RESEARCH PAPER

Biosynthesis Novel Approach of Silver Nanoparticles Reduced by Aerodramus Fuciphagus Extracts for Antibacterial Applications

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Abstract: *Research on green synthesis methods for silver nanoparticles has significantly risen due to its positive features, such as cost-effectiveness, rapidity, and reduced environmental impact. This study employed an edible bird's nest (EBN), an important economic resource, as the main material for manufacturing silver nanoparticles, utilizing water as the sole solvent. The EBN extract was subjected to metabolite profiling using LC-QTOF-MS in positive mode (ESI+), which identified the presence of lipids, glycosides, peptides, polysaccharides, and disaccharides. The effective production of AgNPs was evidenced by noticeable color changes from translucent to brown upon the addition of silver nitrate to the aqueous EBN extract. Further analysis of these silver nanoparticles was conducted using UV-visible spectroscopy, which identified an absorption peak at 421 nm. Additional characterization was conducted via FESEM, ATR-FTIR spectroscopy, and EDX analysis. The confirmation of the reduction of silver particles was established by the participation of phenolic agents, proteins, and amino acids. The produced nanoparticles had a spherical morphology, with a particle size ranging from 10 to 20 nm. The existence of elemental silver was verified by a prominent and powerful peak at around 3 kilo electron volts (keV) in the energy-dispersive X-ray (EDX) spectrum. The antibacterial efficacy of silver nanoparticles against Escherichia coli and Staphylococcus aureus was assessed using the agar diffusion method to determine their potential.*

Keywords: *Aerodramus fuciphagus, Antibacterial properties, Green synthesis, silver nanoparticles.*

1. INTRODUCTION

Researchers in the field of nanotechnology have shown significant interest in the concept of eco-friendly synthesis. The production of nanoparticles, such as silver (Ag), gold (Au), and platinum (Pt), is typically achieved by the application of chemical and physical techniques [1-3]. Various biological entities, including microbes, algae, bacteria, viruses, and plants, have been studied for their potential to produce ecologically beneficial biological nanoparticles [4-6]. Food plants have gained significance as biological sources due to their ability to produce a wide range of phytochemicals that are non-toxic to organisms and because they enable faster synthesis compared to microorganisms. Therefore, plants are considered to have a highly advantageous role in the production of nanoparticles. Extensive work has been carried out on the synthesis of a wide range of nanoparticles by exploiting several plant species. The stability and production of silver nanoparticles require the presence of phenolic chemicals, flavonoids, alkaloids, proteins, and reducing sugars found in plants. These chemicals have a significant impact on the reduction and capping processes, which are crucial for the successful production of silver nanoparticles [7]. The ecologically sustainable approach to nanoparticle production holds great potential, not only due to its good environmental impact but also due to its wide range of potential uses.

Aerodramus fuciphagus, commonly referred to as the "white-nest swiftlet," is a small bird belonging to the swift family. Southeast Asia is the predominant geographical region where it is commonly found, with the majority of its population concentrated in South China and Southeast Asia. The birds utilize their saliva, which undergoes solidification to form edible bird nests (EBN), as the principal constituent for

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constructing their nests. Aerodramus fuciphagus, commonly referred to as the white nest, and Aerodramus maximus, commonly referred to as the black nest, are the defining features of EBN. The nests include a diverse range of nutritional components, including amino acids, essential trace elements, proteins, carbohydrates, and heavy metals [8]. A recent study conducted in Malaysia investigated the nutritional value of white-nest swiftlets from Pahang and Terengganu. The study emphasized the importance of calcium, sodium glycoprotein, carbohydrates, and potassium in EBN (edible bird's nest) [9-10].

Historically, EBN has been a component of Chinese cuisine. It is often made as a soup by double boiling, and it is typically sweetened with rock sugar. People in China take EBN because of the multiple health benefits it offers, including the promotion of the formation of new skin cells and the maintenance of smooth skin through its consumption. EBN is also well-known for its antiviral and antibacterial characteristics, which it possesses against bacteria and viruses. Sialic acid is a major component that contributes to the antiviral activities of EBN. Experiments conducted on mice have demonstrated that the administration of EBN to the mother during the stages of pregnancy and lactation can result in improved spatial learning abilities in the offspring [11]. In addition, there is evidence that suggests that the ingestion of EBN extract can either improve bone mass or decrease the aging process of the skin in women who have gone through menopause [12]. According to the findings of research carried out by Khalid and colleagues, it is possible to improve the process of cell repair from damage by increasing the rate of cell multiplication and ensuring that correct functioning is maintained in the process of wound healing of corneal tissues [13].

