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LETTER

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Extract of cassava waste as a lixiviant for gold leaching from electronic waste

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ABSTRACT

Conventional gold leaching from electronic waste requires the use of strong acid and threatens environment. Alternatively, gold can be extracted from electronic waste by the cyanide secreted by bioleaching microorganisms. However, bioleaching microorganisms generally have slow growth rates and require specific growth conditions, restricting their industrial applications. Cassava, a cyanogenic plant containing cyanogenic glucosides, is not currently exploited as a bio-lixiviant source. Cassava is a staple food, and its production is increasing to meet global food requirements. In this work, we developed a protocol to extract cyanide from fresh cassava leaves, an agricultural waste. After multiple extractions, the cyanide concentration reached 120 ppm, higher than the concentration of cyanide produced by cyanogenic microorganisms. Finally, we demonstrated that the extract can be used to leach gold from electronic waste with an efficiency of 69% compared with the control (KCN solution). After optimization of the amount of electronic waste, the recovery reached 26.9%, comparable to that of bioleaching by cyanogenic bacteria. The leaching reaction is selective for gold in the presence of high amounts of Ni and Cu in the electronic waste. The results suggest that cassava leaves are a promising bio-lixiviant source for gold leaching from electronic waste.



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Cassava; electronic waste; gold bioleaching; cyanide

Introduction

Electronic devices have enabled the global population to benefit from a higher standard of living. However, the large amounts of electronic devices create electronic waste (E-waste). The amount of E-waste is growing rapidly due to the high consumption of electronic devices along with their short life cycles and limited options for repair (1-3). According to the Global Ewaste Monitor 2020 report (4), the amount of E-waste is expected to increase from 53.6 million metric tons (Mt) in 2019 to 74 Mt by 2030. Because E-waste contains toxic metals such as lead, mercury, cobalt, and nickel, metal recycling from E-waste is critical to protect the

environment (5-7). The recycling of E-waste is also driven by the fact that E-waste contains significant amounts of precious metals such as gold (Au) (8, 9). Compared with natural Au ores, which contain 0.5-13.5 g Au per ton of Au ore, the Au content in E-waste (10 g to 10 kg Au per ton of E-waste) is much higher. Currently, the metal recycling industry uses pyrometallurgy (10), which requires high energy input (temperatures over 1000°C) or hydrometallurgy (11), which uses chemical lixiviants such as agua regia and cyanide, generating toxic pollutants. Therefore, these methods of recycling metals from E-waste are not environmentally sustainable.

Bioleaching is a sustainable recycling method in which lixiviant-secreting microbes are used for precious metal recovery from E-waste without the emission of harmful gases or particles (12-14). The most successful example of bioleaching is the use of Chromobacterium violaceum, a cyanogenic microorganism. C. violaceum can biosynthesize glycine de novo, which is converted into hydrogen cyanide (HCN) under the catalysis of HCN synthase (15, 16). C. violaceum was further engineered to increase the cyanide yield from 20 ppm (wild type) to 54 ppm (17, 18). The produced lixiviant, cyanide, aerobically reacts with Au to form $Au(CN)_{2}^{-}$, which is termed gold cyanidation (Figure 1A) (19). Under the optimized growth and leaching conditions, the gold recovery reached 30% at a pulp density of 0.5% w/v (18). Other bacteria such as Pseudomonas aeruginosa (20, 21), Bacillus megaterium (21, 22), and Micrococcus species (23) are capable of generating cyanide from glycine, which must be added into the growth medium. The pH of the medium affects the stability of free cyanide. When the pH is lower than 10.5, free cyanide is protonated to form gaseous HCN, thereby reducing the amount of cyanide available to leach gold. However, the abovementioned cyanogenic bacteria cannot tolerate high pH. Roseovarius tolerans, an iodide-oxidizing bacterium, can oxidize iodide to iodine and triiodide for the extraction of gold (24, 25). Heterotrophic bacteria can secrete organic acids (e.g. amino acids) to form gold complexes for gold extraction (26). However, most of these bacteria are not model microorganisms (27), and genetic tools are not available to engineer them to increase lixiviant yield. In addition, most growth media used for the microbial leaching of gold are costly because they require the addition of specific nutrients for bacterial growth or metabolic precursors for lixiviant production. In an alternative approach, bio-derived materials (28) such as chitosan derivatives (29, 30) and proteins (31, 32) have been used to absorb gold or other precious metals. Although these materials show high metal absorption efficiency and reusability, their preparation involves chemical modification (29, 30) or protein immobilization (31, 32), which often requires expensive chemicals and generates chemical waste or byproducts.

