# Optical studies on human hair fibres treated with a natural extract of red tulip flowers

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Valorization of natural wastes in view of some interesting applications is in the spotlight of scientific research. Hair is a valuable biological material and its wastes could be used in various applications including optoelectronics. Therefore, the treatment of these natural fibres with phyto-derived bioactive agents is of great interest. This paper describes a "green" strategy to treat hair wastes with an extract of red flowers of tulip (*Tulipa gesneriana* L.). The tulip extract presents impressive antioxidant and antibacterial properties. FTIR-ATR and reflectance spectra proved the insertion of natural extract into hair fibres. These findings are promising, with great potential in biomedical and biophotonics fields.

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## 1. Introduction

Human hair is a filamentous biomaterial consisting mainly of proteins especially keratins which are characterized by the presence of many disulfide bonds [1]. This valuable biomaterial was used to develop high-performance ultraviolet (UV) photodetectors [2] while its optical properties [3], [4] could be exploited for optoelectronics applications.

Hair wastes were used to develop keratin nanoparticles as environmental friendly and inexpensive adsorbent for the removal of Cr(VI) ions which are mutagenic and carcinogenic to humans and other living organisms, while Cr(III) is a basic nutrient for human beings as well as animals and plants [5]. Moreover, keratin extracted from hair wastes was converted into value-added products such as biomaterials for tissue regeneration [6] or as biofertilizers with a key role in sustainable agriculture [7]. Furthermore, the hair pigments such as trichochromes [8], melanin [9] or eumelanins [10] were used for (bio)optoelectronics applications.

Long exposure to sunlight and oxidative colouring can produce seriously oxidative damage of hair. During the last years, the harmful effects of oxidative stress, prevented by antioxidants, have gained an increasing interest [11].

Very common plants in our country (Romania) have antioxidant and antimicrobial properties due to their specific content in active substances. The garden tulip (*Tulipa gesneriana* L.) belonging to *Liliaceae* family, is a wonderful spring ornamental plant. The antibacterial properties of garden tulip have been known for a long time [12], [13]. Responsible for the antimicrobial activity of garden tulips is a secondary metabolite (6-Tuliposide B) which is found specifically in the flower anthers. The mechanism of action of this metabolite has not yet been determined [14]. It is also known that the content in phenolic compounds and organic acids leads to a high antioxidant and antimicrobial activity [15], [16].

Krzyminska *et al*. [17] have determined the content of phenolic compounds and organic acids in the flowers of selected *Tulipa gesneriana* cultivars.

Nieuwhof *et al*. [18] showed that all tulips with red flowers contained cyanidin. Also, plants with red flowers contained carotenoids, a high amount of pelargonidin, and delphinidin. As well, a secondary metabolite with antimicrobial activity, namely 6-Tuliposide B, was found in tulip anthers [14]. Another component with antimicrobial activity found in tulips is Tulipalin B (α-methylene-β-hydroxy-γ-butyrolactone, PaB) which can be used as a natural preservative agent for cosmetics and pharmaceuticals (instead of synthetic parabens) [19].

Tulipalin A, known as α-methylene-γ-butyrolactone [20] is also found in the composition of red tulips but is known as allergenic agent and can only be used as the monomer of bioplastics [21].

To benefit from the properties of tulip (a seasonal plant), all the rest of the year they are grown in greenhouses.

Due to the chemical composition of the tulips mentioned above (tulip extract it is considered the next big skin care ingredient), the extracts from these flowers are also used in cosmetics such as creams, facial masks [22] and shampoos against hair loss [23].

Also, *Tulipa gesneriana* phytoplacenta extract has antioxidant properties and it is used in cosmetics as a moisturizing agent or as a hair care agent [24].

An innovation in the field of cosmetics was made by the South Korean company "Sognap" which uses an ice cream tulip extract in cosmetics composition [25].

This research work presents optical studies regarding the treatment of human hair with an aqueous extract of red tulip petals.

Our research team previously reported the use of ornamental plants in development of antioxidant silver nanoparticles [26] or of DNA/phytoextract complexes [27] with potential applications in biophotonics.

Besides hair degradation for various reasons listed above, there is also a disease of the hair follicles called "scalp folliculitis" (quite common hair affection). Various microorganisms such as bacteria and yeasts can also be responsible for this disease. The most common microorganisms that cause this disease are those of *Staphylococcus* class, especially of *Staphylococcus aureus* [28]. For these reasons, the aqueous maceration of red tulips has been tested against *Staphylococcus aureus*.

