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Chemical composition and antioxidant activity of stamens in four species of the genus *Opuntia*

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Abstract. Currently, information on the phytochemical composition and functional activity of *Opuntia* genus stamens remains limited, but recent studies have shown promising results. Further research on this topic is necessary to apply it to the differentiation between species and evaluate their chemical richness and antioxidant potential. In this context, the present study analyzed the chemical profile and antioxidant activity of the stamens of four *Opuntia* species. Aqueous extraction with methanol was performed, and various phytochemicals, including pigments, phenolic compounds, flavonoids, condensed tannins, and reducing sugars, were quantified using spectrophotometric methods. Antioxidant activity was also assessed using FRAP and DPPH assays. Principal components analysis explained 97.9% of the total variation. Reducing sugars strongly correlated with component 1 (0.99), while chlorophyll b (0.67), the antioxidant activity as determined by FRAP (0.45), and carotenoids (0.38) were associated with component 2. The stamens of *O. microdasys* exhibited the highest levels of reducing sugars and carotenoids, while *O. macrocentra* and *O. arenaria* showed the lowest values, respectively. The highest antioxidant capacity (FRAP) was observed in *O. arenaria* and *O. microdasys*, and the lowest in *O. engelmannii*. Overall, the results suggest that the stamens of *O. microdasys* constitute a promising source of bioactive compounds, with potential for applications in pharmacology and nutrition.

Keywords: *Cacti, plant pigments, natural antioxidants, flavonoids, phenols, principal components analysis*

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Introduction

Over the past decade, there has been increasing interest in identifying natural sources of bioactive compounds with antioxidant potential, particularly those derived from plants. These compounds play a crucial role in preventing cellular damage caused by reactive oxygen species and free radicals, which are associated with the development of various chronic diseases (Hoang and Kim, 2021; Rangel-Huerta *et al.*, 2015).

The Cactaceae family is recognized for producing a variety of antioxidants in response to environmental stresses. These antioxidants include pigments such as carotenoids and betalains, phenolic compounds, fibers, mucilage, and other related molecules (Agostini-Costa, 2022; Aispuro-Hernández *et al.*, 2022; Márquez-Rangel *et al.*, 2025).

Variations in the concentration of these compounds across tissues and species reflect the adaptive strategies of the plants to their environments (Agostini-Costa, 2022). Recent studies have primarily examined two aspects: the ecological role of those antioxidants in arid ecosystems and their potential as bioactive agents in pharmacological and nutraceutical applications, given their antioxidant, anti-inflammatory, and anticancer properties (Martínez *et al.*, 2024; Ramírez-Rodríguez *et al.*, 2020; Slimen *et al.*, 2016). Furthermore, natural antioxidants are considered a promising and sustainable alternative to synthetic antioxidants, the safety and side effects of which remain under discussion (Orozco-Barocio *et al.*, 2024).

The genus *Opuntia* is one of the most studied of the cactus family (Cactaceae). Several studies have characterized the nutraceutical benefits of fruits (including pulp, seeds, and peel) as a potential source of pigments, phenols, polyphenols, betalains, antioxidant activity, and pectin's production (Betancourt *et al.*, 2017; Chahdoura *et al.*, 2015; Chauhan *et al.*, 2013; Ciriminna *et al.*, 2019; Oniszczuk *et al.*, 2020; Vázquez-Espinosa *et al.*, 2022). In addition, these phytochemicals have been described as anti-inflammatory, neuroprotective, anticancer, and hepatoprotective compounds (González-Ponce *et al.*, 2016; Kang *et al.*, 2016; Tilahun and Welegerima, 2018). These advances have been studied primarily in the fruits of the commercial *O. ficus-indica* (L.) Mill species. Consequently, the comparative analyses of these bio-compounds across wild *Opuntia* species and other tissues, such as the stamens, are limited.

The primary purpose of pigmented flowers is plant-animal interactions for pollination and seed dispersal. Moreover, plant pigment concentrations serve as indicators of environmental adaptation, mediating responses to variations in light conditions, nutrient availability, and abiotic stresses. Among these pigments, chlorophylls are essential for photosynthesis in leaves. Chlorophylls are also found in flowers, petals, and pollen, where they facilitate light absorption, protect against excessive light, aid pollination, and provide stress defense (Jaleel *et al.*, 2009; Manrique-Reol, 2003; Narbona *et al.*, 2014).

