Silver nanoparticles obtained by eco-friendly method using *Galanthus nivalis* plant for wood conservation

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The phytosynthesis of metallic nanoparticles (MNPs) represents an interesting domain of research, with promising perspectives especially in medicine and food protection. A special area represents biosynthesis of MNPs by vegetal materials. In the present research, synthesis of silver nanoparticles (AgNPs) was obtained through a simple method, using different parts (flowers and stems) of snowdrop plant (*Galanthus nivalis* L.). The AgNPs were formed by reaction of biomass of *Galanthus nivalis* extract with aqueous solution of $AgNO_3$ at room temperature and dark conditions. The synthesized nanoparticles were confirmed by UV–VIS spectroscopy (between 250-750 nm), optical microscopy and dynamic light scattering – DLS (indicated that all the particles are nano-sized with average diameters between 200-400 nm). To demonstrate the presence of bioactive components of snowdrop plant (flowers and stems), the extract was quantitatively (polyphenols, flavonoids) and qualitatively (alkaloids, carbohydrates, terpenoids) characterized. The antioxidant activity (AA%) of snowdrop extracts and AgNPs samples was evaluated using DPPH method. AA% showed an increase at AgNPs samples compared to the extract. The antimicrobial activity of AgNPs samples was demonstrated by testing on *Candida albicans* and *Staphylococcus aureus* and it was resulted a good activity against these two pathogens. Next step of this research was to find an application of snowdrop-derived AgNPs for the wood treatment or preservation of wood objects.

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Keywords: Plant extract, Green methods, Silver nanoparticles, Bioactivity (antioxidant and antibacterial) properties

1. Introduction

In the last years, the scientific area of nanotechnology has presented an important interest in obtaining and characterizing the composition, shape and size of nanomaterials using eco-friendly methods, aiming to reduce the substances dangerous to human health and environment. So, techniques for obtaining the nanoparticles using toxic substances or organic solvents must disappear. The alternative is the *phytosynthesis* of nanomaterials using *Green Chemistry*. From this point of view, the future perspectives of ecologist people are interested to innovative new ways for obtaining extracts and photosynthesized nanometric architectures, beneficial for healthy or environment engineering fields. In recent period, it has been observed an increase for alternative medicine with plants, due to their therapeutic antioxidant agents [1-4].

The *Galanthus* genus from the *Amaryllidaceae* family, consisted of approximately 1000 species in 85 genera, is well known due to alkaloids with different chemical structures and biological activities [5]. Snowdrops are tiny plants, 3 to 6 inches tall, with white flowers. Each snowdrop bulb produces two linear narrow grassy leaves and a single flower with a delicate small white drooping bell shaped flower. This plant contains an important variety of flavonoids, phenolics, terpenoids and some important alkaloids (galantamine and lycorine) which are very useful in medicine, pharmacological and therapeutical areas. The discovery of galanthamine identified in snowdrop became an interest of scientific area to explore in next period the relations between pharmacological properties of snowdrops (including the antiviral, anti-inflammatory, antimicrobial, antioxidant and anticancer activities) and its chemical content [6--8]. It is important to tell that the flavonoids from this plant have the capacity to chelate and actively reduce metal ions from nanoparticles, due to numerous hydroxyl and carbonyl groups [4]. Flavonoids and polyphenols have several properties, like free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action. Antioxidant activity of *Galanthus nivalis* is due to phenolic compounds [9] existent in this vegetal material. E.B. Ay *et al*. [10] revealed that phenolic content (42,63 mg of GA/g in the bulb and 18,15 mg of GA/g in the root) and flavonoid content (58,63 mg of QE/g in the bulb and 19,46 mg of QE/g in the root) have good results. Luis Marco *et al*. [11] demonstrated the fact that the chemical component, galantamine, used today in the treatment of

Alzheimer's disease is a natural product of the *Amaryllidaceae*.

Taking into account the benefits of snowdrops, we have used an eco-friendly method to obtain silver nanoparticles. This green synthesis of nanomaterials was demonstrated due to the active compounds from plant extracts, which contribute to reduction of metallic ions [12]. The reaction is described in the Fig. 1, when organic molecules from *Galanthus nivalis* plant extract conducts to bioreduction of silver metallic ions in silver nanoparticles (AgNPs).

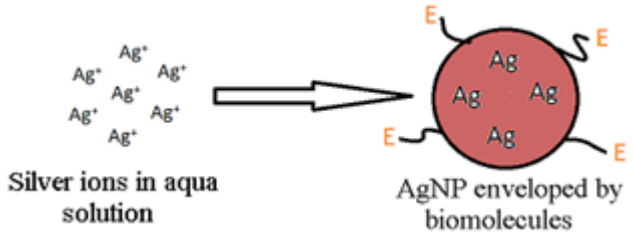


Fig. 1. Conversion of silver ions (Ag^+) into silver nanoparticles (AgNPs), by biomolecules arising from aqueous vegetal extract (E)

The addition of metal nanoparticles to wood could be a benefit to preserve old wooden objects that degrade over time. It is well known that external factors (like fire, low or high temperature, light or microbiological agents) produce wood degradation, which is often irreversible [13]. The scientific literature offers us different methods of preserving wooden objects, but many are expensive or harmful to the environment [14-16]. Wood is an organic material whose structure undergoes continuous degradation processes caused by fire, weathering, or organisms [15]. Always, the specialists have been trying different methods to improve wood durability. In order to delay or stop the degradation processes, the wooden artefacts are subjected to conservation treatments with different material types (polyethylene oxide (PEG); melamine-formaldehyde; silicon-containing polymers; consolidants such as dicarboxylic acid; phenol-formaldehyde (PF), cellulose, chitosan, lignin, biomimetics) [17, 18] to stabilize the wood structure and to avoid contraction, collapse for a longer wood object life [19, 20].

