

Rapid synthesis and characterization of Gold and Silver nanoparticles using exopolysaccharides and metabolites of *Wesiella confusa* as an antibacterial agent against *Esherichia coli*

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Abstract

Characterization and the antibacterial potential of gold (AuNPs) and silver nanoparticle (SNPs) biosynthesized greenly using exopolysaccharides (EPS) and Culture Free Supernatant (CFS) of *Wesiella confusa* against some multidrug resistance (MDR) *E. coli* was investigated. The biosynthesized nanoparticles were characterized by UV-visible spectra, Fourier Transformed Infrared Spectroscopy and Scanning Electron Microscopy. The formation of AuNPs and SNPs were confirmed by changes in colour: Colour change from yellow to wine red and yellow to bluish purple, that indicate the formation of AuNPs from the EPS and CFS and colour change from colourless to brown that indicate the formation of SNPs from the EPS and CFS. Surface Plasmon Resonance (SRP) peaks were observed at 400nm. The particles were aggregate with size ranged from 0.5–2.6 μm and 0.08 - 1.00 μm for WCEPSAuNPs and WCCFSAuNPs and 0.2-3.0 μm and 0.2-2.8 μm for WCEPSSNPs and WCCFSSNPs. The AuNPs and SNPs had antibacterial activity against the tested pathogens. The SNPs exhibited a broad spectrum of activity against the MDR *E. coli* strains tested pathogens. In conclusion EPS and CFS of *Wesiella confusa* were able to bio-reduced and bio-oxidized silver and gold for the biosynthesis of SNPs and AuNPs. The SNPs had broad spectra of activity against *E. coli* strains compared to the AuNPs.

Keywords: Antibacterial Activity; Culture Free Supernatant; Exopolysaccharides; *E. coli*; Gold and Silver Nanoparticle; *Wesiellaconfusa*.

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INTRODUCTION

Weissella confusa is a member of the Lactic Acid Bacteria (LAB), a group of microbes that ferment an array of nutrients into lactic acid primarily [1]. They are found in environments rich in carbohydrates such as plants, fermented foods and the mucosal surfaces of humans, terrestrial and marine animals [2]. They are classified as Generally Recognized As Safe (GRAS) because of their long history of safe use, particularly in dairy foods [3].

LAB has been employed in food preservation and for the alteration of the organoleptic characteristics of foods, for example flavors and

texture [4]. Moreover, nowadays, LAB performs an important role in the industry during the synthesis of chemicals, pharmaceuticals, or other useful products. LAB also produces a wide range of biomolecules which can be applied in the field of nanotechnology in the synthesis of nanoparticles. Organic acids, hydrogen peroxides, bacteriocins, diacetyl and exopolysaccharides are among these biomolecules. These biomolecules has been utilized by different researchers in synthesizing nanoparticles. Exopolysaccharides from LAB was used by Pradeepa *et al.* [5] in the synthesis of gold nanoparticles.

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Nanotechnology is the science which deals with the synthesis, characterization, exploration and application of nano-sized materials for the development of science [6]. The use of nanoparticles for drug delivery have the following advantages: decrease drug resistance, decrease toxicity [7], enhance oral bioavailability [8], enhance the rate of dissolution, enhance solubility [9], increase the stability of drug and formulation [10], increase drug targeting ability [11-12], increase patient compliance [10], increase surface area, reduce the dose needed [12].

Historically, colloidal Au was thought to possess healing ability when taken orally. It was the first recognized form of AuNPs. They possess unique optical properties that make them attractive potential tools in biomedicine. AuNPs are inert, biocompatible, and have a high surface-to-volume ratio. AuNPs possess properties that differ very well from the bulk form [13]. AuNPs are biologically inert, but they can be modified to have various functional groups, such as chemical or photothermal functionalities. Au nanorods are reported to have anti-cancerous and antimicrobial activity following photo-thermal heating [14].

Silver ions and silver compounds possess antimicrobial activities and they are highly toxic to microorganisms [15]. Silver nanoparticles (SNP) are good antimicrobial agents with antioxidant, anti-inflammatory, anticancer and antiangiogenic activities. They are presently regarded as an alternative antibacterial agent to Ag ions due to limited duration of the effect of Ag ion. SNPs exert better antimicrobial activities by the production of reactive oxygen species (ROS) including hydrogen peroxides. They also possess larger surface-to-volume ratios which make it to interact better with the cell membrane and allow easy penetration into the cell, resulting in complete destruction of microbial cell [16]. Among noble metals, silver is the metal of preference in the field of biological systems, living organisms and medicine [17].

