

## Green synthesis of selenium nanoparticles using aloe leaf extract and evaluation of acute toxicity of materials

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### ABSTRACT

*The element selenium (Se) is of great importance in many fields, such as physics, chemistry, and biology. Selenium is also an important trace element, which has a great influence on biological systems due to its antioxidant, anticancer and antiviral activities. Selenium deficiency can lead to a number of serious diseases such as cancer, cardiovascular disease, immune disorders, or cause immunosuppression. Selenium nanoparticles are much more biologically active than other forms of selenium that exist in the form of inorganic salts or in organic compounds. In this paper, selenium nanoparticles are synthesized using a green method using aloe vera leaf extract which acts as a reducing agent and dispersion stabilizer. The properties of the post-synthetic materials analyzed by UV-vis spectroscopy, selected area diffraction (SAED), scanning electron microscopy (SEM), transmission electron microscopy (TEM), energy dispersive X-ray spectroscopy (EDX), X-ray photoelectron spectroscopy (XPS) showed that the synthesized selenium nanoparticles were in the range of 30 - 70 nm. The results of the assessment of acute toxicity in rats showed a lethal concentration (50% death of mice) of 10,374 mg/kg.*

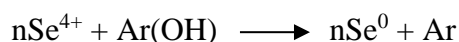
**Keywords:** Selenium nanoparticles; Aloe leaf extract; Acute toxicity.

### 1. INTRODUCTION

Selenium (Se) is an important trace element in the human diet and one of the most important essential trace minerals for all mammals. Selenium has a wide range of effects on biological systems, including antioxidant effects, cancer prevention, and antiviral activities [1]. Selenium deficiency can lead to some serious diseases such as cancer, cardiovascular and immune disorders or cause immunosuppression, while low-dose selenium supplementation can increase or restore immune functions [2-4]. The required selenium content in the nutritional diet of adults is 50 - 200 µg/day [5]. However, selenium can have many side effects if overdosed in the body and even fatal to humans. Therefore, the search for a suitable form of selenium to optimize the advantages of selenium use, while avoiding the side effects of overuse of this trace element becomes urgent [6]. Compared to selenium that exists in the ionic state, selenium nanoparticles (SeNPs) have higher bioavailability, higher bioactivity, and lower toxicity, the biological properties of selenium nanoparticles depend on their size: the smaller the size, the higher the activity [7, 8].

Currently, there are many methods for synthesizing selenium nanoparticles, including methods using chemical agents to deionize selenium, physical methods such as laser ablation, plasma in solution, biological methods using microorganisms that play a role in transforming the precursor forms of selenium to the zero-valent selenium state, etc [9-12]. In particular, the green synthesis method using plant extracts as a reducing agent is attracting the attention of scientists because of its environmental friendliness, low toxicity,

and relatively fast reaction time compared to methods using microorganisms. In plant extracts contain enzyme cofactors, flavonoids, proteins and terpenoids to make good and safe reducing agents and stabilizers. Secondary substances such as polyphenols and flavonoids are able to reduce metal ions, including Se to 0 valence according to the reaction equation as follows [13]:



Where Ar(OH) is a polyphenol or flavonoid.

Aloe vera, also known as aloe vera in the Aloeaceae family, is commonly grown in Vietnam and has many applications in pharmaceuticals, food and cosmetics thanks to its many bioactive ingredients that are anti-inflammatory, anti-stretch marking, anti-ultraviolet rays as well as promote wound healing [14]. Aloe vera contains a large amount of bioactive compounds, such as flavonoids, terpenoids, lectins, fatty acids, anthraquinones, and polysaccharides, which are chemical compounds that can help deionize selenium ( $\text{Se}^{+4}$ ,  $\text{Se}^{+6}$ ) to 0 valence ( $\text{Se}^0$ ) [15-17].