2. Experimental

The reagents used: $NaNO_2$, NaOH, Na_2CO_3, DPPH, Folin–Ciocalteu reagent, $AlCl_3$ were supplied from Merck (Darmstadt, Germany) and $AgNO_3$ from Chimreactiv. The calibration etalon standards (Gallic acid, Catechin) were from Sigma Aldrich. For qualitative determinations, Benedict's and Millon's reagents were purchased from Sigma – Aldrich (Darmstadt, Germany). The distilled water used to prepare all the solutions was freshly obtained in our laboratory, using a Liston A 1125 distiller.

Preparation of flowers extracts

Fresh snowdrops were purchased from local flower shop market. The flower petals and the stems used in this research, were cleaned in distilled water and cut into small pieces, and were weighed about 5 grams. Over the petals/stems was putted distilled water (20 mL) and these mixtures were boiled approximate 15 minutes in order to release the intracellular material into solution. The aqueous petal extracts obtained were filtered through a filter paper, blue band 110 mm, to obtain a clear extract (Fig. 2).

Silver nanoparticle preparation

The snowdrop extracts were used as reducing agents for Ag^+ as well as capping agents for silver nanoparticles. In order to obtain silver nanoparticles, 10 mL of flower petals extracts were added and mixed with 10 mL $AgNO_3$ 10^{-3} M using a Biolab ultrasonic bath (1 h/ 400 rpm) and kept in the dark, in fridge. The sample colours have changed from yellow to brown in approximately 2 hours after the addition of $AgNO_3$ 10^{-3} M. This is the first step in indicating the formation of silver nanoparticles.

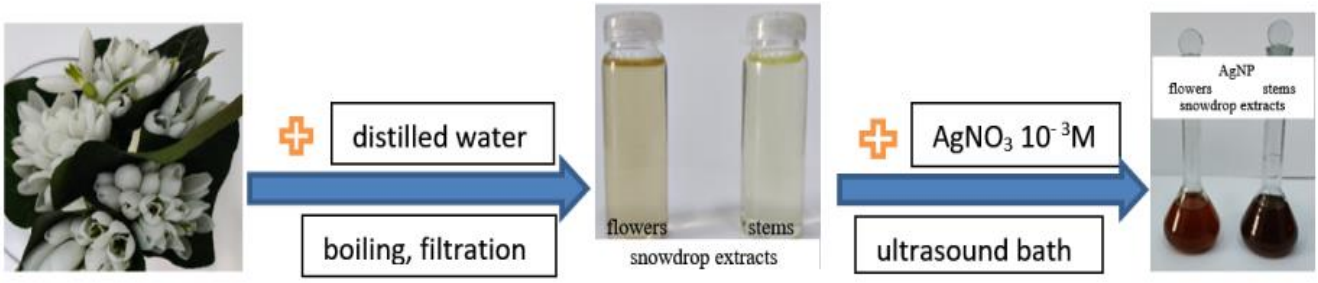


Fig. 2. Synthesis of silver nanoparticles using snowdrop (Galanthus nivalis L.*) plant (color online)*

It is important to specify that these plant materials were chosen because it was observed that the total flavonoids and polyphenols content and antioxidant activity showed very high values, which indicates that they can fight against oxidative stress. The wood was from young hazel tree (approx. 10 years), from the Prahova Valley, which is located in the south-eastern part of Romania, in northern Muntenia. The cut twigs were cleaned of leaves and transported to the laboratory in optimal conditions (Fig. 3).

Fig. 3. Hazel wood (Corylus avellana) (color online)

The branch was cut 5 years ago and kept in optimal conditions at room temperature, in dark and dry place. The samples were immersed in 10 mL AgNPs-snowdrop suspension and were subjected to ultrasounds on the Bioblock Scientific ultrasonic bath (30 min/50°C), then kept for 48 hours in the dark, at room temperature (Fig. 4).

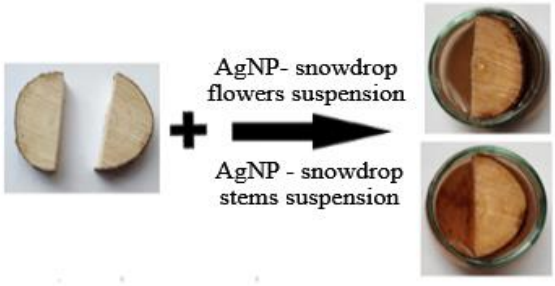


Fig. 4. The image of wood in AgNPs suspensions (color online)