Nanotechnology also provides significant systems, devices and materials for better pharmaceutical applications [18]. Nanotechnology offers the better safety profile against drugs with high harmful potential and these nanoforms can be coordinated to act particularly at the objective tissue by passive and active means.

The use of microorganisms and their metabolites as a tool for the synthesis of new functional nanomaterials has gained much interest in recent

times [19]. A simple and viable biosynthetic method has been employed in production of nanomaterials as an alternative to complex chemical synthesis processes. This process involved the use of microorganisms such as bacteria and fungi and the use of plants extract [20-23]. Biosynthesis of nanoparticles is a kind of bottom up approaches where the main reaction occurring is reduction/oxidation [24]. Exopolysaccharides and Cell free culture of microbes may act both as reducing and capping agents in nanoparticle biosynthesis. Biosynthesis of SNPs using exopolysaccharides and culture free supernatant from lactic acid bacteria has been reported [25, 26]. Microbial metabolites have been reported to contain environmentally benign, complex biomolecules such as enzymes or proteins, amino acids, polysaccharides, and vitamins which may be responsible for the bio-reduction of Ag⁺ ions [27]. This research aimed at biosynthesis of gold and silver nanoparticles using EPS and CFS from *Wesiella confusa*, to characterize the nanoparticle and to check the antibacterial activity of the nanoparticles against *E. coli*.

MATERIALS AND METHODS

Culture Collection

Wesiella confusa culture was collected from the culture collection of the Microbial Physiology and Biochemistry Laboratory, Department of Microbiology, University of Ibadan, Nigeria. The culture was kept in maintenance medium (MRS broth with 12% v/v glycerol) and the stock culture was stored at 4°C and sub-cultured from time to time to regulate its viability. *E. coli* ATCC 35218, *E. coli* 700728, *E. coli* 11775, *E. coli* MDRD4 and *E. coli* MDR D15 was collected from Environmental Unit of the Department of Microbiology and Department of Pharmacy, University of Ibadan.

Production of EPS and Culture Free Supernatant (CFS) by *Wesiella confusa*

EPS production by *W. confusa* was done using Modified Exopolysaccharide Selection Medium (mESM) [28]. The sterile mESM was inoculated with the broth culture of the isolate, and the medium was incubated at 35°C for 72 hrs. The fermentation broth was centrifuge at 16,000 rpm for 30 min and the EPS in the supernatant was precipitated using cold absolute ethanol at 4°C for 24 hrs. The precipitate EPS was recovered by centrifugation at 12,000 rpms for 15 mins. The total sugar concentration was determined by phenol-sulfuric acid method using

glucose as a standard [29]. The exopolysaccharide production was expressed as mg/L.

The CFS was produced by culturing *W. confusa* in sterile MRS broth. The sterile broth was inoculated with pure culture of the isolate and incubated microaerobically at 35°C for 24 hrs. The supernatant were treated with 17% (w/v) of 80% trichloroacetic acid solution and centrifuged at 5000 rpm for 20 minutes. The clarified supernatant obtained was labeled CFS. The EPS and the CFS were used for nanoparticle biosynthesis [30].

Biosynthesis of AuNPs and SNPs using the EPS from Wesiella confusa

Biosynthesis of AuNPs using EPS from *Wesiella confusa* was done using modified method of Sathiyarayanan et al. [31]. Chloroauric acid (HAuCl₄) was prepared at the concentration of 10⁻³ M (1 mM) using sterile distilled water. 5 mg/mL solution of EPS was added to 30 mL of 10⁻³ M (1 mM) of HAuCl₄ solution. The mixture was incubated at a temperature of 40°C for 24 hrs in the dark. The colour change from pale yellow to wine red indicates the formation of AuNPs.

Biosynthesis of SNPs using EPS from *Wesiella confusa* was done using modified method of Kanmani and Lim, [32]. 20 ml of EPS solution was mixed with 20 ml of 10 mM aqueous solution of freshly prepared silver nitrate (AgNO₃). The mixture was incubated at room temperature in a dark place for 24-48 hrs. Formation of yellowish brown colour indicates the SNPs formation.

