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THE GREEN SYNTHESIS OF SILVER AND SELENIUM NANOPARTICLES USING THE PLANT *STELLARIA MEDIA* (L.) VILL

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ABSTRACT

Currently, the interest in the synthesis of metallic nanoparticles has increased a lot because these systems are suitable to be used in distinct fields such as medical, pharmaceutical, agricultural, environmental protection, etc. Their synthesis can be done using different agents such as bacteria, fungi, and yeasts or using plant extracts and fresh plants. This paper aims to synthesize metallic silver (AgNPs) and selenium (SeNPs) nanoparticles using the fresh plant *Stellaria media* (L.) Vill (SM). Also, the synthesis of the metal nanoparticles is demonstrated by using the spectrophotometric method, then being characterized in terms of their size and distribution by DLS. Thus, it was demonstrated that the obtained AgNPs are smaller than SeNPs with dimensions between 28 nm and 255 nm from which 81.56% presented a diameter lower than 100 nm. In this paper, the total content of polyphenols was determined by using the Folin-Ciocalteu method, and the DPPH and FRAP methods demonstrated the antioxidant capacity of the SM plant. The obtained results were correlated with other results from the specialized literature and thus it was demonstrated that the SM plant acted as a reducing agent having an essential role in reducing the metal from the two salts ($AgNO_3$ and Na_2SeO_3), thus taking place the synthesis of the metal nanoparticles. The antimicrobial effect of the SM plant extract has also been demonstrated, so in the future, we want to test *in vitro* the antimicrobial and repairing effect on the skin of the synthesized AgNPs and SeNPs.

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Introduction

Nanoparticles represent systems for transporting various molecules of interest that are used for formulation or are embedded. The formulation of these systems is of major interest due to their use in various distinct fields such as medical, pharmaceutical, and food [1-3].

The formulation of transport or encapsulation systems by classical methods such as those used to obtain liposomes or lipid nanoparticles has some disadvantages [4, 5]. For example, for the formulation of lipid vesicles by the method of hydration of the lipid film, there are used solvents such as methanol and dichloromethane which have toxic properties [6-8]. Also, the use of alginate or chitosan polymers has some disadvantages in the formulation, among which we can mention the difficulty of obtaining nanometric dimensions [9].

In the case of metal particle synthesis using a "green" method, these disadvantages no longer exist. The "green" synthesis of metal particles has as a basic principle the formation of nano or micro particles due to the reaction between phytochemical compounds present in the plant (compounds with antioxidant, antimicrobial, enzyme, and protein properties) and the metal used [10]. In the "green" synthesis, salts of different metals (Ag, Se, Au, Zn) can be used and the method is an eco-friendly one based on the direct reaction between the components mentioned above, while the presence of other elements not being necessary [11].

Therefore, the advantages of this method are multiple: no toxic solvents are used, the costs are low, the method is environmentally friendly, and it is a relatively simple method (performed in one step - the reduction reaction of the metal ion put in contact with reducing agent from the fresh plant or its extract, bacteria, enzymes or proteins) [12, 13].

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Until now, according to the research literature, the plant *Stellaria media* (L.) Vill. (SM) has been incorporated into distinct delivery systems such as liposomes and polymeric microcapsules using the sodium alginate polymer [6, 14].

The SM plant is of major interest due to its composition rich in polyphenols, antimicrobial activity, powerful antioxidant capacity, and due to the biological healing activity of dermal scars studied *in vitro* on normal human dermal fibroblasts by scratch method [15, 16].

The main objective of this study was to highlight the ability of fresh SM extract to biosynthesize Ag and Se nanoparticles and to identify the optimal conditions to obtain the smallest possible size of nanoparticles. To our knowledge, the SM plant has not been used for the synthesis of metal nanoparticles, this part of the work being thus the novelty element.

Also, another objective of the paper was to characterize the SM plant in terms of total polyphenols, antioxidant capacity, and antimicrobial activity on gram-positive and gram-negative bacteria. The experimental design of our study is shown in **Figure 1**.