Cyanogenic plants (33) producing cyanogenic glycosides can be exploited because gold cyanidation is simple with high dissolution efficiency of gold (19). Among cyanogenic plants, cassava (Manihot esculenta Crantz, Euphorbiaceae) attracted our attention. According to the report of the Food and Agriculture Organization of the United Nations (34), cassava is the fourth most important food staple worldwide with a global production of approximately 300 million tons in 2020. Cassava is a particularly important crop in the tropics and subtropics, including Africa, Asia, the Pacific Islands, and Central and South America (35). Cassava contains two cyanogenic: linamarin and a small amount of lotaustralin (36). When cassava tissue is crushed, linamarin is catalytically hydrolyzed by linamarase (37) to generate acetone cyanohydrin followed by the spontaneous release of HCN and acetone (Figure 1B). The total cyanide contents produced by different cassava species and parts range from 1 to 2000 ppm (mg HCN equivalent/kg material) (36), similar to or much higher than the amounts produced by cyanogenic microbes (17, 18). Therefore, cassava is a potential biolixiviant source that has not yet been exploited.

In this work, we successfully developed a protocol to extract cyanide from fresh cassava leaves. After three extractions of cassava leaves, the cyanide concentration was approximately 120 ppm, more than that produced by cyanogenic microorganisms. The extract containing 77 ppm cyanide was used to leach gold from central processing unit (CPU) E-waste. Approximately 38.8 ppm of gold was obtained, corresponding to a recovery of 26.9%, similar to that obtained by bioleaching with cyanogenic bacteria (17, 18, 20). Based on energy-dispersive X-ray spectroscopy (EDX) and inductively coupled plasma-optical emission spectrometry (ICP-OES) analyses, we demonstrated that the leaching reaction was selective for Au in the presence of large amounts of Ni and Cu in the E-waste. The findings indicate that cassava leaves are a promising new bio-lixiviant source for gold leaching from E-waste.

Materials and methods

Materials

Ninhydrin, Folin-Ciocalteu reagent, iron(III) chloride hexahydrate, and the gold standard for ICP (1000 mg/L Au in hydrochloric acid) were purchased from Sigma-Aldrich. The nickel standard for ICP (1000 mg/L Ni in nitric acid) was purchased from CARLO ERBA Reagents. The copper standard for ICP (1000 mg/L Cu in nitric acid) was purchased from Fisher Scientific. Gold foil (99.99%) was purchased from a gold shop in Nakhon Ratchasima, Thailand. Gallic acid was purchased from TCI Chemicals. Cassava samples were harvested on the farm in Nakhon Ratchasima, THAILAND. Activated charcoal and cation-exchange resin were purchased from HomePro, Thailand. CPU waste was purchased from a local computer recycling shop. Nitrilotriacetic acid (NTA) resin was purchased from QIAGEN.

For the ninhydrin assay, 0.5% ninhydrin solution was prepared by the addition of 50 mg of ninhydrin to 10 mL

(A)
$$4 \text{ Au}_{(s)} + 8 \text{ CN}^{-}_{(aq)} + O_{2(g)} + 2 \text{ H}_{2}O_{(l)} \longrightarrow 4 \text{ Au}(\text{CN})_{2}^{-}_{(aq)} + 4 \text{ OH}^{-}_{(aq)}$$
(B)
$$HO \longrightarrow OH \longrightarrow C \longrightarrow OH \longrightarrow OH$$

$$Linamarine \longrightarrow OH \longrightarrow OH \longrightarrow OH$$

$$Linamarine \longrightarrow OH \longrightarrow OH \longrightarrow OH$$

$$C \longrightarrow OH \longrightarrow OH \longrightarrow OH$$

$$C \longrightarrow OH$$

Figure 1. (A) Gold cyanidation reaction. (B) Linamarase catalyzes the hydrolysis of linamarin to generate acetone cyanohydrin, which releases HCN spontaneously. (C) Pictures of cassava leaves (left) and stem (right).

of 2% sodium carbonate (pH 10.4). Ninhydrin solution was freshly prepared and stored in the dark before measurements. For the preparation of every metal standard solution, the metal standard for ICP was diluted by 0.5% HCl solution to make various concentrations. These solutions were used to construct the standard calibration curve for each ICP-OES measurement.

