


# Comparative analysis of the antioxidant compounds of raw edible flowers and ethanolic extracts of *Cucurbita pepo*, *Tagetes erecta*, and *Erythrina americana* during storage

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## Abstract

This work determined the antioxidant compounds of the raw flowers of the zucchini plant, Mexican marigold, and colorin and their ethanolic extracts, which were stored by 270 days for their potential use as functional food. The DPPH<sup>•</sup> radical scavenging capacity was in a range of 50%–95% for the three raw flower species, but the ethanolic extracts of flowers (EEF) of the zucchini plant and colorin lost up to 80% of these compounds. The total phenols of raw flowers and EEF were of 0.3–2.5 mg gallic acid equivalents (GAE)/g. The total flavonoids were up to 85% higher in the EEF of the nondehydrated Mexican marigold than in the raw flowers. In the remaining treatments, there had no notable changes (~0.5 mg quercetin/g). Rutin, quercetin, myricetin, and lucenin were in the EEF of the nondehydrated samples. In conclusion, EEF of nondehydrated Mexican marigold showed higher antioxidant composition than remaining extracts.

## Practical applications

This study demonstrates the presence of antioxidant compounds in three edible flowers produced worldwide for ornamental and industrial purposes, except in Mexico, where they are endemic plants and are also consumed as food. Antioxidant compounds of the evaluated edible flowers can be found in nondehydrated samples and dehydrated, and they can be extracted and preserved for 270 days using cane alcohol as the solvent.

## 1 | INTRODUCTION

Mexico ranks fourth in the world in terms of plant diversity, and it has been reported that of the 26,000 plant species found worldwide, 9,500 are endemic to this country. Currently, there are no official reports about edible and nonedible flowers published by an international organization, including the Food and Agriculture Organization (FAO), the Food and Drug Administration (FDA), or the European Food Safety Authority (EFSA). Exclusively, European Regulation No. 258/97 relating to novel foods and food ingredients explains the safety of flowers. Thus, there are no authorized requisites concerning

the trading of edible flowers (Fernandes et al., 2017). Nowadays, it is known that edible flowers may have important applications providing health benefits based on fresh products and extracts due to their content of vitamins, minerals, and antioxidants compounds such as polyphenols and flavonoids (Pinakin et al., 2020; Pires et al., 2018; Zheng et al., 2021). Fernandes et al. (2017) pointed out that the nutritional components of edible flowers are not different from those of other plant organs; particularly, petals flowers are rich in vitamins, minerals, and antioxidants. However, reports on the nutritional composition and the development and implementation of technologies to preserve edible flowers collected and consumed locally are scarce until now.

The flowers of the zucchini plant (*Cucurbita pepo* L.), Mexican marigold (*Tagetes erecta*), and colorin (*Erythrina americana*) are three popular edible flowers in Mexico, and they are also known in other parts of the world. They are attractive to humans for their color, shape, odor, and medicinal and functional properties. The zucchini plant flowers were domesticated in Mexico and have become a main dietary ingredient (Zhou et al., 2017). Before using them as food, the pistils and the stamens are taken out from female or male flowers. Baljeet et al. (2016) reported that *C. pepo* flowers have phenols, flavonoids, and antioxidant capacity. These compounds may increase or decrease depending on the cooking method used during their preparation. In addition, the authors found that flavonoids are extracted in more significant quantities (56.8 mg quercetin equivalents/100 g) when ethanol is used as the solvent. Aquino-Bolaños et al. (2013) determined 334.6 mg/100 g of phenols and 62  $\mu\text{mol}$  of ascorbic acid equivalent/100 g as antioxidant capacity in the corolla of zucchini flower.

Following studies of flowers and the composition of their extracts, Siddhu and Saxena (2015) stated that *T. erecta* flowers are a good source of antioxidants, such as phenolics and flavonoids. The extraction of these compounds depends on the solvents used, being ethyl alcohol extract/water, the one that contains the highest content. Additionally, Moliner et al. (2018) found an important composition of phenols and antioxidants in two different cultivars of *T. erecta* (orange and yellow flowers), reporting 77 and 81 mg pyrogallol equivalent/g of extract, respectively, and 87% of DPPH<sup>\*</sup> inhibition in both cultivars. Fu et al. (2018) quantified 84.6 mg/g of phenols in *T. erecta* flowers using a microwave and enzyme coassisted aqueous two-phase extraction. Amadioha and Chidi (2019) showed that ethanolic and aqueous extracts (100 g sample/L solvent) of the dry leaves of *T. erecta* were an essential source of flavonoids (3.17% and 2.58%, respectively), although their stability over time was not evaluated. Kurniawan et al. (2019) also indicated that the lutein content decreases by up to 30% in the petals of dehydrated *T. erecta* at more than 40°C.

Another remarkable genus is *Erythrina*, which includes around 130 edible flowers species, including the colorin plant. Colorin is cultivated as ornamental trees and is widely disseminated in tropical and subtropical regions worldwide. The whole colorin plant or a part of it is employed in traditional folk medicine and as food. As a case in point, the methanolic extract of *E. indica* leaves possesses antiulcer properties associated with its phenols (Sachin & Archana, 2009). According to Ordaz et al. (2014), *E. herbacea* root extracts (aqueous, acetone, and hexane) are not carcinogenic and could be used for medicinal purposes. In other studies, carboxylic, gallic, and caffeic acids, alkynes, diterpenes, triterpenes, alcohols, and hydrocarbons were found in ethanol extract of *E. variegata* leaf, becoming a high potential source of natural remedies and for feeding (Muthukrishnan et al., 2016).

Based on the previously outlined reports, establishing the process conditions to preserve the antioxidants content of the above-mentioned edible flowers represents an important contribution

which eventually would favor their consumption as a functional food. Another contribution in the field of study is the implementation of statistical models for data analysis to obtain predictive equations that describe the correlation between a dependent and an independent variable. In this sense, as Mahmoudi (2019) reported, the use of nonlinear regression models represents an alternative because it provides sufficient judgment elements for decision-making under uncertain conditions.

