

Green Synthesis of Biogenic Gold Nanoparticles Using *Crassula Ovata* and *Bryophyllum Pinnatum* Leaf Extracts: Characterization and In-Vitro Evaluation of Antioxidant, Cytotoxic and Anti-Inflammatory Activities

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Abstract

Ethanollic leaf extracts of *Crassula ovata* and *Bryophyllum pinnatum* were used as reducing and capping systems for biogenic gold nanoparticles, and the products were carried through three unrelated in vitro endpoints under one protocol. Extract was reacted with 1 mM chloroauric acid at a 1:9 extract-to-salt ratio (pH 6.0, 70 °C, 1 h, 24 h dark maturation) to give CO-AuNPs and BP-AuNPs, and a 1:1 (w/w) physical mixture of the two purified powders (MIX-AuNPs) was carried as a combination arm. Both batches gave a single surface plasmon resonance band, at 531.87 ± 0.45 nm for CO-AuNPs and 535.70 ± 0.50 nm for BP-AuNPs, with no band in the solvent, salt or extract controls. Hydrodynamic diameter was 69.13 ± 1.25 and 63.03 ± 1.20 nm, polydispersity index 0.228 ± 0.009 and 0.205 ± 0.007 , and zeta potential -25.37 ± 0.65 and -30.33 ± 0.85 mV. Diffraction peaks indexed to face-centred cubic gold with mean crystallite sizes of 25.83 ± 3.04 and 19.22 ± 2.04 nm; EDX returned 76.91 and 79.02 wt% gold with carbon and oxygen signals consistent with an adsorbed phytochemical layer. Cumulative gold-equivalent release over 24 h in phosphate-buffered saline pH 7.4 reached $79.23 \pm 2.85\%$ and $85.27 \pm 2.80\%$. The potency order was the same on every endpoint: MIX-AuNPs > BP-AuNPs > CO-AuNPs > BP-EXT > CO-EXT. DPPH IC₅₀ fell from 143.11 µg/mL for the *C. ovata* extract to 45.42 µg/mL for MIX-AuNPs, against 10.34 µg/mL for ascorbic acid. At 24 h the MTT IC₅₀ for MIX-AuNP was 110.15 µg/mL (MCF-7), 122.25 µg/mL (HCT116) and 142.68 µg/mL (A549). In lipopolysaccharide-stimulated RAW 264.7 macrophages held above 84% viability, MIX-AuNPs lowered TNF-α by $77.55 \pm 5.11\%$ and IL-6 by $79.55 \pm 5.12\%$ at 50 µg/mL. After Holm adjustment the mixture was not significantly superior to either individual system at matched concentrations, so the combination result is reported as enhanced activity and not synergy.

Keywords: green synthesis; gold nanoparticles; *Bryophyllum pinnatum*; *Crassula ovata*; DPPH radical scavenging; MTT cytotoxicity; TNF-α; IL-6

1. Introduction

The Crassulaceae contain roughly 1,400 succulent species, and two of them, *Crassula ovata* (Mill.) Druce and *Bryophyllum pinnatum* (Lam.) Oken, carry long ethnobotanical records for wound care, inflammatory complaints and infection. Their chemistry is not the same. *B. pinnatum* is defined by bufadienolide cardiac glycosides together with quercetin- and kaempferol-type flavonol glycosides; *C. ovata* is dominated by flavonoid aglycones and pentacyclic triterpenoids. Both share the crassulacean acid metabolism route, which leaves the leaf tissue loaded with organic acids and the mineral salts that neutralise them (Fürer et al., 2013; Oufir et al., 2015; Fürer et al., 2016; Gilman & Edwards, 2020). Controlled pharmacology on *B. pinnatum* has kept the two chemistries apart: the flavonoid fraction carries most of the inhibition of porcine detrusor contractility (Fürer et al., 2015; Bachmann et al., 2017), the bufadienolide fraction is the stronger inhibitor of human myometrial contraction (Santos et al., 2019; Santos et al., 2021), and a cream carrying the major leaf flavonoid matches the whole aqueous extract in a rat excision wound model (Coutinho et al., 2021).

Gold differs from silver in ways that matter for a green-synthesis comparison. Au³⁺ reduction requires three electrons rather than one, gold nucleation is far more sensitive to alkalinity, and metallic gold is chemically inert under physiological conditions, so a biological effect cannot be attributed to dissolved metal ion in the way it can for silver. Any activity measured on a gold colloid therefore reports on the particle surface and its adsorbed phytochemical layer rather than on ion release. In both metals the reduction is driven by the phenolic and carbonyl-bearing fraction, and the same molecules stay adsorbed and set the final size, charge and dispersion state (Sharma et al., 2009; Patil & Kim, 2017; Huq et al., 2022; Thomas et al., 2024). Plant-mediated gold synthesis and its characterisation are well described (Bharadwaj et al., 2021; Paiva-Santos et al., 2021), and the bio-inertness of the metallic gold surface is the basis of its use as a biocompatible carrier (Taghizadeh et al., 2019).

This makes the gold system the cleaner test of the question the silver phase raised: does the choice of source extract, and not the synthesis process, set the properties of the product? Two chemically distinct extracts were pushed through one fixed reaction, purified identically, and tested on the same panel, so any downstream difference traces back to the plant. Reports on *Kalanchoe pinnata*, the accepted synonym of *B. pinnatum*, describe selective cytotoxicity through ROS-mediated apoptosis and propose the extract as a candidate adjuvant (Hernández-Caballero et al., 2022; Faundes-Gandolfo et al., 2024).

A 1:1 physical mixture of the two independently synthesised colloids was carried as a third arm. Mixing two separately formed and purified particle populations separates a combination effect from a co-reduction product, because the two populations meet only in the assay well. A lower mean or a lower fitted IC50 at one fixed ratio is not evidence of interaction, so the claim is kept inside what the design supports and is judged against the median-effect framework rather than against a difference of means (Chou, 2010).

The work reported here had four aims: to synthesise gold nanoparticles from ethanolic leaf extracts of *C. ovata* and *B. pinnatum* under one fixed protocol; to characterise both batches by UV-visible spectroscopy, dynamic and electrophoretic light scattering, X-ray diffraction, transmission electron microscopy, infrared spectroscopy and EDX, and to quantify gold-equivalent content and release; to compare the two nanoparticle systems, their parent extracts and the 1:1 mixture on radical scavenging, cytotoxicity against three human cancer cell lines and cytokine suppression in stimulated macrophages; and to state the interaction result at the level the design allows.

2. Materials and methods

2.1 Chemicals and reagents

Chloroauric acid trihydrate, sodium hydroxide, absolute ethanol, methanol (HPLC grade), 2,2-diphenyl-1-picrylhydrazyl, ascorbic acid, MTT, DMSO, high-glucose DMEM, foetal bovine serum, penicillin-streptomycin, lipopolysaccharide from *Escherichia coli* and dexamethasone were of analytical or cell-culture grade. Mouse TNF- α and IL-6 sandwich ELISA kits were used as supplied.

2.2 Plant material, authentication and raw-material quality control

Fresh mature leaves were collected in February 2025 from a botanical supplier in Meerut, Uttar Pradesh, India (altitude 219 m), washed, shade-dried at $25 \pm 5^\circ\text{C}$ for 15 days to a residual moisture below 12%, milled and passed through a #40 sieve (425 μm) then stored in the dark in closed containers. Voucher specimen were deposited to Botanical survey of india ,Central National Herbarium Howrah that Ref. No. CNH/Tech.II/2026/21 and Specimen Numbers ITM/SK-01 (*C. ovata*) and ITM/SK-02 (*B. pinnatum*).

Physicochemical constants of both powders were determined in triplicate per Indian Pharmacopoeia 2022 appendices 2.2.32, 2.3.19 and 2.4.16 (Indian Pharmacopoeia Commission, 2022) and are reported in the companion raw-material dataset.

2.3 Extraction

Powdered leaf (1:10 w/v) was macerated in 70% (v/v) ethanol for 72 h at room temperature with intermittent shaking, filtered through Whatman No. 1 paper and concentrated on a rotary evaporator at 40°C to a dry residue. The 70% ethanol system gave a higher extractive value than 95% ethanol for both species ($15.17 \pm 0.35\%$ and $17.90 \pm 0.30\%$ w/w against $10.03 \pm 0.25\%$ and $11.43 \pm 0.31\%$ w/w). Dried extracts are referred to as CO-EXT and BP-EXT.

2.4 Extract working solution

For each synthesis, 100 mg of dried extract was dispersed in 10 mL of deionised water to give a 10 mg/mL working solution, sonicated for 10 min, centrifuged at 5,000 rpm for 10 min and filtered through a 0.45 μm membrane. The clear filtrate was used immediately.

2.5 Synthesis of gold nanoparticles

Chloroauric acid solution (1 mM) was prepared fresh by dissolving 39.39 mg of the trihydrate in deionised water and making up to 100 mL. For each batch, 90 mL was transferred to a 250 mL conical flask, heated to 70°C on a magnetic stirrer, and 10 mL of the 10 mg/mL extract working solution was added dropwise under continuous stirring, fixing the extract-to-salt ratio at 1:9 and the final volume at 100 mL. Reaction pH was adjusted to 6.0 with 0.1 N sodium hydroxide. A mildly acidic to near-neutral pH was held because Au^{3+} reduction and gold nucleus formation are sensitive to excess alkalinity, which drives uncontrolled aggregation and bulk precipitation. The mixture was stirred at 500 rpm for 1 h at 70°C in a foil-wrapped flask, then left undisturbed in the dark at room temperature for 24 h.

2.6 Purification and drying

Each suspension was centrifuged at 12,000 rpm for 20 min, the supernatant discarded and the pellet washed three times with deionised water. After the final wash the pellet was redispersed in 10 mL of deionised water; a portion was dried at 40 °C to constant weight and stored in amber airtight vials at room temperature.

2.7 Preparation of the combination arm

MIX-AuNPs was prepared immediately before each assay by weighing equal masses of dried CO-AuNPs and BP-AuNPs and dispersing them together in the assay vehicle. Concentrations quoted for MIX-AuNPs are total nanoparticle concentrations: 100 µg/mL contains 50 µg/mL of each component. No co-reduction or co-milling step was used.

