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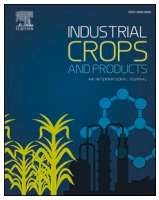

Untargeted metabolomics and in vitro functional analysis unravel the intraspecific bioactive potential of flowers from underexplored Camellia japonica cultivars facing their industrial application

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ABSTRACT

The *Camellia* genus comprises a vast array of underexplored medicinal plants that merit a systematic valorization to exploit their potential as natural sources of phytochemicals with associated health-promoting properties. In this work, flower extracts from eight poorly characterized *Camellia japonica* L. cultivars were subjected to polyphenol profiling through untargeted metabolomics combined with *in vitro* functional analysis. Anthocyanins, mostly represented by cyanidin 3-*O*-glycosides, flavones, and flavonols, were found as the major constituents of *C. japonica* flowers, together with hydroxycinnamic acids, tyrosols, alkylphenols, and stilbenes, which were detected for the first time in this species. The application of multivariate statistics revealed a flower color-dependent fingerprint of *C. japonica* cultivars, featuring anthocyanins and other flavonoids as metabolite markers associated with color-flowered cultivars with respect to white-flowered ones. The accumulation of anthocyanins, especially reported in 'Eugenia de Montijo' flowers, was highly correlated with the cytotoxic and anti-inflammatory properties of the derived extracts, including AGS, Caco-2, and MCF7 cancer cell lines. Moreover, the flavones accumulation reported in 'Carolyn Tuttle' extracts was also associated with high rates of free-radical scavenging activity, as well as a potent cytotoxicity against the Caco-2 cell line. In general, *C. japonica* anthocyanin-enriched flower extracts were revealed as promising candidates for the industrial production of polyphenols with associated biological activities of high interest for critical sectors in the food, pharmaceutical, and cosmetic industries.

1. Introduction

A significant number of species belonging to the genus *Camellia* L. (Theaceae) have focused much attention on their healthy-associated properties, as is the case of *Camellia sinensis*, whose leaves are worldwide employed in the tea industry, or *Camellia oleifera*, whose seeds are primarily exploited in the production of edible and essential oils (Teixeira and Sousa, 2021). The properties associated with these natural resources are a consequence of the accumulation of specialized metabolites with associated bioactivities, like alkaloids, polyphenols, and terpenoids, which are responsible for the development of antioxidant, anti-inflammatory, antimicrobial, antiviral, and antitumoral activities reported to camellias (Teixeira and Sousa, 2021). Besides those well-known species, *Camellia japonica* has been largely exploited for ornamental purposes, thanks to the colorful petals of its flowers, motivated by the accumulation of flavonoids, especially anthocyanins, which play a major role as a result of their natural coloration (Fu et al., 2021), as well as carotenoids (Fernandes et al., 2020). From an industrial perspective, *C. japonica* flowers are largely exploited for their horticultural properties, being considered a widespread ornamental plant across

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tropical and subtropical regions, and it is recognized as an underutilized resource (Majumder et al., 2022), thus urging novel strategies facing their industrial profit. Additionally, the leaves and seeds of *C. japonica* have been reported to present a wide range of phytochemicals, including flavonoids, other exclusive polyphenols like camellianoside, saponins, terpenoids, and polyunsaturated fatty acids (Pereira et al., 2022). Despite this chemical heterogeneity reported in *C. japonica*, scarce efforts have been made to valorize its flowers as a source of bioactive compounds, even though its leaves have been widely used in Asian traditional medicine to treat inflammatory-related diseases (Majumder et al., 2020). Although *C. japonica* flowers are considered edible (Fernandes et al., 2020), they are still underexplored from an agronomical and industrial point of view as it presents more than 32,000 different cultivars. Consequently, this medicinal plant possesses an unraveled health-promoting and cosmeceutical potential still to be determined (Sousa et al., 2019).

Metabolomics has emerged as a promising high-throughput technology to provide the chemical fingerprinting of biological matrices (Garcia-Perez et al., 2023). Thanks to its high analytical performance, it is able to simultaneously identify a vast array of chemical entities, which can be further interpreted by multivariate statistics and other chemometrics-based bioinformatic tools to characterize the metabolic profile of either food, animal or plant samples. Different approaches have been performed with that aim, mostly relying on liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS) to achieve metabolome-wide profiling of bioactive compounds from different plant materials and tissues (Rivera-Pérez et al., 2022). Thus, in recent years, metabolomics has become a useful analytical tool to achieve the phytochemical valorization of underexploited medicinal plants and related matrices, especially by applying untargeted approaches that enable the coverage of a wide range of metabolites. Indeed, the leaves of *C. japonica* have already been subjected to metabolomics-based approaches to shed light on the metabolites responsible for their anti-inflammatory properties (Majumder et al., 2020). In this work, a combinatorial approach integrating untargeted metabolomics (*via* ultra-high performance liquid chromatography coupled to quadrupole-time-of-flight high-resolution mass spectrometry, UPLC/QTOF-HRMS) and *in vitro* determination of antioxidant, cytotoxic, and anti-inflammatory activities was employed to decipher the cultivar influence of *C. japonica* flowers on their phenolic profile and the bioactivities associated with these compounds. Overall, the proposed workflow is expected to contribute to the valorization of this unknown plant matrix, paving the road to its eventual exploitation with industrial purposes and, thus, diversifying its economic profit.

2. Materials and methods

2.1. Plant material and extraction conditions

Flowers from eight different *Camellia japonica* L. cultivars (Theaceae) were botanically identified by official germplasm banks and identification guide resources, and collected in NW Spain (42.431° N, 8.6444° W) by Viveiros Moreira in January 2020, harvesting a total quantity of 200 g of fresh flowers (around 40 flowers for each cultivar). The cultivars involved in this study were: 'Conde de la Torre' (CT), 'Elegans variegated' (EV), 'Donation dentada' (*Camellia japonica* × *Camellia saluenensis,* DD), 'Dr. Tinsley' (DT), 'Eugenia de Montijo' (EM), 'Grandiflora superba' (GS), 'Hagoromo' (HA), and 'Carolyn Tuttle' (CT). An overview of the flowers from the eight cultivars involved in this research is provided in Fig. S1. Flowers were immediately frozen in liquid nitrogen and stored at − 80 °C. Later, the plant material was frozen-dried and powdered for extraction. Lyophilized samples (1 g of dry weight, dw) were extracted by maceration at a solid:liquid ratio of 1:25 (w/v) using 60% (v/v) aqueous methanol for 1 h at 50 °C. Extracts were further centrifuged at 4000 *g* for 15 min, filtered using 0.2 μm pore-sized filter paper and further concentrated by a rotatory evaporator to remove methanol, lyophilized until complete dryness and stored at − 80 °C until subsequent analyses. This extraction procedure was carried out in triplicate.

2.2. Untargeted metabolomic analysis of C. japonica flowers via UPLC/QTOF-HRMS

The phenolic profile of *C. japonica* flower extracts was determined by an untargeted metabolomics approach through ultra-high performance liquid chromatography coupled to quadrupole-time-of-flight high-resolution mass spectrometry (UPLC/QTOF-HRMS), as previously described (García-Pérez et al., 2023). To this aim, 100 mg of concentrated extracts were resuspended in 1 mL of the solvent mixture MeOH:H_2O:HCOOH (80.0:19.9:0.1 v/v/v) and syringe filtered (pore size, 0.22-μm) into vials for analysis. The chromatographic separation was performed in a 1290 Infinity II LC system (Agilent® Technologies, Santa Clara, CA, USA) equipped with a reverse-phase ZORBAX Eclipse Plus C-18 column (Agilent® Technologies, 2.1 × 100 mm, 1.8 μm). The mobile phase included two phases, acetonitrile (solvent A) and water (solvent B), both acidified with 0.1% HCOOH (v/v), and elution was performed following a continuous linear binary gradient for 32 min, from 6%A to 94%A. The injection volume was set at 6 μL and the flow rate was adjusted at 200 μL/min. The chromatographic system was coupled to the mass spectrometer *via* electrospray ionization (ESI) source. The mass spectrometer employed was a 6550 iFunnel QTOF system (Agilent® Technologies), operating in SCAN mode and positive polarity (ESI+). The extended dynamic range was 100 – 1200 *m/z* and a nominal resolution of 40,000 FWHM was employed. Nitrogen was used as both sheath gas (10 L/min, 350 °C) and drying gas (8 L/min, 330 °C). The ionization source operated at a nebulizer pressure of 60 psi, the nozzle voltage was set at 300 V, and the capillary voltage was 4000 V. The injection pattern was completely randomized, including two blanks, and each extract was injected and analyzed twice, making a total of 6 replicates for each *C. japonica* cultivar (n = 6).

