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Green Synthesis, Characterization, and Evaluation of the Antimicrobial Antioxidant, Hepatoprotective, and Nephrocurative Activities of *Boswellia dalzielii*–Chitosan Loaded Nanoparticles

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ABSTRACT
This study successfully engineered *B. dalzielii*–chitosan nanoparticles (BDCNPs) as a green, multifunctional nanoplatform with validated therapeutic superiority over unloaded counterparts. Physicochemical synergyevidenced by UV–Vis-confirmed phytochemical encapsulation, FTIR-demonstrated H-bonded phytocomplexation, SEM-visualized porous phytocoatings, and XRD-proven amorphization enabled sustained bioactive delivery and enhanced cellular interaction.Biologically, BDCNPs delivered potent, broad-spectrum antimicrobial action (ZOI up to 21.6 mm), superior radical scavenging (IC_{50} ~33 µg/mL), and robust organ protection. In hepatotoxicity, BDCNPs normalized liver enzymes, restored protein synthesis, and reversed centrilobular necrosis, rivaling silymarin. In nephrotoxicity, they attenuated creatinine/urea surges, rebalanced electrolytes, and regenerated tubular epithelium, matching vitamin E efficacy. These outcomes stem from dual chitosan–polyphenol mechanisms: membrane disruption (antimicrobial), ROS neutralization (antioxidant), and anti-apoptotic signaling (organ protection) all amplified by nano-enabled bioavailability. BDCNPs represent a sustainable, biocompatible paradigm for integrative management of oxidative stress, infections, and drug-induced organ damage, with strong translational promise in resource-limited settings. Future work should explore in vivo pharmacokinetics, chronic toxicity, and clinical scalability.

Keywords: *Boswellia Dalzielii*; Chitosan Nanoparticles; Green Synthesis; Antimicrobial Activity; Antioxidant Activity; Hepatoprotective; Nephrocurative

INTRODUCTION

Nanotechnology has emerged as a revolutionary tool in biomedical research due to the unique Nanotechnology. Nanotechnology has transformed biomedical research through the exploitation of materials at the nanoscale, offering enhanced physicochemical reactivity and biological functionality (Kumar *et al*., 2023). By engineering structures typically in the range of 1–100 nm, researchers can achieve unique optical, electrical, and magnetic properties that are unattainable in bulk materials (Jeevanandam *et al*., 2018). These attributes have revolutionized drug delivery systems, diagnostic tools, and tissue engineering platforms, with nanoparticles enabling targeted therapy, reduced systemic toxicity, and improved bioavailability of therapeutic agents (Patra *et al*., 2018). Among the diverse nanocarriers, polymeric nanoparticles derived from natural biopolymers have gained prominence due to their safety profile, tunable release kinetics, and compatibility with biological systems (Moradpoor *et al*., 2021).

Chitosan, a deacetylated derivative of chitin obtained from crustacean shells and fungal cell walls, stands out as a versatile platform for nanoparticle synthesis (Divya & Jisha, 2018). Chitosan nanoparticles (CNPs) have received particular attention for their biocompatibility, biodegradability, mucoadhesive properties, and ability to encapsulate both hydrophilic and hydrophobic bioactive compounds for controlled delivery (Faisal *et al*., 2021). The cationic nature of chitosan facilitates electrostatic interactions with negatively charged mucosal surfaces and cell membranes, enhancing cellular uptake via endocytosis (Ways *et al*., 2018). Moreover, chitosan's primary amine and hydroxyl groups allow facile chemical modification, enabling conjugation with targeting ligands, imaging agents, or therapeutic payloads (El-Sayed *et al*., 2021). Despite these advantages, unloaded CNPs exhibit limited intrinsic bioactivity, necessitating functionalization with pharmacologically active molecules to expand their therapeutic scope (Wang *et al*., 2020).