In this study, the results of the synthesis of selenium nanoparticles by aloe vera leaf extract are presented, the properties of selenium nanoparticles are analyzed and evaluated by UV-vis spectroscopy, selected area diffraction (SAED), scanning electron microscopy (SEM), transmission electron microscopy (TEM), energy dispersive X-ray spectroscopy (EDX), X-ray photoelectron spectroscopy (XPS), acute toxicity in mice via oral passage.

## **2. MATERIALS AND METHODS**

### *Materials*

Selen dioxide ( $\text{SeO}_2$ ) 99,9%, Sigma-Aldrich, Ethanol 99,7% Xilong China,  $\text{FeCl}_3$  0,02 M Sigma-Aldrich,  $\text{H}_3\text{PO}_4$  85% Xilong China, 0.9% NaCl solution Xilong China, Folin-Ciocalteu's phenol reagent Merck.

### *Synthesis of materials*

Aloe vera leaves from 8-10 months old collected in Tan Lac, Hoa Binh areas are washed, dried at 105 °C and then ground into a fine powder. Take 10 g of dry powder and put it in 200 ml of 96% ethanol at a temperature of 60 °C for 2 hours. The extract is then filtered, separated and analyzed for total polyphenol content. The polyphenols in the extract were determined by colorimetry, using the Folin-Ciocalteu reagent. This reagent contains an oxidizing agent which is phospho-vanframic acid, during the reduction process, hydroxy phenol groups are easily oxidized, this oxidizing substance produces a blue color with maximum absorption at the wavelength of 760 nm. This reaction is due to the blue formation of vonfram and molybdenum. The Folin-Ciocalteu reagent reacts with a variety of polyphenol compounds. The polyphenols will react with the reagent and form a solution that is spectral blue. From the color intensity of each experiment and the benchmark graph, we can infer the concentration of polyphenols present in the sample to be analyzed. The total polyphenol content in aloe vera leaf extract was 10,83 mg/ml.

$\text{H}_2\text{SeO}_3$  solution is prepared by mixing  $\text{SeO}_2$  in distilled water 2 times with a concentration of 10 mM. Aloe vera leaf extract after being diluted with distilled water in the ratio of 1:5 and put into a 3-neck spherical flask stirred from heating. The  $\text{H}_2\text{SeO}_3$  solution is slowly dripped into the aloe vera leaf extract, when it is completely dripped,

continue to stir for 1 hour at a temperature of 50 °C.

#### Characterization of SeNPs

The structural morphology of postsynthetic materials is determined by UV-vis spectrophotometer (Hitachi-UH4150, Japan), transmission electron microscopy (JEM2100, JEOL), a scanning electron microscope (SEM) from HITACHI S-4800 (Japan). The chemical composition was analyzed by X-ray energy scatterer (EDX, S-4800, Hitachi), X-ray photoelectron spectroscopy (XPS AXIS Supra<sup>+</sup>, Japan).

#### Acute Toxicity

Forty-two healthy BALB/c white rats, with a mass of about 19 - 21 g, regardless of breed, were kept in animal cages under standard temperature and light conditions, then they were divided into seven lots (six rats/lot) and completely starved for 16 hours before drinking the study sample. Selenium nanoparticles dispersed in 0.9% pathogen-free NaCl are administered orally at different doses. The animals were observed for survival and clinical signs of toxicity on the day of administration and then daily for 14 days. The cumulative mortality rate within 72 hours of treatment is used to calculate the average fatal dose (LD<sub>50</sub>).

