



From Kiwi Peels’ “End-of-Life” to Gold Nanoparticles: the Upcycling of a Waste

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Abstract

Following a green approach, kiwi peels (a waste) were washed in hot water to obtain a water-based polyphenolic extract (KPWW) used to reduce Au³⁺ (coming from a HAuCl₄ water-based solution) for forming gold nanoparticles (AuNPs). Indeed, KPWW, as shown after performing high-performance liquid chromatography-mass spectrometry (HPLC/MS-MS) analysis, is mainly composed by different polyphenols acting as reductant agents, accomplishing a red-ox reaction and decorating the AuNPs-KPWW surface. Spectroscopic and morphologic techniques were used in synergy for investigating the AuNPs-KPWW main features. Polyhedral-shaped plasmonic nanoparticles with a mean size of 30±10 nm and a negative charge of −40 mV were thus obtained. The AuNPs’ stability was assessed under different working conditions, investigating the role of ionic strength, pH, and temperature. The photostability was also assessed by irradiating AuNPs-KPWW with a solar simulator lamp. Both temperature and solar light did not perturb AuNPs-KPWW. Thanks to the presence of polyphenols, the antioxidant and skin-lightening properties were positively demonstrated. Moreover, the protective role of AuNPs in scavenging H₂O₂ and ·OH was also investigated by inhibiting the oxidation of a biomolecule. The sunscreen ability of AuNPs-KPWW was also estimated, and the theoretical calculation of the sun protection factor (SPF) was determined. Finally, the AuNPs-KPWW biocompatibility was tested on endothelial colony-forming cells and normal dermal fibroblasts as human cell lines, revealing that AuNPs-KPWW did not affect cell viability and did not alter cell morphology, demonstrating their safety and their potential application in nanomedicine.

Keywords Gold nanoparticles · Kiwi peel extract · Polyphenols · Green chemistry · Circular economy · Waste management · Biomedicine · Cosmetics

1 Introduction

In the last decades, the high rates of resource consumption and the huge amounts of waste products have been considered to be likely the driving force toward an ecological collapse [1]. Indeed, around 20% of food produced in the European Union (EU) is wasted each year, causing worsening social, environmental, and economic problems. Therefore, the EU is trying to solve the problem by incentivizing food waste management to develop sustainable paths leading to innovative recycling approaches. In this context, the European Waste Framework Directive (WFD) started to mention the concept of end-of-waste and the related criteria, which states the requirements for specific waste to cease to be waste and become a product or a secondary raw material to valorize, giving them a “second life” [2, 3]. Accordingly, the circular economy has become the focus of a recent major

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EU policy program that lists biomass and bio-based products as interesting resources to be upcycled to “new input products” [2–6]. Hence, the sustainable way to treat waste should lower the environmental impact, with the concrete possibility of obtaining high gains in terms of novel productions that would positively affect human health and well-being.

On this ground, this paper assesses the possibility of recovering kiwi peels among agri-food wastes, conferring alternative reuse following a green approach, and avoiding their dumping, thus considering them as value-added by-products. Not surprisingly, Chamorro et al. [7] reported that in 2019, kiwi cultivation occupied a surface of $\sim 270 \times 103$ ha around the world, and Europe contributed with $\sim 43 \times 103$ ha. The paper by Silva et al. [8] accounted that in 2020, worldwide kiwifruit production reached 4.86 million tons, with 923 thousand tons from the EU. In addition, from 5 to 20% of the fruit produced cannot be marketed based on size or appearance [8]. Moreover, beyond the discarded fruits, the kiwis' processing industry also generates many by-products, including seeds, peels, marc, and pruning residues [8]. Indeed, kiwi waste production is high, accounting for $\sim 4.5 \times 10^6$ tons globally, whereas Europe produces $\sim 1 \times 10^6$ tons [7]. Consequently, this implies a huge global production of these wastes with dangerous environmental consequences, encouraging a bio-circular economy approach as the most pertinent. In this context, kiwifruit wastes (such as seeds, peels, and pulp discards), as materials particularly rich in bioactive compounds, have already been used in many different fields (nutraceutical, pharmaceutical, cosmetic, and biomedical). Interestingly, peels have a higher bioactivity than pulp, and it is considered a natural source of biomolecules, especially polyphenols [7]. On the other hand, polyphenols have started to be carefully monitored due to their high risk of contaminating ecosystems, particularly aquatic ones [9]. A strategy for avoiding their disposal into the environment could be found in employing them for innovative uses. So, for these reasons, in this work, wasted kiwi peels were specifically employed as starting raw by-product material for obtaining a KPWW extract, subsequently used for the green synthesis of AuNPs-KPWW to be proposed for biomedical applications. Due to the polyphenols' antioxidant properties [10], they were involved in forming AuNPs through the spontaneous reduction of Au^{3+} to Au^0 . In this way, hybrid organic/inorganic nanoparticles were obtained, with a gold metallic core wrapped by an organic polyphenol shell that could act as skin-lightening and natural sunscreen agents, not only as antioxidants. This green production of AuNPs, if compared with the commonly used methods, would respect the sustainability principles as predicted by the European Green Deal, foreseeing very low associated costs, as in the case of the present work, in which 0.6 € for kilogram was estimated. Moreover, this process would respect the principles of green chemistry production and

sustainability [11–16]: it is one pot (obtaining the vehicle and the functional biomolecules on the same system after a single step of reaction), requires a very short time of reaction, mild working conditions, zero contaminants, and by-products, with the great advantage to attain biocompatible nanomaterials. For this purpose, this latter feature was assessed on two human cell lines: endothelial colony-forming cells (ECFCs) and normal human dermal fibroblasts (NHDF). The results revealed that AUNPs-KPWW appeared biocompatible, thus opening the possibility of using them in biomedicine or cosmetics.

Focusing the attention on kiwi fruit, it is worth mentioning that the green synthesis of AuNPs involving kiwifruit was exploited in the past literature by directly using the kiwifruit juice, not peel extract. Zuorro et al. [17] reported the antioxidant activity of the juice without any information about the potential properties and uses of the derived AuNPs. Another example is the work of Gao et al. [18]. In this case, the synthesized AuNPs were not stable (they aggregated after several hours of storage) until adjusting the pH value to 8, stabilizing the nanoparticles for more than 2 months, but in conditions far from the biological ones. The interest in using kiwi juice as a green reagent can also be demonstrated by considering the synthesis of AgNPs [18]. Instead, to the best of our knowledge, the only example of the use of kiwi peels available in the literature regards the green synthesis of ZnO NPs [19]. So, this work presents a new usage of peels in synthesizing green AuNPs, demonstrating interesting features useful for clinical research that, in the next years, should prioritize the green approaches.

Another important and innovative aspect that is worth to be mentioned is the recycling of the exhausted solid raw waste, after extracting the polyphenolic content, as green adsorbent material suitable for water remediation technology, as described in our recent work [6]. So, the current research plan would offer a bio-circular economy strategy and an intrinsic, circular perspective where a secondary by-product (the exhausted solid waste) is also outputted in the bio-circular production for other eco-friendly environmental applications.

2 Materials and Methods

2.1 Chemicals

HAuCl_4 , ABTS (2,2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt), $(\text{NH}_4)_2\text{S}_2\text{O}_8$ (ammonium persulfate), DPPH (2,2-diphenyl-1-picrylhydrazyl), tyrosinase (from mushroom lyophilized powder, ≥ 1000 unit/mg solid), tyrosine (reagent grade, $\geq 98\%$), NaCl, LiCl, KCl, MgCl_2 , CaCl_2 , NaBr, NaI, NaClO_4 , KH_2PO_4 , KOH, HCl, and PerdrogenTM 30% H_2O_2 (w/w) were purchased

from Sigma-Aldrich (Milan, Italy); 4-thio (2'-deoxy) thymidine (S⁴TdR), having 99+ % purity, was purchased from Carbosynth (Compton, Berkshire, UK); Iron(II) sulfate heptahydrate (FeSO₄ • 7 H₂O), ≥ 99.0%, was purchased from ACS reagent. PhytoLab provided analytical standards for kaempferol-3-glucoside and quercetin-3-glucoside (Vestenbergsgreuth, Germany). Sigma-Aldrich provided the reference materials for the remaining 34 of the 36 bioactive compounds (Milan, Italy). Stock solutions of each analyte (1000 mg/L) were made by dissolving pure reference materials in methanol (HPLC-grade) and then keeping them at 5 °C in glass stoppered bottles until analysis. Working solutions of standards at different concentrations were made fresh by diluting stock solutions with methanol (HPLC-grade). Merck provided formic acid at a concentration of 99% (Darmstadt, Germany). Methanol of HPLC quality was acquired from Sigma-Aldrich, located in Milan, Italy. A Milli-Q SP Reagent Water System filtered deionized water to give ultrapure water with a resistivity of >18 M cm (Millipore, Bedford, MA, USA). Sartorius Stedim provided 0.2-μm polyamide filters which were used to filter all liquids (Goettingen, Germany). PhenexTM RC 4-mm 0.2-μm syringeless filters purchased from the company of Phenomenex, located in Castel Maggiore, BO, Italy, were used to filter all samples before injection into the HPLC instrument.

2.2 Kiwi Peel Wastewater Extract Preparation

Kiwi fruits were provided by local Italian producers (Bari, Apulia, Italy). The ones discarded because damaged were well washed and peeled. The obtained kiwi peels were placed in hot water and boiled for 10 min. The derived KPWW extract was centrifuged using a Thermo Scientific Heraemus Multifuge X3R Centrifuge, and the supernatant was stored at −19°C before use. The spent kiwi peel solid residual was separated, collected, dried in an oven at 50°C, and stored. The remained pulp was also collected, centrifuged, and stored at −19°C (see also Scheme S1).

2.3 Green AuNPs-KPWW Synthesis Protocol

A HAuCl₄ solution (1 × 10^{−3} M) was prepared in deionized water. 0.250 mL of this solution was mixed with 1.750 mL of KPWW extract to reach a final HAuCl₄ concentration of 1.25 × 10^{−4} M. Two milliliters was adopted as the total batch volume. The resulting colloidal solution was then moderately stirred by adopting different contact time intervals (from 1 to 24 h) to find the best experimental conditions for NP formation. Subsequently, each batch was centrifuged at 8000 rpm for 20 min using a D2012 High Speed Mini Centrifuge and washed twice with deionized water to remove unreacted Au³⁺ and KPWW. Before collecting UV-Visible

absorption spectra, the samples were diluted 1:5 with deionized water (see also Scheme S2).

2.4 UV-Visible Measurements

The UV-Visible absorption spectra of KPWW extract and AuNPs-KPWW colloidal solutions were collected using a Varian CARY 5 UV-Vis-NIR spectrophotometer (Varian Inc., now Agilent Technologies Inc., Santa Clara, CA, USA) in the range 200–800 nm, at a 1 nm/s scan rate. Measurements were performed by using a cuvette with a 1-cm path length. The AuNPs-KPWW colloidal stock solution had a concentration of 2 × 10^{−12} M.

