Perfil fenólico y capacidad antioxidante de *Pithecellobium dulce* (Roxb) Benth: una revisión. Ángel Félix Vargas Madriz 2020-

Universidad Autónoma de Querétaro
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Artículo

Que como parte de los requisitos para obtener el grado de

Maestro en Ciencias de la Nutrición Humana

Presenta

Ángel Félix Vargas Madriz

dirigido por

Dr. Jorge Luis Chávez Servín

Octubre 2020, Querétaro Qro.

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TESIS

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Presenta:
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Dirigido por:
Dr. Jorge Luis Chávez Servín

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Dedicatorias

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Resumen

Pithecellobium dulce (Roxb) Benth (*P. dulce*) es un árbol perteneciente a la familia de las leguminosas nativo de México que se encuentra principalmente en zonas tropicales, esta planta fue introducida en Filipinas y en la India en el siglo XVI. entre los meses de enero hasta agosto proporciona una vaina con cinco arilos comestibles, dentro de los arilos contienen una semilla de color negra y en ciertos lugares es consumida, sus ramas son alargadas compuesta por dos pares de foliolos en forma de bipinnadas. Este árbol se encuentra principalmente en las zonas tropicales y se ha reportado en el sur de América. Todas las partes de *P. dulce* son utilizadas principalmente en zonas rurales, el arilo es comestible, ornamental y etnobotanicamente el arilo, semilla y hoja son utilizados para tratar enfermedades estomacales como ulcera, gastritis, actualmente existen pocas investigaciones relacionadas con la caracterización de sus compuestos fenólicos y su capacidad antioxidante. Sin embargo, la mayoría de los estudios han reportado variabilidad en los resultados, esto debido a diversos factores como las condiciones agroclimáticas, tratamiento postcosecha de la muestra, almacenamiento, tipos de extracciones, uso de solventes entre otros. Por otro lado, también la cuantificación de los compuestos fenólicos por medio de HPLC varían debido por estos factores.

Palabras clave: *P. dulce,* fenoles, capacidad antioxidante, HPLC, extracción.

Abstract

Pithecellobium dulce (Roxb) Benth (*P. dulce*) is a tree belonging to the family of legumes native to Mexico that is mainly found in tropical areas, this plant was introduced in the Philippines and India in the XVI century. Between the months of January and August, it provides a pod with five edible arils, within the arils they contain a black seed and in certain places it is consumed, its branches are elongated, composed of two pairs of bipinnate-shaped leaflets. This tree is found mainly in the tropics and has been reported in southern America. All parts of *P. dulce* are used mainly in rural areas, the aril is edible, ornamental and ethnobotanically the aril, seed and leaf are used to treat stomach diseases such as ulcer, gastritis, currently there is little research related to the characterization of its compounds phenolics and their antioxidant capacity. However, most studies have reported variability in results, this due to various factors such as agroclimatic conditions, post-harvest treatment of the sample, storage, types of extractions, use of solvents, among others. On the other hand, also the quantification of phenolic compounds by means of HPLC variation due to these factors.

Keywords: *P. dulce,* phenols, antioxidant capacity, HPLC, extraction.

Indice

INTRODUCCIÓN

Las frutas y vegetales procedentes de algunas plantas, son un importante alimento en la dieta humana obteniendo de estos ciertos nutrimentos necesarios para realizar nuestras funciones vitales (Dos Santos et al., 2012); las plantas principalmente contienen nutrimentos y compuestos bioactivos que son utilizados con fines terapéuticos principalmente en localidades rurales (Drago et al., 2006; Zambrano-Intriago et al., 2015); estos compuestos conocidos como fitoquímicos se han adentrado en la industria alimentaria y farmacéutica, utilizándolos para la prevención y tratamiento de ciertas enfermedades crónicas (Drago et al., 2006).

Los compuestos fenólicos son metabolitos secundarios que sintetizan las plantas, sus funciones son de protección contra depredadores, rayos ultra violeta, de reproducción y crecimiento; los compuestos fenólicos químicamente están conformados por uno o más grupos hidroxilos unidos a uno o más anillos aromáticos, estos compuestos se pueden clasificar en ácidos fenólicos, flavonoides, y taninos (Ozcan et al., 2014; Peñarrieta et al., 2014). Sus propiedades como antioxidantes naturales han proporcionado un gran interés en el área de la nutrición humana, estudiando sus efectos sobre la salud (Cieślik et al., 2006; Porras & López-Malo, 2009).

Pithecellobium dulce (Roxb) Benth conocido en México como “guamúchil”, es un árbol que tiene uso de cerco vivo, ornamental, maderable, medicinal, forraje y sombra (Martínez-De La Cruz et al., 2015), el arilo que contiene este árbol es consumido por los seres humanos en diferentes preparaciones, en crudo, en agua fresca, en salsas y en atole (Monroy & Colín, 2004), existen algunas investigaciones que mencionan que la semilla puede ser consumida en crudo, asado y cocida (Nagmoti & Juvekar 2013; Rao, 2013); tanto en arilo como en la semilla se han reportado la presencia de aminoácidos esenciales, ácidos grasos, vitaminas y minerales (Longvah et al., 2017; Singhal, 2014).

Actualmente gracias a su gran distribución y disponibilidad, la corteza, hojas, arilo y semillas de *Pithecellobium dulce* (Roxb) Benth son utilizadas en forma de etnomedicina principalmente en enfermedades gastrointestinales (Srinivas et al., 2018).

Cerca de 10 000 compuestos fenólicos son los que se encuentran en la naturaleza principalmente en las frutas y vegetales (Muñoz et al., 2015). En Asia es donde existen más estudios de los compuestos fenólicos y actividad antioxidante de *Pithecellobium dulce* (Roxb) Benth, los compuestos fenólicos reportados en arilo son el ácido elágico, ácido gálico, ácido mandélico, ácido ferúlico, ácido vanílico y ácido p-cumárico; y los flavonoides reportados son rutina, quercitrina, naringenina, kaempferol, daidzeina por medio de HPLC, en semilla se ha detectado alcaloides, contenido total de flavonoides y contenido total de fenoles; los ensayos DPPH, NO, SO, TBARS, TAC, son reportado en la literatura en extractos con agua y metanol (Megala & Geetha, 2010; Nagmoti & Juvekar, 2013).

Debido a que los estudios sobre los compuestos fenólicos y su capacidad antioxidante de *P. dulce* indican variabilidad incluso en la cuantificación por HPLC; probablemente a diversos factores como las condiciones agroclimáticas, cosecha, madurez de la planta entre otras. Por esta razón se realizó una revisión exhaustiva de la literatura sobre los compuestos fenólicos y capacidad antioxidante.

Phenolic profile and antioxidant capacity of Pithecellobium dulce *(Roxb) Benth: a review*

Ángel Félix Vargas-Madriz, Aarón Kuri-García, Haidel Vargas-Madriz, Jorge Luis Chávez-Servín, Roberto Augusto Ferriz-Martínez, et al.

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REVIEW ARTICLE

Phenolic profile and antioxidant capacity of *Pithecellobium dulce* (Roxb) Benth: a review

Ángel Félix Vargas-Madriz[1] · Aarón Kuri-García[1] · Haidel Vargas-Madriz[2] · Jorge Luis Chávez-Servín[1] · Roberto Augusto Ferriz-Martínez[1] · Luis Gerardo Hernández-Sandoval[3] · Salvador Horacio Guzmán-Maldonado[4]

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Abstract *Pithecellobium dulce* (Roxb) Benth (*P. dulce*), known as "guamúchil", is a tree native to the American continent. Various parts of the tree are used in traditional medicine, primarily for treating gastrointestinal disorders. The phenolic compounds and antioxidant capacity of this plant are largely responsible for the beneficial health effects attributed to it. A number of authors have studied the antioxidant capacity and phenolic compounds of the aril, seed, leaf and root of *P. dulce* using various methodologies, which can differ considerably in variables such as environmental factors, type of drying, temperature, the way the sample is stored, and the use of different solvents in the various extraction methods. Even methods of quantification by HPLC vary tremendously. This paper summarizes the existing research carried out to date on determining the phenolic profile and antioxidant capacity of *P. dulce*.

Keywords *Pithecellobium dulce* · Guamúchil · Phenolic compounds · Antioxidant capacity · Phenolic profile

✉ Jorge Luis Chávez-Servín
jorge.chavez@uaq.mx

[1] Laboratory of Cellular and Molecular Biology, School of Natural Science, Autonomous University of Queretaro, Av. de las Ciencias S/N, Juriquilla, CP 76230 Querétaro, QRO, Mexico

[2] Department of Agricultural Production, University Center of the South Coast, UDG, Av. Independencia Nacional, No 151, CP 48900 Autlán, JAL, Mexico

[3] Botanical Laboratory, School of Natural Science, Autonomous University of Queretaro, Av. de las Ciencias S/N, Juriquilla, CP 76230 Querétaro, QRO, Mexico

[4] Food Laboratory, Bajio Experimental Field, Regional Research Center, INIFAP, Km 6. Carr., Celaya - San Miguel Allende AP 112, CP 38110 Celaya, GTO, Mexico

Introduction

Since ancient times, people have taken advantage of the biological effects of plants as traditional remedies for certain diseases. Approximately 80% of the world's population uses medicinal plants as traditional treatment, mainly in developing countries (Lee et al. 2019). Empirical knowledge in some regions have identified plants useful for the treatment of certain diseases, but the use of a given plant as a traditional remedy also depends on popular beliefs in that region (Adeniyi et al. 2018). Mexico is home to the highest number of medicinal plants in the world: it is estimated to hold approximately 3000 plants with ethnobotanical uses. In 2012, 57.4% of the population used plants for traditional medicinal purposes (Alonso-Castro et al. 2017).

The food and pharmaceutical industries have become increasingly interested recently in analyzing some medicinal plants, mainly their phenolic profile. Phenolic compounds are secondary metabolites produced in plants, which protect them against biotic and abiotic stress; some also have beneficial effects for the organisms that consume them (Szajdek and Borowska 2008; Porras-Loaiza and López-Malo 2009; Li et al. 2018). Their chemical structure consists of one or more aromatic rings linked to at least one hydroxyl group, and they are divided according to this structure into phenolic acids, simple phenols, flavonoids, coumarins, lignans, and tannins. Several of these compounds have a high antioxidant capacity, believed to assist in the prevention of certain chronic diseases (Gallegos-Zurita 2016; Chutipaijit and Sutjaritvorakul 2018; Xu et al.

2019; Valduga et al. 2019). A wide range of publications in the scientific literature have reported on the extraction processes used to determine the phenolic compounds and antioxidant capacity of such plants, but comparisons between them are difficult because of differences in their methods for obtaining of the raw material, their processing, the different types and mixtures of solvents used, and expression of units of antioxidant capacity and content of phenolic compounds (Kuri-García et al. 2017). The objective of this work is to provide a review of the literature in which the phenolic profile and the antioxidant capacity of *P. dulce* are discussed in detail.

P. dulce

P. dulce is a tree belonging to the family Leguminosae and the subfamily Mimosoideae. It is native to tropical America and is widely distributed in Mexico in states such as Tamaulipas, San Luis Potosí, Jalisco and Querétaro (Parrotta 1991; CONABIO 2013). *P. dulce* has also been reported in some parts of the Asian continent, mainly in India in the states of Tamil Nadu, Haryana, Maharashtra and West Bengal (Megala and Geetha 2010; Pal et al. 2012; Kumar et al. 2013; Nagmoti and Juvekar 2013; Preethi and Saral 2014). It is known in rural areas as *guamúchil* (monkeypod or Madras thorn in English). The leaves are formed by a pair of leaflets which are considered evergreen. The bark is rough and gray. In the months of February to August it produces a coiled green-and-red variegated pod, containing 5 to 12 white and pink arils inside of which are black seeds (Fig. 1). Nearer the equator of the American continent—in countries like Puerto Rico—the aril is available year round (Parrotta 1991; Monroy and Colín 2004; Pío-León et al. 2013; Wall-Medrano et al. 2016; Rao et al. 2018).