Biosynthesis of AuNPs and SNPs using CFS from Wesiella confusa

Biosynthesis of AuNPs using CFS from *Wesiella confusa* was done [33]. Equal volume of 1mM HAuCl₄ solution was mixed with CFS of *Wesiella confusa* and the mixture was incubated at 40°C in the dark. Color change from pale yellow to bluish purple indicates AuNPs formation.

Biosynthesis of SNPs using CFS was done [34]. 50 ml of AgNO₃ solution (10 mM) was added to 10 mLs of the CFS and the mixture was incubated at room temperature for 48 hrs in the dark.

Characterization of the Synthesized SNPs

Visual detection, UV-visible spectrophotometric and Scanning Electron Microscopic (SEM) analysis of the SNPs

The greenly synthesized AuNPs and SNPs using the EPS and CFS were observed for a change in

colour in comparison to control as a visual method of detection of nanoparticles biosynthesis.

UV-Visible spectra of the nanoparticles solutions were analyzed at room temperature using UV-Vis spectrophotometer (a Lambda 25-Perkin Elmer, Waltham, MA, USA) with a resolution of 0.5 nm. The absorbance of the sample was read at the wavelengths of 200-800 nm.

The morphological compositional structure of the AuNPs and SNPs was analyzed by Scanning Electron Microscopy. The chemical structures of the nanoparticles were determined using FTIR and the functional groups obtained were used for the nanoparticles characterization. The FTIR spectra of the nanoparticles were analyzed using FTIR spectroscopy (Shimadzu) operated at resolution of 4 cm⁻¹. For the measurement the dried samples were powdered with KBr pellets and pressed on a mold. The spectra were recorded at a wave range of 500-4000 cm⁻¹.

Antibacterial Potential of the Nanoparticles against some pathogens

Antibacterial activity of the EPS and CFS stabilized AuNP and SNP was measured using agar well diffusion method [35]. The following clinical pathogens: *E.coli* ATCC 35218, *E. coli* 700728, *E.coli*11775, *E. coli* MDRD4, *E. coli* MDR D15, *S. aureus* 29213, *Staphylococcus* sp. MDR B24, *P. aeruginosa* ATCC 27853, and *Pseudomonas* sp. MDR B24 were used as indicator microorganisms. A 24 hrs old cultures of the pathogens grown on nutrient agar at 37°C were suspended in saline. A lawn of the indicator strain was made by spreading the cell suspension over the surface of Mueller - Hinton agar plates with a sterile cotton swab. The plates were allowed to dry and a sterile cork borer of diameter 7 mm was used to cut uniform wells in the agar. Each well was filled with 20 µL of the biosynthesized AuNPs and SNPs. HAuCl₄ and AgNO₃ solution (1 mM) and EPS were put into respective wells as negative controls. The plates were incubated appropriately. After incubation at 37°C for 24 hrs, the plates were observed for zones of inhibition (ZOI) around the wells. Results were considered positive if the diameter (mm) of the ZOI was greater than 1 mm [36].

RESULTS AND DISCUSSION

Biosynthesis of AuNPs and SNPs using EPS and CFS from Wesiella confusa

The biosynthesized AuNPs and SNPs using EPS and CFS from *Wesiella confusa* were characterized.

Characteristic of biosynthesized AuNPs and SNPs
Visual detection of AuNPs and SNPs

The visual observation of the AuNPs produced using EPS and CFS from *W. confusa* after incubation for 72hrs at 40°C is shown in Fig. 1a-b. Colour changes from yellow to wine red and yellow to bluish purple that indicates the formation of WCEPSAuNP and WCCFSAuNP. The visual observation of the SNPs produced using EPS and CFS from *W. confusa* after 72hrs of incubation at room temperature is shown in Fig. 1c-d. Colour changes from white to brown from yellow to brown that indicate the synthesis of WCEPSSNPs and WCCFSSNPs

The colour change indicates the synthesis of silver nanoparticle and gold nanoparticles. The colour change is reported to be as a result of oscillation of electrons on the surface of the nanoparticles as light rays incidence on it. This phenomenon is known as the Surface Plasmon Resonance (SPR) and it is a characteristic property of nanoparticles [37].

