

ORIGINAL RESEARCH ARTICLE

Antibacterial potency of biosynthesized aluminum–potassium hybrid nanoparticles using *Polyalthia longifolia* leaf extract against multidrug-resistant clinical isolates

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Abstract

Antimicrobial resistance (AMR) poses an escalating global health threat, with projections of up to 10 million annual deaths by 2050 unless alternative therapeutic strategies are developed. Green nanotechnology offers a sustainable approach to combating multidrug-resistant (MDR) pathogens by harnessing plant-derived phytochemicals as biocompatible reducing and capping agents. This study aims to synthesize, characterize, and evaluate the antibacterial efficacy of biogenic aluminum–potassium hybrid nanoparticles (P@Al-KNPs) fabricated using aqueous leaf extract of *Polyalthia longifolia* against MDR clinical isolates of *Staphylococcus aureus*, *Streptococcus* sp., and *Salmonella* sp. P@Al-KNPs were synthesized by reacting aqueous *P. longifolia* leaf extract (30 mL) with a mixed AlCl_3/KCl precursor solution (70 mL) at 60 °C–70 °C (pH 6.2). Physicochemical characterization employed UV–visible (Vis) spectroscopy, attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR), scanning electron microscopy (SEM), and energy-dispersive X-ray (EDX) analysis. Antibacterial activity was assessed using the Kirby–Bauer disc diffusion method at concentrations of 50, 75, 100, and 150 µg/mL. Data were analyzed using one-way ANOVA with Duncan's multiple range test at $\alpha = 0.05$ using SPSS v.19. Successful nanoparticle formation was confirmed using a colorimetric transition and UV–Vis absorption at 408 nm (absorbance: 0.970). ATR-FTIR identified phenolic, carbonyl, and hydroxyl groups as principal mediators of biosynthesis. SEM revealed a predominantly spherical morphology (70–103 nm), and EDX confirmed Al (49.11%) and K (24.99%) as the primary constituents. Antibacterial testing demonstrated significant dose-dependent inhibition zones of 16.4 mm (*S. aureus*), 16.0 mm (*Streptococcus* sp.), and 14.3 mm (*Salmonella* sp.) at 150 µg/mL. The one-way ANOVA reported significant differences among concentrations ($p < 0.05$). In conclusion, P@Al-KNPs constitute a novel, phyto-mediated dual-metal nanoplatform with significant antibacterial activity against MDR pathogens. Their facile green synthesis

and superior activity at subminimum inhibitory concentrations position them as promising candidates for biomedical antimicrobial applications.

Keywords: Aluminum–potassium hybrid nanoparticles; Antimicrobial resistance; Disc diffusion; Energy-dispersive X-ray; Green synthesis; Multidrug-resistant bacteria; *Polyalthia longifolia*

1. Introduction

Antimicrobial resistance (AMR) represents one of the most formidable public health challenges of the 21st century, threatening the gains of modern medicine and compromising the management of routine infectious diseases.¹ The misuse and overuse of conventional antibiotics in healthcare, veterinary medicine, and agriculture have precipitated the emergence and rapid dissemination of multidrug-resistant (MDR) bacterial strains, including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE).² Murray *et al.*³ estimated that nearly 4.95 million deaths globally in 2019 were associated with bacterial AMR, and projections indicate that, without decisive intervention, AMR could account for up to 10 million annual fatalities by 2050.⁴ Nanotechnology has emerged as a transformative strategy in addressing AMR, offering nanoscale materials with unique physicochemical properties that circumvent conventional resistance mechanisms.^{5,6} Among various nanomaterials, metal and metal-oxide nanoparticles have demonstrated broad-spectrum antibacterial activity through multiple concurrent mechanisms, including disruption of bacterial cell membrane integrity, generation of reactive oxygen species (ROS), and inhibition of key metabolic enzymes.^{7,8} Critically, this poly-mechanistic action substantially reduces the probability of developing resistance, an inherent limitation of single-target conventional antibiotics.^{9,10}

Aluminum and potassium represent pharmaceutically relevant elements with established biomedical utilities. Aluminum-based compounds serve as vaccine adjuvants and antacids¹¹, while potassium-based compounds are employed in electrolyte management and cardiac arrhythmia prevention.¹² Their nanoparticulate forms exhibit enhanced antibacterial activity, with aluminum nanoparticles reported to suppress *S. aureus* at minimum inhibitory concentrations (MICs) as low as 10 µg/mL.¹³ The combination of aluminum and potassium into a hybrid nanoparticle system is a novel approach that may leverage complementary ionic interactions to enhance antibacterial efficacy; however, this specific dual-metal formulation remains largely unexplored in the literature.

Green synthesis using plant-derived extracts offers an environmentally benign, cost-effective, and scalable alternative to conventional physicochemical fabrication methods that employ hazardous reducing agents.^{14,15} *Polyalthia longifolia* (Sonn.) Thwaites (family Annonaceae) is a medicinal tree widely distributed across South Asia and sub-Saharan Africa. Its leaves are rich in alkaloids, flavonoids, polyphenols, and terpenoids—phytochemicals capable of reducing metal ions and stabilizing the resulting nanoparticles.^{16,17} Previous studies have demonstrated the utility of *P. longifolia* extracts in silver nanoparticle synthesis with potent antifungal activity¹⁷ and copper nanoparticle fabrication with antibacterial efficacy¹⁶; however, no study has reported the phyto-mediated co-synthesis of aluminum–potassium hybrid nanoparticles using this plant.

