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The In vitro Biological Activity of Biosynthesized Silver Nanoparticles Produced Using Mangifera *indica* Stem Bark Extract and Properties of Its Pharmaceutical Gel Formulation

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Abstract
This study reports the production of silver nanoparticles using *Mangifera indica* stem bark (aqueous and methanol) extracts as capping agents and formulation of pharmaceutical gel loaded with the nanoparticles. The extracts were prepared using standard procedures and utilized in biosynthesizing silver nanoparticles. Biosynthesis was ascertained through colour changes, UV–Visible and FTIR spectroscopy. Antioxidant activity of the extracts and biosynthesized nanoparticles were examined by DPPH method. The antimicrobial evaluation was carried out on *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Pharmaceutical gels were produced (F1–F5), and loaded with the nanoparticles. Nanoparticles exhibited maximum absorption under UV–visible spectroscopy between 315 and 320 nm. FTIR spectrum showed that alkene and ester functional groups were conferred on the silver nanoparticles by the extracts used. The nanoparticles demonstrated antimicrobial activity against the organisms, which was significantly higher ($p < 0.05$) than for extracts and reference drug. The antioxidant capacity was in a concentration-dependent manner but significantly lower ($p < 0.05$) than that of the reference drug. Formulated gels had acceptable organoleptic profiles, pH range of 6.8–7.1, high viscosity, and pseudoplastic flow patterns. The in vitro release profiles of the gels showed was gradual, with t_{90} higher than 2 h. The release seemed to be influenced by the viscosity of the gel systems. In addition, the release kinetics of the nanoparticle-loaded gel systems followed Higuchi model with r^2 ranging from 0.9958 to 0.9980. Mangifera indica extracts were successfully used as bio-reducing agents in the synthesis of silver nanoparticles. The gel formulations had acceptable physical properties and release profiles.

Keywords *Mangifera indica* extracts · Aqueous extract-mediated silver nanoparticles · Methanol extract-mediated silver nanoparticles · Antioxidant activity · Antibacterial activity · Pharmaceutical gel

1 Introduction

The skin is the largest organ of the human body, and as a region of drug administration, it provides large contact surface to achieve both local and systemic effects. Topical formulations are designed to penetrate some extent into the skin layers where their effects are exerted for the treatment of skin diseases and microbial infections, pain and such like [1]. It is known that the stratum corneum usually poses a barrier for the absorption of drugs applied topically; however, new advances in the design of formulation have successfully achieved the administration of a large number of drugs through the skin. Drugs can be designed in such a way that improves the pharmacological and therapeutic properties of the drug, many of the pharmacological properties of conventional free drugs have been improved by the use of drug delivery systems which alter the pharmacokinetics and bio-distribution of drugs for better therapeutic outcome [2].

Nanotechnology is a term derived from the Greek word "nano" meaning dwarf. Albert Franks defined it as an area of Science and Technology where dimensions and tolerances are in the range of 0.1–100 nm [3]. Nanotechnology is applicable in gene therapy, drug delivery, imaging, and novel drug discovery techniques. In drug delivery, nano particulate systems such as nano suspensions, nano emulsions, and nano micelles have been able to achieve enhancement

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in therapeutic effect by increasing dissolution rate and drug bioavailability [4]. In particular, various solubility-related problems of poorly soluble drugs have been solved [5]. Patient acceptance and compliance have also been improved. It can be generally stated that nano formulations have improved the physicochemical properties of the active pharmaceutical ingredients to achieve better effects. Nanoparticles include particles with a diameter of less than 1 µm consisting of various biodegradable materials such as natural or synthetic polymers, lipids, phospholipids, and even metals [3]. They have been used as drug carriers in which drug can be attached to the periphery or incorporated in the matrix for example, silver and carbon nanoparticles to mention a few. The potential areas of use for nanoparticles include medicine, catalysis, bio-sensors, chemical sensors, environmental remediation, semi-conductors, and photovoltaic devices [6].

Nanoparticles have been found to help in pro-drug absorption via the skin. Nano particles below 6–7 nm or 36 nm in size may be absorbed through the aqueous pores or lipidic transepidermal route, while those between 10 and 210 nm take the transfollicular route [7]. Although the mechanism of skin-particle penetration and interaction is yet to be fully understood, some of this pattern has been discovered and exploited as particles can interact with the skin on a cellular level as adjuvant. This nanoparticle skin interaction can be used to enhance immune reactivity for vaccine application.

The synthesis of silver nanoparticles can be carried out using physiochemical techniques like gamma ray radiation, microemulsions, electrochemical techniques, chemical reduction, photochemical reduction, laser ablation, and microwave irradiation. However, green synthesis recently developed is mostly used [6].

In recent times, there has been a growing interest in the synthesis of metallic nanoparticles by green methods. For this purpose, biomass or extracts of different plants have been successfully tried as reducing and capping agents for such metals. For instance, in the literature, there are reports of the synthesis of silver, copper and gold nanoparticles using extracts of different plants [8]. This work is part of this new line of research. Green synthesis is a method of synthesizing nanoparticles using plant extracts; it also utilizes biological organisms, enzymes, algae, yeast, mold, and metabolites from arthropods [9]. This method helps to reduce the danger of toxicity of products from chemical methods and the high cost of physical methods. It has gained a lot of attention over the years because of its simplicity, for instance, the basic requirement for the green synthesis of silver nanoparticles is silver nitrate and a natural reducing agent [6]. Green synthesis has diverse advantages including, low energy consumption, use of moderate temperature and pressure, cost effectiveness, eco-friendliness, mass productivity, and therapeutic usefulness [10].