Compound annotation was performed computationally based on the isotopic pattern, according to monoisotopic mass, isotopic spacing, and isotopic ratio, applying the "find-by-formula" algorithm by the MassHunter Profinder software (version 10.0; Agilent® Technologies) and using the Phenol-Explorer database (version 3.6; available at http://phenol-explorer.eu/) in compliance with Level 2 of the COSMOS Metabolomics Standards Initiative, i.e.: putatively annotated compounds. The annotated features were filtered according to several post-acquisition parameters: 5-ppm mass accuracy tolerance, 0.1-min retention time shift, a minimum abundance of 5000 units and the presence in at least 80% of replicates.

After annotation, chemical features were classified into different phenolic classes and subclasses and quantified following a semi-quantitative approach. Each class was quantified using a calibration curve obtained from pure analytical standards (all of them HPLC-grade purchased from Extrasynthese®, Lyon, France) of a representative compound, and the results for each class were expressed as equivalents (in μg) of the representative compound per gram of dry weight (μg of equivalents/ g dw). Thus, anthocyanin content was expressed as cyanidin equivalents (CyE); flavanol content was expressed as (+)-catechin equivalents (CaE); flavone, flavanone, isoflavones, chalcone, and dihydrochalcone content was expressed as luteolin equivalents (LE); flavonol content was expressed as quercetin equivalents (QE); lignan content was expressed as sesamin equivalents (SE); low-molecular-weight (LMW) phenolic and other polyphenol content was expressed as tyrosol equivalents (TE); phenolic acid content was expressed as ferulic acid equivalents (FE); and stilbene content was expressed as *trans*-resveratrol equivalents (RE). The results were expressed as the mean ± standard deviation (n = 6).

2.3. Determination of biological activities of C. japonica flower extracts

2.3.1. Antioxidant activity

The antioxidant activity of *C. japonica* flower extracts was determined by different methods. In all cases, the concentrated extracts of *C. japonica* flowers were resuspended in water at a concentration of 8 mg/mL and tested in terms of inhibition of lipid peroxidation, reducing power, and free-radical scavenging activity. In all cases, concentrated *C. japonica* flower extracts were resuspended at a final concentration of 1.6 mg/mL using 60% aqueous methanol, and decreasing concentrations of flowers extracts were tested, performing 1:2 serial dilutions of the extracts, from 320 to 5 μg/mL, against a blank containing only the solvent. The spectrophotometric determinations were recorded using an ELX800 Microplate Reader (Bio-Tek Instruments Inc., Winooski, VT, USA). All determinations were carried out in triplicate (n = 3), and results were expressed as the mean ± standard deviation. Experimental conditions were previously determined elsewhere (Aylanc et al., 2023; Vega et al., 2023).

The inhibition of lipid peroxidation of extracts was evaluated by the thiobarbituric acid reactive substances method (TBARS) using porcine brain cell homogenate (*Sus scrofa*). Briefly, this method determines the inhibition of the formation of malondialdehyde-thiobarbituric acid complex by the tested extracts by measuring spectrophotometrically the presence of this complex at 532 nm. Results were expressed as inhibitory concentration 50, IC_{50} (in μg/mL), which indicates the extract concentration required to inhibit lipid peroxidation by 50%.

The reducing power of extracts was determined by the ferric-reducing antioxidant power (FRAP) method. For this purpose, 250 μL of extract was mixed with 1.25 mL of sodium phosphate buffer (pH = 6.6) and 1.25 mL of 1% (w/v) potassium ferricyanide, and incubated at 50 °C for 20 min. Later, 1.25 mL of 10% (v/v) trichloroacetic acid were added and the mixture was centrifuged at 7000 × *g*. Finally, 1.25 mL of supernatants were collected and mixed with 1.25 mL of water and 0.25 mL of 0.1% (w/v) ferric chloride. The absorbance was measured at 700 nm, and the results were expressed as IC_{50} (in μg/mL), indicating the extract concentration reducing by 50% the oxidation of ferric ions.

The free-radical scavenging activity was determined by the 2,2-diphenyl-1-pycrilhydrazyl (DPPH) method. Briefly, 150 μL of extract were mixed with 150 μL of 24 μg/mL DPPH solution in methanol. The absorbance was spectrophotometrically measured at 515 nm, and the results were expressed as IC_{50} (in μg/mL), representing the extract concentration quenching 50% of the absorbance attributed to the DPPH radical.

2.3.2. Cytotoxic activity

The cytotoxic activity of *C. japonica* flower extracts was assessed by the spectrophotometric sulforhodamine B (SRB) *in vitro* cellular assay applied for 6 cell lines. Four of them (AGS gastric adenocarcinoma, Caco-2 colorectal adenocarcinoma, MCF7 breast adenocarcinoma, and NCI-H460 lung carcinoma cell lines) are considered as tumor cell lines and were employed to evaluate the antitumor activity of extracts. Two additional lines are considered non-tumor cell lines and were used to test both the hepatotoxicity, using the PLP2 hepatocyte cell line, and the cytotoxicity of the extracts using the Vero kidney-derived cell line. The AGS, Caco-2, MCF7, NCI-H460 and Vero cell lines were purchased from ATCC® (Manassas, VA, USA), whereas the PLP2 cell line was obtained following an in-house protocol (Mandim et al., 2019). The experimental details for the determination were thoroughly described in previous works (Aylanc et al., 2023; Cassani et al., 2022). To conduct this determination, concentrated *C. japonica* extracts were resuspended at a concentration of 8 mg/mL in DMSO, and ellipticine (Enzo Life Sciences, Inc., NY, USA) was used as positive control. The spectrophotometric determinations were recorded using an ELX800 Microplate Reader (Bio-Tek Instruments Inc.). The results were expressed as growth inhibitory concentration 50 (GI_{50}), representing the extract concentration (in μg/mL) required to inhibit cell proliferation by 50%. All determinations were carried out in triplicate (n = 3), and results were expressed as the mean ± standard deviation.

2.3.3. Anti-inflammatory activity

The anti-inflammatory activity of *C. japonica* flower extracts was evaluated by the inhibition of nitric oxide production by lipopolysaccharide (LPS)-induced RAW 264.7 macrophages, as explicitly explained in previous works (Aylanc et al., 2023; Cassani et al., 2022). The RAW 264.7 line was purchased from ECACC (Salisbury, UK). For this aim, concentrated *C. japonica* extracts were resuspended at 8 mg/mL concentration in DMSO, and dexamethasone (Sigma-Aldrich®, Saint Louis, MO, USA) was used as positive control. NO production was determined using the Griess Reagent System kit (Promega®, WI, USA), following the manufacturer's guidelines. The spectrophotometric determinations were recorded using an ELX800 Microplate Reader (Bio-Tek Instruments Inc.). Results were expressed as IC_{50} (in μg/mL), indicating the extract concentration required to inhibit by 50% the production of NO by LPS-induced macrophages. All determinations were carried out in triplicate (n = 3), and results were expressed as the mean ± standard deviation.

