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Review

Phenolic profile and antioxidant capacity of *Cnidoscolus chayamansa* and *Cnidoscolus aconitifolius*: A review

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Research into ancient cultures has yielded a large body of evidence on the use of medicinal plants for preventive and/or therapeutic purposes. Such plants may have many metabolic activities and functions in the body-antioxidant, anti-inflammatory, platelet aggregation inhibitory and immunological and they can act at different molecular levels. This work offers a comprehensive review of research into the phenolic profile and antioxidant capacity of a plant used since the pre-Columbian era, native to southeast Mexico, commonly known as "chaya". The most prevalent phytochemicals in this plant are its phenolic compounds, and their antioxidant capacity is responsible for many of its health benefits, specifically in controlling chronic diseases. In the chaya leaf, there is a general trend toward the presence of different phenolic groups, such as coumarin, flavonoids, phenols, tannins, anthraquinones and flobotanins in aqueous and alcoholic extracts. Aside from environmental factors, there are differences in the ways samples are treated before the extraction process, such as the treatment type and the drying conditions. There are also differences in the solvents used and in the methods of extraction and concentration of compounds. Finally, a diversity of techniques is used, and even the data are quantified and expressed differently. Chaya has great potential for production as food and as a medicinal plant, but much more research is needed on the composition of its leaf and the biological effects of its components.

Key words: Chaya, *Cnidoscolus aconitifolius*, *Cnidoscolus chayamansa*, phenolic compounds, antioxidant capacity.

INTRODUCTION

The use of plants in medicine goes back to the beginnings of human civilization. Substantial evidence has been found on the use of plants for preventive and/or therapeutic purposes in ancient cultures (Mwine and Van

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Damme, 2011). According to the World Health Organization (WHO), a medicinal plant is one that contains substances that can be used for therapeutic purposes and/or can serve as active ingredients for the synthesis of new drugs (WHO, 2005). The use of traditional medicines and medicinal plants has been widely observed in most developing countries, where they are seen as therapeutic agents for the maintenance of good health (Soetan and Aiyelaagbe, 2009).

For several decades, various lines of research have been pursued into medicinal plants and their components. One of them focuses on the study of the composition of minority compounds, mainly phenolic compounds, given their various benefits in battling chronic disease, including cardiovascular disease, neurodegeneration, and cancer (Del Rio et al., 2013). They cover a wide range of metabolic activities and have many functions in the body: antioxidant, anti-inflammatory, platelet aggregation inhibitory and immunological; and they can act at different molecular levels. Thus, the consumption of phenolic compounds is associated with health benefits (Rangel-Huerta et al., 2015). Also, several studies in plants report on their antioxidant capacity. There are a large number of publications on different plants, applying a variety of methods for extracting and measuring phenolic compounds and antioxidant capacity (Gutiérrez-Grijalva et al., 2016). These publications differ considerably in the types of processing used for the raw material, and also the solvents used (for example, aqueous, alcoholic and non-polar, as well as different mixtures thereof), as well as the times, temperatures, concentration, and other factors. Finally, there are diverse ways of expressing the content of phenolic compounds and antioxidant capacity. This work offers a comprehensive review of the literature on the phenolic profile and antioxidant capacity of a plant that has been in use since the pre-Columbian era, native to southeast Mexico, commonly known as "chaya".

CHAYA (*CNIDOSCOLUS* SPECIES)

Chaya refers to any group of plants of the genus *Cnidoscolus*, which is a part of the family Euphorbiaceae (Cifuentes et al., 2010). This genus is composed of 50 species, 20 of which are endemic to Mexico. They are distributed in tropical and subtropical areas, mainly in regions of low deciduous forest and xerophilous scrub of Mexico (Kolterman et al., 1984). Some species of *Cnidoscolus* are of interest for their nutritional potential, particularly the most commonly used for both consumption and traditional uses such as medicinal and ornamental plants, *Cnidoscolus aconitifolius* and *Cnidoscolus chayamansa* (Kolterman et al., 1984). *C. aconitifolius* has pentalobulated leaves, with lobed, serrated edges, with a long petiole length, without pubescences, with sagittate base, with the presence of glands and with white flowers (Adebiyi et al., 2012). In contrast, *C. chayamansa* has three-lobed leaves, with smooth lobed edges, with short petiole length, without pubescence, and similarly with sagittate base, with the presence of glands and with white flowers (Standley and Steyerman, 1949) (Figure 1). These two species originated in the Yucatán region of southern Mexico and are fast-growing perennial shrubs (Grubben and Denton, 2004).

The chaya plant is a domesticated shrub, highly valued by people in rural communities of central and southern Mexico as food, as a medicinal plant and as an ornamental. Chaya has been used as food since pre-Columbian times and is currently consumed regularly in some populations (Ross-Ibarra and Molina-Cruz, 2002). In addition, chaya leaves have been found to be an important source of protein, β-carotene, vitamins, ascorbic acid, calcium, potassium, and iron (Jiménez-Arellanes et al., 2014; Kuti and Kuti, 1999).

Chaya is consumed in a manner similar to spinach, which is why it is also called “Mayan spinach” (Ross-Ibarra, 2003). But its nutrient content is far superior to spinach: 78% more proteins, 111% more fiber, 100% more iron and 242% more vitamin C (Kuti and Torres, 1996) (Table 1).

Chaya leaves contain a cyanogenic glycoside called Linamarin. Linamarin is a glucoside conjugate of an acetone and a cyanide (Kuti and Konuru, 2006). It is a secondary metabolite of plants that performs defense functions, since when it is hydrolyzed by enzymes it releases hydrogen cyanide, a process called cyanogenesis. The content of cyanogenic glycosides according to Gonzalez-Laredo et al. (2003) is 2.37 to 4.25 mg/100 g dry matter (DM). These authors tested various thermal treatments to remove this compound from the leaves and reported that 5 min in boiling water is sufficient to remove any residue of cyanide (Figure 2).

The use of chaya leaves has been reported in traditional medicine for various pathologies, where it is believed to have antidiabetic, antioxidant, hepatoprotective, and hormone-related properties on the pituitary-gonadal axis (García-Rodríguez et al., 2014; Hitchcock et al., 1997; Jiménez-Arellanes et al., 2014; Kulathuran et al., 2012; Kuti and Konuru, 2006; Kuti and Torres 1996; Loarca-Piña et al., 2010; Lucky and Festus, 2014; Miranda-Velasquez et al., 2010).