The present study therefore addresses two critical knowledge gaps: (i) the absence of a green synthetic route for dual-metal aluminum–potassium hybrid nanoparticles (P@Al-KNPs), and (ii) the limited evaluation of *P. longifolia*-mediated nanoparticles against clinically relevant MDR pathogens. The specific objectives of this study are to: (i) synthesize P@Al-KNPs using *P. longifolia* aqueous leaf extract; (ii) comprehensively characterize the nanoparticles using UV–visible (Vis) spectroscopy, attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR), scanning electron microscopy (SEM), and energy-dispersive X-ray (EDX); and (iii) evaluate their dose-dependent antibacterial activity against MDR isolates of *S. aureus*, *Streptococcus* sp., and *Salmonella* sp. using the Kirby–Bauer disc diffusion method with appropriate statistical validation.

2. Materials and methods

2.1. Materials and reagents

Analytical-grade aluminum chloride (AlCl₃), potassium chloride (KCl), and concentrated sulfuric acid (H₂SO₄) were sourced from the Department of Chemistry Laboratory, Kaduna State University, Nigeria. Mueller–Hinton Agar (MHA), standard antibiotic discs (amoxicillin 25 µg, septrin 25 µg, streptomycin 10 µg, ampiclox 10 µg, chloramphenicol 30 µg), and deionized water were procured

from standard laboratory suppliers. Clinical isolates of *Salmonella* sp., *Streptococcus* sp., and *S. aureus* were obtained from the Microbiology Diagnostic Laboratory of Oxford Hospital, Kakuri, Kaduna State, Nigeria, and from the Agricultural Microbiology Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt. All isolates were confirmed as MDR based on resistance to ≥ 3 antibiotic classes per Clinical and Laboratory Standards Institute (CLSI) guidelines.¹⁸

2.2. Collection and preparation of plant extract

Fresh leaves of *P. longifolia* were collected from a fadama ecosystem adjacent to the Kaduna Refining and Petrochemical Company (KRPC), Oil Village, Kaduna State, Nigeria. Botanical identity was confirmed by a qualified taxonomist (voucher specimen deposited at the institution's herbarium). Leaves were washed thoroughly under running tap water to remove surface contaminants, then rinsed twice with deionized water. The washed leaves were oven-dried at 60 °C for 24 h (SLW 75 POL-EKO oven, POL-EKO, Poland) until constant weight was achieved, then pulverized into a fine powder using a laboratory mill and sieved through a 250 μ m mesh. Twenty grams of the powder were added to 200 mL of deionized water in a 500 mL beaker and heated to boiling for 30–60 min. The extract was cooled to ambient temperature and filtered through Whatman No. 1 filter paper (Cytiva, Germany). The filtered extract was stored at 4 °C and used within 48 h as the bioreducing and capping agent.

2.3. Preparation of precursor solution

Aluminum chloride (AlCl_3 , 1.33 g, 10 mmol) and potassium chloride (KCl, 1.77 g, 24 mmol) were accurately weighed using an analytical balance (FA1204N, ± 0.0001 g, Changzhou XingYun Electronic Equipment Co., Ltd., China) and dissolved sequentially in approximately 70 mL of deionized water in a 100 mL volumetric flask, consistent with the procedure of James *et al.*¹⁹ The flask was sealed and agitated on an orbital shaker until complete dissolution was confirmed visually, after which the volume was adjusted to exactly 100 mL with deionized water to yield a combined precursor concentration of 0.1 M AlCl_3 and 0.24 M KCl.

2.4. Biosynthesis of P@Al-KNPs

Thirty milliliters of the *P. longifolia* leaf extract was combined with 70 mL of the precursor solution in a 250 mL beaker (extract:precursor ratio = 3:7 v/v). The mixture was heated on a calibrated hot plate–magnetic stirrer (IKA RCT Basic S1, IKA-Werke GmbH & Co. KG, Germany) to 60 °C–70 °C under continuous stirring at 300 rpm, and pre-equilibrated at the target temperature for 10 min before the extract was introduced, to ensure a stable and reproducible

reaction temperature at the point of mixing. Concentrated H_2SO_4 (0.1 mL) was added dropwise under a fume hood, with appropriate personal protective equipment, to adjust and maintain the pH at 6.2 (monitored continuously using a calibrated pH meter; HI2020 Edge, Hanna Instruments, Romania). The pH was rechecked every 5 min throughout the reaction to confirm stability. The colorimetric transition from dark brown to light brown, documented photographically at fixed time points (Figure 1), was used as the primary visual indicator of nanoparticle nucleation, attributed to surface plasmon resonance (SPR). The full transition was complete within 1–2 h of mixing. The suspension was then allowed to age undisturbed for 24 h at 24 °C to assess colloidal stability, with visual inspection for sedimentation or aggregation performed at 0, 6, 12, and 24 h. The resulting P@Al-KNPs were harvested by centrifugation (10,000 rpm, 15 min; KA-1000 centrifuge, Shanghai Anting Scientific Instrument Factory, China). The supernatant was discarded, and the pellet was washed twice with 20 mL of deionized water per wash to remove unbound phytochemicals and residual ions. The pellet was then dried in an oven at 45 °C for 24 h to constant weight. The synthesis was performed in triplicate independent batches to confirm reproducibility of the colorimetric transition and yield.