2.5 ATR-FTIR Spectroscopic Measurements

ATR-FTIR spectra were recorded within the 400–4000 cm^{−1} range using a Fourier transform infrared spectrometer (FTIR spectrum two from Perkin Elmer, Waltham, MA, USA), whose resolution was set to 4 cm^{−1}. Sixteen scans were summed for each acquisition.

2.6 Transmission Electron Microscopy (TEM)

The samples subjected to TEM analysis were prepared by casting a drop of AuNPs-KPWW aqueous colloidal solution (10 μL from the stock solution having a concentration of 1 × 10^{−11} M) onto a carbon-coated copper TEM grid (400 mesh) and letting the solvent dry at room temperature.

2.7 Zeta Potential and Size Measurements

The average hydrodynamic diameters of AuNPs-KPWW were measured by dynamic light scattering (DLS) using a Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, UK) with a He–Ne red-light-emitting laser (wavelength, λ = 633 nm). The AuNP suspensions were diluted to obtain a concentration of 2 × 10^{−12} M. All measurements were performed in triplicate with the samples remaining in equilibrium for 2 min, considering the refractive indices of AuNPs (0.2) and the dispersant (1.33). During the DLS studies, the scattered light was detected at an angle of 173° (i.e., backscatter mode), and data processing was performed with the Zetasizer software 6.32 (Malvern Instruments). In addition, the same instrument measured the surface charge of AuNPs-KPWW as Zeta potential through laser Doppler velocimetry.

2.8 HPLC Analysis

The quantification of 35 bioactive analytes was carried out using a modified version of our previously described method [20]. The HPLC-MS/MS investigations were carried out

with an Agilent 1290 Infinity series and a Triple Quadrupole 6420 bought from Agilent Technology located in Santa Clara (CA, USA) and linked to an electrospray ionization (ESI) source that operated in negative and positive ionization modes. Employing Optimizer Software, each standard's MS/MS parameters were optimized by flow injection analysis (FIA). The separation of phenolic compounds was obtained by direct injection of diluted KPWW extract (1:5) applying a gradient elution mode on a Phenomenex Synergi Polar–RP C18 column (250 mm × 4.6 mm, 4 µm), using a mixture of water and methanol as solvents A and B, respectively, both with 0.1% formic acid. A Polar RP security guard cartridge preceded the column (4 mm × 3 mm ID) for column protection. The mobile phase composition was made up of the following components: 0–1 min, isocratic condition, 20% B; 1–25 min, 20–85% B; 25–26 min, isocratic condition, 85% B; 26–32 min, 85–20% B. A 0.2-µm polyamide filter was used to filter all solutions and solvents. The injection volume was 2 µl, and the flow rate was kept at 0.8 mL min^{−1}. The temperature of the column was set to 30 °C, and the drying gas temperature in the ionization source was set to 350 °C. The flow rate of the gas was set to 12 L/min, the capillary voltage was 4000 V, and the nebulizer pressure was 55 psi. The peak areas were integrated for quantitation after detection in the dynamic-multiple reaction monitoring (dynamic-MRM) mode. Each analyte's most abundant product ion was employed for quantification, while the other ions were used for qualitative analysis. Each compound's unique time window (Δ retention time) was set at 2 min.

2.9 AuNPs-KPWW Thermo-stability

A heating magnetic stirrer (Arex, Velp Scientifica) controlled by an MGW Lauda R42/2 digital thermometer was used to perform thermostability experiments. An appropriate dilution of the AuNPs-KPWW colloidal solution was prepared to obtain a concentration of 2×10^{−12} M and heated at different temperature values before collecting the corresponding UV-Vis absorption spectra, useful for monitoring if the temperature affected the AuNPs-KPWW stability.

2.10 AuNPs-KPWW Photostability

The AuNPs-KPWW photostability was assessed by irradiating the samples with a solar simulator lamp purchased from Oriel Corporation, Stratford, Conn, USA, Model 6684, equipped by a Xenon lamp (150 W) with an E_0 : 1482 mW/cm²~1.48 suns. A 1-cm path-length quartz cuvette containing the sample was placed at 6.5 cm from the source. The AuNPs-KPWW aqueous suspension was spectrophotometrically monitored and irradiated for 180 min. The same protocol was also followed for monitoring the photostability of

the raw KPWW extract. The concentration value of the used AuNPs-KPWW suspension was 2×10^{−12} M.

2.11 Determination of the Theoretical Sun Protection Factor (SPF)

As already reported in the literature [11–13, 21], a mathematical expression (Eq. 1) for calculating the theoretical SPF of the proposed AuNPs-KPWW as potential sunscreen ingredients in the UVB region was used and, for the purpose, the UV spectrophotometry was employed.

$$\text{SPF}_{\text{Spectrophotometric}} = \text{CF} \times \sum_{290}^{320} \text{EE}(\lambda) \times I(\lambda) \times \text{Abs}(\lambda) \quad (1)$$

where EE(λ) is the erythemal effect spectrum; I(λ) is the solar intensity spectrum; Abs(λ) is the absorbance of standard solutions of the tested AuNPs-KPWW samples in the wavelength region 290–320 nm; and CF is the correction factor (=10). The EE(λ)×I(λ) values obtained from the literature are constants [22, 23].

2.12 ABTS Assay

The ABTS was solubilized in water until reaching 7 mM as the final concentration for the stock solution. ABTS radical cation (ABTS^{•+}) derived from the reaction of the ABTS stock solution after adding 500 µL of ammonium persulfate (0.6 mg/mL). The mixture was left for 12 h in the dark at room temperature before its use. Subsequently, the solution was diluted 1:10 in water. By starting from this mixture, the studies were performed by diluting the latter solution (1:6) in water, also in the presence of different amounts of AuNPs-KPWW or KPWW extract samples, as specified during the discussion of the paper [11]. Equation 2 was used to calculate the % of the ABTS^{•+} bleaching due to the antioxidant activities of the explored samples:

$$\% \text{ of bleaching} = \frac{A_{\text{ABTS}} - A_{\text{sample}}}{A_{\text{ABTS}}} \times 100 \quad (2)$$

where A_{sample} is the absorbance value of the solution containing AuNPs-KPWW and ABTS after 1 h as incubation time, read at 800 nm, while A_{ABTS} is the absorbance value of the ABTS solution read at the same wavelength before adding AuNPs-KPWW. Before collecting the UV-Visible spectra, the samples were centrifuged to eliminate the contribution of the SPR band related to the AuNPs-KPWW presence.

2.13 DPPH Assay

The antioxidant activity of AuNPs-KPWW was also evaluated through the DPPH assay. The AuNPs-KPWW suspensions were adequately diluted in the presence of DPPH

radical for finally reaching a concentration of 8.0×10^{-4} M, utilizing methanol as solvent. The samples were taken in the dark, at room temperature, for 15 min and 60 min as contact time, respectively, and afterward, the absorption spectra of DPPH were recorded, focusing attention, particularly on the band settled at 517 nm [11–13]. In this case, before collecting the UV-Vis spectra, the samples containing AuNPs-KPWW were centrifuged after the incubation to eliminate the contribution of the SPR band related to the AuNPs-KPWW presence. Instead, the UV-Vis spectra referred to KPWW extract samples were collected as prepared. The calculation of the % DPPH bleaching, due to the antioxidant activities of the explored samples, was obtained by applying Eq. 3:

$$\% \text{ of bleaching} = \frac{A_{\text{DPPH}} - A_{\text{sample}}}{A_{\text{DPPH}}} \times 100 \quad (3)$$

A_{sample} is the DPPH solution absorbance at 517 nm after 15min/60min in contact with AuNPs-KPWW, and A_{DPPH} is the DPPH solution absorbance before adding AuNPs-KPWW.

2.14 Determination of the Antioxidant Activity in the Presence of 4-Thiothymidine, a Biomolecule

4-thiothymidine ($S^4\text{TdR}$) stock solution with a concentration of 1.0×10^{-3} M was prepared in deionized water and used for experiments. The hydrogen peroxide, H_2O_2 , was chosen as a model oxidant agent by performing experiments also when working in Fenton conditions. The nucleoside oxidation was progressively monitored by achieving UV-Vis spectroscopic measurements, following the intensity of the main absorption band at 337 nm, which collapses in favor of by-products. The $S^4\text{TdR}$ degradation induced by H_2O_2 and $\cdot\text{OH}$ was monitored in the presence and absence of AuNPs-KPWW to evaluate the antioxidant activity of the proposed nanoparticles.

2.15 Tyrosinase Assay

A tyrosinase stock solution of 1000 U/mL in phosphate buffer 5.0×10^{-2} M, pH 7.5, and properly diluted at 1:600, was used. A 2 mM tyrosine solution was prepared by adding HCl to dissolve the amino acid in water. The assay was performed in the dark by adopting 4 mL as the final volume. Two milliliters of the diluted tyrosinase solution was reacted with 160 μL of tyrosine and 1 mL of the AuNPs-KPWW sample. After adding tyrosine to the tyrosinase solution, the reaction was immediately analyzed by measuring the absorbance at 475 nm, indicative of the dopachrome formation [11–13]. The effect of the contact time was also

investigated. Equation 4 was used to calculate the % of dopachrome inhibition:

$$\% \text{ of inhibition} = \frac{A_{475(B)} - A_{475(S)}}{A_{475(B)}} \times 100 \quad (4)$$

where $A_{475(S)}$ is the absorbance value at 475 nm of solutions containing NPs and tyrosine/tyrosinase at different contact times, and $A_{475(B)}$ is the absorbance value of solutions in the absence of AuNPs-KPWW. Before each analysis, the samples containing NPs were centrifuged to remove the contribution of AuNPs-KPWW in the spectra. In addition, the raw KPWW extract was also subjected to the test.

2.16 Cell Culture

Endothelial-colony forming cells (ECFCs), a subpopulation of endothelial progenitor cells (EPCs), were isolated from >50 mL human UCB (umbilical cord blood) of healthy newborns after maternal informed consent and in compliance with Italian legislation. Cells were analyzed for the expression of surface antigens CD45, CD34, CD31 (BD Biosciences), CD105, ULEX, vWF, and KDR (Vector Laboratories) by flow cytometry as previously described [24, 25]. ECFCs were cultured in EGM2 (endothelial cell growth medium) medium (Euroclone) supplemented with 10% (v/v) fetal bovine serum (FBS, Euroclone). Cells were incubated at 37 °C with 5% CO_2 saturation. NHDF (normal human dermal fibroblast) (Lonza) were cultured in Dulbecco's modified Eagle's medium (DMEM, Euroclone) supplemented with 10% (v/v) fetal bovine serum (Euroclone).

