Phytofabrication of Iron Nanoparticles for Hexavalent Chromium Remediation

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ABSTRACT: Hexavalent chromium is a genotoxic and carcinogenic byproduct of a number of industrial processes, which is discharged into the environment in excessive and toxic concentrations worldwide. In this paper, the synthesis of green iron oxide nanoparticles using extracts of four novel plant species [Pittosporum undulatum, Melia azedarach, Schinus molle, and Syzygium paniculatum (var. austral)] using a “bottom-up approach” has been implemented for hexavalent chromium remediation. Nanoparticle characterizations show that different plant extracts lead to the formation of nanoparticles with different sizes, agglomeration tendencies, and shapes but similar elemental makeup. Hexavalent chromium removal is linked with the particle size and monodispersity. Nanoparticles with sizes between 5 and 15 nm from M. azedarach and P. undulatum showed enhanced chromium removal capacities (84.1–96.2%, respectively) when compared to the agglomerated particles of S. molle and S. paniculatum with sizes between 30 and 100 nm (43.7–58.7%, respectively) in over 9 h. This study has shown that the reduction of iron salts with plant extracts is unlikely to generate vast quantities of stable zero valent iron nanoparticles but rather favor the formation of iron oxide nanoparticles. In addition, plant extracts with higher antioxidant concentrations may not produce nanoparticles with morphologies optimal for pollutant remediation.

INTRODUCTION

Chromium is a valuable resource in a number of different industries, having essential roles in refractories, alloys, electroplating, pigments, catalyst generation, and leather tanning, to name a few applications. It is most commonly found in the environment as the trivalent (Cr III) cation Cr\(^{3+}\) or the hexavalent (Cr VI) oxyanion, that is, HCrO\(_4^-\) and CrO\(_4^{2-}\). Trivalent chromium is an essential macronutrient, which aids in the regulation of lipid, carbohydrate, and protein metabolism; however, overabundance may lead to erythrocyte membrane disturbances and cause skin irritation. Hexavalent chromium is considerably more toxic than its trivalent counterpart. Unlike Cr(III), it can penetrate the skin and kill cells and/or damage DNA through the generation of reactive oxygen species. It is genotoxic and recognized as a human carcinogen (group 1) by the World Health Organization. In addition, Cr(VI) is considerably mobile in soils and waterways, increasing its potential for human and widespread ecosystem exposure.

More than 1760 industrial facilities have been listed by the USEPA’s “Toxic Release Inventory-2003” for releasing chromium into the environment with a total discharge of approximately 52,600 metric tons. For example, in India, it is estimated that greater than 2000 tons of chromium is released into the environment annually. Wastewater from some of these regions contains up to 5000 ppm of chromium, far exceeding the discharge limit of 2 ppm.

As a consequence of the amounts of Cr being released into the environment and the potential environmental impact, a number of different techniques have been implemented for the removal of Cr(VI) species from the environment. The environmental remediation of chromium is faced with a plethora of different factors and conditions, which must be considered, that is, pH, Cr(VI) concentration, temperature, interfering species in aqueous environments, absence of oxygen in groundwater, likelihood of contamination to pose a health risk, time-sensitive remediation occurrences, and so forth. Considering this, different remediation technologies may be required in different situations. The benefits and disadvantages of each technology are compared in Table 1.

Recently, the use of nanoparticles for the remediation of hexavalent chromium species has been investigated. Zhou et
in sugars, and lipids) are likely to interact with iron precursors to found within plant cells (e.g., polyphenols, proteins, reducing pollutants has shown encouraging results.

Although the generation of reductones, which are free radical chain effects have been linked with antioxidative e

taking into account antioxidative properties (i.e., size, shape, reactivity, monodispersity, oxidation potential, crystallinity, etc.) presents a challenge. Many different active molecules found within plant cells (e.g., polyphenols, proteins, reducing sugars, and lipids) are likely to interact with iron precursors to influence nanoparticle formation and properties. The application of iron and bimetallic nanoparticles is vast; however, their use in the environmental remediation of organic and inorganic pollutants has shown encouraging results.

