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"BIOMIMICRY: Study of daisy photoprotection strategies for the development of new cosmetic products"

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1. Introduction

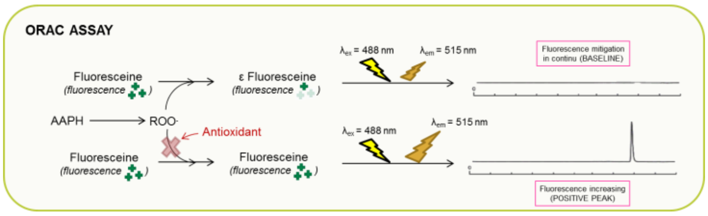
Biomimicry is a method to draw inspiration from the ingenuity of natural strategies to develop sustainable innovation to overcome contemporary challenges. By observing, abstracting and transferring the strategies developed by living systems over billions of years of evolution, we can design highly efficient, sustainable, and nature friendly solutions. The success stories of biomimicry are numerous and demonstrate its potential to revolutionize various sectors, especially cosmetics. In terms of solar protection, plants are an inspiring role model due to their need for sunlight and lack of mobility, in particular the daisy, *Bellis perennis*, which has a remarkable affinity to the sun and great whiteness. These characteristics make it a competitive candidate to replace the controversial titanium dioxide (TiO_2) [1] with a bio-based and environmentally friendly photoprotective agent. The originality of our study lies in the multidisciplinary characterization of daisy petals, with a dual approach: chemical and physical.

2. Materials and Methods

Biomimetic methodology: After analyzing the problem of solar protection, especially the chemical impact of current solar filters, the team decided to search for biological inspiration since living organisms also need to shield themselves from radiation. The daisy and particularly its petals became a biological system of interest due to their whiteness, a color known for solar protection. The lack of scientific knowledge to comprehend the mechanisms at play in daisy petals led to this multi-angle analysis including characterization, abstraction and simulation, presented in this paper. Following the steps of the biomimetic design process [2;3], the main strategies of interest will then be abstracted and implemented in the design of more eco-friendly solar protection systems. The discoveries will therefore be used to inform our solar protection industry.

Chemical: We performed an accelerated solvent extraction of the different parts of the daisy flower (stem, corolla, and petals). The extracts were analyzed by liquid chromatography with biodetection (LC-BCD) coupled with online mass spectrometry (HRMS) to identify antioxidant properties of the compounds present in a complex matrix. This method has been developed by the analytical chemistry department [4] to enable a coupling between liquid chromatography and biodetection (specified as Anti-ox). This system is composed of an HPLC/DAD (UV/Vis) chain coupled to a biodetection system (bioreactor – post-column system coupled to a fluorescence detector) via a flow divider directing 10% of the mobile phase towards the biodetection system. The ORAC protocol was transposed to the BCD system in order to identify antioxidant substances stabilizing peroxyl radicals (ROO.) in complex mixtures (figure 1).

<u>Biodetection: ORAC test (Oxygen Radical Absorbance Capacity)</u>



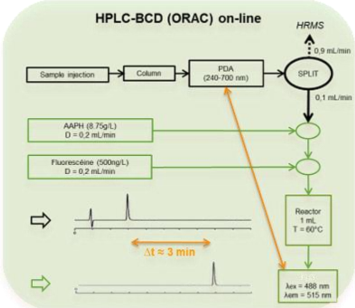


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Figure 1. (1) Descriptive diagram of the ORAC protocol for detecting molecules with antioxidant activity; (2) Schematic of the HPLC-BCD setup – reprinted from [4]

Physical:

Preparation of petal samples for Scanning Electron Microscope (SEM) analysis:

The images obtained previously were taken from fresh petals of flowers picked directly on the Aulnay-sous-Bois campus. For the various experiments (chemical analysis, microscopy, imaging, etc.), dried daisies from Bosnian cultures were used. It is therefore important to confirm that the geographical origin of the flower does not impact its structure, as verified by the chemical composition.Fresh daisies were collected, the petals were immediately separated from the flower, and then a necessary quantity was introduced onto an SEM plate.Cross-sections of fresh petals were observed using secondary electrons in a FEI Quanta 400 field emission scanning electron microscope. The samples were previously frozen on a copper block cooled with liquid nitrogen, then gradually brought back to room temperature over 12 to 20 hours under secondary vacuum in order to slowly freeze-dry them while preserving the biological structures as much as possible.

We performed optical modeling and simulation of UV-visible reflectance of the petal by Finite-Difference Time-Domain (FDTD) method [5].

