



Biosynthesis and Characterization of Copper-Based Particles Using Green Betel Leaf (*Piper betle* L.) Aqueous Extract as a Bioreductant

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ABSTRACT

Green synthesis provides an alternative route for preparing copper-based materials using plant-derived reducing and stabilizing agents. This study evaluated copper-based particles obtained by reacting 0.1 M copper(II) nitrate trihydrate ($\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$) with an aqueous extract of green betel leaves (*Piper betle* L.). The extract was screened for secondary metabolites and combined with the precursor at extract-to-copper-solution volume ratios of 1:1, 1:2, 1:3, 1:4, and 1:5 at a fixed total volume of 50 mL, so that the extract fraction decreased from 50.0 to 16.7% (v/v) while the Cu^{2+} content increased from 2.50 to 4.17 mmol. After 24 h, the particle fraction was recovered by centrifugation, washed, redispersed in distilled water, and analyzed by UV-Vis spectrophotometry. The dried product from the ratio showing the highest measured absorbance was characterized by SEM-EDX. The extract showed maximum absorption at 280.0 nm (absorbance 1.034) and tested positive for flavonoids, alkaloids, phenols, saponins, and tannins. The 1:1 ratio gave the highest measured absorbance (3.390 at 207.0 nm); however, the differences among ratios were small (3.212–3.390), the band is not specific to copper, and no replicate data were available, so this value served only as a screening criterion. SEM revealed irregular surfaces and nonuniform spherical particle-like features with apparent sizes of 74.2–392 nm, indicating substantial agglomeration. EDX detected Cu (57.96 wt%), O (29.44 wt%), and C (12.59 wt%). The results support the formation of a copper-based particulate product, whose oxidation state and crystalline phase remain to be confirmed by XRD or XPS.

Keywords: Copper-Based Particles, Green Synthesis, *Piper Betle* L., Copper Nitrate, Bioreductant.

ABSTRAK

Sintesis hijau merupakan pendekatan alternatif untuk menghasilkan material berbasis tembaga dengan memanfaatkan senyawa pereduksi dan penstabil yang berasal dari tumbuhan. Penelitian ini mengevaluasi partikel berbasis tembaga yang diperoleh melalui reaksi larutan tembaga(II) nitrat trihidrat ($\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$) 0,1 M dengan ekstrak air daun sirih hijau (*Piper betle* L.). Ekstrak diuji kandungan metabolit sekundernya dan dicampurkan dengan prekursor pada perbandingan volume ekstrak:larutan tembaga 1:1, 1:2, 1:3, 1:4, dan 1:5 dengan volume total tetap 50 mL, sehingga fraksi ekstrak menurun dari 50,0 menjadi 16,7% (v/v) sedangkan jumlah Cu^{2+} meningkat dari 2,50 menjadi 4,17 mmol. Setelah 24 jam, fraksi partikel dipisahkan dengan sentrifugasi, dicuci, didispersikan kembali dalam akuades, dan dianalisis menggunakan spektrofotometri UV-Vis. Produk kering dari perbandingan dengan absorbansi terukur tertinggi dikarakterisasi menggunakan SEM-EDX. Ekstrak menunjukkan serapan maksimum pada 280,0 nm (absorbansi 1,034) serta positif mengandung flavonoid, alkaloid, fenol, saponin, dan tanin. Perbandingan 1:1 memberikan absorbansi terukur tertinggi (3,390 pada 207,0 nm); namun, perbedaan antarperbandingan kecil (3,212–3,390), pita tersebut tidak spesifik untuk tembaga, dan tidak tersedia data ulangan, sehingga nilai ini hanya digunakan sebagai kriteria penapisan. Hasil SEM menunjukkan permukaan tidak merata dan fitur menyerupai partikel bulat yang tidak seragam dengan ukuran tampak 74,2–392 nm, yang mengindikasikan aglomerasi yang cukup besar. EDX mendeteksi Cu (57,96% b/b), O (29,44% b/b),



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dan C (12,59% b/b). Hasil ini mendukung terbentuknya produk partikulat berbasis tembaga, yang tingkat oksidasi dan fase kristalnya masih perlu dikonfirmasi dengan XRD atau XPS.