2.5. Physicochemical characterization

UV–Vis absorption spectroscopy was performed on the colloidal nanoparticle suspension using an Agilent Cary 5000 UV–Vis–NIR spectrophotometer (Agilent Technologies, United States of America [USA]) over a wavelength range of 300–800 nm, with deionized water as the baseline blank, to confirm SPR absorption and compare it with the plant extract spectrum.^{20,21} ATR–FTIR spectroscopy (Agilent Cary 630 FTIR, Agilent Technologies) was performed between 650 and 4,000 cm^{-1} (resolution: 8 cm^{-1} ; background scans: 16; sample scans: 30) on both the dried plant extract and the dried P@Al-KNPs powder to identify functional groups mediating biosynthesis.^{22,23} The ATR crystal was cleaned with ethanol, and a background spectrum was re-collected between samples. Surface morphology was determined using SEM (JEOL FFM-7401F, JEOL Ltd., Japan) after sputter-coating dried samples with gold to minimize charging, operated at an accelerating voltage and a working distance of 2.97 mm at 100,000 $\times$ magnification (as recorded in the instrument overlay). Particle size measurements were performed on calibrated SEM micrographs using ImageJ 1.54 software ($n = 50$ particles measured across at least three separate fields of view; National Institutes of Health, USA), with size expressed as mean $\pm$ SD Feret diameter. Elemental composition was determined using EDX (Oxford 7585, Oxford Instruments, United Kingdom) coupled to the

SEM, with spectra acquired over a 15 s live-time count (22,288 total counts) at an accelerating voltage consistent with the SEM imaging conditions.²¹

2.6. Antibacterial susceptibility testing

The disc diffusion (Kirby–Bauer) method was employed in accordance with CLSI guidelines.¹⁸ MDR status was confirmed using susceptibility profiling against 10-disc multidrug panels containing amoxicillin, septrin, streptomycin, ampiclox, and chloramphenicol (10–30 µg each), applied to freshly swabbed MHA plates and read after 18–24 h at 37 °C. Bacterial inocula were prepared in sterile 0.9% NaCl and adjusted to a 0.5 McFarland turbidity standard ($\sim 1.5 \times 10^8$ CFU/mL) using a calibrated turbidimeter/McFarland comparator (DEN-1, Biosan, Latvia). Suspensions were uniformly applied to MHA plates using the lawn/swab technique (sterile cotton swab rotated across the entire agar surface in three directions at 60° to one another) within 15 min of standardization to maintain inoculum density. Sterile 6 mm filter-paper discs were impregnated with 20 µL of P@Al-KNPs suspension at 50, 75, 100, and 150 µg/mL *P. longifolia*, then applied to the agar surface with sterile forceps, spaced at least 24 mm apart (center-to-center) and 15 mm from the plate edge to avoid zone overlap. Plates were incubated at 37 °C for 18–24 h in an inverted position, after which zones of inhibition (ZOI), measured as the diameter of complete growth inhibition including the 6 mm disc, were recorded in millimeters using a calibrated digital caliper/ruler at two perpendicular diameters per zone. All experiments were performed in triplicate ($n = 3$ independent plates per concentration per isolate), with a fresh bacterial suspension prepared for each replicate.

2.7. Statistical analysis

All quantitative data are expressed as mean $\pm$ standard deviation (SD) of three independent replicates ($n = 3$). The effect of P@Al-KNPs concentration on antibacterial activity (ZOI, mm) was assessed using one-way analysis of variance (ANOVA). Where ANOVA indicated statistically significant differences ($p < 0.05$), post-hoc pairwise comparisons were performed using Duncan's multiple range test (DMRT) at the 5% significance level, with different superscript letters indicating statistically distinct groupings. All analyses were conducted using IBM® SPSS® Statistics software (version 19; USA).²⁴ The threshold for statistical significance was set at $\alpha = 0.05$ throughout.

3. Results

3.1. Biosynthesis and visual confirmation of P@Al-KNPs

The reaction of *P. longifolia* aqueous leaf extract with the AlCl_3/KCl precursor solution at 60 °C–70 °C and pH 6.2

produced a distinct colorimetric transition from dark brown to light brown within 1–2 h, indicating progressive reduction of Al^{3+} and K^+ ions by phytochemical reducing agents in the extract (Figure 1). The colloidal suspension remained stable for 24 h post-synthesis without visible precipitation, confirming nanoparticle formation and the stabilizing efficacy of the plant extract.

3.2. UV-visible spectroscopy

UV-Vis spectroscopy confirmed the formation of P@Al-KNPs through a characteristic SPR absorption peak at 408 nm (absorbance: 0.970), distinct from the *P. longifolia* extract absorption at 334 nm (absorbance: 2.890). The red shift of the plant extract absorption peak at 408 nm is consistent with the formation of nanoscale metal particles with collective electron oscillations at the particle surface, corroborating established optical signatures for aluminum-based nanoparticles within the 300–800 nm characterization range.^{20,21}

3.3. Attenuated total reflectance Fourier transform infrared spectroscopy analysis

Attenuated total reflectance Fourier transform infrared spectra of the *P. longifolia* extract and the synthesized P@Al-KNPs revealed significant peak shifts and intensity changes that identify the functional groups involved in nanoparticle biosynthesis (Figure 2; Tables 1 and 2). In the plant extract, broad O–H/N–H stretching vibrations at 3,928.6, 3,574.5, and 3,507.4 cm^{-1} , indicative of phenols, alcohols, and amines, respectively, were substantially attenuated and shifted to a single broadened peak at 3,291.2 cm^{-1} in the P@Al-KNPs spectrum. This attenuation indicates donation of electrons from the hydroxyl groups of phenolic and alcoholic moieties to facilitate $\text{Al}^{3+}/\text{K}^+$ ion reduction and surface coordination.^{25,26} C–H stretching vibrations at 2,922.2 and 2,855.1 cm^{-1} , attributable to aliphatic alkane chains in the extract, were markedly diminished or absent in the P@Al-KNP spectrum, consistent with the formation of a non-polar aliphatic stabilizing shell around the nanoparticles.¹⁴ Notably, the C=O stretching at 1,628.8 cm^{-1} was retained in the P@Al-KNPs spectrum at the same position, suggesting that carbonyl-containing compounds (e.g., flavonoids, terpenoids) retained their coordination roles as stabilizing ligands on the nanoparticle surface.^{27,28} N–H and C–H bending vibrations at 1,520.8 and 1,438.8 cm^{-1} , respectively, showed shifts in position and reduced intensity, indicating amine-mediated surface interactions.