2.4. Data processing of metabolomics data and statistical analysis

The raw data proceeding from the untargeted metabolomics approach were processed statistically using the Mass Profile Professional software tool (version 12.6; Agilent® Technologies). Firstly, compounds' abundance values were transformed to $\log_2$ values, normalized at the 75th percentile, and further baselined to the median of all samples. Later, multivariate statistics were carried out to decipher the effect of the cultivar on the phenolic profile of *C. japonica* flower extracts. Two unsupervised multivariate statistical analyses were performed: hierarchical cluster analysis (HCA) and principal component analysis (PCA). To develop the HCA, a fold change-based heatmap combined with dendrogram analysis (Euclidean distance, Ward's linkage rule) was built to naively assist in the establishment of similarities and/or dissimilarities between cultivars according to a metabolome-wide perspective. In parallel, a supervised orthogonal projection to latent structures discriminant analysis (OPLS-DA) was carried out by SIMCA (version 16.0, Umetrics®, Malmö, Sweden) to elucidate the discrimination between cultivars according to their phenolic profile. The resulting OPLS predictive model was combined with variable importance in projection (VIP) analysis to identify the metabolites showing the strongest influence in the projected discrimination between cultivars, the so-called VIP markers (only the compounds presenting a VIP score < 1.15 were considered). The quality of the OPLS model obtained was assessed according to their goodness-of-fit (represented by the R^2Y coefficient) and goodness-of-prediction (represented by the Q^2 coefficient). Furthermore, the OPLS model was statistically validated by cross-validation analysis of variance (CV-ANOVA), and permutation test (n = 100) was carried out to assess the absence of model over-fitting.

The analysis of $\log_2$-transformed abundances of VIP markers was performed through two-tailored t-test and results were expressed as boxplots, using the R software tool (version 4.2.0) and the results obtained from the semi-quantification of phenolic compounds and the different biological activities determination were analyzed statistically by one-way ANOVA and Duncan's *post hoc* test using the SPSS software tool (version 25.0, IBM Corp., Armonk, NY, USA), considering statistical differences at a significance level $\alpha = 0.05$. All variables from phenolic compounds quantification and biological activities determination were subjected to Pearson's correlation analysis using the R software tool, and the results were displayed as a heatmap-based correlation matrix, setting a significance level of $\alpha = 0.01$ for statistically significant correlations.

3. Results and discussion

3.1. Untargeted metabolomics reveals a heterogeneous phenolic profile of C. japonica flower extracts

Flower extracts from different *C. japonica* cultivars were subjected to phenolic profiling *via* UPLC/QTOF-HRMS untargeted metabolomics. This high throughput technology allowed the annotation of 246 different phenolic compounds throughout the flower extracts of the eight *C. japonica* cultivars investigated. The comprehensive list of annotated compounds, together with their class, raw abundance values, retention time (min), mass (u), and molecular formula is included in Table S1. The most representative classes of phenolic compounds reported in *C. japonica* flower extracts were flavonoids, phenolic acids, and low-molecular-weight and other polyphenols. (Table S1). Considering subclasses, a heterogeneous flavonoid profile was reported, featuring 34 anthocyanin glycosides, represented mainly by cyanidin 3-*O*-glycosides. The presence of anthocyanins in the flowers of *C. japonica* was reported previously by other metabolomics-based investigations as responsible for the coloration of the petals of this ornamental species in five cultivars grown in China, i.e.: 'Niuxiaomeiyu', 'Sishaluo', 'Zaohongyang', 'Dahongmudan', and 'Huangjiatianerong' (Fu et al., 2021). For instance, these authors highlighted cyanidin-3-*O*-(6″-*O*-malonyl) glucoside, cyanidin-3-*O*-rutinoside, cyanidin-3-*O*-glucoside, and pelargonidin-3-*O*--glucoside as the main contributors for the red petal phenotypes and depth coloration of the petals. In parallel, Li and co-workers elucidated that cyanidin-3-*O*-glucoside and cyanidin 3-*O*-(6''-*p*-coumaroyl-galactoside) play a key role in the red coloration of *C. japonica* flowers, as it was reported among 22 different cultivars (Li et al., 2019). These findings follow the present results, where all these compounds showed high abundance values among the investigated cultivars (Table S1).

Beyond anthocyanins, 32 flavones were reported within the flavonoid profile of *C. japonica* flowers, essentially represented by apigenin and luteolin glycosides (Table S1). The presence of flavones was marginally reported in the petals of different *C. japonica* cultivars (Fu et al., 2021), and they were recently found absent in *C. japonica* fruit shell extract from Korean cultivars (You et al., 2022), thus suggesting a more diversified polyphenolic fingerprint associated with the flowers from the cultivars investigated here. As well, 20 flavonols were reported in *C. japonica* flower extracts, from which kaempferol and quercetin glycosides were found to a greater extent in all eight cultivars investigated (Table S1). The presence of kaempferol and quercetin was reported previously in the shoots of several *C. japonica* cultivars, showing high kaempferol content in the shoots of *Camellia japonica donkelarri*, whereas the shoots of *Camellia japonica* Red. *Camellia rosaflora* exhibited high quercetin contents (Jeganathan et al., 2016). This observation suggests that flavonols produced by *C. japonica* may be synthesized by some tissues and be further mobilized towards other organs, such as the flowers. Nevertheless, the combined presence of flavones and flavonols, considered colorless pigments, may contribute to the phenotypical color expression in the flowers of *C. japonica*, as they are recognized to secondarily contribute to the flower pigmentation attributed to anthocyanins in *Chrysanthemum morifolium* (Wang et al., 2021).

Besides flavonoids, 32 hydroxycinnamic acids were significantly found in *C. japonica* flower extracts (Li et al., 2019), whereas only 8 hydroxybenzoic acids were reported (Table S1). The higher presence of hydroxycinnamic acids contrasts with the previous results performed in flower extracts of Korean *C. japonica* cultivars, where hydroxybenzoic acids were mostly reported, especially gallic acid and protocatechuic acids (Lee et al., 2011; Trinh et al., 2018), both present in the flower extracts analyzed here. The great variety of hydroxycinnamic acids reported in the flower extracts opens a new perspective in the field of *C. japonica* research as a promising and still underutilized source of these compounds. To a lesser extent, a wide range of LMW phenolic was also recorded in the flower extracts of *C. japonica*, highlighting tyrosols and alkylphenols as the most represented according to the UPLC/QTOF-HRMS-based profile (Table S1). Interestingly, tyrosol was recently reported for the first time in brewed *C. japonica* petal wine, from plants cultivated organically (Majumder et al., 2022). The partially-hydrophobic nature attributed to alkylphenols makes them suitable candidates to be further investigated as functional ingredients of *C. japonica* essential oil, as structurally-related compounds were already reported in this matrix, considered as one of the principal products obtained from *C. japonica* flowers cultivated in China (Kong et al., 2021). Other reported compounds in the flowers of the investigated cultivars include lignans, stilbenes and catechins (flavanols), showing a lower presence in the corresponding extracts. Although catechins have been largely described in other *Camellia* species, in particular *C. sinensis* (Gong et al., 2020), to the best of our knowledge, this is the first report reflecting the presence of these polyphenolic subfamilies in the flowers of *C. japonica*, thus justifying the application of untargeted metabolomics approaches for the metabolome-wide discovery of functional metabolites from poorly investigated medicinal plants.