2.8 UV-visible spectroscopy

Purified suspensions were diluted 1:10 with deionised water and scanned from 300 to 800 nm at 1 nm intervals in a 1 cm quartz cell against a deionised water blank ($n = 3$). Deionised water, 1 mM chloroauric acid and each extract were scanned as controls. Full width at half maximum was read from the mean spectrum and the A650/Amax ratio calculated as an aggregation indicator.

2.9 Calibration and gold-equivalent content

Dried nanoparticles were dispersed and diluted to 5, 10, 20, 30, 40 and 50 µg/mL, and absorbance recorded at 534 nm in triplicate. Limit of detection and quantitation were calculated as $3.3\sigma/S$ and $10\sigma/S$. For content determination, weighed dried nanoparticles were diluted to a theoretical 25 µg/mL and the measured concentration obtained from the regression equation.

2.10 Particle size, polydispersity and zeta potential

Hydrodynamic diameter and polydispersity index by dynamic light scattering, zeta potential by electrophoretic light scattering, on diluted aqueous dispersions at 25 °C in triplicate.

2.11 X-ray diffraction

Diffraction patterns were recorded over $2\theta = 20-80^\circ$ with Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$), indexed against the face-centred cubic gold lattice, and crystallite size calculated from the Debye-Scherrer relation.

2.12 Transmission electron microscopy and infrared spectroscopy

For electron microscopy, purified dispersion was diluted, sonicated for 2 min, drop-cast on a carbon-coated copper grid and air-dried, then imaged on a Hitachi HC-1 at 120 kV at $\times 40k$ with a 200 nm scale bar; aspect ratio and circularity were computed from the same micrographs and are dimensionless. Infrared spectra were recorded on a Bruker instrument with a diamond attenuated total reflectance accessory over $4000-400 \text{ cm}^{-1}$, each nanoparticle batch against its own parent extract in the same session. For EDX, dried nanoparticles were mounted on carbon conductive tape and analysed at 15 kV on a JED-2300 analysis station with ZAF standardless quantitation, reported as elemental confirmation rather than as an assay.

2.13 In vitro release

Dialysis membrane method. Nanoparticle equivalent to 2 mg in 2 mL inside a 12–14 kDa cut-off membrane, immersed in 50 mL of phosphate-buffered saline pH 7.4 at $37 \pm 0.5^\circ\text{C}$, 100 rpm. Samples of 2 mL withdrawn at 0.5, 1, 2, 4, 6, 8, 12 and 24 h and replaced with fresh medium. Gold-equivalent concentration read at 534 nm against the calibration equation. Cumulative amount corrected for withdrawal as $Q_n = C_n V_r + \sum C_i V_i$, and expressed against the measured gold-equivalent content rather than nominal mass.

2.14 DPPH radical scavenging

Test materials at 6.25, 12.5, 25, 50, 75, 100, 150 and 200 µg/mL against 0.1 mM DPPH in methanol, 30 min in the dark, read at 517 nm ($n = 3$). Ascorbic acid (2.5–80 µg/mL) was the reference. Every concentration carried a matched sample blank without DPPH, and corrected absorbance is reaction minus blank.

2.15 MTT cytotoxicity

MCF-7, HCT116 and A549 cells were maintained in high-glucose DMEM with 10% foetal bovine serum and 1% penicillin-streptomycin at 37°C in 5% CO_2 , seeded at 1×10^4 per well in 100 µL in flat-bottom 96-well plates, left 24 h to attach and exposed for 24 h to 6.25–800 µg/mL. Vehicle DMSO was held at or below 0.5% v/v. MTT (0.5 mg/mL in PBS, 20 µL per well) was added for 4 h at 37°C in the dark and formazan dissolved in 100 µL DMSO before reading at 570 nm (Mosmann, 1983). Viability is expressed against the matched corrected untreated control, following the reduction-of-viability convention on which the ISO 10993-5 cytotoxicity categories rest (International Organization for Standardization, 2009). Because plant extracts and metallic nanoparticles interfere with tetrazolium assays (Holder et al., 2012; Tournebise et al., 2013), each concentration was run as a cell-free blank. Each concentration of

each test material was run as a cell-free blank in complete assay matrix and carried through the identical sequence; corrected absorbance is raw minus matched blank.

2.16 Anti-inflammatory evaluation

RAW 264.7 macrophages were pre-treated for 2 h at 3.125, 6.25, 12.5, 25 and 50 µg/mL then challenged with lipopolysaccharide at 100 ng/mL for 24 h. Dexamethasone at 1 µM was the reference. Clarified supernatants were diluted 1:2 and assayed for TNF-α and IL-6 by sandwich ELISA (OD450 corrected at 570 nm), fitted with a four-parameter logistic model and back-calculated by inverse interpolation. Inhibition was calculated as (LPS mean – treatment) / (LPS mean – unstimulated mean) × 100. A parallel MTT viability plate with material-matched optical blanks was run under identical conditions, with 80% viability set in advance as the interpretability threshold.

2.17 Statistical analysis

Results are mean ± SD of three replicates. Concentration-response data were fitted with a normalised variable-slope logistic model to individual replicate observations, and IC50 values are reported with 95% confidence intervals, Hill slope and replicate-level R². Treatment and concentration effects were tested by two-way ANOVA on replicate-level values. Release profiles were compared by two-way ANOVA with Šidák's test, and cytokine data by two-way ANOVA followed by Welch tests with Holm adjustment against the LPS control. Significance codes are ns for p > 0.05, * for p < 0.05 and ** for p < 0.01.

3. Results

3.1 Synthesis and yield

Colour change was visible within minutes in both reactions. CO-AuNPs moved from pale yellow to ruby red with onset at 4 min; BP-AuNPs developed faster, with onset at 3 min and a final purple-red. No precipitation was seen during the reaction or over the 24 h maturation. Reaction pH drifted down slightly from the 6.0 set point, to 5.81 and 5.76. Dry yield after purification was 26.13 ± 0.29 and 28.27 ± 0.29 mg per 100 mL, higher than the corresponding silver batches, which follows from the higher atomic mass of gold at equal molar charge. Both pellets redispersed in water and supernatants were colourless by the third wash.

Table 1. Synthesis conditions, visual observations and dry yield

Parameter	CO-AuNPs	BP-AuNPs
Extract	C. ovata ethanolic	B. pinnatum ethanolic
Metal salt / concentration	HAuCl ₄ ·3H ₂ O, 1 mM	HAuCl ₄ ·3H ₂ O, 1 mM
Extract-to-salt ratio (v/v)	1:9	1:9
Extract concentration / volume	10 mg/mL, 10 mL	10 mg/mL, 10 mL
Final reaction volume	100 mL	100 mL
Set pH / pH after reaction	6.0 / 5.81	6.0 / 5.76
Temperature / stirring / time	70 °C, 500 rpm, 1 h	70 °C, 500 rpm, 1 h
Maturation	24 h, dark, room temperature	24 h, dark, room temperature
Onset of colour change	4 min	3 min
Colour after maturation	Ruby red	Purple-red
Visible precipitation	Not observed	Not observed
Pellet character	Ruby-red to maroon, soft compact	Purple-red to wine-red, dense soft
Redispersion behaviour	Freely redispersed	Redispersed after mild vortexing
Dry yield (mg/100 mL)	26.13 ± 0.29	28.27 ± 0.29

3.2 UV-visible spectra

Each purified suspension gave one symmetric surface plasmon resonance band and no secondary feature, with the three scans superimposable. Peak position was 531.87 ± 0.45 nm for CO-AuNPs and 535.70 ± 0.50 nm for BP-AuNPs, both inside the 520-560 nm window characteristic of colloidal gold. Peak absorbance was 0.776 ± 0.007 and 0.824 ± 0.008, with coefficients of variation below 1.0%. Band width was 62.03 ± 0.65 and 66.73 ± 0.60 nm, and the A650/Amax ratio 0.073 and 0.086, low enough to exclude substantial aggregation

and, for gold specifically, to exclude a significant anisotropic population, which would carry a longitudinal band above 600 nm (Table 2, Fig. 1a-b).

Table 2. UV-visible spectral parameters of the purified suspensions (mean $\pm$ SD, n = 3)

Batch	λ_{max} (nm)	A _{max}	CV of A _{max} (%)	FWHM (nm)	A ₆₅₀	A ₆₅₀ /A _{max}	Band profile
CO-AuNPs	531.87 $\pm$ 0.45	0.776 $\pm$ 0.007	0.85	62.03 $\pm$ 0.65	0.057	0.073	Single SPR band
BP-AuNPs	535.70 $\pm$ 0.50	0.824 $\pm$ 0.008	0.91	66.73 $\pm$ 0.60	0.071	0.086	Single SPR band

Controls: deionised water, 1 mM chloroauric acid and both ethanolic extract blanks gave no band between 520 and 560 nm; extract absorbance was confined below 380 nm.

