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Research Article

Green Synthesis of Silver Nanoparticles Using Extracts of *Ehretia cymosa* and Evaluation of Its Antibacterial Activity in Cream and Ointment Drug Delivery Systems

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Objectives. The use of antibacterial drugs for the treatment of infections has been on for several decades but not without some challenges such as resistance. Research on natural products is on-going to mitigate this challenge. The aim of this study was to synthesize silver nanoparticles (SNPs) with aqueous and methanol extract of *Ehretia cymosa* leaf and to explore its antibacterial potentials in semisolid dosage delivery system as topical antibacterial cream and ointment. *Methods*. *E. cymosa* leaf was extracted by macerating in distilled water and methanol. The extracts were used to synthesize SNPs. SNPs were characterized and confirmed by visual observation, UV-visible spectroscopy, FTIR, atomic absorption spectroscopy, scanning electron microscopy, and energy dispersive X-ray spectroscopy. SNPs were used to formulate cream and ointment, and the antibacterial activity of the formulations was evaluated against *Staphylococcus aureus* and *Escherichia coli*. *Results*. Absorption band was observed at 450 nm for aqueous extract SNPs and 420 nm for methanol extract SNPs due to surface plasmon resonance. SNPs were agglomerated with the irregular size of 55 nm and 90 nm. The formulations had acceptable physicochemical properties with good drug-excipient compatibility. The antibacterial activity of cream formulations had a significantly ($p < 0.0001$) higher antibacterial activity compared to ointment formulations. Both formulations with SNPs had higher antibacterial activity than ciprofloxacin. *Conclusion*. Cream and ointment formulations loaded with green synthesized *E. cymosa* leaf extract SNPs present a potential for a more efficient and effective antibacterial drug delivery to ameliorate the impact of antibacterial drug resistance.

1. Introduction

The world's need for antibacterial drugs has continued to grow exponentially since the 20th century when antibacterial infections were discovered; they have been put to use successfully in the treatment of infectious diseases [1, 2]. The combined positive impacts of these "Miracle Products" coupled with advances in immunizations and other proper environmental conditionings such as sanitation, housing, and food have resulted in a dramatic drop in deaths from diseases that sometime ago were widespread and often fatal [3]. However, the continued usage of these products has led to the challenge of increasing resistant pathogenic organisms which limits the effectiveness of these products in the treatment of infectious diseases [4, 5]. Infections caused by these resistant microorganisms do not respond to hitherto antibacterial drugs that have been used to eliminate them previously; these have resulted in prolongation of these organisms on their host; consequently, the organisms become very virulent [6]. More people will likely be affected by these resistant strains since it will have become widely spread and actively circulating in the community. Second and third generation antibiotics have been used promptly to mitigate the development of resistance but it is not hundred percent effective [7]. With this situation at hand, there is a need for continual research into new antibacterial drugs.

Historically, natural products have been used in traditional medicine successfully for centuries in the treatment of diseases [8–12]. Several studies are available in the literature comparing antimicrobial properties of natural products with commercially available synthetic drugs [13–17].

Ehretia cymosa (*E. cymosa*) is a deciduous shrub in the family Boraginaceae. The leaves are simple, broad in shape, glossy, and dark green in colour [18]. The leaves and other parts of the plant have been reported to be used traditionally for the treatment of measles, diarrhea, epilepsy, convulsions, spasm, venereal diseases, dry cough, respiratory infections, asthma, malaria, tonsils, mental problems, typhoid, wounds, and aphrodisiac [19–23]. Pharmacological studies of the crude extracts or individual compounds from *E. cymosa* confirmed antioxidant, antiinflammatory, antiallergic, antibacterial, and antitubercular activities, as well as antisnake venom property [24]. The antibacterial activity of the ethanol extract of the plant on Gram-negative and Gram-positive bacteria was reported by Sarkodie et al. [25].

Biomolecules present in plant extracts and microorganisms can be used to reduce metal ions to metal nanoparticles (MNPs) in a single-step and biosynthetic process [26]. Biosynthesis of MNPs has always been more beneficial economically because it requires less energy and is more ecofriendly; therefore, it will be free of toxic contaminants as required in therapeutic applications (Madkour, 2018). Recently, it was established that biosynthesis of nanomaterial-based antimicrobials is effective against a wide range of microorganisms and also to combat the emergence of multidrug resistant [27]. Silver nanoparticles (SNPs) are obtained by the reduction of silver ion to silver atom, and it has been reported to possess the ability of preventing the development of drug resistance because of its high antibacterial efficiency [28–30]. Creams and ointment are semisolid preparations intended for topical application to the skin or mucous membrane usually for local or systemic use. They possess numerous advantages including ease of access, self-administration, noninvasiveness, and reduced adverse reaction over other dosage forms.

The objective of the study was to characterize SNPs synthesized with aqueous and methanol extract of *E. cymosa* leaves and to explore its antibacterial potentials in the semisolid dosage delivery system as topical antibacterial creams and ointment.

The hypothesis that guides the study is how to explore natural products in developing an efficient and effective antibacterial drug delivery product that will prevent the development of antibacterial drug resistance.

2. Materials and Methods

2.1. Medicinal Plant Collection and Identification. *E. cymosa* plant leaves were collected in May 2019 from the wild in Ibadan, Oyo State, Nigeria, and identified at the Forest Research Institute of Nigeria (FRIN), Ibadan, with voucher No. 112440.

2.2. Bacterial Strain. Bacterial strains *Staphylococcus aureus* (*S. aureus* ATCC 25923) and *Escherichia coli* (*E. coli* ATCC 25922) and a total of 20 clinical isolates were collected from Ogun State University Teaching Hospital (OOUTH), Sagamu (Ten (10) isolates each of *S. aureus* and *E. coli*, respectively).

2.3. Extraction of the Crude Extract. *E. cymosa* leaves were dried and milled into powder with a blender (Model 857 Williamette Industries, Bowing Green Kentucky USA). The powdered leaves (400 mg) were then extracted by macerating separately in 2,400 mL of distilled water and methanol, respectively, for 72 h. The filtrates were concentrated to dryness with a rotary evaporator (Buchi Model R210, Switzerland).

2.4. Phytochemical Screening. Phytochemical screening of the crude extract was performed to test for the presence of alkaloids, tannins, saponins, glycosides, steroids, antraquinones, flavonoids, and steroids using standard methods [31, 32].

2.5. Preparation of Culture. Two samples of typed culture of *E. coli* and *S. aureus* and ten (10) clinical samples each for *E. coli* and *S. aureus* were collected from Ogun State University Teaching Hospital (OOUTH) Sagamu Microbiology Laboratory Department. The microorganisms were collected as probable samples of *S. aureus* and *E. coli* submitted for medical investigations. The microorganisms were further confirmed by standard biochemical tests and morphological studies. The microorganisms were cultured on nutrient agar from where their microbial suspensions were made using sterile relevant broths at a concentration that was adjusted to 0.5 McFarland standards, of approximately 10^6 CFU/mL.

2.6. Antimicrobial Activity of Crude Extract. The method used by Sarkodie et al. [25] was adapted. Three different concentrations of sterile solutions of the plant extract were prepared (10, 15 and 20 mg/mL) using water and methanol, respectively. Molten agar stabilized at 45°C was then seeded with 0.1 mL inocula of the 24 hour nutrient broth cultured of the test organism, and then the mixtures were properly mixed. The resultant mixture was aseptically transferred into a Petri dish and allowed to set. Thereafter, a 10 mm cork borer was used to bore 6 holes in the agar equidistant from each other. The holes were filled with 200 μL of the respective concentrations of the test extract, distilled water (as the negative control), and 2 mg/mL ciprofloxacin infusion (as the positive control). The Petri dishes were then incubated at 37°C for 24 h. This experiment was repeated in triplicate for *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922). The 3 different concentrations of both the aqueous and the methanol extracts were tested.

2.7. Synthesis of Silver Nanoparticles. About 0.1 g of the powdered extract was dissolved in 100 mL of distilled water at 60°C for 1 h to give 0.1% W/V concentration; it was allowed to cool and then filtered with Whatman No. 1 paper. The filtrate was centrifuged at 4000 rpm for 15 min. Thereafter, 1 ml of the centrifuged filtrate was transferred into 40 mL of 1 mM aqueous silver nitrate solution in 250 mL Erlenmeyer flasks and incubated for 72 h. The mixture was observed for colour change every 24 h. After incubation, the mixture containing the biosynthesized SNP was centrifuged and the pellets obtained were freeze-dried and stored [33].

2.8. Characterization of E. cymosa-Synthesized Silver Nanoparticles

2.8.1. Visual Observation. SNPs synthesized were observed visually for colour change every 24 h for 72 h as the synthetic reaction progresses.

2.8.2. UV-Visible Spectroscopy Analysis. The synthesis of *E. cymosa* SNPs was monitored by measuring the absorption spectra in the wavelength range of 300–700 nm using the T90+ UV/Vis spectrometer, and the spectrum was recorded, and the maximum absorption wavelength was determined.

2.8.3. Fourier Transform Infrared (FTIR) Spectroscopy Analysis. SNPs synthesized were also characterized by Transform infrared (FTIR) spectroscopy using the PerkinElmer spectrum spectrometer version 10.03.02 to detect the biomolecules responsible for the biosynthesis of the SNPs.

2.8.4. Atomic Absorption Spectroscopy (AAS) Analysis. The concentration of silver ions (Ag^+) as the synthetic reaction progresses was examined by atomic absorption spectroscopy (AAS Model 200 series Buck Scientific, USA) to confirm its conversion into silver atom Ag° nanoparticle. A standard solution of 10 ppm of silver nitrate solution was analyzed with AAS initially before adding the crude extracts of *E. cymosa* leaf. The concentration of the silver ion in the reaction solution after adding the crude extract was analyzed every 24 h for 72 h.

2.8.5. Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray Spectroscopy (EDX) Analysis. The size, morphology, and elemental analysis of SNP was conducted using Phenom Pro X (Phenom-World BV, Netherlands) SEM/EDX. The microscope is fitted with a CeB6 electron source and a 4-segment back-scattered electron detector at a resolution of ≤10 nm. A silicon drift detector is fitted for EDX analysis at energy resolution of Mn K$\alpha \leq 137$ eV.