Kata kunci: Partikel Berbasis Tembaga, Sintesis Hijau, *Piper Betle* L., Tembaga Nitrat, Bioreduktan.

1. Introduction

Copper is widely used in electronic, coating, catalytic, and antimicrobial applications; however, excessive copper can be toxic to microorganisms and other living systems. Converting copper into nanoscale materials can expand its potential uses, particularly in catalytic and antimicrobial applications. Nanoparticles are generally described as materials with at least one primary dimension of approximately 1–100 nm, whereas aggregation or agglomeration can generate larger secondary structures [1,2].

Conventional physical and chemical routes for nanoparticle synthesis may require high costs, long processing times, and chemical reagents that can generate environmentally undesirable residues. Green chemistry therefore promotes processes that minimize hazardous substances, reduce waste at the source, and use renewable raw materials. Plant extracts are attractive in this context because their secondary metabolites can act as reducing and stabilizing agents during the formation of copper and copper oxide nanoparticles [3–5].

Green betel leaf (*Piper betle* L.) is widely recognized as a medicinal plant and contains secondary metabolites such as flavonoids, phenolic compounds, alkaloids, tannins, and saponins [6,7]. Functional groups in these compounds, particularly hydroxyl and carbonyl groups, may participate in metal-ion reduction and surface stabilization. Published studies show that *P. betle* has already been used in several copper-based nanomaterial systems. Praburaman et al. reported *P. betle*-mediated CuO nanoparticles in 2016 [8]. Kasi et al. subsequently synthesized CuO nanoparticles from *P. betle* leaf extract using a copper sulfate precursor [9], and Omran et al. reported *P. betle*-derived CuO nanoparticles using an aqueous extract and copper sulfate route [10]. More recent work has extended *P. betle*-mediated copper materials to environmental CuO applications [11], bimetallic zero-valent Cu–Fe particles [12], and copper-based nanoparticles associated with betel leaves for dye degradation and Al³⁺ sensing [13]. Thus, the use of *P. betle* itself is not the novelty of the present study.

In plant-mediated synthesis, the proportion of extract to metal precursor is one of the main variables governing the product, because it sets the balance between the available phytochemical reductants and capping agents and the Cu²⁺ ions to be converted. A higher relative extract content generally accelerates Cu²⁺ reduction and nucleation and supplies more capping molecules, which favors smaller and better-stabilized particles, whereas a higher relative precursor content tends to favor particle growth, larger sizes, and agglomeration; an excessive extract fraction may, in turn, promote aggregation or leave more residual organic matter in the product [4,14,15]. The extract-to-precursor ratio is therefore scientifically critical because it directly influences particle size, product yield, colloidal stability, and the organic content of the final material. Within the literature reviewed here, the *P. betle*–copper studies cited above focused mainly on the properties and applications of their products [8–13], and comparative data on how this proportion affects the *P. betle*–copper system under a common protocol remain limited.

The present study does not claim novelty from the use of *P. betle* or copper nitrate precursor alone. Instead, its specific contribution lies in the systematic comparative screening of five aqueous extract-to-precursor volume ratios (1:1, 1:2, 1:3, 1:4, and 1:5) under the same reaction protocol. This range was chosen because it lies within the range commonly screened in plant-mediated copper synthesis (for example, 1:2–1:4 extract:CuSO₄ for *Fortunella margarita* [14]) and because, at a fixed total volume of 50 mL, it spans a stepwise transition from an equal-volume, extract-rich mixture (50.0% v/v extract) to a precursor-rich mixture (16.7% v/v extract) in which the phytochemical supply per Cu²⁺ ion is progressively reduced. The recovered particle fractions were comparatively evaluated by UV-Vis spectrophotometry, while the product from the treatment showing the highest measured absorbance was further characterized by SEM-EDX. The study is therefore positioned as an exploratory screening that provides baseline comparative data on the optical response, morphology, and elemental profile of *P. betle*-mediated copper-based products, rather than as a definitive optimization.