UV-vis spectroscopy of the biosynthesized AuNPs and SNPs

Fig. 2 a-d shows the UV-vis spectra of WCEPSAuNP, WCCFSAuNP, WCEPSSNPs and WCCFSSNPs. Fig. 2a-b shows the UV-vis spectra of WCEPSAuNP and WCCFSAuNP at 24hrs, 48hrs and 72hrs of incubation. Absorption peaks were observed at 400 nm at 24hrs, 48hrs and 72hrs of incubation.

Fig. 2c-d shows the UV-vis spectra of WCEPSSNP and WCCFSSNP at 24hrs, 48hrs and 72hrs of incubation. The peak was also observed at 400 nm at 24hrs, 48hrs and 72hrs.

Nanoparticles exhibits strong absorbance in the UV-region due to SPR phenomenon and the wavelength at which absorbance occurs is a characteristics property of a particular metal nanoparticles and this depends on the size and shape of the particles [38]. Both SNPs and AuNPs in this work showed a single broad Plasmon Resonance band between 400-500 nm. Shankar et al.,[39] suggested that the peak at 400nm is



Fig. 1 a-d: visual observation of the WCEPSAuNP (a), WCCFSAuNP (b), WCEPSSNPs (c) and WCCFSSNPs (d).

due to the excitation of the longitudinal Plasmon vibrations. Shahverdi *et al.*, [40] stated that as the particle size increases the peaks becomes narrower with a decreased bandwidth and increased band intensity.

Fourier Transform Infra-Red (FT-IR) analysis of the AuNPs AND SNPs

Fig. 3a shows the FT-IR spectrum of WCEPSAuNPs. The FT-IR spectrum shows six (6) characteristics peaks ranging from 3389.57-833.58 cm^{-1} . The broadest and strongest peak was observed at 3389.57 cm^{-1} , indicating a stretching vibration of hydroxyl groups (O-H). The absorption peak at 2384.8-2109.6 cm^{-1} indicates the C-H

stretching frequency. The intense absorption peaks at 1648.40 cm^{-1} indicate the C=C stretching frequency. The absorption peak at 1354.06-1078.21 cm^{-1} indicates the S-O and C-O stretching and the intense peak at 990.67 cm^{-1} indicates the presence of carbohydrates. The absorption peak at 833.58 cm^{-1} could be attributed to CH bend of aromatic amine.

Fig. 3b shows the FT-IR spectrum of WCCFSAuNPs. The FT-IR spectrum shows seven (7) characteristics peaks ranging from 3379.57-833.58 cm^{-1} . The broadest and strongest peak was observed at 3389.57 cm^{-1} , indicating a stretching vibration of hydroxyl groups (O-H). The absorption peaks at 2384.8-d2109.6 cm^{-1} indicates the C-H

