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Research article

Silver nanoparticle synthesis by *Acalypha wilkesiana* extract: phytochemical screening, characterization, influence of operational parameters, and preliminary antibacterial testing

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A B S T R A C T

Single pot green synthesis of silver nanoparticles (AgNPs) was successfully carried out using medicinal plant extract of *Acalypha wilkesiana* via bottom-up approach. Five imperative operational parameters (pH, contact time, concentration, volume ratio and temperature) pivotal to the synthesis of silver nanoparticles were investigated. The study showed pH 9, 90 min contact time, 0.001 M Ag^+ concentration, volume ratio 1:9 (extract: Ag^+ solution), and temperature between 90 – 100 °C were important for the synthesis of *Acalypha wilkesiana* silver nanoparticles (AW-AgNPs). Phytochemical screening confirmed the presence of saponins, flavonoids, phenols and triterpenes for A. *wilkesiana*. These phytomolecules served as both capping and stabilizing agent in the green synthesis of silver nanoparticles. AW-AgNPs was characterized by UV-Vis Spectroscopy, Fourier Transform Infrared (FTIR) Spectroscopy and Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM) and Energy Dispersive X-ray (EDX). The surface Plasmon resonance (SPR) was observed at 450 nm which is a characteristic absorbance region of AW-AgNPs formation as a result of the collective oscillation of free electron of silver nanoparticles. FTIR Spectroscopy confirmed the presence of functional groups responsible for bioreduction of Ag^+. SEM and TEM results confirmed a well dispersed AW-AgNPs of spherical shape. EDX shows the elemental distribution and confirmed AgNPs with a characteristic intense peak at 3.0 keV. AW-AgNPs showed significant inhibition against selected Gram negative and Gram positive prevailing bacteria. AW-AgNPs can therefore be recommended as potential antimicrobial and therapeutic agent against multidrug resistant pathogens.

1. Introduction

Nanotechnology has its origin from various fields of Science and Engineering where interestingly, new ideas have been produced to alter molecules and single atoms (Prathna et al., 2011). In the rapidly improving field of nanotechnology, nanomaterials are on the prominent application in environmental and medical science. According to Dada et al. (2018a), of all the metallic nanoparticles, silver nanoparticles are exceptional and they are most explored by researchers globally because of their various versatilities, simplicity of synthesis, adaptability,

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morphology and high surface areas. Generally, nanoparticles are prepared by a variety of chemical and physical methods which are quite expensive and potentially hazardous to the environment. This involves the use of toxic and unsafe chemicals that are responsible for several biological risks recently reported (Ahmed and Ikram, 2015). The reduction by borohydride is gradually facing out because of its toxicity, thus the major reason why green synthesis has been preferable.

In recent years, the application of green chemistry for the synthesis of metal nanoparticles (NPs) that is bio-compatible has gained considerable attention for potential applications in biomedicine (Zhang et al., 2016). In the global efforts to reduce generated hazardous waste, "Green Chemistry" and chemical processes are progressively integrated with modern developments in science and industry. In this study, green synthesis of silver nanoparticles was carried which involved the use of medicinal plant extract through bottom-up approach in a single pot synthesis by wet chemistry method. This approach is ecofriendly, low cost and silver nanoparticles formed are stable and well dispersed with limited aggregation and good size control (Shiv et al., 2003; Yong and Kim, 2008). Extract of *Acalypha wilkesiana* was used as reducing and stabilizing agent in this study. This plant is commonly called Irish petticoat, it is native to the south pacific islands and belongs to the family Euphorbiaceae.

Acalypha wilkesiana is a plant readily available in Landmark University, Nigeria, West Africa. It has proven medicinal properties. This plant is often called "copperleaf or Jacob's coat thrives in partial shade or sun. The leaves, which may be flat or crinkled, are large and broad with teeth around the edge. It has the following dimensions: height is about 2–3 m, length 10–20 cm and 15 cm wide. It is a widely cultivated ornamental, outdoor plant due to its attractive red colouration. It is valued for its wide range of variegated cultivars and hedge (Nagarajan et al., 2003). In spite of the advancement in medical science, millions of people still resort to the use of naturally occurring, low cost and environmentally benign medicinal plant such as *Acalypha wilkesiana*. Both the leaves and seeds of *A. wilkesiana* are medicinally potent. In the developing world, it has been used for healing of different ailments. Researchers have reported it effectiveness in treatment of malaria, skin diseases, gastro-intestinal disorders, hypertension and diabetes mellitus, breast tumor in Western Nigeria (Udobang et al., 2010; Akinyemi et al., 2006; Oyelani et al., 2003).

