Green synthesis and characterization of silver nanoparticles from *Euphorbia kamerunica* latex, and the synergistic antimicrobial effects of their functionalization with Co-Amoxiclav

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Abstract

Antimicrobial resistance is a growing global health concern, prompting the need for alternatives to synthetic antibiotics. One potential solution is the use of plant-derived antimicrobials. This study investigates the enhancement of these antimicrobials by synthesizing silver nanoparticles (AgNPs) and functionalized AgNPs from the latex of *Euphorbia kamerunica*, a plant rich in phytochemicals. It evaluates their antimicrobial activity against various pathogens. The main objective was to develop a green synthesis approach that enhances the effectiveness of AgNPs through functionalization with co-amoxiclav, a commonly used antibiotic. The biosynthesis of AgNPs was achieved by mixing *Euphorbia kamerunica* latex with silver nitrate as the precursor. The formation of AgNPs was monitored visually by the colour change of the reaction mixture and confirmed by UV-visible spectroscopy. FTIR analysis was performed to identify the functional groups responsible for reducing and stabilizing the nanoparticles. The AgNPs were then functionalized with co-amoxiclav, and their morphology was characterized using Scanning Electron Microscopy (SEM). Antimicrobial activity was assessed using the disk diffusion method against gram-positive and gram-negative bacteria. The results indicated successful biosynthesis of AgNPs, confirmed by a characteristic SPR peak at 428 nm and FTIR spectra showing the presence of phytochemicals. SEM analysis revealed nanoparticle sizes ranging from 50 to 500 nm with some agglomeration. Functionalized AgNPs exhibited enhanced antimicrobial activity, particularly against *Salmonella subspecie3b* and *Staphylococcus aureus*, compared to non-functionalized AgNPs and latex alone. The study highlights the remarkable potential of *Euphorbia kamerunica* latex as a sustainable, eco-friendly resource for the green synthesis of silver nanoparticles (AgNPs). Furthermore, the functionalization of these AgNPs with co-amoxiclav significantly enhances their antimicrobial efficacy, demonstrating superior performance against resistant bacterial strains. This innovative approach showcases the synergy between phytochemicals and antibiotics and positions these functionalized nanoparticles as promising candidates for advanced biomedical applications, particularly in combating antibiotic-resistant infections.

Keywords: **Silver nanoparticles, *Euphorbia kamerunica*, green synthesis, antimicrobial activity, co-amoxiclav**

1. Introduction

THE growing challenge of antibiotic resistance underscores the need to identify new drugs derived from plants (Lautié *et. al.*, 2020). Salam *et al.* (2023) highlighted considerable public health threats from antimicrobial resistance (AMR). Numerous efforts are underway to thoroughly investigate and pinpoint new antimicrobial agents that extend beyond the current arsenal to mitigate the effects of pathogenic microorganisms (Shinu *et al.*, 2021).

Historically, medicinal plants have served as effective cures for human sickness and have demonstrated efficacy. These plants are renowned not only for their antimicrobial properties but also for their non-toxic nature, systemic action, and biodegradability. As a result, plant extracts have emerged as promising candidates for alternative or supplementary antimicrobial strategies (Alajeeli *et al*., 2023). Their established antibacterial qualities make them valuable in medical interventions (Akbar *et al.*, 2022).

Nano-science has been incorporated into the screening of medicinal plants to enhance their therapeutic qualities and effectiveness as antimicrobials. This approach leverages their unique properties by manipulating materials at the atomic or molecular level (1 to 100 nm). Nano-materials, like gold nanoparticles used in stained glass, have been unknowingly employed and have shown effectiveness in treating certain illnesses (Boomi *et al.*, 2020).

Recent research highlights the varied applications of metal nanoparticles across biomedical, agricultural, environmental, and physicochemical fields (Ahmad *et al.*, 2024). Silver nanoparticles (Ag-NPs) are noteworthy for their unique properties, which enable their use in diagnostics, biosensing, antifungal and antibacterial treatments, and their anti-inflammatory, anti-permeability, and anti-angiogenic effects (Naganthran *et al.*, 2022).

AgNPs are used as diagnostic tags, biosensors, antifungal, and antibacterial agents in textiles and exhibit anti-inflammatory, anti-permeability, and anti-angiogenic activities (Goel *et al*., 2023). They are ideal for wound and

antimicrobial coatings due to their biocompatibility, nontoxicity, and skin-friendly attributes (Vasile *et al.*, 2020).

