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Article

Contenido de polifenoles y capacidad antioxidante de *Physalis chenopodifolia* Lam. silvestre y cultivo

Polyphenols content and antioxidant capability of wild and under cultivation *Physalis chenopodifolia* Lam.

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Resumen:

La composición de polifenoles así como la actividad antioxidante en *Physalis chenopodifolia* (Solanacea) ha sido poco estudiada en México. En este trabajo se evaluó el contenido de fenoles totales, flavonoides totales y actividad antioxidante por dos métodos DPPH y ORAC, en hojas y frutos de plantas silvestres y bajo cultivo de dicha especie. Los resultados mostraron que las hojas y frutos de las plantas silvestres tienen más contenido de polifenoles [196.46 y 9.44 mg 100 g^{-1} respectivamente de peso seco (PS)] y flavonoides totales (148.52 y 23.1 mg 100 g^{-1} respectivamente de PS) que las plantas cultivadas. La actividad antioxidante por el método DPPH reveló mayor porcentaje en hojas de las plantas cultivadas (4.19-11.5 %) en todas las concentraciones, que las de plantas silvestres (2.14-8.28 %). En frutos, los resultados fueron similares, tanto en los dos tipos de plantas; sin embargo, mediante el método ORAC, las hojas de plantas silvestres tuvieron más actividad que las de plantas cultivadas: de 1 396 y 156 μM Etx 100 g^{-1} de PS, respectivamente, datos que se correlacionaron con los altos valores de polifenoles totales (R^2 = 0.953). Por lo tanto, se concluye que la contribución al conocimiento de la presencia de polifenoles en diferentes solanáceas es importante para el uso potencial de la especie estudiada como antioxidante.

Palabras clave: Antioxidantes, DPPH, fenoles, flavonoides, ORAC, *Physalis chenopodifolia* Lamb.

Abstract:

The composition of polyphenols as well as the antioxidant activity in *Physalis chenopodifolia* (Solanacea) has been little studied in Mexico. In this work the content of total phenols, total flavonoids and antioxidant activity was evaluated by two methods DPPH and ORAC, in leaves and fruits of wild plants and under culture of said species. The results showed that the leaves and fruits of the wild plants have more polyphenols content [196.46 and 9.44 mg 100 g^{-1} respectively of dry weight (PS)] and total flavonoids (148.52 and 23.1 mg 100 g^{-1} respectively of PS) than the cultivated plants. The antioxidant activity by the DPPH method revealed higher percentage in leaves of the cultivated plants (4.19-11.5 %) in all the concentrations, than those of wild plants (2.14-8.28 %). In fruits, the results were similar, both in the two types of plants; however, through the ORAC method, the leaves of wild plants had more activity than those of cultivated plants: of 1 396 and 156 μM Etx 100 g^{-1} of PS, respectively, data that correlated with the high values of total polyphenols (R^2 = 0.953). Therefore, it is concluded that the contribution to the knowledge of the presence of polyphenols in different Solanaceae is important for the potential use of the species studied as an antioxidant.

Key words: Antioxidants, DPPH, phenols, flavonoids, ORAC, *Physalis chenopodifolia* Lam.

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Introduction

At present, there is a great interest in the search for ingredients that provide health benefits, such as functional foods (Robertfroid, 2000). Among them, those of plant origin have a wide content of nutrients and phytochemical components with a variety of chemical structures with different physiological effects in the human body. Epidemiological studies refer to an association between the consumption of fruits, vegetables and grains and the prevention of the risk of chronic-degenerative diseases (Ferrari *et al.,* 2003; Ilow *et al*., 2008). In this context, it is well-known that phenols are compounds that, from their antioxidant properties, are considered as preventive of diseases related to the imbalance of the oxidative system, and also able to destroy cancer cells (Scarpa and Ninfali, 2015).

On the other hand, the species of the genus *Physalis*, of the Solanaceae family, are of great economic and cultural importance; and they are cultivated mainly for their nutritional characteristics, such as the green tomato (*Physalis philadelphica* Lam.) that was domesticated in Mexico and taken to Europe and other parts of the world (Zamora *et al.,* 2015); Archaeological findings have confirmed that its use in the diet of the Mexican population goes back to pre-Columbian times. Different parts of the plant (leaves, root, chalice and fruits) of several species are used in traditional medicine as well as in the industrial, ornamental and forage sector (Santiaguillo and Blas, 2009). Despite this, few species have been cultivated, including *P. ixocarpa* Brot. ex Horm., *P. peruviana* L. and *P. alkekengi* L.