3.4. Scanning electron microscopy morphological characterization

Scanning electron microscopy micrographs of the biosynthesized P@Al-KNPs revealed predominantly

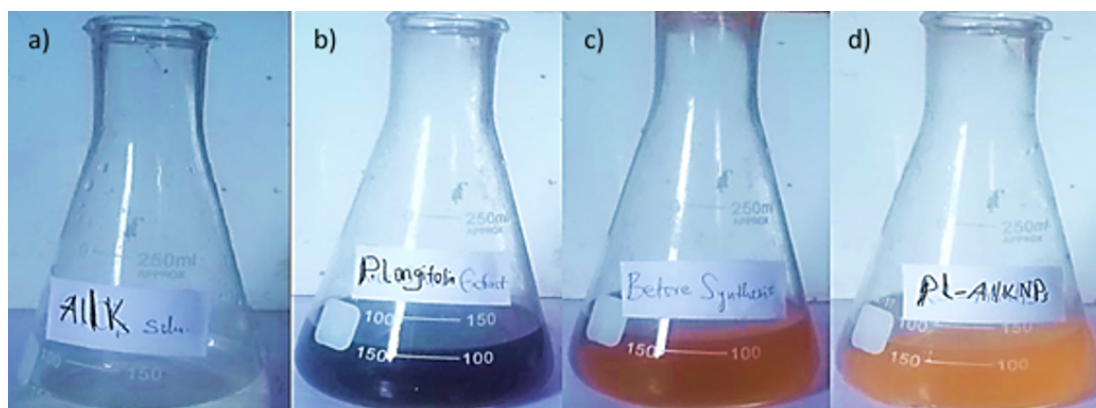


Figure 1. Colorimetric progression during P@Al-KNPs biosynthesis: (a) AlCl_3/KCl precursor solution; (b) *Polyalthia longifolia* aqueous leaf extract; (c) reaction mixture prior to heating; (d) reaction mixture following nanoparticle formation, showing the characteristic transition from dark brown to light brown indicative of surface plasmon resonance

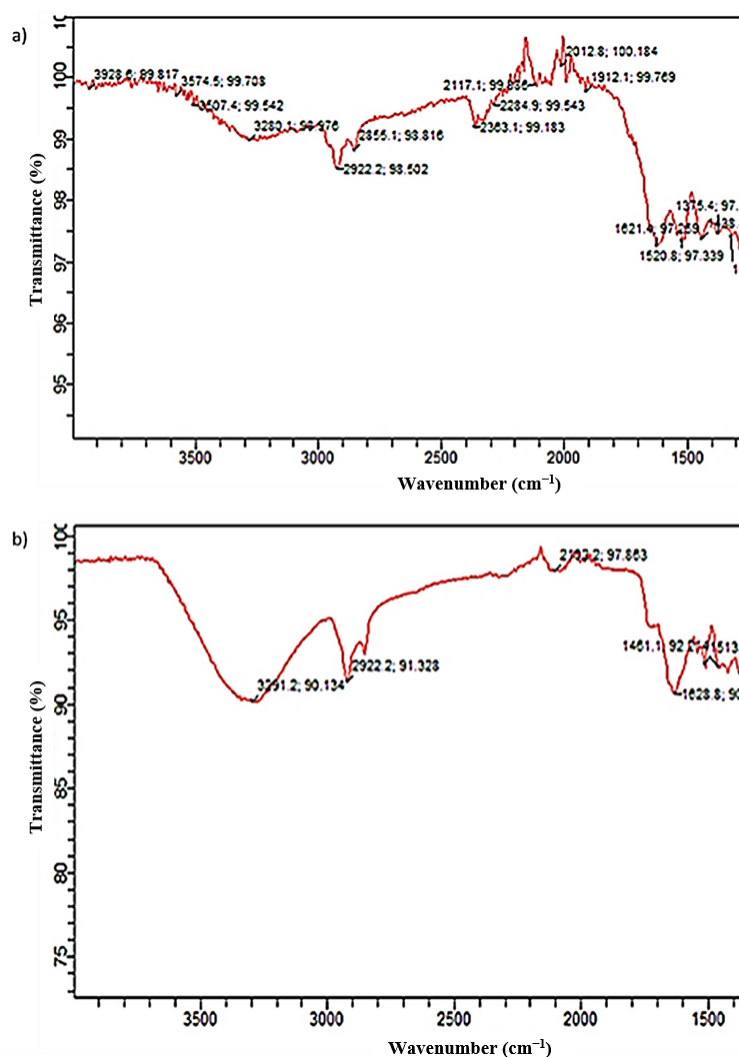


Figure 2. Attenuated total reflectance Fourier transform infrared spectra of (a) *Polyalthia longifolia* aqueous leaf extract and (b) biosynthesized P@Al-KNPs ($650\text{--}4,000\text{ cm}^{-1}$).