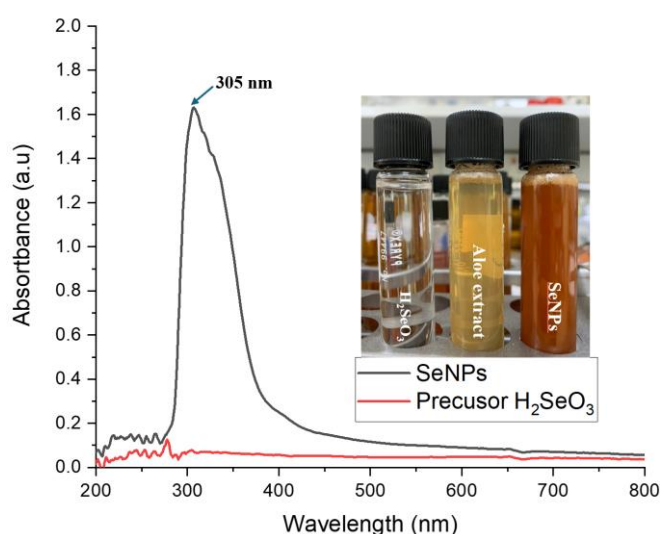
The determination of LD<sub>50</sub> was carried out according to the Karber Behrens method as follows:

$$LD_{50} = LD_{100} - (\Sigma a \times b) / N$$

- LD<sub>50</sub>: Dose of 50% death of animals
- LD<sub>100</sub>: The lowest dose causes 100% mortality in laboratory animals
- N: Number of animals in a group
- a: Dosage differences between two consecutive doses
- b: The average mortality rate of two consecutive groups

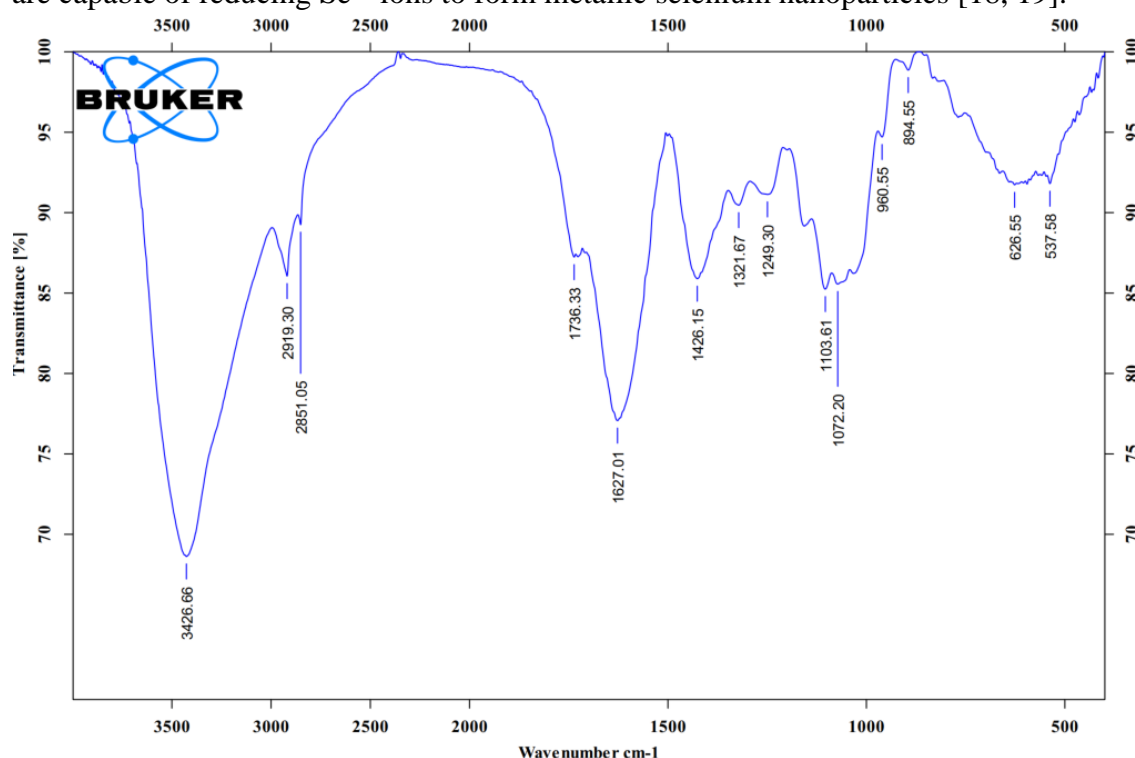
### 3. RESULTS AND DISCUSSION

#### Characterizations of materials



**Figure 1.** UV-vis absorption spectrum of selenium nanoparticle dispersion solution, and solution color change before and after the reaction.