2. Materials and Methods

2.1. Materials and Instrumentation

The materials were distilled water, green betel leaves (*Piper betle* L.), copper(II) nitrate trihydrate ($\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$, pro analysis grade), Whatman No. 42 filter paper, HCl, concentrated H_2SO_4 , 10% NaOH, Mg, 1% and 5% FeCl_3 , Dragendorff reagent, and Wagner reagent.

The main instruments were a JEOL JCM-6000 Plus scanning electron microscope-energy dispersive X-ray system, a Varian Cary 50 Conc UV-Vis spectrophotometer, a Zenith Lab (Jiangsu) centrifuge, a D-Lab hotplate stirrer, a magnetic stirrer, analytical balances, a thermometer, a blender, and standard laboratory glassware.

2.2. Source, Preparation, and Aqueous Extraction of Green Betel Leaves

Green betel leaves were collected from Kampung Beru Mawang, Bontomarannu District, Gowa Regency, South Sulawesi, Indonesia. The leaves were washed with distilled water, cut into small pieces, and air-dried at room temperature for 3–7 days until sufficiently dry for grinding. The dried leaves were ground, and 100 g of powder was prepared. For aqueous extraction, 25 g of powder was mixed with 250 mL of distilled water in a 500 mL beaker and heated at 55–60 °C while stirring at 600 rpm. The mixture was filtered through Whatman No. 42 paper, and the extract filtrate was scanned at 250–550 nm by UV-Vis spectrophotometry [10,16–18].

2.3. Phytochemical Screening

The aqueous extract was qualitatively tested for flavonoids, alkaloids, phenols, saponins, and tannins. Flavonoids were tested using Mg–HCl, 10% NaOH, and concentrated H_2SO_4 . Alkaloids were examined with Dragendorff and Wagner reagents. Phenols and tannins were tested with 5% and 1% FeCl_3 , respectively, while saponins were evaluated by the persistence of foam after vigorous shaking with distilled water [19–22].

2.4. Preparation of Copper Precursor and Biosynthesis of Copper-Based Particles

Copper(II) nitrate trihydrate ($\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$; 24.16 g) was dissolved in distilled water and diluted to 1000 mL to prepare a 0.100 M precursor solution, followed by homogenization at 300 rpm. The green betel leaf extract and precursor solution were combined to a total volume of 50 mL at extract:precursor volume ratios of 1:1, 1:2, 1:3, 1:4, and 1:5, corresponding to extract/precursor volumes of 25.00/25.00, 16.67/33.33, 12.50/37.50, 10.00/40.00, and 8.33/41.67 mL, respectively. Because the total volume was fixed, the extract fraction decreased from 50.0 to 16.7% (v/v) while the Cu^{2+} content increased from 2.50 to 4.17 mmol (0.050–0.083 M in the reaction mixture) (Table 2). After mixing, the reaction mixtures were left to stand (static incubation) at room temperature for 24 h without stirring. During this period, dark-green coloration and a suspended brown precipitate were observed. The mixtures were then centrifuged at 2000 rpm for 15 min. The recovered particle fraction was purified by washing with distilled water and redispersed in distilled water, following the general purification-by-redispersion approach used in *P. betle*-mediated copper synthesis [8]. The purified copper-based particle dispersions were analyzed at 200–500 nm by UV-Vis spectrophotometry. A portion of the recovered solid was dried at room temperature until a dry solid was obtained for SEM-EDX analysis [8,16].

2.5. SEM-EDX Characterization of Copper-Based Particles

The ratio that produced the highest measured UV-Vis absorbance in the purified redispersed particle samples was selected for further characterization. SEM was used to evaluate surface morphology and apparent feature size at 3000× and 10000× magnification, while EDX was used to determine the elemental composition of the dried product.

3. Results and Discussion

3.1. Characteristics of the Green Betel Leaf Extract

The aqueous extract was brownish and exhibited a maximum absorption wavelength of 280.0 nm with an absorbance of 1.034 (Figure 1). This band is consistent with absorption reported for flavone-type compounds in the 250–280 nm region [18,23]. The result is compatible with the qualitative phytochemical findings and indicates the presence of plant-derived compounds that may participate in metal-ion reduction or surface stabilization.