These plants can grow up to 6 m high, with lobed leaves, milky sap and small dichotomous white flowers at the tip of the branches. It is propagated by planting stem cuttings or woody stem cuttings. Within the chaya subspecies there is a considerable morphological and phenological variation. In research carried out by Ross-Ibarra and Molina-Cruz (2002), four cultivated varieties of chaya were identified, with easily separable and quite consistent morphological differences, but their taxonomy is not yet assigned. These are classified as star, beaked, chayamansa and round. Seeds and ripe fruit are rare and unknown (McVaugh, 1994). Given the ease of its

Table 1. Comparison of nutritional compositions of chaya leaves (*Cnidoscolus chayamansa* McVaughn) and spinach (*Spinacia oleraceae* L.) per 100 g fresh weight.

Component	Chaya	Spinach	Δ (%)
Water (%)	85.3	90.7	-6
Protein (%)	5.7	3.2	78
Lipid (%)	0.4	0.3	33
Fiber (%)	1.9	0.9	111
Calcium (mg/100 g)	199.4	101.3	96
Phosphorus (mg/100 g)	39.0	30.0	30
Potassium (mg/100 g)	217.2	146.5	48
Iron (mg/100 g)	11.4	5.7	100
Ascorbic acid (mg/100 g)	164.7	48.1	242

Δ (delta) represents the change (increase or decrease) of the value of a variable, using as reference the values of spinach. Adapted from (Kuti and Torres, 1996).

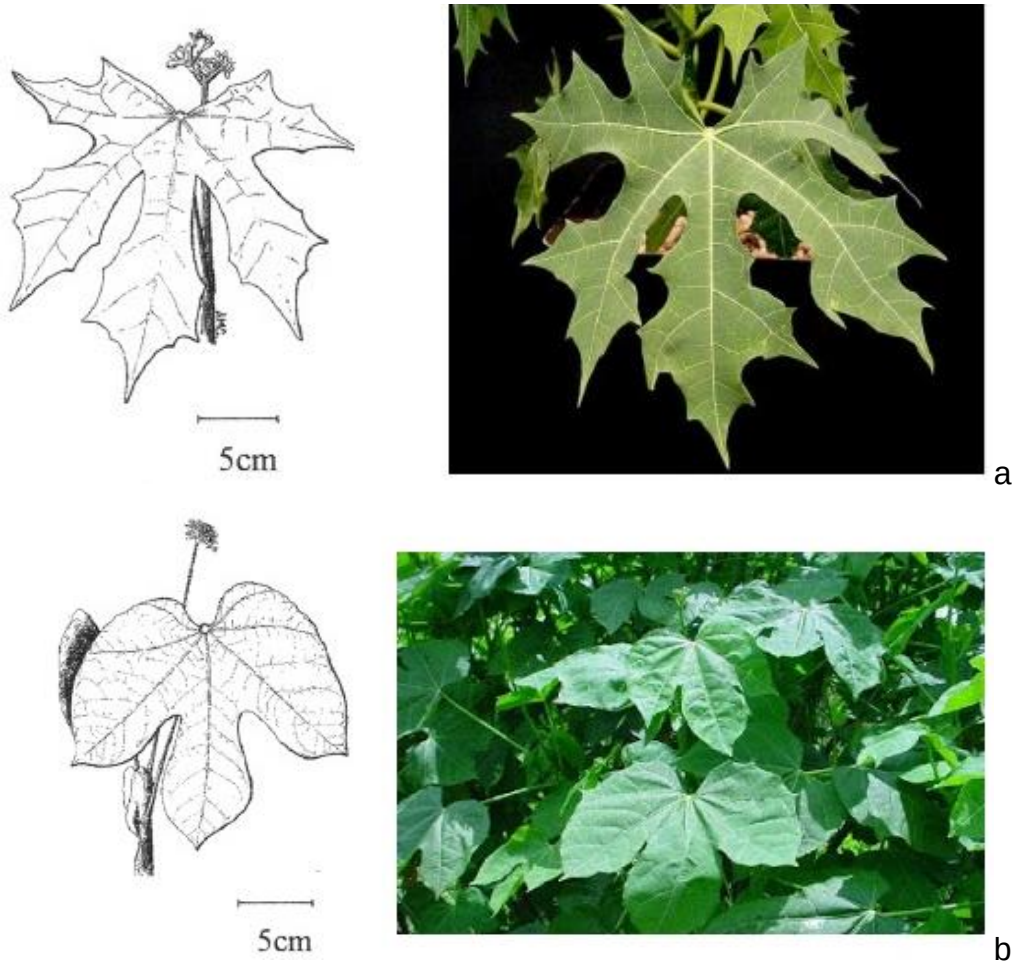


Figure 1. Images of (a) *C. aconitifolius* and (b) *C. chayamansa*, known locally as chaya. Source: Adebiyi et al. (2012); Cifuentes et al. (2010).

cultivation, its potential productivity, and above all its high nutritional value, chaya has been proposed as a potential crop for regions outside of Mesoamerica (Kuti and Torres, 1996; Molina-Cruz et al., 1997; Ross-Ibarra and

Molina-Cruz, 2002).

IMPORTANCE OF PHENOLIC COMPOUNDS AND ANTIOXIDANT CAPACITY

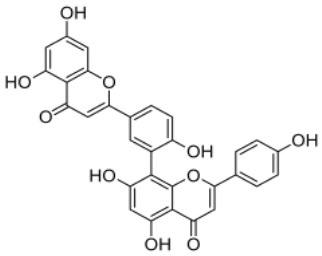
Atoms or molecules containing one or more unpaired electrons are called free radicals. Free radicals are responsible for tissue degeneration through damage to DNA, proteins and lipid peroxidation through oxidative stress, which has been implicated in the pathophysiology of different diseases. Some authors have found that the degree of damage caused by free radicals can be mitigated by supplementation with one or more antioxidants (Marchioli et al., 2001). Several compounds with differential antioxidant properties are found in plants and these plants are considered to have high biological potential in the context of the prevention and treatment of damage caused by free radicals. Several medicinal plants have been examined and evaluated for their properties in antagonism toward free radicals induced by oxidative stress (Esparza-Martínez et al., 2016; Vinson et al., 2001).