Carotenoids and xanthophylls, along with chlorophylls, are the main pigments responsible for plant coloration; however, their functions transcend the chromatic purpose. These compounds play a fundamental role in light energy absorption, photoprotection of photosynthetic apparatus, and biosynthesis of signaling molecules to mitigate photooxidative damage (Demmig-Adams and Adams, 1996; Lu *et al.*, 2001). In non-photosynthetic tissues, carotenoids and xanthophylls contribute to the coloration of flowers and fruits, facilitating pollination and seed dispersal, and are involved in the cellular antioxidant system maintenance (Giuliano *et al.*, 2008). Furthermore, these pigments play an essential role in protecting reproductive structures —such as flowers and pollen grains— from photooxidative stress, a particularly relevant condition due to their exposure to intense light and ultraviolet radiation (Demmig-Adams and Adams, 2002). In this sense, high concentrations of xanthophylls and carotenoids in stamens and pollen have been associated with physiological adaptation to high-irradiance environments, protecting against light-induced damage, and the antioxidant defense against photooxidation, respectively (Nadot and Carrive, 2021).

Betalains are water-soluble pigments classified into two classes: the red-to-violet betacyanins and the yellow betaxanthins (Chauhan *et al.*, 2013). Some betalains serve multiple physiological and ecological functions, acting as osmolytes that support cellular homeostasis, stabilizing subcellular structures, mitigating nitrogen toxicity, functioning as efficient radical scavengers due to their strong

antioxidant capacity, and contributing to flower coloration, thereby attracting pollinators (Gandía-Herrero *et al.*, 2016).

The wild species *O. microdasys* Engelm. (1849), *O. macrocentra* Engelm. (1856), *O. engelmannii* Salm-Dyck ex Engelm. (1850), and *O. arenaria* Engelm. (1857) are widely distributed in the Sierra de Samalayuca, Ciudad Juárez municipality, Chihuahua, Mexico. Previous studies have shown that fruit consumption offers potential nutritional and functional benefits due to its phytochemical content and antioxidant capacity (Núñez-Gastélum *et al.*, 2018; Valero-Galván *et al.*, 2021). However, few comparative studies have examined these biocompounds in wild *Opuntia* species and different tissues, such as flower stamens. In this context, this study aimed to evaluate the chemical profile and antioxidant activity of the stamens in four *Opuntia* species. Spectrophotometric measurements assessed pigments, total phenolics, flavonoids, reducing sugars, and antioxidant activity to identify interspecific differences and determine which species contained the highest chemical richness and antioxidant potential.

Material and Methods

Biological material and collection site

The species analyzed were *O. microdasys, O. macrocentra, O. engelmannii*, and *O. arenaria*. They are native to Ciudad Juárez, Chihuahua, Mexico. Flowers were collected in early April 2023 in the Sierra de Samalayuca (31° 18' 02.5" N and 106° 30' 01.5" W). The collection followed the methodology of González-Fernández *et al.* (2025), with modifications. Briefly, ten individuals per species were randomly selected and monitored every ten days, from March 1 to April 30, 2023. During this period, two flowers per plant were randomly collected from different branches of the same individual, until a total of 20 flowers per species were gathered. Flowers were cut at the pre anthesis stage to prevent opening and anther dehiscence and minimize pollen contamination. Sampling occurred on the same day, between 7:00 and 10:00 a.m. The flowers were carefully placed in individual airtight polyethylene bags and transported to the laboratory within one hour. Fixatives were not used. Previous observations revealed that flowers cut in the pre anthesis stage retain pollen and anther integrity without noticeable alterations. In the laboratory, each flower was sectioned longitudinally with a scalpel, and stamens (with anthers and pollens) were separated. All stamens by species were placed in a mortar and dried at 28 °C for 96 h in an incubator (Thermo Scientific, Germany). This low temperature allowed tissue dehydration and preserved thermosensitive pigments. In leafy matrices, carotenoid concentrations remain stable below 25 – 30 °C, with degradation increasing sharply above 50 – 75 °C (Lefsrud *et al.*, 2008). Studies confirm carotenoids degrade significantly even at 40 °C (Moura *et al.*, 2023). Betalain pigments are most stable between 10 and 30 °C, degrading rapidly at higher temperatures (Ahmad-Fadzillah *et al.*, 2019). Betalains processed at 30 °C retain their integrity better than those processed at 40 °C, where degradation increases significantly (Chaux-Gutiérrez *et al.*, 2021). Subsequently, the dried samples were manually ground with a mortar and pestle, and the resulting material was placed into properly labeled paper envelopes and stored at 27 °C until analysis (24 h after grinding).