## 2. Materials and methods

### 2.1. Materials

Tris [tris (hydroxymethylaminomethane base)], hydrochloric acid (HCl), hydrogen peroxide ($H_2O_2$), luminol (5-amino-2,3-dihydro-phthalazine-1,4-dione), and peptone were purchased from Merck (Germany). Sodium chloride (NaCl) was supplied from Sigma Aldrich (Germany). Agar was obtained from Fluka (Switzerland) and the yeast extract was purchased from Biolife. The brown natural coloured hair fibres were collected from one of the authors (CU) aged of 40 years. The hair fibres were washed with a mild shampoo, rinsed with distilled water, dried at room temperature and stored in clean boxes.

### 2.2. Preparation of tulip extract

The tulip flowers (Fig. 1) have been harvested on April and have been washed and used in optimal storage conditions at room temperature, away from light. The tulips were harvested from Dărăști-Vlașca area, Giurgiu County (44°17′30″ N 26°0′34″ E), from one of the authors' private garden.

The useless parts of the plant, namely the stem, leaves and root were removed, keeping only the floral part of interest. After a careful selection, the fresh petals were washed in distilled water which was removed with clean dry napkins. Further, we proceeded to the weighing procedure, to meet the optimal quantitative conditions in the recipe for preparing the extract. Accordingly, a volume of 100 mL of distilled water was added to an amount of 20 g of dried fresh tulip petals which were previously crushed. Then, this mixture was boiled for 5 minutes and passed through a filter paper no. 4 and preserved in brown bottles, in freezer.

*Fig. 1. Tulipa gesneriana red flowers (color online)*

### 2.3. Ultrasound treatment of hair fibres

The hair fibres were subjected to ultrasound treatments in an ultrasonic bath (BRANSON 1210, Marshall Scientific, Hampton, NH, USA) for 30 minutes, in the absence and in the presence of tulip extract. The technical ultrasound parameters were: 47 kHz frequency and power of 80 W; the work temperature was kept at 25 $^{o}$C.

### 2.4. Characterization methods

**UV-Vis absorption spectrum** of the aqueous extract of tulip petals was recorded in the wavelength range of 200-800 nm, at the resolution of 1 nm, on a double beam Lambda 2S Perkin Elmer spectrophotometer.

The natural hair fibres were studied by **reflectance spectroscopy** by using UV-Vis-NIR spectrophotometer, Jasco V-570 (Tokyo, Japan) equipped with an integrating sphere, in the spectral range 190-2,000 nm with a resolution of 1 nm, and a scanning speed of 100 nm/min. To measure the reflection on hair, the sample has to consist of several hairs bundled in one small bundle. The small bundle of hair was placed on a microscope slide and spread directed on one direction (the small bundle was duct taped on both edges on the microscope slide).

**FTIR-ATR spectroscopy** was used to get deep insights about treatment of hair fibres with tulip extract. Fourier transformed IR (**FTIR**) spectral analyses were carried out on a Perkin Elmer SpectrumGX instrument with attenuated total reflectance (**ATR**) diamond crystal, at a spectral resolution of 4 $cm^{-1}$, in the range of 500–4,000 $cm^{-1}$.

***In vitro* evaluation of antioxidant properties** of the aqueous extract of red tulips was assayed by **chemiluminescence (CL) technique**, on a Chemiluminometer Turner Design TD 20/20 (USA). The oxidative stress was simulated by generating reactive oxygen species (ROS) by using a system containing luminol ($10^{-3}$ M), $H_2O_2$ ($10^{-5}$ M), in TRIS-HCl buffer

solution (pH 8.6). These experiments were carried out in triplicate, and the values of *in vitro* antioxidant activity (AA%) were expressed as:

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

where $I_0$ and $I$ represent the maximum CL intensity at $t = 5$ s, for the reaction mixture without and with the sample, respectively [29].

**For antibacterial assay**, the test bacterium was *Staphylococcus aureus* ATCC 25923 [Gram(+) bacterium]. This bacterium culture was maintained on Luria Bertani Agar (LBA) purchased from VWR (Darmstadt, Germany) and maintained at 4 °C. *Staphylococcus aureus* was sub-cultured onto LBA plates and incubated overnight at 37 °C (Laboshake Gerhardt, Germany). After 24 hours, three bacterial colonies with similar morphology were inoculated into 10 mL sterile Mueller Hinton Broth [MHB, purchased from VWR (Darmstadt, Germany)] and incubated overnight at 37 °C. After this incubation, the bacterial suspensions were adjusted to 0.5 McFarland Standard with sterile MHB broth [30]. The antibacterial activity was determined by the agar well diffusion method as previously described in [31].

