Photophysical aspects regarding the effects of *Paeonia officinalis* flower extract on DNA molecule labelled with methylene blue

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This paper reports *in vitro* studies of bio-performances of an aqueous extract of *Paeonia officinalis* fresh petals and its interaction with methylene blue/ deoxyribonucleic acid (MB/DNA) complex. Peony extract was characterized by spectral methods (UV-Vis absorption and FTIR-ATR spectroscopy). The phytoextract exhibited good antioxidant and antibacterial properties. Spectral investigations (UV-Vis absorption and emission spectra) on DNA stained with methylene blue as an optical probe, revealed that the peony extract replaced the dye in the biomolecule. The results could be exploited as an ecological strategy to design novel materials based on DNA and natural extracts.

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1. Introduction

In the last years, the ecological methods using the natural materials, gained a huge interest in the scientific world, due to their safety, biodegradability, low-cost and high abundance in nature. From this point of view, there are some interesting topics in the spotlight of scientists, regarding the use of vegetal raws for obtaining biomaterials with applications in optoelectronics and biophotonics [1-3], nanomedicine [4], agriculture [5], in cosmetics [6] or to inhibit the carbon steel corrosion in acidic media [7-8]. Recently, the scientists focused on novel economic and ecological concepts to remove different pollutants from wastewater. A most common pollutant arising from industrial processes is methylene blue (MB), a planar, cationic dye molecule, belonging to the phenothiazinum family of compounds, with wide range of applications, especially as a photosensitizer in optoelectronic devices, solar cells [9], photodynamic therapy [10], in genetics as stain for nucleic acids, in microbiology, surgery, diagnostics, or paint production and wool dyeing [11]. MB is an attractive low-cost and efficient dye, but its consumption may cause difficulty in breathing, mental disorder, vomiting, diarrhea, nausea, eye burning [11]. The liquid effluents containing dyes arising from industrial processes, result in major environmental problems. Thus, Lorkit and co-workers prepared eco-friendly TiO_2/ activated carbon double-layered film with enhanced photocatalytic activity towards methylene blue degradation [12]. Other researchers synthesized Cu/ZnO nanorods for photocatalytic degradation of methylene blue [13].

The aim of this paper is the removal of methylene blue from deoxyribonucleic acid (DNA) molecule and its replacing by an aqueous extract of *Paeonia officinalis* (the common peony) flowers, to achieve DNA-plant extract complexes with potential applications in photonics.

Since its discovery, DNA, *the informational molecule of life*, continues to stimulate the interest of scientific world. Its unique 3D structure consisting of a double helix offers many exciting applications in various fields, especially medicine, nanotechnology, photonics, organic electronics [1], or to the development of DNA-based bio-lasers [14]. It was shown also that chromophore doped DNA can be used to achieve electrochromic devices [2]. Moreover, Manea and co-workers [15] reported the preparation of complexes of DNA with green tea extract, for photonics application.

The flowers represent a rich source of bioactive compounds with antioxidant, anti-inflammatory, antitumor or antimutagenic properties [16, 17]. In addition, there are some encouraging results on the use of ornamental flowers in culinary arts [16, 18]. An attractive ornamental plant usually occurred in Romanian gardens is *Paeonia officinalis* L., belonging to the family of *Paeoniaceae*. The study of its benefits on human health are still a challenge. Peony was used as a therapeutic agent in liver diseases, including hepatitis C [19], kidney injury [20], as anti-inflamatory [21], and recently for the treatment of multiple sclerosis [22]. *Paeonia officinalis* has wonderful flowers rich in many active phyto-compounds: tannins,

monoterpene ester glucosides, proteins, sugars, organic acids, phenolics, flavonoids [19, 23] with high therapeutic potential, but insufficiently exploited.

This paper reports also *in vitro* studies of bio-performances of an aqueous extract of fresh petals of peony flowers, and spectral investigation of its effect on MB/DNA complex.

