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Original article

Phytochemical content and antioxidant potential of tropical sapodilla fruit (*Manilkara zapota*)

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Summary

Introduction – **Mexico is a country with a wide diversity of fruits and vegetables that have been consumed, since ancient times, as part of its traditions and custom; however, today, the nutritional and nutraceutical value is unknown despite its current consumption in the primary diet of rural populations. The sapodilla or chicozapote (*Manilkara zapota*) is a tropical fruit of the Sapotaceae family; it is found in some Southern Mexico areas, and has a high content of starch and coloring pigments which represents secondary metabolites from interest. The objective of the present work was to analyze the phytochemical content, and determine the antioxidant potential of the sapodilla.** *Materials and methods* – **Materials used in this work included ABTS, DMPD and DPPH free radicals used to determine the antioxidant activity and standard methods were used for the phytochemical analysis.** *Results* – **The content of flavonoids (4.12 mg EQ 100 g^{-1} fresh weight), total phenols (118.06 mg EAG 100 g^{-1} fw), vitamin C (0.08 mg EVC 100 g^{-1} fw), carotenoids (0.05 mg E 100 g^{-1} fw), and antioxidant activity (AOA 33.4% inhibition of free radicals as the highest value) were obtained.** *Conclusion* – **The sapodilla presented a high content of phenols and flavonoids, which reflected in the antioxidant potential that may function as a free radical inhibitor.**

Keywords
flavonoids, free radicals, nutraceutical, phenols, zapote, chicozapote

Significance of this study

What is already known on this subject?

- The sapodilla (or chicozapote) is an endemic fruit of the southern region of Mexico. With the passage of time its use and consumption have been lost, however, it is a potential source of bioactive compounds because it can avoid free radicals attack on cells, and thus prevent some diseases. Some studies have demonstrated that the habitual consumption of this endemic plants improves the life expectancy of people but did not quantify their potential. Derived from this, this work aims to quantify the antioxidant potential to motivate people to consume this fruit as frequent as possible to improve their quality of life.

What are the new findings?

- This work aims to rescue the use of ancestral foods and makes it public knowledge that the sapodilla has bioactive compounds that act as antioxidants and prevent the human body from suffering from some chronic degenerative diseases.

What is the expected impact on horticulture?

- Taking into consideration the health benefits offered by this product, it is possible to share the knowledge with producers and industries that can take full advantage of the fruit for the benefit of society. Thus, sapodilla crops can be increased, benefiting the farmer.

Introduction

Mexico has a great plant diversity, which has favored the exploitation of plants and fruits for medicinal and nutritional purposes since pre-Hispanic times (Gazel, 2002; Rodrigues *et al.*, 2019). However, the fast and sedentary lifestyle of most of the population has led to an increment in the consumption of sugars, processed or canned food, which is related to an increase in the occurrence of numerous diseases (Popkin *et al.*, 2012; Sharifi-Rad *et al.*, 2020). A healthy diet in the early stages of life is of the utmost importance to prevent the onset of chronic degenerative diseases in the long term (Čakar *et al.*, 2018a). The WHO estimates that ¾ of the population in developed countries uses fruits, leaves, roots, and other parts of plants in various preparations as part of traditional medicine to relieve several ailment symptoms (Kaneria *et al.*, 2009).

Oxidative stress is one of the main factors in the appearance of various diseases (Kaneria *et al.*, 2009; Battino *et al.*, 2020) and defined as a disproportion between the concentration of free radicals and the production of antioxidants that repair the damage caused by oxidant molecules (Sies, 2015). Some studies indicate that free radicals can damage biomolecules (lipids, proteins, and nucleic acids), altering their structure and modifying their functions (Kaminskyy and Zhivotovsky, 2014; Coronado *et al.*, 2015; Di Meo and Venditti, 2020).

Normal biochemical reactions are increased by environmental pollution, high levels of dietary xenobiotics, self-medication, excessive exercise, radiation (UV, IR, X-rays), smoking, chemical additives, stress, pathogenic microorganisms (bacteria, viruses, parasites), processed food (Pendyala *et al.*, 2008; Coronado *et al.*, 2015; Nimse and Pal, 2015), among others, which results in the generation of reactive oxygen

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(ROS) and nitrogen (RNS) species that are responsible for oxidative stress, and consequently different pathophysiological conditions (Coronado *et al*., 2015; Gupta, 2015; Nimse and Pal, 2015; Battino *et al*., 2020). Among the types of free radicals that exist, those that stand out are the hydroxyl radical (•OH), the superoxide anion (O_2•), hydrogen peroxide (H_2O_2), peroxynitrite (ONOO-), and nitric oxide (NO•) (Kunwar and Priyadarsini, 2011; Yan *et al*., 2020).