Some of these plants' medicinal properties are attributed to their phytochemical composition, specifically a variety of minority compounds derived from the secondary metabolism of plants, which have attracted interest recently for their bioactive effects. Phenolic compounds are among these, and are ubiquitous in foods of plant origin. The main functions of phenolic compounds in plants have to do with pigmentation and protection against pathogens and predators. They are chemical compounds having at least one aromatic ring to which one or more hydroxyl groups are attached to aromatic or aliphatic structures (Bravo, 1998). There are over 10,000 different phenolic compounds, ranging from the simplest to the most complex, and their wide diversity in nature is evident upon analysis of their characteristics (Gutiérrez-Grijalva et al., 2016; Neveu et al., 2010; Rothwell et al., 2014; Zare et al., 2014). Many constituents of these plants can contribute to their protective properties, including: vitamins C and E; selenium and other mineral micronutrients; carotenoids; phytoestrogens; glucosinolates and indoles; ditholthiones; isothiocyanates; protease inhibitors; fiber; and folic acid. These compounds may act alone or in combination, as anticarcinogenic or cardioprotective agents, through a variety of mechanisms. One of these protective mechanisms, attributed to vitamins C and E and to carotenoids, is antioxidant activity (radical barrier) (Rice-Evans et al., 1997).

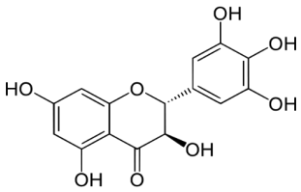
There are several classes of flavonoids, which differ in the level of oxidation and saturation of ring C, and individual compounds within each class differ in the substitution pattern of rings A and B (Wojdyło et al., 2007). Researchers have been looking into the antioxidant properties of many plant species for at least 50 years. There is currently a great deal of interest in the commercial production of plants as sources of antioxidants that can enhance the properties of food, both for nutritional and medicinal purposes. Numerous epidemiological studies have shown an inverse relationship between consumption of fruits, vegetables and cereals and the incidence of coronary heart disease and certain cancers (Gunjan et al., 2011). The plant kingdom is vast, with thousands of species and varieties that demand study. The phenolic composition and antioxidant activity of plants, both wild and cultivated traditionally, are a particularly rich area for future research. The antioxidant capacity of various plants is generally studied with respect to the content of total phenolic compounds using traditional methods, and only one test is used to determine free radical scavenging ability. Although extensive studies of bioactive compounds and their content of total phenolic compounds have been carried out in many species, the phenolic identification data are still insufficient and incomplete. In particular, quantitative data on specific phenolic compounds in plant species remains a pending task. There are also few comparisons of the phenolic constituents identified in several species of different plant families. Further research is required into the structure-activity relationships of phenolic compounds present in plant species (Czapecka et al., 2005; Ivanova et al., 2005). The objective of this work is to review the literature on phenolic composition and the antioxidant capacity of different extracts derived from the leaves of *C. aconitifolius* and *C. chayamansa*. A comprehensive search was performed using the terms "*Cnidoscolus chayamansa*" and "*Cnidoscolus aconitifolius*" without reducing or limiting the search elements. A total of 57 publications were consulted on the main scientific portals (Scopus, PubMed, Science Direct, Springer-Link, Wiley, Redalyc, Google Scholar, and Web of Science). The information was subsequently analyzed and classified as described subsequently.

PREPARATION FOR PHENOLICS EXTRACTIONS

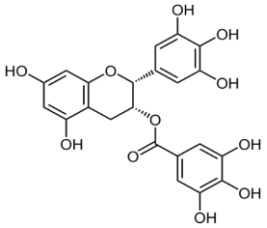
Plant extracts are a complex mixture, with a multitude of chemical compounds obtained by physical and chemical processes from a natural source and usable in almost any technological field. The WHO estimates that 80% of developing country populations rely on traditional medicines, mostly plant drugs, for their primary health care needs (Soetan and Aiyelaagbe, 2009). Plant extracts have been used since the beginning of civilization because they increase the useful life of the compound. There are few synthetic chemicals that can be used without toxicity or side effects, but nature is a potential source for discovering new structures that may have therapeutic qualities. Various phenolic compounds such as flavonoids can be extracted from fresh or dry



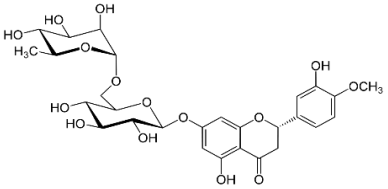
Amentoflavone (Figueroa-Valverde et al., 2004; González-Laredo et al., 2003)



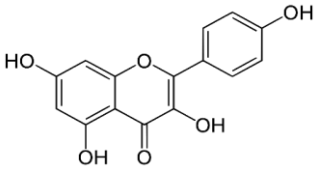
Dihydromyricetine (Figueroa-Valverde et al., 2004; González-Laredo et al., 2003)



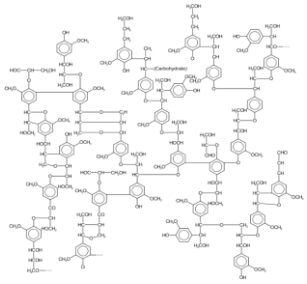
Epigallocatech gallato (Numa et al., 2015; Ramos-Gómez et al., 2016)



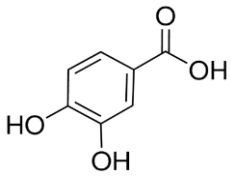
Hesperidin (Figueroa-Valverde et al., 2004; Ramos-Gómez et al., 2016)



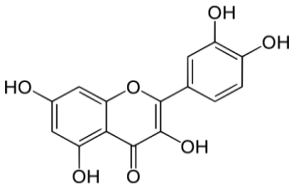
Kaempferol (Kolterman et al., 1984; Kuti and Konuru, 2004)



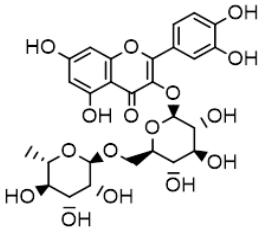
Lignin (Numa et al., 2015; Sarmiento-Franco et al., 2003)



Protocatechic acid (Loarca-Piña et al., 2010; Ramos-Gómez et al., 2016).



Quercetin (Figueroa-Valverde et al., 2004; Kolterman et al., 1984; Kuti and Konuru, 2004; Ramos-Gómez et al., 2016; Valenzuela-Soto et al., 2015)



Rutine (Figueroa-Valverde et al., 2004; Loarca-Piña et al., 2010; Ramos-Gómez et al., 2016; Valenzuela-Soto et al., 2015)

Figure 2. Structures of the most reported phenolic compounds in chaya leaves.

material, as long as proper methods and care are used to avoid significant alteration of their contents and composition. Nonpolar or slightly polar solvents are initially used to separate chlorophylls, gums and aglycones from highly methoxylated flavonoids. Flavonoids, which have many unsubstituted hydroxyl groups or sugars, are considered polar, so they are slightly soluble in polar solvents such as methanol, ethanol, acetone, dimethyl sulfoxide (DMSO) or water. The final filtrate is usually concentrated and the solvent is removed (Sarker and Nahar, 2012; Skerget et al., 2005). Most phenolic compounds are found within plant cells in aglyconated or in glycosylated form. This protects them from degradation, diminishes their toxic effects and at the same time aids transport through membranes, increasing their aqueous solubility. These compounds, in any of their forms, are already aglyconated or glycosylated, are in the vacuoles of plant cells and are in a soluble polar fraction.

