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CHARACTERIZATION OF ANTIOXIDANT ACTIVITY AND PHYTOCHEMICAL COMPOUNDS, METAL NANOPARTICLES OBTAINED BY *SIDERITIS SCARDICA* EXTRACTS

Ioana-Raluca SUICA-BUNGHEZ,[a*] Sofia TEODORESCU,[b] Ioan-Alin BUCURICA,[b] Raluca SOMOGHI[a] and Rodica-Mariana ION[a,c]

[a] National R&D Institute for Chemistry and Petrochemistry – ICECHIM, Splaiul Independentei Street, Bucharest, 060021, Roumania
[b] Multidisciplinary Science and Technology Research Institute of Valahia University of Targoviste - ICSTM-UVT, 13 Sinaia Alley, Targoviste, 130004, Roumania
[c] Valahia University of Targoviste, Materials Engineering Dept, 13 Sinaia Alley, Targoviste, 13004, Roumania

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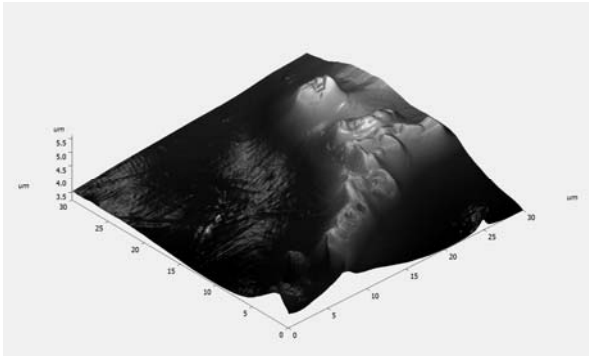
An eco-friendly method for obtaining metallic nanoparticles using *Sideritis Scardica* plant is presented in our research. The first step was conducted to investigate and compare the composition, total phenolic, flavonoid, tannins and terpenoids contents in flowers and strains *of Sideritis Scardica* plant. For phytochemicals analyses it was used the UV-VIS Specord M400, using different wavelengths and the results were calculated using standard calibration curves. Antioxidant activity was determined using DPPH method. The infrared spectral analysis was carried out to characterize the type of functional groups existent in different parts of *Sideritis Scardica* plant (flowers and strains).
The second step of this research study was to use a green method for obtaining metallic nanoparticles due to the biomolecules present in plant extracts, which can be used to reduce metal ions to nanoparticles. This study presents the use of *Sideritis Scardica* flowers and strains for the biosynthesis of metal nanoparticles. The formation and characterization of metal nanoparticles (PtNP, AgNP, Au,NP) in *Sideritis Scardica* extracts was confirmed by UV-Vis and FTIR spectroscopy, dynamic light scattering (DLS), optical microscopy (OM), atomic force microscopy (AFM) and scanning electron microscopy (SEM).



INTRODUCTION

Sideritis scardica (ironwort, mountain tea) is an endemic plant which is found in Balkan Peninsula (Bulgaria, Greece, Crete, Macedonia).[1,2] *Sideritis Scardica,* named tea of longevity due to their benefits for human body, being considered in traditional medicine as a treatment to bronchial asthma, the common cold, lung emphysema, gastrointestinal problems, inflammation or rheumatic disorders. Also, is an active constituent of dietary supplements for the prevention of anemia.[3,4] This plant is adapted to survive without more water and soil. Active components (flavonoids, terpenoids, phenols) existent in *Sideritis Scardica* plays a great role due to their anti-inflammatory, antioxidant and antimicrobial properties.[4,5] The noble metal nanoparticles (Au, Ag, and Pt) present an important role in pharmaceuticals, photocatalysts and sensors area. In our days, scientific research studies confirmed the fact that a number of metallic nanoparticles

* Corresponding author: raluca_bunghez@yahoo.com

based terapeutic bioactive agents have been developed for the treatment of different diseases (cancer, diabetes, asthma).[6,7]

MATERIAL AND METHODS

1. Materials

Silver nitrate (10^{-3} M $AgNO_3$), chloroplatinic acid hydrate (10^{-3} M $H_2PtC_{l6}xH_2O$) and (10^{-3} M $HAuCl_4$) were purchased from Sigma-Aldrich. DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate stable free radical) was supplied by Sigma-Aldrich (Germany) and ethanol (C_2H_6O) by Scharlau (Spain). Distilled water was obtained in our laboratory, using a Liston distiller. *Sideritis Scardica* plant was purchased from a market in Bulgaria country.