Plant extracts contain abundant natural compounds such as alkaloids, flavonoids, saponins, steroids, tannins, and other nutritional compounds. These compounds are obtainable from various parts of plants such as leaves, stems, roots, shoots, flowers, barks, and seeds [11]. Plant parts such as fruit exocarp and latex are used for metallic nanoparticle synthesis because of the presence of active agents such as polyphenols and antioxidants [12]. *Mangifera indica* belongs to the family Anacardiaceae that consists of about sixty genera and six hundred species. It is a tropical fruit indigenous to south Asia and Central America [13] Mango varieties differ not only in fruit shapes, but also in flavor which is an important trait that makes the seed oil useful in cosmetics as a deodorizing agent [14]. This has been explored in the production of lotion and soaps. It's an herb commonly used in ayurvedic medicine. It is widely used as a source of food, medicine and timber. In Nigeria, different parts of mango tree are commonly used as herbal preparations in the treatment of toothache, gastrointestinal disorders, dysentery, and diarrhea [15]. The leaf extracts were investigated for antibacterial activity against Staph aureus, Escherichia coli, and Pseudomonas aeruginosa [16]. In addition, the antidiabetic property of the plant was investigated by [17].

2 Materials and Methods

Magnifera indica indica stem bark was collected at the University of Ibadan environment and authenticated at the Department of Botany, University of Ibadan. It was cleaned with distilled water, chopped into small pieces and dried. The dried bark was milled using a laboratory mill and stored in an airtight container.

Cold extraction method was used, in which 100 g of the well dried bark was soaked in 1L each of the solvents (methanol and distilled water) using amber glass bottles. The mixture was periodically shaken to improve the extraction of phytochemicals. The aqueous mixture remained for 24 h while the methanol mixture was left for 48 h. The mixture was filtered thrice using a Whatmann filter paper. A clear solution was obtained as filtrate, this was transferred into an evaporating dish. The filtrate was concentrated using a water bath set at 40 °C. The dried extracts obtained were wrapped in aluminum foil and stored in amber glass containers for further investigations [18, 19].

2.1 Qualitative Phytochemical Screening of Plant Extracts

Phytochemical screening for tannins, saponins, alkaloids, flavonoids, glycosides, quinones, phenols, terpenoids, cardiac glycosides, coumarins, anthraquinone, steroids, and anthocyanins was conducted using standard methods as described by [20].

2.2 Preparation of 1 mM of Silver Nitrate Solution

The molecular weight of silver nitrate ($AgNO_3$) is 169.87 g/mol. This means that 169.87 g in 1000 mL will yield 1 M solution and 0.16987 in 1000 mL will yield 1 mM solution [21, 22]. $AgNO_3$ (0.16987 g) was dissolved in 1000 mL of distilled water to obtain 1 mM silver nitrate solution which was used in the biosynthesis of silver nanoparticles [23].

2.3 Biosynthesis of Silver Nanoparticles

The crude methanol and aqueous extracts of *Mangifera indica* stem barks were used for the biosynthesis of silver nanoparticles. Aqueous solution (1000 ml of 1 mM) of silver nitrate (AgNO3) was prepared in a volumetric flask, thereafter 40 mL (0.1 g/10 mL) and 20 mL (0.1 g/10 mL) of the plant extracts solutions were prepared and added each to 100 mL of silver nitrate for the bio-reduction of $AgNO_3$ to silver ions. The solutions were placed in an incubator (water bath DK-600A) for complete bio-reduction at 37 °C for 24–72 h. The silver nanoparticles produced were lyophilized using a freeze dryer (Lab kits -FD-10-MR) and stored in dark glass bottles for further analysis [24].

2.4 Visual Observation of the Biosynthesized Silver Nanoparticles

Visual observation of the plant extract and silver nitrate mixture was done to check for color changes at 24, 48, and 72 h of incubation.

2.5 UV–Visible spectroscopy of the biosynthesized SNPs

The biosynthesized biosynthesized silver nanoparticles (SNPs) were characterized using UV–visible spectroscopy. Absorption spectra was taken between the ranges of 200–400 nm.

2.6 Determination of DPPH-Free Radical Scavenging Ability

The free radical scavenging ability of the extracts against 2,2-diphenyl-1-picrylhdrazyl was evaluated as described by Gyamfi et al. [25]. In brief, appropriate dilution of the samples (extracts and silver nanoparticles) (1 mL) was mixed with 1 mL of 0.4 mM methanol solution containing DPPH solution, the mixture was left in the dark for 30 min and the absorbance of the remaining DPPH was measured at 516 nm. The percentage DPPH scavenging ability of the extracts was subsequently calculated as percentage of control as shown in Eq. 1 below. Ascorbic acid, a potent antioxidant, was used as a reference drug in the study.

$$\% \ Scavenging = \left[(1 - ABS/ABC)\right] \times 100 \quad (1)$$

where "ABS" and "ABC" are absorbance of sample and control respectively.

2.7 Antimicrobial Evaluation of Extracts and Biosynthesized Silver Nanoparticles

Agar-well diffusion method was used to evaluate the antimicrobial activities of the biosynthesized SNPs as well as the extracts. Pathogenic organisms that are implicated in skin infections (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) were used as indicator strains. Mueller Hinton agar solution was prepared according to the manufacturer's instruction using distilled water. The agar was autoclaved at 20 psi for 20 min. After autoclaving, it was allowed to cool to about 40 °C poured into petri dishes and allowed to solidify.