2. Experimental part

2.1. Materials

Tris (hydroxymethylaminomethane base), hydrochloric acid (HCl), hydrogen peroxide (H_2O_2), luminol (5-amino-2,3-dihydro-phthalazine-1,4-dione), potassium dihydrogen phosphate (KH_2PO_4), sodium hydrogen phosphate (Na_2HPO_4), and peptone were purchased from Merck (Germany). Herring DNA and agar were supplied from Fluka (Switzerland) and the yeast extract from Biolife. Methylene blue (MB, $C_{16}H_{18}ClN_3S$, M=315.5 g/mol) was purchased from Loba Fein Chimie, and sodium chloride (NaCl) from Sigma Aldrich (Germany).

All reagents were of analytical grade and all solutions were prepared using purified water (conductivity ≤ 0.1 $\mu S \cdot cm^{-1}$) from a Millipore Milli-Q system (USA).

Herring DNA was labelled with methylene blue (MB) dye in a MB/DNA molar ratio of 0.06. The concentrations of the stock solutions of herring DNA and of MB were determined by absorption spectroscopy by using the Lambert-Beer law, and the following molar absorption coefficients: ε_{260}=6600 $M^{-1}cm^{-1}$ for DNA [24], ε_{664}=76000 $M^{-1}cm^{-1}$ for MB [25].

The pink flowers of *Paeonia officinalis* were purchased from a local garden.

The peony water extract was tested against pathogenic *Escherichia coli* ATCC 8738 bacterium. *Escherichia coli* was grown in Luria Bertani Agar (LBA) plates at 37°C with following composition: yeast extract (5 g/L), peptone (10 g/L); NaCl (5 g/L) and agar (20 g/L). The stock culture was maintained at 4°C.

2.2. Preparation of *Paeonia officinalis* aqueous extract

An amount of 20 g of petals of peony pink flowers (Fig. 1) were boiled for 5 minutes in 100 mL of hot distilled water. This mixture was cooled and filtered through a Whatman filter paper no.1 resulting in a clear extract.

Fig. 1. Romanian Paeonia officinalis flowers

2.3. Characterization methods

UV-Vis absorption spectra were recorded at the resolution of 1 nm, on a double beam spectrophotometer Lambda 2S Perkin Elmer, in the wavelength range of 200-800 nm, with 1 nm slit width and 0.3 nm/s scan rate.

The fluorescence emission spectra of MB in samples were collected in the wavelength range of 670-800 nm, on a LS55 Perkin Elmer fluorescence spectrometer, by illuminating the samples with 662 nm excitation light.

FTIR-ATR analysis was done on FTIR/ATR Perkin Elmer Spectrum 400, in the wavenumber range of 650-4000 cm^{-1}.

Testing of antioxidant capacity of the samples was achieved by chemiluminescence (CL) assay, on a Chemiluminometer Turner Design TD 20/20 (USA), using the free radical generator system containing: luminol (1 mM), H_2O_2 (10 μM) in Tris-HCl buffer solution (pH 8.6). The *p*H value was measured with InoLab 7110 *p*H-meter. The *in vitro* antioxidant activity (AA%) of plant extract was estimated by the equation:

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

where I_0 is the maximum CL intensity at t = 5 s, for the reaction mixture without the sample, and I is the maximum CL intensity for *Paeonia* extract at t = 5 s [26].

The agar disc diffusion method [27] was used to evaluate the antibacterial activity of peony extract against *Escherichia coli* ATCC 8738 bacterium. A suspension of *Escherichia coli* bacterium (1000 μL) was spread on a solid agar medium in Petri dishes. The wells made using a sterile Durham tube 6 mm diameter was loaded with 50 μL of peony extract; the pure distilled water was used as a control. The antimicrobial activity was estimated by measuring the diameter (in mm) of inhibition zone (IZ) against test micro-organism. Experiments were performed in triplicate. The mean zone of inhibition was calculated with standard deviation procedure. The data were presented as mean ± standard deviation (SD). SD was calculated as the square root of variance using STDEV function in Excel 2010.

