. *Klebsiella oxytoca, E. coli, P. mirabilis and K. pneumoniae* all showed resistant to ampiclox. The anti-bacterial and anti-fungal activity of each nanoparticles against the test organisms are depicted in Figure 3-4. Three organisms showed resistant to AgNP-SM. The highest zone of inhibition was shown in *Proteus mirabilis* at 100 mg/ml and in *Klebsiella pneumoniae* at 50 mg/ml. *Alchornea laxiflora* mediated nanoparticles (AgNP-AL) showed more and better activities than AgNP-SM.

The MIC and MBC of AgNPs against Gram-negative bacteria and *Candida albican*

The MIC value of AgNPs of 50 and 25 mg/ml which were observed for bacterial isolates as shown in Table 2. The MCB exhibited by the nanoparticles on the organisms were determined at 50 and 25 mg/ml concentration as well as the MIC (50 and 25 mg/ml) while few were not determined.

Table 1: Qualitative phytochemicals constituents of ethanolic extracts of *Spondia mombin* and *Alchornea laxiflora*

Phytochemicals	ATH	PHN	SPN	QNE	STR	TPN	PLB	FLD	AKD	GLY	ACY
Spondia mombin	-	+	+	-	-	+	-	+	++	-	+
Alchornea laxiflora	-	++	++	++	-	-	-	++	++	+	-

Footnotes: + (Positive); ++ (abundance), – (Negative); ATH-Anthraquinone; PHN-Phenol; SPN-Saponin; QNE-Quinone; STR-Steriods; TPN-Terpenoids; PLB-Pholobatannins; FLD-Flavoniods; AKD-Alkaloid; GLY-Glycosides; ACY-Anthocyanin

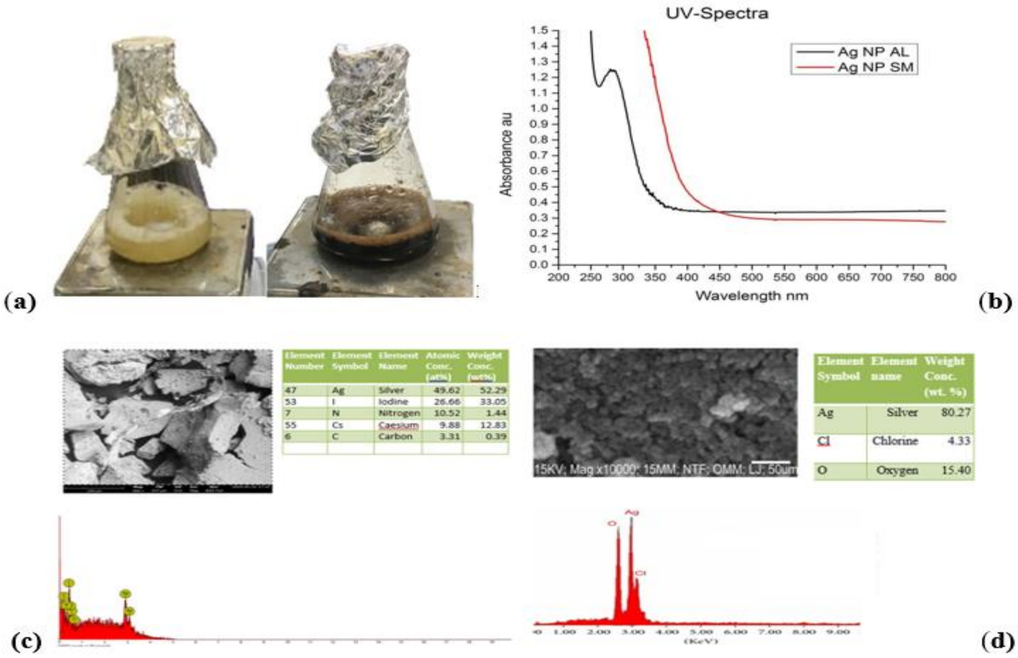

Figure 1: (a) $AgNO_3$ Solution before and after adding extracts, (b) UV-vis spectra of the AgNP-AL and AgNP-SM (c) SEM/EDX spectra of AgNP-SM and (d) SEM/EDX spectra of AgNP-AL

