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ANTIBACTERIAL ACTIVITY POTENTIAL OF PHYTO-SYNTHESIZED SILVER NANOPARTICLES USING COCOA (*THEOBROMA CACAO)* SEED EXTRACT ON MULTIDRUG RESISTANT *E. COLI* ISOLATED FROM POULTRY ENVIRONMENT

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ABSTRACT
Phyto-synthesised silver nanoparticles (AgNPs) have gained significant attention from researchers recently due to their non-toxic (eco-friendly) nature, cost-effectiveness, and readily available synthesis materials (plant extracts). This study assessed the antimicrobial activity potential of phyto-synthesised silver nanoparticles using cocoa (*Theobroma cacao*) seed extract on multidrug-resistant *Escherichia coli* isolated from a poultry farm environment. The phyto-synthesised silver nitrate was characterised using spectroscopy, transmission electron microscopy (TEM), UV-visible spectroscopy, and Fourier transform infrared (FTIR) spectroscopy. The antimicrobial potentials of the AgNPs were then tested on eight (8) multidrug-resistant *Escherichia coli* strains collected from ecotoxicological laboratory, at the department of microbiology, university of Ilorin Nigeria, which were molecularly identified during the course of this study as *E. coli* O157:7 TR01, *E. coli* 0157:H7 TR01, *E. coli* H17, *E. coli* ST2747, *E. coli* NCTC9733, *E. coli* 210221272, *E. coli* EGE, and E. coli FORC081. Disk diffusion method was employed. An absorbance peak at 450 nm was observed in UV-visible spectroscopy, and the FTIR spectral peak indicated the presence of the O-H stretch of amide (3632.72) in the synthesised AgNPs. The particle sizes ranged from 7.56 to 14.96 nm, and the AgNPs demonstrated antibacterial activity against 37.5 % of the test isolates, including *E. coli* 0157:H7 TR01, *E. coli* 210221272, and *E. coli* EGE.

Keywords: Silver nanoparticle; *E. coli*; Poultry waste; Multidrug resistance; Phyto-synthesis.

INTRODUCTION

The poultry industry and public health are facing significant challenges due to the emergence of multidrug-resistant (MDR) *Escherichia coli* strains in poultry farms (Islam *et al.,* 2023). These strains have the potential to cause infections that are difficult to treat and may transfer resistance genes to human pathogens (Aworh *et al.,* 2019). The potent antimicrobial properties of silver nanoparticles (AgNPs) have attracted attention. An eco-friendly and cost-effective approach to combat MDR pathogens can be achieved through the green synthesis of AgNPs using plant extracts, such as cocoa seed extract (Otuyelu *et al*., 2025).
Metal nanoparticles exhibit exceptional properties compared to their bulk counterparts. They are known for their large surface area, quantum confinement effects, small particle sizes, and other extraordinary features that enable their use in a wide range of applications. Silver nanoparticles (AgNPs) have been synthesised using various methods (Otuyelu *et al.,* 2025). However, biological methods can produce AgNPs without compromising environmental sustainability. Among these methods, plants are simple and attractive sources for AgNP synthesis (Sharma *et al*., 2023). Compared to AgNPs produced via other synthesis modes, phyto-synthesised AgNPs are considered safer and advantageous for a variety of applications, especially in biological fields. Significant research efforts have been dedicated to exploring applications of phyto-synthesised AgNPs (Pattanyak *et al.,* 2022). Scientists are developing phyto-synthetic AgNPs for innovative applications in science and technology to benefit humans (Otuyelu *et al*., 2025).
A study by Thatikayala *et al.* (2019) demonstrated that AgNPs could be successfully synthesised using extracts from different parts of Theobroma cacao. This research focuses on phyto-synthesis of AgNPs using cocoa seed extract on various strains of *E. coli* isolated from poultry waste.

