

Article

Investigation of Selected Compounds in Nectar and the Antioxidant, Amylase and Glucosidase Inhibitory Capabilities of Canna Flower Nectar (*Canna × generalis*)

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Abstract

Floral nectar is not only an important nutritional source for pollinators but also contains numerous biologically valuable compounds. This study evaluated the nectar characteristics, phytochemical composition, and biological potential of four *Canna × generalis* cultivars (CG-1, CG-2, CG-3, and CG-4) at different times throughout the day. Nectar volume and soluble solids content (°Brix) were measured. Phytochemical constituents were screened using spectrophotometric methods, while total phenolic, flavonoid, and alkaloid contents were determined using the Folin–Ciocalteu, aluminum chloride, and bromocresol green methods, respectively. Antioxidant activity was evaluated using DPPH, ABTS•⁺, and FRAP assays. In addition, α-amylase and α-glucosidase inhibitory activities and *in vitro* cytotoxicity against human liver cancer cells (HepG2) and breast cancer cells (MCF-7) of the flower nectar were also determined. The results showed that floral nectar volume ranged from 40.76 to 92.39 µL per flower and reached the highest value in the morning, whereas soluble solids content ranged from 18.76 to 30.81 °Brix and gradually increased from midday to afternoon. Phytochemical analyses revealed the presence of various groups of secondary metabolites, including polyphenols, flavonoids, tannins, coumarins, carotenoids, saponins, quinones, and terpenoids. The nectar collected in the morning from the CG-4 cultivar exhibited the highest phenolic content (12.02 ± 0.06 µg GAE/mL) and alkaloid content (2.66 ± 0.00 µg AE/mL), while the highest flavonoid content was recorded in the nectar of CG-4 recorded in the afternoon (8.57 ± 0.09 µg QE/mL). All samples exhibited antioxidant activity and inhibitory activity against α-amylase and α-glucosidase. The nectar of CG-2 collected in the afternoon showed the strongest DPPH radical scavenging activity (IC₅₀ = 19.60 ± 0.09 µg/mL), whereas CG-2N and CG-3M exhibited the strongest ABTS•⁺ radical scavenging activity, with no significant difference between them (CG-2N: IC₅₀ = 13.04 ± 0.08 µg/mL; CG-3M: 13.91 ± 0.30 µg/mL). The nectar of CG-2M at morning showed the strongest α-glucosidase inhibitory activity (IC₅₀ = 0.11 ± 0.00 µg/mL). In addition, CG-4M and CG-4A exhibited the strongest ferric reducing activity, which exceeded the linear range of the assay at the 50% concentration, while CG-4M showed the strongest α-amylase inhibitory activity (IC₅₀ = 78.49 ± 0.14 µg/mL). CG-2 showed no significant cytotoxicity against HepG2 and MCF-7 cells (IC₅₀ > 128 µg/mL). Overall, these findings warrant further investigation of *Canna × generalis* floral nectar as a potential source of bioactive compounds with *in vitro* antioxidant and digestive-enzyme inhibitory activities.



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Keywords: floral nectar; phenolic; flavonoid; alkaloid; antioxidant activity; α -amylase; α -glucosidase; HepG2; MCF-7

1. Introduction

Canna × generalis is a group of ornamental hybrid plants belonging to the genus *Canna*, the only genus in the family Cannaceae [1]. These plants are widely cultivated because of their brightly colored flowers and their good adaptability to various environmental conditions. They are mainly used as ornamental plants and also have certain values in food and medicinal applications [2]. In addition, *Canna* flowers produce a relatively large amount of nectar, indicating their *in vitro* antioxidant and digestive-enzyme inhibitory activities and supporting further investigation of their potential functional properties.

Floral nectar is a secretion produced by floral nectaries, playing an important role in attracting pollinators and maintaining interactions between plants and pollinating organisms [3–6]. In addition to carbohydrates, floral nectar contains various secondary metabolites, such as phenolics, flavonoids, and alkaloids [7–10]. These compounds not only participate in the ecological functions of flowers but also exhibit a wide range of important biological activities [11,12].

Phenolics and flavonoids in floral nectar are known as natural antioxidants that can neutralize reactive oxygen species (ROS), thereby contributing to the reduction in cellular damage associated with many chronic diseases [13,14]. In addition, bioactive compounds have also been reported to inhibit α -amylase and α -glucosidase, two enzymes that play important roles in carbohydrate digestion and glucose absorption [15,16]. Therefore, the search for natural sources rich in bioactive compounds has attracted increasing attention in the fields of functional foods and blood glucose management.

Although many studies have investigated the chemical composition and biological activities of different parts of plants belonging to the genus *Canna*, information on floral nectar remains very limited. In particular, data regarding the chemical composition, antioxidant activity, and enzyme inhibitory activity of *Canna × generalis* floral nectar have not yet been fully elucidated. In addition to antioxidant and digestive-enzyme inhibitory activities, a preliminary *in vitro* cytotoxicity assessment was conducted to provide complementary information on the biological activity of the floral nectar. HepG2 and MCF-7 cell lines were selected as model systems for an initial evaluation of cell-growth inhibitory effects under the experimental conditions used. Therefore, this study was conducted to determine the contents of the major bioactive compounds and to evaluate the antioxidant activity as well as the α -amylase and α -glucosidase inhibitory activities of floral nectar collected from four *Canna × generalis* cultivars.

2. Materials and Methods

2.1. Materials

This study used floral nectar samples collected from four *Canna × generalis* cultivars grown in Lai Vung district, Dong Thap province, Vietnam. The cultivars were designated as CG-1, CG-2, CG-3, and CG-4, in which “CG” stands for *Canna × generalis* and the numbers represent different cultivars (Figure 1). Floral nectar from each cultivar was collected at three different times of the day: morning (M), noon (N), and afternoon (A), resulting in 12 sample groups coded CG-1M to CG-4A. Sample codes were assigned according to the cultivar and collection time, where the number (1–4) identifies the cultivar and the letters M, N, and A represent morning, noon, and afternoon, respectively. Thus, CG-1M, CG-1N,

and CG-1A represent nectar samples collected from cultivar CG-1 in the morning, noon, and afternoon, respectively.

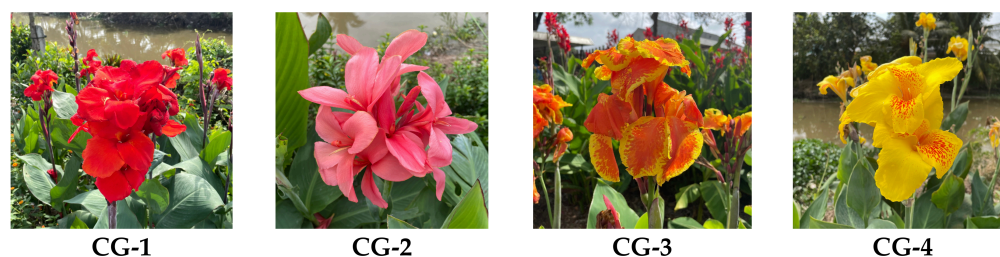


Figure 1. Four *Canna × generalis* cultivars used in this study.

2.1.1. Sample Collection

Floral nectar was collected from flowers at the fully open stage (Figure 2) on sunny days at three sampling times: morning (07:30–08:30), noon (11:30–12:30), and afternoon (16:30–17:30). Three healthy clumps of each cultivar were used as independent biological replicates. At each sampling time, nectar was collected from five flowers per clump over seven consecutive days. The five flowers were treated as subsamples within each clump rather than independent biological replicates. Measurements obtained across the five flowers and seven sampling days were averaged within each clump for each sampling time, and the resulting clump-level values were used for statistical analysis ($n = 3$ biological replicates per cultivar $\times$ sampling time). The total volume of nectar collected from each clump was at least 10 mL.

