Deshi et al. Journal of Analytical Science and Technology (2016) 7:23
DOI 10.1186/s40543-016-0104-7

Journal of Analytical Science and Technology

RESEARCH ARTICLE Open Access

Antimicrobial efficacy of biosynthesized silver nanoparticles from different solvent extracts of *Waltheria americana* root

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Abstract

Background: The extensive application of silver compounds especially in nanomedicine, has increased the need to develop environmental friendly and cost effective route to synthesizing silver nanoparticles (AgNPs).

Methods: Water, diethyl ether, and ethanol were used in the extraction of *Waltheria americana* root. Silver nanoparticles (AgNPs) were synthesized by reacting 0.001 M $AgNO_3$ solution with the different crude extracts of *W. americana* root in the ratio of 10:1. The synthesized AgNPs were analyzed using UV–visible spectrophotometer, X–ray diffraction (XRD), scanning electron microscope (SEM), and FTIR techniques. The different crude extracts and their synthesized colloidal AgNPs were tested against *Proteus* species, *Streptococcus* species, *Klebsiella* species, *Staphylococcus aureus*, and ciprofloxacin (control).

Results: UV–vis results showed surface plasmon resonance (SPR) at 415, 435, and 425 nm for synthesized colloidal AgNPs from water, diethyl ether, and ethanol extracts, respectively. When screened against all test organisms, the synthesized colloidal AgNPs from diethyl ether extract of *W. americana* root (WARDEEP) showed more improved antimicrobial efficacy than other crude extracts and their synthesized AgNPs. The strongest antimicrobial activity of WARDEEP against all test organisms were at 400, 100, and 200 mg/mL concentrations for *Proteus* species and *Staphylococcus aureus*, *Klebsiella* species, and *Streptococcus* species, respectively. From minimum inhibitory concentration (MIC) results, it was observed that WARDEEP exhibited a strong antibiotic activity against *Proteus* and *Streptococcus* species at a least value of 12.5 mg/mL concentration. Minimum bactericidal concentration (MBC) results showed that WARDEEP exhibited a minimum antibiotic activity at 25 mg/mL concentration against *Proteus* and *Streptococcus* species.

Conclusions: Therefore, silver nanoparticles were sucessfully synthesized from all the crude extracts. The synthesized silver nanoparticles could comparatively provide better alternative treatment to both gram–positive and gram–negative bacteria than the crude plant extracts.

Keywords: *Waltheria americana*, Antimicrobial efficacy, Silver nanoparticles, Biosynthesis

Background

Nanotechnology is a significant field of modern research dealing with design, synthesis, and utilization of particles structure ranging from approximately 1–100 nm (Shameli et al. 2012). These unique properties of size, shape, and controlled disparity of nanoparticles make them attractive for commercial and medical development (Kumar 2012). Nanomedicine finds extensive application as a result of the fusion of nanotechnology and medicine. Medicine is no longer an exclusive job of a physician because materials and devices designed at nanoscale level are used for diagnosis, treatment, and prevention of diseases that are antibiotic resistant, traumatic, injury pain relief, and also the overall preservation and improvement of health (Savithrama et al. 2011). The new-age drugs are nanoparticles of polymers, ceramics, and metals which can combat conditions like cancer and bacteria (Singh et al. 2008).

Silver compounds and their derivatives are commercially employed as antimicrobial agents in the medical field to treat burns and a variety of infections (Singh et

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al. 2008). Medical, biological, and pharmaceutical preparations containing the silver ions in creams, solution electrodes, ligatures, biological skin, and catheters have been developed over the past decades (Lei 2007). The bactericidal effect of silver ions on micro-organisms is very well known; nevertheless, the design of a synthetic method of metal colloidal particles in which size, morphology, stability and properties are controlled, has become a major field of interest (Sharma et al. 2009). The use of colloidal silver nanoparticles in the treatment of infection has the advantage of being eco-friendly because of its low cost and less toxic to both human and the environment (Mallikarjuna et al. 2011). Singh and Tiwari (2015) reported that nanomaterials are key to future solution to the fields of technological, medical, and environmental challenges.

Despite the vast publicity, development and application of nanoparticles in various areas of human life across the world, its understanding, development and application is a serious challenge in many developing countries like Nigeria. This has caused most research in Africa to be focused on the antibacterial activities of biosynthesized colloidal silver nanoparticles, for the optimization of the medicinal healing properties of both the synthetic and medicinal plants (Lei 2007).

Olajuyigbe et al. (2011) studied the phytochemicals of *Waltheria americana* root, stem, and leaf extracts and reported that it contained alkaloids, anthraquinones, cardiac glycosides, phenols, tannins, and saponins. High amount of saponins and anthraquinones was present in the three different parts of the plant than other phytochemicals. Tannins and cardiac glycosides were observed to be more in the roots and leaf extracts than in the stem extracts. *W. americana* plant is widely used in traditional medicine in various cultures worldwide. Generally, it is utilized therapeutically for the treatment of malaria, sore throat, arthritis, asthma, neuralgia, wound healing, sexual stimulation, cold and cough, painful mensuration, fatigue, diarrhea, skin lightening, antisyphilic, antiseptic, laxative, and anti-inflammatory (Mohammed et al. 2007; Jansen et al. 2010; Pavithra et al. 2010; Olajuyigbe et al. 2011; Zongo et al. 2013). This work, therefore, intends to synthesize colloidal silver nanoparticles from the different solvent extracts of *W. americana* root so as to produce nanoparticles with higher potency against microbes than the pure plant extract.