2.16.1 Cell Viability

Cell viability was performed according to WST-8 assay. WST-8 utilizes the highly water-soluble tetrazolium salt WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt] that produces a water-soluble formazan dye upon reduction in the presence of an electron carrier. WST-8 is reduced by dehydrogenases in cells to give a yellow-colored product (formazan), which is soluble in the tissue culture medium. The amount of the formazan dye generated by the activity of dehydrogenases in cells is directly proportional to the number of living cells. 10000 cells were plated in 96 wells plate and treated for 24h with increasing doses (1:100, 1:50, 1:20, 1:10, 1:5 v/v) of AuNPs-KPWW. The day after, cells were washed with PBS (1 $\times$) three times and incubated again with 100 μL of media. Ten microliters of WST-8 stock solution (Merck) was added to each well, followed by incubation for 3 h at 37 °C under 5% CO_2 . Absorbance was recorded at 450 nm using a microplate reader (Biorad). The cell viability (percentage of control) was calculated according to Eq. 5.

$$\% \text{ of cell viability} = \frac{\text{OD}_{\text{Control}}}{\text{OD}_{\text{Treated}}} \times 100 \quad (5)$$

where OD is the optical density, and control (CTRL) are cells treated with the vehicle (water).

2.16.2 May-Grunwald-Giemsa Staining

Cells were plated in a p30 Petri dish and treated for 24 h with increasing doses of AuNPs-KPWW before performing May-Grunwald-Giemsa staining to visualize cellular morphology. May-Grunwald-Giemsa stain is a combination of two stains: May-Grunwald is an alcohol-based stain composed of methylene blue and eosin; Giemsa stain is an alcohol stain composed of methylene blue, eosin, and azure B. The solvent methanol initially fixes the cells. The basic dyes carry net positive charges; consequently, they stain nuclei (because of the negative charges of phosphate groups of DNA and RNA molecules). The eosin carries a net negative charge and stains cytoplasm.

3 Results and Discussion

3.1 Green AuNPs-KPWW: an Overview of Their Synthesis

This work aims to obtain a one-pot, low-cost synthesis of AuNPs functionalized with polyphenols. The goal was pursued by mixing an appropriate amount of KPWW extract with an aqueous HAuCl₄ solution. Thus, the formation of AuNPs-KPWW was observed, and the best working conditions were searched by changing the ratio between KPWW extract and HAuCl₄. The color of the derived colloidal

solution changed from light yellow (the typical color of HAuCl₄ solution) to violet, indicative of the AuNPs' occurrence [11].

Hence, the TEM analysis (Fig. 1A, B) showed polyhedral-shaped NPs having a mean size of 30 ± 10 nm. In excellent agreement with the previous literature [10, 11] describing natural extracts as reducing agents for forming green AuNPs, the TEM analysis revealed the presence of gold nanostructures wrapped with an organic coating. This organic layer can be attributed to the polyphenols in the KPWW extract that functionalize the AuNP surface. Indeed, by accomplishing a redox reaction, these biomolecules reduced gold from Au³⁺ to Au⁰, contemporary decorating the AuNP surface through coordination bonds and stabilizing AuNPs in water (see Scheme S2) [11, 12, 15, 16]. After the agglomeration of the obtained gold atoms (Au⁰), the formation of AuNPs-KPWW was obtained. Not surprisingly, by looking at the UV-VIS spectrum of AuNPs-KPWW reported in Fig. 2A, the clear presence of the SPR band at 555 nm, typical of metal nanomaterials [11–13], can be observed. A signal at around 280 nm was also observed and attributed to the presence of polyphenols from KPWW that wrapped AuNPs (Fig. 2A) [12, 13]. Based on these results, as the first step to searching for the best reaction time for obtaining AuNPs-KPWW, the SPR band position and shape were monitored during the AuNPs-KPWW formation (from 1 to 24 h). Figure 2B reports the AuNPs-KPWW SPR band time evolution, focusing the attention on wavelength position read at the maximum absorption values, along with the correspondent FWHM. Indeed, the position and shape of the SPR band are diagnostic to infer information about the particle size, dispersity, and aggregation [14, 16]. Usually, if, on the one hand, the maximum of the SPR shifts toward lower wavelengths when the particle size decreases, on the other hand, the increase in FWHM can be observed when the particle

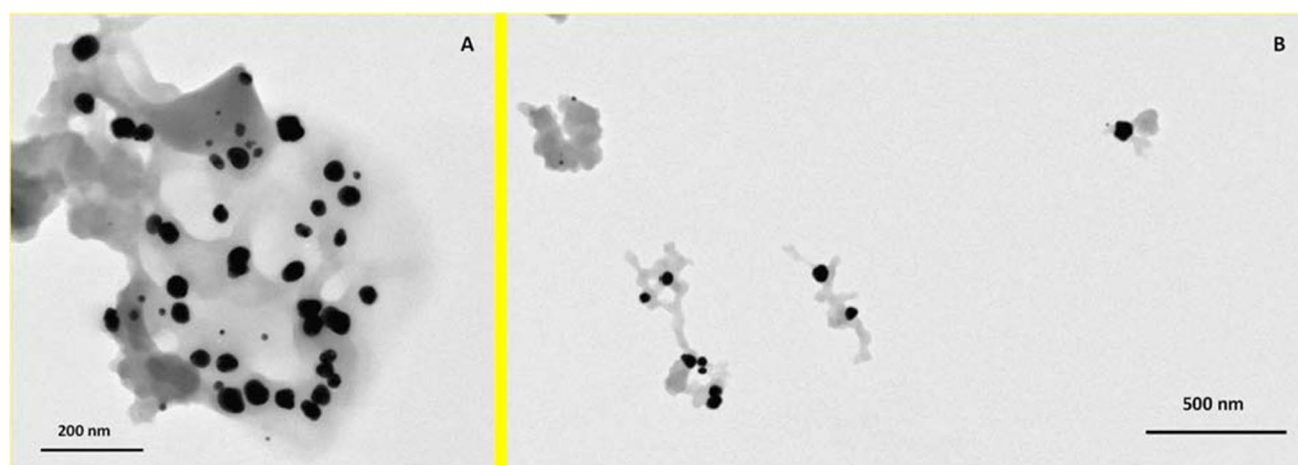


Fig. 1 TEM images of AuNPs-KPWW at different magnification ratios (A, B)

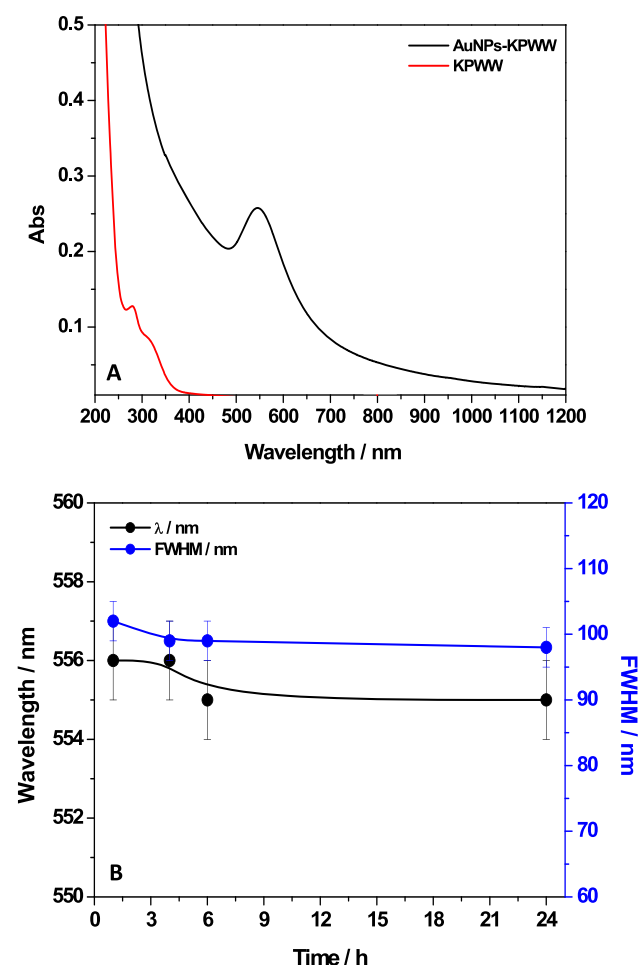


Fig. 2 UV-VIS spectra of KPWW extract and AuNPs-KPWW colloidal solutions (dilution 1:5 from the stock solution, for finally reaching an NP concentration of 2×10^{-12} M) (A); wavelength and FWHM time evolution of the AuNPs-KPWW SPR band in a water medium (B)

dispersity increases [14]. So, Fig. 2B shows that the SPR position moved from 556 to 555 nm after 24 h, adopted as incubation time, and the related FWHM changed from around 100 to 97 nm. The increased incubation time reduced the AuNPs-KPWW dispersity in favor of slightly smaller NPs. At the same time, the absorption intensity of AuNPs-KPWW was monitored, and, as reported in the *inset* of Fig. 2B, this value increased and leveled off after 24 h, suggesting the complete formation of AuNPs-KPWW. The characterization was completed by measuring the surface charge of the nanoparticles through Z-potential measurements. AuNPs-KPWWs are negatively charged with a Z-potential value of -40 ± 5 mV in an aqueous medium at pH 6.

To infer the concentration of AuNPs-KPWW, a molar absorption coefficient (ϵ) of 3.36×10^9 M $^{-1}$ cm $^{-1}$ was employed and related to the observed size [14]. Using the UV-Visible spectroscopy and the Lambert-Beer law, an AuNPs' mean concentration of 1×10^{-11} M from each

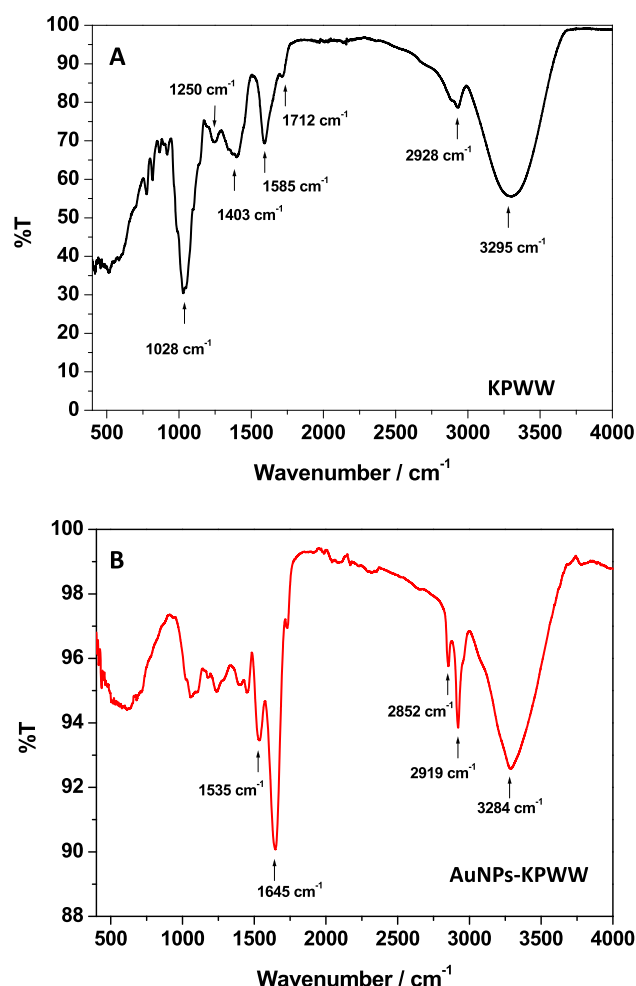


Fig. 3 ATR-FTIR spectra of KPWW extract (A) and AuNPs-KPWW (B)

synthesis batch (obtained after 24 h) was inferred, corresponding to 1 mg/mL of NPs. Particularly, by considering both the ϵ and the AuNPs-KPWW mean size, 1.79×10^{11} nanoparticles/mL could be accounted for.