2.9. Antibacterial Activity of SNP. Three different concentrations of synthesized SNP were obtained using different concentration of the crude extract (10, 15, and 20 mg/mL). Molten agar stabilized at 45°C was then seeded with 0.1 mL inocula of the 24 h nutrient broth cultured of the test organism. The mixture of the agar and organisms were properly mixed. The resultant mixture was aseptically transferred into a Petri dish and allowed to set. Thereafter, a 10 mm cork borer was used to bore 6 holes in the agar equidistant from each other. The holes were filled with 200 μL of the respective concentrations of the test extract, distilled water (as the negative control), and 2 mg/mL ciprofloxacin infusion (as the positive control). The Petri dishes were then incubated at 37°C for 24 h. This experiment was repeated in triplicate for *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922).

The experiment was carried out using both the aqueous and methanol SNP.

2.10. Preparation of Formulation

2.10.1. Cream Preparation. Thirty (30) grams of emulsifying ointment was heated on a water bath at 60°C. 0.1 g of chlorocresol was dissolved with the required quantity of distilled water in a beaker heated at 60°C. At same temperature of 60°C, chlorocresol solution was added into the emulsifying ointment with vigorous stirring then followed by the addition of different concentrations (1, 2, and 3%) of the crude extract or the SNP. Similar procedure was used to formulate 1% ciprofloxacin creams.

2.10.2. Ointment Preparation. Five (5) grams of wool fat, hard paraffin, cetostearyl alcohol, and required quantity of white soft paraffin were melted together in a crucible dish at 70°C with continuous stirring. Before the melted mixture sets, different concentrations (1, 2, and 3%) of the crude extract or SNP were added and stirred. Similar procedure was used to formulate 1% ciprofloxacin ointment.

2.11. Evaluation of Formulations

2.11.1. Physical Evaluation of Formulations. The physical properties of the formulations such as the texture, homogeneity, colour, and washability were evaluated by visual

examination. Small quantity of both the cream and ointment was rubbed between the thumb and index finger to determine their texture and homogeneity [34, 35].

2.11.2. pH Determination. About 500 mg of the cream formulation was mixed with 50 mL of water in a beaker. The ointment formulation was heated on water bath at 60–70°C [35]. The pH of the formulations was determined by using a pH meter (pH600 digital pocket sized, Milwaukee). The determinations were carried out in triplicate.

2.11.3. Spreading Time Analysis of Formulations. Two glass slides were compressed with a uniform weight of the creams/ointments for 5 min. The time required for the upper glass slide to move over the entire length of the lower glass slide was recorded as the spreading time [36].

2.11.4. Viscosity of Formulations. Brookfield viscometer (Model-DV-11 + Pro, Brookfield Eng. Labs Inc Middleboro, MA, USA) was used to measure the viscosity of the formulated creams and the ointments. The spindle size used was number 6 at 20 rpm, 50 rpm, and 100 rpm at 25°C [37].

2.11.5. Dye Test. The dye test was performed using amaranth solution. The dye was mixed with the cream and ointment. A drop of each of the mixture was placed on a microscope slide and covered with a cover slip. It was then examined using a light microscope, Model CX21FS1, Olympus Corporation. If the drop appears red in a colourless background (continuous phase), the formulation is water-in-oil type and on the hand, if the drop appears colourless in a red background, the formulation is oil-in-water type [38].

2.11.6. Compatibility Studies. The method described by Adeleye et al. [39] was used. FTIR spectroscopic was used to analyze the synthesized SNP and the medicated creams/ointment formulation by the KBr method with PerkinElmer Spectrum spectrometer version 10.03.02 to detect any remarkable shift in absorption peaks.

2.12. Determination of the Antibacterial Activity of the Formulations. The test samples, namely, ACO1, ACO2, and ACO3 represented 1%, 1.5%, and 2% aqueous crude extract ointment, respectively; MCO1, MCO2, and MCO3 represented 1%, 1.5%, and 2% methanol crude extract ointment, respectively; ASO1, ASO2, and ASO3 represented 1%, 1.5%, and 2% aqueous SNPs ointment, respectively, and MSO1, MSO2, and MSO3 represented 1%, 1.5%, and 2% methanol SNP ointment, respectively; Cipro represented 1% ciprofloxacin (positive control) tested against *E. coli* and *S. aureus* typed/clinical samples. Microorganism culture was prepared as stated in Section 2.5, and the antibacterial activity of the formulations was determined using the method described by Sarkodie et al. [25].

2.12.1. Agar Diffusion Method. The cream and ointment samples were tested for their antibacterial activities. The agar diffusion method was used for antibacterial screening as follows: a sterile Mueller–Hinton agar medium was used for the antibacterial assay. The media were prepared and allowed to solidify in the plates, and then 0.1 mL of the microbial suspension (10^6 CFU/mL) was streaked over the surface of the medium using a sterile glass spreader. The well was made by using cork borer (6 mm) under aseptic conditions, and then each of the samples was put in each well. The plates were then incubated for 24–48 hours at 37°C in order to get reliable microbial growth. The resultant microbial inhibition zones were measured using a ruler.

2.12.2. Determination of Minimum Inhibitory Concentration (MIC). MIC was determined for the *E. cymosa* leaf crude extracts which were extracted by water/methanol (ACC and MCC) and the water/methanol crude extract loaded with *E. cymosa* leaf extract mediated-SNPs (ASC and MSC) for both *E. coli* and *S. aureus* typed/clinical samples by broth dilution method as described by Cheesbrough [40].

2.13. Statistical Analysis. The experimental data were expressed as the mean ± standard error of mean, and the statistical significance of the antibacterial properties of the formulations was evaluated by Student's *t*-test and one-way analysis of variance followed by Tukey's post hoc test with GraphPad Prism 5 version 5.01 software. Values with $p < 0.05$ were considered to be statistically significant.

3. Results

3.1. Phytochemical Screening. The phytochemical screening of the extracts of *E. cymosa* leaf showed the presence of alkaloids, tannins, saponins, glycosides, anthraquinones, and flavonoids in the aqueous extract while alkaloids, tannins, saponins, glycosides, anthraquinones, flavonoids, and steroids are present in the methanol crude extract.

3.2. Antibacterial Screening of the Crude Extract. The zone of inhibition of the antibacterial activity of the aqueous and methanol crude extract of *E. cymosa* leaf as presented in Table 1 revealed a significantly increase in activity ($p < 0.0001$) with increase in concentration of the extracts. However, methanol extract showed significant higher activity ($p < 0.0001$) compared with aqueous extract. The positive control had significantly higher activity ($p < 0.0001$) on both organisms than both extracts.

3.3. Synthesis of Silver Nanoparticles. The mixture of *E. cymosa* leaf with silver nitrate solution resulted in colour change from light green to brown with the aqueous extract while there was a colour change from oxblood to greyish green with the methanol extract which was visually observed indicating the synthesis of SNPs. The intensity of the colours deepened as the reaction time progresses (Figures 1(a) and 1(b)).

Table 1: The zone of inhibition (mm) obtained with different concentrations of *E. cymosa* crude extracts in aqueous and methanol solvent.

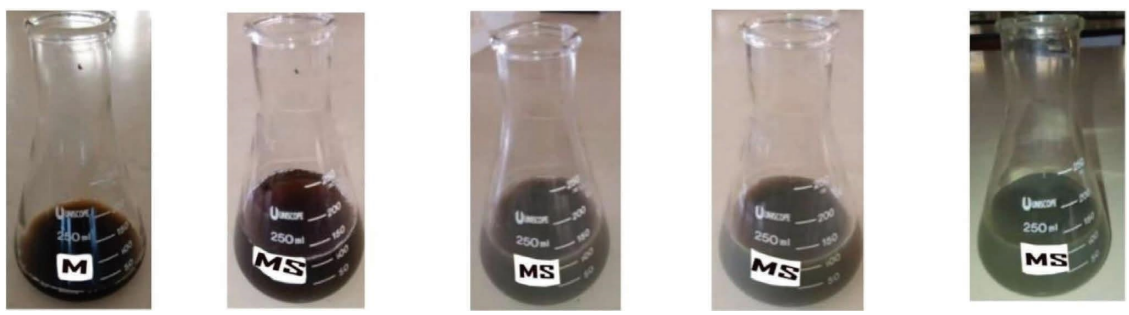
Crude extract	Concentration (% w/v)	Microorganisms	
		S. aureus ATCC 25923	*E. coli* ATCC 25922
DS	0	—	—
AC1	1	12.8 ± 0.11	10.2 ± 0.04
AC2	1.5	13.5 ± 0.03	10.9 ± 0.59
AC3	2	14.1 ± 0.20	11.4 ± 0.02
MC1	1	13.9 ± 0.30	10.8 ± 0.14
MC2	1.5	15.3 ± 0.12	11.9 ± 0.10
MC3	2	17.4 ± 0.07	13.8 ± 0.05
CI	1	21.2 ± 0.03	19.1 ± 0.06

Note. DS: distilled water (negative control), AC1, AC2, and AC3: 1%, 1.5%, and 2% aqueous crude extract, MC1, MC2, and MC3: 1%, 1.5%, and 2% methanol crude extract, respectively, and CI: 2 mg/mL ciprofloxacin infusion (positive control).



Aq. Extract | Aq. Ext+$AgNO_3$ (0 hr) | Aq. Ext+$AgNO_3$ (24 hr) | Aq. Ext+$AgNO_3$ (48 hr) | Aq. Ext+$AgNO_3$ (72 hr)

(a)



Met. Extract | Met. Ext+$AgNO_3$ (0 hr) | Met. Ext+$AgNO_3$ (24 hr) | Met. Ext+$AgNO_3$ (48 hr) | Met. Ext+$AgNO_3$ (72 hr)

(b)

Figure 1: Progressive colour changes as evidence of synthesis of silver nanoparticles. Synthetic progress of SNPs with (a) aqueous extract and (b) methanol extract. Aq, aqueous; Met, methanol.