Methods

Sample location, identification, collection, and pretreatment

The roots of *W. americana* plant were located and collected from Girei, Girei Local Government Area of Adamawa State, Nigeria. The botanical name of the plant was identified and confirmed by a taxonomist in the Biological Science Department of Modibbo Adama University of Technology, Yola.

Fresh roots of *W. americana* plant sample were collected, and the voucher specimen was numbered and kept in Chemistry Research Laboratory of Modibbo Adama University of Technology, Yola, for further reference. The collected sample (roots of *W. americana*) was freed from twigs and extraneous matter. Soil, grit, sands, and dirt were removed by sifting. In order to remove the remnants of adhering foreign matter, the samples were thoroughly washed under tap water, rinsed with distilled water and then, shade dried at room temperature for 15 days. The dried samples were pulverized to a fine powder using a porcelain pestle and mortar. *Proteus* species, *Streptococcus* species, *Klebsiella* species, and *Staphylococcus aureus* were collected from the Microbiology Research Laboratory of Modibbo Adama University of Technology, Yola.

Preparation of plant extracts

The heat and cold extraction methods were used in the preparation of extracts from the prepared sample. In the heat extraction method, about 5 g of the powdered sample was boiled with 100 mL of distilled water in a 250-mL conical flask for about 10 min. The mixture was stirred and allowed to stand and cool. The cooled mixture was filtered using Whatman No. 1 filter paper. The extract was stored in the refrigerator until use.

For cold extractions, about 5 g of the powdered sample was soaked in 100 mL of ethanol in a 250-mL conical flask, for 2 h. The mixture was filtered using Whatman No. 1 filter paper. The extract was stored in a refrigerator until use. The same procedure was repeated using diethyl ether (Barminas et al. 2014).

Synthesis and characterization of colloidal silver nanoparticles

Silver nanoparticles (AgNPs) were synthesized by reacting 100 mL of 0.001 M $AgNO_3$ with 10 mL of the plant extract (i.e., 10:1) at room temperature for 2 h. The time of addition of extract into the aqueous $AgNO_3$ solution was considered as the beginning of the reaction. The method used by Roy and Barik (2010) was adopted in monitoring the reduction of Ag^+ to Ag^0 by measuring the absorption spectrum of each reaction mixture (silver nitrate solution + plant extracts) with a T-60 UV–vis spectrophotometer. The reduction of silver ions was confirmed by qualitative reaction with NaCl.

The different crude extracts and their bio-reduced colloidal AgNPs solution were tested for their antimicrobial efficacy. The synthesized colloidal AgNPs solution that showed the best antimicrobial efficacy was further characterized using FTIR, XRD, and SEM. Thus, the synthesized colloidal AgNPs solution was centrifuged using Cole centrifuge machine (model 0412-1) at 4000 rpm for 30 min. The sediment was washed and rinsed with 5 mL

of deionized water to get rid of the free proteins or enzymes that were not capping the colloidal AgNPs. The rinsed sediment was dried at 55 °C and stored for analysis (Priya et al. 2011).

Instrumental characterization

The absorption spectrum of the sample was measured on a T-60 UV–vis spectrophotometer. The phase identity and crystalline size of the synthesized AgNPs were characterized with Empyrean model of X–ray diffractometer. Morphological features were studied by using Phenom–World model of scanning electron microscope (SEM). In order to determine the functional groups and possible bio reductants, the samples were mixed with potassium bromide and pelletized for infra-red analysis on a Shimadzu – 8400S FTIR spectrophotometer, in the wave number range of 4000–400 cm^{-1}.

Preparation of the nutrient agar

The method used by Shagal et al. (2012) was adopted. Nutrient agar powder of 51.55 g was measured and dissolved in 100 mL of distilled water in a conical flask. The mixture was heated to dissolve the medium completely, and this was further sterilized by autoclaving at 121 °C and 151 lbs pressure for 15 min. It was allowed to cool to 47 °C and was then dispensed into the sterilized plates which was used for culturing and sensitivity test of the organism.