3.2 ATR-FTIR Spectroscopic Measurements

ATR-FTIR experiments were performed to better identify the main nature and role of biomolecules on the AuNPs-KPWW surface (Fig. 3A and B). At first, the FTIR analysis was focused on KPWW extract to establish the possible class of water-soluble molecules involved in the NP formation. In this regard, it is worth mentioning that kiwi peels are lignocellulosic materials containing various species, such as alcohols, aldehydes, ketones, carboxylic, and ether compounds. But they are also particularly rich in polyphenols [7], which main presence was already supposed to be as evidenced in the KPWW extract during the UV-VIS analysis. Indeed, by looking

at the ATR-FTIR spectrum of KPWW extract, a typical profile attributed to polyphenols was observed [12, 13]. The spectrum reported in Fig. 3A shows a band at 3295 cm^{-1} due to polyphenols' O-H vibration with the possible contribution of water-soluble polysaccharides and carboxylic groups [26, 27]. Indeed, the band at 1028 cm^{-1} arose from C-OH bonds with the partial contribution of sugars and sugar-like structures. The bands at around 2928 cm^{-1} can be mainly attributed to asymmetric and symmetric C-H bonds stretching of methyl and methylene groups present in polyphenols [26, 27].

Accordingly, the CH_3 out-of-plane bending and scissoring were observed at around 1400 cm^{-1} . The band at 1712 cm^{-1} was identified as stretching vibration of C=O groups from aldehydes, ketones, carboxylic acids, and esters groups of polyphenols [26, 28]. The aromatic C=C stretching was detected at around 1585 cm^{-1} . The small contribution of amine moieties at 1590 cm^{-1} could also be taken into account, evidencing the occurrence of water-soluble amino acids and peptides [10, 11]. Additionally, the band at around 1250 cm^{-1} would be due to vibrations of C-O and C-OH groups, respectively, ascribed to hydroxyflavonoids [28]. Therefore, from the ATR-FTIR analysis, it was possible to assess the main presence, in KPWW, of aqueous soluble polyphenols that might play a key role in stabilizing AuNPs-KPWW as capping agents, wrapping their surface. Indeed, the synthesized AuNPs-KPWW (Fig. 3B) and KPWW extract showed a similar FTIR spectrum profile, retaining important features. The band at 3295 cm^{-1} shifted to 3284 cm^{-1} , appearing less wide and sharper, suggesting [29] the possible involvement of OH groups, derived from polyphenols, in the AuNP formation according to the proposed Scheme S2 [29]. The band at 1585 cm^{-1} , previously observed in the KPWW extract, was shifted toward small wavenumbers (1535 cm^{-1}); at the same time, the relative intensities of C-H vibrations at around 2900 cm^{-1} appeared intense and well-defined with respect to the same signals in KPWW extract. All these results could be ascribed to a different arrangement of polyphenols when on the NPs' surface. If the band at 1712 cm^{-1} attributed to C=O vibration occurred less evident, on the other hand, the band at 1645 cm^{-1} appeared very intense, and these outcomes could be attributed to the stretching and bending vibration of the hydroxyls on the AuNP surface, and the further possible involvement of C=O functionalities [15]. So, it is possible to assess that mainly -OH and -COOH groups from polyphenols of KPWW extract can be involved during the AuNPs-KPWW formation, capping the surface of Au through coordination bonds that should favor the reduction of gold, stabilizing the AuNPs in water medium (see Scheme S2).

3.3 HPLC/MS-MS Measurements

To unveil and better identify the nature of the main involved biomolecules, already qualitatively detected by ATR-FTIR analysis, HPLC/MS-MS measurements were additionally achieved, focusing the attention on the polyphenols detection. Table 1 reports the obtained results that refer to the analysis performed on KPWW extract before and after the AuNPs-KPWW formation and on the AuNPs-KPWW colloidal solution. Polyphenols belonging to different classes were detected in the KPWW extract, which exhibited a total phenolic content of 61.87 mg/L . Significantly, this value was reduced at 11.04 mg/L after the AuNPs-KPWW formation, indicating the clear involvement of polyphenols in the NP synthesis. Indeed, most of the detected biomolecules reported in Table 1 reduced their relative amount after the AuNPs' formation, confirming the finding. Moreover, the analysis was performed on the AuNPs-KPWW sample. Specifically, the colloidal solution containing AuNPs was filtered to remove them to demonstrate that the polyphenols not retrieved in the KPWW (after the AuNP formation) were on the AuNPs' surface and not free in the water. Indeed, the HPLC/MS-MS analysis performed on this latter water solution did not reveal the presence of polyphenols, undoubtedly confirming the findings.

3.4 AuNPs-KPWW Stability

With the aim of assessing the stability of AuNPs-KPWW, the effect of different parameters was investigated and reported as follows.

3.4.1 pH Effect

The explored pH values ranged from 2 to 12. Once again, the first screening of the possible effects induced by the pH change was carried out by monitoring the λ of the SPR signal and the related FWHM (Fig. 4A), considered diagnostic for nanoparticles' stability [14]. It is evident how both λ position and FWHM were significantly affected. If, on the one hand, a certain stability was observed in the pH range from 4 to 12, on the other hand, at pH 2, the λ and FWHM moved to higher values. The SPR signal shifts toward higher wavelengths, denoting the important contribution of aggregated AuNPs-KPWW that also tended to raise their polydispersity according to the corresponding FWHM increase [13]. This observation suggested that the large presence of H^+ can neutralize the negative NPs' surface charge, affecting their stability [14]. Indeed, these results were confirmed by Zeta potential and size measurements (Fig. 4B). The reported AuNPs-KPWW, when dispersed in a water medium in the range of pH 4–12, were negatively charged, with a Zeta potential ranging from -30 to -40 mV , suggesting

Table 1 List of polyphenols retrieved in KPWW extract before/after the AuNPs-KPWW formation and on the AuNPs-KPWW sample

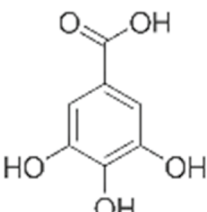
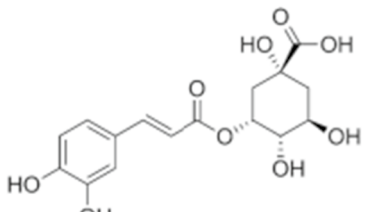
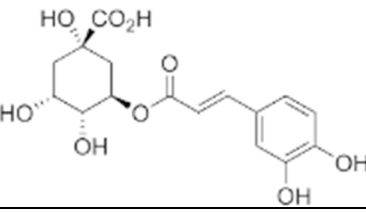
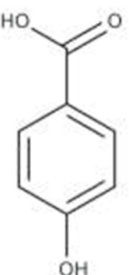
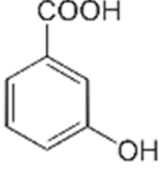
No.	Compound	KPWW extract before the formation of AuNPs (mg L ⁻¹)	AuNPs-KPWW	KPWW extract after the formation of AuNPs (mg L ⁻¹)
	Phenolic acids			
1	Gallic acid 	0.07	n.d.	n.d.
2	Neochlorogenic acid 	1.20	n.d.	0.56
3	Chlorogenic acid 	1.20	n.d.	0.46
4	p-Hydroxybenzoic acid 	0.03	n.d.	n.d.
5	3-Hydroxybenzoic acid 	n.d.	n.d.	n.d.
6	Caffeic acid	2.51	n.d.	2.00

Table 1 (continued)

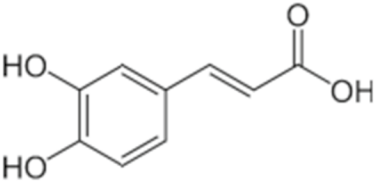
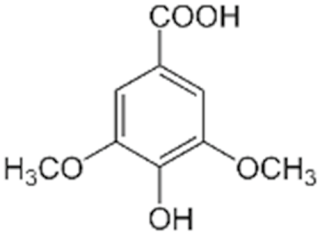
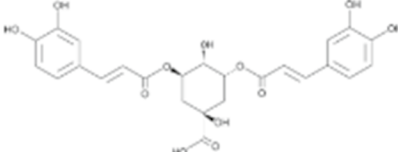
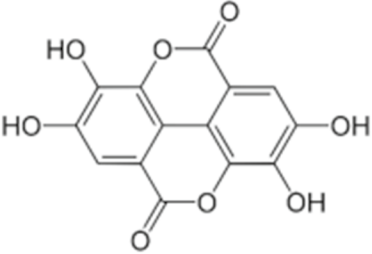
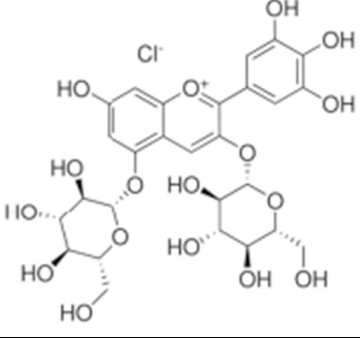
				
7	Syringic acid 	n.d.	n.d.	n.d.
8	3,5-Dicaffeoylquinic acid 	0.01	n.d.	n.d.
9	Ellagic acid 	2.50	n.d.	n.d.
	Flavonoids			
	A) Anthocyanins			
10	Delphinidin 3,5 diglucoside 	0.20	n.d.	0.06
11	Delphinidin3-galactoside	n.d.	n.d.	n.d.

Table 1 (continued)

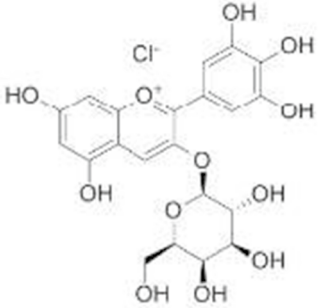
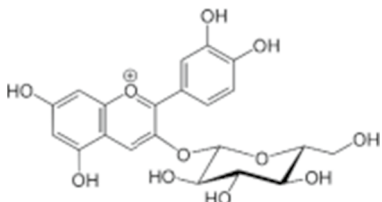
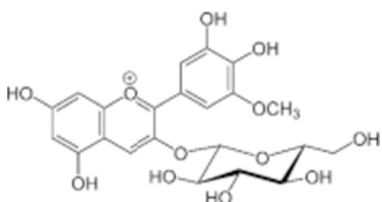
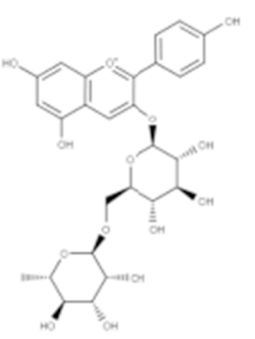
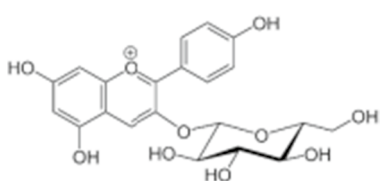
				
12	Cyanidin-3-glucoside 	n.d.	n.d.	n.d.
13	Petunidin-3-glucoside 	n.d.	n.d.	n.d.
14	Pelargonidin-3-rutinoside 	n.d.	n.d.	n.d.
15	Pelargonidin-3-glucoside 	n.d.	n.d.	n.d.
16	Malvidin-3-galactoside	n.d.	n.d.	n.d.

