



Article

Two-Step Non-Food Valorisation of *Phaleria macrocarpa* Fruit Lignin into Lignin Nanoparticles and Quantum Dots for Antibacterial and Bioimaging Applications

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Abstract

Lignin from *Phaleria macrocarpa* (Mahkota Dewa) fruit, a bioactive-rich cultivated medicinal biomass, was employed as a renewable precursor for lignin quantum dots (LQDs). A simple, aqueous, catalyst-free two-step route (lignin to lignin nanoparticles to LQDs) is demonstrated, enabling the valorization of non-food lignin into photoluminescent nanomaterials. The resulting LQDs were predominantly amorphous with short-range graphitic ordering and a narrow particle size distribution (3–5 nm). Structural and chemical analyses indicated a partially graphitized carbon framework enriched with oxygenated surface functionalities, which is consistent with their bright blue–green emission (λ_{em} of 490 nm; average fluorescence lifetime of 4.51 ns). Hydrothermal carbonization induced a blue shift in the UV–Vis absorption profile, resulting in a main band at 288 nm with a shoulder at 312 nm. The LQDs exhibited high cytocompatibility toward L929 mouse fibroblasts ($93.1 \pm 6.5\%$ viability at 24 h) and were readily internalized by cells, facilitating green fluorescence live-cell imaging as a proof-of-concept. Antibacterial activity was observed against both Gram-positive and Gram-negative strains, supporting dual biofunctional performance. Overall, this study established a green and scalable route for converting *P. macrocarpa* fruit lignin into multifunctional LQDs, with potential applications in circular-bioeconomy such as antimicrobial/active coatings and optical sensing in agro-industrial contexts.

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Keywords: lignin-derived nanomaterials; carbon quantum dots; photoluminescent nanodots; antimicrobial coatings; optical sensing

1. Introduction

Lignocellulose is a complex biopolymeric matrix composed mainly of lignin, cellulose, and hemicellulose and represents one of the most abundant renewable resources on Earth. It offers considerable potential for the production of high-value products, including biocomposites, biofuels, and nanomaterials [1]. However, its heterogeneous and recalcitrant structure makes processing challenging, often requiring pretreatment and fractionation in biorefineries to isolate individual components prior to upgrading [2]. Accordingly, significant research efforts have focused on developing sustainable extraction routes for lignin, which is a prerequisite for synthesizing lignin-derived nanomaterials [3]. In this context, green pretreatment strategies have been proposed to improve lignocellulosic fractionation and facilitate its integration into biorefinery schemes [4].

Lignin is an aromatic polyphenolic polymer and the second most abundant biopolymer in lignocellulosic biomass after cellulose [5]. Despite its abundance, less than 2% of lignin is valorized annually, and it is largely burned for energy recovery or used in low-value applications (e.g., adhesives, dispersants, and additives) [6]. This underutilization is primarily attributed to the complex and variable molecular architecture of lignin, which strongly depends on the botanical origin and extraction conditions [7]. Therefore, enhancing lignin valorization is a key objective in modern biorefinery design [8], particularly under the growing demand for sustainable materials and the transition towards a circular bioeconomy.

Among lignin-derived nanotechnological approaches, carbon-based nanomaterials are particularly attractive because of their renewable origin, chemical tunability, and alignment with green chemistry principles. In particular, the conversion of raw lignin into lignin quantum dots (LQDs) offers a route towards photoluminescent nanomaterials with broad technological relevance, spanning electronics, photonics, environmental applications, and biointerfaces. LQDs typically exhibit high surface functionality, enabling versatile tailoring for high-value applications, including antimicrobial agents, stabilizers, emulsions, and sensing platforms [9]. More broadly, LQDs represent an opportunity to transform lignin from a traditionally low-value by-product into a multifunctional nanomaterial platform that bridges materials science and sustainable chemistry.

To map current research trends and identify underexplored areas, a bibliometric analysis of the Web of Science records (2011–2025) was performed. Four dominant thematic clusters were identified: (i) lignin nanoparticles (LNPs) and biocompatibility, (ii) green synthesis and carbon quantum dots (CQDs), (iii) photoluminescence and optical properties, and (iv) nanocomposites and catalytic applications (Figure 1). While substantial progress has been made in the green synthesis of CQDs, comparatively few studies have clearly connected the LNP and LQD domains. Notably, the VOSviewer (v1.6.20) keyword co-occurrence network shows weak linkages between the “lignin nanoparticles” and “carbon quantum dots”, suggesting limited overlap between these research directions and indicating that the transformation of LNPs into LQDs remains insufficiently explored. Furthermore, no bibliographic records were found in the Web of Science for *Phaleria macropora* (Mahkota Dewa) as a lignin source for LQD synthesis.

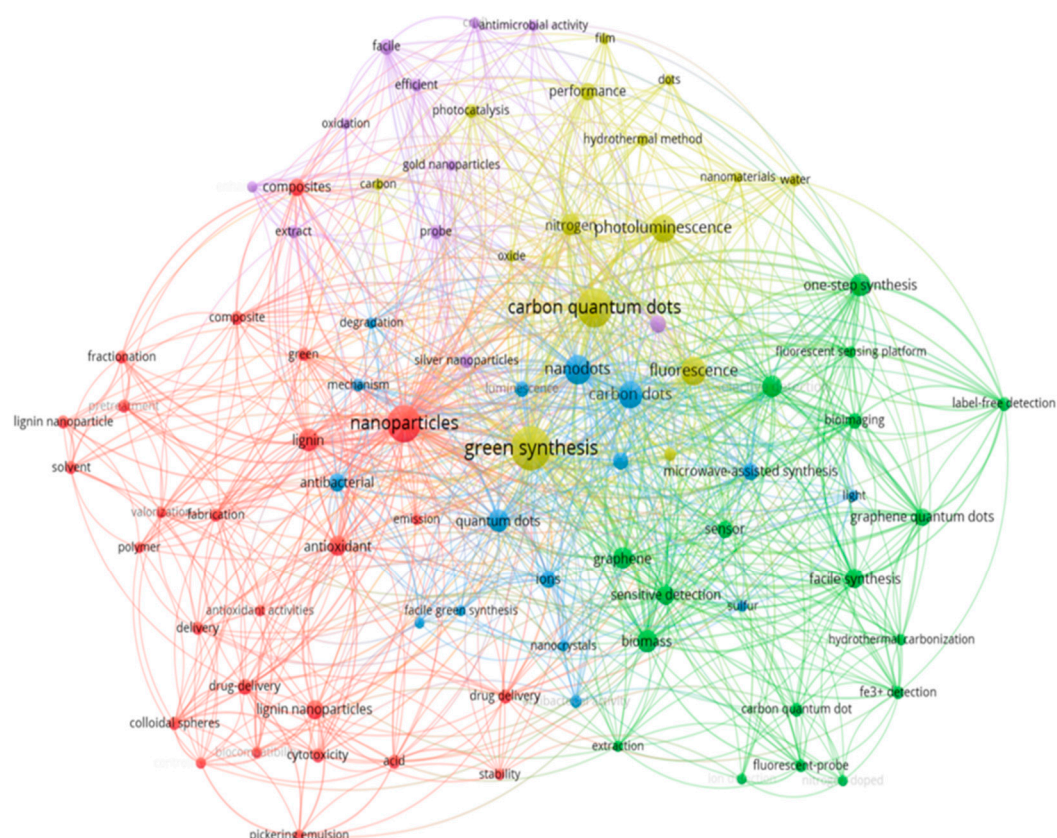


Figure 1. Bibliometric co-occurrence map of keywords extracted from scientific publications indexed in the Web of Science [10] using the query (“*Phaleria macrocarpa*” OR “Mahkota Dewa”) AND (“lignin” OR “nanoparticle” OR “quantum dot”) AND (“hydrothermal” OR “carbonization” OR “green synthesis”) AND (“antibacterial” OR “antimicrobial” OR “biocompatibility” OR “cytotoxicity” OR “bioimaging” OR “biosensing”). The analysis was performed using VOSviewer software [11]. Each node represents a keyword, with node size proportional to its frequency of occurrence, and link thickness corresponding to the strength of co-occurrence with other terms. Colors indicate thematic clusters automatically detected by the software: the red cluster represents lignin nanoparticles and their biological or functional applications (e.g., antibacterial, antioxidant, and drug delivery); the green cluster corresponds to green synthesis and CQDs; the yellow cluster focuses on photoluminescence and optical properties; and the purple cluster is associated with composite materials and photocatalysis. The weak linkage between the “lignin nanoparticles” and “carbon quantum dots” clusters indicates a limited overlap between these research domains, confirming that the transformation of LNPs into LQDs remains underexplored.