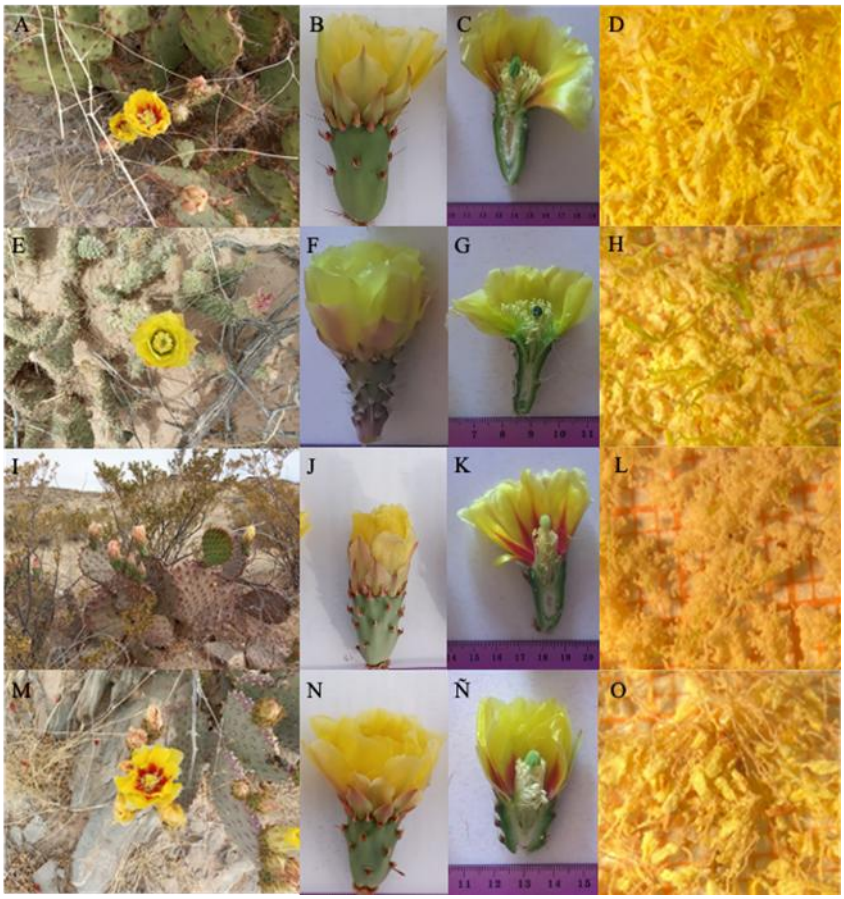

Figure 1. View of plant flowering, entire flower, longitudinal section, and stamens with pollen from *O. engelmannii* (A, B, C, D, respectively), *O. arenaria* (E, F, G, H, respectively), *O. macrocentra* (I, J, K, L, respectively), and *O. microdasys* (M, N, Ñ, O, respectively).

Preparation of the extracts

To reduce inter-individual biological variability and to obtain representative mean values, stamens collected from multiple individuals of each species were pooled to generate a single composite sample per species. Consequently, the three replicates obtained for each species represent technical replicates, as they were derived from the same homogenized biological material rather than from independent biological samples. Three replicates of a standard extract were prepared for each species using the solid-liquid extraction procedure with an 80 % (v/v) aqueous methanol solution (DEQ, Mexico), following the methodology described by Reyes-Corral *et al.* (2022). The 80 % methanol solution is typically used for extracting plant metabolites. In a previous comparative study, this solvent turned out to be the most balanced and efficient for extracting the pigment and phenolic profiles from the stamens of the evaluated species (Madrigal-Hernández *et al.*, 2025). For each species and replicate, 0.03 g of dry stamens were independently placed in a mortar and homogenized with 1.5 mL of 80 % (v/v) methanol solution using a pestle. The homogenate was transferred to 2 mL tubes and preserved at 4 °C in the dark for 12 hours. The extracts were centrifuged at 5,000 rpm for 3 minutes at 4 °C in a centrifuge (Eppendorf, USA). Finally, supernatants were transferred to new tubes and stored at 4 °C until further analysis.

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Pigment quantification

Seventy-five µL of each of the four extracts was deposited into independent cells in triplicate in a 96-well microplate (Corning, Mexico). Wavelength absorbance was measured at 470 *nm*, 490 *nm*, 547 *nm*, 630 *nm*, 632 *nm*, 647 *nm*, 652 *nm*, 649 *nm*, 664 *nm*, 665 *nm*, 691 *nm*, and 696 *nm* using a spectrophotometer coupled to a microplate reader (Hercules, USA) with the Microplate Manager 6.0 program (Bio-Rad, USA). The same solvent for extraction served as the blank. To determine the content of chlorophyll a, chlorophyll b, total chlorophyll, xanthophylls, and carotenoids, the equations proposed by Ritchie (2008) and Lichtenthaler (1987) were used. The equations proposed by Castellanos-Santiago and Yahia (2008) were applied to quantify total betacyanins, betaxanthins, and betalains.