MATERIALS AND METHODS

Sample and Isolate Collection

Cocoa (*Theobroma cacao)* seed extract used in this study was obtained from Ogbondoroko, a rural settlement in Kwara state.
Eight (8) isolates used in this study were obtained from the Ecotoxicology laboratory, Department of Microbiology, University of Ilorin, Kwara state, Nigeria. Isolates (Ecf1, Ecf2, Ecw1, Ecf3, Ecf4, Ecf5, Ecw2, Ecw3) which have previously been cultured and identified from wastewater and faeces of chickens in a poultry in Ilorin metropolis.

Culture Media and chemical

Nutrient Agar, Nutrient broth and Mueller Hinton agar (Hi media, UK) were used in this research. Each of which was prepared in accordance to the manufacturer's guide. Sliver nitrate ($AgNO_3$) Analytical grade used in this study was obtained from Sigma-Aldrich, USA.

Phyto-synthesis of Silver Nanoparticles Using Coco Seed Extract

The procedure described by Tariq *et al*. (2020) was adopted for AgNPs phyto-synthesis. 90 mL of 1 mM silver nitrate ($AgNO_3$) solution was inoculated with 10 mL of cocoa seed extract and mixed thoroughly. The resulting mixture was then exposed to sun light at room temperature for 4 hours, where the gradual reduction of silver ions to silver atoms occurred after 4 hours of exposure. Under the same conditions, control experiments were performed

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using a silver nitrate solution that did not contain coconut seed extract. Observation of colour change from pale brown to dark brown was the sign indicating extracellular AgNP synthesis.

Characterisation of the synthesised Silver Nanoparticles
Different analytical techniques were used in analysing AgNPs mediated by cocoa seed extract. These included FTIR, UV-vis spectrophotometer and TEM. The absorbance and correlation were determined by analysing the absorption spectrum of supernatant aliquots using a UV-Vis spectrophotometer (Analytika Specord 200). Spectral peaks were recorded using an FTIR spectrophotometer (Nicolet 6700) within the range of 400–4000 cm^{-1} and analyzed to identify the functional groups present. TEM (JEM-ARM200F-G Japan (JEM-1010) Model) was used in evaluating and observing morphology and mean sizes of the formed AgNPs. Evaluation of the particle size distribution of AgNPs was done using ImageJ 1.45 software 1493 (Mazzonello *et al.,* 2017).

Antibiotic Susceptibility Profile of *E. coli* Isolates
The antibiotic susceptibility profile of isolates used was determined by using conventional antibiotics for the treatment of bacteria, adopting the disc diffusion method by Kirby-Bauer (Jorgensen and Turnidge, 2015). Antibiotics used are ceftriaxone (30 µg), ceftazidime (30 µg), cefepime (30 µg), amoxicillin-clavulanic acid (30 µg), and imipenem (10 µg). Bacterial lawns were prepared on Mueller-Hinton agar plates using a sterile cotton swab, after which antibiotic discs were carefully placed onto the surface. The plates were then incubated at 37 °C for 18–24 hours and presence of zone of inhibition was observed. Zone of inhibition was measured and recorded accordingly.

Antibacterial Screening of Biosynthesised Silver Nanoparticles
Using the disc diffusion method, the phyto-synthesised AgNPs were tested against the resistant *E coli* isolates (Jorgensen and Turnidge, 2015). Aseptically, MHA plates seeded with resistant *E coli* strains were seeded with sterile filter paper disks impregnated with synthesised AgNPs that had a diameter of 5 mm. After incubation of the plate at 37 °C for 18-24 h, records of the clear zones observed were taken accordingly.

RESULTS AND DISCUSSION

Molecular Identification Result of Recovered Isolates
A total of eight bacterial isolates were successfully identified through molecular characterisation using nucleotide BLAST (BLASTn) analysis. All isolates were confirmed as strains of *E. coli*, showing high sequence similarity to known reference strains in the NCBI database.