Carbon quantum dots (CQDs) were first reported in 2004 as a class of carbon-based nanostructures exhibiting zero-dimensional quantum confinement [12]. Typically, <10 nm in size, CQDs are quasi-spherical nanomaterials comprising sp^2/sp^3 -hybridized carbon cores decorated with functional groups such as hydroxyl, carboxyl and methoxy moieties. Conventional CQD synthesis often relies on petrochemical or hazardous precursors, raising concerns regarding sustainability and environmental impact. This has motivated the use of renewable feedstocks, with lignin emerging as a particularly promising precursor owing to its abundance and intrinsic aromatic framework, which favours the carbonization and formation of functionalized carbon nanodots [13,14].

Recent studies have highlighted the versatility of lignin as a precursor for CQDs. For instance, a recent study has identified kraft lignin as an effective carbon source for CQD synthesis [15]. Another study reported that LQDs can exhibit strong luminescence and surface-dependent functional adaptability, which supports their potential as sustainable

nanomaterials for diverse applications [16]. Nevertheless, many reported LQD systems originate from industrial technical lignins that are substantially modified during pulping and can display pronounced process-dependent heterogeneity, which constrains opportunities for design functionally enriched nanodots without post-synthetic modifications. This motivates the exploration of alternative lignin feedstocks and isolation routes that enable sulfur-free lignin and distinct functional group profiles, while maintaining a rigorous link between the precursor chemistry and the surface/defect states formed after conversion.

In this context, the present study introduces lignin derived from *P. macrocarpa* fruit as a novel precursor for LQD synthesis. The selection is lignin-centred and does not rely on the presence of low-molecular-weight extractives in the raw biomass, since such compounds are targeted for removal during lignin isolation and are not assumed to contribute directly to the LQD properties. Lignin is the most abundant renewable aromatic polymer, and its structural motifs and functional group distributions can vary with botanical origin and isolation conditions, which may influence the chemical evolution during the hydrothermal conversion. Here, lignin was isolated by alkaline extraction and acid precipitation, providing a sulfur-free aromatic framework suitable for conversion into photoluminescent nanodots. Upon hydrothermal conversion, such lignin-derived aromatic/phenolic motifs and oxygenated substituents can reorganize into nanoscale sp²-rich domains while generating oxygen-functionalized surface states (e.g., -OH, C=O, -COOH) and defect sites, which may contribute to the optical response and interfacial biointeractions of the resulting LQDs. This approach supports the rational design of bio-based nanomaterials by integrating renewable carbon frameworks with process-generated surface functionalities.

To our knowledge, this is the first report on the use of *P. macrocarpa* fruit lignin as a precursor for LQDs synthesis. The objectives of this study are to: (i) develop a green, aqueous and catalyst-free two-step route to convert *P. macrocarpa* lignin into lignin nanoparticles and subsequently LQDs; (ii) elucidate the structural and chemical evolution during hydrothermal carbonization using complementary characterization techniques; (iii) establish structure–optical property relationships, focusing on absorption and photoluminescence behaviour; (iv) evaluate biofunctional performance, including antibacterial activity against Gram-positive and Gram-negative bacteria; and (v) demonstrate proof-of-concept applicability through cellular fluorescence imaging.

Overall, this study framed a non-food lignin valorization pathway from a cultivated medicinal crop (*P. macrocarpa* is traditionally used in medicinal contexts [17,18]) towards multifunctional photoluminescent nanomaterials, supporting circular bioeconomy applications in antimicrobial/active coatings and optical sensing.

2. Results and Discussion

The transformation of lignin extracted from the medicinal fruit *P. macrocarpa* into nanostructured carbon materials was comprehensively investigated to elucidate the chemical, structural, thermal, and morphological changes accompanying the formation of nanoparticles and quantum dots. *P. macrocarpa* is traditionally recognized for its wide range of pharmacological properties, yet its lignocellulosic residues remain largely unexplored as a carbon-rich feedstock. Therefore, this study introduces, for the first time, a sustainable valorization approach that converts this underutilized medicinal biomass into functional nanomaterials via sequential alkaline extraction, acid precipitation, and hydrothermal carbonization. This dual-stage process not only advances the green synthesis of lignin nanostructures but also demonstrates the untapped potential of medicinal fruit by-products for generating high-value, carbon-based nanomaterials. The integrated

characterization presented here establishes a direct relationship between molecular reorganization, degree of carbonization, and nanoscale morphology.

2.1. Characterization of LNPs and LQDs

The chemical evolution from raw fruit powder to LNPs and finally to LQDs was examined by FTIR spectroscopy (Figure 2a). The spectrum of LNPs retained the characteristic features of lignin, with a broad band at 3300 cm^{-1} corresponding to O–H stretching from phenolic and aliphatic hydroxyl groups, and absorptions at 2920 and 2850 cm^{-1} associated with C–H stretching of methylene and methyl groups. The band at 1630 cm^{-1} was assigned to aromatic C=C stretching (with possible contribution from the bending mode of adsorbed water), while the signals at 1420 and 1367 cm^{-1} correspond to aromatic skeletal vibrations with C–H in-plane deformation and aliphatic C–H/phenolic O–H in-plane bending, respectively, indicating preservation of the aromatic backbone. In the fingerprint region, ether-related C–O stretching bands typically reported for lignin around 1265 – 1270 cm^{-1} (often associated with guaiacyl-related C–O stretching) and 1030 – 1080 cm^{-1} (C–O stretching in side chains/ether linkages) display a lower relative intensity in LQDs than in LNPs (Figure 2a, inset), consistent with progressive C–O–C bond cleavage and chemical reorganization during hydrothermal conversion. In contrast, the LQD spectrum revealed marked structural reorganization; the aliphatic C–H stretching bands were significantly reduced, and a carbonyl band near 1700 cm^{-1} (C=O) became more intense, indicating oxidation and condensation during hydrothermal carbonization. Additional absorptions at 1157 and 1056 cm^{-1} were attributed to C–O stretching modes, consistent with the oxygenated surface functionalities typical of CQDs. These features corroborate previous reports describing the oxidative fragmentation and aromatization of lignin into nanoscale graphitic domains [19–22].

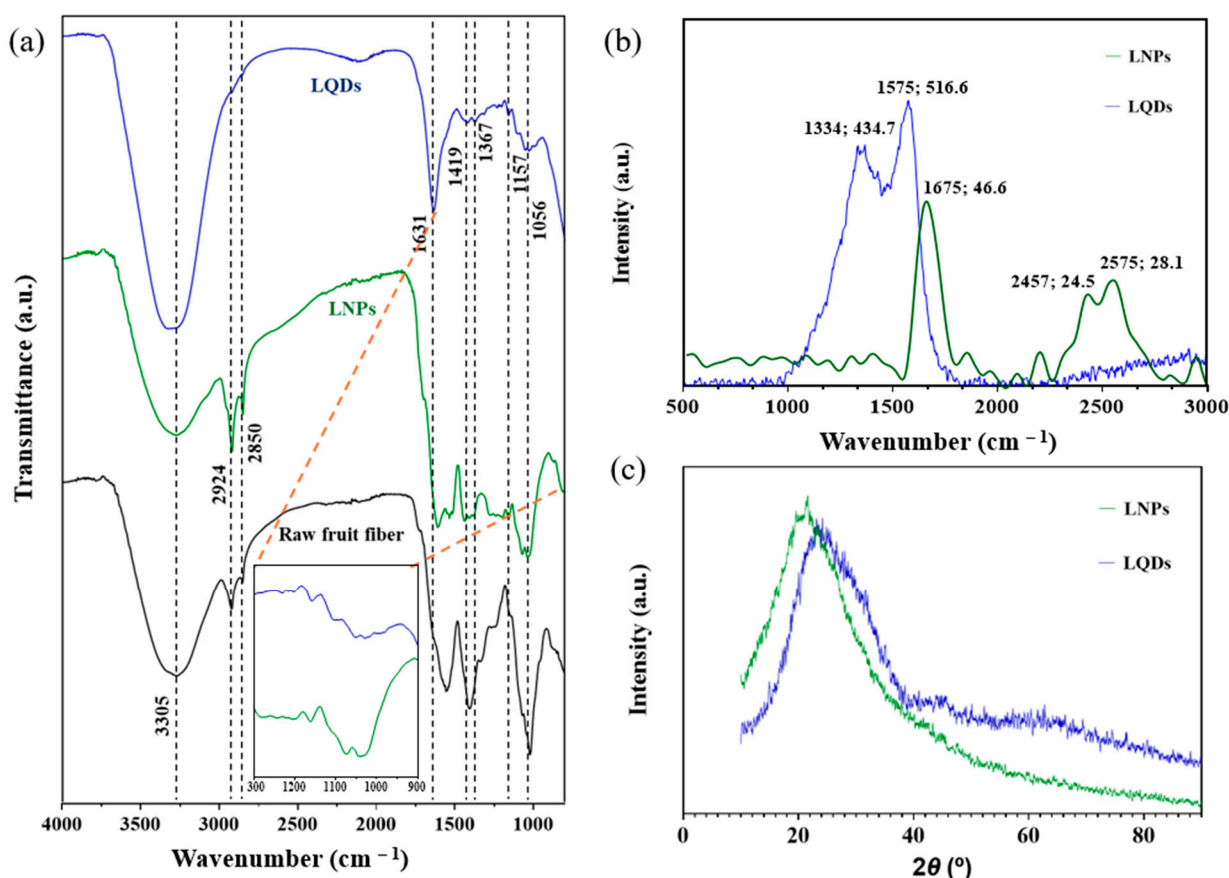


Figure 2. Structural characterization of lignin-based materials: (a) FTIR spectra of raw fruit powder, lignin nanoparticles (LNPs), and lignin quantum dots (LQDs); inset: normalized 1300–900 cm^{-1} fingerprint region; (b) Raman spectrum of LNPs and LQDs; (c) XRD patterns of LNPs and LQDs.

