ejpmr, 2019,6(3), 151-169

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EVALUATION OF ANTIOXIDANT POWER, PHYSICOCHEMICAL PROPERTIES AND PHYTOCHEMICAL COMPOSITION OF *BACTRIS GASIPAES* SEED EXTRACTS FOR DERMOCOSMETIC FORMULATIONS

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Article Received on 13/01/2019 **Article Revised on 03/02/2019** **Article Accepted on 23/02/2019**

SUMMARY

Introduction: *Bactris Gasipaes* is a palm tree from which a fruit—commonly known in Costa Rica as Pejibaye— is obtained. Is native to Central America and very common in local meals because of its high content of starch in its fruit, but also, its wood is used for various utensils, and the tender stems are cut in half to obtain its marrow, commonly known as palmito, is widely used in salads and other dishes, the seed is an industrial waste product, which has high potential to be used as a raw material for the production of cosmetic products and drugs because of its content of antioxidants, fats and other compounds that have been linked to biological actions. The objective of the present study is to identify the main phytochemical groups and to quantify the main markers in order to associate them with cosmetic or medicinal functions in pharmaceutical products. **Materials and methods:** The ripe fruits of *Bactris gasipaes* were peeled, the seeds were separated from the mesocarp, then dried at 75 °C for 3 hours, milled, fractionated with low, medium and high polar solvents, followed by phytochemical screening according to the Guidelines for Quality Control of Natural Origin Products of the World Health Organization. The antioxidant properties were evaluated by means of the H-ORAC, and DPPH assay, the total phenol concentration was determined by Follin ciocalteau, and total carotenoids by visible spectroscopy. The content and type of saturated and unsaturated fatty acids were determined by gas chromatography with mass detector, finally the physicochemical properties of the aqueous extract, pH, specific gravity, Brix degrees, conductimetry, and osmometry were determined, and an absorption spectrum was performed in the range from 260 nm to 800 nm. **Results and Conclusions:** The study found little concentration of phenolic compounds such as condensed and non-condensed tannins. The existence of anthocyanins was not determined; carotenoids, terpenes, flavonoids, saponins, fats, carbohydrates, and alkaloids were found as major phytochemical groups. A medium to high antioxidant power was found, measured in a H-ORAC 13.08 ± 0.57 µg mol Trolox Equivalent/g dry seed, and an EC 50 1014 mg/L for seed oil. The total phenols 0.447 ± 0.020 mg/gram dry sample of gallic acid equivalents verify the qualitative results of the phytochemical screening and show a low presence of antioxidant phenolic compounds and a humidity of about 6% in the seed, in addition terpenoid compounds, alkaloids, tannins, flavonoids, saponins, carbohydrates and fats were found, which makes *Bactris gasipaes* an excellent active for antioxidant, anti-aging, anti-wrinkles, nourishing for the skin, moisturizers, and emollient formulations. The seed oil consists mainly of saturated fat 84.8% ± 1.7, the presence of lauric acid, myristic and palmitic acid was found, and a 15.2% ± 1.7 mainly composed of oleic and linoleic acids, the pH is practically neutral 6.32 ± 0.10, the aqueous extract is hyperosmotic (2174 ± 9.54 mOs/kg) and has a high electrical conductivity (1513 ± 18.48 µS/cm) due to the presence of polyelectrolytes and a high concentration of sugars, mainly sucrose, this was verified according to the obtained brix degrees (3.43 ° ± 0.02). Finally the 2% w/w seed oil shows an absorption of ultraviolet radiation of 20% in the wavelengths between 260 nm to 400 nm, which could also be useful to formulate compositions for sun protection.

KEYWORDS: *Bactris gasipaes*, carotenoids, Pejibaye, Antioxidant, Phytochemical screening, Seed, Cosmetic.

INTRODUCTION

Bactris Gasipaes is a palm tree from which a fruit—commonly known in Costa Rica as Pejibaye— is obtained. It is indigenous to Central America, very common in Costa Rica, due to its fruit's high content of starch. The wood is used for various utensils, and the tender stems are cut in half to obtain their marrow, which is called palmito and commonly used in salads and other dishes (Mora-Urpí, Weber, Clement, & C, 1997).

Table I: *Bactris Gasipaes* taxonomic classification.

Taxonomic Class	Name
Domain	Eukaryote
Kingdom	Plantae
Division	Magnoliophyta
Class	Liliopsida
Subclass	Commelinidae
Family	Arecaceae
Subfamily	Arecoideae
Tribe	Cocoeae
Genus	Bactris
Species	Gasipaes

Source (Global Biodiversity Information Facility, 2017).

This plant is widely distributed from Central America to South America. Common names include tembé (Bolivia), pupunha (Brazil), chontaduro (Colombia), pejibaye (Costa Rica and Dominican Republic), chonta (Ecuador), manaco, Pibá or pifá (Panama) pijuayo (Peru), pijiguao (Venezuela), peewah (Trinidad and Tobago). It's a subsoil species that grows on the edges of the primary forest between sea level and 1,200 meters in height with average annual rainfall of 2000 to 5000 mm and average annual temperatures of 24 to 28 ° C. Bactris Gasipaes was domesticated by the Indians of South American influence, widely used in the culture of Chibchense (Mora-Urpí, Weber, Clement, & C, 1997; Hernández Ugalde, Mora Urpí, & Rocha Nuñez, 2008).