Wang et al., synthesized iron nanoparticles with green tea and eucalyptus leaves, which were able to remove 59.7% and 47.2%, respectively, from an initial concentration of 20 mg/L. Luo et al. showed that grape leaf-mediated synthesis of Fe/Pd bimetallic nanoparticles removed 81.7% of Cr(VI) from an aqueous solution in an ultrasound-assisted system across pH 2–7. Luo et al. used Fe/Ni bimetallic particles to remove Cr(VI) from a solution was captured or transformed in over 30 h of results, the antioxidant concentrations for P. undulatum, M. azedarach, S. melle, and S. paniculatum were 12.6, 9.9, 7.2, and 7.5 mg ascorbic acid (AA) (equiv)/g, respectively, indicating the highest and lowest reducing powers of the same plant extracts were 5.6, 6.0, 4.7, and 3.5 mg ascorbic acid (AA) (equiv)/g, respectively, showing statistically higher concentration. Recycling antioxidant powers (Table 2). Antioxidative effects have been linked with interesting phytochemical compositions, which may lead to novel interactions between biological and inorganic species. The differences in nanoparticle morphologies and physicochemical properties, along with their capacity to remove Cr(VI) species from solution, were investigated.

### RESULTS AND DISCUSSION

**Characterization of Plant Extracts.** In the search for plant species capable of generating “green” nanoparticles with optimal morphologies/properties, much of the selection process has focused on species containing high plant antioxidant concentrations. Although increasing antioxidant concentrations are often trended with increasing reducing powers, their ability to instill optimum morphological nanoparticle features has not been ascertained. From our results, the antioxidant concentrations for P. undulatum, M. azedarach, S. melle, and S. paniculatum were 12.6, 9.9, 7, and 8.1 mM Trolox (equiv)/g, respectively, with only S. paniculatum showing statistically higher concentration. Reducing powers of the same plant extracts were 5.6, 6.0, 4.7, and 7.5 mg ascorbic acid (AA) (equiv)/g, respectively, indicating that the species with the highest and lowest antioxidant concentrations also possessed the highest and lowest reducing powers (Table 2). Antioxidative effects have been linked with the generation of reductones, which are free radical chain

| plant extract   | reducing powers [mg AA (equiv)/g] | antioxidants [mM Trolox (equiv)/g] | phenolics [mg GA (equiv)/g] | reducing sugars [mg glucose (equiv)/g] | iron-chelating capacity [mg EDTA (equiv)/g] |
|-----------------|-------------------------------|-----------------------------------|-----------------------------|----------------------------------------|------------------------------------------|
| P. undulatum    | 5.6 ± 0.2                     | 12.6 ± 0.4                        | 1.9 ± 0.2                   | 42.9 ± 9.9                             | 2.6 ± 0.2                                |
| M. azedarach    | 6.9 ± 0.1                     | 9.9 ± 0.9                         | 1.9 ± 0.4                   | 90.9 ± 16.4                            | 2.1 ± 0.3                                |
| S. melle        | 4.7 ± 0.5                     | 7.2 ± 0.5                         | 2.3 ± 0.1                   | 64.3 ± 7.0                             | 5.8 ± 0.5                                |
| S. paniculatum  | 7.5 ± 0.2                     | 4.8 ± 0.5                         | 4.0 ± 0.4                   | 158.4 ± 14.5                           | 0.3 ± 0.0                                |

References 8 and 9.
reaction terminators. Therefore, antioxidant activity has, in some cases, a relationship with the reductive capacity.\textsuperscript{25} Phenols are known antioxidant agents, and therefore, it is no surprise that phenolic content followed the same trend as antioxidant concentration. \textit{P. undulatum}, \textit{M. azedarach}, \textit{S. molle}, and \textit{S. paniculatum} possessed phenolic concentrations of 1.9, 1.9, 2.3, and 4.0 mg gallic acid (GA) (equiv)/g with only \textit{S. paniculatum} showing statistically higher concentration (Table 2).

Reducing sugars, as their name suggests are reducing agents with the capacity to transform iron salts to nanoparticles.\textsuperscript{26} \textit{P. undulatum} and \textit{S. molle} possessed the lowest reducing sugar concentrations (42.9 and 64.3 mg glucose (equiv)/g, respectively) and were not significantly different from one another. \textit{M. azedarach} possessed significantly higher reducing sugars than \textit{P. undulatum} but was not statistically different from \textit{S. molle}. \textit{S. paniculatum} possessed considerably higher concentrations of reducing sugars than all other plant extracts with 158.4 mg glucose (equiv)/g [\textgreater{}1.5 times that of \textit{M. azedarach}, which contained 90.9 mg glucose (equiv)/g]. It is interesting to note that although \textit{S. paniculatum} contained higher reducing sugar and antioxidant concentrations when compared to the other species, its reducing powers was not significantly greater than those of \textit{P. undulatum} and \textit{M. azedarach} (Table 2).