## 3. Results and discussion

### 3.1. Optical and biological properties of *Tulipa gesneriana* extract

Optical characterization of the aqueous extract of petals of *Tulipa gesneriana flowers* was carried out by UV-Vis absorption and FTIR-ATR spectroscopy.

The UV-Vis absorption spectrum of tulip extract (Fig. 2) displays the following absorption peaks located at: 262 nm with a shoulder at 287 nm, corresponding to the absorption of amino acid residues of proteins and of A-ring flavonoids (benzoyl system, band II); 350 nm attributed to the B ring portion (cinnamoyl system, band I) of flavonoids [32], [33].

FTIR-ATR spectrum of tulip extract (Fig. 3) displays main bands at 3,275 $cm^{-1}$ (strong and sharp) assigned to O−H stretching vibration of phenolic compounds [34] and N−H bonds [35]; 2,925 $cm^{-1}$ (weak and narrow) attributed to C–H anti-symmetric stretching vibration [36]; 1,599 $cm^{-1}$ (strong and sharp) characteristic for Amide I band, arising due to carbonyl stretch in proteins [31], [35]; 1,407 $cm^{-1}$ (sharp) assigned to phenol or tertiary alcohol, O–H bend groups [34] and bending of $-CH_2-$ [37]; 1,250 $cm^{-1}$ (weak) attributed to vibrations of the –C–O–C– and –C–O–H groups [34], [38]; and 1,039 $cm^{-1}$ associated to –C–O–C− stretching vibration [34].

Optical characterization of the tulip extract by UV-Vis (Fig. 2) and FTIR-ATR (Fig. 3) spectra showed the presence of proteins, flavonoids, and other phenolic compounds in tulip petals' composition.

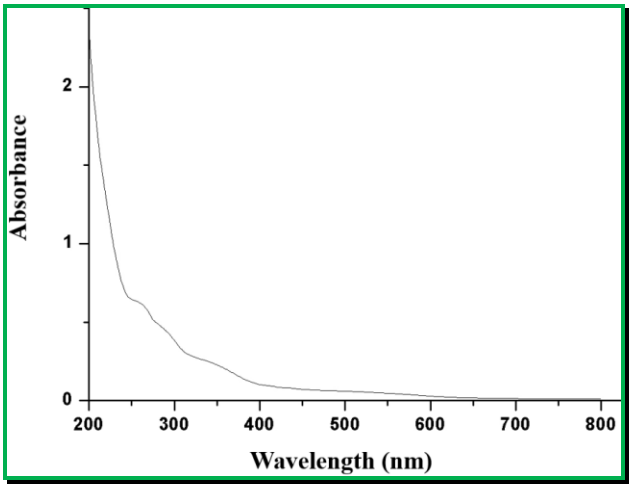


*Fig. 2. The UV-Vis spectrum of tulip petals' extract*

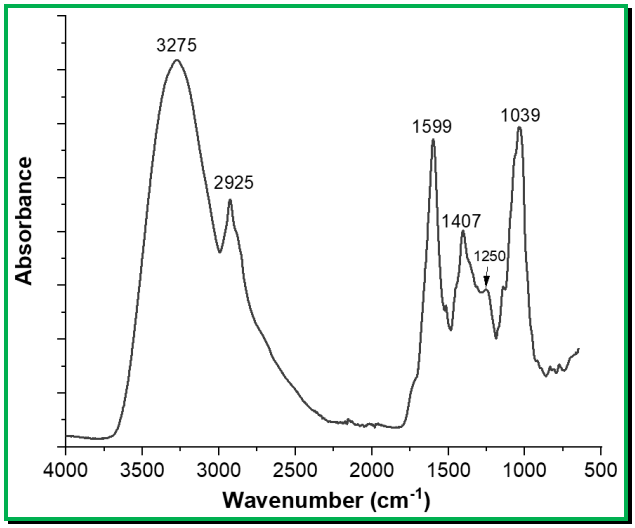


*Fig. 3. The FTIR-ATR spectrum of tulip petals' extract*

The tulip extract was further biologically characterized in terms of antioxidant and antibacterial properties.

The chemiluminescence technique revealed the impressive antioxidant activity (99.24 ± 0.28) % of aqueous extract of red tulip flowers.

Fig. 4 shows a photograph of a Petri dish containing agar inoculated with *Staphylococcus aureus*, and loaded with tulip extract introduced into the well. A mean diameter of inhibition zone (IZ) of 20 ± 0.47 mm is observed around the well. This value is comparable with similar results obtained with another flower extract against *Staphylococcus aureus* [39] and also with extracts from petals of the tulip (*Tulipa gesneriana* L.) [40].