Instruments

An ultraviolet-visible (UV-Vis) spectrometer (Thermo Scientific Multiskan GO) was used to determine the cyanide concentrations in ninhydrin assay samples. The amounts of Au, Ni, and Cu in each sample were determined using an ICP-OES instrument (PerkinElmer Optical Emission Spectrometer Optima 8000). The pH values of solutions were measured using a METTLER TOLEDO pH meter. The samples were spun down using a centrifuge (Sorvall Legend XFR Centrifuge). A field-emission scanning electron microscope (SU-8030, Hitachi) equipped with an energy-dispersive X-ray spectrometer was used for scanning electron microscopy (SEM) and EDX analyses.

Cyanide extraction from cassava sample

Cassava samples (1.0 g of stem or leaf) were shredded into small pieces using scissors. The cassava samples were then ground and homogenized in 20 mL of 0.1% (w/v) NaHCO₃ using a mortar and pestle. The mixture was transferred in a 50-mL falcon tube, which was centrifuged at 4500 rpm at 25°C for 10 min. The supernatant was filtered through a 0.2-µm nylon filter or passed through a cation-exchange resin (7 g). The filtered supernatant was used to conduct the next extraction, after which gold leaching and cyanide concentration measurements were conducted.

Determination of cyanide concentration by ninhydrin assay

Five microliters of extract was added into 95 µL of 0.5% ninhydrin solution in a 96-well plate. The mixture was incubated at room temperature for 5 min followed by recording the UV-Vis spectrum. The intensity at 485 nm after the subtraction of the blank signal was used to calculate the cyanide concentration according to the calibration curve of cyanide standard solutions. The final cyanide concentration in the extract was multiplied by 20 to account for the sample dilution in the ninhydrin assay.

E-waste preparation and metal analysis

The pins of the CPU were disassembled using a heat gun and collected (Figure S1). The sample was prepared by adding 100 mg of pins into 1 mL of aqua regia at room temperature for 2 h to dissolve all metals. The solution was then diluted with DI water to a final volume of 15 mL. Finally, the solution was filtered through a 0.2µm nylon filter and analyzed by ICP-OES to determine the contents of Au, Ni, and Cu. According to the



calibration curve of each metal (Figure S2), the contents of Au, Ni, and Cu were 0.36%, 14.49%, and 6.68% by weight, respectively.

Characterization of E-waste by SEM and EDX

The morphologies and metal compositions of the CPU samples were explored by SEM and EDX, respectively. The crushed CPU samples before and after leaching were loaded onto a copper stub with the help of carbon tape. The surface morphology of the CPU sample was recorded by field-emission SEM (SU-8030, Hitachi) without any further metal coating. EDX was used to reveal the metal constituents in the CPU samples.

Gold leaching using gold foil and CPU samples

The pH of the extract was adjusted to 11 by the addition of KOH solution. Approximately 3 mg of gold foil or 800 mg of CPU sample was immersed in the extract. The mixture was stirred vigorously at 25°C. After the first and second days, 4 mL of the solution was taken out and filtered through a 0.2-µm nylon filter. The flow through was digested with 7.5 mL of 10% HCl and 100 μL of 7 M HNO₃ for 1 h. The solution was then diluted with DI water to a final volume of 15 mL. Finally, the Au concentration in the solution was analyzed by ICP-OES according to the calibration curve of gold (Figure S2). The final gold concentration in the gold leaching experiment accounted for the dilution during ICP-OES sample preparation.

Preparation of ferric-nitrilotriacetic acid (Fe-NTA) resin

Twenty milliliters of 0.2 M FeCl₃ in water was added to a 5-mL column volume (CV) of NTA-agarose to prepare the Fe-NTA resin. Water (5 CV) was then added to remove unbound Fe³⁺. Next, the resin was washed with 5 CV of 6% acetic acid (pH 3.5) to remove loosely bound Fe³ ⁺ followed by another washing with 5 CV of water. Finally, the Fe-NTA resin was equilibrated with 0.1% NaHCO₃. Cassava extract was passed through the Fe-NTA resin, and the flow through was collected for gold leaching experiments.

Determination of total phenolic content (TPC) by Folin-Ciocalteu assay

Twenty microliters of the sample or gallic acid standard solution (0.005, 0.025, 0.05, 0.10, or 0.14 mg/mL in ethanol) was added to 100 µL of 6% (v/v) Folin-Ciocalteu reagent in DI water. The mixture was incubated at room temperature for 5 min. Next, 80 µL of 7% (w/v) Na₂CO₃ in DI water was added into the mixture and incubated in the dark at room temperature for 90 min. Finally, 100 µL of the mixture was transferred into a 96-well plate, and its absorbance was measured at 760 nm. The calibration curve of gallic acid for Folin-Ciocalteu assay is shown in Figure S4. TPC was calculated according to Figure S4.