The beneficial properties of fruits and vegetables are due to the presence of various nutraceutical ingredients and/or secondary metabolites (phenolic compounds, terpenoids, vitamins, anthocyanins) that help to prevent some chronic and terminal diseases (cancer) (Young *et al*., 2005), found and distributed in various parts of plants such as the fruit, bark, leaf, flower, root, rhizome, *etc.* (Jain *et al*., 2019; Čakar *et al*., 2018b; Yang *et al*., 2018).

Nutraceuticals are food products that have a beneficial effect on health or prevent diseases. Antioxidants are a specific nutraceutical type (Dutta *et al*., 2018; Santini *et al*., 2018). Antioxidants are defined as that substance found in foods of regular consumption that can prevent harmful effects of reactive species on physiological functions in living beings (Coronado *et al*., 2015).

The additive and synergistic effects between antioxidants and nutrients can contribute beneficial health effects (Veurink *et al*., 2020); increasing scientific interest in clinical and epidemiological studies that try to find the correlation between the intake of food or its components with the prevention of some cardiovascular, visual, neurological diseases, cancer, aging, diabetes, *etcetera* (Dutta *et al*., 2018; Čakar *et al*., 2017; Milani *et al*., 2017). Interest in antioxidants has spread to other areas such as pharmaceuticals and the cosmetics industry, which makes very important the investigation of the diversity of compounds present in plant species (Almeida *et al*., 2011).

In traditional medicine are used the different parts of the sapodilla: the seed has purgative, diuretic, and tonic properties, thereby helping with ailments of the digestive system; on the other hand, the bark of the tree is with antidiarrheal, astringent, and antibiotic effects attributed; and the extracted gum that was for many years used as chewing gum, the fruit, and the leaves are used for the treatment of respiratory and digestive diseases (Ahmed *et al*., 2011; Barbalho *et al*., 2015; Pravin and Shashikant, 2019).

According to the Mexican National Commission for the Knowledge and Use of Biodiversity (CONABIO), in some states like Yucatán this plant species is protected by its commercial fruits, in addition to the fact that timber exploitation currently has been prohibited because it has the great advantage of being very resistant to different species of insects. The Agrifood and Fisheries Information Service (SIAP) reports a sapodilla production of 57.8 million pesos, being the largest producer of this fruit in Campeche, with 43% of all the national production.

Based in the hypothesis that sapodilla (*M. zapota*) has natural bioactive compounds that prevent diseases derived from oxidative stress; the objective of the present work is to analyze the phytochemical content and to determine the antioxidant potential of this fruit.

Materials and methods

Obtaining plant material

The sapodilla, chicle, zapote, chicozapote or zapotillo, as the *Manilkara zapota* species is commonly known, is a significant food of pre-Hispanic gastronomy that grows in the tropical and subtropical regions of Mexico; crops of this fruit in the Pacific and the Gulf coasts can also be found, especially in the Yucatán Peninsula (O'Farrill *et al*., 2006; Lim *et al*., 2018; Arrieta-Ramos *et al*., 2020). The fruit is edible. It is considered a round-looking berry, 10 cm in diameter and weighing up to 200 g; the rind or skin is thin, rough, and brown in color, and the sweet pulp is yellow to reddish-brown; inside are distributed 3 to 12 oval and flat seeds, up to 23 mm long (Ahmed *et al*., 2011; Vargas y Vargas *et al*., 2015). It is worth mentioning that the tree from which this fruit grows resists stressful ecological conditions; that is, it supports soils of a calcareous, stony nature, plains, *etc*., but for good development of the fruit, 2,000 mm of annual rainfall and temperatures of 24 °C are required. It is difficult to determine the degree of maturity of the sapodilla; if the skin can be easily detached and the fruit separates from the stem is said to be a ripe fruit; however, it must be stored at room temperature for a few days for consumption; it is worth mentioning that fully ripe fruit may be frozen for up to a month (Pravin and Shashikant, 2019).

The plant species sapodilla was obtained in the Municipal Market "General Adrian Castrejon" located at Iguala, Guerrero, Mexico. Those fruits that did not present any visible morphological alteration or signs of pathogenic conditions were carefully selected. Each sample was washed to remove soil excess, then rinsed in distilled water (3 times); finally, dried to proceed with the corresponding analysis.