3.2. Multivariate statistics decipher the cultivar-dependent profile of C. japonica flowers and identify key discriminant metabolites

The application of untargeted metabolomics requires the performance of multivariate statistics to provide a reliable biological interpretation of the obtained analytical results and add value to the metabolic fingerprint of *C. japonica* flowers according to their phenolic profile. First, to naively discriminate among the investigated cultivars, an unsupervised HCA was performed according to their untargeted phenolic profile (Fig. 1A). The results, based on the fold-change heatmap show a clear separation into two subclusters, grouping the cultivars EM, EV, TU, apart from the rest of the cultivars, i.e.: DD and CT in one subgroup, and DT, GS and HA in another subgroup (Fig. 1A). Interestingly, the results from HCA align with those obtained by principal component analysis (PCA; Fig. 1B), where a clear group merging the cultivars CT, HA, DT, and GS was observed. Meanwhile, the rest of the cultivars (i.e., EM, EV, TU, and DD) were independently reported, thus suggesting the existence of specific phenolic signatures associated with these cultivars (Fig. 1B). According to the 'International Camellia Register' (International Camellia Register, 2022), the results from the unsupervised multivariate analysis of *C. japonica* cultivars follow a color-based pattern of flower classification: the close relationship reported between the cultivars CT, DT, GS, and HA (reported by both HCA and PCA; Fig. 1) may owe to the coloring of their flowers, as CT and GS are considered as white-flowered cultivars, whereas DT and HA exhibit white-to-pinkish flowers (International Camellia Register, 2022). In contrast, the rest of the cultivars, the performance of PCA revealed unique phenolic profiles despite being grouped together by HCA. This observation could be explained by the different coloration observed in the flowers of these cultivars: EM flowers are considered red, meanwhile EV and TU are considered pink-flowered cultivars, as well as the cultivar DD (International Camellia Register, 2022). In this regard, these results suggest that a flower-based color pattern, associated with their phenolic profile, could be established as a significant factor in discriminating between different cultivars of *C. japonica*, as previously hypothesized (Pereira et al., 2022).

Besides unsupervised analyses, the untargeted phenolic profile of *C. japonica* flower extracts was subjected to supervised multivariate analysis through OPLS discriminant analysis. The obtained predictive model (Fig. 2A) shows high-quality parameters in terms of fitness and predictability, as indicated by their corresponding coefficients: $R^2Y = 0.988$ and $Q^2 = 0.974$, respectively. Moreover, the model was statistically validated by CV-ANOVA ($p < 0.001$). The model exhibits a clear separation that is likely in accordance with those reported by unsupervised analyses: GS, DT, and HA cultivars were grouped together, as part of white to pinkish-flowered cultivars, although in this case CT was separated. On the other hand, the color-flowered cultivars exhibited

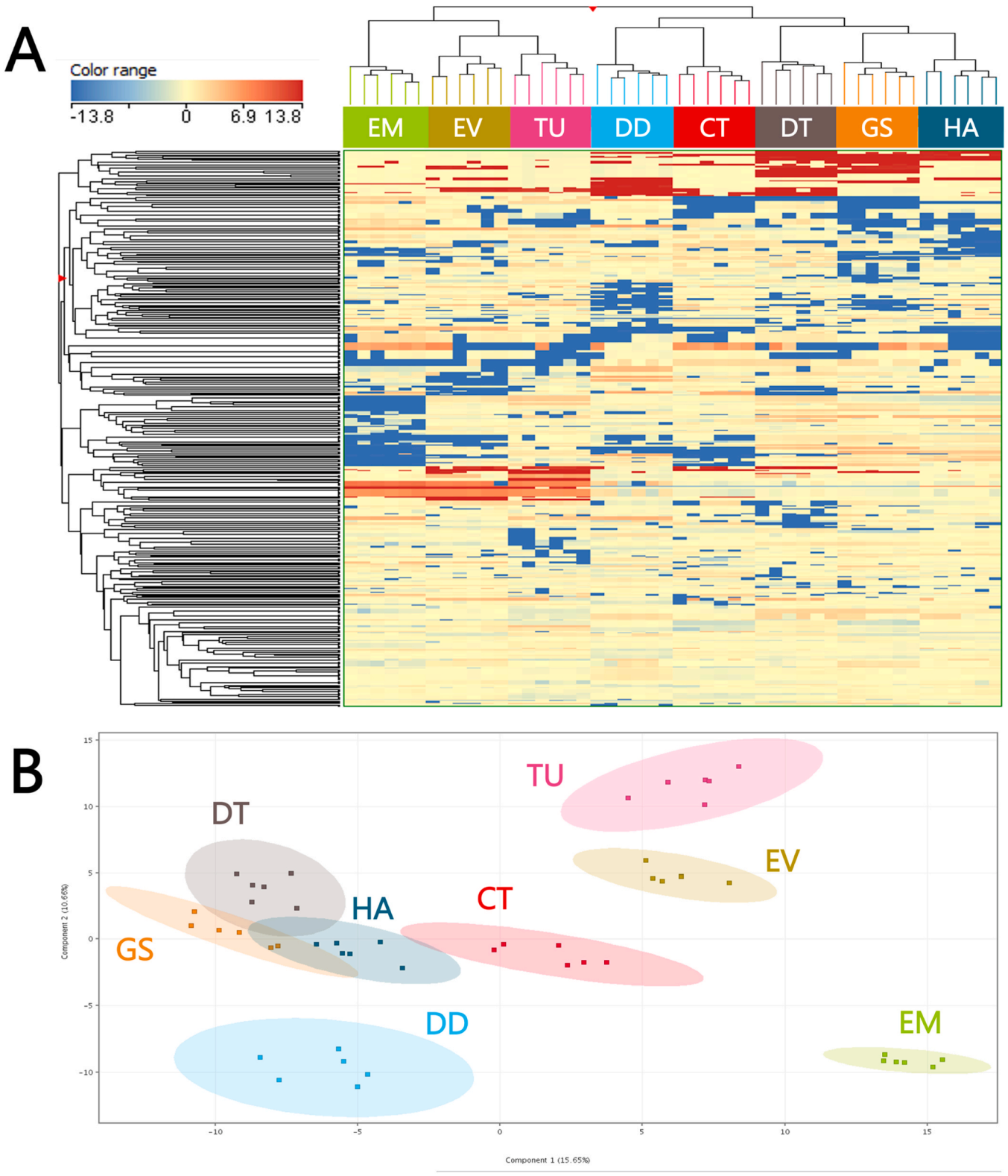

Fig. 1. Unsupervised multivariate statistical analysis on the phenolic profile of *C. japonica* L. flower extracts. The investigated cultivars were 'Conde de la Torre' (CT), 'Elegans variegated' (EV), 'Donation dentada' (DD), 'Eugenia de Montijo' (EM), 'Grandiflora superba' (GS), 'Dr. Tinsley' (DT), 'Hagoromo' (HA), and Carolyn Tuttle (TU). A. Hierarchical cluster analysis (HCA) derived from fold change-based heatmap (Euclidean distance, Ward's linkage rule). B. Principal component analysis (PCA): PC1 = 15.65%, PC2 = 10.66%.