Table 1: Molecular Identification of Isolate

Isolates Tag	NBC Accession no.	Organism Identified
Ecf1	CP033605.1	*E. coli* 0157:7 TR01
Ecf2	CP033605.1	*E. coli* 0157:H7 TR01
Ecf3	CP021193.1	*E. coli* H17
Ecf4	CP007392.1	*E. coli* ST2747
Ecf5	CP026845.1	*E. coli* NCTC9733
Ecw1	CP016404.1	*E. coli* 210221272
Ecw2	KY65509.1	*E. coli* EGE
Ecw3	CP02905075.1	*E. coli* FORC081

This result confirms the genetic identity of all isolates as distinct strains of *E. coli*.

Silver Nanoparticles Phyto-synthesis
The potential of coca seed extract for the phyto-synthesis of silver nanoparticles is highlighted in Fig. 1, showing a dark brown colouration after 48 hours of synthesis as an indication of AgNP formation. The colour change from pale brown to dark brown was visually observed, in line with Ahmed *et al*. (2024). Similarly, Gowda *et al.* (2024) conducted a study that reported the bio-reduction and phyto-synthesis of AgNPs using sandalwood (Santalum album L.) leaf extract, which also resulted in a dark-brown colouration signifying synthesis. The colour formation has been attributed to surface plasmon resonance excitation of metal nanoparticles, as documented by Wang *et al*. (2022).

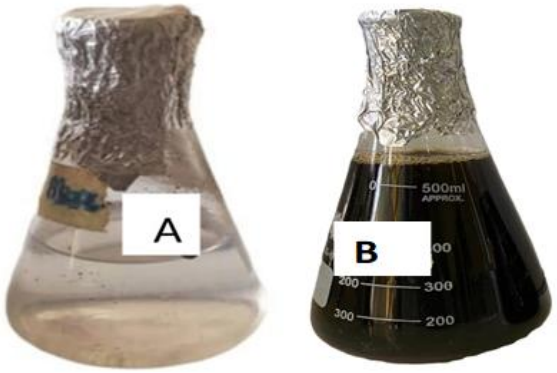

Figure 1: (A) Silver nitrate solution ($AgNO_3$); (B) Phyto-synthesised AgNPs with cocoa seed.

Characteristics of Phyto-synthesised Silver Nanoparticles
AgNPs synthesis was characterised using a UV spectrophotometer in the range of 250-700 nm with peak absorbance of 2.42 A at 450 nm, as shown in (Fig. 2). Otuyelu *et al.* (2025) reported a peak at 440 and 385, a similar peak range that was obtained in this study. However, the synthesised nanoparticles' peak absorbance has been reported to range between 350 to 450 nm (Genc, 2021). Fourier transform infrared spectroscopy (FTIR) spectrum revealed possible biomolecules that were involved in the bio-reduction of silver salt and stabilisation of AgNPs. Figure (3) revealed spectral peaks at 3632.72, 3205.20, 2062.27, 1644.33, 1182.26 and 736.62 cm^{-1} which are attributed to O–H stretching of amide group and stretching vibrations of phenol/carboxylic group CH_2 stretching, bending vibration of the amide I band of the protein (C=C group), stretching vibrations of C–O groups in the phenol, ether, or ester group and OCN bending in the amide IV band arising (Aisida *et al*., 2019; Otuyelu *et al*., 2025).

The TEM micrograph, Fig. 4, revealed that the silver nanoparticle was monodispersed and spherical in shape when observed. It shows particle sizes to be between 7.56 – 14.96 nm (Figure 4). The spherical shape and sizes reported in this study are consistent with the report of Wan *et al*. (2016).