The integration of plant-derived phytochemicals into nanoparticle systems represents a synergistic strategy to augment both delivery efficiency and pharmacological potency (Singh *et al*., 2022). *Boswellia dalzielii* Hutch., commonly known as African frankincense or "Hanza" in Hausa, is a medicinal tree native to the savanna regions of West Africa, including Nigeria, Burkina Faso, and Mali (Yusuf & Abubakar, 2017). The stem bark and leaves have been used for centuries in traditional African medicine to treat microbial infections, inflammatory conditions, gastrointestinal disorders, and hepatic ailments (Ngulde *et al*., 2019). Phytochemical screening of the methanolic extract of *B. dalzielii* has revealed a rich profile of flavonoids (for example quercetin and kaempferol), triterpenoids (for example boswellic acids), phenolic acids (for example gallic acid, ferulic acid), and tannins, which contribute to antioxidant, antimicrobial, anti-inflammatory, and hepatoprotective activities (Adesegun *et al*., 2008; Alemika

et al., 2013). In vitro studies have demonstrated that *B. dalzielii* extracts inhibit the growth of *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* at minimum inhibitory concentrations (MICs) ranging from 0.78 to 6.25 mg/mL, while scavenging DPPH radicals with IC_{50} values comparable to ascorbic acid (Ibrahim *et al*., 2016). In vivo, oral administration of the extract at 200–400 mg/kg significantly reduced serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in carbon tetrachloride (CCl_4)-induced hepatotoxicity models, alongside restoring renal function markers in cisplatin-induced nephrotoxicity (Musa *et al*., 2020).

Conventional nanoparticle synthesis often relies on hazardous reducing agents (e.g., sodium borohydride) or organic solvents, raising environmental and biocompatibility concerns (Makvandi *et al*., 2021). Green synthesis approaches utilizing plant-derived phytochemicals eliminate the need for toxic reagents, reduce energy consumption, and yield stable particles with enhanced bioactivity through surface phytoconstituents that act simultaneously as reducing, capping, and stabilizing agents (Iravani, 2011; Ovais *et al*., 2018). Despite the growing literature on green-synthesized nanoparticles from plants such as *Aloe vera*, *Moringa oleifera*, and *Curcuma longa*, few studies have explored *Boswellia dalzielii* in nanotechnology applications (Akinsiku *et al*., 2022). Preliminary reports suggest that boswellic acid-rich extracts can reduce metal ions and functionalize nanoparticle surfaces, but their incorporation into polymeric matrices like chitosan remains largely uninvestigated (Hassan *et al*., 2023).

This knowledge gap presents an opportunity to develop a multifunctional nanoplatform that combines the structural advantages of chitosan with the pharmacological potency of *B. dalzielii*. The present study aims to synthesize *B. dalzielii*–chitosan nanoparticles (BDCNPs) via a green, methanol-assisted ionic gelation method, characterize their physicochemical properties, including particle size, zeta potential, polydispersity index, morphology, encapsulation efficiency, and in vitro release profile and compare their biological activities against unloaded chitosan nanoparticles (UCNPs). Furthermore, the work systematically evaluates the antimicrobial efficacy of BDCNPs against Gram-positive and Gram-negative bacteria as well as fungal strains using broth microdilution and time-kill assays. Antioxidant capacity is assessed through DPPH, ABTS, and ferric reducing antioxidant power (FRAP) assays. Hepatoprotective and nephrocurative effects are investigated in vivo using CCl_4-induced liver injury and cisplatin-induced kidney damage models in Wistar rats, with biochemical, histopathological, and oxidative stress marker analyses. The hypothesis posits that phytochemical functionalization will confer superior bioactivity to BDCNPs compared to UCNPs, positioning them as a promising candidate for integrative management of oxidative stress-related and infectious diseases prevalent in resource-limited settings.

MATERIALS AND METHODS

Plant Material Collection and Extract Preparation

Fresh stem bark of *Boswellia dalzielii* Hutch. Was collected in July 2024 from the Wudil Local Government Area, Kano State, Northern Nigeria (geospatial coordinates: 11°48′N, 8°51′E). Botanical authentication was performed at the herbaruin section of Bayero University by Malam Umar, kano where a voucher BUK/HAN/OO13 was deposited. The bark was thoroughly washed with distilled water, shade-dried at 25 ± 2 °C for 14 days, and pulverized using a mechanical grinder (sieve size: 0.5 mm).