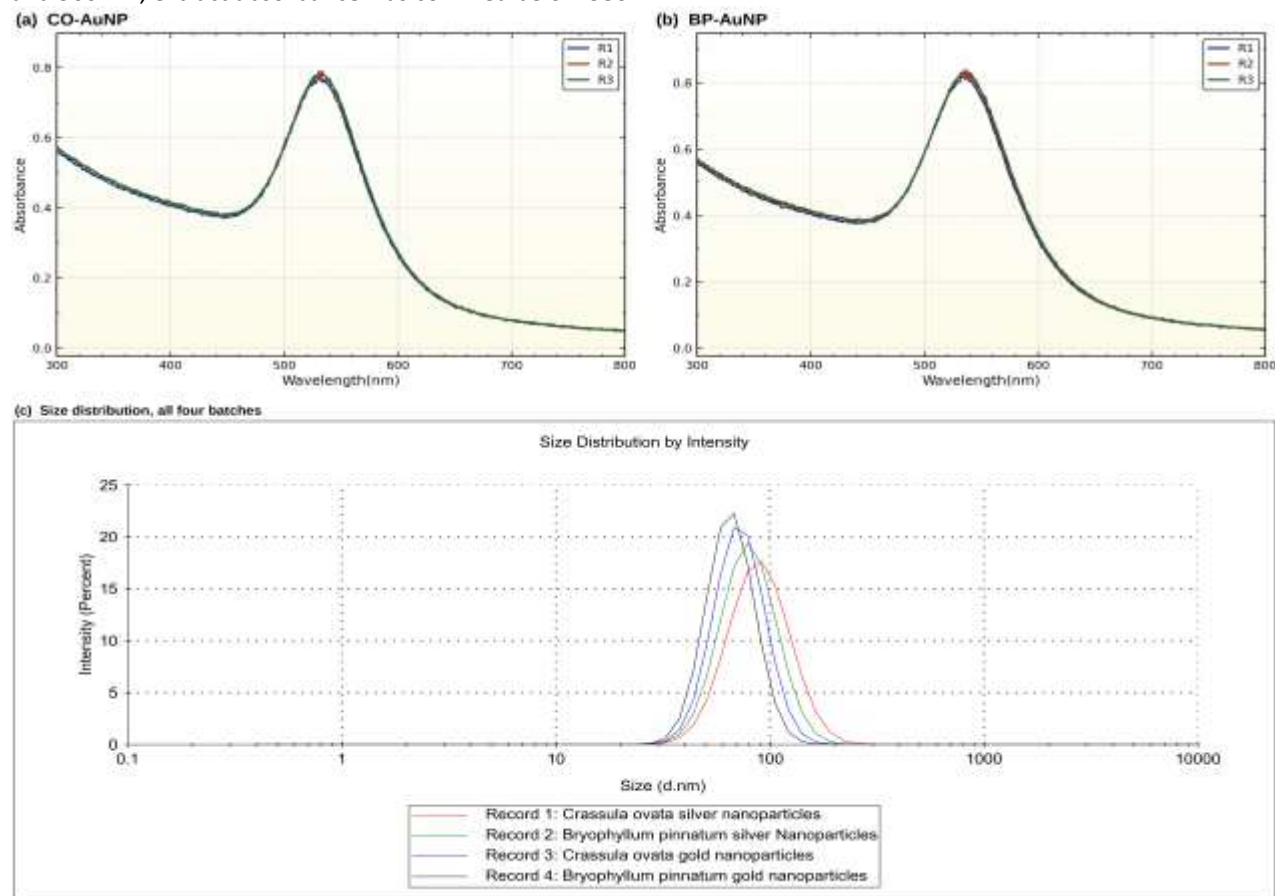


Figure 1. Optical and colloidal characterisation. UV-visible absorption spectra of the purified suspensions, 300-800 nm at 1 nm interval, three scans overlaid: (a) CO-AuNPs, mean peak 531.87 nm; (b) BP-AuNPs, mean peak 535.70 nm. A single surface plasmon resonance band is present in each case and the three scans are superimposable. No band was recorded in the deionised water, 1 mM chloroauric acid or ethanolic extract controls. (c) Intensity-weighted size distribution by dynamic light scattering in water at 25 °C; records 3 and 4 are the gold batches reported here, records 1 and 2 the silver batches from the same two extracts in the companion study, shown for reference only. All distributions are monomodal.

3.3 Calibration and gold-equivalent content

Absorbance at 534 nm was linear from 5 to 50 $\mu\text{g/mL}$, giving $y = 0.01482x + 0.00766$ with $R^2 = 0.9998$ and residual standard deviation 0.00431, from which the limit of detection was 0.96 $\mu\text{g/mL}$ and the limit of quantitation 2.91 $\mu\text{g/mL}$. Back-calculated accuracy ranged from 96.74% to 103.58% and residuals showed no trend with concentration. Measured gold-equivalent content was 96.28 $\pm$ 1.36% for CO-AuNPs and 99.61 $\pm$ 1.44% for BP-AuNPs, with relative standard deviations of 1.42% and 1.45% (Table 3).

Table 3. Calibration performance at 534 nm and gold-equivalent content

Parameter	Value
Concentration range	5–50 µg/mL
Regression equation	$y = 0.01482x + 0.00766$
Coefficient of determination	0.9998 (exact 0.99978)
Residual standard deviation, S_y/x	0.00431
Limit of detection	0.96 µg/mL
Limit of quantitation	2.91 µg/mL
Back-calculated accuracy across six levels	96.74–103.58%
CO-AuNP content (theoretical 25 µg/mL)	$96.28 \pm 1.36\%$ (RSD 1.42%)
BP-AuNP content (theoretical 25 µg/mL)	$99.61 \pm 1.44\%$ (RSD 1.45%)
CO-AuNP versus BP-AuNP content	$t(4) = 2.90$, $p = 0.044$ (unpaired t-test on replicates)
Content uniformity window	Both batches within 95–105% of theoretical

3.4 Particle size, polydispersity and zeta potential

BP-AuNPs was the smaller and the more highly charged of the two. Hydrodynamic diameter was 63.03 ± 1.20 nm against 69.13 ± 1.25 nm, polydispersity index 0.205 ± 0.007 against 0.228 ± 0.009 , and zeta potential -30.33 ± 0.85 mV against -25.37 ± 0.65 mV. Intensity distributions were monomodal for both, with distribution widths of 17.8 and 20.9 nm. Both gold batches were smaller and more narrowly distributed than the corresponding silver batches prepared from the same two extracts, consistent with the shorter reaction time and the higher nucleation rate of Au³⁺ reduction (Table 4, Fig. 1c).

Table 4. Particle size, polydispersity index and zeta potential (mean $\pm$ SD, $n = 3$, 25 °C)

Batch	Hydrodynamic diameter (nm)	Polydispersity index	Zeta potential (mV)	Distribution width (nm)
CO-AuNPs	69.13 ± 1.25	0.228 ± 0.009	-25.37 ± 0.65	20.9
BP-AuNPs	63.03 ± 1.20	0.205 ± 0.007	-30.33 ± 0.85	17.8

Both batches gave monomodal intensity distributions with a single peak accounting for 100% of scattered intensity.

3.5 X-ray diffraction

Both samples gave four reflections indexing to the face-centred cubic gold lattice. CO-AuNPs peaked at 38.24° , 44.47° , 64.67° and 77.62° , assigned to (111), (200), (220) and (311), with a mean crystallite size of 25.83 ± 3.04 nm. BP-AuNPs peaked at 38.31° , 44.55° , 64.78° and 77.70° with broader lines and a mean crystallite size of 19.22 ± 2.04 nm. The (111) reflection was the most intense in both. Low-intensity broad background between 20° and 35° , stronger in BP-AuNPs, was not indexed to metallic planes and is attributed to the amorphous organic capping layer (Table 5, Fig. 2a-b).

Table 5. X-ray diffraction parameters (Cu K α , $\lambda = 1.5406$ Å)

Batch	2θ (°)	hkl	Relative intensity (%)	FWHM (°)	d-spacing (Å)	Crystallite size (nm)
CO-AuNPs	38.24	(111)	100	0.28	2.352	30.03
CO-AuNPs	44.47	(200)	46	0.33	2.036	26.01
CO-AuNPs	64.67	(220)	24	0.39	1.440	24.11
CO-AuNPs	77.62	(311)	17	0.44	1.229	23.17
BP-AuNPs	38.31	(111)	100	0.38	2.348	22.13
BP-AuNPs	44.55	(200)	43	0.45	2.032	19.08
BP-AuNPs	64.78	(220)	22	0.52	1.438	18.09
BP-AuNPs	77.70	(311)	14	0.58	1.228	17.59

Mean crystallite size: CO-AuNPs 25.83 ± 3.04 nm; BP-AuNPs 19.22 ± 2.04 nm. Broad unindexed background between 20° and 35° , stronger in BP-AuNPs, is attributed to the amorphous organic capping layer.

3.6 Transmission electron microscopy

Counted micrographs placed the metal core below the hydrodynamic diameter and above the crystallite size in both batches. The number-mean core diameter was 29.85 ± 14.16 nm for CO-AuNPs across 124 particles and 27.65 ± 13.57 nm for BP-AuNPs across 125 particles, with geometric standard deviations of 1.567 and 1.537. The ratio of Z-average to counted mean was 2.32 and 2.28, lower than the silver batches, which is consistent with the narrower gold distributions. Both means exceed the corresponding Debye-Scherrer crystallite size, giving 1.54 and 2.98 coherent domains per particle on a cubed diameter basis, so BP-AuNPs is the most polycrystalline batch in the series and also the one with the broadest diffraction profile. Shape descriptors were computed without a length calibration: 7.3% of CO-AuNPs and 0.8% of BP-AuNPs particles exceeded an aspect ratio of 2.0, with median aspect ratios of 1.24 and 1.20 and median circularities of 0.84 and 0.85. Both populations are quasi-spherical to polyhedral, and the anisotropic fraction is small enough to be consistent with the single symmetric plasmon band and the low A650/Amax ratios in Table 2 (Table 7, Fig. 3).

Table 7. Transmission electron microscopy: counted particle size and shape census

Parameter	CO-AuNPs	BP-AuNPs
Particles counted	124	125
Number-mean core diameter (nm)	29.85 ± 14.16	27.65 ± 13.57
D10 / D50 / D90 (nm)	15.47 / 27.48 / 42.83	14.93 / 25.71 / 42.78
Geometric standard deviation	1.567	1.537
Debye-Scherrer crystallite size (nm)	25.83 ± 3.04	19.22 ± 2.04
Ratio, DLS Z-average : TEM mean	2.32	2.28
Aspect ratio > 2.0 (% of particles)	7.3	0.8
Median aspect ratio	1.24	1.20
Median circularity	0.84	0.85

Counted from two non-overlapping fields per batch at $\times 40k$. Shape descriptors are dimensionless and were computed without a length calibration. Both gold batches are narrower in distribution than the silver batches prepared from the same extracts, and BP-AuNP carries the lowest anisotropic fraction of any batch in the series.