Table 1 (continued)

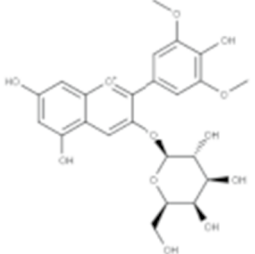
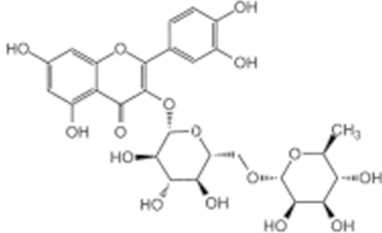
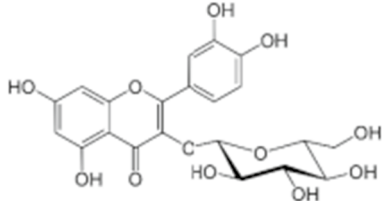
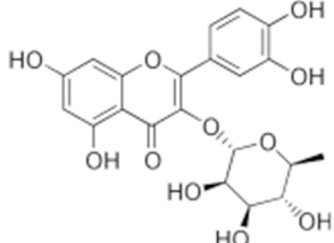
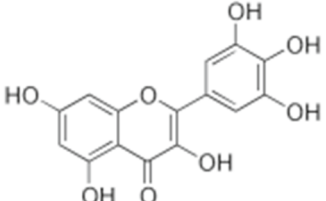
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	B) Flavonols			
17	Rutin  <chem>Oc1cc(O)c2c(c1)c3c(O)c(O)c(O)c3O[C@H]4[C@@H](O[C@H]5[C@@H](O)[C@H](O)[C@H]5O)[C@H](O)[C@H](O)[C@H]4O</chem>	0.88	n.d.	0.37
18	Isoquercitrin  <chem>Oc1cc(O)c2c(c1)c3c(O)c(O)c(O)c3O[C@H]4[C@@H](O[C@H]5[C@@H](O)[C@H](O)[C@H]5O)[C@H](O)[C@H](O)[C@H]4O</chem>	0.22	n.d.	0.07
19	Quercitrin  <chem>Oc1cc(O)c2c(c1)c3c(O)c(O)c(O)c3O[C@H]4[C@@H](O[C@H]5[C@@H](O)[C@H](O)[C@H]5O)[C@H](O)[C@H](O)[C@H]4O</chem>	0.93	n.d.	0.59
20	Myricetin  <chem>Oc1cc(O)c2c(c1)c3c(O)c(O)c(O)c3O</chem>	n.d.	n.d.	n.d.
21	Kaempferol-3-glucoside	0.01	n.d.	n.d.

Table 1 (continued)

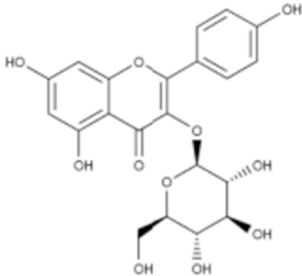
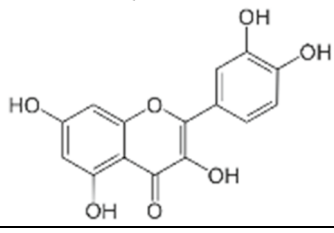
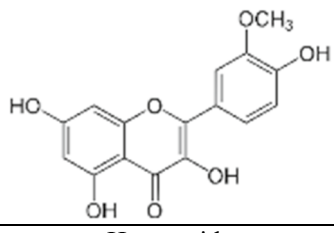
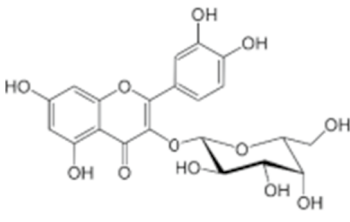
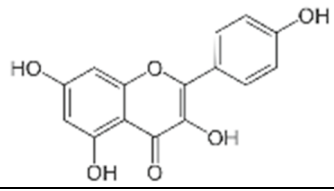
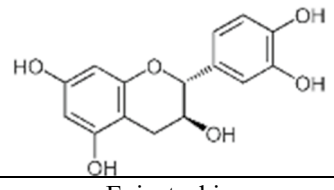
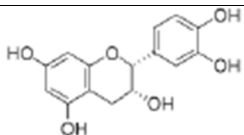
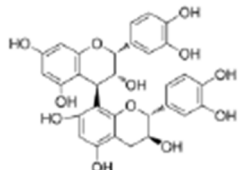
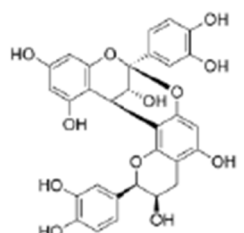
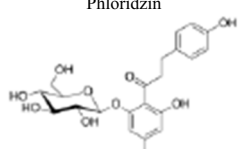
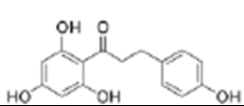
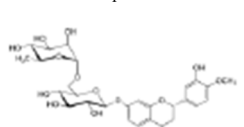
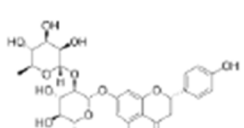
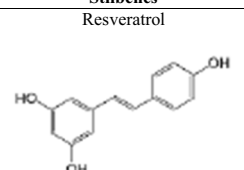
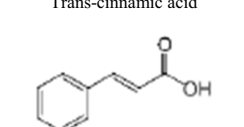
				
22	Quercetin 	0.12	n.d.	n.d.
23	Isorhamnetin 	n.d.	n.d.	n.d.
24	Hyperoside 	0.45	n.d.	0.07
25	Kaempferol 	0.01	n.d.	n.d.
	C) Flavan-3-ols			
26	Catechin 	4.12	n.d.	0.88
27	Epicatechin 	4.95	n.d.	0.77

Table 1 (continued)

				
28	Procyanidin B2 	40.76	n.d.	n.d.
29	Procyanidin A2 	0.24	n.d.	n.d.
D) Dihydrochalcones				
30	Phloridzin 	0.20	n.d.	0.14
31	Phloretin 	0.002	n.d.	n.d.
E) Flavanones				
32	Hesperidin 	n.d.	n.d.	n.d.
33	Naringin 	n.d.	n.d.	n.d.
Stilbenes				
34	Resveratrol 	n.d.	n.d.	n.d.
Non-phenolic acids				
35	Trans-cinnamic acid 	0.03	n.d.	n.d.
Total phenolic content		61.87	0	11.04

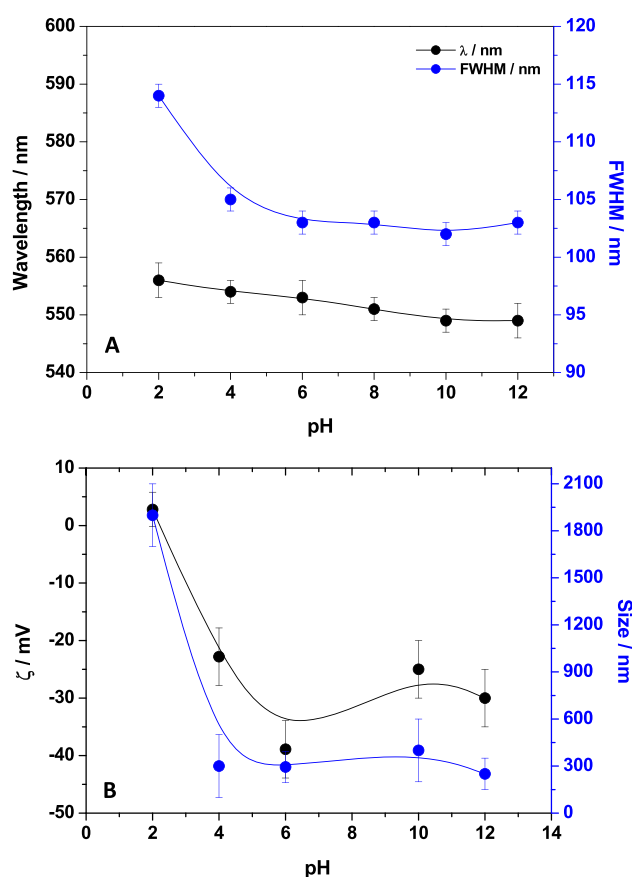


Fig. 4 Wavelength and FWHM time evolution of the AuNPs-KPWW SPR band (A) with the related Zeta potential and size (B), at different pH values. The adopted NP concentration was 2×10^{-12} M

good stability [14, 26]. At pH < 4, the Zeta potential values collapsed to zero. Accordingly, the measured sizes appeared constant, with a hydrodynamic diameter of 300 ± 100 nm, in the pH range of 4–12. Conversely, at pH 2, aggregated AuNPs were observed. In this regard, it is worth remembering that nanoparticles' size information provided by TEM analysis regarded the averaged diameter of single “dry” nanoparticles applied to a grid. However, while allowing the determination of the size and shape of nanoparticles, TEM measurements cannot produce information about NPs' properties in solution (such as aggregation), which is often important for assessing their suitability in many practical applications. On the contrary, size measures obtained using dynamic light scattering (DLS) in integrated instruments consider the organic shell and the environment in which nanoparticles are dispersed, providing their average hydrodynamic size. Therefore, the hydrodynamic size obtained by DLS measurement was considerably greater than that obtained by TEM. In addition, this technique is highly sensitive to the degree of particle aggregation [30–34].

These results, in synergy with ATR-FTIR and HPLC analyses, suggested the large contribution of molecules

having negatively charged functionalities, such as carboxylic moieties, in affecting the AuNPs-KPWW behavior. Indeed, carboxylic groups are negatively charged ($-\text{COO}^-$) up to pH 4, conferring to NPs a negative electrical charge in water, as experimentally observed. On the other hand, at pH values below 4, these organic moieties are in their protonated form ($-\text{COOH}$). Hence, at pH < 4, the AuNPs-KPWW rapidly tended to cluster due to surface charge neutralization [12, 13].