Therefore, these aglyconated and glycosylated compounds can be extracted relatively easily using polar solvents (Jones and Vogt, 2001).

EXTRACTIONS FROM THE CHAYA LEAF WITH DIFFERENT SOLVENTS

Table 2 shows different forms of extraction of chaya leaf compounds reported in the literature.

Water extraction

Awoyinka et al. (2007) report that the aqueous extraction was performed from the dry leaves of *C. aconitifolius* that had been processed with a mortar and pestle. At the end, the substance was heated in an oven at 45°C until it reached a constant weight, although the proportion of the extraction is not specified. Musa et al. (2008) allowed the leaves of *C. aconitifolius* to dry at 40°C for 48 h. The reported extraction rate was 20 g of dried ground leaf to 1 L of cold distilled water, mixing for 48 h at a constant

Table 2. Studies that have reported on analysis of chaya leaf composition.

Solvent species	Plant samples	Extraction process	Elimination of cyanogenic glucoside	Reference
		Water extraction		
C. aconitifolius	Leaves dried at 45°C	NR	NR	Awoyinka et al. (2007)
C. aconitifolius	Leaves dried at 40°C for 48 h	20 g of DM in 1000 ml of distilled water	NR	Musa Toyin Yakubu et al. (2008)
C. aconitifolius	Sun-dried leaves	218 g of DM in 500 ml of distilled water	NR	Mordi and Akanji (2012)
C. aconitifolius	Leaves air dried at 28°C for 28 days	NR	NR	Obichi et al. (2015)
C. chayamansa	Leaves air dried for 15 days	5 g of DM in 100 ml of distilled water at 19°C for 10 min.	10 min at 90°C	Valenzuela et al. (2015)
C. aconitifolius	Leaves divided into 5 groups: fresh, bleached, boiled, extract and extract residue	Bleached leaves, 65°C for 10 min; leaves boiled, 100°C for 15 min. Juice was extracted from the leaf and juice residue	15 min at 100°C	Babalola and Alabi (2015)
C. chayamansa	Ethno-botanical information available (without quotation)	20 g of DM in 1000 ml of boiling water for 20 min	Boiling water during 20 min	Ramos-Gomez et al. (2016)
		Extraction using ethanol and mix polar solvents		
C. aconitifolius	NR	5 g DM in 20 ml ethanol/acetone/water/acetic acid (40:40:20:0.1 v/v)	heating in a microwave oven for 2 min	Kuti and Konuru (2004)
C. aconitifolius	Dried leaves ground in a mortar	96% ethanol for 3 h rotaevaporated at 30°C for 25 min and dried in an oven at 45°C	NR	Awoyinka et al. (2007)
C. aconitifolius	NR	5 g DM in 40 ml in ethanol/acetone/water/acetic acid (40:40:20:0.1 v/v) dried in a 60°C water bath for 1 h	NR	Johnson et al. (2008)
C. aconitifolius	Leaves air-dried at room temperature	1000 g of mature leaves in 70% ethanol, reduced by evaporation at 50°C and defatted with *n*-hexane	NR	Mordi and Akanji (2012)
C. chayamansa	NR	135 g of DM using 9.44 g of ethanol, the solvent was rotaevaporated and allowed to dry at 25°C in an oven	NR	García-Rodríguez et al. (2014)
C. aconitifolius	Dried and macerated leaves	7 days in 96% ethanol with solvent change daily	NR	Numa et al. (2015)
		Extraction using methanol and mix polar solvents		
C. aconitifolius	Ground dried leaves	70% methanol and 30% water	NR	Kolterman et al. (1984)
C. chayamansa	Leaves dried at 60°C for 6 h	It was extracted with methanol (x2) and rotaevaporated (<40°C), finally separated with hexane, ethyl ether and ethyl acetate (x2)	Boiled in water for 1, 5, 10, and 15 min, soaked in water at 20°C, 60 min; 70°C, 30 min, and sun-dried for 4 days	Gonzalez-Laredo et al. (2003)

Table 2. Contd.

C. chayamansa	Leaves previously dried	20 g in 250 ml of 80% methanol for 8 h	NR	Figueroa-Valverde et al. (2009)
C. chayamansa	Dried and macerated leaves.	500 g DM in 1000 ml using hexane-acetone (1:1 v/v) at room temperature for 5 days 2 times a day. The material was extracted with 100% methanol under the same conditions	NR	Loarca-Piña et al. (2010)
C. aconitifolius	Dry leaves (3 kg) in an extractor at 30°C	Methanol for 5 h, rotaevaporated at 35°C for 30 min.	NR	Adaramoye et al. (2011)
C. aconitifolius	Air dried in the laboratory for 5 days at room temperature followed by oven drying at 40°C followed by grinding to powder form using an electric mill.	1000 g DM in 2500 ml methanol. Rotaevaporated at 40°C	NR	Ikpefan et al. (2013)
		Other solvents		
Ethyl acetate *C. aconitifolius*	NR	135 g of DM was treated with 5.27 g of ethyl acetate. It was rotaevaporated to dryness and dried in an oven at 25°C	NR	García-Rodríguez et al. (2014)
Dichloromethane *C. aconitifolius*	20 freshly cut leaves	1000 ml of methylene chloride for 20 secs and evaporated to obtain 5333.3 mg of residue	NR	Escalante-Erosa et al. (2004)
Hexane *C. aconitifolius*	NR	135 g of DM treated with 5.68 g of hexane. It was rotaevaporated to dryness and oven dried at 25°C	NR	García-Rodríguez et al. (2014)
		Studies that do not report extraction		
C. aconitifolius fresh matter	The leaves from each plant were stored in plastic bags and frozen at −10 ◦C until analysis.	NR	NR	Sarmiento-Franco et al. (2003)
C. aconitifolius dry matter	Leaves cooked at 80 and 90°C for 19 min and allowed to dry	NR	Cooked 10 min at 90°C	Aye (2012)
C. aconitifolius dry matter	Leaves dried in oven at 40°C	NR	Boiled for 20 min.	Akachukwu et al. (2014)
C. aconitifolius dry matter	Leaves dried in oven at 70°C for 3 days to constant weight	NR Fresh or dry matter is related with preparation of leaf	NR	Jiménez-Aguilar and Grusak (2015)
		Studies that do not report extraction		
C. aconitifolius fresh matter	The leaves from each plant were stored in plastic bags and frozen at −10 ◦C until analysis.	NR	NR	Sarmiento-Franco et al. (2003)

Table 2. Contd.