Fig. 1 Aril and seed from *Pithecellobium dulce*

The aril is consumed raw, roasted or mixed into *agua fresca* (a fruit-flavored beverage or cold tea) or *atole* (a hot beverage made from cornstarch) (Monroy and Colín 2004). The seed may be consumed raw, roasted, cooked or curried, according to research carried out in India (Nagmoti et al. 2012; Rao 2013). *P. dulce* aril has exceptional nutritional qualities given its high carbohydrate and protein content (Table 1) (Khanzada et al. 2013). Likewise, seed protein flour is high in fat and protein and minerals like copper, iron, magnesium, phosphorus, potassium and zinc (Rao 2013).

Ethno-botanical studies mention that the different parts of the P. *dulce* tree are used for their analgesic, anti-inflammatory, antibacterial, antidiarrheal, antiulcer, antioxidant, hypoglycemic, and hepatoprotective properties, in treating cardiovascular and gastrointestinal diseases (Kulkarni and Jamakhandi 2018; Rao et al. 2018). Various researchers have conducted work on analyzing the phenolic composition and antioxidant capacity of *P. dulce* (Megala and Geetha 2010; Kulkarni and Jamakhandi 2018; Rao et al. 2018).

Sample treatment prior to extraction

The treatment of the collected sample is important for the analysis of the compounds present in the plant material. The studies reviewed in this paper analyze the phenolic compound content and antioxidant capacity of aril, leaves, pericarp and seed. The different treatments reported in *P. dulce* analyses are described in Table 2.

Table 1 Proximate analysis of the seed protein flour and aril of *Pithecellobium dulce* dry matter

Component	Seed protein flour Rao (2013)	Aril Bhati and Jain (2016)
Moisture (%)	10.3	84.7
Protein (%)	39.2[a]	18.6
Ethereal extract (%)	ND	1.4
Carbohydrates (%)	ND	70.9
Total ash (%)	3.0	5.1
Fiber (%)	ND	3.9

The information is presented as described by the authors. All values are expressed on a dry basis, except for moisture in the aril

ND Not determined

[a] % (N × 6.25)

Table 2 Sample treatment prior to extraction of the different parts of *Pithecellobium dulce* (Roxb) Benth

Plant Sample	Sample treatment	References
Aril	Aril is washed and later cut in half	Samee et al. (2006)
Aril	Aril is washed and dried at room temperature and subsequently the dry sample is pulverized and homogenized	Megala and Geetha (2010)
Aril	Aril is freeze dried for extraction	Kubola et al. (2011)
Aril	Aril is dried at room temperature in low light conditions. Sample is ground and sieved	Preethi and Saral (2014)
Aril	Aril is dried at room temperature in low light conditions for a few days. The dried fruits are then ground and sieved (10/60) for coarse powder	Raju and Jagadeeshwar (2014)
Aril	Aril is washed and dried in an oven at 60 °C and subsequently ground and sieved to a fine powder	Cheema et al. (2017)
Aril	Aril is washed with tap water. Sample is then separated into fresh matter and dry matter. The fresh sample is stored in polyethylene bags sealed at − 18 ± 5 °C. Another sample is dried in a hot air oven at 45 ± 5 °C. The dried sample is ground using a 1.0 mm mesh and finally stored	Bhati and Jain (2016)
Aril	Sample is cleaned and the aril is separated from the seed. The sample is freeze dried in low light conditions, ground and stored at − 20 °C	Wall-Medrano et al. (2016)
Aril	The Aril is freeze dried and ground in a mill, then passed through a 0.44 mm sieve. The sample is stored at − 20 °C in low light conditions	López-Angulo et al. (2018)
Aril	Aril is cut into pieces and dried at room temperature. The sample is then ground in a mortar	Suganthi and Josephine (2018)
Leaves	Leaves are cleaned with water and the sample allowed to dry at room temperature, then ground in a mill	Sugumaran et al. (2008)
Leaves	Leaves are dried using a tray dryer at 55 °C for 24 h. Once the moisture is removed, the sample is milled and defatted with petroleum ether. The plant material is dried at a temperature in a hot air oven of 40 °C	Katekhaye and Kale (2012)
Leaves	Leaves are cleaned with tap water and with distilled water, left to dry in an oven at 40 °C until they reached a constant weight, then milled and stored in airtight containers at room temperature	Kumar et al. (2013)
Leaves	Fresh leaves (100/500 mg) are ground with distilled water (1.5 mL) in a mortar and pestle	Krishnaveni et al. (2014)
Leaves	Leaves are dried in low light conditions at room temperature, then ground using a mill	Poongodi and Hemalatha (2015)
Leaves	Leaves are washed with tap water and then with distilled water. The plant sample is dried in a 40 °C oven and subsequently milled in a mill and stored in airtight containers at room temperature	Kalavani et al. (2016)
Leaves	Leaves are cleaned with tap water and then with distilled water, and then dried at room temperature (25 °C) for 5 days in the in low light conditions. The dried samples are ground to a fine powder which is stored in airtight containers at room temperature	Vanitha and Manikandan (2016)
Leaves	Leaves are washed first with tap water then with distilled water, and the sample allowed to dry in low light conditions. It is then ground to a fine powder and stored in airtight containers at room temperature	Kumari (2017)
Pericarp	Pericarp plant sample is collected from the market and stored at − 20 °C	Ponmozhi et al. (2011)
Seed	Seeds are cleaned and dried in the absence of sunlight. The plant sample is dried in a hot air oven at 40 °C, then powdered in mixer grinder and used for solvent extraction	Nagmoti et al. (2012)
Seed	Fresh seeds are cleaned to remove adhering dust and then dried in the shade. The dried samples are powdered in mixer grinder to a coarse powder and used for solvent extraction	Nagmoti and Juvekar (2013)

The information is presented as described by the authors

The sample can be analyzed as fresh matter or dry matter. In the case of drying there are conventional methods, such as oven drying with hot air and sun drying, that are used for their simplicity and low cost (Qiu et al. 2018), and modern methods such as freeze drying that require special equipment, although this can be expensive and has limited capacity to handle large volumes of samples (Wojdyło et al. 2016). It is useful to consider variables that impact the degradation and loss of the compounds of interest, for example water activity, temperature, drying time, and light exposure. The moisture content of the plant material can cause degradation and prooxidation of the components by microbial growth and enzymatic reactions (Rahman Nur et al. 2018). High temperature and long drying times can generate undesirable compounds, degradation of compounds and oxidation of phenolic compounds. Therefore, a balance must be sought between drying temperature and drying time (Gunel et al. 2018).

When possible, it is preferable to use a freeze-drying system, since this type of drying causes changes in the tissue structure of the plant material, making it porous and improving the extraction efficiency of phytochemical compounds. In addition, because it involves very low temperatures, it better preserves bioactive compounds (Karaman et al. 2014; Mccullum et al. 2019).

For dried samples, the reports in *P. dulce* (Table 2) indicate that this process may be carried out at room temperature, in the shade, or in low light conditions. Since many compounds in plant matter are susceptible to light, it is not advisable to dry the plant matter directly in the sun (Bachir et al. 2016). The use of amber or foil-lined containers is recommended to avoid exposure to light and loss of compounds of interest. Also, many of the components are heat-labile, so high drying temperatures are not recommended. It is preferable to dry at temperatures ranging from 30 to 60 °C and in low light conditions. (Złotek et al. 2019). The authors who carried out oven drying in *P dulce*, reported temperatures ranging from 40 to 60 °C (Table 2).

After drying, most authors report grinding the sample in a mill or with a mortar. Finally, the sample is stored at room temperature or at freezing temperature, usually at − 20 °C. None of the studies compared the way in which the sample is treated (type of drying) with the content of phenolic compounds or the antioxidant capacity identified in the study (Que et al. 2008; Mediani et al. 2014).

Normally, dried samples are put through a process of grinding and homogenization to obtain an adequate particle size. Many mills have sieves of different sizes. The smaller the size of the sieve, the smaller the particle that can pass through it. With a 0.5 mm sieve, particle sizes < 0.05 mm are obtained. This particle size allows a good interaction with the solvent and offers a suitable contact surface to carry out the extraction (Lucas-González et al. 2018). As can be seen in Table 2, most of the studies do not report the particle size, or the sieve used (mm). This is a considerable limitation in analyzing the extraction conditions in a methodology, comparing the results, reproducing or optimizing the method mentioned.

Extraction of phenolic compounds

Extraction is the second step carried in identifying and quantifying the compounds contained in plant vegetable matter (Cong-Cong et al. 2017). The preparation and extraction of phenolic compounds depends mostly on the nature of the sample matrix and the chemical properties of the phenolics, including molecular structure, polarity, concentration, number of aromatic rings and hydroxyl groups (Khoddami et al. 2013). Other variables in the extraction process are: temperature, pH, the relationship between the solid:liquid ratio of the sample and solvent, and the extraction time (Stalikas 2007; Altemimi et al. 2017).

As mentioned before, after grinding, the compounds of interest are extracted through procedures involving solvents. The solvents most commonly used due to their polarity and affinity with the compounds of interest are methanol, ethanol, acetone, diethyl ether and ethyl acetate. Some phenolic compounds are highly polar and require a mixture of alcohol and water.

Phenolic compounds in the plant can be highly polymerized or linked with other phenolic and non-phenolic components such as carbohydrates, proteins, fat and organic acids. During the extraction process, the compounds of interest must migrate from the plant matrix to the solvent (Dai and Mumper 2010). Subsequently, the compounds of interest must be isolated or purified in order to be analyzed. Liquid sample extractions are generally centrifuged and filtered, and the solvent is removed. The conditions present during the purification process are directly related to the amount of phenolic compounds present in an extract. The purer the extract, the more phenolic compounds it will contain. On the other hand, if a process of purification or removal of the residue of vegetable matter in the sample is not carried out, there will be a lower content of phenols per sample unit (Khoddami et al. 2013). As can be seen in Table 3, most of the authors do not specify how the extracts were obtained. Ideally, they would tell us whether the sample was filtered, what were the conditions under which the filtering process was carried out, the type of filter paper, its porosity and whether the residue was subjected to washing or re-extraction. Similarly, the centrifugation and solvent removal conditions should be specified. All these variables influence the reported content of phenolic compounds in a plant sample (Stalikas 2007; Khoddami et al. 2013).

Extraction using water, ethanol, methanol and other solvents

Various extraction methods are reported in the studies analyzed. Some authors use single solvents to extract phenolic compounds from one or more parts of *P. dulce* (arils, leaves and seeds) as reported by Kumar et al. (2013). Others use water-alcohol solvents at different dilutions, such as Wall-Medrano et al. (2016), which are shown in Table 3. Among the solvents used for the extraction of *P. dulce* compounds are: water, ethanol, methanol, petroleum ether, acetone, chloroform and hexane.