As far as we know, no prior studies have examined the association between silver nanoparticles and the bioactive compounds present in edible bird nests. We offer a unique biological extract for producing stable silver nanoparticles (AgNPs) by utilizing EBN products as a substrate. This is a response to the increasing potential of EBN extracts to serve as efficient reducing and capping agents for nanoparticles. By employing this method, we achieved the successful synthesis of nanoparticles without the need for a stabilizing ingredient. This was achieved by employing a certain amount of silver nitrate ($AgNO_3$) while adjusting the concentration of EBN accordingly. An EDX analysis, FTIR analysis, FESEM analysis, and UV-visible spectrophotometry were applied to carry out the process of characterizing the silver nanoparticles that were generated.

2. EXPERIMENTAL PROCEDURES

2.1. Materials

Silver nitrate, $AgNO_3$ was purchased from Sigma-Aldrich (M) Sdn. Bhd. The edible bird nests (EBN) were purchased from Prosper Food & Beverages Sdn. Bhd. in Kuala Lumpur in their raw, cleaned form. All chemicals were used without any further purification.

2.2. Synthesis of EBN-AgNP Solution

A solid crystal of $AgNO_3$ with a molecular weight of 169.87 g/mol was produced in a solution with a concentration of 0.05 M in deionized water. Edible bird nests were made at a concentration of 6% weight/volume by dissolving them in deionized water and then heating the mixture at 90°C. The mixture was stirred until no further changes in the texture of the solution occurred. Once the solution had completely cooled, the EBN solution was filtered using Whatman filter paper. Subsequently, 800 µl of a 0.05 M $AgNO_3$ aqueous solution was combined with 10 mL of EBN extract at room temperature in a light-restricted environment. The solution contained in the bottle was covered with aluminum foil to prevent additional evaporation. No color transformation was observed or recorded. The solution of EBN-AgNP was kept in a desiccator for future use.

2.3. Characterization of EBN-AgNP Nanoparticles

The UV-visible spectrophotometer (Shimadzu UV-1800) was used to measure the absorbance of a reduced solution of $AgNO_3$ in the wavelength range of 200-800 nm. This was done to detect the existence of AgNP.

The FTIR analysis, specifically using the Perkin Elmer Spectrum 100 instrument, was employed to identify the specific functional groups present in Aerodramus fuciphagus that are responsible for the reduction and capping of AgNP. The prepared sample was positioned on an attenuated total

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reflection (ATR) sample cell and subjected to analysis within the wavelength range of 4000-650 cm^{-1}.

The FESEM (JSM-7800F) was utilized to measure the particle size and morphological structures of AgNP. The nanoparticle sample was deposited onto carbon-coated copper grids to create a film, which was then left to dry in the chamber for 2 hours. The morphological photos of AgNP were generated at a resolution of 1.3 nm under a high vacuum of 30 kV. The identification of elements in silver nanoparticles was accomplished by the utilization of EDX analysis, which was conducted in conjunction with the FESEM analyzer. The identical specimen utilized in FESEM was also employed in EDX analysis.

The non-volatile chemicals in the EBN extract were screened using an LC-QTOF mass spectrophotometer (Vion IMS LCQTOF MS) in positive ion mode. A 5-minute linear gradient was employed, with flow rates of 0.6 mL/min, transitioning between solvent A (water with 0.1% Formic acid) and solvent B (Acetonitrile). The mass spectra were acquired within the m/z range of 50 to 100. The cone gas, desolvation gas, and capillary voltage were set at 50 L/h, 800 L/h, and 2.50 kV, respectively, using high-purity nitrogen. The initial temperature of the source was 120 degrees Celsius, while the temperature at which desolvation occurred was 550°Celsius.

2.4. Antibacterial Activity of AgNPs

The antibacterial activity of EBN-AgNP nanofibers, which were generated through biological processes, was assessed against two types of bacteria: gram-positive Staphylococcus aureus (S. aureus) and gram-negative Escherichia coli (E. coli), using the agar diffusion assay. Bacterial cultures were preserved at 4°C using nutrient agar (NA). The bacterial suspension was prepared by transferring a solitary bacterial colony from NA onto a sterile loop and subsequently introducing it into 10 mL of sterile saline water. The optical densities of the resulting microbial culture solutions were adjusted and compared to the 0.5 McFarland standards. Subsequently, a volume of 100 µL of bacterial solution was evenly distributed onto a sterile plate containing potato dextrose agar (PDA). The sterilized nanofiber disc was relocated to the central surface of the plate. The zone of inhibition was subsequently established following the incubation of the plates at a temperature of 37°C for 24 hours.

