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Hybrid Nanoaggregates from Plant-Based Noble Metallic Nanoparticles and Functionalized Macrocyclic Derivatives †

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Abstract: Noble metallic nanoparticles, silver (AgNPs) and gold (AuNPs) mainly, exhibit good antimicrobial, antibacterial, and antifungal properties and, therefore, have a significant contribution to the constant growing field of nanomedicine. They can be synthesized using both conventional or unconventional methods and, in the last decades especially, the unconventional routes that use plants as raw vegetal materials are studied with proven results. On the other hand, macrocyclic derivatives such as phthalocyanines (Pcs) have photoactive properties and numerous applications. The conjugation between silver and gold nanoparticles with phthalocyanine derivatives considerably increases both the photochemical activity of Pcs as well as the stability of noble metallic nanoparticles. This paper describes recent research in the field of green-synthesized AgNPs and AuNPs from different plants with important pharmacological applications in two different temperature conditions: at room temperature, for 12 h, and at 50 °C for 30 min. AgNPs and AuNPs then react with tetracarboxamido-zinc phthalocyanine $ZnPc(CONH_2)_4$ and octacarboxamido–zinc phthalocyanine $ZnPc(CONH_2)_8$ to obtain two hybrid nanoaggregates, confirmed by spectroscopic analyses and by determining their antioxidant and antimicrobial activity.

Keywords: nanoaggregates; metallic nanoparticles; green synthesis; macrocyclic compounds

1. Introduction

Noble metallic nanoparticles, silver (AgNPs) and gold (AuNPs) especially, exhibit novel and unique properties due to their reduced size, characteristic morphology and distribution, and, therefore, are of special importance in the constant growing scientific field of nanotechnology [1]. AgNPs and AuNPs have antimicrobial and antioxidant activity that makes them excellent candidates for different applications in medicine (photodynamic therapy, implantology), pharmacy (minimize the toxicity of different drugs), and diagnosis (cell bioimaging, molecular diagnosis) [2].

Both AgNPs and AuNPs are obtained via conventional and unconventional methods, the main disadvantage of the conventional routes being that they involve toxic chemicals, require high energy consumption and hazardous secondary products are obtained [3]. As a consequence, unconventional methods based either on different microorganisms (bacteria, fungi) or on plant extracts are constantly gaining importance and are intensively studied [4]. Plant-based methods are preferred to other green synthesis methods, because they are the most affordable living organism, are easy to procure, and

they react with either silver nitrate ($AgNO_3$) to form AgNPs or tetrachloauric acid ($HAuCl_4$) to form AuNPs [5].

Sea buckthorn (*Hippophae rhamnoides* L.), fruit packed with vitamins (especially A and C), also known as "Romanian ginseng", reduces oxidative stress, protects the brains, boosts the immune system, protects the liver, etc. [6]. The edible fruits of European cornel (*Cornus mas*) are a significant source of natural antioxidants (e.g., polyphenols, anthocyanins, flavonoids, tannins, etc.) and are also a very good natural source of vitamin C, and, therefore, are used to treat bronchitis, urinal infections, and helps retain fluids [7]. Peony (*Paeonia officinalis*), a "super flower" according to many specialists, has the ability to help ease numerous medical issues ("peony" in Greek culture originates from the god Paean, the god of healing) [8].

Phthalocyanines (Pcs), compounds with good thermal stability and proven photoactive capacity, are versatile macrocycles able to tailor multiple surfaces [9]. The hybrid nanoaggregates formed with AgNPs and AuNPs increase the catalytic activity of Pcs, especially after irradiation with UV light.

This paper describes recent research in the field of green-synthesized AgNPs and AuNPs from different plants with important pharmacological applications in two different temperature conditions: at room temperature, for 12 h, and at 50 °C for 30 min. AgNPs and AuNPs then react with tetracarboxamido-zinc phthalocyanine $ZnPc(CONH_2)_4$ and octacarboxamido–zinc phthalocyanine $ZnPc(CONH_2)_8$ to obtain two hybrid nanoaggregates, confirmed by spectroscopic analyzes and by determining their antioxidant and antimicrobial activity.