Table 3 Studies that have reported extraction processes for different parts of *Pithecellobium dulce* (Roxb) Benth

Plant Samples	Extraction process	References
Water extraction		
Aril	10 g of cut fruit in 50 mL of water; using an extractor to obtain a juice and then heating at 96 °C for 1 min and the sample stored at − 18 °C	Samee et al. (2006)
Aril	Aril is cut into small pieces and homogenized in 50 mM phosphate buffer, with a pH of 7.2 and at a temperature of 4 °C, centrifuged at 12,000 g for 30 min then freeze dried	Manna et al. (2011)
Aril	10 g of powdered sample in 100 mL distilled water for 2 h at 60 °C, then the sample is filtered, freeze dried and stored at 4 °C	Megala and Geetha (2010)
Aril	Aril is cut into small pieces and homogenized in 50 mM phosphate buffer, with a pH of 7.2 and at 4 °C, and centrifuged at 12,000 g for 30 min and freeze dried	Pal et al. (2012)
Aril	Using dry powder of mature arils, extracted for 18 h, and after 24 h the extract is filtered and dried at 45 °C	Raju and Jagadeeshwar (2014)
Leaves	Powder is degreased with petroleum ether at 60–80 °C. The extraction is carried out for 18 h, after which the extract is filtered and rotoevaporated to an optimal extraction value of 18.58% w/w	Sugumaran et al. (2008)
Leaves	500 g of powdered sample in 1500 mL of distilled water for 24 h at room temperature; the extract is then filtered and rotoevaporated, and stored at 4 °C	Kumar et al. (2013)
Leaves	100/500 mg of fresh leaves are ground with 1.5 mL distilled water in a mortar and pestle	Krishnaveni et al. (2014)
Leaves	25 g of powdered sample in 250 mL of water, and allowed to extract for 48 h; the extract is then filtered and rotoevaporated, and finally the extract is stored at 4 °C	Poongodi and Hemalatha (2015)
Leaves	50 g of powdered sample in 1000 mL of water for 2 h. The extract is filtered and centrifuged at 10,000 rpm at 25 °C; the extract is later rotoevaporated and freeze dried	Vanitha and Manikandan (2016)
Leaves	500 g of powdered sample in 1500 mL of distilled water for 72 h at room temperature in a shaker with a rotation of 250 rpm; the extract is then filtered; this process is repeated twice. Finally, the extracts are evaporated and stored at − 20 °C	Kumari (2017)
Seed	Air-dried powdered seed are extracted in Soxhlet extractor successively with pet ether followed by distilled water. Extracts are then concentrated by rotary vacuum evaporator and dried	Nagmoti et al. (2012)
Extraction using ethanol and mixed polar solvents		
Aril	10 g of powdered sample in 100 mL of 70% ethanol for 8 h at 60 °C; the sample is then filtered, evaporated, freeze dried and stored at 4 °C	Megala and Geetha (2010)
Aril	20 g of powdered sample in 200 mL of 80% ethanol by microwave-assisted Soxhlet extraction, at a temperature of 50% (350 Watts) for 3 h; finally, the extract is evaporated and stored at 4 °C	Preethi and Saral (2014)
Aril	Using a dry powder of mature arils, extraction is performed using a proportion of (1:6) ethanol for 18 h, and after 24 h the extract is filtered and dried at 45 °C	Raju and Jagadeeshwar (2014)
Leaves	Dry powder sample is degreased with petroleum ether at a temperature of 60–80 °C. The extraction is performed with alcohol (95% v/v) for 18 h, after which the extract is filtered and rotoevaporated to the optimal extraction value of 17.93% w/w	Sugumaran et al. (2008)
Leaves	25 g of powdered sample in 250 mL of ethanol, allowed to extract for 48 h, after which the extract is filtered and rotoevaporated, and finally stored at 4 °C	Poongodi and Hemalatha (2015)
Leaves	500 g of powder sample in 1500 ml of ethanol for 24 h at room temperature, then extract is filtered. This process is repeated three times and the extract is then evaporated and stored at 4 °C	Kalavani et al. (2016)
Extraction using methanol and mixed polar solvents		
Aril	1 g freeze-dried material is extracted in 10 mL of 80% methanol at room temperature using an orbital shaker set at 180 rpm for 2 h. The mixture is centrifuged at 1400 g for 20 min and the supernatant is decanted into a 30 ml vial. The sediment is re-extracted as previously described and the supernatants are used for the subsequent analyses	Kubola et al. (2011)
Aril	1 g powder sample is extracted in 20 mL and sonicated for 10 min, then centrifuged at 20,000 g for 10 min at 4 °C; the supernatant was recovered and the pellet was re-extracted one more time and both supernatants combined. An aliquot of the supernatant was taken to evaluate the antioxidant activity and the other part was concentrated a 39 °C in a vacuum	(Pío-León et al. 2013)
Aril	0.5–1 g powder sample (freeze-dried) in different methanol solutions: methanol (5 mL); 20 mL of hydro-methanol in a ratio of 20:80 (20 mL); and acidified methanol (20 mL); all processed at room temperature. Extracts are hydrolyzed with MetOH: H2SO4 (20:2 v/v) for 20 h at 85 °C, the sample are either centrifuged 3000 rpm for 15 min at 2–5 °C	Wall-Medrano et al. (2016)

Table 3 continued

Plant Samples	Extraction process	References
Aril	1 g of freeze dried sample is mixed with the solvent 1:20 (w/v), sonicated for 15 min and centrifuged at 10,000 rpm for 20 min at 4 °C. The resulting pellet is again extracted and the solvent is evaporated in a vacuum at 38 °C	López-Angulo et al. (2018)
Aril	1 g of powder sample in 50 mL of solvent with 1% HCl for 24 h at room temperature, and the contents are centrifuged for 5 min	Suganthi and Josephine (2018)
Leaves	Powder sample is extracted by Soxhlet apparatus with solvent for 3 h; the extract is then dried in hot air oven at 40 °C, and finally rotoevaporated	Katekhaye and Kale (2012)
Leaves	500 g of powdered sample in 1500 mL of methanol for 24 h at room temperature, after which the extract is filtered and rotoevaporated, then stored at 4 °C	Kumar et al. (2013)
Leaves	25 g of powdered sample is extracted in 250 mL of methanol for 48 h. The extract is filtered and rotoevaporated, then stored at 4 °C	Poongodi and Hemalatha (2015)
Leaves	500 g of powdered sample is combined with 1500 mL of methanol and eleft for 72 h at room temperature in a shaker with a rotation of 250 rpm. The extract is filtered, evaporated and stored at −20 °C	Kumari (2017)
Pericarp	500 mg is extracted in 10 mL of 2 different solvents (methanol and acidified methanol) and the mixture is centrifuged at 10,000 rpm for 10 min	Ponmozhi et al. (2011)
Seed	Air dried powdered seeds are extracted in Soxhlet extractor successively with pet ether followed by methanol. Extracts are concentrated by rotary vacuum evaporator and dried	Nagmoti et al. (2012)
Seed	100 g of powdered sample is extracted by Soxhlet apparatus and the extract is rotoevaporated and stored in refrigeration	Nagmoti and Juvekar (2013)
Other solvents		
Aril	Dry powder of mature arils is extracted using a 1:6 ratio of two solvents, petroleum ether and chloroform, using a Soxhlet apparatus for 18 h. After 24 h the extract is filtered and dried at a temperature of 45 °C	Raju and Jagadeeshwar (2014)
Leaves	Dry powder sample is extracted using a Soxhlet apparatus with 70% acetone for 3 h, then the extract is rotoevaporated	Katekhaye and Kale (2012)
Leaves	500 g of powdered sample is mixed into 1500 mL of different solvents (acetone, benzene and chloroform) and left for 24 h at room temperature, after which the extract is filtered and rotoevaporated, then stored at 4 °C	Kumar et al. (2013)
Leaves	25 g of powdered sample is mixed in 250 mL of two solvents, chloroform and petroleum ether, and left for 48 h after the extract is filtered and rotoevaporated, and finally the extracts are stored at 4 °C	Poongodi and Hemalatha (2015)
Leaves	500 g of powdered sample is mixed into 1500 mL of three different solvents (acetone, chloroform and hexane) for 72 h at room temperature in the shaker with the rotation of 250 rpm, and the extract is then filtered. This process is repeated twice, and finally the extracts are evaporated and stored at −20 °C	Kumari (2017)

Only the information available in each of the references is mentioned

NR Not reported

Extraction using water

Samee et al. (2006) use a solid-solvent ratio of 1:5 (w/v) (10 g of fresh aril in 50 mL) using an extractor to obtain a juice and then heating it at 96 °C for 1 min to inactivate the enzymes. It is then cooled to room temperature and filtered. The volume of the filtered extract is adjusted to 100 mL of water and finally the sample of the extract is stored at − 18 °C. Manna et al. (2011), on the other hand, report cutting the arils into small pieces and homogenizing them in 50 mM phosphate buffer, at a pH of 7.2 and at a temperature of 4 °C. The homogenized mixture is centrifuged at 12,000 g for 30 min and finally freeze dried. However, they do not specify whether they are using fresh or dried arils. Megala and Geetha (2010) report using a solid-solvent ratio of 1:10 (w/v) (10 g of aril powdered sample mixed in 100 mL) of distilled water, with constant agitation for 2 h at 60 °C. The extract is filtered, freeze dried and stored at 4 °C. The methodology reported by Pal et al. (2012) is the same as Manna et al. (2011). In the research of Raju and Jagadeeshwar (2014), an aqueous extract of aril is prepared through a maceration process using a soxhlet extractor for 18 h. After 24 h, the extract is filtered and dried in a hot air oven at 45 °C. This study does not mention the proportions of solid and liquid ratio in the extraction, however. Sugumaran et al. (2008) defat the powdered leaf sample with petroleum ether (95% v/v) at a temperature between 60 and 80 °C. The extraction with

water is carried out in a soxhlet extractor for 18 h. The extract is filtered and then a rotoevaporator is used to concentrate the sample, until reaching 18.58% (w/w). The authors do not mention the ratio of the extraction, however, Kumar et al. (2013) use a solid-solvent ratio of 1:15 (w/v) (500 g of leaf powder sample mixed in 1500 mL) of distilled water for 24 h at room temperature. The aqueous extract is filtered and the whole process is repeated twice more. Subsequently, the filtrate is evaporated and finally the residue is stored at 4 °C. Krishnaveni et al. (2014) mention only using fresh leaves which are ground in a mortar using a solid-solvent ratio 1:10 (w/v) of distilled water (100/500 mg of fresh leaves which are ground in a mortar using 1.5 mL of distilled water), however, the extraction time and temperature are not mentioned. Poongodi and Hemalatha (2015) use a solid-solvent ratio of 1:10 (w/v) (mix 25 g of leaf powder sample in 250 mL) of water for 48 h. The extract is filtered, then evaporated and finally the residue is stored at 4 °C. Vanitha and Manikandan (2016) use a solid-solvent ratio of 1:20 (w/v) (50 g of dry sample in 1000 mL) of water for 2 h and then the extract is filtered. The filtered extract is centrifuged at 10,000 rpm at 25 °C, dried by means of a rotoevaporator and subsequently freeze dried. Kumari (2017) use a solid-solvent ratio of 1:15 (w/v) (500 g of leaf powder sample in 1500 mL) of distilled water for 72 h at room temperature and an agitation of 250 rpm. The extract is then filtered (the author mentions that the process is repeated twice) and after filtration, the solution is evaporated by means of a rotoevaporator. The aqueous extract is stored at − 20 °C. Nagmoti et al. (2012) use a soxhlet extractor and successively with pet ether followed by distilled water; they then use a rotary vacuum to dry the extract. The authors do not mention the ratio of the extractant solution or the solid–liquid range.