Table 1. ATR-FTIR peak assignments comparing *Polyalthia longifolia* extract and biosynthesized P@Al-KNPs, illustrating functional group involvement in nanoparticle reduction and stabilization

Functional group	<i>P. longifolia</i> extract (cm ⁻¹)	P@Al-KNPs (cm ⁻¹)	Assignment
O–H / N–H stretching	3,928.6, 3,574.5, 3,507.4	3,291.2 (broadened, reduced intensity)	Phenols, alcohols—reductive capping
C–H stretching	2,922.2, 2,855.1	Diminished/Absent	Alkane stabilization shell
C=O stretching	1,628.8	1,628.8 (retained)	Carbonyl coordination—stabilizing ligand
N–H/C–H bending	1,520.8, 1,438.8	Shifted/Reduced	Amine & aliphatic capping interactions

Abbreviation: ATR-FTIR: Attenuated total reflectance Fourier transform infrared.

spherical nanoparticles with measured diameters ranging from 70 to 103 nm (mean: 86.5 ± 10.2 nm, $n = 50$; Figure 3a). The particles exhibited minor polydispersity and some degree of aggregation, attributed to the heterogeneous distribution of stabilizing biomolecules inherent to biological reducing agents.²⁹ Spherical morphology is characteristic of biogenic metal nanoparticles and reflects the isotropic templating action of plant polyphenols during nucleation and growth.^{30,31}

3.5. Energy-dispersive X-ray elemental composition

Energy-dispersive X-ray analysis confirmed that aluminum (49.11 wt.%) and potassium (24.99 wt.%) are the predominant elemental constituents of P@Al-KNPs, consistent with the AlCl_3 and KCl precursors employed (Figure 3b). The high-intensity characteristic EDX peaks at 3.3 keV (Al K α and K K α , separated by the detector resolution) validated the dual-metal hybrid composition. Additional elements, including silicon (6.82%), magnesium (11.26%), and trace quantities of chlorine, calcium, sodium, phosphorus, sulfur, iron, and titanium, were detected and are attributed to the elemental matrix of *P. longifolia* biomass.³² Full elemental assignments are presented in Table 2.

3.6. Antibacterial activity and statistical analysis

The antibacterial activity of P@Al-KNPs against the three MDR clinical isolates demonstrated a clear, significant dose-dependent response at concentrations of 50–150 $\mu\text{g/mL}$ (Table 3, Figures 4 and 5). The plant extract control (1,000 $\mu\text{g/mL}$) produced no ZOI for any isolate (0.0 mm), confirming that the observed antibacterial effects are attributable to the nanoparticles rather than residual phytochemicals.

One-way ANOVA confirmed significant inter-concentration differences for all three pathogens ($p < 0.05$). DMRT post-hoc analysis revealed statistically significant groupings denoted by superscript letters (Table 3): the highest concentrations (100 and 150 $\mu\text{g/mL}$) consistently produced the largest ZOIs, with 150 $\mu\text{g/mL}$ yielding maximum ZOIs of 16.4 ± 0.4 mm (*S. aureus*, Duncan group a), 16.0 ± 0.3 mm (*Streptococcus* sp., Duncan group a), and 14.3 ± 0.3 mm (*Salmonella* sp., Duncan group b). No inhibition was detected for *Streptococcus* sp. and *S. aureus* at ≤ 75 $\mu\text{g/mL}$ and ≤ 50 $\mu\text{g/mL}$, respectively, indicating a concentration threshold for effective antibacterial action.

4. Discussion

4.1. Biosynthesis mechanism and colloidal stability

The colorimetric transition from dark brown to light brown during P@Al-KNPs synthesis provides direct visual evidence of nanoparticle nucleation, consistent with the SPR phenomenon, in which conduction electrons at the particle surface oscillate resonantly with incident electromagnetic radiation, producing characteristic absorption in the visible spectrum.²² The UV–Vis SPR peak at 408 nm confirms nanoscale particle formation within the reported range for aluminum-based nanoparticles (200–800 nm characterization window).²¹ The 24-h colloidal stability without sedimentation indicates effective nanoparticle capping by phytochemicals from *P. longifolia*.

The biosynthetic mechanism is driven by the rich phytochemical composition of *P. longifolia*, particularly flavonoids, polyphenols, and terpenoids, which function as both reducing agents (donating electrons to reduce $\text{Al}^{3+}/\text{K}^+$ ions) and capping/stabilizing agents (forming protective organic corona layers).^{16,32} Hydrogen bonding and

electrostatic interactions between aluminum ions and the capping molecules further facilitate colloidal stabilization, preventing aggregation consistent with literature reports on green-synthesized metal nanoparticles.^{19,27}

4.2. Physicochemical characterization

The ATR-FTIR data corroborate the multifaceted role of *P. longifolia* phytochemicals in P@Al-KNPs synthesis. The significant attenuation of O–H stretching bands (from three distinct peaks at 3,928.6–3,507.4 cm⁻¹ in the extract to a single broadened peak at 3,291.2 cm⁻¹ in P@Al-KNPs) is consistent with the involvement of phenolic and alcoholic hydroxyl groups as electron donors during metal ion reduction.^{33,34} The retention of C=O stretching at 1,628.8 cm⁻¹ in the P@Al-KNPs spectrum indicates that carbonyl-containing ligands remain surface-coordinated in the final nanoparticle form, serving as stabilizing groups—consistent with coordination chemistry principles

described by Ahmed *et al.*²⁷ and Mittal *et al.*²⁸ The diminished C–H stretching peaks suggest the formation of a protective aliphatic shell, as documented by Iravani¹⁴ for plant-mediated nanoparticle synthesis.

