AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Phytochemical profile and phenolic content of traditional milpa vegetables

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Citation: Ramos-Cuamatzi, Y. A., Delgado-Alvarado, A., Herrera-Cabrera, B. E., Bustamante-González, A., & Fuentes-Herrera, P. B. (2025). Phytochemical profile and phenolic content of traditional milpa vegetables *Agro Productividad*. https://doi.org/10.32854/pz46g387

Academic Editor: Jorge Cadena Iñiguez
Associate Editor: Dra. Lucero del Mar Ruiz Posadas
Guest Editor: Juan Francisco Aguirre Medina

Received: February 5, 2025.
Accepted: September 19, 2025.
Published on-line: November XX, 2025.

Agro Productividad, 18(10). October. 2025. pp: 49-61.

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ABSTRACT

Objective: To analyze qualitatively the variation of phytochemicals, and to determine the content of total phenolic compounds (TPC) and total flavonoids (TF) in nine leafy vegetables and two fruits traditionally harvested from the milpa in Tlaxcala, Mexico.
Design/methodology/approach: The eleven samples were selected for their relevance in consumption in six localities belonging to four municipalities of Tlaxcala. The identification of phytochemicals by thin layer chromatography (TLC), and the quantification of TPC and TF, determined by UV/VIS spectrophotometry, were performed from methanol and hexane extracts.
Results: Qualitative analysis showed the presence of phenolic acids, flavonoids, saponins, tannins, terpenes and alkaloids in all the samples analyzed, except for tannins in *huazontle* and alkaloids in *apipisco*. TPC and TF were detected in the highest amount in *lengua de vaca* (22.91 mg GAE/g DM and 43.89 mg QE/g DM) and *lengüita* (15.6 mg GAE/g DM and 56.05 mg QE/g DM), while *tlalayote lampiño* (4.35 mg GAE/g DM and 5.06 mg QE/g DM) and *apipisco* (5.21 mg GAE/g DM and 7.5 mg QE/g DM) had the lowest contents.
Implications: The phytochemical analysis of these scarcely documented plants is significant for the pharmaceutical area and potential production of new drugs.
Findings/conclusions: The results of the qualitative profile evidenced the presence of different groups of phytochemicals of interest to further identify, quantify and possibly conduct pharmacological studies of these traditionally consumed vegetables, in addition to their valorization as functional foods.

Keywords: bioactive compounds, edible plants, functional foods, milpa, thin layer chromatography.

INTRODUCTION

Wild vegetables have carried out an essential role and are frequent in the traditional diet of indigenous peoples and rural communities in many parts of the world. They have an important role for subsistence of many people, since, in addition to being an important source of food, people also depend on the plants in their natural environment for other practical applications. For example, several of them have medicinal properties and are used to treat common ailments due to the presence of bioactive compounds (Sanjay, 2023). Among the more than 50,000 secondary metabolites discovered in plants (Teoh, 2016),

coumarins, lignans, anthocyanins, glycosides, saponins, phenolic compounds, terpenoids, and alkaloids are the phytochemicals that have been widely studied for their benefits to health and their pharmacological potential (Samal *et al.*, 2024).

The state of Tlaxcala is located in the central zone of Mexico and has a total surface area of 3,996.6 km^2, from which more than 59% of its territory is devoted to agriculture, mainly to cultivation of maize (*Zea mays*), bean (*Phaseolus vulgaris*), barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), and potato (*Solanum tuberosum*) (INEGI, 2021); and, where a large part of its agrobiodiversity is preserved thanks to its *in situ* conservation within traditional agricultural systems, such as the milpa system, which integrates a diversity of useful plants that promote food sovereignty and ecological resilience of the region (Altieri and Toledo, 2011).

Recent studies have emphasized the importance of vegetables traditionally collected from the milpa, not only for their nutritional value, but also because of their phytochemical content with bioactive properties (Méndez-Flores *et al.*, 2021). For example, Arrieta-Flores *et al.* (2022) point out that *verdolaga* (*Portulaca oleracea*), a species of common consumption in Tlaxcala, presents high contents of total phenolic compounds (TPC), in leaf (25.4 mg GAE/g DM) and stem (21.8 mg GAE/g DM), compared to what was reported for the same species by Alam *et al.* in 2014 (0.96 to 9.1 mg GAE/g DM). In turn, González-Amaro (2008) identified 109 species of weeds in milpas from three communities in Nanacamilpa, Tlaxcala, two as new reports for quelites, 90 with documented uses, and 19 that do not have a use. This underscores the wealth of local agrobiodiversity available and its potential to contribute to food security and health, which refers that inside the state there are still vegetables in the milpa that are not taxonomically and phytochemically documented. Therefore, those resources could have potential functional properties of interest for the innovation of family and regional agricultural production, as well as for healthcare through their consumption. However, more research about their phytochemical and pharmacological composition is still needed.

