SUPPORTING INFORMATION

Size Fractionation of Titania Nanoparticles in Wild *Dittrichia Viscosa* Grown in a Native Environment

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S1. Extended Experimental Details

S1.1 Processing of Harvested Plant Materials

All water used in the sample preparation and nanoparticle extraction processes was deionized water (DI) with a conductivity of 18.0 MΩ·cm provided by a Waters NanoDiamond water dispenser. After harvesting, each plant organ was thoroughly rinsed with water and soaked in sterile DI water containing sodium hypochlorite (1.25%, v/v) for 20 min to help release the remaining debris and bacteria from the surfaces of each section of plant.[1] After three additional rinses with DI water for each plant organ (250 mL each time), the plant material was dried for 10 days. These samples were kept away from any source of heat or light. The separate organs and sections of the *Dittrichia viscosa* plant species were cut into small pieces (~1 cm^2) in preparation for separately grinding each of the plant parts using a mortar and pestle. After this grinding step, the plant parts were dry sieved through a 0.5 mm mesh.

The rhizosphere samples were collected to a depth of 20 cm and dried for 10 days. These samples were subsequently ground and dry sieved through a 2 mm mesh. About 10 g of the sieved rhizosphere sample was soaked in 200 mL of DI water. After 1 h of vigorous shaking, the pH of the solution containing the soil material was measured at using a calibrated pH meter.

S1.2. Extraction and Purification of Nanoparticles from Plant and Soil Materials

A procedure was established for the extraction and the purification of nanoparticles from these dried, ground and sieved samples. Special care was taken to avoid contamination at all steps. For example, the surfaces of all vessels and tools were cleaned with isopropanol immediately before and after use. Between 20 g and 25 g of each dry sieved plant part, and 50 g of the corresponding rhizosphere were separately soaked in 200 mL aliquots of DI water containing 1%

w/v sodium dodecyl sulfate (SDS, Sigma Aldrich), which was used as a surfactant to help release nanomaterials from the cell walls and other organelles. These mixtures were vigorously shaken for 48 h at room temperature on an orbital shaker at 250 rpm. The suspensions obtained from this process were separately filtered through a medium sieve (1/16 inch mesh) and centrifuged at 3,000 rpm to remove large debris, which appeared as a thick pellet after centrifugation. A 2 mL portion of each supernatant was passed through filters with a 50 nm nominal pore size (Whatman polycarbonate membrane filters) with the assistance of a Büchner vacuum filtration device. Each of these filters were subsequently dried while held in clean petri dishes at room temperature for 24 h. The filters with the retentate material were stored in individual clean plastic bags in the dark and within a dry container at room temperature before being processed further. Concentrating and storing the samples on these filters enabled both a pre-concentration of the isolated material, as well as simplification of transport to the electron microscopy facilities.

One week before the electron microscopy analyses, each filter was soaked for 24 h in 750 µL of ultrapure water in a clean, sealed glass vial at room temperature while protected from light. After this incubation period, the vial containing both the filter and the aqueous solution was placed on an orbital shaker operating at 250 rpm while maintained at room temperature for 6 h and protected from light. At this point, scanning electron microscopy (SEM) analyses were performed on each of the samples to assess the presence of particulate materials released from each of the plant samples, such as from the stems or the leaves. This assessment was performed using an FEI Nova NanoSEM operating at 15 kV equipped with a secondary electron detector.

The SEM analyses revealed that the samples collected from the stems contained flakes with micrometer size dimensions. The large amount of micrometer-sized organic debris present in the sample prevented a direct analysis by transmission electronic microscopy (TEM) techniques of

some of these larger components (Figures S1A, S1B, and S1C). A sample containing particles isolated from the leaves was processed and prepared for SEM analysis using similar techniques. Three different suspensions of the samples from the leaves were prepared for SEM analyses. These included an aliquot of the mixed supernatant obtained right after treatment of the sample by centrifugation at 300 rpm (Figure S1D), or through examining the isolated phases of the same supernatant obtained after settling for 24 h [e.g., supernatant (Figure S1E), and the isolated pellet (Figure S1F)]. All three of these samples exhibited particulate matter with a similar morphology. Micrometer-sized debris was present as flakes and fibers that needed to be removed from the solutions for further analysis of nanoparticles present by scanning TEM (STEM) techniques.