Tests using the FDTD tool allowed us to create a model to simulate the reflectance resulting from the nanostructuring of the petals. The physical parameters of the structure were gathered from SEM image analysis to reproduce the petal as well as possible.

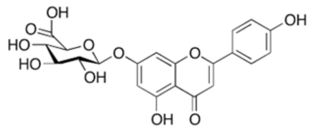
Of course, some modeling simplifications were operated, for instance, the constant refractive index of 1.51 over the whole spectrum (from UV to Visible range) especially because it is very complicated to obtain easily precise data. We assume the variation of the real part of the complex refractive index to be negligible in comparison to the optical effects at stake. Also, in these simulations the imaginary part of the refractive index is reduced to 0 to simulate only the refractive behavior of the structure. An “infinite” material is simulated via periodic boundary limits

in the (x,y) plane because in real life the total length of the petal is very large in comparison to the small simulated volume chosen for computation efficiency. In the same spirit only one polarization (perpendicular to the structure) is simulated though in real life light is at an unpolarized state.
The upper structure exhibits reflection in the UV when the material (index 1.51) is surrounded by air (index 1). The lower structure does not reflect in the UV, unlike the upper structure (for a cross-polarization of light relative to the structure). In addition, the reflectance bands of the upper structure seem to have a complementary spectral position in regards to the absorption band of the molecules within. A key remark, this reflectance property is likely to fade away in a cosmetic composition due to typical refractive index of cosmetic formulations (around 1.4-1.45). This is due to the refractive index gap between the petal (or fragment of petal) and its surrounding which decreases in that case.
FDTD simulations done with Ansys FDTD software (https://www.ansys.com/products/optics/fdtd), an Optical software used in the photonics industry, but transferred for cosmetic applications.
Optical software used in the photonics industry, but transferred for cosmetic applications.
It consists in electromagnetic modeling and resolution of Maxwell Equations thanks to a FDTD solver. It relies also on tools for creating complex structures, as well as on analyzing electromagnetic fields. The resolution does not depend on the geometry of the structure nor its size (close to λ), it is also independent of the materials.

Chemical / physical combination:
The study focused on the detection of the molecule of interest by matrix-assisted laser desorption/ionization, at atmospheric pressure, coupled with high-resolution mass spectrometry (AP-MALDI / HRMS Orbitrap) on each of the two petal faces.
A sample of the molecule of interest (standard) was used to perform MALDI HRMS [6] mass spectra and define the ionization conditions adapted to the molecule.
Each of the two petal faces will then be analyzed by AP-MALDI / HRMS to determine whether the molecule is preferentially detected on the upper or lower face.

3. Results

Chemical: Under these conditions, we detected a major peak exhibiting a significant response in the ORAC system, therefore indicating strong antioxidant activity. We identified this compound using high-resolution mass spectrometry (HRMS). After interpretation, we identified the peak as Apigenin-7-O-β-D-glucuronide (APG) [7] (figure 2). We injected commercially available APG under the same analytical conditions as the daisy extract. The retention times, UV/Vis spectra, MS2 spectra in positive/negative mode, and MS3 spectra in positive/negative mode are similar between the com-mercial compound and the compound primarily detected in the daisy extract (figure 2 (2)).