Chemical quantification

The content of total phenolics and flavonoids was quantified using the method proposed by Georgé *et al.* (2005) with adjustments suggested by Valero-Galván *et al.* (2024). The Folin-Ciocalteu method quantifies total phenolic compounds by a reduction-oxidation reaction. In this reaction, the hydroxyl groups of phenols reduce phosphomolybdic and phosphotungstic acids in the Folin-Ciocalteu reagent in an alkaline medium. This process forms a blue complex, the intensity of which is proportional to the phenol concentration (Muñoz-Bernal *et al.*, 2017). For phenolic quantification, 25 µL of each of the four extracts was deposited into independent cells in triplicate in a 96-well microplate (Corning, Mexico). Then, 40 µL of 7.5 % Na_2CO_3 (m/v) (Sigma-Aldrich, Mexico) and 125 µL of Folin-Ciocalteu 2 N reagent (Sigma-Aldrich, Mexico) were added to each extract. The same solvent used for extraction was used as the blank. The mixture was incubated for 15 min at 25 °C in the absence of light, and the absorbance was measured at 710 *nm* in a spectrophotometer (Hercules, USA) coupled to the Microplate Manager 6.0 program (Bio-Rad, USA). A calibration curve was generated using gallic acid (concentrations ranging from 0.0125 to 0.2 $mg \cdot mL^{-1}$) (Sigma-Aldrich, Mexico) as the standard. Results were expressed as mg of gallic acid equivalents per g of dry mass (mg $GAE \cdot g^{-1}$) .

The aluminum chloride method quantifies total flavonoids by taking advantage of the formation of colored complexes between these compounds and aluminum chloride ($AlCl_3$): the hydroxyl groups of the flavonoids, especially at the C-3 and/or C-5 positions, react with the Al^{3+} to form chelates, resulting in a yellow complex with a bathochromic shift in the absorption spectrum, whose intensity is proportional to the concentration of flavonoids (Georgé *et al.*, 2005). For flavonoid quantification, 62.5 µL of each extract was deposited into independent cells in triplicate in a 96-well microplate (Corning, Mexico). Then, 46.5 µL of 5 % $NaNO_2$ (m/v) (Sigma-Aldrich, Mexico), 46.5 µL of $AlCl_3$ at 10 % (m/v) (Sigma-Aldrich, Mexico), and 62.5 µL of 0.5 M NaOH (Sigma-Aldrich, Mexico) were added to each extract. The same solvent used for extraction was used as the blank. The mixtures were incubated for 15 min at 25 °C in darkness, and the absorbance was measured at 510 *nm* under the same conditions as in the previous case.

Total condensed tannins were quantified using a colorimetric method based on the 4-(dimethylamino) cinnamaldehyde (DMAC) assay, as described by Reyes-Corral *et al.* (2022). Briefly, 50 µL of each extract or standard was individually mixed with 200 µL of 0.1% DMAC reagent in a 96-well microplate, and the reaction was allowed to develop for 5 min at 25 °C in the dark. Absorbance was recorded at 640 *nm*.

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For both total flavonoids and condensed tannins, a calibration curve was performed using catechin (concentrations ranging from 0.12 to 0.2 $mg \cdot mL^{-1}$) (Sigma-Aldrich, Mexico) as the standard. Results were expressed as mg of catechin equivalents per g of dry mass (mg $CE \cdot g^{-1}$).

The determination of reducing sugars was performed using the method proposed by Ávila-Núñez *et al.* (2012) with adjustments suggested by Valero-Galván *et al.* (2024). For quantification, 50 µL of each extract was deposited in independent 1.5 mL tubes in triplicate. Then, 150 µL of 3,5-dinitrosalicylic acid (DNS) solution (43 mM) was added to each extract, and the mixture was incubated for 5 min at 95 °C in a thermoblock (Scientific, USA). Finally, the tubes were left to sit on ice for 2 min. The same solvents used for extraction were used as the blank. Subsequently, each extract was deposited into independent cells in a 96-well microplate (Corning, Mexico) and absorbance was measured at 540 nm using a spectrophotometer (Hercules, USA) with the Microplate Manager 6.0 program (Bio-Rad, USA). A calibration curve was performed using glucose (concentrations ranging from 0.6 to 10 $mg \cdot mL^{-1}$) (Sigma-Aldrich, Mexico) as the standard. Data were expressed as mg glucose equivalents per g dry weight (mg $GE \cdot g^{-1}$).

Quantification of antioxidant activity

The antioxidant activities of reducing ferric ion (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were quantified using the method proposed by Moreno-Escamilla *et al.* (2017) with some modifications (Valero-Galván *et al.*, 2024). For FRAP quantification, 24 µL of each extract was deposited into independent cells in triplicate in a 96-well microplate (Corning, Mexico). The same solvent used for extraction was used as the blank. Next, 180 µL of FRAP reagent (10 mM 2,4,6-tri[pyridyl]-s-triazine TPTZ) (Sigma-Aldrich, Mexico); 300 mM $C_2H_3O_2Na$ (Sigma-Aldrich, Mexico); 20 mM $FeCl_3$ (Sigma-Aldrich, Mexico) was added, and the mix was incubated for 15 min at 25 °C in darkness. Absorbance was measured at 595 *nm* in a spectrophotometer (Hercules, USA) with the Microplate Manager 6.0 program (Bio-Rad, USA). For DPPH quantification, 25 µL of each extract was placed into independent cells in triplicate in a 96-well microplate (Corning, Mexico). Next, 200 µL of a 190 µM DPPH solution (Sigma-Aldrich, Mexico) was added and incubated for 15 min at 25 °C in the dark. The same solvent used for extraction was used as the blank. Subsequently, absorbance was measured at 517 *nm* using a spectrophotometer (Hercules, USA) with the Microplate Manager 6.0 program (Bio-Rad, USA). For both activities, a calibration curve was performed using TROLOX (concentrations ranging from 8 to 130 mM for FRAP and from 25 to 400 mM for DPPH) (Sigma-Aldrich, Mexico) as the standard. Results were expressed in µM of TROLOX equivalents per g of dry mass (µM $TE \cdot g^{-1}$).