The use of plant extract as reducing and stabilizing agent in the green synthesis of silver nanoparticles is a novel method which has triggered several researchers. Rivera-Rangel et al. (2018) worked on green synthesis of silver nanoparticles in oil-in-water microemulsion and nano-emulsion using *geranium* leaf aqueous extract as a reducing agent. Ajayi and Afolayan (2016) synthesized silver nanoparticles (AgNPs) from the alkalinized leaf extract of *Cymbopogon citrates*. Another study carried out by Umadevi et al. (2012) was on the biosynthesis of silver nanoparticles (NPs) using *Dillenium carota* extract and this was investigated for various concentrations of *D. carota* extract. Ahmed and Ikram (2015), used the one-pot green synthesis approach as a simple, cost effective bio-reduction on silver nanoparticles using the *Terminalia arjuna* plant extract. Kaumeel Chokshi et al. (2016), studied the demonstration of a sustainable approach for the biogenic synthesis of silver nanoparticles using lipid extracted residual biomass of microalgae *Acutodesmus dimorphus* cultivated in dairy wastewater. Shankar et al. (2016), implemented the biosynthesis of gold and silver nanoparticles using Algae. *Calotropis procera and Tithonia diversifolia plants* extracts were used in our previous studies for the synthesis of silver nanoparticles (Dada et al., 2016, 2018b). Other list of studies on the green synthesis of silver nanoparticles using different plant extracts are as follows: *Syzygium aromaticum* extract (Vijayaraghavan et al., 2012); *Acalypha indica* leaf extract (Krishnaraj et al., 2010); *Punica granatum* peel extract (Edison & Sethuraman, 2013); banana peel extract (Ibrahim, 2015); *Thevetia peruviana Juss* (Oluwaniyi et al., 2015); *Calotropis procera* extract (Dada et al., 2017b).

During the course of this study, various operational parameters such as; effect of concentration and volume ratio were studied as a justification for ascertaining the minimum concentration leading to the feasibility of growth of nanoparticle. Despite all these studies carried out, experimental optimization of operational parameters which are the factors influencing the synthesis of silver nanoparticles have not been given a total consideration. Therefore, phytochemical screening of *Acalypha wilkesiana* leaves extract, experimental optimization of operational parameters imperative to the green synthesis of *Acalypha wilkesiana* silver nanoparticles (AW-AgNPs), the characterization and application of AW-AgNPs on Multi-Drug Resistance Micro-organisms have not been reported hence the need for this study.

2. Materials and methods

2.1. Synthesis of AW-AgNPs, phytochemical screening and characterization

Acalypha wilkesiana (AW) plant (Fig. 1) was collected in the vicinity of Landmark University, Nigeria. Thereafter, it was cleaned to remove the farm land soil and air-dried to avoid losing vital volatile molecules. Extract preparation and synthesis of AW-AgNPs was carried out following the procedure in our previous studies (Dada et al., 2017b, 2018b). In a typical procedure, 10 mL of the leaf extract was measured and poured into a clean 250 mL beaker and reacted with 90 mL of 1×10^{-3} M $AgNO_3$ at room temperature. The resulting solution was stirred on the mechanical shaker at optimum operational conditions. *Acalypha wilkesiana* silver nanoparticles (AW-AgNPs) formed was separated by centrifugation at 4000 rpm at optimum contact time. Phytochemical screening was carried out to identify the presence of phenols, saponins, triterpenes, flavonoids, alkaloids and steroids in the AW leaves extract. These various tests were done following the procedure in our previous study (Dada et al., 2018b and in the literature (Senguttuvan et al., 2014). The synthesized AW-AgNPs was characterized using a combination of spectroscopic techniques vis-à-vis Ultraviolet Visible (UV-Vis) spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, Scanning Electron Microscopy (SEM) coupled with Energy Dispersive X-ray (EDX), and Transmission Electron Microscopy (TEM).

Fig. 1. Typical *Acalypha wilkesiana* plant (snapped in Landmark University environment).

Table 1
Phytochemical screening test results on *Acalypha wilkesiana* extracts.

S/N	Phytochemical Screening	*Acalypha wilkersiana leaf* extract
1	Test for Phenols ($FeCl_3$)	+
2	Test for Triterpenes	++
3	Test for Saponins (Froth's test)	+
4	Test for Steroids (Salkowski's test)	-
5	Test for Alkaloids (Mayer's test)	-
6	Test for Flavonoids	
	(a) Lead Acetate test	+
	(b) Alkali test (NaOH)	+

Table Key: - = Absent, + = Present, ++ = Present in abundance.

