

## Polyphenols in five varieties of *Euphorbia pulcherrima* native to Mexico

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### Abstract

Poinsettia (*Euphorbia pulcherrima* Willd. ex Klotsch) grows wild in the Mexican tropical forests in a shrubby form and with striking red bracts and less often it has white bracts. The main use of the native poinsettia is ornamental, little has been studied about the nutritional or nutraceutical properties of the plant. The objective of the present study was to determine the profile of flavonoids and phenolic acids present in the methanolic-aqueous extracts in bracts of five varieties of sun poinsettia in order to know their nutritional properties and promote their consumption. Dry bracts (0.5 g per sample) were used. The extraction of the compounds was done from a solution of methanol/water (80:20 v/v). A profile of phenolic acids and flavonoids was made by high-performance liquid chromatography (HPLC) coupled to a diode-array detector. Gallic and syringic acids were the ones that had the highest concentrations in the varieties analyzed. The flavonoid rutin was found in high concentrations in all varieties, however, in the Juan Pablo variety, it was not possible to detect phlorizin and phloretin. These results suggest that poinsettia bracts may provide important antioxidants when consumed.

**Keywords:** euphorbiaceae, flavonoids, HPLC, phenolic acids.

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## Introduction

Poinsettia (*Euphorbia pulcherrima* Willd. ex Klotsch) is a plant that grows wild and shrubby in the Mexican tropical forests, with a small stem and with striking red bracts and very rare with white bracts (Trejo *et al.*, 2012). A variant of wild poinsettias is sun poinsettia. According to Galindo-García *et al.* (2012), sun poinsettia is an ornamental and traditional shrub that can have several colors such as red, pink, white, of great economic and social importance and has been produced conventionally in an open-air nursery for more than thirty years.

At present, nine varieties of sun poinsettia registered in the National Catalogue of Plant Varieties (Snics CNVV, 2021) are recognized: ‘Valenciana’ (red), ‘Juan Pablo’ (pink), ‘Rehilete’ and ‘Belén’ (red) and ‘Amanecer navideño’ (white), they are called varieties of public domain and are the result of the selection, reproduction and modifications made by some nurserymen from the native poinsettias from: Tetela del Monte, Tepoztlán, Oaxtepec, Jiutepec and Ahuatepec, in the state of Morelos (Colinas *et al.*, 2015; CNVV, 2021). This study focused on the varieties: ‘Amanecer Navideño’, ‘Juan Pablo’, ‘Valenciana’, ‘Corona’ and one not yet registered, ‘Valenciana Superior’.

According to Steinmann (2002), many of the endemic species of the family Euphorbiaceae, to which poinsettia belongs, are cultivated for medicinal, industrial, food and ornamental use, however, there are very few studies about the nutritional or nutraceutical properties of sun poinsettia. Poinsettia leaves and bracts have begun to be introduced in different dishes. In Taxco Guerrero, where December 8 is celebrated as the day of poinsettia, there is a tasting of different dishes using the bracts of sun poinsettia.

Likewise, recipes using bracts are shared at the annual Christmas festival of the Ecological Park of Xochitla, State of Mexico. Edible ornamental plants can be attractive for their smell, color and taste; however, the evaluation of their nutritional properties is required to include them in the diet. The use of flowers for edible purposes has been reactivated through the consumption of different parts of the flower, such as stems and nectar, including them in the diet as spices, dyes and additives, in addition a great interest has been generated due to their nutraceutical properties (Janarny *et al.*, 2021). Edible flowers and ornamental plants can be considered as a new source of nutraceuticals (Mlcek and Rop, 2011).

On the other hand, edible flowers may contain phenolic compounds with different chemical structures, mainly phenolic acids, flavonoids and anthocyanins, which can provide antioxidant capacity. Phenolic acids are distributed in different parts of plants, these aromatic secondary metabolites are responsible for providing color, flavor and astringency, thus contributing to the organoleptic characteristics of foods (Rashmi and Negi, 2020). The functions of phenolic acids have been the subject of many studies related to agriculture, biology, chemistry and medicinal studies. Mahomoodally *et al.* (2020), studying three species of *Euphorbia* (*E. hirta*, *E. heterophylla* and *E. convolvuloides*), report a high content of total phenols, between 35.84 and 441.9 mg g<sup>-1</sup>, and indicate their potential as antioxidants.