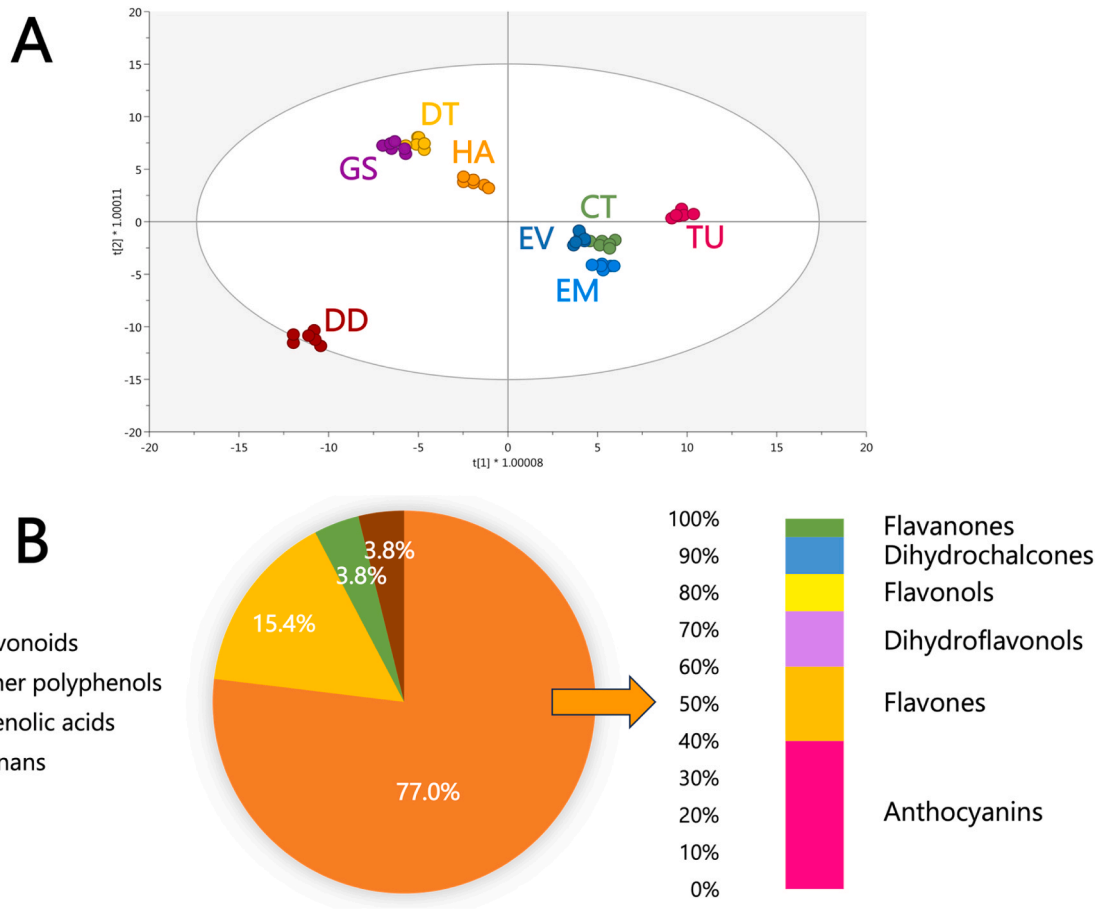

Fig. 2. Supervised orthogonal projection to latent structures discriminant analysis (OPLS-DA) modeling on the phenolic profile of *C. japonica* L. flower extracts. The investigated cultivars were 'Conde de la Torre' (CT), 'Elegans variegated' (EV), 'Donation dentada' (DD), 'Eugenia de Montijo' (EM), 'Grandiflora superba' (GS), 'Dr. Tinsley' (DT), 'Hagoromo' (HA), and Carolyn Tuttle (TU). A. OPLS-DA model. Quality parameters: $R^2Y = 0.988$; $Q^2 = 0.974$; $p < 0.001$ (CV-ANOVA, 100 permutations). B. Pie chart indicating the proportion of VIP markers according to their class (left), and proportion of flavonoid subclasses represented (right).

exclusive phenolic patterns, being independently separated among them, especially in the case of DD cultivar. Such a distinctive pattern attributed to DD could be related to the genetic background of this cultivar, that is considered a hybrid between *C. japonica* with *C. saluenensis* (*Camellia* × *williamsii*), thus reflecting a differential profile at a metabolic level, as defined by the UK's Royal Horticultural Society (Royal Horticultural Society, 2023).

To provide insight into the differential study of *C. japonica* cultivars, the OPLS model was combined with the variable importance in projection (VIP) analysis to indicate those metabolites with the highest contribution in the discrimination. The complete list of discriminating compounds, known as VIP markers (VIP score < 1.15), is shown in Table S2. As observed in Figs. 2B, 77.0% of VIP markers were classified as flavonoids, from which anthocyanins represented 40%, whereas other flavonoids like flavones and flavonols were represented much less. This result reinforces the previous hypothesis that flower anthocyanins play a major role in the discrimination of *C. japonica* cultivars, whereas other flavonoids, like flavones and flavonols, play a secondary role as co-pigments (Fu et al., 2021; Wang et al., 2021). To elucidate the biological significance of VIP markers, Fig. 3 shows the abundance of each metabolite marker, analyzed according to the previously established groups of *C. japonica* cultivars: CT, DT, GS, and HA were grouped as white-flowered cultivars, while EM, EV, TU, and DD were classified as color-flowered varieties. Among the anthocyanins featured as VIP markers, the great majority of them were found at statistically significant higher abundance in colored flower extracts (Fig. 3), essentially pelargonidin- and malvidin-3-*O*-glycosides, as observed for pelargonidin 3-*O*-sambubioside ($p < 0.01$), pelargonidin 3-*O*-glucosyl-rutinoside ($p < 0.001$), malvidin 3-*O*-galactoside ($p < 0.05$), and malvidin 3-*O*-arabinoside ($p < 0.01$). In contrast, delphinidin 3-*O*-feruloyl-glucoside exhibited a higher abundance in white-flowered cultivars ($p < 0.01$), being only reported in the DT extracts (Table S1), whereas malvidin 3-*O*-(6″acetyl-galactoside) was also found at a higher abundance in these cultivars ($p < 0.001$; Fig. 3). Although cyanidin glycosides, together with pelargonidin glycosides were previously featured as responsible for the coloration of *C. japonica* flowers (Fu et al., 2021), the cultivars investigated in this work depict other anthocyanin markers. Interestingly, a metabolomics approach applied to *Camellia sasanqua* flowers from different cultivars indicated the importance of cyanidins and delphinidins regarding their discrimination, thus concluding that the combination of different anthocyanins rules the phenotypical expression of flower color (Fan et al., 2023). Hence, the differential accumulation of anthocyanins in flowers has a direct impact on their coloration, since cyanidins present reddish attributes, pelargonidins exhibit orange coloration, and delphinidins present bluish-purplish colors (Y. Liu et al., 2022), thus supporting the identification of pelargonidin and delphinidin glycosides as discriminant markers of *C. japonica* cultivars.

Besides anthocyanins, other flavonoids identified as VIP markers showed a significantly higher abundance in color-flowered cultivars (Fig. 3), as it is the case of flavonols and dihydroflavonols, represented by kaempferol 3-*O*-(2″-rhamnosyl-6″-acetyl-galactoside) 7-*O*-rhamnoside ($p < 0.01$), dihydroquercetin ($p < 0.001$), dihydromyricetin 3-*O*-rhamnoside ($p < 0.001$); as well as flavones, like rhoifolin 4′-*O*-glucoside