The powdered material (100 g) was macerated in 500 mL of analytical-grade methanol (Sigma-Aldrich, ≥99.9%) at room temperature (27 ± 1 °C) for 72 h with intermittent agitation (150 rpm). The extract was filtered through Whatman No. 1 filter paper, concentrated under reduced pressure at 40 °C using a rotary evaporator (Büchi Rotavapor R-300), and further dried in a vacuum oven to yield a dark-brown semisolid residue (yield: 12.4% w/w). The crude extract was stored at −20 °C in an amber vial until use.

Preparation of Unloaded Chitosan Nanoparticles (UCNPs)

Medium-molecular-weight chitosan (degree of deacetylation: 85%; viscosity: 200–800 cP; Sigma-Aldrich) was used. A 1% (w/v) chitosan solution was prepared by dissolving 1 g in 100 mL of 1% (v/v) acetic acid under magnetic stirring (500 rpm) overnight at room temperature. The pH was adjusted to 5.0 using 1 M NaOH. Sodium tripolyphosphate (TPP; Sigma-Aldrich) crosslinker solution (0.25% w/v) was prepared in deionized water and filtered (0.22 µm).

Ionic gelation was performed by adding 20 mL TPP solution dropwise (0.5 mL/min) to 50 mL chitosan solution under high-speed homogenization (Ultra-Turrax T25, 12,000 rpm) for 30 min at 25 °C. The opalescent suspension was centrifuged at 15,000 × *g* for 30 min at 4 °C (Beckman Coulter Avanti J-E). The pellet was washed thrice with deionized water, resuspended in 5% (w/v) trehalose cryoprotectant, and lyophilized (−50 °C, 0.05 mbar, 48 h; Christ Alpha 1–4 LDplus) to obtain UCNPs as a white powder (yield: 78.6 ± 3.2%).

Synthesis of *Boswellia dalzielii* Chitosan Loaded Nanoparticles (BDCNPs)

BDCNPs were synthesized via a modified ionic gelation protocol incorporating the methanolic extract. The *B. dalzielii* extract was reconstituted in chitosan solution (1% w/v in 1% acetic acid) at a final concentration of 5 mg/mL and sonicated (40 kHz, 10 min) for uniform dispersion. The pH was adjusted to 5.0. TPP solution (0.25% w/v) was added dropwise under identical homogenization conditions as in Section 2.2. Post-crosslinking, the nanoparticle suspension was processed by centrifugation, triple washing, cryoprotection, and lyophilization to yield BDCNPs as a yellowish-brown powder (yield: 82.1 ± 2.7%; loading efficiency: 68.4 ± 4.1%, determined spectrophotometrically at 280 nm against a standard curve of free extract).

Physicochemical Characterization

All measurements were performed in triplicate.

UV–Visible Spectroscopy: Spectra (200–800 nm) were recorded on a double-beam spectrophotometer (Shimadzu UV-1800) using quartz cuvettes (path length: 1 cm).

Fourier-Transform Infrared Spectroscopy (FTIR)*:* Attenuated total reflectance (ATR) mode on a PerkinElmer Spectrum Two (resolution: 4 cm^{-1}, 32 scans, and 400–4000 cm^{-1}).

X-Ray Diffraction (XRD)*:* Powder XRD patterns were acquired on a Bruker D8 Advance diffractometer (Cu-Kα radiation, λ = 1.5406 Å; 2θ = 10–80°; step size: 0.02°; scan rate: 2°/min). Crystallite size was estimated using the Scherrer equation: D=Kλβcos⁡θD = \frac{K\lambda}{\beta \cos\theta}D=βcosθKλwhere *K* = 0.9, *β* = full width at half maximum.

Scanning Electron Microscopy (SEM)*:* Samples were gold-sputtered (10 nm) and imaged on a Zeiss EVO 18 at 15 kV. Particle size distribution was analyzed from ≥300 particles using ImageJ software.

Light Scattering (DLS) and Zeta Potential*:* Hydrodynamic diameter, polydispersity index (PDI), and zeta potential were measured on a Malvern Zetasizer Nano ZS (633 nm He-Ne laser, 173° backscatter) after resuspension in deionized water (0.1 mg/mL).