Many species of *Physalis* emerge within established crops, and have developed characteristics of adaptation and resistance to adverse conditions, so they thrive as ruderals or weeds; thus, they are collected for family feeding and in the economy through the sale of their fruits (Santiaguillo *et al*., 2000).

Phytochemical studies have identified the presence of witaesteroids (fisalins) as the secondary metabolite of the genus (Ray and Grupta, 1994, Pérez-Castorena *et al*., 2004), as well as flavonols and various phenolic acids with antioxidant properties (Medina-Medrano *et al*., 2015).

66 wild species of *Physalis* have been reported in Mexico, from which 37 are endemic. In addition, this country is considered the center of diversity of the genus (Martínez, 2017), which has great phytochemical potential and medical properties. *P. chenopodifolia* stands out for its ethnobotanical use; it is a semi-cultivated taxon and it is used for several purposes (Santiaguillo and Blas, 2009).

Thus, this species concentrates a high content of minerals, especially iron in leaves of wild plants (243.5 mg kg^{-1}) and cultivated (272.27 mg kg^{-1}); in a phytochemical screening, Salcedo-Pérez *et al.* (2015) recorded the presence of terpenes / steroids and phenols, particularly in leaves and stems of wild and cultivated plants.

Based on the foregoing, the objective was the evaluation of the phenolic composition and antioxidant activity in leaves and fruits of wild plants and under cultivation of *Physalis chenopodifolia.*

Materials and Methods

Plants of *P. chenopodifolia* were collected in an oak forest of the *San Nicolás de los Ranchos* farm, municipality of *Cholula*, in the state of *Puebla* in November 2015. The specimens were deposited in the *Herbario del Instituto de Botánica de la Universidad de Guadalajara (IBUG)* (Herbarium of the Institute of Botany of the University of Guadalajara) (IBUG) and recorded with registration number 1 469.

Mature seeds were separated from these plants, which were grown in an experimental field of the *Centro Universitario de Ciencias Biológico y Agropecuarias (CUCBA) de la Universidad de Guadalajara* (University Center of Biological and Agricultural Sciences (CUCBA) of the University of G*uadalajara*). The seeds were planted in rows with plastic mulch and drip irrigation strips. They were incorporated NPK fertilizer (30:30:30) in the vegetative stage and another in the reproductive stage (15:45:25); In addition, systemic insecticide was mainly applied to whitefly, and weeded manually (Valdivia-Mares, 2016). The cultivation was carried out from January to July 2016.

Experimental material

The leaves and whole fruits of the wild and cultivated plants were dehydrated at 45 °C for 48 h, in a forced air oven (*Novatech* HS35-EA) and milled to a particle size of 0.5 mm in diameter. With 0.5 g of each sample of leaves and fruits separately, a mixture was prepared with 50 mL of methanol / water (80/20) in an ultrasonic bath (Branson B-200) for 20 min; of the extract, 2 mL were separated, portion that was filtered and centrifuged at 14 000 rpm for 5 min in a Labogen MINI centrifuge (Atanassova *et al.,* 2011).

Determination of phenols and total flavonoids

The content of total phenols was determined with the Folin-Cilocalteu method. One mL of each extract in triplicate was mixed with 9 mL of HPLC grade water and one mL of the Folin-Ciocalteu reagent. After 5 min, 10 mL of sodium carbonate (Na_2CO_3) was added at 7 % (w / v) and at the end the mixture was adjusted to 25 mL with HPLC grade water. They were incubated in the dark for 90 min at room temperature and the absorbance was read at 750 nm in a JENWAY model 6320D spectrophotometer. Different concentrations of gallic acid (0, 20, 40, 60, 80 and 100 mg L^{-1}) were used as standard to calculate the content of total phenols, expressed as mg of gallic acid equivalents (EAG) 100^{-1} g of sample in dry weight (mg EAG 100 g^{-1} PS) (Atanassova *et al*., 2011).

The total content of total flavonoids was analyzed by the aluminum chloride ($AlCl_3$) method, for which an aliquot was taken in triplicate of one mL of the extract and mixed with 4 mL of distilled water and 0.3 mL of 5 % $NaNO_2$. (p / v). After 5 min, 0.3 mL of 10 % $AlCl_3$ and six minutes later, 2 mL of a 1M of sodium hydroxide (NaOH) solution were added and complemented to a total volume of 10 mL with water. It was mixed on a vortex shaker (OHAUS OHS-30392115) and the absorbance was read at 510 nm in a JENWAY 6320D model spectrophotometer. Different concentrations of (+) catechin (0, 20, 40, 60, 80 and 100 mg L^{-1}) were used for the realization of a

standard curve to calculate the total flavonoid content expressed as mg equivalents of catechin per 100^{-1} g of dry weight (mg EC 100 g^{-1} PS) (Atanassova *et al.*, 2011).