On the origin and genetic variety of the pejibaye (*Bactris Gasipaes*), Mora urpí indicates that the common ancestor was the Guillelma native of South America, the wild populations were domesticated individually, but then they came into contact and formed the cultivated species *Bactris Gasipaes*, Varieties are *Bactris Gasipaes* utilis, and *Bactris Gasipaes* tuira (Hernández Ugalde, Mora Urpí, & Rocha Nuñez, 2008).

The following is an outline of the origin and the cultivated and primitive varieties of *Bactris Gasipaes*:

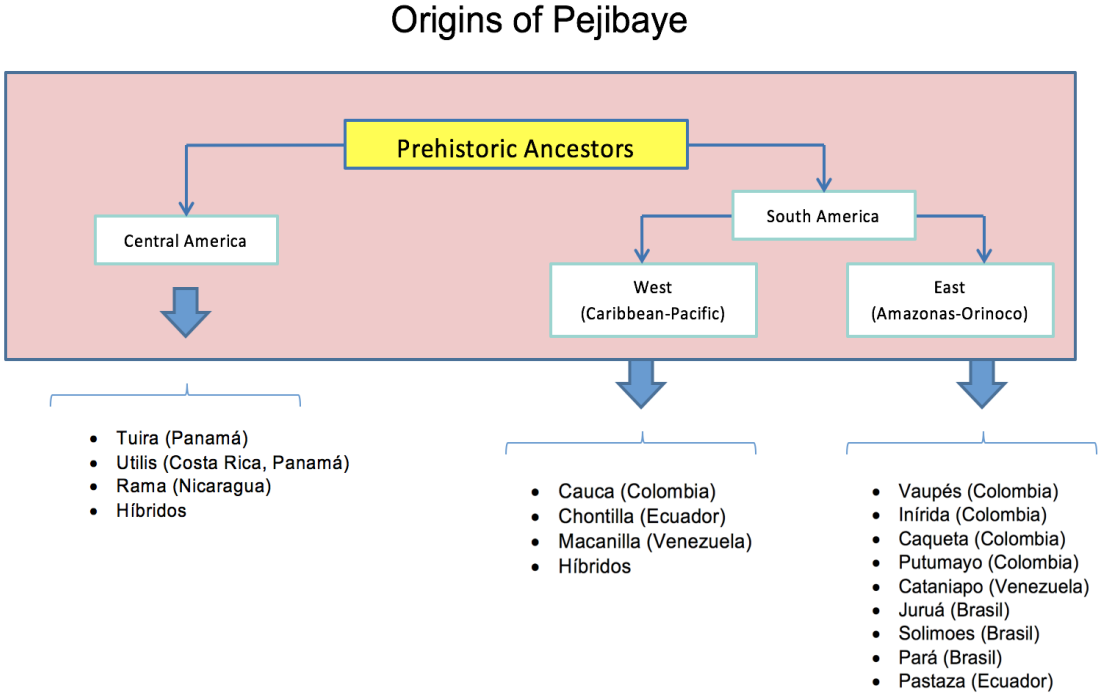


Figure 1: Scheme on the origin of the cultivated Pejibaye *Bactris Gasipaes* and their varieties (Mora-Urpí J., 2003).

Currently in Costa Rica it is cultivated mainly in Tucurrique Carthage, Guápiles province of Limón and Perez Zeledón in the southern region, where there is even a town called Pejibaye. In the market three qualities can be obtained depending on the size of the fruit— first, second, and third— however some of the third fruits appear to come from wild or old cultivated varieties. In addition, the tilis variety is normally also characterized by its place of origin due to some variations in the color of the fruit, the leaves and the size of these. The more common subvarieties are Guapiles, Tucurrique, Guatuso and Yurimaguuas (Corporación Bananera Nacional, 2000).

Figure 2: *Bactris Gasipaes* and Frutos palm with and without shell (Mora-Urpí, Weber, Clement, & C, 1997).

The oldest archaeological evidence of *Bactris Gasipaes*. cultivation is from Costa Rica between the dates of 2300 to 1700 BC. The historical records during the European contact indicate that this species was one of the main cultivated in Costa Rica at that time. The exact origin of the domestication is not known, for example the indigenous Malekus of the northern part of the country have been in Costa Rica for at least 600 years, before the domestication of the plant, and began the cultivation of the species *Bactris Upalensis*, which was then contaminated with the genotype Gasipaes. It's known for genetic studies that consists of 28 chromosomes, and varieties are being cultivated in Peru and Colombia without thorns to extract the palm heart. The cultivation for the consumption of its fruit is a derivative of its cultivation for palmito; most countries, with the exception of Costa Rica and parts of Nicaragua and Panama, do not consume the fruit regularly. The cultivars vary widely, especially in fruit size, ranging from 20 to 70 grams in different cultivars (Bermejo & León, 1994; Mora-Urpí, Weber, Clement, & C, 1997; Graefe, Dufour, Van Zonneveld, Rodriguez, & Gonzalez, 2013).

It is a palm whose stem is multiple and grows up to 20 meters in height. The internodes of the stems (mother regions between the leaves) and leaf pods are armed with spines that can be up to 5 centimeters long. The leaves measure up to 2.5 meters long, and are divided into small leaflets that arise from the middle vein and grow up to 50 centimeters long. The species is monoecious. The flowers are small, yellow and the feminine are arranged irregularly around the masculine ones. The development of inflorescence bud in *Bactris gasipaes* triggers foliar abscission, therefore, only the base of the leaf stem remains in the stem at the moment when the inflorescence develops. Up to nine clusters of flowers are produced by stem. Each inflorescence is associated with a bract that is brown and prickly on the outside and white and without spines in the interior. Staminated (pollen-producing) and pistillate (ovum producing) flowers are produced within the same group of flowers in this species and many more male flowers are produced from pistillate flowers (Mora-Urpí, Weber, Clement, Dufour, Van Zonneveld, Rodriguez, & Gonzalez, 2013).

