

## Production of green nano zero-valent iron (G-nZVI) particles using polyphenol extracts of Tawa-tawa (*Euphorbia hirta linn*) leaves and green tea (*Camelia sinensis*) leaves

Anabella Vilando<sup>1\*</sup>, Erison Roque<sup>1</sup>, Maria Aira Benitez<sup>1</sup>, Jonathan Rey Diva<sup>1</sup>, and Jhenea Ellice Ragindin<sup>1</sup>

<sup>1</sup>Department of Chemical Engineering, College of Engineering, Adamson University, 900 San Marcelino St., Ermita, Manila 1004, Philippines

**Abstract.** Green synthesis of nano zero-valent iron (nZVI) can be used for environmental remediation. In this study, nZVI was produced from the extracts of Tawa-tawa (*Euphorbia hirta*) and Green tea (*Camelia sinensis*) leaves using Fe<sup>2+</sup> and Fe<sup>3+</sup> precursors. Solid-liquid extraction was used to obtain the extract of oven-dried Tawa-tawa and Green tea leaves with 60% v/v ethanol at a fixed solid-liquid ratio (1:20) and varying time (30, 60, 90, 120, and 150 min). Total phenolic content (TPC) of the extracts was determined by Follin Ciocalteu method. The extraction time of 60 min yielded the highest average TPC values of 7.26 ± 0.01 mg GAE/ g of Tawa-tawa dried sample and 5.23 ± 0.13 mg GAE/g of Green tea dried sample. To produce 0.1 gram of nZVI, 12 mL and 6 mL tawa-tawa extract were needed for Fe<sup>2+</sup> and Fe<sup>3+</sup>, respectively. For green tea, 24 mL and 20 mL extracts were needed for Fe<sup>2+</sup> and Fe<sup>3+</sup> to obtain the same amount of particles. G-nZVI has a particle diameter ranging from 50 nm to 130 nm, and mainly composed of iron and oxygen. Synthesized nZVIs from Tawa-tawa and Green tea reduced the nitrates (as nitrogen) concentration from 99.35 mg-L<sup>-1</sup> to 42.32 mg-L<sup>-1</sup> and 48.77 mg-L<sup>-1</sup>, respectively.

### 1 Introduction

Nanotechnology is one of the most active areas of research in modern material science. New applications of nanoparticles (NPs) and nanomaterials especially the ones from green synthesis are emerging rapidly. Green synthesis provides an advancement over chemical and physical method as it is cost effective, environment friendly, and easily scaled up for large-scale synthesis. One of the most considered methods in green synthesis is the production of metal NPs using biological systems such as microbes, fungi and several plant extracts. NPs produced by plants are more stable and the rate of synthesis is faster in the case of microorganisms [1].

The frequently used nanomaterials in remediation are zero-valent iron nanoparticles (nZVIs). Zero-valent iron started to be used in permeable reactive barriers (PRBs) in the beginning of the 1990s for the treatment of water, wastewater, groundwater, soil, sediments, and gaseous streams, mostly contaminated with chlorinated contaminants (trichloroethylene and/or perchloroethylene), but also with heavy metals (chromium or lead) or organochlorine pesticides [2-3]. Iron nanoparticle technology represents perhaps, one of the first generation nano-scale environmental technologies.

One of the reducing agents that can be used for the production of nano zero-valent iron is the polyphenolic compounds found in medicinal plants [4]. Among these plants known to have high polyphenolic compounds is the Tawa-tawa (*Euphorbia hirta linn*). Another plant known to have polyphenolic compound and is said to have an antioxidant, an antimicrobial, an anti-carcinogenic and anti-inflammatory properties is the Green Tea (*Camellia sinensis*) plant [5-6].

To utilize polyphenolic compound in the plant, many extraction methods are available. Among all existing methods, Solid-Liquid Extraction (SLE) technique is one of the easiest, most common, and most relevant techniques in the environmental extraction field [7-8].

In this paper, synthesizing zero-valent iron nanoparticles (nZVIs) that is capable of reducing nitrates using the polyphenolic extracts from the leaves of Tawa-tawa (*Euphorbia hirta linn*) leaves and Green tea (*Camelia sinensis*) leaves via solid-liquid extraction was being studied.