Determination of antioxidant capacity

The antioxidant activity was evaluated using two different methods. The activity of inhibiting free radicals with DPPH, according to Atanassova *et al.* (2011) using DPPH (1,1-diphenyl-2-picrylhydrazyl), 0.004% (in methanol, w / v), the extracts (n = 3) were diluted at different concentrations (100, 200 and 500 μmL mL^{-1}) and mixed with 4 mL of DPPH, in the dark. After 60 min of incubation in the dark and at room temperature, the absorbance was read against a blank (only reagents without sample with methanol) at 517 nm in a JENWAY 6320D UV-VIS spectrophotometer. The antioxidant activity was expressed as a percentage (%) of inhibition of free radicals by DPPH, according to the following equation:

$$I\% = [(A\ blank - A\ sample)/A\ blank] \times 100.$$

The evaluation of the capacity of donation of hydrogen atoms was determined by the ORAC method (Ou *et al*., 2001) with some modifications. It is one of the most accepted techniques for the measurement of antioxidant capacity, since it measures the oxidation inhibition induced by peroxide radicals, generated by the thermal decomposition of 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH), which reacts with a fluorescent probe (fluorescein), to form a colorless non-fluorescent product. A Trolox calibration curve was designed as a reference standard in concentrations of 0.02 to 3 μM of Trolox (μM ETx mL^{-1}). The antioxidant activity was calculated from the following equation:

$$y = 6.812x + 2.797;\ R^2 = 0.98)$$

Obtained from the Trolox calibration curve.

The assay was carried out in a 96-well microplate reader (Tecan infinite M200PRO Fluorometer) where 20 µL of the extracts or standard were placed (Trololox 5 mM), 120 µL of fluorescin (120 nM) were added.) and AAPH (2,2-azobis (2-methylpropionamidine) dihydrochloride) 40mM, were incubated in a kiln (*Novatech* HS35-EA) at 37 °C for 30 min and the readings were recorded with excitation 485 nm and λ emission 538 nm respectively with 70 cycles each 5 minutes. The protective effect of the antioxidant was calculated using the differences of areas under the decay curve of the fluorescein between the blank and the sample. The area under the curve (AUC) was calculated as follows:

$$AUC = 1 + f_1/f_0 + f_2/f_0 + f_3/f_0 + f_4/f_0 + \ldots + f_{34}/f_0 + f_{35}/f_0$$

Where:

f_0 = Reading of the initial fluorescence at minute 0

f_1 = Fluorescence reading at time *i*.

The net AUC was obtained by subtracting the AUC from the AUC blank of the sample:

$$\text{net AUC} = AUC_{sample} - AUC_{blank}$$

Results are expressed in µM equivalent of Trolox in 100 g of dry weight (µM ETx 100 g^{-1} PS).

Statistical analysis

The results were expressed as the mean ± standard deviation; in addition, with the Microsoft Excel 2016 for Mac program, the polyphenol content data were compared with the antioxidant activity to know if there was a correlation between these values.

Results

Content of phenols and total flavonoids

In Table 1 it can be observed that the wild plants had higher content of phenolic compounds (196.46 ±0.02 mg EAG 100 g^{-1} of PS) both in leaves and in fruits, in comparison with the cultivated ones (23.58 ±0.12 EAG 100 g^{-1} of PS). In fruits, the values obtained were 9.44 in wild and 6.21 in cultivated plants. The same happens with the total polyphenols; the flavonoids were obtained in greater quantity in the leaves and fruits of wild plants (148.52 ±0.1 and 23.1 ± 0.01 mg EC 100 g^{-1} of PS, respectively) than in those cultivated (6.01 and 3.79 respectively) (Table 1).

Table 1. Content of polyphenols and total flavonoids in leaves and fruits of wild and cultivated plants of *Physalis chenopodifolia* Lam.