Figure 3: Fruit Ratio of *Bactris Gasipaes* (Mora-Urpí, Weber, Clement, & C, 1997).

The fruits are red or yellow drupes, very variable in dimensions with a weight in the range of 4-186 grams, and grow in clusters of up to several hundred units that can weigh up to 20 kg. Each fruit contains a single seed (sometimes contains two), surrounded by a dark fibrous endocarp. (Bermejo, JEH and J. Leon 1994). The fruits are highly perishable; can be kept in good condition only for about 4 days and under refrigeration for up to 8 days. The whole fruit is separated from the bunch and boiled in salted water for 1-3 hours to remove the oxalate crystals and inhibit trypsin. The pericarp, with a high content of starch, is consumed after removing the peel. It's also used to make flour for bakery and for other purposes; it is also used to smoke the fruits, or to dry them.

Table II: Nutritional content of Bactris *Gasipaes* pericarp in 100 grams of fruit.

Characteristic	Value
Energy	185-196 kilocalories
Humidity	50.5-52.2 grams
Dietary fiber	1.0-1.4 grams
Proteins	2.6-3.3 grams
Grease	4.4-4.6 grams
Total carbohydrates	37.6-41.7 grams
Ashes	0.8-0.9 grams
Calcium	14-23 milligrams
Magnesium	20 milligrams
Potassium	196 milligrams
Phosphorus	46-47 milligrams
Iron	0.7-1.0 milligrams
Vitamin A	1117-7300 UI
Riboflavin	0.11-0.16mg
Niacin	0.9-1.4 mg
Vitamin C	3.5-20 mg
Vitamin B6	0.06 milligrams
Folic Acid	34 milligrams

Source: (Camacho, 1972).

A more recent report on the composition of carotenoids and tocopherols in the fleshy part after boiling shows trans lycopene 20 µg/100 g cis lycopene 63.7 µg/100 g, alpha carotene 423 µg/100 g, trans beta carotene 591 µg/100 grams, cis beta carotene 391 µg/grams, and 01 mg/100 grams, of alpha tocopherol (Furtado, Siles, & Campos, 2004, Basto et al., 2016). The first to study the composition of the mesocarp fatty acids and pejibaye seeds was Jorge Mora Urpí, in his work published in 1982 an identification of the acids from the seed and the mesocarp was made (Hammond, Pan, & Mora-Urpí, 1982).

Pejibaye peel and seed composition have also been studied, both of which are industrial wastes of their production. Recently the commercialization in canning has been initiated, which generates a waste of shells and seeds. The composition of the shell on dry basis of *Bactris Gasipaes*, determined an ethereal extract of between 23.35%-24.14%, the percentage of dry matter varied between 37.8%-42.33%, the ash varied between 2.1%-2.3%, lignin between 1.71%-2.46%, the main fatty acids present were oleic (540.77-557.84 mg/g) and palmitic (253.15-255.31 mg/g), then palmitoleic acid (66.98-80.68 mg/g) and linoleic acid (84.85-94.7 mg/g) (Márquez Salinas, 2014).

Another study analyzed the composition of four varieties of *Bactris Gasipaes* (Restrepo, J., Vinasco, LE, & Estupiñán, JA 2013), it was concluded that the lipid extract of the mesocarp of *Bactris gasipaes* from the Colombian Pacific reveals a composition of Saturated and unsaturated fatty acids very similar to those of the varieties analyzed in the Central Amazonia of Brazil and those of Costa Rica. At the same time, this description of saturated and unsaturated fatty acids in *Bactris Gasipaes* can be compared to olive oil, palm oil and other commercial oleaginous products, due to the high content of oleic acid, but it has a low content of lauric and myristic acid (Restrepo, Vinasco, & Estupiñán, 2013).

MATERIALS AND METHODS

Materials

The fully ripe fruits (stage 3) ripening scale of Ó. Acosta-Montoya et al., directly from the farm in the Copey area of Dota, 2 kg of plant material was obtained.

The reagents were fluorescein, quercetin, ellagic acid, gallic acid, Follin Ciolcateu reagent, DPPH reagent (2,2-diphenyl-1-picrylhydracil) dihydrochloride), Drangerdof, Prussian blue, Trolox (6-hydroxy- 7,8-tetramethyl-2-carboxylic acid) are from Sigma Aldrich Chemical Company (St. Louis, MO, USA); (AAPH) Cyanidin-3-O-glucoside from Extrasynthesis (Genay, France); Other reagents used are analytical grade and HPLC grade solvents.

The equipment used was: Thermo Scientific UV-Visible spectrophotometer model Evolution 600 with integrating sphere device, Thermo Scientific UV-visible spectrophotometer model Genesys 10 s, Ohaus analytical balance model Abventurer 310, Advanced Instruments Osmometer model 2200, Hanna Instruments model pH211 pH-meter, Metler Toledo conductivity meter model seven easy, NIndustrias Nury constant temperature bath model LL630, Thelco drying kiln, Labconco freeze dryer, Fisher Scientific centrifuge 225, Labconco rotary evaporator, Oster blender, Rudoph Instrument Refractometer, Brookfiel Model DV III ULTRA Viscometer, Corning PC-420 Stirrer-Heater.