The antimicrobial action of tulip extract probably is based on various mechanisms including its surface binding on the bacterial cell wall and membrane followed by a further penetration into the cytoplasm with the addition of disruption of organelles that ultimately leads to cell death. Another explanation is the presence of flavonoids in tulip

extract (as shown above) which is a key factor for antioxidant and antibacterial properties of tulip extract [32].

*Fig. 4. The antibacterial activity of the Tulipa gesneriana extract against Staphylococcus aureus ATCC 25923, estimated by agar well diffusion method. IZ = 20 ± 0.47 mm (color online)*

### 3.2. Optical characterization of human hair subjected to various treatments

The human hair samples were divided into 3 batches: 1) pristine hair; 2) hair treated with the natural extract of red tulip petals, and 3) hair subjected to natural extract and ultrasound irradiation.

A comparison of the FTIR-ATR spectra of the three hair batches is presented in Fig. 5.

The untreated hair showed the characteristic absorbance peaks of proteins (especially keratins): amide A (3,280 $cm^{-1}$), amide I (1,635 $cm^{-1}$), amide II (1,526 $cm^{-1}$), and amide III (1,237 $cm^{-1}$) [41], [42].

The absorbance ratio at 2,918 $cm^{-1}$ and 2,850 $cm^{-1}$ attributed to asymmetric and symmetric stretching vibrations of $CH_2$ [43] belonging to keratin polymeric network, had changed after hair treatments.

The weak absorption at 3,071 $cm^{-1}$ could be assigned to aromatic C–H stretching [34] of amino acid residues (Tyr or Phe) contained in hair proteins or to stretch vibrations of C–H groups from fatty acid moiety [44] of hair lipids.

Another band at 1,455 $cm^{-1}$ corresponds to aromatic ring stretch [34] and to bending vibrations of the methyl and methylene groups of fatty acids in lipids [45].

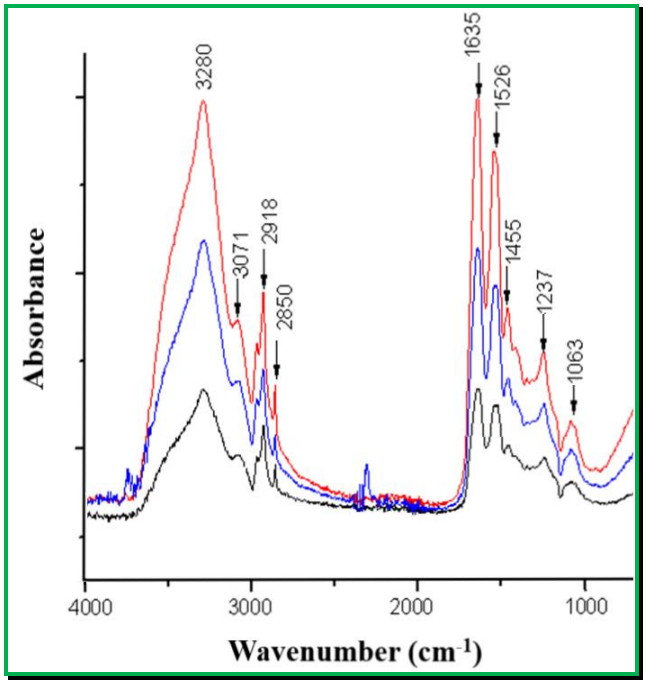


*Fig. 5. The FTIR-ATR spectra of pristine hair (black line, ―), hair treated with natural extract (red line, ―), and hair subjected to ultrasounds irradiation in tulip extract (blue line, ―) (color online)*

The height of the FTIR absorbance peaks and some FTIR absorbance ratios ($A^{3280}/A^{3071}$; $A^{2918}/A^{2850}$; Amide I/Amide II) of hair fibres subjected to ultrasound treatment in tulip extract are intermediate between those of pristine hair and those of hair treated with natural extract.

Spectral differences between the three hair batches were also observed in the reflectance spectra (Fig. 6). Some spectral changes are observed in the reflectance patterns, especially in the regions 200-350 nm and 1,200-2,000 nm, after ultrasound irradiation of hair fibres treated with phyto-extract.

These spectral changes indicate that tulip extract penetrated into the hair fibres after ultrasound treatment.