Iron chelation relates to the ability of plant extract constituents to bind to iron ions and form complex ring structures called chelates. Low and high pH compromise the stability of the chelating agents.\textsuperscript{27} Although the formation of nanoparticles resulted in highly acidic solutions following mixing of precursors, the original pH of the plant extract may also alter the chelation capacity. The pH of plant extracts of \textit{P. undulatum}, \textit{M. azedarach}, \textit{S. molle}, and \textit{S. paniculatum} were 5.95, 5.11, 6.10, and 3.87, respectively (Table 2). The iron-chelating capacity for the aforementioned plant extracts was 2.6, 2.1, 5.8, and 0.3 mg ethylenediaminetetraacetic acid (EDTA) (equiv)/g, which shows a pH-dependent trend, with lower pH resulting in lower chelation capacity. Satue-Gracia et al.\textsuperscript{28} showed that the anthocyanin antioxidants, delphinidin and malvidin, were unable to bind to copper ions under acidic conditions. \textit{S. paniculatum} contains the glycosidases of malvidin and delphinidin (i.e., malvidin and delphinidin 3,5-diglucoside),\textsuperscript{29} and its extract is also acidic, and this may explain its low metal chelation capacity. In addition, the type of phytochemicals present within the plant extract may react differently to pH or other competing influences. Interestingly, \textit{S. molle} possessed the lowest antioxidant concentration but more than twice the iron-chelating capacity of any other extract. Freeze-dried samples of this extract yielded a saplike substance, which may not possess high antioxidative properties but may still bind to the particles. \textit{S. molle} was the only sample to possess a sap/resin following the lyophilization process.

**Synthesis of Iron Nanoparticles.** The introduction of iron chloride solution to the plant extract resulted in a color change from a yellow/brown extract to a jet-black nanoparticle solution instantaneously. This indicated that iron nanoparticles had been formed. The pH of the nanoparticle solutions dropped to 1.5 in all cases; however, nanoparticles were separated from their solution with the aid of dialysis centrifuge tubes [molecular-weight cutoff 30 000 Da] and freeze-dried prior to being used in Cr(VI) removal studies. The nanoparticles were not magnetic and did not settle out of solution under atmospheric conditions.

Current studies on plant extracts for the synthesis of nanoparticles tend to select plant candidates based on high antioxidant concentrations;\textsuperscript{30−32} however, this may not be beneficial for optimal nanoparticle morphologies. In the current study, antioxidant concentrations along with phenolic content and reducing power capacity have shown little to no statistical differences between \textit{P. undulatum}, \textit{S. molle}, and \textit{M. azedarach}. However, the morphologies of the nanoparticle are markedly different (Figure 1). \textit{P. undulatum} showed a mixed nanoparticle morphology with the majority of particles between 5 and 10 nm in size. However, a few larger particles with size ranges between 20 and 70 nm are infrequently present (Figure 1). In addition, there are flat plates that fold into rods with nanometer dimensions. The presence of numerous phytoactive compounds acting on the iron chloride precursor in different ways may be responsible for the numerous different nanoparticle morphologies present. \textit{M. azedarach} possessed particles between 5 and 15 nm in size with little variation in the nanoparticle morphology. \textit{S. molle} formed chainlike structures with particles often falling outside of the nanoparticle size range (>100 nm in all particle dimensions). Nanoparticle structures generated from \textit{S. paniculatum} were\textsuperscript{33} also chainlike in appearance. However, particle sizes were considerably smaller than that of \textit{S. molle} (sizes ranging between 30 and 60 nm) (Figures 1 and S1).

If the generation of maximum nanoparticle concentration is the only parameter of interest, then high antioxidant concentration and reducing powers may be beneficial. However, if specific nanoparticle properties are required, high antioxidant concentration may not be a major contributing factor in achieving this. Optimal nanoparticle properties for pollution remediation include particles that are small, monodisperse, possess no particle–particle attraction, and do not passivate readily under aerobic and aqueous conditions.\textsuperscript{34} Our findings, along with other studies that utilize
antioxidant rich green tea extracts to generate iron nanoparticles, have shown that high antioxidant concentrations lead to particles that agglomerate and form chainlike structures,34−38 thereby reducing the nanoparticle surface area available for remediative interactions.