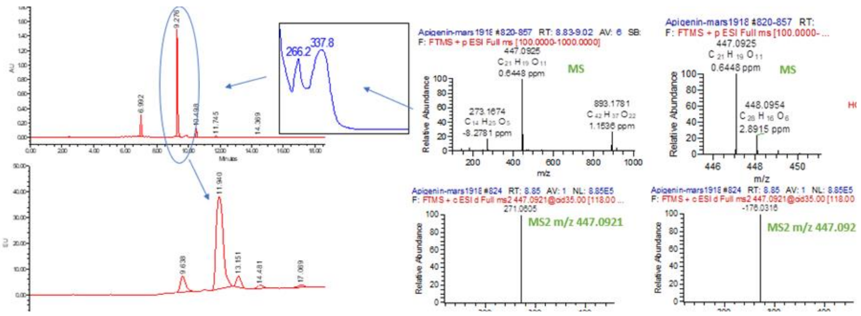


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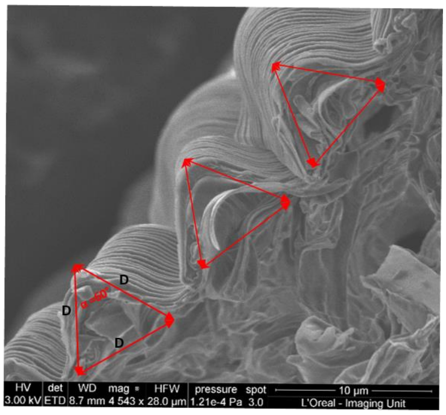
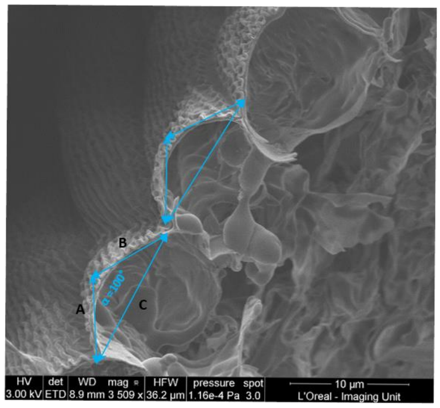
Figure 2. (1) Identification of Apigenin-7-O-β-D-glucuronide; (2) Chromatogram of mixture, Rays UV absorbance of compound (nm) and mass spectrum of molecule.

Apigenin-7-O-β-D-glucuronide (APG), an active flavonoid derivative isolated from agricultural residue, is a metabolite of Apigenin. This compound possesses multiple pharmacological activities [8], including antioxidant [9], anti-complement [10], and aldose reductase inhibitory activities [11]. To date, no reports have identified the anti-inflammatory mechanisms of APG. Studies highlight the action of apigenin-7-O-glucopyranoside (APG), a flavonoid located in flowers, which could have a modulatory role in the treatment of neurovegetative diseases, particularly Alzheimer's disease [12]. This flavonoid appears to act as an acetylcholinesterase inhibitor [13]. Results from some studies suggest that AG can be used as a source of anti-inflammatory agents as well as a dietary supplement for health promotion [14].To date, no studies have also highlighted any potential UV filtering properties. Its absorption spectrum has two maxima: 266nm and 337nm.

Physical: we observed that the nanostructuring is different between the top and the bottom of the petal (figure3). Microscopic studies have allowed the study of the structural organization of the petals with well-organized networks in 2 dimensions [15]. These structural properties could reflect certain UV rays in order to protect from the sun [16].



D=7µm



A =7µm B =7µm C=10µm

(1) (2)

Figure 3. (1) Top of the petal; (2) Underside of the petal. We observed that the nanostructuring is different between the top and the bottom of the petal. Microscopic studies have allowed the study of the structural organization of the petals with well-organized networks in 2 dimensions. These structural properties could reflect certain UV rays in order to protect from the sun.

Thanks to data of the structural organization by microscopy and FDTD technical we are able to identify a simulated reflectance on top of the petal (face expose on sun). However, we did not detect simulated reflectance on underside of the petal of daisy (figure 4).

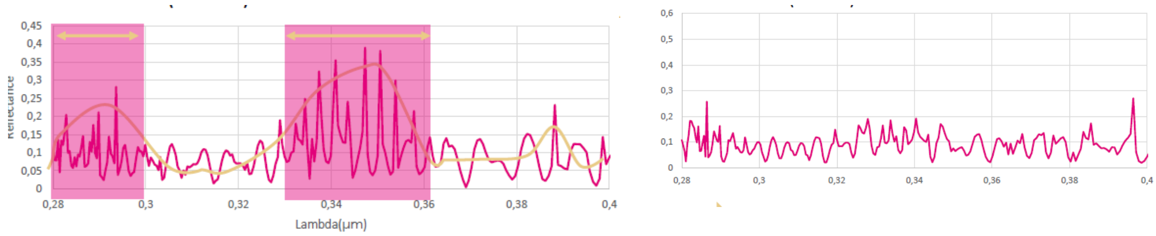


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Figure 4. Study by Optical simulation by FDTD (Finite Difference Time Domain). (1) Reflectance simulated of top of the petal; (2) Reflectance simulated of Underside of the petal.

Association Chemical & Physical:
We observed a difference in active concentration between a decoction of whole petals and crushed petals, The hypothesis put forward is that the active is present in the canals.
Hypothesis that the association of the two UV protection strategies developed by the daisy could be effective in finding a new way to protect the skin against UV rays
A collaboration with the Luxembourg Institute of Science and Technology (LIST) made it possible to use MALDI imaging techniques to verify the hypothesis that the active is on the upper canals of the petals [17] (figure 5).