2.2. *Experimental optimization of operational parameters*

Effects of five operational parameters which are concentration, contact time, volume ratio, pH and temperature on the formation of AW-AgNPs were investigated and the study was monitored using Biochrom Libra PCB 1500 UV-VIS spectrophotometer. Batch operational parameter was studied in a batch technique following the procedure reported in our previous studies (Dada et al., 2018a,b). Synthesis of AW-AgNPs was optimized by operational parameters variation. The concentrations of reacting $AgNO_3$ to *A. wikesiana* aqueous leaf extract were 0.001 M, 0.002 M, 0.004 M, 0.006 M, 0.008 M and 0.01 M. To study the optimum contact time for the synthesis of silver nanoparticles, the reaction mixture was monitored from 0 to 90 min. More so, the effect of volume was monitored by varying the volume ratio of $AgNO_3$ to *A. wikesiana* leaf extract. In addition, effect of pH was investigated in the synthesis of silver nanoparticles from pH 2–11 and effect of temperature was investigated at different temperatures (30, 45, 60, 90 and 100 °C).

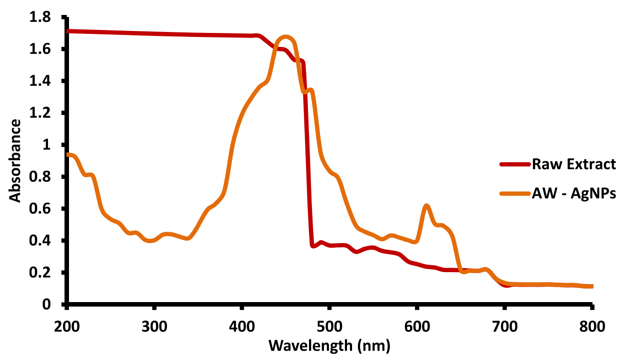

Fig. 2. UV-Vis Spectra of AW-AgNPs with SPR peak at 460nm.

Table 2
FTIR Vibration bands of Raw AW & *AW-AgNPs*.

Functional Group	Frequency/Vibrational Bands of Raw AW (cm^{-1})	Frequency/Vibrational Bands of AW-AgNPs (cm^{-1})
-OH-	3566	3477
-C-H-	2068	2059
-C=C-	1641	1641
(- CH_3-)	1384	1349
(- C–N-)	694	772

2.3. *Antibacterial studies*

The antimicrobial activity of AW-AgNPs on *Escherichia coli* and *Staphylococcus aureus* (obtained from the University of Ilorin Teaching Hospital) was tested. Agar well diffusion method was employed and Muller Hinton Agar (Lab M) was used (Oluwaniyi et al., 2015). The medium was prepared as directed by the manufacturer and sterilized. One ml of standardized inoculum (1.5×10^8 CFU/ml) was introduced into 20 ml of molten agar. This was swirled gently and transferred into a Petri dish. Furthermore, 0.9 cm diameter wells were made in the agar and 100 μl of test sample was introduced into the well. The same was done for the positive and negative controls; distilled water was used as positive while chloramphenicol served as negative control. Plates were allowed to stand for about an hour and then incubated at 37 °C for 24 h. Clear zones around the well was taken as positive result, and the diameter of the clear zone was measured. Mean values were reported (Dada et al., 2018b).

3. Results and discussion

3.1. *Phytochemical screening*

Phytochemical screening analysis was done to qualitatively determine the presence of phytomoelcules present in *Acalypha wilkesiana* (Fig. 1) medicinal leaf extract. The key under Table 1 shows that negative sign indicates absence while positive sign (+) indicates presence and double positive signs indicate abundance of phytomolecules. The result illustrated on Table 1 shows the presence of phenols, triterpenes, saponins, and flavonoids responsible for bioreduction of Ag^+ to Ag^0 and stability of AW-AgNPs. This finding is supported by the report of