Phytochemical analysis

1. Preparation of fresh plant material. 1 g of the fresh fruit pulp was with 10 mL of 80% aqueous methanol (80% v/v aq. MeOH) added; the suspension was sonicated for 15 min at room temperature and allowed to stand for 24 h. Finally, the ethanolic extracts were filtered and centrifuged for 10 min at 1,409 × g. The supernatants were collected and kept at 4 °C for subsequent use in the next 24 h (Wojdylo *et al*., 2007).

2. Quantification of anthocyanins. The pH difference method, described by Giusti and Wrolstad (2001), was used with some modifications; two buffer systems were used: HCl/KCl at pH = 1.0 and $CH_3COOH/C_2H_3O_2Na*3H_2O$ at pH = 4.5. Two test tubes were prepared with 200 µL of the previously prepared ethanolic extracts; 1.8 mL of the buffer solution with pH = 1.0 was added to one of them, and to another 1.8 mL of the buffer solution with pH = 4.5; the mixtures were vortexed, and the absorbance measured at a wavelength of 510 and 700 nm, the corresponding buffer solution was used as blanks. The total absorbance of the sample was calculated using the following equation:

$$A_t = [(A_{510} - A_{700})_{pH=1.0}] - [(A_{510} - A_{700})_{pH=4.5}].$$

The concentration of monomeric pigments in the extract is expressed as mg of cyanidin-3-glucoside equivalent to 100 g of fresh weight, and it was calculated employing the following equation:

$$Monomeric\ anthocyanins\left(\frac{mg}{L}\right) = \frac{A_t * MW * FD * 1000}{(\varepsilon * 1)}.$$

3. Estimation of flavonoid content. The colorimetric method with aluminum chloride was used where 0.5 mL of the supernatant were taken from the previously prepared ethanolic extracts, 1.5 mL of 95% methanol (v/v), 0.1 mL of 10% aluminum chloride (w/v), 0.1 mL of potassium acetate (0.1 M), and 2.8 mL of distilled water (Chang *et al*., 2002).

The mixture was shaken and incubated for 30 min at room temperature. Then, the absorbance was measured at 415 nm in a Genesys 10S spectrophotometer. For its quantification, a standard curve of quercetin flavone of 0–300 mg L^{-1} was run. The results of the total flavonoid content are reported as equivalent mg of quercetin 100 g^{-1} of fresh weight. The total content of flavonoids was quantified using the following formula:

$$\frac{mg\ of\ quercetin}{100\ g\ fw} = \left[\frac{\left(\frac{Abs-b}{m}\right)}{C}\right] * 100.$$

4. Determination of total phenols. The spectrophotometric method described by Folin and Ciocalteu (1927) was used with some modifications from And and Shahidi (2005). From the previously prepared samples, 0.5 mL were taken and were added 0.5 mL of Folin-Ciocalteu reagent 0.2 N and 4 mL of Na_2CO_3 0.7 M; the mixture was vigorously vortexed and incubated at room temperature and in the dark for about 2 hours. Then, absorbance was measured on a spectrophotometer at 765 nm. Gallic acid (0–400 mg L^{-1}) was used as standard. Results were expressed as equivalent mg of gallic acid 100 g^{-1} fresh weight, calculated by the following formula:

$$\frac{\left(\frac{Abs-b}{m}\right)}{[C]}[=]\ \frac{mg\ of\ gallic\ acid}{g\ of\ fresh\ weight}.$$

5. Quantification of vitamin C content. It was carried out by determining ascorbic acid according to the method described by Robinson and Stotz (1945). 3 mL of 3% v/v metaphosphoric acid solution were added to 1 g of sample, the mixture was macerated for 3 min and filtered. 1 mL of the filtrate was taken and made up to 10 mL with the 3% v/v metaphosphoric acid solution. 2 mL of the extract, and 2 mL of acetate regulator, 3 mL of dichloroindophenol, and 15 mL of xylene were added; it was vigorously stirred. The organic phase was separated, and absorbance was read in a spectrophotometer at 520 nm. Using the standard curve, the concentration of ascorbic acid present in each sample was obtained using the following equation:

$$\frac{mg\ of\ C\ Vit}{mg} = \frac{(C \times V \times 100)}{(A \times W)}.$$

The concentration of vitamin C was expressed as mg equivalent of ascorbic acid per 100 g of fresh weight.