Statistical analysis

Data were analyzed using SPSS statistical software (IBM, version 15.0, United States) to identify potential statistically significant differences in pigment and phytochemical content, as well as in antioxidant activity. A one-way analysis of variance (ANOVA) was performed, considering species as the independent variable. Multiple comparisons of means were also carried out using Tukey's method ($p \leq 0.05$). Canonical discriminant function analysis was performed to visualize species differentiation and explain variance. Finally, variables contributing to the species separation within this genus were identified using the canonical multiple correlation coefficients in the structure matrix.

Results and Discussion

Pigments contents

Significant interspecific variation in the stamen of pigments was observed among the *Opuntia* species. Specifically, levels of chlorophyll a (p = 0.04) and b (p = 0.04) ranged from 7.10 to 14.76 mg·g^{-1} and from 11.32 to 25.02 mg·g^{-1}, respectively, with *O. macrocentra* showing the highest values and *O. engelmannii* the lowest. In contrast, for xanthophyll content (p = 0.04), *O. arenaria* stamens had the highest values, whereas *O. macrocentra* had the lowest. Regarding carotenoids (p = 0.01), *O. microdasys* and *O. arenaria* stamens showed the highest and lowest values, respectively. Finally, with respect to betalains, *O. macrocentra* stamens exhibited the highest amounts of betacyanins (p = 0.02), betaxanthins (p = 0.01), and total betalains (p = 0.02), while *O. arenaria* stamens had the lowest concentrations of these pigments (Table 1).

Table 1. Pigment content in stamens of the four *Opuntia* species from the Sierra Samalayuca, Chihuahua, Mexico.

Pigments	Species			
	O. engelmannii	***O. arenaria***	***O. macrocentra***	***O. microdasys***
Chlorophyll a*	5.84±0.21^{a}	12.71±2.34^{b}	14.76±0.35^{b}	7.10±0.92^{a}
Chlorophyll b*	11.82±2.93^{a}	16.15±2.08^{ab}	25.02±2.82^{d}	21.3±1.03^{c}
Total chlorophyll*	23.95±4.08^{a}	28.86±4.42^{a}	29.22±4.36^{a}	28.39±0.77^{a}
Xanthophyll*	3.62±1.49^{ab}	4.85±1.21^{b}	1.66±0.27^{a}	2.70±1.80^{ab}
Carotenoids*	6.37±0.18^{a}	6.04±0.52^{a}	11.24±0.35^{b}	13.91±0.71^{c}
Betacyanins*	0.15±0.05^{ab}	0.14±0.01^{a}	0.22±0.08^{c}	0.18±0.71^{b}
Betaxanthins*	0.01±0.00^{a}	0.02±0.00^{a}	0.02±0.01^{a}	0.02±0.01^{a}
Betalains*	0.16±0.05^{a}	0.16±0.02^{a}	0.23±0.04^{b}	0.20±0.01^{b}

*Pigment content is expressed as mg per g of dry mass (mg·g^{-1}). Values represent means ± SD (n= 3) and were subjected to one-way analysis of variance (ANOVA, p < 0.05). Different letters within a row (a-d) indicate significant differences according to Tukey's test (p < 0.05).

Specific studies that quantify chlorophyll content in the stamens of the *Opuntia* genus are lacking. However, research on related cacti such as *Cylindropuntia* provides a useful comparative framework. In these species, chlorophyll a (12.58 - 49.51 mg·g^{-1}) and total chlorophyll (43.73 - 89.10 mg·g^{-1}) have been quantified in the stamens of *C. imbricata*, *C. spinosior*, *C. kleiniae*, and *C. arbuscula*. These values were lower than those observed in *Opuntia*, suggesting that *Opuntia* stamens may possess a greater photosynthetic pigment load or distinct tissue properties that favor higher chlorophyll accumulation (Madrigal-Hernández *et al.*, 2025).

In contrast, xanthophyll (1.66 - 4.85 mg·g^{-1}) and carotenoid (6.04 - 13.91 mg·g^{-1}) content in *Opuntia* were lower than those found in *Cylindropuntia*, where xanthophyll ranged from 2.98 to 29.37 mg·g^{-1} and carotenoids from 16.63 to 23.95 mg·g^{-1} (Madrigal-Hernández *et al.*, 2025). This may reflect species-specific differences in pigment allocation associated with stress tolerance or flower physiology, since xanthophylls and carotenoids are closely linked to photoprotective and antioxidant function in reproductive tissues.