Complementary insights into the carbon framework were obtained using Raman spectroscopy (Figure 2b). The LNPs (prior to hydrothermal conversion) showed a spectrum dominated by broad aromatic features and background fluorescence, without distinct D and G bands, consistent with an amorphous aromatic biopolymer prior to carbonization. In contrast, the LQDs exhibited two pronounced bands at 1330 cm^{-1} (D band) and 1575 cm^{-1} (G band), corresponding to the defect-activated mode of sp^2 rings (D) and in-plane stretching of sp^2 carbon (G), respectively. The D-to-G intensity ratio, I_D/I_G , of 0.84 indicates a partially graphitized structure with abundant defect sites, which is typical of biomass-derived CQDs [23,24]. The coexistence of ordered and disordered carbon phases reflects incomplete graphitization, in which oxygenated defects and edge sites likely contribute to the electronic and photoluminescent properties of the materials.

The Raman spectrum of the LNPs did not display distinct D and G bands. Instead, it revealed a weak band at 1675 cm^{-1} , which can be attributed to aromatic C=C and/or conjugated C=O vibrations. Additionally, low-intensity bands were observed at 2457 cm^{-1} and 2575 cm^{-1} in the higher-wavenumber region, likely associated with overtone or combination modes. These spectral characteristics suggest a predominantly amorphous, non-graphitized aromatic structure in the LNPs. Together, the Raman trends from lignin/LNPs to LQDs support progressive aromatization and partial graphitization during hydrothermal conversion, complementing the FTIR evidence of chemical reorganization discussed above and providing a coherent structural basis for the optical fingerprints discussed below.

XRD analysis confirmed the predominantly amorphous character of both the LNPs and LQDs (Figure 2c). The LNPs displayed a broad diffraction feature centered near 22° (2θ), which is typical of amorphous lignin and indicative of the short-range order within the aromatic domains. Conversely, the LQDs exhibited a broader and less intense halo at a similar location, suggesting a higher degree of structural disorder and nanoscale carbonization. The absence of sharp peaks suggests a lack of long-range crystalline order and supports the Raman evidence of mixed sp^2/sp^3 hybridization. Such amorphous and turbostratic structures are common among lignin and biomass-derived carbon nanomaterials and are associated with enhanced dispersibility and defect-mediated optical properties [25].

TGA and DTG analyses were performed to evaluate the effect of nanoparticle formation on the thermal behavior of lignin (Figure 3a,b). Both the raw fruit powder and LNPs exhibited an initial mass loss below 100 $^\circ\text{C}$, which was attributed to the desorption of physically adsorbed water. A major decomposition event occurred between 200 and 400 $^\circ\text{C}$, which was associated with the depolymerization of lignocellulosic constituents and cleavage of ether and carbon-carbon linkages within the lignin matrix. The LNPs exhibited a shift in the main DTG peak to a higher temperature (360 $^\circ\text{C}$) compared to the precursor (310 $^\circ\text{C}$), indicating enhanced thermal stability. This improvement can be attributed to increased aromatic condensation and crosslinking induced by alkaline extraction and acid precipitation, which yield a more compact molecular network, together with the expected depletion of water-soluble low-molecular-weight fractions and carbohydrate-rich components during extraction and repeated washing/neutralization. A higher degradation temperature reflects the formation of a structurally reinforced lignin framework with improved resistance to thermal scission. Consistent with previous reports on LNPs from other lignocellulosic sources, this shift confirms that nanoparticle formation enhances the intrinsic thermal robustness of lignin [26].

The DTG curve of the LNPs further supports this observation, exhibiting a broader and less intense peak than that of the raw fruit powder. This broadening indicates a more gradual degradation process, consistent with chemical reorganization and reduced contributions from non-lignin constituents in the starting biomass. Because no dedicated compositional assays were performed (e.g., residual carbohydrate or extractives quantification), these interpretations are presented qualitatively. Overall, the improved thermal behaviour supports the suitability of LNPs for incorporation into thermally stable nanocomposites, polymer reinforcement, and bio-based coatings.

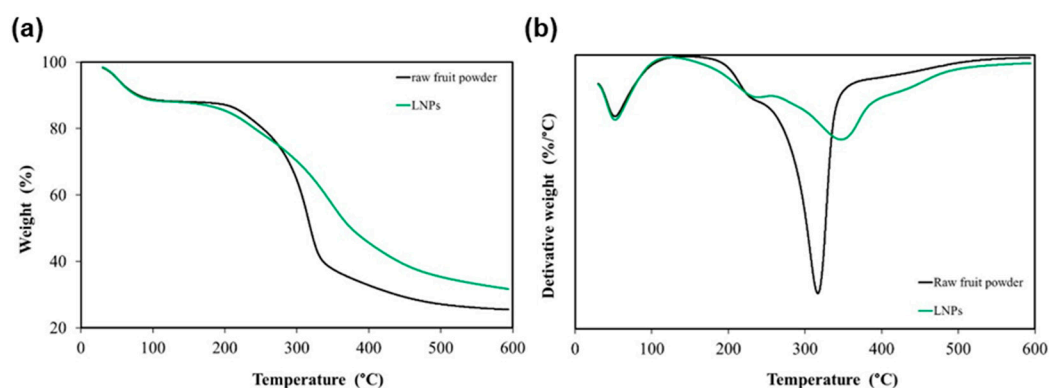


Figure 3. Thermogravimetric analysis of raw fruit powder and lignin nanoparticles (LNPs): (a) TG curves and (b) DTG curves.

The morphological features of the synthesized materials were examined using HR-TEM (Figure 4) and FE-SEM analyses (Figure 5). The limited resolution of FE-SEM for particles smaller than 10 nm necessitates confirmation of discrete quantum dots by HR-TEM rather than FE-SEM [24,27]. HR-TEM images revealed that the LNPs consisted of irregular, aggregated nanostructures (Figure 4(a1,a2)) with diameters in the range of 80–150 nm, consistent with lignin-derived nanoparticles (Figure 4c). The observed aggregation is typical of LNPs due to residual hydrogen bonding and π - π stacking interactions among aromatic units [28].

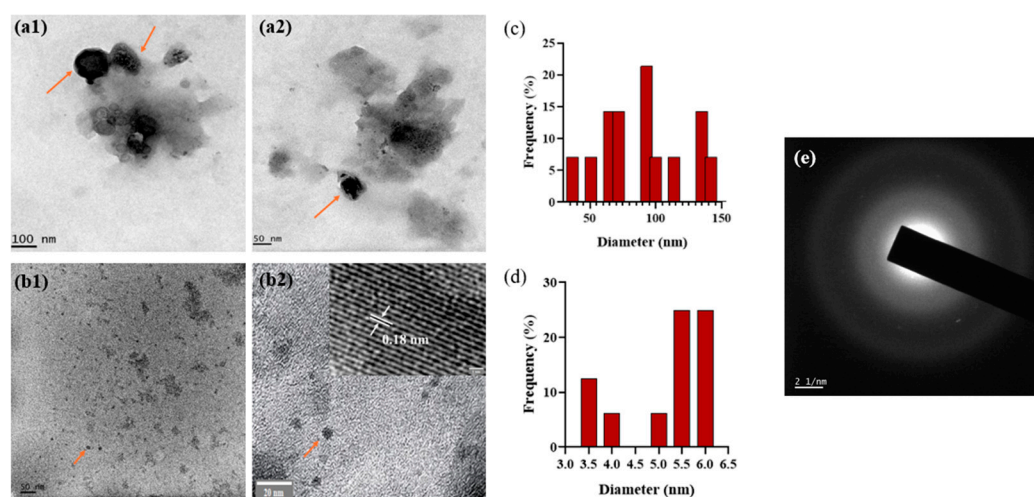


Figure 4. Morphology and structure of lignin-based nanomaterials: (a1,a2) HR-TEM images of lignin nanoparticles (LNPs) the orange arrows indicate individual spherical LNPs; (b1,b2) HR-TEM images of lignin quantum dots (LQDs), with short-range graphitic fringes visible in (b2); the orange arrows highlight individual LQDs, and short-range graphitic fringes (0.18 nm) are visible in (b2); (c,d) particle size distribution histograms for LNPs and LQDs, respectively; (e) SAED pattern of

LQDs showing diffuse concentric rings, characteristic of nanocrystalline graphitic domains within the carbon matrix, confirming the semi-crystalline structure of the LQDs.