X-ray Photoelectron Spectroscopy (XPS) and Powder X-ray Diffraction (XRD). There is a common misconception that the production of black iron nanoparticles upon reaction of plant extracts with iron salt precursors confirms the presence of nZVI (Fe0).39 Reactive iron is likely to interact with active phytochemicals within plant extracts making it difficult to form repeating lattice structures required for crystallinity. This is supported by the amorphous nature of nanoparticles generated in this study and several other studies producing iron nanoparticles with plant extracts.37,39−41 In addition to crystallinity, Fe0 nanoparticles are ferromagnetic in nature42 and show a representative XPS peak at 706 eV (Figure 2).43,44 XPS analysis of freshly synthesized iron nanoparticles with plant extracts and the C 1s, O 1s, and Fe 2p core levels revealed that the surface of the particles were composed of a mixture of carbon, oxygen, and iron species (Figure 2). Carbon peaks for the representative sample showed peaks at 284.6, 285.5, 286.6, and 288.6 eV corresponding to C=C, C–O, C–OH, and O=C–O bonds, respectively.45−48 These bonds show that plant bioactive compounds are bound to the surface of the iron oxide nanoparticles. Oxygen was also present on the surface of the particles, with peaks 532.1, 533.2, and 534.2 relating to oxygen in oxyhydroxides, O=C=O, and O=C–OH/ O–N, respectively.49−52 (Figure 2). Iron signature peaks were numerous and , indicating the presence of mixed iron oxide species. Oxygen was also present on the surface of the particles, with peaks 532.1, 533.2, and 534.2 relating to oxygen in oxyhydroxides, O=C=O, and C–OH/O–N, respectively39−52 (Figure 2). Iron signature peaks were numerous and , indicating the presence of mixed iron oxide species. However, no Fe0 was identified. The peaks identified around 710.4 and 711.9 relate to Fe3+ species representative of ferric compounds such as Fe2O3, Fe3O4, and FeOOH.53 Peaks at 715.1, 728.5, and 718.7 are representative of Fe3O4;54,55 however the peak at 711.9 together with OH groups (peaks at 534.2 and 286.6 eV) point toward the presence of hydrous iron oxides, such as FeOOH and/or Fe(OH)3.56

Fourier Transform Infrared (FTIR) Spectroscopy. S. paniculatum showed strong representative carboxylic acid signature peaks with O–H stretching bonds between 3200 and 3400 cm−1 and C–O bonds located between 1725 and 1710 cm−1. P. undulatum also possessed these peaks, but its representation in the spectra was weak (Figure 3). S. molle and S. paniculatum also showed the existence of phenolic compounds by the presence of O–H stretching bonds between 3000 and 2850 cm−1, and C–O stretching bonds between 1000 and 1100 cm−1.57−59 Peaks located above 3000 cm−1 are unsaturated and are willing to take part in hydrogen bonding.60,61 S. molle and S. paniculatum (and to a minor extent P. undulatum) possessed well-defined peaks in this region, and this may be responsible for the particles’ tendency to agglomerate.

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Representative XPS and XRD fingerprints of green nanoparticles (from S. paniculatum). (A) Carbon XPS C1 core level, (B) oxygen XPS O1 core level, (C) iron XPS Fe 2p core level, and (D) XRD spectra (2θ°).

![Figure 3](https://example.com/figure3.png)

**Figure 3.** FTIR spectra of iron nanoparticles generated with different plant extracts.
All nanoparticle species contained peaks located in the regions of 1635–1545, 1430–1350, and ~1000 cm$^{-1}$ (Figure 3), which are representative of the stretching of the carboxylate group (COO$^-$), bending of O–H bonds from carboxylate groups, and C–O stretching bonds, respectively. Peaks relating to N–H bending are also found within the region of 1650–1500 cm$^{-1}$ and may be masked by the presence of the carboxylate signature.$^{62–64}$

Nanoparticles from $P. undulatum$ and $M. azedarach$ showed an absence of strong signatures above 3000 cm$^{-1}$ and possessed carboxylate groups, which have repulsive forces with other carboxylate groups.$^{65}$ This may explain the formation of monodispersed nanoparticles generated within these samples.