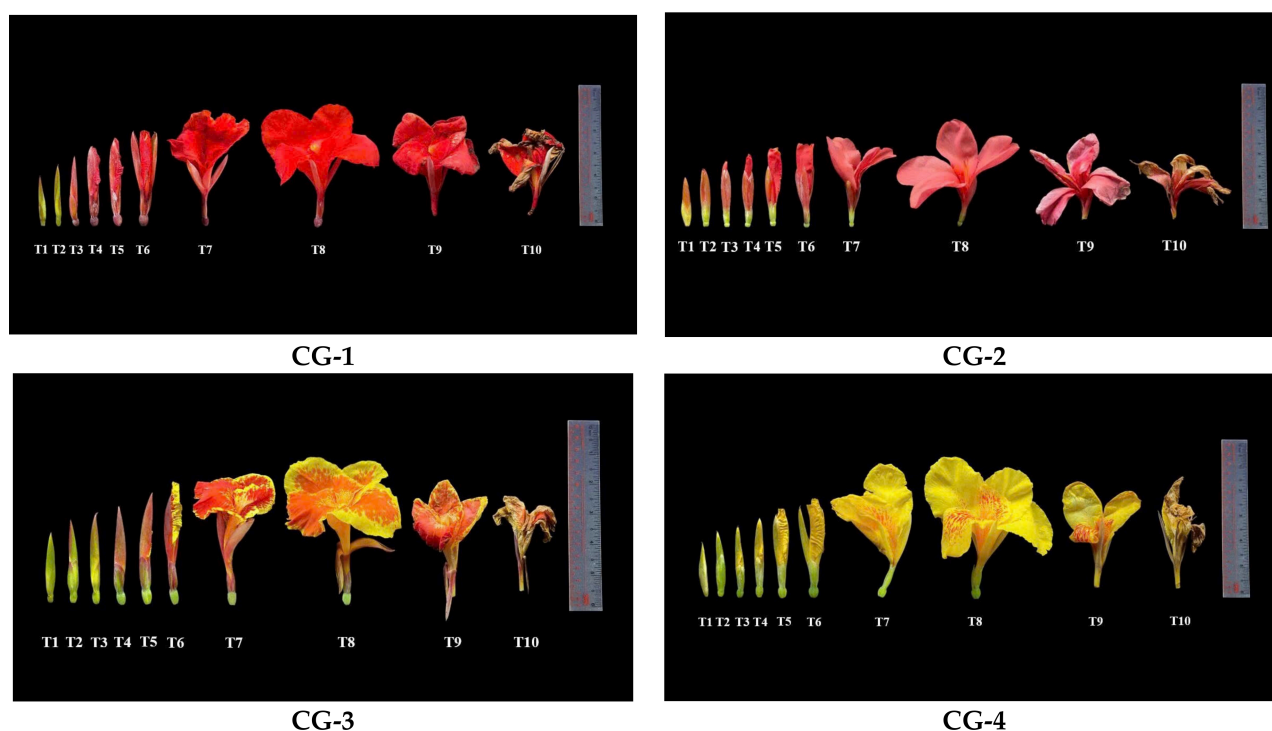


Figure 2. Flower developmental stages of the four *Canna × generalis* cultivars (T1–T10), from young flower buds to fully opened flowers. Stage T7 marks the appearance of floral nectar, whereas stage T8 corresponds to the fully opened flower stage selected for nectar collection.

The samples were transported to the laboratory under refrigerated conditions within 12 h after collection. Before storage at $-20\text{ }^{\circ}\text{C}$, each nectar sample was aliquoted into micro-centrifuge tubes according to the intended analyses to avoid repeated freeze–thaw cycles

and preserve the integrity of bioactive compounds. In this study, one biological replicate was defined as floral nectar collected from one plant clump at a specific sampling time.

For the bioassays, the original liquid floral nectar was used directly without drying or lyophilization. Briefly, 1 mg of freshly collected liquid nectar was accurately weighed and diluted to 1 mL with the appropriate assay solvent to prepare a stock solution equivalent to 1000 µg/mL of original liquid nectar. The stock solution was subsequently diluted with the corresponding assay solvent to obtain the required test concentrations. For the DPPH and ABTS•⁺ assays, the final concentrations were 5, 10, 20, 40, 80, and 160 µg/mL. Thus, the concentrations expressed in µg/mL represent the mass-equivalent concentration of the original liquid floral nectar in the assay solution and are not based on dry matter, lyophilized residue, or soluble-solids content.

2.1.2. Determination of Floral Nectar Volume at Different Developmental Stages

Floral nectar volume was determined at each developmental stage using a micropipette. Five flowers were used for nectar volume measurement at each stage.

The nectar volume of each flower was recorded individually, and the average nectar volume was calculated using the following equation:

$$V_{mean} = \frac{V_{total}}{n}$$

in which:

V_{mean} is the average nectar volume per flower (µL); V_{total} is the total nectar volume collected from flowers at the same developmental stage (µL); and n is the number of flowers examined.

2.2. Chemicals and Reagents

Methanol, chloroform, distilled water, and other necessary chemicals and reagents were used in this study. The main chemicals included BCG, DPPH, ABTS, gallic acid, quercetin, atropine, acarbose, α-amylase, α-glucosidase, pNPG, MTT, DMSO, ellipticine, and other chemicals.

2.3. Experimental Methodology

2.3.1. Determination of Nectar Volume and °Brix in Four *Canna × generalis* Cultivars

Floral nectar volume (µL) and soluble solids content (°Brix) were determined from fully opened *Canna × generalis* flowers. Floral nectar was collected at three time points during the day (morning, noon, and afternoon) over seven consecutive days. Nectar volume was measured using a micropipette, whereas soluble solids content was determined using a calibrated handheld refractometer. Each measurement was performed on five flowers at each sampling time, and the results were expressed as the mean values obtained over the seven-day sampling period. No pollinator-exclusion or flower-bagging treatment was applied during nectar collection.

2.3.2. Determination of Phytochemical Constituents in Floral Nectar

Qualitative Phytochemical Analysis of Floral Nectar

The phytochemical constituents of *Canna × generalis* floral nectar were subjected to a preliminary spectrophotometric screening, in which the absorbance of each sample was measured at the characteristic absorption wavelength of each compound class (Table 1), adapted from Harborne [17]. The sample diluent was used as the blank. Because absorbance at a single wavelength is not specific to a single compound class, this screening is semi-quantitative and indicative only, and definitive identification requires chromatographic and spectroscopic confirmation.

Table 1. Characteristic absorption wavelengths used for the spectrophotometric screening of phytochemical compounds.

Compound	Absorption Wavelengths (nm)	Reference
Polyphenol	215	[18]
Coumarin	255	[18]
Tannin	265	[19]
Flavonoid	300	[18]
Carotenoid	450	[18]
Saponin	545	[20]
Alkaloid	550	[18]
Quinone	260	[18]
Terpenoid	230	[21]

The presence of phytochemical compounds was determined based on the blank-corrected absorbance (*A*) values (Table 1), categorized as follows:

$A < 0.1$: very low

$0.1 \leq A \leq 0.5$: Low (+)

$0.5 < A \leq 1.0$: High (++)

$A > 1.0$: Very high (+++)

Quantitative Determination of Phytochemical Compounds in Floral Nectar

Total phenolic content (TPC). TPC was determined using the Folin–Ciocalteu colorimetric method according to Yadav and Agarwala [22], with modifications for floral nectar. Briefly, 1.0 mL of floral nectar sample or gallic acid standard was mixed with 2.5 mL of 10% (*v/v*) Folin–Ciocalteu reagent and allowed to react for 5 min at room temperature. Subsequently, 2.0 mL of 2% (*w/v*) Na_2CO_3 was added, and the mixture was incubated for 45 min at room temperature. The absorbance was measured at 765 nm against a reagent blank. Gallic acid was used as the calibration standard over the concentration range of 1–10 $\mu\text{g/mL}$. TPC was calculated from the linear regression equation of the gallic acid calibration curve and expressed as μg gallic acid equivalents (GAE) per mL of original floral nectar ($\mu\text{g GAE/mL}$). The calibration curve for gallic acid was $y = 0.01605x - 0.0195$ ($R^2 = 0.9936$), where *y* is absorbance at 765 nm and *x* is gallic acid concentration ($\mu\text{g/mL}$). **Total flavonoid content (TFC).** TFC was determined using the aluminum chloride colorimetric method [23], with modifications. Briefly, 1.0 mL of floral nectar sample was diluted ten times with distilled water, followed by addition of 0.30 mL of 5% (*w/v*) NaNO_2 . After 5 min at room temperature, 2% (*w/w*) AlCl_3 was added. After a further 6 min, 2.0 mL of 1.0 M NaOH was added. The absorbance was measured at 430 nm against an appropriate reagent blank. Quercetin was used as the calibration standard, and TFC was expressed as μg quercetin equivalents (QE) per mL of original floral nectar. The quercetin calibration curve was $y = 0.0071167x + 0.0015$ ($R^2 = 0.9998$), where *y* is absorbance at 430 nm and *x* is quercetin concentration ($\mu\text{g/mL}$).