This work aimed then (1) to evaluate the antioxidant capacity, total phenols, and total flavonoids of raw flowers of zucchini plant, Mexican marigold, and colorin (dehydrated and nondehydrated) and their corresponding ethanolic extracts, (2) to predict the content of total phenols as important antioxidants compounds in the ethanolic extracts of flowers (EEF) using nonlinear regression models, and (3) to identify and quantify, using Thin-Layer Chromatography (TLC) and high performance liquid chromatography (HPLC), the significant flavonoids in the ethanolic extract of the nondehydrated three flowers species.

## 2 | MATERIALS AND METHODS

### 2.1 | Plant material characteristics and experimental conditions

The flowers of the zucchini plant, Mexican marigold, and colorin were organically grown in Yautepec, Morelos, Mexico (18°53'00" Latitude and -99°04'00" Longitude). These were collected in October 2017 (25.3°C and 216 mm of precipitation) and colorin in March 2018 (29.1°C and 12 mm of precipitation). Flowers were transported to the Postharvest Laboratory of Agricultural Products of CEPROBI-IPN. Later, the petals were separated and removed from the flowers, washed, and disinfected in sodium hypochlorite solution at 200 ppm for 3 min. The petals were cut into 4- to 5-mm squares. One hundred fifty grams of petals for each flower species were then weighed, of which 75 g were dehydrated at 28°C, and the remaining petals were used as the nondehydrated flower. Three repetitions per treatment were used, and the experimental unit was 25 g. Two treatments were evaluated for each flower: (1) dehydrated flowers and (2) nondehydrated flowers.

### 2.2 | Moisture content determination in petals of flowers

This variable was determined by the thermogravimetric method described by Horwitz and Latimer (2005). The method is based on the difference between the initial weight of flowers and the weight reached after drying at 28°C, using a convection oven (Olg-Científica, Mexico). The results were reported as moisture content and expressed as a percentage.

### 2.3 | Elaboration of EEF

EEF was elaborated as recommended by Silva (2012) to obtain ethanolic extracts measurable by spectrophotometric techniques. Sugar cane alcohol (Milab, Mexico) as the extraction solvent was used, of which 125 ml was added to the petals of the zucchini plant and colorin and 300 ml to the Mexican marigold petals. The solvent covered the 25 g of the nondehydrated flower petals completely; for dehydrated flowers, 1.13 g of zucchini plant, 1.78 g of Mexican marigold, and 2.13 g of colorin were used. The blend was placed in the dark for a week to obtain the EEF and then filtered to remove the nondehydrated flower petals or dehydrated ones. The extract was pasteurized for 20 min at 65°C and stored at 28°C in darkness. The antioxidant content was evaluated every 15 days for the first 6 months and then every month until 270 days.

### 2.4 | DPPH<sup>•</sup> radical scavenging capacity assay in flower petals and EEF

The method proposed by Sharma et al. (2015) was followed for quantifying the DPPH<sup>•</sup> radical scavenging capacity. Two grams of nondehydrated flower petals and, for dehydrated flowers, 0.09, 0.152, and 0.17 g for zucchini plant, Mexican marigold, and colorin, respectively, were considered. Flower petals and 5 ml of methanol, analytical grade (Hycel, Mexico), were homogenized, and the resulting blend was centrifuged for 10 min at 3,448 g (8,000 rpm, Eppendorf centrifuge, USA). For quantifying the DPPH<sup>•</sup> radical scavenging capacity of EEF and previous to its analysis, 2.5 µl of the EEF was blended with 247.5 ml of methanol, analytical grade. Later, the final reaction involved blending of 750 µl of 133-µM DPPH<sup>•</sup> radical and 250 µl of the sample used as an antioxidants source. Subsequently, the blend was reserved for 30 min without light, and after, it was evaluated at 517 nm (Thermo Scientific, USA). The results were reported as a DPPH<sup>•</sup> scavenging percentage.

### 2.5 | Total phenols and flavonoids in flower petals and EEF

The total phenols analysis was made following the methodology reported by Singleton et al. (1999). For nondehydrated flower petals, two grams were used and, in the case of dehydrated flower petals of the zucchini plant, Mexican marigold, and colorin, 0.09 g, 0.152 g, and 0.17 g, respectively. The flower petals were macerated with 5 ml of 80% methanol, analytical grade (Hycel, Mexico). The homogenate was centrifuged at 3,448 g (8,000 rpm) at 25°C for 10 min, and the aqueous phase was recuperated as a source of phenols. On the other hand, 150 µl of EEF was blended with 3.85 ml of distilled water, and it was considered the phenols extract.

For the phenols extract (0.02 ml), 0.25 ml of Folin-Ciocalteu (Hycel, Mexico) and 0.75 ml of sodium carbonate at 20% were blended and made up to 5 ml using distilled water. The blend was reserved for

2 hr in the dark at 28°C. Later, it was evaluated at 760 nm (Genesys 10s spectrophotometer). For its quantification, a standard curve of gallic acid was employed as a standard compound. Each sample was made in triplicate, and the results were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight using a standard curve of gallic acid (10–1,000 µg;  $y = 0.0009x + 0.0315$ ;  $R^2 = 0.99$ ).

On the other hand, the total flavonoids were evaluated in flower petals and EEF, according to Chougui et al. (2013). For the extraction in the nondehydrated and dehydrated flower petals, 2 g of the sample (raw or equivalent value after the drying process) was macerated with 5 ml of 80% methanol, analytical grade (Hycel, Mexico). The homogenate was centrifuged at 3,448 g (8,000 rpm; Eppendorf centrifuge, USA) and 25°C for 12 min, and the aqueous phase was recovered. In addition, EEF was directly recovered for analysis. After that, the reaction consisted of blending 1.5 ml of supernatant or EEF and 1.5 ml of AlCl<sub>3</sub>. At the end of 30 min, the reaction was evaluated at 430 nm (Spectrophotometer Genesys 10s). The total flavonoids were quantified through a standard curve of quercetin (20–110 µg;  $y = 0.2062x - 0.0009$ ;  $R^2 = 0.98$ ) and expressed as milligrams of quercetin per gram of dry weight.