3.4.2 Salt Effect

As the second step, the effect of ionic strength on AuNPs-KPWW stability was studied. This aspect is very important for potential application in biomedicine. Due to their intrinsic thermodynamic instability, nanoparticles' colloidal suspensions tend to flocculate. Therefore, controlling AuNPs-KPWW aggregation under the experimental condition typical of many biological systems is important to evaluate possible practical applications. Indeed, as happens in many biological systems, sufficient ionic strength of the aqueous medium might induce NP aggregation by screening the electrostatic repulsion (a well-known process in metal nanoparticle suspensions) [35, 36].

Again, the λ and FWHM of the SPR band, the AuNP size, and Zeta potential were considered in synergy, and Figs. 5 and S1 show the results. In this context, it is worth mentioning that, in general, the presence of electrolytes reduces the colloidal stability of the systems when electrolyte concentration is high. Indeed, the absorption spectra acquisition was difficult at high ionic strength ($I > 0.5$ M) because nanoparticles tended to precipitate (*data not shown*). So, the spectrum acquisition needed to be performed within 2 min from the sample preparation.

NaCl was chosen as a model among salts, and its effect on AuNPs was evaluated. As reported in Fig. 5A, by increasing the salt concentration from 0.01 to 3 M, a slight red shift of the SPR band was observed, along with a mild increment in the FWHM. The Zeta potential and size measurements are reported in Fig. 5B. The Zeta potential progressively incremented its values from the solution in absence of salt to those in the presence of NaCl. Particularly, zero-charged AuNPs-KPWW were obtained above NaCl 1 M due to the screened surface charges by Na^+ [37]. This might indicated a trend toward NPs' aggregation due to a prevalence of van der Waals attraction forces between nanoparticles according to the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory. However, looking at both the maximum absorption position and corresponding FWHM values (Fig. 5A), this not seemed to be the case in the short time from sample preparation. FWHM and wavelength varied within a very low range, about 5 and 3 nm, respectively. The absence of a significant peak broadening and wavelength red-shift in the presence

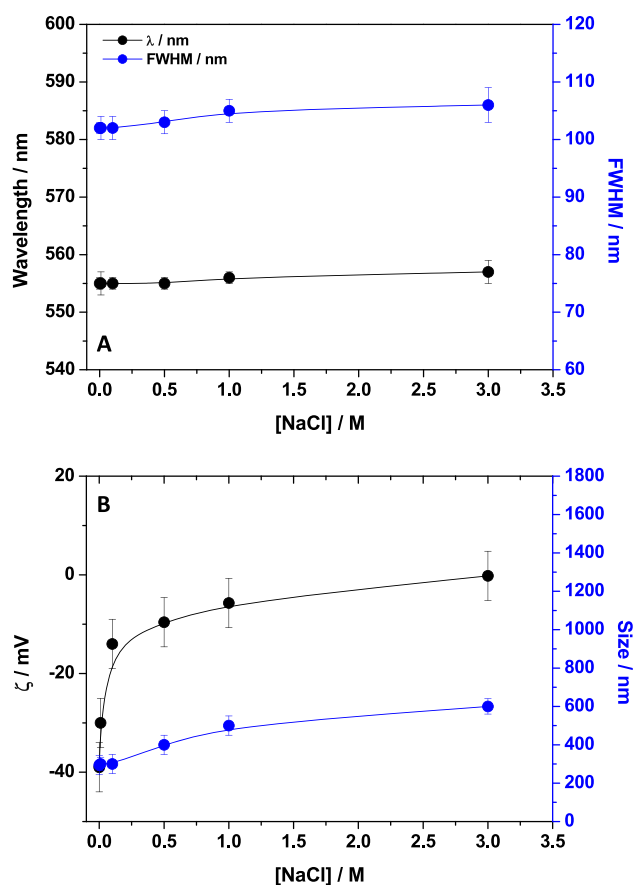


Fig. 5 Wavelength and FWHM time evolution of the AuNPs-KPWW SPR band (A) with the related Zeta potential and size (B), at different NaCl concentrations. The adopted NP concentration was 2×10^{-12} M

of NaCl concentrations up to 3 M suggested resistance to aggregation at least in the first 5 min after preparation [36].

In detail, for all values of NaCl concentration, no significant modification of the NPs' size was observed in the short time. However, this stability was maintained over time for low concentrations (up to 0.5 M), while at higher salt concentrations, the suspension became unstable within 10 min (*data not shown*). Likely, NaCl concentrations less than 0.5 M were not enough to completely screen AuNPs-KPWW surface charges, and the colloidal suspension is long-term stable. This is also confirmed by the trend of Zeta potential values (Fig. 5B), which becomes greater than -30 mV while still keeping negative values at 0.5 M of NaCl.

With a further increase in the salt concentration, a gradual and slow aggregation process probably occurred over time, as suggested by the increment of the AuNPs-KPWW hydrodynamic diameter from 300 to ~ 600 nm at a Zeta potential value of almost zero (Fig. 5B). This was consistent with the previous literature stating that colloidal particles electrostatically stabilized can be considered long-term stable when Zeta potential $\geq |30|$ mV. Conversely, as Zeta potential $\leq$

$|30|$ mV or approached zero, the colloidal suspension became unstable and begins to flocculate [35, 37, 38].

Thus, the AuNPs-KPWW aggregation was favored at high ionic strengths because the electrostatic double layer (EDL) on the AuNP-KPWW surface was compressed and neutralized, inducing the AuNPs aggregation under the effect of van der Waals forces [38, 39].

The phenomenon was further studied by changing both the nature of cations and anions (Fig. S1). The nature of the cation (by fixing Cl^- as the anion) was explored, and Fig. S1A shows the obtained results. Based on the previous observations, a rapid screening was performed by primarily focusing on FWHM, monitoring its values when changing the type and concentration of the electrolyte. In detail, increasing the monovalent cation size (from Li^+ to K^+), the FWHM slightly increased. This indicated that the dispersity and aggregation of AuNPs-KPWW were favored, especially at higher salt amounts, even if in different ways depending on cation type. In particular, Li^+ , Na^+ , and K^+ worked dissimilarly due to their different size and hydration capability. Specifically, their radii of hydration are $\text{Li}^+ = 3.4$ Å, $\text{Na}^+ = 2.76$ Å, and $\text{K}^+ = 2.32$ Å [39]. So, Li^+ strongly retained water molecules due to its smaller size with respect to K^+ or Na^+ , reducing the interaction with the NP surface [37]. The EDL thickness and, thus, the AuNPs-KPWW aggregation were probably slightly affected. Indeed, the effect became evident only if increasing the Li^+ concentration. On the other hand, when passing from Li^+ to K^+ , the effect was more pronounced, mostly influencing the EDL, and the use of the largest cation showed the greatest effect. Not surprisingly, the effect was more evident by adopting bivalent cations, such as Mg^{2+} and Ca^{2+} . Mg^{2+} and Ca^{2+} would shorten the Debye length, being strongly attracted to the NPs' surface, enhancing clusterization [40]. According to the divalent cation bridging theory, bivalent cations should have a nonspecific binding capacity with negatively charged functional groups, greatly affecting the NPs' stability [41]. The differences between monovalent and bivalent cations were further evidenced by comparing the Zeta potential and the size of AuNPs-KPWW in the presence of NaCl and MgCl_2 . Figures S1C and D report the obtained results. As expected, MgCl_2 greatly influenced the stability of AuNPs-KPWW. Indeed, the nanoparticles were characterized by a very small Zeta potential, near zero, when the MgCl_2 concentration was low if compared with NaCl under the same condition (Fig. S1D). The simultaneous increment of the hydrodynamic radius of NPs can be also observed (Fig. S1) at the same concentration of NaCl. In particular, comparing the electrolyte concentration of 0.5 M, the size of NPs in MgCl_2 increased up to about 700 nm, whereas in NaCl was ~ 350 nm, highlighting the role of MgCl_2 in the NPs clustering. To infer other details, the same analysis was performed by changing the anions' type. Again, the FWHM was

monitored, and the study was focused on Cl^- , Br^- , I^- , and ClO_4^- (Fig. S1B), fixing the nature of the cation (Na^+). The obtained results clearly showed the expected minor role of anions in the AuNPs-KPWW stability due to the negatively charged AuNPs-KPWW surface. On this ground, the Zeta potential and size measurements were carried out. Although the anions were changed, the results appeared similar to each other's (*data not shown*).

3.4.3 Thermostability and Photostability: AuNPs-KPWW as Sunscreen

Experiments were performed to assess the thermostability and photostability of as synthesized AuNPs-KPWW. The AuNPs-KPWW colloidal solution was heated in a temperature range from 20 to 80°C (Fig. 6A) and irradiated with solar light (Fig. 6B), respectively. Once again, the λ and the FWHM of the SPR band were monitored. As previously discussed, since the position and shape of the SPR band are strongly related to particle size, dispersity, and aggregation, the observed lack of significant changes during the

experiments suggested the AuNPs-KPWW thermostability and photostability.

Basing on these considerations, the possibility of using AuNPs-KPWW as potential physical and chemical filters arises. Indeed, AuNPs-KPWW, due to their photostability, should prevent sunlight damage by absorbing and scattering the radiation [11–13]. In particular, the polyphenols on the AuNPs-KPWW surface, due to their absorption, have the features of a typical sunscreen. They can potentially absorb in the UV-A and UV-B regions of the solar spectrum (see Fig. 2A, where a high absorption in the region below 400 nm is evident) [11, 12]. Accordingly, this feature indicated the AuNPs-KPWW potential use as a sunscreen, preventing sunburns and other skin damages. So, the theoretical SPF value, through absorption spectroscopy, was evaluated to obtain a tangible result [11–13]. Equation 1 was thus used for the purpose. For example, by adopting the spectrum reported in Fig. 2A corresponding to AuNPs-KPWW 2×10^{-12} M, an SPF value of 5 was inferred. This concentration value was chosen according to the cytotoxicity assessment discussed in the paper, which suggested the absence of toxic effects on the adopted cell lines under the tested condition. However, the amount of AuNPs could be potentially increased when used as a functional ingredient in a cosmetic formulation. To pursue this aim, work is in progress. Hence, based on the obtained results, the synthesized nanostructures could be proposed as SPF boosters for increasing the sunscreen features of commercial products and preventing skin photodamages, along with the already-known TiO_2 and ZnO . About this topic, according to previous literature [11, 42–44], skin photodamage is usually favored by the reactive oxygen species (ROS) formation induced by UV light. A series of negative effects could be observed, such as a reduced antioxidant defense system and cutaneous disorders. AuNPs-KPWW, due to their intrinsic antioxidant properties (demonstrated in the next paragraph), could be suitable as additional ingredients for formulating sunscreen cosmetic products, ensuring an “ancillary photoprotection” [11–13].

3.5 Antioxidant Properties of AuNPs-KPWW

3.5.1 ABTS and DPPH Assays

Another aspect investigated was the antioxidant features of AuNPs-KPWW. As described, polyphenols are considered the main biomolecules responsible for the AuNPs-KPWW synthesis, capping and stabilizing them in a water medium. Since the antioxidant properties of polyphenols are well-known [10, 12, 13], the synthesized AuNPs-KPWW were tested to evaluate if polyphenols' antioxidant properties were retained by the NPs. For this purpose, the ABTS and DPPH assays were carried out both on KPWW extract and AuNPs-KPWW.