2. Methods

2.1. Preparation of plant extract

Sideritis scardica plant (Figure 1 a)) was purchased dried. It was separated flowers by strains. Approximate, 2 g from flowers and 1 g from strains were weighed and transferred into a 25 mL Berzelius flask which contained hidroalcoholic solution (ethanol: distilled water) and were boiled for 10 minutes in order to release the intracellular material into solution. The aqueous extract obtained (figure 1 b)), was cooled and filtered through a filter paper to obtain a clear extract.

2.2. Synthesis of silver,gold and platinum nanoparticles

An aqueous solution of silver nitrate (10^{-3} M $AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. *Sideritis Scardica* (SS) aqueous extract (10 mL) was agitated with 10^{-3} M $AgNO_3$ solution (10 mL). The new solution, AgNP-SS, it was also agitated in ultrasound bath at 50°C, for 30 minutes.

To obtain gold nanoparticles, have been mixed 5 mL extract sample and 5 mL $HAuCl_4$ (10^{-3} M), which were heated and agitated in ultrasound bath at 50°C, for 30 minutes, then were left over night. The next day, were heated and agitated in ultrasound bath at 35°C, for 20 minutes. For platinum nanoparticles solution, it was prepared 5 mL $HgPtCl_6$ (10^{-2} M) and 5 mL of extract plant (flowers and strains) sample. The working procedure was the same used for silver nanoparticles preparation.

2.3. Characterization methods

The absorption spectra of the plant extracts and of the metallic nanoparticles were obtained using a M400 Carl Zeiss Jena UV-VIS spectrophotometer, in the wavelength range of 350-650 nm.

Optical microscopy (OM) was obtained using a Novex microscope with digital camera.

Fourier transform infrared spectroscopy (FTIR) is a technique for measuring infrared spectra and for the spectrum acquisition a FT-IR spectrometer Vertex 80 and high-resolution microscope Hyperion 3000, in the range of 8000–400 cm^{-1} it was used.

The Scanning Electron Microscope (SEM) SU-70 (Hitachi, Japan) is very sensitive research equipment, with field emission and is based on a Schottky electron source. Applications field of SEM (coupled with EDS, WDS and EBL) was utilized for characterization of micro- and nanomaterial, qualitative and quantitative analysis of samples.

For dynamic light scattering (DLS), it was used a Zetasizer Nano SZ – Malvern instrument, which has a computer connected to the device, with Zetasizer software, used to control measurement sample.

Fig. 1 – a) *Sideritis Scardica* dried plant and 1b) extract solutions of *Sideritis Scardica;* strains (T) and flowers (F).

Table 1

Preparation methods of phytochemicals analyses[8]

No.	Assay	Reagents	Conditions	Monitoring and calibration
1	Total Tannins Content	0.5 mL extract + 3 mL 4% vanillin-MeOH and 1,5 mL HCl	15 minutes of incubation at room temperature	Absorbance at 500 nm; Catechin curve calibration standard
2	Total Flavonoids Content	1 mL extract + 4 mL distilled water and 0,3 mL $NaNO_2$ (5%); After 5 min, 0,3 mL $AlCl_3$ (10%); After other 5 min., 2 mL 1M NaOH and 2,4 mL distilled water	30 minutes of incubation at room temperature	Absorbance at 510 nm; Catechin curve calibration standard
3	Total Polyphenols Cointent	1 mL diluted extract and 5 mL Folin-Ciocalteau reagent. After 8 min., 4 mL Na_2CO_3	60 min of incubation at room temperature	Absorbance at 765 nm; Gallic acid curve calibration standard
4	Total Terpenoids Content	- 2 mL extract and 1 mL 2% vanillin-H_2SO_4	- heated at 60 ºC/20 min, cooled at 25 ºC/5 min	Absorbance at 608 nm; Linalool curve calibration standard

Atomic Force Microscope (AFM) used in this research is from Ntegra Prima by NT-MDT, a device capable to scan micrometric areas on the sample surface and obtain 2D and 3D topographic images at nano and micro scale. For surface determination have been used a polysilicon cantilever (HA_NC) with mono-crystal silicon tip coated by 20nm gold layer. The tip length is ~ 10μm and cantilever resonance frequency is 235 kHz. The obtained images quantify a 256x256 lines scanned on sample at 1.0 scanning speed ratio.