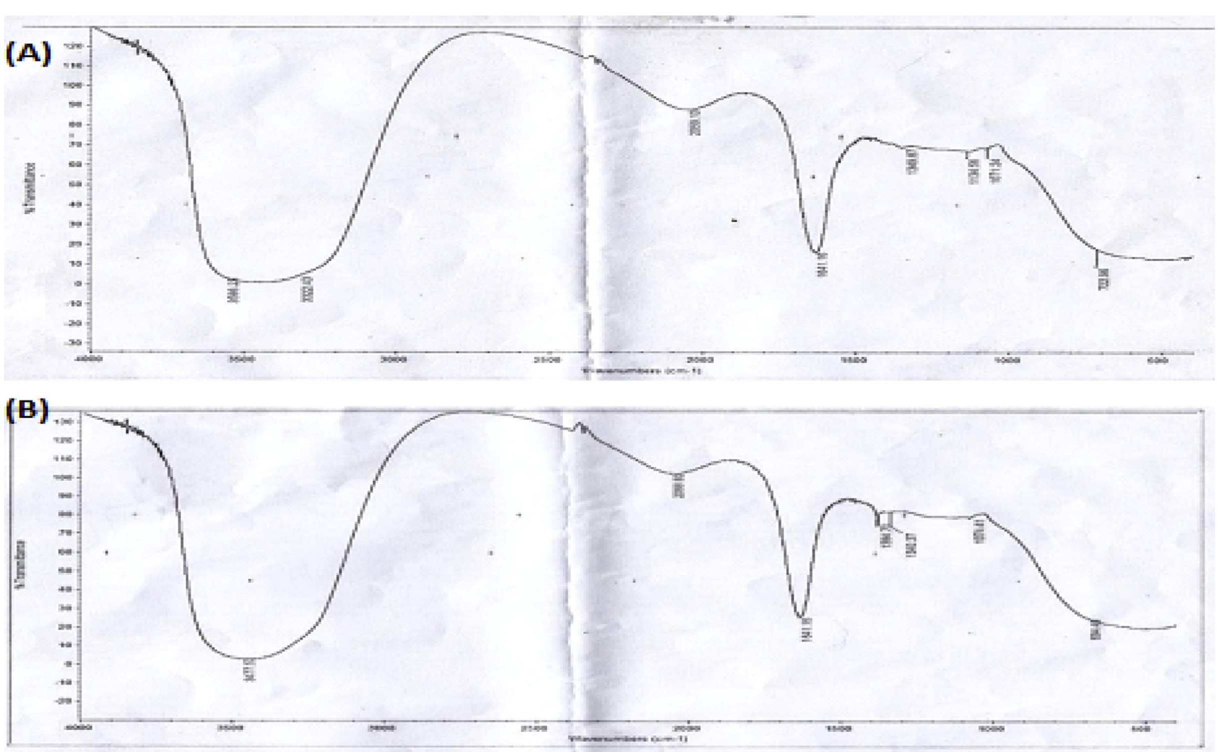

Fig. 3. FTIR spectra of (A) Extract of AW and (B) Synthesized AW-AgNPs.

Pochapski et al. (2011) and Dada et al. (2018b).

3.2. Characterization of AW-AgNPs

3.2.1. UV-Vis Spectroscopy

Surface plasmon resonance was determined using a double beam Biochrom Libra PCB 1500 UV-VIS spectrophotometer. The UV-Vis measurements (Fig. 2) were taken for both *A. wikesiana* plant and silver nanoparticles formed. Change in colour from red to brown was an indication of the formation of silver nanoparticles confirming the surface plasmon resonance (SPR). The SPR is due to the free electron arising from the conduction and valence bands lying close to each other in metal nanoparticles. It is as a result of the collective oscillation of free electron of silver nanoparticles in resonance with the light wave in silver nanoparticle synthesis (Dada et al., 2018a, b; Femi-Adepoju et al., 2019). Stable SPR peak was observed at 450 nm (Fig. 2) which gives a spontaneous spectroscopic sign for the formation of nanostructures (Tran et al., 2013; Dada et al., 2017a).

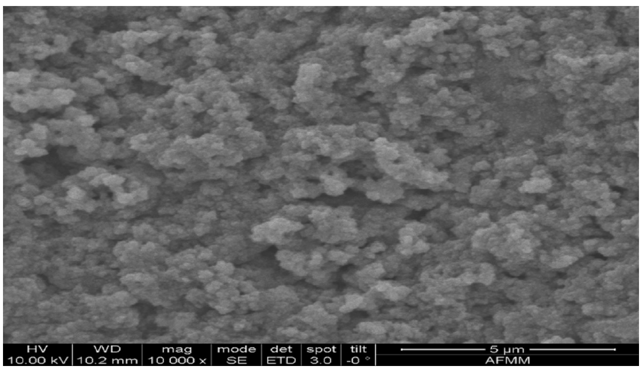

Fig. 4. SEM micrograph of AW-AgNPs.

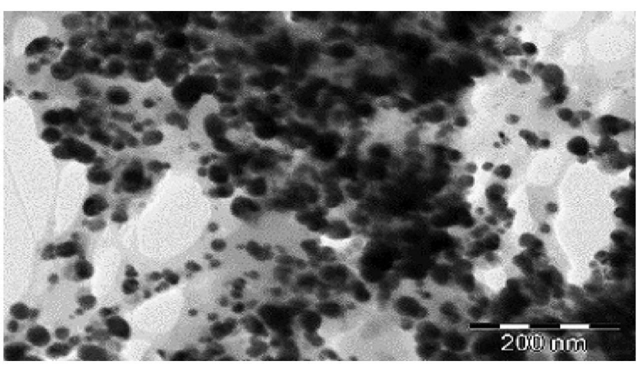

Fig. 5. TEM micrograph of AW-AgNPs.