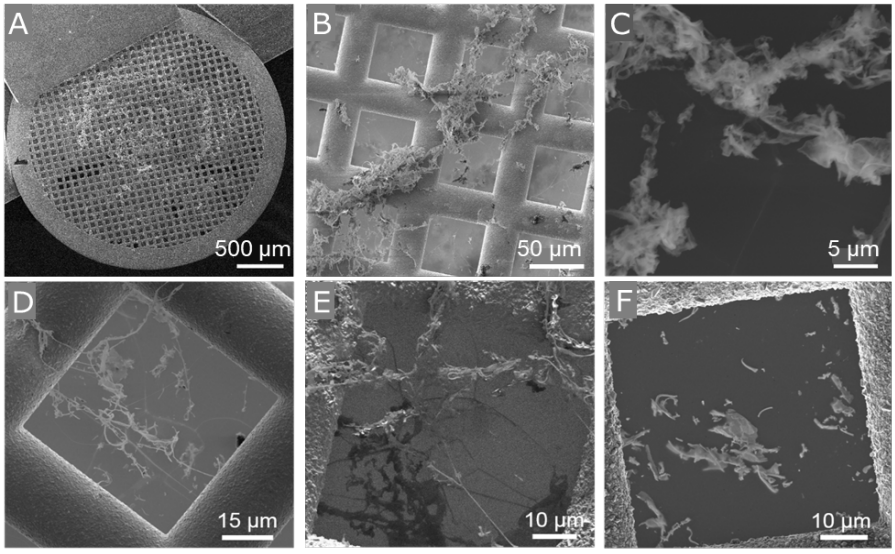

Figure S1. Representative scanning electron microscopy (SEM) micrographs with an increasing magnification from (A) to (C) of particulate samples isolated from the stems. These samples were drop-cast onto a transmission electron microscopy (TEM) grid from a suspension containing the materials released from a filter over 24 h followed by a mild agitation on an orbital shaker. (D–F) Representative SEM micrographs of the particles isolated from a sample of leaves, which were each drop-cast onto TEM grids from: (D) a supernatant obtained right after treatment of the sample by centrifugation at 300 rpm; (E) a supernatant obtained after settling for 24 h; or (F) from the sediment obtained after settling for 24 h.

Based on this initial analysis of the samples (Figure S1), it was determined that additional purification steps were necessary to prepare the samples for imaging at higher magnifications. The solutions obtained after agitating the filters in water for 6 h were each centrifuged at 300 rpm for 10 min at room temperature. The isolated supernatants were transferred to an Eppendorf tube and allowed to settle overnight at 4 °C. The resulting supernatants were treated by ultracentrifugation

at 80,000 rpm at room temperature for 7 h in a Beckman Optima Max using polypropylene microtubes. The solid fractions obtained after this process for each plant part or organ (e.g., rhizosphere, roots, stems, or leaves) were separately suspended into 500 µL aliquots of DI water with the assistance of agitation prior to further analysis (Figure S2). These solutions were each evaluated for the size and shape of the TiO_2 particles present in each sample.