Characterization methods

- The absorption spectra of the plant extracts and of the silver nanoparticles were obtained with UV-VIS spectrophotometer T60U, in the wavelength range of 250-750 nm.
- Fourier transformed IR spectroscopy (FT-IR ATR) spectra were collected using a Perkin Elmer Spectrum Duo, in range of 400–4500 cm^{-1} wavenumbers.
- Preparation of samples for Dynamic Light Scattering (DLS) measurements consisted in 0.5 mL of the sample diluted with distilled water in a 25 mL volumetric flask in the presence of 0.2 g NaCl. The samples thus prepared were ultrasonicated for 3-4 minutes in the ultrasound bath (Bandelin Sonotex Digitec DT 31 H, Bandelin Electronic, Berlin, Germany), before being subjected to analysis. At least 5 measurements were performed for each sample. The measurement with the values closest to the average value was selected.
- The microscope images were taken using a Novex trinocular optical microscope from Euromex Microscope BV Holland, at different magnifications (40x, 100x). The instrument has a digital video camera attached (model CMEX DC.1300), and the microscope software (Image Focus Professional) allowed real-time data acquisition. For a good viewing, the obtained images have been easily converted from 2D in 3D format.
- *Qualitative analyses* are based on the colour change reaction of the studied extracts after different chemical reagents are added. These qualitative assays give information only regarding the presence and/or absence of the most common bioactive compounds found in the studied plants (e.g.: carbohydrates, alkaloids, proteins, etc.) and do not quantify the amount of bioactive compounds. Test for saponins: 2 mL extract and 2 mL distilled water are vigorously shaken lengthwise, using a graduated cylinder, for 15 minutes. The formation of a 1 cm foam layer confirms the presence of saponins. Mayer test for alkaloids: to 2 mL extract, 2 mL of concentrated HCl were added and 5 drops of Mayer reagent (potassium mercuric iodide). A green solution or white precipitate indicates the presence of alkaloids.

-*Quantitative methods* [10, 14, 15] are presented in Table 1.

Table 1. Phytochemical assays effectuated for snowdrop (Galanthus nivalis) plant

Assay	Reagents	Conditions	Monitoring and calibration
Total Flavonoids Content	1 mL extract + 4 mL distilled water and 0,3 mL $NaNO_2$ (5% w/w); After 5 min, 0,3 mL $AlCl_3$ (10% w/w); After other 5 min., 2 mL of 1M NaOH and 24 mL distilled water	30 minutes of incubation at room temperature	λ = 510 nm at UV-VIS; Catechin curve calibration standard
Total Polyphenols Content	1 mL diluted extract and 5 mL Folin-Ciocalteu reagent. After 8 min., 4 mL Na_2CO_3	60 min of incubation at room temperature	λ = 765 nm at UV-VIS; Gallic acid curve calibration standard

- The *antioxidant activity* of the extracts was evaluated spectrophotometrically using the DPPH method [21]. Each plant extract was evaluated at 100 mg/L concentration, by mixing 0.5 mL of them with 1 mL of DPPH solution (2 mg/100 mL). Each sample was mixed 30 minutes and then kept in the dark for 30 minutes, at room temperature. After that, each mixture sample was tested for the DPPH radical-scavenging activity by measuring the absorbance at 517 nm on the T60 UV-VIS spectrophotometer. As blank was used a solution prepared by mixing 0.5 mL of bidistilled water with 1 mL of the DPPH solution (2 mg/100 mL). The antioxidant activity (AA%) was calculated using the formula:

$$AA\% = [(A_{Control} - A_{Extract}) / A_{Control}] \times 100 \quad (1)$$

where: $A_{Control}$ is the absorbance of a DPPH solution without extract, $A_{Extract}$ is the absorbance of the plant extract treated with DPPH (2 mg/100 mL). As positive control it was used hydroquinone at 100 mg/L.

- The *antimicrobial activity* was evaluated by diffusimetric method through spot inoculation. The tests were performed on *Staphylococcus aureus* (ATCC 29213) on agar Muller Hinton medium (Scharlau, Spain) with the following composition (in $g \cdot L^{-1}$): 17.5, peptone; 1.5, starch; 2.0, and meat infusion solids) and on *Candida albicans* (ATCC 10231) on Sabouraud Dextrose Agar (SDA) with the following composition (in $g \cdot L^{-1}$): glucose; 40, peptone; 10, agar; 15) in Petri plates. From the tested strains was prepared a microbial suspension in sterile physiological water from a young culture of about 24 hours and inoculum turbidity was adjusted nephelometrically (McFarland standard 0.5 = 1.5×10^8 UFC/mL). The plates were inoculated with the microbial suspension, using a sterile swab by spreading. Subsequently, the plates were incubated for 24 hours at 37°C for *Staphylococcus aureus* and at 28°C for *Candida albicans*. The antimicrobial activity was evaluated by measuring the diameter of the clear area (halo) appearing around the inoculation area (spot) and the test was performed in duplicate [22].

- *Colorimetric analysis* was performed using an instrument from Konica Minolta (Japan), under a D65 light source and an observer angle of 10° [23]. Values recorded for each point, were an average of three measurements.

The colouring density of the hazelnut wood, in absolute value, in points, Δb, was calculated using the formula:

$$\Delta b = b_{x\ treated} - b_{x\ untreated} \quad (2)$$

where: $b_{x\ treated}$ = colour degree for treated hazelnut wood; $b_{x\ untreated}$ = colour degree for untreated hazelnut wood.