Flavonoids are phenolic substances widely distributed in all vascular plants and can be present in their free form, glycosylated or as methylated derivatives, they have a powerful antioxidant, anti-inflammatory, antimutagenic, antimicrobial and anticancer activity, in addition to abilities to trap free radicals and other medicinal properties related to the ability to modulate enzymatic functions (Karak, 2019). Phenolic compounds are an essential part of the human diet and are considered of great interest due to their antioxidant properties and potential health effect (Shahidi and Ambigaipalan, 2015). The objective of this study was to analyze the profile of phenolic acids and flavonoids of five varieties poinsettia of public domain, in order to know their nutritional properties and promote their consumption.

## Materials and methods

### Plant material

Bracts of five poinsettia varieties of public domain, free of mechanical or pathogens damage, were collected in January 2019, the varieties were: Amanecer Navideño (AN), Juan Pablo (JP), Valenciana (V), Valsu (VS) and Corona (C), which are kept under greenhouse conditions in the experimental field of the Chapingo Autonomous University (UACH, for its acronym in Spanish). The bracts were dehydrated at a low temperature in a freeze dryer (Free Zone 2.5 Liter Benchtop Labconco. USA), for their further analysis.

### Method of extraction of phenolic acids and flavonoids

The obtaining of the extracts was carried out according to the methodology of Wang *et al.* (2010). In each poinsettia variety, 0.5 g of freeze-dried bracts were weighed and 10 ml of a methanol/water solution (80:20 v/v) was added. The mixture was stirred in a Vortex (VWE International) for 3 min at 3 000 rpm, sonicated for 15 min with a sonicator (Ultrasonic Cleaner 8890, Cole Palmer) and stirred for 30 min at 27 °C. This procedure was performed in triplicate. The extracts were stored in amber bottles for their further analysis.

### Identification of phenolic acids and flavonoids

The analyses were performed in a high-performance liquid chromatograph (HPLC) (Hewlett Packard, mod. 1100) coupled to a diode-array detector. For the quantification of phenolic acids, a Nucleosil 100 column at 125 x 4 mm id 5 µm (Marcherey-Nagel, Fisher Scientific, USA) was used. The eluents used were: water (pH 2.5) with trifluoroacetic acid (A) and acetonitrile (B). For the mobile phase, the following elution gradient (v/v) was used: 85%A-15%B (0-10'), 65%A-35%B (20'), 65%A-35%B (23'). The elution flow was kept constant at 1 ml min<sup>-1</sup> and the injection volume was 20 µl. The temperature of the column was kept at 30 °C. Retention times and spectra were obtained at 280 nm.

Regarding the quantification of flavonoids, a Hypersil ODS column 5µm particle diameter, 125 x 4 mm id. (Agilent Technologies, USA) was used. The eluents were: water (pH 2.5) with trifluoroacetic acid (A) and acetonitrile (B). For the mobile phase, the following elution gradient (v/v) was used: 85%A-15%B (0-10'), 65%A-35%B (20'), 65%A-35%B (23'). The elution flow was kept constant at 1 ml min<sup>-1</sup> and the injection volume was 20 µl. The temperature of the column

was kept at 30 °C. Retention times and spectra were obtained at 254, 316 and 365 nm. For both phenols and flavonoids, the identification was made based on a calibration curve, using the respective standards of the Merck brand, Dalmsdat, Germany.

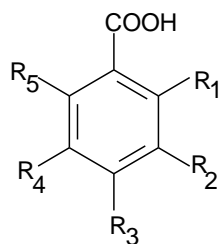
### Statistical analysis

The analyses were done in triplicate and were expressed in milligrams of phenolic acids or flavonoids per gram of sample in dry matter ( $\text{mg g}^{-1} \text{dm}$ ). All data were analyzed with the SAS statistical package for Windows 9.0, statistical differences were determined in an analysis of variance (Anova). The means were expressed using Tukey's HSD statistic with a minimum significant difference of  $p \leq 0.05$ .