### 2.6 | Nonlinear regression model

Only the experimental data of the phenols quantified from EEF elaborated with nondehydrated and dehydrated samples were adjusted to a nonlinear regression model. Data were analyzed with Statgraphics Centurion 18 software. The following equation was used to estimate phenols content in the EEF:

$$f(x) = \text{Log } y\sqrt{x}$$

### 2.7 | Identification of flavonoids in EEF elaborated with nondehydrated samples using TLC and HPLC

The EEF was freeze dried, and then 2 g of the sample was purified by column chromatography using a glass column (400 × 22 mm) packed with silica gel 60 F254 F254 (Merck, silica gel 60 RP-18 F254 S, USA). A blend of water (25 ml) and acetonitrile suitable for HPLC (5%–50%, 5% added every five fractions) was used for eluting the sample. All the recovered fractions were set up on silica gel 60 F254 chromatographic plates (10 × 5 cm). Samples were eluted with a blend of water/acetonitrile (70:30). Polyethylene glycol was used as the developer for TLC. Fractions that showed a yellow–orange color characteristic of flavonoids were analyzed by HPLC (Hertog et al., 1992).

The chromatographic analysis was fulfilled by Waters 2695 separation module system. It was supplied with a Waters 996 photodiode array detector and Pro empowerment software (Waters Corporation, USA). Supelcosil LC-F column of 4.6 mm × 250 mm internal diameter, 5-µm particle size (Sigma-Aldrich, USA) to the extract separation was employed. The 0.5% aqueous solution of trifluoroacetic acid as

Solvent A (Sigma Aldrich, USA) and acetonitrile as Solvent B (BDH Chemicals, USA) were the mobile phases. The gradient systems are as follows: 0–1 min, 0% B; 2–4 min, 10% B; 5–7 min, 20% B; 8–14 min, 30% B; 15–18 min, 40% B; 19–22 min, 80% B; 23–26 min, 100% B; and 27–28 min, 0% B. The initial flow conditions (0% B, 0.9 ml/min) were continued for 30 min. Ten microliters of a sample was injected.

The identification and quantification of flavonoids were made from a standard curve of rutin ( $y = 8,290.1x - 26,698$ ;  $R^2 = 0.994$ ), and quercetin glycoside ( $y = 16,214x - 61,310$ ;  $R^2 = 0.999$ ) is evaluated in the range of values from 0 to 100  $\mu\text{g/ml}$  and of myricetin (0–250  $\mu\text{g/ml}$ ;  $y = 4,233.2x - 56,325$ ;  $R^2 = 0.979$ ), myricetin glycoside (0–250  $\mu\text{g/ml}$ ;  $y = 11,745x + 83,075$ ;  $R^2 = 0.976$ ), and lucenin (0–500  $\mu\text{g/ml}$ ;  $y = 3,755.7x - 15,315$ ;  $R^2 = 0.999$ ). The standard compounds were acquired of Sigma-Aldrich, USA, and assessed using 1 mg/ml (Wagner & Bladt, 1996).

## 2.8 | Statistical analysis

Data were analyzed in triplicate by analyzing variance (ANOVA) with a factorial arrangement (InfoStat Statistical version 2017). Tukey's test was employed for comparing means ( $p < .05$ ).

## 3 | RESULTS

### 3.1 | Moisture content

There were no significant differences in the moisture content of the petals of the three tested flowers, with corresponding values from 91% to 96% (Table 1).

### 3.2 | DPPH<sup>•</sup> radical scavenging capacity assay

All the nondehydrated raw flowers and the EEF showed higher DPPH<sup>•</sup> radical scavenging capacity than the dehydrated ones. The highest loss of DPPH<sup>•</sup> radical scavenging capacity was observed in the raw flowers of the zucchini plant (up to 50%) after the drying process (Figure 1a). In comparison, the raw flowers of Mexican marigold and colorin lost approximately 10% and 20%, respectively, compared with dehydrated flowers. The EEF of the zucchini plant (nondehydrated and dehydrated) had a very low concentration

**TABLE 1** Moisture content determination of three edible flowers

Flower petals	Moisture content (%)
Zucchini plant	95.5 ± 0.4 <sup>a</sup>
Mexican marigold	92.9 ± 0.9 <sup>a</sup>
Colorin	91.5 ± 3.4 <sup>a</sup>

Note: The different letters indicate significant differences ( $p < .05$ ).

of antioxidants with values of approximately 5% during storage (Figure 1b,c). Conversely, the EEF of Mexican marigold (nondehydrated) showed fivefold and fourfold more antioxidant capacity than the EEF of the zucchini plant and colorin, respectively, for up to 180 days of storage and similar content to that of the nonstored flowers. Additionally, although EEF of Mexican marigold was prepared with 2.4-fold more solvent than the remaining ones, their antioxidant composition was the highest. On the other way, the EEF of dehydrated colorin preserved their DPPH<sup>•</sup> radical scavenging capacity for 270 days, but in the nondehydrated samples, the DPPH<sup>•</sup> radical scavenging capacity was about 100% for 75 days only.