The synthesis of nanoparticles can be broadly categorized into physical, chemical, and biological methods. Biological or "green" synthesis, which utilizes biological materials such as plant extracts, bacteria, and fungi, is particularly appealing due to its cost-effectiveness, non-toxic nature, and environmental benefits (Mathur and Bahadur, 2024). This method offers a sustainable alternative to traditional physical and chemical synthesis techniques. The green synthesis approach leverages biological agents' natural reducing and stabilizing properties to produce nanoparticles, thus minimizing environmental impact and reducing reliance on harmful chemicals.

Plant-based synthesis of nanoparticles is increasingly recognized for its advantages, mainly because plant extracts can provide a rich source of secondary metabolites, amino acids, and proteins that act as reducing and capping agents. This helps to prevent particle aggregation and ensures the stability of the nanoparticles. Plant latex is notable for its rich phytochemical content and diverse biological activities, including anti-inflammatory, antiseptic, antiviral, antibacterial, antifungal, nematicidal, and insecticidal effects. Essential compounds such as glycosides, tannins, phytosterols, flavonoids, acetogenins, and saponins contribute to its therapeutic potential (Goel *et al.*, 2023).

Over 20,000 plant species across 40 families produce latex as a defence mechanism (Castelblanque *et al.*, 2017). In particular, the Euphorbiaceae family, with its extensive range of genera and species, is notable for its latex production. The Euphorbiaceae family includes over 317 genera and about 7,500 species, making it one of the largest and most economically significant plant families (Abbas, 2023). Members of this family, such as *Euphorbia kamerunica*, are found in tropical and temperate regions and have been traditionally used for various medicinal purposes, including the treatment of coughs, ulcers, wounds, rheumatism, respiratory infections, and sexually transmitted diseases (Chaudhary *et al.*, 2023).

Research has demonstrated the antimicrobial efficacy of numerous plant extracts, including those from the *Euphorbia* genus, against a range of pathogens such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Salmonella typhii*, *Staphylococcus aureus*, and *Klebsiella pneumonia* (Metin and Bürün, 2023). Plant extracts from Euphorbia thymifolia have been incorporated into pharmaceutical formulations to enhance antimicrobial activity (Fuad *et al.,* 2024).

Plant extracts can be integrated into formulations, encapsulating or combining them with conventional drugs for synergistic effects (Oseni *et al.,* 2021). The utilization of plants for nanoparticle synthesis appears promising due to the absence of pathogenicity, as Adeyemi *et al*. (2022) suggested. In the green synthesis of Silver nano-particles, a key advantage lies in that secondary metabolites, amino acids, or proteins in the reaction medium serve as reducing and capping agents, preventing particle aggregation.

Plant extracts, rich in phytochemicals and derived from various plant parts, have significant applications in medical sciences. Phytochemicals are metabolites produced by different plant parts, and plant latex stands out as particularly significant. Plant latex is a milky fluid produced by some plants when injured or cut. It is an antiseptic, anticoagulant, anti-inflammatory, antioxidant, and anti-proliferative agent.

Scanning Electron Microscopy (SEM) provides high-magnification images to analyze the surface morphology (Yadav *et al.,* 2023) and structure of phytochemicals (Sharma *et al.,* 2022), revealing their physical appearance, particle size, and distribution (Patel *et al.,* 2021), which is crucial for understanding their characteristics and applications.

Functional groups and chemical bonds in phytochemicals are identified using methods such as FTIR Spectroscopy, NMR Spectroscopy, Mass Spectrometry, UV-Vis Spectroscopy and chromatography (Yallappa *et al.,* 2015; Zhang *et al.,* 2021), each providing unique insights into their molecular structures. FTIR spectroscopy is an essential technique for identifying and characterizing functional groups and chemical bonds in phytochemicals.

It is extensively used to confirm the presence of bioactive compounds in plant extracts, validate traditional medicinal applications, and link chemical composition with biological activities. For instance, studies conducted by Verma *et al.* (2022), Lee *et al.* (2022) and Ali *et al.* (2023) illustrate how this technique can uncover variations in phytochemical content due to factors such as geographical location and extraction techniques in plant extracts. Habila *et al.* (2024) used FTIR spectral analysis to identify key functional groups in ethanol and acetone leaf extracts of *Apium graveolens,* indicating its relevance in both traditional and modern medicine.