3.4. UV-Visible Analysis. The UV-Vis absorption spectra of the synthesized SNPs as shown in Figures 2(a) and 2(b) revealed peak absorption wavelength of *E. cymosa* aqueous and methanol leaf extract which are 450 nm and 420 nm, respectively.

3.5. Fourier Transform Infrared (FTIR) Spectroscopy Analysis. The FTIR spectra of the aqueous and methanol extract SNPs are shown in Figures 3(c) and 3(f), respectively. There is a noticeable peak around 3400 cm^{-1} which is attributed to the stretching frequency. A little broadness observed in these peaks is due to the intermolecular hydrogen bonding. The prominent peak around 1620 cm^{-1} is due to the -C=O (carbonyl) stretching frequency while the peak observed around 1050 $cm^{-}$is due to -C-O- bonding frequency. The result of this study is in agreement with previous work [41, 42].

3.6. Atomic Absorption Spectroscopy (AAS) Analysis. A steady decline in the silver ion concentration in the presence of the aqueous and methanol extracts-silver nitrate solution mixture was observed as a function of time. Lowest concentrations of 0.83 ppm and 0.04 ppm were recorded after 72 h of reaction for aqueous extract-silver nitrate solution and methanol extract-silver nitrate solution, respectively. This indicates a faster bioreduction rate of reaction for methanol extract than the aqueous extract component.

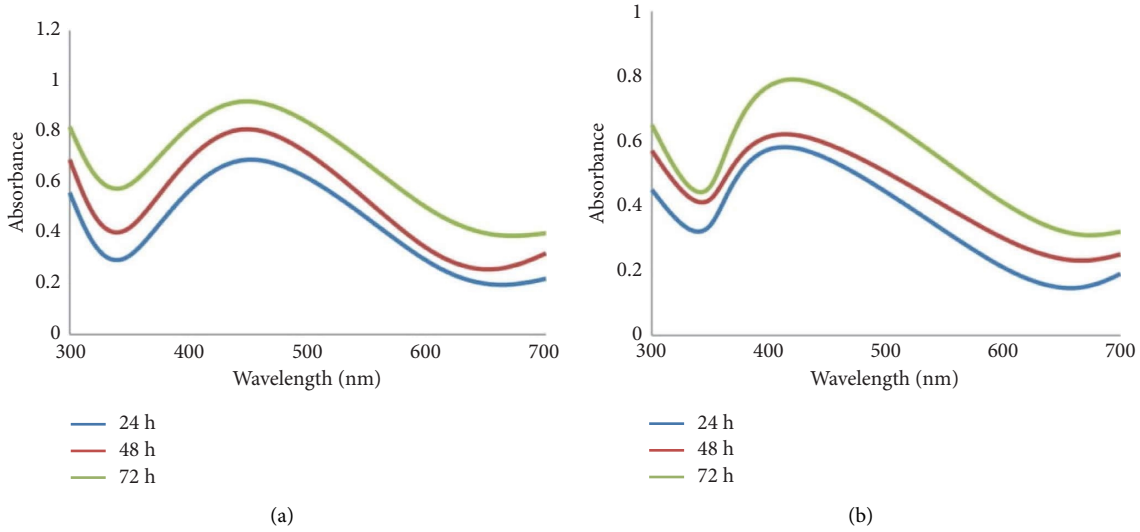


Figure 2: UV-Vis spectrum of (a) aqueous extract SNP and (b) methanol extract SNP.

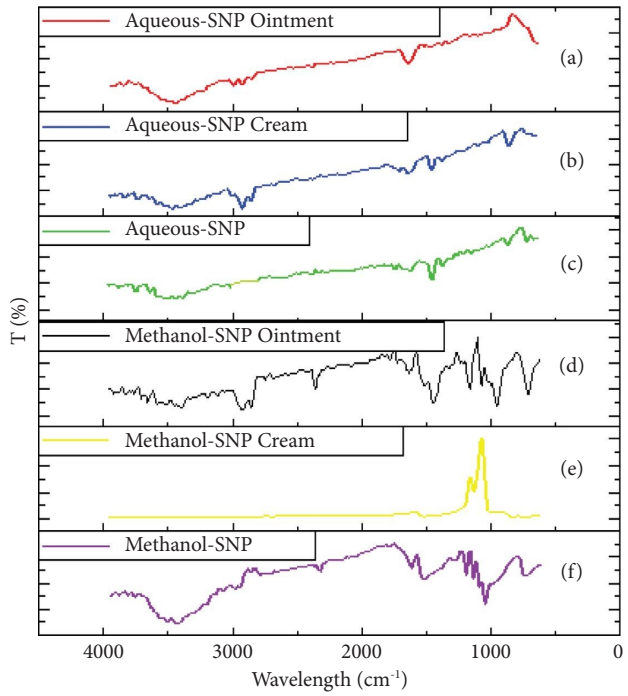
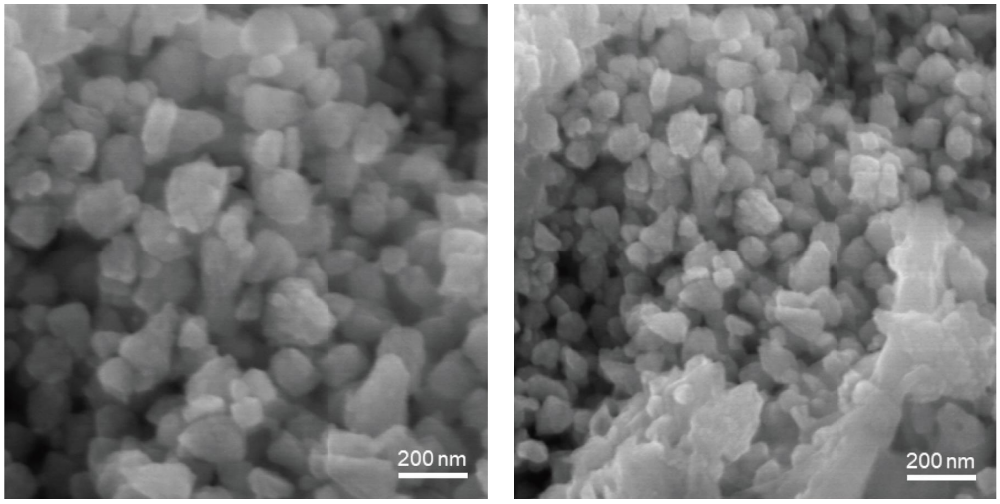


Figure 3: Wavelength cm^{-1}.

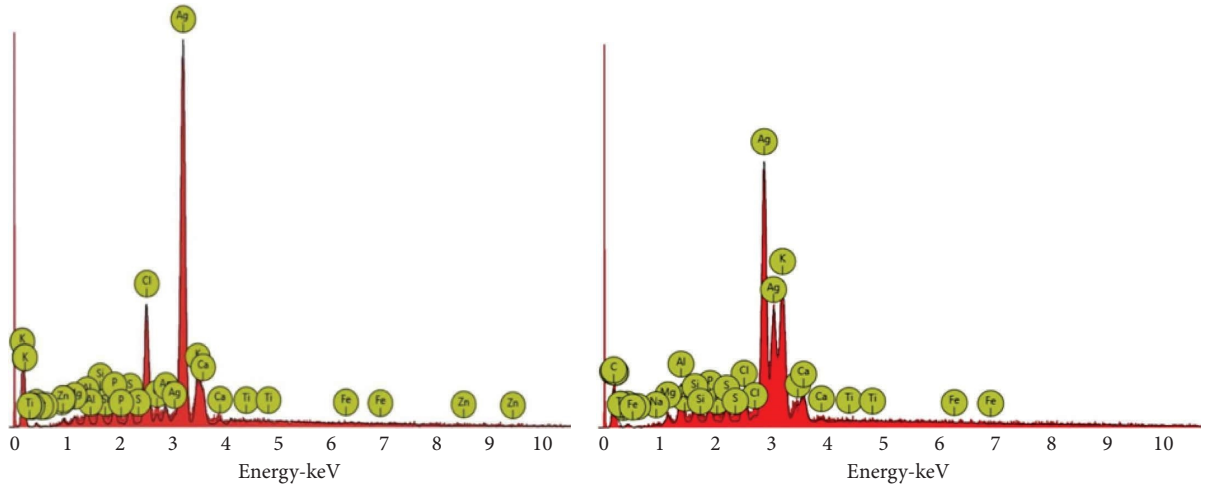
3.7. Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray Spectroscopy (EDX) Analysis. The particle sizes and morphologies of the SNPs are shown in Figure 4. The particle sizes ranged between 50 and 100 nm as a result of agglomeration of the particles, and the methanol SNP particle sizes were smaller when compared with that aqueous extract SNP. The particles were aggregated as a cluster with an irregular shape. Figures 5(a) and 5(b) show the EDX spectrum of the SNPs revealing silver as the major element present in the nanoparticle. The aqueous and methanol extract SNPs have silver element weight concentration of 51.26% and 66.43%, respectively. Other elements present in both extract SNPs are potassium, oxygen, nitrogen, calcium, carbon, and sulphur.

3.8. Antimicrobial Activity of Silver Nanoparticles. The zone of inhibition of the antibacterial activity of the aqueous and methanol extract SNPs of *E. cymosa* is presented in Table 2. There was a significant increase in antibacterial activity ($p < 0.0001$) of both the aqueous and methanol extract SNPs with increase in concentration. Methanol extract SNPs generally showed significant higher activity ($p < 0.0001$) than aqueous extract SNPs. Although the positive control



(a) (b)

Figure 4: SEM micrographs of (a) aqueous extract SNPs and (b) methanol extract SNPs.



(a) (b)

Figure 5: EDX spectrum of (a) aqueous extract SNPs and (b) methanol extract SNPs.

Table 2: The zone of inhibition (mm) obtained with different concentrations of the synthesized silver nanoparticles.