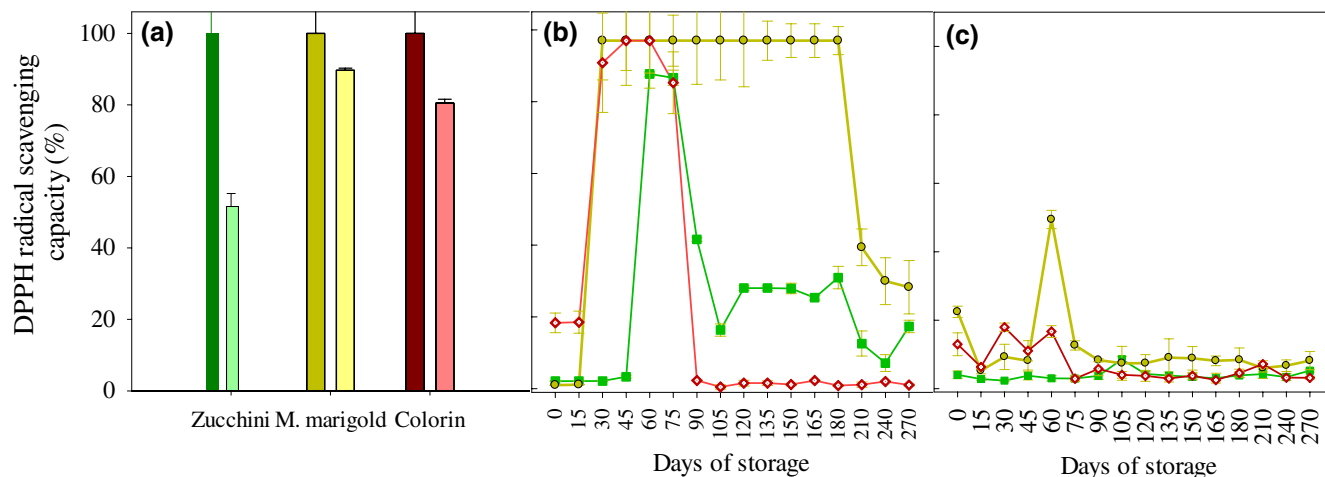
### 3.3 | Total phenols and flavonoids

The total phenols and flavonoids were higher in the nondehydrated flowers and EEF than the dehydrated ones. Initially, the total phenols content of the three plant species exhibited significant differences ( $p < .05$ ) concerning the dehydration process and type of flower. The dehydrated flowers of Mexican marigold, zucchini plant, and colorin had 8-, 50-, and 100-fold lower total phenols than the nondehydrated ones (Figure 2a). On the other hand, the EEF of the nondehydrated samples showed from 90% to 95% higher phenols than dehydrated ones, being notably different. For the EEF of the three nondehydrated flowers, the highest values (1–2 mg GAE/g dry weight) were obtained until 75 days of storage (Figure 2b); after, there was a decrease in the content of these compounds. The EEF (dehydrated) did not show a significant difference ( $p < .05$ ) in the phenolic content during storage (~0.025 mg GAE/g dry weight; Figure 2c).

For the raw flowers, the total flavonoids in the dehydrated Mexican marigold, colorin, and flowers of the zucchini plant were 52-, 21-, and 30-fold higher than those in the nondehydrated ones. Specifically, the Mexican marigold raw flowers (nondehydrated and dehydrated) had up to sevenfold more flavonoids content than the remaining flowers (Figure 2d). On the other hand, the EEF of the zucchini plant and colorin (nondehydrated and dehydrated) were almost similar during storage. Overall, the corresponding average values were 0.02–1.8 mg quercetin/g dry weight, respectively (Figure 2e,f). Concerning the EEF of the Mexican marigold, significant differences ( $p < .05$ ) were observed in terms of dehydration processing of the flowers but no during the given storage days. After 30 days of storage, the nondehydrated EEF of these plant species had higher values (7–22 mg quercetin/g dry weight) than the dehydrated ones (0.4–0.7 mg quercetin/g dry weight).

### 3.4 | Modeling phenols content in EEF using nonlinear regression

The nonlinear regression model had coefficients of determination in the range of values from 0.71 to 0.91. This suggests that the nonlinear regression model could estimate phenols content in EEF over storage in all cases (using dehydrated and nondehydrated samples).



**FIGURE 1** DPPH<sup>\*</sup> radical scavenging capacity of raw edible flowers (a), and nondehydrated (b), and dehydrated EEF (c) of zucchini plant (■), Mexican marigold (■), and colorin (■) stored for 270 days. All data were calculated based on the dry weight of the samples. In Figure (a), the bar with the highest color intensity represents the nondehydrated raw flowers and the dehydrated ones with the lowest color intensity

Additionally, it was observed that all experimental data of EEF with nondehydrated samples had a maximum peak during the 100 days of storage (Figure 3a,c,e); however, in the dehydrated ones, this was no evident (Figure 3b,d,f).

### 3.5 | Identification and quantification of flavonoids by TLC and HPLC

Flavonoids composition was different for each plant species. For example, the EEF obtained from the nondehydrated flowers of the zucchini plant showed 30 fractions. It was confirmed in the 22 and 25 flavonoids by TLC (Figure 4a), using ultraviolet light to observe their fluorescence (yellow–orange coloration). On the other hand, the EEF of the nondehydrated Mexican marigold showed flavonoids in the 21 and 32 fractions (Figure 4b), whereas in terms of the EEF of colorin, the flavonoids were observed in the 22, 23, 25, and 26 fractions (Figure 4c).

Under other conditions, different flavonoids in each EEF were detected by HPLC. In the EEF of the zucchini plant, rutin and quercetin glycoside were identified as significant flavonoids. The Mexican marigold extracts had myricetin and myricetin glycoside, and, in the case of colorin, the flavonoid lucenin was also detected (Table 2). Additionally, the EEF of Mexican marigold had the highest flavonoid content. The identification of flavonoids was followed using reference standards (Figure 5).