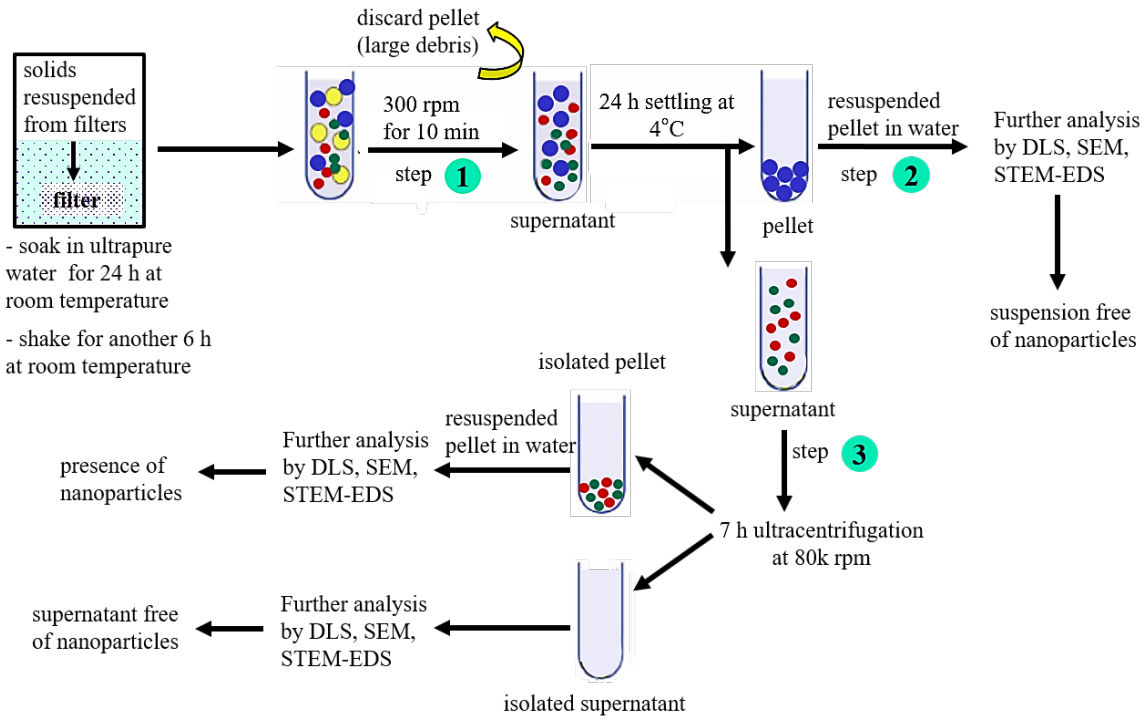

Figure S2. Representative steps of the workflow adapted for the extraction and purification of nanoparticles from the plant parts (e.g., leaves, stems or roots) and the rhizosphere.

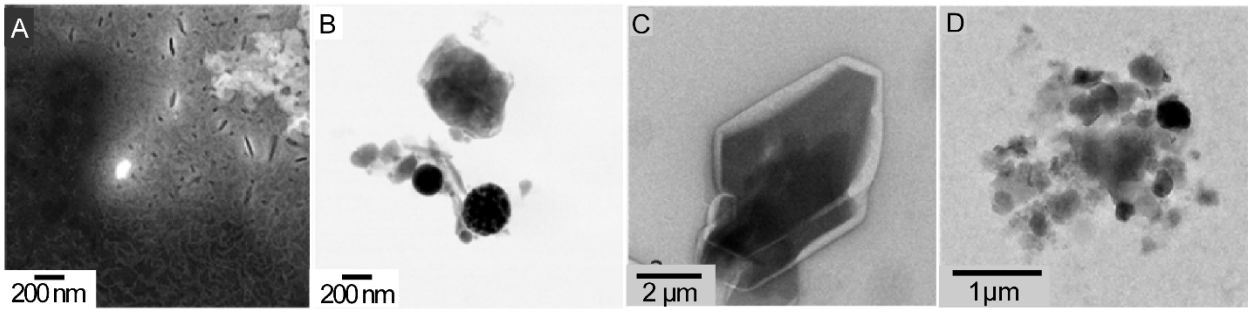

Figure S3. (A) Typical TEM image of the particles found within the supernatant of a sample prepared from a rhizosphere after step 3 of the adapted workflow (Figure S2; treatment of a suspension of the sample by ultracentrifugation for 7 h). (B) Typical TEM image of the particulate matter within the pellet isolated by ultracentrifugation from the rhizosphere sample (also step 3 in Figure S2). (C) A representative TEM image of the particles isolated from the supernatant of the root sample after treatment by ultracentrifugation (step 3 in Figure S2), and (D) the particulate matter within the pellet of the root sample after treatment by ultracentrifugation (also step 3 in Figure S2). Note that the particles observed within the supernatant of these samples following ultracentrifugation were seemingly of an organic composition, in contrast the particles within the isolated pellets.

S2. Further Assessment of the Isolated Nanoparticles

Particles isolated at each of the steps described in the workflow (Figure S2) were also assessed by dynamic light scattering (DLS) measurements at room temperature using a Malvern Zetasizer NanoZS (Malvern Instruments DSC1060C). Examples of the results from these DLS measurements are plotted in Figures S4 and S5. The DLS measurements were obtained at different steps of the extraction and purification processes, which demonstrated the ability of the procedure

to isolate the nanoparticles from the microparticles present within the same sample. The large particles are easily distinguished in the DLS data. Examples are shown here for samples from the roots and the rhizosphere. It was expected that, even after the initial purification steps, the resuspended solid fractions belonging to the rhizosphere samples could exhibit a persistent aggregation of the particles therein. The presence of these aggregates may be due to their natural state in the soil that usually contains large aggregates likely due to the inevitable interactions of the nanoparticles with organic matter (e.g., humic acid species). The coexistence of the nanoparticles and organic matter makes the separation of these materials difficult. Additionally, the isolated fractions of nanoparticles may form large aggregates due to the processes of sample purification. The same limitations could apply to samples isolated from the roots since it is the plant organ that is directly in contact with the surrounding soil. The dimensions of the particles isolated for both types of samples were heterogeneous as shown in Figures S4 and S5.