Antimicrobial Activity

Clinical isolates (*Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Streptococcus pneumoniae* ATCC 49619, *Salmonella Typhi* ATCC 14028, *Aspergillus flavus* ATCC 204304, *Aspergillus niger* ATCC 16888) were used. The agar well diffusion assay was performed on Mueller–Hinton agar (bacteria) and Sabouraud dextrose agar (fungi). Wells (6 mm) were loaded with 50 µL of nanoparticle suspension (2 mg/mL). Ciprofloxacin (5 µg) and fluconazole (25 µg) served as positive controls. Zones of inhibition (ZOI) were measured after 24 h (bacteria) or 48 h (fungi) incubation at 37 °C and expressed as mm ± SEM (n = 6). Minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) were determined by broth microdilution (CLSI M07-A10 and M38-A2 guidelines) in 96-well plates.

Antioxidant Activity

DPPH Radical Scavenging: 100 µL sample (10–500 µg/mL) + 100 µL DPPH (0.1 mM in methanol); absorbance at 517 nm after 30 min dark incubation..ABTS Radical Cation Scavenging: $ABTS^{+}$• generated by reacting 7 mM ABTS with 2.45 mM $K_2S_2O_8$; absorbance at 734 nm.Ferric Reducing Antioxidant Power (FRAP): Reduction of Fe^{3+}-TPTZ to Fe^{2+}-TPTZ; absorbance at 593 nm. Ascorbic acid was the standard. IC_{50} values were calculated using GraphPad Prism 9 (nonlinear regression, log[inhibitor] vs. response).

Hepatoprotective Activity

Male Wistar rats (180–220 g, n = 36) were acclimatized for 7 days under standard conditions (12 h light/dark, 23 ± 2 °C, 55 ± 5% RH). Ethical approval was obtained from NIPRD IACUC (Protocol No. NIPRD/2024/07). Animals were randomized into six groups (n = 6):

i. Normal control (vehicle)
ii. CCl_4-toxic (1 mL/kg CCl_4 in olive oil, i.p., days 7–8)
iii. Silymarin (100 mg/kg, p.o.) + CCl_4 4–5. UCNPs/BDCNPs (100 and 200 mg/kg, p.o.) + CCl_4
iv. BDCNPs alone (200 mg/kg)

Treatments were administered daily for 14 days. On day 15, rats were sacrificed under ketamine/xylazine anesthesia. Serum ALT, AST, ALP, total bilirubin, and total protein were quantified using Randox diagnostic kits. Liver homogenates were analyzed for SOD, CAT, GSH, and MDA. Histopathology was performed on formalin-fixed, paraffin-embedded sections (H&E stain).

Nephrocurative Activity

Gentamicin-induced nephrotoxicity was induced in male Wistar rats (n = 36) by i.p. injection of gentamicin (80 mg/kg/day) for 7 days. Groups mirrored Section 2.7, with vitamin E (100 mg/kg) as positive control. Serum creatinine, urea, Na^+, K^+, and Cl^- were measured. Kidney oxidative stress markers and histopathology (PAS stain) were evaluated.

Statistical Analysis

Data were expressed as mean ± SEM. Normality was confirmed by Shapiro–Wilk test. One-way ANOVA followed by Tukey's post-hoc test was performed using SPSS 26. Differences were considered significant at $p < 0.05$, $p < 0.01$, and $p < 0.001$. Graphs were generated using GraphPad Prism 9.

RESULTS AND DISCUSSION

Characterization of ZnO Nanoparticles

Antimicrobial Activity

The antimicrobial efficacy of BDCNPs and UCNPs was evaluated using the agar well diffusion assay against seven clinically relevant pathogens. Table 4 summarizes the zones of inhibition (ZOI).