By contrast, the LQDs exhibited well-dispersed, nearly spherical nanodots with diameters of 3–5 nm and a narrow size distribution (Figure 4(b1,b2,d)). The substantial size reduction relative to the LNPs reflects fragmentation of lignin macromolecules and subsequent carbonization into nanoscale domains. HR-TEM revealed distinct lattice fringes with an interplanar spacing of 0.21 nm, consistent with graphitic (100) in-plane spacing, confirming short-range graphitic ordering within an amorphous carbon matrix. Selected-area electron diffraction (SAED) displayed diffuse rings at 0.21–0.24 nm, attributable to graphitic in-plane reflections ((100)/(110)), supporting the presence of nanosized sp^2 domains embedded in an amorphous matrix. Similar fringe spacings and particle sizes have been reported for lignin- and cellulose-derived quantum dots obtained under mild hydrothermal conditions. The size confinement and increased local ordering are expected to influence the electronic and optical characteristics of the LQDs. In addition, the relatively narrow size distribution observed by HR-TEM is consistent with the aggregate-removal purification workflow (centrifugation and 0.2 μm filtration) applied prior to characterization.

The SAED pattern of the LQDs (Figure 4e) shows diffuse concentric rings rather than discrete spots, indicating the absence of long-range crystallinity. The rings at 0.21–0.24 nm are attributable to graphitic in-plane reflections ((100)/(110)), consistent with short-range ordering in sp^2 -hybridised carbon and with nanosized sp^2 domains embedded in an amorphous matrix.

FE-SEM imaging (Figure 5) provided complementary morphological evidence across the synthesis stages, from raw fruit powder to LNPs and LQDs. The raw fruit powder exhibited a fibrous, porous architecture typical of lignocellulosic biomass. Following alkaline extraction and acid precipitation, the LNPs displayed a markedly different morphology composed of fragmented, quasi-spherical aggregates, consistent with conversion of the bulk material into nanoparticles. At the FE-SEM scale, large micron-sized particulates were not evident in the imaged fields. The nanoscale morphology obtained here for *P. macrocarpa* fruit is consistent with previous studies on LNPs derived from other biomass sources [28].

Following hydrothermal carbonization, FE-SEM revealed thin, wrinkled, sheet-like carbonaceous domains, which are commonly observed after drying ultrasmall colloids on a substrate and are compatible with a colloidal nanodot dispersion.

Overall, the multi-technique characterization demonstrated a clear structural progression from lignocellulosic biomass to LNPs and, finally, to LQDs. FTIR indicates pronounced chemical reorganization after hydrothermal conversion, with reduced aliphatic signatures and enhanced oxygenated functionalities (Figure 2a), consistent with oxidation/condensation processes and lignin linkage cleavage. Raman (D and G bands) together with the broad XRD halo supports the formation of defective sp^2 -rich domains within a predominantly amorphous carbon framework (Figure 2b,c). In parallel, the higher DTG maximum observed for LNPs is consistent with increased condensation/crosslinking relative to the precursor (Figure 3a,b). HR-TEM/SAED further confirms the conversion into size-confined nanodots (3–5 nm) displaying short-range graphitic fringes (Figure 4e). Taken together, these complementary results provide a coherent structure–property basis for the optical changes discussed in Section 2.3 (UV-Vis/PL; Figure 6), rather than isolated observations from individual techniques.

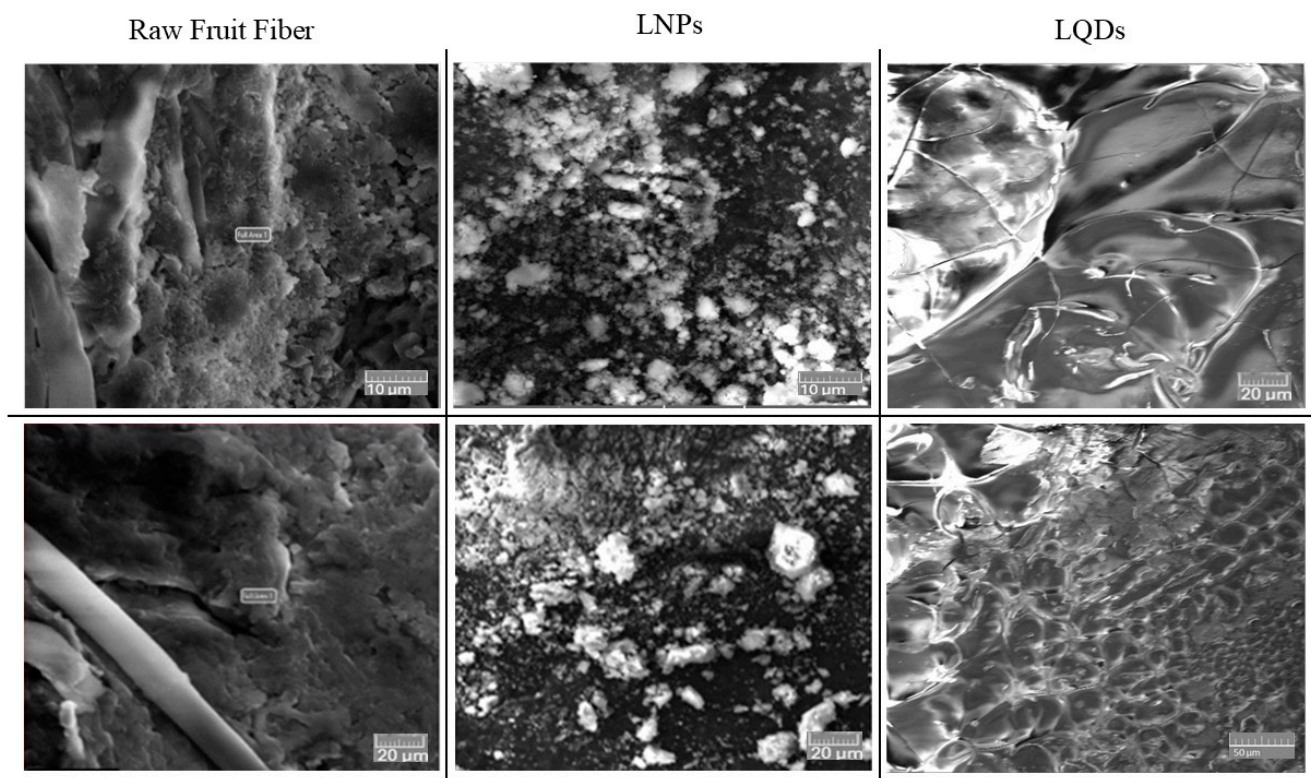


Figure 5. FE-SEM micrographs at different magnifications of raw fruit powder, lignin nanoparticles (LNPs), and lignin quantum dots (LQDs).