Regarding betalains, interspecific variation was also observed. Betacyanins (0.14 - 0.22 mg·g^{-1}) and total betalains (0.16 - 0.23 mg·g^{-1}) in *Opuntia* stamens fell within the range previously reported for

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Cylindropuntia stamens (0.010 - 0.190 mg·g^{-1} for betacyanins and 0.020 - 0.270 mg·g^{-1} for betalains) (Madrigal-Hernández *et al.*, 2025). This congruence suggests that reproductive tissues in both genera share similar pathways for betalain synthesis and accumulation, likely shaped by shared evolutionary histories within the Cactaceae family.

Phytochemical content and antioxidant activity

No significant differences in phenolic content (p = 0.06) were observed among the four species analyzed (Table 2). Regarding flavonoid content (p = 0.01), the stamens of *O. arenaria* and *O. microdasys* exhibited significantly higher values than those of the other two species, with *O. engelmannii* displaying the lowest content (Table 2). Tannin levels (p = 0.001) were higher in *O. macrocentra* and *O. arenaria*, whereas *O. engelmannii* had the lowest values (Table 2). When measuring reducing sugars (p = 0.01), the stamens of *O. microdasys* showed significantly higher levels than the other species, while *O. macrocentra* had the lowest levels.

Table 2. Chemical content and antioxidant activity in stamens of the four *Opuntia* species from the Sierra Samalayuca, Chihuahua, Mexico.

Chemicals and antioxidant activity	**Species**			
	O. engelmannii	***O. arenaria***	***O. macrocentra***	***O. microdasys***
Phenolics*	6.31±0.22^{a}	5.07±0.60^{a}	6.17±0.63^{a}	6.56±1.42^{a}
Flavonoids**	3.66±0.50^{a}	4.81±0.41^{b}	4.26±0.27^{ab}	4.78±0.95^{b}
Condensed tannins**	0.001±0.00^{a}	0.03±0.01^{b}	0.04±0.00^{b}	0.002±0.00^{a}
Reducing sugar***	123.6±3.04^{b}	111.75±1.02^{b}	86.14±0.66^{a}	159.28±1.63^{d}
FRAP****	18.46±1.19^{a}	26.76±2.41^{b}	25.29±1.63^{ab}	27.47±4.86^{b}
DPPH****	10.64±1.59^{a}	17.42±2.27^{b}	13.95±0.84^{ab}	12.99±1.73^{a}

Values represent mean ± standard deviation (n= 3) and were subjected to one-way analysis of variance (ANOVA, p < 0.05). Different letters within a row (a-d) indicate significant differences according to Tukey's test (p < 0.05). *mg GAE·g^{-1}; **mg CE· g^{-1}; ***mg GE· g^{-1}; ***µM TE· g^{-1}.

Assessment of antioxidant activity using the FRAP method revealed significant differences among the species (p = 0.03) (Table 2). The stamens of *O. arenaria* and *O. microdasys* demonstrated the highest antioxidant capacity, with no significant difference between them. *O. macrocentra* exhibited intermediate values, and *O. engelmannii* had the lowest (Table 2). Additionally, analysis of antioxidant capacity using the DPPH method revealed that *O. arenaria* exhibited the highest activity, while *O. engelmannii* and *O. microdasys* showed the lowest, and *O. macrocentra* presented intermediate values (p = 0.004) (Table 2).

In the present study, the phenolic content in the stamens of the analyzed *Opuntia* species ranged from 5.07 to 6.56 mg GAE·g^{-1}, values lower than those documented for *Cylindropuntia* stamens (7.37-10.97 mg GAE· g^{-1}) and far below those documented in species such as *Nymphaeaceae antares* (110.61 mg GAE· g^{-1}) and *Crocus sativus* (126.31 mg GAE· g^{-1}) (Madrigal-Hernández *et al.*, 2025; Mamri *et al.*, 2024; Mohd-Zin *et al.*, 2021). However, phenolic levels in *Opuntia* were higher than those reported in stamens of *C. sativus* (0.034 mg GAE· g^{-1}), *Mangifera indica* (0.16 mg GAE· g^{-1}), *Terminalia catappa* (0.22 mg GAE· g^{-1}), and *Delonix regia* (0.32 mg GAE· g^{-1}) (Adeonipekun *et al.*, 2023; Jadouali *et al.*, 2016).