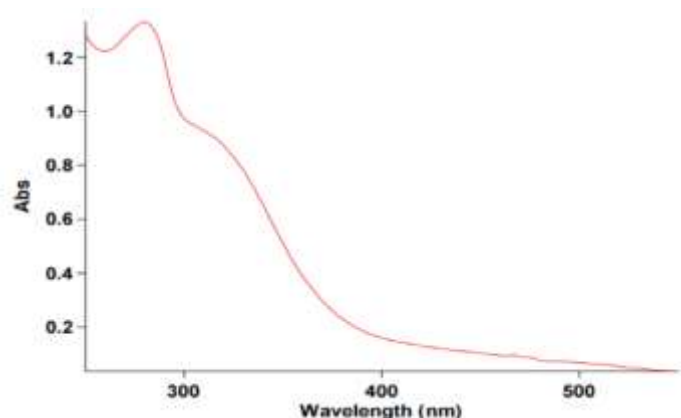


Figure 1. UV-Vis absorption spectrum of the aqueous green betel leaf extract

Table 1. Phytochemical screening of the aqueous green betel leaf extract.

Compound	Reagent	Positive criterion	Observation	Result
Flavonoid	Concentrated H_2SO_4	Yellow, red, or brown	Yellow-brown	+
Flavonoid	Mg and HCl	Brick-red, yellow, or orange	Yellow	+
Flavonoid	10% NaOH	Yellow, red, brown, or green	Dark yellow	+
Alkaloid	Dragendorff	Red, brick-red, or orange precipitate	Brick-red precipitate	+
Alkaloid	Wagner	Brown precipitate	Brown precipitate	+
Phenol	5% $FeCl_3$	Dark green	Dark green	+
Saponin	Distilled water	Stable foam	Foam observed	+
Tannin	1% $FeCl_3$	Blue-black or dark green	Dark green	+

All tested metabolite groups gave positive responses (Table 1). Flavonoids produced yellow to yellow-brown colors with the three reagents. Alkaloids formed brick-red and brown precipitates with Dragendorff and Wagner reagents. Phenols and tannins produced dark-green complexes with $FeCl_3$, while saponins generated persistent foam. These observations agree with the qualitative criteria described in the methods and indicate that the extract contains several classes of compounds with hydroxyl, carbonyl, or nitrogen-containing groups that may contribute to reduction and capping [19–22].

3.2. Biosynthesis and UV-Vis Response of Copper-Based Particles

After addition of the copper(II) nitrate precursor, the extract–precursor mixtures changed from green to dark green during the 24 h incubation, and a suspended brown precipitate formed. The dried product was brownish black (Figure 2). The observed color change is interpreted only as a preliminary indication that an interaction or reaction occurred between constituents of the plant extract and the copper precursor [8,24,25].

Figure 2. (a) Green betel leaf extract and (b) color change of the extract- $Cu(NO_3)_2 \cdot 3H_2O$ mixtures after 24 h.

The purified and redispersed particle samples from the five extract:precursor ratios showed different maximum wavelengths and absorbance values (Figure 3 and Table 2). The 1:1 ratio produced the highest measured absorbance (3.390 at 207.0 nm), followed by the 1:3, 1:2, 1:5, and 1:4 ratios. In a related *Piper-*

mediated copper system, Amaliyah et al. reported an absorption near 207 nm for polyphenolic components and a second band at 234–255 nm associated with the copper-particle system [26]. In addition, the scanned range (200–500 nm) does not cover the visible region in which the surface plasmon resonance of metallic copper nanoparticles is usually reported (approximately 560–600 nm) [2], and residual nitrate ions, if incompletely removed by washing, also absorb strongly below about 230 nm. The 207.0 nm band therefore cannot be assigned specifically to copper, and its intensity is not interpreted as a measure of nanoparticle formation or synthesis efficiency.