2. Materials and Methods

2.1. Materials

Tetrachloroauric acid ($HAuCl_4$), DPPH, (2,2-diphenyl-1-picryl-hydrazyl-hydrate stable free radical), hydrochloric acid (HCl), sulphuric acid (H_2SO_4), Benedict reagent, phthalonitrile and glacial acetic acid (CH_3COOH) were purchased from Sigma-Aldricht. Ethanol (C_2H_5OH) was purchased from Scharlau, and silver nitrate ($AgNO_3$) from ChimReactiv. Sea buckthorn was purchased from the local natural shop "Plafar" and used as such while European cornel and Peony were purchased from the local market and dried in the laboratory. The two phthalocyanine derivatives were chemically synthesized using an original method.

2.2. Preparation of Aqueous Plant Extracts

Sea buckthorn, European cornel, and Peony were used to prepare aqueous extracts following the same protocol: 25 g of plant were weighted, transferred into an extractor and to that 250 mL of distilled water were added; the mixture was left for 24 h, at 4 °C, to extract until all intracellular material was infused and the aqueous extract was thoroughly filtered.

2.3. Green Synthesis of Noble Metallic Nanoparticles

A 10^{-3} M aqueous solution of metallic salt ($AgNO_3$ or $HAuCl_4$) was mixed with either of the three aqueous extracts using two different temperature conditions: room temperature, in the dark for 12 h, or at 50 °C for 30 min under a continuous stirring of 600 rpm.

2.4. Synthesis of Hybrid Nanoaggregates from Green Metallic Nanoparticles and Phthalocyanine Derivatives

The two phthalocyanine derivatives tetracarboxamido-zinc phthalocyanine $ZnPc(CONH_2)_4$ and octacarboxamido–zinc phthalocyanine $ZnPc(CONH_2)_8$ were chemically synthesized and used for the synthesis of two hybrid nanoaggregates as follows: the green-synthesized AgNPs and AuNPs were dried, a specific amount was weighted and mixed for 96 h under continuous stirring with a solution of phthalocyanine in dimethylsulphoxide (DMSO).

2.5. Characterization Methods

The absorption spectra were recorded using a M 400 Carl Zeiss Jena UV—Vis spectrophotometer in the wavelength range of 210–800 nm. Fourier transform infrared spectroscopy (FTIR) spectra were recorded using a Vertex 80 FT-IR spectrometer in the range of 8000–400 cm^{-1}. Dynamic light scattering (DLS) spectra were recorded using a Zetasizer Nano SZ—Malvern instrument with a computer connected/equipped with preinstalled Zetasizer software. Antioxidant activity (AA, %) was tested using a standard method: a DPPH solution was prepared in ethanol and 0.5 mL aqueous extract was mixed with 1 mL 0.02 mg/mL DPPH solution. The resulted mixtures were tested by recording and marking the absorbance at 517 nm [10]. The antioxidant activity was calculated according to the formula:

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

where $A_{Control}$ is the absorbance of the blank DPPH solution, and A_{Sample} is the absorbance of the aqueous extracts mixed with 0.02 mg/mL DPPH solution.

The antibacterial activity was determined using the disk-diffusion method [11]. The data obtained were compared to the ones obtained from antibiotics. The microorganisms were either isolated in medical facilities from different patients (*Escherichia coli*, *Bacillus subtilis*) or were purchased from DSMZ collection (*Candida rugosa*).

3. Results and Discussions

3.1. Antioxidant Activity

The results obtained for the AA (%) using the DPPH method are presented in comparison between all the aqueous extracts and their corresponding noble metallic nanoparticles (Tables 1 and 2).

Table 1. Antioxidant activity of aqueous extracts and AgNPs.

Aqueous Extract	AA (%) of Aqueous Extracts	AA (%) AgNPs Room Temperature	AA (%) AgNPs 50 °C
Sea buckthorn	81, 63	91, 92	92, 59
European cornel	80, 52	90, 49	91, 25
Peony	81, 55	89, 78	90, 09

Table 2. Antioxidant activity of aqueous extracts and AuNPs.