3.2.2. FTIR spectroscopy

Fourier Transform Infrared (FTIR) analysis was done using SHIMADZU FTIR model IR8400s spectrophotometer. FTIR is relevant in determining the functional groups present in both the leaves extract of *Acalypha wilkesiana* (AW) and *Acalypha wilkesiana* silver nanoparticles (AW-AgNPs) as presented in Table 2 and Fig. 3(A &B). These functional groups are responsible for the formation of Ag nanoparticles. A shift in band as seen Table 2 is an indication that functional groups actively participated in the bioreduction of Ag^{+} to Ag^{0}. Signal between 3477 – 3566 cm^{-1} corresponds to –OH band, 2059–2068 cm^{-1} is ascribed to –CH- band, –C=C- band is found at 1641 cm^{-1}. The functional groups show the presence of phyto molecules (phenols, terpenoids, saponins and flavonoids) suggesting that the biomolecules are acting as reducing, capping and stabilizing agents. This finding was supported by the report of researcher in the literature (Prathna et al., 2011; Jyoti et al., 2016; Dada et al., 2017b).

3.2.3. SEM, TEM and EDX

SEM analysis was carried out using a TESCAN Vega TS 5136LM SEM typically at 20 kV at a working distance of 20 mm coupled with EDX analyzer. TEM analysis was on Zeiss Libra 120 @ 80 kV. It is evident from the SEM micrograph (Fig. 4) that the morphology of AW-AgNP is spherical and this is in good agreement with the shape of Surface Plasmon Resonance (SPR) band in the UV–Vis spectra (Singh et al., 2013; Dada et al., 2017a, b). The characteristic spherical shape of AW-AgNPs was further confirmed from the TEM image presented in Fig. 5. A good dispersion of small spherical size between 10 – 26 nm was observed (Babu and Prabu, 2011). Elemental chemical distribution of AW-AgNPs is seen in EDX spectrum presented in Fig. 6 (Dada et al., 2017c). A strong and pronounced signal at 3 keV confirmed the presence of silver nanoparticle. This is supported by the finding of other researcher that metallic silver nanocrystals generally show typical optical absorption peak at 3 keV due to surface plasmon resonance (Dada et al., 2016; Tippayawat et al., 2016).

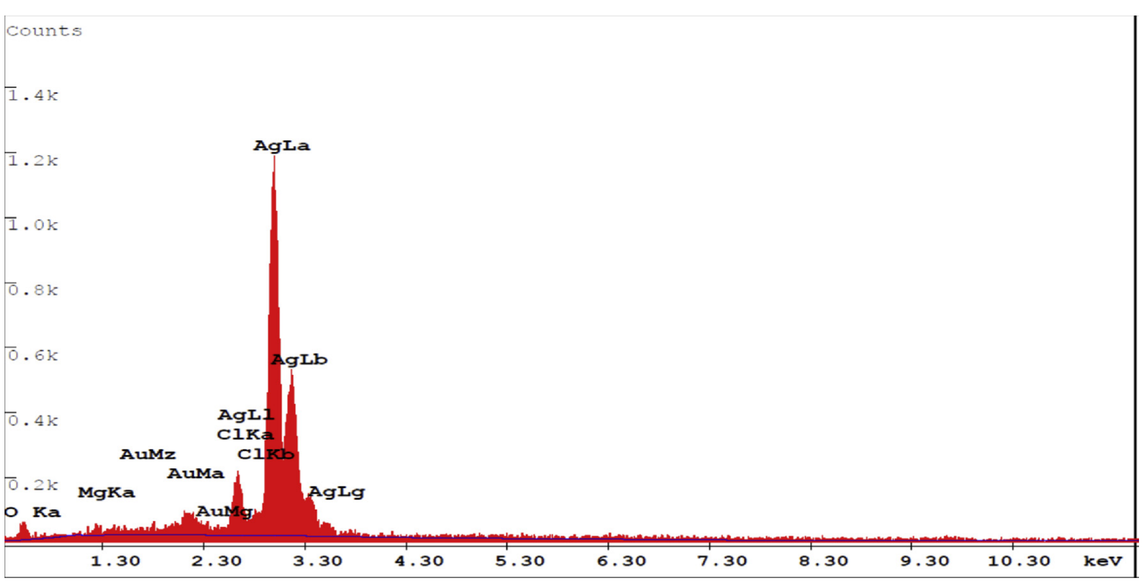

Fig. 6. EDX spectrum of AgNPs.