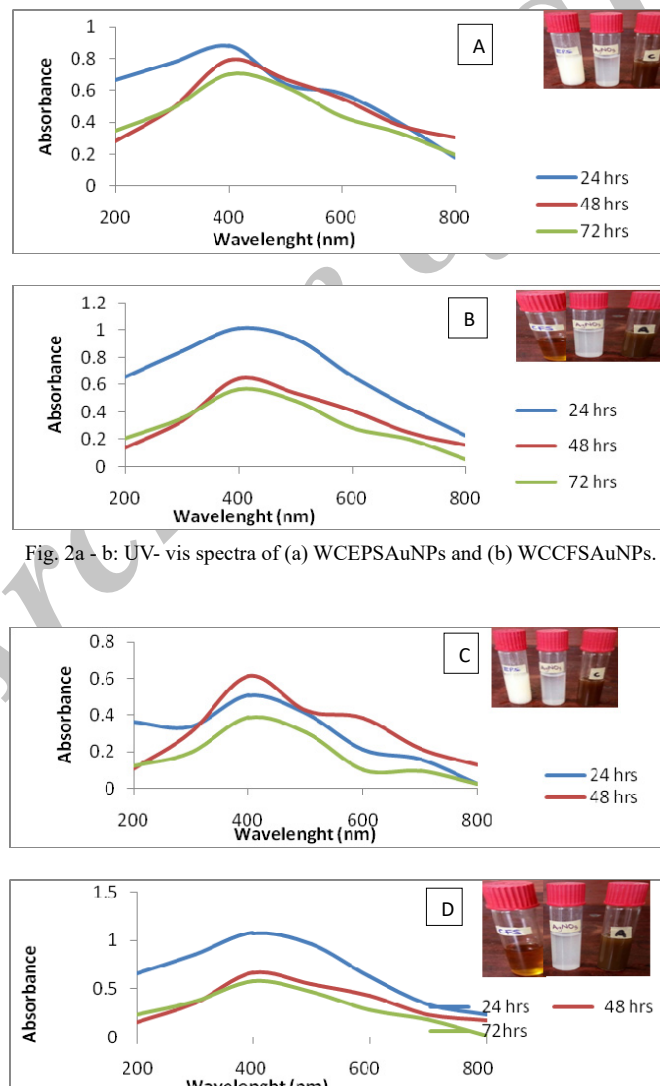


Fig. 2a - b: UV- vis spectra of (a) WCEPSAuNPs and (b) WCCFSAuNPs.

Fig. 2 c- d: UV- vis spectra of (c) WCEPSSNPs and (d) WCCFSSNPs.

stretching frequency. The intense absorption peaks at 1648.40 cm^{-1} indicate the C=C stretching frequency. The absorption peak at 1354.06-1078.21 cm^{-1} indicates the S-O and C-O stretching and the intense peak at 990.67 cm^{-1} indicates the presence of carbohydrates. The absorption peak at 833.58 cm^{-1} could be attributed to CH bend of aromatic amine.

Fig. 3c shows the FT-IR spectrum of WCEPSSNP. The FT-IR spectrum shows eleven (11) characteristics peaks ranging from 3390.01-833.71 cm^{-1} . The broadest and strongest peak was observed at 3390.01 cm^{-1} , indicating a stretching vibration of hydroxyl groups (O-H). The absorption peaks at 2935 cm^{-1} indicate the C-H stretching frequency. The peak at 2381.09 cm^{-1} indicates strong carbon dioxide (O=C=O) stretching. The absorption peak at 2077 cm^{-1} indicates isothiocyanate (N=C=S) stretching. The intense absorption peaks at 1647.94 cm^{-1} indicate the amine (N-H) stretching frequency. The absorption peak at 1357.29-1052.4 cm^{-1} indicates the S-O and C-O stretching and the intense peak at

833.71-553.43 cm^{-1} indicates the presence of halo compounds.

Fig. 3d shows the FT-IR spectrum of WCCFSSNP. The FT-IR spectrum shows sixteen (16) characteristics peaks ranging from 3379.08-632.27 cm^{-1} . The broadest and strongest peak was observed at 3379.08 cm^{-1} , indicating a stretching vibration of hydroxyl groups (O-H). The absorption peaks at 2992.5-2940.7 cm^{-1} indicate the C-H stretching frequency. The peak at 2054.1 cm^{-1} indicates strong isothiocyanate (N=C=S) stretching. The intense absorption peaks at 1720.2-1647.27 cm^{-1} indicate the amine (N-H) stretching frequency. The absorption peak at 1359.49-1052.4 cm^{-1} indicates the S-O and C-O stretching. The intense peak at 925.7 cm^{-1} indicates the presence of carbohydrates and the intense peak at 862.38-632.27 cm^{-1} indicates the presence of halo compounds.

The FT-IR spectrum of AuNPs and SNP showed that some organic biomolecules from EPS and CFS had become associated with the surface of the silver and gold nanoparticles. All the peaks