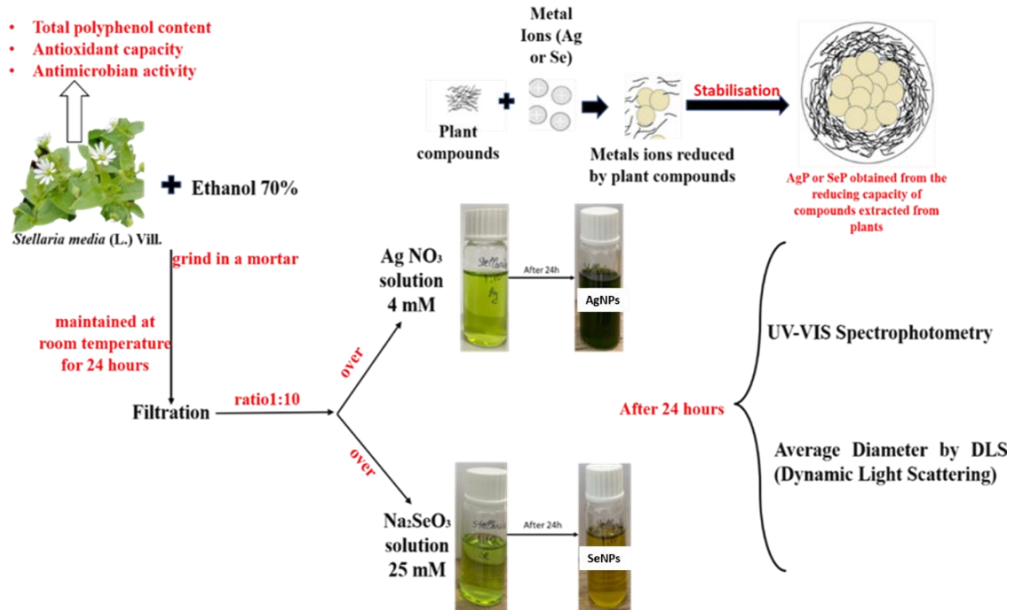


Figure 1. The experimental design consists of two steps. The first, the SM extract was characterized from the point of view of total phenols content, antioxidant capacity, and antimicrobial activity. In the second step, the extract was used for the green biosynthesis of metal nanoparticles (AgNPs and SeNPs). The metal nanoparticles were characterized by UV-Vis spectrophotometry and DLS.

Materials and Methods

Sodium Selenite (Sigma Aldrich), Silver Nitrate (Sigma Aldrich), Gallic Acid (Sigma Life Science), Folin-Ciocalteu Reagent (Merck), Sodium Carbonate (Ingen Laboratory), 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were purchased from Sigma Aldrich (St. Louis, MO, USA). All the chemical reagents were of analytical grades.

Preparation of Stellaria Media Extract

The SM plant was harvested on 15.03.2022 from Oradea, Bihor County, Romania. The temperature at harvest was 12 ºC. 525 g of the plant were harvested and dried at room temperature to constant weight. After drying, 50 g of the plant were crushed and 70% ethanol was added in a ratio of 1:20 (w / v). The mixture was kept in the dark under continuous stirring for 24 h, then filtered and ethanol was removed by using a rotary evaporator (Heidolph Rotary Evaporator, Laborota 4000) at 45 °C and 90 rpm. The SM extract was kept at -80 ° C overnight and then lyophilized (Christ Alpha 1–2 Ldplus lyophilizer). The SM powder was used for the determination of polyphenols (Folin-Ciocalteu method), antioxidant capacity (DPPH, FRAP), and antimicrobial properties. The aqueous stock solution of SM extract of concentration 1mg / mL was used. All analyzes were performed in triplicate.

Total Polyphenols Content of the SM Plant

The total polyphenols content of the SM plant extract was determined by applying the Folin-Ciocalteu method according to the literature [15, 17], as follows: 0.1 mL SM extract stock solution (1mg / mL) was mixed with 1.7 mL distilled water and 0.2 ml of the Folin-Ciocalteu reagent (freshly prepared). Then, 1 mL of 7.5% Na_2CO_3 solution was added, stirred by using a vortex, and kept in the dark for 2 hours. The absorbance was recorded by using the UV-VIS spectrophotometer Shimadzu MiniUV-VIS at a wavelength of 765 nm.