Thin layer chromatography (TLC) is a valuable qualitative technique in exploratory studies, such as the one presented here, due to its versatility and efficacy to quickly detect secondary metabolites and bioactive compounds in vegetable extracts. This technique allows the preliminary evaluation of possible bioactive properties (antioxidants, antimicrobial, enzyme inhibitors, among others) to study the relationships and similarities between plants according to their chemical composition, approach known as chemotaxonomy. In addition, TLC is also implemented for the analysis of quality control in medicinal, culinary, cosmetic or psychoactive plants (Zahiruddin *et al.*, 2021; Kowalska and Sajewicz, 2022).

Until now, there is no documentation about the phytochemical composition determined from thin layer chromatography of vegetables associated to milpas in Tlaxcala, and for this reason, the hypothesis of this study maintains that within this traditional agricultural system there are plants and fruits with important diversity of bioactive compounds (such as phenolic acids, flavonoids, saponins, tannins, terpenes, or alkaloids), which could be of interest through their consumption because they have beneficial properties to health, beyond a nutritional benefit. Therefore, this study had the objective of analyzing the qualitative profile of secondary metabolites (phytochemicals) in eleven specimens of vegetables traditionally collected in Tlaxcala, Mexico, and quantifying the content of TPC and TF to evaluate their pharmacological potential and their use as food resource with functional properties.

MATERIALS AND METHODS

Leafy vegetables and fruits were obtained from the milpa in six locations of the state of Tlaxcala, Mexico, where preferably there is no use of agrochemicals. Their collection was carried out prior to the flowering stage during the summer of 2023. The plant species collected, in addition to being identified by their common name, were authenticated taxonomically in the Herbarium of the Benemérita Universidad Autónoma de Puebla (Table 1).

The plant material was dehydrated in a Triad Freeze Dryer Labconco at 0.22 mBar and −81 °C, in a period between 24 and 48 hours depending on the type of tissue. Then, it was pulverized in an electrical Hamilton Beach Fresh Grind™, sieved with N° 40 mesh to obtain a particle size of 420 microns, and stored in amber containers at −20 °C until use.

Qualitative analysis by thin layer chromatography (TLC)

Extract preparation. To 250 mg of freeze-dried sample, 5 mL of solvent were added: methanol or hexane depending on the phytochemical group to be extracted (Table 2). The sample was shaken for 30 seconds in vortex and placed in ultrasonic bath (Ultrasonic Cleaner, modelo AS5150B) for 30 min at a power and degasification of 5. Then, the extract was left in maceration for 12 hours at room temperature and stored under refrigeration at 4 °C until its use (no longer than 24 hours).

Analysis of phytochemical groups. The analyses were conducted on silica gel plates with fluorescence indicator (Silica gel 60, F_{254}) of 20×10 cm (Merck, Catalog 1.05554), to which 25 μL of extract from each sample were added. Preparation of the mobile phase (eluent), as well as the controls and development protocol were performed

Table 1. Nomenclature of eleven types of milpa vegetables collected at six locations in Tlaxcala, Mexico.

Common name	Scientific name	Locality
Lengua de vaca	*Rumex obtusifolius* L.	Tepetitla
Verdolaga	*Portulaca oleracea* L.	Tepetitla
Malva	*Malva parviflora* L.	Tepetitla
Lengua de pájaro	*Calandrinia ciliata* (Ruiz & Pav.) DC	San Felipe Cuauhtenco
Apipisco	*Jaltomata procumbens*	San Felipe Cuauhtenco
Huazontle cimarrón	*Chenopodium album* L.	Tepetitla
Quintonil blanco	*Amaranthus powellii* S. Watson	Jesús Huitznahuac
Quintonil jaspeado	*Amaranthus*	Guadalupe Tlachco
Lengüita	*Calandrinia ciliata* (Ruiz & Pav.) DC	San Lorenzo Techalote
Tlalayote lampiño	*Chthamalia decumbens* (W. O. Stevens) L. O. Alvarado & E. B. Cortez	Hueyotlipan
Verdolaga	*Portulaca oleracea* L.	Hueyotlipan

Source: Prepared by the authors.

