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# Gold and silver geranium biocomposites

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**ABSTRACT**

Gold and silver nanoparticles green synthesized using an aqueous extract of geranium (*Pelargonium graveolens*) leaves were embedded in cholesterol-containing dipalmitoyl phosphatidylcholine lipid vesicles by a simple eco-friendly method, resulting in bio-based composites with antioxidant and antibacterial properties. The artificial lipid bilayers were loaded with chlorophyll *a*, in order to get a deeper insight into the biocomposite formation monitored by fluorescence emission spectroscopy. These silver and gold-based biohybrids exhibited good stability and antioxidant activity. Antimicrobial investigations against *Escherichia coli* ATCC 8738 revealed that these bio-based composites were the most potent biocides.

**KEYWORDS**

Liposomes; chlorophyll; gold and silver nanoparticles; geranium; antioxidant and antimicrobial activity; biocomposites

## 1. Introduction

"Green" nanotechnology has recorded a rapid progress in the last years because it keeps clean the environment by using eco-friendly methods and raw materials resulting in safe recyclable wastes. A special interest is given to metal nanoparticles (MNPs) owing their interesting properties (enhanced absorption of visible light, antimicrobial activity) caused by the large surface-to-volume ratio of the particles, and thus they could have many applications in different domains [1, 2]. Vegetal extracts are often used to produce MNPs, the plants being secure and cheap raw materials, which are found in abundance in nature. Special attention of scientists was focused on the phyto-synthesis of MNPs as carriers for different phyto-active molecules, thus can be used in biomedical field [2–7].

Our previous work [8] demonstrated that ornamental plants can be used to biosynthesize noble metal nanostructures with antioxidant activities. One of such decorative plant is *Pelargonium graveolens* (Geranium), name given by the botanist Charles Louis L'Héritier de Brutelle (1746–1800), due to the similarity of its capsules to a stork's bill [9, 10].

*Pelargonium graveolens* (*P. graveolens*), a perennial flowering plant belonging to the Geraniaceae family, is well known for the special fragrance of leaves and its use as an aromatic and as a medicinal plant or insect repellent. This herb could be used in cosmetics and in food,

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alcohol and beverage industry, and in biomedical field to treat many diseases: diabetes, diarrhea, dysentery and colic, gallbladder problems, gastric ulcers, liver problems, inflammations, skin disorders, depression, anxiety and panic, blood disorders, cancer etc. [11–13].

In 2003, Shankar and co-workers reported for the first time, the biosynthesis of silver [14] and gold nanoparticles [15] from Geranium leaves. The aqueous leaf *Pelargonium* extract was used also by other scientists, as a bio-reducing agent for fabrication of MNPs [16].

*Pelargonium graveolens* leaves contain many active constituents responsible for the reduction of noble metal ions: proteins, alkaloids, flavonoids etc., which work also as capping agents that form a layer covering the metal nanoparticles and thus stabilizing them [16, 17].

This paper presents for the first time, a "green" approach to build-up biocomposites based on chlorophyll-loaded biomimetic membranes and noble metallic nanostructures. In our study, an aqueous extract of geranium (*Pelargonium graveolens*) was employed for gold (GG) and silver (GS) nanoparticle synthesis. The resulted bio-based composites were characterized by fluorescence emission spectroscopy using chlorophyll *a* (Chl*a*) as a spectral marker. This photo-pigment was successfully used in our previous research [18–21]. An antimicrobial investigation of the samples was performed against *Escherichia coli* ATCC 8738. The stability of the samples was checked by zeta potential measurements and their antioxidant activity was evaluated by chemiluminescence method.

## 2. Materials and methods

### 2.1. *Materials*

Tris (hydroxymethylaminomethane base), luminol (5-amino-2,3-dihydro-phthalazine-1,4-dione), HCl, $H_2O_2$, $KH_2PO_4$, and $Na_2HPO_4$ were supplied from Merck (Germany). Silver nitrate ($AgNO_3$), $HAuCl_4$, dipalmitoyl phosphatidylcholine (DPPC) and cholesterol (Chol) were purchased from Sigma Aldrich (Germany).