**Hexavalent Chromium Reduction.** Within this study, an equal weight of nanoparticles (5 mg/mL Cr$^{6+}$ solution) was added to each treatment vessel as opposed to an equal volume of crude nanoparticle reaction solution. In this way, we were able to assess the efficiency of the nanoparticles on Cr$^{6+}$ removal alone without the unreacted iron salt and unbound plant extracts interfering with the degradation kinetics. Mystrioti et al.$^{66}$ showed that pomegranate and clove plant extracts alone could remove approximately 25% Cr(VI) from solution over a 2-day period. This study also showed that clove extract possessed a low polyphenol content, however, it was still efficient at reducing Cr(VI). The authors mention that the type of polyphenols present within the plant may be a more important factor to focus on rather than the total polyphenol content of specific extracts.

In this current study, the plant extract of $P. undulatum$ was the only plant extract, which had an impact on hexavalent chromium reduction with 100 µL of extract resulting in approximately 35% removal of Cr(VI) in the same concentration and volumes as the nanoparticle Cr(VI) removal assays (data not shown). However, as capping of the particles only ranged from 3.3 to 5.4% of the total weight of the particles (determined by differences in weight following the removal of organic matter in a blast furnace at 500 °C for 12 h), their influence is likely to have a negligible contribution to the overall Cr(VI) degradation within the nanoparticle treatment microcosms.

Although antioxidant concentration may show trends with increased reducing powers and hence in the formation of more concentrated nanoparticle solutions, when the ability of the resulting nanoparticles is assessed for Cr(VI) removal efficiency, they may not be the best performers.

This current study assessed the capacity of iron nanoparticles generated from plant extracts with different antioxidant profiles to remove Cr(VI). Plant-generated nanoparticles (100 mg) with residual precursors removed were added to aqueous Cr(VI) solutions (20 mL of 50 mg/L solutions). Results showed that $P. undulatum$- and $M. azedarach$-generated nanoparticles had significant and high Cr(VI) removal capacities with rates of 96.2 and 84.1%, respectively, in over 9 h (Figure 4). Nanoparticles generated from $S. molle$, which showed the lowest antioxidant concentration and reducing power capacity had the lowest Cr(VI) removal efficiency (43.7%). However, $S. paniculatum$, which possessed the highest antioxidant concentration and reducing power capacity was only able to achieve 58.7% Cr(VI) removal after 9 h. As all nanoparticles were made up of mixed oxides, Cr(VI) removal dynamics was expected to be similar. As Cr(VI) removal after the first 15 min was slow and steady, an absorption mechanism is suggested. This is supported by Jiang et al.$^{67}$ who showed that absorption of Cr(VI) to maghemite nanoparticles was spontaneous and highly favorable.

Nanoparticle crystallinity, composition, surface bonds, and concentrations were similar (or the same), but their Cr(VI) removal capacities were different. This may be attributed to the size of the particles. It is widely documented that a decrease in the nanoparticle size greatly increases its surface area and in turn increases the available surface available for interactions to occur.$^{68–70}$ The nanoparticles that showed the highest Cr(VI) removal capacities had the smallest nanoparticle sizes, with most particles falling within the 5–15 nm size range. In addition, these nanoparticles were also quite monodispersed, further maximizing the particle surface area. Conversely, the two worst performing nanoparticle types possessed chainlike structures. Although the individual particles were nanometric in size, the linking of particles reduces their surface area, and as a result, they behave like millimetric-sized particles with reduced Cr(VI) removal capacity. It should also be mentioned that the type of phytochemicals capping the nanoparticles may also slow down the interactions between the pollutant and the particle. To enhance the nanoparticle capacity for removing Cr(VI) from wastewater, functionalization of nanoparticles may aid in both monodispersity and selective binding to Cr(VI).$^{71}$ Both nanoparticles may be applied to nanoparticles, this may aid in elevated remediation rates.