## Results and discussion

### Phenolic acids

The phenolic acids identified in the methanolic-aqueous extracts of bracts of *Euphorbia pulcherrima* varieties are shown in Table 1. Their chemical structures vary according to the number and position of free radicals present in them (Figure 1).



**Figure 1.** Position of the radical groups of phenolic acids present in varieties of *Euphorbia pulcherrima*.

**Table 1.** Phenolic acids identified in the bracts of five varieties of *Euphorbia pulcherrima*.

Phenolic acid	IUPAC Name	Structure
Gallic	3,4,5-trihydroxybenzoic	$R_3 = R_4 = R_5 = \text{OH}$
Chlorogenic	(1S,3R,4R,5R)-3-[[[(2E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-1,4,5-trihydroxycyclohexanecarboxylic	$R_1 = R_4 = R_5 = \text{OH}$
Syringic	4-hydroxy-3,5-dimethoxybenzoic	$R_4 = \text{OH}; R_3 = R_5 = \text{CH}_3\text{O-}$
Vanillic	4-hydroxy-3-methoxybenzoic	$R_4 = \text{OH}; R_3 = \text{CH}_3\text{O-}$
P-hydroxybenzoic	4-hydroxybenzoic	$R_4 = \text{OH}$
Caffeic	(E)-3-(3,4-dihydroxyphenyl)prop-2-enoic	$R_3 = R_4 = \text{OH}$
Ferulic	(E)-3-(4-hydroxy-3-methoxy-phenyl)propyl-2-enoic	$R_4 = \text{OH}; R_3 = \text{CH}_3\text{O-}$
P-coumaric	(E)-3-(4-hydroxyphenyl)-2-propenoic	$R_4 = \text{OH}$

The concentrations of gallic and syringic acid were the highest in the five poinsettia varieties, with the Corona variety standing out with 50% more gallic acid than the Amanecer Navideño and Valenciana varieties, which rank second and third, respectively. While for syringic acid, the highest contents were in the Corona and Valenciana varieties. On the other hand, p-coumaric acid was found in lower concentrations (0.028-0.54 mg g<sup>-1</sup> dm) in all varieties, particularly in Juan Pablo and Valenciana.

Considering the eight phenolic acids evaluated in this research, overall, the highest content of phenolic acids was obtained in the Corona variety, which with 57.14 mg g<sup>-1</sup> dm represents the highest value, followed by the Valenciana variety with 33.08% and Amanecer Navideño with 37.73% less, respectively. Overall, the phenolic acid content was lower in the Valsu variety (11.48 mg g<sup>-1</sup> dm).

Phenolic compounds are present in virtually all plant-based foods, because they provide useful building blocks with excellent functionality and biocompatibility, their use has been promoted as a replacement for synthetic antioxidants commonly used in the food industry (Dias *et al.*, 2020). In the research conducted by Ertas *et al.* (2015), gallic acid was one of the most abundant phenolic acids in two species of *Euphorbia* (*E. gaillardotii* and *E. macroclada*) analyzed, a result similar to that reported in the poinsettia varieties of the present study.

Florkiewicz *et al.* (2019) analyzed the content of gallic acid in varieties of the genus *Brassica*, such as brussels sprouts (*Brassica oleracea* var. *gemmifera*) and broccoli (*Brassica oleracea* var. *italica*), obtaining concentrations of 0.0027 mg g<sup>-1</sup> dm and 0.0025 mg g<sup>-1</sup> dm respectively, while in the poinsettia varieties analyzed, the values of gallic acid are in a range of 0.13 mg g<sup>-1</sup> dm to 23.76 mg g<sup>-1</sup> dm. Gallic acid can also be found in beverages such as coffee, tea, and wine (Nayeem *et al.*, 2016). According to Badhani *et al.* (2015), gallic acid provides efficient protection against oxidative damage caused by reactive species found in biological systems including hydroxyl, superoxide and peroxy and non-radicals such as hydrogen peroxide and hypochlorous acid.