C. aconitifolius dry matter	Leaves cooked at 80 and 90°C for 19 min and allowed to dry	NR	Cooked 10 min at 90°C	Aye (2012)
C. aconitifolius dry matter	Leaves dried in oven at 40°C	NR	Boiled for 20 min.	Akachukwu et al. (2014)
C. aconitifolius dry matter	Leaves dried in oven at 70°C for 3 days to constant weight	NR Fresh or dry matter is related with preparation of leaf	NR	Jiménez-Aguilar and Grusak (2015)

Only the information available in each of the references is mentioned. NR: Not reported.

temperature. The mixture was then filtered and concentrated in a steam bath until 4.88 g of residue remained. Mordi and Akanji (2012) dried the *C. aconitifolius* leaves in the sun, and then macerated them. The proportion was 218 g of dry matter to 500 ml of distilled water using a rotoevaporator at 50°C. This residue was then lyophilized. Obichi et al. (2015) mentioned that only *C. aconitifolius* leaves were harvested, cleaned and air dried at 28°C for 28 days before use. Valenzuela et al. (2015) reported drying the *C. chayamansa* leaf for 15 days at room temperature in a closed and ventilated area, where the sample was then ground with a mortar and stored at room temperature. The sample was prepared by mixing 5 g of dry matter into 100 ml of distilled water at 90°C for 10 min. This was then filtered with Whatman paper (No. 4, 110 mm) and the extract was stored at 5°C for analysis. Babalola and Alabi (2015) reported four different processes: in the first group, the leaves of *C. aconitifolius* were bleached at 65°C for 10 min, in the second they were boiled at 100°C for 15 min, in the third the juice was extracted from the leaf, and in the fourth the residue of the juice was collected after extraction. Ramos-Gomez et al. (2016) used a technique gathered from available ethno-botanical information for *C. chayamansa*, which was to boil 20 g in 1 L of drinking water for 20 min, then to pass this mixture through a 0.5-mm pore size filter.

Extraction using ethanol and mix polar solvents

Kuti and Konuru (2004) mention that the extraction of *C. aconitifolius* was 5 g DM in 20 ml of ethanol/acetone/water/acetic acid (40:40:20:0.1 v/v). This is the only study that reports using a microwave oven (1.3 cu ft Panasonic microwave 1000-W), in which the sample was heated for 2 min, to remove the cyanogenic glycoside from the plant. Awoyinka et al. (2007) mention that *C. aconitifolius* dried leaves were ground in a mortar and that the extraction was carried out with 96% ethanol for 3 h. The resulting solution was placed in a rotoevaporator at 30°C for 25 min, then placed in a drying oven at 45°C until a constant weight was reached. Johnson et al. (2008) reported placing a mixture of 5 g of *C. aconitifolius* dried leaf in 40 ml of an ethanol/acetone/water/acetic acid solution (40:40:20:0.1 v/v), in a water bath at 60°C for 1 h. Mordi and Akanji (2012) mention that the air-dried powder from *C. aconitifolius* leaves (1 kg) of fresh matured *C. aconitifolius* were extracted by percolation at room temperature with 70% ethanol (EtOH). A leaf extract from *C. aconitifolius* was concentrated under reduced pressure (bath temperature 50°C) and finally defatted with n-hexane. The extract was evaporated to dryness. This yielded 69.9 g of dried mass. García-Rodríguez et al. (2014) mentioned that approximately 135 g of *C. aconitifolius* dried leaves were extracted by maceration using ethanol (9.44 g) at room temperature (25°C). The samples were kept in the dark at room temperature for successive testing during the course of the research reported. The solvent was removed by rotary evaporation to dryness and the resulting material dried completely in an oven at 25°C. Numa et al. (2015) mention that to prepare the soluble extract in ethanol, the *C. aconitifolius* leaf was dried, ground and macerated for 7 days in a solution of 96% ethanol, changing the solvent daily.

Extraction using methanol and mix polar solvents

The first report was by Kolterman et al. (1984), in which the dry matter of *C. aconitifolius* was extracted in 70% methanol/30% water. Later, González-Laredo et al. (2003) reported drying the leaves of *C. chayamansa* at 60°C for 6 h. These

authors also performed a duplicate extraction with methanol and rotary evaporation at <40°C. Subsequently a separation was performed in duplicate using hexane, ethyl ether and ethyl acetate. Figueroa-Valverde et al. (2009) performed their extraction by placing 20 g of previously dried leaves of *C. chayamansa* in 250 ml of 80% methanol for 8 h, then performing a rotary evaporation of the mixture. They then added a chloroform: water solvent mixture (4:1 v/v) to remove the organic phase from the aqueous. The volume of the organic phase was reduced to dryness and the obtained mixture was reconstituted with 70% ethanol to be used as stock solution. Loarca-Piña et al. (2010) reported drying and macerating the *C. chayamansa* leaves, then performing the extraction by placing 500 g of dry leaf in 1000 ml of solvent (hexane-acetone, 1:1) at room temperature for 5 days, twice daily. Subsequently, the material was extracted with methanol under the same conditions. It was then dried in a rotoevaporator and stored at 4°C. In Adaramoye et al. (2011), approximately 3 kg of dry *C. aconitifolius* leaves were placed in an extractor at 30°C using methanol for 5 h and the extract was concentrated in a rotary evaporator at 35°C for 30 min. In Ikpefan et al. (2013), *C. aconitifolius* leaves were air dried for 5 days in a laboratory at room temperature. Oven drying was then carried out at 40°C, followed by milling in powder form, using an electric mill. 1 kg of the dry matter was extracted in 2.5 L of methanol. The extracted liquid obtained was concentrated using a rotoevaporator at a steady temperature of 40°C then kept in refrigeration afterwards.

Other solvents

García-Rodríguez et al. (2014) reported extraction from approximately 135 g of dried leaves of *C. aconitifolius* by maceration, using 5.27 g of ethyl acetate at room temperature (25°C). The solvent was removed by rotary evaporation to dryness and completely dried in an oven at 25°C. Escalante-Erosa et al. (2004) reported that they used 20 freshly cut *C. aconitifolius* leaves. Subsequently, they added 1 L of methylene chloride for 20 s. Afterwards, the mixture was subjected to rotary evaporation to produce 533.3 mg of wax. García-Rodríguez et al. (2014) reported that approximately 135 g of dried leaves were extracted by maceration using 5.68 g of hexane at room temperature (25°C). The solvent was removed by rotary evaporation to dryness and dried completely in an oven at 25°C.