Silver nanoparticle	Concentration	Microorganisms	
		S. aureus ATCC 25923	*E. coli* ATCC 25922
DS	0	—	—
AS1	1	15.9 ± 0.30	14.8 ± 0.43
AS2	1.5	16.4 ± 0.70	15.7 ± 0.33
AS3	2	17.6 ± 0.35	16.5 ± 0.19
MS1	1	18.4 ± 0.16	16.2 ± 0.67
MS2	1.5	22.0 ± 0.12	18.3 ± 0.02
MS3	2	23.1 ± 0.27	19.7 ± 0.11
CI	1	21.2 ± 0.03	19.1 ± 0.06

Note. DS: distilled water (negative control), AS1, AS2, and AS3: 1%, 1.5%, and 2% aqueous SNPs, MS1, MS2, and MS3: 1%, 1.5%, and 2% methanol SNPs, respectively, and CI: 2 mg/mL ciprofloxacin infusion (positive control).

had higher activity on both organisms than aqueous extract SNPs, the methanol extract SNPs had comparative activity against *E. coli* ATCC 25922 and significantly higher activity against *S. aureus* ATCC 25923.

3.9. Physical Evaluation of Formulations. All the formulations prepared with aqueous crude extract were white in colour, all those formulated with aqueous SNPs were light brown, and those formulated with ciprofloxacin were white. All the formulations prepared with methanol crude extract were light brown in colour, all those formulated with methanol SNPs were light green, and those formulated with ciprofloxacin were white. The formulations were soft and smooth in texture, homogenous in appearance, easy to apply, and easy to wash except the ointment formulations.

3.10. pH of Formulations. The pH of the formulations as presented in Tables 3 and 4 was between 5.2 ± 0.10 and 6.8 ± 0.51. The pH of the ointment formulations was relatively higher than the pH of the cream formulations.

3.11. Spreading Time of Formulations. The spreading times of the formulations were found to be in the range of 7.6 ± 0.09 and 18.2 ± 2.22 for the cream formulations and 13.1 ± 0.62 and 28.8 ± 1.86 for the ointments formulations as presented in Tables 3 and 4.

3.12. Viscosity of Formulations. The viscosities of the cream and ointments formulations are shown in Figure 6. The viscosities of both formulations decreased with increase in shear speed. The viscosities of ointment formulations were higher than viscosities of cream formulations.

3.13. Dye Test. The cream formulation appeared in a red background indicating an oil-in-water type formulation while the ointment formulation appeared in a colourless background indicating a water-in-oil type formulation.

3.14. Compatibility Studies. The positions of the characteristic peaks in the FTIR spectra as shown in Figure 3 were considered to analyze the compatibility between the synthesized SNPs with the cream and ointment bases. Figures 3(a) and 3(b) are the spectra of ointment and cream formulated with aqueous extract SNPs, respectively, while Figures 3(d) and 3(e) are the spectra of ointment and cream formulated with methanol extract SNPs, respectively. The result revealed no obvious significant shift in the positions of the characteristic peaks in the spectrum of the synthesized SNPs with the cream and ointment bases.

3.15. Antimicrobial Activity of the Formulations. The antibacterial activity of the formulations was analyzed from the zones of inhibition obtained from the wells on plate. The zone of inhibition of the cream and ointment is presented in Tables 5 and 6, respectively. For both formulations, there was significant increase in antibacterial activity with increase in concentration of the extract and SNPs. The antibacterial activity of the cream formulations was significantly ($p < 0.0001$) higher than that of the ointment formulations.

Table 3: pH and spreading time of cream formulations.

Formulations	pH	Spreading time (sec)
CB	5.4 ± 0.12	18.2 ± 2.22
ACC1	5.6 ± 0.65	17.9 ± 0.78
ACC2	5.8 ± 0.02	16.3 ± 1.47
ACC3	5.9 ± 0.00	14.0 ± 3.05
MCC1	5.5 ± 0.14	14.3 ± 1.63
MCC2	5.9 ± 0.07	13.7 ± 0.80
MCC3	5.7 ± 0.02	11.7 ± 0.14
ASC1	6.2 ± 0.16	11.4 ± 4.42
ASC2	6.0 ± 0.30	10.8 ± 3.07
ASC3	5.8 ± 0.05	9.5 ± 5.71
MSC1	5.9 ± 0.08	9.3 ± 2.22
MSC2	5.6 ± 0.21	8.9 ± 2.80
MSC3	5.2 ± 0.10	7.6 ± 0.09
CC	6.4 ± 0.62	13.5 ± 1.63

Note. CB: cream base (negative control), ACC1, ACC2, and ACC3: 1%, 1.5%, and 2% aqueous crude extract cream, respectively, MCC1, MCC2, and MCC3: 1%, 1.5%, and 2% methanol crude extract cream, respectively, ASC1, ASC2, and ASC3: 1%, 1.5%, and 2% aqueous SNPs cream, respectively, MSC1, MSC2, and MSC3: 1%, 1.5%, and 2% methanol SNPs cream, respectively, and CC: 1% ciprofloxacin cream (positive control).

Table 4: pH and spreading time of ointment formulations.

Formulations	pH	Spreading time (sec)
OB	6.3 ± 0.62	28.8 ± 1.86
ACO1	6.2 ± 0.02	25.9 ± 3.22
ACO2	6.5 ± 0.17	24.6 ± 6.03
ACO3	6.8 ± 0.13	22.9 ± 1.27
MCO1	6.6 ± 0.22	22.2 ± 3.16
MCO2	6.8 ± 0.51	21.4 ± 0.10
MCO3	6.7 ± 0.16	21.2 ± 0.73
ASO1	6.5 ± 0.11	18.9 ± 2.27
ASO2	6.3 ± 0.32	18.1 ± 5.14
ASO3	6.1 ± 0.72	16.6 ± 1.04
MSO1	6.0 ± 0.27	15.0 ± 2.30
MSO2	6.1 ± 0.09	15.7 ± 2.02
MSO3	5.9 ± 0.24	13.1 ± 0.62
CO	6.8 ± 0.18	22.3 ± 4.31

Note. OB: ointment base (negative control), ACO1, ACO2, and ACO3: 1%, 1.5%, and 2% aqueous crude extract ointment, respectively, MCO1, MCO2, and MCO3: 1%, 1.5%, and 2% methanol crude extract ointment, respectively, ASO1, ASO2, and ASO3: 1%, 1.5%, and 2% aqueous SNPs ointment, respectively, MSO1, MSO2, and MSO3: 1%, 1.5%, and 2% methanol SNPs ointment, respectively, and CO: 1% ciprofloxacin ointment (positive control).

3.15.1. Minimum Inhibitory Concentration. The MIC results showed that the minimum concentration of the formulated *E. cymosa* leaf loaded SNPs, the positive control drug (ciprofloxacin), and the aqueous extract that is needed to inhibit the test microorganisms is the same. A decrease value as low as four fold of the concentration was required by the methanol extract loaded creams. This is presented in Tables 7 and 8.

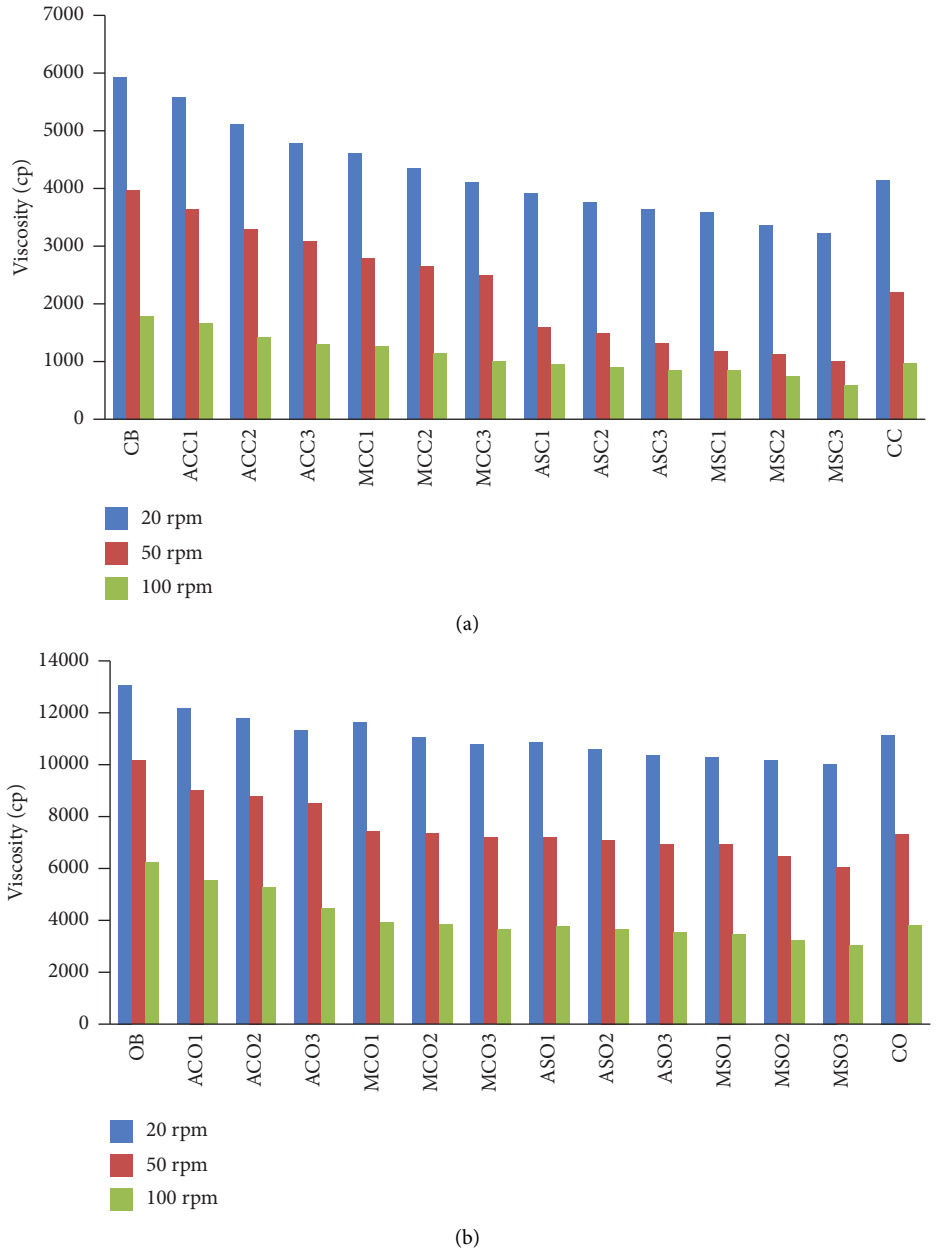


Figure 6: Viscosities of formulations: (a) cream formulation and (b) ointment formulation.