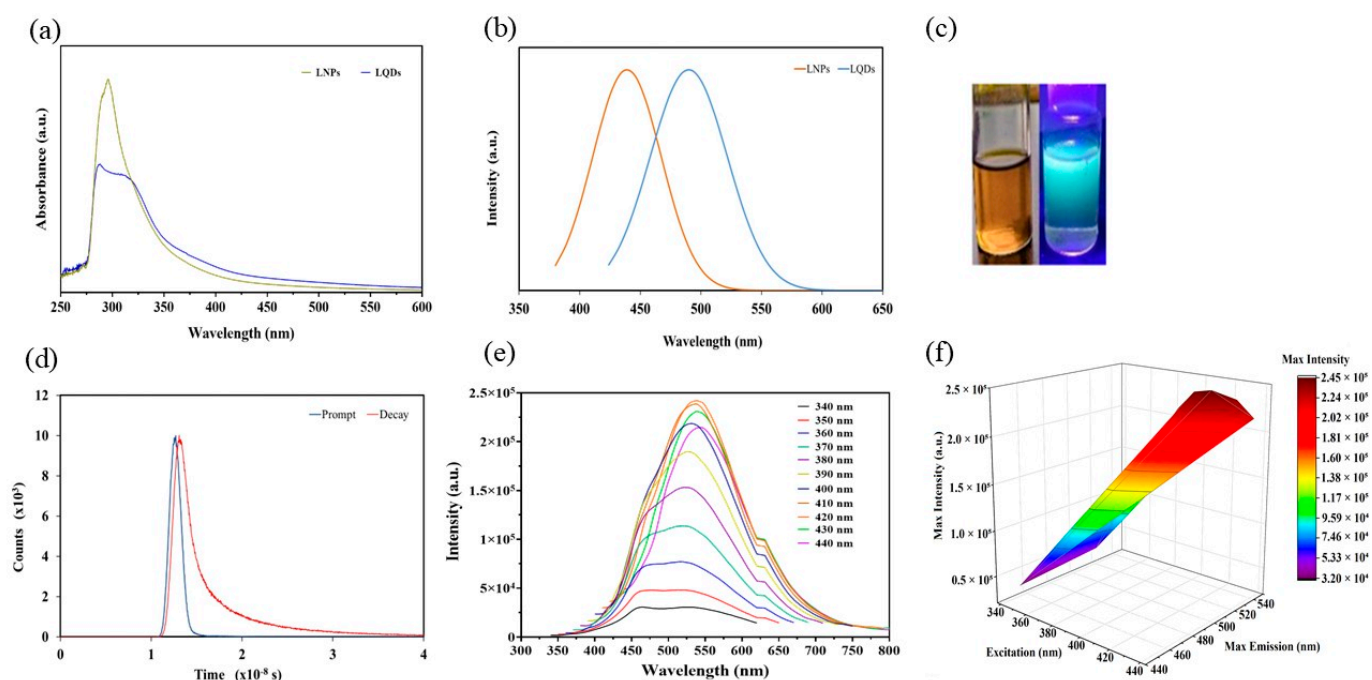


Figure 6. Optical properties of lignin-based nanomaterials. (a) UV-Vis absorption spectra of lignin nanoparticles (LNPs) and lignin quantum dots (LQDs); (b) Photoluminescence (PL) spectra of LNPs and LQDs; (c) Photographs under ambient and 365 nm UV; (d) Time-resolved fluorescence of LQDs (TCSPC, time-correlated single-photon counting; prompt and decay). (e) Excitation-dependent PL spectra of LQDs (λ_{ex} = 340–440 nm, 10 nm steps); (f) 3D surface plot showing the emission maximum ($\lambda_{\text{em, max}}$) and corresponding maximum intensity as a function of excitation wavelength.

2.2. Biological Activity of LQDs

2.2.1. Antibacterial Activity

LQDs produced measurable inhibition zones in both the test organisms (Table 1). After 24 h, the LQDs reached approximately 22 mm in *S. aureus* and up to 20 mm in *K. pneumoniae*. Taken together, these results indicate activity against Gram-positive and Gram-negative model strains. From an application perspective, the observed 24 h inhibition suggests the potential for non-food bio-based products, such as antimicrobial/active coatings, functional papers, or sensor inks within lignin matrices.

Table 1. Zones of inhibition (ZOI, mm; mean $\pm$ SD, $n = 3$) for LQDs against *Staphylococcus aureus* and *Klebsiella pneumoniae*; erythromycin used as positive control.

Bacteria	<i>S. aureus</i> (+)		<i>K. pneumoniae</i> (–)	
	24 h	72 h	24 h	72 h
Erythromycin	19.75 $\pm$ 0.18 ^{a,A}	7.75 $\pm$ 0.41 ^{a,B}	4.42 $\pm$ 0.09 ^{a,A}	2.50 $\pm$ 0.19 ^{a,B}
LQDs	21.77 $\pm$ 0.57 ^{a,A}	29.23 $\pm$ 0.54 ^{b,B}	20.27 $\pm$ 1.30 ^{b,A}	19.54 $\pm$ 0.38 ^{b,B}

Assays used 6 mm disks. Different letters represent significantly different means between treatments (small letters) and different incubation times (capital letters) ($p < 0.05$).

The species-dependent response is consistent with differences in the bacterial cell envelope: Gram-positive bacteria such as *S. aureus* lack an outer membrane and have a thick peptidoglycan layer, whereas *K. pneumoniae* possesses an outer membrane with a lipopolysaccharide-rich outer leaflet that acts as a selective permeability barrier, which can limit the accumulation of exogenous agents [29,30]. In addition, *K. pneumoniae* often expresses an extracellular capsular polysaccharide, which may further hinder access of antimicrobials to the cell surface [31].

The time-dependent inhibition observed here may be influenced by the physicochemical features of the LQDs determined in this work, namely their ultrasmall size (3–5 nm, HR-TEM) and oxygen-containing surface functionalities (FTIR), which can favour interfacial interactions with bacterial surfaces. In the broader carbon-dot literature, antibacterial activity has been associated with membrane perturbation and/or oxidative stress pathways depending on surface chemistry and experimental conditions; however, these mechanisms were not directly assessed in the present study [32].

The comparatively stable inhibition zone observed for *K. pneumoniae* between 24 and 72 h may therefore reflect the combined effect of Gram-negative surface barriers (outer membrane and, potentially, capsule) together with the qualitative nature of the disk diffusion assay (diffusion in agar and growth-related factors). Accordingly, the present discussion is framed as a structure-informed interpretation, and dedicated MIC/MBC and mechanistic assays are required to confirm the dominant antibacterial pathway.

Overall, these results support the potential use of LQDs derived from *P. macrocarpa* fruit as antimicrobial agents, with higher apparent efficacy against *S. aureus* under the tested conditions and support their integration into non-food bio-based products (e.g., antimicrobial/active coatings) where metal-free photoluminescent additives are of interest.

2.2.2. Cytotoxicity of LQDs

The cytocompatibility of LQDs derived from *P. macrocarpa* fruit was evaluated using the L929 mouse fibroblast cell line. The assay showed a mean viability of $93.1 \pm 6.5\%$ at 24 h, indicating low cytotoxicity. Materials are generally considered non-cytotoxic when cell viability remains above 70% of that of the untreated control [33,34]. Under these conditions, the high viability suggests that essential cellular functions were maintained.

The biogenic origin of LQDs contributes to their safety profile. *P. macrocarpa* is rich in phenolics, flavonoids, and lignans, which are associated with antioxidant and antimicrobial activities [18,35], whereas some seed extracts show cytotoxic or anticancer effects at high concentrations [36]. Reports on “green” or LQDs typically describe $\geq 85\%$ viability in fibroblasts and epithelial lines [37,38]. Taken together, the literature reports and the present viability data support low cytotoxicity under the conditions tested; however, a direct link between the phytochemical profile of the precursor and the surface chemistry/bioreactivity of hydrothermally produced LQDs has not yet been established.

The observed low cytotoxicity, combined with the previously demonstrated antibacterial activity, highlights the dual functionality of *P. macrocarpa*-derived LQDs. Their favourable cytocompatibility supports their potential use in biomedical applications, such as antimicrobial coatings, wound dressings, and bioimaging systems, where low toxicity toward mammalian cells is essential.

2.3. Optical Properties of LNPs and LQDs

The UV-Vis absorption spectra of the LNPs (Figure 6a) show a strong absorption band in the UV region, which is characteristic of the conjugated π systems within the lignin structure. This feature arises from $\pi \rightarrow \pi^*$ transitions in sp^2 hybridised aromatic carbon domains and $n \rightarrow \pi^*$ transitions from oxygen-containing functional groups, in agreement with previous reports for lignin-based nanomaterials [39,40]. The preservation of these chromophoric bands after nanoparticle formation confirms that the aromatic framework of lignin remains structurally intact, maintaining its electronic conjugation and optical properties.

Following the synthesis pathway, LQDs were obtained from LNPs via a controlled hydrothermal carbonization step that induced further carbonization and size reduction. This secondary transformation led to marked changes in the UV-Vis response, consistent with increased graphitization and the emergence of new emissive domains in the solid state. The LNPs displayed a distinct absorption peak at 295 nm, which was attributed to the $\pi \rightarrow \pi^*$ transitions in the aromatic rings conjugated with ether (C–O–C) groups. In contrast, the LQDs exhibited a broadened, blue-shifted band centered at 288 nm with a shoulder at 312 nm, consistent with the partial scission of C–O–C linkages and the formation of smaller sp^2 domains. The reduced intensity and broadening are consistent with C–O–C cleavage observed in the FTIR spectrum (Figure 2a), reflecting the partial depolymerization and graphitization of lignin during carbonization. Raman spectra (Figure 2b) further supported this, revealing characteristic D (defect) and G (graphitic) bands typical of amorphous carbon networks [41]. The XRD pattern (Figure 2c) also displayed a broad halo, confirming the formation of a disordered sp^2 carbon matrix. Collectively, these observations indicate partial deoxygenation via ether (C–O–C) scission, accompanied by increased sp^2 content and π -electron delocalization, yielding a more graphitic, yet oxygen-functionalized surface.