Depending on the degree of wood colouring, the following stability classes are divided into 3 categories: Stable ≤ 3; Moderately stable > 3 - ≤ 8; Unstable > 8 of absolute value of b_x.

- *The moisture content* (M%) was calculated at the equilibrium point according to the next equation [13]. Where: W_t = wet weight and W_0 = initial dry weight.

$$M\ \% = 100\ x\ \frac{(Wt - W0)}{W0} \quad (3)$$

- *Hardness tests* were performed with a Silver Schmidt & Hammer (Proceq, Switzerland). The hammer hit the sample and the hardness result was displayed on the device screen [13].

3. Results and discussions

Quantitative and qualitative results presented in Table 2, confirmed that snowdrop plant have great values of polyphenols and flavonoids. Table 3 contains results regarding the presence (+) or absence (-) of phytochemicals using qualitative analyses.

Table 2. Quantitative contents in snowdrop (Galanthus nivalis) plant

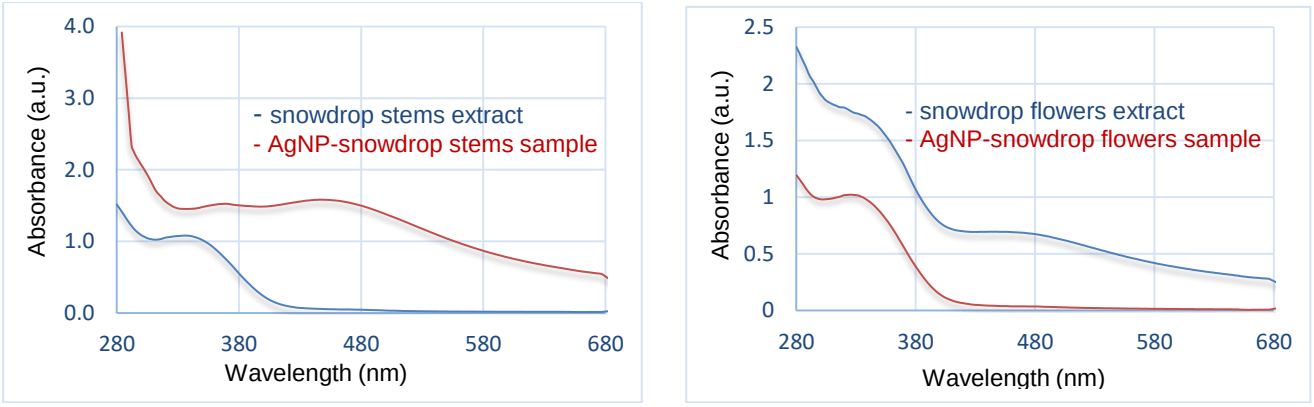
Determinations	Snowdrop	
	flowers	stems
Total polyphenols content (mg/L gallic acid equivalents; GAE)	100144	98703
Total flavonoids content (mg/L catechin equivalents; CAE)	403100	339200

Table 3. Qualitative results of snowdrop (Galanthus nivalis) plant

Phytochemical test	Snowdrop	
	flowers	stems
Carbohydrates (general) - Molisch	Pink – beige solution (+)	Violet – beige solution (+)
Carbohydrates (reducing sugars) - Benedict	Cold: emerald – green solution; upon boiling: brick – orange precipitate, blue greenish solution (+)	Cold: turqoise solution; upon boiling: brick – orange precipitate, blue greenish solution (+)
Carbohydrates (hexose sugars) - Seliwanoff	Red orange solution (+)	Brown orange solution (+)
Saponins	0.6 cm foam layer (+)	0.2 cm foam layer (+)
Anthraquinones	Yellow – brown solution (-)	Yellow solution (-)
Alkaloids - Mayer	Yellow – brownish solution (+)	Opalescent yellow – brown suspension (+)
Proteins and aminoacids - Millon	Opalescent orange solution (+)	Light orange solution (+)
Aminoacids – Ninhydrin test	Opalescent white-yellow solution (+)	Opalescent yellow solution (+)
Proteins – biuret test	Green solution (+)	Green - blueish solution (+)
Steroids	Colourless layer, brown ring, colourless upper layer (-)	Colourless layer, brick red ring, colourless upper layer (-)
Glycosides – $FeCl_3$ reagent	Brown – orange solution (-)	Brown solution (-)

UV-VIS results

The first indication of the silver nanoparticles formation was the colour change of solutions, from pale yellow to dark brown, due to excitation of surface plasmon vibrations in the metal nanoparticles. This aspect was confirmed by UV–VIS absorption spectroscopy. The bioreduction of Ag^+ ions was monitored by recording the UV-VIS spectra of the samples. UV–VIS absorption spectra at 470 nm for snowdrop flowers-AgNP (Fig. 5 a) and at 460 nm for the snowdrop stems-AgNP (Fig. 5 b) being characteristic for silver nanoparticles formation as highlighted in scientific literature [24]. The absorption band between 320-380 nm is attributed to phenolic acids and their derivatives (flavonols, flavones, quinones) [25, 26] arising from plant extracts.



a) b)

Fig. 5. UV-VIS spectra of snowdop extracts and AgNPs (color online)