**CONCLUSIONS**

Reduction of iron salt precursors with different plant extracts can generate iron nanoparticles with different morphological features. However, the phytochemical fingerprints examined in this study were not comprehensive enough to link the compounds to morphology. All particles were composed of mixed iron oxide species and were amorphous in nature. Zero
valent iron was not identified and is unlikely to be present in nanoparticles because of the lack of representative XRD and XPS peaks along with an absence of magnetism. Nanoparticles possessed C=C, C–O, O–C=O, and C–OH bonds on their surface, which was confirmed with XPS and FTIR analysis, indicating that they were capped with plant-based phytochemicals. Nanoparticles, which possessed strong O–H bonds peaks in the FTIR analysis above 3000 cm$^{-1}$, also showed agglomerated nanoparticles. The antioxidant concentration of plant extracts was linked with the reducing power capacity but not with optimum nanoparticle morphologies for chromium degradation. Plant extracts with the lowest and highest antioxidant concentrations showed the lowest Cr(VI) removal capacity for all nanoparticles tested. This was attributed to the nanoparticle-chained structures formed, which reduced the nanoparticle surface area and interaction with Cr(VI). P. undulatum and M. azedarach possessed the highest Cr(VI) removal capacity of 96.2 and 84.1%, respectively. Nanoparticles of these species were between 5 and 15 nm in size and were monodispersed. This research presents a green, cost-effective, and efficient technology for the removal of Cr(VI) from aqueous solutions. Further research into the fractionation of plant extracts is required to determine the specific phytochemicals responsible for instilling specific nanoparticle traits. In addition, modification of nanoparticles using ligands with a strong affinity for specific pollutants is expected to deliver elevated remediation capacities. The immobilization of nanoparticles onto a solid support may be of interest for removing pollutants and nanoparticles from the environment following remediation.

**MATERIALS AND METHODOLOGY**

**Dry Weight Determination.** The water content of the plant leaves was determined by drying 10 g of fresh plant leaf biomass at 40 °C for 5 h or until no further weight change was apparent. Percentage moisture content was determined by the following equation

\[
\text{% moisture content} = \frac{\text{plant biomass following drying}}{\text{initial weight of fresh biomass}} \times 100
\]

**Preparation of Plant Extracts.** Dried plant leaves of interest (10 g) were added to 100 mL of Milli-Q water and were put into a water bath at 80 °C for 1 h and then removed and allowed to cool at room temperature. The solution containing water-soluble phytochemicals was filtered through a 0.2 μm filter and stored at −20 °C until further use.

**Phytochemical Analysis of Extracts. Trolox Equivalent Antioxidant Capacity (TEAC) Assay.** The antioxidant capacity of the extract was determined using a modified method of that described by Stratil et al.\textsuperscript{74} and Re et al.\textsuperscript{75} ABTS$^+$ cation stock solution was created by reacting 1:1 (v/v) of 7 mM ABTS$^•$ solution to 4.90 mM potassium persulfate solution (2.45 mM final concentration). This solution was left to stand for 15 h at 25 ± 2 °C in the dark to form the radical cation ABTS$^{**}$. To determine the TEAC, the ABTS$^+$ solution was diluted with phosphate-buffered saline (PBS, pH 7.4) to get an absorbance reading [optical density (OD)] between 1.0 and 1.5 at 734 nm when 200 μL of the sample was measured on a 96-well plate reader (BMG POLARstarOmega).

A Trolox standard curve was created by making serial dilutions of an initial 1.5 mM Trolox solution in PBS. Ten microliters of the Trolox solutions was added to 19 0 μL of ABTS$^{**}$ solution in a 96-well plate (COSTAR). Spectrophotometric measurements at 734 nm were taken exactly 6 min after initial mixing, and increased antioxidant concentration was determined by a decrease in the OD values.

The aforementioned procedure was repeated with 10 μL of plant extract. Dilutions of plant extract (diluted in PBS, pH 7.4) were made if values fell outside the measurable range, and OD values were multiplied by the dilution factor. Findings are expressed as Trolox equivalents/g plant biomass [mM Trolox (equiv)/g].

PBS (pH 7.4) and Trolox mixed with each corresponding plant extract in volumes used (without ABTS$^{**}$) served as controls and standards.