2. MATERIALS AND METHODS

The fresh stem of *Euphorbia kamerunica* was identified by Dr Faboyede from the Department of Biological Sciences, Crawford University, Ogun State, Nigeria. The plant's latex was collected by injuring the stem of a *Euphorbia kamerunica* tree on the premises of Crawford University, Faith City, Igbesa, Ogun State (Longitude 3056'E, latitude 6034'46N).

PREPARATION OF PLANT LATEX

The latex was collected directly from the plant and introduced into water inside a 10ml measuring cylinder, creating a 3% aqueous solution]. For future use, a stock solution was formulated by combining 10ml of the latex extract with 90ml of distilled water in a conical flask, and it was subsequently stored in a refrigerator.

PHYTO-MEDIATED SYNTHESIS OF THE SILVER NANOPARTICLE

The aqueous latex extract was introduced to a silver nitrate solution at an extract-to-precursor ratio of 1:10. The addition of the latex extract was done drop-wise under agitation for a duration of 1 h. As a result, the solution, initially white (milky white), transitioned to a dark brown colour, indicating the formation of *AgNPs*. The particles were centrifugated to eliminate the supernatant, which was conducted at 4000 rpm for 30 min, followed by thorough washing with ethanol. This process of centrifugation and washing was repeated three times, and the resulting nanoparticles were then dried in an oven at 60°C for 3 h. The obtained nanoparticles were subsequently ground in a crucible using a smooth-surfaced mortar and stored

in an Eppendorf tube at 4°C.

FUNCTIONALIZATION OF AgNPs

The antibiotic Coamoxiclav was utilized as the functionalizing agent. A 2 mg/ml aqueous solution of Coamoxiclav was prepared by dissolving 1 g of the tablet in 500 ml of distilled water to create the stock. From the stock solution, 10 ml was measured and transferred into a conical flask. Subsequently, 10 ml of 0.7 mg/ml AgNP was dissolved in water, added to the Coamoxiclav solution, and then incubated at ambient temperature for 24 h. The resulting mixture underwent centrifugation at 4000 rpm for 5 minutes, followed by a wash with ethanol. This process of centrifugation and washing was repeated three times, and the obtained particles were air-dried at ambient temperature for 24 h. The functionalized nanoparticles were ground using a smooth-surfaced mortar in a crucible and stored in an Eppendorf tube at 4°C.

CHARACTERIZATION OF SILVER NANOPARTICLE ULTRAVIOLET-VISIBLE (UV-VIS) SPECTROSCOPIC ANALYSIS

In assessing the optimum point for silver nanoparticle production, the solution was monitored by measuring the absorption spectra of the sample at 560 nm using a UV-Vis spectrophotometer. The duration and progress of the reaction between metal ions and the latex were observed; distilled water was used as the blank.

FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPIC ANALYSIS

FTIR spectroscopy, spanning the frequency range of 400-4000 cm, was employed to analyze the diverse functional groups within the solution, each absorbed at its characteristic frequency. The FTIR peak values were documented, and the readings were replicated twice to confirm the spectrum accuracy.

SCANNING ELECTRON MICROSCOPY (SEM) ANALYSIS

Details of AgNPs, such as morphology and size, were analyzed using SEM. The suspension above the precipitate of plant extract biomass was taken as SEM samples and was dropped on sterile electric stubs to remove excess water before being introduced into SEM. SEM focused on the particle cluster, and an image was observed.

SOURCE OF TEST ORGANISMS

The following test organisms: *Staphylococcus aureus, Bacillus mycloides, Escherichia coli, Pseudomonas aeruginosa, Klebisella oxytoca,* and *Salmonella sp.* were obtained from the stock culture in the Microbiology laboratory of the Department of Biological Science, Crawford University, Faith City, Igbesa Ogun State, Nigeria.

PREPARATION OF ISOLATES

The bacteria species from the stock were sub-cultured on Nutrient agar to obtain a pure culture of each organism. These cultures were then suspended in sterile distilled water. The strains were inoculated on Mueller-Hinton agar plates for antimicrobial susceptibility testing.