Extraction using ethanol

Megala and Geetha (2010) use a solid-solvent ratio of 1:10 (w/v) (10 g of the aril milled sample in 100 mL of 70% ethanol) at 60 °C for 8 h. The extract is filtered, dried by evaporation and finally freeze dried. The sample is stored at 4 °C for further analysis. Preethi and Saral (2014) use a soxhlet extractor assisted by a microwave oven. They use a solid-solvent ratio of 1:10 (w/v) (20 g of dry matter powder in 200 mL of 80% ethanol) by microwave-assisted Soxhlet extraction at a temperature of 50% (350 Watts) for 3 h. Then the extract is dried by means of the rotoevaporator and finally the residue is stored at 4 °C. This is one of the few studies reporting the use of a microwave oven. Raju and Jagadeeshwar (2014) prepare the extract with ethanol at a solid-solvent ratio of 1:6 (w/v) in a soxhlet extractor for 18 h. After 24 h of extraction, the filtration is carried out and then the filtered extract is dried in a hot air oven at 45 °C, which generates a semi-solid mass. Sugumaran et al. (2008) defat the milled leaf sample with petroleum ether at a temperature between 60 and 80 °C. The extraction is carried out by means of a soxhlet extractor with a 95% (v/v) ethanol concentration for 18 h. Subsequently the extracts are filtered and dried by rotoevaporator, obtaining a value of 17.93% (w/w). The authors do not mention the amount of dry sample used in the extraction. Poongodi and Hemalatha (2015) perform the extraction process with a solid-solvent ratio of 1:10 (w/v) (25 g of leaf dry ground sample in 250 mL) of ethanol for 48 h. The extract is then filtered. The filtered extract is evaporated and finally the extract residue is stored at 4 °C. Kalavani et al. (2016) use a solid-solvent ratio of 1:15 (w/v) (500 g of leaf ground dry matter mixed in 1500 mL) of ethanol for 24 h at room temperature. The extract is filtered and the process repeated three times. The extract is then evaporated by rotoevaporator, and the residue is stored at 4 °C.

Extraction using methanol

Kubola et al. (2011) use a solid-solvent ratio of 1:10 (w/v) (1 g of the freeze-dried sample in 10 mL) of 80% methanol with an agitation of 180 rpm at room temperature for 2 h. Subsequently, the extract is centrifuged at 1400 g for 20 min and the excess is decanted in a 30 mL vial. The sediment obtained from the process is re-extracted in the same manner mentioned above. Pío-León et al. (2013) use a solid-solvent ratio of 1:20 (w/v) and sonicate the mixture for 10 min, then centrifuge it at 20,000 g for 10 min at 4 °C. The supernatant is recovered and the pellet is re-extracted one more time. Both supernatants are combined. An aliquot of the supernatant is taken to evaluate the antioxidant activity and the other part is concentrated a 39 °C in a vacuum to obtain the methanolic extract which was stored at − 20 °C in darkness.

Wall-Medrano et al. (2016) use a solid-solvent ratio of 1:20 (w/v) (0.5 to 1 g) of aril freeze dried sample in different methanol solutions: methanol (5 mL); hydromethanol in a ratio of 20:80 (20 mL); acidified methanol (20 mL); processed at room temperature. Then, the extracts are hydrolysed with MetOH: H2SO4 (20:2 v/v) for 20 h at 85 °C to estimate hydrolysable phenolic compounds content, under dark conditions and cooled at room temperature. Samples are either centrifuged (3000 rpm for 15 min between 2 and 5 °C) or directly filtered (0.22–0.45 μM) prior spectophotometric or HPLC analyses. López-Angulo et al. (2018) use 1 g of freeze-dried aril sample with particle size < 0.5 mm, mixed in methanol with a ratio of 1:20 (w/v); the extract is sonicated for 15 min and centrifuged at

10,000 rpm for 20 min at 4 °C. The sediment obtained is re-extracted again as mentioned above. The methanolic extract is dried by rotoevaporator at 38 °C. Suganthi and Josephine (2018) use a solid-solvent ratio of 1:50 (w/v) (1 g of ground dry matter in 50 mL of solvent) with 1% HCl for 24 h at room temperature. Subsequently, the contents are centrifuged for 5 min. Katekhaye and Kale (2012) perform the extraction from leaf powder samples by means of a soxhlet extractor for 3 h at 40 °C. Subsequently, the extract is dried by rotoevaporator. Kumar et al. (2013) use a solid-solvent ratio of 1:15 (w/v) (500 g of the ground dry sample in 1500 mL) of methanol for 24 h at room temperature. The extract is then filtered and the whole process is repeated three times. Subsequently the resultant solution is dried by means of a rotoevaporator and finally the extract is stored at 4 °C. Poongodi and Hemalatha (2015) use a solid-solvent ratio of 1:10 (w/v) (25 g of dry ground leaf sample mixed in 250 mL of methanol) for 48 h. The extract is filtered, evaporated, and the extract stored at 4 °C. Kumari (2017) use a solid-solvent ratio of 1:15 (w/v) (500 g powdered sample in 1500 mL of methanol) with an agitation of 250 rpm at room temperature for 72 h. It is then filtered and the same process is repeated twice. The filtered extract is dried by a rotoevaporator and finally the extracts are stored at − 20 °C. Ponmozhi et al. (2011) use a solid-solvent ratio of 1:20 (w/v) (500 mg of fresh plant material in 10 mL) of different solvents: methanol and acidified methanol. They then centrifuge it at 10,000 rpm for 10 min. Nagmoti et al. (2012) use a soxhlet extractor successively with pet ether followed by methanol. It is then dried by a rotoevaporator. In this study, the proportion of the extraction is not mentioned. Nagmoti and Juvekar (2013) report that 100 g of dry powdered seed sample are extracted with pet ether (60–80 °C) and methanol using a sohxlet extractor. The sample is then dried in a retoevaporator and the extract obtained is stored in refrigeration. This study does not mention the amount of ground dry matter used or the ratio of the solvent.

Sample processing considerations

As will be seen later, the studies analyzed show wide variability in the reported data. This may be due to various factors, which could include geographical location, plant biology and the sample treatment process. The first of these implies different agro-climatic conditions such as air pollution, UV rays, precipitation, temperature, insect attack, the season of the year, and availability of water and soil nutrients, all of which affect the expression of secondary metabolites (Rezende et al. 2015). There is also is the way in which the biological material is processed to identify phenolic compounds and antioxidant capacity in plants. This can in turn be divided into the drying process (which has been discussed in previous paragraphs) and the extraction process.

The biology of the plant creates different proportions of proteins, lipids and carbohydrates which can interfere in the extraction or determination of the compounds of interest. Therefore, the extraction method is essential to be selective and avoid interference according to the analysis technique (Rajha et al. 2014; Kafkas et al. 2018). According to the literature reported on extraction methods in aril, leaves and seeds of *P. dulce* are diverse. This makes it difficult to compare the results. Some authors use an orbital shaker (Megala and Geetha 2010; Kubola et al. 2011); others rely on microwave assisted soxhlet extraction (Preethi and Saral 2014), sonication (López-Angulo et al. 2018), or soxhlet apparatus (Katekhaye and Kale 2012), and others mention only the use of centrifugation (Manna et al. 2011). The extraction process is essential for separating the compounds of interest from the plant matrix. One of the most commonly reported traditional systems is the soxhlet apparatus. The limitations of this type of extraction are that it requires large amounts of solvents, the sample cannot normally be shaken, and it requires a lengthy period of time, which causes the decrease of compounds due to oxidation (Arceusz et al. 2013).

In the case of the orbital shaker, the plant sample is mixed with the volume of solvent to be used at a specific stirring speed. This process provides for greater surface interaction between the solvent and the plant particles, and thus favors the extraction of phenolic compounds, which migrate from the plant matrix to the solvent. Compared to the traditional soxhlet method, the orbital shaker uses less solvent and lower temperatures (Arceusz et al. 2013). However, due to the relationship between the solute and the solvent, sometimes large volumes of solvent are used, which increases the cost and time required to extract the compounds of interest. With the aim of improving extraction performance, the time required and the amount of solvents, less traditional methods have recently become more popular, such as ultrasound extraction (De Souza et al. 2018). Ultrasound extraction constitutes one of the most simple and convenient extraction processes. It employs mechanical vibrations generated by sound waves (> 20 kHz) to extract bioactive compounds (Tzima et al. 2018). This method is a good alternative to traditional methods, since it is very simple, more environmentally-friendly, and reduces extraction times (Tanase et al. 2019). In one study, the extraction yield, content of phenolic compounds and antioxidant activity of the bark of *F. religiosa* are evaluated according to the extraction method (Ashraf et al. 2016). The authors use three techniques (orbital shaker, sonication and magnetic stirrer) and four

solvents (absolute ethanol, absolute methanol, 80% aqueous ethanol and 80% aqueous methanol). They report a higher concentration of TPC, TFC and antioxidant capacity by the application of sonication using 80% methanol, compared to the other techniques. This is probably due to the force exerted by the sonicator waves, which allow for better separation of these compounds compared to the other two techniques. In another study, the influence of sonication and solvent extraction treatments on phenolic and antioxidant compounds in star fruits (*Averrhoa carambola* L.) is evaluated (Annegowda et al. 2012). These authors use sonication treatments at different intervals (0, 15, 30, 45 and 60 min.) extracted in methanol and water. The time in which they report the highest yield of phenolic compounds and antioxidant capacity is 30 min in methanolic extracts. They also observe that at longer times, both phenolic compounds and antioxidant capacity begin to decrease. In some cases, it has been reported that a prolonged sonication (> 40 min) in frequencies above 20 kHz could have a detrimental effect on the components of interest. This effect is attributed to the reduction of diffusion area and rate, but also the increase of the diffusion distance, which can lead to a minimum yield of phenols and flavonoids. In addition, possible free radical formation can occur (Tzima et al. 2018).

Another possible reason for the variability of the results obtained by the different authors may be factors intrinsic to the same extraction method, such as solvents or solvent mixture, and the relationship between solid and liquid, particle size, and extraction temperature and time (Rajha et al. 2014). In relation to the solvents found in this review, the hydroalcoholic solvent mixtures, mainly 80/20 (v/v) ethanol–water, presented a greater amount of phenolic compounds compared to methanolic and aqueous extracts. This may be due to the polarity and solubility generated by the ethanol–water mixture, as well as its affinity with the phenolic compounds of the plant (Kim et al. 2007). On the other hand, aqueous extracts are related to hydrophilic compounds, but water alone can extract other types of impurities such as sugars, organic acids and soluble proteins, which can interfere in the analysis of results of phenolic compounds by means of spectrophotometric tests (Mokrani and Madani 2016).