	Total polyphenols (mg EAG 100 g^{-1} PS)	**Flavonoids (mg EC 100 g^{-1} PS)**
Wild plants		
Leaves	196.46 ±0.2	148.52 ±0.1
Fruits	9.44 ±0.01	23.10 ±0.01
Cultivated plants		
Leaves	23.58 ±0.12	6.01 ±0.03
Fruits	6.21 ±0.03	3.79 ±0.02

Antioxidant activity by DPPH

By this method, a higher percentage of free radical blocking was observed in leaves of cultivated plants than in wild plants, despite the fact that the content of polyphenols and flavonoids was higher in leaves of the latter (Table 2). The antioxidant activity improved with increasing concentrations, both in leaves and in fruits. The highest value corresponded to 500 μL mL^{-1} with 11.5± 0.4 % and 8.28 ± 0.3 %, for cultivated and wild plants, respectively. The other concentrations (200 and 100 μL mL^{-1}) were 6.45 ± 0.3 and 4.19 ± 0.2 for the cultivated plants and 3.91 ± 0.1 and 2.14 ± 0.1 in wild plants.

Table 2. Uptake of free radicals (%) with DPPH in leaves of wild plants and under cultivation of *Physalis chenopodifolia* Lam.

Concentration (μL mL^{-1})	**Wild plants**	**Cultivated plants**
500	8.28 ± 0.3	11.50 ± 0.4
200	3.91 ± 0.1	6.45 ± 0.3
100	2.14 ± 0.1	4.19 ± 0.2

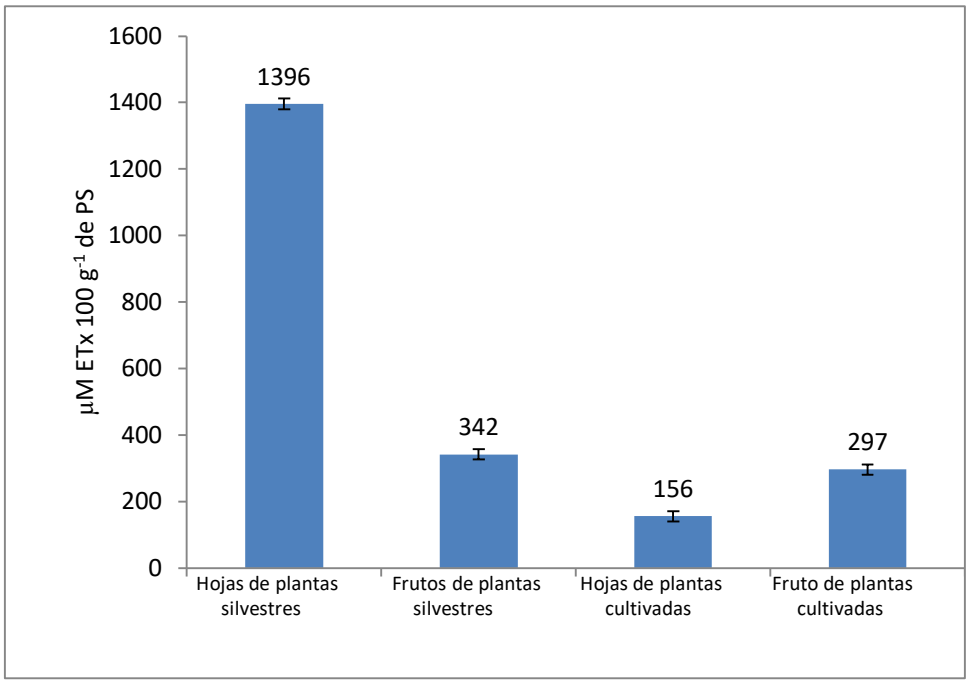
In the fruits, the free radical blockage percentages were similar in both types of plants (Table 3), and, as in the leaves, the higher concentration of the samples increased the antioxidant activity; at 500 μL mL^{-1} the highest of 61.78 ± 1.2 and 62.69 ± 1.5 %, respectively, were recorded; in 200 μL mL^{-1} were 20.7 ± 0.9 and 17.42 ± 0.6, and 100 (μL mL^{-1}), the lowest with 9.86 ± 0.4 and 8.22 ± 0.3 %.

Table 3. Uptake of free radicals (%) DPPH of *Physalis chenopodifolia* Lam. in fruits of wild plants and under cultivation.

Concentration (μL mL^{-1})	**Wild**	**Cultivated**
500	61.78 ± 1.2	62.69 ± 1.5
200	20.70 ± 0.9	17.42 ± 0.6
100	9.86 ± 0.4	8.22 ± 0.3

Antioxidant activity with the ORAC-Fluorescein assay (ORAC-FL

The highest values were found in leaves and fruits of the wild plants of 1 396 ± 15.5 and 342 ± 10 μM ETx 100 g^{-1}, respectively, in comparison with the cultivated plants (156 ± 5 and 297 ± 8 μM ETx 100 g^{-1} for leaves and fruits, respectively (Figure 1).