The highest absorbance was nevertheless used as the selection criterion for a practical reason. Because all five dispersions were purified and measured in the same way, the absorbance at the band maximum reflects the overall optical density of each purified dispersion, which may arise from the amount of recovered particulate solid (including light scattering by suspended particles), from plant-derived organic matter bound to the particles, or from both. In the absence of a copper-specific optical marker, this value was used solely as an operational criterion to select one representative sample for SEM-EDX, on the assumption that the dispersion with the highest optical density contained the largest amount of recovered material. It was not used to identify an optimum formulation, and the 1:1 treatment is accordingly described only as the ratio that showed the highest measured absorbance.

The absorbance values also lie within a narrow interval (3.212–3.390), corresponding to a maximum relative difference of about 5.5% between the 1:1 and 1:4 samples, and the ranking (1:1 > 1:3 > 1:2 > 1:5 > 1:4) does not change monotonically with the extract fraction. Moreover, absorbance values above approximately 3 lie close to the upper limit of the reliable photometric range of conventional UV-Vis spectrophotometers, where stray light reduces accuracy. Because replicate measurements were not available, the variability of each value cannot be estimated and the differences cannot be shown to be statistically significant. The ranking is therefore regarded as indicative only; replicate syntheses, measurements of appropriately diluted dispersions, and statistical testing are required before any ratio can be designated as optimal.

The experimental design must also be considered when interpreting these differences. Because the total volume was fixed at 50 mL, changing the ratio simultaneously changed the absolute amounts of both reactants: the extract volume decreased from 25.00 to 8.33 mL, whereas the Cu²⁺ content increased from 2.50 to 4.17 mmol (Table 2). The observed UV-Vis differences thus reflect the combined effects of the extract amount, the Cu²⁺ amount, and their proportion, and cannot be attributed to the ratio alone. Isolating the ratio effect would require a design in which one reactant is held constant while the other is varied, or in which the ratio is varied at a constant total amount of reactants.

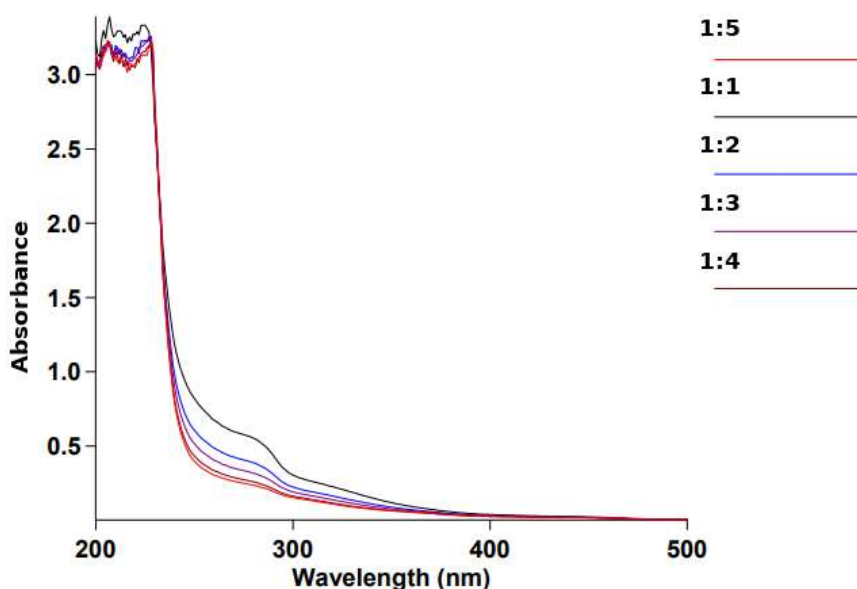


Figure 3. UV-Vis spectra of purified and redispersed copper-based particle samples obtained at five extract-to- $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ ratios.

Table 2. Reaction composition, maximum wavelength, and absorbance of purified and redispersed copper-based particle dispersions.