Determination of antimicrobial activity

Antimicrobial activities of the crude extracts and their synthesized colloidal AgNPs were determined using the agar well diffusion assay method reported by Prasad et al. (2011). Approximately 20 mL of molten and cooled media (NA/SDA) was poured into sterilized Petri dishes. The plates were left overnight at room temperature to check for sterility. The test organisms were grown in selected broth for 24 h. About 1 mL broth culture of each test organism containing approximately 1×10^5 cfu/mL was used to prepare bacterial lawns. Agar wells of 5-mm diameter were prepared with the help of a sterilized stainless steel cork borer labeled as A and B. "A" well was loaded with 30 μL of colloidal AgNPs suspended "hydrosols", and "B" well was loaded with 30 μL of positive control drugs (ciprofloxacin) used as a positive controls. The plates containing the test organism and colloidal AgNPs were incubated at 37 °C for 24–48 h. The plates were examined for evidence of zones of inhibition, which appeared as a clear area around the wells. The diameter of such zones of inhibition was measured using a meter ruler, and the mean value for each organism was recorded and expressed in millimeter.

Determination of minimum inhibitory concentration (MIC)

The MIC of the extracts and synthesized colloidal AgNPs that showed antimicrobial activity was determined by using double serial dilution technique of Sahm and Washington (1990). The stock solution of *W. americana* root extract was prepared by adding 1 mL of the extract to 1 mL of sterile nutrient broth contained in a 5-mL test tube. This is to obtain an extract concentration of 400 mg/mL which was further diluted to get extract concentrations of 200, 100, 50, 25, 12.5, and 6.25 mg/mL in different test tubes. A portion (1 mL) of an 18-h culture of *Proteus* species previously diluted to 0.5 McFarland turbidity standard (1.0×10^8 cfu/mL) was introduced or inoculated into each of the test tube and incubated at 37 °C for 18 h. The eighth test tube containing 1 mL of nutrient broth and sterile plant served as the negative control while the positive control test tube contained ciprofloxacin antibiotic. MIC was determined by visual observation of growth. The minimum concentration of the extract that showed no detectable growth was taken as the minimum inhibitory concentration. The above procedure was repeated for the rest of the bacteria: *Streptococcus* species, *Klebsiella* species, and *Staphylococcus aureus*.

Determination of minimum bactericidal concentration (MBC)

The method described by De and Ifeoma (2002) was adopted. For each of the test tube in the MIC determination that showed a visible growth, a loopful of the broth was inoculated on a sterile nutrient agar plates using agar streaked method. The inoculated plates were incubated for 24 h at a temperature of 37 °C. After incubation, the highest dilution that yielded no single bacterial colony on the plates was recorded as minimum bactericidal concentration (MBC).

Statistical data analysis

Descriptive analysis was performed on the results obtained using SPSS 17.0 while charts were drawn using Microsoft Excel 2007.

Results and discussion

UV-visible spectra analysis

The synthesized colloidal AgNPs were first confirmed through visual observations. During the reaction of different solvent extracts of *W. americana* root with silver nitrate solution, the colorless silver nitrate solution changed to reddish brown, gray, and pale yellow in ethanol, water–heat, and diethyl ether extracts, respectively. This indicates the formation of AgNPs. According to Wiley et al. (2006), surface plasmon vibrational phenomenon and the reduction of silver nitrate ions to silver atom (Ag^0) could be tracked by change in color. This change in color might be as a result of the absorption of ultraviolet radiation in the wavelength ranges of 400–500 nm which is the range at which colloidal AgNPs absorbs ultraviolet radiation.

Figures 1, 2, and 3 show the absorption peaks of UV-visible for the plant extracts and their respective synthesized colloidal AgNPs. None of the extracts showed

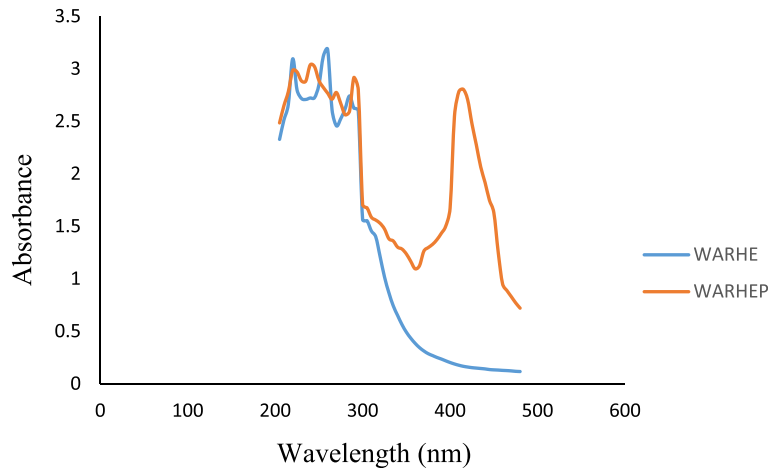

Fig. 1 UV–visible absorption spectra for *W. americana* root water–heat extract (WARHE) and its synthesized colloidal silver nanoparticles (WARHEP)

absorption band within 400–500 nm, and this indicates that none of the extract is a colloidal nanoparticles. The synthesized AgNPs from water–heat, ethanol, and diethyl ether extracts showed absorption maxima at 415, 425, and 435 nm, respectively, due to surface plasmon resonance (SPR) of AgNPs. This suggests that the substrates are good bio-reducing agent because of the presence of sufficient amount of reductive biomolecules in the solvents used for their extraction. Also, the different solvents used in the extraction resulted to the occurrence of different vibrational transitions in the synthesized colloidal AgNPs which is responsible for the observed variation in their absorption maxima. Olajuyigbe et al. (2011) reported that extract of *W. americana* root contains bioactive molecules such as alkaloids, anthraquinones, cardiac glycosides, phenols, tannins, and saponins. These biomolecules can be used in the reduction of silver ion and capping of colloidal nanoparticles.