**Folin–Ciocalteu Assay.** The phenolic content of plant extracts was determined by the Folin–Ciocalteu method, as outlined by Meda et al.\textsuperscript{76} and Moein et al.\textsuperscript{77} Briefly, the plant extract (50 μL) was added to 450 μL of Milli-Q water and filtered through a Whatman no. 1 filter paper. An aliquot (50 μL) of this solution was then mixed with 250 μL of 0.2 M Folin–Ciocalteu reagent (Sigma-Aldrich Chemie, Steinheim, Germany) for 5 min, and 200 μL of 75 g/L sodium carbonate (Na$_2$CO$_3$) (Labosi, Paris, France) was added. After incubation at room temperature for 2 h, the absorbance of 150 μL from the reaction mixture was measured at 760 nm against methanol blank in a 96-well plate reader. Phenolic content is expressed as GA equivalents/g plant biomass [mg GA (equiv)/g].

The linear region of a standard curve, generated from the reaction of the above solution with GA (0–0.50 mg/mL), was used to determine the phenol content of plant extract samples (GA equivalents).

**Reducing Power Assay.** The Fe$^{3+}$ reducing power (antioxidant capacity assay) of the extract was determined using the methods outlined by Berker et al.\textsuperscript{78} with modifications relating to the incubation method and the volumes of reagents. Briefly, 100 μL of Milli-Q H$_2$O, 30 μL of 0.20 M HCl, 30 μL of ferricyanide solution (1%), 10 μL of sodium dodecyl sulfate (1%), and 10 μL of FeCl$_3$ 6H$_2$O (0.2%) were combined to make a total of 180 μL solution.

The plant extract or the standard to be tested was made up to 20 μL with ethanol (96%). The volume of the sample added to ethanol needed to be optimized to fit within measurable parameters, and this differed between samples tested.

Both the aforementioned final solutions were mixed together to make a 200 μL solution, which was incubated at 50 °C within a thermocycler (Bio-Rad, T-100) for 20 min and then held at room temperature until further use. An aliquot (150 μL) of the resulting sample was transferred to a 96-well plate (COSTAR), and the absorbance was measured at 700 nm ($A_{700}$) against a reagent blank within 25 min.

AA solution (0.0–100.0 μg/mL) was used as the standard. Increased absorbance of the reaction mixture indicated increased reducing power. Findings are expressed as i-AA equivalents/g plant biomass [mg AA (equiv)/g]. All measurements were conducted in triplicate in a 96-well plate reader, BMG Labtech, Germany.

**3,5-Dinitrosalicylic Acid (DNS) Assay.** The reducing sugar content of plant extracts was determined using the DNS method, as outlined by King et al.\textsuperscript{79} Briefly, DNS working solution was prepared by combining 10 g of DNS with 403 g of KNaC$_5$H$_7$O$_6$.4H$_2$O and then made up to 1 L in 0.4 M NaOH
(heated at 50 °C till dissolved). The solution was then filtered through a 0.45 μm filter.

Sixty microliters of glucose standard (0.0–3.0 mg/mL) prepared in 0.1 M sodium acetate buffer pH 5.0 or filtered plant extract was added to 120 μL of DNS working solution in PCR tubes. PCR tubes were then placed into a PCR thermocycler (Bio-Rad, T-100), and the incubation cycle was set at 95 °C for 5 min, cooling to 48 °C for 1 min, and holding at 20 °C.

Fifty microliters of the completed DNS reaction product was then added to 150 μL of Milli-Q H2O in flat-bottom microplates (COSTAR), and the absorbance was measured at 540 nm. Findings are expressed as glucose equivalents/g plant biomass [mg glucose (equiv)/g].

**Ferrous Ion-Chelating Activity.** The capacity of plant extracts to chelate Fe2+ ions was determined using the protocol described by Decker and Welch80 with some minor modifications. Briefly, to a flat-bottom 96-well plate (COSTAR), the following were added: 67.5 μL of Milli-Q water, 50.0 μL of the standard or plant extract, and 2.5 μL of FeCl2·4H2O (2 mM). The reaction was initiated by addition and thorough mixing of 5.0 μL of ferrozine solution (5 mM), and the reaction was allowed to develop in the dark for 10 min. Absorbance values were measured using a 96-well plate reader (BMG POLARstarOmega, BMG Labtech, Germany) at 562 nm. The linear region of a standard curve, generated from the reaction of the above solution with EDTA (0–250 mg/mL) was used to determine the metal chelation capacity of the plant extract samples. Results are expressed as EDTA equivalents/g plant biomass [mg EDTA (equiv)/g].