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Regarding flavonoids, values ranged from 3.66 to 4.81 mg CE· g^{-1}, comparable to those found in *Cylindropuntia* (2.58 - 3.78 mg CE· g^{-1}) (Madrigal-Hernández *et al.*, 2025). These concentrations exceeded those of *M. indica* (0.28 mg CE· g^{-1}) and *D. regia* (0.57 mg CE· g^{-1}) (Adeonipekun *et al.*, 2023), but were lower than the significantly higher levels in species such as *C. sativus* (14.10 mg CE· g^{-1}), *N. antares* (82.23 mg CE· g^{-1}), *N. lotus* (29.64 - 230.74 mg CE· g^{-1}), and *Punica granatum* (99.10 mg CE· g^{-1}) (Jadouali *et al.*, 2016; Mohd-Zin *et al.*, 2021; Tungmunnithum *et al.*, 2020; Zhang *et al.*, 2023).

Condensed tannin values ranged from 0.001 to 0.040 mg CE· g^{-1}, being also lower compared with those of *M. indica* (0.08 mg CE· g^{-1}), *T. catappa* (0.06 mg CE· g^{-1}), and *D. regia* (0.15 mg CE· g^{-1}) (Adeonipekun *et al.*, 2023).

Reducing sugar contents (86.14–159.28 mg GE· g^{-1}) were comparable to those reported in *Cylindropuntia* stamens (98.22 - 136.89 mg GE· g^{-1}) (Madrigal-Hernández *et al.*, 2025).

The antioxidant activity determined by FRAP (18.46 - 27.47 µM TE· g^{-1}) was also comparable to *Cylindropuntia* (18.08 - 26.00 µM TE· g^{-1}), but much lower than those obtained in *P. granatum* (963.18 µM TE· g^{-1}) and *N. lotus* (2405.25 µM TE g^{-1}) (Madrigal-Hernández *et al.*, 2025; Tungmunnithum *et al.*, 2020; Zhang *et al.*, 2023). Likewise, the antioxidant activity determined by DPPH (10.64 - 17.42 µM TE· g^{-1}) was lower than that obtained in *Cylindropuntia* (20.86 - 23.84 µM TE· g^{-1}), *P. granatum* (489.16 µM TE· g^{-1}), and *N. lotus* (3167.85 µM TE· g^{-1}) (Madrigal-Hernández *et al.*, 2025; Tungmunnithum *et al.*, 2020; Zhang *et al.*, 2023).

The variations observed in phytochemical content may arise from multiple interacting factors, including tissue type, environmental condition, genetic background, and the methods used for the extraction and quantification (Adeonipekun *et al.*, 2023; Astello-García *et al.*, 2015; Berrabah *et al.*, 2019). Secondary metabolites, such as phenolics, flavonoids, and tannins, often accumulate in response to biotic and abiotic stresses (Cárdenas-Sandoval *et al.*, 2012; Yang *et al.*, 2018). Their concentration is also influenced by nutrient availability; for instance, nutritional deficiencies frequently promote flavonoid accumulation (Sampaio *et al.*, 2016). Moreover, phenolic profiles vary among the plant organs and developmental stages (Ayan *et al.*, 2007; Cárdenas-Sandoval *et al.*, 2012).

Although multiple environmental and physiological drivers can modulate metabolite accumulation, the patterns observed in this study suggest that genetic variation among species plays a predominant role in determining phenolic, flavonoid, and tannin levels in *Opuntia* stamens. This is supported by previous studies showing consistent interspecific differences in stamen phytochemistry across genera (Cárdenas-Sandoval *et al.*, 2012; Feng *et al.*, 2024; Zhang *et al.*, 2023). The present results, therefore, highlight species-specific biochemical strategies that likely show intrinsic metabolic regulation rather than direct environmental alteration of floral tissues.

Hierarchical clustering and Principal Component Analysis

Hierarchical clustering revealed clear patterns in pigment and phytochemical compositions among the four *Opuntia* species (Figure 2A). Using pairwise clustering and Euclidean distance as the similarity metric, the species were grouped into three main clusters. The first cluster consisted of *O. arenaria* (Oa) and *O. engelmannii* (Oe), suggesting that these species share closely related pigment and phytochemical profiles. The second cluster consisted solely of *O. macrocentra* (Om), which displayed

a moderate degree of dissimilarity relative to the first group. The third cluster corresponded to *O. microdasys* (Omic), the most divergent species in the analyzed set, indicating that it has a clearly differentiated pigment and phytochemical compounds.