Extract:Cu solution ratio	Extract fraction (%) v/v)	Cu^{2+} (mmol)	λ_{max} (nm)	Absorbance
1:1	50.0	2.50	207.0	3.390
1:2	33.3	3.33	226.9	3.253
1:3	25.0	3.75	228.0	3.261
1:4	20.0	4.00	206.1	3.212
1:5	16.7	4.17	206.1	3.234

3.3. Morphology and Elemental Composition of Copper-Based Particles

SEM images of the 1:1 product showed an uneven surface at $3000\times$ magnification and predominantly spherical particle-like features at $10000\times$ magnification (Figure 4). The observed shapes and apparent sizes were not uniform. Among the features annotated on the SEM image (Section 2.5), the smallest was 74.2 nm, whereas the largest was 392 nm. Only the lower end of this range falls within the conventional nanoscale, while the larger features are consistent with aggregation or agglomeration. Brownian motion and van der Waals interactions can promote aggregation, producing irregular surfaces and broader apparent dimensions [17]. Comparable green-synthesized copper systems have also reported larger apparent dimensions when agglomeration occurs [14,16,27]. The present SEM images cannot unambiguously distinguish primary particles from agglomerates; accordingly, 74.2–392 nm is reported as an apparent SEM feature-size range and not as a primary nanoparticle-size distribution.

The EDX spectrum contained a prominent copper signal near 8 keV (Figure 5). The measured characteristic X-ray energy for Cu was 8.040 keV [28]. Quantitative EDX analysis gave 57.96 wt% Cu, 29.44 wt% O, and 12.59 wt% C (Table 3), with the compositional percentages summing to 100.00%. An additional intense signal is visible at approximately 2.1 keV. This energy lies within the M-alpha line region commonly produced by conductive coating materials such as Au, Pt, or Os; JEOL reports that these materials generate EDS M-alpha signals at approximately 1.9–2.4 keV [29]. Because the available specimen-preparation record does not identify a coating material and the quantitative EDX table reports only C, O, and Cu, the approximately 2.1 keV signal is treated as a possible specimen-preparation contribution rather than assigned to the copper-based product. The quantitative discussion is therefore restricted to the elements reported in Table 3. These data establish that copper is the dominant detected metallic element in the analyzed region and support the designation of the recovered material as a copper-based product. The oxygen and carbon signals may originate from oxygen-containing copper species, residual or surface-bound plant-derived organic constituents, the

specimen support, or combinations of these sources. EDX provides elemental composition but does not determine oxidation state or crystalline phase. Consequently, the present data cannot distinguish metallic copper (Cu^0), Cu_2O , CuO , copper hydroxide or hydroxynitrate species, or Cu–organic complexes, and no claim is made that Cu^{2+} was reduced to metallic Cu^0 . The atomic O/Cu ratio calculated from Table 3 (approximately 2.0) exceeds those of CuO (1.0) and Cu_2O (0.5), indicating that a substantial part of the oxygen is associated with organic matter, hydroxyl groups, adsorbed water, or other non-oxide species, although EDX quantification of light elements is only semi-quantitative.

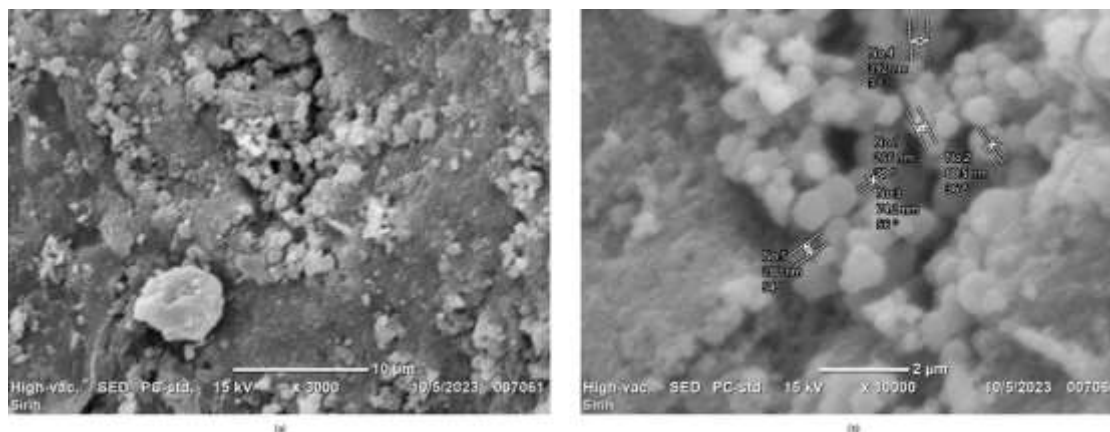


Figure 4. SEM images of the 1:1 copper-based product at (a) 3000× and (b) 10000× magnification.