Table 2. Thin layer chromatography systems for the identification of phytochemicals in plants and edible fruits from milpas in Tlaxcala, Mexico.

Phytochemical	Solvent	Eluent	Control	Developer
Phenolic acids	Methanol	Ethyl acetate: formic acid: acetic acid: water (10:8:8:1)	Gallic acid 0.5 mg/mL	$Na_2(CO)_3$ 20% and Folin-Cioucalteu 50%
Flavonoids	Methanol	Ethyl acetate: formic acid: acetic acid: water (7.5:1:1:0.5)	Quercetin 0.5 mg/mL	NP 1% and PEG 5%
Saponins	Methanol	Butanol: glacial acetic acid: water (4:1:4)	Saponin 10 mg/mL	Vanillin 1% and H_2SO_4 6%
Tannins	Methanol	Ethyl acetate: formic acid: acetic acid: water (10:8:8:1)	Tannic acid 0.5 mg/mL	$FeCl_3$ 5% in 0.5 N HCl
Terpenes	Hexane	Hexane: ethyl acetate (7:3)	*Syzygium aromaticum* (clove) 0.1 mg/mL	Vanillin 1% and H_2SO_4 10%
Alkaloids	Methanol	Ethyl acetate: methanol: water (3:1:1)	*Camellia sinensis* (black tea) 200 mg/mL	Buchard reagent: I 2%:KI 2%

Adapted from Andrade-Andrade (2016).

following the methodology by Andrade-Andrade (2016), based on Wagner and Bladt (1996), and summarized in Table 2. To ensure the stability of the technique, previous assays were carried out with a control and three repetitions of each sample, according to the phytochemical group to identify, until securing the reproducibility of the results and on clearly defined bands. Observation of the bands was conducted with visible light, except for flavonoids, which were visualized in ultraviolet light at 365 nm (UVP lamp, UVLMS-38, series 3UV™ Lamp). Finally, the displacement of the compounds through the plaque was expressed through calculation of the retention factor (Rf), which involves the distance traveled by the sample over the distance traveled by the eluent.

Quantitative analysis by spectrophotometry

The extracts were prepared in the same way as described for the qualitative analysis, but at a concentration of 20 mg/mL, and the moisture percentage was determined for its expression based on dry matter (DM).

Total phenolic compounds. Their quantification was based on the Folin-Ciocalteau colorimetric method described by Herald *et al.* (2012) with modifications. In a microplate, 75 μL of deionized water and 25 μL Folin-Ciocalteau phenol reagent (1:1 with deionized water) were added to 25 μL of extract, shaken for 10 seconds and rested for 6 minutes at room temperature in the dark. Then, 100 μL of Na_2CO_3 at 7.5% were added, shaken for 10 seconds and left resting for 60 minutes under the same conditions. Finally, its absorbance was recorded at a wavelength of 725 nm in a Varioskan flash UV/VIS (Thermo-Scientific) spectrophotometer, and a standard curve ($y=0.0072x-0.0012$, $R^2=0.9993$) prepared with gallic acid at 1 mg/mL. The results were expressed in mg of gallic acid equivalents per gram of dry matter (mg GAE/g DM).

Total flavonoids. They were determined based on the method described by Silva-Beltrán *et al.* (2015) with modifications. The reaction was carried out at room temperature, in the dark, and brief agitation of 8 seconds was carried out after the addition of each reagent: in microplate, 25 μL of extract were mixed with 100 μL of deionized water, 10 μL of $NaNO_2$ (5% w/v) were added, shaken and rested for 5 minutes. Then, 15 μL of $AlCl_3$ (10% w/v) were added and rested for 6 minutes; 50 μL of NaOH 1M were added, shaken and 50 μL of deionized water were added, and shaken again for 15 seconds, rested for 5 minutes, and its absorbance was determined at 415 nm in a Varioskan flash UV/VIS (Thermo-Scientific) spectrophotometer. The results were calculated with the equation of the standard curve obtained with a standard of quercetin dissolved in ethanol at 1 mg/mL ($y=0.0011x+0.036$, $R^2=0.995$) and were expressed in mg of quercetin equivalent per gram of dry matter (mg QE/g DM).