The surface plasmon absorption in the nanoparticles is due to the collective oscillation of the free conduction band electrons which is excited by the incident electromagnetic radiation, and this type of resonance is seen when the wavelength of the incident light far exceeds the particle diameter (Naika et al. 2015; Elemike et al. 2016).

Antimicrobial activity

The antimicrobial activities of the different solvent extracts of *W. americana* roots and their synthesized colloidal AgNPs on test organisms using agar plates showed the diameter of zone of inhibition reflecting the magnitudes of susceptibility of the micro-organisms. The strains susceptible to antimicrobial agent exhibit larger diameter of zone of inhibition, whereas resistant strains exhibit smaller diameter of zone of inhibition.

Table 1 shows the result of the antimicrobial analysis of the different solvent extracts of *W. americana*

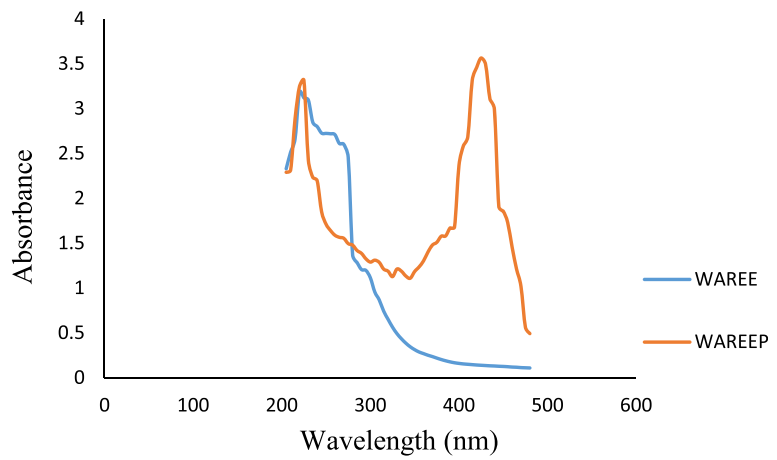

Fig. 2 UV–visible absorption spectra for *W. americana* root ethanol extract (WAREE) and its synthesized colloidal silver nanoparticles (WAREEP)

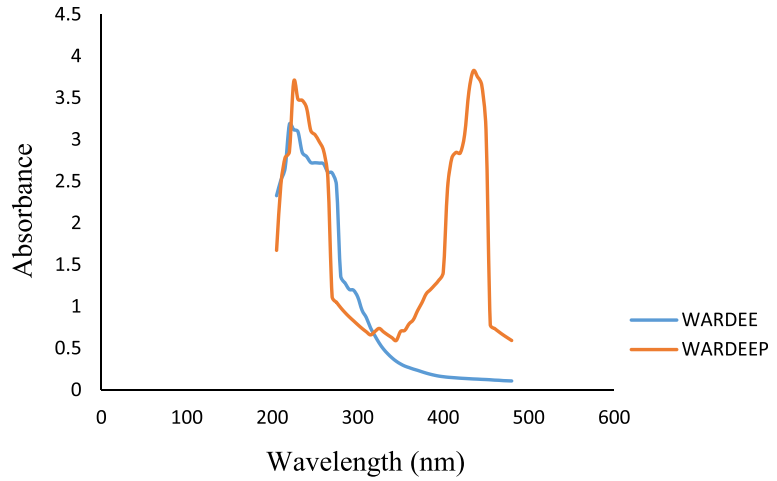

Fig. 3 UV–visible absorption spectra for *W. americana* root diethyl ether extract (WARDEE) and its synthesized colloidal silver nanoparticles (WARDEEP)

root and their synthesized colloidal AgNPs which were examined against selected test organisms. When screened against the test organisms, the performance of the synthesized colloidal AgNPs from diethyl ether extract of *W. americana* root (WARDEEP) showed a more improved efficacy than other solvent extracts or their synthesized AgNPs. Compared to diethyl ether extract of *W. americana* root (WARDEE), WARDEEP exhibits better antibacterial activity against gram–negative bacteria (*Proteus* species and *Streptococcus species*) than gram–positive bacteria.

The trend of the potency rises gradually from 50 mg/mL concentration of AgNPs to 100 mg/mL, but drops at 200 mg/mL (Table 1). The strongest performance of the WARDEEP against all the test organisms were at 400, 100, and 200 mg/mL concentrations for *Proteus* species and *Staphylococcus aureus*, *Klebsiella* species, and *Streptococcus* species, respectively.