Chlorophyll *a* (Chl*a*) was extracted from fresh spinach leaves as previously described [22].

The antibacterial activity was tested against *Escherichia coli* ATCC 8738 bacteria. *E. coli* was grown in Luria Bertani Agar, LBA, plates at 37°C containing: peptone (Merck) 10 g/L, NaCl (Sigma-Aldrich) 5 g/L, yeast extract (Biolife) 5 g/L, and agar (Fluka) 20 g/L.

### 2.2. *Preparation of noble metal nanoparticles*

Silver and gold nanoparticles were bio-synthesized by mixing equal volumes of geranium leaf aqueous extract with silver nitrate solution (1mM) and, respectively, $HAuCl_4$ solution (1mM), following the procedure described in reference [23].

### 2.3. *Preparation of liposomes*

Cholesterol-containing dipalmitoyl phosphatidylcholine multilamellar lipid vesicles (Chol-DPPC-MLVs, sample LB) were prepared by hydrating the thin lipid film (Chol/DPPC molar ratio = 1/4) with a phosphate buffer solution (PB), $KH_2PO_4$–$Na_2HPO_4$ pH 7.4, as previously described [20, 24]. The preparation procedures were performed above the critical temperature ($T_c$) of phase transition of lipids resulting in liquid crystalline artificial lipid bilayers. During the lipid film preparation, the artificial lipid bilayers were marked with Chl*a* as a spectral marker, to monitor the formation of biocomposites.

**Table 1.** The codes of the samples prepared and tested.

| Sample | Code |
|---|---|
| Chl*a*-Chol-DPPC-MLVs | LB |
| Geranium-AgNPs | GS |
| Geranium-AuNPs | GG |
| Geranium-AgNPs / Geranium – AuNPs mixture | GGS |
| Geranium-AgNPs - Geranium AuNPs/ Chl*a*-Chol-DPPC-MLVs bio-composites | BH |

### 2.4. *Preparation of liposomes/MNPs biocomposites*

An appropriate amount of AuNPs (GG) and AgNPs (GS) has been added to lipid vesicle suspensions, in a final concentration of 1.5% each, related to the liposomal suspension volumes, and the resulted mixture was subjected to ultrasound irradiation using a titanium probe sonicator (Hielser, UP 100 H), giving rise to bio-composites BH.

In Table 1 are listed the samples prepared and tested.

### 2.5. *Characterization methods*

The absorption spectra of samples were recorded on a double beam Lambda 2S Perkin Elmer spectrophotometer, in 200–800 nm wavelength range, operated at a resolution of 1 nm.

The fluorescence emission spectra of Chl*a* in artificial lipid membranes and in biocomposites were collected on a LS55 PERKIN-ELMER spectrophotofluorimeter, at 600 – 700 nm, by illuminating the samples with a 430 nm excitation.

The dimension of metallic nanoparticles was estimated by Dynamic Light Scattering (DLS) technique (Zetasizer Nano ZS, Malvern Instruments Ltd., U.K.), as hydrodynamic diameters, $Z_{average}$ (the particle diameter plus the double layer thickness), at 25°C temperature and at a scattering angle of 90°. For each sample, the particle size analysis data were evaluated using the intensity distribution, and the mean diameter and the polydispersity index (PdI) were calculated from three individual measurements, thus the mean values (±standard deviation, SD) will be further reported.

The physical stability of each sample was estimated by zeta potential (ZP) values measured using an appropriate dispositive of Zetasizer Nano ZS (Malvern Instruments Ltd., U.K.) by applying an electric field across the analyzed aqueous suspensions; all these measurements were performed in triplicate and the mean values (± SD) were reported.

The *in vitro* antioxidant activity of the samples has been determined by chemiluminescence (CL) method on a Chemiluminometer Turner Design TD 20/20, USA, using luminol/$H_2O_2$ in Tris-HCl alkaline buffer pH 8.6 as a generator of free radicals. The mean values of antioxidant activities (AA %) were obtained from three different measurements using the equation (1):

$$AA = [(I_0 - I)/I_0] \cdot 100\% \quad (1)$$

where $I_0$ is the maximum CL intensity for standard at t = 5 s and $I$ is the maximum CL intensity for each sample at t = 5 s [25].