FT-IR ATR analysis

Fourier transform infrared spectroscopy (FT-IR ATR) was used to study the obtaining of AgNPs using snowdrop flowers and stems and to identify the possible biomolecules responsible for the reduction of the Ag^+ ions and capping the bioreduced silver nanoparticles synthesized by the plant extract. The FT-IR ATR spectra (Fig. 4) indicate the presence of active functional groups in the synthesized silver nanoparticles. In order to obtain good signal/noise ratio, the FT-IR ATR transmission spectra of snowdrop extract before and after bioreduction

of Ag^+ ions were recorded in the region 400-4000 cm^{-1}. In snowdrop extract, bands at 1641, 1423 and 1057 cm^{-1} have been attributed to amides, proteins and enzymes, which seems to be responsible for the reduction of metal ions when it is using vegetal materials for the synthesis of metal nanoparticles [25, 27, 28].

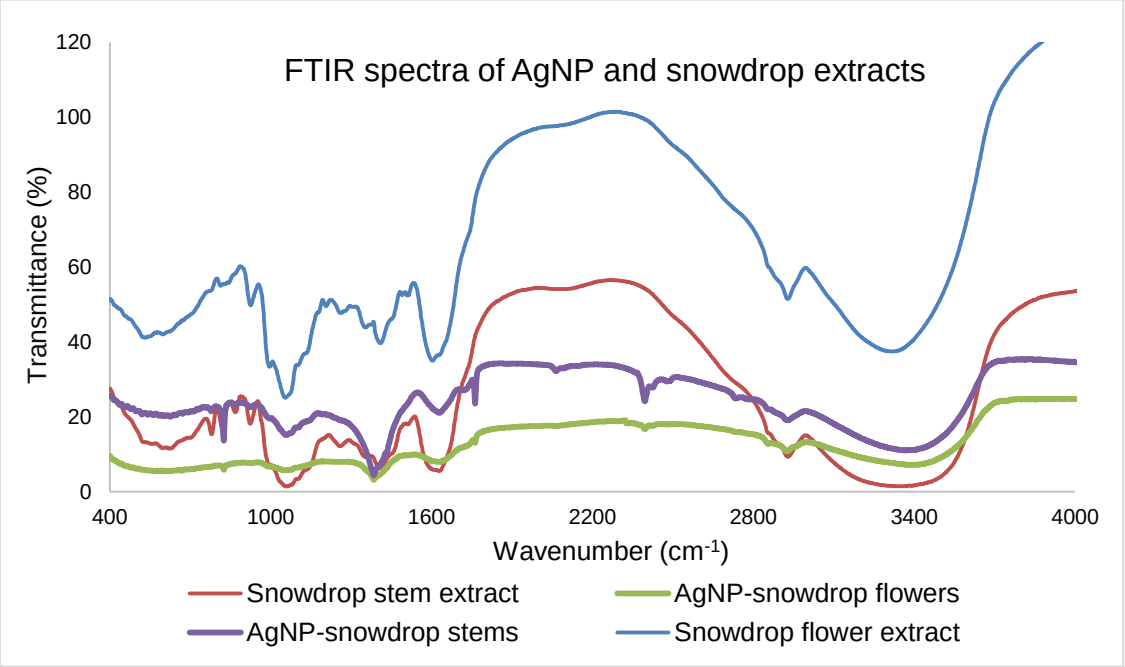
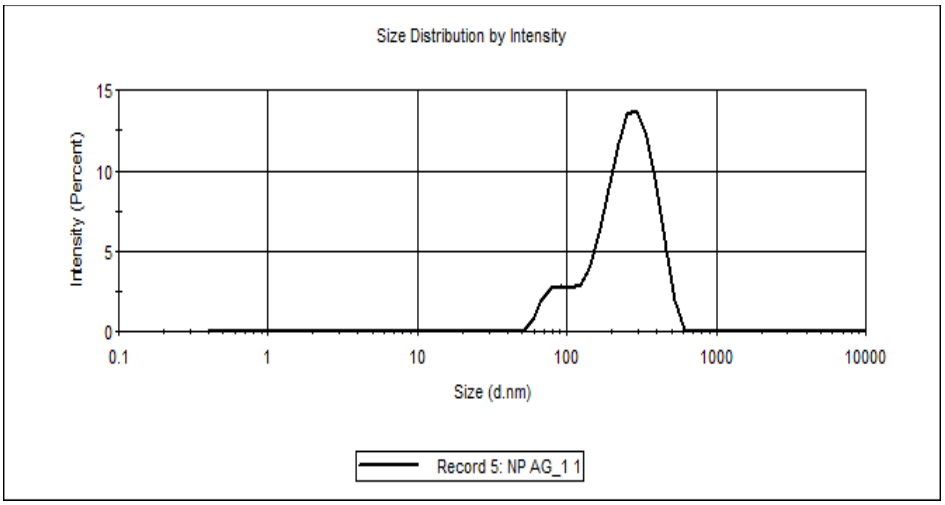


Fig. 6. FT-IR ATR spectra of AgNPs and snowdrop (flowers and stems) (color online)