Statistical analysis

The evaluation of qualitative results of TLC was carried out through contingency tables. For the quantification of TPC and TF, an experimental design by completely random blocks was used. The eleven specimens were considered as treatments, each one constituted by four independent blocks (corresponding to four samples of each specimen). For each block, three extracts (repetitions) were carried out and each repetition was determined by triplicate. The data were processed through analysis of variance and means test (Tukey $\alpha=0.05$) with the statistical package SAS version 9.0 (SAS Institute Inc., 2002).

RESULTS AND DISCUSSION

Qualitative analysis by thin layer chromatography (TLC)

The qualitative detection of the different groups of phytochemical components confirmed the presence of phenolic acids, flavonoids, saponins, tannins, terpenes and alkaloids in the eleven specimens analyzed, except for tannins in *huazontle cimarrón* and alkaloids in *apipisco* (Table 3). The saponins were the phytochemical group of greatest presence in this collection of vegetables (50 bands), followed by flavonoids (46 bands), which suggests that these compounds could be key in the bioactive properties of these plants. Thus, the evaluation of their biological properties and studies focused on the quantification of these metabolites are necessary to take advantage of their pharmacological potential in practical applications.

The visualization of the plates developed under visible light and UV light allowed observing a notable diversity of phytochemicals in the extracts analyzed (Figure 1).

Phenolic acids. This phytochemical group was identified in all the plants analyzed, with 1 or 2 bands. The Rf values ranged between 0.61 and 0.87, which suggests the presence of compounds with relatively small molecular weight, for example, hydroxycinnamic acids, common in vegetables and of great interest due to their wide range of antioxidant activity documented *in vitro* (Shahidi and Ambigaipalan, 2015). Within the pattens identified, *lengua de pájaro* and *lengüita* have bands with similar Rf and densities; Rf=0.63 and 0.66; Rf=0.73 and 0.74, respectively (Figure 1, Table 4). According to the density of the bands, it is possible for *huazontle* to have a lower content of phenolic acids, followed by malva and both varieties of *quintonil*. The importance of confirming the presence of phenolic acids

Table 3. Number of bands observed in thin layer chromatography by phytochemical group, from extracts of eleven traditionally collected vegetables in milpas from the localities in the state of Tlaxcala, Mexico.

Specimen	Phe. ac.	Flav	Sap	Tann	Ter	Alk	Total
	Number of bands						
Control	1	1	2	1	1	1	7
(1) Lengua de vaca	1	9	6	1	3	4	24
(2) Verdolaga from Tepetitla	1	6	5	2	3	3	20
(3) Malva	2	6	3	1	4	4	20
(4) Lengua de pájaro	2	2	4	1	4	1	14
(5) Apipisco	1	2	3	1	2	0	9
(6) Huazontle cimarrón	1	2	5	0	3	1	12
(7) Quintonil blanco	1	4	4	1	1	4	15
(8) Quintonil jaspeado	1	4	4	1	2	4	16
(9) Lengüita	2	2	6	1	2	4	17
(10) Tlalayote lampiño	1	2	4	2	3	1	13
(11) Verdolaga from Hueyotlipan	1	6	4	1	4	4	20
Total	15	46	50	13	32	31	187

Phe. ac.=phenolic acids, Flav=flavonoids, Sap=saponins, Tann=tannins, Ter=terpenes, Alk=alkaloids. Prepared by the authors.