The SEM analysis confirmed a predominantly spherical morphology (70–103 nm), consistent with biogenic synthesis reports using *P. longifolia* extracts for silver nanoparticles¹⁷ and copper nanoparticles¹⁶. The spherical morphology reflects isotropic nucleation directed by the symmetric distribution of phytochemical templating agents. The observed polydispersity and minor aggregation are inherent limitations of biological synthesis, arising from heterogeneity in plant extract composition, and are similarly reported in other green-synthesis studies.^{30,31} Importantly, the 70–103 nm size range of P@Al-KNPs falls within the biomedically relevant nanoparticle window, favoring bacterial membrane interaction and cellular uptake.¹³

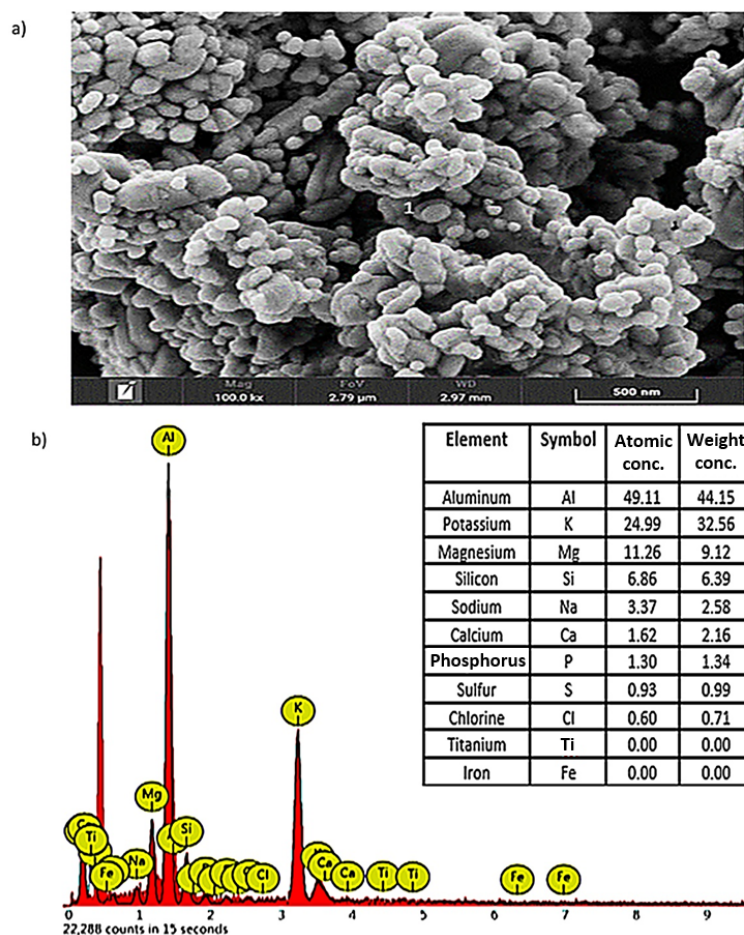


Figure 3. Morphological characterization. (a) Scanning electron microscopy micrograph of P@Al-KNPs displaying predominantly spherical morphology with particle sizes of 70–103 nm. Scale bar: 500 nm; magnification: 1,000×. (b) Energy-dispersive X-ray spectrum confirming Al (49.11%) and K (24.99%) as principal elemental constituents, with characteristic peaks at 3.3 keV.

Table 2. Elemental composition of biosynthesized P@Al-KNPs as determined by EDX spectroscopy, with probable origins of detected elements

Element	Weight (%)	EDX peak (keV)	Probable origin
Aluminum (Al)	49.11	3.30 (primary peak)	Precursor: AlCl_3
Potassium (K)	24.99	3.30 (secondary peak)	Precursor: KCl
Silicon (Si)	6.82	1.74	Plant silica matrix
Magnesium (Mg)	11.26	1.25	Plant macronutrient
Chlorine (Cl)	Trace	2.62	Residual precursor salt
Calcium (Ca)	Trace	3.69	Plant mineral content
Sodium (Na)	Trace	1.04	Plant mineral content
Phosphorus (P)	Trace	2.01	Biomass residue
Sulfur (S)	Trace	2.31	Biomass residue
Iron (Fe)/Titanium (Ti)	Trace	6.40/4.51	Environmental contamination

Abbreviation: EDX: Energy-dispersive X-ray.