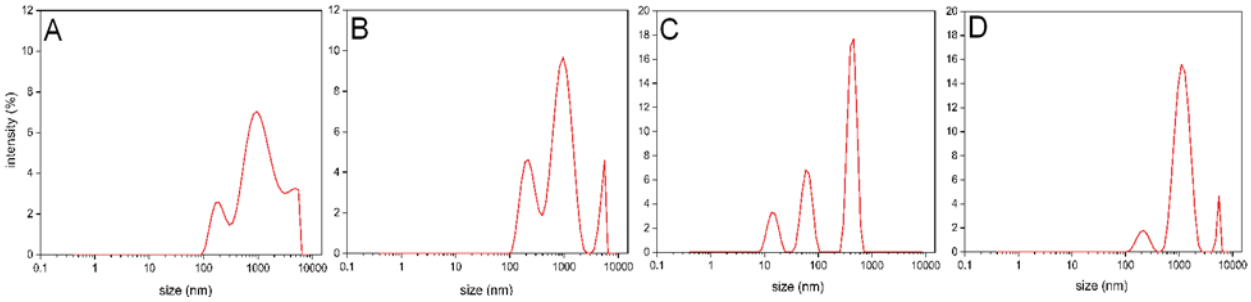

Figure S4. Dynamic light scattering (DLS) measurements of the material isolated from the root organ of *Dittrichia viscosa*. These samples were obtained at different stages of the process to extract and purify the fractions containing nanoparticles. (A) The DLS data for the supernatant after treatment by centrifugation at 300 rpm for 10 min. (B) The DLS measurements for the supernatant in (A) after 24 h of settling. (C) The DLS response of the supernatant after ultracentrifugation. (D) The DLS results for the resuspended pellet obtained after ultracentrifugation.

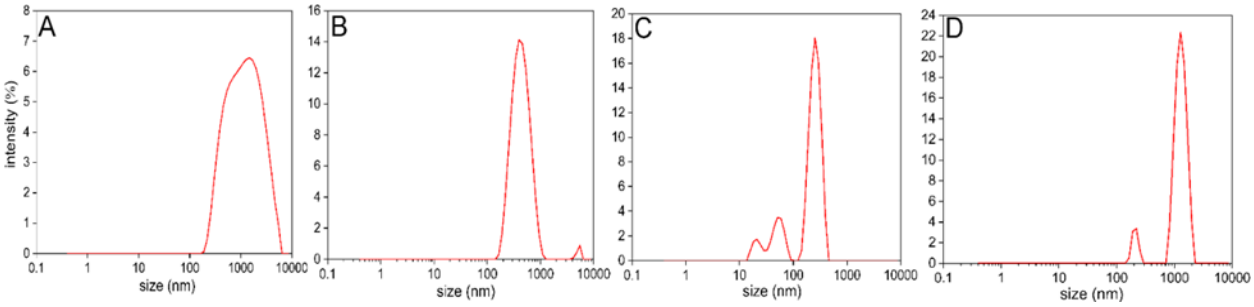

Figure S5. The DLS measurements of the material isolated from the rhizosphere part of *Dittrichia viscosa*. These samples were obtained at different stages of the process to extract and purify the fractions containing the nanoparticles. (A) The DLS data of the particles in the supernatant after treatment by centrifugation at 300 rpm for 10 min. (B) The DLS measurements on the supernatant that was obtained after 24 h of settling. (C) The DLS response of the supernatant after ultracentrifugation. (D) The DLS results for the resuspended pellet obtained after ultracentrifugation.

The final fractions obtained by ultracentrifugation were also analyzed for their composition and to further confirm the potential presence of nanomaterials (Figures S6, S7, S8 and S9). The recovered supernatant contained organic materials as shown in Figures S3A and S3C, but the solids resuspended from the isolated pellet contained nanoparticles (Figures S3B and S3D). This fraction of nanoparticles was characterized by scanning transmission electron microscopy (STEM) and energy dispersive X-ray spectroscopy (EDS) techniques. A TEM analysis was performed only on the isolated solid fractions after 7 h of ultracentrifugation since the supernatant otherwise exhibited only the presence of organic materials during the electron microscopy analyses.