3. RESULTS AND DISCUSSION

3.1. UV-Visible Analysis of AgNPs

The experiment involved the manufacture of AgNPs through the reduction of silver ions using an extract derived from an edible bird nest (EBN). An investigation was conducted to examine the impact of EBN concentration. A preliminary investigation was conducted within the illumination of a laboratory. After 15 minutes of adding $AgNO_3$ to the EBN solution, the color of the reaction mixture changed from colorless to reddish-brown, as shown in Fig. 1. The alteration in color and its subsequent stabilization for 15 minutes signified the eco-friendly production of silver nanoparticles through the biotransformation of Ag^+ to Ag nanoparticles. A previous study suggests that the presence of proteinase in spiderwebs and paper wasp nests could potentially lead to the creation of silver nanoparticles. The presence of color in the reaction mixture is a result of the intense absorption of electromagnetic waves known as surface plasmon resonance (SPR). AgNPs were identified using UV-visible spectroscopy in the wavelength range of 300 nm to 800 nm following a 15-minute reaction. The phenomenon of surface plasmon resonance (SPR) originated from the application of an electromagnetic field to nanoparticles. The excitation peaks observed in the UV-visible spectrum indicated the formation of AgNPs. The observed SPR bands are contingent upon the size of the nanoparticles. This work shows that the metabolites of edible bird nests can be used to environmentally-friendly synthesize silver nanoparticles.

The surface plasmon resonance (SPR) bands seen in the ultraviolet-visible (UV-Vis) spectra of the AgNPs samples were depicted in Fig. 2. The absorption spectra were measured within the wavelength range of 300 to 800 nm. Surface plasmon vibration was excited, resulting in absorbance at a wavelength of 421 nm following a 15-minute synthesis of EBN combined with silver nitrate. In contrast, no peak was detected in this wavelength area when EBN was used alone (see Fig. 2).

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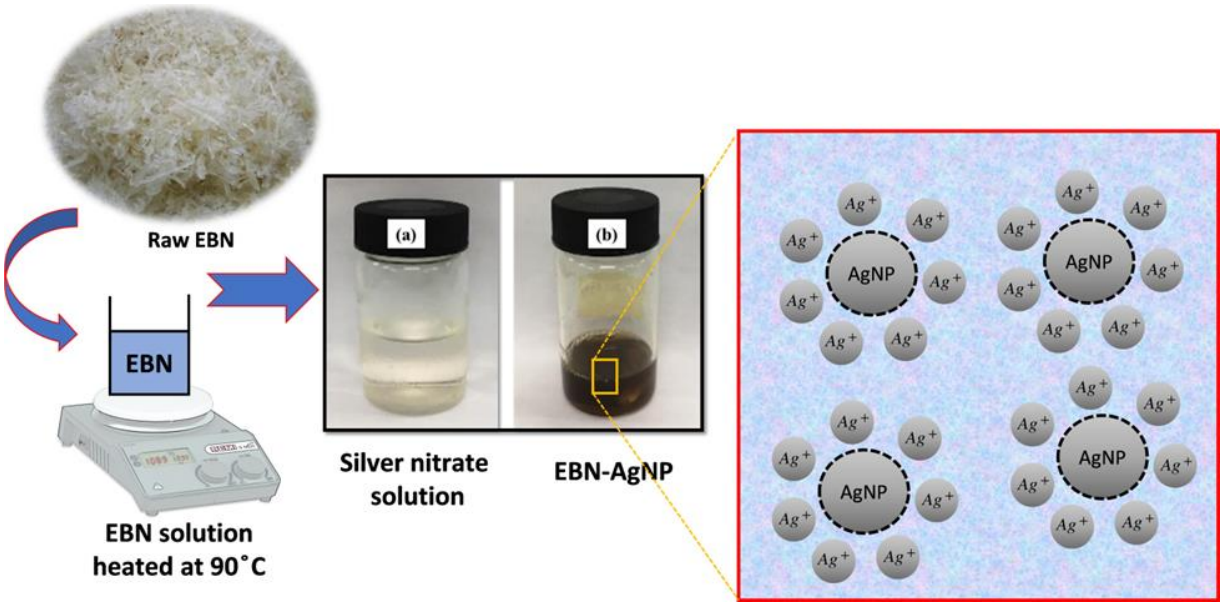

Fig. 1. Photograph images of (a) 0.05 M silver nitrate clear solution and (b) silver nanoparticles dispersion solution which was chemically reduced from Ag^+ to Ag nanoparticles by edible bird nest (EBN) extract solution for 15 minutes at room temperature.

This suggests that EBN possesses phenolic agents and other chemicals that are responsible for capping and stabilizing the production of silver, reducing silver ions (Ag^+) by acquiring electrons to produce silver atoms (Ag^0). As the reactivity increased, silver atoms were aggregated to minimize surface energy, forming minuscule clusters or nuclei, leading to the expansion of the nanoparticles [14].