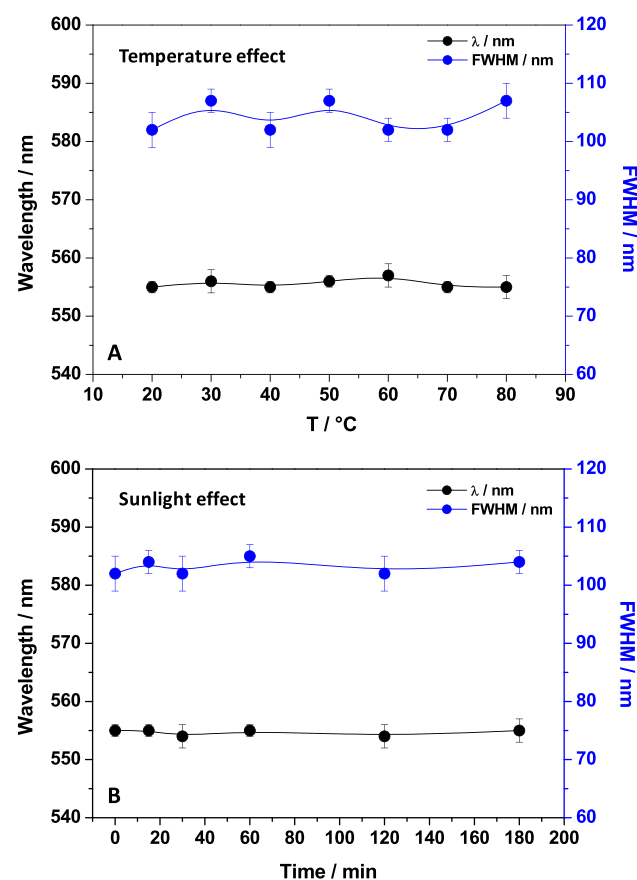


Fig. 6 Wavelength and FWHM time evolution of the AuNPs-KPWW SPR band in water medium at different temperatures (A) and sunlight exposure (B). Experiments were performed in an AuNPs-KPWW colloidal suspension 2×10^{-12} M

In detail, the ABTS test was performed in water, monitoring spectrophotometrically (from 500 to 900 nm) the bleaching of the $\text{ABTS}^{\bullet+}$ solution when in the presence of different amounts of KPWW extract or AuNPs-KPWW. The contact time was fixed at 15 min for the KPWW extract due to its fast reactivity and 60 min for AuNPs-KPWW. Equation 2 was used to infer the % of ABTS bleached, indicative of the potential antioxidant activity. The obtained results are reported in Fig. 6. KPWW extract in different amounts, ranging from 25 to 200 mg/L, showed a dose-response effect (Fig. 7A): the ABTS bleaching changed from 50 to around 100% when in the presence of the highest KPWW amount. Additionally, if the incubation time was extended to 60 min, the differences between the samples level off, i.e., all the concentrations of KPWW extracts produced 100% of ABTS bleaching. The behavior of AuNPs-KPWW was

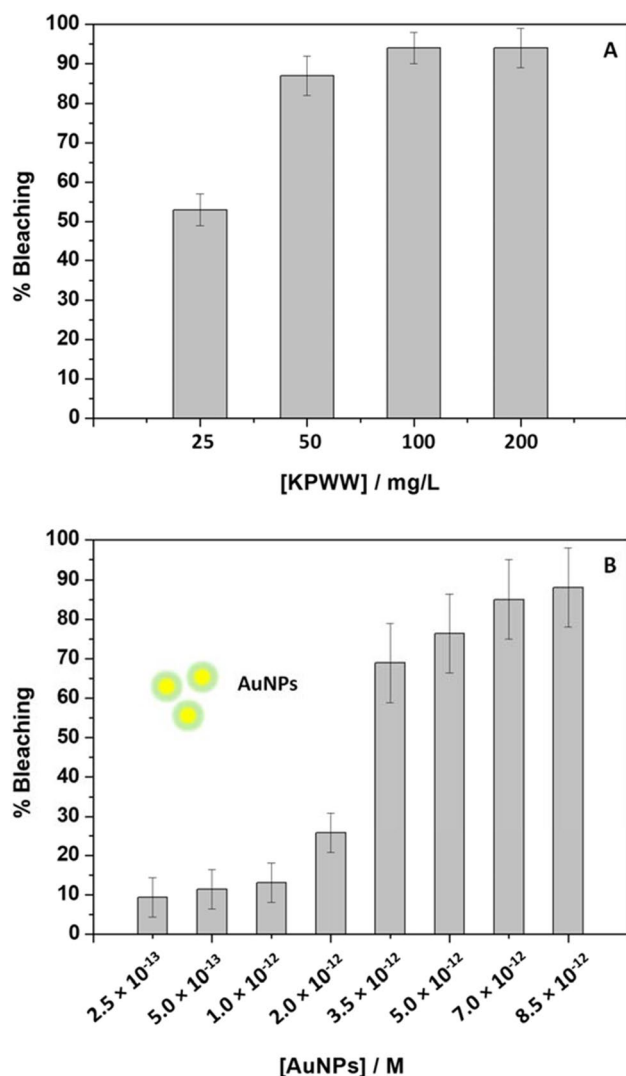


Fig. 7 % of ABTS bleaching referred to KPWW extract (A) and AuNPs-KPWW explored at different concentrations (B)

subsequently investigated, working in the same conditions (Fig. 7B). Once again, a typical dose-response histogram was observed. However, since the contact time used in the nanoparticle experiment was 60 min, this indicated that the $\text{ABTS}^{\bullet+}$ bleaching was slowed down after diluting the sample. Notwithstanding, the obtained results are worth to be mentioned because the NP concentrations were much lower than the KPWW extracts. Indeed, a high % of bleaching, around 100%, comparable with KPWW, was obtained thanks to the pool of polyphenols retained on the NPs' surface.

To further confirm the AuNPs-KPWW antioxidant activity, the DPPH assay was subsequently performed. In this case, the bleaching of the radical $\text{DPPH}^{\bullet}$ was monitored. The related methanolic solution was purple, absorbing around 517 nm, and changed to yellow if reduced by an antioxidant. Figure S2 reports the obtained results, once again, in terms of a dose-response histogram. However, it should be noted that, as reported elsewhere [45], the DPPH test, compared to the ABTS one, is less sensitive and slower, probably due to the methanolic environment in which the test was performed.

3.5.2 Testing Antioxidant Properties of AuNPs-KPWW in the Presence of H_2O_2 and $\cdot\text{OH}$

To further confirm the previous results, additional experiments were performed in the presence of AuNPs-KPWW and H_2O_2 (0.5 M), adopted as model oxidant specie. The Fenton reaction was also studied to produce the hydroxyl radical $\cdot\text{OH}$ [46]. More specifically, the SPR band (Fig. S3A) was monitored for 180 min, and the shape of the signal appeared slightly changed in the presence of H_2O_2 . Particularly, the SPR intensity was mildly reduced by extending the incubation time, and the FWHM showed the same trend. Likewise, it happened when working under Fenton conditions (*data not shown*). These observations suggested a reduced stability of the AuNPs-KPWW when H_2O_2 or Fenton reagents were added. This could be rationalized by considering the mechanism of AuNPs-KPWW formation, as previously discussed in the paper. The organic layer constituted by the KPWW polyphenols surrounding NPs, behaving as capping for stabilizing them in water, was progressively oxidized by H_2O_2 and/or $\cdot\text{OH}$, favoring the observed instability. This finding was confirmed if looking at the ATR-FTIR spectra of AuNPs-KPWW collected before and at the end of the experiments (Fig. S3B). The effect of the used oxidants agents was the same: the band at 3284 cm^{-1} ascribed to OH groups moved toward lower wavelength values, 3278 cm^{-1} , and at the same time, the ratio between this signal and those ascribed to C-H vibration changed. The OH band reduced its intensity by appearing also broadened. Moreover, the ratio between the signals at 1645 cm^{-1} and 1535 cm^{-1} changed in favor of the latter. Indeed, their band position moved to 1632 cm^{-1} and 1527 cm^{-1} , respectively. Overall, these results

indicate a clear reaction of H_2O_2 and $\cdot\text{OH}$ with polyphenols that acted as antioxidants being partially degraded under the adopted working conditions [10, 12, 13].

3.5.3 Antioxidant Activity of AuNPs-KPWW Toward a Biomolecule When in the Presence of H_2O_2 and $\cdot\text{OH}$

To better appreciate the AuNPs-KPWW antioxidant activity, the oxidation of a biomolecule, the 4-thiothymidine (S^4TdR), a sulfur-modified nucleoside, was monitored in the presence of AuNPs-KPWW. In particular, the ability of AuNPs-KPWW to prevent the degradation of this nucleoside in the presence of H_2O_2 and $\cdot\text{OH}$ was evaluated. S^4TdR was adopted because some of the authors of this paper proposed it as a suitable chemical probe to detect H_2O_2 and $\cdot\text{OH}$ among ROS [41, 43]. In detail, the nucleoside oxidation was studied using the UV-Vis spectroscopy, and the results are reported in Fig. S4. Moreover, the reaction with H_2O_2 was monitored after 180 min, whereas the same reaction under Fenton conditions was followed up after 60 min due to the higher reactivity toward $\cdot\text{OH}$. As well described by Rizzi et al. [47, 48], the nucleoside degradation determines a decrease of the absorption intensity at 337 nm, the signal characteristic of this nucleoside in a water medium at neutral pH. Indeed, the intensity reduction of that band is diagnostic for S^4TdR oxidation with the formation of other signals, denoting the presence of by-products [46, 49]. In particular, when H_2O_2 oxidizes S^4TdR , several products can be obtained: thymidine (TdR) and a hydroxylated intermediate (R-SOH) are the main ones [46, 49]. If TdR shows an absorption band in UV-Vis spectrum at around 265 nm, R-SOH can be detected by looking at the absorption at 360 nm [46, 49]. In particular, the R-SOH hydrolysis induces the TdR formation. On this basis, the control samples in the absence of oxidant agents were compared with the same samples in the presence of AuNPs-KPWW (Fig. S4A). Starting from the reaction in the presence of only H_2O_2 , after 180 min, an intense shoulder was observed at 360 nm, indicating the outcoming formation of R-SOH. Accordingly, its presence was also confirmed by the slight wavelength red-shift of the S^4TdR main absorption due to the contribution of this specie at 360 nm. At the same time, since the R-SOH started hydrolysis, TdR was detected at 265 nm [46, 47, 49]. Significant changes were observed when the same reaction was followed in the presence of AuNPs-KPWW. Since the absorption band at 337 nm appeared more intense than the same signal in the absence of NPs, this finding indicated that the oxidation was slowed down. So, the R-OH formation occurred retarded, and the related signal was less pronounced. Accordingly, also the band at 265 nm, due to the formation of TdR, was less evident. Overall, these results confirmed the antioxidant capacity of AuNPs-KPWW in preventing the oxidation of the biomolecule. This is better

observed by testing the NPs' behavior in the Fenton reaction conditions. Particularly, Fig. S4B shows the same spectroscopic features. However, the differences between the samples in the absence and presence of AuNPs-KPWW were more evident and already detectable after 60 min.