Total alkaloid content (TAC). TAC was determined using the bromocresol green (BCG) spectrophotometric method according to Prajapati et al. [24], with modifications. Briefly, 1000 μL of floral nectar sample or atropine standard was reacted with 1000 μL of BCG reagent under acidic conditions at pH 4.7 to form a yellow ion-pair complex. The complex was extracted with 2 mL chloroform, and the absorbance of the chloroform phase was measured at 415 nm against the corresponding blank. Atropine was used as the calibration standard over the range of 4–13 $\mu\text{g/mL}$ in chloroform. TAC was calculated from the atropine calibration curve and expressed as μg atropine equivalents (AE) per mL of original

floral nectar. The atropine calibration curve was $y = 0.02823x + 0.00173$ ($R^2 = 0.9855$), where y is absorbance at 415 nm and x is atropine concentration ($\mu\text{g/mL}$)

2.3.3. Evaluation of Antioxidant Activity of Floral Nectar

DPPH radical-scavenging assay. DPPH radical-scavenging activity was evaluated according to Ghasemzadeh et al. [25], with modifications. Briefly, 2.5 mL of floral nectar at different concentrations (5, 10, 20, 40, 80, 160 $\mu\text{g/mL}$) was mixed with 1.0 mL of 0.3 mM DPPH solution. The reaction mixtures were incubated in the dark at room temperature for 30 min, and absorbance was measured at 518 nm. A sample blank containing the corresponding volume of floral nectar and ethanol without DPPH was used to correct for sample background, while a DPPH control containing DPPH solution and ethanol was used to represent the maximum radical absorbance. BHT were used as positive antioxidant controls. Radical-scavenging activity was calculated as:

$$\text{DPPH inhibition (\%)} = [1 - (A_s - A_{sb})/A_c] \times 100$$

where A_s is the absorbance of the sample with DPPH, A_{sb} is the absorbance of the sample blank without DPPH, and A_c is the absorbance of the DPPH control. The IC_{50} value was estimated from the concentration–response curve as the concentration of floral nectar required to achieve 50% DPPH radical scavenging. **ABTS $\bullet^+$ radical-scavenging assay.** ABTS $\bullet^+$ radical-scavenging activity was determined according to Re et al. [26], with modifications. ABTS was prepared at 7 mM, and ABTS $\bullet^+$ was generated by reacting ABTS with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12–16 h. Before use, the ABTS $\bullet^+$ solution was diluted with the appropriate solvent to obtain an absorbance of 0.700 ± 0.020 at 734 nm and equilibrated at 30 °C. 10 μL of floral nectar at different concentrations (5, 10, 20, 40, 80, 160 $\mu\text{g/mL}$) was mixed with 1 mL of diluted ABTS $\bullet^+$ solution. The absorbance was recorded at 734 nm after 30 min. Appropriate solvent and sample blanks were included. Trolox was used as the reference antioxidant control. The percentage of ABTS $\bullet^+$ inhibition was calculated as:

$$\text{ABTS inhibition (\%)} = [1 - (A_s - A_{sb})/A_c] \times 100$$

where A_s is the absorbance of the sample with ABTS $\bullet^+$, A_{sb} is the absorbance of the sample blank, and A_c is the absorbance of the ABTS $\bullet^+$ control. The IC_{50} was estimated from the concentration–response curve as the concentration of floral nectar required to inhibit 50% of ABTS $\bullet^+$ absorbance.

FRAP assay. Ferric reducing antioxidant power was determined according to Ferreira et al. [27], with modifications. The FRAP reagent was freshly prepared by mixing 300 mM acetate buffer, 10 mM TPTZ prepared in 40 mM HCl, and 20 mM FeCl_3 in a 10:1:1 ($v/v/v$) ratio. 150 μL of floral nectar at different concentrations was mixed with 2.85 mL of FRAP reagent and incubated in the dark for 30 min at room temperature. Absorbance was measured at 593 nm. A reagent blank was prepared using the solvent in place of the nectar sample. Gallic acid was used to construct the calibration curve, and the reducing capacity was calculated from the corresponding regression equation and expressed as $\mu\text{g GAE/mL}$ of original floral nectar.

2.3.4. Evaluation of α -Amylase and α -Glucosidase Inhibitory Activities of Floral Nectar

α -Amylase inhibitory activity. α -Amylase inhibitory activity was determined according to Telagari and Hullatti [28,29], with modifications. Briefly, 20 μL of floral nectar at different concentrations was mixed with 50 μL of 100 mM sodium phosphate buffer (pH 6.8) and 10 μL of α -amylase (2 U/mL) and pre-incubated at 37 °C for 20 min. Subsequently,

20 µL of 1% (*w/v*) soluble starch prepared in sodium phosphate buffer was added, and the reaction mixture was incubated at 37 °C for 30 min. The reaction was terminated by addition of 100 µL DNS reagent, followed by heating at 95 °C for 10 min. After cooling, the absorbance was measured at 540 nm. A negative control was prepared using the assay mixture without floral nectar, and a reagent blank without enzyme was included to correct for background absorbance. Acarbose was used as the positive control. Enzyme inhibition was calculated as:

$$\text{Inhibition (\%)} = [1 - (A_s - A_{sb}) / (A_c - A_{cb})] \times 100$$

where A_s is the absorbance of the sample reaction, A_{sb} is the sample blank, A_c is the control reaction, and A_{cb} is the control blank. The IC_{50} was estimated from the concentration–response curve as the concentration of floral nectar required to inhibit 50% of α -amylase activity.

α -Glucosidase inhibitory activity. α -Glucosidase inhibitory activity was determined according to Telagari and Hullatti [29], with modifications. Briefly, 20 µL of floral nectar at different concentrations was mixed with 50 µL of 100 mM sodium phosphate buffer (pH 6.8) and 10 µL of α -glucosidase (1 U/mL) and pre-incubated at 37 °C for 15 min. Subsequently, 20 µL of 5 mM p-nitrophenyl- α -D-glucopyranoside (pNPG) was added as substrate, and the reaction mixture was incubated at 37 °C for 20 min. The reaction was terminated by adding 50 µL of 0.1 M Na_2CO_3 , and the absorbance of the released p-nitrophenol was measured at 405 nm. A negative control containing the enzyme without floral nectar and a reagent blank without enzyme were included. Acarbose was used as the positive control. Enzyme inhibition was calculated as:

$$\text{Inhibition (\%)} = [1 - (A_s - A_{sb}) / (A_c - A_{cb})] \times 100$$

where A_s is the absorbance of the sample reaction, A_{sb} is the sample blank, A_c is the control reaction, and A_{cb} is the control blank. The IC_{50} was estimated from the concentration–response curve as the concentration of floral nectar required to inhibit 50% of α -glucosidase activity.

2.3.5. Evaluation of the Cytotoxic Activity of Floral Nectar Against HepG2 and MCF-7 Cell Lines

The cytotoxic activity of floral nectar was evaluated against human liver cancer cells (HepG2) and breast cancer cells (MCF-7) using the MTT assay [30]. Cell growth inhibition was expressed as IC_{50} values.

2.4. Statistical Methods and Data Processing

All measurements were performed in triplicate, and the results were expressed as mean $\pm$ standard deviation (mean $\pm$ SD, $n = 3$). Differences among samples were evaluated by two-way analysis of variance (ANOVA; cultivar $\times$ sampling time) followed by Tukey's honestly significant difference (HSD) test at a significance level of $p < 0.05$ using SPSS 21 software; different letters in the figures denote significant differences. Relationships among the phytochemical contents and biological activities were further examined by Pearson correlation analysis, principal component analysis (PCA), and hierarchical clustering (Ward's method) using Python (SciPy, scikit-learn, and Matplotlib). Before PCA, variables were standardized to zero mean and unit variance using z-score standardization. PCA was performed as an exploratory analysis because of the limited number of sample-level observations. Values that fell outside the working range of an assay were reported as not detected (<LOD), below the lowest tested concentration (<LCL), or above the upper limit of

quantification ($>LOQ$), and were excluded from the corresponding statistical analyses. The LOD and LOQ were calculated as $3.3\sigma/S$ and $10\sigma/S$, respectively, where σ is the standard deviation of the response and S is the slope of the calibration curve. Censored observations were not imputed. Graphs were generated using Microsoft Excel and Python.

3. Results

3.1. Nectar Volume and Soluble Solids Content of Four *Canna × generalis* Cultivars

The nectar volume and °Brix of *Canna × generalis* flowers at the fully open stage are presented in Figures 3 and 4.