The photoluminescence (PL) spectra (Figure 6b) show a pronounced emission shift from 439 nm (LNPs) to 490 nm (LQDs). This bathochromic shift is consistent with the formation of additional emissive surface states after hydrothermal carbonization, as reflected by the excitation-dependent PL (Figure 6e,f). Time-resolved fluorescence measurements (Figure 6d) revealed a multiexponential decay with lifetimes of $\tau_1 = 3.11$ ns, $\tau_2 = 0.542$ ns, and $\tau_3 = 10.3$ ns, yielding an amplitude-weighted mean lifetime of 4.51 ns, which is typical of CQDs and comparable to bright organic fluorophores [42,43]. The photoluminescence quantum yield (QY) was 5.33%, consistent with the reports for hydrothermally prepared LQDs (QY = 5.36%) [44]. This value, together with the lifetime, indicates that non-radiative pathways dominate under the present conditions, leaving scope for further surface passivation to increase QY.

To benchmark the overall performance of the LQDs against representative lignin-based quantum dots reported in the literature, a comparison table (Table 2) is provided, integrating key morphological, biological, and optical indicators.

The benchmarking highlights that only a limited number of studies report a combined dataset including size, optical metrics and biological endpoints, supporting the relevance of the present characterization for application-oriented evaluation.

Table 2. Performance comparison of lignin-derived quantum dots (LQDs) reported in the literature and in this study.

Precursor (Extraction/Processing)	Particle Size (nm)	Antibacterial Activity (ZOI, mm)	Cell Viability	Fluorescence Lifetime (ns)	Quantum Yield (%)	Reference
<i>P. macrocarpa</i> fruit lignin (alkaline extraction + acid precipitation; HTC to LQDs)	3–5	21.77 (<i>S. aureus</i>); 20.27 (<i>K. pneumoniae</i>)	93.1 (L929, 50 µg/mL)	4.51 (mean)	5.33	This work
Alkali lignin (two-step HTC + oxidation/exfoliation)	3.61–3.92	nr.	nr.	19.49–24.39	2.32–18.00	[45]
Alkali lignin (AL; hydrothermal + acid additive)	4.86	nr.	nr.	nr.	23.68	[46]
Aminated alkali lignin (AL; functionalised lignin precursor)	4.42	nr.	nr.	4.59	15.28	[47]
Alkali lignin + DES (DES processing; N-doped CDs)	4–12	nr.	nr.	nr.	7.95	[20]
Alkaline-extracted lignin	4.4	nr.	biocompatibility (qualitative)	nr.	22	[48]
Alkali lignin (acid additives for heteroatom-doped CQDs)	nr.	nr.	nr.	nr.	30.5	[24]
Kraft lignin (technical lignin)	3.7–4.3	nr.	nr.	2.73–3.65	4.34–10.55	[49]
Kraft softwood lignin	5–10	nr.	cell viability/adhesion observed (qualitative)	nr.	nr.	[22]
Kraft lignin (eucalyptus)	2 to 5	nr.	nr.	nr.	nr.	[50]
Lignosulfonate (sulfonated lignin)	2.03–3.53	16 mm (<i>S. aureus</i>) 16 mm (<i>C. albicans</i>)	nr.	nr.	nr.	[51]
Bio-waste lignin (technical lignin; source/waste not specified)	6.9	nr.	90% (A549, 300 µg/mL)	4.29	22.2	[52]
Lignin (type not specified)	<10	nr.	Good biocompatibility (qualitative)	nr.	43	[53]
Technical lignin in GVL/H ₂ O (type not specified; solvent-assisted processing)	4.9	nr.	nr.	nr.	47.3	[54]
Solvent-fractionated lignin (ethanol sieving + DCM/H ₂ O fractionation)	<10	nr.	92.8% and 99.4% (L02, 0.5 mg/mL)	3.51–4.88	1.32–9.57	[55]
G/S lignin; G/S–C lignin	2.80–3.01	nr.	nr.	1.61–1.75	1.17–4.98	[56]
Lignin/lignin-char (rapid air-assisted pyrolysis)/hydrothermal oxidation	nr.	nr.	nr.	0.05	nr.	[57]
Spruce biomass lignin (acidolysis-derived fragments; hydrothermal)	<10	nr.	nr.	4.8	7	[58]
Supernatant component after lignin acidolysis	3.17	nr.	nr.	nr.	19.5	[59]

Cracked lignin fragments (ethanol supercritical catalysis; noble-metal catalysis)	4.01–2.81	nr.	nr.	nr.	16.60–19.45	[60]
Unbleached mechanical pulp (lignocellulosic biomass)	9.73 ± 1.64	nr.	nr.	nr.	nr.	[61]
Softwood sawdust (lignocellulosic biomass; HTC)	nr.	nr.	nr.	nr.	3.4	[62]

nr., not reported; ZOI, zone of inhibition; AL, alkali lignin; DES, deep eutectic solvent; GVL, γ -valerolactone; HTC, hydrothermal carbonization; DCM, dichloromethane; G/S, guaiacyl/syringyl. Quantum yield, inhibition zones and cell viability depend on experimental conditions (e.g., solvent/excitation wavelength; strain/concentration/incubation time; cell line/dose/exposure time). Values are reported as stated in the original sources.

Excitation-dependent PL (Figure 6e,f) was observed, where the emission maximum red shifted from 455 nm (at $\lambda_{\text{ex}} = 340$ nm) to 538 nm (at $\lambda_{\text{ex}} = 440$ nm). Quantitatively, this corresponds to an apparent slope of 0.83 (83 nm red shift in emission per 100 nm increase in excitation wavelength). The Stokes shift decreased from 115 nm at $\lambda_{\text{ex}} = 340$ nm to 98 nm at $\lambda_{\text{ex}} = 440$ nm, consistent with the progressive access to lower-energy emissive sub-populations as the excitation energy decreased. The PL band remained broad (FWHM on the order of 10^2 nm) with only a weak dependence on excitation, indicative of ensemble emission from a distribution of core/surface states rather than a single electronic transition [41,63].

The blue-shifted UV-Vis absorption and excitation-dependent PL are consistent with the sp^2 enrichment and oxygenated surface states identified by FTIR/Raman spectroscopy.

The integrated PL intensity increased with excitation and reached a maximum at $\lambda_{\text{ex}} = 430$ –440 nm (Figure 6e,f), providing a practical excitation window for imaging and sensing. Combining the QY (5.33%) with the average lifetime (4.51 ns) yields a low radiative rate constant, indicating that non-radiative pathways dominate, and leaving headroom for further passivation/functionalization to increase the QY.

Overall, the significant Stokes shift, excitation-tunable emission, and nanosecond lifetimes support applications in multiplexed detection, optical encoding, and fluorescence anti-counterfeiting. Simultaneously, the quantitative metrics above facilitate comparison with other biomass-derived carbon dots [25].

2.4. Bioimaging

Fluorescence imaging was performed on MCF-7 cells incubated with LQDs. As shown in Figure 7, uniform green fluorescence was observed predominantly in the cytoplasm, with negligible nuclear signal, indicating intracellular localization without evident morphological changes.

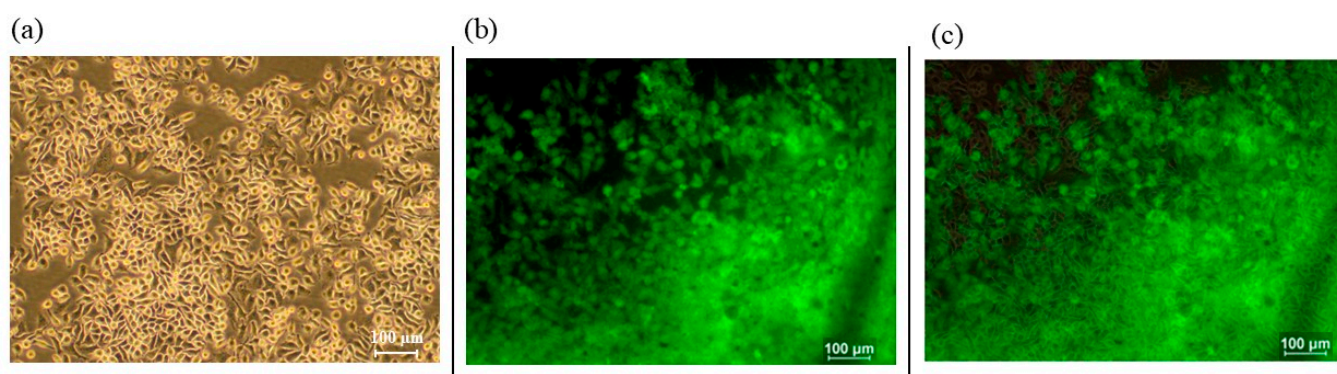


Figure 7. Fluorescence imaging of MCF-7 cells incubated with lignin quantum dots (LQDs): (a) bright-field, (b) fluorescence, (c) merged bright-field/fluorescence.