ANTIBIOTIC RESISTANCE TESTING

Antibiotic resistance tests were conducted using OXOID antibiotic discs employing the modified Kirby-Bauer disc diffusion technique. After allowing the agar surface to dry for approximately 5 minutes, antibiotic discs were placed on the plate using forceps and lightly pressed down. The agar plate was incubated at 37^0C for 16-18 hours and examined for the presence or absence of a zone of inhibition. The diameters of these inhibition zones were measured and recorded using a metric rule.

ANTIBACTERIAL SUSCEPTIBILITY TESTING

This was carried out according to Gajic *et al.* (2022). The different organisms were sub-cultured on Nutrient agar plates to obtain pure cultures. Mueller-Hinton agar was prepared, autoclaved at 121°C for 15 min and allowed to cool. The sterilized Agar media was allowed to cool and then poured into eight Petri dishes. Each organism was streaked on two specific plates each (duplicate). A size "4" cork borer was then used to bore holes in the plate. A total of six holes were bored on each plate. Silver nanoparticles at different concentrations of 50, 25, 12.5, 6.25, and 3.125 mg/ml were inserted with a micro-pipette in each hole. The sixth hole contained the plant latex placed in the plate's middle. The plates were then left to stand for 15 min before incubation. The plates were incubated at 37°C for 24 h, after which the plates were brought out and the zones of inhibition measured.

3. RESULTS AND DISCUSSION

VISUAL OBSERVATION

The formation of AgNPs was initially monitored by observing the colour changes in the reaction mixture.

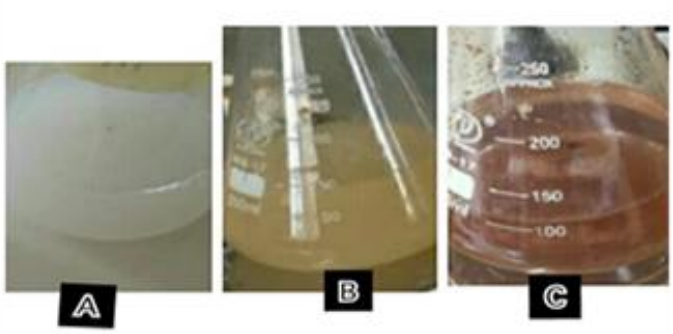

Plate 1: Phyto-mediated synthesis showing the colour change (a) Milky (b) Pale brown (c) Dark brown

Within ten minutes, the colour evolved from milky white to a pale brown and deepened to a dark brown hue upon completion of the reaction. These progressive colour changes, detailed in Plate 1, visually indicate the successful synthesis and maturation of the AgNPs.

UV-VISIBLE SPECTROSCOPIC ANALYSIS

The UV-visible spectroscopy spectrum (Fig. 1) indicates that the reduction of silver ions (Ag^+) to AgNPs was successfully monitored by observing the characteristic surface plasmon resonance (SPR) peak at 428 nm. The SPR peak at this wavelength is a typical signature of AgNPs, confirming their formation during the reaction. The gradual increase in absorbance up to 20 min suggests that the concentration of AgNPs increases as the reaction progresses, indicating successful nucleation and growth of the nanoparticles. This result supports the submission of Yallappa *et al.* (2015).

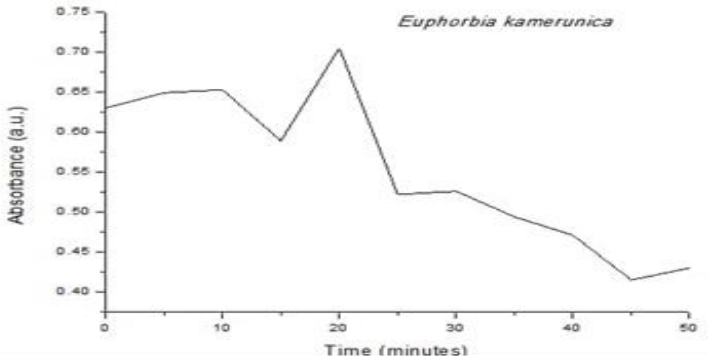

Fig. 1: Absorption peak of silver nanoparticles using latex of *Euphorbia kamerunica* under UV-Vis spectroscopy.

The subsequent decline in absorbance after 20 min likely points to the agglomeration of the AgNPs. Agglomeration occurs when individual nanoparticles start to coalesce into larger clusters, leading to a reduction in the number of discrete particles. This aggregation can cause a decrease in SPR intensity, as larger particles scatter light differently than smaller, isolated nanoparticles, leading to diminished absorbance in the UV-visible spectrum. The described trend in absorbance is consistent with typical nanoparticle synthesis, where an initial increase in absorbance corresponds to particle formation, followed by a decrease due to agglomeration if stabilization is inadequate.