4. Discussion

The phytochemical analysis of the aqueous and methanol extracts of *E. cymosa* leaf showed the presence of alkaloids, tannins, saponins, glycosides, and flavonoids, while only the methanol extracts indicated the presence of steroids. The presence of these phytochemicals in crude extracts of plants accounts for their biological activity as antibacterial agents [39, 43], and they may also be involved in the reduction of silver ions to silver nanoparticles by acting as reducing and stabilizing agents [44–46].

The synthesis of SNPs was observed visually as the colour of the reaction mixture changed indicating the formation of SNPs [47, 48]. UV-visible spectrophotometric analysis was then used to confirm the synthetic reaction. Absorption band was visible in regions of 300 nm–700 nm, and peak absorbance was observed at 450 nm for aqueous extract SNPs and 420 nm for methanol extract SNPs due to surface plasmon resonance (SPR) of the SNPs. The SPR absorption band due to free electrons from metal nanoparticles results in the mutual vibration of electrons of nanoparticles in resonance with light wave [49]. This band corresponds to the absorption of SNPs in the region of 400–450 nm. The synthesized SNPs were consequently confirmed by the UV-visible spectrophotometric analysis with absorption peaks of 420 nm and 450 nm.

FTIR spectroscopy was used to identify the functional groups responsible for the reduction and capping of silver

Table 5: The zone of inhibition (mm) obtained with different concentrations of the *E. cymosa* for the crude extracts and silver nanoparticles loaded cream formulations.

Drugs tested	% concentration of the active ingredients	Strains of microorganisms used			
		S. aureus		*E. coli*	
		Clinical isolates	ATCC 25923	Clinical isolates	ATCC 25922
CB	0	2.4 ± 0.12	2.1 ± 0.10	2.2 ± 0.20	2.0 ± 0.08
ACC1	1	7.5 ± 0.16	7.2 ± 0.16	4.8 ± 0.15	4.8 ± 0.15
ACC2	1.5	7.4 ± 0.15	7.2 ± 0.10	5.0 ± 0.32	4.8 ± 0.32
ACC3	2	9.2 ± 0.05	8.9 ± 0.25	5.9 ± 0.05	5.6 ± 0.82
MCC1	1	8.8 ± 0.21	8.4 ± 0.90	5.6 ± 0.28	5.4 ± 0.10
MCC2	1.5	10.4 ± 0.22	10.5 ± 0.22	7.1 ± 0.17	6.9 ± 0.16
MCC3	2	12.2 ± 0.30	11.9 ± 0.35	8.6 ± 0.30	8.7 ± 0.30
ASC1	1	12.4 ± 0.13	12.0 ± 0.22	8.8 ± 0.56	8.6 ± 0.42
ASC2	1.5	13.0 ± 0.20	13.1 ± 0.82	9.5 ± 0.08	9.5 ± 0.08
ASC3	2	14.8 ± 0.18	14.2 ± 0.44	10.2 ± 0.46	10.1 ± 0.10
MSC1	1	14.6 ± 0.15	14.4 ± 0.50	11.0 ± 0.51	10.9 ± 0.50
MSC2	1.5	15.7 ± 0.72	15.0 ± 0.10	11.6 ± 0.80	11.5 ± 0.32
MSC3	2	17.5 ± 0.61	17.2 ± 0.50	12.8 ± 0.07	12.5 ± 0.15
CC	1	14.8 ± 0.08	14.6 ± 0.26	12.2 ± 0.17	12.2 ± 0.26

Note. CB: cream base (negative control), ACC1, ACC2, and ACC3: 1%, 1.5%, and 2% aqueous crude extract cream, respectively, MCC1, MCC2, and MCC3: 1%, 1.5%, and 2% methanol crude extract cream, respectively, ASC1, ASC2 and ASC3: 1%, 1.5%, and 2% aqueous SNPs cream, respectively, MSC1, MSC2, and MSC3: 1%, 1.5%, and 2% methanol SNPs cream, respectively, and CC: 1% ciprofloxacin cream (positive control).

Table 6: The zone of inhibition (mm) obtained with different concentrations of the *E. cymosa* for the crude extracts and silver nanoparticles loaded ointment formulations.

Drugs tested	% concentration of the active ingredient	Strains of microorganisms used			
		S. aureus		*E. coli*	
		Clinical isolates	ATCC 25923	Clinical isolates	ATCC 25922
OB	0	—	—	—	—
ACO1	1	5.4 ± 0.13	5.2 ± 0.15	2.9 ± 0.15	2.8 ± 0.05
ACO2	1.5	6.6 ± 0.09	6.2 ± 0.12	3.0 ± 0.61	2.8 ± 0.30
ACO3	2	7.9 ± 0.21	7.6 ± 0.06	4.2 ± 0.12	4.0 ± 0.22
MCO1	1	7.5 ± 0.33	7.4 ± 0.24	4.6 ± 0.17	4.3 ± 0.10
MCO2	1.5	8.1 ± 0.26	8.0 ± 0.12	6.1 ± 0.30	5.8 ± 0.21
MCO3	2	9.4 ± 0.18	9.2 ± 0.16	7.4 ± 0.08	7.3 ± 0.14
ASO1	1	9.1 ± 0.33	8.9 ± 0.27	6.7 ± 0.42	6.5 ± 0.05
ASO2	1.5	10.4 ± 0.17	10.2 ± 0.35	7.4 ± 0.31	7.3 ± 0.60
ASO3	2	12.2 ± 0.32	12.1 ± 0.21	8.2 ± 0.18	8.1 ± 0.26
MSO1	1	12.6 ± 0.15	12.5 ± 0.53	8.1 ± 0.63	7.9 ± 0.30
MSO2	1.5	13.2 ± 0.22	13.0 ± 0.16	9.5 ± 0.12	9.3 ± 0.34
MSO3	2	14.5 ± 0.62	14.4 ± 0.40	10.3 ± 0.16	10.1 ± 0.10
CO	1	12.5 ± 0.13	12.3 ± 0.08	10.6 ± 0.06	10.2 ± 0.22

Note. OB: ointment base (negative control), ACO1, ACO2, and ACO3: 1%, 1.5%, and 2% aqueous crude extract ointment, respectively, MCO1, MCO2, and MCO3: 1%, 1.5%, and 2% methanol crude extract ointment, respectively, ASO1, ASO2, and ASO3: 1%, 1.5%, and 2% aqueous SNPs ointment, respectively, MSO1, MSO2, and MSO3: 1%, 1.5%, and 2% methanol SNPs ointment, respectively, and CO: 1% ciprofloxacin ointment (positive control).

Table 7: Minimum quantity of the test sample loaded into the ointments that are required to inhibit the test microorganisms.

Test sample	Minimum inhibitory concentrations (% μg/ml)			
	S. aureus		*E. coli*	
	Clinical isolates	ATCC 25923	Clinical isolates	ATCC 25923
ACO	0.031	0.675	0.125	0.125
MCO	0.031	0.031	0.675	0.675
ASO	0.016	0.016	0.016	0.016
MSO	0.008	0.016	0.016	0.016
CO	0.016	0.016	0.016	0.016

Note. ACO: aqueous crude extract ointment; MCO: methanol crude extract ointment; ASO: aqueous SNPs ointment, MSO: methanol SNP ointment, and CO: ciprofloxacin ointment (positive control).

Table 8: Minimum quantity of the test sample loaded into the cream that is required to inhibit the test microorganisms.

Test sample	Minimum inhibitory concentrations (% μg/ml)					
	S. aureus		*E. coli*		*C. albicans*	
	Clinical isolates	ATCC 25923	Clinical isolates	ATCC 29196	Clinical isolates	ATCC 10231
CB	**2**	**2**	**2**	**2**	***2***	***2***
ACC	0.016	0.016	0.031	0.031	0.008	0.008
MCC	0.008	0.008	0.016	0.016	0.004	0.004
ASC	0.008	0.008	0.008	0.008	0.004	0.004
MSC	0.002	0.002	0.008	0.008	0.002	0.002
Cipro	0.016	0.016	0.016	0.016	0	0
Keto	0	0	0	0	0.008	0.008

Note. ACC: aqueous crude extract cream, MCC: methanol crude extract cream, ASC: aqueous SNPs cream, MSC: methanol SNPs cream, and CC: ciprofloxacin cream (positive control).

ions into nanoparticles. The main functional groups present in both aqueous and methanol SNPs are carboxylic acid (O-H), phenol (C-O), and carbonyl (C=O).

The atomic absorption spectroscopy analysis showed a decrease in concentration of silver ion as reaction time progresses indicating the conversion of silver ion into SNPs. This decrease further confirmed the formation of SNPs. Methanol extract of *E. cymosa* was more efficient in the synthesis of SNPs than the aqueous extract probably due to the presence of steroid in methanol extracts acting as a more efficient reducing and stabilizing agents [50]. The antibacterial activity of the biosynthesized SNPs showed that *S. aureus* was more sensitive than *E. coli* which is in agreement with the previous report by Abdel-Raouf et al. [51] which reported that Gram-positive bacteria are more sensitive than Gram-negative bacteria to SNPs. The mechanism of bactericidal activity of SNPs is most likely due to the attachment of SNPs to the cell wall and the generation of free radicals [52]. SNPs disturb the permeability of the membrane by penetrating to the cell membrane and causing intracellular ATP leakage and cell death. The silver ions released from SNPs act as reservoir causing antibacterial activity of SNPs [53].