Results and discussion

Cyanide extraction from cassava leaves

Tuberous root, stem, and leaf are the three primary parts of cassava. Farmers typically harvest the roots to manufacture flour and starch for food using a cyanide-removal method (38). The massive amount of cassava produced globally results in enormous quantities of cassava waste, including stems and leaves (Figure 1C). Although this waste could be used for animal feed (39, 40), cassava waste cannot supply the required nutrients, and it also contains toxic cyanide. As a result, farmers frequently throw away the cassava waste. The application of this waste may help farmers increase profit. While cassava extract has been shown to leach gold from gold ore concentrates (41), that previous report focused on the characterization of gold complexes after the leaching process. In this study, we systematically developed a process to extract cyanide from cassava leaves. The extract can be used to leach Au from E-waste for a circular economy.

The most valuable part of cassava for consumption is the root, which was not employed in this study. Cassava stems and leaves are significant waste products. As a result, we investigated the amount of cyanide recovered from fresh and dried stems and leaves. Using a pestle to break the plant tissue, 1 g of stem or leaf was ground and homogenized in 20 mL of 0.1% NaHCO₃ in a mortar (42). In NaHCO₃ buffer, the enzyme linamarase has been found to hydrolyze linamarin and produce cyanide. To eliminate debris, the solution was centrifuged and filtered. The cyanide content in each solution was determined by the ninhydrin assay using a UV-Vis spectrophotometer at 485 nm (Figure 2) (42). Among the samples (extracts from fresh leaves, dried leaves, fresh stem, and dried stem), fresh cassava leaf extract had the greatest cyanide concentration (almost two times greater than the other three samples; Table 1). As a result, fresh cassava leaves were used in following experiments.

Different amounts of leaf were used in the same volume (20 mL) of 0.1% NaHCO₃ to compare the

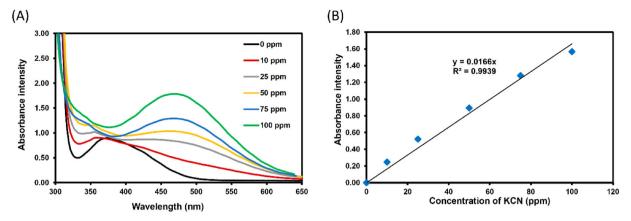


Figure 2. (A) UV-Vis spectra of the ninhydrin assay for different concentrations of KCN. (B) Standard calibration curve of cyanide based on the ninhydrin assay.

contents of extracted cyanide (Figure 3). As the amount of leaf increased, the content of extracted cyanide increased; however, the increase in cyanide content was not proportional. In addition, the leaf quantity affected the homogenization efficiency, and some extract was inevitably lost during debris removal. Considering the yield and efficiency, the optimal scenario was 1 g of leaf extracted in 20 mL of 0.1% NaHCO₃, which produced 63 ppm cyanide. This concentration is similar to the concentration of cyanide produced by the engineered *C. violaceum* (54 ppm) (18).

The amount of cyanide used in gold cyanidation is critical (Figure 1A). A multi-step extraction procedure in which the extract was reused as a buffer to extract the following leaf sample was applied to enhance the cvanide concentration. However, the cvanide content did not increase as expected as the number of extractions increased (data not shown). We hypothesized that some metabolites in the extract prevented the extraction. Thus, we tried to pass the extract through two different resins (activated charcoal and a cationexchange resin) to remove these metabolites. These two resins are widely employed in water purification, and their costs are reasonable. Activated charcoal can absorb organic compounds, and cation-exchange resins absorb charged molecules. After the extracts were passed through the resin, the cyanide content was determined before conducting the next extraction

Table 1. Cyanide concentrations in extracts from different cassava parts in 20 mL of 0.1% NaHCO₃.

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Cassava part	Cyanide concentration (ppm) ^a
Dried stem	30.6 ± 5.1
Fresh stem	31.9 ± 6.3
Dried leaf	33.9 ± 4.7
Fresh leaf	72.5 ± 8.0

^aDetermined by the ninhydrin assay.

steps (Figure 4). Surprisingly, after passing through the cation-exchange resin, the cyanide content in the extract increased from 45 to 62 ppm; no significant change in cyanide content was observed after the extract was passed through activated charcoal.