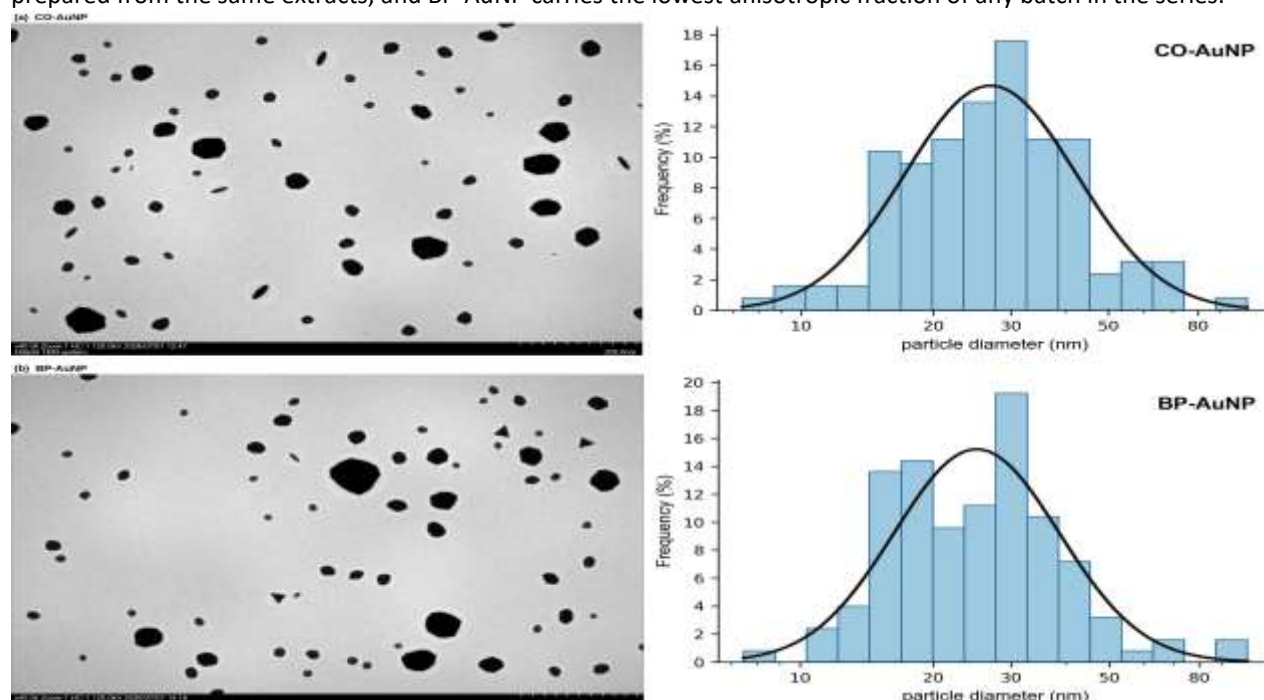


Figure 3. Transmission electron micrographs and number-weighted size distributions: (a) CO-AuNPs and (b) BP-AuNPs (Hitachi HC-1, 120 kV, $\times 40k$; scale bar 200 nm). Histograms are the counted particle diameters with a lognormal fit overlaid.

3.7 Infrared spectroscopy of the capping layer

The parent extract and its nanoparticle were run as a matched pair for each species. Aliphatic C-H stretching moved by no more than 8 cm⁻¹ in either system, fixing the internal reference. Against that reference, the hydroxyl stretch of BP-EXT moved from 3478 to 3428 cm⁻¹ and the second hydroxyl feature from 3284 to 3236 cm⁻¹, the carbonyl from 1738 to 1722 cm⁻¹, and the glycosidic C-O-C envelope from 1036 to 1016 cm⁻¹, all to lower wavenumber and all consistent with coordination of oxygenated groups to the metal surface. Every shift is larger than the corresponding silver value by 2 to 6 cm⁻¹.

Two carboxylate bands appear in the nanoparticle spectra that are absent from both extracts: an asymmetric stretch at 1582 cm⁻¹ in both gold batches, paired with a symmetric stretch at 1390 and 1388 cm⁻¹. The separation is 192 and 194 cm⁻¹, in the bridging bidentate range, identifying the binding geometry. Two further features are present in the gold spectra and in neither extract nor either silver batch: a sharp non-hydrogen-bonded hydroxyl stretch at 3584 and 3580 cm⁻¹, and a band at 432 cm⁻¹ in the metal-oxygen region. As in the silver system, the carbonyl band is no longer resolved in the *C. ovata* nanoparticle while *B. pinnatum* retains it, so the carbonyl-bearing fraction of the *C. ovata* extract is more completely consumed during reduction regardless of which metal is used (Table 8, Figs. 4 and 5).

Table 8. ATR-FTIR band positions and shifts on nanoparticle formation (cm⁻¹)

Band	BP-EXT	BP-AuNPs	$\Delta\nu$	CO-AuNPs	Role
$\nu(\text{O-H})$, free	absent	3580	new band	3584	Diagnostic
$\nu(\text{O-H})$	3478	3428	-50	3430	Coordination
$\nu(\text{O-H})$	3284	3236	-48	3240	Coordination
$\nu_{\text{as}}(\text{C-H})$	2924	2916	-8	2916	Reference
$\nu_{\text{s}}(\text{C-H})$	2854	2848	-6	2846	Reference
$\nu(\text{C=O})$	1738	1722	-16	not resolved	Coordination
$\nu_{\text{as}}(\text{COO}^-)$	absent	1582	new band	1582	Diagnostic
$\nu_{\text{s}}(\text{COO}^-)$	absent	1388	new band	1390	Diagnostic
$\nu(\text{C-O-C})$	1036	1016	-20	1018	Coordination
Metal-oxygen region	absent	432	new band	432	Confirmatory

Diamond ATR, 4000-400 cm⁻¹; positions are peak-picked from the instrument output. Shifts are tabulated for the *B. pinnatum* pair, for which both members carry a peak-picked list. CO-AuNPs positions are given as absolute values against the *C. ovata* extract spectrum in Figure 4; the peak-picked list for that extract is outstanding, so the corresponding shift column is not tabulated. Carboxylate separation $\Delta\nu(\text{as-sym})$ is 192 cm⁻¹ for CO-AuNPs and 194 cm⁻¹ for BP-AuNPs, both in the bridging bidentate range. Two features are present in the gold spectra and in neither extract nor either silver batch: a sharp non-hydrogen-bonded hydroxyl stretch near 3580 cm⁻¹ and a band at 432 cm⁻¹.

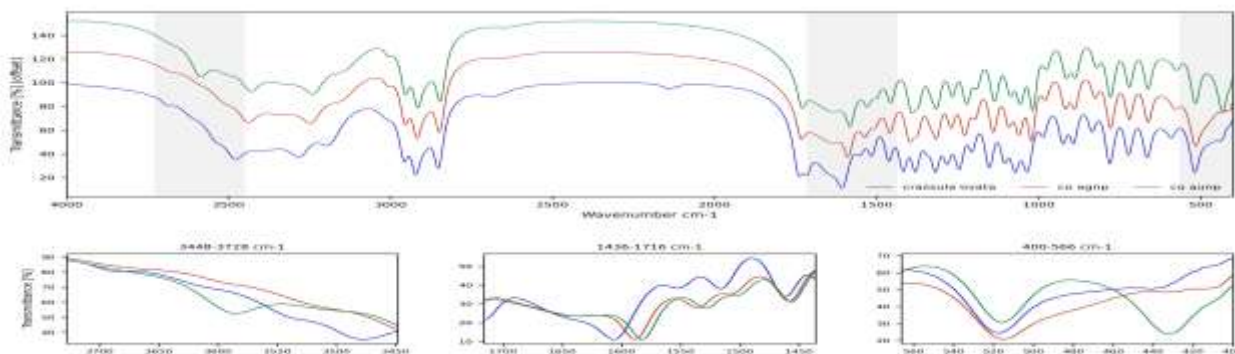


Figure 4. ATR-FTIR spectra of the *Crassula ovata* system, 4000-400 cm⁻¹, offset for clarity, with three regions expanded below. The trace for the silver batch prepared from the same extract is included for reference only and is reported in the companion study.

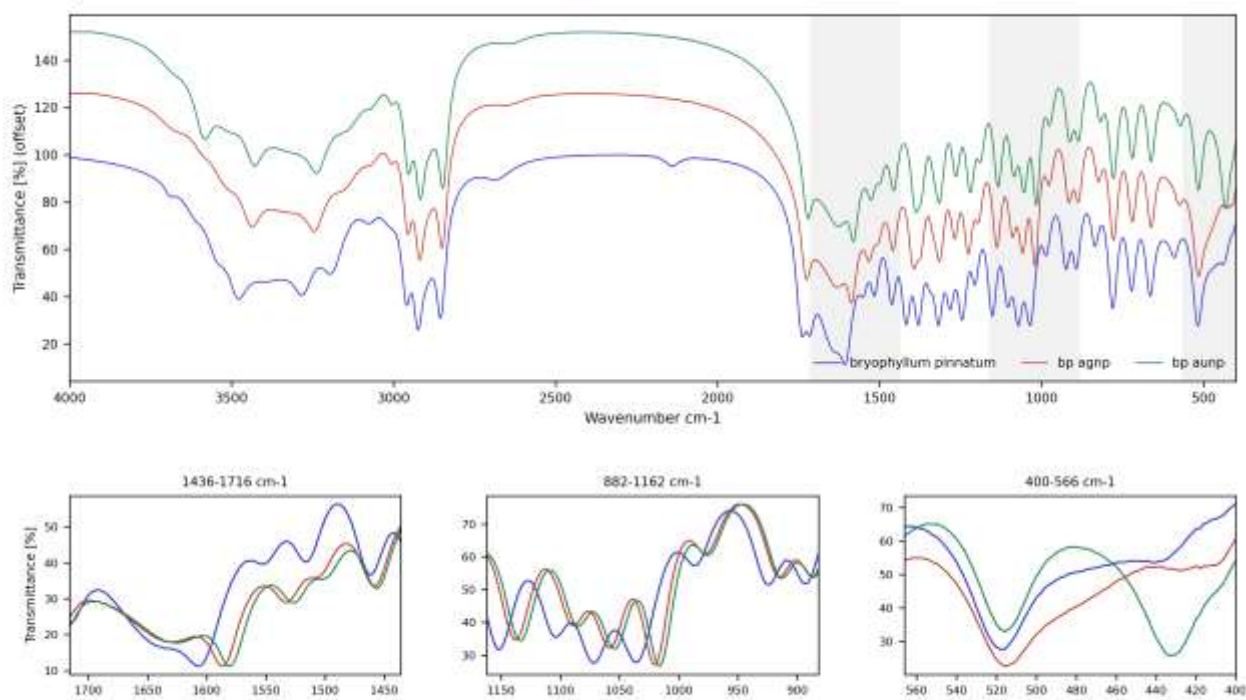


Figure 5. ATR-FTIR spectra of the *Bryophyllum pinnatum* system, 4000-400 cm^{-1} , offset for clarity, with three regions expanded below. The trace for the silver batch prepared from the same extract is included for reference only.