The results of the initial SEM and TEM based analyses further indicated the importance of the described workflow to isolate the nanoparticle fractions from the other suspended matter prior

to further analysis by STEM and related techniques. The STEM analyses were performed using an FEI Tecnai Osiris (Hillsboro, OR, USA) operating at 200 kV and equipped with an energy dispersive X-ray spectrometer (Super-X EDS detection system). To prepare the samples for each of these TEM based analysis, a 3 µL portion of the desired suspension was drop-cast onto a copper TEM grid. The grids were pre-coated with a thin film of Formvar/carbon (Part No. 01753-F; Electron Microscopy Sciences, Hatfield, PA, USA). Each drop of solution was dried for at least 3 h under reduced pressure (~230 Torr) prior to analysis by STEM and EDS techniques.

S3. Additional Electron Microscopy Results for the Isolated Nanoscale Particles of TiO_2

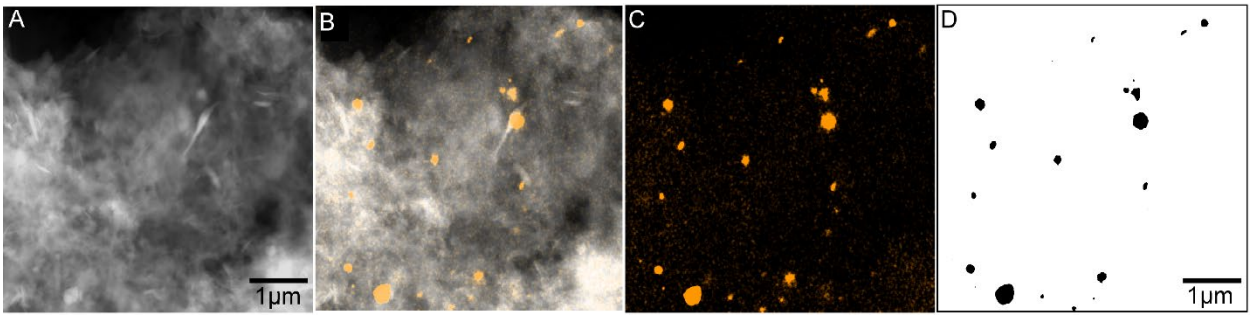

Figure S6. Representative micrographs of nanoscale TiO_2 extracted from the leaves of *Dittrichia viscosa*. (A) Scanning transmission electron microscopy (STEM) image of the isolated particles obtained using a high-angle annular dark-field (HAADF) detector. (B) Energy dispersive X-ray spectroscopy (EDS) map of Ti species (orange) overlapped with the HAADF image (gray). (C) The EDS map of only the Ti species in this same region. (D) Thresholded, binarized image of the Ti containing particles used for the calculation of average particle sizes.

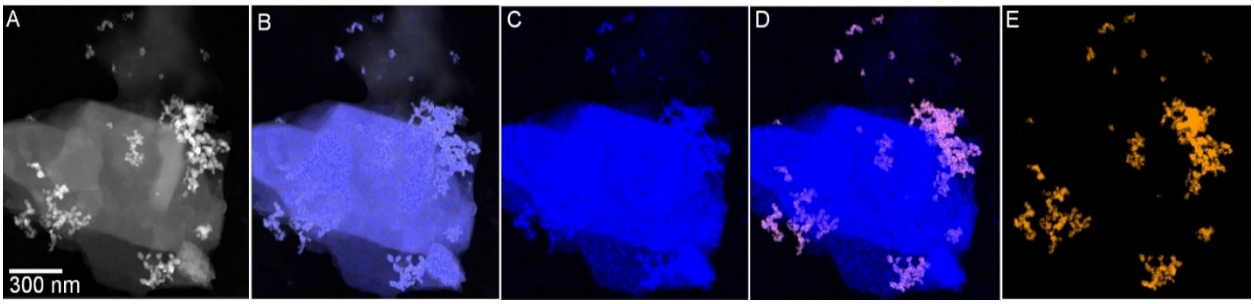

Figure S7. Representative micrographs of nanoscale TiO_2 extracted from the stems of *Dittrichia viscosa*. (A) A STEM image of the isolated particles obtained using a HAADF detector. (B) An EDS map of the O containing species (blue) overlapped with the HAADF image (gray). (C) The EDS map of only the O species in this same region. (D) The EDS map of the O species overlapped with the Ti species (pink). (E) The EDS map of just the Ti species (orange) in the same region.