The inoculum for isolates was prepared using those previously stored in slants, which were picked, sub-cultured on nutrient agar plates and incubated for 48 h at 37 °C. A colony of the freshly sub-cultured isolate was then picked into McCartney bottles containing 10 ml of sterile water and shaken to obtain a suspension of the organisms. Inoculum standardization was done using 0.5 McFarland standards. Broth culture of the test pathogen was prepared and streaked with the swab stick on labeled sterile Mueller Hinton Agar (MHA) plates. The inoculated plates were allowed to dry and wells were bored using 8 mm sterile cork-borer. About 200 µL of the fabricated silver nanoparticles, Streptomycin 10 mg/mL, and AgNO3 solution (1 mM) was placed into the wells. The plates were incubated and clear zones of inhibition around the wells was measured.

2.8 FTIR Spectroscopy

A small amount of the sample was put in mortar, using a pestle, potassium bromide (KBr) was added to blend it. With the aid of a spatula, a small amount of sample was carefully taken out and put inside the sample holder to make a pellet; this was hung on to the machine (Buck scientific, model: M530, infrared spectrophotometer) to scan it and the spectra formed (at 40,000 to 1000 cm^{-}) was observed.

2.9 Pharmaceutical Gel Formulation

The biosynthesized nanoparticles were used as drug models in this study. The drug concentration was kept constant at 1% w/w in the gel formulations; propylene glycol was also kept constant at 2% w/w. The polymers hydroxypropylmethylcellulose (HPMC) and carboxymethylcellulose (CMC) were combined in the first formulation (F1), each of which was

used at 1%w/w. Formulations 2 to 5 contained the drugs; triethanolamine was used to neutralize and adjust the pH of the gel systems. The preparations were made up to 100 g using distilled water as shown in Table 2. Finally, proper mixing was done with the aid of a magnetic stirrer (MS300 model) at room temperature [26].

Determination of the Physical Properties of Unloaded and Nanoparticle-Loaded Pharmaceutical Gel Formulations All the formulated gels were visually inspected for color, consistency and homogeneity; this was done after they have been transferred into transparent containers where the presence of any aggregate could be easily detected.

The pH of the gel formulation was determined using a pH meter at room temperature. An amount (2.5 g) of the gel was weighed and dispersed in 25 mL distilled water and the pH was read. The pH was done in triplicates for all the formulations [24].

The measurement of viscosity was done using a Brookfield Viscometer (DV-II + Pro model). The speed of rotation were 5, 10, 20, 50, and 100 rpm. The spindle size used was spindle 3. At each speed, the corresponding dial readings were noted in triplicates.

In vitro Release Measurement for Nanoparticle-Loaded Pharmaceutical Gel The release measurement in 900 mL buffer (pH 7.4) was conducted in a dissolution test apparatus using a seamless high retention dialysis tubing. The dialysis tubing was soaked in distilled water for 30 min before use; it was sealed at one end and the gel formulations (3 mL) was placed inside it while the other end was attached to the rotating paddle. The medium in the receptor compartment was maintained at 37 °C and rotated at 100 rpm. Samples (5 mL) of the receptor compartment fluid were withdrawn at various time interval (5, 10, 15, 30, 45, 60, 90, 120, and 150 min) and the percentage of the extract-mediated silver nanoparticles released was analyzed using UV–Visible Spectrophotometer at 315 nm. The volume withdrawn each time was replaced with drug-free receptor fluid. The cumulative percentage drug released at various time intervals was calculated and plotted against time.

3 Results

3.1 Phytochemicals Present in Extracts

The phytochemical screening as presented in Table 1 shows that the methanol extract contained more phytochemicals than the aqueous. However, aqueous extracts revealed the presence of saponins which was not so in the methanol extracts.

Table 1 Phytochemical composition of *Mangifera indica* stem bark extract

Phytochemical	Aqueous extract	Methanol extract
Saponins	+ +	−
Tannins	+ +	+ + +
Flavonoids	+ + +	+ +
Cardiac glycosides	−	+ +
Anthraquinones	−	−
Terpenoids	−	+ +
Steroids	+	+
Alkaloids	+	+
Phenol	+ +	+ + +
Glycoside	−	+ +
Quinones	+ +	+ + +
Coumarine	+ +	+ + +
Anthocyanin	+ +	+ +

+ present, + + abundant, − absent

3.2 Visual Observation of Biosynthesis

The color changes during the green synthesis of silver nanoparticles using *Mangifera indica* stem bark extracts are shown in Fig. 1. The change in color deepened as time increased.

3.3 UV–Visible Spectroscopy of Biosynthesized Silver Nanoparticles

The UV–visible spectrum as presented in Fig. 2 showed that the wavelength of maximum absorption was at 315 nm.

3.4 FTIR Spectroscopy

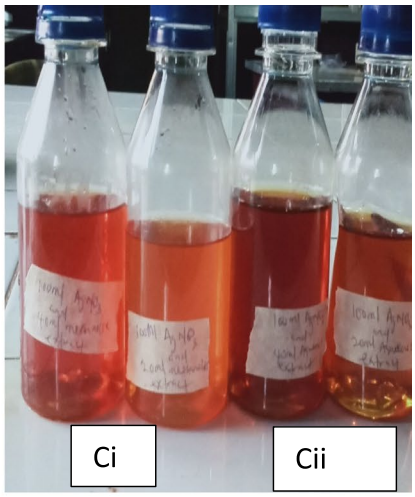
The results of FTIR as presented on Fig. 3 shows the presence of alkene and esters, which was conferred on the biosynthesized nanoparticles by the plant extracts.