After passing through the cation-exchange resin, the extract was used to extract the subsequent cassava leaf sample. The cyanide concentration increased as the number of extractions increased (Figure 5). After the third extraction, the cyanide content had doubled (from 62 to 129 ppm). The loss of extract during cassava debris removal is one downside of this multi-step extraction technique and might explain why the increase in cyanide concentration was not proportional to the number of extractions. Overall, we extracted over 100 ppm of cyanide from fresh cassava leaves using the cation-exchange resin and the three-step extraction method. This cyanide concentration is higher than that produced by cyanogenic microorganisms. For cyanide production by the engineered *C. violaceum*, 1 mM

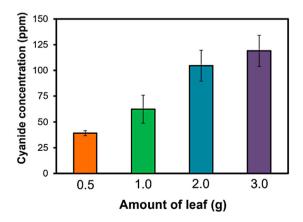


Figure 3. Cyanide concentrations extracted from different amounts of fresh cassava leaves in 20 mL of 0.1% NaHCO₃.

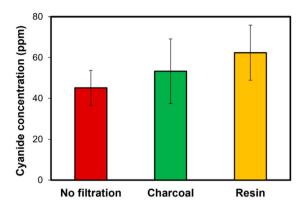


Figure 4. Cyanide concentrations in extracts subjected to different filtration methods (no filtration, activated charcoal, and cation-exchange resin).

isopropyl β -*D*-1-thiogalactopyranoside (IPTG) 0.002% arabinose are required to induce cyanide generation (17,18), resulting in a significantly higher cost compared with cassava leaves.

Gold leaching using cassava leaf extract

After the successful extraction of cyanide from fresh cassava leaves, the pH of the extract was adjusted to 11 for gold cyanidation. Because the pKa value of HCN in water is 9.2, the pH value for efficient gold cyanidation must be greater than 10.5 to produce non-volatile ionic cyanide (19). Certified gold foil (3 mg, 99.99% purity) was added to 20 mL of extract containing 72 ppm cyanide and rapidly agitated at various temperatures for one day. To assess the gold concentration, each mixture was filtered through a 0.22-µm nylon filter before ICP-OES analysis (Figure 6). Gold leaching occurred in all samples. The gold concentration in the sample leached at 25°C was 52.2 ppm, while the gold concentration in

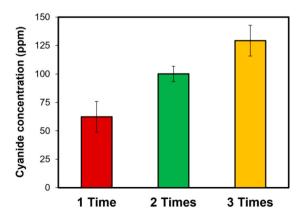


Figure 5. Cyanide concentration in the extract after different extraction times.

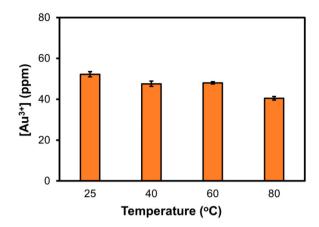


Figure 6. Gold cyanidation of gold foil using 20 mL of cassava extract containing 72 ppm cyanide at different temperatures for one day. The data shown are derived from triplicate experiments.

the sample leached at 80°C was 40.5 ppm. The leaching activity and rate were reported to increase with increasing temperature (19). However, as temperature increases, gas solubility decreases, causing the amount of oxygen in solution to decrease. Furthermore, higher temperatures also intensify the HCN volatilization in extraction process (43). One option to overcome this issue is to conduct gold leaching at high temperature in a pressure reactor with an oxygen supply (44). In subsequent experiments, a temperature of 25°C was used for simplicity.

Although several studies have been published on gold bioleaching, each study employed different conditions and approaches to maximize recovery and/or efficiency (15-18, 25). As a result, it is difficult to compare our study to past works. Thus, for each

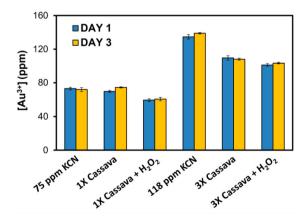


Figure 7. Comparison of the gold cyanidation efficiencies of 20 mL of different cassava extracts and the corresponding control solutions containing the same cyanide concentrations. For H₂O₂ addition, the final H₂O₂ concentration was 0.1%. All experiments were conducted in triplicate.



Table 2. Contents of the main metals in CPU pins.