6. Carotenoid content. The carotene content was quantified according to the method proposed by Lichtenthaler (1987). 10 mL of 80% acetone were added to 10 mL of fruit juice to be analyzed, the solution was filtered, and the absorbance at 663, 646, and 476 nm was obtained using acetone as blank. The carotene concentration was calculated using the following formulas:

Chlorophyll *a* (C*a*) = 12.25A_{663} - 2.79A_{646};

Chlorophyll *b* (C*b*) = 21.50A_{646} - 5.10A_{663};

Total chlorophyll (C*a*+ *b*) = 7.15A_{663} + 18.71A_{646};

Carotenes = (1000A_{476}-1.63C*a*-104.96C*b*) (221-1).

Antioxidant activity

1. ABTS method (2,2'-azino-bis (3-ethylbenzothiazolin) -6-sulfonic acid). The antioxidant activity (AOA) and the inhibition percentage were determined using the methodology described by Re *et al.* (1999), based on the generation of the radical cation $ABTS^+$ (blue-green chromophore) through the oxidation of the reagent with $K_2S_2O_8$. A solution of the $ABTS^+$ radical (7 mM) was prepared with $K_2S_2O_4$, 2.45 mM; the mixture was left at rest at room temperature (± 25 °C), in the dark for approximately 16 h before being used for the following analysis. Once the $ABTS^+$ free radical has been formed, it is diluted with a sufficient amount of ethanol until an absorbance of 0.7 ± 0.1 is obtained at 734 nm. One mL of the radical formed was taken, placed in test tubes, and added 10 µL of the previously prepared samples (ethanolic extracts). The mixture was vigorously vortexed and subsequently incubated in a water bath at 30 °C. Finally, absorbance was measured at 734 nm at 1 and 7 min. Trolox (0–2.0 mM) was used as control. The results were expressed as equivalent mg of Trolox per 100 g of fresh weight (mg ET 100 g^{-1} fw). The AOA was calculated using the following formula:

$$mg\ ET\ 100\ g^{-1} fw = \frac{\left(\frac{Abs-b}{m}\right) * MW}{C}.$$

And inhibition percentage was calculated using the following formula:

$$\%\ Inhibition\ of\ free\ radicals = \left(\frac{A_0 - A_F}{A_0}\right) * 100.$$

2. DMPD (N, N-dimethyl-p-phenylenediamine dihydrochloride) method. The method used was the one described by Fogliano *et al.* (1999), based on free radical discoloration due to the antioxidants (sample) presence. The free radical $DMPD^+$ was produced by preparing a solution with a concentration of 100 mM. Once prepared, was taken 1 mL, to which 100 mL of a buffer mixture, prepared with acetic acid/sodium acetate at pH = 5.25 and 200 µL of $FeCl_3$*$6H_2O$, 0.05 M were added. The mixture was vigorously stirred for 10 min to measure initial absorbance at 506 nm (adjusted to 0.9 ± 0.1). Aliquots of 1 mL were taken and placed in test tubes, to which 50 µL of the test sample or the reference were added. The mixture was stirred vigorously and subsequently incubated at 25 °C for an approximate period of 10 min, then absorbance was measured at 506 nm. Acetate buffer pH = 5.25 was used as blank. Gallic acid (0–1.2 mM) was used as control. The results were expressed as mg equivalent of gallic acid per 100 g of fresh weight (mg EAG 100 g^{-1} fw). The percentage of inhibition of free radicals was calculated using the following formula:

$$\%\ Inhibition\ of\ free\ radicals = \left(1 - \frac{A_F}{A_I}\right) * 100.$$

3. DPPH (2,2-diphenyl-1-picrylhydrazyl) method. The colorimetric technique described by Kim *et al.* (2002) was used, based on the discoloration of the $DPPH^+$ radical when is added an antioxidant agent; this compound is commercially available. Aliquots of 2.9 mL of the free radical solution at a concentration of 100 µM were added to 100 µL of the samples. The mixture was vortexed and incubated for 30 and 60 min at room temperature in the dark, then, absorbance was measured using a spectrophotometer at 517 nm. The reference blank was 80% aqueous methanol and quercetin (0–0.9 mM) was used as control. The results were reported as mg equivalents of quercetin in 100 g of fresh weight (mg EQ 100 g^{-1} fw). The inhibition of free radicals was calculated as described in DMPD section.