The post-reaction solution is carried out with a spectral scan from the range from 190 - 1100 nm, the formation of selenium nanoparticles in the solution is determined by absorption peaks between 250 and 350 nm. Figure 1 shows the UV-Vis spectroscopy of the samples before and after the synthesis was pointed. It has been well-known in the literature that the UV-vis spectrum of  $\text{Se}^{4+}$  ions shows no absorption peak, indicating no surface plasmon vibration effect occurs in this state, the post-reaction solutions with a characteristic absorption peak at  $\lambda_{\text{max}} \sim 305$  nm, this absorption peak is given to oscillate the surface plasmon in the excited state of the selenium nanoparticle (figure 1). This result also corresponds to the color change of the pre- and post-reaction solution, the colorless  $\text{H}_2\text{SeO}_3$  precursor solution, the aloe vera leaf extract is yellow, and the post-reaction solution is red. This proves that the polyphenol compounds present in aloe vera leaf extract are capable of reducing  $\text{Se}^{4+}$  ions to form metallic selenium nanoparticles [18, 19].

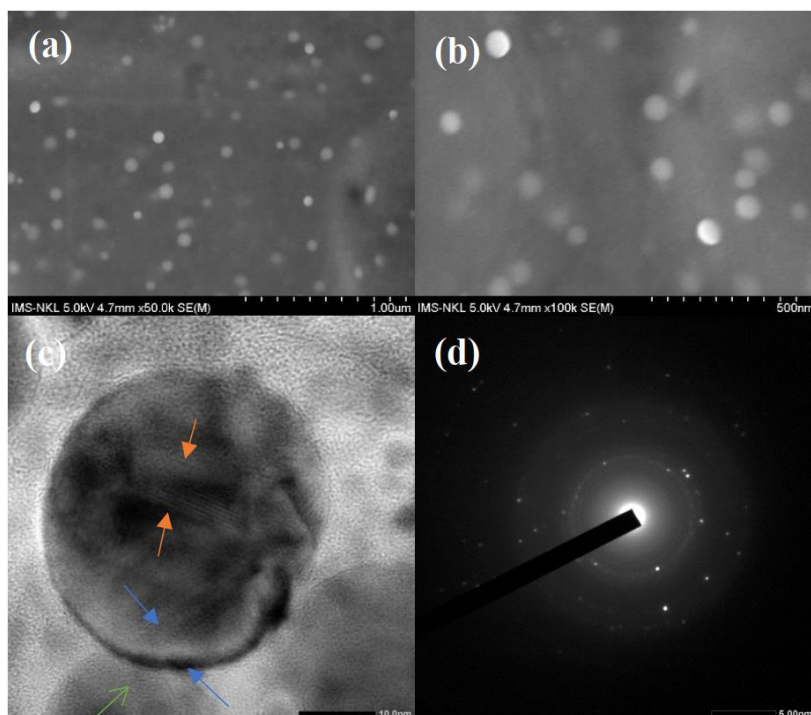


**Figure 2.** FTIR spectrum of aloe vera leaf extract.

FTIR spectroscopy was used to test for chemically bonded aloe vera leaf extract; the results are shown in figure 2. The O-H bonds of moisture, alcohol, and phenol groups in the extract are responsible for the wide absorption range in the FTIR spectrum at a peak of  $3427\text{ cm}^{-1}$ . The vibration detected at  $2919\text{ cm}^{-1}$  is thought to be caused by the alkynes of the C-H elongated bond. The FTIR spectrum also showed the C=O elongation group in the aldehyde and ketone groups at  $1736\text{ cm}^{-1}$ . Strong vibration peaks at  $1627\text{ cm}^{-1}$  and  $1426\text{ cm}^{-1}$  are attributed to the N-O asymmetric bond in nitro compounds and the C-C bond in the aromatic ring, respectively. The strong absorption peak at  $1321\text{ cm}^{-1}$  is attributed to the prolonged C-H in the alkane. Some of the vibrational bands at  $1103$  and  $1072\text{ cm}^{-1}$  are thought to be due to the C-N bending of the amines. In carboxylic acid, the O-H group is also visible in the  $960\text{ cm}^{-1}$  vibration band. This result shows that different