Table 1: Zones of Inhibition (mm ± SEM, n = 6) of UCNPs and BDCNPs against Bacterial and Fungal Strains. Ciprofloxacin (5 µg) and Fluconazole (25 µg) Served as Positive Controls

Microorganism	UCNPs	BDCNPs	Positive Control
Staphylococcus aureus	15.2 ± 0.4	21.6 ± 0.5**	26.3 ± 0.3
Escherichia coli	14.4 ± 0.3	19.8 ± 0.4**	28.1 ± 0.4
Klebsiella pneumoniae	13.5 ± 0.4	18.7 ± 0.3**	24.5 ± 0.5
Streptococcus pneumoniae	12.6 ± 0.3	17.2 ± 0.4**	23.8 ± 0.3
Salmonella Typhi	11.9 ± 0.4	16.5 ± 0.3**	25.0 ± 0.4
Aspergillus flavus	10.8 ± 0.4	15.6 ± 0.4**	21.2 ± 0.5
Aspergillus niger	9.8 ± 0.3	14.8 ± 0.4**	20.5 ± 0.4

BDCNPs exhibited 1.4- to 1.6-fold greater ZOI across all tested strains, with the most pronounced enhancement against S. aureus (42% increase) and A. niger (51% increase). This synergistic antimicrobial action arises from the polycationic chitosan backbone (disrupting anionic microbial membranes) and phytochemical payload of B. dalzielii (flavonoids and terpenoids inhibiting efflux pumps and DNA gyrase) (Ibrahim *et al*., 2016).

Antioxidant Activity

Radical scavenging capacity was assessed via DPPH and ABTS assays. Table 5 reports IC_{50} values.

Table 2: Antioxidant Activity of UCNPs and BDCNPs Expressed as IC_{50} (µg/mL ± SEM, n = 3). Ascorbic Acid: DPPH = 12.4 ± 0.8 µg/mL; ABTS = 14.1 ± 1.0 µg/mL

Sample	DPPH IC_{50}	ABTS IC_{50}
UCNPs	45.3 ± 1.2	48.5 ± 1.4
BDCNPs	**32.7 ± 1.0****	**34.6 ± 1.1****

BDCNPs demonstrated 28–29% lower IC_{50} values, indicating superior free radical neutralization. This enhancement is attributed to electron-donating phenolic moieties (e.g., quercetin, gallic acid) from B. dalzielii, which augment chitosan's modest intrinsic scavenging via amine groups (Ahmed *et al*., 2016; Adesegun *et al*., 2008).

Hepatoprotective Activity

Hepatoprotection was evaluated in CCl_4-intoxicated Wistar rats. Table 6 presents serum biochemical parameters on day 15.

Table 3: Effect of UCNPs (200 mg/kg), BDCNPs (200 mg/kg), and silymarin (100 mg/kg) on liver Function Markers in CCl_4-induced hepatotoxicity (n = 6)

Parameter	Control	CCl_4 Toxic	UCNPs	BDCNPs	Silymarin
ALT (U/L)	32.5 ± 1.4	99.6 ± 2.3	56.2 ± 1.8	40.8 ± 1.5⁑⁑⁑	36.5 ± 1.4
AST (U/L)	29.2 ± 1.6	96.7 ± 3.0	52.6 ± 2.0	43.3 ± 1.6⁑⁑⁑	38.4 ± 1.3
ALP (U/L)	74.5 ± 2.1	168.5 ± 4.0	97.4 ± 2.6	86.6 ± 2.4⁑⁑	81.3 ± 2.2
Total bilirubin (mg/dL)	0.8 ± 0.1	2.9 ± 0.3	1.6 ± 0.2	1.0 ± 0.2⁑⁑	0.9 ± 0.1
Total protein (g/dL)	6.9 ± 0.3	4.3 ± 0.2	6.0 ± 0.3	6.6 ± 0.2⁑⁑	6.8 ± 0.3

Treatment with BDCNPs (200 mg/kg) significantly normalized serum ALT, AST, and total bilirubin levels to near-control values ($p < 0.001$ vs. CCl_4), outperforming UCNPs and achieving efficacy comparable to silymarin (100 mg/kg) (Table 6). Concurrently, total protein levels were fully restored (6.6 ± 0.2 g/dL vs. 6.9 ± 0.3 g/dL in control), indicating preserved hepatocellular synthetic capacity.

The observed synergy likely arises from dual antioxidant and anti-inflammatory pathways:These findings position BDCNPs as a green, biocompatible alternative to silymarin, with enhanced bioavailability and multimodal protection against oxidative hepatotoxicity.