To test antioxidant activity (AA %), it was made a DPPH solution (2, 2-diphenyl-1-picryl-hydrazyl-hydrate stable free radical), supplied by Sigma-Aldrich and ethanol (C_2H_6O) by Scharlau. So, 0.5 mL of *Sideritis scardica* extract sample was mixed with 1 mL of 0.02 mg/mL DPPH solution. The mixtures were tested by reading the absorbance at 517 nm. As a blank, it was prepared 0.5 mL of distilled water with 1 mL of 0.02mg/mL DPPH solution, which was read at the same wavelength.[9,10]

The antioxidant activity (AA %) percentage was calculated using the formula:

$$AA\% = [A_{Control} - A_{Sample} / A_{Control}] \times 100$$

where: $A_{Control}$ is the absorbance of a DPPH solution without sample and A_{Sample} is the absorbance of the sample mixed with 0.02 mg/mL DPPH solution.

The phytochemical quantification procedures were used for the determination of total tannins, total flavonoids, total pholyphenols and total terpenoids in the extracts. The assays are presented in Table 1.

RESULTS AND DISCUSSION

Antioxidant activity results are presented in the table 2. It is observed the fact that *Sideritis Scardica* (flowers or/and strains) has good antioxidant properties.

Results of total tannins content

The amount of total condensed tannins is expressed as mg catechin/L. All samples were analyzed in triplicate. The total tannins results are presented in table 3.

Results of total flavonoids content

It was used catechin as standard for calibration curve, useful for calculation of total flavonoids content (table 4).

Results of total polyphenols content

To calculate total polyphenols results, it was it was used gallic acid as standard, necessary for the calibration curve (table 5).

Table 2

Antioxidant activity results

Sample	Antioxidant activity AA %
Sideritis Scardica flowers extract	79.46
Sideritis Scardica strains extract	79.73

Table 3

Total tannins content (T.T.C.) in *Sideritis Scardica* plant extract

Sample	T.T.C. mg/L Catechin
Sideritis Scardica flowers extract	19.133
Sideritis Scardica strains extract	19.563

Table 4

Total flavonoids content (T.F.C.) in *Sideritis Scardica* plant extract

Sample	T.F.C. mg/L Catechin
Sideritis Scardica flowers extract	153.1
Sideritis Scardica strains extract	232.42

Table 5

Total phenolics content (T.P.C.) in *Sideritis Scardica* plant extract

Sample	T.P.C. mg /L Gallic acid
Sideritis Scardica flowers extract	766.101
Sideritis Scardica strains extract	673.728

Table 6

Total terpenoids content (T.Tp.C.) in *Sideritis Scardica* plant extract

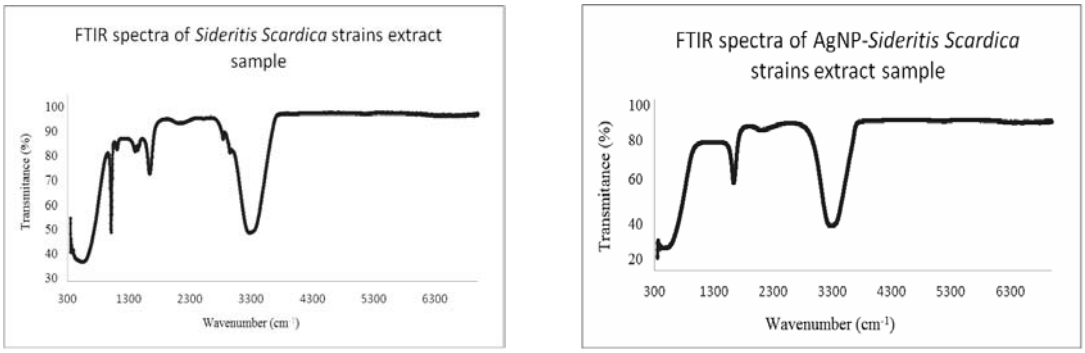
Sample	T.Tp.C. mg /L Linalool
Sideritis Scardica flowers extract	33.928
Sideritis Scardica strains extract	75.155

Results of total terpenoids content (table 6)

For preparation of the calibration curve, it was used linalool as standard.