3.8 Elemental composition

Energy-dispersive X-ray analysis put gold as the dominant element by mass in both batches: 76.91 wt% (20.06 at%) for CO-AuNPs and 79.02 wt% (22.22 at%) for BP-AuNPs, in each case from an intense Au $M_{\alpha 1}$ line near 2.12 keV with characteristic $M_{\beta 2}$ and $L_{\alpha 1}$ lines. Carbon and oxygen accounted for 8.78 and 12.34 wt% in CO-AuNPs and 7.92 and 11.06 wt% in BP-AuNPs, lower than the corresponding silver batches. Residual chlorine (0.69-0.76 wt%) and potassium (1.21-1.31 wt%) carried over from the extract and the precursor. Quantitation by the ZAF standardless method is treated here as elemental confirmation rather than as an assay; the gold-equivalent content in Table 3 is the quantitative figure (Table 9, Fig. 2c-d).

Table 6. EDX elemental composition (ZAF standardless quantitative analysis)

Batch	Element	Mass (%)	Atom (%)
CO-AuNPs	C	8.78	37.61
	O	12.34	39.67
	Cl	0.76	1.11
	Au	76.91	20.06
	K	1.21	1.59
BP-AuNPs	C	7.92	36.57
	O	11.06	38.27
	Cl	0.69	1.08
	Au	79.02	22.22
	K	1.31	1.86

Au M_{α} signal at approximately 2.12 keV with characteristic M_{β} and L_{α} lines. Carbon arises partly from the conductive tape and partly from adsorbed phytochemicals; chlorine and potassium are extract carry-over.

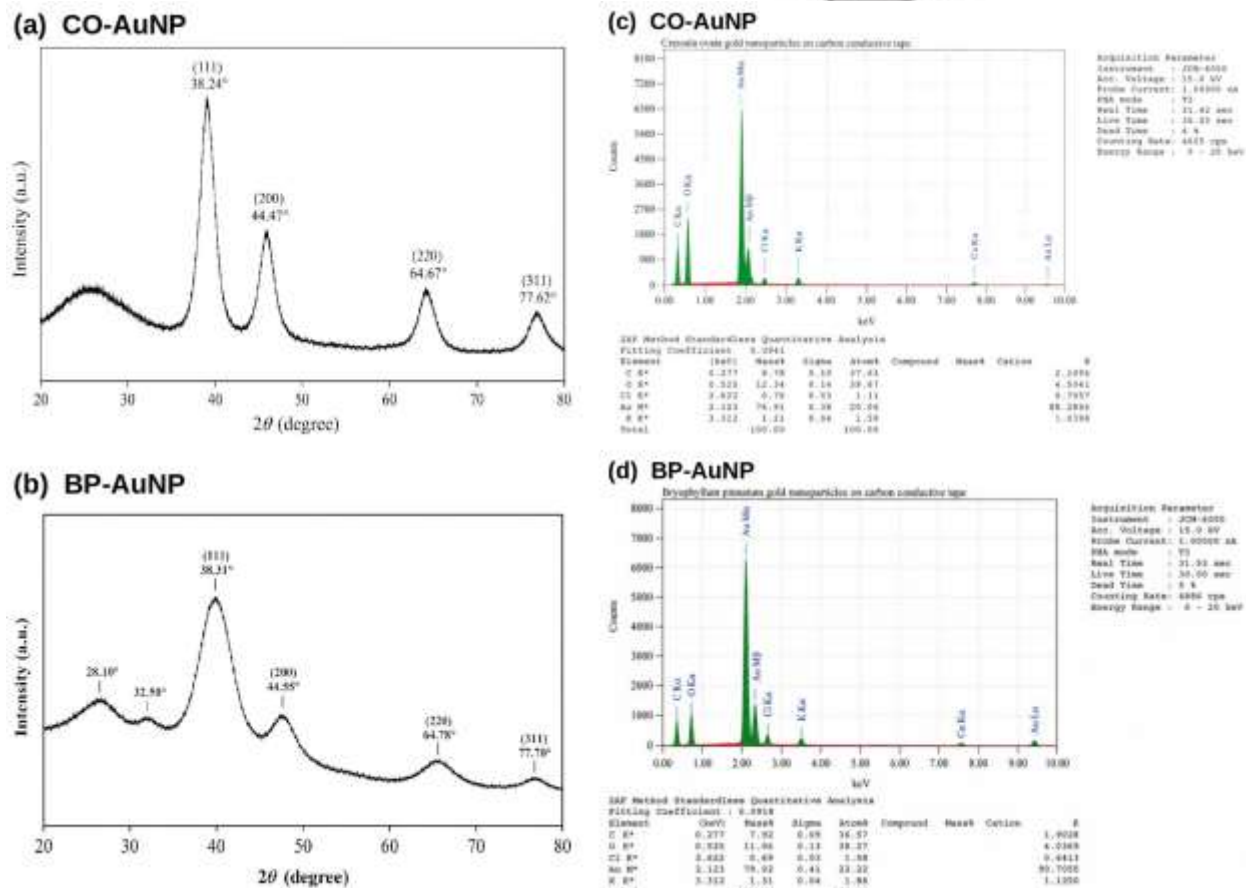


Figure 2. Crystalline phase and elemental composition. X-ray diffractograms over $2\theta = 20\text{--}80^\circ$ ($\text{Cu K}\alpha$, $\lambda = 1.5406 \text{ \AA}$) for (a) CO-AuNPs and (b) BP-AuNPs with the (111), (200), (220) and (311) reflections of face-centred cubic gold indexed; panels are assigned from the 2θ values printed within each plot. Energy-dispersive X-ray spectra with ZAF standardless quantitation for (c) CO-AuNPs and (d) BP-AuNP at 15 kV, with the Au $\text{M}\alpha$ line near 2.12 keV dominant in both.

3.9 In vitro release

Both batches released gold-equivalent material steadily over 24 h with no burst phase. At 30 min cumulative release was $7.07 \pm 0.65\%$ and $8.17 \pm 0.65\%$; by 8 h $55.73 \pm 2.60\%$ and $63.77 \pm 2.65\%$, and at 24 h $79.23 \pm 2.85\%$ and $85.27 \pm 2.80\%$. Two-way ANOVA gave $F(1,32) = 81.411$ for formulation and $F(7,32) = 1012.44$ for time, both $p < 0.0001$, with a near-significant interaction, $F(7,32) = 2.269$, $p = 0.0539$. $\text{Tukey's } b$ -adjusted comparisons placed the difference at 4, 6, 8, 12 and 24 h, and the area under the curve was 1383.00 ± 57.92 against 1541.33 ± 58.84 ($p = 0.0293$), so the separation between the two gold profiles persisted to the endpoint (Table 10, Fig. 6a).

Table 9. Cumulative in vitro gold-equivalent release in phosphate-buffered saline pH 7.4 (mean $\pm$ SD, $n = 3$)

Time (h)	CO-AuNPs (%)	BP-AuNPs (%)	Difference (percentage points)
0.5	7.07 ± 0.65	8.17 ± 0.65	1.10
1	12.20 ± 0.92	14.93 ± 0.96	2.73
2	21.17 ± 1.35	25.23 ± 1.45	4.06
4	32.80 ± 1.75	39.13 ± 2.06	6.33
6	44.47 ± 2.30	52.40 ± 2.66	7.93
8	55.73 ± 2.60	63.77 ± 2.65	8.04
12	67.90 ± 2.76	75.53 ± 2.70	7.63
24	79.23 ± 2.85	85.27 ± 2.80	6.04

Two-way ANOVA: formulation $F(1,32) = 81.411$, $p < 0.0001$; time $F(7,32) = 1012.44$, $p < 0.0001$; formulation $\times$ time $F(7,32) = 2.269$, $p = 0.0539$. Šidák-adjusted comparisons were significant at 4, 6, 8, 12 and 24 h. AUC_{0–24h} 1383.00 ± 57.92 (CO-AuNPs) versus 1541.33 ± 58.84 (BP-AuNPs), $p = 0.0293$. The difference column is computed from the tabulated means.

3.10 DPPH radical scavenging

Converting either extract into its nanoparticle form improved potency. CO-EXT scavenged with an IC₅₀ of $143.11 \mu\text{g/mL}$ (95% CI $132.36\text{--}153.86$) and BP-EXT with $92.78 \mu\text{g/mL}$ ($88.21\text{--}97.34$); the corresponding nanoparticles gave $78.95 \mu\text{g/mL}$ ($75.32\text{--}82.57$) and $60.43 \mu\text{g/mL}$ ($57.97\text{--}62.89$). MIX-AuNPs was the most active test material at $45.42 \mu\text{g/mL}$ ($43.55\text{--}47.28$). Ascorbic acid remained the strongest scavenger at $10.34 \mu\text{g/mL}$. Hill slopes rose from 0.896 for CO-EXT to 1.182 for MIX-AuNPs, and replicate-level R^2 fell between 0.9786 and 0.9940. Two-way ANOVA returned $F(4,80) = 284.333$ for treatment, $F(7,80) = 1680.234$ for concentration and $F(28,80) = 9.125$ for the interaction, all $p < 0.0001$ (Table 11, Fig. 6b).