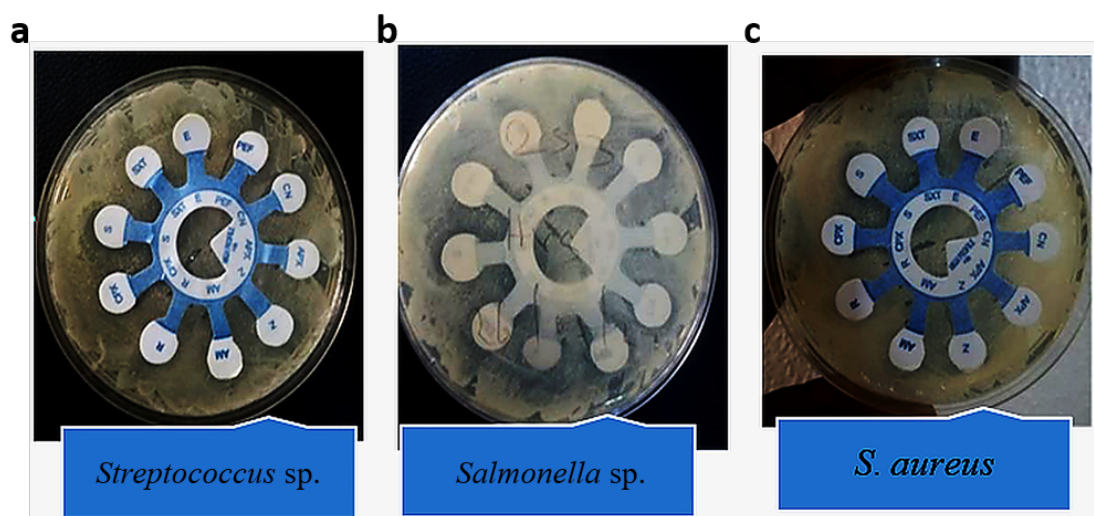


Figure 4. Reference antibiotic discs (amoxicillin, septrin, streptomycin, ampiclox, and chloramphenicol) produced zones of inhibition of 0–6 mm, confirming the multidrug-resistant phenotype of all isolates: (a) *Streptococcus* sp., (b) *Salmonella* sp., (c) *Staphylococcus aureus*

Table 3. ZOIs of P@Al-KNPs against MDR clinical isolates at varying concentrations

MDR pathogen	ZOI (mm; mean $\pm$ SD)				
	<i>Polyalthia longifolia</i> extract (1,000 μ g/mL)	P@Al-KNPs concentration (μ g/mL)			
		50	75	100	150
<i>Streptococcus</i> sp.	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	13.2 $\pm$ 0.4 ^c	16.0 $\pm$ 0.3 ^a
<i>Staphylococcus aureus</i>	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	5.3 $\pm$ 0.6 ^g	11.1 $\pm$ 0.5 ^d	16.4 $\pm$ 0.4 ^a
<i>Salmonella</i> sp.	0.0 $\pm$ 0.0	6.2 $\pm$ 0.5 ^f	9.1 $\pm$ 0.4 ^e	11.8 $\pm$ 0.4 ^d	14.3 $\pm$ 0.3 ^b

Notes: $n = 3$. Data were compared using one-way ANOVA followed by DMRT at $\alpha = 0.05$. Within each row, values sharing at least one superscript letter are not significantly different from one another ($p > 0.05$); values that do not share any superscript letter differ significantly ($p < 0.05$). Superscript letters are assigned independently within each row (i.e., letter groupings are not comparable across rows/pathogens) and are ranked in descending order of mean ZOI, such that “a” denotes the statistically highest-performing group for that pathogen. Values of 0.0 indicate no detectable inhibition. Abbreviations: ANOVA: Analysis of variance; DMRT: Duncan’s multiple range test; MDR: Multidrug-resistant; SD: Standard deviation; ZOI: Zone of inhibition.

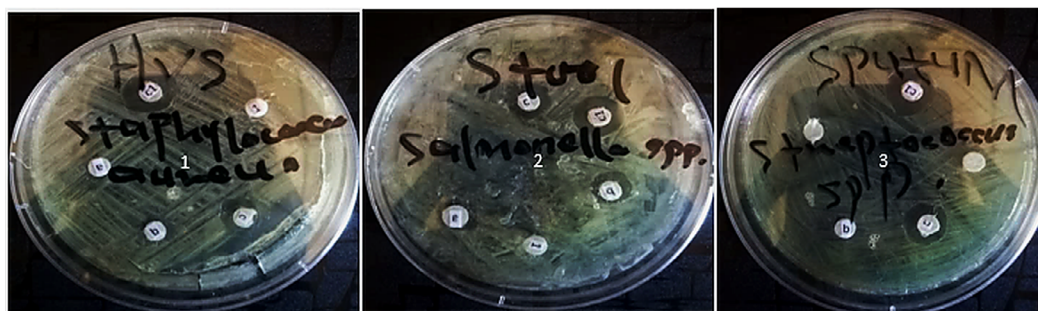


Figure 5. Representative images of dose–response experiment for P@Al-KNPs antibacterial activity: zone of inhibition (mm) as a function of nanoparticle concentration (μ g/mL) for *Staphylococcus aureus*, *Streptococcus* sp., and *Salmonella* sp

The EDX data confirm the successful incorporation of both aluminum (49.11%) and potassium (24.99%) into the nanoparticle matrix—the first report of such a dual-metal hybrid composition achieved via green synthesis. The proportions reflect the molar ratio of the precursors employed (1:2.4 AlCl_3 :KCl), with the higher aluminum mass fraction attributable to the greater atomic mass of Al^{3+} in the reaction. Trace elements (Si, Mg, Cl, Ca, Na, P, S) are consistent with the mineral composition of *P. longifolia* biomass and have been similarly reported in green-synthesized potassium nanoparticles from *Sideroxylon capiri*³⁰ and biogenic potassium nanoparticles from Sheoran *et al.*³¹

4.3. Antibacterial activity and mechanistic considerations

The statistically validated, dose-dependent antibacterial

activity of P@Al-KNPs (one-way ANOVA, $p < 0.05$; DMRT) against all three MDR pathogens demonstrates their significant antimicrobial potential. The maximum ZOIs at 150 μ g/mL—16.4 mm (*S. aureus*), 16.0 mm (*Streptococcus* sp.), and 14.3 mm (*Salmonella* sp.)—are clinically meaningful and comparable to or exceeding results reported for aluminum nanoparticles against *S. aureus* by Dassanayake *et al.*³³ and for copper nanoparticles using *P. longifolia* by Maulana *et al.*¹⁶ A comparative literature summary is presented in Table 4.