Another phenolic compound of great abundance in the plant kingdom is syringic acid and is present in olives, dates and spices, it also exhibits useful properties in the biomedical sector as it acts as an antioxidant, antimicrobial, anti-inflammatory and anticancer agent (Srinivasulu *et al.*, 2018). Pioro-Jabrucka *et al.* (2011) analyzed the methanolic extracts of *Euphorbia hirta*, reporting values of syringic acid of 0.61 mg g<sup>-1</sup> dm, a value lower than that found in the Juan Pablo, Valenciana, Valsu and Corona varieties of the present study. The Corona variety presented the highest concentrations of gallic, syringic and vanillic acids. The latter is also known as 4-hydroxy-3-methoxybenzoic, it is a phenolic compound derived from edible plants and fruits (Prince *et al.*, 2011), several studies have provided evidence of the effectiveness of vanillic acid in managing immune or inflammatory responses (Kim *et al.*, 2010).

As for the Juan Pablo and Valenciana varieties, it was not possible to detect vanillic acid and in the Valenciana Superior variety, p-hydroxybenzoic and caffeic acids were not detected because they were found in very low concentrations (Table 2). Hydroxycinnamic acids are more commonly found in nature, compared to hydroxybenzoic acids, and are usually available either

in their soluble form conjugated with sugars and organic acids or bound to cellular constituents. The most common hydroxycinnamic acids are p-coumaric, caffeic and ferulic acids (Rashmi and Negi, 2020). Jahan *et al.* (2013) analyzed five phenolic acids (gallic, chlorogenic, p-coumaric, ferulic and caffeic) present in methanolic extracts of *Euphorbia tirucalli* by liquid chromatography, gallic, chlorogenic and p-coumaric acids were the most representative; obtaining lower values of gallic (0.01 mg g<sup>-1</sup> dm) and chlorogenic acids (0.04 mg g<sup>-1</sup> dm) unlike those reported in the varieties analyzed in this study.

**Table 2. Quantification of phenolic acids by HPLC in methanolic-aqueous extracts of *Euphorbia pulcherrima*.**

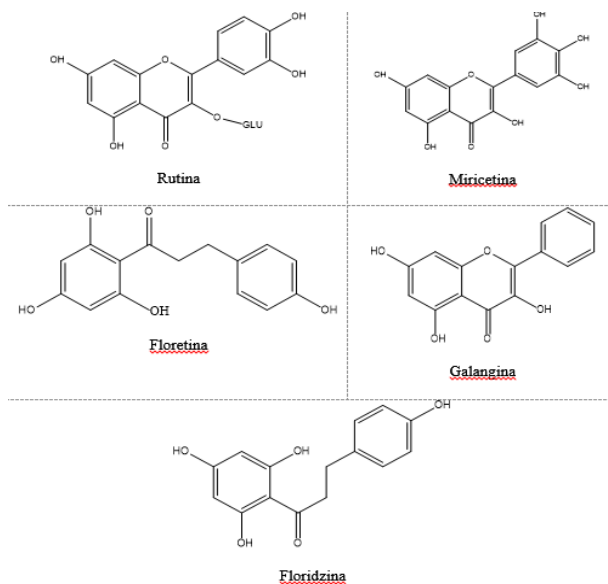
Phenolic acid (mg g <sup>-1</sup> dm)	Variety				
	AN <sup>1</sup>	JP	V	VS	C
Gallic (1.635)*	10.65a ±0.76	1.81cb ±0.16	11.5ba ±4.28	0.13b ±0.03	23.76a ±3.02
Chlorogenic (2.491)*	1.37bc ±0.11	2.54cb ±2.11	3.75ba ±6.3	4.57a ±4.5	2.75b ±3.2
Syringic (3.00)*	0.27c ±0.06	2.45b ±2.11	12.63a ±5.76	6.36b ±0.08	17.37ba ±0.91
Vanillic (3.387)*	0.28c ±0.09	ND	ND	0.17b ±0.26	12.3ba ±1.58
P-hydroxybenzoic (11.271)*	2.41bc ±0.21	1.65cb ±1.08	0.15ba ±0.14	ND	0.18b ±0.09
Caffeic (3.387)*	9.47ba ±3.1	8.7a ±0.34	9.8ba ±0.26	ND	0.37b ±0.15
Ferulic (6.056)*	2.49bc ±0.38	1.41cb ±0.63	0.32ba ±0.06	0.14b ±0.01	0.07b ±0.003
P-coumaric* (5.289)	0.54c ±0.08	0.03c ±0.02	0.09ba ±0.03	0.11b ±0.15	0.34b ±0.14

\* = retention time. Values with the same letters within each column are statistically similar (Tukey's HSD,  $p \geq 0.05$ ); <sup>1</sup> = standard deviation; AN= Amanecer Navideño; JP= Juan Pablo; V= Valenciana; VS= Valsu; C= Corona; ND= not detected.