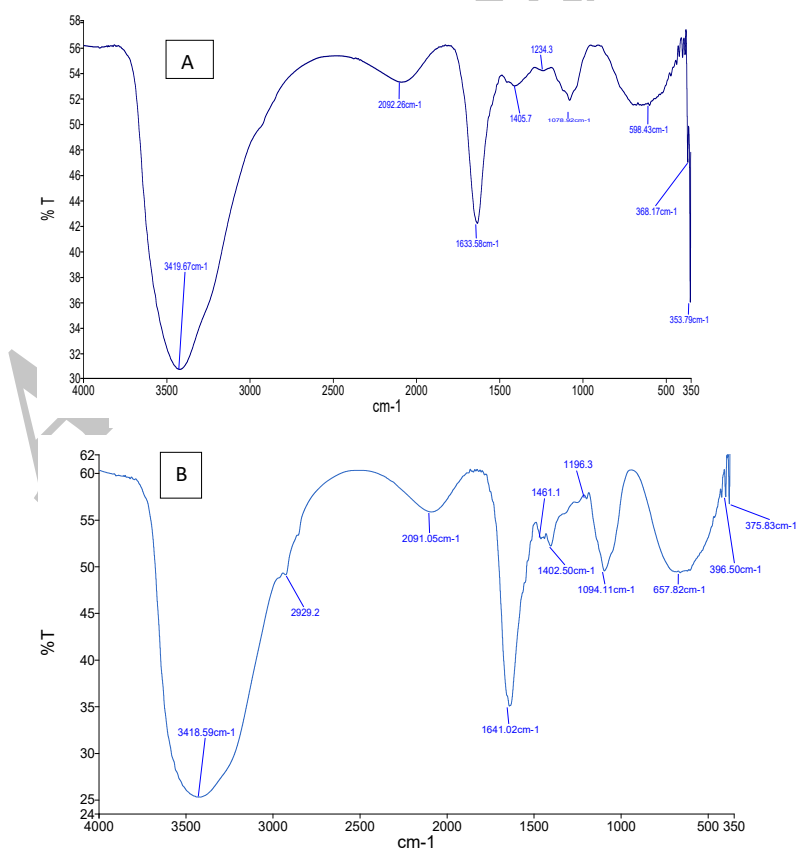


Fig. 3a-b: FT-IR spectrum of WCEPSAuNP (a) and WCCFSAuNP (b).

observed in the FT-IR spectrum of EPS-stabilized SNP and AuNPs differ from the FT-IR spectrum of pure EPS. This suggests a strong interaction of the metals with the EPS functional groups [32]. Also, the FT-IR spectrum of CFS AuNPs and SNPs reveals that amide linkages between amino acid residue and proteins give rise to well-known characteristics band of the electromagnetic spectrum. Balashanmugam *et al.*, [41] reported that from FTIR spectrum analysis, their SNPs were surrounded by proteins and amino acids which may be responsible for the stability of the SNPs. In addition, the capped proteins have previously been reported to retain their secondary structure on nanoparticles surface to make them biocompatible [42].

Scanning Electron Microscopic analysis of AuNPs AND SNPs

The AuNPs and SNPs were further characterized by scanning electron microscopy. The micrographs of the samples are given in Fig. 4a-d. The SEM analysis showed that the biosynthesized

nanoparticles are aggregated with size ranging from 0.5-2.6 μm for WCEPSAuNPs, 0.08-1.00 μm for WCCFSAuNPs, 0.2-3.0 μm for WCEPSSNPs and 0.2-2.8 μm for WCCFSSNPs.

The shape of the nanoparticles was not clear because they are aggregated. The aggregation observed may be due to the drying process. Sadowski *et al.*, [43] observed a similar situation in their work on synthesis of SNPs using *Penicillium* strains isolated from the soil. They concluded that sample preparation including drying can affect the shape and size of SNPs [43].

Antibacterial activity of the biosynthesized AuNPs and SNPs against *E. coli*

Table 1 shows the antimicrobial activity of WCEPSAuNPs and WCCFSAuNPs, The antimicrobial activity for WCEPSAuNPs ranged from 14-33 mm. The antimicrobial activity of WCCFSAuNPs ranged from 20-30 mm. The antimicrobial activity of gentamicin, which was used as positive control ranged 27-28 mm. HAuCl_4 solution did not show any antimicrobial activity against the pathogens.