At 400 mg/mL concentration, WARDEEP exhibited the maximum antimicrobial activity of 26 ± 0.10 mm of zone of inhibition against *Proteus* species. At the same concentration, it also exhibited an intermediate antimicrobial activity of 24 ± 1.45 mm of zone of inhibition

Table 1 Determination of the antimicrobial activity of the different solvent extracts and their synthesized colloidal silver nanoparticles of *W. americana* root

Conc.	50 mg/mL				100 mg/mL				200 mg/mL				400 mg/mL			
Bacteria	PSP	SSP	KSP	SA	PSP	SSP	KSP	SA	PSP	SSP	KSP	SA	PSP	SSP	KSP	SA
WAREE	14 ± 1.00[a]	17 ± 0.00[a]	15 ± 0.66[a]	16 ± 0.64[a]	20 ± 0.35[a]	19 ± 0.90[a]	20 ± 0.71[a]	18 ± 0.60[a]	21 ± 0.10[a]	19 ± 0.50[a]	20 ± 0.70[a]	19 ± 0.32[a]	22 ± 0.00[a]	20 ± 0.30[a]	22 ± 0.10[a]	20 ± 0.70[a]
WARDEE	12 ± 0.75[a]	18 ± 0.66[a]	17 ± 0.66[a]	16 ± 1.50[a]	17 ± 0.50[a]	19 ± 1.00[a]	18 ± 0.50[a]	17 ± 0.00[a]	18 ± 0.20[a]	20 ± 0.30[a]	19 ± 0.10[a]	18 ± 0.50[a]	19 ± 0.15[a]	21 ± 1.10[a]	20 ± 1.20[a]	20 ± 1.57[a]
WARHE	16 ± 0.55[a]	15 ± 1.00[a]	16 ± 0.66[a]	16 ± .50[a]	17 ± 1.00[a]	18 ± 1.50[a]	17 ± 0.50[a]	18 ± 1.00[a]	18 ± 0.00[a]	19 ± 1.00[a]	18 ± 0.30[a]	19 ± 0.10[a]	18 ± 1.20[a]	19 ± 0.34[a]	18 ± 1.25[a]	19 ± 0.35[a]
WAREEP	19 ± 0.33[a]	20 ± 0.22[a]	20 ± 1.00[a]	20 ± 1.00[a]	20 ± 1.00[a]	21 ± 2.00[a]	20 ± 1.00[a]	21 ± 0.00[a]	19 ± 1.00[a]	21 ± 1.00[a]	20 ± 1.20[a]	21 ± 1.20[a]	20 ± 1.15[a]	22 ± 1.75[a]	21 ± 1.45[a]	22 ± 1.16[a]
WARDEEP	18 ± 1.25[a]	21 ± 1.00[a]	21 ± 1.66[a]	20 ± 0.50[a]	20 ± 0.00[a]	22 ± 1.50[a]	23 ± 5.00[a]	21 ± 0.50[a]	19 ± 1.30[a]	23 ± 0.00[a]	21 ± 1.00[a]	20 ± 1.50[a]	26 ± 0.10[a]	20 ± 0.14[a]	22 ± 1.18[a]	24 ± 1.45[a]
WARHEP	16 ± 1.00[a]	20 ± 0.75[a]	16 ± 0.20[a]	18 ± 0.00[a]	17 ± 1.00[a]	21 ± 0.65[a]	20 ± 0.00[a]	19 ± 0.00[a]	17 ± 0.20[a]	21 ± 0.00[a]	17 ± 1.00[a]	19 ± 0.60[a]	18 ± 0.15[a]	22 ± 1.25[a]	18 ± 1.50[a]	20 ± 0.35[a]
Control (Ciprof)	30 ± 1.33[a]	31 ± 0.66[a]	32 ± 2.00[a]	33 ± 1.00[a]	30 ± 2.00[a]	35 ± 1.00[a]	34 ± 2.00[a]	30 ± 0.00[a]	33 ± 0.00[a]	32 ± 1.00[a]	33 ± 1.00[a]	31 ± 1.50[a]	32 ± 2.00[a]	33 ± 1.40[a]	35 ± 0.15[a]	31 ± 1.45[a]

Result is presented in mean ± SD

Key: *PSP Proteus* spp., *SSP Streptococcus* spp., *KSP Klebsiella spp.*, *SA Staphylococcus aureus*, *WAREE Waltheria americana* root ethanol extract, *WARDEE Waltheria americana* diethyl ether root extract, *WARHE Waltheria americana* root water–heat extract, *WAREEP Waltheria americana* root ethanol extract silver nanoparticles, *WARDEEP Waltheria americana* diethyl ether root extract silver nanoparticles, *WARHEP Waltheria americana* root water–heat extract silver nanoparticles, and *Ciprof* ciprofloxacin

[a]Standard deviation of three composite readings. Microbial diameter of inhibition zone measured in millimeter

against *Staphylococcus aureus.* Minimum antimicrobial activity of 12 ± 0.75 mm diameter of zone of inhibition was exhibited by WARDEE against *Proteus* species at 50 mg/mL concentration. In comparison to ciprofloxacin tablet as a reference standard at 400 mg/mL, the WARDEEP showed significant antibacterial activity against *Proteus* species. This could be attributed to the influence of solvent used in extracting the plant metabolites (El-Mahmood et al. 2008; Al-bayati and Sulaiman 2008).