The absence of inhibition by the *P. longifolia* extract alone (0.0 mm at 1,000 μ g/mL) underscores that the antibacterial effects are nanoparticle-mediated rather than attributable to phytochemical bioactivity. The Gram-positive pathogens (*S. aureus*, *Streptococcus* sp.) demonstrated higher susceptibility at intermediate concentrations than the

Table 4. Comparative analysis of P@Al-KNPs with selected green-synthesized nanoparticles reported in the literature, highlighting the novelty of the dual-metal hybrid system

Study	Precursor	Size (nm)	Morphology	Max ZOI (mm)	Method
P@Al-KNPs (This study)	AlCl ₃ /KCl	70–103	Spherical	16.4 (<i>Staphylococcus aureus</i>)	Disc diffusion, ANOVA
Judith & Vasudevan ³⁰	<i>Sideroxylon capiri</i> extract/K	200–360	Spherical	N/R	Disc diffusion
Sheoran <i>et al.</i> ³¹	K ₂ SO ₄ biogenic	21–30	Spherical	N/R (wheat growth)	Spectrophotometry
Dashora <i>et al.</i> ¹⁷	AgNO ₃ / <i>Polyalthia longifolia</i>	<18	Spherical	N/R (antifungal)	MIC/Disc diffusion
Maulana <i>et al.</i> ¹⁶	CuSO ₄ / <i>P. longifolia</i> roots	~30	Spherical	15.0 (<i>S. aureus</i>)	Disc diffusion
Dassanayake <i>et al.</i> ³³	γ-Al ₂ O ₃ NPs	<100	Irregular	16.2 (<i>S. aureus</i> equiv.)	MIC/MBC

Abbreviations: MBC: Minimum bactericidal concentration; MIC: Minimum inhibitory concentration; NP: Nanoparticle; N/R: Not reported.

Gram-negative *Salmonella* sp., consistent with differences in cell wall architecture—Gram-positive bacteria lack the outer lipopolysaccharide membrane barrier, facilitating nanoparticle-mediated disruption of the peptidoglycan layer and cytoplasmic membrane.^{5,34}

The proposed antibacterial mechanisms of P@Al-KNPs include: (i) disruption of bacterial cell membrane integrity through direct nanoparticle–membrane electrostatic interactions; (ii) generation of ROS leading to oxidative damage to lipids, proteins, and nucleic acids; (iii) interference with bacterial membrane potential and ATP synthesis; and (iv) inhibition of key metabolic enzymes through metal ion release (Al³⁺, K⁺). The polyvalent nature of this dual-metal nanopatform, which engages multiple concurrent mechanisms, substantially reduces the probability of bacterial resistance development, distinguishing P@Al-KNPs from single-mechanism conventional antibiotics.^{9,33}

The inefficacy of conventional antibiotic discs (0–6 mm ZOI) against all three isolates confirms the MDR phenotype of the clinical strains employed, thereby validating P@Al-KNPs as an effective nanotherapeutic against true MDR bacteria. These findings align with the framework of Pushpalatha *et al.*¹⁰, who identified nanoparticle-based strategies as essential novel approaches for combating antibiotic-resistant infections.

5. Conclusion

This study reports, for the first time, the successful green

synthesis of aluminum–potassium hybrid nanoparticles (P@Al-KNPs) using *P. longifolia* aqueous leaf extract as both a reducing and capping agent—a novel dual-metal nanopatform not previously described in the literature. Comprehensive physicochemical characterization confirmed SPR-attributed UV–Vis absorption at 408 nm, FTIR-verified phytochemical-mediated biosynthesis through phenolic, carbonyl, and hydroxyl group interactions, predominantly spherical SEM morphology (70–103 nm), and EDX-confirmed hybrid composition (Al: 49.11%; K: 24.99%).

Antibacterial evaluation by the Kirby–Bauer disc diffusion method, statistically validated by one-way ANOVA and DMRT post-hoc analysis ($p < 0.05$), demonstrated significant dose-dependent activity against MDR *S. aureus*, *Streptococcus* sp., and *Salmonella* sp., with maximum ZOIs of 16.4, 16.0, and 14.3 mm, respectively, at 150 µg/mL. These results position P@Al-KNPs as promising candidates for addressing the global AMR crisis, with the green synthesis approach providing environmental, economic, and scalability advantages aligned with principles of sustainable nanotechnology and green chemistry. Future research should prioritize: (i) determination of MICs and minimum bactericidal concentrations using broth microdilution; (ii) *in vitro* cytotoxicity and biocompatibility assessment on mammalian cell lines; (iii) optimization of synthesis parameters (temperature, pH, extract:precursor ratio) to achieve monodisperse nanoparticle populations; and (iv) *in vivo* efficacy and safety evaluation in animal infection

models to establish the translational potential of P@AI-KNPs as a biomedical antimicrobial agent.

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Conflict of interest

Mathew Gideon is an employee of Sustainable Organic Innovation Limited but was not involved in any activities that could constitute a conflict of interest in relation to this study. The authors declare that they have no competing interests.

Author contributions

Conceptualization: All authors

Formal analysis: All authors

Investigation: All authors

Supervision: Basma T. Abd-Elhalim

Writing–original draft: All authors

Writing–review & editing: Basma T. Abd-Elhalim

Ethics approval and consent to participate

Not applicable. This study did not involve human participants, human tissue, or animal subjects.

Consent for publication

Not applicable.

Availability of data

All data generated and analyzed during this study are included in this article. Raw datasets are available from the corresponding author upon reasonable request.

Further disclosure

During the preparation of this manuscript, the author(s) did not use any artificial intelligence (AI) or AI-assisted technologies in the generation of scientific content, data, analysis, or conclusions. The authors confirm that ChatGPT was used for language editing; we therefore take full responsibility for the content of this publication.

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