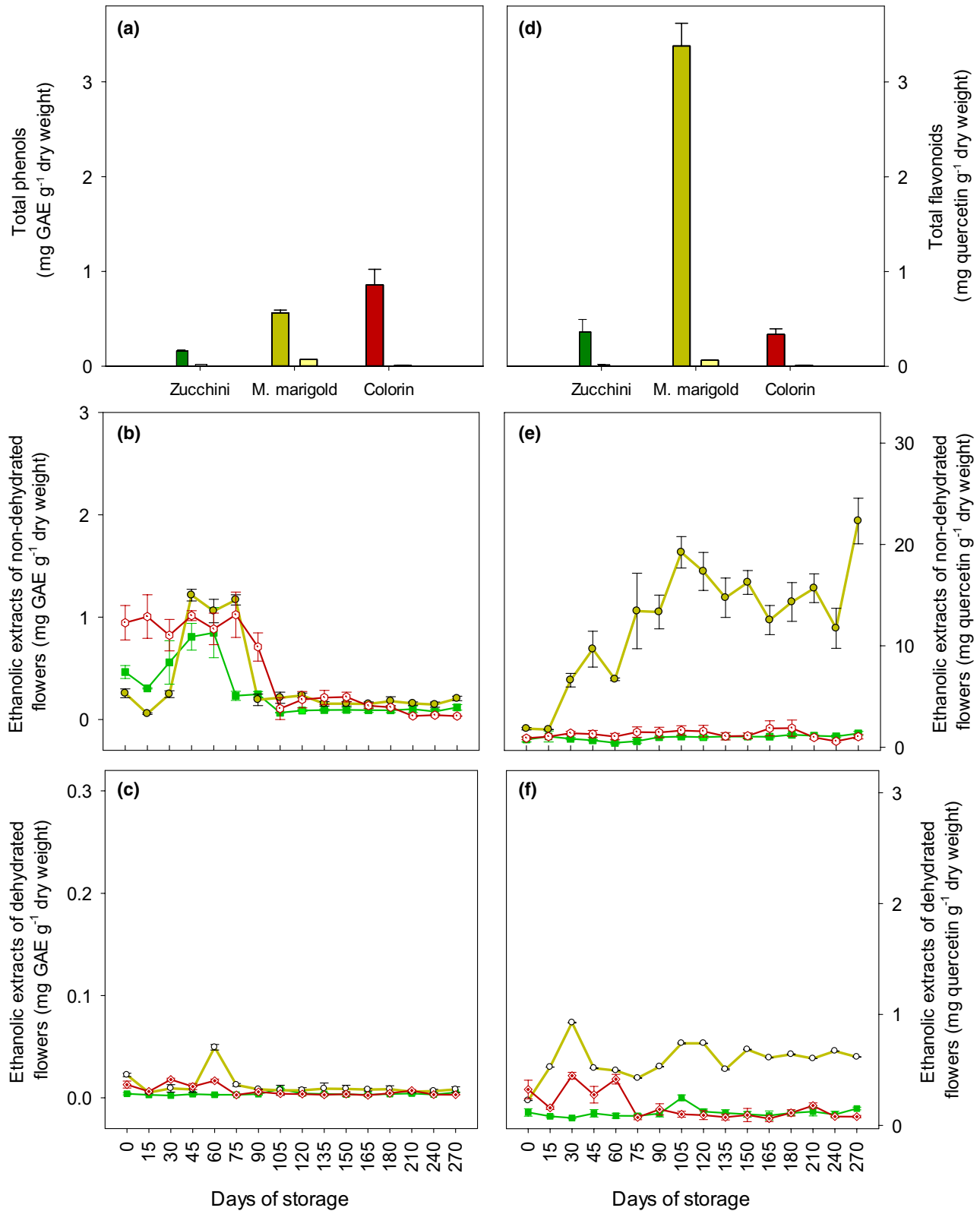
## 4 | DISCUSSION

For the moisture content, it is possible that the similar values obtained in the three flowers species be attributed to that only petals were used, which are not water reserve plant organs. Alike, Sotelo et al. (2007) reported a water content of 93.2% and 86.6% in the

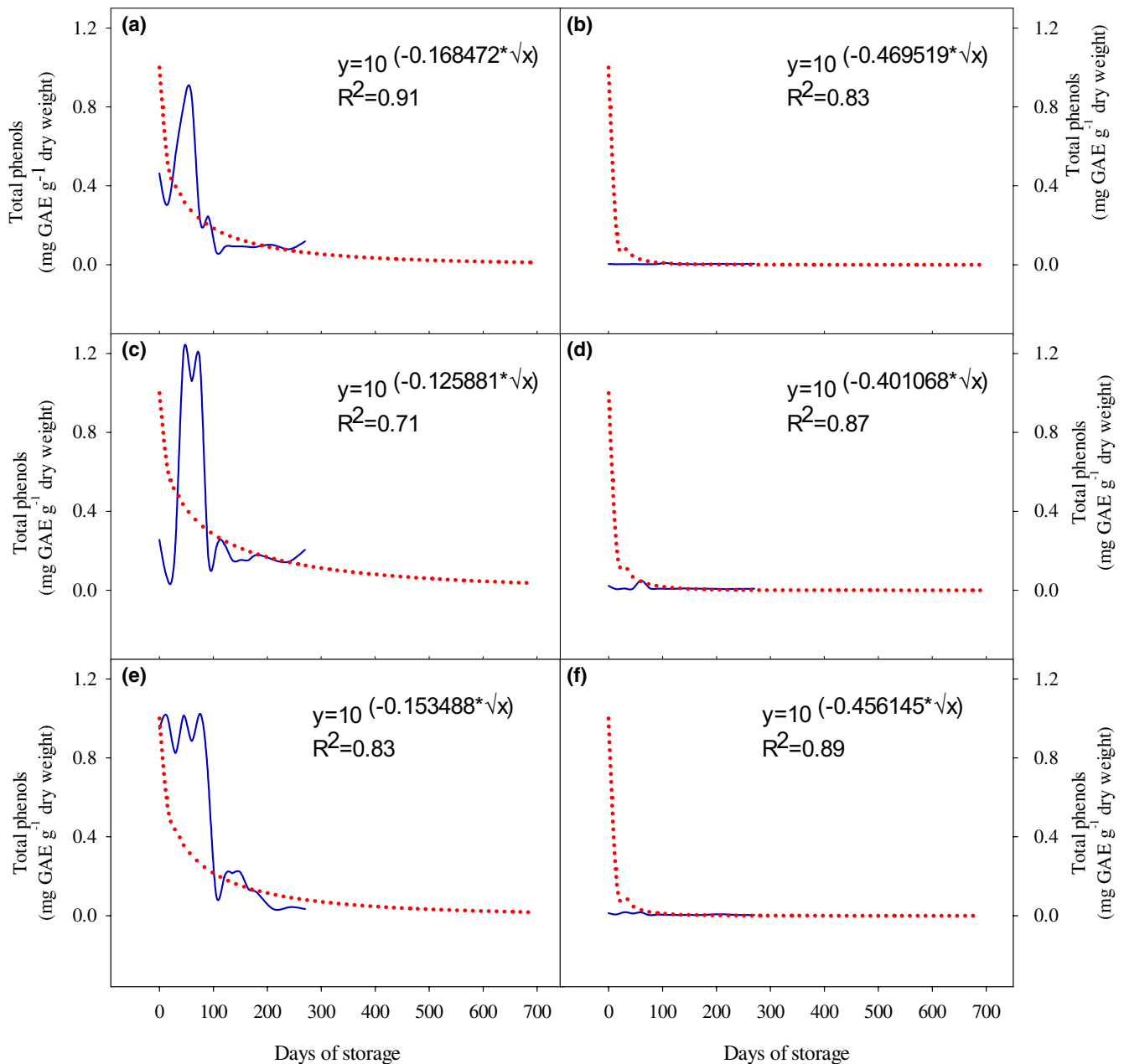
flowers of the zucchini plant and colorin, respectively. Apart from adding freshness and exotic aroma, delicate taste, and attractive appearance, edible flowers also provide health benefits for the consumer. They contain biologically active compounds, including phenols. This group of compounds is in part responsible for the color and antioxidant content (Lara-Cortés et al., 2013, 2014). According to the literature, yellow and red flower petals could be associated with content of phenols and flavonoids. These compounds are primarily responsible for the flowers' antioxidant capacity, as Fernandes et al. (2017) reported.