FTIR ANALYSIS

The FTIR spectrum of *Euphorbia kamerunica* latex (Fig. 2a) displays a broad peak at 3245 cm^{-1}, indicative of C–OH stretching vibrations. Peaks at 1634, 2374, and 1051 cm^{-1} correspond to –C=C– (aromatic), C-H (aromatic), and C–O functional groups, respectively, suggesting the presence of phytochemicals such as polyphenols, flavonoids, saponins, and tannins. This supports the observations of Zhang *et al.* (2021).

For the *AgNPs* (Fig. 2b), a broad peak at 3465 cm^{-1} also indicates C–OH stretching, with characteristic peaks at 1643, 2955, and 1050 cm^{-1} corresponding to –C=C– (aromatic), C-H (aromatic), and C–O groups.

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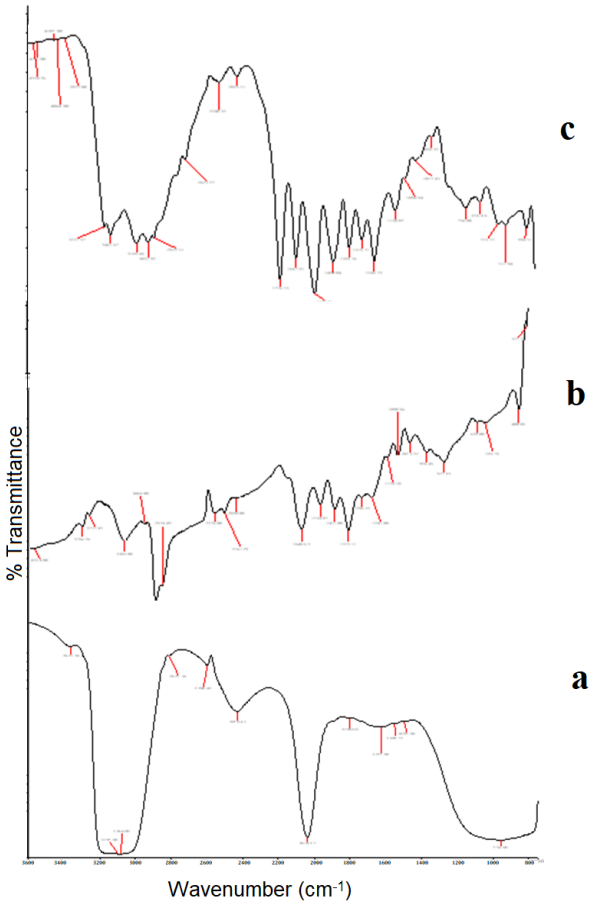

Fig. 2: FTIR analysis of (a) crude latex of *Euphorbia kamerunica (b)* AgNPs *(c)* co-amoxiclav functionalized AgNPs.

The co-amoxiclav functionalized *AgNPs* (Fig. 2c) show an O-H stretching band at 3351 cm^{-1}, C-H stretching at 2980 cm^{-1}, and a C=C stretching band at 1617 cm^{-1}. Additional bands at 1453, 1392, and 1368 cm^{-1} are likely due to CH_2 and CH_3 groups and bands at 1220-1130 cm^{-1} correspond to C-O groups. These peaks match the FTIR bands of pure co-amoxiclav, confirming the successful functionalization of the *AgNPs* with co-amoxiclav. These observations support previous works such as those of Verma *et al.* (2021), Lee *et al.* (2022) and Faud *et al.* (2024).

SEM ANALYSIS

SEM image of the phytosynthesized *AgNPs* (Plate 2) shows the agglomeration of the nanoparticles with individual nanoparticles having particle diameters ranging from 50 nm to 500 nm. UV/visible studies support the agglomeration. While this may affect the available surface area of the nanoparticles, it suggests a solid inter-particle interaction due to the type of phytochemicals bound to the nanoparticle surface.