Hojas de plantas silvestres = Leaves of wild plants; *Frutos de plantas silvestres* = Fruits of wild plants; *Hojas de plantas cultivadas* = Leaves of cultivated plants; *Frutos de plantas cultivadas* = Fruits of cultivated plants.

Figure 1. Oxygen radical absorption capacity (ORAC) μM equivalents of Trolox 100 g^{-1} PS in leaf and fruit of *Physalis chenopodifolia* Lam. in wild and cultivated plants.

Discussion

Phenols and flavonoids

The values of total phenols in the leaves of the wild plants of *Physalis chenopodifolia* (196.46 mg of EAG 100 g^{-1} PS) are higher than those of the leaves of species with medicinal use in Poland (0.15 to 15.15 mg of EAG 100 g^{-1} PS) (Wojdylo *et al.,* 2007); of Bulgaria, with values of 27.94 to 48.9 mg de EAG 100 g^{-1} PS (Attanasova *et al*., 2011), and of some plants of traditional Chinese medicine such as *Pinellia ternata* (Thunb.) Makino, with 12 mg of EAG 100 g^{-1} PS, up to 166 mg of EAG 100 g^{-1} PS in *Trichosanthes kirilowii* Maxim (Song *et al*., 2010), with the same Folin-Ciocalteu test and gallic acid as standard. Likewise, of edible vegetables, considered as important sources of phenols such as asparagus, cabbage, carrot, celery, squash, garlic, lettuce, onion, radish, spinach, chili, tomato and beet with values between 13.5 to 154.1 mg 100 g^{-1} PS (Ninfali *et al.,* 2005).

However, they are inferior to other commercial medicinal plants reported by Yoo *et al.* (2008), with values of 464.2 to 844.4 mg 100 g^{-1} PS and by Pourmorand *et al.* (2006) in medicinal plants of Iran with 3 100 to 28 950 mg 100 g^{-1}, as well as some other popular ones in Serbia for therapeutic ethnobotanical use in diseases such as diabetes, rheumatism, urinary tract, ulcerations, hypertension, arteriosclerosis, for example, with values from 960 to 6 200 mg of EAG 100 g^{-1} (Žugić *et al.,* 2014).

Compared to other Solanaceae, the species under study has a lower content than *Solanum madrense* Fernald, as described by Fernández y Ruiz (2017), which reached leaf values of 202 to 377 mg EAG 100 g^{-1}. In other *Physalis* species, such as *P. patula* Mill, *P. subulata* L., *P. solanacea* L. and *P. hederifolia* A. Gray var. *hederifolia*, Medina-Medrano *et al.* (2015) estimated a leaf content of 58.75 to 12 90.06 mg EAG g^{-1} and established that the most common are phenolic acids, quercetin and glycosylated kaempferol, determined by high pressure chromatography (HPLC).

The polyphenols of *Physalis chenopodifolia* fruits are similar to commercial fruits such as *Musa cavendish* L. (7 mg EAG 100 g^{-1} (Singh *et al.,* 2016) but inferior to mango fruits (78.3), guava (87.5), papaya (88.2) and dates (304.24 to 563.71) (Amira *et al*., 2012;

Patthamakanokporn *et al.,* 2008;), as well as fruits from Ecuador from 25 to 2 167) and *Physalis peruviana* from different localities of Peru (106 to 149.3 mg EAG 100 g^{-1}) (Jurado *et al.,* 2016; Vasco *et al.,* 2008).

The content of flavonoids in the leaves of the species of interest was above those of the leaves of spices and medicinal plants according to Ninfali *et al*. (2005), Žugić *et al.* (2014) and Attanasova *et al*. (2011). Likewise, the figures of the present study are higher to those corresponding to edible vegetables (7.0 to 89.1 mg EC 100 g^{-1}) (Ninfali *et al.,* 2005).

However, they are inferior to spices and medicinal herbs according to Žugić *et al.* (2014), Ninfalli *et al.* (2005) and Yoo *et al.* (2008), whose values are go from 600 to 1 321.2 mg EC 100 g^{-1}. The same thing is extensive for other Solanaceae such as *Solanum madrense* (389 to 531 mg EC 100 g^{-1}) (Fernández and Ruiz, 2017) and other Mexican species of *Physalis*, with leaf values of 6.543 to 21.265 mg of EC g^{-1} of sample (Medina-Medrno *et al.,* 2015).