3. Results and discussions

3.1. Spectral characterization of *Paeonia officinalis* aqueous extract

The peony aqueous extract was characterized by UV-Vis absorption and FTIR-ATR spectroscopy.

The UV-Vis absorption spectrum of peony petals' extract (Fig. 2) showed the following absorption bands: 240 nm, characteristic for transitions arising from the A ring portion (benzoyl system, band II) of flavonoids [28]; 257 nm attributed to the L-phenylalanine, with a shoulder at 270 nm, due to the absorption of polyphenols, and of aminoacid residues of proteins; 317 nm, with a shoulder at 370 nm assigned to the B ring portion (cinnamoyl system, band I) of flavonoids [28].

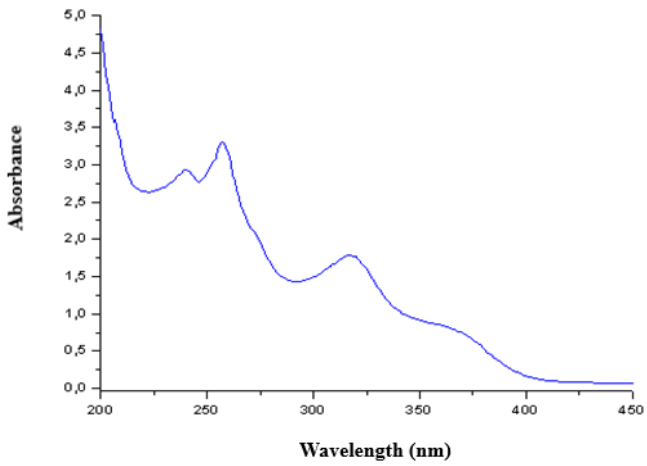

Fig. 2. The UV-Vis spectrum of Paeonia officinalis flowers' aqueous extract

The FTIR-ATR spectrum of peony extract (Fig. 3) exhibited a strong sharp peak at 3304 cm^{-1} assigned to N–H stretching vibrations and to the bending and stretching vibrations of hydroxyl groups in polysaccharides, alcohols and phenolic compounds [29, 30].

The weak peak at 2932 cm^{-1} is attributed to the aldehydic C–H anti-symmetric stretching mode [31].

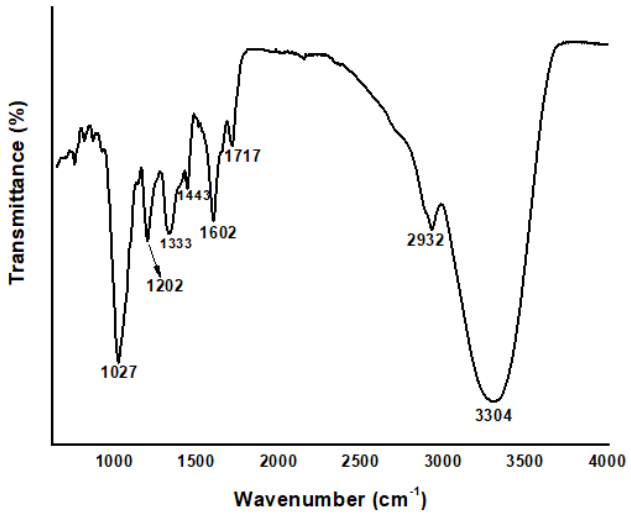

Fig. 3. FTIR-ATR spectrum of peony petal aqueous extract

The peak observed at 1717 cm^{-1} is characteristic for frequencies of carbonyl stretching vibrations of compound groups belonging to esters, carboxylic acids, ketones, aldehydes derived from proteins and aminoacids [32].

The strong and sharp peak at 1602 cm^{-1} and the weak peak at 1443 cm^{-1} are attributed to aromatic ring stretch [32].

Strong sharp peaks at 1333, 1202 and 1027 cm^{-1} are associated with –C–O–C stretching vibration of phenolic compounds, and flavonoids [33, 34].

The peaks located at: 688, 704, 758 and 817 cm^{-1}, can be assigned to aromatic C–H out-of-plane bending [32].