3.3. Operational parameters

The synthesis of silver nanoparticles depends largely on some operational parameters. These are factors that influence its synthesis irrespective of the technique used. In this study, five different operational parameters were studied which are: effect of contact time, initial concentration, volume ratio, temperature and pH. Each of these parameters was monitored by UV-Vis spectroscopic measurements.

Effect of concentration was determined by varying the various Ag^{+} concentrations (0.01 M, 0.001 M, 0.002 M, 0.004 M, 0.006 M). At higher concentrations, the increase in particle size yields increase in the intensity of the spectrum (Kokila et al., 2015). Optimum concentration of 0.001 M gave the best surface plasmon resonance (SPR) was observed at 450 nm as shown in Fig. 7 (a).

Contact time is one of the parameters that controls the size of silver nanoparticles because of the blue shift of the adsorption peaks. At optimum concentration, effect of contact at various time interval from 30, 45, 60 and 90 min on the reaction was investigated (Fig. 7(b)). This is important in order to study the kinetics and rate of formation of AW-AgNPs. A colour change from green to red and finally a stable brown colouration was observed as the time interval increased. The intensity of the peak is function of the contact time therefore it increases with increase in time. At the early stage between zero and 30 min, the SPR band was broadened due to the slow conversion of silver ion (Ag^{+}) to zerovalent silver (Ag^{0}) nanoparticles (Wen et al., 2012; Ravindran et al., 2013). As the contact increase, more of the Ag^{+} was converted to zerovalent silver (Ag^{0}) resulting in excellent plasmon band. At 90 min, maximum absorption peak was obtained together with best surface plasmon resonance and all other studies were carried out at this optimum time.

In the formation of *Acalypha wilkesiana* silver nanoparticles (AW-AgNPs), the ratio the volume of raw extract of *Acalypha wilkesiana* to solution of Ag^{+} investigated are 1:9, 2:8, 4:6, 6:4, 8:2 (Fig. 7(c)) respectively. Since extract of AW are functioning as reducing, capping and stabilizing agent, excess Ag^{+} solution is needed for well dispersed formation of AW-AgNPs without agglomeration. Volume ratio of 1 mL of