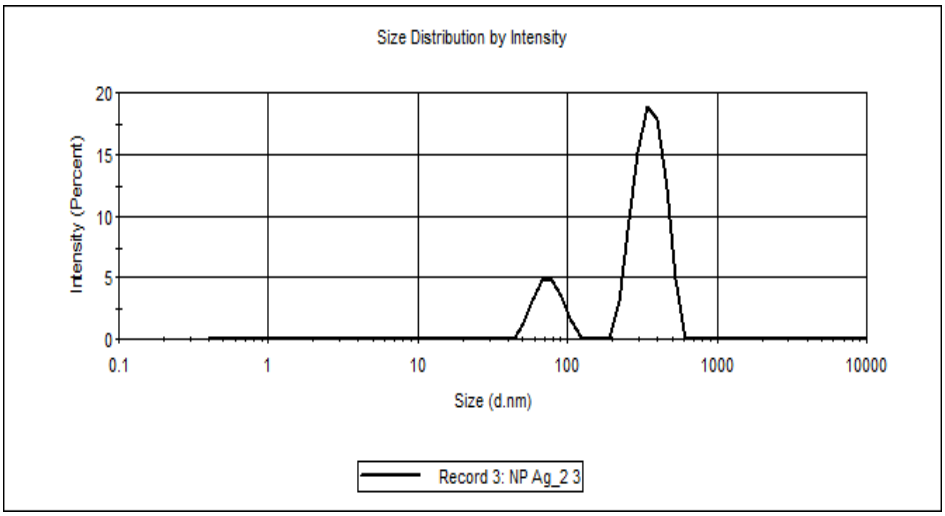
IR bands common to snowdrop extract and AgNPs, are attributed to O–H stretching (3432 cm^{-1} - AgNP and 3240 cm^{-1} - snowdrop extracts), at 1636 cm^{-1} (AgNPs and snowdrop extracts) are assigned to amide I, arising due to carbonyl stretch in proteins; the peaks at 1084 cm^{-1} – AgNPs and 1069 cm^{-1} - snowdrop extracts, corresponding to C-O, C-N stretching vibrations of the aliphatic amines or alcohols/phenols, indicating the presence of polyphenols in the plant extracts [29-31]. Specific to AgNPs sample is the peak identified at 2395 cm^{-1} (alkyls C–H stretching). The silver ions are surrounded by various phytochemical constituents present in *Galanthus nivalis*, creating a coating on silver ions which receive electrons from these phytocompounds (electron donors) [25]. In conclusions, FT-IR ATR results demonstrated that bioactive compounds responsible for silver bioreduction could be alkaloids, proteins and polyphenols from snowdrop sample extracts presumed to act as reducing and capping agents for the silver nanoparticles preventing the agglomeration of the particles and thereby stabilizing the nanoparticles.

DLS results

The size of the silver nanoparticles was determined by Dynamic Light Scattering measurements. Fig. 7 shows the size distribution by intensity, of AgNPs prepared. The hydrodynamic diameters (Dm) and polydispersity index (PdI) are presented in Table 4. The polydispersity index is the measure of the distribution of nanoparticle population and high values for PdI indicate a large size distribution with multiple AgNPs aggregates. Also, in Table 4 are presented te mean values and standard deviation for 3 measurements per sample.



a)



b)

Fig. 7. a) AgNP-snowdrop flowers, b) AgNP-snowdrop stems

Table 4. Dynamic Light Scattering data of AgNPs

Sample	**Dm (nm)**	**PdI**	**Peak (Intensity)**	**Peak (Volume)**	**Peak (Number)**
AgNP- snowdrop flowers (SF)	257	0.463	P1 = 274 P2 = 85	P1 = 294 P2 = 84	P1=87
AgNP- snowdrop stems (SS)	456	0.441	P1 = 360 P2 = 75	P1 =371 P2 = 67	P1 = 320 P2 = 61

Table 5. Mean values and standard deviations for 5 measurements per sample

Sample		**Z-Ave (d.nm)**	**PdI**	**P1 (d.nm)**	**P2 (d.nm)**
AgNP- snowdrop flowers (SF)	Mean	399.7	0.527	398	79.7
	Std. Dev.	53.17	0.081	90.93	6.77
AgNP- snowdrop stems (SS)	Mean	254.2	0.396	272.3	79.75
	Std. Dev.	7.601	0.059	2.401	7.84

The majority of the particle population (indicated by the P2 peak) of AgNPs-snowdrop flowers sample, has an average diameter of ~ 85 nm. The AgNPs-snowdrop flowers sample is very polydisperse. For the next sample, AgNPs-snowdrop stems sample, the majority of the particle population (indicated by the P2 peak) has an average diameter of ~ 70 nm. Larger particles are also present, indicated by the P1 peak, with an average diameter of ~ 320-360 nm. These can be attributed to the aggregates of smaller particles. In addition, there are a few even larger aggregates whose dimensions exceed the detection limit of the device.

Antioxidant activity results

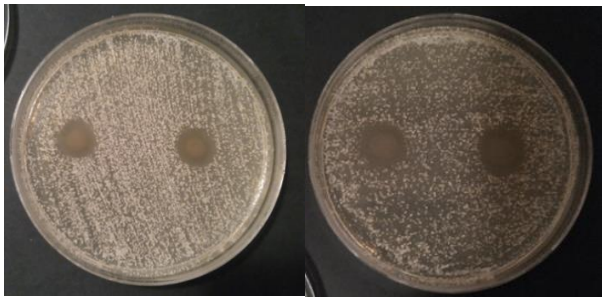
The antioxidant properties of snowdrop extracts and AgNPs samples were determined by DPPH method. Antioxidant activity (AA%) results showed an increase in antioxidant activity on silver nanoparticles samples compared to the snowdrop extracts (Table 6). This behaviour was in accordance with other previous reports [25, 26].