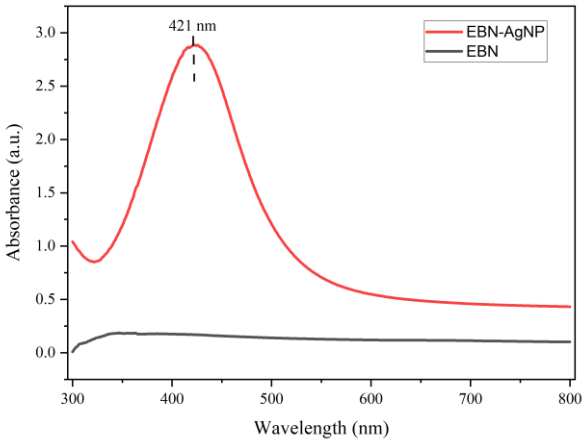

Fig. 2. UV-visible spectra of sample synthesised by the reaction of $AgNO_3$ (800 µL, 0.05 M) reacted with EBN extracted at a concentration of 6 wt. %.

The finding of this study was similar to previous research which has indicated that the widening of the highest point in the 400 to 450 nm region could be caused by spherical nanoparticles [15]. Other publications have reported the presence of AgNPs in the absorption spectra within the range of 425 to 460 nm [16]. A study found that silver produced with green tea extracts exhibited a prominent peak at a wavelength of 410 nm [17]. The extract's stabilizing effect was compromised by the formation of metal nanoparticles.

3.2. Evaluation of FT-IR Analysis Data

The FTIR analysis data on both EBN and AgNPs was used to define the surface composition of the solutions involved in the process of reducing and stabilizing synthesized silver nanoparticles. FTIR can detect functional groups present on the surface of silver nanoparticles and analyze the chemical composition of capping agents or stabilizers utilized in their manufacture [18]. This aids in comprehending the relationship between nanoparticles and adjacent molecules. The analysis displayed in Fig. 3 (a) revealed three distinct absorbance peaks at wavelengths of 3321 cm^{-1}, 2136 cm^{-1}, and 1635 cm^{-1} for the extract obtained from edible bird nests. The wavenumber 3321 cm^{-1} indicates the stretching of the O-H bond in alcohol and phenolic metabolites. The wavenumber 2136 cm^{-1} corresponds to the stretching of the C≡N bond in the alkyne group. Lastly, the wavenumber 1635 cm^{-1} corresponds to the stretching of the C=O bond in the carbonyl group. The presence of a chelating agent capable of absorbing and binding to the nano-sized silver

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ions was confirmed by the carbonyl group derived from the amino acid.

Fig. 3 (b) demonstrated a noticeable shift in the peaks, along with the appearance of additional weaker peaks. This suggests that the molecule included in EBN played a role in reducing the production of AgNPs. The peaks at 3321 cm^{-1} and 2136 cm^{-1} saw a shift to 3338 cm^{-1} and 2141 cm^{-1} respectively, following the synthesis process. However, the peak at 1635 cm^{-1} remained unchanged, maintaining its strong and sharp characteristics. The emergence of a new peak at 1414 cm^{-1} and 1092 cm^{-1} indicates the presence of O-H bending, whereas the C-N stretching in the amine group may contribute to the capping of AgNPs.

3.3. Evaluation of FESEM and EDX Analysis Data

The AgNPs that were synthesized underwent additional characterization using FESEM and EDX analysis to determine the size and form of the nanometals. To conduct the FESEM analysis, a small amount of the synthesized AgNPs solution was carefully placed on the carbon-coated grid disk using a pipette. The solution was then allowed to dry in the chamber, resulting in the formation of a thin layer. The surface characteristics and chemical composition of EBN-AgNPs were examined using a Field Emission Scanning Electron Microscope (FESEM) equipped with Energy Dispersive X-ray (EDX) analysis. The distribution of spherical-shaped silver nanoparticles, spanning from 10 to 20 nm, is depicted in the FESEM images shown in Fig. 4 (b). The mean diameter of spherical silver nanoparticles ranged from 10 to 35 nm. The results indicate that the nanomaterials had a nearly spherical shape, were of nano-scale size, and formed unattached clusters. Therefore, it can be affirmed that the AgNPs were in a state of stability.