SEM micrographs indicated that SNPs obtained from *E. cymose* extract occur as agglomerated cluster with irregular morphology. SEM analysis from a previous report shows that SNPs exhibited agglomeration [54]. Our prepared SNP from *E. cymosa* extract is consistent with literature. The methanol SNPs have smaller individual average size estimated to be 55 nm compared to aqueous extract SNPs individual average size estimated to be 90 nm due to the chemical reduction using methanol extract as a surfactant [55].

It has been established by EDX spectroscopy that SNPs generally show characteristic absorption peak in the range of 2.5–4 keV due to SPR [49, 56]. In the present investigation, SNPs showed absorption peak within this range revealing the existence of elemental silver and confirming the silver character of the synthesized nanoparticle. However, methanol extract SNPs showed a stronger absorption peak and elemental concentration by weight compared with aqueous extract SNPs. The results of EDX spectroscopy support the results of UV-visible spectrophotometric analysis and the atomic absorption spectroscopy analysis, all confirming the formation of SNPs by both aqueous extract and methanol extract of *E. cymosa*.

The formulations were soft and smooth in texture, homogenous in appearance, and easy to apply and easy to wash except the ointment formulations that is not easy to wash due to its water-in-oil nature. The pH of the formulations was within the usual pH range of the skin indicating compatibility with the skin.

The viscosities of both formulations decreased with increase in shear rate due to structural breakdown of bonds which is regained after shearing stops as expected [57]. Viscosity and spreading time have a direct relationship; the lower the viscosity, the lower the spreading time. The viscosity and spreading time of both formulations were satisfactory; however, they were higher in the ointment formulations.

Drug-excipient compatibility testing is a necessary preformulation procedure during the development of new drug delivery systems to ensure safety, bioavailability, and stability of the drug product [46, 58, 59]. FTIR spectroscopy was used in this investigation. Shift in peak position, disappearance of a peak, or appearance of new peak is an indication of incompatibility of the drug with the excipient [59]. As shown in Figure 3, there was no significant shift in the position of the absorption peak of the pure SNPs when compared with the formulations and also there was no disappearance of a peak or appearance of new peak confirming compatibility of SNPs with the excipient.

The aqueous and methanol crude extract of *E. cymosa* leaf was screened for antibacterial activity as a preformulation parameter against *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) to ascertain its activity. The methanol extract exhibited a significantly higher antibacterial activity than the aqueous extract. This may likely be due to the quality of the phytochemicals present in these extracts. Sarkodie et al. [25], Bagaje et al. [60], and Sori et al. [61] in their studies confirmed the antibacterial activity of *E. cymosa* plant. The same trend was observed with the antibacterial activity of aqueous extract SNPs and methanol extract SNPs against *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922). The methanol extract SNPs exhibited a significantly higher antibacterial activity than the aqueous extract SNPs. The reason for this may be correlated to the particle size of the methanol extract SNPs (55 nm) being smaller than the

particle size of aqueous extract SNPs (90 nm). Several reports have established the antibacterial effects of nanoparticles being dependent on size [62–64]. This nanosize has the advantage of increase in specific surface area and penetrability into bacterial cell. The antibacterial activity of SNPs is significantly higher than the crude extracts. This also may be as a result of the size of the materials and also because silver itself naturally possess antimicrobial activity. There are several silver formulations available from time immemorial used in medicine for the treatment of microbial infections and other diseases [65–68]. From the results of the antibacterial activity of the formulations, cream formulations had higher antibacterial activity compared to ointment. This may be attributed to the lower viscosity and spreading time of the cream as result of the presence of water in the cream formulation. It has been reported that viscosity of semisolid topical products may directly influence their drug delivery [69]. Another factor that may have contributed to the high antibacterial activity of the cream is the composition of the ingredients used in formulation of the products. The cream base used in this study is aqueous cream base while the ointment base used is simple ointment base. The hydrophilicity or hydrophobicity nature of the cream base or ointment base, respectively, may be highly implicated in the antibacterial activity of these products [70]. The aqueous cream base due to its hydrophilicity will allow faster and better diffusion of the active ingredient to exert its activity. In contrast, the hydrophobic nature of the simple ointment base will retard the diffusion of the active ingredient.

The small antibacterial activity observed with the cream base could possibly be due to the presence of 0.1% chlorocresol in the cream base. This is expected since chlorocresol is used in most cosmetics and topical products as a preservative at a maximum concentration of 0.2% [71]. Interestingly, the antibacterial activity of the cream and ointment formulations was significantly higher when compared ($p < 0.05$) to the positive control antibiotic-ciprofloxacin.

In this study, the biological method was used to synthesize SNP. The chemical and physical methods of silver nanoparticle synthesis had been practiced for decades, but their formation was found to be expensive and it also involved the use of various toxic chemicals for their synthesis [72]. The biological methods are preferred because they are easier, more reliable, and nontoxic in nature [73].

Gudikandula and Charya Maringanti [72] reported that the reducing agents for the biological methods are collection of enzymes, such as nitrate reductase which were reported to play predominant roles but for chemical synthesis, it consists of chemical solutions such as polyol, N2H4, NaBH4, sodium citrate, and N,N-dimethyformamide [74]. Furthermore, in the case of chemical synthesis methods, a stabiliser (surfactant) is added to the first solution to prevent the agglomeration of silver nanoparticles, whereas in this experiment no stabilizing agent was added [75]. The experiment was also carried out at normal environmental conditions so we could testify to the eco-friendly experience, and there were no major concern against the safety of the researchers. In similar experiment, it was reported that nanoparticles synthesized from biological means showed better antimicrobial activity against pathogenic bacteria comparatively with the chemical synthesis [72]. As discussed above, our experiment also showed susceptibility against both typed and clinical cultures of *E. coli* and *S. aureus*. The clinical cultures were collected from patients attending OOU Teaching Hospital in Sagamu, Ogun State, Nigeria. Due to these advantages, Gudikandula and Charya Maringanti [72] suggested that nanoparticles with biocompatibility and antimicrobial potency may be exploited for several biomedical applications including development of catheters, topical antimicrobial gel formulations, food packaging ingredients, food processing equipment, and so on. The past few decades has witnessed many synthetic methods of producing silver nanoparticles. The major challenge with the physical and chemical methods is that highly toxic and expensive chemicals are needed, some of which are difficult to use on large scale; these cause the biosynthesis of nanoparticles very difficult; some of these toxic substances are also been absorbed into the final product [76]. The green synthesis approach that was used in this study is one of the solutions being advocated to overcome this limitation. The biological synthesis of AgNPs has been reported to exhibit desirable properties, some of which includes as high yield, solubility, and stability [77].

Nanotechnology involves precise manipulation of materials at molecular level, and there have been a number of ethical and safety issues that have been raised. Studies about the safety and possible measures to reduce its impacts are still ongoing. However, it has been reported that silver has low toxicity when used in medical applications such as wound stitches, tracheostomy, surgical equipment, and bone prosthesis. At the same time, due to their small size, SNPs exhibit different chemical, physical, and biological properties to bulk materials so the toxicity may be negligible. However, there is need for further research on the safety and toxicity of SNPs [78].

The mechanism of action of *E. cymosa* in this study could be as reported by Brandelli et al. [79]; SNPs are able to exert their effect on microbial cells by generating membrane damage, oxidative stress, and injury to proteins and DNA. In another similar study, Shaikh et al. [80] explained further that SNPs generate ions that interfere with the cell granules of the bacteria leading to the formation of condensed particles. Burdusel et al. [81] also stated that SNPs interact with the bacterial membrane and penetrate the cell, thus producing a drastic disturbance regarding proper cell function, structural damage, and cell death.

5. Conclusion

The phytochemicals in the aqueous and methanol crude extracts of *E. cymosa* were responsible for the reduction of silver ions to SNPs. The green synthesized SNPs of both solvent were confirmed by various characterization parameters. The methanol crude extract and methanol extract SNPs displayed a higher antibacterial activity than the aqueous crude extract and aqueous extract SNPs. The formulated cream and ointment loaded with the SNPs had

acceptable physicochemical properties with good drug-excipient compatibility. The antibacterial activity of cream formulations had a significantly ($p < 0.0001$) higher antibacterial activity compared to the ointment. The cream and ointment formulations had higher antibacterial activity in comparison with ciprofloxacin.

Cream and ointment formulations loaded with green synthesized *E. cymosa* leaf extract SNPs present a potential for a more efficient and effective antibacterial drug delivery to ameliorate the impact of antibacterial drug resistance.

Data Availability

The data used in this study are included in the manuscript.

Ethical Approval

This article does not contain any studies with human and animal subjects performed by any of the authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

[1] C. A. Michael, D. Dominey-Howes, and M. Labbate, "The antimicrobial resistance crisis: causes, consequences, and management," *Frontiers in Public Health*, vol. 2, p. 145, 2014.

[2] E. Martens and A. L. Demain, "The antibiotic resistance crisis, with a focus on the United States," *Journal of Antibiotics*, vol. 70, no. 5, pp. 520–526, 2017.

[3] N. Chandra and S. Kumar, "Antibiotics producing soil microorganisms," *Antibiotics and Antibiotics Resistance Genes in Soils: Monitoring, Toxicity, Risk Assessment and Management*, Springer, Singapore,pp. 1–18, 2017.

[4] D. M. Livermore, "Fourteen years in resistance," *International Journal of Antimicrobial Agents*, vol. 39, no. 4, pp. 283–294, 2012.

[5] A. Akilimali, W. A. Awuah, H. Cakwira et al., "Antimicrobial resistance in Democratic Republic of Congo: the way forward," *International Journal of Surgery: Globalization and Health*, vol. 6, no. 3, Article ID e0150, 2023.

[6] T. M. Viertel, K. Ritter, and H. P. Horz, "Viruses versus bacteria—novel approaches to phage therapy as a tool against multidrug-resistant pathogens," *Journal of Antimicrobial Chemotherapy*, vol. 69, no. 9, pp. 2326–2336, 2014.

[7] S. R. Modi, J. J. Collins, and D. A. Relman, "Antibiotics and the gut microbiota," *Journal of Clinical Investigation*, vol. 124, no. 10, pp. 4212–4218, 2014.