Studies that do not report extraction

Sarmiento-Franco et al. (2003) mentioned only the use of ground dry matter from *C. aconitifolius*. Unlike Oyagbemi et al. (2011), they mentioned that the leaves of *C. aconitifolius* were collected, cleaned and air dried at room temperature. Aye (2012) reported that the preparation of *C. aconitifolius* leaves was washed, weighed and cooked in batches of 80 and 90°C for 10 min, and then allowed to dry. Akachukwu et al. (2014) mentioned only that the leaves of *C. aconitifolius* were dried in an oven at 40°C and subsequently ground. Jiménez-Aguilar and Grusak (2015) reported that *C. aconitifolius* leaves were dried in an oven at 70°C for 3 days to maintain a constant weight.

Phenolic compounds detected in chaya leaf

In an aqueous extraction, Musa et al. (2008) found different phenolic compounds in different concentrations: 1.86% phenols, 0.93% tannins, 0.30% flavonoids, 0.072% anthraquinones, and 0.065 % flobotannins (Table 3). Mordi and Akanji (2012), also using an aqueous extraction, found a moderate presence of phenols (++), a low presence of tannins (+), and a high presence of flobotannins (+++). In an aqueous extraction of chaya leaf, Obichi et al. (2015) found 5.7% of tannins and 23.7% of flavonoids. Babalola and Alabi (2015) also tested an aqueous extraction of chaya leaf and found 15.17 gallic acid equivalents (GAE)/100 g fresh matter (FM) of total phenolic compounds, and 243.33 mg/100 g FM of flavonoids. Valenzuela et al. (2015) performed a chaya leaf infusion and reported a total phenolic compound concentration of 6.34 mg GAE/ml. Kuti and Konuru (2004) performed leaf extractions of chaya using ethanol as solvent, reporting on the concentration of total phenolic compounds in raw and cooked leaves, finding values of 2906.2 and 2241.4 mg chlorogenic acid equivalents (CAE/kg) FM, in raw and cooked leaves, respectively. Various researchers analyzed ethanolic extracts: Awoyinka et al. (2007) reported a mean presence of tannins; Johnson et al. (2008) reported a total phenolic compound concentration of 5.6 mg GAE/g DM; Mordi and Akanji (2012) in an ethanolic extract reported a high presence of phenols and tannins and a low presence of flobotanins and flavonoids; García-Rodríguez et al. (2014) reported a low presence of coumarin and flavonoids, and a total phenolic compound concentration of 35.7 mg GAE/g DM. These authors also tested a hexanoic extract, in which reported a total phenolic compound concentration of 22.3 mg GAE/g DM. On the other hand, Loarca-Piña et al. (2010) tested a methanolic extract, and reported a concentration of phenolic compounds of 71.3 mg GAE/g extract, and a total flavonoid concentration of 42.7 mg catequin equivalents (CE)/g extract. Among other authors who tested a methanolic extract, Oyagbemi et al. (2011) reported a high presence of flavonoids and a low presence of tannins. Adaramoye et al. (2011), reported a high presence of flavonoids and a moderate presence of tannins. Aye (2012) reported a total phenolic compound concentration of 3.78% TE. Akachukwu et al. (2014) reported a tannin concentration of 0.14%, a phenol of 0.19% and a flavonoid of 2.36%. Jimenez-Aguilar and

Table 3. Phenolic compounds reported in chaya leaves.

Solvent system used/species	Phenolic compounds reported	Reference
Aqueous		
C. aconitifolius	Phenols: 1.86%, Tannins: 0.93%, Flavonoids: 0.30%, Anthraquinones: 0.072%, and Flobotanins: 0.065%	Musa et al. (2008)
C. aconitifolius	Phenols (++) Tannins (+) Flobotanins (+++)	Mordi and Akanji (2012)
C. aconitifolius	Tannins 5.7% and Flavonoids 23.7%	Obichi et al. (2015)
C. aconitifolius	TFC 15.17 GAE/100 g DM and Flavonoids 183.33 mg/100 g DM	Babalola and Alabi (2015)
C. chayamansa	TFC 6.34 mg GAE/ml infusion	Valenzuela-Soto et al. (2015)
Ethanolic		
C. aconitifolius	TFC Crude: 2906.2 and Cooked: 2241.4 mg CAE/kg FM	Kuti and Konuru (2004)
C. aconitifolius	Tannins (++)	Awoyinka et al. (2007)
C. aconitifolius	TFC 5.6 mg GAE/g DM	Johnson et al. (2008)
C. aconitifolius	Phenols (+++), Tannins (+++), Flobotanino (+), Flavonoids (+)	Mordi and Akanji (2012)
C. aconitifolius	Coumarin (+), Flavonoids (+), TFC : 35.7 GAE/g DM	García-Rodríguez et al. (2014)
Methanolic		
C. chayamansa	TFC 71.3 mg GAE/g extract; Total flavonoids 42.7 mg CE/g extract	Loarca-Piña et al. (2010)
C. aconitifolius	Flavonoids (+++), Tannins (+)	Oyagbemi et al. (2011)
C. aconitifolius	Flavonoids (+++) and Tannins (++)	Adaramoye et al. (2011)
C. aconitifolius	TFC 3.78% TE (average)	Aye (2012)
C. aconitifolius	Flavonoids (+++) and Tannins (+++)	Ikpefan et al. (2013)
C. aconitifolius	Tannins: 0.14%, Phenols: 0.19% and Flavonoids: 2.36%	Akachukwu et al. (2014)
C. aconitifolius	TFC 5.66 mg GAE/g FM; Total flavonoids 332.8 µg CE/g FM	Jiménez-Aguilar and Grusak (2015)
Other solvents		
Ethyl acetate *C. aconitifolius*	Coumarin (+), Flavonoids (+), TFC 13.2 GAE/g DM.	García-Rodríguez et al. (2014)
Hexanoic *C. aconitifolius*	TFC 22.3 GAE/g DM	García-Rodríguez et al. (2014)

Results presented as reported by the authors.

Grusak (2015) reported total phenolic compounds content of 5.66 mg GAE/g FM, and a total flavonoid content of 332.8 µg CE/g FM. Finally, using an ethyl acetate extractant, García-Rodríguez et al. (2014) found a weak presence of coumarin and flavonoids. They also found a concentration of 13.2 mg GAE/g DM of total phenolic compounds in a hexanoic extract.

Determination of individual compounds

Figure 2 presents the structures of the most reported phenolic compounds in chaya leaves. Valenzuela et al. (2015) reported the presence of quercetin and rutine in an aqueous extraction of *C. chayamansa* leaf (Table 4). Ramos-Gómez et al. (2016) performed an aqueous extraction of *C. chayamansa* leaf and analyzed by high performance liquid chromatography with a diode-array detector (HPLC-DAD)/mass spectrometer

Table 4. Identification of specific phenolic compounds in chaya leaves.