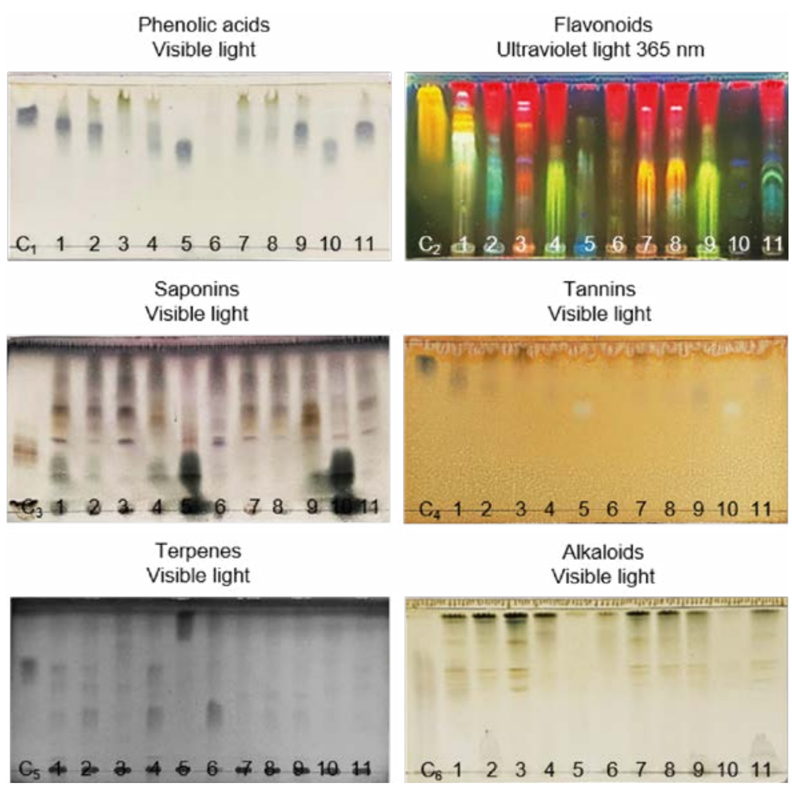

Figure 1. Detection of bands of phytochemicals from thin layer chromatography of edible plant extracts from Tlaxcala, Mexico. C_1=Gallic acid; C_2=Quercetine; C_3=Saponin; C_4=Tannic acid; C_5=*Syzygium aromaticum* (clove); C_6=*Camellia sinensis* (black tea); 1=Lengua de vaca; 2=Verdolaga from Tepetitla; 3=Malva; 4=Lengua de pájaro; 5=Apipisco; 6=Huazontle cimarrón; 7=Quintonil blanco; 8=Quintonil jaspeado; 9=Lengüita; 10=Tlalayote lampiño; 11=Verdolaga from Hueyotlipan. Prepared by the authors.

in these samples lies mainly in that they are multipurpose secondary metabolites that are widely distributed in the plants and represent an important group of phytochemicals with antioxidants, anti-inflammatory, antimicrobial properties, among others (Xu *et al*., 2008; Shahidi and Ambigaipalan, 2015).

Flavonoids. Because of the light density of the bands, it is possible that the fruits of *apispico* and *tlalayote lampiño*, as well as *huazontle cimarrón*, have a low concentration of flavonoids. However, different tones were detected in the development of this phytochemical group, where the sample of *lengua de vaca* stands out, which presented up to nine clearly defined bands with orange, white, lilac and blue colors; this reflects a wide variety of different flavonoids. In some of the specimens, an intense yellow color was observed which coincides with what was reported by Gomathi *et al*. (2012), although Wagner and Bladt (1996) mention the orange color as characteristic of flavonoids. In this study, it was detected that plants of the same species, such as *lengua de pájaro* and *lengüita*, have a predominant type of flavonoid, responsible for the green-yellow color band, with Rf=0.48 (Table 4). Likewise, the similar profile of both samples of *quintonil* points to the presence of four types of flavonoids with different particle size and characteristic colors, where the bands in orange color stand out in intensity, at Rf=0.49.

Saponins. The size and color of the bands were quite varied, which reflects a great variety of types of saponins. Among them, tones of lilac, pink, blue, green, brown and purple were identified, all in opaque intensity (Figure 1). The phytochemical screening of the samples from both fruits, *apipisco* and *tlalayote lampiño*, reflect a greater possibility of having saponins in a concentration of interest. Although in specific concentrations they are considered as anti-nutrients, they are also molecules of great interest for commercial applications and in the research sphere for their wide range of bioactive properties; they can act as antimicrobial, antihyperglycemic, antiviral, antioxidant, anti-inflammatory, anticancer, immunomodulating molecules, among others, as described by Osbourn *et al*. (2011).

Tannins. In the control, for which tannic acid was used, the bands were identified with a blue tone, but in the samples the bands were purple and white. In the results of this phytochemical group, in the samples from fruits of *apipisco* and *tlalayote lampiño*, the presence of a specific type of tannin, with white bands (Figure 1), and Rf=0.62 stands out (Table 4). In the phytochemical screening of *huazontle cimarrón*, the presence of bands corresponding to this phytochemical group was not identified (Table 4). This result contrasts with what was described by Arora *et al*. (2020), who confirm the presence of tannins in methanol, acetone, and ethyl acetate extracts based on plant material of *huazontle cimarrón* (*Chenopodium album* L.), dehydrated, ground and degreased.