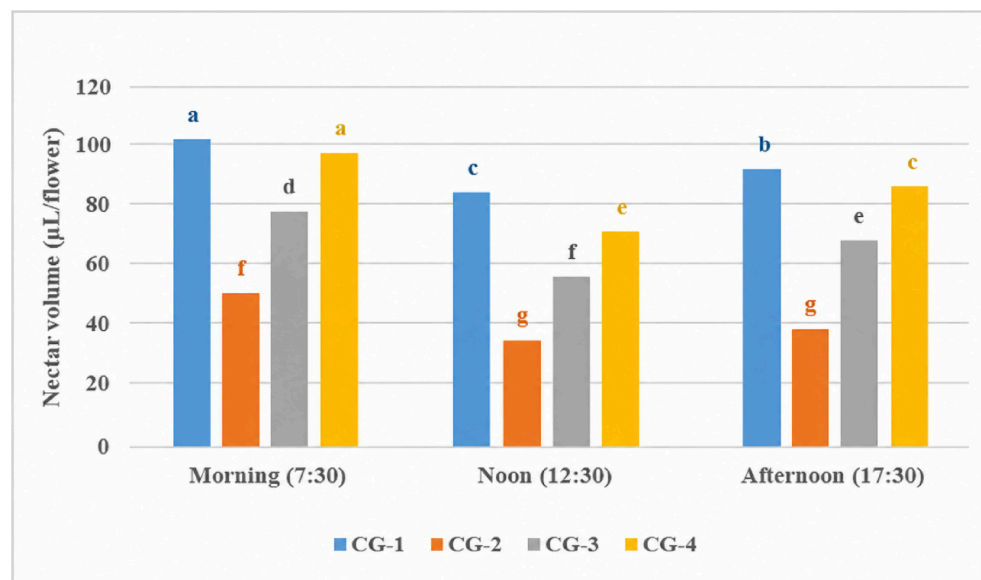


Figure 3. Nectar volume ($\mu\text{L}/\text{flower}$) of *Canna × generalis* flowers at the fully open stage. Columns with the same letter on the top are not significantly different.

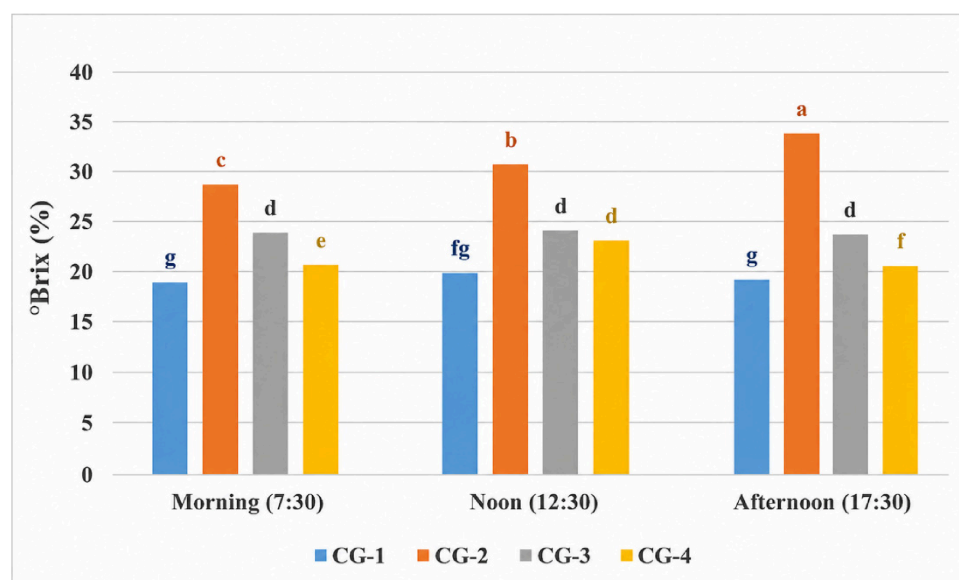


Figure 4. °Brix (%) of *Canna × generalis* flowers at the fully open stage. Values are presented as the mean of three replicates. Different letters within the same column indicate significant differences ($p < 0.05$).

Nectar volume and soluble solids content differed significantly among cultivars, sampling times, and the interaction between these two factors ($p < 0.05$). The mean nectar

volume ranged from 40.76 to 92.39 $\mu\text{L}/\text{flower}$ and was highest in CG-1. Nectar volume showed a decreasing trend from morning to noon. In contrast, the mean soluble solids content ranged from 18.76 to 30.81%, with the highest value observed in CG-2, and showed an increasing trend from morning to noon before remaining stable in the afternoon. The highest individual values recorded were 101.40 $\mu\text{L}/\text{flower}$ (CG-1, morning) for nectar volume and 33.85% (CG-2, afternoon) for $^{\circ}\text{Brix}$.

3.2. Qualitative Analysis of Phytochemical Constituents in Floral Nectar

The preliminary spectrophotometric screening indicated that *Canna* $\times$ *generalis* floral nectar gave absorbance signals consistent with the presence of various groups of secondary metabolites, including phenolics, coumarins, tannins, flavonoids, carotenoids, quinones, and terpenoids, in every sample examined (Table 2). On the basis of relative absorbance intensity, flavonoids and terpenoids gave the strongest signals. As this screening relies on non-specific single-wavelength absorbance, these assignments are indicative rather than confirmatory.

Table 2. Preliminary spectrophotometric screening of phytochemical constituents in *Canna* $\times$ *generalis* floral nectar.

Sample	Presence of Compounds								
	Phenolic	Coumarin	Tannin	Flavonoid	Carotenoid	Saponin	Alkaloid	Quinone	Terpenoid
CG-1M	+	+	+	++	+	+	+	+	+++
CG-1N	+	+	+	++	+	—	—	+	+++
CG-1A	+	+	+	+++	+	+	+	+	+++
CG-2M	+	++	++	+++	+	+	+	+	+++
CG-2N	+	+	+	+++	+	+	+	+	+++
CG-2A	+	+	+	+++	+	—	—	+	+++
CG-3M	+	+	+	+++	+	—	—	+	+++
CG-3N	+	+	+	++	+	—	—	+	+++
CG-3A	+	+	+	+++	+	+	+	+	+++
CG-4M	+	+	+	++	+	+	+	+	+++
CG-4N	+	+	+	++	+	+	+	+	+++
CG-4A	+	+	+	+++	+	+	+	+	+++

Note: —: very low; +: low; ++: high; +++: very high. Scoring reflects the relative absorbance intensity at the characteristic wavelength (Table 1) and is indicative, not a specific compound identification.

3.3. Quantitative Determination of Phytochemical Constituents in Floral Nectar

Standard calibration curves of gallic acid, quercetin, and atropine were established to quantify the total phenolic, total flavonoid, and total alkaloid contents in the floral nectar of the four *Canna* $\times$ *generalis* cultivars (Figure 5). The contents of these compounds were expressed as $\mu\text{g GAE}/\text{mL}$, $\mu\text{g QE}/\text{mL}$, and $\mu\text{g AE}/\text{mL}$, respectively.

Based on these regression equations, the contents of the compounds in each sample were calculated and are presented in Figure 6.

The total phenolic content (TPC), total flavonoid content (TFC), and total alkaloid content (TAC) differed significantly among the floral nectar samples ($p < 0.05$). TPC, TFC, and TAC ranged from 3.03 to 12.02 $\mu\text{g GAE}/\text{mL}$, 2.56 to 8.57 $\mu\text{g QE}/\text{mL}$, and not detected to 2.66 $\mu\text{g AE}/\text{mL}$, respectively. The highest TPC and TAC were recorded in CG-4M (12.02 $\pm$ 0.06 $\mu\text{g GAE}/\text{mL}$ and 2.66 $\pm$ 0.00 $\mu\text{g AE}/\text{mL}$, respectively), whereas the highest TFC was observed in CG-4A (8.57 $\pm$ 0.09 $\mu\text{g QE}/\text{mL}$). In contrast, the lowest TPC, TFC, and TAC were found in CG-1N (3.03 $\pm$ 0.03 $\mu\text{g GAE}/\text{mL}$), CG-3N (2.56 $\pm$ 0.00 $\mu\text{g QE}/\text{mL}$), and CG-1N (not detected, <LOD), respectively.