Solvent system used/species	Technique used	Phenolic compounds identified	Reference
Aqueous/ *C. chayamansa*	Only one chromatogram is shown	Quercetin and Rutine	Valenzuela-Soto et al., 2015
Aqueous/ *C. chayamansa*	HPLC-DAD/MSD	Epigallocatech gallato 27.4 mg/g FM Rosmarinic Acid 26.8 mg/g FM Hesperidin 16.2 mg/g FM Vanillin 11.3 mg/g FM Rutine 10.6 mg/g FM Chlorogenic Acid 8.6 mg/g FM 4-Hydroxybenzoic acid 8.1 mg/g FM Coffeic Acid 5.4 mg/g FM Ferulic Acid 4.7 mg/g FM Catechin 4.3 mg/g FM Protocatechic acid 4.2 mg/g FM P-coumaric acid 3.0 mg/g FM Naringenin 2.7 mg/g FM Synaptic Acid 1.7 mg/g FM Quercetin 1.4 mg/g FM Ellagic Acid 0.8 mg/g FM Galocatequin gallate 0.5 mg/g FM	Ramos-Gómez et al. (2016)
Ethanol/*C. aconitifolius*	HPLC-DAD	Crude: Kaempferol 58.2, Quercetin 16.9 and Cooked: Kaempferol 50.0, Quercetin 12.6 µg/g FM	Kuti and Konuru (2004)
Ethanol/*C. aconitifolius*	HPLC–DM	Hispidulin Sulfate and Eucalyptine, Epigalocatechin di-O-gallate, Epicatequin di-O-gallate, Acutifoline D and Tiegusanin F Lignin and Coumarin Fraxetin.	Numa et al. (2015)
Fresh matter/*C. aconitifolius*	AOAC Method	Lignin 39.6 g/kg FM	Sarmiento-Franco et al. (2003)
Methanol/*C. aconitifolius*	Gas-liquid Comatography	Glucosidated flavonols present are Galactosidized, Glucosidized, Ramnosididated and Ramnosylglucosidates of Quercetin and Kaempferol, and two triglycosides of Quercetin	Kolterman et al. (1984)
Methanol/*C. chayamansa*	Nuclear magnetic resonance	Dihydromyricetine was observed in the stem, and in the leaves, the biflavonoid (3 '→ 8) - Diapigenin (Amentoflavone) and the Kaempferol-3-O-glucoside (Astragalin) and Kaempferol-3-O-rutinoside glycoside	González-Laredo et al. (2003)
Methanol/*C. chayamansa*	Ultraviolet spectrophotometric analysis	Dihydromyricetine, Amentoflavone, Rutin, Quercetin, Naringin, Hesperidin, Nobiletine	Figueroa-Valverde et al. (2009)
Methanol/*C. chayamansa*	HPLC-DAD	Protocatecuic Acid 0.24 mg/g and Rutine 2.00 mg/g freeze-dried.	Loarca-Piña et al. (2010)

Results presented as reported by the authors.

(MSD). They reported the concentration of different phenolic compounds: epigallocatequin gallate 27.4 mg/g FM, rosmarinic acid 26.8 mg/g FM, hesperidin 16.2 mg/g FM, vanillin 11.3 mg/g FM, rutin 10.6 mg/g FM, chlorogenic acid 8.6 mg/g FM, 4-hydroxybenzoic acid 8.1 mg/g FM, caffeic acid 5.4 mg/g FM, ferulic acid 4.7 mg/g FM, catechin 4.3 mg/g FM, protocatechic acid 4.2 mg/g FM, p-coumaric acid 3.0 mg/g FM, naringenin 2.7 mg/g FM, synapic acid 1.7 mg/g FM, quercetin 1.4 mg/g FM, ellagic acid 0.8 mg/g FM, and galocatequin gallate 0.5 mg/g FM. Kuti and Konuru (2004) analyzed raw and boiled chaya leaf extracts and analyzed them by HPLC-DAD. They reported kaempferol 58.2 µg/g FM and 50.0 µg/g FM, quercetin 16.9 µg/g FM and 12.6 µg/g FM in the raw and boiled extracts, respectively. Numa et al. (2015) analyzed an ethanolic extract of *C. aconitifolius* leaf by HPLC-DM, finding the presence of hispidulin sulphate, eucalyptine, epigallocatechyne di-O-gallate, epicatequin di-O-gallate, acutifolin D, lignin Tiegusanin F, and coumarin fraxetin. Sarmiento-Franco et al. (2003) harvested *C. aconitifolius* by cutting off all the leaves first, and then allowing the young stems to reach approximately 1 m in height. The leaves from each plant were stored in plastic bags and frozen at -10°C until analysis. By this process, they determined the presence of lignin: 39.6 g/kg FM carried out according to AOAC methods (AOAC, 1980). Kolterman et al. (1984) performed a methanolic extraction of *C. aconitifolius* leaf and analyzed it by gas-liquid chromatography, identifying glucosidic flavonols, such as galactosidated, glucosidized, rhamnosididated and rhamnosylglucosidates of quercetin and kaempferol, and two quercetin triglycosides. Gonzalez-Laredo et al. (2003) performed methanolic extractions from the stem and leaf of *C. chayamansa* and analyzed them by nuclear magnetic resonance. They reported dihydromyricetin in the stem, and in the leaves, biflavonoid (3 '→ 8) - diapigenin (amentoflavone), glycoside kaempferol-3-o-glucoside (astragalin) and kaempferol-3-o-rutinoside. Figueroa-Valverde et al. (2009) examined a methanolic extract of *C. chayamansa* leaf using ultraviolet spectrophotometric analysis, and found the presence of dihydromyricetine, amentoflavone, rutin, quercetin, naringin, hesperidin, and nobiletin. Loarca-Piña et al. (2010) found a protocatecuic acid (0.242 ± 0.001 mg/g of extract) and rutin (2.00 ± 0.097 mg/g) in a methane extract from *C. chayamansa* leaf, analyzed by HPLC-DAD.