functional groups effectively cover the surface of the synthetic NPs, which is the cause of the subsequent NPs stabilizing and decreasing. It has been proven that the organic components of the extract act as a stabilizer for metal nanoparticles.

The surface morphology and structure of selenium nanoparticles synthesized by aloe vera leaf extract were observed by SEM and TEM images at different magnifications (figure 3). Figure 3a and figure 3b are SEM images of the post-synthetic material showing that selenium nanoparticles are spherical, fairly evenly distributed in the size range from 30-70 nm. The size and shape of this material are further observed by the TEM image shown in figure 3c, the TEM image shows that the selenium nanoparticles are covered with a thin film on the surface, the thin film is formed by the organic compounds present in the extract composition. In addition, the TEM image also observed the lattice faces of the selenium nanoparticles, which were also received by selected area diffraction (SAED) method, which showed that the selenium nanoparticles have a polycrystalline structure (figure 3d) with easily recognizable peaks (100), (101), (110), (102), (112), (202) (figure 3d) [20].

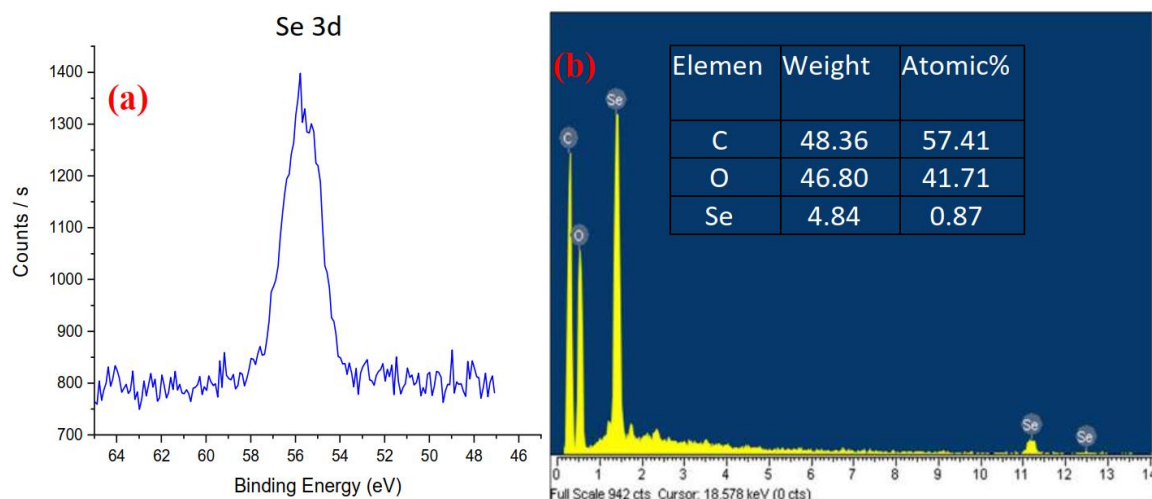


**Figure 3.** SEM (a), (b) and TEM (c) images of the SeNPs, SAED (d).

The chemical composition of the post-synthetic sample was analyzed by X-ray energy scattering spectroscopy (EDX). The presence of selenium nanoparticles has been determined by characteristic peaks of around 1.4 keV and 11.2 KeV attributed to the  $SeL_{\alpha}$  and  $SeK_{\beta}$  of metallic selenium (figure 4b). There are also high-intensity peaks in the presence of elements C and O, which appear to be of an organic coating on the surface of selenium nanoparticles, a coating that stabilizes particle size, and protects the particles from oxidation [21].