The results are expressed in milligrams of gallic acid equivalent (GAE)/ gram of dry vegetable product (dw).

Determination of the Antioxidant Capacity of SM Extract

DPPH Method

SM plant extract was tested for antioxidant capacity by using the DPPH method according to the authors Miere (Groza) *et al.* Briefly, 0.1 mL of SM extract (1 mg / mL) was homogenized with 2.8 mL of DPPH methanolic solution (80 pM). The sample was kept in the dark for 30 minutes and the absorbance was measured at 517 nm. The antioxidant capacity expressed as % DPPH radical scavenging was calculated according to equation (1) [14, 18].

$$\% \text{ Radical Scavenging Activity (RSA)} = [(A_0 - A_1) / A_0] \times 100 \quad (1)$$

where: A_0 = the absorbance of DPPH free radical solution in methanol
A_1 = the absorbance of the sample.

FRAP Method (Ferric Reducing Antioxidant Power)

The method is based on the reduction reaction of the Fe^{3+} ion from the tripyridyltriazine complex to Fe^{2+} under the influence of the compounds with the antioxidant capacity of the tested extract [14].

Briefly, 0,1 mL of SM extract from the stock solution is combined with 0,5 mL FRAP reagent and 2 mL distilled water. The sample is kept in the dark for 1 hour at room temperature, and the absorbance was read at a wavelength of 595 nm using a spectrophotometer [13]. The results are expressed in µmol Trolox equivalents (TE) /g dw.

Determination of the Antimicrobial Activity of SM Extract

To test the antimicrobial activity of the SM extract, plates with Mueller-Hinton type medium with a layer thickness of 80 mm were used. The test method used was diffusion [19]. The antimicrobial activity of the SM extract was tested on *Staphylococcus aureus* and *Escherichia coli*, the results being compared with those obtained for different antibiotics such as Doxycycline 30 µg, Gentamicin 10 µg, Nitrofurantoin 300 µg.

The SM extract was applied in concentrations of 10 µg / mL and 15 µg / mL, and after 24 hours at 37 ºC, the diameter of the inhibition zone was measured.

Synthesis of AgNPs and SeNPs by Using the Plant Stellaria Media (L.) Vill.

4 mM aqueous solution of silver nitrate ($AgNO_3$) and a 25 mM aqueous solution of sodium selenite (Na_2SeO_3) were used for the synthesis of AgNPs and SeNPs [20-24].

The SM plant was used in fresh form, weighing 10 g which is triturated with 70% ethanol in a ratio of 1:10 (w / v). This mixture was kept in the dark for 3 hours, then it was filtered and the obtained extract was added to the solution of 4 mM $AgNO_3$ and 25 mM Na_2SeO_3 respectively, in a ratio of 1:10 (v / v). After this stage, the samples were kept at room temperature, in the dark for 24 hours, and then were characterized in **Figure 1** [25].

Characterization of AgNPs and SeNPs

UV-VIS Spectroscopy

To highlight the synthesis of AgNPs and SeNPs, the samples were screened by UV-VIS spectroscopy. The Shimadzu MiniUV-VIS spectrophotometer was used to carefully evaluate the wavelength at 420 nm for AgNPs and 296 nm for SeNPs. The reading was performed after 24 hours and a dilution of 1:10 was performed [23].

Average Diameter by DLS (Dynamic Light Scattering)

The dynamic light scattering (DLS) method was applied to determine the diameter and distribution of the synthesized AgNPs and SeNPs using a Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK). Polystyrene cells with an optical path of 1 cm were used for diameter measurements which were done in triplicate [26].

Results and Discussion

According to the literature, the extract of the SM plant is rich in phenolic compounds, flavonoids, and vitamin C which are responsible for its remarkable antioxidant properties [6, 15].

Among the compounds from the SM plant extract with a strong antioxidant character, we can mention ferulic acid, caffeic acid, vanillic acid, chlorogenic acid, luteolin, apigenin, vincenin-2, genistein [6, 27].