[8] E. M. Abdallah, "Plants: an alternative source for antimicrobials," *Journal of Applied Pharmaceutical Science*, vol. 1, pp. 16–20, 2011.

[9] S. Hosseinzadeh, A. Jafarikukhdan, A. Hosseini, and R. Armand, "The Application of Medicinal Plants in Traditional and Modern Medicine: A Review of *Thymus vulgaris*," *International Journal of Clinical Medicine*, vol. 6, no. 9, pp. 635–642, 2015.

[10] F. S. Li and J. K. Weng, "Demystifying traditional herbal medicine with modern approach," *Nature Plants*, vol. 3, no. 8, pp. 1–7, 2017.

[11] S. Bernardini, A. Tiezzi, V. Laghezza Masci, and E. Ovidi, "Natural products for human health: an historical overview of the drug discovery approaches," *Natural Product Research*, vol. 32, no. 16, pp. 1926–1950, 2018.

[12] I. Süntar, "Importance of ethnopharmacological studies in drug discovery: role of medicinal plants," *Phytochemistry Reviews*, vol. 19, no. 5, pp. 1199–1209, 2020.

[13] P. Garg, E. Maccune, V. Malik, U. P. Singh, D. Sinha, and S. Tyagi, "Comparison of antimicrobial efficacy of propolis, *Morinda citrifolia, Azadirachta indica*, triphala, green tea polyphenols and 5.25% sodium hypochlorite against *Enterococcus fecalis* biofilm," *Saudi Endodontic Journal*, vol. 4, no. 3, pp. 122–127, 2014.

[14] K. Abbas, U. Niaz, T. Hussain et al., "Antimicrobial activity of fruits of Solanum nigrum and Solanum xanthocarpum," *Acta Poloniae Pharmaceutica*, vol. 71, no. 3, pp. 415–421, 2014.

[15] G. Evangelopoulo, N. Solomakos, A. Ioannidis et al., "Assessment of genotoxicity and cholinesterase activity among women workers occupationally exposed to pesticides in tea garden," *Mutation Research: Genetic Toxicology and Environmental Mutagenesis*, vol. 841, pp. 1–7, 2019.

[16] U. Anand, N. Jacobo-Herrera, A. Altemimi, and N. Lakhssassi, "A comprehensive review on medicinal plants as antimicrobial therapeutics: potential avenues of biocompatible drug discovery," *Metabolites*, vol. 9, no. 11, p. 258, 2019.

[17] N. Puvača, E. Lika, V. Tufarelli et al., "Influence of different tetracycline antimicrobial therapy of mycoplasma (*mycoplasma synoviae*) in laying hens compared to tea tree essential oil on table egg quality and antibiotic residues," *Foods*, vol. 9, no. 5, p. 612, 2020.

[18] F. Maroyi, "Evaluation of medicinal uses, phytochemistry and biological activities of *E. cymosa* Thonn. (Ehretiaceae)," *International Journal of Research in Pharmacy and Science*, vol. 12, pp. 1521–1528, 2021.

[19] M. K. Oladunmoye and F. Y. Kehinde, "Ethnobotanical survey of medicinal plants used in treating viral infections among Yoruba tribe of South Western Nigeria," *African Journal of Microbiology Research*, vol. 5, no. 19, pp. 2991–3004, 2011.

[20] L. Li, Y. Peng, L. J. Xu, M. H. Li, and P. G. Xiao, "Flavonoid glycosides and phenolic acids from Ehretia thyrsiflora," *Biochemical Systematics and Ecology*, vol. 36, no. 12, pp. 915–918, 2008.

[21] P. Jeruto, C. Lukhoba, G. Ouma, D. Otieno, and C. Mutai, "Herbal treatments in Aldai and Kaptumo divisions in Nandi district, Rift valley province, Kenya," *African Journal of Traditional, Complementary and Alternative Medicines*, vol. 5, no. 1, pp. 103–105, 2007.

[22] P. Jeruto, C. Mutai, C. Lukhoba, and G. Ouma, "Phytochemical constituents of some medicinal plants used by the Nandis of South Nandi district, Kenya," *Journal of Animal and Plant Sciences*, vol. 9, pp. 1201–1210, 2011.

[23] P. Jeruto, E. Tooa, L. A. Mwamburia, and O. Amuka, "An inventory of medicinal plants used to treat gynaecological-obstetric-urino-genital disorders in South Nandi Sub Country in Kenya," *Journal of Natural Sciences Research*, vol. 5, pp. 136–152, 2015.

[24] A. Shukla and A. Kaur, "A systematic review of traditional uses bioactive phytoconstituents of genus ehretia," *Asian Journal of Pharmaceutical and Clinical Research*, vol. 11, no. 6, pp. 88–100, 2018.

[25] J. A. Sarkodie, S. A. Squire, B. E. Oppong, I. A. Kretchy, C. Y. Domozoro et al., "The antihyperglycemic, antioxidant and antimicrobial activities of E. cymosa," *Journal of Pharmacognosy and Phytochemistry*, vol. 4, pp. 105–111, 2015.

[26] L. H. Madkour, "Biogenic-biosynthesis metallic nanoparticles (MNPs) for pharmacological, biomedical and environmental nanobiotechnological applications," *Chronicles of Pharmaceutical Science Journal*, vol. 2, no. 1, pp. 384–444, 2018.

[27] A. Gupta, S. Mumtaz, C. H. Li, I. Hussain, and V. M. Rotello, "Combatting antibiotic-resistant bacteria using nanomaterials," *Chemical Society Reviews*, vol. 48, no. 2, pp. 415–427, 2019.

[28] H. Yousaf, A. Mehmood, K. S. Ahmad, and M. Raffi, "Green synthesis of silver nanoparticles and their applications as an alternative antibacterial and antioxidant agents," *Materials Science and Engineering: C*, vol. 112, Article ID 110901, 2020.

[29] A. Panáček, M. Smékalová, M. Kilianová et al., "Strong and nonspecific synergistic antibacterial efficiency of antibiotics combined with silver nanoparticles at very low concentrations showing no cytotoxic effect," *Molecules*, vol. 21, no. 1, p. 26, 2015.

[30] A. Panáček, M. Smékalová, R. Večeřová et al., "Silver nanoparticles strongly enhance and restore bactericidal activity of inactive antibiotics against multiresistant Enterobacteriaceae," *Colloids and Surfaces B: Biointerfaces*, vol. 142, pp. 392–399, 2016b.

[31] A. Trease and W. C. Evans, *Pharmacognosy*, Balliene Tindiall, London, UK, 13 edition, 1989.

[32] B. O. Obadoni and P. O. Ochuko, "Phytochemical studies and cooperative efficacy of the crude extract of some homeostatic plant in Edo and Delta State of Nigeria," *Journal of Pure and Applied Sciences*, vol. 8, pp. 203–208, 2001.

[33] O. A. Adeleye, A. O. Badru, O. E. Oyinloye et al., "Green synthesized silver nanoparticles for cream formulation: its anti-inflammatory and healing activities," *IOP Conference Series: Materials Science and Engineering*, vol. 805, no. 1, Article ID 12016, 2020.

[34] V. Kilor, N. Sapkal, and G. Vaidya, "Design and development of novel microemulsion based topical formulation of hesperidin," *International Journal of Pharmacy and Pharmaceutical Sciences*, vol. 7, pp. 142–148, 2015.

[35] D. M. Avish and R. L. Swaroop, "Formulation and evaluation of ointment containing sunflower wax," *Asian Journal of Pharmaceutical and Clinical Research*, vol. 12, pp. 115–120, 2019.

[36] El-Gied, Amgad A. Awad, Abdelkareem M. Abdelkareem, and Elnazeer I. Hamedelniel, "Investigation of cream and ointment on antimicrobial activity of Mangifera indica extract," *Journal of Advanced Pharmaceutical Technology & Research*, vol. 2, no. 6, p. 53, 2015.

[37] O. A. Adeleye, C. O. Babalola, M. N. Femi-Oyewo, and G. Y. Balogun, "Antimicrobial activity and stability of Andrographis paniculata cream containing shea butter," *Nigerian Journal of Pharmaceutical Research*, vol. 15, pp. 9–18, 2019.

[38] R. Gyawali, R. K. Gupta, S. Shrestha, R. Joshi, and P. N. Paudel, "Formulation and evaluation of polyherbal cream containing *Cinnamomum zeylanicum* Blume, *Glycyrrhiza glabra* I and Azadirachta indica A. Juss. extracts to topical use," *Journal of Institute of Science and Technology*, vol. 25, no. 2, pp. 61–71, 2020.

[39] O. A. Adeleye, L. G. Bakre, O. A. Bamiro et al., "Formulation and evaluation of *E. cymosa* biosynthesized silver nanoparticle anti-inflammatory ointment," *International Journal of Pharmacology Research*, vol. 13, pp. 3662–3670, 2021.

[40] M. Cheesbrough, *District Laboratory Practice in Tropical Countries Part-1*, Cambridge University Press, Cambridge, England, 2009.

[41] K. O. Sodeinde, A. M. Ojo, S. O. Olusanya et al., "Cellulose isolated from Delonix regia pods: characterisation and application in the encapsulation of vitamin A," *Industrial Crops and Products*, vol. 160, Article ID 113138, 2021.

[42] M. Rasheed, M. Jawaid, B. Parveez, A. Zuriyati, and A. Khan, "Morphological, chemical and thermal analysis of cellulose nanocrystals extracted from bamboo fibre," *International Journal of Biological Macromolecules*, vol. 160, pp. 183–191, 2020.

[43] M. N. Femi-oyewo, O. A. Adeleye, C. O. Babalola, O. B. Banjo, M. N. Adebowale, and F. O. Odeleye, "In vitro evaluation of antimicrobial activity of *Distemonanthus benthamianus* chewing stick extract mouthwash," *İstanbul Journal of Pharmacy*, vol. 51, no. 1, pp. 105–110, 2021.