Table 10. DPPH radical-scavenging concentration-response parameters

Treatment	IC ₅₀ ($\mu\text{g/mL}$)	95% CI	Hill slope	Replicate-level R^2	Fold change versus CO-EXT
CO-EXT	143.11	132.36–153.86	0.896	0.9786	1.00
BP-EXT	92.78	88.21–97.34	1.063	0.9882	1.54
CO-AuNPs	78.95	75.32–82.57	1.085	0.9902	1.81
BP-AuNPs	60.43	57.97–62.89	1.057	0.9931	2.37
MIX-AuNPs	45.42	43.55–47.28	1.182	0.9940	3.15
Ascorbic acid	10.34	9.94–10.74	1.294	0.9964	13.84

Ascorbic acid is an analytical reference control, not a test material. Fold change is computed from the tabulated IC₅₀ values. Two-way ANOVA across the five study treatments: treatment $F(4,80) = 284.333$; concentration $F(7,80) = 1680.234$; interaction $F(28,80) = 9.125$; all $p < 0.0001$. DPPH control absorbance 0.7128 ± 0.0032 .

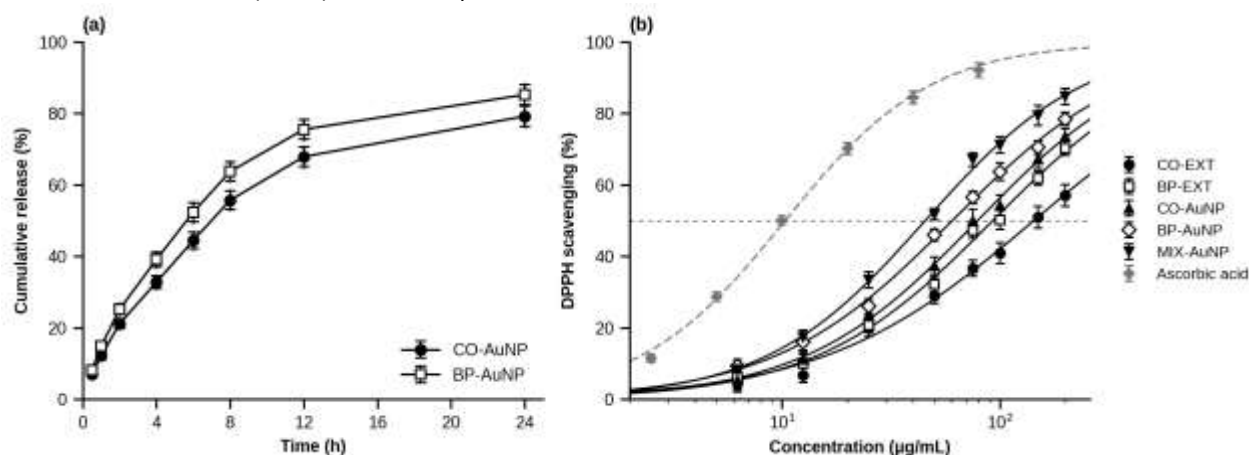


Figure 6. (a) Cumulative in vitro gold-equivalent release in phosphate-buffered saline pH 7.4 at $37 \pm 0.5^\circ\text{C}$; small grey points are individual replicates. (b) DPPH radical-scavenging concentration-response curves for the five study treatments with ascorbic acid as the analytical reference (grey, dashed); the horizontal dashed line marks 50% scavenging. Symbols are means with SD bars, $n = 3$, and curves are normalised variable-slope logistic fits.

3.11 Cytotoxicity

The same rank order held on all three cell lines. Against MCF-7, IC₅₀ fell from $346.11 \mu\text{g/mL}$ (CO-EXT) and $216.65 \mu\text{g/mL}$ (BP-EXT) to $159.75 \mu\text{g/mL}$ (CO-AuNPs), $131.78 \mu\text{g/mL}$ (BP-AuNPs) and $110.15 \mu\text{g/mL}$ (MIX-AuNPs). HCT116 ran 468.86 , 285.04 , 205.42 , 168.59 and $122.25 \mu\text{g/mL}$, and A549 530.50 , 365.66 , 254.64 , 212.73 and $142.68 \mu\text{g/mL}$. Every value was bracketed inside the tested $6.25\text{--}800 \mu\text{g/mL}$ range. MCF-7 was the most responsive line and A549 the least, on every test material. Hill slopes were shallower for the extracts (0.837–0.953) than for the nanoparticle systems (0.976–1.220), and replicate-level R^2 ranged from 0.9563 to 0.9840 (Table 12, Fig. 7).

Table 11. MTT cytotoxicity after 24 h exposure: IC₅₀ (µg/mL) with 95% confidence intervals and Hill slopes

Treatment	MCF-7	HCT116	A549
CO-EXT	346.11 (301.65–390.57); h = 0.880	468.86 (411.55–526.18); h = 0.909	530.50 (457.59–603.40); h = 0.953
BP-EXT	216.65 (195.04–238.26); h = 0.918	285.04 (254.88–315.20); h = 0.837	365.66 (324.35–406.98); h = 0.869
CO-AuNPs	159.75 (136.01–183.49); h = 1.037	205.42 (184.36–226.48); h = 1.140	254.64 (221.40–287.88); h = 0.978
BP-AuNPs	131.78 (119.30–144.27); h = 1.154	168.59 (153.25–183.94); h = 1.010	212.73 (188.97–236.48); h = 0.976
MIX-AuNPs	110.15 (97.24–123.06); h = 1.161	122.25 (110.88–133.61); h = 1.220	142.68 (129.93–155.42); h = 1.065

All IC₅₀ values are bracketed within the tested 6.25–800 µg/mL range; none is extrapolated. Replicate-level R² ranged from 0.9563 to 0.9840. Two-way ANOVA was significant for treatment, concentration and their interaction on all three lines: MCF-7 F(4,80) = 53.338, F(7,80) = 875.849, F(28,80) = 7.386; HCT116 F(4,80) = 104.834, F(7,80) = 1097.360, F(28,80) = 10.391; A549 F(4,80) = 90.766, F(7,80) = 881.629, F(28,80) = 7.651; all p < 0.0001. Untreated corrected controls: MCF-7 0.9200 ± 0.0040, HCT116 0.9800 ± 0.0060, A549 0.8800 ± 0.0040.

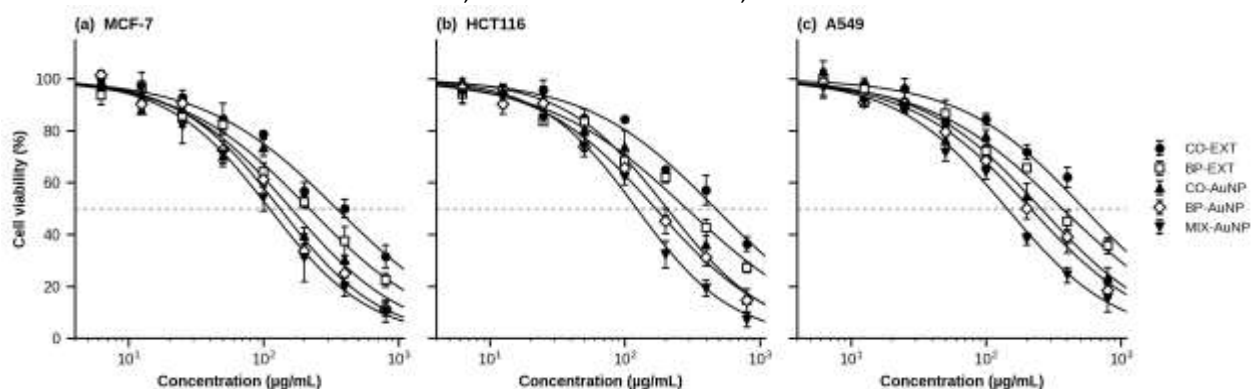


Figure 7. Cell viability after 24 h exposure: (a) MCF-7, (b) HCT116, (c) A549. Symbols are means with SD bars, n = 3; curves are normalised variable-slope logistic fits and the horizontal dashed line marks 50% viability. [FIGURE TO BE REPLACED ON RECEIPT OF THE SUPPLIED MTT IMAGE SET.]

3.12 Macrophage viability

The viability plate was read first. Across the 3.125–50 µg/mL range every treatment held mean viability above 84%; the lowest value was MIX-AuNPs at 50 µg/mL, 84.90 ± 3.74%, comfortably above the prespecified 80% threshold. Lipopolysaccharide alone left viability at 96.23 ± 0.34% and dexamethasone at 97.95 ± 0.35% (Table 13).

Table 12. RAW 264.7 macrophage viability under the anti-inflammatory treatment conditions (mean ± SD, n = 3)

Treatment	3.125 µg/mL	6.25 µg/mL	12.5 µg/mL	25 µg/mL	50 µg/mL
CO-EXT	101.20 ± 1.68	99.10 ± 2.05	98.50 ± 2.43	95.50 ± 2.80	92.90 ± 3.18
BP-EXT	100.70 ± 1.82	98.60 ± 2.20	97.30 ± 2.57	93.50 ± 2.94	90.40 ± 3.32
CO-AuNPs	101.00 ± 1.96	98.80 ± 2.34	97.50 ± 2.71	93.30 ± 3.08	89.40 ± 3.46
BP-AuNPs	100.40 ± 2.10	98.30 ± 2.48	96.50 ± 2.85	91.50 ± 3.22	87.40 ± 3.60
MIX-AuNPs	100.20 ± 2.24	97.60 ± 2.62	95.30 ± 2.99	89.50 ± 3.36	84.90 ± 3.74

Percentage viability against the unstimulated control. Lipopolysaccharide alone gave 96.23 ± 0.34% and dexamethasone 1 µM gave 97.95 ± 0.35%. The prespecified interpretability threshold was 80%; the lowest value in the whole gold panel was MIX-AuNP at 50 µg/mL, 84.90 ± 3.74%, which clears the threshold by a wider margin than the corresponding silver arm.