Also, the content of total polyphenols was determined by other authors obtaining values between 17.23 ± 2.31 and 19.15 ± 1.13 mg GAE / g dw which are comparable to the values obtained in this study **(Table 1)**. The antioxidant capacity was also presented in the literature, being highlighted by different methods such as FRAP, TEAC, DPPH, and CUPRAC, the results being also comparable with those found in **Table 1** of this study [6, 14, 15].

Table 1. Total polyphenols content and the antioxidant capacity of the SM extract

Total polyphenols mg GAE/ g dw	DPPH (%)	FRAP µmol TE/g dw
18.02 ± 1.27	77.55 ± 1.67	24.59 ± 2.32

Oladeji *et al*. showed a lower antioxidant capacity (76%) than the result obtained in this study using the DPPH method. Miere *et al*., presented the values obtained by the FRAP method, being close to 24.59 ± 2.32 µmol TE / g dw, namely 27.76 ± 11.22 µmol TE / g dw [27].

Also, the antioxidant and anti-inflammatory capacity is strongly supported by the presence of vitamins and minerals such as: vitamin C, magnesium, iron, copper, and zinc and the presence of essential and non-essential amino acids (glycine, alanine, serine, proline, thiamine, etc.) [27-29].

Table 2 shows the results obtained in the case of testing the antimicrobial capacity of the SM plant extract.

Thus, it was shown that compared to the frequently used antibiotics (Doxycycline 30 µg, Gentamicin 10 µg, Nitrofurantoin 300 µg), the SM plant extracts applied in two concentrations of 10 µg / ml and 15 µg / ml showed inhibitory activity on gram-positive and gram-negative bacteria.

The results obtained on gram-positive bacteria (*S. aureus*) were better than those obtained on gram-negative bacteria (*E. coli*): the diameter of the inhibition zone was 12.10 ± 1.50 mm in the case of 10 µg SM extract/ml and 15.20 ± 1.84 mm in the case of 15 µg SM extract/ml on *S. aureus*, meanwhile on *E. coli* the results were 9.20 ± 1.23 mm and 11.40 ± 0.95 mm for SM extract with a concentration of 10 µg / ml and 15 µg / ml, respectively.

Table 2. The antimicrobial capacity of the SM plant extract compared to some antibiotics

Substances applied or SM extract	Inhibition zone diameter (mm)	
	S. aureus	***E. coli***
Doxycycline 30 µg	-	19.45±1.51
Gentamicin 10 µg	24.22±1.45	21.24±1.94
Nitrofurantoin 300 µg	23.50±1.80	22.11±1.74
SM 10 µg/ml	**12.10±1.50**	**9.20±1.23**
SM 15 µg/ml	**15.20±1.84**	**11.40±0.95**

The antimicrobial activity has been tested by other authors for both SM plant extract and other plant extracts from the same family (*Caryophyllaceae*) and the results showed the existence of their antimicrobial activity, especially on the bacteria tested in this paper (*S. aureus* and *E. coli*) [15, 16].

For this reason, the SM plant extract is suitable for use in dermatological treatments such as scratches, burns, or other lesions either as an extract or embedded in other vehicle systems [15, 30]. Also, the anti-inflammatory properties presented in the literature determine even more the future appreciation of this plant in the dermatological field [15].

Due to its phytochemical composition, the SM plant can act as a reducing agent on metals from various salts ($AgNO_3$ and Na_2SeO_3) with the formation over time of metal nanoparticles (AgNPS and SeNPs) **(Figure 2)**.