This investigation agrees with the previous findings of Lee et al. (2007) and Shrivastava et al. (2007), who reported that the antibacterial effect of colloidal AgNPs against microbes were dose-dependent and more pronounced against gram–negative microbes than gram–positive ones.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial agent that inhibits the growth of a microorganism after 18–24 h while minimum bactericidal concentration (MBC) is the lowest concentration of an antibiotic required to kill a microorganism (Pavithra et al. 2010). The antimicrobial potency of both plant extracts and the synthesized colloidal AgNPs against the test organisms are expressed in MIC and MBC. High values of MIC and MBC is an indication of low antimicrobial activity whereas low values means high antimicrobial activity.

The results of MIC and MBC determinations for the extracts of *W. americana* root and their synthesized colloidal AgNPs that was screened against the selected test organisms at 6.25, 12.5, 25, 50, 100, 200, and 400 mg/mL concentrations of the extract or colloidal AgNPs are shown in Table 2. The result showed that the MIC for the extracts of *W. americana* root and their synthesized colloidal AgNPs ranged between 12.5 and 100 mg/mL while the MBC ranged between 25 and 200 mg/mL. It was observed that against *Proteus* and *Streptococcus* species, WARDEEP exhibited the least values of 12.5 and 25 mg/mL as MIC and MBC, respectively, which is a demonstration of a strong antimicrobial activity against both organisms. This result indicates that at lower concentration, WARDEEP is more effective against gram–negative bacteria than against gram–positive bacteria. This could be due to the complete solubility of the bioactive components of the plant in the extraction solvent and the inability of the cell membrane to exhibit permeability barrier (El-Mahmood et al. 2008).

Against *Staphylococcus aureus*, maximum values of 100 and 200 mg/mL concentrations was observed as MIC and MBC, respectively, to the corresponding *W. americana* root ethanol extract (WAREE), *W. americana* root ethanol extract silver nanoparticles (WAREEP) WARDEE, *W. americana* root water–heat extract (WARHE), and *W. americana* root water–heat extract silver nanoparticles (WARHEP). This suggests lower susceptibility to the efficacy of the plant extracts and the synthesized colloidal AgNPs. Thus, a demonstration of minimum antimicrobial activity against *Staphylococcus aureus.* This makes *Staphylococcus aureus* develop a permeability barrier in the cell membrane in resistance against the antibacterial agent.

Generally, the synthesized colloidal AgNPs from the diethyl ether extract of *W. americana* root was the most effective in inhibiting the bacterial growth than the ethanol and water–heat extracts of the *W. americana* root. This suggests that the polar solvent of diethyl ether was successful in extracting secondary metabolites which are responsible for the antimicrobial properties. This is confirmed by the reports of Pavithra et al. (2010).

The possible mechanism for the antimicrobial property of AgNPs against bacteria are due to the large surface area to volume ratio needed for better efficiency; bacteria membrane which contains proteins and DNA that provide preference sites for colloidal AgNPs interaction, affinity of silver for sulfur and phosphorus compounds; continuous release of Ag^+ once in bacterial cell creating free radicals and oxidative stress which enhances their antibacterial properties and the attack of respiratory chain in bacterial mitochondria that leads to death of the cell (Feng et al. 2000; Sharma et al. 2009).

FTIR spectra analysis

FTIR measurements were carried out purposely to identify the possible biomolecules and functional groups responsible for the reduction of silver ions and the capping of

Table 2 Determinations of minimum inhibitory concentration (mg/mL) and minimum bactericidal concentration (MBC) of different solvent extracts and their synthesized colloidal silver nanoparticles from *Waltheria americana* root

Bacteria	WAREE		WARDEE		WARHE		WAREEP		WARDEEP		WARHEP		Control Cipro tab	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Proteus sp.	25	50	25	50	25	50	25	50	12.5	25	25	50	6.25	12.5
Streptococcus sp.	50	100	50	100	50	100	25	50	12.5	25	50	100	12.5	25
Klebsiella sp.	50	100	25	50	50	100	50	100	25	50	50	100	12.5	25
Staphylococcus aureus	100	200	100	200	100	200	50	100	50	100	100	200	25	50

the bio-reduced silver particles synthesized from the plant extracts (Shameli et al. 2012). The absorption peaks for the various functional groups in the diethyl ether extract and its synthesized AgNPs are given in Fig. 4.

