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Functional Iron Oxide Nanocrystals and its Application on the Removal of Arsenic in Origanum Vulgare Herbal Preparations

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Abstract

Origanum vulgare which is commonly known as oregano, is an herbal plant that is usually recommended as alternative medicine. Traditionally, it has been used to treat respiratory and gastrointestinal disorders and menstrual irregularities. Modern herbalists recommend topical application of oregano oil for the treatment of infection. Studies also showed that it has antiviral and antifungal properties. Moreover, oregano is also known as a culinary herb used for flavourings and fragrances.A study on the heavy metal contents of selected medicinal plants including Origanum vulgare was conducted and it was found out that the toxic element arsenic was present in the leaves, barks and roots ranging from 2.85 to 3.39 mg/kg, values which are beyond the permissible level of 1 ppm set by FAO/WHO. The herbal infusion, likewise contains 0.4 µg/L. Arsenic is a semi-metal element, naturally occurring in the earth's crust. It is a white, harmless-looking powder, resembling flour or sugar at a quick glance, is virtually undetectable in hot food and drink and fatal in small doses. Thus, the removal of arsenic in oregano is of great importance.This study was designed to prepare functional iron oxide nanocrystals and aimed to remove arsenic present in three different herbal preparations namely hot infusions, cold infusions and juice. The functional iron oxide nanocrystals were placed in the different herbal preparations namely hot infusion, cold infusion and juice and stirred for 5 minutes. The effect of different amounts of iron oxide nanocrystals was also investigated. The formation of bubbles on the surface of the nanocrystals was observed. The samples, before and after treatment, was analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES). Results of the analysis showed that the functional iron oxide nanocrystals were able to remove 89.45-100% of the arsenic present in hot infusion, 2.70-33.33% in cold infusion, and 29.33-88.22% in oregano juice. The removal of arsenic is through nanomagnetism and based on the results of this study, the higher the amount of the nanorust, the higher the concentration of arsenic removed from the solution. Furthermore, temperature has an effect on the nanomagnetic properties of the functional iron

oxide nanocrytals. Characterization of the nanocrystals before and after addition to the herbal preparation was done through scanning electron microscopy (SEM).
Keywords: Nanocrystal, Arsenic, Rust, Nanomagnet.

1. Introduction

Oregano scientifically named *Origanum vulgare* by Carolus Linnaeus, is a common species of *Origanum*, a genus of the mint family (Lamiaceae). It is native to warm-temperate western and southwestern Eurasia and the Mediterranean region. Oregano is a perennial herb, growing from 20–80 cm tall, with opposite leaves 1–4 cm long. It is characterized of having an aromatic, warm and bitter taste.

Oregano is an important culinary herb such as flavorings in pizzas, barbecues, fishes and meats, removes odors of carabao and buffalo meats, and found on table together with pepper and salt. Moreover, oregano is also known as a medicinal plant, having an antiseptic, antioxidant and antimicrobial properties. It has been used to cure disorders in gastrointestinal tract, respiratory tract and nervous system.

A study on the heavy metal contents of selected medicinal plants including *Origanum vulgare* was conducted and it was found out that the toxic element arsenic was present in the leaves, barks and roots ranging from 2.85 to 3.39 mg/kg, values which are beyond the permissible level of 1 ppm set by FAO/WHO. The herbal infusion, likewise contains 0.4 µg/L. (Arpadjan S. et.al., 2008). Arsenic is a semi-metal element, naturally occurring in the earth's crust. It is a natural element found in water, air and soil. It is a white, harmless-looking powder, resembling flour or sugar at a quick glance, is virtually undetectable in hot food and drink and fatal in small doses. Inorganic arsenic which are commonly found in pesticides and inseticides poses cancer and digestive health risks. Thus, the removal of arsenic in oregano is of great importance.

1.1. Objectives of the Study

This study aimed to the evaluate the efficiency of functional iron oxide nanocrystals in the removal of arsenic in *Origanum vulgare* herbal preparations.