Fourier transform infrared spectroscopy (FTIR) results

Perkin-Elmer spectrometer FTIR Spectrum in the range 8000–300 cm^{-1} was used. The sample was dried and then placed in Fourier Transform Infrared FTIR for the analysis of the nanoparticles. The FTIR spectra (figure 2 a) and b)) indicate various functional groups present at different positions. At 3300 cm^{-1} it is observed a strong band at the both samples (SS strains extract and AgNP-SS strains). This band represents the alkynes. In the 1600 cm^{-1} region, C=O bonds are present. Absorptions between 1300-1500 cm^{-1} regions, observed at *Sideritis Scardica* strains extract sample (fig. 2 a)), were caused by base sugar vibrations[8]. Between 2800-3000 cm^{-1} are medium bands, characteristic for alkyl-methyl bonds are observed just in figure 2 a), in the Sideritis Scardica strains extract sample. The bands at 1465 cm^{-1} characterized the imidazole ring vibration. Between 580 cm^{-1} and 610 cm^{-1} are amines groups and at 1750 cm^{-1} are found esters bands.[9] It is important to explain the peaks located at 1200 cm^{-1} existent in the *Sideritis Scardica* strains extract sample spectrum (fig. 2 a)), correspondent to amides, proteins and enzymes which seem to be responsible for the Ag^{+} reduction, because in the figure 2 b) is not present. Also, this peak at 1200 cm^{-1} present at *Sideritis Scardica* strains extract sample spectrum, can be assigned to C-O, C-N stretching vibrations of the aliphatic amines or alcohols/phenols, indicating the presence of polyphenols in the sample extract.[6] The clear peak at 1640 cm^{-1}, responsible for asymmetric and symmetric stretching vibrations of the nitrate group, is present in the spectrum of AgNP-SS strains sample.



a) b)

Fig. 2 – FTIR spectra of a) *Sideritis Scardica strains* extract and b) AgNP-*Sideritis Scardica* strains

Ultraviolet–visible spectroscopy (UV-VIS) results

The compounds of flowers and strains sample extracts of *Sideritis Scardica* plant, were characterized by UV-VIS (figure 3 a)). The absorption in the UV region 270 nm and 370 nm corresponds to phenolic acids and their derivatives (flavones, flavonols, quinones).[11] Thus, phenolic derivatives are found in our samples at 280 nm and flavonoids around 350 nm.

The reduction of noble metal ions to metallic nanoparticles was firstly analyzed by UV-VIS spectrometry technique, between 220-800 nm wavelengths.[10] The reduction of silver ions in the aqueous solution of silver nanoparticles-*Sideritis scardica* (AgNP-SS) plant (flowers and strains) extracts, presented a maximum absorption at 440 nm[6] as shown in figure 3 b). In the aqueous solution of platinum nanoparticles-*Sideritis Scardica* (PtNP-SS) plant (flowers and strains) samples, it appeared a maximum absorption around 280 nm[12] as shown in figure 4 a). The reduction of gold ions in the gold nanoparticles-*Sideritis Scardica* (AuNP-SS) plant (flowers and strains) samples, it is formed a peak at 550 nm[13] (Figure 4 b)).

Optical microscopy (O.M.) images are presented in figures 5, 6 and 7. Optical microscopy it was utilized to acquire first images with the new aggregates formed. It was observed the fast conglomerate particles obtained. To making SEM, DLS and AFM images, obviously were ultrasounded to break the agglomerates of nanoparticles.