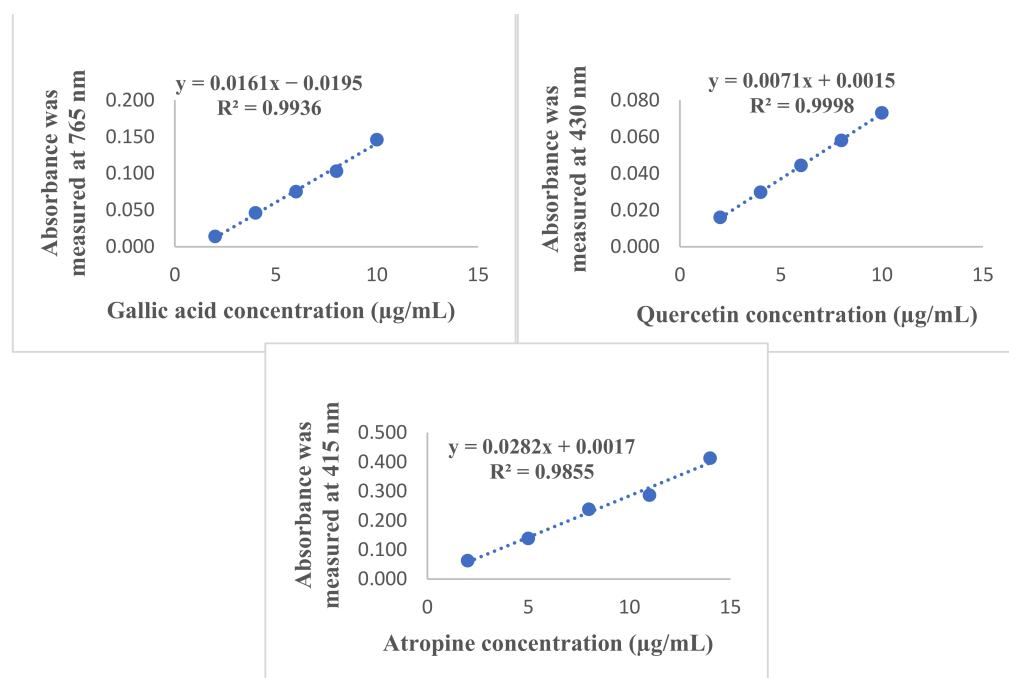


Figure 5. Standard calibration curves of gallic acid, quercetin, and atropine for the determination of total phenolic, total flavonoid, and total alkaloid contents. Absorbance was measured at 765 nm for total phenolics, 430 nm for total flavonoids, and 415 nm for total alkaloids. Values represent the mean of three replicates after blank correction.

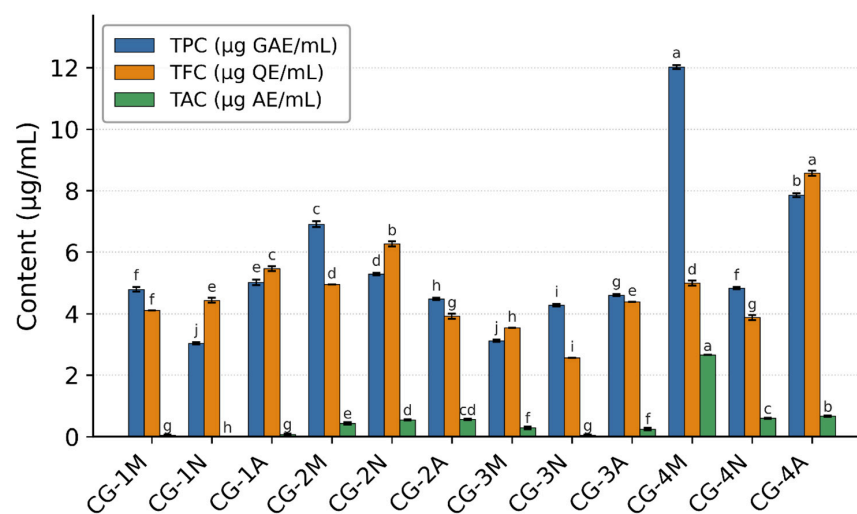


Figure 6. Total phenolic (TPC, µg GAE/mL), flavonoid (TFC, µg QE/mL) and alkaloid (TAC, µg AE/mL) contents of *Canna × generalis* floral nectar samples (mean $\pm$ SD, $n = 3$). The alkaloid content of CG-1N was below the limit of detection. Different letters within the same parameter indicate significant differences ($p < 0.05$).

3.4. Antioxidant Activity

The antioxidant activities of the floral nectar samples, evaluated by DPPH radical scavenging activity, ABTS $\bullet^+$ radical scavenging activity, and ferric reducing capacity, are presented in Figures 7 and 8.

The IC_{50} values showed that the antioxidant activities of the floral nectar samples differed significantly ($p < 0.05$). Sample CG-2A exhibited the strongest DPPH radical scavenging activity with the lowest IC_{50} value (19.60 ± 0.09 µg/mL) and also showed a relatively low ABTS $\bullet^+$ IC_{50} value (22.10 ± 0.11 µg/mL). In the ABTS $\bullet^+$ assay, CG-3M

and CG-2N exhibited the lowest IC_{50} values, with no significant difference between them ($p > 0.05$). Overall, CG-2A exhibited outstanding antioxidant activity due to its high and relatively consistent free radical scavenging capacity in both assay systems.

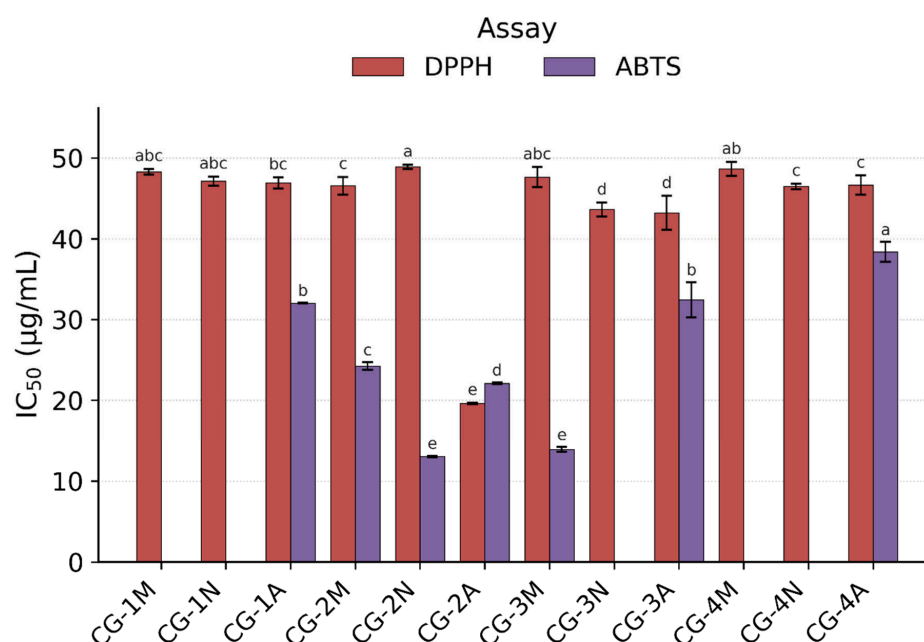


Figure 7. DPPH and ABTS $\bullet^+$ radical-scavenging activities (IC_{50} , $\mu\text{g/mL}$) of *Canna* $\times$ *generalis* floral nectar samples. Values are presented as mean $\pm$ SD ($n = 3$). Different letters within the same assay (DPPH or ABTS $\bullet^+$) indicate significant differences among samples ($p < 0.05$). Lower IC_{50} values indicate stronger radical scavenging activity. Samples without IC_{50} values for the ABTS $\bullet^+$ assay did not achieve 50% radical scavenging activity within the concentration range tested.

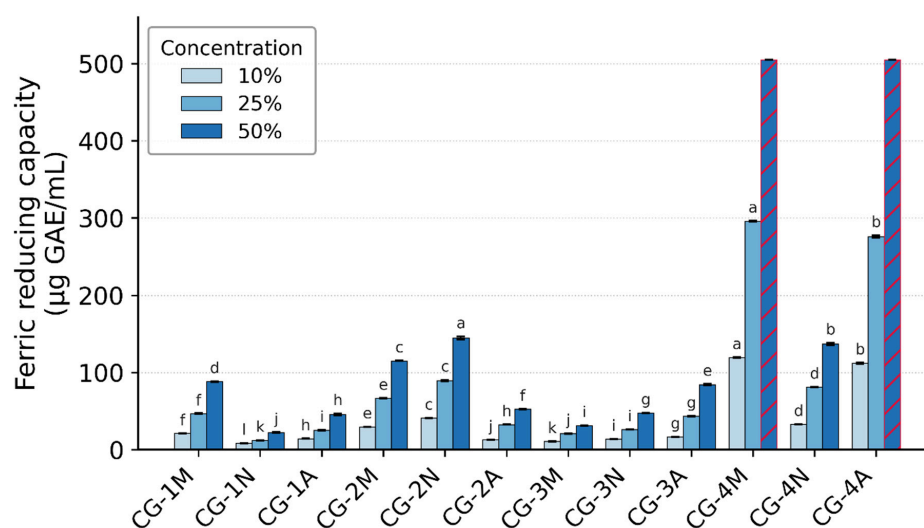


Figure 8. Ferric reducing capacity (FRAP, $\mu\text{g GAE/mL}$) of *Canna* $\times$ *generalis* floral nectar samples at 10%, 25% and 50% concentrations. Values are presented as mean $\pm$ SD ($n = 3$). Different letters within the same concentration indicate significant differences among samples ($p < 0.05$). Hatched bars (CG-4M and CG-4A at the 50% concentration) exceeded the upper limit of quantification, i.e., above the linear range of the assay.