3.6 Tyrosinase Assay

As well confirmed from the literature, polyphenols can play a key role in inhibiting tyrosinase [50], an enzyme involved in melanin synthesis within the first two-steps of the melanogenesis process [12, 13, 51, 52]. As previously done for the antioxidant's activity, the features of KPWW extract were preliminarily investigated (Fig. 8A). The incubation time between KPWW and the enzyme was fixed at 60 min. By adopting different amounts of KPWW (Fig. 8A), the

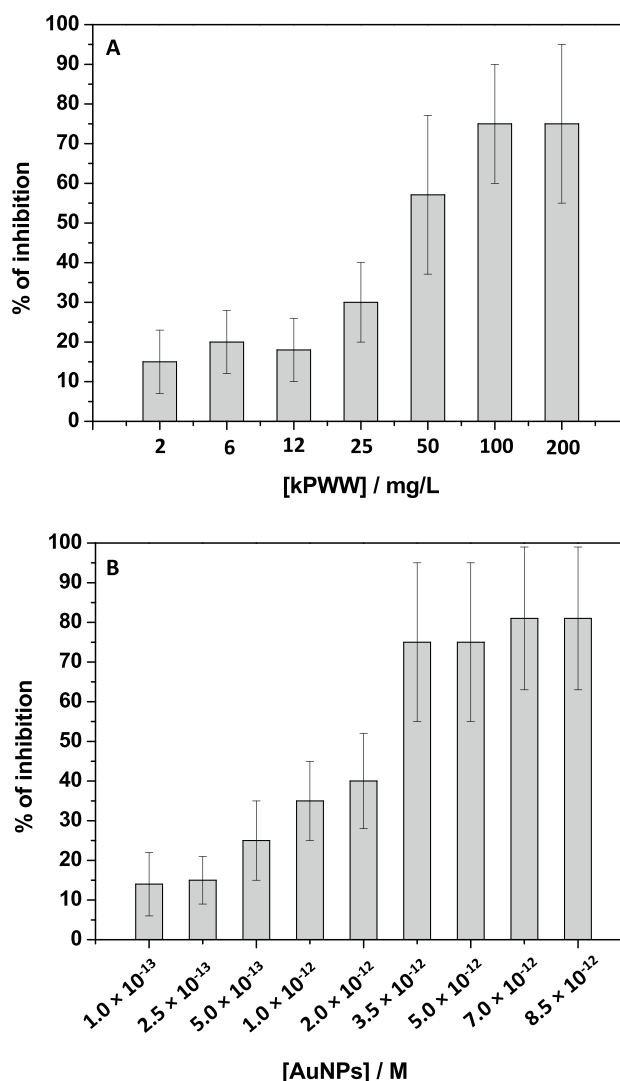


Fig. 8 % tyrosinase inhibition of raw KPWW extract (A) and AuNPs-KPWW (B) at different added amounts

dose-dependent tyrosinase inhibition was observed, being quite complete when KPWW added amount was fixed at 100 and 200 mg/L. A similar behavior was observed for AuNPs-KPWW (Fig. 8B), suggesting that the tyrosinase inhibiting activity ascribed to polyphenols was retained on AuNPs-KPWW, although the amount of NPs was very low, as also previously displayed for testing the antioxidant activity. These results suggest the possibility of proposing AuNPs-KPWW for applications in skin-lightening products [11–13].

3.7 Assessment of AuNPs-KPWW Compatibility with Human Cells

Finally, AuNPs-KPWW biocompatibility was assessed onto two human cell lines, ECFCs, and NHDF, measuring the mitochondrial activity by WST-8. The WST-8 test assesses cell metabolic activity by measuring the ability of the mitochondrial succinate-tetrazolium reductase system to convert negatively charged water-soluble tetrazolium dye into purple-colored formazan in living cells. The results demonstrated very mild cytotoxicity, with a ~5 to 9% decrease in viability on both cell lines with the lowest adopted concentration of AuNPs-KPWW (Fig. 9A, B). However, there was no significant difference in the ECFC viability between the control and treatment at all the increasing AuNPs-KPWW concentrations (from 1×10^{-13} to 2×10^{-12} M) (Fig. 9A), while it was observed an increase in the mitochondrial activity of NHDF (15–28%) (Fig. 9B). Their

effect on cell morphology of ECFCs and NDHF, stained with May-Grunwald-Giemsa, was analyzed to confirm the biocompatibility of AuNPs-KPWW using a light microscope. Indeed, the optical images reported in Fig. 9C and D showed a dose-dependent uptake of nanoparticles, identifiable as the black areas inside the cells and with no changes in cell morphology even at the highest nanoparticle concentration. Considering these results, it is possible to confirm that the proposed nanoparticles are safe and potentially used for biomedical purposes since they do not affect cell viability or alter cell morphology.

4 Conclusions

Advances about the use of green synthesized AuNPs, specifically AuNPs-KPWW, obtained by following a novel green approach starting from an agricultural/food waste, are reported in this paper. Using a water-based polyphenolic extract derived from kiwi peels, KPWW, hybrid polyhedral NPs with a metallic central core wrapped with an organic layer made of polyphenols from KPWW were produced. Thus, a one-pot and low-cost approach for the fast production of AuNPs-KPWW is reported, demonstrating as polyphenols were able to reduce a salt solution of Au^{3+} to Au^0 . UV-Vis and ATR-FTIR spectroscopies were used in synergy to confirm these findings, enabling the possibility of assessing the physical and chemical features

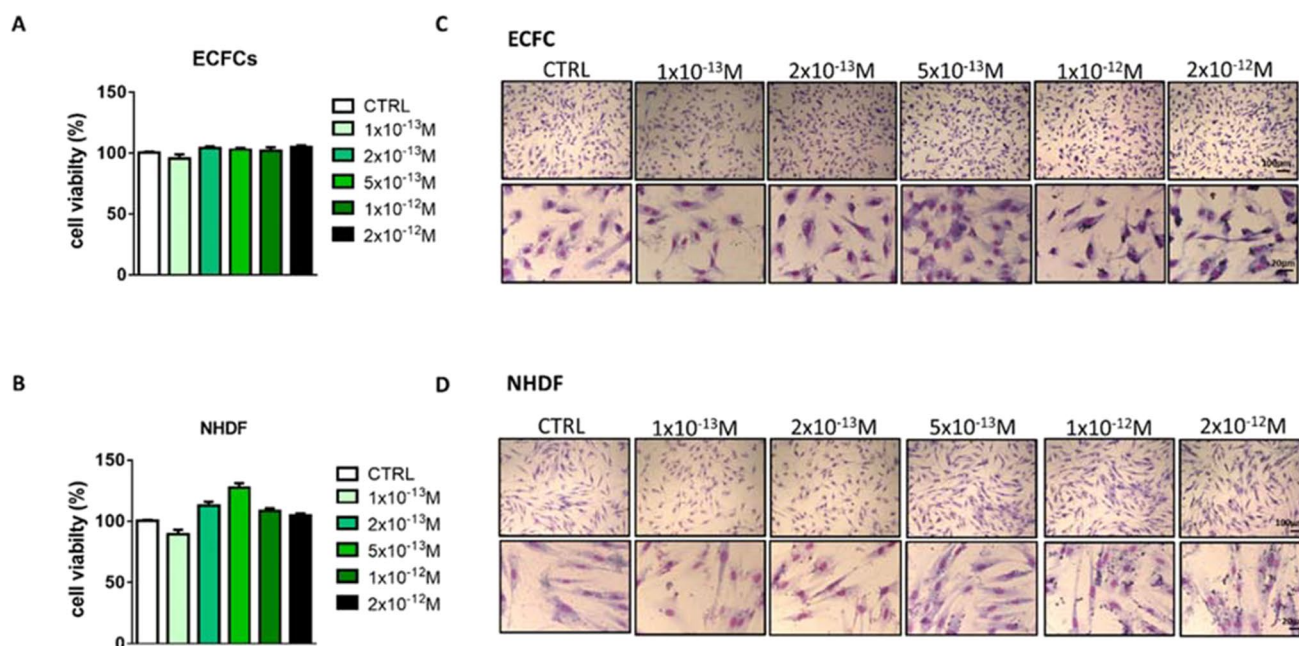


Fig. 9 Effects of AuNPs-KPWW in NHDF and ECFCs. Histograms refer to % of viable cells based on tetrazolium salt (WST-8) assay (A, B); Cell morphology before and after treatments: Images are repre-

sentative of cell cultures photographed using a phase-contrast microscope and stained with Maygrunwald-Giemsa (C, D); Data are presented as the mean $\pm$ standard deviation from triplicate experiments

of AuNPs-KPWW. By monitoring and measuring the SPR band, particularly referring to the wavelength positions and FWHM, information about the AuNPs-KPWW stability was acquired. The role of temperature, light, pH, and ionic strength was shown, and the results revealed that the proposed NPs were stable in a large range of temperatures and under sunlight irradiation. When in the range of pH 4–12, a certain AuNPs-KPWW stability was observed. On the other hand, at pH 2, the NP aggregation occurred. All these findings were also confirmed after performing dynamic light scattering and Zeta potential measurements. When in the presence of monovalent cations, the AuNPs-KPWW aggregation was observed only at high ionic strength concentrations. The results evidenced the interaction of cations with negatively charged NPs, and the effect was more pronounced if bivalent cations were in use. So, EDL thickness changed, affecting the AuNPs-KPWW stability in water. Finally, the antioxidant and tyrosinase inhibition features of AuNPs-KPWW were positively evaluated using ABTS, DPPH, and an enzymatic test, respectively, evidencing their potential use in biomedicine or cosmetic. Indeed, the AuNP biocompatibility was investigated in two human cell lines, namely endothelial colony-forming cells and normal dermal fibroblasts. AuNPs-KPWW were safe and potentially feasible in nanomedicine, not affecting cell viability and morphology. The theoretical SPF was also calculated and resulted dose-dependent. For these reasons, thanks to the organic shell around their surface being able to absorb the UV radiation, the AuNPs-KPWW could be potentially proposed as a sunscreen ingredient for boosting the SPF.

The associated costs were also calculated and mainly ascribed to the gold salt solution. Indeed, the cost associated with kiwi peels is quite null, and the amount of water used and energy cannot be significant on a lab scale. 0.6 €/kg of AuNPs-KPWW was the supposed cost, indicative of a cheap production that could also be attempted at a future large scale by simply scaling up the amount and volume, obtaining AuNPs-KPWW that retained the same features.

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Data Availability The authors declare that the data supporting the findings of this study are available from the corresponding authors upon reasonable request.

Declarations

Ethical Approval Not applicable

Conflict of Interest The authors declare no competing interests.

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