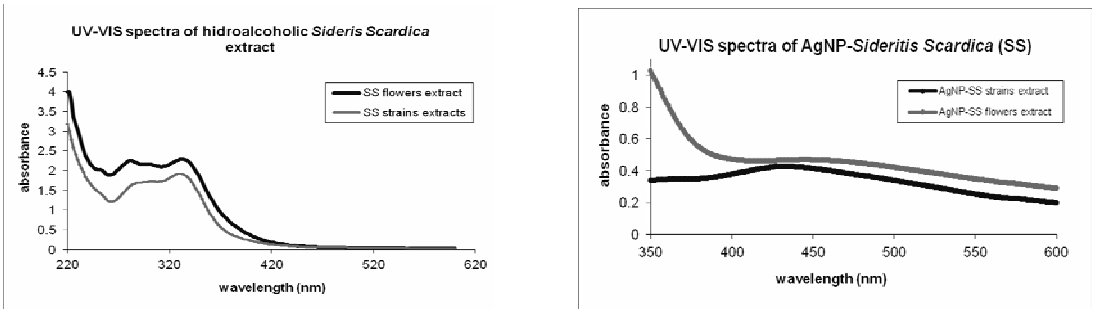


Fig. 3 – UV-VIS spectra of a) *Sideritis Scardica* extract and b) silver nanoparticle AgNP-SS samples.

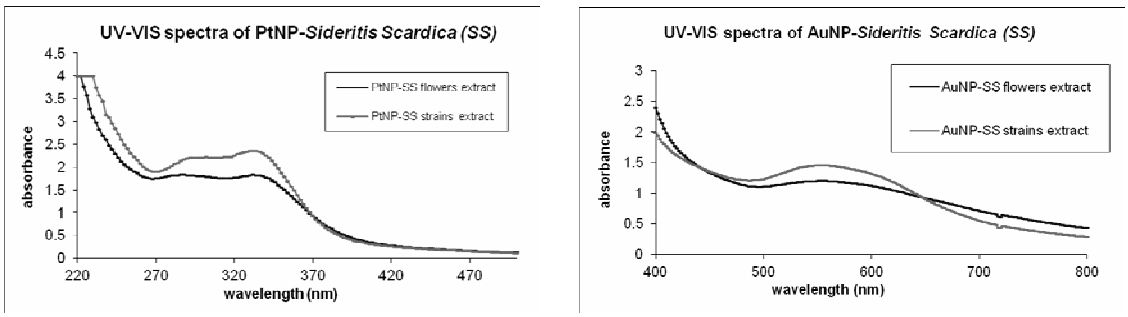
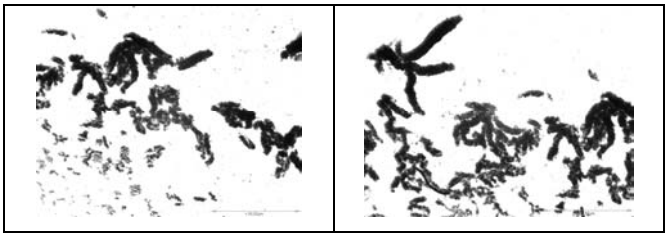
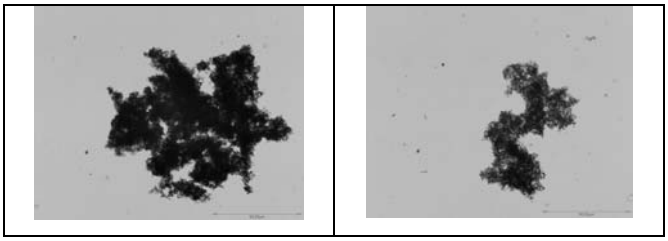


Fig. 4 – UV-VIS spectra of a) platinum nanoparticles PtNP-SS and b) gold nanoparticles AuNP-SS samples.



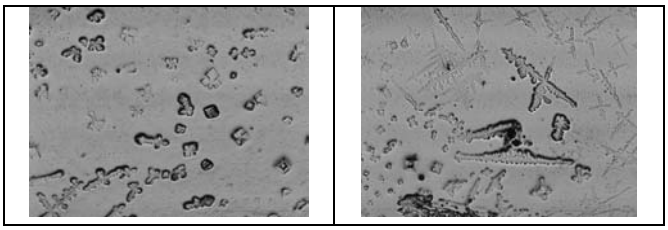
a) b)

Fig. 5 – Optical microscopy images of a) AgNP-SS flowers extract and b) AgNP-SS flowers strains.



a) b)

Fig. 6 – Optical microscopy images of a) AuNP-SS flowers extract and b) AuNP-SS strains extract.



a) b)

Fig. 7 – Optical microscopy images of a) PtNP-SS flowers extract and b) PtNP-SS strains extract.