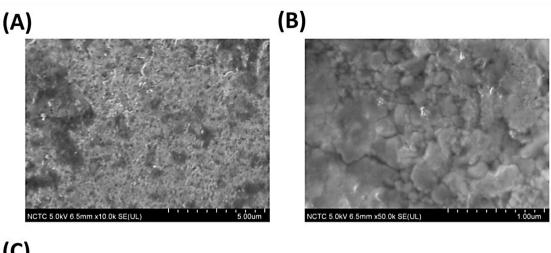
Element	Au	Ni	Cu
Content (wt%)	0.36	14.49	6.68

cassava extract, we developed a control solution with the same volume of 0.1% Na₂CO₃ (pH 11) and the same concentration of cvanide to allow comparison of the gold leaching efficiency. At room temperature, the extract and control solutions were vigorously stirred independently. On the first and third days, 4 mL of solution was spun down and filtered through a 0.22-µm nylon filter. The ninhydrin assay was used to determine the cyanide concentrations in the two filtrates, and ICP-OES was used to determine the gold concentrations in the filtrates after acid digestion based on the calibration curve established by Au standard solutions (Figure S2). After 1 day, more than 90% of the cyanide in each solution had been consumed (Table S1), suggesting that the gold cyanidation process takes nearly 1 day to complete. Furthermore, the pH of the solution remained unchanged. The gold content in the extract was 69.6 ppm after 1 day compared to 73.0 ppm in the control solution (Figure 7). Thus, the leaching efficiency was almost identical. However, the gold concentration in the extract obtained after three extractions (3X cassava) was 109.5 ppm compared to 134.6 ppm in the control solution (Figure 7). Thus, the efficiency of the 3X cassava extract was approximately 81% that of the control solution.

The gold leaching concentration increased with the concentration of cyanide in the extract. However, the gold leaching efficiency of the 3X cassava extract was lower than that of the corresponding control solution; thus, we assumed that reducing metabolites in the cassava extract inhibited gold cyanidation, which is an oxidative reaction. In addition, oxidant additives are frequently used in gold mining to increase gold leaching yield. Hydrogen peroxide (H₂O₂) is a common and inexpensive oxidant (45). Therefore, H₂O₂ was added to the cassava extract at a final concentration of 0.1%. Although H₂O₂ addition did not improve the leaching efficiency of the 3X cassava extract (Figure 6), it did reduce the leaching efficiency of the 1X cassava extract by 19%. Cyanide oxidation by H₂O₂ to create cyanate might be the cause of the reduced efficiency (46).

As a demonstration, the 1X cassava extract was applied to leach gold from E-waste. The CPU was physically sheared to obtain its pins (Figure S1). The pin surfaces contain a significant quantity of gold, whereas the pins' interior cores are mostly copper (47). Nickel in the pin acts as an intermediate layer between Cu and Au, allowing Au to adhere securely to the Cu core more securely. As a result, the pins were digested in aqua regia before ICP-OES analysis to determine the concentrations of Au, Ni, and Cu (Table 2). The morphology and metal composition of the pin's surface were respectively analyzed by SEM and EDX (Figure 8). The SEM images revealed a smooth surface (Figure 8A and 8B). EDX analysis revealed the presence of Au (72.1 wt %), Ni (19.0 wt%), and Cu (5.5 wt%) on the pin surface (Figure 8C), demonstrating that Au was present on the surface. CPU pins (800 mg) were introduced into 20 mL of 1X cassava extract containing 75 ppm cyanide. The mixture was aerobically agitated, and the supernatant was filtered at different time points followed by the determination of cyanide concentration by the ninhydrin assay and the measurement of Au content by ICP-OES. As shown in Figure S3, the cyanide concentration decreased from 75 to 26 ppm in the initial 4 h, and the cyanide was depleted after 8 h. However, we found that gold cyanidation requires a longer time to equilibrate because the gold concentration in the extract continuously increased along with the time and reached 31.3 ppm after 1 day (Figure S3B and Figure 9). After 3 days of leaching, the gold concentration in the 1X cassava extract reached 42.8 ppm (Figure 9). These results suggest that most of the gold leaching occurred in the first day. The gold concentrations in the control solution were 53.2 and 62.1 ppm after the first and third days, respectively (Figure 9). Thus, compared to the control solution, the leaching efficiency of the 1X cassava extract was 69% after the third day of leaching. The surface morphology and metal composition of the CPU pin after leaching were investigated by SEM and EDX, respectively (Figure 10). The SEM images in Figure 10A and B show a rough surface compared to before leaching (Figure 8A and B). The EDX spectrum in Figure 10C shows a decrease in the gold content compared to before leaching (Figure 8C). Overall, the results demonstrate that cassava extract can recover gold from both the CPU waste pins and gold foil.