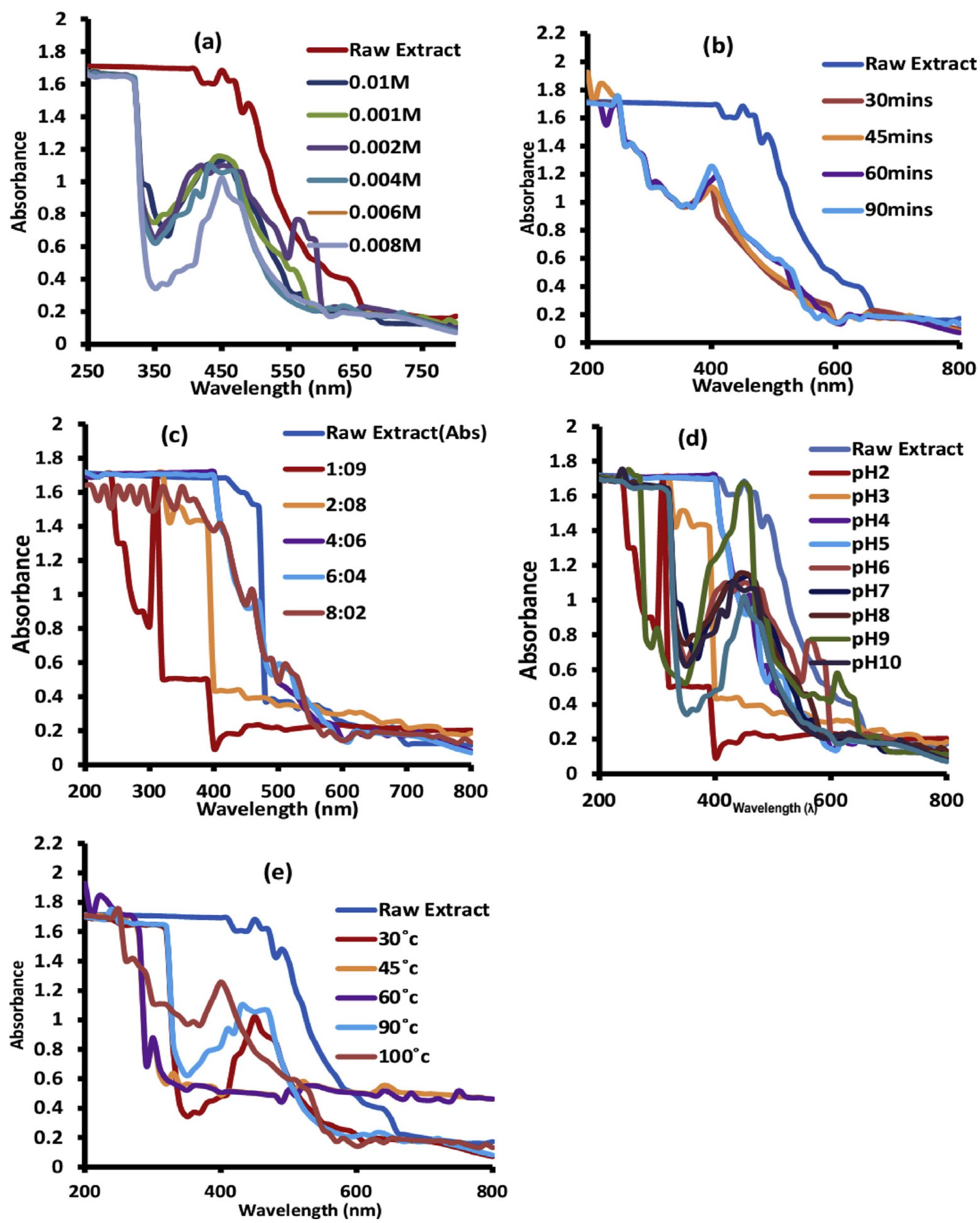

Fig. 7. (a–e): Experimental optimization of various operational parameters on effects of: (a) concentration (b) contact time (c) volume ratio (d) change in pH (e) temperature.

AW extract to 9 mL of Ag^+ solution gave the best SPR at 450 nm as presented in Fig. 7(c).

Effect of pH affects the chemistry of the reaction medium. This was investigated by adjusting the pH of the extract from pH 2–11 and monitoring the reduction process using a UV-Visible spectrophotometer. It was observed that the rate of formation of AgNPs increases with increase in pH up to pH 9 as presented in Fig. 7(d) inferring that OH groups were responsible for a reduction of Ag^+ as supported by Davidović et al. (2017). In addition, the study carried out by Kokila et al. (2015) concurred that the formation of AgNPs depended mostly on the pH of the reaction medium. The results confirmed that the formation of silver nanoparticles was more effective in the basic medium than in acidic medium due excellence SPR obtained in the basic medium (Dada et al., 2018a).

Temperature is another essential factor that should be considered in the synthesis of silver nanoparticles because it controls the reaction kinetics of the synthetic process. The effect of temperature was studied by varying the temperatures. The synthesis was carried out at room temperature, 30 °C – 100 °C (Fig. 7e) while keeping other parameters constant. It was observed that an increase in temperature leads to increase in the intensity of the Plasmon band as a result of bathochromic shift resulting in a decrease in the mean diameter of silver nanoparticle (Bindhu and Umadevi., 2014). Best surface plasmon resonance was attained at 100 °C as shown in Fig. 7(e)

Table 3
Antimicrobial result for Plates 1 & 2.

Samples	*Staphylococcus aureus*			*Escherichia Coli*		
	Plate 1	Plate 2	MD (mm)	Plate 1	Plate 2	MD (mm)
Acalypha wilkersiana extract	0	0	0	0	0	0
AW-AgNPs	20	18	19	18	16	17
Positive Control	30	27	29	32	26	29
Negative Control	0	0	0	0	0	0

Key: MD = Mean Diameter (mm).

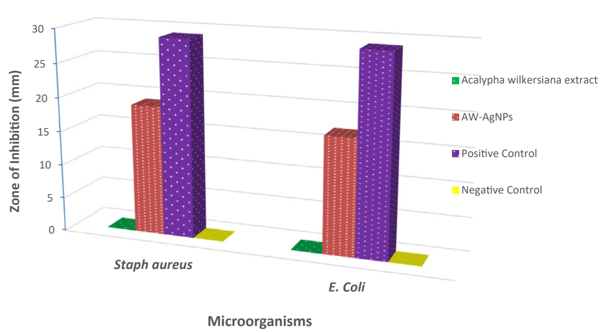

Fig. 8. Antimicrobial activity of AW-AgNPs against *Staphylococcus aureus* and *Escherichia coli*.

3.4. Antimicrobial studies

The antimicrobial activity of AW-AgNPs against *Escherichia coli* and *Staphylococcus aureus* was investigated. This study was carried out using the following samples: *Acalypha wilkesiana* silver nanoparticles (AW-AgNPs), Chloramphenicol (positive control), raw extract of *Acalypha wilkesiana* and distilled water (negative control). The choice of the bacteria cells used was based on the fact that these organisms are implicated in water-borne diseases and common infections respectively especially as we live in developing country. *Staphylococcus aureus* (*S. aureus*) is the most dangerous of all of the many common staphylococcal bacteria. These gram-positive, sphere-shaped (coccal) bacteria often cause infections of the skin, soft tissue, pneumonia, heart valve and bone (Tong et al., 2015). *Escherichia* coli (*E. coli*) are a group of Gram-negative bacteria that normally reside in the intestine of healthy people, but some strains can cause severe infection in the digestive tract, urinary tract, or many other parts of the body (Olivier et al., 2010).