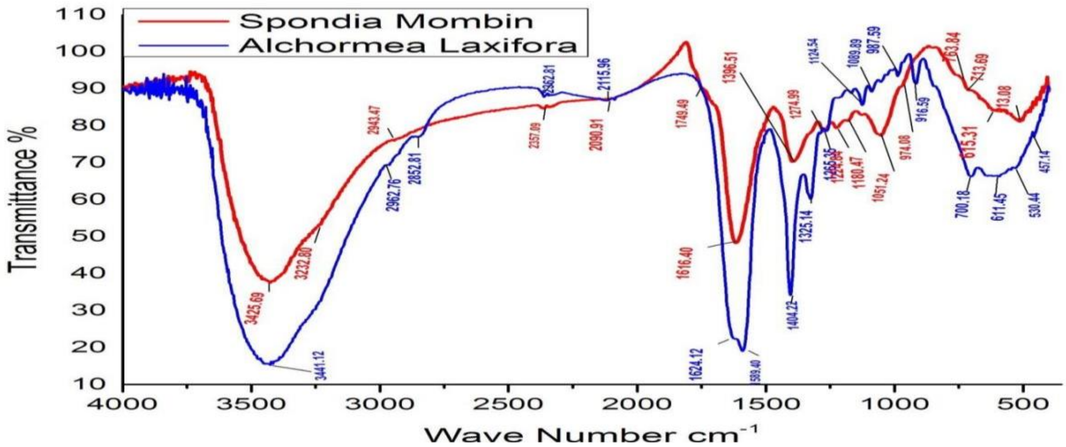

Figure 2: FT-IR spectra of AgNP-AL and AgNP-SM

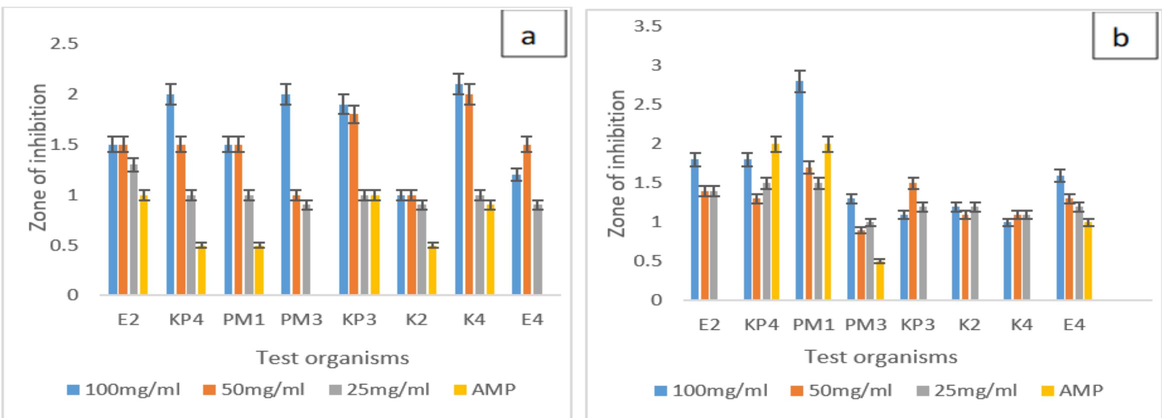

Figure 3: Antibacterial activity of (a) *Alchornea laxiflora* AgNP (b) *Spondia mombin* AgNP against test organisms. Vertical bars represent standard error (n = 3). E2 and E4- *Escherichia coli*, KP4 and KP3- *Klebsiella pneumoniae*, PM1 and PM3- *Proteus mirabilis.* K2 and K4- *Klebsiella oxytoca*