The results of X-ray photoelectron spectroscopy (XPS) clearly show the binding and elemental valence nature of selenium. The Se 3d peak is made up of two components: Se

$3d^{3/2}$  and  $Se\ 3d^{5/2}$  (figure 4a) at 56.07 and 55.25 eV, respectively. The typical  $3d\ Se^0$  peak of Se is found at 55.64, confirming that the valence state of Se in Se-NP is in the basal state because  $Se^{+4}$  is peaked at about 59.4 eV;  $Se^{+6}$  at about 61 eV;  $Se^{+2}$  at about 57.7 eV and  $Se^{-2}$  at a binding energy < 55 eV, which is completely absent from the spectrum.



**Figure 4.** XPS spectra (a), EDX spectrum (b) of the SeNPs.

#### Evaluation of acute toxicity of the material on mice

The dosage of test samples on the experimental batches is arranged:

Lot 1: Physiological control batch using distilled water, from batch 2 to batch 7 is given to drink with doses of 15, 25, 30, 35, 40, 45, 50 ml, respectively.

**Table 1.** External manifestations and number of dead rats.

No	Experimental lots	Number of mice died in 72 hours	External manifestations within 0-72 hours
1	Lot 1	0	Rats move and eat normally, good light and sound reflection
2	Lot 2	0/6	Rats move and eat normally, good light and sound reflection
3	Lot 3	1/6	Normal intake of food and water
4	Lot 4	2/6	Reduced ability to receive food
5	Lot 5	3/6	Reduced ability to receive food
6	Lot 6	4/6	Reduced ability to receive food
7	Lot 7	6/6	After taking the sample, the mice moved less and ate less. Died within 24 hours of ingestion

The results of the above experiment show that selenium at a concentration of 300 ppm can cause the death of mice in this experiment. The lowest dose to cause 100% mortality in laboratory animals was chosen to calculate  $LD_{50}$  at 50 ml/kg.

**Table 2.** Summarize the data obtained.

Experimental lots	Number of mice that died in 72 hours	N	a (g)	b	a × b (g)
Lot 1	0	6	0	-	-
Lot 2	0	6	0	-	-
Lot 3	1	6	10	0,5	5,0
Lot 4	2	6	5	1,5	7,5
Lot 5	3	6	5	2,5	12,5
Lot 6	4	6	5	3,5	17,5
Lot 7	6	6	10	5	50,0
$\Sigma a \times b$					92,5
$LD_{100}$ (g)					50

The LD<sub>50</sub> values of the selenium nanoparticle dispersion sample at a concentration of 300 ppm are:

$$LD_{50} = LD_{100} - (\Sigma a \times b) / N$$

$$LD_{50} = 50 - 92,5 / 6$$

$$LD_{50} = 50 - 15,42$$

$$LD_{50} = 34,58 \text{ (ml/kg)}$$

The lethal dose of 50% of the experimental animals of the selenium sample at a concentration of 300 ppm is about 34.58 ml/kg, equivalent to 10,374 mg/kg body weight. Compared with the lethal dose of SeO<sub>2</sub> of 2,5 g/kg, it can be concluded that the selenium nanoparticles synthesized by aloe vera leaf extract have less solubility, safer chemical form, resulting in a reduction in acute toxicity compared to the selenium ion form [22].