Results and discussion

The sapodilla is a seasonal fruit regularly consumed only in the towns where the tree grows and bears fruit. In the market, there are few or almost no processed products based on this fruit. The biggest challenge is that ascorbic acid and sugars are susceptible to oxidation, therefore, the shelf life of the edible fruit is reduced. That is why studying the phytochemical and antioxidant profile of this fruit is relevant, resulting in the motivation to include it in the daily diet. Moo-Huchin *et al*. (2017), in their study on pulp extracts of tropical fruits in Yucatán, México, reported contents of 36.41, 2.56 ± 0.01, and 1.08 ± 0.06 mg of β-carotene in 100 g of edible portion in mamey sapote, black sapote and sapodilla, respectively; it is worth mentioning that these values are higher than those reported in this study. The results obtained from the phytochemical analysis of the edible sapodilla fruit are shown in Figure 1.

As can be seen, the quantity of phenols is considerably higher in relation to the other compounds analyzed, being 118.06 mg EAG*100 g^{-1} fw. Second is the flavonoid content, followed by anthocyanins, and with less impact the content of vitamin C and carotenoids.

It is worth mentioning that few studies explore the phytochemical content and antioxidant activity in the pulp of the sapodilla fruit, not so in other parts of the plant; for example, Kaneria *et al*. (2009) reported in the methanolic extract of the leaves the content of flavonoids and phenols, registering 35.5 and 194.06 mg g^{-1}, respectively. On the other hand, Shanmugapriya *et al*. (2011) determined the flavonoids content (4.0 mg g^{-1}) and phenols (3.98 mg g^{-1}) in the ethanolic extract of sapodilla seeds. It is also worth mentioning that the sapodilla fruit can be consumed directly as part of the diet in several Mexican regions. These data are not comparative to the carried-out work because they are reported in different parts of the plant and using other solvents; however, they represent evidence of the distribution of phytochemicals in the tree. This means that the pulp has important functional compounds, but the other parts of the sapodilla plant can be used to extract metabolites and probably add them to some other food product.

Lim *et al*. (2018) reported 5.52 mg EVC*100 mL^{-1} fw of juice in sapodilla, a value higher than that reported in this work; it is worth mentioning that this data was obtained from juice added with water (50:50) without considering pulp and skin. Regarding the phenols content, the data reported by these same authors are significantly lower than those obtained in this research because the antioxidant phytochemicals: gallic acid and quercetin, may be contained in the skin and pulp that have synergistic effects and increase the antioxidant potential of the sample.

On the other hand, Lee *et al*. (2013) presented a phenol content of 16.64 mg GA*100 g^{-1} fw of fruit, a lower value than the one obtained in this work. The difference in phenol content is notable, indicating that sapodilla is an important source of this compound. The differences in phenol content could be due to the edaphoclimatic conditions where the fruit was harvested, since the research where the results are compared was carried out with fruits from unequal geographical areas, which implies different types of soil, nutrients, solar energy, humidity, water availability, height above sea level, *etcetera* (Yang *et al*., 2018); remembering that the edaphoclimatic conditions influence the nutrient content of the fruits.

Reyes *et al*. (2005) carried out a study of the loss/gain of phenols during the ripening process of the sapodilla fruit; they observed that the content of phenols decreases as the maturation of the fruits increases due to the oxidation of monohydric and o-dihydric phenols, decreasing its antioxidant potential.

The AOA of sapodilla by the three methods analyzed is shown in Table 1. The antioxidant activity can be different between the chromophores used according to the affinity of the compounds present in the sapodilla pulp, it is thus that there are values from 27.84% (ABTS method) to 33.4% (DPPH method).

Moo-Huchin *et al*. (2017) performed the antioxidant analysis by ABTS and DPPH methods in hexane/acetone/ethanol extracts (70:15:15) of tropical fruit pulp, such as mamey sapote, dragon fruit, green nance, red mombin, black sapote, sapodilla, among others. By the ABTS method,