P-coumaric acid had a value similar to that reported in the varieties analyzed, with a concentration of 0.09 mg g<sup>-1</sup> dm. P-coumaric acid is present in plants and fungi, either in its free or conjugated form, it is also present in fruits (apples, pears, grapes, oranges, tomatoes and berries) and in vegetables (onions, beans and potatoes) or in cereals such as corn, oats and wheat. Several studies have shown that p-coumaric acid conjugates provide antioxidant, anti-inflammatory, antimutagenic, antiulcer and anticancer activity (Pei *et al.*, 2016).

## Flavonoids

The flavonoids identified in the methanolic-aqueous extracts of bracts of the *Euphorbia pulcherrima* varieties are shown in Figure 2 and Tables 3 and 4. High concentrations of rutin were obtained in all the varieties analyzed, the Amanecer Navideño variety had the highest concentration of this flavonoid, followed by the Juan Pablo variety with 16.47% less and, in third place, Valsu with 14.53% less. Corona had the highest content of phlorizin (66.22 ±0.09 mg g<sup>-1</sup> dm), followed by the Amanecer Navideño variety with 32.85% less. On the other hand, myricetin was found in greater concentration in the Amanecer Navideño variety, its content being very low in the other four varieties evaluated. The equipment did not detect the presence of the flavonoids phlorizin and phloretin in the Juan Pablo variety.



**Figure 2.** Structures of flavonoids present in varieties of *Euphorbia pulcherrima*.

**Table 3.** Flavonoids identified in the bracts of five varieties of *Euphorbia pulcherrima*.

Flavonoid	IUPAC Name	Structure
Rutin	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3- {[ (2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-({ [(2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxy }methyl)oxan-2-yl]oxy }-4H-chromen-4-one	R <sub>1</sub> = R <sub>2</sub> = R <sub>3</sub> = OH
Myricetin	3,5,7-trihydroxy-2-(3,4,5-trihydroxyphenyl)-4-chromenone	R <sub>1</sub> = R <sub>4</sub> = R <sub>5</sub> = OH
Phloretin	3-(4-hydroxyphenyl)-1-(2,4,6-trihydroxyphenyl)propan-1-one	R <sub>4</sub> = OH
Galangin	3,5,7-trihydroxy-2-phenylchromen-4-one	R <sub>4</sub> = OH; R <sub>3</sub> = R <sub>5</sub> = CH <sub>3</sub> O-
Phlorizin	1-(2,4-dihydroxy-6-{{ (2" S", 3" R", 4" S", 5 "S", 6" R")-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy }phenyl)- 3-(4-hydroxyphenyl)propan-1-one	R <sub>4</sub> = OH

**Table 4.** Quantification of flavonoids by HPLC in methanolic-aqueous extracts of *Euphorbia pulcherrima*.

Flavonoid mg g <sup>-1</sup> dm	Variety				
	AN <sup>1</sup>	JP	V	VS	C
Rutin (5.734)*	32.68a ±1.43	27.3a ±2.58	22.74a ±3.68	27.93a ±1.14	26.57ba ±0.7
Phlorizin (9.056)*	44.47a ±0.37	ND	0.37b ±0.1	0.32b ±0.06	66.22a ±0.09
Myricetin (9.190)*	9.4a ±0.06	0.21b ±0.12	0.21b ±0.02	0.17b ±0.01	0.14b ±0.02
Phloretin (16.263)*	0.28a ±0.2	ND	0.04b ±0.01	0.28b ±0.22	0.22b ±0.01
Galangin	0.4a ±0.01	0.03b ±0.06	0.42b ±0.04	0.33b ±0.004	0.04b ±0.005

\* = retention time. Values with the same letters within each column are statistically similar (Tukey's HSD,  $p \geq 0.05$ ); <sup>1</sup>= standard deviation; AN= Amanecer Navideño; JP= Juan Pablo; V= Valenciana; VS= Valsu; C= Corona; ND= not detected.