Scanning Electron Microscope (SEM) results

The morphology details, size and shape of PtNP-*Sideritis Scardica* extract were certified by using SEM. The SEM image (figure 8) showed a high density of platinum nanoparticles synthesized by using *Sideritis Scardica* extract. It was observed the development of platinum nanostructures uniform in size and shape with a narrow size distribution. The solution mixture was kept 20 minutes in ultrasonic bath for the morphological nature of the nanoparticles and then the aqueous substance was characterized by (SEM). The nanoparticles formed have the diameter between 40-80 nm.

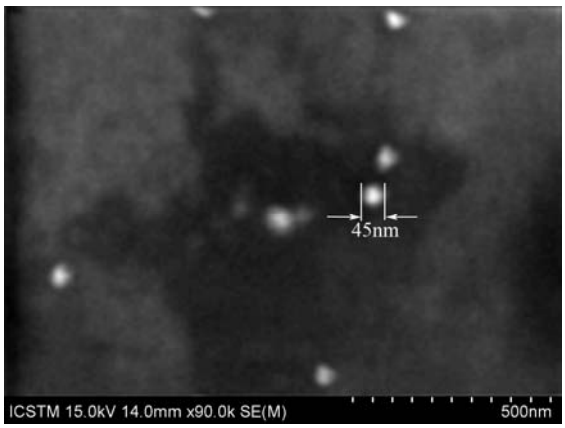


Fig. 8 – SEM PtNP-*Sideritis Scardica* flowers.

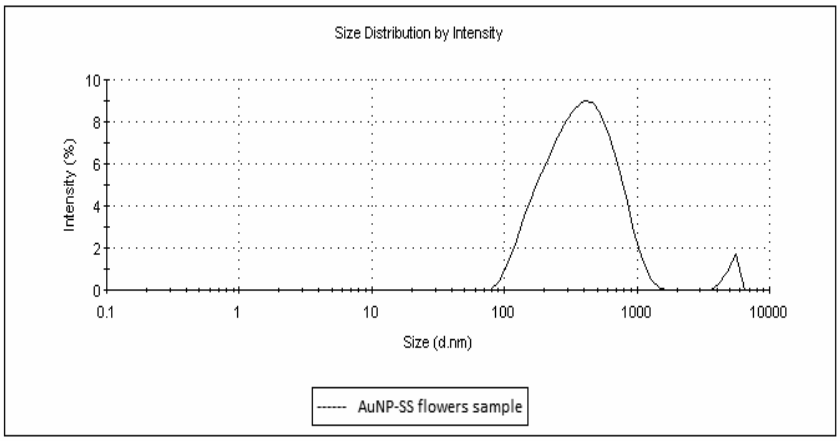
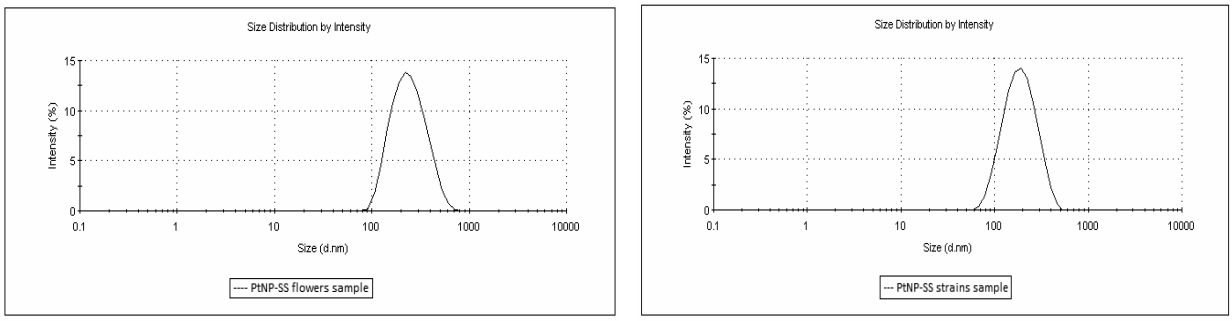


Fig. 9 – DLS results for gold nanoparticles AuNP-*SideritisScardica* flowers extract.



a) b)

Fig. 10 – DLS results for a) PtNP-*Sideritis Scardica* flowers and b) PtNP-*Sideritis Scardica* strains.