The antioxidant activity of herbs, fruits, food supplements and beverages is usually analyzed with *in vitro* tests and generally in relation to the content of polyphenols by traditional methods, and only one technique is applied. The most common methods to measure this capacity are DPPH and ORAC, because they are very easy to reproduce, but they also show significant differences in their response to antioxidants (Roy *et al*., 2010).

Antioxidant activity, DPPH

In general, samples with a higher polyphenol or flavonoid content have greater antioxidant activity (Žugić *et al*., 2014), which was not reflected in the samples of the present study; however, other compounds with antioxidant activity have been reported, such as the phyisalines considered to be the main metabolite in this genus. Hernández (2015) refers to antioxidant activity with DPPH in leaves of *Physalis peruviana*, so it would be interesting to evaluate the content of phyisalines in these samples.

The values obtained in the leaves are superior to other Solanaceae such as *Solanum ferrugineum* Jacq., on which 10 % is calculated at a higher concentration (1 000 mL^{-1}) (Medina-Medrano *et al.,* 2016), but less than the leaves of commercial and medicinal herbs such as *Eucalyptus globulus* Labill. and *Chamaemelum nobilis* L. with values of 60.1 and 91 % at a concentration of 100 µL mL^{-1} also analyzed with DPPH (Yoo *et al.,* 2008). In leaves of *Solanum madrense*, Fernández and Ruiz (2017) estimated 90 % at 500 µL mL^{-1}, by means of DPPH.

At 500 µL mL^{-1} in the samples of the studied fruits, the numbers are higher than those recorded in other Solanaceae such as *Solanum ferrugineum* (with 15 % at a concentration of 1 000 µg mL^{-1}) (Medina-Medrano *et al*., 2016) and *Physalis peruviana* (42.22 % a 800µg mL^{-1}) (Jurado *et al*., 2016).

Antioxidant activity, ORAC-Fluorescein

Unlike the obtained with the DPPH technique, the leaves of cultivated plants showed greater antioxidant capacity; this can be explained by the content of total polyphenols and flavonoids, since there is significant evidence that ORAC values are strictly dependent on the content of phenols and flavonoids; furthermore, while ORAC techniques measure the capacity to reduce oxygen radicals induced by peroxide radicals, DPPH quantifies the percentage of free radical uptake in general (Ninfali *et al*., 2005).

The ORAC values of the leaves of wild plants of *Physalis chenopodifolia* are higher than those of some medicinal plants such as *Petroselium hortensis* L. (13 018 µmol ET 100 g^{-1}) and *Rosmarinus officinalis* L. (29 032 µmol ET 100 g^{-1}). The same happens with some edible vegetables such as chili, tomato, onion squash, carrot, lettuce and radish with values of 252 to 1 240 µmol ET 100 g^{-1} (Ninfali *et al*., 2005), but inferior to other medicinal ones also according to Ninfali *et al*., 2005) with values that reach more than 32 000 µmol ET 100 g^{-1}.

Conclusions

The vast world of medicinal plants, with thousands of species and varieties, demands a lot of scientific research, especially in its content of phenolic compounds and antioxidant activity, in wild and cultivated specimens.

The present study shows, for the first time, the content of phenols, flavonoids and antioxidant capacity in leaves and fruits of *Physalis chenopodifolia* wild and under cultivation, empirically used as medicinal and that is already semi-cultivated. Different results were observed in the antioxidant activity with DPPH and ORAC, the latter showed values more dependent on the content of phenols for our sample. Therefore, it would be important to identify and quantify individual polyphenols by more specific techniques such as HPLC since it is well-known that there is a relationship between the type of polyphenols and their antioxidant activity. The results reported here indicate that the ORAC assay better reflects antioxidant activity for the species under study. The leaves of the wild plants showed the highest content of polyphenols and flavonoids, as well as increased antioxidant capacity by the ORAC method, which means that these could represent a potential source of compounds to develop new drugs in human health.

Conflict of interests

The authors declare no conflict of interests.

Contribution by author

Lucía Barrientos Ramírez: writing of the first manuscript; María Lourdes Arvizu: accomplishment of laboratory techniques; Eduardo Salcedo Pérez: logistic support and field-work; Socorro Villanueva Rodríguez: standardization of antioxidant techniques; j. Jesús Vargas Radillo: information search for sample analysis; Bianca Azucena Barradas Reyes: laboratory sample analysis and standardization of techniques; Mario Alberto Ruiz-López: writing of the manuscript.

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