3.5 Antimicrobial Evaluation of Extracts and Biosynthesized Nanoparticles

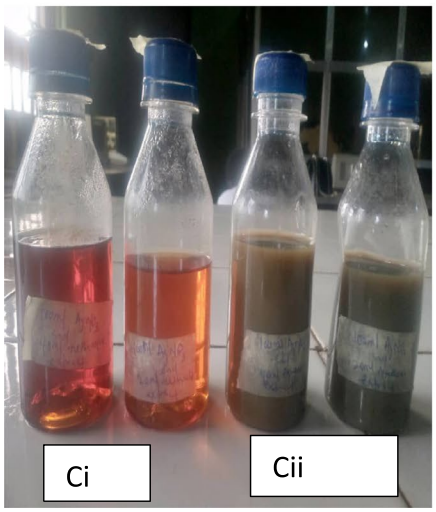
In the antimicrobial evaluation, the results are shown in Fig. 4, using zone of inhibition of the extracts and biosynthesized SNPs. The results revealed that the extracts showed activity on the two microorganisms but lower than that of the biosynthesized nanoparticles. The nanoparticles labelled as 1–4 in plate E and F showed distinct zones of inhibition on both organisms tested. For *Staphylococcus aureus*, the nanoparticles showed less zone of inhibition than the positive control (Streptomycin) while for *Pseudomonas aeruginosa*, the biosynthesized SNPs showed higher inhibition zones than streptomycin. Silver nitrate on its own as seen in plates C and D had much less activity

Silver nitrate solution (A)

Mangifera indica extract in solution (B)



Mixture of extract and silver nitrate (Ci – aqueous extract-mediated; Cii- ethanol extract-mediated)



Extract-mediated silver nitrate nanoparticles after 24 hour (Ci-aqueous extract; Cii-ethanol extract)

Extract-mediated silver nitrate nanoparticles after 48 hour (Ci-aqueous extract; Cii-ethanol extract)

Extract-mediated silver nitrate nanoparticles after 72 hour (Ci-aqueous extract; Cii-ethanol extract)

Fig. 1 The color changes observed during the biosynthesis of silver nanoparticles using *Mangifera indica* aqueous and methanol extracts as reducing agent

on the organisms. The activity of streptomycin was significantly higher on S. aureus while it was lower on P. aeruginosa. For *Staphylococcus aureus*, the ranking order was streptomycin > silver nanoparticles > extract > silver nitrate while for *P. aeruginosa*, it was silver nanoparticles > extract > silver nitrate > streptomycin.

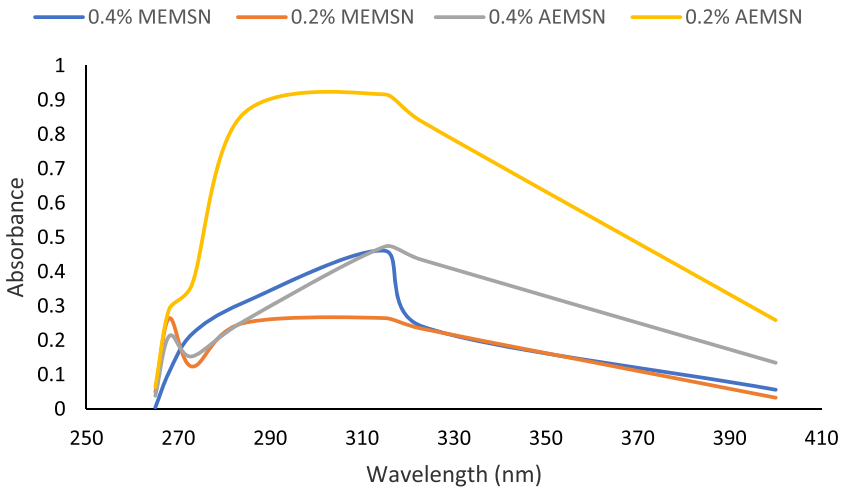


Fig. 2 UV–visible spectra for bio-synthesized silver-nanoparticles

Key
A-Extracts' activity on *Staph aureus.*
B- Extracts' activity on *Pseudomonas aeruginosa.*
C-Activity of Streptomycin and silver nitrate on *Staph aureus.*
D-Activity of Streptomycin and silver nitrate on *Pseudomonas aeruginosa.*
E- Nanoparticles' *activity on Pseudomonas aeruginosa.*
F-Nanoparticles' activity on *Staph aureus.*

3.6 Antioxidant Activity of Extracts and Biosynthesized Silver Nanoparticles

The DPPH scavenging ability of the extracts and nanoparticles is shown in Fig. 5; it was observed that aqueous extract-mediated silver nanoparticles (AEMSN) exhibited the highest antioxidant capacity among the formulations. In comparison with the reference; however, the responses of the silver nanoparticles were significantly ($p < 0.05$) lower. Aqueous extract-mediated silver nanoparticles exhibited higher antioxidant activities than methanol the one produced from methanol extract, this was also observed in the extracts. For the higher concentration, methanol extract-mediated silver nanoparticles showed 27.1% while aqueous extract-mediated nanoparticles showed 33%. For the extracts, aqueous extract highest scavenging activity was 36%, while for the methanol extract it was 33.9%.

3.7 Physicochemical Properties of Formulated gels

The details of ingredients in the formulations are presented in Table 2. The gel formulations were smooth and homogeneous in appearance; the formulation without nanoparticles were white and transparent in appearance while those containing them were light brown and translucent.