Methods

Preparation of Extracts and Phytochemical Screening

The fruits were peeled and the seed was extracted, it was crushed into pieces and dried in an oven at 50°C for 3 hours, then ground in a hand mill. Each section of seed was separated into 250 grams, and was processed as follows: parts of 50 grams of sample were taken, placed in a refluxing soxleth for one hour, with 100 grams of distilled water (for the aqueous extract 400 grams of water were used with 100 grams of pejibaye seed and then filtered) (phase a), 100 grams of ethanol (phase b), 100 grams of acetone (phase c), 100 grams of petroleum ether (phase d), 100 grams of hexane (phase e), all were placed in an independent soxleth for two hours. The hexane phase had to be performed in quadruplicate since other tests were necessary, it was indicated which corresponded to the mesocarp and which to the seed; these extracts were stored in amber jars at -20°C.

Ethanolic and aqueous extracts

The aqueous and ethanolic extracts were made for the following analyzes of Gelatin (Tannins), Gelatin-Salt (Tannins), Formaldehyde-HCl (Tannins), Ferric Chloride (Tannins), Mucilages, Shinoda, Pews (Flavonoids), Dragendorff, Mayer, Rosenheim, (Saponins) Rosenthaler, Fheling, Molish, Tollens, Benedict (Carbohydrates), (Lactones-Coumarines), Nitrogen, Amino Acids, Steroids Lieberman-Burchard, (Quinones) Borntrager, (Anthocyanins) Hydroxycinnamate Ferric Baljet, Kedde, Guinard, Sudan III (Fats), Carr-Price (Carotenes), (Terpenes) Ammonium IV Molybdate, Perchloric Acid, Salkowski, Tortelli-Jaffe.

Extract in Acetone

The acetone extract may form an emulsion, in which case a saturated solution of NaCl is added and allowed to stand until the phases are separated. Once separated the oil is extracted with a pasteur pipette. To the acetone extract were added 100 mL of acidified methanol to a pH of 3 (0.01 molar in HCl) (phase c), then stirred for 15 minutes at room temperature in a vacuum trap, by first bubbling the extract with nitrogen for 1 minute and protected from light, then filtered using vacuum to recover the solvent with the extract. The extracts were concentrated in a rotary evaporator at 40°C, up to 80 mL; the aceto-methanolic extract was taken to a 100 ml volumetric flask and gauged with acidified methanol. 20 mL (phase c1) were taken and separated for the analysis of tannins. Amino acids, and flavonoids, the remaining 80 mL were placed again in a rotary evaporator at 40°C until dry. 150 mL of 0.01 molar aqueous HCl solution was added at 40°C; three washes were performed with 5 mL of 0.01 molar aqueous HCl solution at 40°C. The extract and the washes were transferred to a 250 mL erlemeyer, then centrifuged for 30 minutes at 10 000 rpm. Supernatant was filtered on a 0.45 μm pore membrane (phase c2); the insoluble or precipitated part was separated, then washed with cold water at 5 °C, dried and dissolved in 20 mL of chloroform (phase c3). Lieberman-Burchard and Borntrager analysis of the soluble acid part (phase c4), were performed, then alkalinized to pH 9.0 with 0.1 molar ammonia solution. The alkaline extract was placed in a 250 mL separatory funnel; the alkaline phase was extracted with three 30 mL portions of hexane (phase c.5). The remaining aqueous phase (phase c.6) was saturated with sodium sulfate; the chloroform phase (phase c.5) was washed with three 5 ml portions of cold water 5 °C, of the organic phase (phase c.5), 10 ml were taken and placed on the rotary evaporator at 40 °C to dryness. 2 mL of chloroform (phase c.5.1) were added and the Lieberman-Burchard test was performed; the remaining phase (c5.2) was brought to dryness and 10 mL of HCl are added. The Dragendorf and Mayer, and/or Wagner test was carried out; the remaining aqueous phase was saturated with anhydrous sodium sulfate (phase c.6). Three 50 mL portions of a 3:2 chloroform:ethanol mixture, in weight, were added, then placed in a separating funnel and separated the aqueous phase (phase c.7) to which the Shinoda and Rosenheim test is performed. The chloroform: ethanol mixture (phase c.8) was washed with saturated aqueous sodium sulfate solution; the chloroform:ethanol mixture (phase c.8) was cooled in an amber flask at -20 °C until its analysis for the Shinoda, Rosenheim, Lieberman, Burchard, Dragendorff and Mayer tests.