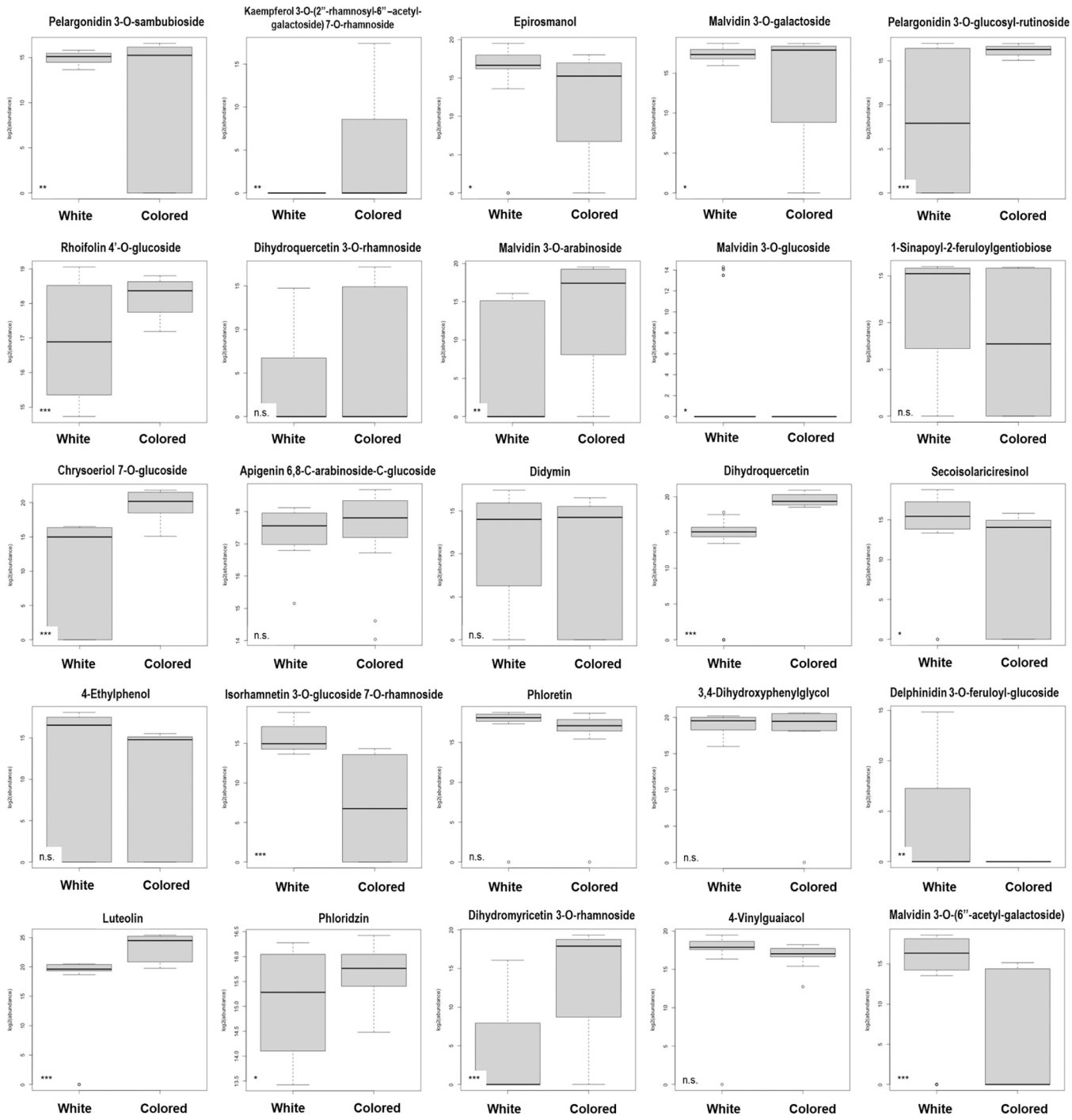

Fig. 3. Box-plot distribution of the log_2-transformed abundance of VIP markers from white-flowered and color-flowered *C. japonica* L. cultivars. White-flowered cultivars included: 'Conde de la Torre' (CT), 'Dr. Tinsley' (DT), 'Grandiflora superba' (GS), and 'Hagoromo' (HA). Color-flowered cultivars included: 'Elegans variegated' (EV), 'Donation dentada' (DD), 'Eugenia de Montijo' (EM), and Carolyn Tuttle (TU). The statistical significance was obtained according to t-test analysis: n.s., not significant; * , significance at $p < 0.05$; * *, significance at $p < 0.01$; * ** , significance at $p < 0.001$.

($p < 0.001$), chrysoeriol 7-*O*-glucoside ($p < 0.001$), and luteolin ($p < 0.001$). Interestingly, the accumulation of flavonols by flowers has been reported in the yellow-flowered *Camellia nitidissima* × *C. japonica* hybrid cultivars, such as 'Yinbai Chalisi' and 'Baifeng' from China (W. Liu et al., 2022), which suggests the contribution of these compounds to the yellowness of color-flowered *C. japonica* cultivars. On the other hand, other discriminant polyphenols were found highly accumulated in white-flowered *C. japonica* cultivars, as observed for epirosmanol ($p < 0.05$) and secoisolariciresinol ($p < 0.05$), indicating that flowers from these cultivars present a specific profile of non-pigment phenolic compounds.

3.3. Semi-quantification of phenolic compounds and biological activity determination of *C. japonica* flower extracts

A semi-quantitative approach to determine the content of the different phenolic subclasses was applied to *C. japonica* flower extracts. The results are shown in Table 1. Among the different subclasses,

Table 1
Semi-quantification of the content of different polyphenol classes on flower extracts of *C. japonica* L. cultivars.

Cultivar	Anthocyanins (CyE, μg/g dw)	Flavanols (CaE, μg/g dw)	Flavones (LE, μg/g dw)	Flavonols (QE, μg/g dw)	Lignans (SE, μg/g dw)	LMW and others (TE, μg/g dw)	Phenolic acids (FE, μg/g dw)	Stilbenes (RE, μg/g dw)
CT	281.9 ± 7.1 d	799.1 ± 81.3 a	42.0 ± 9.7 d	132.9 ± 4.8 d	58.7 ± 0.7 ab	765.6 ± 80.2c	683.8 ± 41.3 ab	11.9 ± 1.3 e
EV	670.1 ± 23.1 b	683.2 ± 48.6 b	55.8 ± 3.1c	1589.7 ± 41.3 a	60.0 ± 1.7 ab	804.3 ± 56.9c	635.6 ± 14.3 bc	17.1 ± 1.4c
DD	89.4 ± 14.4 f	529.4 ± 12.7c	160.5 ± 2.9 a	483.0 ± 16.7c	61.2 ± 3.9 ab	1219.0 ± 48.9 a	714.4 ± 78.1 ab	13.2 ± 1.3 de
DT	197.0 ± 3.5 e	533.0 ± 22.7c	53.5 ± 4.1c	174.9 ± 17.4 d	58.8 ± 0.5 ab	972.3 ± 55.2 b	614.1 ± 34.4 bcd	16.8 ± 1.3 cd
EM	1346.3 ± 45.2 a	843.8 ± 15.3 a	112.1 ± 6.7 b	1048.0 ± 69.8 b	62.4 ± 5.8 ab	880.8 ± 120.5 bc	547.1 ± 50.5 cd	4.4 ± 0.5 f
GS	358.0 ± 19.7c	688.0 ± 116.9 b	52.6 ± 2.1c	134.0 ± 12.6 d	55.1 ± 6.7 ab	796.1 ± 103.4c	683.1 ± 56.0 ab	54.5 ± 0.8 a
HA	205.0 ± 12.3 e	611.5 ± 26.7 bc	54.3 ± 6.5c	129.8 ± 2.4 d	49.2 ± 17.0 b	856.8 ± 20.4 bc	520.2 ± 72.0 d	27.8 ± 2.9 b
TU	660.7 ± 33.4 b	574.0 ± 86.4 bc	108.3 ± 4.0 b	1547.0 ± 81.6 a	67.9 ± 15.4 a	1155.4 ± 120.9 a	758.7 ± 50.4 a	28.8 ± 4.4 b

Results are expressed as equivalents of representative compounds for each polyphenol class (in μg/ g dw) as the mean ± standard deviation (n = 6). The investigated cultivars were 'Conde de la Torre' (CT), 'Elegans variegated' (EV), 'Donation dentada' (DD), 'Dr. Tinsley' (DT), 'Eugenia de Montijo' (EM), 'Grandiflora superba' (GS), 'Hagaromo' (HA), and Carolyn Tuttle (TU). Different letters within the same column indicate statistically significant differences ($\alpha = 0.05$), according to one-way ANOVA and Duncan's *post hoc* test. CyE, cyanidin equivalents; CaE, (+)-catechin equivalents; LE, luteolin equivalents; QE, quercetin equivalents; SE, sesamin equivalents; TE, tyrosol equivalents; FE, ferulic acid equivalents; RE, *trans*-resveratrol equivalents; LMW, low-molecular-weight phenolics.

anthocyanins, flavanols, flavonols, LMW and phenolic acids showed the highest content rates. As expected, the cultivars classified as color-flowered presented the highest rates of anthocyanin content, especially EM, which showed the highest value, 1346.3 ± 45.2 μg/g dw, followed by EV and TU, ~660 – 670 μg/g dw (Table 1). Additionally, these colored cultivars exhibited a differential accumulation of other flavonoids: EM also showed the highest flavanol content 843.8 ± 15.3 μg/g dw; DD presented the highest flavone content, 160.5 ± 2.9 μg/g dw; and EV and TU contained the highest flavonol rates, > 1500 μg/g dw in both cases (Table 1). As previously stated, the differential accumulation of anthocyanins and flavonoid-based co-pigments determine the phenotypical response to flower color (Fan et al., 2023), which is confirmed according to this semi-quantitative approach. Besides flavonoids, the TU cultivar also contained the highest rates of lignans and phenolic acids (67.9 ± 15.4 μg/g dw and 758.7 ± 50.4 μg/g dw, respectively), whereas DD presented the highest content of LMW and other phenolics, 1219.0 ± 48.9 μg/g dw (Table 1). Concerning white-flowered cultivars, they also showed high content of non-pigment polyphenols, as observed for the flavanol content of CT (799.1 ± 81.3 μg/g dw) while GS exhibited the highest rate of stilbenes, 54.5 ± 0.8 μg/g dw. The differential composition of the phenolic subclasses quantified across the different cultivars directly affects the bioactive potential of their derived flower extracts.