**Iron Nanoparticle Synthesis. Preparation of Iron Nanoparticles.** The plant extract was mixed in a beaker at top speed using a magnetic stirrer, and 0.1 M FeCl2·6H2O solution was slowly introduced using a peristaltic pump with a flow rate of 2 mL/min, ensuring a 2:1 ratio v/v of plant extract to FeCl3·6H2O, respectively. The color of the solution changed to black indicating the synthesis of nanoparticles. The resulting solution was centrifuged at 14 000 rpm for 30 min within Amicon Ultra-0.5 Centrifugal Filter Devices with 10 kDa cutoff; the flowthrough was discarded and the nanoparticles pelleted on the filter were resuspended in Milli-Q water, frozen to −80 °C, and lyophilized.

**Characterization of Plant-Generated Nanoparticles.** Transmission Electron Microscopy (TEM). TEM characterization was undertaken for nanoparticles prior to the dye degradation reaction. Plant extract-generated nanoparticle samples were prepared by drop-coating the samples on a carbon-coated copper grid. The grid was left to dry overnight in a dust-free environment, and the morphological characteristics of iron nanoparticles were analyzed with a JEOL1010 TEM operated at 100 kV (Thermoemission cathode).

**FTIR Spectroscopy.** FTIR spectroscopy spectra of freeze-dried plant extracts, plant extract-generated iron nanoparticles, and plant-generated nanoparticles with organic material burnt off at 500 °C for 10 h in a muffle furnace were determined by a Fourier transform infrared spectroscopy (PerkinElmer Frontier). Nanoparticles were freeze-dried, washed in Milli-Q water, and centrifuged at 14 000g and the nanoparticles were retained. Nanoparticles were dried at room temperature before being processed by a spectroscope. An average of 18 scans was collected for each measurement with a resolution of 0.5 cm⁻¹ in the range of 4000–400 cm⁻¹.

**XRD.** To determine the crystalline phases of plant-generated nanoparticle, XRD analysis was conducted using a Bruker AXS D8 Discover diffraction instrument equipped with a Cu Kα radiation source (wavelength 0.1542 nm) operating at 40 kV and 35 mA. All X-ray data were obtained in the 2θ–2θ locked-couple mode over a 2θ interval of 10–60. The identity of the diffraction peaks was assigned through auto-fitting in the instrument software (Bruker Evaluation).

**XPS.** Nanoparticle surface compositions were analyzed by XPS (Thermo K-Alpha XPS). The instrument was equipped with an Al Kα X-ray radiation source with a photon energy of 1486.6 eV. All measurements were conducted at room temperature under ultrahigh vacuum (10⁻⁸ Pa). The C 1s, O 1s, and Fe 2p core level spectra were recorded with an overall resolution of 0.1 eV. The spectra were handled with Avantage 4.88 Surface Chemical Analysis Software, Thermo Scientific.

**Hexavalent Chromium Removal.** Aqueous solutions of Cr6+ (20 mL of 50 mg/L) were prepared with K2Cr2O7 and buffered to pH 3.5 in acetate buffer. The diphenylcarbazide (DPC) method was used to determine Cr6+ species in solution41 with some minor modifications. To each Cr6+ solution, 130 μL of DPC solution (200 mg of DPC/10 mL of acetone) was added. After 10 min, time 0 OD(540nm) measurements were taken using a BMG POLARstarOmega using COSTAR flat-bottom 96-well plates. Following this, 100 mg of plant-generated nanoparticles was added to vessels and periodic OD(540nm) measurements were taken for over 9 h.

Hexavalent chromium removal efficiency (%) was calculated as follows

\[ \text{Hexavalent chromium removal efficiency (\%)} = 100 - \left( \left( C_0/C_1 \right) \times 100 \right) \]

where \( C_0 \) is the OD(540nm) of the hexavalent chromium/DPC complex at time 0 and \( C_1 \) is the OD(540nm) of hexavalent chromium/DPC complex at time \( t \).

**Statistical Analysis.** The concentrations of all bioactive components were compared within plant species and between plant species. Univariate ANOVA pairwise comparison coupled with Tukey and Duncan’s multiple comparison post hoc tests were used to identify correlations at a 0.05 level.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b00410.

Enhanced magnification of nanoparticles and carbon, oxygen, and iron atomic composition from the XPS analysis (PDF)

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**Notes**

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