*In vitro* antioxidant methods and the estimation of total phenolic content using colorimetric assays not only are used for an idea of potential beneficial effects but also for quality control of natural products and foods and as low-cost, high-throughput tools used to discover potential antioxidant sources (Granato et al., 2018). The antioxidants in flowers are water or fat soluble; with this in mind, evaluating the antioxidant capacity was dependent on the type of compounds contained in the extracts. Particularly, DPPH<sup>\*</sup> radical was used in this study to evaluate the ability of flowers and EEF to scavenge this free radical. During the interaction between antioxidants and DPPH<sup>\*</sup> radical, the first transfer an electron or hydrogen atom to DPPH<sup>\*</sup>, neutralizing its free radical character (Naik et al., 2003). The DPPH<sup>\*</sup> radical scavenging capacity of the three edible flowers studied (95%) was reached using 0.4 g/ml (sample/solvent) in comparison with the values obtained for fruit and vegetables such as strawberry (60%), tomato (58%), and bell pepper (54%), which were evaluated at concentrations of 0.1 g/ml, thus, evidencing their possible use as a functional food (Black-Solis et al., 2019; González-Saucedo et al., 2019; Ventura-Aguilar et al., 2018).

For edible flowers, Hamissou et al. (2013) quantified a DPPH<sup>\*</sup> scavenging of 12.19% in zucchini fruit but not in the flowers. Despite that, the DPPH<sup>\*</sup> radical scavenging capacity reported was fivefold lower than that quantified in flowers, evidencing its potential use as an antioxidant source. Additionally, Kaisoon et al. (2011) informed



**FIGURE 2** Total phenols and flavonoids of raw edible flowers (a and d), and EEF elaborated with nondehydrated (b and e), and dehydrated flowers (c and f) of zucchini plant (■), Mexican marigold (■), and colorin (■) stored for 270 days. All data were calculated based on the dry weight of the samples. In (a) and (d), the bars with the highest color intensity represents the nondehydrated raw flowers and the dehydrated ones with the lowest color intensity. GAE, gallic acid equivalents

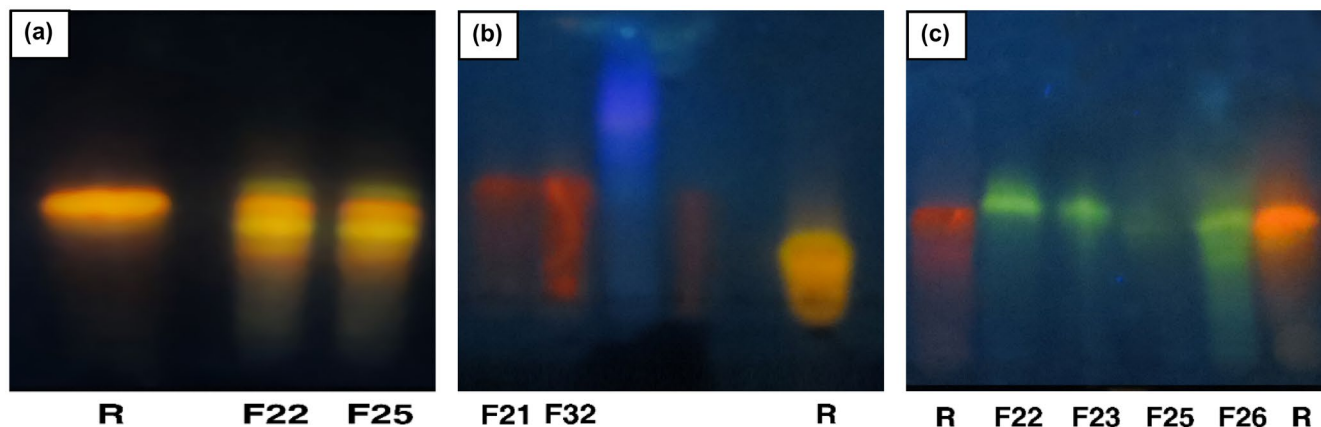


**FIGURE 3** Nonlinear regression model for describing phenols content response in ethanolic extracts of flowers (EEF) to storage. Equations are given in each figure. Experimental data (—). Data predicted by the model (.....). (a), (c), and (e) represent EEF elaborated with nondehydrated flowers of zucchini plant, Mexican marigold, and colorin. (b), (d), and (f) are EEF elaborated with dehydrated flowers. GAE, gallic acid equivalents

that 20 mg/ml of *T. erecta* neutralizes 94.32% of the DPPH<sup>•</sup> radical, which coincides with the obtained values in the present study. For colorin flower, the DPPH<sup>•</sup> radical scavenging capacity was positively affected by the dehydration process at 28°C, contrary to the findings Bernardino-Nicanor et al. (2016). They highlighted that the drying process at 105°C increased by almost eightfold the antioxidant activity of young *E. americana* flowers (IC<sub>50</sub> of 71.4 and 560 mg/ml, dry and fresh flowers, respectively). This could be due to the high temperatures that caused the caramelization of the sugar contained in the sample. Consequently, the DPPH<sup>•</sup> radical scavenging capacity quantified by the colorimetric method such as DPPH<sup>•</sup> was

overestimated. Moreover, previous reports showed that dehydrated Mexican marigold petals are a better choice for elaborating aqueous extracts than those of roots, stems, and leaves (35, 20, and 10 equivalents of Trolox μmol/g, respectively) because they had a higher antioxidant capacity (145 equivalents Trolox μmol/g) (Kazibwe et al., 2017).