Terpenes. The bands in this group of phytochemicals were detected with purple-blue tones. In general, the bands were faint for most of the samples, but in *lengua de vaca* (1), *verdolaga* (2), *malva* (3), *lengua de pájaro* (4) and *huazontle cimarrón* (6), three bands with greater density were revealed (Figure 1). It should be mentioned that in the study carried out by Arora *et al*. (2020) in *huazontle cimarrón*, they did not detect terpenes in methanol extracts, but they did detect the presence of triterpenes in chloroform and petroleum ether extracts; in this study, the detection of these phytochemicals was carried out in hexane extract (Figure 1). This shows that the presence of terpenes can vary in relation with the extraction solvent used, and it could be due to differences in the polarity and efficacy of solvents to solubilize these compounds, in addition to the differences between treatments of the plant material prior to the maceration of extracts.

Alkaloids. Even when the formation of the bands was dim, possibly due to the structural complexity of the molecules, the results confirm the presence of alkaloids in all the vegetables studied except for *apipisco* (Table 4). For this purpose, the yellow-brown bands identified under visible light were considered to be alkaloids, which agrees with what was reported by Gomathi *et al.* (2012). Their detection in vegetables is very important because they have various applications of interest, such as in dermatology in the medical sphere (as anti-inflammatory, sedative, diuretic, anti-cold, anti-spasmodic, among others) (Lombardo and Ortíz, 2009). In addition, there are plants with high toxicity due to the presence of these compounds, although out of the more than 5000 known alkaloids, the minority are toxic. It should be emphasized that to prevent the ingestion of the plant, these compounds have the property of giving a bitter taste (Villar and Díaz, 2006). Therefore, it

Table 4. Retention factor of the bands identified through thin layer chromatography of extracts from plants and edible fruits collected in milpas in Tlaxcala, México.

Phytochemical	Sample*											
	C	1	2	3	4	5	6	7	8	9	10	11
Phenolic acids	0.84	0.77	0.74	0.74 0.87	0.63 0.73	0.62	0.81	0.72	0.72	0.66 0.74	0.61	0.74
Flavonoids	0.95	0.49 0.55 0.60 0.68 0.75 0.80 0.84 0.88 0.93	0.34 0.48 0.54 0.60 0.66 0.92	0.32 0.43 0.53 0.74 0.84 0.88	0.48 0.61	0.54 0.65	0.53 0.74	0.32 0.49 0.72 0.85	0.32 0.49 0.72 0.85	0.48 0.73	0.41 0.64	0.35 0.45 0.50 0.57 0.78 0.87
Saponins	0.37 0.45	0.22 0.28 0.44 0.53 0.64 0.8	0.22 0.43 0.52 0.63 0.8	0.43 0.51 0.8	0.21 0.28 0.57 0.77	0.21 0.27 0.43	0.4 0.44 0.51 0.56 0.84	0.44 0.6 0.77 0.82	0.44 0.6 0.76 0.82	0.19 0.44 0.56 0.63 0.77 0.82	0.21 0.26 0.45 0.63	0.42 0.48 0.53 0.66
Tannins	0.89	0.78	0.76 0.86	0.8	0.71	0.62		0.7	0.73	0.69	0.62 0.74	0.76
Terpenes	0.65	0.34 0.5 0.63	0.36 0.52 0.63	0.36 0.51 0.63 0.74	0.37 0.47 0.52 0.63	0.81 0.93	0.37 0.65 0.85	0.46	0.35 0.46	0.36 0.46	0.51 0.68 0.88	0.36 0.52 0.63 0.88
Alkaloids	0.64	0.5 0.55 0.64 0.81	0.62 0.8 0.84	0.51 0.59 0.63 0.8	0.85		0.81	0.54 0.61 0.65 0.82	0.55 0.61 0.66 0.82	0.55 0.61 0.66 0.82	0.66	0.56 0.63 0.68 0.8

*C=Control, 1=Lengua de vaca, 2=Verdolaga from Tepetitla, 3=Malva, 4=Lengua de pájaro, 5=Apipisco, 6=Huazontle cimarrón, 7=Quintonil blanco, 8=Quintonil jaspeado, 9=Lengüita, 10=Tlalayote lampiño, 11=Verdolaga from Hueyotlipan. Prepared by the authors.

is suggested to approach quantitative studies that determine their concentration and, from this, an evaluation of risks according to the regional patterns of consumption. Likewise, the possible characterization of the alkaloids present.