\* Corresponding author: [anabella.vilando@adamson.edu.ph](mailto:anabella.vilando@adamson.edu.ph)

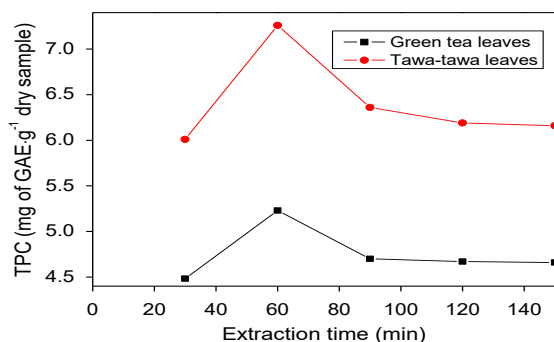
## 2 Materials and methods

Sun-dried Green tea (*Camelia sinensis*) leaves and fresh Tawa-tawa (*Euphorbia hirta linn*) leaves were used. For the extraction of polyphenol from plants, ethyl alcohol with an analytical grade of 60% (v/v) was used as a solvent. For the determination of the polyphenolic content of the plants, Follin-Ciocalteu method was followed. The following reagents were used: Follin-Ciocalteu reagent with anhydrous sodium carbonate and Gallic acid. Gallic acid was used to make the standard calibration curve. For the synthesis of G-nZVI,  $Fe^{2+}$  and  $Fe^{3+}$  iron precursors were used. Ferrous sulfate heptahydrate ( $FeSO_4 \cdot 7H_2O$ ) and ferric chloride hexahydrate ( $FeCl_3 \cdot 6H_2O$ ) for the extraction of polyphenols from the plants, magnetic stirrer was used to maintain the constant stirring of the solution. Solid particles were separated from the liquid after extraction through vacuum filtration with the support of a pump. A rotary evaporator was used to remove the solvent in the extracts. For the determination of total polyphenolic content with Follin Ciocalteu method, UV-VIS spectrophotometry was used. The synthesized G-nZVI was dried using freeze drier. A scanning Electron Microscope with Energy-Dispersive X-Ray (SEM-EDX) was used for the characterization of the particles, specifically, determination of the size particle and the elemental composition.

## 3. Results and discussion

### 3.1 Effect of extraction time on TPC

Values of time and total phenolic content (TPC) (mg of GAE per gram of dry sample) were graphically presented below. In Figure 1, the highest amount of polyphenol in both plant extracts was at 60-minute extraction time. Beyond 60 minutes, the TPC decreased because of the degradation of polyphenols due to prolonged exposure to light and heat [9-10]. Therefore, optimum time for extraction of polyphenol from both plants using ethanol as solvent was 60 minutes. Also, this showed that the TPC naturally found in the Tawa-tawa leaves is higher than the Green tea leaves.

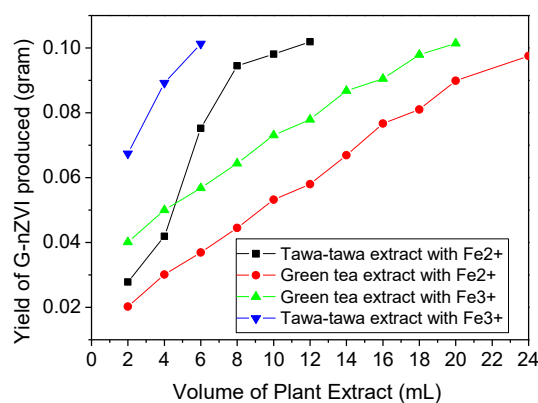


**Fig. 1.** Extraction time vs. total phenolic content of Tawa-tawa leaves and Green tea leaves

### 3.2 Effect of volume of plant extract on nZVI mass yield

**Fig. 2** showed the yield of the varying volume of different samples (plant extracts and iron precursor) in the production of G-nZVI. The volume needed to produce approximately 0.1 g G-nZVI from Tawa-tawa leaves with iron precursors ( $Fe^{2+}$  and  $Fe^{3+}$ ) were 12 and 6 mL, respectively. While, the volume needed to produce an approximately 0.1 g G-nZVI from green tea leaves with iron precursors ( $Fe^{2+}$  and  $Fe^{3+}$ ) were 24 mL and 20 mL, respectively.

Tawa-tawa extract was observed to have a smaller volume needed for both iron precursors than green tea extract to yield the same amount of particles. This implied that Tawa-tawa leaf extract has more effective reducing agent to both precursors than Green tea leaf extract. This was because Tawa-tawa extract has higher amount of TPC as compared with Green Tea leaf extract.