Antioxidant capacity of chaya leaf

García-Rodríguez et al. (2014) performed a non-polar extraction using ethyl acetate as solvent and analyzed antioxidant capacity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reduction activity potential (FRAP) techniques (Table 5). They reported an 11.6% inhibition by DPPH and 387.1 µmol Fe/L by the FRAP technique. Valenzuela et al. (2015) performed an infusion with chaya leaf and found an antioxidant capacity of 5.9 mM Trolox equivalents/ml infusion. In an aqueous extract, Ramos-Gómez et al. (2016) reported an antioxidant capacity of 25.5 µg/ml by DPPH, of 44.3 µg/ml by 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), and 38.5 µg/ml by NO. Kuti and Konuru (2004) performed ethanolic extractions on raw and boiled chaya leaves, testing for antioxidant capacity using the oxygen radical absorbance capacity (ORAC) technique, which yielded values of 15.3 µmol Trolox equivalents/g FM in the extract of raw leaves and 11.8 µmol Trolox equivalents/g FM in the extract of cooked leaves. García-Rodríguez et al. (2014) reported the antioxidant capacity in an ethanolic extract of chaya leaves using the DPPH and FRAP techniques. These authors reported a 10.6% inhibition by DPPH, and 245.0 µmol Fe/L with the FRAP technique. Also, in a hexanoic extract, they reported 0.5% inhibition by DPPH and 239.4 µmol Fe/L by FRAP. Loarca-Piña et al. (2010) analyzed the antioxidant capacity of the chaya leaf, reporting a 45.5% inhibition by DPPH, and a 95% inhibition by ABTS. They reported IC50 of 1693 µg/ml. Finally, Jiménez-Aguilar and Grusak (2015) analyzed the antioxidant capacity of a chaya leaf methanolic extract, reporting 34.38 µmol Trolox equivalents/g FM.

The most commonly used methods for analyzing antioxidant capacity are ABTS+, DPPH, ORAC and FRAP. These are highly reproducible under certain assay conditions, but also show significant differences in their response to antioxidants. The free radical DPPH (DPPH) does not require any special preparation, whereas the radical cation ABTS (ABTS+) must be generated by enzymes or chemical reactions (Arnao, 2000). Another significant difference is that ABTS+ can be dissolved in aqueous and organic media, in which antioxidant activity can be measured, given the hydrophilic and lipophilic nature of the compounds in the samples. In contrast, DPPH can only be dissolved in organic media, especially in ethanol, which is a significant limitation in interpreting the role of hydrophilic antioxidants. In both radicals, however, reductive capacity does not necessarily reflect antioxidant activity, as suggested by Wong et al. (2006), Katalinic et al. (2006) and Wojdyłol et al. (2007). From a scientific standpoint, the best approach is to conduct a variety of tests to evaluate antioxidant capacity, since this yields a more complete and ultimately more accurate analysis.

The content of the phenolic compounds and their antioxidant capacity varies from one extract to another, not only in the environmental factors, but also by the way in which the data are expressed, either in different units or in different states of the sample, for example, lyophilized, dried or fresh matter. The results also vary in that the distinct types of extractions are not usually 100% of a single solvent, but instead use different mixtures

Table 5. Antioxidant capacity reported in chaya leaves.

Solvent system used/species	Antioxidant capacity	Reference
Aqueous		
C. chayamansa	5.9 mM Trolox Eq/ml of infusion	Valenzuela-Soto et al. (2015)
C. chayamansa	DPPH 25.5, ABTS 44.3 and NO 38.5 (IC_{50} µg/mL)	Ramos-Gómez et al. (2016)
Ethanolic		
C. aconitifolius	ORAC raw leaf: 15.3, cooked leaf: 11.8 µmol Trolox Eq/g FM	Kuti y Konuru (2004)
C. aconitifolius	DPPH: 10.6% inhibition and in FRAP: 245.0 µmol Fe/L	García-Rodríguez et al. (2014)
Methanolic		
C. chayamansa	DPPH 45.5% inhibition and ABTS 95% inhibition and 1693 (IC_{50}) µg/mL	Loarca-Piña et al. (2010)
C. aconitifolius	ORAC - APPH 34.38 µmol Trolox Eq/g FM	Jiménez-Aguilar y Grusak (2015)
Other solvents		
Ethyl acetate/*C. aconitifolius*	DPPH 11.6% inhibition and in FRAP 387.1 µmol Fe/L	García-Rodríguez et al. (2014)
Hexanoic/*C. aconitifolius*	DPPH: 10.5% inhibition and in FRAP: 239.4 µmol Fe/L	García-Rodríguez et al. (2014)

Results presented as reported by the authors.

and proportions, in addition to various extraction conditions and various determination methodologies.

Future perspectives

The studies presented in this review do not enable us to clearly determine which is the best extraction method for the phenolic compounds of the chaya leaf. This is because of the highly diverse processes mentioned by the different authors, as can be seen in Tables 2 to 4. Apart from the environmental factors, there are differences in the treatment of the sample before the extraction process, such as the type and the drying conditions. There are also differences in the solvents used and in the methods of extraction and concentration of compounds. Finally, a diversity of techniques are used, and even the data themselves are quantified and expressed differently. Even so, it can be said that the greatest amount and variety of phenolic compounds was obtained with different mixtures of hydroalcoholic proportions. Common knowledge tells us that the best drying method is one in which the conditions used to remove the water are not very aggressive with the biological material, for example, temperatures no higher than 40°C and a short drying time to avoid the degradation of the compounds of interest.

Specific further study is needed to evaluate different types of solvents and mixtures of them for the extraction of phenolic compounds, where the same methodology is used for sample handling, from the harvesting of chaya leaves, the method of drying, grinding and extraction conditions, through the analysis of the compounds to create a phenolic profile. This would enable researchers to determine the best solvent for extracting certain type of phenolic compounds in chaya leaves. It would also be useful to perform the extractions from both raw and boiled leaves since it is known that the raw leaves have a cyanogenic glycoside that is eliminated by boiling the leaves in water, and this heat treatment could affect the phenolic profile.

CONCLUSIONS

In the chaya leaf, there is a general trend toward the presence of different phenolic groups, such as coumarin, flavonoids, phenols, tannins, anthraquinones, and flobotanins in aqueous and alcoholic extracts. The chaya plant has potential for production as food and as a medicinal plant, but the task of comparing the results obtained from the different research articles is complicated by the different processes used by each of the researchers to report the phenolic compounds and the antioxidant capacity of this plant. Apart from the analysis of different extraction methods, solvents and forms of preparation, as well as the diversity of extracted compounds, further research is also important and necessary through *in vitro* and *in vivo* studies of each type of extract in order to evaluate their biological effects on health, for example, in reducing glucose levels, or as a possible chemopreventive or chemoprotector agent against colon cancer.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

Abbreviations

GAE, Gallic acid equivalents; **CAE,** chlorogenic acid equivalents; **CE,** catequin equivalents; **TE,** tannin equivalents; **FM,** fresh matter; **DM,** dry matter.

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