This study specifically aimed to:

1. prepare functional iron oxide nanocrystals using rusts and prepared soaps;
2. remove arsenic present in three different herbal preparations namely hot infusions, cold infusions and juice.
3. determine the effect of different amounts of functional iron oxide nanocrystals on the removal of arsenic in the herbal samples; and;
4. characterize the functional iron oxide nanocrystals before and after it is applied to the herbal samples using Scanning Electron Microscopy (SEM)

This study focuses mainly on the preparation of functional iron oxide and its effect on the removal of arsenic in *Origanum vulgare* herbal preparations. Saponification followed by the separation of the fatty acid mixture from the soap was also done.

The functional iron oxide was produced from the fatty acid mixture that was separated from the prepared soap and rusts. Physical characterization of the nanocrystals was determined by the use of a scanning electron microscopy.

Applications and evaluation on arsenic levels in *Origanum vulgare* herbal preparations were conducted. The functional iron oxide nanocrystals were placed in the different herbal preparations namely hot infusion, cold infusion and juice. The effect of different amounts of iron oxide nanocrystals was also investigated. The arsenic removal was only to be limited on the determination of amounts arsenic at a specified mass of functional iron oxide nanocrystals compared to the specified amounts of arsenic present in the untreated samples (blank).

2. Methodology

The synthesis of the functional iron oxide nanocrystals was divided into three parts: saponification, extraction of oleic acid from the soap, and preparation of nanocrystals from rust and fatty acids.

2.1. Saponification

In a container, 200 mL of the liquid oil was measured. In another container 30 grams of NaOH was dissolved in 100mL water. The sodium hydroxide solution was poured into the liquid oil and stirred for about 15 minutes. The mixture was set aside in an open air in a ventilated area to dry and cure for a few days.

2.2. Extraction of the Fatty Acid

The soap was grated and mixed with 300 mL of 5% acetic acid. It was heated with occasional stirring until the soaps were completely dissolved. The mixture was set aside to cool. There were two layers observed, the upper layer which is the fatty acid and the lower layer which is the inorganic layer. Transfer the mixture in a separatory funnel to complete the separation of the liquids. Discard the lower layer and collect the upper layer. This is the fatty acid mixture.

2.3. Preparation of the Functional Iron Oxide Nanocrystals

The fatty acid mixture was placed in an evaporating dish and heated on med-high flame with occasional stirring. While heating, 5 grams of rusts were mixed with the fatty acid mixture. The container was covered with a loose cap for proper ventilation. There were heavy smokes and steams produced as the reaction occurs. The mixture was evaporated up to dryness.

2.4. Origanum vulgare Herbal Preparations

Hot Infusions. 100 g of oregano leaves were washed thoroughly in running water and cut into small pieces and placed in 200 mL distilled water. The mixture was boiled for thirty minutes with constant stirring. The mixture was filtered and the filtrate was divided into four and labeled as T1,A, T1,B, T1,C and T1,D.

Cold Infusions. 100 g of oregano leaves were washed thoroughly in running water. It was cut into small pieces and placed in 200 mL cold distilled water. The mixture was set aside for thirty minutes with constant stirring The mixture was filtered and the filtrate was divided into four and labeled as T2,A, T2,B, T2,C and T2,D.

Juice. 100 g of oregano leaves were washed thoroughly in running water. It was cut into small pieces and extract the juice by pressing and squeezing. 200 mL of water was added into the juice followed by stirring. Filter the mixture and divide the filtrate into four and labeled as T3,A, T3,B, T3,C and T3,D.