**Fig. 2.** Volume of extract vs mass yield of nZVI

### 3.3. Characterization of the synthesized nZVI

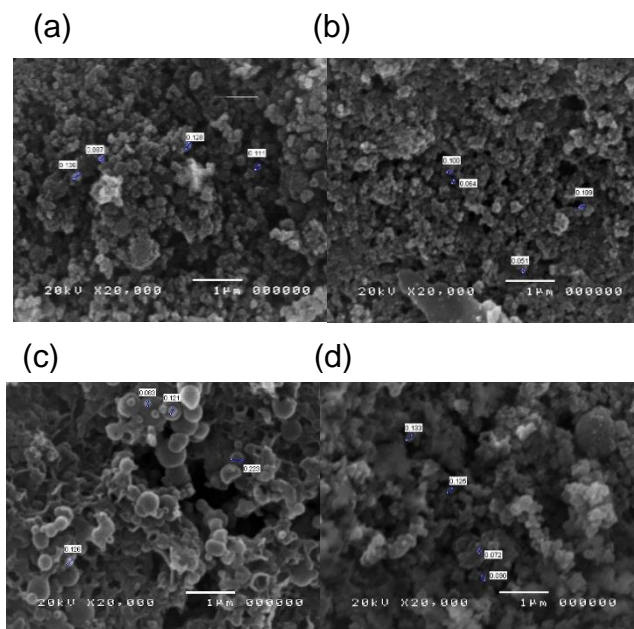
A scanning Electron Microscope (SEM) was used to determine the particle size of the G-nZVI. **Fig. 3** presented the morphology and the particle size of nZVI particles at 20,000 magnifications.

**Fig. 3** showed that the size of the synthesized nZVI particles using Tawa-tawa extract and both precursors were 87 nm, 111 nm, 128 nm, 51 nm, 64 nm, and 100 nm. For Green tea extract and both precursors, the particle sizes were 83 nm, 121 nm, 126 nm, 72 nm, 90 nm, and 125 nm. The basis for nanoparticles was within 10 – 100 nm in particle diameter. However, values not exceeding 200 nm were acceptable for nZVI particles [11]. Therefore, the synthesized zero-valent iron particles were classified as nanoparticles.

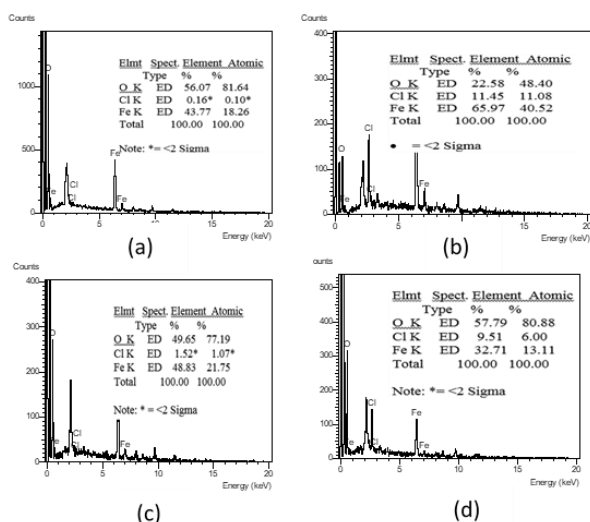
The elemental composition of G-nZVI particles was analyzed using Energy Dispersive X-Ray. This confirmed that the synthesized materials were composed of iron particles.

**Fig. 4** showed that the synthesized nZVI were composed mainly of iron and oxygen. The synthesized nZVI from two precursors and two plant extracts have iron contents of 48.83%, 32.71%, 43.77%, and 65.97%. These implied that the synthesized particles were

composed mainly of iron which comes from the iron precursors. The presence of oxygen, with values of 49.7%, 57.8%, 56.1%, and 22.6% came from the oxygen of the phenolic compounds of plant extracts. The presence of chlorine in the analysis B and D shown in Figure 4-d, with values 9.5% and 11.50%, came from ferric chloride heptahydrate, the iron precursor used for the samples.



**Fig. 3-a** is the SEM image of synthesized G-nZVI particles using Tawa-tawa leaf extract and  $Fe^{2+}$  (**Fig. 3-b**) synthesized G-nZVI particles using Tawa-tawa leaf extract and  $Fe^{3+}$  (**Fig. 3-c**) synthesized G-nZVI particles using Green Tea leaf extract and  $Fe^{2+}$  (**Fig. 3-d**) synthesized G-nZVI particles using Green Tea leaf extract and  $Fe^{3+}$ .