A functional analysis was subsequently performed to determine the biological properties of *C. japonica* flowers in terms of *in vitro* antioxidant, cytotoxic, and anti-inflammatory activities (Table 2). A Pearson correlation analysis was also performed to elucidate concealed patterns and relations between the semi-quantified phenolic families and the reported biological activities (Fig. 4). Concerning antioxidant activity, differential results were reported according to the methodology applied

Table 2
Biological activities attributed to flower extracts of different *C. japonica* L. cultivars.

	Antioxidant activity (IC_{50}, μg/mL)			Cytotoxic activity (GI_{50}, μg/mL)						Anti-inflammatory activity (IC_{50}, μg/mL)
Cultivar	**TBARS**	**Reducing power**	**DPPH**	**AGS**	**Caco-2**	**MCF7**	**NCI-H460**	**PLP2**	**Vero**	**RAW264.7**
CT	50.3 ± 0.2 ef	67.3 ± 0.1 d	71.8 ± 0.5 d	61.6 ± 0.5 e	134.7 ± 4.3 e	92.6 ± 0.6 e	140.0 ± 7.9c	141.3 ± 10.4 a	67.0 ± 2.3c	33.2 ± 1.4 b
EV	54.9 ± 0.2 g	86.5 ± 0.1 h	136.5 ± 4.5 f	44.7 ± 1.2c	19.0 ± 0.2 a	75.1 ± 3.0c	113.2 ± 2.6 b	> 400	48.7 ± 2.0 a	> 400
DD	48.8 ± 0.3 de	82.0 ± 0.5 e	54.3 ± 0.2 a	44.2 ± 1.6c	47.1 ± 2.7 b	79.0 ± 3.7c	169.6 ± 5.1 d	> 400	212.7 ± 4.7 f	> 400
DT	51.8 ± 1.9 f	84.8 ± 0.1 g	62.2 ± 0.6 b	79.4 ± 2.1 f	92.9 ± 0.9 d	135.2 ± 1.2 f	201.3 ± 6.7 f	187.2 ± 7.3c	75.7 ± 4.7 de	66.7 ± 0.9 d
EM	35.0 ± 0.1 b	48.7 ± 0.1c	62.6 ± 1.1 b	14.1 ± 0.1 a	13.8 ± 0.6 a	69.6 ± 3.7 b	183.3 ± 5.1 e	174.0 ± 2.4 b	77.7 ± 4.3 e	22.8 ± 0.2 a
GS	48.3 ± 0.1 d	84.2 ± 0.1 f	86.8 ± 0.6 e	51.6 ± 0.5 d	56.9 ± 0.5c	67.8 ± 2.2 b	78.9 ± 1.0 a	135.0 ± 7.5 a	56.4 ± 2.1 b	57.7 ± 0.4c
HA	22.5 ± 0.1 a	42.1 ± 0.2 a	68.7 ± 0.4c	24.5 ± 0.8 b	239.5 ± 7.0 f	85.4 ± 3.6 d	185.8 ± 2.6 e	> 400	69.7 ± 4.5 cd	65.9 ± 1.5 d
TU	39.1 ± 2.4c	44.8 ± 0.1 b	70.2 ± 0.4 cd	63.5 ± 1.8 e	62.4 ± 3.8c	49.4 ± 3.6 a	80.0 ± 5.3 a	> 400	48.0 ± 3.1 a	95.8 ± 1.7 e
C+	-	-	-	1.23 ± 0.03	1.21 ± 0.02	1.02 ± 0.02	1.01 ± 0.01	1.40 ± 0.1	1.41 ± 0.06	6.30 ± 0.4

Results are expressed as inhibitory concentration 50 (IC_{50}, in μg/mL) for antioxidant and anti-inflammatory activity and growth inhibitory concentration 50 (GI_{50}, in μg/mL) for cytotoxic activity. In all cases values are indicated as the mean ± standard deviation (n = 3). The investigated cultivars were 'Conde de la Torre' (CT), 'Elegans variegated' (EV), 'Donation dentada' (DD), 'Dr. Tinsley' (DT), 'Eugenia de Montijo' (EM), 'Grandiflora superba' (GS), 'Hagaromo' (HA), and Carolyn Tuttle (TU). Different letters within the same column indicate statistically significant differences ($\alpha = 0.05$), according to one-way ANOVA and Duncan's *post hoc* test. The highest activity rates correspond to the lowest IC_{50} and GI_{50} values. C+ represent positive controls: ellipticine was used for cytotoxic activity determination and dexamethasone was used for anti-inflammatory activity determination.

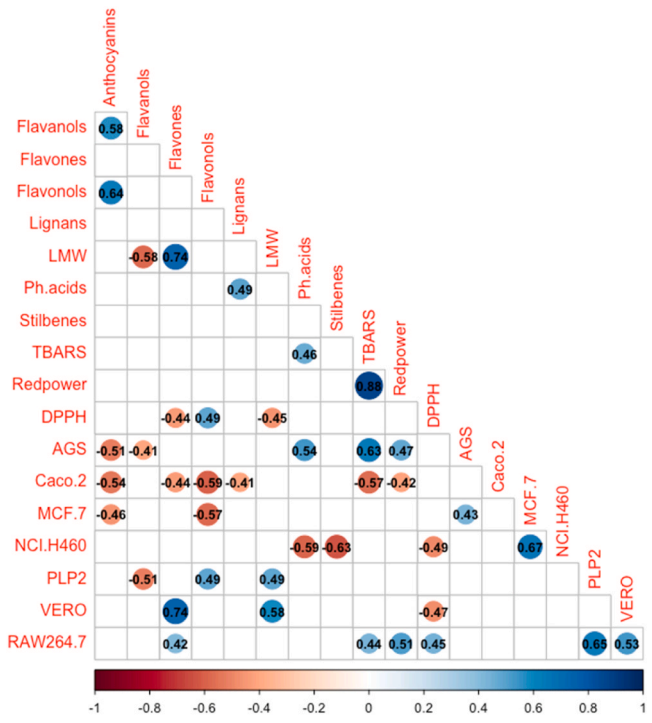

Fig. 4. Pearson correlation heatmap between phenolic compounds content (according to different classes: anthocyanins, flavanols, flavones, flavonols, lignans, phenolic acids, and stilbenes), and biological activities of *C. japonica* L. flower extracts (antioxidant activity, through TBARS, reducing power, and DPPH methods; cytotoxic activity towards AGS, Caco-2, MCF7, NCI-H460, PLP2, and Vero cell lines; and anti-inflammatory activity by the inhibition of NO production by LPS-induced RAW 264.7 macrophages). Only statistically significant coefficients were displayed ($\alpha = 0.01$).

since HA extracts promoted the strongest lipid peroxidation (IC_{50} = 22.5 ± 0.1 μg/mL by TBARS method) and reducing power (IC_{50} = 42.1 ± 0.2 μg/mL by FRAP method), whereas DD extracts exhibited the strongest free-radical scavenging activity (IC_{50} = 54.3 ± 0.2 μg/mL by DPPH method; Table 2). These results highlight the importance of developing several assays to provide a more comprehensive perspective of the antioxidant capacity of plant extracts. Indeed, the antioxidant capacity of *C. japonica* flower extracts from a Korean cultivar was reported to involve a heterogeneous system combining reactive oxygen species scavenging and the induction of the antioxidant enzymatic system, promoting the activity of superoxide dismutase, catalase and glutathione peroxidase (Piao et al., 2011). Although HA extract did not show the highest rates of phenolic compounds, it was found to promote an intense antioxidant activity, which suggests an efficient synergism of polyphenols in terms of lipid peroxidation inhibition and reducing power. Meanwhile, the strongest free-radical scavenging activity (RSA) attributed to DD extracts may be associated with their highest flavone and LMW contents (Table 1), supported by the highly significant correlation among these families and DPPH-derived IC_{50} values ($p < 0.01$; Fig. 4). Among the compounds reported in DD extracts, the flavone apigenin 6-*C*-glucoside and the LMW polyphenol 4-hydroxycoumarin showed the highest abundances (Table S1), being suggested as promoters of the RSA in these extracts. Interestingly, apigenin 6-*C*-glucoside (also known as isovitexin) is largely known for its associated RSA activity, reporting IC_{50} values < 20 μg/mL, determined by the DPPH method of the pure compound (He et al., 2016). As well, 4-hydroxycoumarin and its derivatives have been deeply investigated as potent free-radical scavengers, thanks to their ability to donate their -OH-containing hydrogen atom to counter unpaired electrons *via* single-step hydrogen-atom transfer (HAT) mechanism (Rodríguez and Baumgartner, 2014).