The detection of phenolic acids, flavonoids, saponins, tannins, terpenes and alkaloids in all the samples analyzed (Figure 1) suggests that these vegetables and fruits under study could have antioxidant properties and benefits for the health of those who consume them, which supports their traditional use in the diet and as medicine. Although, qualitatively, phenolic acids and tannins do not seem to be the most abundant compounds in the samples (Table 4), their constant presence indicates that they could contribute synergically with other secondary metabolites that are more present, such as flavonoids, to potentiate their mechanisms of action and biological effects. Previous studies have shown that phenolic compounds act in an additive and synergic way, improving their bioactive properties (Liu, 2003; Pereira *et al.*, 2009). For example, phenolic acids or tannins can stabilize free radicals, while flavonoids act on other metabolic pathways, which result in a stronger combined effect than each compound separately (Rice-Evans *et al.*, 1996). In addition, two compounds sharing biosynthetic paths and being found together in the plants can help to improve the bioavailability and biological efficacy of both compounds (Shahidi and Ambigaipalan, 2015). In other cases, the presence of phenolic acids (such as gallic acid), increases the antioxidant activity of flavonoids, since they protect them from oxidative degradation (Rice-Evans *et al.*, 1996).

Quantitative analysis

The analysis of variance showed significant differences both for TPC and for TF between the species of plants evaluated. The concentration of TPC varied from 4.3 to 22.9 mg GAE/g DM; within the samples, the leaf vegetable known as *lengua de vaca* showed the highest concentration, followed by *lengüita* (15.6 mg GAE/g DM), *lengua de pájaro* (12 mg GAE/g DM), and both samples of *verdolaga* (Figure 2). In this study, the lowest

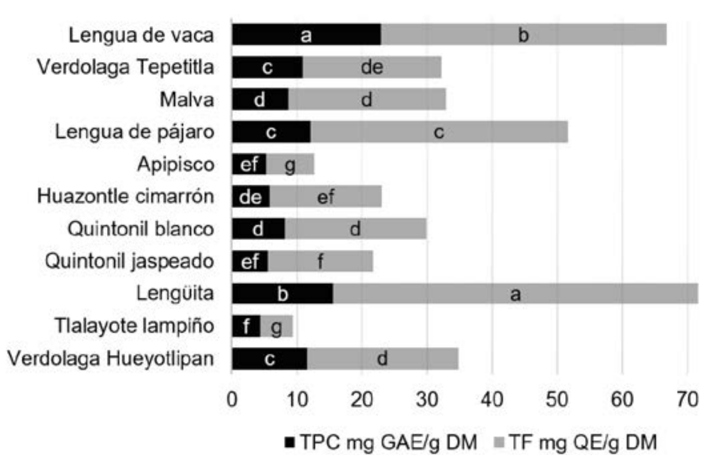

Figure 2. Content of total phenolic compounds (TPC) and flavonoids (TF) in eleven specimens of plants and edible fruits traditionally collected in milpas in Tlaxcala, Mexico. Average values with the same letter in the same phytochemical group were not statistically different (Tukey p≤0.05). Prepared by the authors.

determinations of TPC corresponded to *apipisco* (5.5 mg GAE/g DM), *quintonil jaspeado* (5.4 mg GAE/g DM) and *tlalayote lampiño* (4.3 mg GAE/g DM), which shows the functional potential of these, and the rest of the species evaluated from the milpa. The results obtained in *verdolaga* (10.9-11.6 mg GAE/g DM) were on average higher than what was reported by Cantú-López *et al.* (2022) in samples from this same species collected in Tepetitla, Tlaxcala (3.7 mg GAE/g DM). In turn, Román-Cortés *et al.* (2018) describe a concentration of TPC of 1.4 mg GAE/g DM for *verdolaga* obtained from the Supply Center in Ecatepec, State of Mexico. *Quintonil* and *huazontle* were also considered as *quelites* of important ancestral use in Mexico, where Román-Cortés *et al.* (2018) detected concentrations of 3.99 and 4.5 mg GAE/g DM, respectively, which are slightly lower levels than the ones obtained in this study for the same plants: *huazontle* (5.77 mg GAE/g DM), *quintonil jaspeado* (5.45 mg GAE/g DM) and *quintonil blanco* (8.11 mg GAE/g DM). In *malva*, the value of TPC (8.6 mg GAE/g DM) detected was nearly double the concentration it had in this species (4.7 mg GAE/g DM) collected in Hidalgo, Mexico, and reported by Sandoval-Gallegos (2022). In this regard, Jiménez-Aguilar and Grusak (2015), in their study of nutritional and phytochemical analysis of four species of green leafy vegetables native to Mexico, Central America and Africa, describe the content of TPC in a range of 2.29 to 5.66 mg GAE/g based on fresh weight; these authors indicate the native species as foods with higher content of TPC, compared to other vegetables of high consumption, such as some spinach, lettuce and cilantro.