Although cassava extract could be used to recover gold from the CPU waste, its efficiency was only 69% that of the control solution (Figure 9). Recent studies have prepared gold nanoparticles from plant extracts because tannin, a polyphenol found in the extracts, acts as a chelator and reductant (48, 49). Because tannin forms a purple complex with ferric ion (Fe³⁺) (50, 51), we hypothesized that a resin loaded with Fe³⁺ may remove tannin from the extract. We used Fe-NTA, which is often employed for phosphopeptide enrichment via ionic interaction between phosphate and Fe3+ in a basic environment (52, 53). After passing the extract through Fe-NTA resin, the resin



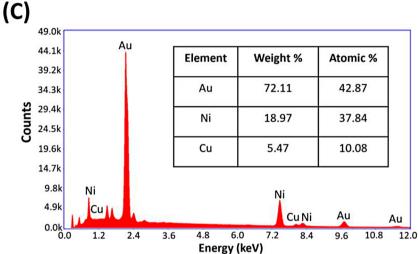


Figure 8. (A and B) SEM images of the CPU pin surface before leaching. (C) Metal composition of the CPU pin surface before leaching determined by EDX.

turned dark purple, indicating the development of Fe³ ⁺-tannin complexes (Figure S4). Total phenolic content (TPC), including tannin, was quantified using Folin-

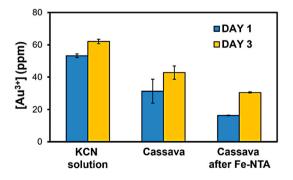


Figure 9. Gold concentrations leached from 800 mg of CPU pins in 20 mL of different solutions. The cyanide concentrations in the KCN solution and cassava extract were 75 ppm. The cyanide concentration in cassava extract after passing through the Fe-NTA resin was 52 ppm. All experiments were conducted in triplicate.

Ciocalteu assay based on the gallic acid calibration curve (Figure S5) (54). The contents of TPC were 0.18 mg/mL in the extract and 0.08 mg/mL in the flow through. Thus, the Fe-NTA resin successfully removed phenolic compounds from the extract. The cyanide concentration in the flow through, on the other hand, decreased from 75 to 52 ppm after passing through the Fe-NTA resin, possibly due to complexation between cyanide and Fe3+. Finally, the flow through was used for gold leaching from CPU waste. The ratio of the gold concentration to the cyanide concentration in the cassava sample was 0.57, similar to that in the Fe-NTA sample (0.59; Figure 9). To examine gold cyanidation efficiency, different quantities of gallic acid, a tannin equivalent, were added to the cyanide control solution. No differences in outcomes were observed after the addition of gallic acid (Figure S6). These findings indicate that tannin does not limit gold cyanidation as we had hypothesized.

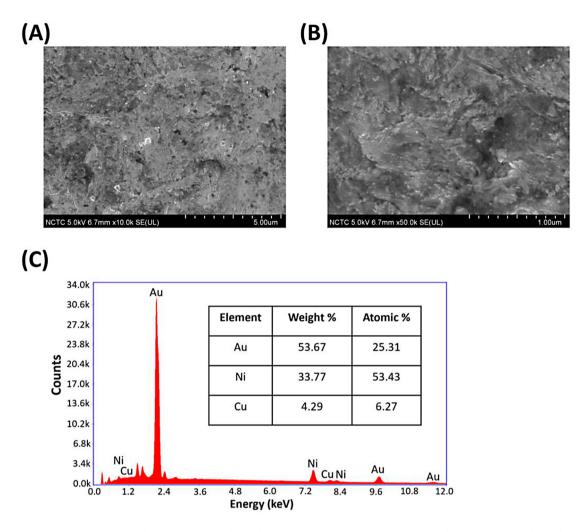
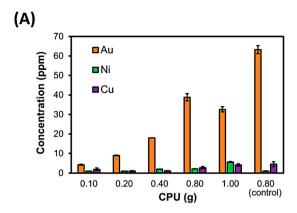


Figure 10. (A and B) SEM images of the CPU pin surface after leaching. (C) Metal composition of the CPU pin surface after leaching determined by EDX.