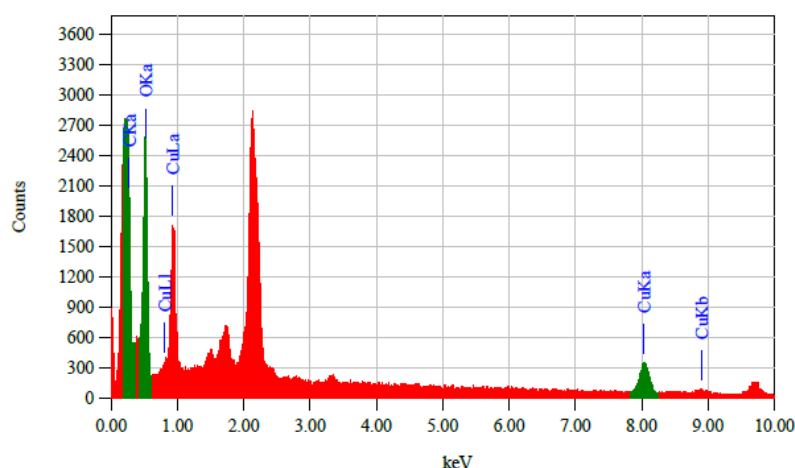


Figure 5. EDX spectrum of the biosynthesized copper-based product.

Table 3. Elemental composition of the biosynthesized copper-based product obtained by EDX.

Element	Characteristic X-ray energy (keV)	Mass fraction (wt%)
C K	0.277	12.59
O K	0.525	29.44
Cu K	8.040	57.96
Total		100.00

Taken together, phytochemical screening, the preliminary color change, the UV-Vis response of the purified redispersed particle fraction, SEM morphology, and EDX composition support the formation of a copper-based particulate product in the presence of aqueous green betel leaf extract, although its oxidation state and phase remain undetermined. Within the tested series, the extract-richest formulation (1:1) showed the highest measured UV-Vis absorbance; however, because the differences were small and unreplicated and both reactant amounts varied together, this result is regarded as a screening outcome rather than evidence of an

optimum. The broad SEM feature-size range indicates substantial agglomeration under the applied conditions, in line with other plant-mediated copper systems [8–13].

4. Conclusion

Aqueous green betel leaf (*Piper betle* L.) extract containing flavonoids, alkaloids, phenols, saponins, and tannins produced a copper-based particulate product from 0.1 M $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$. Among the five extract-to-precursor volume ratios, the 1:1 treatment gave the highest measured absorbance (3.390 at 207.0 nm), and its product consisted of agglomerated, nonuniform particle-like features (apparent size 74.2–392 nm) containing 57.96 wt% Cu, 29.44 wt% O, and 12.59 wt% C. The main scientific implication is that the extract-to-precursor proportion is a practical control variable in the *P. betle*–copper system: the extract-richest formulation gave the strongest optical response of the purified product, whereas the agglomeration observed even at this ratio shows that the ratio alone is insufficient to produce discrete nanoscale particles and that additional stabilization control is needed. These conclusions are limited by the small, unreplicated absorbance differences and the non-specific 207 nm band, the simultaneous variation of extract and Cu^{2+} amounts at a fixed total volume, the absence of XRD/XPS phase and oxidation-state analysis, and several undocumented reaction parameters. Future work should therefore include replicate syntheses with statistical testing, a design that varies one reactant at a time, and XRD, XPS, and TEM characterization.

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6. Conflicts of Interest

The authors declare no conflict of interest.

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