3.13 Cytokine inhibition

Lipopolysaccharide raised TNF-α from 41.16 ± 2.55 pg/mL in unstimulated cells to 755.97 ± 20.04 pg/mL, and IL-6 from 27.26 ± 2.44 to 911.99 ± 29.44 pg/mL; dexamethasone brought them to 201.66

8.52 and 242.33 $\pm$ 10.60 pg/mL. At 50 μ g/mL, TNF- α inhibition was 37.36 $\pm$ 4.53% for CO-EXT, 47.41 $\pm$ 4.68% for BP-EXT, 60.46 $\pm$ 4.82% for CO-AuNPs, 68.50 $\pm$ 4.97% for BP-AuNPs and 77.55 $\pm$ 5.11% for MIX-AuNPs, with IL-6 inhibition running 35.36 $\pm$ 4.53%, 48.41 $\pm$ 4.67%, 61.45 $\pm$ 4.82%, 70.51 $\pm$ 4.97% and 79.55 $\pm$ 5.12% (Table 14).

Table 13. Cytokine inhibition in lipopolysaccharide-stimulated RAW 264.7 macrophages (mean $\pm$ SD, n = 3)

Treatment	Conc. (μ g/mL)	TNF- α inhibition (%)	Code	IL-6 inhibition (%)	Code
CO-EXT	3.125	3.24 $\pm$ 3.15	ns	2.24 $\pm$ 3.15	ns
	6.25	5.58 $\pm$ 3.49	ns	5.58 $\pm$ 3.49	ns
	12.5	14.48 $\pm$ 3.84	ns	13.48 $\pm$ 3.84	ns
	25	23.70 $\pm$ 4.18	*	22.70 $\pm$ 4.18	*
	50	37.36 $\pm$ 4.53	*	35.36 $\pm$ 4.53	**
BP-EXT	3.125	5.27 $\pm$ 3.30	ns	5.27 $\pm$ 3.30	ns
	6.25	9.53 $\pm$ 3.64	ns	10.52 $\pm$ 3.64	ns
	12.5	20.54 $\pm$ 3.98	*	21.54 $\pm$ 3.98	*
	25	31.66 $\pm$ 4.33	*	33.66 $\pm$ 4.33	**
	50	47.41 $\pm$ 4.68	**	48.41 $\pm$ 4.67	**
CO-AuNPs	3.125	7.31 $\pm$ 3.44	ns	8.31 $\pm$ 3.44	ns
	6.25	14.47 $\pm$ 3.79	ns	15.47 $\pm$ 3.79	ns
	12.5	28.61 $\pm$ 4.13	*	29.61 $\pm$ 4.13	*
	25	43.62 $\pm$ 4.47	**	44.62 $\pm$ 4.47	**
	50	60.46 $\pm$ 4.82	**	61.45 $\pm$ 4.82	**
BP-AuNPs	3.125	10.34 $\pm$ 3.59	ns	11.34 $\pm$ 3.60	ns
	6.25	19.41 $\pm$ 3.94	*	20.41 $\pm$ 3.93	*
	12.5	34.67 $\pm$ 4.28	*	36.67 $\pm$ 4.28	**
	25	50.58 $\pm$ 4.62	**	52.58 $\pm$ 4.62	**
	50	68.50 $\pm$ 4.97	**	70.51 $\pm$ 4.97	**
MIX-AuNPs	3.125	14.36 $\pm$ 3.74	ns	15.37 $\pm$ 3.73	ns
	6.25	25.35 $\pm$ 4.08	*	26.36 $\pm$ 4.09	*
	12.5	43.73 $\pm$ 4.42	**	45.74 $\pm$ 4.43	**
	25	60.54 $\pm$ 4.77	**	62.54 $\pm$ 4.77	**
	50	77.55 $\pm$ 5.11	**	79.55 $\pm$ 5.12	**

Codes are Holm-adjusted Welch comparisons against the LPS control: ns $p > 0.05$, * $p < 0.05$, ** $p < 0.01$. Reference values: LPS alone raised TNF- α to 755.97 $\pm$ 20.04 pg/mL and IL-6 to 911.99 $\pm$ 29.44 pg/mL against unstimulated values of 41.16 $\pm$ 2.55 and 27.26 $\pm$ 2.44 pg/mL; dexamethasone 1 μ M gave 201.66 $\pm$ 8.52 and 242.33 $\pm$ 10.60 pg/mL.

3.14 Apparent inhibitory concentrations and multiplicity-adjusted comparisons

Apparent 50% inhibitory concentrations followed the same order, but both extracts extrapolate beyond the highest tested concentration on both analytes and are labelled as such. Two-way ANOVA gave $F(4,50) = 102.181$ for treatment, $F(4,50) = 357.237$ for concentration and $F(16,50) = 4.170$ for the interaction on TNF- α , and $F(4,50) = 120.139$, $F(4,50) = 363.126$ and $F(16,50) = 4.679$ on IL-6, all $p < 0.0001$. Against the LPS control after Holm adjustment, MIX-AuNPs and BP-AuNPs separated from control from 6.25 μ g/mL on both analytes, CO-AuNPs from 12.5 μ g/mL, and the two extracts from 12.5 to 25 μ g/mL. Direct comparison of MIX-AuNPs against either individual system at matched concentrations produced no adjusted p value below 0.05 on either analyte; the smallest was 0.0908 (Table 15, Fig. 8).

Table 14. Apparent cytokine-inhibition concentrations

Analyte	Treatment	Apparent IC ₅₀ (μ g/mL)	95% CI	Hill slope	R ²	Range status
TNF- α	CO-EXT	82.44	63.47–101.40	0.998	0.935	Extrapolated
	BP-EXT	54.90	46.09–63.71	0.976	0.953	Extrapolated

Analyte	Treatment	Apparent IC ₅₀ (µg/mL)	95% CI	Hill slope	R ²	Range status
	CO-AuNP	32.33	28.78–35.88	1.036	0.968	Bracketed
	BP-AuNP	23.88	21.52–26.24	1.046	0.972	Bracketed
	MIX-AuNP	16.33	14.81–17.85	1.089	0.975	Bracketed
IL-6	CO-EXT	89.45	66.84–112.06	0.993	0.930	Extrapolated
	BP-EXT	51.92	43.84–60.00	0.963	0.955	Extrapolated
	CO-AuNP	31.02	27.66–34.37	1.019	0.969	Bracketed
	BP-AuNP	22.01	19.88–24.14	1.051	0.973	Bracketed
	MIX-AuNP	15.14	13.74–16.54	1.108	0.975	Bracketed

Values above the highest tested concentration of 50 µg/mL are extrapolated estimates and are not treated as experimentally bracketed potencies. Two-way ANOVA: TNF-α treatment $F(4,50) = 102.181$, concentration $F(4,50) = 357.237$, interaction $F(16,50) = 4.170$; IL-6 $F(4,50) = 120.139$, $F(4,50) = 363.126$, $F(16,50) = 4.679$; all $p < 0.0001$.

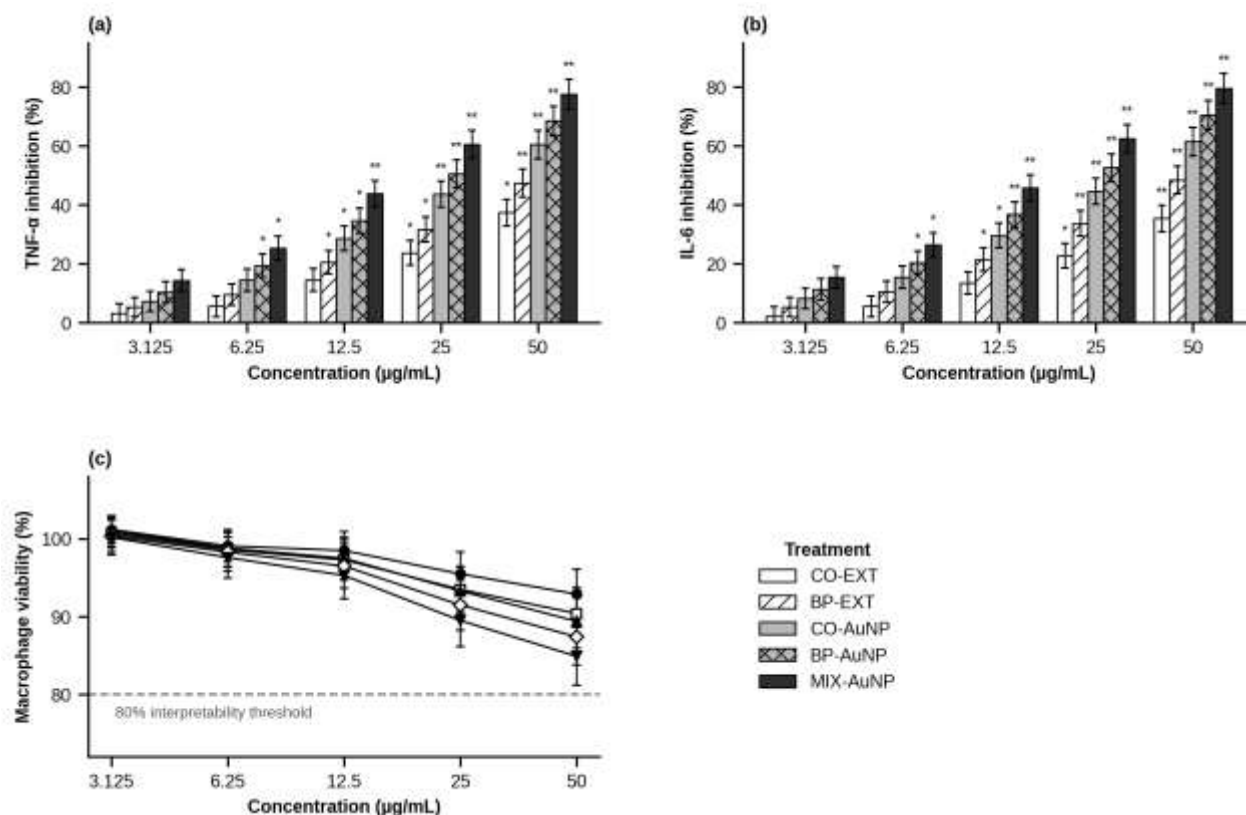


Figure 8. Anti-inflammatory evaluation in lipopolysaccharide-stimulated RAW 264.7 macrophages: (a) TNF-α inhibition, (b) IL-6 inhibition, (c) parallel macrophage viability with the 80% interpretability threshold marked. Bars and symbols are means with SD bars, $n = 3$. Significance codes above bars are Holm-adjusted Welch comparisons against the LPS control (* $p < 0.05$, ** $p < 0.01$).