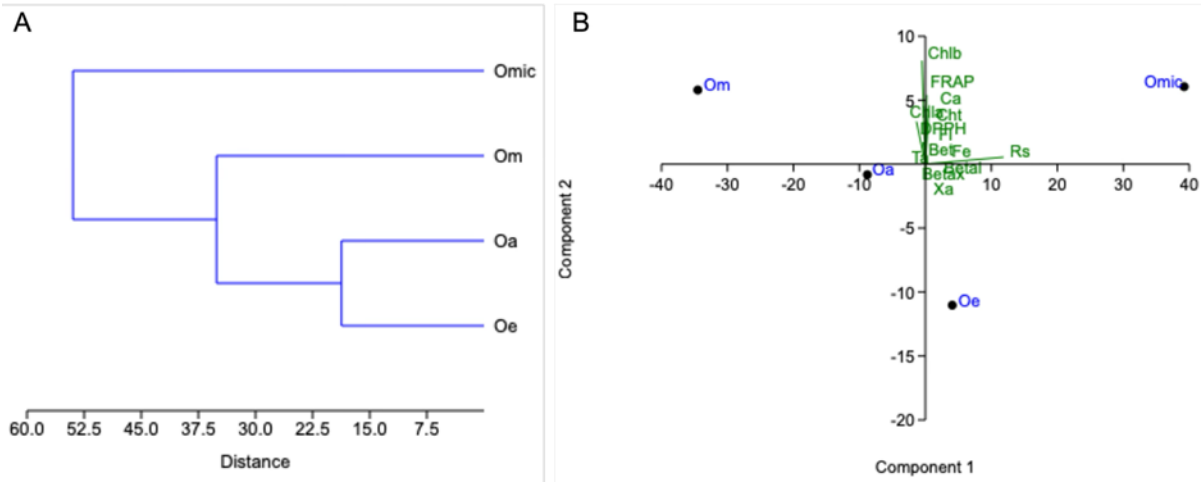

Figure 2. Hierarchical clustering analysis (A) and the biplot of the principal components analysis (B). *O. engelmannii* (Oe); *O. arenaria* (Oa). *O. macrocentra* (Om); *O. microdasys* (Omic).

Principal component analysis (PCA) was performed, and components were selected based on the Kaiser criterion (eigenvalues greater than 1). Under this criterion, the first two components were retained, together explaining 97.9% of the total variance (Figure 2B). The PCA revealed a clear separation among the four *Opuntia* species.

The first component (PC1) explained 91.8% of the total variance and showed a strong positive association with reducing sugar content (r = 0.99), indicating that this variable was the primary driver of interspecific differentiation. The second component (PC2) explained 6.2% of the variation and was positively correlated with chlorophyll b content (r = 0.676), carotenoids (r = 0.384), and antioxidant activity measured by the FRAP method (r = 0.453). These variables contributed secondary gradients that complement the dominant metabolic axis detected in PC1.

The PCA biplot (Figure 2B) further illustrates how these variables shape species differentiation. Species positioned on the positive side of PC1 exhibit higher concentrations of reducing sugars, whereas species on the negative side show lowered levels of these metabolites. Along PC2, species located higher in the plot display relatively elevated levels of chlorophyll b, carotenoids, and FRAP antioxidant activity. This vertical separation captures subtler biochemical differences not fully explained by PC1 alone.

The biplot configuration reflects the groupings identified by hierarchical cluster analysis. *O. arenaria* and *O. engelmannii* occupy overlapping regions of the multivariate space, *O. macrocentra* exhibits an intermediate but distinct biochemical profile, and *O. microdasys* is the most divergent species in the dataset. The agreement between PCA and clustering confirms the robustness of the identified phytochemical patterns.

The results obtained are consistent with those reported by Madrigal-Hernández *et al.* (2025), who observed similar patterns in the analysis of pigments and phytochemical compounds in stamens of four *Cylindropuntia* species. In that study, variables such as chlorophylls, reducing sugars, and antioxidant activity contributed to interspecific differentiation; however, it is important to note that the association of reducing sugars with antioxidant activity is indirect, as sugars do not act as antioxidants themselves but may reflect physiological adjustments related to carbon metabolism under stress conditions. Similarly, Berrabah *et al.* (2019) reported that soluble sugars and antioxidant activity were key determinants in the separation of *O. ficus-indica* populations, suggesting that both traits may serve as complementary indicators of the species' metabolic and ecological responses rather than representing a direct functional relationship. This supports the importance of these variables as indicators of secondary metabolism and adaptive capacity in *Opuntia* species.

Conclusions

This study constitutes the first phytochemical and functional characterization of *Opuntia* stamens from the Ciudad Juárez germplasm. Multivariate analysis revealed clear differentiation among species, highlighting both shared traits and distinctive phytochemical signatures. Among them, the stamens of *O. microdasys* emerged as a promising source of bioactive compounds, reinforcing the relevance of this species within the genus. Overall, these findings provide a solid foundation for advancing the identification and quantification of key secondary metabolites in *Opuntia* stamens. Future studies should focus on the extraction of specific compounds and the evaluation of their potential applications in functional foods, natural colorants, and biotechnological formulations.

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Not applicable

CONSENT FOR PUBLICATION

Not applicable

AVAILABILITY OF SUPPORTING DATA

Nor applicable

COMPETING INTERESTS

The authors declare that they have no competing interests.

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AUTHOR CONTRIBUTIONS

J.V.G, M.Q.M., and R.G.F. contributed to the design and implementation of the research, to the analysis of the results, and to the writing of the manuscript.

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