The FTIR absorption spectra of WARDEE (red spectra) show strong absorption band at 1026, 1423, 1647, 2927, and 3431 cm^{-1} which indicates the presence of ether groups (–C–O–C–), C–H deformation in –O–C=O–CH_3 group, amide I group due to C=O stretching in proteins, C–H stretching vibrations of methyl and methylene groups, and O–H groups of water molecules, respectively (Morrison and Boyd 2002). The weak absorption band observed at 2108 and 2528 cm^{-1} denotes –C≡C– stretching and S–H stretching (Mercaptans), respectively. These absorption bands in the FTIR spectra of WARDEE indicate stretching vibrational bands responsible for bioactive molecules like alkaloids, anthraquinones, glycosides, phenols, tannin, saponins, flavonoids, and terpenoids, which can be responsible for efficient reduction of silver ions to AgNPs.

From the absorption spectra of WARDEEP (black spectra), very weak absorption bands were observed at 1089, 1377, 1647, and 3470 cm^{-1} which are characteristic absorption peaks of ether groups, germinal methyls, C=O stretching in amide I group, and O–H groups, respectively (Elemike et al. 2016). This weak absorption bands observed in the spectra of WARDEEP is also a clear evidence that these functional groups are responsible for the reduction of Ag^{+} to AgNPs, efficient capping and stabilization of the synthesized AgNPs. The absorption around 424 cm^{-1} in WARDEEP spectra could be attributed to silver metal (Shameli et al. 2012).

Scanning electron microscope (SEM) analysis

The SEM images of the extract (WARDEE) and synthesized AgNPs (WARDEEP) at different magnifications are shown in Figure 5. The SEM image of the extract (Fig. 5a, b) showed a rough portion on the surface of the extract which could possibly be the site of the bioactive molecules. From the morphology of the AgNPs (Fig. 5c, d), it was observed that AgNPs in the form of rectangular flakes with large surface area was synthesized. This is as a result of hydrogen and electrostatic interaction between the bioactive capping molecules in the extract and the silver ions in the silver nitrate solution. The smooth surface morphology of the synthesized AgNPs is an evidence of its high stability and also explains the weak absorptions observed in the FTIR spectra of WARDEEP in Fig. 4. The nature of AgNPs observed in the SEM micrograph further supports the XRD result that crystalline AgNPs was synthesized.

Powder X–ray diffraction (XRD) analysis

Figure 6 displays XRD patterns of the synthesized AgNPs from diethyl ether extract of *W. americana* root (WARDEEP). The synthesized AgNPs showed particle sizes of about 7–24 nm. A number of Bragg's reflection was observed in the X–ray diffraction pattern of the AgNPs, showing 2 values of 38.67°, 44.95°, 65.45°, and 78.63° which corresponds to (111), (200), (220), and (311) sets of plane lattice, respectively. This may be indexed as the band for face-centered cubic structure of silver (Dare et al. 2015). Thus, the diffraction pattern attests to high purity and crystalline nature of the synthesized AgNPs (WARDEEP). The other observed peaks and noise could be related to the effect of nanosized particles and the presence of various crystalline biological macromolecules in the diethyl ether extract (Elemike et al. 2016).

The obtained results illustrate that silver ions had indeed been reduced to colloidal AgNPs under the reaction conditions.

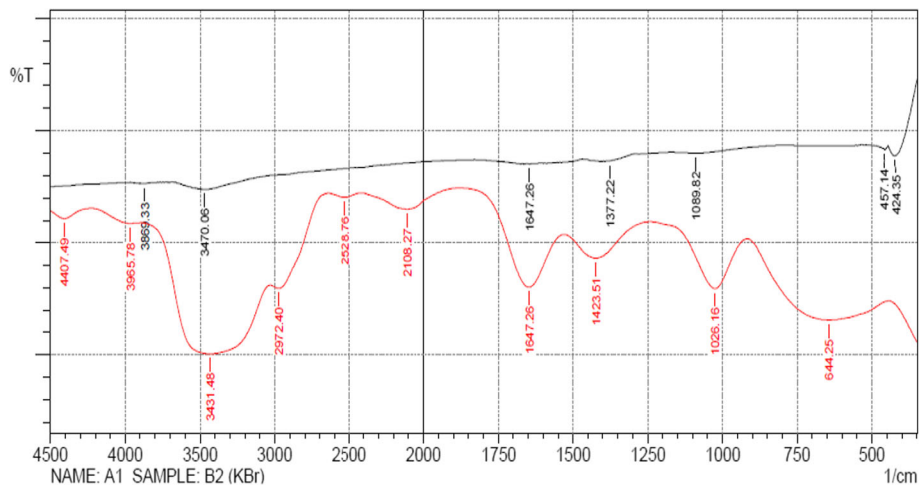

Fig. 4 FTIR absorption spectra for *W. americana* root diethyl ether extract (WARDEE) (*red*) and its synthesized silver nanoparticles (WARDEEP) (*black*)