The pH and viscosity of the gel systems are presented in Table 3. The pH ranged between 6.8 and 7.1, while viscosity ranged from 983. 15 ± 21.40 to 1189.00 ± 18.40 cP. The flow pattern as revealed in Fig. 6 showed that as the shear rate increased, the viscosity decreased. The release profile of the nanoparticle-loaded pharmaceutical gels showed immediate

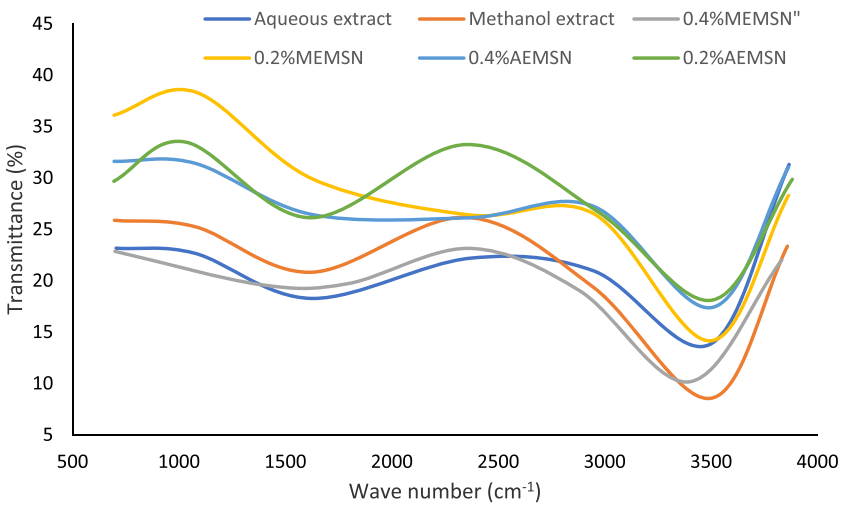


Fig. 3 Fourier transform infrared spectra for extracts and bio-synthesized silver-nanoparticles

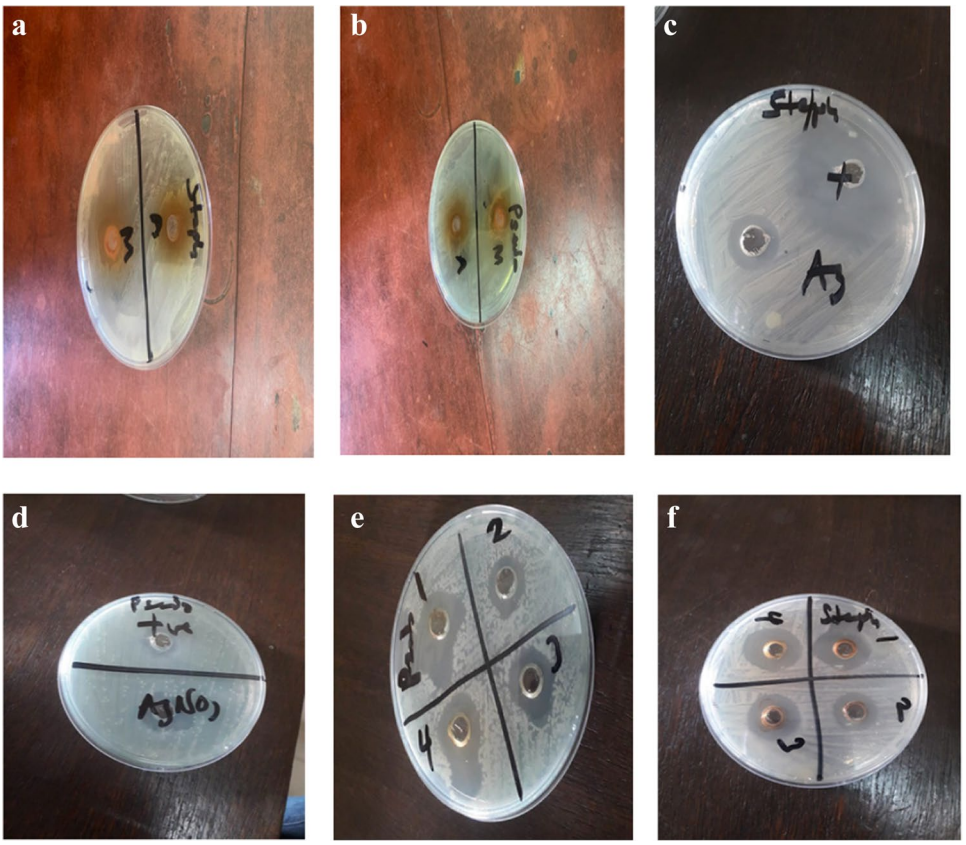


Fig. 4 Zones of inhibition of extracts and biosynthesized SNPs on the pathogens

release which then became prolonged after the first 25 min; this is shown in Fig. 7. The release kinetics of the gel formulations are shown in Table 4 and the kinetics followed Higuchi pattern and n value > 0.5 but < 1.0. The release times of the gel formulations are presented in Table 4.

4 Discussion

The extraction of *Mangifera indica* stem bark using water and methanol have shown that methanol gave a higher yield of 9.2% than water which gave 4.3% yield. This is in accordance with a study by Sanusi et al. [15] where methanol also gave a higher yield. This can partly be attributed to longer extraction time (48 h) of the methanol; this is in accordance with [24]. Water cannot be used for a longer period in extraction because of the possibility of contamination with microbes.