The ferric reducing capacity of the floral nectar samples increased with increasing concentration and differed significantly among samples ($p < 0.05$) (Figure 8). The FRAP responses of CG-4M and CG-4A at the 50% concentration exceeded the validated upper

limit of quantification (ULOQ) and were therefore not assigned quantitative values. These observations were not used for quantitative comparison or statistical analysis.

3.5. α -Amylase and α -Glucosidase Inhibitory Activities of Floral Nectar

The inhibitory activities of the floral nectar samples against α -amylase and α -glucosidase are presented in Figure 9.

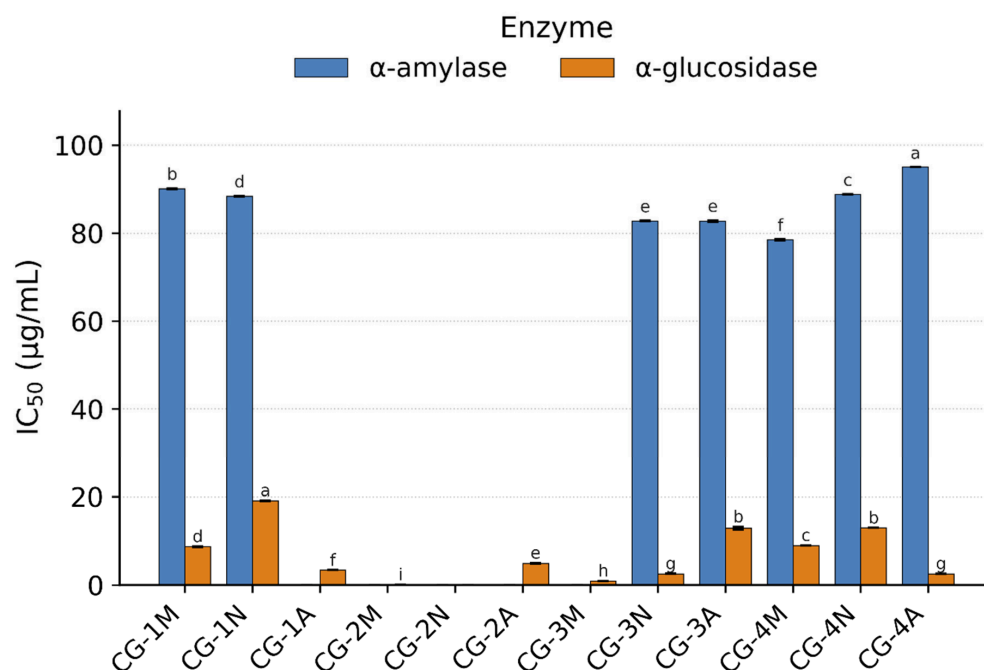


Figure 9. α -Amylase and α -glucosidase inhibitory activities (IC₅₀, µg/mL) of *Canna × generalis* floral nectar samples. Values are presented as mean $\pm$ SD (n = 3). Different letters within the same assay (α -amylase or α -glucosidase) indicate significant differences among samples ($p < 0.05$). Lower IC₅₀ values indicate stronger enzyme inhibitory activity. Samples without IC₅₀ values for the α -amylase assay did not reach 50% enzyme inhibition within the concentration range tested. The CG-2N α -glucosidase value fell below the lowest tested concentration and is not reliably quantifiable.

The IC₅₀ values showed that the α -amylase and α -glucosidase inhibitory activities differed significantly among the samples ($p < 0.05$). CG-4M exhibited the strongest α -amylase inhibitory activity with the lowest IC₅₀ value (78.49 ± 0.14 µg/mL), whereas CG-4A showed the highest IC₅₀ value (95.01 ± 0.00 µg/mL). For α -glucosidase inhibition, among the samples with quantitatively estimated IC₅₀ values CG-2M exhibited the lowest IC₅₀ value (0.11 ± 0.00 µg/mL), while the highest value was recorded for CG-1N (19.10 ± 0.04 µg/mL). Overall, the samples tended to inhibit α -glucosidase more effectively than α -amylase, as indicated by their considerably lower IC₅₀ values.

3.6. Cytotoxicity of Floral Nectar Against HepG2 and MCF-7 Cell Lines

Due to the limited amount of floral nectar available, a pooled CG-2 nectar sample comprising nectar collected in the morning, noon, and afternoon was used for cytotoxicity evaluation against HepG2 and MCF-7 cell lines.

The cell growth inhibitory activity of the floral nectar sample is presented in Table 3 and Figure 10.

The results in Table 3 showed that under the present experimental conditions, the tested CG-2 nectar sample showed no appreciable cytotoxicity within the tested concentration range against HepG2 and MCF-7 cells, with IC₅₀ values > 128 µg/mL. In contrast, the

positive control, ellipticine, exhibited strong activity, with IC_{50} values of $0.59 \pm 0.01 \mu\text{g/mL}$ for HepG2 cells and $0.57 \pm 0.03 \mu\text{g/mL}$ for MCF-7 cells.

Table 3. IC_{50} values ($\mu\text{g/mL}$) of the floral nectar sample (CG-2) and the positive control (ellipticine) against HepG2 and MCF-7 cell lines (mean $\pm$ SD, $n = 3$).

Sample	IC_{50} ($\mu\text{g/mL}$)	
	HepG2	MCF-7
Floral nectar (CG-2)	>128	>128
Ellipticine (positive control)	0.59 ± 0.01	0.57 ± 0.03

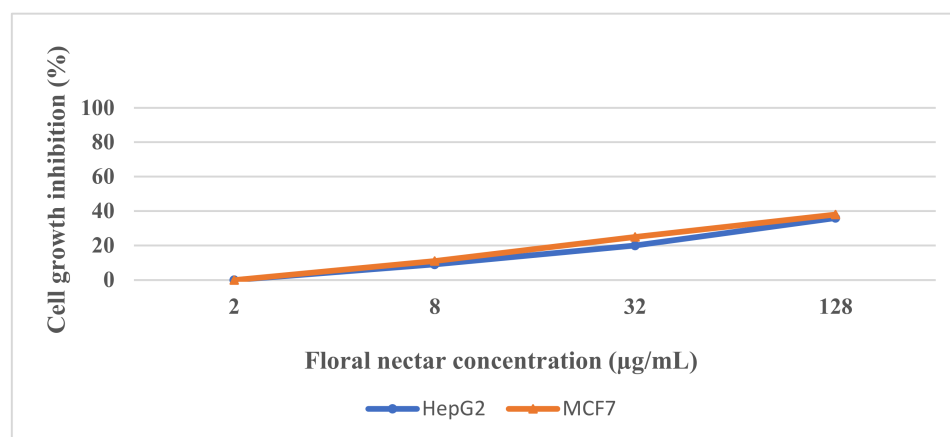


Figure 10. Cell growth inhibitory activity of the floral nectar sample (CG-2).

3.7. Correlation and Multivariate Analysis of Phytochemical and Bioactivity Data

Pearson correlation, principal component analysis (PCA), and hierarchical clustering were applied to explore relationships among phytochemical contents and bioactivities across the 12 floral nectar samples.

Pearson correlation analysis showed that the total phenolic content was strongly and positively correlated with the ferric reducing capacity ($r = 0.90$, $p < 0.001$) and with the total alkaloid content ($r = 0.89$, $p < 0.001$), while the total alkaloid and total flavonoid contents were also positively correlated with the ferric reducing capacity ($r = 0.80$, $p < 0.01$ and $r = 0.65$, $p < 0.05$, respectively) (Figure 11). In contrast, DPPH radical-scavenging activity was not significantly correlated with the phenolic, flavonoid or alkaloid contents ($|r| \leq 0.24$), and neither α -amylase nor α -glucosidase inhibitory activity was significantly correlated with any phytochemical group. These results indicate that phenolic and alkaloid contents were strongly associated with ferric reducing capacity, whereas DPPH scavenging and enzyme inhibition showed weaker relationships with the measured phytochemical groups.