It should be mentioned that the studies referred from the literature do not specify the agricultural context where the samples were collected, so the suggestion is to evaluate the impact of traditional and conventional agricultural practices in the phytochemical profile of the vegetables and to complement the information with studies that consider the amount of biomass required to consume to have better use of these bioactive compounds obtained through the diet. For example, in the review by Mercado-Mercado *et al.* (2013) a wide variety of traditional foods used as spices in Mexico was documented, which have great potential of antioxidant properties due to their high content of polyphenols and flavonoids, but, in general, the amount of biomass used tends to be small. This leads us to question up to what point the functional or nutraceutical property of a food depends on the amount that is consumed.

Regarding the content of TF, the highest concentration was identified in *lengüita* (56.1 mg QE/g DM), followed by *lengua de vaca* (43.9 mg QE/g DM), *lengua de pájaro* (39.7 mg QE/g DM) and *malva* (24.3 mg QE/g DM). The concentration of flavonoids obtained in this study in the *verdolaga* (plants 21.3 and 23.3 mg QE/g DM), was at least four times higher than what was found by Cantú-López *et al.* (2022) in samples of this species collected in Huaquechula, Puebla (5.1 mg QE/g DM) and Tepetitla, Tlaxcala (3.3 mg QE/g DM). The determinations of FT carried out by Román-Cortés *et al.* (2018) for *huazontle*, *verdolaga* and *quintoniles*, included 0.47 to 1.14 mg QE/g DM, concentrations quite lower than those obtained in this study for the same species, which varied from 16.23 to 23.23 mg QE/g DM. As presented in the results in this study, and what is suggested by Cantú-López *et al.* (2022), the environment where plants develop plays a defining role in the concentration of TPC and TF, since these variations could be attributed to environmental factors such as water stress, the content of nitrogen in the soil, and other elements that influence the biosynthesis of secondary metabolites. In fact, these authors observed that, under certain stress conditions, the plants increased production of these compounds as a defense mechanism, which could explain the differences obtained in the concentration of both phytochemicals between species of the same genera or between individuals of the same species.

This study provides guidelines to promote future qualitative and bioactivity studies in compounds that these and other available plants in the milpa could contribute, to inquire about their therapeutic potential, as well as foster, validate, and support their conservation and traditional use in the localities of study and others where there is the intention to implement the milpa system.

CONCLUSIONS

The study of the qualitative profile by thin layer chromatography in the nine leafy vegetables and two fruits collected in the milpa system revealed a wide diversity of compounds in the phytochemical groups evaluated, primarily, in the flavonoid and saponin compounds. The results evidence that these dietary resources are not only important from the nutritional point of view for the communities but could also have a pharmacological potential of interest and significant phenolic compounds. Quantification of total phenolic compounds and total flavonoids confirms the presence of these bioactive compounds in relevant concentrations for their continuous study, primarily in *lengua de vaca* (*Rumex obtusifolius* L.), *lengüita* (*Calandrina ciliata* [Ruiz & Pav.] DC) and *verdolaga* (*Portulaca oleracea* L.). This provides guidelines to new perspectives for their use in the diet, preventive medicine and industrial areas, such as cosmetics, among others.

ACKNOWLEDGEMENTS

This study is product of the thesis of the first author, who thanks the Secretaría de Ciencia, Humanidades, Tecnología e Innovación (SECIHTI) for scholarship number 22233051 supporting Master of Science studies. In addition, we would like to thank all the people from the localities in Tlaxcala who contributed by donating plant material to perform this study.

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