The phytochemical screening of *Mangifera indica* stem bark aqueous and methanol extracts showed the presence of tannins, flavonoids, phenol, quinones, alkaloids, steroids, coumarins, and anthocyanins; this is in accordance with [27]. A study by Okwu et al. [28] showed the presence of flavonoids, alkaloids, saponins, tannins, and phenols in *Mangifera indica* stem bark. In addition to the aforementioned, El-Mahmood [29] reported the presence of cardiac glycosides while another study by Gitonga et al. [30] of methanol extracts of *Mangifera indica* shows presence of coumarins, terpenoids, and flavonoids. These phytochemicals are responsible for the activity of the plant and its use as medicine. The aqueous extracts showed the presence of

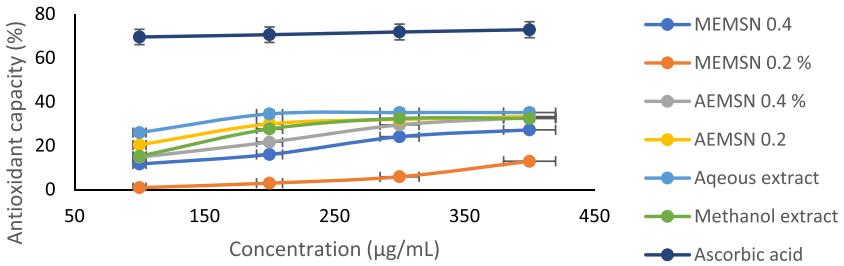


Fig. 5 The antioxidant profiles of the extracts and extract-mediated silver nanoparticles

Table 2 Details of ingredients in pharmaceutical gel formulations

Ingredients	F1	F2	F3	F4	F5
Hdroxypropylmethylcelulose	1	1	1	1	1
Carboxyl methylcellulose	1	1	1	1	1
Propylene glycol	2	2	2	2	2
0.4% MEMSN	–	1	–	–	–
0.2% MEMSN	–	–	1	–	–
0.4% AEMSN	–	–	–	1	–
0.2% AEMSN	–	–	–	–	1
Triethanolamine	Qs	Qs	Qs	Qs	Qs
Water to	100 g	100 g	100 g	100 g	100 g

MEMSN methanol extract-mediated silver nanoparticle, *AEMSN* aqueous extract-mediated silver nanoparticles

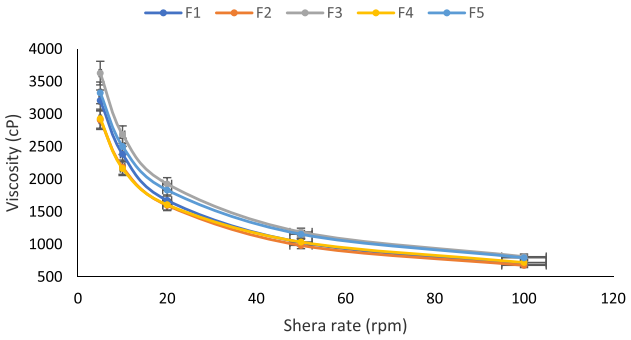


Fig. 6 Rheological profiles of unloaded (F1) and nanoparticle-loaded (F2-F5) pharmaceutical gel formulations

saponins contrary to the methanol extracts; also, methanol extracts showed abundance of glycosides whereas the aqueous extract showed its absence. Knowledge of phytochemicals present in a plant assists scientists in the synthesis of more complex chemical substances; it also explains the reason behind other analytical results obtained during research on such plants [31]. Tannins are known to be useful in the treatment of inflamed or ulcerated tissues; they also have activity in cancer prevention [32]. Additionally, tannins are known to have antiviral and antibacterial properties, and certain tannins are able to inhibit HIV replication [33]

Phenolic compounds are very important constituents because they exhibit antioxidant activity by inactivating free radicals, they also prevent decomposition of hydrogen peroxide into free radicals [34]. Cardiac glycoside are useful as natural drugs for the treatment of heart failure [32]. Coumarins are known for their anti-inflammatory, anticoagulant, bactericidal and fungicidal activities to mention a few [35]; these activities cut across the several types of coumarins. Anthocyanins belong to the flavonoid class, they are also found in foods and beverages like red wine and juices. They are highly water-soluble and are potent antioxidants [36]. In a study by Kalt et al. higher intake of anthocyanins was associated with reduction in hypertension risk [37].

Table 3 pH and viscosity of unloaded and nanoparticle-loaded pharmaceutical gel formulations

Codes	Description of contents	pH	Viscosity @ 50 rpm
F1	Without nanoparticle	6.90 ± 0.02	1029.00 ± 11.40
F2	0.4% MEMSN	7.11 ± 0.09	983. 15 ± 21.40
F3	0.2% MEMSN	6.91 ± 0.03	1189.00 ± 18.40
F4	0.4% AEMSN	6.80 ± 0.05	1035.00 ± 14.20
F5	0.2% AEMSN	6.82 ± 0.05	1155.00 ± 24.00

MEMSN methanol extract-mediated silver nanoparticle, *AEMSN* aqueous extract-mediated silver nanoparticles

During the biosynthesis of the extract-mediated silver nanoparticles (AEMSN), there were notable color changes from light-yellow to brown within 24 h. This is presented in Fig. 1. The color change while using methanol extract was slower than that of aqueous extract. The delay in color change while using the methanol extract can be attributed to lack of free hydrogen which should react with the silver nitrate, this is in accordance with Odeniyi et al. [24]. The color change signifies the reduction of silver ions to silver nanoparticles [38]; it occurs as a result of excitation of surface plasmons vibration in the metal nanoparticle [21].