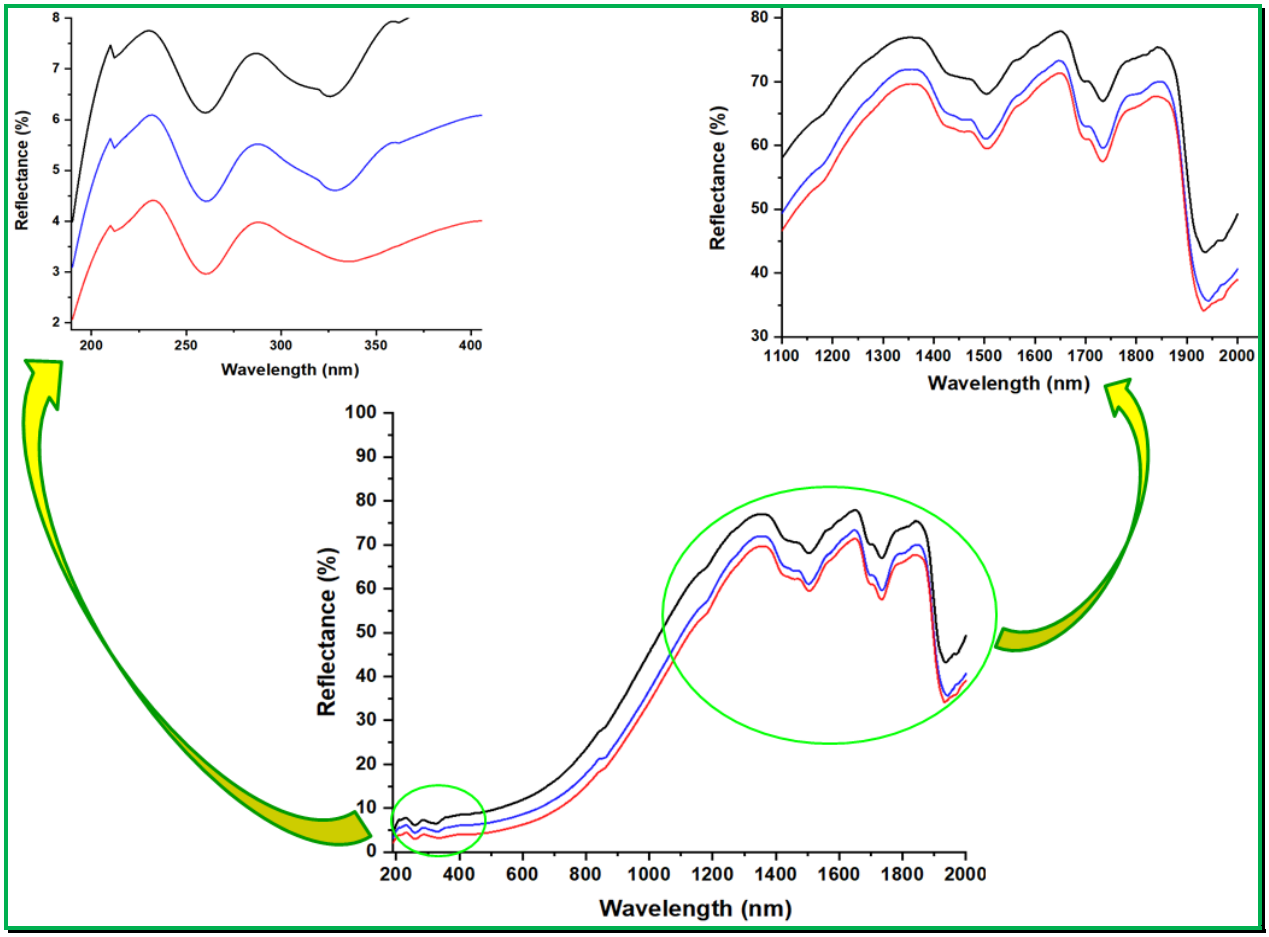


*Fig. 6. The reflectance spectra of pristine hair (black line, ), hair treated with natural extract (red line, ), and hair subjected to ultrasounds irradiation in tulip extract (blue line, ). Insets show the magnified regions of the reflectance spectra of hair samples (color online)*

## 4. Conclusions

Human hair is a complex and thin biostructure, the investigation on this valuable biomaterial being still a challenge. The hair treatment with natural extracts is beneficial for improving its value, considering that phyto-extracts contain a cocktail of biocompounds with antioxidant and antimicrobial activities.

The tulip extract presented high scavenging activity of reactive oxygen species. More than that, this phyto-extract was an effective biocidal agent against *Staphylococcus aureus*.

FTIR-ATR and reflectance spectra demonstrated that the extract of tulip petals affected the optical properties of human hair. Moreover, it was observed that ultrasound treatment of hair facilitated insertion of active ingredients of tulip extract into the hair structure.

In the future, various antioxidant natural agents will be tested to improve cosmetics with a hair protective role.

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