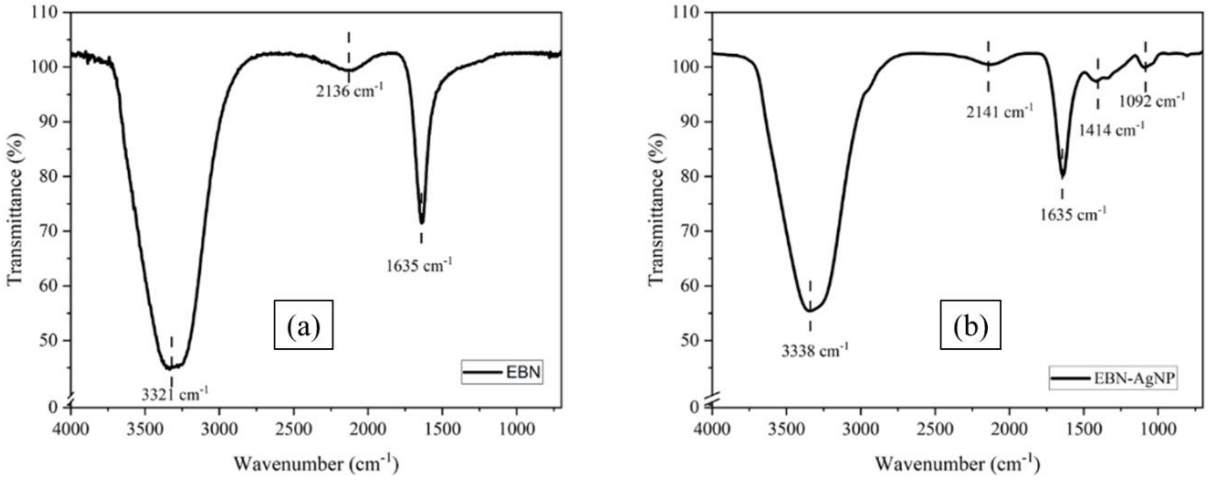

Fig. 3. ATR-FTIR spectra data of (a) EBN extract and (b) synthesised of EBN-AgNPs.

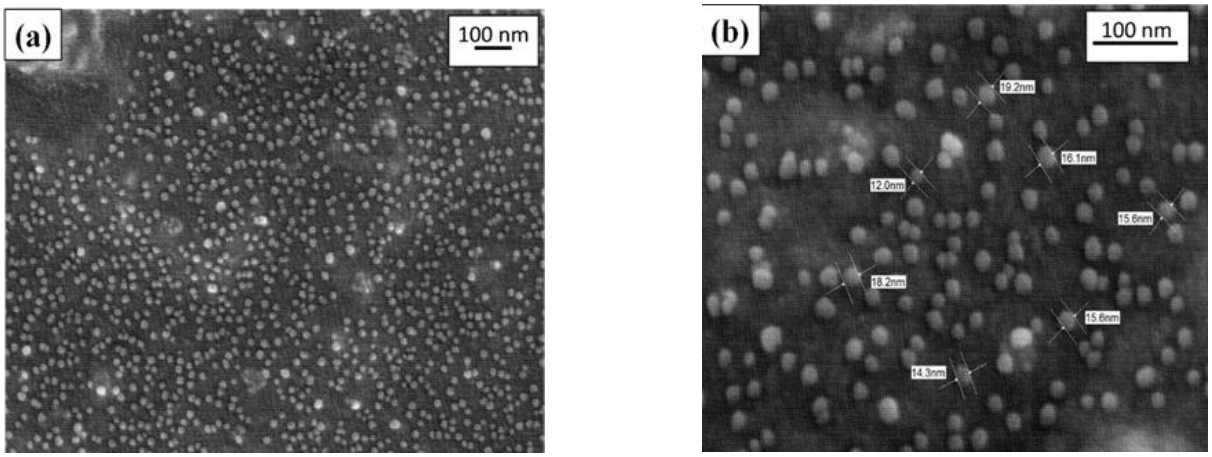

Fig. 4. FESEM image of EBN-AgNPs in 80 k× (a) and 200 k× (b).

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The elemental composition of the AgNPs was confirmed by analyzing them using EDX analysis along with FESEM EDX profile images. The analysis indicated a peak at 3 keV, indicating the presence of silver (Fig. 5). Additionally, additional components such as carbon (C), oxygen (O), and nitrogen (N) were also discovered. The phytochemical components present on the surface of AgNPs exhibit distinct peaks on the spectrum. The presence of biomolecules on AgNPs resulted in the recording of carbon and oxygen peaks on spectral pictures. Each element exhibited distinct peaks on the X-ray spectra as a result of their unique atomic structures. The EDX investigation verified the presence of silver nanoparticles at around 3 keV, which can be attributed to surface plasmon resonance. The existence of silver was confirmed by analyzing spectral pictures that detected peaks at 2.98 keV corresponding to the silver element. The yield percentage was calculated at 18.63% by considering the elemental value of Ag at 17.91% in Table 1. The concentration of silver nanoparticles (AgNPs) exhibited a clear correlation with the atomic percentage. The weight of the silver element has an impact on the quantity of phenolic agent used to reduce and stabilize nanoparticles.