To optimize the leaching conditions, different amounts of CPU waste were added to 20 mL of cassava extract containing 77 ppm cyanide. Each mixture was aerobically stirred for 3 days. The supernatant was filtered through a 0.22-µm nylon filter before determining the concentrations of Au, Ni, and Cu (abundant metals in CPU waste) by ICP-OES (Figure 11A). The leaching solution containing 0.80 g CPU waste yielded the maximum gold concentration (38.8 ppm), and each leaching solution had fewer than 5 ppm Ni and Cu. The ICP-OES (Table 2) and EDX (Figure 8C) analyses of the CPU samples revealed high levels of Ni and Cu, suggesting that the cyanide in the cassava extract selectively reacted with Au. This was confirmed by the high selectivity for Au observed in experiments with the control solution. The gold recovery for each leaching experiment was calculated based on the gold concentration in the supernatant and the original gold content in the CPU sample (Figure 11B). The

gold recovery ranged from 18.1% to 26.9%, while that for the control solution with 0.80 g of CPU waste was 43.9%. The recovery achieved by the cassava extract was comparable to that of gold cyanidation by engineered C. violaceum (17, 18, 20). Although the leaching solution containing 0.80 g of CPU waste provided the highest gold concentration (Figure 11A), its recovery yield was not significantly higher than those of the other samples (Figure 11B). To consider the downstream process of leaching solution to recycle the most Au after Au³⁺ reduction (9), the leaching solution containing 0.80 g of CPU waste in 20 mL of cassava extract was considered the optimal solution because it resulted in the highest gold concentration. We scaled up the leaching experiment with this optimal solution by three times (total volume of 60 mL) to evaluate the effect on recovery. In this 60-mL experiment, the gold content was 58.9 ppm, slightly higher than that in the 20-mL experiment (42.8 ppm; Figure 12). It was suggested that cassava



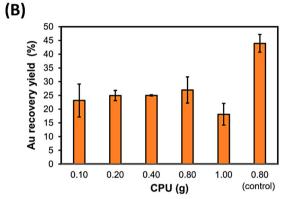


Figure 11. Results of gold leaching experiments with different amounts of CPU waste in 20 mL of cassava extract containing 77 ppm cyanide and the control solution after 3 days of stirring. All experiments were conducted in triplicate. (A) Au, Ni, and Cu concentrations (ppm) in each supernatant determined by ICP-OES. (B) Au recovery (%) in each experiment calculated according to the Au content in the CPU waste before leaching.

extract may be used for large-scale gold recovery. However, the recovery in our system did not satisfy the requirement for industrial use. To increase the total gold recovery, the CPU sample after one day of leaching

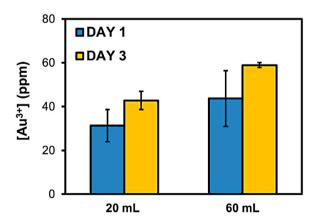
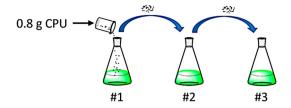


Figure 12. Gold concentrations measured during gold leaching experiments using different volumes of cassava extract. The gold concentrations after leaching using 20 and 60 mL of cassava extract were similar.



Leaching	Gold concentration (ppm)	Au recovery yield ^a (%)
Round 1	26.8 ± 1.1	18.6
Round 2	28.6 ± 3.0	19.9
Round 3	29.7 ± 1.8	20.6
	Total	59.1

^aThe yield is calculated according to the initial gold amount in CPU.

Figure 13. Continuous gold leaching of the same CPU waste. The CPU sample after one day of leaching was transferred to new cassava extract for more leaching. Each extract (20 mL) contained approximately 75 ppm cyanide. The gold concentration (ppm) in each supernatant was determined by ICP-OES. All experiments were conducted in triplicate.

was transferred to freshly prepared 1X cassava extract (20 mL) for continuous leaching. The cyanide concentration in each extract was approximately 75 ppm, and the cyanide was depleted after one day of leaching. As shown in Figure 13, the gold concentration in each of the three supernatants was approximately 30 ppm. Thus, using new cassava extract to continuously leach the same CPU sample is an effective approach to increase gold recovery.

Conclusions and perspective

We developed a feasible protocol to extract cyanide from fresh cassava leaves, an agricultural waste. The resulting extract can be used to leach gold from E-waste. In our demonstration of CPU waste, the gold leaching efficiency was approximately 69% that of the control KCN solution. Under the optimal conditions, the gold recovery was 26.9%. The leaching reaction was selective for gold in the presence of considerable amounts of Ni and Cu in the CPU waste. Continuous leaching was demonstrated as an approach to increase total recovery yield of Au from CPU. Although tannin was reported to reduce Au³⁺ during gold nanoparticle formation, tannin had no effect on gold cyanidation in this study.

In conclusion, cassava is a promising new bio-lixiviant source for leaching gold from E-waste. Our findings pave the way for developing a long-term bioleaching strategy based on agricultural waste for E-waste recycling.



Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

Data available on request from the authors.

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