The agar disc diffusion method [26, 27] was used to investigate the antimicrobial activities of the samples against *Escherichia coli* ATCC 8738 bacterium. The wells were made according to the number of samples (using a sterile Durham tube 6 mm diameter) and then were loaded with 50 $\mu$L of each sample. The pure solvent (PB) was used as a control. All the test plates were incubated at 37°C for 24 h to allow microbial growth. The antimicrobial activity was

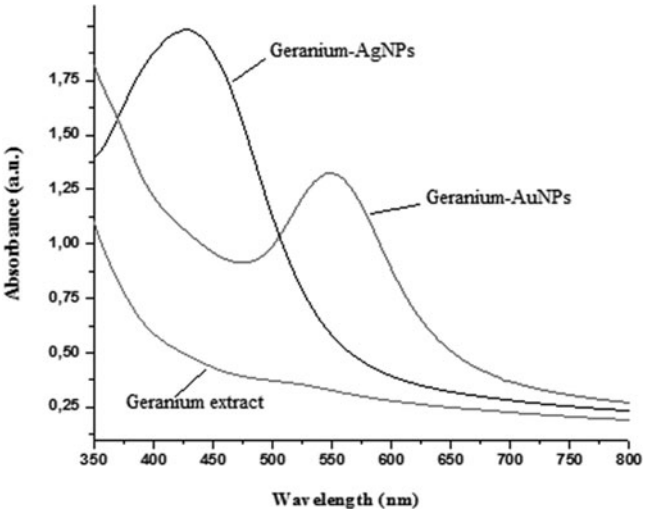


**Figure 1.** The Vis absorption spectra of Geranium extract, Geranium-AgNPs and Geranium-AuNPs.

evaluated by measuring the zone of inhibition (ZOI) against test organism. Experiments were performed in triplicate.

The experiments were carried out in dark to avoid the sample photo-damage.

## 3. Results and discussion

### 3.1. *Monitorization of noble metal nanoparticle phyto-synthesis by visible absorption spectroscopy and DLS measurements*

The Vis absorption spectroscopy was used to monitor the phytoformation of silver and gold nanoparticles from geranium leaf extract (Figure 1).

The phyto-synthesis of noble metallic nanoparticles was confirmed by the appearance of a peak at 430 nm in the spectrum of *Geranium*-AgNPs, characteristic for the silver nanoparticle formation [28–30] and a peak at 550 nm in the spectrum of *Geranium*-AuNPs, characteristic for the synthesized gold nanoparticles [15].

Given these aspects, and the fact that these bands does not appear in the spectrum of the *Geranium* extract, we can conclude that these peaks are assigned to the SPR bands of the noble metal nanoparticles.

The biosynthesis of nano-scaled metallic particles was checked also by DLS measurements. As illustrated in Figure 2, geranium-AgNPs exhibited a mean diameter of 59 nm, geranium-AuNPs an average size of 61 nm, and for biohybrid, the mean particle size was 312 nm.

### 3.2. *The fluorescence emission spectra of Chla in biocomposites*

The biocomposite formation was monitored by fluorescence analysis, as depicted in Figure 3.

The emission peak of Chl*a* incorporated in liposomes is located at the same wavelength as in biocomposites, at 680 nm. The fluorescence intensity decreased by 13.45% in the case of biohybrids, due to the interaction of porphyrin ring of Chl*a* (inserted in artificial lipid membranes as a fluorescence marker) with the noble metal nanoparticles. This fluorescence quenching is in agreement with other scientific studies [31].