3.4. Liquid Chromatography Quadrupole Time of Flight Mass Spectrometry (LC-QTOF-MS) Analysis

The LC-QTOF-MS analysis of EBN revealed the identification of metabolite groups in the EBN extract. The equipment was used to screen the non-volatile compounds included in EBN during the testing of solution EBN. LC-QTOF-MS is utilized to analyze and characterize metabolites and proteins found in biological materials. The LC-QTOF-MS offers useful insights into the molecular composition of substances via fragmentation analysis, which could aid in silver reduction. The high-resolution mass spectra enable the identification of elemental composition, whereas fragment ions offer information about the structural characteristics of the molecules. Table 2 displays the detected compounds together with their retention time (in minutes), compound name, retention drift (in milliseconds), chemical type, and response. The metabolites found in this research pertain to distinct chemical categories. Identifying the metabolites composition in EBN is crucial for determining the specific composition that can provide health benefits. This study documented the metabolic compositions found in EBN, each of which possesses distinct activities.

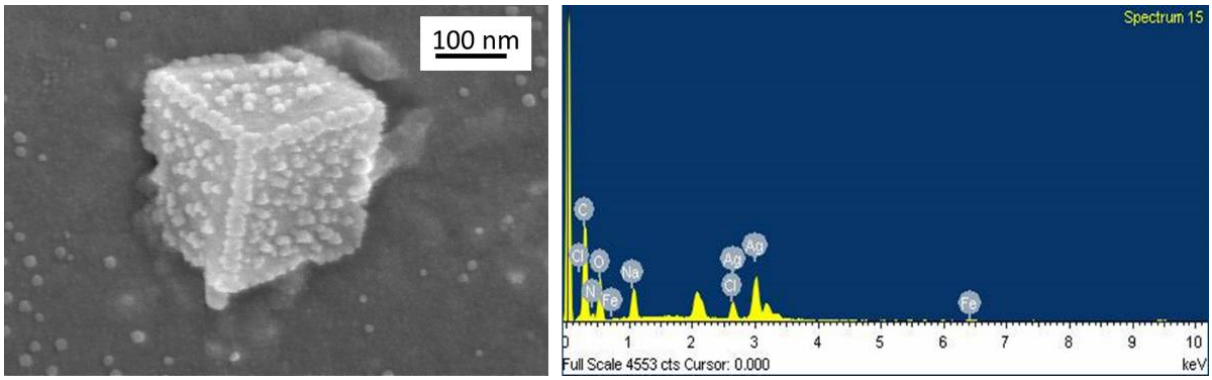

Fig. 5. Elemental analysis of nano-Ag confirmed by EDX.

Table 1. Elements analysed on synthesised AgNPs.

Element	Weight%	Atomic%
C K	34.70	48.88
N K	13.28	16.04
O K	25.87	27.36
Na K	4.55	3.35
Cl K	2.53	1.21
Fe K	1.17	0.35
Ag L	17.91	2.81
Total	100.00	100.00

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Cortisone, a steroid medication, is a chemical lipid that is utilized for the treatment of allergic reactions, and autoimmune diseases, and to reduce inflammation and immunological response. This composition will inhibit the release of inflammatory chemicals. In addition, sulfatide is often abundant in the myelin sheath. Its crucial role encompasses the regulation of cellular development and adhesion. Tricosanoic acid belongs to the group of fatty acids that have carboxylic acids as alternate parents. These compounds have been reported to exhibit antibacterial activity against E.coli and S.aureus. Furthermore, it has been discovered that cholesteryl ester plays a role in the process of intestinal cholesterol absorption. The presence of peptide clusters inside the protein structure was identified, resembling Thymidine in the EBN extract. Thymidine is employed in the process of DNA replication and is generated by cells. In addition, Citrusin C is classified as a phenolic glycoside, which is a type of organic chemical. It has been demonstrated that EBN possesses a phenolic structure, which aids in the natural reduction of silver ions for the creation of silver nanoparticles in this work. The polysaccharide mannotriose has been proposed as a means to improve cell viability.

3.5. Effect of AgNPs against Bacteria

Due to their prevalence as wound-infecting microbes, the antimicrobial properties were tested against Staphylococcus aureus and Escherichia coli. The antimicrobial effects of the 6% nanoparticles (EBN-AgNPs) were compared to those of the control sample (pure EBN extract) using the agar diffusion method.