In the studies analyzed, the time and temperature differs in each of the extraction methodologies. Extraction times range from 1 min to 72 h, while the temperature used varies from 4 to 96 °C. In addition, some authors report carrying out the extraction of phenolic compounds at room temperature. In the case of methanolic extractions, most of the studies were carried out at room temperature (Table 3). The time and temperature of extraction are essential, as already mentioned, since prolonged time and high temperatures can cause oxidation and decrease the yield of the compounds of interest (Dai and Mumper 2010; Khoddami et al. 2013). Likewise, the relationship between the solute and the solvent may be affect the determination of phenolic compounds and the antioxidant capacity of *P. dulce*. The solute and solvent ratio varies in the different studies from 1: 5 (w/v) to 1:50 (w/v). In different investigations it has been observed that the greater the volume of solvent, the better the extraction yields of phenolic compounds and their antioxidant capacity. This is due to mass transfer, where the concentration gradient between the solid and the solvent is the driving force for the mass transfer (Pinelo et al. 2005; Nayak et al. 2015; Predescu et al. 2016). In one study, the authors evaluate different relationships between solute and solvent ranging from 1:20 to 1:60 (w/v) in the extraction of *Polygonum multifl orum Thunb*. root by means of different mixtures of solvents and temperatures (Le Pham and Van Muoi 2018). They observe that the 1:30 (w/v) ratio between solute and solvent extracts the greatest amount of phenolic compounds and antioxidant capacity. Also as the solute and solvent ratio increase, the phenolic extraction and antioxidant capacity decrease. Similar results are shown in other studies (Elboughdiri 2019). However, the solid–liquid ratio used depends on the extraction method, as well as its intrinsic factors, such as the extraction time, the solvent and its mixtures, the temperature and the particle size of the sample (Pinelo et al. 2005; Le Pham and Van Muoi 2018). The solvent ratio is directly related to the temperature. In a study on the effects of temperature, time and the ratio of solvents on the extraction of phenolic compounds in leaves of *Clinacanthus nutans* Lindau, the authors mention that the 90:10 (v/v) ratio of water–ethanol solvent shows a greater quantity of phenolic compounds at 60 °C than at 80 °C. This is because some compounds are thermolabile (Sulaiman et al. 2017). They also report that the 70:30 (v/v) water–ethanol ratio obtained a higher concentration of compounds at 80 °C. This indicates that a concentration of the hydroalcoholic solvents with a higher percentage of alcohol can be used at temperatures higher than 60 °C to extract a higher concentration of phenolic compounds. Some researchers mention that mixtures of ethanol and water in different proportions can be used to obtain an optimal extracting solution for phenolic compounds compared to other solvents (Alothman et al. 2009; Drinić et al. 2018).

In the last years, a wide diversity of unconventional methods for extracting phenolic compounds have become increasingly prevalent. The most common of these are: Ultrasound Assisted Extraction (UAE), Microwave Assisted Extraction (MAE), Ultrasound-Microwave Assisted Extraction (UMAE), Supercritical Fluid Extraction (SFE), Subcritical Water Extraction (SCWE), and Processing of high hydrostatic pressure (HHPP). Compared to traditional methods, these new techniques require less time and less

solvent (Kafkas et al. 2018). However, many laboratories use traditional equipment accompanied by a modern technique. One study reports that using microwave-assisted soxhlet extraction for 3 h at a temperature of 50% (350 Watts) with 80% ethanol obtains more phenolic compounds than what was reported by other authors in aqueous, methanolic and ethanolic extracts (Preethi and Saral 2014).

Phenolic compounds reported in *P. dulce*

Table 4 shows the phenolic compounds found in the arils, leaves, and seeds of *P. dulce*. The analyses are carried out using extracts prepared with the following solvents: water, ethanol, methanol, chloroform and acetone; and with different parts of *P. dulce* (aril, leaf, pericarp, seed).

In the aqueous extract, Samee et al. (2006) report a content of total phenolic compounds (TPC) of 230.1 ± 31.8 µg equivalents of gallic acid (GAE)/g in fresh weight (FW) in aril. Manna et al. (2011) report 3.1 ± 0.08 mg GAE/mL dry weight (DW) in TPC and 55.4 ± 1.5 mg/g DW of flavonoids in the aril, as well as the presence of phenols and flavonoids. Megala and Geetha (2010) obtain 10.4 ± 1.0 mg GAE/g of TPC in the aqueous extract of the aril. Pal et al. (2012) report amounts of 45.1 ± 1.0 mg GAE/g DW of phenols and 55.4 ± 1.5 mg/g DW of flavonoids in aqueous aril extract. Sugumaran et al. (2008) report a concentration of 0.21 mg tannic acid equivalents (TAE)/g of TPC in a leaf extract. Krishnaveni et al. (2014) report quantities of total flavonoid compounds (TFC) and TPC in aqueous extract of *P. dulce* leaf from two locations: in the first location they find 2.1 ± 0.05 mg of quercitine equivalents (QE)/of sample of TFC and 8.4 ± 0.05 mg GAE/g DM of TPC, while the second location they obtain values of 4.2 ± 0.2 mg QE/g of sample of TFC and 8.9 ± 0.1 mg GAE/g DM of TPC. Nagmoti et al. (2012) report a concentration of 1.3 ± 0.006 mg GAE/g of extract of TPC in aqueous extraction of *P. dulce* seed.

Megala and Geetha (2010) report 26.5 ± 1.3 mg GAE/g of TPC in DM in an ethanolic extract of *P. dulce* aril. Rao et al. (2011) classify the arils of *P. dulce* separately, as white and red. In the ethanolic extracts from white aril they report values of 1370.5 ± 0.6 mg/100 g of TPC in DM, while the red aril yields values of 50.5 ± 0.5 mg/100 g of anthocyanins and 993.3 ± 0.4 mg/100 g of TPC. Using an ethanolic extract of aril, Preethi and Saral (2014) report a TFC concentration of 2.8 mg QE/g dry extract and TPC of 622.5 mg GAE/g dry extract. Cheema et al. (2017) report flavonoid concentrations of 85.6 ± 0.04 mg QE/100 g DW in ethanolic extract of aril, along with TPC of 516.3 ± 0.07 mg/100 g DW. Sugumaran et al. (2006) report a low presence of phenols (+) and flavonoids (+) in an ethanolic leaf extract of *P. dulce*, while Sugumaran et al. (2008) report TPC amounts of 0.2 mg TAE/g of the plant extract.

Using a methanol extract of aril, Kubola et al. (2011) report TPC values of 3.8 ± 0.1 mg GAE/g dried sample and TFC of 2.1 ± 0.2 mg GAE/g dried sample. Pío-León et al. (2013) also classify arils into red and white, and perform a methanolic extraction, finding, in white aril, quantities of anthocyanins of < 1 mg C3GE/100 g FW, tannins of 148.2 ± 48 mg CE/100 g FW, TFC of 50.0 ± 2.7 mg QE/100 g FW, and TPC values of 392.2 ± 5 mg GAE/100 g FW. In red aril, they report anthocyanins of 25.9 ± 0.5 mg C3GE/100 g FW, tannins of 309.2 ± 49 mg CE/100 g FW, TFC of 86.6 ± 9.5 mg QE/100 g FW and TPC of 517.8 ± 42 mg GAE/100 g FW. Suganthi and Josephine (2018) report concentrations of 0.2 ± 0.2 g/100 and TPC of 1.3 ± 0.2 g/100 g in a methanol extract of aril tannin. Katekhaye and Kale (2012) perform methanolic extractions of the leaves of *P. dulce*. finding TPC values of 0.084 ± 0.2 µg GAE/mg extract and TFC of 0.9 ± 0.01 µg QE/mg extract. Ponmozhi et al. (2011) prepare a methanolic extract of the pericarp and report anthocyanin values of 29 ± 0.2 mg/g of extract, TFC of 2.03 ± 0.01 mg QE/100 g FM, and TPC of 204 ± 0.3 mg/g FM. These same authors analyze the pericarp using 1% HCl, obtaining anthocyanin results of 32 ± 0.3 mg/g of extract, 6.2 ± 0.1 mg QE/g FM of TFC, and TPC of 200 ± 0.3 mg/g of extract. Nagmoti et al. (2012) prepare a methanol extract of *P. dulce* seed, reporting TPC values of 1.7 ± 0.0035 mg GAE/g of extract. In a similar study, Nagmoti and Juvekar (2013) report TFC amounts of 6.3 ± 0.1 mg of rutin equivalent (ER)/g of extract and TPC of 1.7 ± 0.0035 mg GAE/g DW of extract. Katekhaye and Kale (2012) perform the extraction of compounds from the leaves of *P. dulce* using acetone as a solvent. In the leaf extract, they report TFC values of 0.2 ± 0.01 µg QE/mg of extract and TPC of 0.1 ± 0.2 µg GAE/mg of extract.

The studies analyzed report the presence of phenols, flavonoids, coumarins, tannins, anthraquinones, anthocyanins, phlobatannins, polyphenols and total flavonoids. Other authors analyze extracts of aril and leaves using other solvents such as petroleum ether, benzene and hexane, reporting the presence of anthraquinones, tannins and phlobatannins.

Some studies show variability in Total Phenolic Compounds (TPC) and Total Flavonoid Compounds (TFC) values when comparing aril extracts obtained using different solvents (Megala and Geetha 2010) as well as in leaves (Krishnaveni et al. 2014). There is even a variation in the results obtained using the same solvent (methanolic) for extracts of red and white arils (Pío-León et al. 2013).

Table 4 Reported phenolic compounds of *Pithecellobium dulce* (Roxb) Benth

Solvent/part of the plant	Reported phenolic compounds	References
Aqueous		
Aril	TPC: 230.1 ± 31.8 µg GAE/g of FW	Samee et al. (2006)
Aril	TPC: 3.1 ± 0.08 mg GAE/mL dry weight; Flavonoids: 55.4 ± 1.5 mg/g dry weight; Phenols: (+), Flavonoids: (+)	Manna et al. (2011)
Aril	TPC: 10.4 ± 1.0 mg GAE/g	Megala and Geetha (2010)
Aril	TPC: 45.1 ± 1.0 mg GAE/g dry weight, Flavonoids: 55.4 ± 1.5 mg/g dry weight; Phenols: (+), Flavonoids: (+)	Pal et al. (2012)
Aril	Flavonoids: (+), Tannins: (+)	Raju and Jagadeeshwar (2014)
Leaves	Flavonoids: (+), Tannins: (−)	Sugumaran et al. (2006)
Leaves	TPC: 0.21 mg TAE/g plant extract	Sugumaran et al. (2008)
Leaves	Location 1: TFC: 2.1 ± 0.05 mg QE/g of sample, TPC: 8.4 ± 0.05 mg GAE/g DM Location 2: TFC: 4.2 ± 0.20 mg QE/g of sample, TPC: 8.9 ± 0.1 mg GAE/g dry mass	Krishnaveni et al. (2014)
Leaves	Phenols: (+), Flavonoids: (+),Tannins: (+)	Poongodi and Hemalatha (2015)
Leaves	Anthraquinones: (−), Coumarins (++),Flavonoids: (++), Phlobatannins: (−), Tannins: (+)	Kumari (2017)
Leaves	Phenols: (+), Flavonoids: (+), Tannins: (+)	Sivakumar and Srikanth (2018)
Seed	TPC: 1.3 ± 0.006 mg GAE/g of extract, Flavonoids: (+)	Nagmoti et al. (2012)
Ethanolic		
Aril	TPC: 26.5 ± 1.3 mg GAE/g	Megala and Geetha (2010)
White Aril	TPC: 1370.5 ± 0.6 mg/100 g aril powder	Rao et al. (2011)
Red Aril	Anthocyanins: 50.5 ± 0.5 mg/100 g aril powder, TPC: 993.3 ± 0.4 mg/100 g aril powder	
Aril	Flavonoids: (+), Tannins: (+)	Raju and Jagadeeshwar (2014)
Aril	TPC: 622.5 mg GAE/g of dry extract, TFC: 2.8 mg QE/g of dry extract; Phenols: (+++), Flavonoids: (+)	Preethi and Saral (2014)
Aril	Flavonoids: 85.6 ± 0.04 mg QE/100 g dry weight, TPC: 516.3 ± 0.07 mg/100 g dry weight	Cheema et al. (2017)
Leaves	Flavonoids: (+), Tannins: (−)	Sugumaran et al. (2006)
Leaves	TPC: 0.20 mg TAE/g of the plant extract	Sugumaran et al. (2008)
Leaves	Phenols: (+), Flavonoids: (−), Tannins: (+)	Poongodi and Hemalatha (2015)
Leaves	Phenols: (++), Flavonoids: (++), Tannins: (++)	Kalavani et al. (2016)
Leaves	Anthraquinones: (+++), Flavonoids: (++), Tannins: (++)	Vanitha and Manikandan (2016)
Methanolic		
Aril	TPC: 3.8 ± 0.1 mg GAE/g, TFC: 2.1 ± 0.2 mg GAE/g of dried sample	Kubola et al. (2011)
White aril	Anthocyanins: < 1 mg C3GE/100 g FW, Tannins: 148.2 ± 48 mg CE/100 g FW, TFC: 50.0 ± 2.7 mg QE/100 g FW, TPC: 392.2 ± 5 mg GAE/100 g FW	Pío-León et al. (2013)
Red aril	Anthocyanins: 25.9 ± 0.5 mg C3GE/100 g FW, Tannins: 309.2 ± 49 mg CE/100 g FW, TFC: 86.6 ± 9.5 mg QE/100 g FW, TPC: 517.8 ± 42 mg GAE/100 g FW	Pío-León et al. (2013)
Aril	Tannins: 0.2 ± 0.2 g/100 g, TPC: 1.3 ± 0.2 g/100 g	Suganthi and Josephine (2018)
Leaves	TPC: 0.084 ± 0.2 µg GAE/mg extract, TFC: 0.9 ± 0.01 µg QE/mg extract	Katekhaye and Kale (2012)
Leaves	Anthraquinones: (+++), Flavonoids: (++), Tannins: (++)	Kumar et al. (2013)