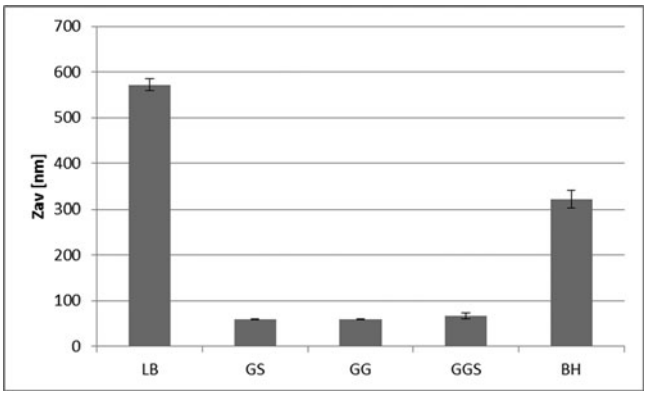


**Figure 2.** Mean particle size of geranium nanometal-based samples.

### 3.3. *Zeta potential of the liposomes and biocomposites*

The physical stability of the biocomposites was estimated in terms of ZP. As it can be observed in Figure 4, all the samples carried a negative electric charge, so it is necessary a great negative ZP value to ensure the stability of a suspension. The multilamellar lipid vesicles exhibited poor stability (ZP = −19 mV) and the geranium-metallic nanoparticles a moderate stability in time (ZP = −26.7 mV for AgNPs and −22.2 mV for AuNPs). In order to enhance their stability, the geranium-MNPs were stabilized with the artificial lipids membranes, LB. The biocomposites BH showed a good stability (ZP = −30 mV) as compared to lipid vesicles and MNPs alone.

### 3.4. *Evaluation of antioxidant properties of the bio-based composites*

As depicted in Figure 5, the lipid vesicles (sample LB) presented weak antioxidant properties (30%); their antioxidant action is due to the presence of the chlorophyll photo-pigment. The biogenic silver and gold nanoparticles exhibited good antioxidant activities: 64% (GS) and,

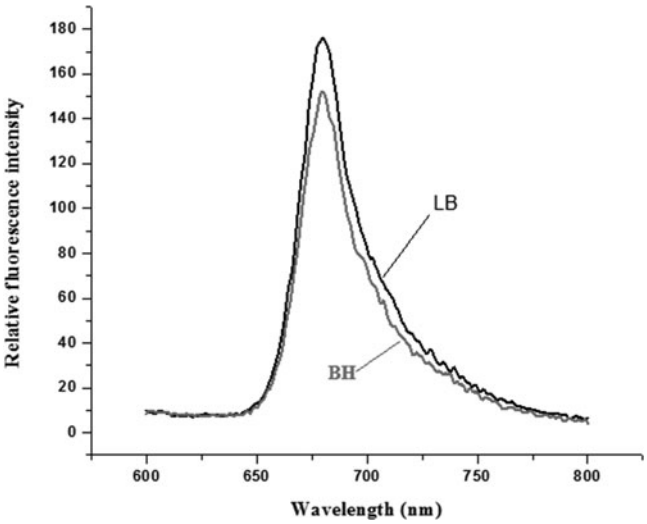


**Figure 3.** Fluorescence emission spectra of Chl*a* in liposomes and in biocomposites (the excitation wavelength was 430 nm).

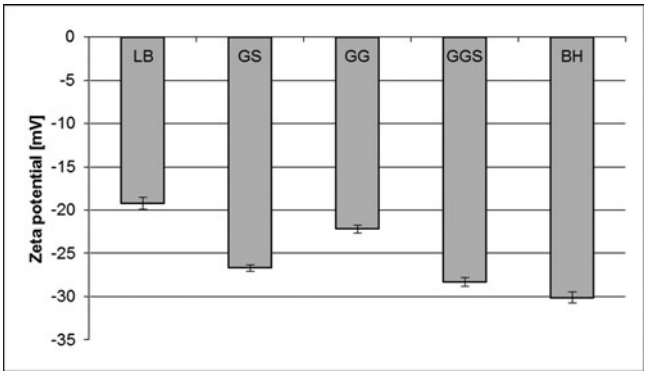


**Figure 4.** Evaluation of physical stability of the samples.

respectively, 59% (GG), highlighting the role and effectiveness of nanoparticle-based extract of geranium plant to capture free radicals. The mixture of GS (Geranium-AgNPs) and GG (Geranium-AuNPs) exhibited an improvement of AA%, reaching the value of 72% (see sample GGS). The most effective free radical scavenging system proved to be the biocomposite BH showing an antioxidant activity of 85%.