MCF-7 cells were selected as an adherent human cell model widely used in proof-of-concept fluorescence bioimaging studies, enabling direct visualization of cellular internalization and intracellular fluorescence distribution of luminescent nanomaterials under standard microscopy conditions. To support biosafety under imaging conditions, cytocompatibility was evaluated in L929 fibroblasts using the MTT assay, which showed $93.1 \pm 6.5\%$ viability after 24 h at the same LQDs concentration used for imaging. This result supports low acute cytotoxicity under the conditions applied and strengthens the suitability of the LQDs as a cellular imaging probe within the tested dose/time window.

The cytoplasmic pattern suggests endocytic uptake and retention in membrane-bound compartments (e.g., endosomes/lysosomes), as commonly reported for carbon-based nanomaterials [3,40,64], and similar predominantly cytoplasmic fluorescence with low nuclear signal has been reported for biomass-derived carbon quantum dots used for cellular imaging (e.g., cyanobacteria-derived CQDs) [39,65]. The absence of a nuclear signal may be beneficial for fluorescence labelling applications where preferential cytoplasmic localization is desired. Cytoplasmic retention may facilitate subcellular labelling and qualitative monitoring of intracellular distribution, although no co-localization assays were performed here. These imaging data therefore serve as a proof-of-concept for live-cell fluorescence labelling under the tested conditions.

2.5. Outlook and Future Directions

Building on the present proof-of-concept, the next phase of work will focus on translation-oriented validation and process refinement to support realistic deployment scenarios. The first priority is the systematic assessment of the colloidal and photoluminescence stability of LQDs under application-relevant complexity. This includes evaluating dispersion stability, surface accessibility, and fluorescence response across variations in ionic strength and pH, as well as in the presence of natural organic matter and protein-containing/biological media, where adsorption layers can alter the interfacial interactions and optical output. Stability mapping under storage- and use-relevant conditions (time, temperature, and light exposure) will further define the operational windows and inform formulation strategies.

In parallel, functional persistence should be examined under realistic matrices rather than simplified laboratory media. Antibacterial performance over time can be assessed in representative aqueous environments (e.g., natural water, wastewater-impacted water, and saline media) and in soil-related matrices (e.g., soil extracts or model slurries), where adsorption, aggregation, and interactions with organic matter may modulate bioavailability and contact efficiency. These studies can be complemented by quantitative antimicrobial metrics (e.g., MIC/MBC) and targeted mechanistic assays (e.g., membrane integrity and oxidative stress readouts), enabling a clearer linkage between LQDs physicochemical features and antimicrobial outcomes under practical conditions.

Targeted compositional characterization of the extracted lignin (e.g., residual carbohydrate content and extractives) will further refine structure–property correlations and support process optimization. The second development axis concerns the process efficiency, robustness, and scalability of the two-step route. The optimization of key parameters across both stages, lignin extraction (e.g., alkali concentration, extraction temperature/time, and solid-to-liquid ratio) and hydrothermal conversion (e.g., temperature, residence time, precursor loading, and post-treatment/neutralization conditions), can be implemented using a structured design-of-experiments framework, such as the response surface methodology. The optimization targets should include overall yield and batch-to-

batch reproducibility while constraining critical performance attributes (e.g., particle size distribution, optical response, and antibacterial activity). Defining robust process windows will support scale-up decisions and enable efficiency improvements (e.g., reduced energy input, streamlined washing/neutralization, and recovery/reuse of process streams) without compromising the proof-of-concept functionality established in this study.

3. Materials and Methods

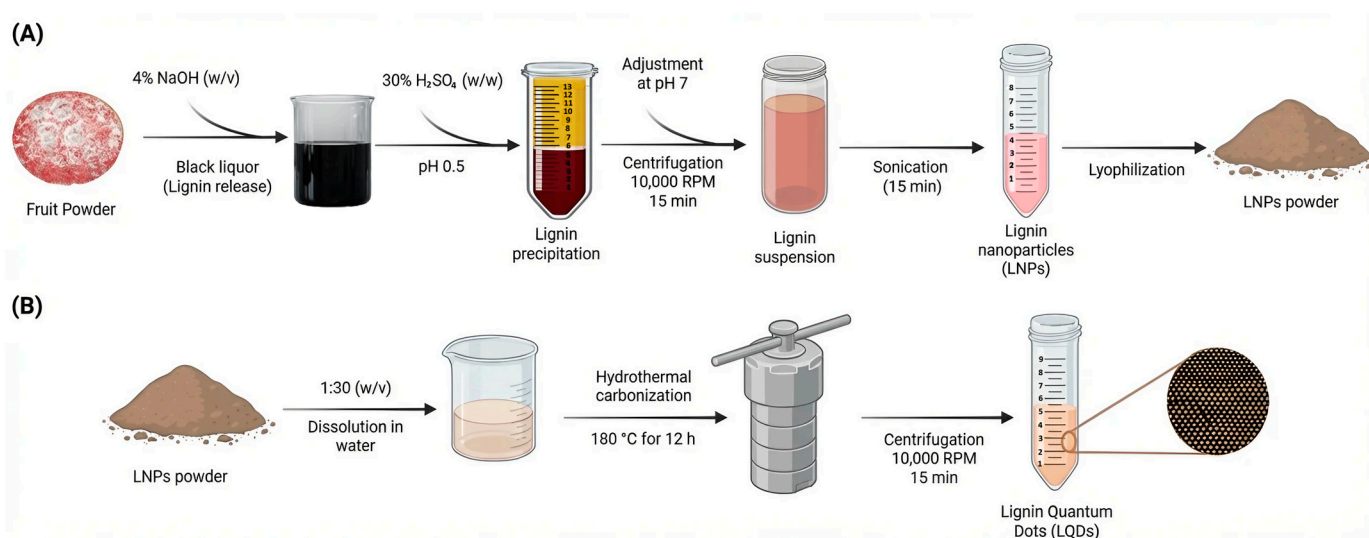
3.1. Materials

P. macrocarpa fruit was collected from the local regions of Kollam district (Latitude: 9.0985965° N, Longitude: 76.5469143° E) in Kerala, India. The fruit was used as the raw material for lignin extraction. The collected fruit was thoroughly washed with distilled water several times, air-dried for 7 days, then cut into 10 mm pieces and ground to a fine powder. Sodium hydroxide (NaOH), sulfuric acid (H₂SO₄), and isopropyl alcohol were obtained from Isochem Laboratories (Angamaly South, Kochi, Ernakulam, India). Poly (tetrafluoroethylene) (PTFE) microporous membrane filter discs with a pore diameter of 0.2 µm were obtained from Merck Life Science Private Limited, India. All chemicals employed were of analytical reagent grade and used as received, without further purification.

3.2. Methods

3.2.1. Extraction of Lignin Nanoparticles (LNPs)

LNPs were prepared from *P. macrocarpa* fruit via alkaline extraction, followed by acid precipitation (Scheme 1A). Briefly, dried fruit powder was treated with 4% (*w/v*) NaOH solution at a solid-to-liquid ratio of 1:20 (g:mL) and heated at 90 °C for 6 h in a water bath under continuous stirring at 400 rpm. The resulting black liquor was filtered through Whatman No. 40 filter paper to remove the insoluble residues. The filtrate was cooled in an ice bath, and H₂SO₄ (30% *w/w*) was slowly added under stirring until the pH reached ca. 0.5, which led to lignin precipitation. The suspension was then cooled to room temperature and centrifuged at 10,000 rpm for 15 min to remove residual impurities. The precipitate was repeatedly washed with distilled water and centrifuged until the supernatant reached a neutral pH. The resulting neutral lignin suspension was sonicated for 15 min to obtain a homogeneous LNP dispersion. Finally, the suspension was freeze-dried to yield LNP powder, which was stored for subsequent studies.



Scheme 1. Process flow diagram outlining the preparation of (A) lignin nanoparticles (LNPs) and (B) lignin quantum dots (LQDs) from *P. macrocarpa* fruit.

3.2.2. Synthesis of Lignin Quantum Dots (LQDs)

LQDs were synthesized via hydrothermal carbonization (Scheme 1B), a sustainable process that converts the aromatic structures of lignin into graphitic domains [66]. Briefly, LNP powder was dispersed in distilled water at a mass-to-volume ratio of 1:30 (g:mL) under continuous magnetic stirring until a uniform suspension was formed. The suspension was then transferred to a Teflon-lined stainless-steel autoclave and heated at 180 °C for 12 h. After natural cooling to room temperature, the reaction mixture was filtered through Whatman No. 40 filter paper to remove coarse particulates, and the filtrate was centrifuged at 10,000 rpm for 15 min to obtain the supernatant. The resulting dark yellow supernatant was further purified using a 0.2 µm membrane filter to eliminate residual impurities. The purified LQDs solution was freeze-dried at −40 °C for 48 h to obtain a dry powder. Both the aqueous solution and freeze-dried LQDs were stored at 4 °C until further use.