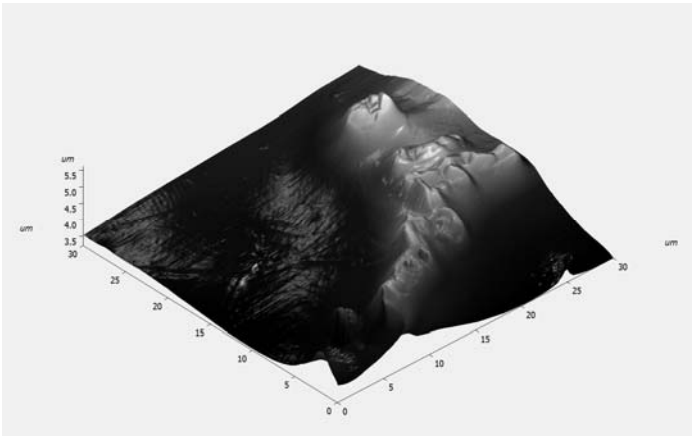


Fig. 11 – AFM image of PtNP-*Sideritis Scardica* strains extract.

Dynamic light scattering (DLS) results

Figure 9 presented DLS for AuNP-*Sideritis scardica* flowers, Z-Average = 348.1 nm and Pdl= 0.929.

Figure 10 a) presented DLS for PtNP-*Sideritis scardica* flowers, Z-Average = 222 nm and Pdl = 0.155. In Fig. 10 b) it is presented DLS for PtNP-*Sideritis scardica* strains, Z-Average = 164.8 nm and Pdl = 0.153.

Atomic-force microscopy (AFM) results

The surface topography and morphology of platinum nanoparticles PtNP was confirmed by AFM imaging. It is a very important investigation tool used to characterize the platinum nanoparticles obtained in *Sideritis Scardica* strains extract. The figure 11 shows the 3-D view of PtNP deposited on the glass surface. It can be observed the size and the height distribution of NPs deposition. So,

the AFM results show that the platinum nanoparticles are agglomerated in clusters.

CONCLUSIONS

In this research it were utilized aqueous extract solutions from flowers and strains of *Sideritis Scardica, a* balkanic plant, to characterize the phytochemical compounds (using UV-VIS and FTIR) and obtain metallic nanoparticles. This plant presents a good antioxidant activity and an important quantity of phytochemical components. *Sideritis Scardica* plant extract have good properties for obtaining an "eco friendly" new method of metallic nanoparticles. The color of silver nanoparticles (AgNP) solution became brown after few hours and presented an intense absorption band at 430 nm. The color of gold nanoparticles (AuNP) became purple and an important peak it was observed at 550 nm. Also, the yellow PtNP solution presented an intense absorption band at 280 nm. Optical microscopy (OM) it was utilized to acquire and register first images with the new aggregates formed. Scanning Electron Microscope (SEM) analysis of PtNP-SS extract sample presented the formation of spherical platinum nanoparticles between 40-80 nm diameters. The atomic force spectroscopy (AFM) results show that the platinum nanoparticles are agglomerated in clusters. Dynamic light scatering (DLS) analysis demonstrated the presence of metallic nanoparticles in the tested samples. Antioxidant activity (AA %) of AgNP-SS flowers=81,35 % and AgNP-SS strains=80,94 % demonstrated the fact that the antioxidant level increased after it were formed silver nanoparticles. Analytical techniques utilized in the research and phytochemical results demonstrated that bioactive plant responsible for metallic bioreduction could be flavonoids and polyphenols of *Sideritis Scardica* suposed to act as reducing and capping agents for the metal nanoparticles preventing the agglomeration of the particles and thereby stabilizing the nanoparticles.

In conclusion, a simple green method has been applied for the synthesis of metallic nanoparticles, using hidroalcoholic extract plant solutions. The techniques used in this research, suggest that the *Sideritis Scardica* played an important role in the reduction and stabilization of silver, gold and platinum nanoparticles.

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