Aqueous Extract	AA (%) of Aqueous Extracts	AA (%) AuNPs Room Temperature	AA (%) AuNPs 50 °C
Sea buckthorn	81, 63	86, 56	87, 95
European cornel	80, 52	85, 69	86, 98
Peony	81, 55	84, 87	85, 83

Comparing the results presented in Table 1, it can be concluded that all the values for the antioxidant activity of AgNPs are considerably higher than the ones measured for the aqueous extracts. AA (%) for AgNPs—Sea buckthorn has the highest value (92, 59%) in the case of AgNPs green-synthesized at 50 °C, followed by AA (%) of AgNPs—European cornel and AgNPs—Peony that were also green-synthesized at 50 °C. The values for AgNPs green-synthesized at room temperature are slightly lower but, whatever the temperature conditions, it is clear that the values for AA (%) are higher for AgNPs than those recorded for the aqueous extracts. In the case of green-synthesized AuNPs, the recorded values for the AA (%) are lower than the corresponding AgNPs. AuNPs—Sea buckthorn has the highest value (87, 95%) at 50 °C, with a slight decrease for those green-synthesized at room temperature.

3.2. Ultraviolet–Visible (UV–Vis) Results

UV–Vis spectra were recorded for all three aqueous extracts in the range of 210–600 nm. as a general conclusion, the absorptions recorded at approximately 270 nm and 370 nm for all the aqueous extracts can be ascribable to phenolic acids and their derivates (flavones, quinones) (Table 3).

Table 3. UV–Vis absorptions for aqueous extracts.

Aqueous Extract	Phenolic Acids	Flavonoids
Sea buckthorn	275 nm	365 nm
European cornel	278 nm	368 nm
Peony	274 nm	371 nm

The bioreduction of silver and gold ions can be first easily observed by the visual change in color of the resulted colloidal solutions and then confirmed by the UV–Vis spectra. As a general rule, the maximum absorption for AgNPs is between 440 and 460 nm (Table 4) and for AuNPs is in the region of 520–545 nm (Table 5). The colour for AgNPs ranges from light brown to grey-brown (depending on the size of the green-synthesized AgNPs) and for AuNPs from cherry red to violet-red.

Table 4. UV–Vis absorptions for the green-synthesized AgNPs.

Aqueous Extract	AA (%) AgNPs Room Temperature	AA (%) AgNPs 50 °C
Sea buckthorn	445 nm	448 nm
European cornel	450 nm	449 nm
Peony	453 nm	457 nm

Table 5. UV–Vis absorptions for the green-synthesized AuNPs.

Aqueous Extract	AA (%) AuNPs Room Temperature	AA (%) AuNPs 50 °C
Sea buckthorn	520 nm	522 nm
European cornel	531 nm	535 nm
Peony	541 nm	545 nm

UV–Vis spectra were also recorded for the two phthalocyanine derivatives (Table 6). Also, preliminary UV–Vis spectra were recorded for the nanoaggregates chemically synthesized from octacarboxamido–zinc phthalocyanine and green-synthesized AgNPs.

Table 6. Characteristic absorptions for ZnPc derivatives.

Compound	λ/ε (nm/mol·cm^{-1})
Tetracarboxamido-zinc phthalocyanine	337/2,1487; 610/2,8470; 674/2,8941
Tetracarboxi-zinc phthalocyanine	325/3,1907; 610 (*aggregation appears, on-going studies)/1,6778; 662 */3,2555
Octacarboxamido-zinc phthalocyanine	628/3,0240; 653/3,1140, 700/3,0820
Octacarboxi-zinc phthalocyanine	353/7,949; 626/4,057; 696/2,0690