Principal component analysis (PCA) of the phytochemical and bioactivity variables explained 70.4% of the total variance in the first two components (PC1 = 51.9%, PC2 = 18.5%) (Figure 12). PC1 was defined mainly by the total phenolic, flavonoid and alkaloid contents and the ferric reducing capacity, clearly separating the phenolic-rich CG-4 samples (CG-4M and CG-4A) from the remaining samples, whereas PC2 was defined mainly by the DPPH and α -glucosidase activities, reflecting variation that was independent of phenolic content. Consistently, hierarchical clustering grouped CG-4M and CG-4A apart from the other samples on the basis of their higher phenolic content and reducing capacity (Figure 13). Together, the multivariate analyses confirm that the antioxidant and enzyme-inhibitory activities of the nectar were not governed by the total phenolic content alone, but depended on the specific composition of each sample.

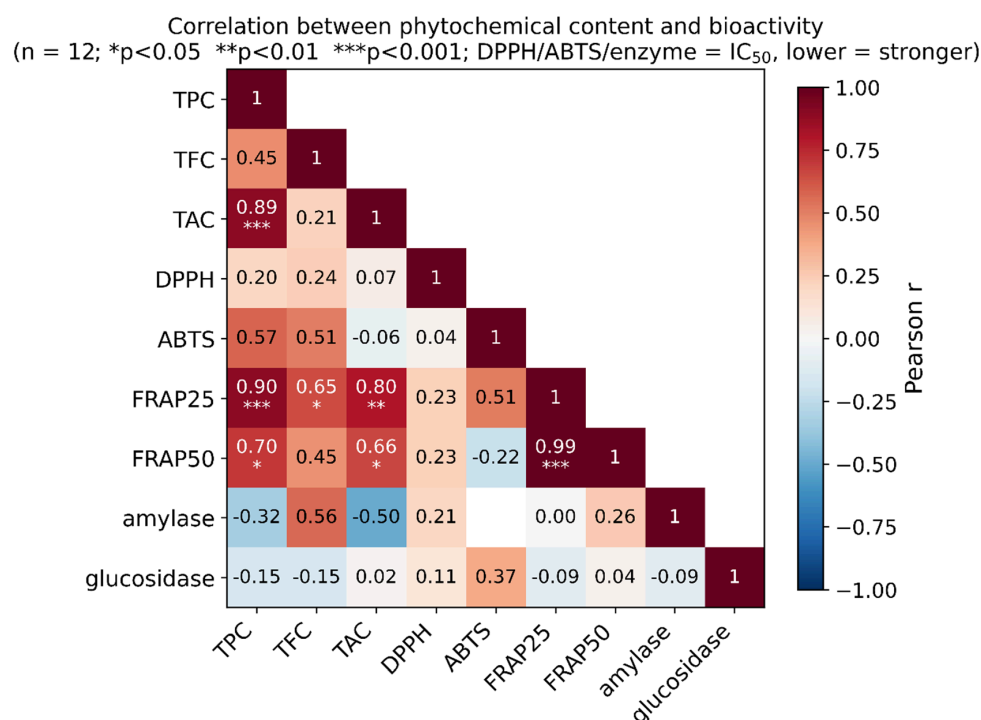


Figure 11. Pearson correlation matrix among phytochemical contents (TPC, TFC, TAC) and antioxidant/enzyme-inhibitory activities (DPPH, ABTS, FRAP, α -amylase, α -glucosidase) across the 12 *Canna × generalis* nectar samples (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; DPPH/ABTS/enzyme are IC₅₀, lower = stronger).

The results of the two-way ANOVA evaluating the effects of cultivar, sampling time, and their interaction on the characteristics and bioactivities of *Canna* flower nectar are summarized in Table 4.

PCA biplot (n = 11; CG-2N excluded as censored; 70.4% of total variance)
Colour = cultivar, shape = sampling time

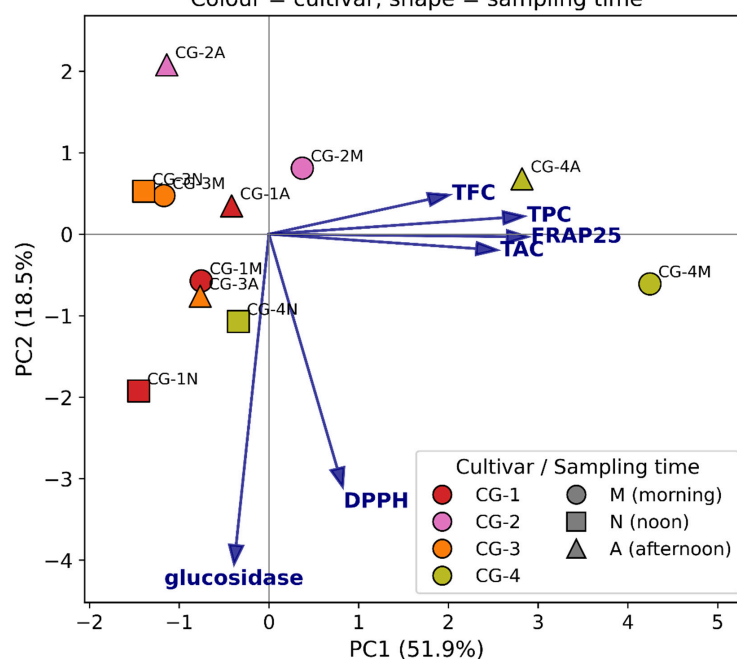


Figure 12. PCA biplot of the nectar samples based on phytochemical and bioactivity variables (color = cultivar, shape = sampling time; sample CG-2N excluded as a censored α -glucosidase value).

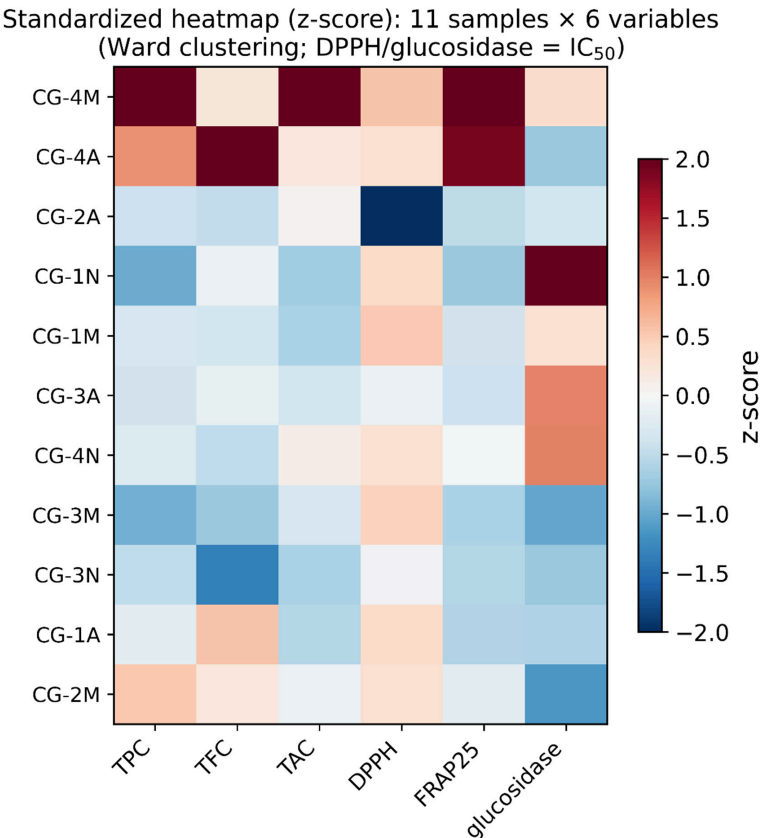


Figure 13. Hierarchical clustering heatmap (z-scores) of nectar samples × variables (Ward’s method; DPPH and α-glucosidase expressed as IC₅₀).

Table 4. Two-way ANOVA of the effects of cultivar and sampling time on the characteristics and bioactivities of *Canna* flower nectar.