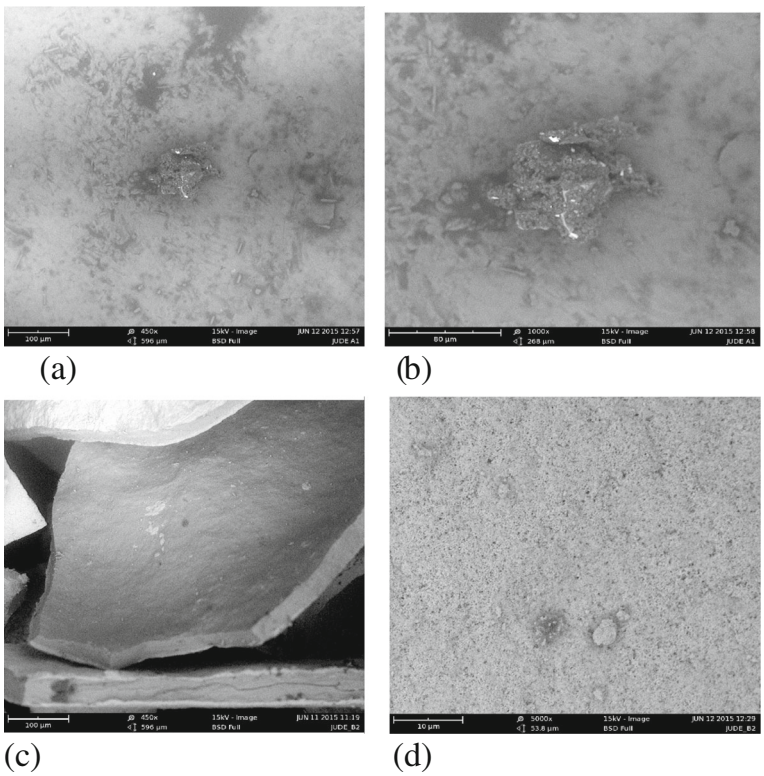

Fig. 5 Scanning electron micrographs of *W. americana* root diethyl ether extracts (WARDEE) (**a**, **b**) and its synthesized silver nanoparticles (WARDEEP) (**c**, **d**) at different magnifications

Conclusions

This research revealed that the medicinal property of *W. americana* root aided in successful synthesis of AgNPs from its extracts, and the synthesized colloidal AgNPs enhanced the therapeutic efficacy of the plant extracts. The synthesized colloidal AgNPs from the diethyl ether extract of *W. americana* root (WARDEEP) has more improved antimicrobial efficacy than other crude extracts and their synthesized colloidal AgNPs. The antimicrobial efficacy of WARDEEP against all test organisms is dose-dependent and more pronounced against gram–negative bacteria than gram–positive bacteria. The minimum inhibitory concentration (MIC) results showed that WARDEEP exhibited a strong antibiotic activity against *Proteus* and *Streptococcus* species at a least value of 12.5 mg/mL concentration. Minimum bactericidal concentration (MBC)

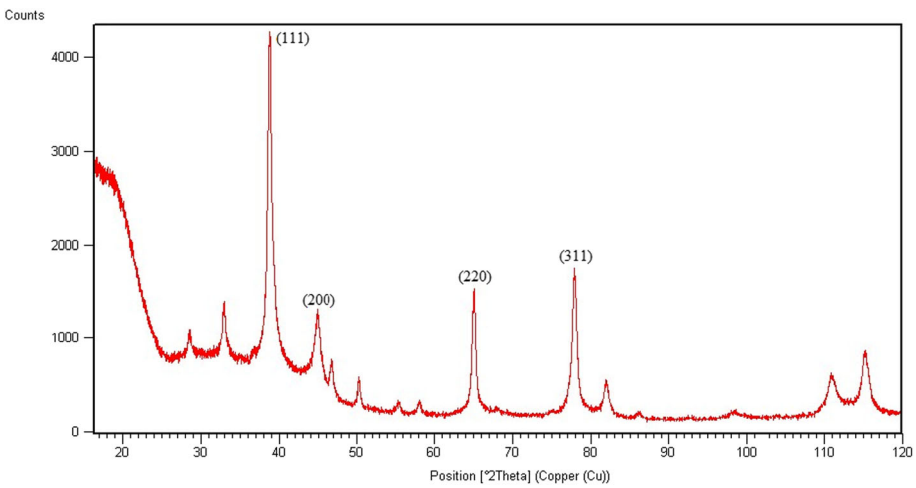

Fig. 6 Representative XRD diffraction patterns for synthesized silver nanoparticles from diethyl ether extract of *W. americana* Root (WARDEEP)

results revealed that WARDEEP exhibited minimum antibiotic activity at 25 mg/mL concentration against *Proteus* and *Streptococcus* species. UV–vis spectrophotometer, FTIR, XRD, and SEM confirm the synthesis of a stable AgNPs with large surface area.

Acknowledgements
The authors wish to thank Mr Afolabi Onipede S. and other technologists in the Department of Chemistry, Federal University Lafia, Nasarawa State – Nigeria and Department of Chemistry, Modibbo Adama University of Technology Yola; for their immense contribution towards the Laboratory work.

Authors' contributions
DJ and BJ designed the research. OJ and MI carried out the Laboratory work while DJ, BJ, OJ, DP, and MO interpreted the results. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests. All authors participated in this research and have endorsed the publication of this work.

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Received: 30 August 2016 Accepted: 3 November 2016
Published online: 11 November 2016

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