The total phenols in raw Mexican marigold and colorin, as well as their EEF obtained in the nondehydrated samples (0.5–2 mg GAE/g dry weight), were similar to than reported in broccoli (0.88 mg/g), lettuce (1.07 mg/g), spinach (1.12 mg/g), pear (1.25 mg/g), and pineapple (0.94 mg/g) (Reis, 2013). Phenols could be the essential



**FIGURE 4** Identification of flavonoids in ethanolic extracts of nondehydrated flowers of zucchini plant (a), Mexican marigold (b), and colorin (c) using Thin-Layer Chromatography. R = positive control (quercetin) and F = fractions containing flavonoids

**TABLE 2** Flavonoids identified in the nondehydrated EEF of zucchini plant, Mexican marigold, and colorin, using HPLC

Flavonoids	Retention time (min)	mg/ml ethanolic extract of flowers
Flowers of the zucchini plant		
Rutin	8.9	7.3
Quercetin glycoside	9.3	6.2
Flowers of Mexican marigold		
Myricetin	8.5	71.2
Myricetin glycoside	9.1	4.6
Flowers of colorin		
Lucenin	8.5	0.114

EEF = ethanolic extracts of flowers; HPLC = high performance liquid chromatography.

contributor to the antioxidant capacity of flowers, as reported on *E. variegata* by Li et al. (2014). According to Koffi et al. (2010), phenols are soluble in organic solvents, ethanol being the most efficient solvent for their extraction in *Terminalia catappa*, *Combretum molle*, *Annona senegalensis*, *Arachis hypogea*, and *Hibiscus sabdariffa* plants; all of which are widely recognized for their content of phenols. Therefore, it could explain why a similar phenols content was quantified in flowers and the EEF in our research. In addition, the preservation of these compounds during the storage of ethanolic extracts of dehydrated flowers could be associated with the pasteurization process, which avoids enzymatic activity in samples. On the other side, the zucchini plant flowers have a high water content that increased the phenols' solubility in the EEF and allowed a greater quantification of these compounds than in the flowers. It could be due to that the addition of water to organic solvents weakens the hydrogen bonds among polyphenols and compounds such as the proteins or sugars present in the plants (Rajbhar et al., 2015).

Furthermore, it was observed that the phenols from flowers of the zucchini plant were higher significant than those described by Tarhan et al. (2007), who obtained 98.8% fewer total phenols using the complete flower, compared with our results, where only

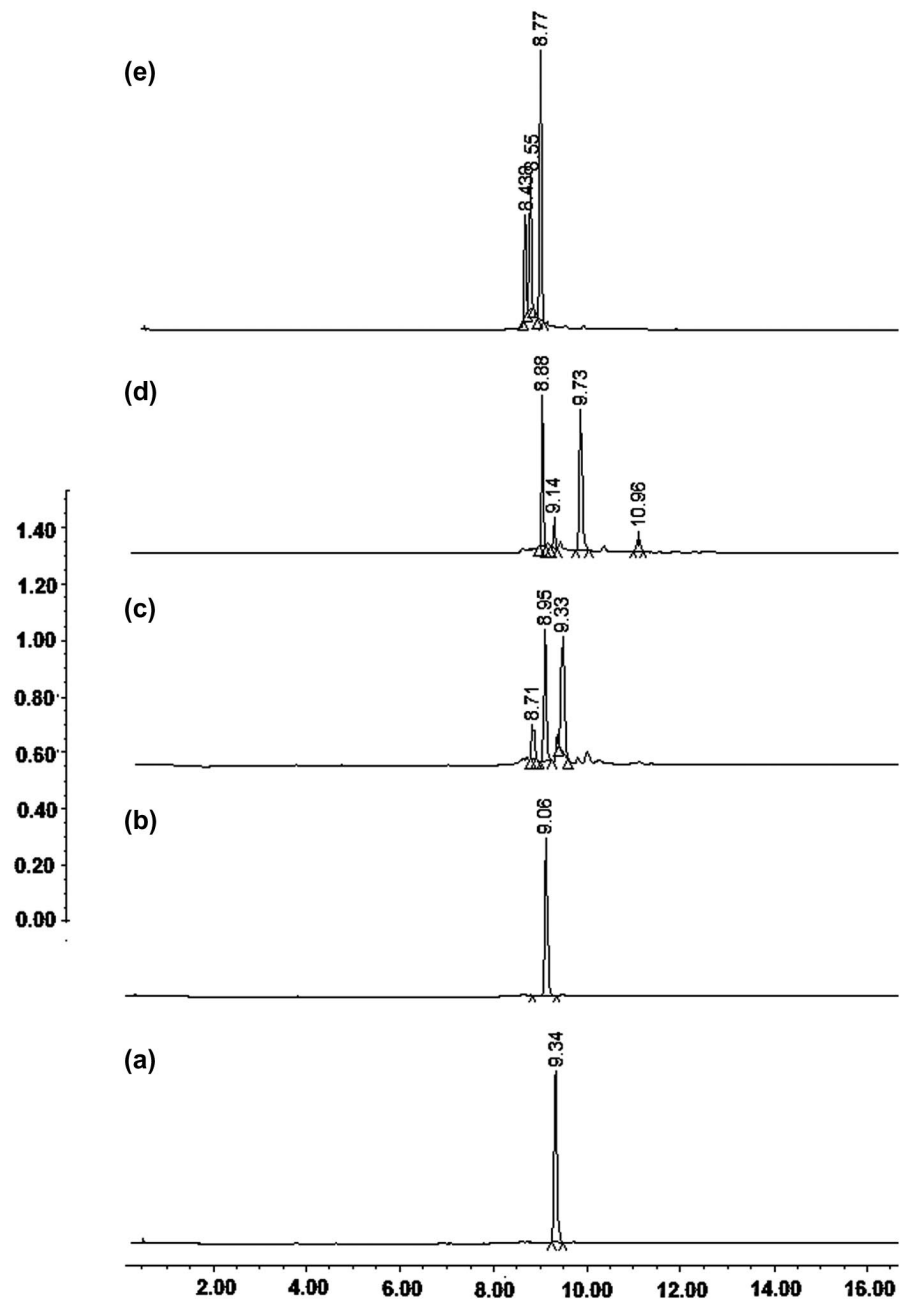
the flower petals were used (0.117 and 10  $\mu\text{g}$  GAE/ $\mu\text{g}$  dry biomass, respectively). Another critical behavior observed in this work was the content of phenols when dehydrating them. On this, Bernardino-Nicanor et al. (2016) found that the floral bud of colorin lost 54% of its phenols when dehydrated (362–167 mg/ml). In this case, some phenols could be subjected to degradation (thermal or oxidative) during the dehydration process. Therefore, the phenols in flowers and EEF offer an alternative for their use in the food industry; to date, they are only required as natural dyes, for development of bioactive films, as compounds with prebiotic properties, and as enhancers of physicochemical properties of starch, which is added to different foods (de Araújo et al., 2021).