#### 4. CONCLUSIONS

This study successfully synthesized selenium nanoparticle dispersion solution by green synthesis method using aloe vera leaf extract as a reducing agent. Synthesized selenium nanoparticles are in the 30-70 nm range with polycrystalline structures. The results of the oral toxicity test on mice showed that the lethal dose of 50% of the experimental animals of the selenium sample at a concentration of 300 ppm was about 34.58 ml/kg, equivalent to 10,374 mg/kg body weight.

#### REFERNECES

- [1]. Zhang JS, Gao XY, Zhang LD, Bao YP. "Biological effect of nano red elemental selenium". *Biofactors*;15(1): 27–38, (2001).
- [2]. Aribi M, Meziane W, Habi S, Boulatika Y, Marchandin H, Aymeric JL. "Macrophage bactericidal activities against staphylococcus sereus are enhanced In vivo by selenium supplementation in a dose- dependent manner". *PLoS One*; 10 (9): e0135515, (2015).
- [3]. Kumar S, Tomar MS, Acharya A. "Carboxylic group-induced synthesis and characterization of selenium nanoparticles and its anti-tumor potential on Dalton's lymphoma cells". *Colloids Surf B: Biointerfaces*; 126: 546-552, (2015).
- [4]. Kumar A, Prasad S. "Role of nano-selenium in health and environment". *J Biotechnol*; 325: 152-163, (2021).

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- [5]. Jing Gao et al. "Daily selenium intake in a moderate selenium deficiency area of Suzhou, China". Food Chemistry. Volume 126, Issue 3, Pages 1088-1093, (2011).
- [6]. C. Ramamurthy et al. "Green synthesis and characterization of selenium nanoparticles and its augmented cytotoxicity with doxorubicin on cancer cells". Bioprocess Biosyst. Eng. vol. 36, no. 8, pp. 1131-1139, (2013).
- [7]. Khanna P et al. "Selenium nanoparticles: a review on synthesis and biomedical applications". Mater Adv.; 3: 1415-1431, (2022).
- [8]. Wang H, Zhang J, Yu H. "Elemental selenium at nano size possesses lower toxicity without compromising the fundamental effect on seleno-enzymes: comparison with selenomethionine in mice". Free Radic Biol Med; 42(10): 1524-1533, (2007).
- [9]. Sieber F et al. "Elemental selenium generated by the photobleaching of seleno-merocyanine photosensitizers forms conjugates with serum macro-molecules that are toxic to tumor cells", Phosphorus Sulfur Silicon Relat. Elem.; 180: 647–657, (2005).
- [10]. Saini D, Fazil M, Ali MM, Baboota S, Ameeruzzafar A, Ali J. "Formulation, development and optimization of raloxifene-loaded chitosan nanoparticles for treatment of osteoporosis". Drug Deliv.; 22(6):823-836, (2015).
- [11]. Yanhua Huang, Qingli Chen, Hai Zeng, Chen Yang, Guan Wang, Li Zhou. "A Review of Selenium (Se) Nanoparticles: From Synthesis to Applications". Particle & Particle Systems Characterization Volume 40, Issue 11, (2023).
- [12]. Neha Bisht, Priyanka Phalswal and Pawan K. Khanna. "Selenium nanoparticles: a review on synthesis and biomedical applications". Materials Advances, 3, 1415–1431, (2022).
- [13]. Phuong Thi Mai Nguyen et al. "Green synthesis of selenium nanoparticles with augmented biological activity using *Smilax glabra* Roxb extract combined with electrochemical plasma". Nano-Structures & Nano-Objects. Volume 38, 101185, (2024).
- [14]. Marta Sánchez, Elena González-Burgos, Irene Iglesias, and M. Pilar Gómez-Serranillos. "Pharmacological Update Properties of Aloe Vera and its Major Active Constituents". Molecules; 25(6): 1324, (2020).
- [15]. Borna Fardsadegh and Hoda Jafarizadeh-Malmiri. "Aloe vera leaf extract mediated green synthesis of selenium nanoparticles and assessment of their *In vitro* antimicrobial activity against spoilage fungi and pathogenic bacteria strains". Green Process Synth.; 8: 399-407, (2019).
- [16]. Mustafa H.N, Nnas S.mohammed. "Synthesis of Silver Nanoparticles by Using Aloe Vera and Bio Application". Journal of Nanostructures. Volume 13, Issue 1, Pages 59-65, (2023).
- [17]. Prashant J. Burange et al. "Synthesis of silver nanoparticles by using Aloe vera and *Thuja orientalis* leaves extract and their biological activity: a comprehensive review". Bull Natl Res Cent. 45, 181 (2021).
- [18]. José A. Hernández-Díaz et al. "Antibacterial Activity of Biosynthesized Selenium Nanoparticles Using Extracts of *Calendula officinalis* against Potentially Clinical Bacterial Strains". Molecules, 26, 5929, (2021).
- [19]. Nahid Shahabadi, Saba Zendehehshem, Fatemeh Khademi. "Selenium nanoparticles: Synthesis, *in-vitro* cytotoxicity, antioxidant activity and interaction studies with *ct-DNA* and *HSA*, *HHb* and *Cyt c* serum proteins". Biotechnology Reports. Volume 30, e00615, (2021).
- [20]. M. Salah, Nesreen A. S. Elkabbany & Abir M. Partila. Salah, M., Elkabbany, N.A.S. & Partila, A.M. "Evaluation of the cytotoxicity and antibacterial activity of nano-selenium prepared via gamma irradiation against cancer cell lines and bacterial species". Sci Rep. 14, 20523 (2024).
- [21]. Muhammad Aamir Ramzan Siddique et al. "Ascorbic acid-mediated selenium nanoparticles as potential antihyperuricemic, antioxidant, anticoagulant, and thrombolytic agents". Green Processing and Synthesis; 13: 20230158, (2024).
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- [22]. M. Shakibaie, A. R. Shahverdi, M. A. Faramarzi, G. R. Hassanzadeh, H. R. Rahimi, and O. Sabzevari. "Acute and subacute toxicity of novel biogenic selenium nanoparticles in mice". Pharm. Biol. vol. 51, no. 1, pp. 58-63, (2013).