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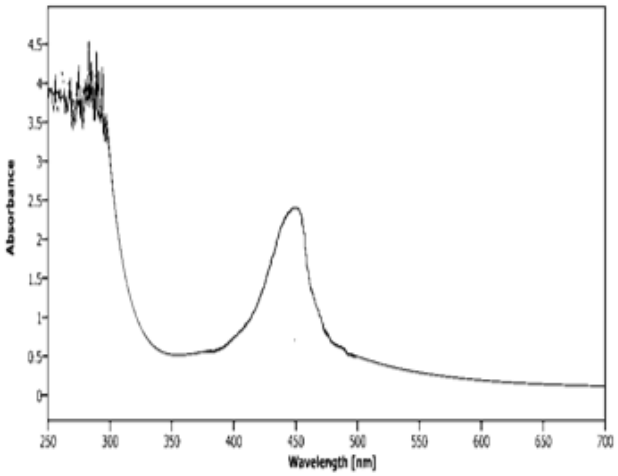

Figure 2: UV-vis spectroscopy of phyto-synthesised AgNPs

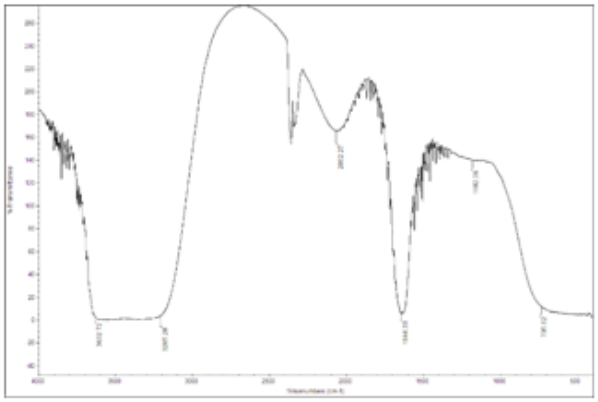

Figure 3: FTIR spectrum of Phyto-synthesised AgNPs

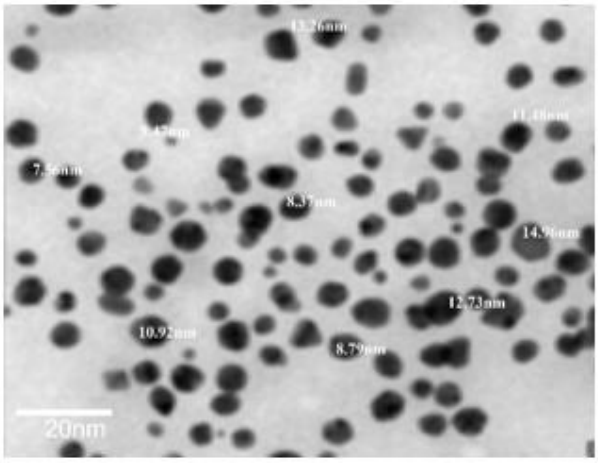

Figure 4: TEM micrograph of Phyto-synthesized AgNPs

Antimicrobial Activity of Silver Nanoparticles

The antibacterial activities of Phytosynthesized silver nanoparticles with cocoa seed extract and conventional antibiotics against the clinical pathogens are shown in Table 2. The assay showed activity against three of the test isolates, as represented in the table. Four out of five (80 %) antibiotics used displayed activity against at least one of the test isolates, with no single activity recorded by CAZ. This result confirms the antimicrobial activity of phyto-synthesised AgNPs with coca seed extract.

Table 2: Antibacterial activity of Phyto-synthesised AgNPs and conventional antibiotics on Isolates

Isolates	Zone of Inhibition (mm)					
	AgNPs	CRO	CAZ	FEP	AMC	IMP
E. coli O157:7 TRO1	0	0	0	0	0	0
E. coli O157:H7 TR01	25.67 ± 0.71	0	0	0	0	14.33 ± 0.33
E. coli H17	0	0	0	0	21.33 ± 0.33	0
E. coli ST2747	0	17.66 ± 0.33	0	34. 67 ± 0.67	0	0
E. coli NCTC9733	0	0	0	0	0	0
E. coli 210221272	27.33 ± 1.08	0	0	0	0	0
E. coli EGE	17.67 ± 0.01	0	0	0	0	0
E. coli FORC081	0	0	0	0	0	0