Table 6. Antioxidant activity of snowdrop extracts and AgNPs

Sample	AA %
Snowdrop flowers extract	78.102
Snowdrop stems extract	78.563
AgNPs- snowdrop flowers	91.471
AgNPs- snowdrop stems	91.932

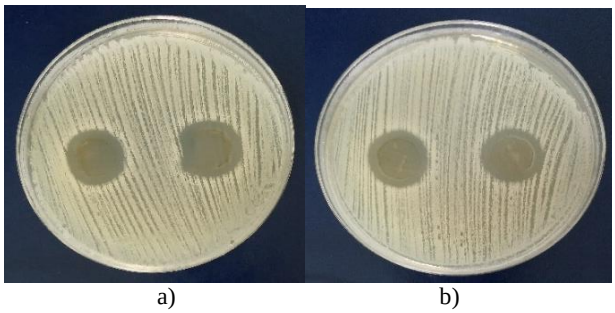
Antimicrobial activity results

The antimicrobial activity of silver nanoparticles obtained using plant extracts, is due to chemical constituents, such as alkaloids, polyphenols, saponins and essential oils [8, 26, 34, 35]. One of the aims of this research was to highlight the in vitro antibacterial and antifungal potential of silver nanoparticles obtained from snowdrop extracts. A qualitative method was used. After 24 h of incubation it was observed on the inoculated culture media, the presence of a clear zone (halo) around the spot area, both in the case of the AgNPs-snowdrop flowers sample and in the AgNPs-snowdrop stems suggesting that the tested samples possessed antimicrobial activity against the strains selected in the test. In the case of *Staphylococcus aureus*, the diameter of the inhibition zone, for AgNPs-snowdrop flowers (a) and and AgNPs-snowdrop stems (b) was 18 mm as shown in Fig. 8. For *Candida albicans* strain, the diameter of the inhibition zone in the case of the AgNPs-snowdrop flowers (a) and AgNPs-snowdrop stems (b) was 20 mm, as seen in Fig. 9.



a) b)

Fig. 8. Antimicrobial activity of AgNPs-snowdrop flowers (a) and AgNPs-snowdrop stems (b) against S. aureus



a) b)

Fig. 9. Antimicrobial activity of AgNPs-snowdrop flowers (a) and AgNPs-snowdrop stems (b) against C. albicans

Colorimetric analysis

The colorimetric analysis was performed for dried hazelnut tree wood and for hazelnut slices impregnated with AgNPs-snowdrop suspension (Table 7). Defined as a three-dimensional colour space system, CIE L*a*b* separates the colour information into lightness-darkness (L*), red-green (a*) and yellow-blue axes (b*) [13]. It could be observed that the AgNPs-wood was more stable than the wood untreated, or impregnated just with $AgNO_3$. A stabilization to weathering due to the space filling network formed on the wood with silver nanomaterials is responsible for this statement [23].

Table 7. Colorimetric results of hazelnut wood untreated and treated with AgNPs suspensions

Sample	Lx	ax	bx	ΔLx	Δax	Δbx	$\lvert\Delta bx\rvert$ ($b_{x\ treated}$-$b_{x\ untreated}$)	ΔEx	ΔE ($E_{x\ treated}$-$E_{x\ untreated}$)
hazelnut wood untreated	88.49	1.84	0.29	61.47	-0.34	0.33	-	61.47	-
hazelnut wood+$AgNO_3$ 0,01 M	87.55	1.89	0.15	50.53	-0.28	0.19	0.14	60.53	0.94
hazelnut wood-AgNPs-snowdrop flowers (SF)	78.39	1.90	0.13	49.36	-0.27	0.17	0.16	49.36	12.11
hazelnut wood-AgNPs-snowdrop stems (SS)	77.43	2.10	0.59	50.41	-0.08	0.63	0.3	50.41	11.06

Humidity tests

The samples were weight before umecting, then were kept 30 min in distilled water (W_t), 1h/60°C + 12h/room temperature (W_0). The results demonstrated the fact that in the presence of silver nanoparticles, a protective layer has been formed on the wood surface. The changes of the wood humidity are presented in Fig. 10, where it could be observed that presence of AgNPs strongly decreased the wood humidity.