### TÓM TẮT

#### **Tổng hợp xanh hạt nano selen bằng dịch chiết lá nha đam và đánh giá đặc độc tính cấp của vật liệu**

Nguyên tố selen (Se) có tầm quan trọng lớn trong nhiều lĩnh vực như vật lý, hóa học và sinh học. Selen cũng là nguyên tố vi lượng quan trọng, có ảnh hưởng nhiều đến các hệ thống sinh học do có hoạt tính chống oxy hoá, chống ung thư và kháng virus. Sự thiếu hụt selen có thể dẫn đến một số bệnh nghiêm trọng như ung thư, tim mạch, rối loạn miễn dịch hoặc gây ức chế miễn dịch. Hạt nano selen có hoạt tính sinh học cao hơn hẳn so với các dạng selen tồn tại ở dạng muối vô cơ hoặc trong các hợp chất hữu cơ. Trong bài báo này, các hạt nano selen được tổng hợp bằng phương pháp xanh sử dụng dịch chiết lá nha đam đóng vai trò là chất khử và chất ổn định hệ phân tán. Đặc trưng tính chất của vật liệu sau tổng hợp được phân tích các phương pháp quang phổ UV-vis, nhiễu xạ điện tử lựa chọn vùng (SAED), kính hiển vi điện tử truyền qua (TEM), phổ tán sắc năng lượng tia X (EDX) cho thấy hạt nano selen tổng hợp được có kích thước nằm trong dải 30 đến 70 nm. Kết quả đánh giá độc tính cấp trên chuột cho thấy liều gây chết 50% động vật thí nghiệm là 10,374 mg/kg.

**Từ khoá:** Hạt nano selen; Dịch chiết lá nha đam; Độc tính cấp.