Table 4 continued

Solvent/part of the plant	Reported phenolic compounds	References
Leaves	Phenols: (+), Flavonoids: (−), Tannins: (+)	Poongodi and Hemalatha (2015)
Leaves	Anthraquinones: (−), Coumarins (++), Flavonoids: (++), Phlobatannins: (−), Tannins: (+)	Kumari (2017)
Pericarp	Methanol: Anthocyanins: 29 ± 0.2 mg/g of extract, TFC: 2.03 ± 0.01 mg QE/100 g fresh matter, TPC: 204 ± 0.3 mg/g of extract	Ponmozhi et al. (2011)
	Acidified methanol (1%): Anthocyanins: 32 ± 0.3 mg/g of extract, TFC: 6.2 ± 0.1 mg QE/g fresh matter, TPC: 200 ± 0.3 mg/g of extract	
Seed	TPC: 1.7 ± 0.0035 mg GAE/g of extract; Flavonoids: (+)	Nagmoti et al. (2012)
Seed	TFC: 6.3 ± 0.1 mg E.R/g DW extract, TPC: 1.7 ± 0.0035 mg GAE/g DW of extract	Nagmoti and Juvekar (2013)
Chloroform		
Aril	Flavonoids: (−), Tannins: (−)	Raju and Jagadeeshwar (2014)
Leaves	Flavonoids: (−), Tannins: (+)	Sugumaran et al. (2006)
Leaves	Anthraquinones: (++), Flavonoids: (−), Tannins: (++)	Kumar et al. (2013)
Leaves	Phenols: (−), Flavonoids: (−), Tannins: (+)	Poongodi and Hemalatha (2015)
Leaves	Anthraquinones: (−), Coumarins (+), Flavonoids: (−), Phlobatannins: (++), Tannins: (+)	Kumari (2017)
Acetonolic		
Leaves	Flavonoids: (−), Tannins: (+)	Sugumaran et al. (2006)
Leaves	TPC: 0.1 ± 0.2 µg GAE/mg of extract, TFC: 0.2 ± 0.01 µg QE/mg of extract	Katekhaye and Kale (2012)
Leaves	Anthraquinones: (+++), Flavonoids: (+++), Tannins: (+++)	Kumar et al. (2013)
Leaves	Anthraquinones: (−), Coumarins (++), Flavonoids: (++), Phlobatannins: (++), Tannins: (+)	Kumari (2017)
Others solvents		
Aril/petroleum ether	Flavonoids: (−), Tannins (−)	Raju and Jagadeeshwar (2014)
Leaves/benzene	Tannins: (−), Flavonoids: (−)	Sugumaran et al. (2006)
Leaves/ petroleum ether	Tannins: (−), Flavonoids: (−)	Sugumaran et al. (2006)
Leaves/Benzene	Anthraquinones: (+), Flavonoids: (−), Tannins: (+)	Kumar et al. (2013)
Leaves/ petroleum ether	Phenols: (−),Flavonoids: (−), Tannins: (−)	Poongodi and Hemalatha (2015)
Leaves/hexane	Anthraquinones: (−), Coumarins (−), Flavonoids: (−), Phlobatannins: (++), Tannins: (+)	Kumari (2017)

C3GE Cyanidin-3-glucoside equivalents, *CE* Catchin equivalents, *E.R* Rutin equivalent, *DM* Dry mass, *DW* Dry weight, *FM* Fresh matter, *FW* Fresh weight, *GAE* Gallic acid equivalent, *QE* Quercetin equivalent, *TAE* Tannic acid equivalent, *TFC* Total flavonoid compounds, *TPC* Total phenolic compounds

However, Nagmoti et al. (2012) present similar results in the TPC when comparing two different extracts of the seed, as Sugumaran et al. (2008) obtain similar results in the content of TPC in two different extracts of leaves, one ethanolic and another aqueous.

One study provides a determination of PTC and TFC in various fruits found in India, including *P. dulce* (Cheema et al. 2017). The concentration of PTC in the aril of *P. dulce* is similar to other fruits such as *Broussonetia papyrifera* (481.94 ± 2.7 mg/100 g dry weight) and

Syzygium cumini (550.89 ± 0.1 mg/100 g dry weight). Likewise, *P dulce* is found to have a higher amount of PTC than other fruits such as *Mimusops elengi* (98.32 ± 0.7 mg/100 g dry weight) and *Artocarpus heterophyllus* (98.60 ± 0.2 mg/100 g dry weight). Flavonoid concentration is similar with to that of the fruit *Artocarpus heterophyllus* (86.93 ± 2.1 mg QE/100 g dry weight), and higher than other plants such as *Broussonetia papyrifera* (36.65 ± 1.2 mg QE/100 g dry weight), *Morus nigra* (42.07 ± 1.4 mg QE/100 g dry weight) and *Terminalia chebula* (16.03 ± 1.0 mg QE/100 g dry weight).

The results of phenolic contents reported by Pío-León et al. (2013) in aril of *P. dulce* are comparable with studies of other fruits found in tropical areas of Mexico (Moo-huchin et al. 2014). For example, *Lucuma hypoglauca Stanley* (373.27 ± 26.7 mg of GAE/100 g FW) and *Annona reticulata* (358.25 ± 17.0 mg of GAE/100 g FW). Likewise, *P dulce* has a higher concentration of TPC than other fruits such as *Chrysophyllum cainito L.* (18.10 ± 4.5 mg of GAE/100 g FW), *Pouteria sapota Jacq.* (14.21 ± 3.1 mg of GAE/100 g FW), *Hylocereus undatus Haworth* (58.89 ± 11.8 mg of GAE/100 g FW), *Byrsonima crassifolia* (240.76 ± 16.6 mg of GAE/100 g FW), and *Diospyros digyna* (158.48 ± 1.0 mg of GAE/100 g FW). On the other hand, the concentration of TFC in the aril was lower than in *Lucuma hypoglauca Stanley* (341.88 ± 1.4 mg of QE/100 g FW) and *Annona reticulata* (418.24 ± 3.7 mg of QE/100 g FW); and similar to that of *Pouteria sapota Jacq.* (65.24 ± 4.5 mg of QE/100 g FW) and *Annacardium occidentale* (59.27 ± 10.0 mg of QE/100 g FW).

In another study, the TPC content of Indian fruits as *Psidium guajava* (374 ± 20.9 mg GAE/100 g FW) is found to be similar than the aril of *P. dulce*, while other fruits present lower concentration of TPC as *Carica papaya* (6.2 ± 9.1 mg GAE/100 g FW), *Vitis vinífera* (126 ± 6.3 mg GAE/100 g FW), *Achras sapota* (57 ± 6.2 mg GAE/100 g FW), and *Citrus aurantifolial* (133 ± 6.3 mg GAE/100 g FW) (Reddy et al. 2010).

The red aril of *P. dulce* reported by Pío-León et al. (2013) has a TPC concentration of 517.8 ± 42 mg GAE/100 g FW, greater than that of the white aril, as well as that of various tropical fruits reported by Moo-huchin et al. (2014). However, this TPC concentration is similar to that of *Annona squamosa L.* (Purple sugar apple) (78.73 ± 1.6 mg QE/100 g FW), and is lower than in red tropical fruits like *Byrsonima crassifolia* (Red nance) (131.98 ± 7.4 mg QE/100 g FW) and *Annacardium occidentale* (Red cashew) (344.61 ± 4.3 mg QE/100 g FW).

Identification of phenolic compounds in *P. dulce* by HPLC

To identify and quantify *P. dulce* phenolic compounds, various authors report the use of RP-HPLC, HPLC–UV, HPLC–DAD-ESI–MS techniques as shown in Table 5. Aqueous and hydroalcoholic extracts from the arils of *P. dulce* have been analyzed and the following phenolic compounds have been reported: caffeic acid, chlorogenic acid, ferulic acid, gallic acid, p-coumaric acid, protocatechuic acid, apigenin, catechin, daidzein, kaemferol, luteolin, quercetin, myricetin, naringin and rutin.

The most widely used traditional techniques for determining the phenolic profile are high performance liquid chromatography (HPLC) with diode array detection (DAD) and liquid chromatography-mass spectrometry (LC–MS). Compared with generic techniques such as the determination of total phenolic compounds and total flavonoids, they are long-lasting and expensive, but it is possible to identify specific phenolic compounds (Jibril et al. 2019). Gas chromatography (GC) has also been used to identify low molecular weight phenolic compounds and mainly volatile compounds (Kivilompolo et al. 2007). In this process, fat removal from the sample can be carried out to subsequently release the phenolic compounds from the glycosidic fraction (Khoddami et al. 2013). For the determination of phenolic compounds, gas chromatography (GC) is usually used in conjunction with mass spectrometry (GC–MS) since this affords higher precision and accuracy than other methods (Kivilompolo et al. 2007). However, one of the limitations of GC is that it requires high temperatures and a derivatization process (Augusto et al. 2011).

Thin layer chromatography (TLC) has been used for the quantification of flavonoids, and is considered an inexpensive and short-lived method. Like CG, TLC is combined with mass spectrometry (MS) to perform precise quantification (Fuchs et al. 2011).