[44] L. Carson, S. Bandara, M. Joseph et al., "Green synthesis of silver nanoparticles with antimicrobial properties using *Phyla dulcis* plant extract," *Foodborne Pathogens and Disease*, vol. 17, no. 8, pp. 504–511, 2020.

[45] S. Bawazeer, A. Rauf, S. Shah et al., "Green synthesis of silver nanoparticles using *Tropaeolum majus*: phytochemical screening and antibacterial studiesTropaeolum majus: phytochemical screening and antibacterial studies," *Green Processing and Synthesis*, vol. 10, no. 1, pp. 85–94, 2021.

[46] O. A. Adeleye, O. Bamiro, M. Akpotu, M. Adebowale, J. Daodu, and M. A. Sodeinde, "Physicochemical evaluation and antibacterial activity of *Massularia acuminata* herbal toothpaste," *Turkish Journal of Pharmaceutical Sciences*, vol. 18, no. 4, pp. 476–482, 2021.

[47] J. Jena, N. Pradhan, B. P. Dash, L. B. Sukla, and P. K. Panda, "Biosynthesis and characterization of silver nanoparticles using microalga *Chlorococcum humicola* and its antibacterial activity," *International Journal of Nanomaterials and Biostructures*, vol. 3, pp. 1–8, 2013.

[48] R. A. Hamouda, W. E. Yousuf, E. E. Abdeen, and A. Mohamed, "Biological and chemical synthesis of silver nanoparticles: characterization, mic and antibacterial activity against pathogenic bacteria," *Journal of Chemical and Pharmaceutical Research*, vol. 11, pp. 1–12, 2019.

[49] K. Anandalakshmi, J. Venugobal, and V. Ramasamy, "Characterization of silver nanoparticles by green synthesis method using *Pedalium murex* leaf extract and their antibacterial activity," *Applied Nanoscience*, vol. 6, no. 3, pp. 399–408, 2016.

[50] A. Rana, K. Yadav, and S. Jagadevan, "A comprehensive review on green synthesis of nature-inspiredmetal nanoparticles: mechanism, application and toxicity," *Journal of Cleaner Production*, vol. 272, Article ID 122880, 2020.

[51] N. Abdel-Raouf, N. M. Al-Enazi, and I. B. Ibraheem, "Green biosynthesis of gold nanoparticles using Galaxaura elongata and characterization of their antibacterial activity," *Arabian Journal of Chemistry*, vol. 10, pp. S3029–S3039, 2017.

[52] R. S. Thombre, V. Shinde, E. Thaiparambil, S. Zende, and S. Mehta, "Antimicrobial activity and mechanism of inhibition of silver nanoparticles against extreme halophilic archaea," *Frontiers in Microbiology*, vol. 7, p. 1424, 2016.

[53] O. Azizian-Shermeh, A. A. Jalali-Nezhad, M. Taherizadeh, and A. Qasemi, "Facile, low-cost and rapid photosynthesis of stable and eco-friendly silver nanoparticles using *Boerhavia elegans* (Choisy) and study of their antimicrobial activities," *Journal of Inorganic and Organometallic Polymers and Materials*, vol. 31, no. 1, pp. 279–291, 2021.

[54] S. Medda, A. Hajra, U. Dey, P. Bose, and N. K. Mondal, "Biosynthesis of silver nanoparticles from aloe vera leaf extract and antifungal activity against *Rhizopus sp.* and

Aspergillus sp," *Applied Nanoscience*, vol. 5, no. 7, pp. 875–880, 2015.

[55] E. Vélez, G. Campillo, G. Morales, C. Hincapié, J. Osorio, and O. Arnache, "Silver nanoparticles obtained by aqueous or ethanolic aloe vera extracts: an assessment of the antibacterial activity and mercury removal capability," *Journal of Nanomaterials*, vol. 2018, Article ID 7215210, 7 pages, 2018.

[56] S. Kaviya, J. Santhanalakshmi, B. Viswanathan, J. Muthumary, and K. Srinivasan, "Biosynthesis of silver nanoparticles using *Citrus sinensis* peel extract and its antibacterial activity," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 79, no. 3, pp. 594–598, 2011.

[57] Z. Long, M. M. Zhao, Q. Z. Zhao, B. Yang, and L. Y. Liu, "Effect of homogenisation and storage time on surface and rheology properties of whipping cream," *Food Chemistry*, vol. 131, no. 3, pp. 748–753, 2012.

[58] E. P. da Silva, M. A. V. Pereira, I. P. de Barros Lima et al., "Compatibility study between atorvastatin and excipients using DSC and FTIR," *Journal of Thermal Analysis and Calorimetry*, vol. 123, no. 2, pp. 933–939, 2016.

[59] A. I. Segall, "Preformulation: the use of FTIR in compatibility studies," *Journal of Innovations in Applied Pharmaceutical Science*, vol. 4, pp. 1–6, 2019.

[60] B. Bagaje, Y. Melaku, and T. Dessalegn, "Chemical constituents and antibacterial activities of theleaves of *E. cymosa*," *Asian Journal of Science and Technology*, vol. 8, pp. 4974–4977, 2017.

[61] C. Sori, R. Sahilu, G. Gutu et al., "Antibacterial triterpenoid from the leaves extract of *E. cymosa*," *Ethiopian Journal of Science and Sustainable Development*, vol. 5, pp. 41–50, 2018.

[62] Y. Dong, H. Zhu, Y. Shen, W. Zhang, and L. Zhang, "Antibacterial activity of silver nanoparticles of different particle size against *Vibrio Natriegens*," *PLoS One*, vol. 14, no. 9, Article ID e0222322, 2019.

[63] D. Swolana, M. Kępa, D. Idzik et al., "The antibacterial effect of silver nanoparticles on staphylococcus epidermidis strains with different biofilm-forming ability," *Nanomaterials*, vol. 10, no. 5, p. 1010, 2020.

[64] V. Thiruvengadam and A. V. Bansod, "Characterization of silver nanoparticles synthesized using chemical method and its antibacterial property," *Biointerface Research in Applied Chemistry*, vol. 10, pp. 7257–7264, 2020.

[65] J. W. Alexander, "History of the medical use of silver," *Surgical Infections*, vol. 10, no. 3, pp. 289–292, 2009.

[66] K. Mijnendonckx, N. Leys, J. Mahillon, S. Silver, and R. Van Houdt, "Antimicrobial silver: uses, toxicity and potential for resistance," *Biometals*, vol. 26, no. 4, pp. 609–621, 2013.

[67] D. J. Barillo and D. E. Marx, "Silver in medicine: a brief history BC 335 to present," *Burns*, vol. 40, pp. S3–S8, 2014.

[68] S. Medici, M. Peana, V. M. Nurchi, and M. A. Zoroddu, "Medical uses of silver: history, myths, and scientific evidence," *Journal of Medicinal Chemistry*, vol. 62, no. 13, pp. 5923–5943, 2019.

[69] W. Siemiradzka, B. Dolińska, and F. Ryszka, "Influence of concentration on release and permeation process of model peptide substance-corticotropin-from semisolid formulations," *Molecules*, vol. 25, no. 12, p. 2767, 2020.

[70] C. I. Igwilo, U. E. Akpan, A. O. Adeoye, and C. N. Ilozor, "Effect of cream bases on the antimicrobial properties of the essential oil of *Aframomum meleguata*," *International Journal of Pharmacognosy*, vol. 29, no. 1, pp. 45–50, 1991.

[71] O. Aremu, O. Olayemi, T. Ajala et al., "Antibacterial evaluation of *Acacia nilotica* Lam (Mimosaceae) seed extract in dermatological preparations," *Journal of Research in Pharmacy*, vol. 24, no. 1, pp. 1–12, 2020.

[72] K. Gudikandula and S. Charya Maringanti, "Synthesis of silver nanoparticles by chemical and biological methods and their antimicrobial properties," *Journal of Experimental Nanoscience*, vol. 11, no. 9, pp. 714–721, 2016.

[73] P. Velusamy, G. V. Kumar, V. Jeyanthi, J. Das, and R. Pachaiappan, "Bio-inspired green nanoparticles: synthesis, mechanism, and antibacterial application," *Toxicological Research*, vol. 32, no. 2, pp. 95–102, 2016.

[74] S. U. R. Qamar and J. N. Ahmad, "Nanoparticles: mechanism of biosynthesis using plant extracts, bacteria, fungi, and their applications," *Journal of Molecular Liquids*, vol. 334, Article ID 116040, 2021.

[75] S. Iravani, H. Korbekandi, S. V. Mirmohammadi, and B. Zolfaghari, "Synthesis of silver nanoparticles: chemical, physical and biological methods," *Research in pharmaceutical sciences*, vol. 9, no. 6, pp. 385–406, 2014.

[76] R. Arif and R. Uddin, "A review on recent developments in the biosynthesis of silver nanoparticles and its biomedical applications," *Medical Devices and Sensors*, vol. 4, no. 1, Article ID e10158, 2021.

[77] H. B. Habeeb Rahuman, R. Dhandapani, S. Narayanan et al., "Medicinal plants mediated the green synthesis of silver nanoparticles and their biomedical applications," *IET Nanobiotechnology*, vol. 16, no. 4, pp. 115–144, 2022.

[78] S. K. Mallineni, S. Sakhamuri, S. L. Kotha et al., "Silver nanoparticles in dental applications: a descriptive review," *Bioengineering*, vol. 10, no. 3, p. 327, 2023.

[79] A. Brandelli, A. C. Ritter, and F. F. Veras, "Antimicrobial activities of metal nanoparticles," *Metal nanoparticles in pharma*, pp. 337–363, 2017.

[80] S. Shaikh, N. Nazam, S. M. D. Rizvi et al., "Mechanistic insights into the antimicrobial actions of metallic nanoparticles and their implications for multidrug resistance," *International Journal of Molecular Sciences*, vol. 20, no. 10, p. 2468, 2019.

[81] A. C. Burduşel, O. Gherasim, A. M. Grumezescu, L. Mogoantă, A. Ficai, and E. Andronescu, "Biomedical applications of silver nanoparticles: an up-to-date overview," *Nanomaterials*, vol. 8, no. 9, p. 681, 2018.