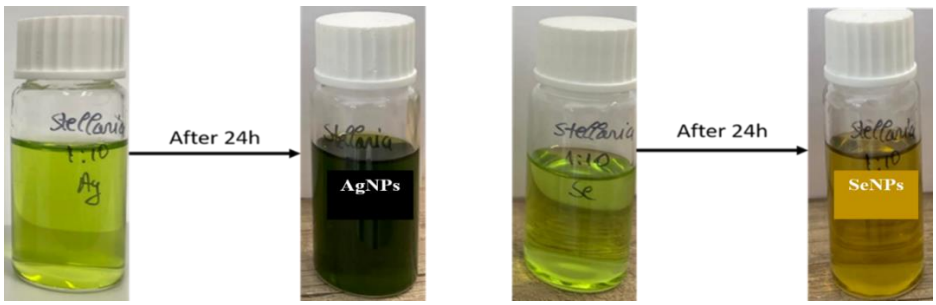
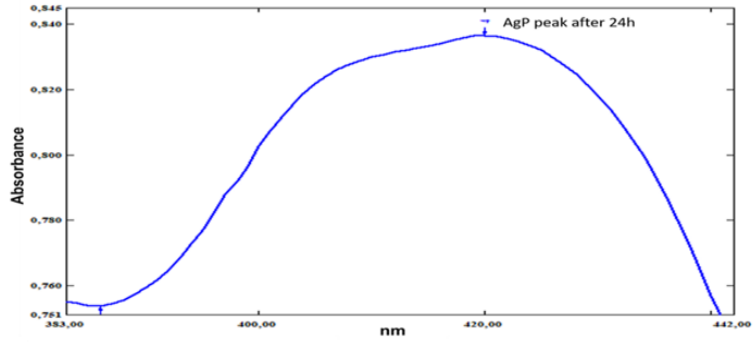


Figure 2. Synthesis of AgNPs and SeNPs after homogenization of the SM extract with the metal salts in a ratio of 1:10 (v / v) after 24 h.

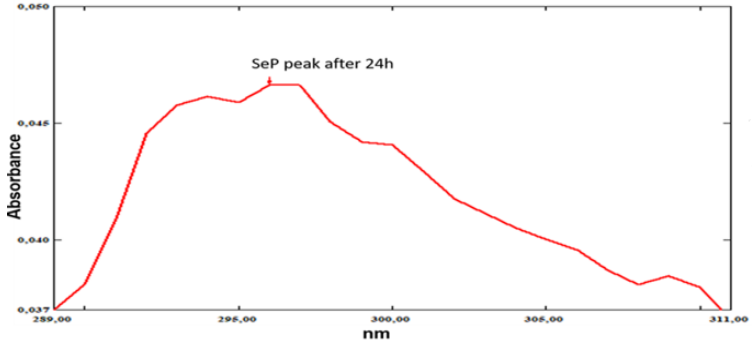
Figure 2 shows the change in color of the mixture containing the SM extract and the salt of the respective metals after 24h which is explained by the fact that the metal nanoparticles (AgNPs and SeNPs) were synthesized.

To demonstrate this, the spectrophotometric analysis of the samples was performed using the Shimadzu MiniUV-VIS spectrophotometer, carefully evaluating the absorbance at the wavelength of 420 nm for AgNPs and 296 nm for SeNPs [31, 32].

The analysis was performed on samples diluted 10 times with ethanol after 24 hours and the obtained results indicated the formation of silver metal nanoparticles (a) and selenium nanoparticles (b) **(Figure 3)**.



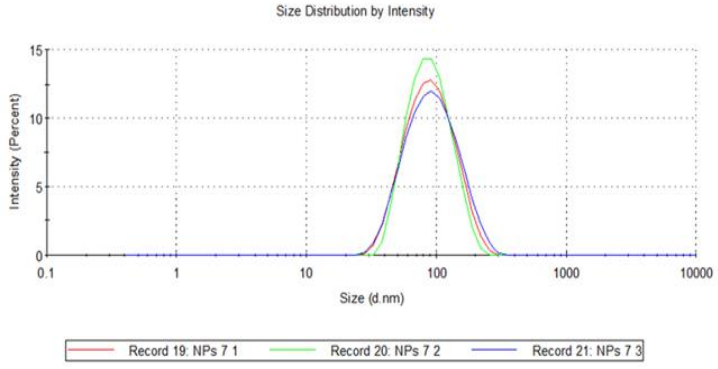
a)