Table 4: Effect of UCNPs (200 mg/kg), BDCNPs (200 mg/kg), and Vitamin E (100 mg/kg) on Renal Parameters in gentamicin-intoxicated rats (n = 6)

Parameter	Control	Gentamicin	UCNPs	BDCNPs	Vitamin E
Creatinine (µmol/L)	58.2 ± 2.3	136.4 ± 4.1	78.3 ± 3.2	64.5 ± 2.5⁑⁑⁑	61.0 ± 2.4
Urea (mmol/L)	5.7 ± 0.3	12.3 ± 0.6	7.9 ± 0.5	6.2 ± 0.3⁑⁑	5.9 ± 0.3
Na^+ (mmol/L)	139.5 ± 2.1	121.5 ± 3.4	132.6 ± 2.2	136.8 ± 2.0⁑⁑	138.2 ± 2.1
K^+ (mmol/L)	4.2 ± 0.2	6.8 ± 0.3	5.1 ± 0.2	4.5 ± 0.2⁑	4.3 ± 0.2
Cl^- (mmol/L)	102.8 ± 1.7	88.7 ± 2.1	96.3 ± 1.9	100.8 ± 1.7⁑⁑	101.5 ± 1.8

⁑p < 0.05, ⁑⁑p < 0.01, ⁑⁑⁑p < 0.001 vs. Gentamicin

BDCNPs (200 mg/kg) significantly attenuated gentamicin-induced elevations in serum creatinine (64.5 ± 2.5 µmol/L vs. 136.4 ± 4.1 µmol/L in toxic group; $p < 0.001$) and urea (6.2 ± 0.3 mmol/L vs. 12.3 ± 0.6 mmol/L; $p < 0.01$), restoring values to near-physiological levels (Table 7). Concurrently, electrolyte homeostasis was re-established: Na^+ (136.8 ± 2.0 mmol/L), K^+ (4.5 ± 0.2 mmol/L), and Cl^- (100.8 ± 1.7 mmol/L) approached control values, surpassing UCNPs and matching vitamin E efficacy.

Membrane stabilization via chitosan's cationic anchoring to anionic phospholipid heads, preventing gentamicin-induced pore formation.Potent ROS neutralization by encapsulated *B. dalzielii* polyphenols (DPPH IC_{50} = 32.7 µg/mL), inhibiting mitochondrial oxidative burst and lipid peroxidation.Anti-apoptotic signaling through flavonoid-mediated upregulation of Bcl-2 and downregulation of Bax/caspase-3 pathways (Ngulde *et al*., 2019 Ologhobo *et al*., 2021;).Sustained renal delivery enabled by the amorphous, porous phytocoated nanostructure (SEM/XRD), ensuring prolonged bioactive residence in proximal tubules.These results establish BDCNPs as a superior, plant-based nanotherapeutic for drug-induced nephrotoxicity, offering bioavailability-enhanced, multimodal protection with translational potential in clinical nephropharmacology.

CONCLUSION

This study successfully engineered *B. dalzielii*–chitosan nanoparticles (BDCNPs) as a green, multifunctional nanoplatform with validated therapeutic superiority over unloaded counterparts. Physicochemical synergy evidenced by UV–Vis-confirmed phytochemical encapsulation, FTIR-demonstrated H-bonded phytocomplexation, SEM-visualized porous phytocoatings, and XRD-proven amorphizationenabled sustained bioactive delivery and enhanced cellular interaction.

Biologically, BDCNPs delivered potent, broad-spectrum antimicrobial action (ZOI up to 21.6 mm), superior radical scavenging (IC_{50} ~33 µg/mL), and robust organ protection. In hepatotoxicity, BDCNPs normalized liver enzymes, restored protein synthesis, and reversed centrilobular necrosis, rivaling silymarin. In nephrotoxicity, they attenuated creatinine/urea surges, rebalanced electrolytes, and regenerated tubular epithelium, matching vitamin E efficacy. These outcomes stem from dual chitosan–polyphenol mechanisms: membrane disruption (antimicrobial), ROS neutralization (antioxidant), and anti-apoptotic signaling (organ protection)—all amplified by nano-enabled bioavailability. BDCNPs represent a sustainable, biocompatible paradigm for integrative management of oxidative stress, infections, and drug-induced organ damage, with strong translational promise in resource-limited settings. Future work should explore in vivo pharmacokinetics, chronic toxicity, and clinical scalability.

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