ANTIMICROBIAL SCREENING OF TEST (AST) ON TEST ORGANISMS

The results of the AST are displayed in Tables 1-6. As observed in Table 1, Ofloxacin has activities against *Escherichia coli*, *Klebsiella oxytoca* and *Salmonella subspecie3b*. For the gram

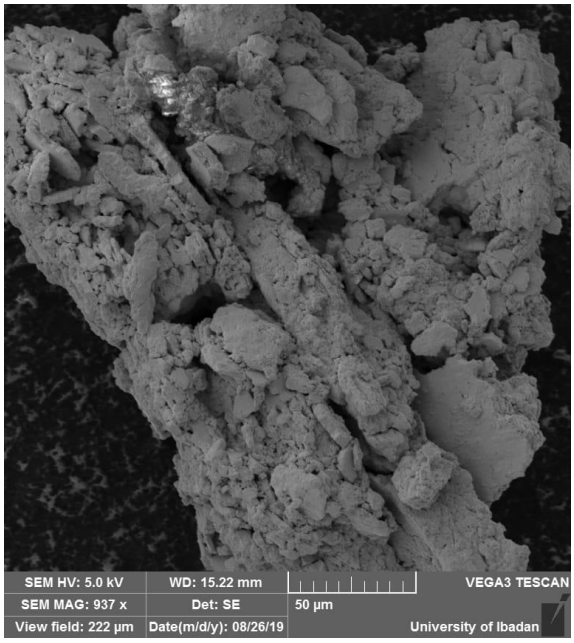

Plate 2: SEM image of AgNPs.

positives in Table 2, Gentamycin was effective and showed antimicrobial activity against *Staphylococcus aureus* and *Bacillus mycoides*. Table 3 shows the zone of inhibition of *Euphorbia kamerunica* latex, where *Bacillus mycoides* and *Escherichia coli* exhibited the highest zone of inhibition, which was followed by *Klebsiella oxytoca*, exhibiting the next highest occurrence zone of inhibition of the 6 organisms introduced to the latex and the remaining showed no inhibition. The series of antibacterial effects of the different latex used in this research supports the claim of Chaudhary *et al*. (2023) about the use of latex in the treatment of diseases.

TABLE 1
Antibiotic resistant profiling for gram negative organisms

S/N	Microorganisms	Ceftazi CTR)	Erythromycin (ERY	Cloxaci CXC	Ofloxacin OFL	Augmentin AUG	Ceftazidime CAZ	Cefuroxime CRX
1	*Escherichia coli*	R	S	R	S	R	R	R
2	*Pseudomonas aeruginosa*	R	R	R	R	R	R	R
3	*Klebisella oxytoca*	R	R	R	S	R	R	R
4	*Salmonella subspecies 3*	R	R	R	S	R	R	R
	Percentage resistance %	100 %	75 %	100 %	25 %	100 %	100 %	10%

Note: R-Resistance S- Susceptible

Table 4 shows the zone of inhibition of EKAgNP on the six organisms, with *Bacillus mycoides* and *Escherichia coli* appearing as the best-inhibited organisms for EKAgNP, respectively, as they showed the highest inhibition zones. *Bacillus mycoides* showed an average zone of inhibition of 13.67 mm, while for *Escherichia coli,* an average zone of inhibition of 12.7 mm was observed. Inhibition of *Salmonella subspecie3b* and *Klebsiella oxytocin were observed with average zones of 12.3 mm and 11.3 mm,* respectively. The EKAgNP showed no inhibitory activity on *Staphylococcus aureus* and *Pseudomonas aeruginosa*. This observation supports Metin and Bürün (2023).

TABLE 2
The resistance profiling of the test organism with known antibiotics

S|N	Microorganisms	Augmentin (AUG)	Amoxicillin (AMX)	Erythromycin (ERY)	Tetracycline (TET)	Gentamycin (GEN)	Cotrimazole (COT)	Cholaphenicol (CHL)
1	*Bacillus mycoides*	R	R	S	S	S	R	S
2	*Staphylococcus aureus*	R	R	R	R	S	R	S

Note: R-Resistance S- Susceptible

TABLE 3
Zone of inhibition of the crude latex of *E. kamerunica*

S/N	Organisms	Zone of inhibition (mm)
1	*Pseudomonas aeruginosa*	0
2	*Klebsiella oxytoca*	4.7±0.6
3	*Salmonella subspecies 3*	0
4	*Bacillus mycoides*	5.7±1.5
5	*Staphylococcus aureus*	0
6	*Escherichia coli*	5.7±0.6