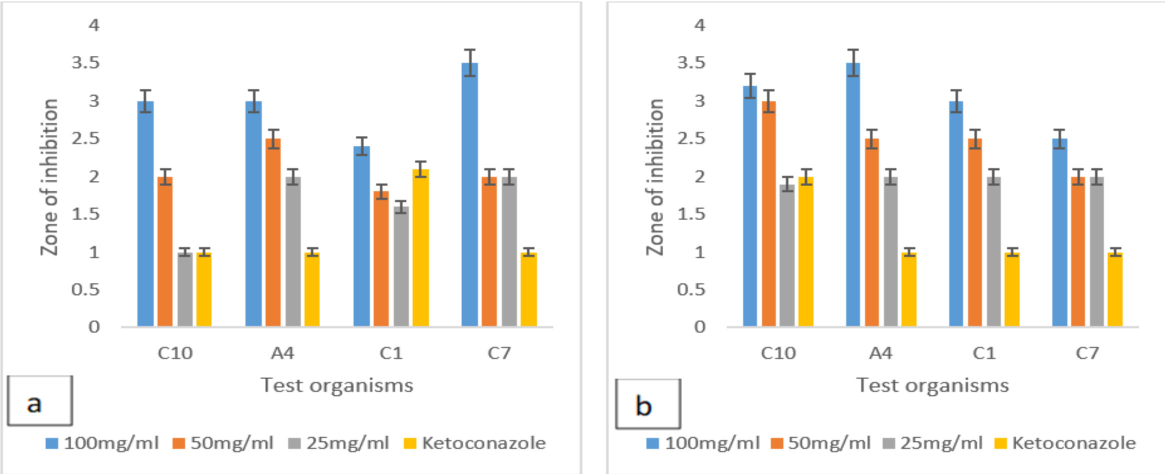

Figure 4: Anticandidal activity of (a) *Alchornea laxiflora* AgNP (b) *Spondia mombin* AgNP against *Candida albicans.* Vertical bars represent standard error (n = 3). C10, A4, C7 and C1-*Candida albican*

Table 2: MIC and MBC values of AgNP-AL and AgNP-SM

Test organisms		E2	KP4	PM1	PM3	KP3	K2	K4	E4	C10	A4	C1	C2
AgNP-NL	MIC	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	50.0
(mg/ml)	MBC	ND	25.0	25.0	ND	25.0	25.0	25.0	25.0	25.0	25.0	ND	25.0
AgNP-SM	MIC	50.0	50.0	50.0	50.0	50.0	50.0	25.0	25.0	25.0	25.0	25.0	25.0
(mg/ml)	MBC	50.0	ND	ND	50.0	ND	50.0	25.0	25.0	25.0	ND	50.0	50.0

Keys: AgNP-AL= *Alchornea laxiflora* nanoparticles, AgNP-SM= *Spondia mombin* nanoparticles, ND= Not Determined, *Escherichia coli* (E2), *Klebsiella pneumoniae* (KP4), *Proteus mirabilis* (PM1), *P. mirabilis* (PM3), *K. pneumoniae* (KP3), *K. oxytoca* (K2), *K. oxytoca* (K4), *Escherichia coli* (E4), *Candida albicans* (C10), *C. albicans* (A4), *C. albicans* (C1), *C. albicans* (C2)

DISCUSSION

The colourless $AgNO_3$ solution became dark-brown in colour, thus facilitating and confirming the synthesis of AgNPs. The increased availability of phytoconstituents in the extracts enhanced reduction potential, which resulted in the rapid production of AgNPs. This clearly indicated that the beneficial phytochemical elements found in the extracts, such as phenolic compounds, saponins, and flavonoids, might be associated with the bioreduction of silver (Ag) ions (Auyeung *et al*., 2016). Both phytochemical compounds are able to function as reducing agent because of the presence of carboxyl and hydroxyl groups.

In addition, these phytoconstituents also acted as capping and stabilizing agents (Zuhrotun *et al*., 2023).