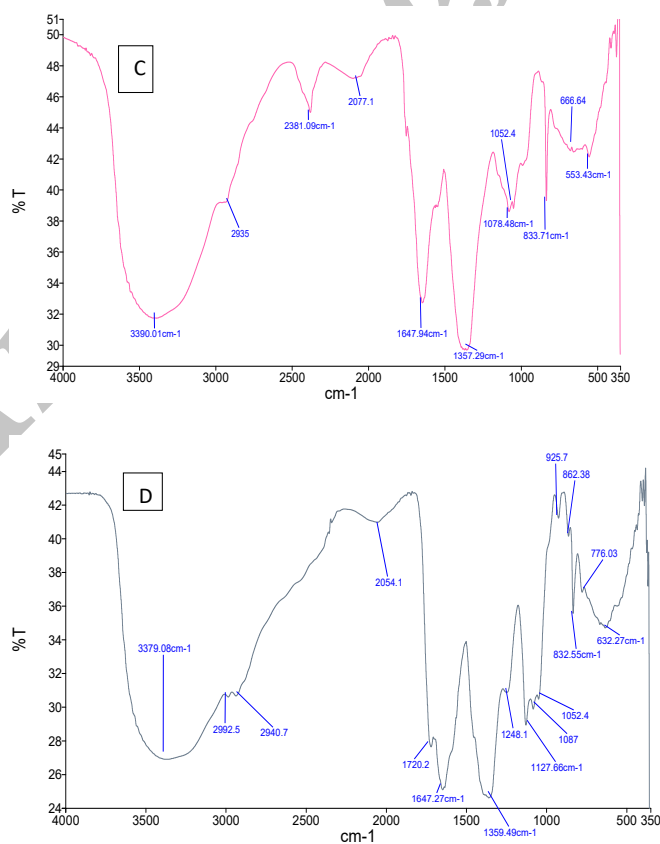


Fig. 3c-d: FT-IR spectrum of (c) WCEPSSNPs and (d) WCCFSSNPs.

WCEPSAuNPs had maximum inhibitory effect against *E. coli* ATCC 700728 (33 mm), followed by *E. coli* ATCC 35218 (27 mm), *E. coli* ATCC 11775 (20 mm), as compared to gentamicin. The least activity was recorded against *E. coli* MDR D4 (14mm). There was no activity against *E. coli* D15.

WCCFSAuNPs also showed maximum inhibitory activity against *E. coli* ATCC 700728 (30 mm), followed by *E. coli* ATCC 35218 (28 mm), *E. coli* ATCC 11775 (20 mm), as compared to gentamicin. The least activity was seen in *E. coli* D4 (14 mm). There was no activity against *E. coli* D15.

Table 2 shows the antimicrobial activity of WCEPSSNPs and WCCFSSNPs, The antimicrobial activity of WCEPSSNPs ranged from 14-37 mm. The antimicrobial activity of WCCFSSNPs ranged from 12-33 mm. The antimicrobial activity of gentamicin, which was used as positive control, ranged 27-28 mm. AgNO₃ had activity which ranged from 12-17 mm

WCEPSSNPs showed maximum activity against *E. coli* ATCC 11775 (37 mm) followed by *E. coli* ATCC

35218 (22 mm) and *E. coli* ATCC 700728 (22 mm) and *E. coli* D4 (17 mm). The least activity was seen in *E. coli* D15 (14 mm) as compared to gentamicin.

WCCFSSNPs also showed maximum activity against *E. coli* ATCC 11775 (33 mm), followed by *E. coli* ATCC 700728 (27 mm), *E. coli* MDR D15as compared to gentamicin. *E. coli* D4 showed the least activity.

The SNPs synthesized with EPS and CFS of LAB showed excellent antibacterial activity against all the test organisms. The biosynthesized SNPs exhibited better antimicrobial activities against *E. coli* strains compare to the AuNPs. Broad spectrum of activity exhibited by the biosynthesized SNPs may be as a result of their extremely large surface area which provides better contact with cell wall of microorganisms [44].

This indicates the broad spectrum of activity of the SNPs [45]. Several studies have reported the interaction of SNPs with bacteria. Baker *et al.*, [46] reported that nanoparticles of low concentration in solution were completely cytotoxic to *E. coli*.

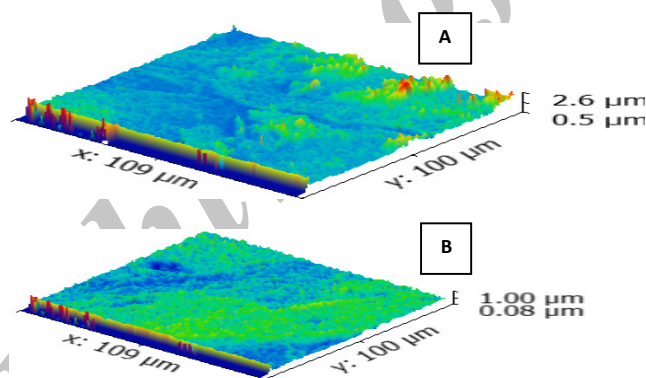


Fig. 4a- b: SEM of (a) WCEPSAuNPs and (b) WCCFSAuNPs.