Extract in Ether

The ethereal phase (phase d) was divided into two phases, d1 and d2, independently evaporated in the rotary evaporator at 40°C. 100 mL of acidified water pH 3 with 0.1 molar HCl were added to phase d1, stirred for 10 minutes, and simultaneously alkalized water was added in the d2 phase to a pH of 12 with 50% w/w NaOH; the latter was refluxed for one hour. Acidified and alkalized (phase d1 and d2) extracts were placed independently in a separatory funnel of 250 mL, and three equal portions of 30 ml of chloroform were added, and the phases were separated. The remaining phases were separated to perform the analysis (phase d1 and d2). The d1 and d2 phases were refrigerated in an amber flask at -20°C until their analysis of Gelatin (Tannins), Gelatin-Salt (Tannins), Formaldehyde-HCl (Tannins), Ferric Chloride (Tannins), Mucilages, Shinoda, Pews (Flavonoids), Dragendorff, Mayer, Wagner (Alkaloids), Nihidrin (Amino Acids), (Steroids) Lieberman-Burchard,

(Quinones), Borntrager, (Anthocyanins) Rosenheim, (Saponinas) Rosenthaler Foam, Fheling, Molish, Tollens, Benedict (Carbohydrates), (Lactonas-Cumarinas), Hydroxycinnamate Ferric Baljet, (Cardiotonic Glycosides) Kedde, (Cyanogenic Glycosides), Guinard, Sudan III (Fats), Carr-Price (Carotenes), (Terpenes) Molybdate Ammonium IV, Perchloric Acid, Salkowski, Tortelli-Jaffe. The organic phases d3 and d4 were tested for carotenoids.

Hexanic Extract

One of the soxleth extracted parts of the hexane phase (phase e) was reconstituted in 100 ml of ethanol. From this phase it's possible to separate the ethanolic phase (e1) from the oily phase (e2). The e1 phase was taken as an aqueous phase and refrigerated in an amber flask at -20°C until their analysis with Gelatin (Tannins), Gelatin-Salt (Tannins), Formaldehyde-HCl (Tannins), Ferric Chloride (Tannins), Mucilages, Shinoda, Pews (Flavonoids), Dragendorff, Mayer, Wagner (Alkaloids), Nihidrin (Amino Acids), (Steroids) Lieberman-Burchard, (Quinones), Borntrager, (Anthocyanins) Rosenheim, (Saponinas) Rosenthaler Foam, Fheling, Molish, Tollens, Benedict (Carbohydrates), (Lactonas-Cumarinas), Hydroxycinnamate Ferric Baljet, (Cardiotonic Glycosides) Kedde, (Cyanogenic Glycosides), Guinard, Sudan III (Fats), Carr-Price (Carotenes), (Terpenes) Molybdate Ammonium IV, Perchloric Acid, Salkowski, Tortelli-Jaffe. The organic phases d3 and d4 were tested for carotenoids and tocopherols.

A part of the hexane phase was reserved for analysis of fatty acid determination, spectroscopy, and for the analysis of carotenoid quantification. This part was placed in a rotary evaporator at 40°C until the dryness, the solid residue was placed in an amber glass bottle with a glass stopper and stored at -20°C. The remaining two parts were reserved for assays of the microemulsion formulations, these parts are placed on a rotary evaporator at 40 °C to dryness, the solid residue is placed in an amber glass bottle with glass stopper and stored at -20°C.

Phytochemical screening and reagents were performed according to Solís, Gattuso, & Cáceres (2003) and World Health Organization, (2007).

Moisture Determination Test

Perform to the freshly cut and peeled mass from the mesocarp and seed.

In a three decimal places balance, 250 grams of seed and mesocarp were weighed independently, placed in a beaker on a tray in an oven for three hours at 50 °C, to dryness. The weight was determined after three hours and the dry matter weight was calculated by difference (Health Organization, 1998; Solís, Gattuso, & Cáceres, 2003; World Health Organization, 2007; Tiwari, Brunton, & Brennan, 2013).

Liposoluble fraction weight test for the hexane phase, for the seed.

Weigh 4 empty flasks, add the hexane and ethereal phases independently. The rotary evaporator is turned on at 40 ° C, the solvent is dried, the weight of the solids amount of the organic phases is calculated by weighing by difference and the percentage of weight of the extracted hexane and ethereal phases is calculated (Organization of the Health, 1998, Solís, Gattuso, & Cáceres, 2003, World-wide Organization of the Health, 2007, Tiwari, Brunton, & Brennan, 2013).

Determination of Total Phenols for the acetone phase of seed oil

Folin-Ciocalteu method: Done in triplicate with the acetone phase, taking 10 grams of dry sample (fresh seed and finely ground, less than 0.5 mm particle size) and leave it at reflux with 100 mL of acetone for three hours at 35 °C, then concentrated to 8 mL in rotary evaporator at 35 °C and brought to mark in a 10 mL volumetric flask with acetone. The standard solution was gallic acid at a concentration of 500 mg/mL in acetone and distilled wáter 1:1. The result was expressed as mg of gallic acid equivalents (GAE) per gram of fresh fruit (Rojano, Cristina Zapata, & Cortes, 2012; Espinosa-Pardo, Martinez, & Martinez-Correa, 2014).

Gallic acid stock solution (500.0 mg/L)

50 mg of gallic acid is accurately weighed, which has previously been dried in the oven at 105-110 °C for 2 hours, and placed in a 100.0 mL volumetric flask. Add 50 mL of distilled water and stir until dissolved. It's brought to mark with distilled water (Rojano, Cristina Zapata, & Cortes, 2012; Espinosa-Pardo, Martinez, & Martinez-Correa, 2014).

Preparation of the calibration curve

A standard solution of gallic acid (500.0 mg/L) was used, from which volumes of 0.1, 0.25, 0.50, 1.00, 2.00 and 4.00 mL of the stock solution of gallic acid were taken and placed in volumetric flasks of 50.0 mL, the volumeof each was completed with 5 mL of 1:1 acetone-water (Rojano, Cristina Zapata, & Cortes, 2012; Espinosa-Pardo, Martinez, & Martinez-Correa, 2014).