From the result obtained (Table 3, Fig. 8), it is obvious that AW-AgNPs exhibited significant antimicrobial activities against the bacteria it was tested. The diameter of the zone of inhibition measured varied from 16 mm to 20 mm as presented in Fig. 9. Both the negative control and the raw extract had no inhibition on the bacteria investigated. The bar chart shows the comparison among the tested samples. Fig. 8 shows that best inhibition was recorded with Chloramphenicol which is an established antimicrobial drug. Also, it was discovered that better inhibitory activity of the synthesized nanoparticles occurred when tested against *S. aureus* with inhibition zones ranging from 18 – 20 mm (Table 3) compared to *E. coli*. It can be inferred from the results in Fig. 9 and Table 3 that an increase in the concentration of AW-AgNPs may result in a better inhibition than chloramphenicol.

4. Conclusion

This understudied report justifies the research that silver nanoparticles can be synthesized using the medicinal plant extract of *Acalypha wilkesiana*. The formation of spherical shaped and highly uniformed silver nanostructures proved this. The effect of the silver nanoparticles (AW-AgNPs) was also tested against some microorganisms as is reported in this work. Phytochemical screening tests identified the presence of some phyto-compounds such as phenol, saponins, triterpenes and flavonoids in *Acalypha wilkesiana*. Experimental optimization studies were also carried out on various parameters. The following optimum conditions were attained: 0.001 M Ag^+ concentration, 90 min contact time, 1:9 volume ratio, pH 9 and 100 °C temperature of the medium. The synthesized AW-AgNPs was characterized by combination of spectroscopic techniques. Excellent surface plasmon resonance (SPR) was attained at 450 nm, FTIR confirms the presence of polyols, flavonoids and terpenoids

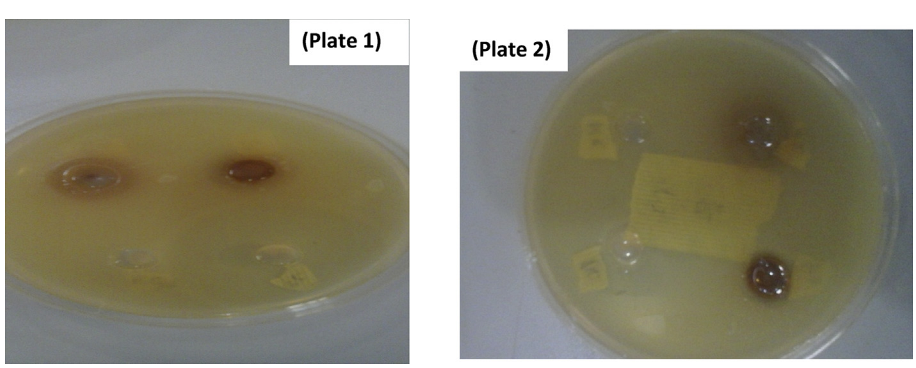

Fig. 9. Plates showing the antimicrobial activities of biosynthesized AW-AgNPs against *Staphylococcus aureus* (plate 1) *and Escherichia coli* (Plate 2).

phyto-constituents responsible for reducing, capping, and stabilization of silver nanoparticles. Both SEM and TEM showed a well dispersed spherical morphology of uniform silver nanoparticles. EDX gave the information on surface atomic distribution and the chemical elemental composition with AW-AgNPs characteristic peak at 3.0 keV. The antimicrobial study also revealed that the synthesized nanoparticles (AW-AgNPs) was effective against both Gram positive and Gram negative bacteria. This finding could be of benefit in the pharmacological and medical fields.

Declarations

Author contribution statement

Adewumi Oluwasogo Dada: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Fehintoluwa E Dada: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Folahan A Adekola: Conceived and designed the experiments; Analyzed and interpreted the data.

Chidiogo Rita Okonkwo & Abimbola P Oluyori: Performed the experiments; Analyzed and interpreted the data.

Adunola Tabitha Adelani-Akande: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Oluwasesan M Bello: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Adejumoke A Inyinbor, Oluwaseun Charles Adetunji & Kola Ajanaku: Analyzed and interpreted the data.

Adeniyi Olayanju: Contributed reagents, materials, analysis tools or data. Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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