2.5. Treatment Descriptions

Treatment 1, A = *Origanum vulgare* Hot Infusion
Treatment 1, B = *Origanum vulgare* Hot Infusion + 0.050g Functional Iron Oxide Nanocrystals
Treatment 1, C = *Origanum vulgare* Hot Infusion + 0.10g Functional Iron Oxide Nanocrystals
Treatment 1, D = *Origanum vulgare* Hot Infusion + 0.50g Functional Iron Oxide Nanocrystals

Treatment 2, A = *Origanum vulgare* Cold Infusion
Treatment 2, B = *Origanum vulgare* Cold Infusion + 0.05g Functional Iron Oxide Nanocrystals
Treatment 2, C = *Origanum vulgare* Cold Infusion + 0.10g Functional Iron Oxide Nanocrystals
Treatment 2, D = *Origanum vulgare* Cold Infusion + 0.5g Functional Iron Oxide Nanocrystals

Treatment 3, A = Origanum vulgare juice
Treatment 3, B = Origanum vulgare juice + 0.050g Functional Iron Oxide Nanocrystals
Treatment 3, C = Origanum vulgare juice + 0.10g Functional Iron Oxide Nanocrystals
Treatment 3, D = Origanum vulgare juice + 0.5g Functional Iron Oxide Nanocrystals

2.6. Treatment Applications

Treatments 1, 2 and 3 were composed of the three methods of medicinal preparations namely hot infusion, cold infusion and juice. In each method of preparation, the effect of the functional iron oxide is added with varying amounts, 0.05 g, 0.10g and 0.5g. Treatments n,1 served as the control with no functional iron oxide nanocrystals added. The mixtures were set aside for 30 minutes. Afterwards, the nanocrystals were removed through filtration. The *Origanum vulgare* samples were then subjected to arsenic determination using inductively coupled plasma optical emission spectrometry (ICP-OES). The separated nanocrystals were set aside for characterization. Each treatment was replicated thrice.

2.7. Characterization of the Nanocrystals

The functional iron oxide nanocrystals before and after treatment were characterized using the Scanning Electron Microscopy (SEM) at the De La Salle University, Manila.

3. RESULTS AND DISCUSSIONS

3.1. The Nanorust and Origanum vulgare Samples

The prepared functional iron oxide nanocrystals were shiny black and in lumps. The nanocrystals were attracted to magnet. Moreover, when it was placed in *Origanum vulgare* samples, bubbles were formed on the surface of the lumps. These bubbles could be attributed to the magnetization of the nanorust to the heavy metal, arsenic.

Qualitatively, before the application of the nanorust, the *Origanum vulgare* solutions were turbid but after treating the solution with nanorust, the solutions became clearer.

3.2. Effect of Nanorust in the levels of arsenic in Oreganum vulgare

Table 1 shows the arsenic concentrations which are naturally present in *Origanum vulgare* solutions. It also shows the effect of the functional iron oxide at different \methods of preparation.

Table-1. Mean initial concentrations of arsenic at different methods of *Origanum vulgare* preparations.

Treatments	Method of Preparation	Concentration of Arsenic (ppb)
T1	Hot infusion	622.60 ± 12.63
T2	Cold Infusion	209.80 ± 7.75
T3	Juice (Squeezing)	209.70 ± 4.87

Table 1 shows that the initial concentration of arsenic varies at different method of preparation used. It is revealed that the *Origanum vulgare* obtained from hot infusion (T1), registered the highest concentration of arsenic (622.60 ppm). It is followed by cold infusion (T2) with 209.80 ppm and the juice with 209.70 ppm. It reveals that heating could enhance the extraction of arsenic which is naturally occurring in the plant. In cold infusion, smaller amounts of arsenic were extracted, 33% lower than that of hot infusion. Generally, solubility of inorganic solutes increases as the temperature increases because of the increased kinetic energy of the molecules. Meanwhile, extracting the juice by squeezing resulted into a concentration of arsenic similar to that of the cold infusion.

Table 2 shows the concentration of arsenic at different methods of herbal preparation and at different amounts of iron oxide. In hot infusion, though it is registered to have the highest concentration of arsenic, it also registered to have the easiest way to remove to impurity. It can be noted that using 0.05g of the functional iron oxide could remove 89.45 – 90.85% of the arsenic while using 0.10g and 0.50g of the functional iron oxide could remove 100% of the impurities.