High Speed Countercurrent Chromatography (HSCCC) is a convenient high-throughput technique with minimum sample loss, high efficiency, high resolution, and ease of sample recovery, without contamination. In the HSCCC the stationary phase is liquid instead of solid, and that provides a lot of advantages over other chromatographic techniques, for example, it does not suffer from the irretrievable adsorption associated with conventional chromatography procedures. It is a good alternative because of its speedier and economically viable separation, ease of scaling-up, ability to be combined with other analytical instruments for establishing on-line hyphenated systems, elevated sample-load capacity, truncated solvent consumption, and availability of a diverse range of solvent-systems and elution modes (Khan and Liu 2018). In this

Table 5 Identification of phenolic compounds of *Pithecellobium dulce* (Roxb) Benth

Solvent/part of the plant	Technique used	Phenolic compounds identified	References
Aqueous/aril	RP-HPLC	Gallic acid, Quercetin and Digitonin	Pal et al. (2012)
Acetonitrile/aril	RP-HPLC	Chlorogenic acid and Catechin	Wall-Medrano et al. (2016)
Ethanol/aril	HPLC–UV	Daidzein: 0.1 mg/100 g Kaempferol: 0.2 mg/100 g Naringin: 0.2 mg/100 g Quericitin: 0.3 mg/100 g Rutin: 0.6 mg/100 g	Megala and Geetha (2010)
Methanol/aril	RP-HPLC	Gallic acid: 12.37 ± 2.36 mg/g dry sample Protocatechuic acid: 3.59 ± 1.69 mg/g dry sample p-hydroxybenzoic acid: NR Chorogenic acid: NR Vanillic acid: NR Caffeic acid: 18.69 ± 1.09 mg/g dry sample Syringic acid: NR p-cormaric acid: 12.36 ± 1.81 mg/g dry sample Ferulic acid: 12.35 ± 1.11 mg/g dry sample Sinapicnic acid: NR Apigenin: 2.6 ± 0.8 mg/g dry sample Kaempferol: 0.8 ± 0.01 mg/g dry sample Luteolin: 120.8 ± 7.4 mg/g dry sample Myricetin: 54.2 ± 4.4 mg/g dry sample Rutin: 2.6 ± 0.1 mg/g dry sample Quercetin: 21.4 ± 4.1 mg/g dry sample	Kubola et al. (2011)
Methanol/red aril	HPLC–DAD-ESI–MS	Cyanidin 3-O-glucoside, pelargonidin 3-O-glucoside	López-Angulo et al. (2018)

Results presented as reported by the authors

NR Not reported

method the compounds are separated according to their partition coefficients between two solvent phases, which are determined by their hydrophobicity. It does not use a solid support so as to allow the permanent adsorption of the compounds in the sample, in addition to isolating the compounds from the plant without having to conduct any prior preparation (Khoddami et al. 2013).

Supercritical fluid chromatography (SFC) is a highly effective method for quick, high-resolution separation of compounds, and it is also environmentally-friendly technique and compatible with different detectors (Khoddami et al. 2013). SFC uses a low-viscosity mobile phase consisting of compressed carbon dioxide to achieve fast and efficient separation. For instance, phenolic compounds have been determined in a few applications using SFC. By using SFC, selectivity is obtained which can be improved by modifying some factors in the mobile phase, such as temperature, pressure, and polarity modifications. Compared to HPLC, the significantly higher diffusion coefficient and lower viscosity exhibited by the CO_2-based mobile phase lead to faster mass transfer and the possibility of using higher flow rates with high efficiency. Also, compared to GC, samples with non-volatile compounds are easier to prepare (Sandahl and Turner 2016).

There are few studies on the use of capillary electrophoresis (CE) to separate and identify phenolics in plant materials. CE is high-resolution technique performed with a solution of ions in a narrow capillary column. It is suitable for identifying charged low and medium-molecular-weight compounds rapidly and efficiently with high resolution, and has low sample and reagent volume requirements. Among the different types of CE separation techniques, the most widely used are Micellar electrokinetic chromatography (MEKC), capillary electrochromatography (CEC) and capillary zone electrophoresis (CZE) coupled with ultraviolet detection (UV), and electrochemistry detection (ECD) or mass spectrometry detection (MS) (Khoddami et al. 2013). CE is a fairly rapid technique, but, there are some factors that must be previously analyzed to obtain optimal results, such as the type of buffer, pH and concentration, temperature, type of capillary, voltage, etc. (Augusto et al. 2011). There are an increasing number of reports using other techniques, such as ultra high performance liquid chromatography

(UHPLC), which requires less time than conventional methods such as LC and also uses less solvents. Ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) and Qualitative tandem liquid chromatography quadrupole time-of-flight mass spectrometry (LC-Q-TOF–MS/MS) has proven to be fast, sensitive and reproducible in the identification of phenolic profiles of different plants. The only disadvantage of this type of equipment is its cost (Kadam et al. 2018; Tzima et al. 2018). Currently, there are no studies reporting the profile of *P. dulce* phenolic compounds using these techniques.

Antioxidant capacity of *P. dulce*

The antioxidant capacity provided by plants can be evaluated using qualitative and quantitative estimates (Ojha et al. 2018). Some of the tests used are based on electron transfer (ET), which evaluate the ability of potential antioxidants to transfer an electron and reduce some compounds, including carbonyls, metals and radicals. Other tests are based on hydrogen atom transfer (HAT), which provides information on the ability of the antioxidant to eliminate free radicals through proton transfer (Lewoyehu and Amare 2019). There are several techniques for determining the antioxidant capacity of food, but the techniques used most often in scientific literature are DPPH, FRAP, TEAC, ABTS + and ORAC. Each of these has advantages and limitations, and there is no one universal method (Han et al., 2014).

DPPH (2,2-dipheyl-1-picrylhydrazyl) is a stable radical, used in an assay based on HAT and ET in which a reduction of the odd nitrogen atom is carried out by means of the donation of a hydrogen from the antioxidant compound (Prior et al. 2005; Kedare and Singh 2011). This reaction causes a color change from purple to yellow, which is proportional to the concentration of the antioxidant in the sample (Apak et al. 2007). The change in coloration is measured by means of a UV–Vis spectrophotometer at a wavelength of 515 to 528 nm. The reaction time for this test varies from 10 min to 6 h, and trolox is regularly used as standard (Prior et al. 2005; Biochem et al. 2011). Results can be expressed as trolox equivalents per sample unit, as percent inhibition and as IC_{50} (concentration of antioxidant causing 50% inhibition of DPPH radical) (Akar et al. 2017). The advantage of this test is that it is quick and simple to perform using a UV–Vis spectrophotometer. It does not present secondary reactions such as chelation of metal ions and enzymatic inhibition. It is a commercially available reagent so it does not have to be synthesized as in other methods. Another advantage is that it can be used in the determination of the antioxidant capacity of any plant in general, either in liquid or solid samples. The disadvantages are that it does not determine the antioxidant capacity in plasma by protein precipitation, it is not similar to a peroxyl radical, the results may be difficult to interpret, and there may be interferences in the reading from other components such as carotenoids (Prior et al. 2005; Lewoyehu and Amare 2019).

FRAP (Ferric reducing antioxidant power assay) is an ET assay, used to measure the reducing power of plasma. It is based on the reduction of ferric ion to ferrous ion by means of tripyridyltriazine (TPTZ) at a pH of 3.6, obtaining a deep blue colored product from 4 to 30 min. It is measured at an absorbance of 513 nm, and the standards used are generally trolox or ascorbic acid (Benzie and Strain 1996; Prior et al. 2005; Apak et al. 2007; Biochem et al. 2011). The advantages of this technique are the ease with which it can be performed, its high reproducibility in a short period of time and its affordability (Thaipong et al. 2006). Both lipophilic and hydrophilic antioxidants are quantified. It is used in biological samples as in plant extracts. The limitation that has been reported in this technique is that it uses a non-physiological pH. Furthermore, because it is a quick technique, it does not detect polyphenolic antioxidants, which react more slowly (Lewoyehu and Amare 2019).

TEAC (Trolox equivalent antioxidant capacity assay) is a spectrophotometric method consisting of TE. It is based on the capture of the cationic radical ABTS (2,2 'azinobis-3-ethylbenzothiazoline-6-sulfonic acid); this radical is generated from potassium persulfate or manganese dioxide over a period of 12 to 16 h, obtaining a dark blue coloration. In the presence of antioxidants, a loss of coloration is caused and a decrease in absorbance is observed, which is analyzed at 658 nm. Trolox is used as standard (Prior et al. 2005; Biochem et al. 2011). This technique is generally practical to perform in aqueous and organic solutions, so the capacity of hydrophilic and lipophilic antioxidants is determined. Also, results are obtained in the first five minutes and different pH ranges can be used. One of its limitations, however, is that the ABTS radical does not represent a biological radical. Also, like the FRAP technique, because it is a technique where the results are read in a short time, the reaction of antioxidants with the ABTS radical may not have ended, which may result in lower values (Prior et al. 2005).

ORAC (Oxygen radical absorbance capacity assay) is a HAT technique, which measures the inhibition of oxidation of a fluorescent molecule (fluorescein and βphycoerythrin) induced by an azo-derivative AAPH (2,2′-az-bis (2-amidino-propane) dihydrochloride) that generates peroxyl radicals. The fluorescent molecule is damaged by peroxyl radicals causing the loss of its fluorescence. Antioxidants protect the fluorescent molecule from oxidative

degeneration. Fluorescence loss is measured with a fluorometer at 520 nm (Amorati and Valgimigli 2014; Ojha et al. 2018). The most commonly used standard is trolox (Lewoyehu and Amare 2019). The ORAC method provides a controllable source of peroxyl radicals that model reactions of antioxidants with lipids in both food and physiological systems, where oxidation reactions are close to the biological system. It can be adapted to detect both hydrophilic and hydrophobic antioxidants by modifying the radical source and solvent. In contrast, the fluorescent molecule (βphycoerythrin) is not photostable, and can be photo bleached after exposure to excitation light, while βphycoerythrin interactions with polyphenols could cause erroneous ORAC values. That is why fluorescein is generally used in this technique. Another difficulty with this technique is that fluorometers are not commonly found in laboratories (Prior et al. 2005; Lewoyehu and Amare 2019).

Evidently, each of the techniques mentioned above has advantages and limitations that must be weighed when deciding which one or more of them to use (Schaich et al. 2015). It is therefore necessary to use different methods to determine antioxidant capacity and obtain a better profile of the analyzed extract (Kuri-García et al. 2017).

Antioxidant capacity has been reported in different extracts of *P. dulce* (arils, leaves, pericarp, and seeds) prepared using various solvents: water, acetone, ethanol and methanol. The data is shown in Table 6. The following techniques are used: ABTS: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); DPPH: 1,1-diphenyl-2-picrylhydrazyl assay; Fe^{+2}: ascorbate induced lipid peroxidation activity; FeSO4 (iron (II) sulfate); FRAP (ferric reducing antioxidant power) assay; H_2O_2 (hydrogen peroxide) scavenging assay, HOCl (hypochlorous acid) scavenging; NO (nitric oxide) scavenging activity; $^1O_{2-}$ (singlet oxygen) scavenging; $O_2^{\bullet}$—(superoxide anion) scavenging; OH (hydroxyl) radical-scavenging activity; SO anion-scavenging activity superoxide; TAC (total antioxidant capacity); TBARS (thiobarbituric acid reactive substances) test; and TEAC (trolox equivalent antioxidant capacity) assay.