Table 2. Metabolite composition of the EBN extracts analyzed by LCQTOF MS

EBN Extract	Retention Time (min)	Retention Drift (ms)	Compound Name	Response	Compound Nature
Pure EBN	16.69	8.76	PE 34:1	1106	Lipids
	16.82	9.24	PE(P-36:2)	1119	
	16.81	9.42	PG 36:2	1137	
	16.78	10.04	C22-OHSulfatide	1256	
	16.71	9.73	PE(P-40:2)	1584	
	7.56	6.86	LPE 18:0	1900	
	16.59	6.34	Cortisone	2121	
	18.52	10.07	PG 40:7	2188	
	16.78	9.78	C20 Sulfatide	2357	
	16.58	6.32	Tricosanoic Acid	4450	
	18.52	10.07	PG 38:6	12173	
	16.82	9.02	PE(P-34:2)	24462	
	16.72	8.94	PE(P-36:5)	47801	
	16.89	10.32	PI 38:4	328870	
	17.06	10.31	PI 38:4	1444359	
	6.02	5.38	Sinapaldehyde glucoside	1306	Glycosides
	0.5	8.58	Mannotriose	1558	Polysaccharides
	0.51	6.88		26209	
	0.46	7.99	β-Gentiobiose	1041	Dissacharides
	0.46	5.55		12608	

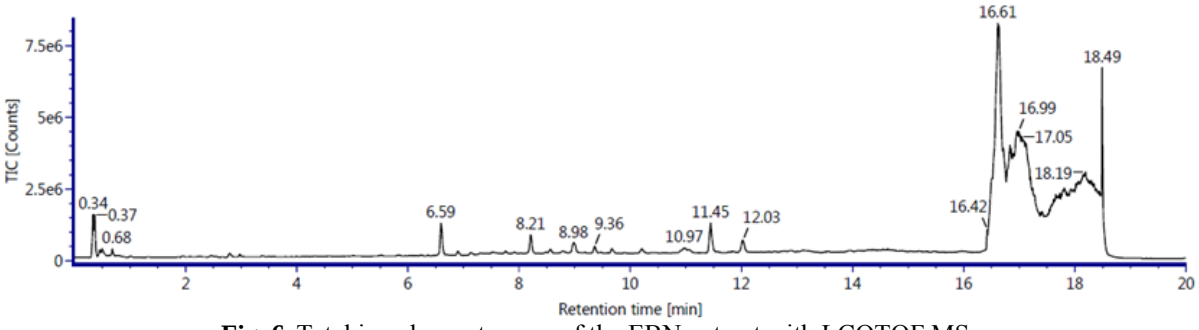

Fig. 6. Total ion chromatogram of the EBN extract with LCQTOF MS.

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The formation of an inhibitory zone on the nanometals sample for both infections, in contrast to the control, indicated that the silver had inhibited the microbe's development. The inhibition zone on S. aureus is larger (10 ± 0.2 mm) (a) in comparison to E. coli (9 ± 0.1 mm) (b) in Fig. 7. Results showed that AgNPs were more efficient against Gram-positive bacteria than Gram-negative ones. Positive bacteria are more vulnerable to AgNPs at higher concentrations of $AgNO_3$, despite the fact that their cell walls are stronger than negative bacteria's owing to the high peptidoglycan content. The antibacterial impact of silver ions released during AgNP production is achieved by interfering with DNA replication and breaking down bacterial cell walls.