**Figure 4.** Energy dispersive X-ray (EDX) spectrophotometry and elemental composition of G-nZVI Particles

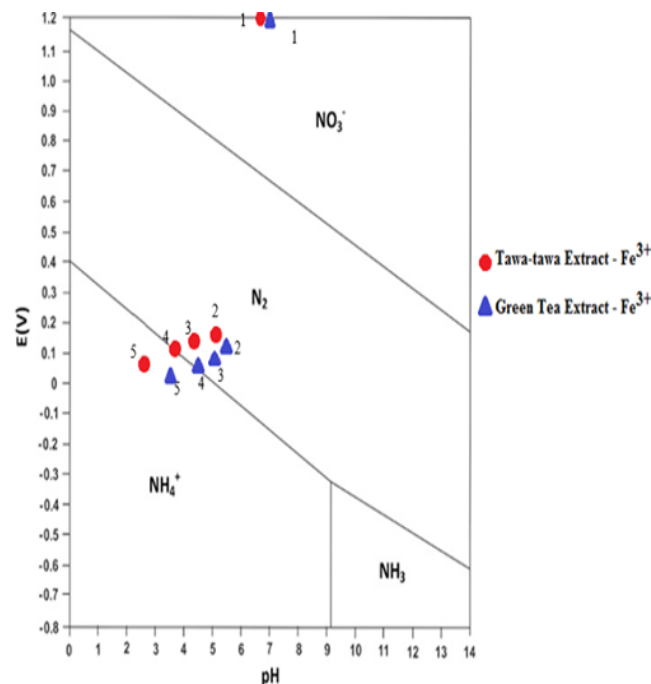
The reading of pH and ORP (oxidation-reduction potential) was done in 60 minutes with 15-minutes interval. The presence of nitrates, nitrites and ammonium were tested in the laboratory. Nitrogen has been computed through mass balance.

It has been observed that as the reaction time increases, the pH decreased from 6.7 to 2.6. Also, the ORP decreased

from 1.200V to 0.047 V. pH and ORP were plotted in Nitrogen Pourbaix Diagram (**Fig. 5**). During the reduction of nitrates in the solution, the complex polyphenol surrounding the iron nanoparticle reacted with hydroxides resulting in carbon dioxide and water. The carbon dioxide further reacted with water producing carbonic acid [12]. Thus, pH of the solution dropped as the nitrates reduction with G-nZVI continued.

The initial concentration of nitrates in solution was 440  $mg\cdot L^{-1}$ , which was equivalent to 99.4  $mg\cdot L^{-1}$  as  $NO_3^-N$ . It has been greatly reduced to 48.3  $mg\cdot L^{-1}$  as  $NO_3^-N$  after 15 minutes of reaction time. Then, a gradual reduction happened from 15 minutes to 60 minutes with a final nitrate concentration of 42.3  $mg\cdot L^{-1}$  as  $NO_3^-N$ . Nitrite (as nitrogen) concentration remained constant at an insignificant amount of less than 0.009  $mg\cdot L^{-1}$ . Small reading of ammonium concentration was obtained from the experiment. This indicated that most of the nitrates removed using synthesized G-nZVI particles were converted to nitrogen gas.

**Fig. 5** showed the data on nitrate reduction using the synthesized G-nZVI particles from both extract and  $Fe^{3+}$  precursor. There was an abrupt decrease of ORP from point 1 to point 2 (15 minutes) for both G-nZVI samples. Then, a gradual decrease followed. At the start, the data points of both G-nZVI samples were in the nitrates ( $NO_3^-$ ) region.



**Fig. 5.** Nitrates removal with pH and ORP

Then, data points 2 and 3 for both samples, lied within the nitrogen ( $N_2$ ) region. Fourth data points were along the boundary of nitrogen ( $N_2$ ) region and ammonium ( $NH_4^+$ ) region. Fifth data points were inside the ammonium ( $NH_4^+$ ) region, but still near nitrogen ( $N_2$ ) region.

Therefore, nitrates were mainly reduced to nitrogen due to the electron transferred from the synthesized G-

nZVI particles. During the reduction of nitrates in the solution, the complex polyphenol surrounding the iron nanoparticle reacted with hydroxides resulting in carbon dioxide and water. The carbon dioxide further reacted with water producing carbonic acid [12]. Thus, the pH of the solution dropped as the nitrate reduction with G-nZVI continued.

## Conclusion

Based on the experimental data obtained, the optimum extraction time using solid-liquid extraction performed at room temperature, with 60% ethanol as solvent and 1:20 solid-liquid ratio, was sixty (60) minutes for both Tawa-tawa (*Euphorbia hirta* linn) leaves and Green tea (*Camelia sinensis*).