TABLE 4
Zone of inhibition of EKAgNP

Micro-organisms	Zone of inhibition (mm) at different Concentrations (mg/ml)			
	50	25	12.5	6.25
Pseudomonas aeruginosa	0	0	0	0
Klebsiella oxytoca	0	0	0	11.3±0.08
Salmonella subspecies 3	9.67 ± 0.08	12.3 ± 1.05	0	0
Bacillus mycoides	12.6± 0.02	13.67±0.53	9.3±0.08	0
Staphylococcus aureus	0	0	0	0
Escherichia coli	12.7±0.01	10.3±0.08	0	0

TABLE 5
Zone of inhibition of Functionalized EKAgNP

Organisms	Zone of inhibition (mm) against pathogenic organisms at different concentration (mg/ml)			
	25	12.5	6.25	3.125
Pseudomonas aeruginosa	0	0	0	0
Klebisella oxytoca	0	0	0	0
Salmonella subspecie 3	12±1	12.3±1.5	0	17±1
Bacillus mycoides	0	0	0	15.7±1.2
Staphylococcus aureus	10±0.6	0	0	12±1
Escherichia coli	14.3±0.58	12±1	10.3±0.58	0

TABLE 6
Comparison of the antimicrobial activities of the latex, EKAgNP and Functionalized

Organisms	Latex	AgNP	FAgNP
Pseudomonas aeruginosa	0	0	0
Klebisella oxytoca	4.7±0.6	0	0
Salmonella subspecies 3	0	12.3±0.15	12±0.5
Bacillus mycoides	5.7±0.5	13.67±0.53	0
Staphylococcus aureus	0	0	0
Escherichia coli	5.7±0.6	10.3±0.58	14.3±0.58

EKAgNP with *Salmonella subspecie3b* appearing as the best-inhibited organism, with an average zone of inhibition of 17 mm and with average zones of 15 mm and 14.3 mm, respectively, *Staphylococcus aureus* preceded by average inhibition zone of 10 mm. The Functionalized EKAgNP showed no inhibitory activity on *Pseudomonas aeruginosa* and *Klebsiella oxytocin*.

Table 6 shows that the Functionalized EKAgNP is more effective than the others, as represented in Fig. 3. This result supports the observation of Oseni *et al.* (2021) and Adeyemi *et al.* (2022) about the synergetic effects of plant extracts and drugs. Plant extracts, including those from *Euphorbia thymifolia*, have been previously incorporated into pharmaceutical formulations to enhance antimicrobial activity (Faud *et al.*, 2024).

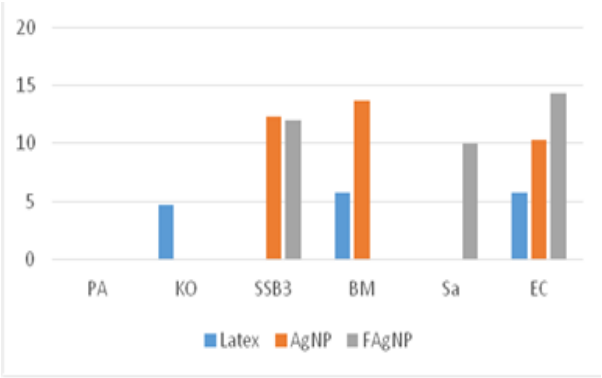

Fig. 3: Comparison of antimicrobial activities of the three latex types

KEY:

PA	*Pseudomonas aeruginosa*	**BM**	*Bacillus mycoides*
KO	*Klebsiella oxytoca*	**SA**	*Staphylococcus aureus*
SSB3	*Salmonella subspecies 3*	**EC**	*Escherichia coli*

4. CONCLUSION

This study successfully synthesized silver nanoparticles (AgNPs) using *Euphorbia kamerunica* latex and silver nitrate. The synthesis was confirmed by visual colour change, UV-visible spectroscopy, and FTIR analysis, which also showed successful functionalization with co-amoxiclav. SEM analysis indicated significant agglomeration of AgNPs, with sizes ranging from 50 nm to 500 nm. Antimicrobial tests revealed that both the synthesized and functionalized AgNPs exhibited strong inhibitory effects, with enhanced activity observed in the functionalized nanoparticles against *Salmonella* and *Staphylococcus aureus*. These results demonstrate the potential of plant-derived AgNPs and their functionalization for effective antimicrobial applications.

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