	Cultivar			Time			Cultivar×Time		
	F	df	p	F	df	p	F	df	p
Nectar volume	734,392	3	<0.0001	174,462	2	<0.0001	4248	6	0.005
Brix	412,109	3	<0.0001	9498	2	0.001	12,852	6	<0.0001
Phenolic Content	10,017,934	3	<0.0001	4,937,530	2	<0.0001	3,213,364	6	<0.0001
Flavonoid Content	2,156,557	3	<0.0001	1,608,557	2	<0.0001	1,707,978	6	<0.0001
Alkaloid Content	4,908,491	3	<0.0001	1,850,962	2	<0.0001	1,803,931	6	<0.0001
FRAP Fe 10	115,830,778	3	<0.0001	14,927,453	2	<0.0001	21,395,672	6	<0.0001
FRAP Fe 25	187,469,466	3	<0.0001	25,750,685	2	<0.0001	30,811,230	6	<0.0001
FRAP Fe 50	299,544,431	3	<0.0001	44,320,953	2	<0.0001	50,873,497	6	<0.0001
DPPH	176,084	3	<0.0001	287,102	2	<0.0001	200,266	6	<0.0001
ABTS+	252,837	3	<0.0001	4,707,690	2	<0.0001	493,579	6	<0.0001
Enzyme glucosidase	77,635,672	3	<0.0001	74,729,571	2	<0.0001	40,718,965	6	<0.0001
Enzyme amylase	1,685,399,915	3	<0.0001	265,616,413	2	<0.0001	611,633,714	6	<0.0001

4. Discussion

Although floral nectar is a characteristic feature of each plant species, the composition and concentration of compounds in nectar may vary among populations, individuals, and even among flowers on the same plant [31,32]. In this study, the differences in nectar characteristics, chemical composition, and biological activities among the *Canna* × *generalis*

cultivars indicate that genetic factors have a marked influence on nectar volume and the accumulation of secondary metabolites. Since all cultivars were grown under the same environmental conditions, the observed differences were most likely attributable to genotypic variation, which is consistent with previous reports on flowering plants [33].

Nectar volume was highest in the morning and generally decreased during the day, whereas °Brix increased toward the afternoon. Because pollinator access was not experimentally excluded, the measured nectar volume should be interpreted as standing nectar crop rather than standing nectar volume alone. The observed diurnal pattern may reflect the combined effects of nectar production and removal, evaporation, and changes in the flower microenvironment. Therefore, the present data do not allow the diurnal pattern to be attributed specifically to nectar volume or overnight accumulation. These findings are consistent with previous studies [34,35].

Preliminary spectrophotometric screening indicated optical responses corresponding to several phytochemical classes, including polyphenols, flavonoids, tannins, coumarin-like compounds, carotenoids, saponins, quinones, and terpenoid-like compounds. Among the samples, CG-4M exhibited the highest phenolic and alkaloid contents, whereas the highest flavonoid content was recorded in CG-4A, reflecting a greater capacity for secondary metabolite accumulation than the other samples. Regarding the sampling time, phenolic content tended to be higher in the morning, whereas flavonoid content increased in the afternoon. The higher phenolic content observed in the morning may be associated with the period of active nectar volume during the early part of the day, when these compounds are simultaneously secreted into the nectar. These compounds not only contribute to attracting pollinators [36,37] but also help protect floral nectar through their antioxidant and antimicrobial properties [38,39]. As nectar volume gradually decreases during the day and nectar is continuously consumed by pollinators, the phenolic content tends to decline. In contrast, the higher flavonoid content observed in the afternoon may be associated with the increased production of antioxidant compounds under conditions of higher light intensity and temperature. Due to their ability to absorb ultraviolet radiation and neutralize free radicals, flavonoids may contribute to protecting floral tissues and maintaining nectar quality during the later part of the day. This trend is consistent with the findings reported in [35], indicating that phenolic and flavonoid compounds may play different roles depending on the time of day.

The antioxidant activities of the samples were clearly dependent on the assay employed. In the DPPH assay, CG-2A exhibited the strongest activity, with the lowest IC₅₀ value, indicating a high capacity to donate hydrogen atoms or electrons for free radical neutralization. This activity is generally associated with the number and position of hydroxyl groups in the phenolic structure, in which compounds containing more hydroxyl groups usually exhibit stronger antioxidant activity [40]. In contrast, CG-2N and CG-3M showed the strongest activity in the ABTS•⁺ assay, with comparable IC₅₀ values, suggesting the presence of various antioxidant compounds capable of scavenging free radicals through both hydrophilic and lipophilic mechanisms [26]. The activity is mainly associated with phenolic compounds rich in hydroxyl groups, which is consistent with the high polyphenol and flavonoid contents of these two samples. Phenolic acids and flavonoids are considered the major contributors to this activity [41]. The differences observed among the antioxidant assays indicate that antioxidant activity depends not only on the total phenolic or flavonoid contents but also on the specific composition and chemical structures of the compounds present in each sample.

Regarding enzyme inhibitory activity, CG-4M exhibited the strongest α-amylase inhibitory activity, whereas CG-2M showed the strongest α-glucosidase inhibitory activity. These activities may be associated with the presence of phenolics, flavonoids, and alkaloids,

which are capable of interacting with enzymes through hydrogen bonding and hydrophobic interactions, thereby reducing their catalytic activity [28,42]. From a physiological perspective, α -amylase is involved in the initial stage of starch hydrolysis, whereas α -glucosidase catalyzes the final step of glucose production for intestinal absorption [43]. Since α -glucosidase plays a decisive role in postprandial blood glucose elevation, it is considered an important target for glucose regulation. Therefore, CG-2M showed stronger *in vitro* α -glucosidase inhibitory activity, whereas CG-4M showed stronger α -amylase inhibitory activity.

The correlation and multivariate analyses provide the first quantitative description of the relationships between nectar composition and bioactivity in *Canna × generalis*. The strong correlation between total phenolic content and ferric reducing capacity ($r = 0.90$), together with their alignment along PC1, confirms that phenolics are the principal electron donors responsible for the reducing power of the nectar. In contrast, the absence of a significant correlation between phenolic content and either DPPH scavenging or α -amylase/ α -glucosidase inhibition—and their separation along PC2—indicates that these activities are not governed by the bulk phenolic pool but by specific constituents or molecular structures. This pattern is consistent with previous reports that phenolic compounds are major contributors to ferric reducing power [41], whereas DPPH and ABTS•⁺ radical-scavenging activities also depend on the specific structures of the antioxidants present rather than on the total phenolic content alone [26,40]. This has two practical implications: (i) bioactivity-guided fractionation, rather than total-phenolic screening, will be needed to identify the compounds responsible for the radical-scavenging and enzyme inhibitory activity; and (ii) a cultivar- and time-specific selection strategy is supported, with CG-4 nectar (phenolic-rich) being most promising for reducing-power antioxidant applications and CG-2 nectar showing the strongest α -glucosidase inhibition relevant to enzyme inhibitory activity. More broadly, these results establish the floral nectar of *Canna × generalis*—a previously uncharacterized matrix—as a chemically and functionally heterogeneous source of bioactive compounds, and provide a multivariate framework to guide future isolation and structure-activity studies.

In contrast, the CG-2 floral nectar sample showed no appreciable cytotoxicity against HepG2 and MCF-7 cells within the tested concentration range, with IC₅₀ values above 128 μ g/mL. These findings indicate only that the pooled CG-2 nectar sample did not produce appreciable cell-growth inhibition under the present experimental conditions. Because the compounds responsible for the antioxidant and enzyme-inhibitory activities were not identified, no direct relationship between these bioactivities and cytotoxicity can be established from the present data. Overall, *Canna × generalis* floral nectar serves not only as a carbohydrate source for pollinators but also contains a diverse range of bioactive compounds with considerable antioxidant and enzyme inhibitory activities. However, further studies are required to identify the specific bioactive compounds, clarify their mechanisms of action, and evaluate their biological potential under *in vivo* conditions.

The present study evaluated *in vitro* antioxidant and digestive-enzyme inhibitory activities, but the compounds responsible for these activities were not identified at the molecular level. In addition, the bioavailability, gastrointestinal stability, *in vivo* efficacy, and safety of the nectar constituents were not investigated. Therefore, the observed enzyme-inhibitory activities, particularly the very low α -glucosidase IC₅₀ values, should not be directly interpreted as evidence of antidiabetic efficacy. Further studies involving compound identification, appropriate matrix controls, comparison with reference inhibitors such as acarbose, and *in vivo* validation are required.

5. Conclusions

This study demonstrated that the floral nectar of *Canna × generalis* contains diverse bioactive compounds and exhibits antioxidant and enzyme inhibitory activities. The phytochemical composition and biological activities varied among samples and collection times, indicating that nectar bioactivity is influenced by both chemical composition and sampling conditions. Multivariate analysis suggested that phenolic compounds contributed substantially to ferric-reducing capacity, whereas radical-scavenging and enzyme-inhibitory activities were associated with other constituents, highlighting the multifactorial nature of nectar bioactivity. Overall, *Canna × generalis* floral nectar represents a promising natural source of bioactive compounds with potential for further investigation as a source of *in vitro* antioxidant and digestive-enzyme inhibitory activities. Further LC–MS-based characterization and bioactivity-guided fractionation are warranted to identify the compounds responsible for these activities.

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