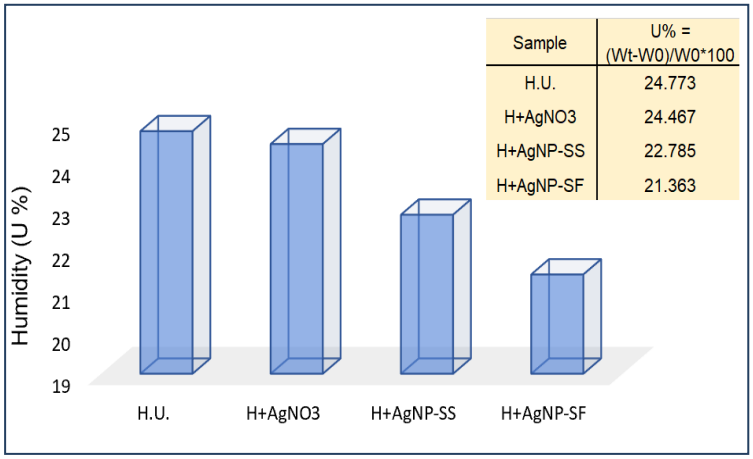
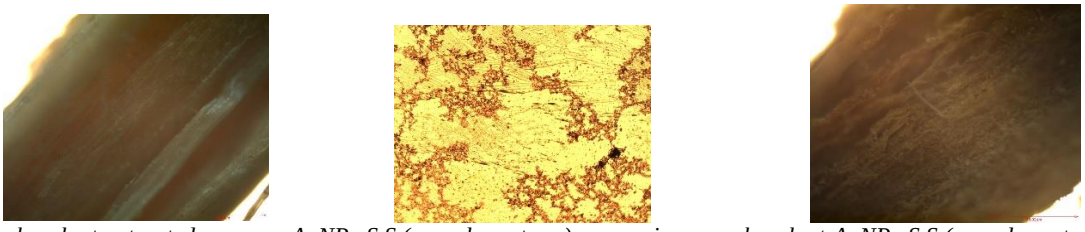


Fig. 10. The humidity changes of hazelnut wood in presence of silver nanoparticles
H.U.= hazelnut untreated; H=hazelnut wood treated; H+AgNP-SS =hazelnut+AgNP-snowdrop stems; H+AgNP-SF=hazelnut+AgNP-snowdrop flowers (color online)

Optical microscopy

The hazelnut wood samples untreated and treated with silver nanoparticles were visualized by optical microscopy (×60) and it were observed differences on the wood surface, before and after addition of AgNPs-snowdrop suspensions (Fig. 11). The hazelnut sample was immersed and kept in the AgNPs suspensions for 48 hours.



hazelnut untreated *AgNPs-S.S (snowdrop stems) suspension* *hazelnut-AgNPs-S.S (snowdrop stems)*

Fig. 11. Optical images of hazelnut wood untreated and treated with AgNPs-snowdrop stems (color online)

Hardness results

The wood tests were performed with a Silver Schmidt Hammer. The surface hardness for the treated samples (Table 8), indicates that the wood hardness is higher for the wood treated with 10 mL of AgNPs-plant samples.

Table 8. Surface hardness of hazelnut wood treated with AgNPs-snowdrop samples

Sample	Surface hardness (N/m^2)
hazelnut wood untreated	34.5
hazelnut wood + $AgNO_3$ 0,01 M	36.5
hazelnut wood - AgNPs snowdrop stems (SS)	43.0
hazelnut wood - AgNPs snowdrop flowers (SF)	44.5

4. Conclusions

Based on the literature data, the main objectives of the current study were the characterization of phytochemical compounds (flavonoids, polyphenols, etc) from *Galanthus nivalis L.* (flowers and stems) and the biosynthesis of AgNPs using snowdrop aqueous extracts (flowers and stems) and using UV-Vis, FTIR, DLS techniques and the evaluation of antioxidant and antibacterial activity for biosynthesized AgNPs.

The biosynthesis of silver nanoparticles using snowdrops is a simple, environmentally-friendly, low cost and non-toxic approach. The fabricated nanoparticles showed antimicrobial activity against *S. aureus* and *C. albicans.*

Development of nanoparticles was monitored by UV-VIS spectroscopy and the primary particle size was measured by DLS. DLS results indicated that all the particles are nano-sized.

FT-IR ATR analysis confirmed the bioreduction of Ag^+ ions to AgNPs by various functional groups, attributed to O-H, C-H, -C=O, C-O. It was demonstrated that the snowdrop plant aqueous extracts used to phytosynthesize silver nanoparticles, have strong reducing properties for "green synthesis" of AgNPs. The phytosynthesis of snowdrop-AgNPs was observed first time by the change of sample colours, from yellow to brown. Next step was the confirmation obtained by spectral analyses (UV-VIS, DLS and FT-IR ATR spectroscopy) that revealed the presence of the polyphenols and proteins in the plant extract, bioactive compounds responsible for bioreduction of silver ions and for stabilization of AgNPs.

UV-VIS analysis revealed the formation of AgNPs, by the presence of specific absorption peak at 470 nm. The antioxidant properties determined by DPPH method, showed that silver nanoparticles exhibited high values of antioxidant activity as compared to aqueous extracts. PdI values indicated polydisperse populations of AgNPs. The bio-reduction of aqueous Ag^+ ions by the snowdrop extract has been demonstrated. This ecofriendly synthesis of silver nanoparticles has many advantages as an easy way to obtain AgNPs and to save material resources. Silver nanoparticles obtained using the *Galanthus nivalis* flowers and stems extracts, were applied to hazelnut wood in order to obtain a method of preserving wooden objects using simple and environmentally-friendly and low-cost method. The tested extracts had both the ability to inhibit the growth of the bacterial strain and the growth of the tested yeast strain. The formation of a clear area of inhibition shows a good antimicrobial activity against the tested strains.

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