NaOH solution (0.35 mol / L)

1.40 g of NaOH are weighed in a granatary scale and placed in an appropriate plastic container. Add 50 mL of distilled water and stir until dissolved. Is brought to an approximate volume of 100 mL with distilled water.

Folin-Ciocalteu Reagent

10 grams of sodium tungstate and 2.5 grams of sodium molybdate are added in 70 mL of water; then 5 mL of 85% w/w phosphoric acid and 10 mL of 36% w/w HCl are added. Heat the solution to reflux for 10 hours (Rojano, Cristina Zapata, & Cortes, 2012; Espinosa-Pardo, Martinez, & Martinez-Correa, 2014).

Reading the calibration curve and the acetone samples
1 mL of each standard or sample, 1 mL of diluted Folin-Ciocalteu reagent and 2 mL of 0.35 mol/L NaOH solution are mixed in a 10 mL test tube. It's agitated for 5 seconds and kept in the dark for 3 minutes. The mixture is stirred for 5 seconds and the absorbance is measured at 765 nm using water and acetone 15:1.

Determination of Antioxidant Power for *Bactris gasipaes* seed DPPH assay
Determination of Antioxidant Power
Hexanic Extract
DPPH (2,2-diphenyl-1-picrylhydrazyl) test
The DPPH solution which was prepared at a concentration of 0.1 mM in methanol, was made immediately prior to use and protected from light to prevent degradation; the standard solution was prepared with 10 mg/mL BHA in acetone. The test procedure was as follows:

Butylated Hydroxy Anisole (BHA) stock solution (500.0 mg/L)
100 mg of BHA are accurately weighed and placed in a 10.0 mL volumetric flask. 5 mL of acetone are added and stirred until dissolved. It's brought to the mark with distilled water.

Note
-This solution must be protected from light and refrigerated at 10°C
BHA Pattern Curve (0.4 mg/mL-1.2 mg/L)

Aliquots of 0.40, 0.60, 0.8, 1.00 and 1.20 mL of the BHA stock solution are taken and placed in volumetric flasks of 10.0 mL, then carried to the mark with acetone. Curve displays *Bactris gasipaes* seed and mesocarp oil (0.4 mg/mL-1.2 mg/L).

100 mg of *Bactris gasipaes* seed or mesocarp oil are accurately weighed in separate containers and placed in a 10.0 mL volumetric flask. 5 mL of acetone is added and stirred until dissolved. It is brought to the mark with acetone.

Aliquots of 0.40, 0.60, 0.8, 1.00 and 1.20 mL of the gallic acid stock solution are taken and placed in volumetric flasks of 10.0 mL. Then carried to the mark with acetone. The supernatant liquid is centrifuged and taken.

Dissolution of DPPH
4-5 mg of DPPH are accurately weighed and placed in a 100.0 mL amber volumetric flask. 50 mL of methanol is added and stirred for 10 minutes to dissolve. It is carried to the calibration mark with methanol.

Note
-This solution must be prepared and used the same day.

Reading pattern curve and samples
In a 10 mL test tube, 3.9 mL of the DPPH solution and 100 µL of each standard or sample are mixed, shaken for 5 seconds and kept in the dark for 30 minutes. Then stirred for 5 seconds and the absorbance is measured at 517 nm using water as a blank. The radical removal activity was reported as EC50 (effective average concentration required to reduce 50% of the DPPH radical) (Acosta-Montoya et al., 2010, Martínez-Cruz N. S. et al., 2011).

The inhibition stock should be calculated by the equation:
% Inhibition = 1- (Sample Absorbance / DPPH Absorbance)) X 100

And the percent inhibition versus the primary standard or sample concentration is plotted.

The C50 is calculated by using the % inhibition equation by the following calculation:
C50 = (50-B) / A

Where B is the intercept of the inhibition percentage curve versus concentration and A is the slope of the percent inhibition vs concentration curve (Radice, et al., 2014).

ORAC test: The ORAC method consists of measuring the decrease in the fluorescence of a protein as a result of the loss of its conformation when it undergoes oxidative damage caused by a source of peroxide radicals (ROO). The method measures the ability of antioxidants in the sample to protect the protein from oxidative damage. The method allows to determine the equivalent moles of Trolox in a range of 5 to 200 µg/ml, where the analyte used as a reference has a linear behavior. The analysis should be performed at room temperature and samples should be protected from light and stored at 4 °C after preparation (Rojano, Cristina Zapata, & Cortes, 2012).

Process
Calibration curve
Exactly 5 mg of trolox were weighed and taken to a volumetric flask of 10mL, then were completely solubilized in the phosphate buffer solution and were brought up to mark. A solution was obtained at 2000 µM (heat the solution slightly to no more than 40°C to facilitate dissolution) (Rojano, Cristina Zapata, & Cortes, 2012).
2. Dilutions were made for the trolox curve by taking 5, 12.5, 25, 50, 75, 100µL of the above solution and bringing to volume of 1 mL with distilled water. Curve 10-200 µg/ml.
3. The following components were added in order to each well: 150 µL of fluorescein, 25 µL of the respective dilution of trolox.
4. In parallel, a blank test was prepared containing: 150 µL of fluorescein and 25 µL of phosphate buffer solution.

5. It was preincubated for 30 min at 37°C
6. 25 µl of 250 mM AAPH solution was added to each well.
7. Fluorescence intensity was measured every 2 min for 2 hours with excitation and emission wavelengths of 485 and 520 nm respectively.