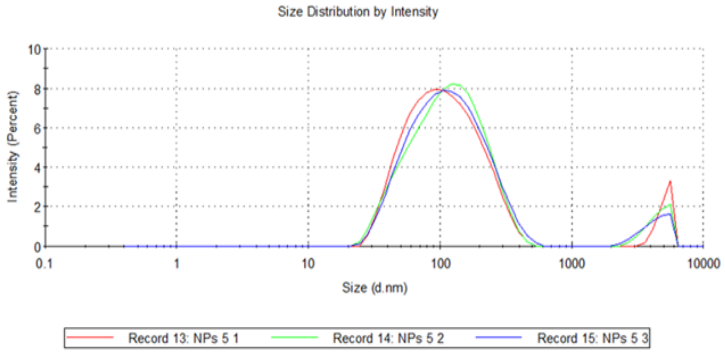
b)

Figure 3. The UV-Vis screening of SM samples after 24 hours of incubation between SM plant and inorganic salts. A. The results of the spectrophotometric screening of the samples after 24h demonstrate the existence of the absorbance at wavelengths characteristic for each metal nanoparticle separately: (a) - the presence of the pick at 420 demonstrates the formation of AgNPs, (b) - the presence of the pick at 296 nm demonstrates the formation of SeNPs.

To determine the size of the synthesized metal nanoparticles, DLS analysis was used, the results being highlighted in **Figures 4a** and **4b**.



a)



b)

Figure 4. Graphical representation of the size of AgNPa (a) and SeNPs (b) obtained by DLS analysis.

The size of AgNPs was between 28 nm and 255 nm with 81.56% of the total AgNPs being up to 100 nm in size **(Figure 4a)**, meanwhile, SeNPs size was between 24 nm and 342 nm with 67.75% of SeNPs having a diameter lower than 100 nm **(Figure 4b)**.

According to the authors Gour *et al.*, the size of the metal nanoparticles is greatly influenced by the reducing agent that acts on the metal involved in the reaction [33].

Thus, it is considered that the smallest sizes of metal particles are obtained when the reducing agent used is a fungus, yeast, or vitamins such as vitamin C or B_2 [33]. It can therefore be explained that a high contribution in the formation of silver or selenium nanoparticles has in this case the high content of the SM plant in vitamin C [27].

According to the literature, the size of metal particles obtained by using a plant extract (by green synthesis) is between 20 nm to 500 nm depending on the metal salt used, but also on the phytochemical composition of the plant [4].

It is also considered that the formation of silver or selenium nanoparticles by using plant extracts as a reducing agent leads to dimensions depending on the concentration of chlorophyll and phytochemicals with antioxidant capacity such as phenols [33].

The phenol-rich composition of the SM plant extract (18.02 ± 1.27 mg GAE / g dw) determines the synthesis of AgNPs and SeNPs because these phytocompounds are electron donors, thus participating in the reduction reaction of the metal (Ag or Se) and the nanoparticles formed are monolayer and very stable [34-36].

The bonds created between metal and plant polyphenols during the synthesis of metal nanoparticles are different depending on the metal used: Ag-polyphenols, Se-polyphenols, Zn-polyphenols, Au-polyphenols, etc., but in all these situations, they are stable at temperature and pH variations, being thus called flexible and stable structures [37].

Conclusion

According to the characterization of the SM plant extract, it has been shown that this plant has a high polyphenol content (18.02 ± 1.27 mg GAE / g dw) and a strong antioxidant capacity (77.55 ± 1.67 % and 24.59 ± 2.32 µmol TE / g dw).

Also, antimicrobial activity on gram-positive and gram-negative bacteria such as *S. aureus* and *E. coli* has been shown to increase the diameter of the dose-dependent inhibition. Besides all these properties, the SM plant proved to be a good reducing agent on metals from salts such as $AgNO_3$ and Na_2SeO_3 leading to the formation of AgNPs and SeNPs. The synthesis of metal nanoparticles was demonstrated using the spectrophotometric method and was characterized using the DLS method.

Thus, this paper proved the synthesis of metallic nanoparticles (AgNPs and SeNPs) using the fresh SM plant, with different sizes (AgNPs between 28 nm and 255 nm and SeNPs between 24 nm and 342 nm).

Taking into account our previous studies on the potential of SM in the treatment of various dermal diseases as future perspectives, we want to test the healing effect on normal human dermal fibroblasts of the synthesized metal nanoparticles by the scratch method

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