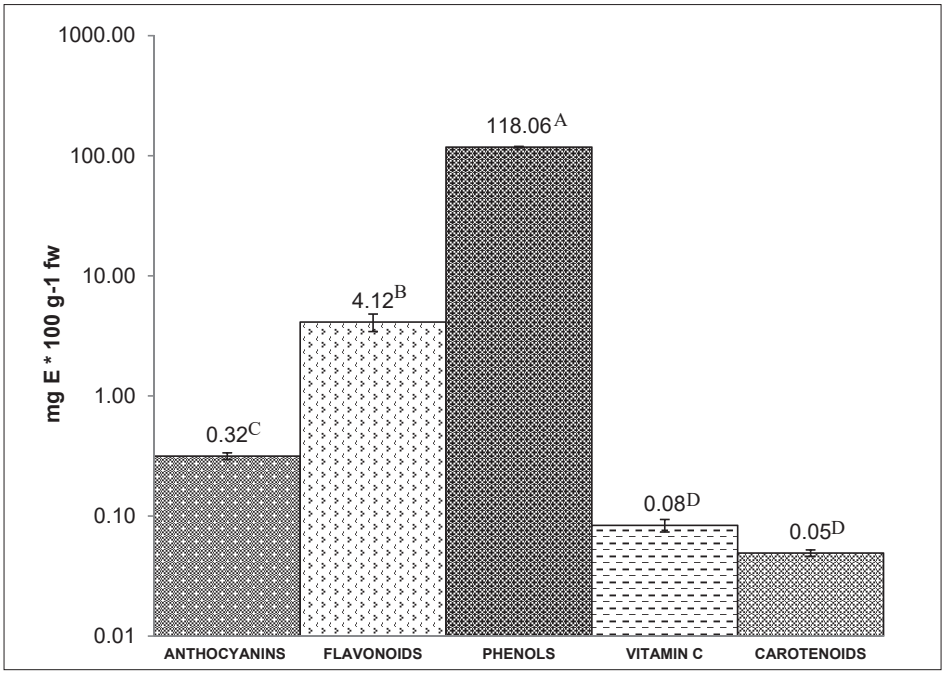

Figure 1. Phytochemical content of the ethanolic extract of sapodilla pulp (mean ± SD, $n = 3$).

Table 1. Determination of the antioxidant activity of the ethanolic extract of *Manilkara zapota*.

Method	AOA	% Inhibition
ABTS 7 min	54.93 ± 1.03[b] mg ET * 100 g^{-1} fw	27.84 ± 0.85[b]
DMPD 10 min	85.12 ± 1.87[a] mg EAG * 100 g^{-1} fw	31.95 ± 0.7[a]
DPPH 30 min	77.51 ± 1.39[a] mg EQ * 100 g^{-1} fw	33.4 ± 1.39[a]

Values with the same letter and same column are not statistically significant based on Tukey's test ($p < 0.05$).

they report values of 11.1 ± 1.4, 19.8 ± 2.8 and 1.3 ± 0.0 µM of Trolox in 100 g of edible portion in black sapote, mamey sapote and sapodilla, respectively. By the DPPH method, values of 2.8 ± 0.0 (black sapote and mamey sapote) and 8.4 ± 0.7 (sapodilla) µM of Trolox in 100 g of edible portion. It is important to mention that these data were carried out in different solvents as used in this study; in addition, other factors such as fruit maturation, the type of growth climate and other environmental factors that could have intervened in the content of phytochemical compounds.

Shanmugapriya *et al*. (2011) report the % inhibition in the ethanolic extract of sapodilla seeds by the ABTS (32.23%) and DMPD (36.07%) methods; values like those presented in this work; however, it is important to mention that the studies cannot be compared because they were not carried out in similar parts of the plant species. These results indicate that the sapodilla fruit provides antioxidants necessary for the prevention of diseases such as diabetes, hypertension, Alzheimer's, *etc*.

Up to date, there are few works that explore the content of other phytochemical compounds in this species, such as the content of anthocyanins or flavonoids. So, this work presents an opportunity to explore compounds as gallic acid, quercetin, ascorbic acid, among others, which offer great health benefits for their consumption. It is important to mention that in recent decades the increase in various chronic and degenerative diseases has led scientists and researchers to explore new sources of potential bioactive compounds against these diseases and some other risks, which are also being recommended by specialists in the health area as part of the human diet.

Conclusion

Sapodilla (*M. zapota*) or chicozapote, an endemic fruit of Mexico, represents a food source rich in phytochemical compounds such as antioxidants, flavonoids and others secondary metabolites. There are several studies that explore biologically active compounds in other parts of the tree, but there are few works that focus on the pulp of the edible fruit. This fruit has a low percentage of inhibition of free radicals compared to other fruits such as grapes, purple tuna or berries; however, it is considered a food with bioactive compounds that can contribute to avoid diseases of the digestive system and the prevention of chronic degenerative diseases caused by the oxidative stress. As mentioned, its traditional use is a source to investigate on its potential in human health.

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