Samee et al. (2006) perform an aqueous extraction of the aril of *P. dulce* and analyze its antioxidant capacity using the 2,2-diphenyl-1-picrilhydrazilo (DPPH) technique, reporting a value of 31.8 ± 5.9 µm TEAC/g FW. (Table 6). Megala and Geetha (2010) analyze the antioxidant capacity of an aqueous extract of aril using different techniques: DPPH, ascorbate-induced lipid peroxidation activity (Fe^{+2}), nitric oxide (NO) radical uptake activity, uptake activity of hydroxyl radicals (OH), and superoxide anion (SO) removal activity. They report DPPH: 41.8% inhibition, Fe^{+2}: 44.1% inhibition, NO: 26.0% inhibition, OH: 554.0 IC_{50} µg/mL, SO: 64.2% inhibition. Similary Kumari (2017) obtain an aqueous extract from the leaf and determine antioxidant activity using DPPH, ferric reduction activity potential (FRAP) and NO. They report DPPH: 35.7 IC_{50} µg/mL, FRAP: 50.7 IC_{50} µg/mL, NO: 81.8 IC_{50} µg/mL. Nagmoti et al. (2012) report the antioxidant capacity of an aqueous extract of the seed using: DPPH, superoxide radical elimination activity ($O_2^{\bullet-}$), OH, NO and thiobarturic acid reactive substances test (TBARS). They report DPPH: 81.9% inhibition, $O_2^{\bullet-}$: 82.1% inhibition, OH: 52.6% inhibition, NO: 49.8% inhibition, TBARS: 42.9% inhibition.

Katekhaye and Kale (2012) perform an extraction from the leaf of *P. dulce* using acetone as solvent and analyze its antioxidant capacity using the: DPPH, OH, SO, NO, elimination of hydrogen peroxide (H_2O_2), collection of singlet oxygen ($^1O_2$), uptake of hypochlorous acid (HOCl), and Fe^{+2} techniqiues. They report DPPH: 83.2% inhibition, OH: 43.9% inhibition, SO: 28.2% inhibition, NO: 41.7% inhibition, H_2O_2: 78.3% inhibition, $^1O_2$: 50% inhibition, HOCl: 34.8% inhibition, Fe^{+2}: 53.8% inhibition.

Kumari (2017) use acetone as a solvent for extraction of the leaf and analyze the antioxidant capacity by means of the DPPH, NO and FRAP techniques. They report DPPH: 49.9 IC_{50} µg/mL, NO: 91.5 IC_{50} µg/mL, FRAP: 72.17 IC_{50} µg/mL.

Megala and Geetha (2010) analyze antioxidant capacity in an ethanolic extract of aril and report DPPH: 44.5% inhibition, Fe^{2+}: 76.9% inhibition, NO: 34.2% inhibition, OH: 415.6 µg/mL, SO: 69.6% inhibition. Preethi and Saral (2014) meanwhile mention only the antioxidant capacity of the ethanolic extract of the aril, showing an inhibitory concentration (IC_{50}%) of 167.0 mg/g.

The following authors also analyze a methanolic extract of the aril and report antioxidant capacity using different techniques: Kubola et al. (2011) report 92.2 ± 0.1% inhibition in DPPH and 0.9 ± 0.04 mmol $FeSO_4$/g in FRAP. In DPPH, Wall-Medrano et al. (2016) report values of 22.3 mg TE/g, ORAC of 159.7 µmol TE/g, and TEAC of 19.9 mg TE/g FDE (freeze dried extract). Bhati and Jain (2016) also use the DPPH technique and report an inhibition of 68.1%. Pío-León et al. (2013) report the antioxidant capacity of the white and red arils. In the white aril, values were reported by the 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) technique, showing 155.9 ± 14 mg of vitamin C equivalents (VCE)/100 g FW, and for DPPH values of 170.9 ± 14 mg VCE/100 g FW. In the red aril they report values of 224.8 ± 16 mg VCE/100 g FW by the ABTS technique, and 223.4 ± 12 mg of VCE/100 g FW by DPPH. López-Angulo et al. (2018) report the antioxidant capacity of a methanolic extract of red aril and also analyze a fraction rich in anthocyanins using 0.01% HCl. In the red aril they report values of 142.2 ± 8.1 µmol TE/g from ABTS, and

Table 6 Reported antioxidant capacity of *Pithecellobium dulce* (Roxb) Benth

Solvent/part of the plant	Antioxidant capacity	References
Aqueous		
Aril	DPPH: 31.8 ± 5.9 µm TEAC/g FW	Samee et al. (2006)
Aril	DPPH: 41.8% inhibition, Fe^{+2}: 44.1% inhibition, NO: 26.0% inhibition, OH: 554.0 IC_{50} µg/mL, SO: 64.2% inhibition	Megala and Geetha (2010)
Leaves	DPPH: 35.7 IC_{50} µg/mL, FRAP: 50.7 IC_{50} µg/mL, NO: 81.8 IC_{50} µg/mL	Kumari (2017)
Seed	DPPH: 81.9% inhibition, $O_2^{\bullet -}$: 82.1% inhibition, OH: 52.6% inhibition, NO: 49.8% inhibition, TBARS: 42.9% inhibition	Nagmoti et al. (2012)
Acetonic		
Leaves	DPPH: 83.2% inhibition, OH: 43.9% inhibition, SO: 28.2% inhibition, NO: 41.7% inhibition, H_2O_2: 78.3% inhibition, $^1O_2$: 50% inhibition, HOCl: 34.8% inhibition, Fe^{+2}: 53.8% inhibition	Katekhaye and Kale (2012)
Leaves	DPPH: 49.9 IC_{50} µg/mL, NO: 91.5 IC_{50} µg/mL, FRAP: 72.17 IC_{50} µg/mL	Kumari (2017)
Ethanolic		
Aril	DPPH: 44.5% inhibition, Fe^{2+}: 76.9% inhibition, NO: 34.2% inhibition, OH: 415.6 µg/mL, SO: 69.6% inhibition	Megala and Geetha (2010)
Aril	The antioxidant activity, IC_{50} % of inhibitory concentration of ethanol extract: 167.0 mg/g	Preethi and Saral (2014)
Methanolic		
Aril	DPPH: 92.2 ± 0.1% inhibition, FRAP: 0.9 ± 0.04 mmol FeSO4/g	Kubola et al. (2011)
Aril	DPPH: 22.3 mg TE/g FDE, ORAC: 159.7 µmol TE/g FDE, TEAC: 19.9 mg TE/g FDE	Wall-Medrano et al. (2016)
Aril	DPPH: 68.1% inhibition	Bhati and Jain (2016)
White aril	ABTS: 155.9 ± 14 mg VCE/100 g FW, DPPH: 170.9 ± 14 mg VCE/100 g FW	Pío-León et al. (2013)
Red aril	ABTS: 224.8 ± 16 mg VCE/100 g FW, DPPH: 223.4 ± 12 mg VCE/100 g FW	Pío-León et al. (2013)
Red aril	ABTS: 142.2 ± 8.1 µmol TE/g, DPPH: 41.4 ± 5.1 µmol TE/g	López-Angulo et al. (2018)
Fraction rich in anthocyanin	ABTS: 884.0 ± 37.8 µmol TE/g, DPPH: 597.8 ± 26.3 µmol TE/g	López-Angulo et al. (2018)
Leaves	DPPH: 28.9% inhibition, Fe^{+2}: 57.9% inhibition, H_2O_2: 61.0% inhibition, HOCl: 40.2% inhibition, $^1O_2$: 57.8% inhibition, OH: 29.9% inhibition, NO: 30.9% inhibition, SO: 31.4% inhibition	Katekhaye and Kale (2012)
Leaves	DPPH: 74.8 IC_{50} µg/mL, FRAP: 13.7 IC_{50} µg/mL, NO: 67.4 IC_{50} µg/mL	Kumari (2017)
Pericarp	DPPH: 40.3% inhibition, Fe^{+2}: 11.0% inhibition, OH: 94.6% inhibition, SO: 55.4% inhibition	Ponmozhi et al. (2011)
Seed	DPPH: 85.4% inhibition, OH: 58.3% inhibition, NO: 52.9% inhibition, SO: 88.0% inhibition, TBARS: 67.3% inhibition	Nagmoti et al. (2012)

Results presented as reported by the authors

ABTS 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), *DPPH* 1,1-diphenyl-2-picrylhydrazyl assay, Fe^{+2} Ascorbate induced lipid peroxidation activity, $FeSO_4$ Iron (II) sulfate, *FDE* Freeze dried extract, *FRAP* Ferric reducing antioxidant power assay, *FW* Fresh weight, H_2O_2 Hydrogen peroxide scavenging assay, *HOCl* Hypochlorous acid scavenging, *NO* Nitric oxide scavenging activity, $^1O_{2-}$ Singlet oxygen scavenging, $O_2^{\bullet -}$ Superoxide anion scavenging, *OH* Hydroxyl radical-scavenging activity *SO* Anion-scavenging activity superoxide, *TAC* Total antioxidant capacity *TBARS* Test of substances reactive to thiobarbituric acid, *TE* Trolox equivalents, *TEAC* Trolox equivalent antioxidant capacity assay, *VCE* Vitamin C equivalents

of 41.4 ± 5.1 µmol TE/g from DPPH. In the fraction rich in anthocyanins they obtained values of 884.0 ± 37.8 µmol TE/g from ABTS, and of 597.8 ± 26.3 µmol TE/g from DPPH. Katekhaye and Kale (2012) analyze a methanolic extract from the leaf. The antioxidant capacity of the leaf measured by DPPH was 28.9% inhibition, by Fe^{+2} 57.9% inhibition, by H_2O_2 61.0% inhibition, by HOCl 40.2% inhibition, by $^1O_2$-

57.8% inhibition, by OH 29.9% inhibition, by NO 30.9% inhibition, and by SO 31.4% inhibition. Kumari (2017) perform a methanolic extraction of the leaf and report in dry matter an antioxidant capacity of 74.8 IC_{50} µg/mL by DPPH, 13.7 IC_{50} µg/mL by FRAP and 67.4 IC_{50} µg/mL by NO.

Ponmozhi et al. (2011) analyze the antioxidant capacity of a methanolic extract of fresh matter from the pericarp. They report an inhibition of 40.3% by DPPH, an inhibition of 11.0% by Fe^{+2}, an inhibition of 94.6% by OH, and an inhibition of 55.4% by OS. Nagmoti et al. (2012) analyze the antioxidant capacity of a methanolic extract from the seed by means of the DPPH, OH, NO, SO, and TBARS techniques. The authors report an inhibition of 85.4% by DPPH, 58.3% by OH, 52.9% by NO, 88.0% by SO, and 67.3% by TBARS.

Conclusion

This review describes the various methodologies that the different authors have used to determine the phenolic profile and antioxidant capacity of *P. dulce*. The presence of several phenolic compounds in different parts of the *P. dulce* tree has been reported using different solvents in different proportions such as water, ethanol, methanol, chloroform and petroleum ether. The reported compounds are: caffeic acid, chlorogenic acid, ferulic acid, gallic acid, p-coumaric acid, protocatechuic acid, apigenin, catechin, daidzein, kaemferol, luteolin, quercetin, myricetin, naringin, and rutin. However, the phenolic profile and antioxidant capacity of *P. dulce* varies in all the investigations found. These variations are due both to agro-climatic factors and to the wide variety of sample preparation methods and forms of extraction of the compounds, including different solvents, solvent combinations and extraction times. The analysis techniques reported for the determination of phenolic compounds and the antioxidant capacity also vary widely. Finally, the results maybe reported from study of fresh matter, dry matter or freeze-dried extract, which makes it even more difficult to interpret and compare results in the different investigations. Currently, there are various types of unconventional extraction techniques (UAQ, MAE, UMAE, SFE, SCWE and HHPP) that use less solvents and offer shorter extraction times. This makes them useful alternatives for future research. Also, to identify specific phenolic compounds, modern techniques such as UHPLC, UHPLC-MS/MS and LC-Q-TOF–MS/MS can be used.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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