Considering the total content of the five flavonoids evaluated, the highest content was obtained in the Corona variety with 93.19 mg g<sup>-1</sup> dm, followed by Amanecer Navideño with 87.23 mg g<sup>-1</sup> dm. While the other three varieties Valsu (29.03 mg g<sup>-1</sup> dm), Juan Pablo (27.54 mg g<sup>-1</sup> dm) and Valenciana (23.78 mg g<sup>-1</sup> dm) had approximately 50% less total flavonoids than the previous varieties.

Flavonoids are an integral part of the diet because they are plant-specific phytochemicals and cannot be synthesized by humans, which is why they must be obtained from different sources, mainly of plant origin (Mahomoodally *et al.*, 2020). According to research conducted by Ertas *et al.* (2015), one of the main constituents of two *Euphorbia* species analyzed was rutin, a result similar to that obtained in the five varieties of *Euphorbia pulcherrima* analyzed in this study.

The flavonoid rutin is constituted by a glycoside of quercetin (flavonol) and rutinose (disaccharide), which is commonly found in the diet and in beverages of plant origin such as tea and wine, it also has various pharmacological properties such as anti-inflammatory, neuroprotective and anticarcinogenic (Imani *et al.*, 2020). Jahan *et al.* (2013) reported myricetin as one of the main flavonoids present in the methanolic extracts of *E. tirucalli*, obtaining a value of 0.82 mg g<sup>-1</sup> dm, higher than those obtained in most of the varieties of *E. pulcherrima* analyzed here, except for the Amanecer Navideño variety, in which a value of 9.4 mg g<sup>-1</sup> dm was found.

Myricetin is a phenolic compound very common in berries, vegetables, teas and wines, it is also produced by several plants, this compound shows a variety of pharmacological activities and acts as an anti-inflammatory, analgesic, antitumor and hepatoprotective agent (Semwal *et al.*, 2016). Mustafa *et al.* (2022) analyzed the content of quercetin and myricetin in the fruits of commercial blueberry (*Vaccinium* spp.), finding values of 0.069 mg g<sup>-1</sup> and 0.003 mg g<sup>-1</sup> respectively, while in the poinsettia varieties analyzed, the values of myricetin are in a range of 0.14 mg g<sup>-1</sup> dm to 9.4 mg g<sup>-1</sup> dm.

On the other hand, chalcones are yellow pigments partly responsible for the color of flowers and fruits (Peñarrieta *et al.*, 2014). This fact could explain why high concentrations of phlorizin (44.47 ± 0.37 mg g<sup>-1</sup> dm) were found in the Amanecer Navideño variety, since its bracts are yellow. According to Haider *et al.* (2020), phloretin is commonly present in apple trees and *Prunus mandshurica*, it also shows various bioactivities such as antioxidant, anticarcinogenic, anti-inflammatory and immunomodulatory. In the study conducted by Zielinska *et al.* (2019), they analyzed the content of phloretin and phlorizin in apple (*Malus* sp., Rosaceae var. Sunrise) in concentrations of 0.0014 and 0.062 mg g<sup>-1</sup> respectively, being values lower than those found in the poinsettia varieties analyzed in this study.

## Conclusions

The concentrations of gallic and syringic acids were the highest, particularly in the Corona variety. It was detected that p-coumaric acid was the one with the lowest concentration in the Juan Pablo variety. The Corona variety stands out in the vanillic acid content. As for flavonoids, high concentrations of rutin were found in all varieties, with Amanecer Navideño standing out. Phlorizin



was found in high levels in Amanecer Navideño and Corona. In the Juan Pablo variety, it was not possible to detect phlorizin and phloretin. It is considered that the bracts of the Corona and Amanecer Navideño varieties, having a high level of phenolic acids and flavonoids, have the potential to be included in the diet.

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