The UV-Vis (Fig. 2) and FTIR-ATR (Fig. 3) spectrum of the studied phytoextract showed the presence of aminoacids, proteins, esters, flavonoids and other phenolic compounds in peony petals' composition.

3.2 Biological performances of peony extract

The antioxidant properties of *Paeonia officinalis* aqueous extract were assessed through chemiluminescence method (Fig. 4) by *in vitro* simulation of oxidative stress. The antioxidant activity calculated using the expression (1) reached the value of 97.47%, demonstrating that peony extract is a powerful free radicals' scavenger.

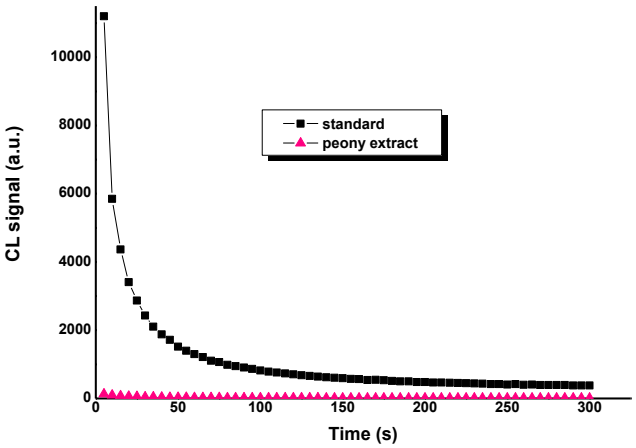

Fig. 4. The chemiluminescence kinetic profiles of CL signal of the phytoextract as compared to standard system (the reaction mixture without the sample)

The extract of *Paeonia officinalis* flowers showed significant antibacterial activity (IZ = 19±0.76 mm) against human pathogen *Escherichia coli* ATCC 8738 bacterium. The clear regions around each bore in the bacto-agar plates inoculated with *E. coli* are the zones of inhibition that indicate the extent of the test organism's inability to survive in the presence of the peony extract (Fig. 5).

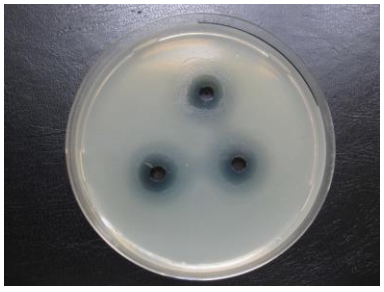

Fig. 5. Biocidal activity of peony flowers extract against Escherichia coli ATCC 8738

Biological value of these wonderful flowers is due to the presence of various bio-active phyto-compounds (like: phenolics, flavonoids, amino acids and others) in their composition, as previously demonstrated by UV-Vis absorption and FTIR-ATR spectra (see *section 3.1*). Li and co-workers pointed out the nutritional value of herbaceous peony petals [23].

3.3. Photophysical aspects of peony extract addition to DNA solution

In order to get deep insights on interaction of peony extract and DNA molecule, methylene blue was used as a spectral probe for DNA, being inserted in a very small concentration (6.2×10^{-7} M), by intercalating mode.

Fig. 6 displays the monitoring of peony extract effect on DNA labelled with MB.

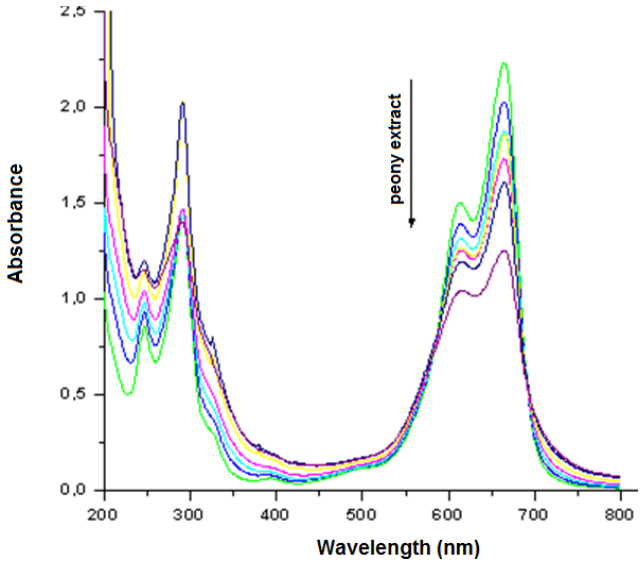

Fig. 6. Effect of peony extract gradual addition on the absorption spectra of MB/DNA in PBS pH 7.4