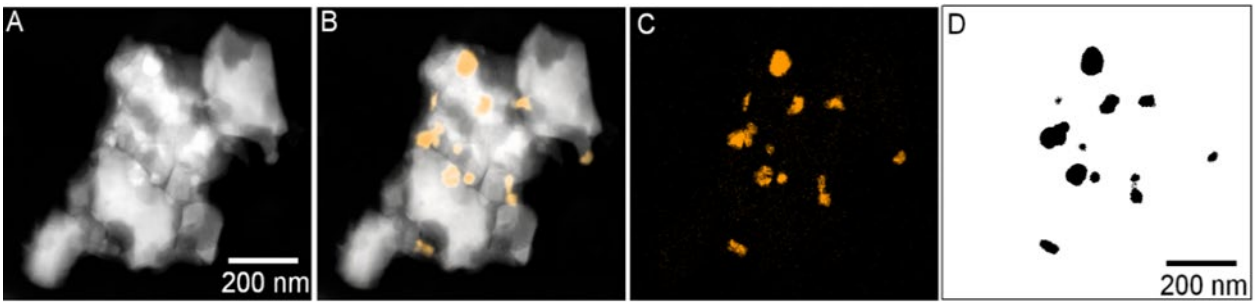

Figure S8. Representative micrographs of nanoscale TiO_2 extracted from the roots of *Dittrichia viscosa*. (A) A STEM image of the isolated particles obtained using a HAADF detector. (B) The EDS map of the Ti species (orange) overlapped with the HAADF image (gray). (C) An EDS map of only the Ti species. (D) A thresholded, binarized image of the Ti containing particles used for the calculation of average particle sizes.

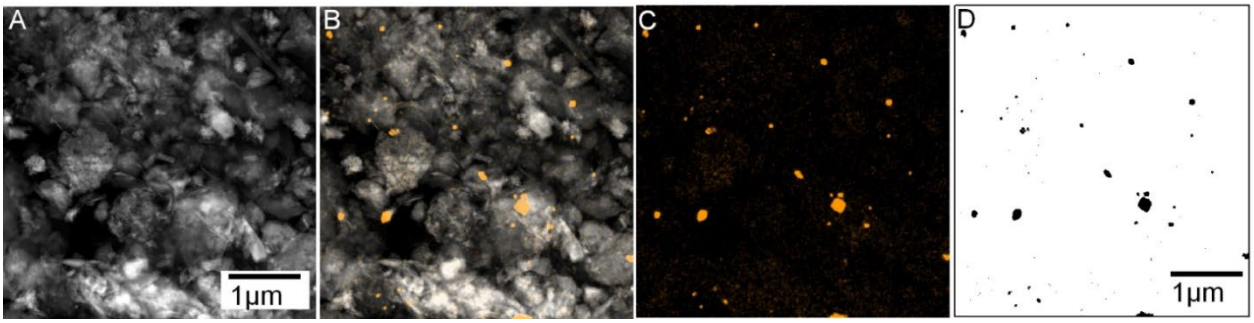

Figure S9. Representative micrographs of nanoscale TiO_2 extracted from the rhizosphere of *Dittrichia viscosa*. (A) A STEM image of the isolated particles obtained using a HAADF detector. (B) The EDS map of the Ti species (orange) overlapped with the HAADF image (gray). (C) An EDS map of only the Ti species. (D) A thresholded, binarized image of the Ti containing particles used for the calculation of average particle sizes.

Abbreviations

DLS dynamic light scattering

EDS energy dispersive X-ray spectroscopy

HAADF high-angle annular dark-field

SEM scanning electron microscopy

STEM scanning transmission electron microscopy

TEM transmission electron microscopy

TiO_2 titanium dioxide

References

(1) Lenaghan, S.C.; Zhu, Q.; Xia L.; Zhang, M. Extraction of organic nanoparticles from plants. In *nanoparticles in biology and medicine, methods and protocols, methods (molecular biology)*; Soloviev, M., Ed.; Humana Press, Totowa, NJ: 2012; 381–391.