The findings of this study revealed the presence of some phyto-chemicals in the extracts under investigation. These embraces phenol, saponins, terpenoids, flavonoids, alkaloids, and anthocyanin present in *A. laxiflolia*. Li *et al.* (2007) reported that these phyto-constituents act as both reductants and good stabilizers for silver ions and AgNPs respectively. Strong reducing agents called flavonoids are found in the leaf extract, an indication that AgNPs are formed through reduction of $AgNO_3$. Flavonoid compounds as one of the compound in the extracts might be actively engaged in facilitating the reduction of silver ion (Ag^+) to silver (Ag^0) (Zuas *et al*., 2014)

The presence and formation of likely spherical AgNPs was relatively associated to this SPR absorption peak (Anandalakshmi *et al*., 2016). The absorbance peak at 400 nm and above that was noticed in this study provides more evidence that Ag nanoparticles are biosynthesized. Several polyphenolic components, such as terpenoids and flavonoids, stabilized the surface of the resulting Ag nanoparticles and facilitated the reduction of silver ions

(Marslin *et al*., 2018). The presence of C=O and O-H indicating carboxyl- and hydroxyl-groups are mainly accountable for reduction and stabilization of AgNPs as reported by Rizwana *et al*. (2023). The functional groups could be due to phenolic compounds capping and stabilizing the AgNPs.

In this study, the presence of clear zone around the AgNPs disk indicated that AgNPs possessed antibacterial and antifungal activity which is able to inhibit the growth of the Gram-negative and fungal pathogens. Earlier reported researches has showed high anticandidal activities of biogenic AgNP against wide range of *Candida* species, such as *C. tropicalis*, *C. albicans* and *C. dubliniensis* (Kamradgi *et al*., 2021) which is comparable to the findings of this study. *Alchornea laxiflora* mediated nanoparticles (AgNP-AL) showed more and better activities than AgNP-SM due to the more phytochemical constituents in *Alchornea laxiflora* than in *Spondia mombin*. This findings is in agreement with previous reports from researchers (Asomie *et al*., 2021), while some studies showed that the produced AgNPs using plant extract or green synthesis were more active against Gram +ve pathogens compared with Gram -ve pathogens (Lee *et al*., 2016). Based on the literature, differences in values of leave extracts may be related to variation in their chemical components present that are volatile in nature and leads to various mechanisms and modes for antimicrobial activities of the leaf extracts against pathogenic microorganisms (Gonelimali *et al*., 2018). Vazquez-Munoz *et al*. (2020) reported preciously that AgNPs has anti-bacterial activities on bacteria with Gram negative cell wall. The aqueous extracts of *Spondia mombin* and *Alchornea laxiflora*, containing silver nanoparticles showed activity in all the concentrations against the test microorganisms. Dalir *et al*. (2020) reported that synthesized AgNps are more efficient and active against Gram negative bacteria cell as compared to the Gram positive bacterial cell which was similar to what is obtained in this study. Silver nanoparticles have emerged as anti-microbial agents against multiple drug resistant pathogens as a reason of their uniqueness of their physico-chemical stick and increment in surface area to volume ratio. The tiny particle size allows AgNPs to adhere to the cell wall and penetrate easily into the cell of bacteria, which then improves their efficacy antimicrobial activity against bacteria (Bruna *et al*., 2020).

CONCLUSION

The synthesized AgNPs used in this study were found to have a pronounced antibacterial and anticandidal activities against different strains of *Escherichia coli, K. pneumoniae, P. mirabilis, Klebsiella oxytoca* and *Candida albicans*. However, *Alchornea laxiflora* mediated nanoparticles exhibited more activities on the pathogens than *Spondia mombin* mediated nanoparticles. Therefore, these plants can be used to synthesize silver nanoparticles for developing new therapeutic substances in future to combat pathogens that are multiple resistant causing gastroenteritis and wound infections. This study therefore recommended that *A. laxiflora* and *S. mombin* are excellent medicinal plants for green synthesis of AgNPs and can be explored for future biomedical and therapeutic applications such as development of new drug and drug combination.

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