UV–visible spectroscopy was carried out to further confirm the formation of nanoparticles, maximum absorption occurred between 315 and 320 nm. Generally, absorbance reduces hours after reaction because the rate of nanoparticle formation has been reduced [39]. The UV–vis. Spectra of the plant extract showed peak at 315 nm; this is in agreement with Pratheesya et al. [40] UV spectroscopy gives information about concentration and structure of the nanoparticles. Plasmonic resonance frequency depends on shape, size, and dielectric environment of nanoparticles while shape and size of particles depends on molar ratio of the silver nitrate and the Nanoparticles [41].

The DPPH assay showed that higher concentration (0.4%w/v) of the biosynthesized silver nanoparticles had improved antioxidant activities than lower ones. The better antioxidant activity in the aqueous preparations can be attributed to their hydrogen donating ability; therefore, they can be used to reduce oxidative stress and associated complication [34]. The extracts and the aqueous nanoparticles showed less antioxidant activities than the standard, comparatively; however, they can be said to possess antioxidant activities.

FTIR spectroscopy is very important to characterize the proteins binding with the silver Nanoparticles. It enables the investigation of surface adsorption of functional groups on Nanoparticles [42]. FTIR analysis of the biosynthesized nanoparticles showed the presence of Aromatics (699 nm), ester (1077 nm), alkene (1628 nm), alkane (2945 nm), and amine (3503 nm) functional groups in the nanoparticles. Alkene and

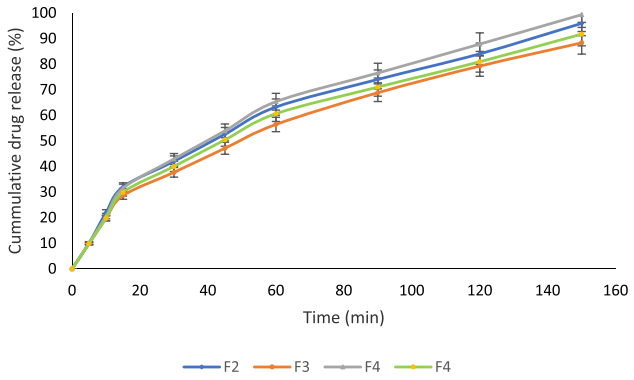


Fig. 7 Release profiles of nanoparticle-loaded pharmaceutical gel formulations

ester functional groups that were originally absent from the silver nitrate were conferred on the aqueous nanoparticles (0.4%w/v); this is because of the presence of the extracts. In addition, amine and alkane peaks were improved as observed in the aqueous and methanol nanoparticles respectively.

Generally, the ranking of the antimicrobial effect was silver nitrate < extracts < biosynthesized nanoparticles < streptomycin. The aqueous extract-mediated silver nanoparticles showed improved activity than the methanol extract-mediated. In addition, from the antimicrobial zone of inhibition of the extract and biosynthesized SNPs, it was observed that for *Staph. aureus*, the nanoparticles showed higher zone of inhibition than the silver nitrate and the positive control (streptomycin) while for *Pseudomonas aeruginosa*, the biosynthesized SNPs showed better inhibition zones than Streptomycin. It may that the strain of *Pseudomonas aeruginosa* used has developed resistance to streptomycin, this justifies the exploration of newer drugs to combat the challenge of antimicrobial resistance as earlier mentioned. Silver nanoparticles have been known to have inhibitory and bactericidal effects, the ones synthesized in this study have also demonstrated the inhibitory activity.

The antibacterial activity of biosynthesized silver nanoparticles has been reported to be dependent on particle size and smaller sizes generally offer higher activity. In addition, interaction between AgNPs and biomolecules which contain silver and phosphorus in the bacterial cell may aid the entrance of the particles into the bacterial cell, leading to cell-killing through the attack of the respiratory chain and cell division [43].

For the formulated gels, the results of the pH showed that the gel systems were within acceptable pH range. The gels showed a pH range of 6.8–7.1 and this is in accordance with Shahtalebi et al. [44]. The effect of pH on skin infections have been studied and it has been observed that at low pH values of 4.7, *Staphylococcus epidermidis* shows growth, while *Staphylococcus aureus* is suppressed at a pH value of 7 [45]. From Table 3, or concentration of extract used in the biosynthesis did not have effect on the viscosity of the gel formulation. The viscosity was high as expected of semi solid dosage forms. In addition, a plot of viscosity against shea rate as observed in Fig. 4 showed that as shear rate increased, viscosity decreased. This implies a non-Newtonian (pseudo-plastic) flow, which is in accordance with a study by Yuan et al. [46]. Many polymer gels exhibit reversibility between the gel state and the sol, this is desirable for ease of drug administration [47].