Keys: ceftriaxone (CRO), ceftazidime (CAZ), cefepime (FEP), amoxicillin-clavulanic acid (AMC), imipenem (IMP)

Antibiotic susceptibility test result in Table 2 indicates that each pathogen was resistant to at least 3 of the test antibiotics used, with only *E. coli* ST2747 showing susceptibility to ceftriaxone and cefepime, while *E. coli* 0157:H7 TR01, *E. coli* H17 were resistant to one each of the test antibiotics (imipenem and amoxicillin-clavulanic acid), respectively, making the *E coli* strains used in this study multidrug-resistant strains. The observed resistance patterns are likely due to various mechanisms, including the production of β-lactamases (ESBLs, AmpC, or carbapenemases), porin loss, and efflux pump activity (Gauba & Rahman, 2023). These mechanisms are often mediated by plasmid-borne genes acquired through horizontal gene transfer. Selective pressure from antibiotic misuse further drives the emergence and spread of multidrug-resistant strains (Muteeb *et al.,* 2023). These findings underscore the critical need for routine susceptibility testing, responsible antibiotic use, and continuous molecular surveillance to manage and prevent antimicrobial resistance effectively.

Growth inhibitory potentials shown by phyto-synthesised AgNPs against some of the isolates are confirmation that AgNPs are good

antimicrobials and are in agreement with Otuyelu *et al* (2025). Effect on the integrity of *E coli* cells through despoliation and destabilisation of the cell membrane has also been reported (Lateef et al., 2015). The size of metallic nanoparticles allows a large surface area of particles to get in contact with the bacterial cells, enhancing the extent of bacterial damage (Hameed *et al.,* 2020). AgNPs' activity in the study demonstrated their effectiveness in combating antibiotic-resistant pathogens with *E. coli* 0157:H7 TR01, *E. coli* 210221272 and *E. coli* EGE all showing susceptibility.

Antibiotic-resistant bacteria in poultry farming are emerging pathogens with resistance that pose a great challenge in treating and controlling their spread (Abreu *et al.,* 2023). Otuyelu *et al*. (2025) presumed that antimicrobial activity exhibited by AgNPs is a factor of the continuous release of silver ions implicated in the bactericidal process. These ions have a strong affinity to sulphur-containing proteins, which are attracted by electrostatic forces that make them adhere easily to the cytoplasm and cell walls. Ji *et al. (*2007) reported that silver nanoparticles inhibit the respiratory chain dehydrogenase, therefore halting the metabolic activity and growth of bacterial cells. It was also reported that through depolarisation and destabilisation of the cell membrane, silver nanoparticles can compromise the integrity of *E coli.* It is guaranteed that a substantial surface area of the particles will interact with bacterial cells due to the small size of the metallic nanoparticle. This large contact surface likely increases the effectiveness of bacteria elimination (Chand *et al.,* 2021).

Phyto-synthesised silver nanoparticles is a promising alternative antimicrobial compared to chemically synthesised AgNPs. It has shown stronger antimicrobial activity against multidrug-resistant *E coli* as observed in this study. It is also an eco-friendly and sustainable green method of synthesis with no fear of toxic byproducts compared to chemical synthesis. Studies have also highlighted the production of controllable size and shape of biosynthesised AgNPs with no harm to healthy human cells compared to chemical-based synthesis (Otuyelu *et al.,* 2025).

Conclusion

This study has established the antibacterial activity of biosynthesised cocoa seed silver nanoparticles. The phytosynthesized nanoparticles exhibited highly potent antibacterial activities and also enhanced efficacy of antibiotics against multidrug resistant isolates of bacteria. The outstanding activities of cocoa seed silver nanoparticles and successful inhibition of microbial growth will be of great service in both the biomedical and materials industries. This highlights the potency of Phyto-syntheses as essential as bacteria and fungi's synthesis of nanoparticles.

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