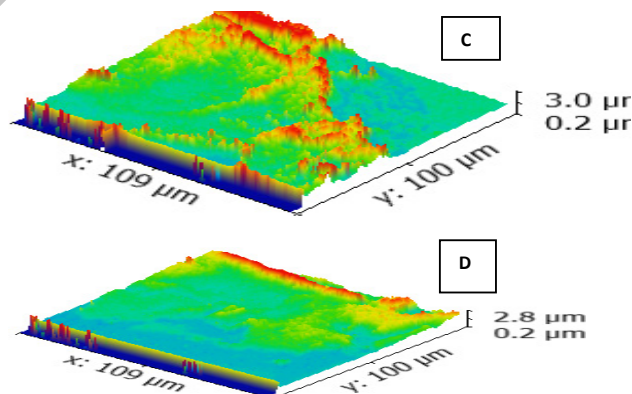


Fig. 4c -d: SEM of (c) WCEPSSNPs and (d) WCCFSSNPs.

Table 1: Antimicrobial activity of AuNPs synthesized with EPS and CFS produced by *W. confusa* against some clinical isolates.

Indicator Organism	Zone of Inhibition(mm)			
	WCEPSAuNPs	WCCFAuNPs	HAuCl ₄	GEN
<i>E. coli</i> ATCC 35218	27	28	-	27
<i>E. coli</i> ATCC 700728	33	30	-	27
<i>E. coli</i> ATCC 11775	20	20	-	28
<i>E. coli</i> (MDR D4)	14	-	-	27
<i>E. coli</i> (MDRD15)	-	-	-	28

Table 2: Antimicrobial activity of SNPs synthesized with EPS and CFS produced by *W. confuse* against some clinical isolate.

Indicator Organism	Zone of Inhibition(mm)			
	WCEPSSNP	WCCFSSNP	GEN	AgNO ₃
<i>E. coli</i> ATCC 35218	22	17	27	17
<i>E. coli</i> ATCC 700728	22	21	27	15
<i>E. coli</i> ATCC 11775	37	33	28	17
<i>E. coli</i> (MDR D4)	17	12	27	14
<i>E. coli</i> (MDRD15)	14	15	28	12

Mendis *et al.*, [47] state that the formation of free radicals from the surface of the silver nanoparticles was responsible for the antibacterial function. In addition, the formation of excess reactive oxygen species (ROS) may lead to the breakdown of membrane function and increased permeability of the cell membrane or leakage of cell matters and morphological changes of the bacterial cells. Martinez-Castanon *et al.*, [48] reported that SNPs interacts with the thiol groups of bacterial proteins and may retard the replication of DNA. Positive charge-containing SNPs are believed to bind with negative charge containing bacterial cell membranes and disrupt cell walls and surface proteins leading to cell death [49].

AuNPs did not have antimicrobial activity against the test organism. This may be attributed to the fact that AuNP is a weak antibacterial agent and does not have a broad spectrum of activity. Nazari *et al.*, [50] also reported that gold nanoparticles had minimum inhibition against *P. aeruginosa*, *Staphylococcus aureus* and *E. coli* by disk diffusion method. Balashanmugam [41] found that AuNPs could target metabolism and transcription of bacteria without triggering ROS reaction, which may be at the same time harmful for the host when killing bacteria.

CONCLUSION

The EPS and CFS of *Wesiella confusa* were able to bio-reduced and bio-oxidized silver and gold respectively for the rapid biosynthesis of SNPs and AuNPs. The SNPs had a broad spectrum of activity against the MDR *E. coli* strains compared to the

AuNPs. These nanobiocomposites can be used in the formulation of novel antibacterial agent to combat Multi drug resistant strains. These nanobiocomposites have potential application in various fields including pharmaceutical, biomedical and nanotechnology.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this review article.

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