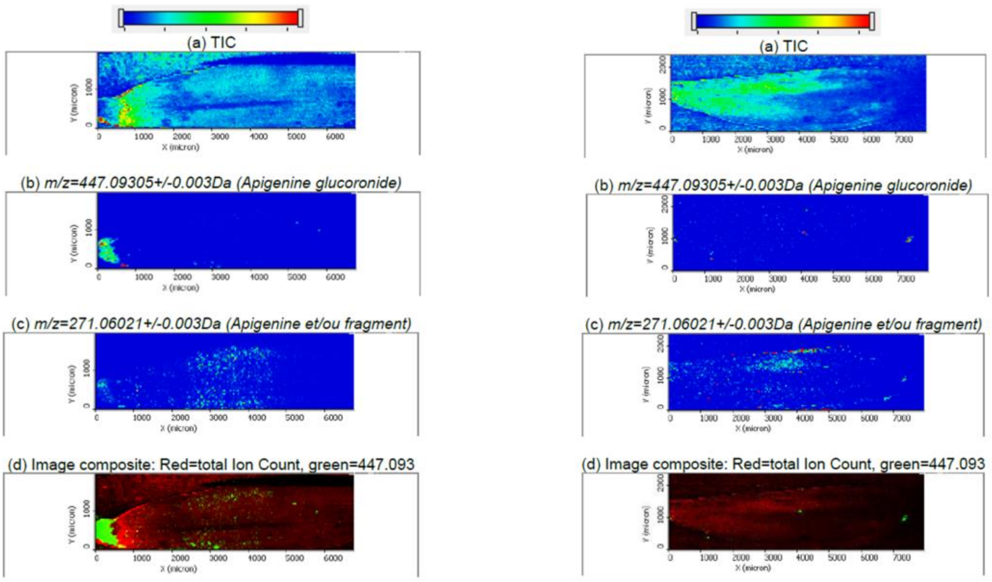

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Figure 5. Study by imaging AP-MALDI HRMS. (1) For top of the petal, total ion count image (a) and 2D images of the molecular ion intensity of the molecule of interest (b) Apigenin-7-O-β-D-glucuronide m/z=447.09305+/-0.003Da and (c) Apigenin and/or fragment

m/z=447.09305+/-0.003Da. (d) Composite image: Red=total ion count, green=447.093. (2) For bottom of the petal, total ion count image (a) and 2D images of the molecular ion intensity of the molecule of interest (b) Apigenin-7-O-β-D-glucuronide m/z=447.09305+/-0.003Da and (c) Apigenin and/or fragment m/z=447.09305+/-0.003Da. (d) Composite image: Red=total ion count, green=447.093.

On the physical side, the morphology of the nanostructures of the upper face of daisy petals led to a simulated reflectance (figure 4) with the same absorbance bands of APG (figure 1), while almost no reflectance was observed for the lower part of the petals. In addition, mass spectrometry imaging revealed that the upper face and base of the petal show a strong APG signal, while no signal was detected on its lower part (figure 5)

4. Discussion

Liquid chromatography-biodetection-mass spectrometry analysis revealed the presence of apigenin-7-O-β-D-glucuronide (molecular mass: 446.4 g/mol, (Figure 2) in Bellis perennis (daisy) petals. This flavonoid, identified as a secondary metabolite [14], has not yet been studied for cosmetic applications [18]. Our results suggest a potential role of this molecule in sun protection, particularly in association with the nanostructures observed on the upper surface of the petals. Reflectance analysis [5] confirmed an increase in UV reflection on this surface, in accordance with our initial hypothesis [19]. Apigenin-7-O-β-D-glucuronide was investigated in Leucanthemum vulgare (daisy) petals and this metabolite was not detected with the LC-BCD system [4], APG is only present in daisies. Further studies, including UV exposure simulations, are needed to explore the combined effect of nanostructures and apigenin-7-O-β-D-glucuronide. In addition, it would be relevant to study other potential functions of the striations observed on the petals. Finally, it would be relevant to evaluate the effectiveness of APG and nanostructures from daisy petals with that of UV filters commonly used in cosmetics. This research is part of a biomimetic approach aimed at developing innovative and sustainable solutions for sun protection, in accordance with the principles of the circular economy [20].

5. Conclusion

The daisy flower, Bellis perennis, is known in the literature for several properties such as firming, healing, anti-inflammatory and antibacterial effects. For the first time, through a biomimicry research process, we explored the whiteness of daisy petals and their potential filtering properties. We demonstrated that daisy petals have a dual strategy (chemical and physical) for UV photoprotection, combining an antioxidant active ingredient (APG) and a UV-reflecting nanostructuration on the upper face of the petal.

These findings not only demonstrate the potential of this biological model but also highlights the potential of biomimicry to revolutionize cosmetics in a sustainable way and the role of nature-inspired design in addressing contemporary challenges through a better understanding of natural strategies.

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