Measurement of Samples
Preparation of solid samples
1. The samples were prepared taking into account that for samples with a high fat content it was necessary to degrease by making the previous saponification (see determination of Carotenoids). Another option is to make a 1 in 100 dilution in methanol with 50% w/w NaOH, reflux 30 minutes, and extract with Ether, then dry the extract and reconstitute with ethanol.
2. A 5 mL aliquot was filtered through a nylon membrane (0.45 µm), stored at 4°C and protected from light.
3. Dilutions of 1:10 and / or 1:100 were made in water.

Preparation of liquid sample
1. 10 mL of sample were taken and centrifuged at 3000 rpm/15 min to remove solid waste.
2. A 5 mL aliquot was filtered through a nylon membrane (0.45 µm), stored at 4°C and protected from light.
3. Dilutions of 1:10 and / or 1:100 were made in water.

Preparation of reagents
Sodium Phosphate/Biphosphate buffer solution 10 Mm: 0.276 grams of Na2HPO4.12H2O and 0.035 g of NaH2PO42H2O were weighed, brought to volume in a 100 mL volumetric flask.

Fluorescein 1µM
A 1mM solution of fluorescein was initially prepared; for this 3.76 mg of Fluorescein were weighed and brought to mark with the phosphate Buffer solution in a 10 mL volumetric flask (heat to a temperature no higher than 50 °C if necessary). Then make the dilution at 1 µM.

Solution of 250 mM AAPH (2,2'-azobis (2-amidinopropane))
678 mg were weighed and brought to volume with the phosphate buffer solution in a 10 mL volumetric flask (Acosta-Montoya, et al, 2010; Gancel, Feneuil, Acosta, & Vaillant, 2011; Tiwari, Brunton, & Brennan, 2013; Thangaraj, 2016).

Specific Gravity
For aqueous extract only
The empty pycnometer at 25°C was weighed in an analytical balance, then weighed with distilled water at 25°C, and finally weighed with the extract at 25°C. The assay was performed in triplicate. The weight of extract is calculated by dividing by weight of water at 25°C, and the specific gravity and its standard deviation were determined (Berrocal, Fonseca, Vargas, & Madrigal, 2012, United States Pharmacopeia Convention, 2016).

Determination of pH
Aqueous extract only
Three 20 mL samples of the extract are placed each into three Beakers and the pH is measured in triplicate at 25°C. Before measuring each sample the electrode must be washed with distilled water and dried with a dry towel. It should be verified that the pH meter was previously calibrated. The average and its standard deviation are subsequently determined (Berrocal, Fonseca, Vargas, & Madrigal, 2012, United States Pharmacopeia Convention, 2016).

Determination of Conductivity
Aqueous extract only
Three 20 mL samples of the extract are placed each into three Beakers and the conductivity is measured in triplicate at 25 °C. Before measuring each sample the electrode must be washed with distilled water and dried with a dry towel.

It should be verified that the conductivity meter was previously calibrated, then the average and its standard deviation are determined (Berrocal, Fonseca, Vargas, & Madrigal, 2012, United States Pharmacopeia Convention, 2016).

Determination of Pharmacopoeial Indexes for Fats and Oils
The test was performed only on the pejibaye seed and mesocarp hexane extracts. The indices to be determined are acidity indicating the amount in grams or milligrams of free fatty acids per gram of sample (United States Pharmacopeia Convention, 2016).

Preparation of the sample
If the sample is liquid and presents a turbid or sedimentary appearance, it is placed inside a beaker in a heater until it reaches a temperature of 50°C; if it's transparent, proceed with the test. If the turbidity remains is filtered and proceeded with the test (United States Pharmacopeia Convention, 2016).

If the sample is solid or semi-solid, place it inside a beaker in a heater until it reaches a temperature of between 40°C to 60°C; if it's transparent, proceed with the test. If the turbidity remains is filtered and proceeded with the test (United States Pharmacopeia Convention, 2016).

Acidity index: one gram of sample is weighed, and dissolved in 30 mL of methanol or 1:1 ethanol/diethyl ether mixture. Three aliquots of 5 mL are taken and titrated with 0.1 molar potassium hydroxide; the indicator is phenolphthalein. A blank solution is made with methanol or ethanol/diethyl ether mixture 1/1. The amount of mg of free fatty acids is calculated subtracting the volume consumed by the white to the consumed by the sample and multiplying it by the molecular weight (United States Pharmacopeia Convention, 2016).

The saponification index measures the amount of milligrams of potassium hydroxide (KOH) required to saponify 1 gram of fat or oil (United States Pharmacopeia Convention, 2016).

Saponification Index Test

One gram of oil or fat is weighed and dissolved in a 100 mL volumetric flask with 2 molar alkaline potassium hydroxide solution and brought to capacity. Three aliquots of 5 mL are taken and titrated with 0.1 molar HCl; the indicator is phenolphthalein. A blank solution is made with methanol. The amount of mg of free fatty acids is calculated by subtracting the volume consumed by the white to the consumed by the sample and multiplying it by the molecular weight (United States Pharmacopeia Convention, 2016).

Peroxide index

It's the number of milliequivalents of oxygen per kilogram of oil or fat (United States Pharmacopeia Convention, 2016).

Test: Reagents solution of acetic acid and chloroform. Mix three volumes of glacial acetic acid with two volumes of chloroform. Freshly prepared saturated potassium iodide solution, 0.1 N sodium thiosulfate solution, duly standardized, starch solution. Dissolve 1 gram of soluble starch in cold distilled water (forming a paste), add 100 mL of 90 °C water, stir the solution rapidly and cool (United States Pharmacopeia Convention, 2016).