In the case of cell-based assays, a cultivar-dependent cytotoxic and anti-inflammatory activity of *C. japonica* flower extracts was highlighted, especially concerning color-flowered cultivars (Table 2). Thus, potent cytotoxic activity against AGS and Caco-2 cell line was reported by EM extracts (GI_{50} ~14 μg/mL in both cases), as well as the strongest anti-inflammatory activity by inhibiting the NO production by LPS-induced RAW264.7 (IC_{50} = 22.8 ± 0.2 μg/mL in both cases; Table 2), whereas the highest cytotoxicity against MCF7 and Vero cell lines was reported to TU extracts (GI_{50} = 49.4 ± 3.6 μg/mL and 48.0 ± 3.1 μg/mL, respectively). Concerning white-flowered cultivars, GS extracts promoted the strongest cytotoxicity against the NCI-H460 cell line (GI_{50} = 78.9 ± 1.0 μg/mL), as well as the most potent hepatotoxicity, as observed for the PLP2 cell line (GI_{50} = 135.0 ± 7.5 μg/mL; Table 2). Following these results, a significant correlation between anthocyanin content and AGS, Caco-2, and MCF7 cytotoxicity was observed (Pearson's coefficients = −0.51, −0.54, and −0.46, respectively; $p < 0.01$; Fig. 4), indicating the anti-tumor potential of anthocyanin-enriched *C. japonica* cultivars. Cyanidin 3-*O*-glucoside, considered a marker of red and pink-flowered *C. japonica* cultivars, is known to be the main active form of anthocyanin against AGS, as it was found to inhibit their proliferation (Sun et al., 2023). In the same way, Chatthongpisut and co-workers determined that extracts enriched with cyanidin 3-*O*-glucoside and peonidin 3-*O*-glucoside (the latter being the monoglycosylated form of the VIP marker peonidin 3-*O*-glucosyl-rutinoside associated with color-flowered cultivars, Fig. 3) promoted an efficient inhibition of Caco-2 proliferation, with GI_{50} rates similar to those reported for EM extracts, 16.1 μg/mL (Chatthongpisut et al., 2015). Besides anthocyanins, other *C. japonica* flower polyphenols were found to be highly correlated with Caco-2 toxicity, i.e.: flavones, flavonols, and lignans (Pearson's coefficients = −0.44, −0.59, and −0.41, respectively; $p < 0.01$; Fig. 4), suggesting that *C. japonica* flower extracts show an intense anti-tumor activity towards this human colorectal adenocarcinoma cell line, probably motivated by the collective action of these subclasses. Furthermore, anthocyanins showed a high correlation with MCF7 toxicity (−0.46; $p < 0.01$; Fig. 4), apparently caused by the highest cytotoxic activity reported by TU extracts (Table 2), supporting the hypothesis that anthocyanin-enriched *C. japonica* flowers can be recognized as promising sources of diversified natural anti-tumor agents.

In parallel, phenolic acids and stilbenes were also significantly correlated with the cytotoxicity against the NCI-H460 cell line, suggesting that the white-flowered GS cultivar could be responsible for the reported effect, as it promoted the highest stilbene content rates together with the lowest GI_{50} values against this cell line (Tables 1 and 2). Although, to the best of our knowledge, little is known about the presence of stilbenes in *C. japonica* extracts, the NCI-H460 cytotoxicity attributed to stilbenes was already described in previous reports, as *trans*-resveratrol (reported in *C. japonica* extracts; Table S1) was found to induce apoptotic events combined with the activation of pro-oxidant responses in this cell line (Khayat et al., 2022). Equally, viniferin, present in *C. japonica* flower extracts (Table S1), was found to exert potent cytotoxicity towards NCI-H460 cell line by caspase-mediated apoptosis induction, whose effects were also reported *in vivo* (Huang et al., 2023). In general, it is noteworthy that all correlations described between phenolic subclasses and *in vitro* biological activities were spotted as statistically significant ($p < 0.01$), and moderate Pearson's correlation coefficients were reported. This observation suggests that phenolics from *C. japonica* flower extracts are involved in the biological activities analyzed, although there are several factors that also contribute to the reported responses. Therefore, it can be hypothesized that a deep interaction occurs between the different phenolic compounds identified in *C. japonica* flower extracts, as well as other families of bioactive compounds. Thus, further studies should be directed in this way, in order to elucidate specific relationships between *C. japonica* phytoconstituents and the related bioactivities reported in this study.

4. Conclusions

The combination of untargeted metabolomics and *in vitro* functional analysis was found to effectively unravel the potential of *Camellia japonica* L. flowers as natural sources of polyphenols with associated antioxidant, cytotoxic and anti-inflammatory properties facing their industrial exploitation. A broad diversity of anthocyanins, mostly represented by cyanidin 3-*O*-glycosides, and other flavonoids, essentially flavones and flavonols, were abundantly reported in flower extracts. Furthermore, a vast array of other polyphenols, such as alkylphenols, hydroxycinnamic acids, lignans, and stilbenes were also reported for the first in these matrices. The application of multivariate statistics allowed deciphering intraspecific variations among color-flowered and white-flowered cultivars, involving not only differences at the phytoconstituents level but also in the functional outcome of extracts, revealing unique chemical fingerprints associated with color-flowered cultivars. Anthocyanins were discriminately identified as markers of pink-flowered cultivars, displaying antitumoral and anti-inflammatory potential against different cancer cell lines, namely AGS gastric adenocarcinoma, Caco-2 colorectal adenocarcinoma, and MCF7 breast adenocarcinoma cell lines. Together with anthocyanins, flavones, and flavonols were found to secondarily assist in the discrimination of *C. japonica* cultivars and their bioactive functionality, as they promoted an efficient free-radical scavenging activity, and their accumulation was correlated with the cytotoxicity towards Caco-2 cell line. Overall, anthocyanin-enriched flower extracts from *C. japonica* constitute an underexplored horticultural resource representing a promising source of polyphenols with associated biological activities of high interest for food, cosmetic and pharmaceutical industries. Future studies should be focused on the elucidation of synergistic and/or antagonistic effects among *C. japonica* constituents to better understand the bioactivities reported here for flower extracts, thus paving the road to their industrial exploitation.

CRediT authorship contribution statement

A.G. Pereira: Conceptualization, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **L. Cassani**: Validation, Formal analysis, Investigation, Writing – review & editing. **T. Oludemi**: Methodology, Software, Validation, Formal analysis, Writing – review & editing. **F. Chamorro**: Validation, Formal analysis, Investigation, Writing – review & editing. **R. C. Calhelha**: Methodology, Software, Validation, Formal analysis, Writing – review & editing. **L. Barros:** Validation, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. **M. A. Prieto**: Conceptualization, Validation, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. **J. Simal-Gandara**: Conceptualization, Validation, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. **L. Lucini**: Validation, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. **P. Garcia-Perez**: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2023.117389.

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