5 In-vitro Drug Release

Drug release evaluation was done for two and half hours, and the extract-mediated silver nanoparticles showed controlled release pattern throughout the duration, this supports the advantage of gels in providing prolonged release of active ingredients [48]. Dissolution testing is being used to measure the quantity of active pharmaceutical ingredients bioavailable in the skin. It can also be employed as a quality control procedure because a dissolution test can reveal changes in a product's composition [49]. The nanoparticle release profile of the four formulations was compared with each other. The dissolution of F_3 was the slowest, following this order $F_3 < F_5 < F_2 < F_4$. This release studies show that the lower the concentration of the nanoparticle in the gel for both MEMSN and AEMSN, the slower the dissolution and vice versa. There was slower dissolution of F_3 (0.2% MEMSN) and F_5 (0.2% AEMSN) from its gels because the 0.2% concentration of nanoparticles was interlocked and

Table 4 Release kinetics of nanoparticle-loaded pharmaceutical gel formulations

Code	Description of content	Zero order		First order		Korsmeyer-Peppas			Hixson-Crowell		Higuchi	
		r^2	K_0	r^2	K_0	N	r^2	K_0	r^2	K_0	r^{2*}	K_0
F2	0.4% MEMSN	0.9662	0.757	0.9923	0.017	0.522	0.9952	7.046	0.9921	0.005	**0.9958**	7.781
F3	0.2% MEMSN	0.9729	0.699	0.9953	0.014	0.539	0.9972	6.022	0.9951	0.004	**0.9980**	7.154
F4	0.4% AEMSN	0.9675	0.784	0.9929	0.018	0.540	0.9956	6.725	0.9934	0.005	**0.9968**	8.030
F5	0.2% AEMSN	0.9667	0.726	0.9947	0.016	0.529	0.9956	6.540	0.9936	0.004	**0.9965**	7.448

MEMSN methanol extract-mediated silver nanoparticle, *AEMSN* aqueous extract-mediated silver nanoparticles

*Highest correlation coefficients; the figures are highlighted to indicate the best fit of kinetic models for the formulations

Table 5 Release times of nanoparticle-loaded pharmaceutical gel formulations

Code	Description of content	$T_{25\%}$ (min)	$T_{50\%}$ (min)	$T_{75\%}$ (min)	$T_{90\%}$ (min)
F2	0.4% MEMSN	10.323	41.292	92.907	133.786
F3	0.2% MEMSN	12.211	48.843	109.896	158.250
F4	0.4% AEMSN	9.693	38.770	87.233	125.616
F5	0.2% AEMSN	11.268	45.072	101.411	146.032

MEMSN methanol extract-mediated silver nanoparticle, *AEMSN* aqueous extract-mediated silver nanoparticles

enclosed inside the matrix of the gel-slow dissolution profile, compared to the 0.4% concentration of nanoparticles (F_2 (0.4% MEMSN) and F_4 (0.4% AEMSN)) which were not totally enclosed inside the matrix of the gel-rapid dissolution profile. The time taken for 25 ($t_{25\%}$), 50 ($t_{50\%}$), 75 ($t_{75\%}$), and 90% ($t_{90\%}$) extract-mediated silver nanoparticles to be released from the gel is shown in Table 5 and were generated from the dissolution plots [Fig. 7]. The release of the nanoparticle was immediate because 25% of the extract-mediated silver nanoparticles were released in less than 15 min for all the four formulations. Formulation F_3 had the longest duration of drug release, in which 90% of the nanoparticles were released after 150 min, which is the maximum duration for the dissolution study. A careful observation of the timing of each release of the extract-nanoparticles at $t_{25\%}$, $t_{50\%}$, $t_{75\%}$, and $t_{90\%}$ showed that the release profile of the four formulations is quite close.

Different mathematical models such as zero-order, first-order, Korsmeyer-Peppas, Hixson-Crowell, and Higuchi models were used to study the drug release kinetics. This study showed that the gel dissolution data is best fitted to the Higuchi equation for all four formulations [Table 4], which describes the quantity of drug release from a semi-solid is a linear function of the square root of time [49]. The Higuchi model had the highest correlation coefficient-r^2 (0.9958–0.9980). Therefore, the mechanism of extract-mediated nanoparticle release is diffusion controlled from the matrix of the gel, which is also its rate limiting step. The release rate of the nanoparticle from the gel matrix revealed the extract-mediated nanoparticle is hydrophilic or highly soluble and are partitioned more into the perimicellar microchannels and less in the micelles of the gel-matrix, thus available for release. Hydrophobic drugs molecules are poorly soluble and are usually more partitioned to the micelles and less partitioning into the perimicellar microchannels of the gel-matrix, thus they are not available for release [50, 51].

6 Conclusion

The aqueous and methanol extracts of Mangifera indica stem bark were successfully used as bio-reducing agents in the synthesis of silver nanoparticles. The nanoparticles demonstrated significantly higher antimicrobial activity compared to the extracts. The antioxidant capacity was in a concentration-dependent manner but significantly lower than that of the reference drug. The nanoparticle-loaded gel formulations were within acceptable pH range for topical preparations and high viscosity with pseudoplastic rheological patterns. The in-vitro release profiles showed that the release of the gel systems was gradual with $t_{90} > 2$ h. In addition, the release kinetics of the nanoparticle-loaded gel systems followed Higuchi model with r^2 ranging from 0.9958–0.9980 and n value > 0.5 but < 1.0.

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Author Contribution OEA (Oluwatosin Elizabeth Adeyemi) conducted some experiments and wrote the first draft of the manuscript, OAO (Omobolanle A. Omoteso) conducted the kinetic studies, the release times, wrote the concerned part in the manuscript and formatted the manuscript to Journal specification. TOA (Tolulope Omolola Ajala) designed the research concept, provided some of the materials for the experiments, supervised the conduct of all experiments, and reviewed the manuscript severally alongside other authors.

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Declarations

Research Involving Humans and Animals Statement This research did not involve human and animal participants.

Informed Consent The informed consent was not needed in this study.

Conflict of Interest The authors declare no competing interests.

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