3.15 Cross-assay summary

Six independent readouts, one order. The potency sequence MIX-AuNPs > BP-AuNPs > CO-AuNPs > BP-EXT > CO-EXT held for radical scavenging, for cytotoxicity on each of MCF-7, HCT116 and A549, and for suppression of both TNF-α and IL-6. The ratio between the weakest and strongest test material was 3.15-fold for DPPH, 3.14-fold for MCF-7, 3.84-fold for HCT116, 3.72-fold for A549, 5.05-fold for TNF-α and 5.91-fold for IL-6 (Table 15).

Table 15. Cross-assay IC₅₀ summary (µg/mL); lower values indicate greater activity

Assay	CO-EXT	BP-EXT	CO-AuNPs	BP-AuNPs	MIX-AuNPs	Weakest / strongest
DPPH	143.11	92.78	78.95	60.43	45.42	3.15

Assay	CO-EXT	BP-EXT	CO-AuNPs	BP-AuNPs	MIX-AuNPs	Weakest / strongest
MTT, MCF-7	346.11	216.65	159.75	131.78	110.15	3.14
MTT, HCT116	468.86	285.04	205.42	168.59	122.25	3.84
MTT, A549	530.50	365.66	254.64	212.73	142.68	3.72
TNF- α inhibition	82.44	54.90	32.33	23.88	16.33	5.05
IL-6 inhibition	89.45	51.92	31.02	22.01	15.14	5.91

Cytokine values are apparent inhibitory concentrations; the two extract values on each analyte are extrapolated above the 50 $\mu\text{g/mL}$ ELISA range. The final column is computed from the tabulated values.

4. Discussion

The two extracts produced measurably different particles from an identical reaction, and the direction of every difference matches what the silver phase showed. BP-AuNPs was smaller by hydrodynamic diameter, narrower by polydispersity index, more negatively charged, smaller by crystallite size and higher in metal content than CO-AuNPs. That the same ranking survives a change of metal, a change of reaction pH from 8.0 to 6.0, a change of temperature from 60 to 70 $^{\circ}\text{C}$ and a halving of reaction time is the strongest available evidence that the source extract, not the process, is setting the product.

The 3.8 nm red shift and wider band for BP-AuNPs point to a broader distribution of shapes rather than larger particles, since light scattering places BP-AuNPs as the smaller of the two. For gold the band-shape argument is sharper than for silver: anisotropic gold structures carry a longitudinal resonance above 600 nm, and the A650/Amax values of 0.073 and 0.086 are low enough to exclude that population at any meaningful number fraction, since anisotropic gold structures are defined by exactly that longitudinal resonance (Mannelli & Marco, 2010). Surface charge is not a cosmetic parameter here either: it governs colloidal stability and, once the particles reach a protein-containing medium, the composition of the adsorbed corona and hence cellular uptake (Bhattacharjee, 2016; Arezki et al., 2022).

Both gold batches were smaller and more narrowly distributed than the silver batches from the same extracts. Au^{3+} reduction is a three-electron process and proceeds faster once initiated, which favours a burst of nucleation over prolonged growth, and the 1 h reaction gave less time for Ostwald ripening than the 2 h silver reaction.

The organic fraction seen by EDX was lower for gold than for silver, 20.4–21.1 wt% carbon plus oxygen against 27.6–30.7 wt%. Part of that is arithmetic rather than chemistry: gold is 1.83 times heavier than silver per atom, so an identical organic layer occupies a smaller mass fraction of a gold particle. The gravimetric balance makes the point directly. Ninety millilitres of 1 mM chloroauric acid carries 17.727 mg of gold, which against dry yields of 26.13 and 28.27 mg per 100 mL puts the retained organic fraction at 32.2–45.7 wt% for CO-AuNPs and 37.3–49.8 wt% for BP-AuNPs over the plausible range of conversion. The capping layer is therefore roughly two-fifths of the pellet by mass, not a trace, which is consistent with the infrared spectra retaining the full fingerprint region of the parent extract. The release profiles behaved differently from the silver arm. In silver the two profiles converged by 24 h and the interaction term was clearly non-significant; in gold the separation persisted to the endpoint, the interaction term sat at $p = 0.0539$ and the area-under-curve difference reached significance at $p = 0.0293$. Read this as mobile-fraction behaviour, not dissolution: metallic gold is inert under these conditions, so the quantity crossing the membrane reports on the capping-mediated dispersion state rather than on ionic release, which is the opposite of the situation for silver, where oxidative dissolution to Ag^+ governs the mobile fraction (Liu & Hurt, 2010).

On the biological side the useful feature is again that one rank order survived six readouts with no shared assay chemistry. Both parent extracts were active in the range expected for flavonol-glycoside-rich material, and the higher activity of BP-EXT matches the structure-activity pattern for flavonoids, where the B-ring catechol arrangement of quercetin-type compounds confers stronger scavenging than kaempferol-type substitution (Silva et al., 2002; Torres Castañeda et al., 2016; Gulcin, 2020); crude polar plant extracts commonly occupy this band when DPPH and tetrazolium endpoints are run side by side (Ahmad et al., 2005). Conversion of either extract to its gold nanoparticle form improved potency by 1.54- to 2.88-fold depending on the endpoint, and the mixture was the most active material on every one. The cytotoxicity values sit within the band reported for plant-mediated gold nanoparticles against carcinoma lines, though comparison across studies is loose because exposure time, seeding density and dose spacing vary (Devi & Sathishkumar, 2017; Wu et al., 2019; Wang et al., 2019; Al-Sarraj et al., 2023). Mechanistic work on biogenic gold converges on reactive oxygen species generation and caspase-dependent apoptosis (Bharadwaj et

al., 2021); none of those endpoints was measured here, so the present study establishes a ranking and not a mechanism.

Placing the two phases side by side is where this dataset earns its keep, because both were run under one protocol on the same cell lines and the same concentration series. The gold systems were consistently less potent than the matched silver systems on radical scavenging and on all three cell lines, by factors of 1.31 to 1.68. On cytokine suppression the two metals were indistinguishable, with gold-to-silver apparent IC₅₀ ratios of 0.92 to 1.03. That dissociation is informative. If activity were carried mainly by the retained phytochemical layer, the two metals should track together everywhere; if it were carried mainly by the metal, they should differ everywhere. The pattern instead suggests that the cytotoxic and antioxidant readouts are metal-sensitive while the anti-inflammatory readout is dominated by the surface layer. The few studies that prepare both metals from one extract and test them on one line report the same direction, with gold the less cytotoxic of the pair (Devi & Sathishkumar, 2017; Khedr et al., 2024). Independent work on gold in stimulated or injured macrophage systems shows cytokine suppression comparable to that seen with silver, and the lipopolysaccharide challenge used here signals through TLR4 and NF- κ B, a pathway at which plant flavonoids act in the same cell model (Fu et al., 2021) (de Carvalho et al., 2018; Yin et al., 2019; Wang et al., 2020; Xu et al., 2021; Fujita et al., 2021; Yang et al., 2022; Deng et al., 2024), which is consistent with a surface-driven rather than a metal-driven mechanism on that endpoint. Testing this directly, by stripping the capping layer or by transplanting it between metals, is the obvious next experiment.

Two further points bear recording. *B. pinnatum* contains bufadienolides, and genotoxicity signals have been reported for ethanolic leaf extract and, more weakly, for fresh leaf juice (Saravanan et al., 2020; Bhavsar & Chandel, 2022; Sharma et al., 2024), so any translational development should carry a genotoxicity package rather than rely on cytotoxicity data alone. Separately, metabolite content in Crassulaceae leaf varies with harvest date, leaf age and growing location by factors of three or more for bufadienolides (Oufir et al., 2015), which is the standard argument for fingerprint-based release testing of plant starting material (Tetali et al., 2021). Several limitations bear on interpretation. The physical mixture was characterised only by the properties of its two components, so hetero-aggregation on mixing cannot be excluded. Cytotoxicity was assessed against three cancer lines without a matched non-tumour line, so no selectivity index is available, and no reference cytotoxic drug was run alongside. Colloidal stability was measured at a single time point rather than over storage or in serum-containing medium. Mechanistic endpoints for both the cytotoxic and the anti-inflammatory effect were outside the present scope, and formal spike-recovery and dilution-parallelism validation of the ELISA was not performed.

5. Conclusion

Ethanolic leaf extracts of *C. ovata* and *B. pinnatum* both reduced chloroauric acid to face-centred cubic gold nanoparticles under one fixed protocol, and the two products differed in ways that track the chemistry of the source plant: BP-AuNPs was smaller (63.03 ± 1.20 nm against 69.13 ± 1.25 nm), more narrowly distributed (0.205 against 0.228), more negatively charged (-30.33 against -25.37 mV), of smaller crystallite size (19.22 against 25.83 nm) and higher in gold content. Across radical scavenging, cytotoxicity on MCF-7, HCT116 and A549, and TNF- α and IL-6 suppression in stimulated RAW 264.7 macrophages, the potency order was identical on every readout: MIX-AuNPs > BP-AuNPs > CO-AuNPs > BP-EXT > CO-EXT. Conversion of either extract into its nanoparticle form improved potency by 1.5- to 2.9-fold. The 1:1 physical mixture was the most active material tested but was not statistically superior to either individual system at matched concentrations after multiplicity adjustment, and is reported as showing enhanced activity rather than synergy.

Declarations

Funding

No external funding is declared at this stage.

Conflicts of interest

The authors declare no competing interests.

Ethics approval

This study used established immortalised cell lines and required no ethical approval for human or animal subjects.

Data availability

Replicate-level absorbance data, fitted curve parameters and the statistical output supporting all tables and figures are available from the corresponding author on reasonable request.

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