Table-2. Percentage removal of arsenic at different method of medicinal preparations and varying amounts of functional iron oxide nanocrystals.

Treatments	Concentration of Arsenic (ppb)	Percentage Removal of Arsenic
Hot Infusion		
T1,A	622.60 ± 12.63	
T1,B	61.33 ± 4.34	89.45 - 90.85
T1,C	-0.89 ± 2.62	99.58 - 100
T1,D	-0.15 ± 0.54	99.99 - 100
Cold Infusion		
T2,A	209.80 ± 7.75	
T2,B	204.20 ± 18.28	2.70 - 11.39
T2,C	155.90 ± 16.03	25.69 - 33.33
T2,D	151.10 ± 4.52	27.98 - 30.00
Juice (Squeezing)		
T3,A	209.70 ± 4.87	
T3,B	141.40 ± 5.34	32.57 - 35.12
T3,C	148.20 ± 11.90	29.33 - 35.00
T3,D	28.76 ± 4.04	86.28 - 88.22

A – control B – with 0.05g nanocrystals
C - with 0.10g nanocrystals D – with 0.50g nanocrystals

In cold infusion, percentage ranging from 2.70 – 11.39% of arsenic is removed by using 0.05g of the functional iron oxide. Comparing the percentage removal of arsenic using 0.10g and 0.50g of the functional iron oxide, 0.10g of the functional iron oxide can remove higher percentage (25.69 – 33.33%) than the 0.50g of functional iron oxide which only removed 27.98 – 30.00% of the arsenic.

In the juice obtained by squeezing, it can be noted that using the highest amount of functional iron oxide (0.50g) could remove 86.28 - 88.22% of the arsenic.

Results could be attributed to the differences of temperatures on the methods of preparations. The hot infusion having the solution with highest temperature, while the cold infusion having the lowest temperature among the solutions. The removal of arsenic by the functional iron oxide in the herbal preparations can be attributed to nanomagnetism. Nanomagnetism deals with the magnetic properties of objects that have at least one dimension in the nanoscopic range from 1 to 100 nm (1 nm = 10^{-9} m).

4. Conclusions and Recommendations

Based from the results of the study, it is concluded that functional iron oxide nanocrystals is effective in the removal of arsenic in herbal preparations especially the hot infusion and the juice. The amount of the nanocrystals can affect the percentage removal of arsenic, in hot infusion 0.10 g can remove 100% of As, in the juice 0.50 g can remove up to 88.22%. In cold infusion, a higher amount of the nanocrystals is needed to remove As. It is therefore recommended that further studies be done to remove arsenic in the herbal preparations of the different parts of *Origanum vulgare* plant. It is also noteworthy to study the other medicinal plants which are known to accumulate arsenic.

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References

Arpadjan S, Celik G, Taşkesen S, Güçer S. Arsenic. 2008. Arsenic, Cadmium and Lead in Medicinal Herbs and their Fractionation. Food Chem Toxicol. 2008 Aug; 46(8):2871-5. doi: 10.1016/j.fct.2008.05.027. Epub 2008

Coles, T. 2013. Oil Of Oregano Benefits: 11 Things To Know About Oregano Oil. The Huffington Post Canada. http://www.huffingtonpost.ca/2013/11/14/oil-of-oregano-benefits_n_4274795.html

VanSant, M. 2013. Harvesting and preserving herbs. http://www.statesman.com/news/news/local/harvesting-and-preserving-herbs/ncGLb/

http://www.epa.gov/pesticides/reregistration/organic_arsenicals_fs.html

http://www.huffingtonpost.com/sandra-hempel-/arsenic-the-nearperfect-m_b_4398140.html_Arsenic -- The Near-Perfect Murder Weapon

http://en.wikipedia.org/wiki/ Oregano

http://www.oreganofromitaly.com/oreganouses.htm. The Many Uses of Oregano

http://www.medicalnewstoday.com/articles/266259.php What are the health benefits of oregano? (2013)

World Health Organization. http://www.who.int/mediacentre/factsheets/fs372/en/