3.3. Fourier Transform Infrared Spectroscopy (FTIR) Results

The FTIR spectra allows the identification of functional groups at specific wavelengths. FTIR spectra showed specific peaks for Sea buckthorn and European cornel that appeared as bands situated at 3337 cm^{-1} (S. buckthorn) and 3325 cm^{-1} (E. cornel) assigned to hydroxyl (-OH) groups. The

band from 2948 cm^{-1} (S. buckthorn) and 2945 cm^{-1} (E. cornel) is specific to methine (-CH) groups, while the bands C=C and C=O were easily identified at 1590 cm^{-1} and 1453 cm^{-1} (S. buckthorn), respectively, 1586 cm^{-1} and 1458 cm^{-1} (E. cornel). The aromatic amide I and amide II groups were found in the range of 1386 cm^{-1} and 1321 cm^{-1} (S. buckthorn), respectively, 1385 cm^{-1} and 1320 cm^{-1} (E. cornel). The C=O groups specific for esters, catechins, and type III amides were situated between 1262 and 1120 cm^{-1} (S. buckthorn), respectively, 1265–1126 cm^{-1} (E. cornel). Specific bands between 1500 and 1300 cm^{-1} were attributed to amides, proteins, and enzymes that contribute to the reduction of Ag ions. All the green-synthesized AgNPs exhibited FTIR bands attributed to polyphenols in the range of 1655 cm^{-1} and 1659 cm^{-1}. In the FTIR spectra recorded for Peony–AgNPs, the peaks at 3335 cm^{-1} were assigned to hydroxyl (-OH) groups and the band at 2945 cm^{-1} is specific for methine (-CH). The bands C=C and C=O were identified at 1588 cm^{-1} and 1455 cm^{-1}. The aromatic amide I and amide II were found in the range of 1388 cm^{-1} and 1323 cm^{-1}. The C=O groups characteristic for esters, catechins, and/or type III amides were found between 1262 and 1125 cm^{-1}. Specific bands between 1500 and 1297 cm^{-1} were attributed to amides, proteins, and enzymes that ease the reduction of metal ions. All the green-synthesized AgNPs exhibited specific FTIR bands for polyphenols in the range of 1650 cm^{-1} and 1659 cm^{-1}.

FTIR spectra were also recorded for the phthalocyanine derivatives (Table 7).

Table 7. FTIR characteristic absorptions for ZnPc derivatives.

Compound	Absorption (cm^{-1})
Tetracarboxamido-zinc phthalocyanine	3432, 3160, 1657, 1651, 1613, 1568, 1522, 1384, 1322, 1150, 1085, 1056, 940, 741, 718
Tetracarboxi-zinc phthalocyanine	3432, 1704, 1695, 1615, 1589, 1522, 1490, 1404, 1380, 1334, 1276, 1148, 1087, 1059, 917, 943, 741
Octacarboxamido-zinc phthalocyanine	3283, 3172, 3031, 1768, 1653, 1457, 1375, 1304, 1153, 1057, 944, 896, 754, 721, 634, 560
Octacarboxi-zinc phthalocyanine	3499, 3322, 2790, 1700, 1655, 1582, 1506, 1426, 1296, 1254, 1115, 1081, 925, 797, 728, 619, 549

3.4. Dynamic Light Scaterring (DLS) Results

All the green-synthesized AgNPs were analyzed using dynamic light scattering measurements (Table 8).

Table 8. DLS and seta potential for the green-synthesized AgNPs.

Crt. No.	Dm (d.nm)	P_1...i (d.nm)	PdI	PZ (mV)
AgNPs—Sea buckthorn	61	$P_1 = 91$; $P_2 = 11$	0.297	−20.5
AgNPs—European cornel	644	$P_1 = 474$; $P_2 = 72$	0.551	−26.3
AgNPs—Peony	3686	$P_1 = 1492$; $P_2 = 123$	0.543	−15.4

4. Conclusions

This paper describes recent research studies of the green synthesis of noble metallic nanoparticles (AgNPs and AuNPs), in two different temperature conditions, from three different plants (Sea buckthorn, European cornel, and Peony). Also, two phthalocyanine derivatives, tetracarboxamido-zinc phthalocyanine and octacarboxamido–zinc phthalocyanine, were chemically synthesized and their UV–Vis and FTIR characterization showed the presence of different functional groups. Antioxidant activity of both aqueous extracts and noble metallic nanoparticles was measured and the results clearly proved an increased value for the green-synthesized AgNPs compared to both aqueous extracts and green-synthesized AuNPs.

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