The synthesis of 0.1 grams of nZVI for both plants with different iron precursors,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  and  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ , was successful: To produce 0.1 grams of nZVI particles, 12mL of Tawa-tawa leaf extract was needed for  $\text{Fe}^{2+}$  precursor. On the other hand, 24mL of Green tea leaf extract was needed to produce the same amount with the same precursor. The synthesized G-nZVI particles had a particle size within the range of 20 nm – 200 nm, and were mainly composed of iron and oxygen. Likewise, to produce 0.1 grams of nZVI particles, 6mL of Tawa-tawa extract was needed for  $\text{Fe}^{3+}$  precursor. Whereas, 20mL of Green tea leaf extract was needed to produce the same amount with the same precursor. The synthesized G-nZVI particles had a particle size within the range of 20 nm – 100 nm, and were mainly composed of iron and oxygen.

Also, the synthesized G-nZVI particles from Tawa-tawa leaves and Green tea leaves were capable of reducing nitrates to nitrogen.

From the conclusions drawn, the following recommendations were suggested. Further research on other polyphenolic-rich plants, extraction parameters and extraction techniques, especially modern extraction techniques, were highly recommended to achieve a better amount of TPC for the production of G-nZVI particles. And the effectivity of synthesized G-nZVI particles on contaminants such as lead, arsenic, selenium, mercury, chromium, etc, present in the actual waste water sample can be studied.

The researchers would like to acknowledge the chairperson and faculties of the Chemical Engineering Department of Adamson University for making this research possible.

## References

- [1] Iravani, S. Green synthesis of metal nanoparticles using plants. *Green Chem.* 13, 2011, 2638–2650.
- [2] Caliman, F.A., Robu, B.M., Smaranda, C. et al. Soil and groundwater cleanup: benefits and limits of emerging technologies. *Clean Technologies and Environmental Policy.* 13(2), 2011, 241-268.

- [3] Huang, W., Bai, Z., Hoefel D., Hu, Q, Lv, X, Zhuang, G., Xu, S., Qi, H., Zhang, H. Effects of cotton straw amendment on soil fertility and microbial communities. *Front. Envir. Sci. Eng.* 6 (3), 2012 336-349
- [4] Sandeep, T., Amit, P., Manisha, C., Kumar, J., Raj, M. Alteration in Thyroid Hormones and Vitamins as Early Makers of Aluminum Induced Neurodegeneration in Rats. Department of Biotechnology, NIET, NIMS University, Shobha Nagar, Jaipur-303121, India, 2009
- [5] De Mejia, Gonzalez., E., Chandra S. Polyphenolic compounds, antioxidant capacity, and quinone reductase activity of an aqueous extract of *Ardisia compressa* in comparison to mate (*Ilex paraguariensis*) and Green (*Camelia sinensis*) tea. *J. Agric. Food Chem.* 52, 2009, 3583-3589.
- [6] Pekal, A., Drozd, P., Pyrzynska, K. Comparison of the Antioxidant Properties of Commonly Consumed Commercial Teas, *International Journal of Food Properties*, 15(5), 2012, 1101-1109.
- [7] Salas, P., Morales-Soto, A., Segura-Carretero, A. Fernández-G. Phenolic-Compound-Extraction Systems for Fruit and Vegetable Samples. Department of Analytical Chemistry, University of Granada, E-18071 Granada, Spain, 2010.
- [8] De Castro, L., Gracia, G.L. Continuous subcritical water extraction of medicinal plant essential oil: comparison with conventional techniques. Analytical Chemistry Division, Faculty of Sciences, University of Córdoba, E-14004 Córdoba, Spain, 2000.
- [9] Howard, L.R., Xu, Z. Analysis of Antioxidant-Rich Phytochemicals. John Wiley & Sons. (2000):
- [10] Jokic, S., Cvjetko, M., Durdica, B., Fabek, S., Toth, N., Vorkapic´-Furac, J.V., Ivana Radojčić Redovnikovic. Optimisation of microwave-assisted extraction of phenolic compounds from broccoli and its antioxidant activity. *International Journal of Food Science and Technology.* Faculty of Food Technology, University J. J. Strossmayer of Osijek, Osijek, Croatia, 2012.
- [11] Labeeuw, V. 2013 In Situ Chemical Reduction using Zero-Valent Iron Injection – A Technique for the Remediation of Source Zones. Retrieved from [www.rwsenvironment.eu](http://www.rwsenvironment.eu)
- [12] Saif, S., Tahir, A., Chen, Y., Green Synthesis of Iron Nanoparticles and Their Environmental Application and Implications. *Nanomaterials.*