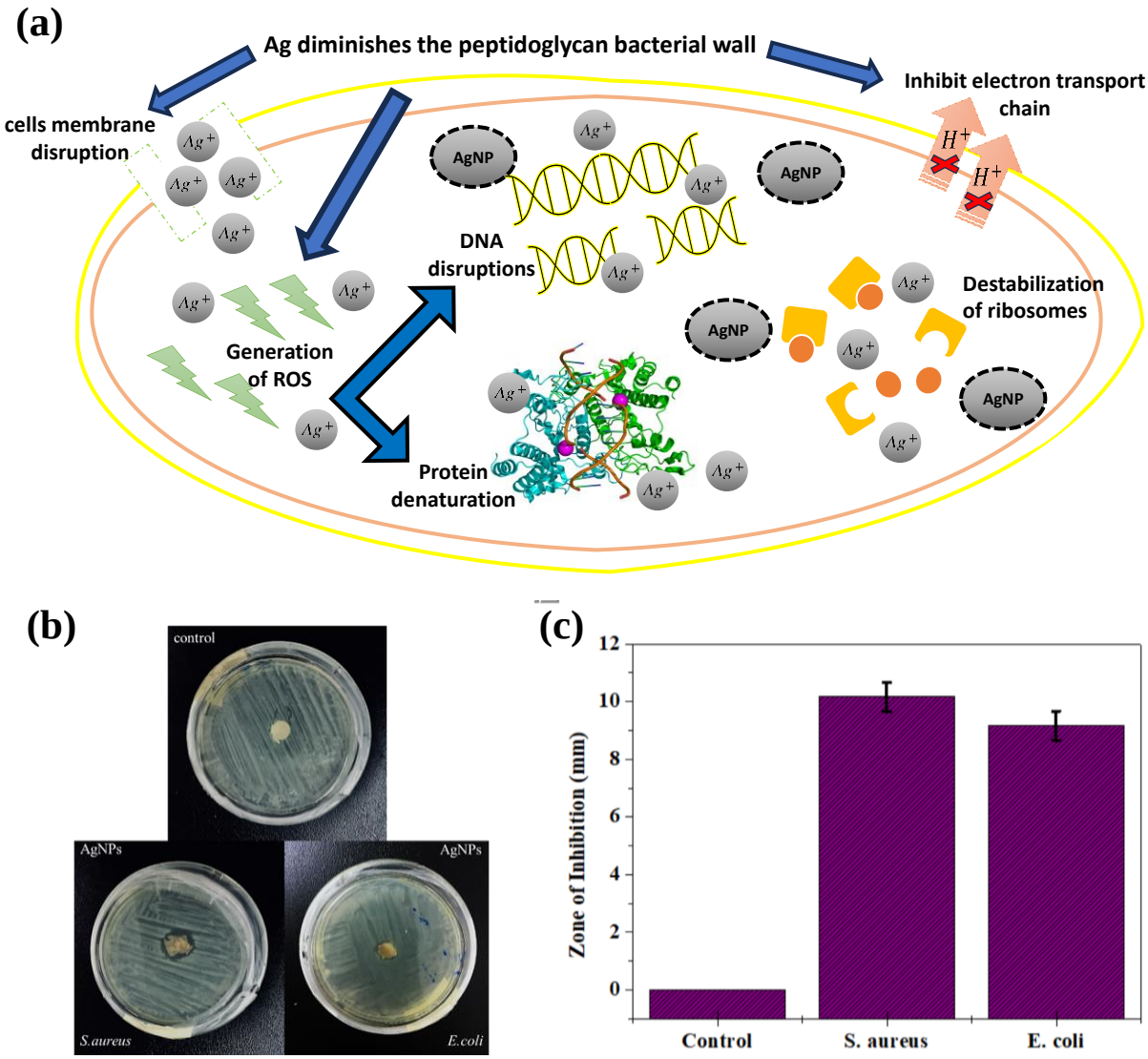

Fig. 7. (a) Theoretical framework explaining the mechanism behind the antibacterial uses of AgNPs. When nanoparticles contact with Ag-cell membranes, they disrupt the cell membrane's structure, causing alterations in its ability to allow substances to pass through and resulting in leaking of the cytoplasm. Additionally, it hampers the integrity of bacterial cell walls by impeding peptidoglycan, resulting in compromised bacterial structure. Silver ions promote the production of reactive oxygen species (ROS) inside cells, causing oxidative stress. This leads to two main effects: i) DNA damage, which results in genetic mutations that hinder replication and transcription processes, and ii) disruption of protein denaturation, affecting crucial bacterial metabolic pathways. Furthermore, the disruption of the electron transport chain, hinders the generation of energy and disturbs the functioning of ribosomes, leading to inaccurate protein synthesis. (b) Micrograph depicting the antibacterial activity, and (c) Figure illustrating the zone of inhibition of pure PVA as a negative control, and EBN/AgNPs, against S.aureus and E.coli.

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4. CONCLUSIONS

In this study, the researchers utilized the extract of the white-nest swiftlet (Aerodramus fuciphagus) to produce silver nanoparticles in a manner that is environmentally friendly, non-hazardous, and uncomplicated. The rapid and eco-friendly synthesis of durable AgNPs was achieved in just 15 minutes by introducing a solution containing 0.05 M of $AgNO_3$. The synthesized AgNPs were characterized using a UV-Visible spectrophotometer, FTIR method, FESEM, and EDX analysis. The chemical found in EBN has been shown to aid in the reduction and stabilization of AgNPs. Furthermore, the surface morphology seen using FESEM predominantly exhibited spherical forms with sizes ranging from 10 to 20 nm. The data analysis conducted by EDX revealed the presence of silver (Ag) in the EBN-AgNPs solution. Studies on bacteria have shown that Gram-positive bacteria were more efficiently eradicated by AgNPs compared to Gram-negative bacteria. Furthermore, the study sought to understand the mechanism of action and synthesis of AgNPs from the EBN extract, which offers a cost-effective and ecologically friendly approach to producing nanometals using green technology.

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