3.3. Material Characterization

3.3.1. Morphological Characterization

Morphological characterization was performed using high-resolution transmission electron microscopy (HR-TEM) and field emission scanning electron microscopy (FE-SEM). HR-TEM was conducted using Philips Tecnai-12 microscope (FEI Company, Hillsboro, OR, USA) operating at 120 kV, and imaging was performed using a TVIPS F224 CCD camera (TVIPS GmbH, Gauting, Germany), enabling detailed visualization of the internal nanostructure of the samples. FE-SEM images were obtained using a MAIA3 XMH system (TESCAN, Brno, Czech Republic). The dried samples were mounted on aluminum stubs with conductive carbon tape and coated with a thin carbon layer (approximately 5 nm) to minimize charging. Micrographs were recorded under high vacuum at accelerating voltages of 5–10 kV, using secondary electron detection.

3.3.2. Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra were recorded using a PerkinElmer PC1600 spectrometer (PerkinElmer, Shelton, CT, USA) equipped with an attenuated total reflectance (ATR) mode, within the spectral range of 4000–500 cm^{−1}, at a resolution of 4 cm^{−1} and 32 scans. Before analysis, the samples were dried at 40 °C for 72 h.

3.3.3. Thermogravimetric Analysis (TGA)

TGA analyses of the samples were performed using an SDT Q600, a simultaneous differential thermal analyser (TA Instruments, New Castle, DE, USA). The samples were heated from room temperature to 600 °C at a constant heating rate of 10 °C/min under a controlled nitrogen atmosphere.

3.3.4. X-Ray Diffraction (XRD)

XRD analysis was performed using a Bruker AXS D8 Advance diffractometer (Bruker AXS GmbH, Karlsruhe, Germany). XRD measurements were performed in the 2θ range of 10° to 50°.

3.3.5. Raman Spectroscopy

Raman spectra were recorded on a WITec alpha300 RA system (WITec GmbH, Ulm, Germany) equipped with a back-illuminated CCD detector and a 532 nm DPSS laser coupled through a single-mode optical fiber. Spectra were acquired in backscattering geometry under 532 nm excitation after wavenumber calibration with the Si band at 520.7 cm^{−1}.

3.3.6. Biological Activity

Antibacterial Activity

The antibacterial activity of LQDs was assessed using the disk diffusion (Kirby-Bauer) method on Mueller-Hinton (MH) agar, in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines. *S. aureus* (Gram-positive) and *K. pneumoniae* (Gram-negative) were cultured and adjusted to a 0.5 McFarland standard (1.5×10^8 CFU/mL) in sterile saline. MH agar plates were inoculated with bacterial suspensions using sterile swabs, and sterile 6 mm filter paper disks were loaded with 50 µg of LQDs (sample), erythromycin (15 µg, positive control), or solvent (negative control), maintaining a centre-to-centre distance of 24 mm between disks. The plates were incubated aerobically at 37 °C for 24 and 72 h.

Cell Viability and Cytotoxicity Studies

Cell viability was assessed using the L929 mouse fibroblast cell line. Cells were seeded at 5.4×10^4 cells per well in 24-well plates and incubated at 37 °C with 5% CO₂ for 24 h. Following the initial incubation, the cells were exposed to LQDs (50 µg/mL) and incubated for an additional 24 h. At the end of the incubation period, the culture medium and test substances were removed, and the cells were treated with 500 µL of fresh medium containing 100 µL of MTT solution [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]. After 2 h, the absorbance was measured at 570 nm.

3.3.7. Optical Properties

Ultraviolet-Visible (UV-Vis) Spectroscopy

UV-Vis absorption spectra were recorded on a PerkinElmer Lambda 650 spectrophotometer (PerkinElmer, Shelton, CT, USA) in the 200–800 nm. Measurements were performed in quartz cuvettes with a 1 cm path length using the corresponding solvent as a blank.

Photoluminescence (PL) Spectroscopy

The PL spectra of the LQDs were recorded using a Horiba Fluorolog-3 TCSPC instrument (HORIBA Jobin Yvon, Edison, NJ, USA) at room temperature (23 ± 2 °C). Samples were prepared at a concentration of 50 µg/mL in Milli-Q water to ensure adequate fluorescence intensity and stability during the measurements. Emission spectra were collected across different wavelengths, and the peak emission wavelengths were determined. Fluorescence lifetimes were obtained from decay curves using a multi-exponential fitting method, where the decay times (τ_1 , τ_2 , τ_3) and their respective amplitudes (a_1 , a_2 , a_3) were used to calculate the mean lifetime (τ_m) according to Equation (1).

$$\tau_m = (a_1\tau_1 + a_2\tau_2 + a_3\tau_3)/(a_1 + a_2 + a_3) \quad (1)$$

Bioimaging Analysis

Bioimaging experiments were conducted using the MCF-7 human breast cancer cell line. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% (*v/v*) fetal bovine serum (FBS) and a standard antibiotic/antimycotic solution. For imaging assays, 4000 cells were seeded per well and incubated at 37 °C for 24 h. LQDs were then added to the cells at a final concentration of 500 µg/mL, followed by an additional 24 h of incubation. After treatment, unbound LQDs were removed by washing the wells thrice with PBS (pH 7.4). Fluorescence images were acquired using a Nikon Eclipse Ti2 microscope (Nikon, Tokyo, Japan) with excitation at 405 nm wavelength.

3.4. Statistical Analysis

Data normality and variance homogeneity were evaluated using the Kolmogorov–Smirnov and D’Agostino–Pearson tests, respectively. Differences among groups were analyzed by one-way ANOVA with Tukey’s post hoc test ($\alpha = 0.05$). Statistical analyses were performed using GraphPad Prism version 9.0, with $p < 0.05$ considered statistically significant.

4. Conclusions

This study establishes *Phaleria macrocarpa* lignin as a viable feedstock for two nanomaterial classes, lignin nanoparticles (LNPs) and lignin quantum dots (LQDs), via a simple, aqueous, catalyst-free sequence, opening a non-food valorization route from a cultivated medicinal crop. Beyond feasibility, it bridges two often-separate domains (LNPs and LQDs) by documenting the sequential conversion and outlining a qualitative structure–property map: partial deoxygenation and increased sp^2 content were associated with blue-shifted absorption, excitation-tunable emission, and nanosecond lifetimes in quantum dots. It should be emphasized that the precursor characteristics are defined by both the biomass source and the isolation route; in this study, alkaline extraction followed by acid precipitation yields a sulfur-free lignin fraction that was subsequently converted into LNPs and LQDs under hydrothermal conditions. Structural characterization revealed the conversion of highly functionalized lignin into amorphous carbon nanostructures with partial graphitization and the reorganization of oxygenated surface groups. These structural transformations were consistent with the quantum confinement effects, enhanced photoluminescence, and nanosecond-scale lifetimes. The resulting LQDs exhibited blue-shifted UV-Vis absorption, intense blue-green emission, and cytocompatibility under the tested conditions. Efficient cellular internalization (imaging proof-of-concept) and antibacterial activity were observed, and application development will focus on non-food, bio-based products (e.g., antimicrobial/active coatings and sensing). Overall, this study contributes a novel crop-derived feedstock, a practicable green processing route, and a clear structure-property framework that can be reused to develop lignin-derived nanostructured functional materials in a circular bioeconomy context. Future studies are expected to advance process optimization for higher yield and robustness and to evaluate LQD stability and antibacterial persistence in complex, application-relevant environments.

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Conflicts of Interest: The authors declare no known competing financial interests or personal relationships that could have appeared to influence the work.

Abbreviations

The following abbreviations are used in this manuscript:

CLSI	Clinical and Laboratory Standards Institute
CQDs	Carbon quantum dots
DCM	Dichloromethane
DES	Deep eutectic solvent
DMEM	Dulbecco's Modified Eagle Medium
DTG	Derivative thermogravimetry
FBS	Fetal bovine serum
FE-SEM	Field-emission scanning electron microscopy
FTIR	Fourier-transform infrared spectroscopy
G/S	Guaiacyl/syringyl
GVL	γ -valerolactone
HR-TEM	High-resolution transmission electron microscopy
HTC	Hydrothermal carbonization
LNPs	Lignin nanoparticles
LQDs	Lignin quantum dots
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
nr.	Not reported
PBS	Phosphate-buffered saline
PL	Photoluminescence
QY	Quantum yield
SAED	Selected-area electron diffraction
SD	Standard deviation
TGA	Thermogravimetric analysis
TCSPC	Time-correlated single-photon counting
UV-Vis	Ultraviolet–visible spectroscopy
XRD	X-ray diffraction
ZOI	Zone(s) of inhibition

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