### 3.5. *Antimicrobial activity of geranium biocomposites*

The antimicrobial investigations were performed on the Gram-negative bacterium *Escherichia coli* chosen for our study because recently this one became more investigated, having new forms and can cause various diseases [32].

Figure 6 shows comparatively, the biocidal properties of the samples against the microorganism *E. coli* ATCC 8738. As it can be seen, the gold and silver work synergically, so the sample GGS (a mixture of phyto-AgNPs and phyto-AuNPs) exhibited a diameter of inhibition zone of 18 mm, while GS and GG presented ZOI diameter of 13 and 11 mm, respectively.

In the case of biocomposites BH, the antibacterial efficiency increased as compared to the mixture phyto-AuNPs-AgNPs (sample GGS); the sample BH proved to be the most potent against tested microorganism, showing inhibition zone of 20 mm. The lipid components of BH can easy fuse with membrane of bacteria cells, perturbing them, and then inserting their content in MNPs, inside the bacteria.

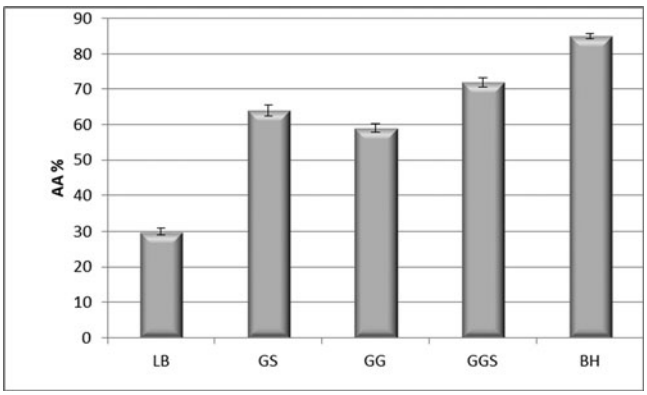


**Figure 5.** The antioxidant properties of the samples.

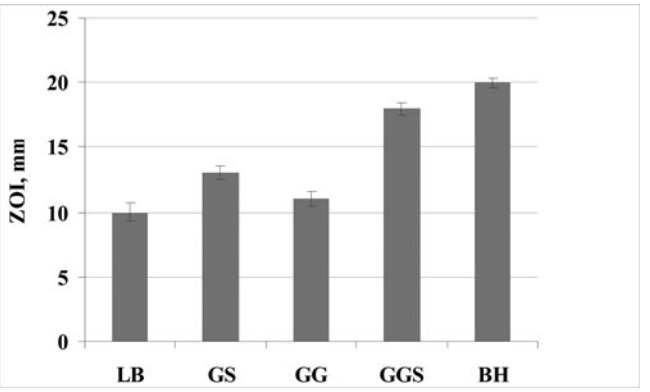


**Figure 6.** The antibacterial activity (evaluated by measuring the zone of inhibition, ZOI) of the samples, against *Escherichia coli* ATCC 8738 bacterium.

The results of antimicrobial investigations are in agreement with those obtained from chemiluminescence experiments.

## Conclusion

A simple and low-cost green *bottom-up* approach was developed to achieve biocomposites based on artificial lipid bilayers and noble nano-metals. Gold and silver nanoparticles were phyto-synthesized using an aqueous extract from geranium (*Pelargonium graveolens*) leaves.

Chlorophyll *a* inserted in artificial lipid bilayers proved to be an excellent spectral tool to rapidly detection of biocomposite formation.

A good correlation between the results of chemiluminescence and antibacterial assays has been observed.

Geranium-based bio-hybrids, BH, presented good antioxidant activity (AA = 85%) and the best antimicrobial properties (offering the highest inhibition zone of 20 mm against *E. coli*) and a good physical stability (ZP = −30 mV).

These results open the door for a new generation of bio-materials based on liquid crystalline lipid membranes and phyto-nanometals, with antioxidant and antimicrobial properties.

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