Methylene blue shows characteristic UV-Vis absorption bands in visible region due to involving of π-π* and n-π* transitions during its interaction with light [35]. The absorption spectra of MB/DNA before and after addition of phytoextract, show MB spectral signatures: a sharp peak assigned to MB monomer at 665 nm which dominates spectra, and a weak peak at 611 nm characteristic for MB dimer [36] (Fig. 6).

Significant changes in the absorption of MB/DNA spectrum, with gradual addition of peony extract, are observed, which means that methylene blue was removed from the complex with DNA, being replaced by phytocompounds of peony extract, fact that is further confirmed by the fluorescence emission study. With increasing amounts of phytoextract, the absorption peaks at 611 nm and 665 nm of MB/DNA exhibited hypochromicity. Contrarely, the absorption peaks at 247 nm and 291 nm showed hyperchromicity, due to the gradual peony extract addition.

The red excitation of MB labelled DNA, by the wavelength of 662 nm, resulted in a fluorescence peak registered at 698 nm (Fig. 7).

After gradual addition of peony extract, the emission spectrum of MB/DNA registered a fluorescence enhancement, revealing that the peony extract bounds to DNA molecule, by displacing the methylene blue dye. The increase of the fluorescence intensity should be due to the fact that MB was released after the addition of phytoextract, being replaced by the flavonoids occurring in peony extract. The formation of peony/DNA complex prevents MB binding to DNA.

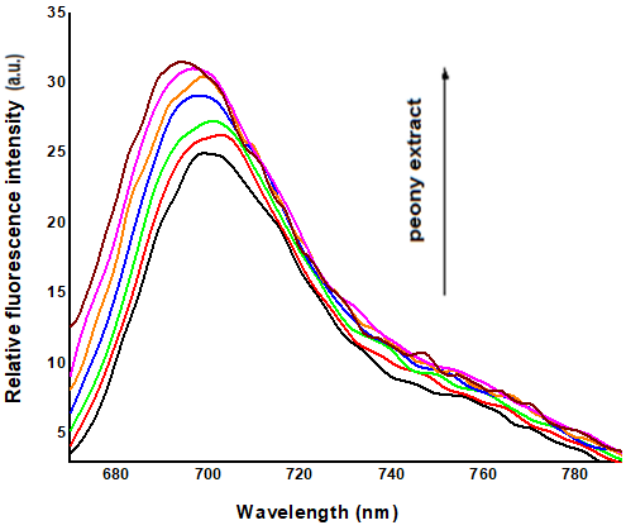

Fig. 7. The effects of peony extract on the fluorescence emission spectra of MB labelled DNA, in PBS pH 7.4 (λex = 662 nm)

These findings suggest that the studied ornamental plant extract could be used to achieve DNA-based carriers for phyto-compounds. On the other hand, the petals of peony could be used as efficient "green" remover of intercalating dyes from DNA molecule.

4. Conclusions

The extract of *Paeonia officinalis* flowers proved to possess high biological value: enhanced antioxidant properties (AA = 97.47%) and good antibacterial activity against *Escherichia coli* ATCC 8738 (IZ = 19±0.76 mm), being able to be used in cosmetics or as antibacterial agent. Spectral investigations (absorption and emission spectra) on DNA stained with methylene blue, revealed that the phytoextract displaced the dye from the DNA biomolecule, and then the phyto-compounds bound to DNA by an intercalative mode. The results could be exploited as „green" method to remove some intercalating agents from DNA molecule. On the other hand, the DNA/phytoextract complexes could be used as building

blocks to design novel materials with potential applications in biophotonics.

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