Following phenols, flavonoids participate in the brilliant blue, scarlet, and orange shades in leaves, flowers, and fruit. From a sensory point of view, it is well documented that these compounds contribute to the bitter and astringent oral sensation of food and beverages and enrich the food models (Bucalossi et al., 2020). These compounds are glycosylated derivatives and are recognized by scavenging reactive oxygen species such as superoxide anion, peroxy, alkoxy, and hydroxyl radicals; hence, they can also function as stress protectants in plants and humans (Pier-Giorgio, 2000), and their presence in edible flowers and EEF were significant. According to our results, similar flavonoid content in raw flowers and their ethanolic extracts prepared with a dehydrated sample were quantified, except for Mexican marigold. Specifically, this plant species showed a considerable increase in flavonoids compared with the other species. Noteworthy, the plant species evaluated are different in terms of their composition. Pot marigold (*Calendula officinalis*) contains carbohydrates, phenols, steroids, terpenes, carotenoids, and tocopherols, and some unusual fatty acids such as calendic acid are accumulated in storage tissues. Fatty acids could then be avoiding the release of flavonoids from the vacuole and cell wall and, consequently, their degradation during the dehydration process and the pasteurization of EEF (Dulf et al., 2013).

On the other hand, the increase of flavonoids in the EEF of non-dehydrated Mexican marigold compared with the raw flowers could be because some flavonoids' solubility increased by the solvent type



**FIGURE 5** Fingerprint of commercial standards: quercetin-3-O-glucoside (a), rutin (b) and flavonoid fractions from ethanolic extract of flowers of the zucchini plant (c), Mexican marigold (d), and colorin (e). Each chromatogram was recorded at a specific wavelength for flavonoids (350 nm)



and the extraction temperature. For instance, Benavente-García et al. (2001) found 30-fold more neohesperidin dihydrochalcone (NHDC) using a temperature of 20°C and ethanol compared with water as a solvent. In addition, they reported that NHDC solubility increased sharply in terms of the effect of temperature, quantifying 85% higher content using a temperature of 75°C than an of 20°C (12 and 790 g/L, respectively). Additionally, He et al. (2015) found that extracts of *Pyrus pashia* flowers modified their flavonoids content according to the solvent used. For example, the highest flavonoid content for this flower was quantified in ethyl acetate extract followed by the butanol fraction, crude extract, petroleum ether fraction, and aqueous fraction (from 278.8 to 51.2 mg rutin equivalents/g dry extract).

Nowadays, there are scarce reports that evidence the use of nonlinear regression models as a mathematical tool to predict the behavior of antioxidant compounds as phenols during storage of edible flowers. Archontoulis and Miguez (2015) informed that nonlinear models are more easily interpretable because the parameters could be biologically meaningful. The results obtained showed an adjustment of the experimental data to the model; hence, it could predict the content of phenols in the EEF using dehydrated and nondehydrated samples. Another characteristic is that their predictions tend to be more robust than competing polynomials, especially outside the range of the observed data (i.e., extrapolation). Chen et al. (2012) studied different linear and nonlinear regression models to explain the antioxidant activity in dry green tea leaves. The nonlinear model

referred to as support vector regression (SVMR) showed an accurate adjustment to experimental data ( $R^2 = 0.98$ ). Alike, Jurinjak et al. (2018) informed that the antioxidant activity assays showed the best agreement between the nonlinear model and experimental data ( $R^2 = 0.69$ ). These evaluations were obtained in the dried plants of lavender (*Lavandula x hybrida*), lemon balm (*Melissa officinalis*), mint (*Metha piperita*), sage (*Salvia officinalis*), and thyme (*Thymus serpyllum*).

Finally, the EEF of the zucchini plant exhibited quercetin and rutin using HPLC. Likewise, Mohamed et al. (2009) identified quercetin and quercetin 4'-O- $\beta$ -D-glucopyranoside in *C. pepo* flowers using fractional chromatography. These flavonoids are widely described and studied because they play important roles in the oxidative stress process associated with food spoilage. About the EEF of Mexican marigold, Navarro-González et al. (2015) identified myricetin, myricetin hexoside, laricitrin, and ellagic acid. Furthermore, in the EEF of colorin, Barrón-Yáñez et al. (2011) found a high glycosylated flavonoids content such as quercetin, isorhamnetin, kaempferol, and lucenin in the ethanolic extract of leaves of *Calia secundiflora*, which agrees with our results. Additionally, Zhao and Tao (2015) suggested that, due to the red, blue, and yellow colors, the content of flavonoids is also high. However, it is essential to highlight that because they have a different mode of action, it would be wrong to compare them. The antioxidant potential of these compounds could be explored as food additives, natural preservatives to inhibit oxidation of food, and nutraceuticals products to prevent diseases associated with oxidative stress, as reported by Takahashi et al. (2020).

## 5 | CONCLUSION

The drying process decreased the DPPH\* radical scavenging capacity, phenols, and flavonoids in the petals of flowers of the zucchini plant, Mexican marigold, and colorin. In the Mexican marigold flowers, despite their ethanolic extract being prepared with 2.4-fold more solvent than the remaining ones, their antioxidant composition was the highest and best preserved because the DPPH\* radical scavenging capacity and flavonoids remained for at least 180 days. Flavonoids such as rutin, quercetin, myricetin, and lucenin were identified in the EEF obtained from the nondehydrated samples. Finally, nonlinear regression models are an effective tool for estimating the behavior of phenolic compounds in EEF.

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## CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

## AUTHOR CONTRIBUTIONS

Isis López-Agama: Methodology; writing-original draft. Margarita de Lorena Ramos-García: Conceptualization; validation; writing-original draft. Alejandro Zamilpa: Methodology; validation. Silvia

Bautista-Baños: Conceptualization; investigation; resources; writing-review & editing. Rosa Isela Ventura-Aguilar: Conceptualization; methodology; writing-review & editing.

## DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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