Saturated potassium iodide solution

It was prepared with potassium iodide and freshly boiled distilled water. It should be ensured that the solution remains saturated, as evidenced by the presence of undissolved crystals. Store in the dark and protected from light. Check 30 mL of the acetic acid solution and chloroform. Add 0.5 mL of the potassium iodide solution and two drops of the starch solution; if a blue color that requires more than one drop of 0.1 N of sodium thiosulfate to disappear, the potassium iodide solution is discarded and a new solution is prepared (United States Pharmacopeia Convention, 2016).

One gram of the sample is placed in an erlermeyer and 10 mL of acetic acid/chloroform solution is added; dissolve with stirring. Upon being solubilized, 0.5 mL of saturated potassium iodide solution is added using the 0.01 N sodium thiosulfate solution titrate gradually and constantly stirring the contents in the Erlenmeyer flask until the yellow color has almost disappeared, then add 0.5 mL of the starch indicator solution and continue the titration near the end point, stirring constantly to release all the iodine from the chloroform layers. Add the sodium thiosulfate solution drop by drop, until the blue color disappears completely (United States Pharmacopeia Convention, 2016).

A blank solution with all the reagents was done without the sample and following the same procedure.

The Peroxide Index is calculated by the following equation:

$I = (v*N/m)*1000$ (6)

Being

I = Peroxide index in meq. of O2 per kilogram of the product.
V = Volume of the sodium thiosulfate solution used in titration of the sample, in mL (corrected for blank solution).
N = Normality of the sodium thiosulphate solution.
M = Analyzed sample mass, in grams (United States Pharmacopeia Convention, 2016).

Iodine Index

Weigh approximately 0.2 mg of sample so that the volume of Wijs solution that is added has an excess of 100% to 150% compared to the amount of iodine that is absorbed by the sample.

The mass of the sample which enables the condition set out in 8.1 can be calculated, approximately, using the following equation:

$M = 26 / i$

M = sample mass for the determination, in g.
I = iodine index expected to be found, in cg / g.

Transfer the weighed amount of the simple to a 500 cm^3 Erlenmeyer flask (or directly weigh the sample in the flask), and add 20 cm^3 of carbon tetrachloride. Then, using a volumetric pipette, add 25 cm^3 of Wijs solution, cover the flask and shake it to get a mixture of its contents.

Store the volumetric flask in a dark place for 1 hour at a temperature of 25 °C. Add 20 cm^3 of potassium iodide solution and 100 cm^3 of freshly boiled and cooled distilled water. Titrate the free iodine with 0.1 N sodium thiosulfate solution (with constant and vigorous stirring), until the yellow color has almost disappeared; add 1 cm^3 to 2 cm^3 of starch indicator solution and continue the titration until the blue color disappears completely (United States Pharmacopeia Convention, 2016).

Near the end point of the reaction, the iodometric flask must be covered and vigorously shaken so that any remaining iodine present in the carbon tetrachloride layer passes into the aqueous solution of potassium iodide (United States Pharmacopeia Convention United States of America, 2016).

Two blank tests should be performed for each determination using all reagents and following the same procedure but without adding the sample. The iodine value is calculated by the following equation:

$I = 12.69 (V-Vi)*N/m$; being:

I = iodine value of the sample, in cg/g.

V = arithmetic mean of the sodium thiosulphate solution volumes used in the titration of the tests, in cm^3
Vi = volume of sodium thiosulphate solution used in titration of the sample in cm^3
N = normality of the sodium thiosulphate solution.
M = mass of the analyzed sample, in grams.

Reagents
Wijs solution
Carbon tetrachloride
15% solution of potassium iodide. Dissolve 150 g of potassium iodide (KI) in approximately 400 cm^3 of distilled water and dilute the solution to 1000 cm^3

0.1 N sodium thiosulfate solution, duly standardized.

Starch indicator solution
Form a homogeneous paste with 1 g of soluble starch and cold distilled water; add 100 cm3 of boiling water, stir the solution rapidly and cool. 125 mg of salicylic acid may be added as a preservative. If the solution is to be stored for a relatively long period of time, it should be refrigerated at a temperature of 4°C to 10°C (United States Pharmacopoeia Convention, 2016).

Refractive index
Three 5 mL samples of the oil are taken, placed in the refractometer tank and the refractive index is measured in triplicate at 25°C. It should be verified that the refractometer was previously calibrated, then the average and its standard deviation are determined (United States Pharmacopeia Convention, 2016).

Seed and mesocarp oil fatty acids determination, hexane phase
0.5 grams of sample was weighed and transesterified using methanol and boron trifluoride. Fatty acids were determined in the dry oil of the hexane phase. Identification of the fatty acids was performed by gas chromatography, using a Variam chromatograph model 3700, model 9176, a stainless steel column 1/8 by 6 inches long, packed with Silar, flame ionization detector (FID) and nitrogen as carrier gas. The temperature of the detector and injector was 220°C and 190°C for the column. Identification of the methyl esters was done by comparing the retention times of the experimental material with the fatty acid standards and were expressed as a percentage (result of the division of the area of each fatty acid between the total area of methyl esters) (Hammond, Pan, & Mora-Urpí, 1982).

UV-Visible Spectroscopy
The test was performed only on pejibaye hexane seed and mesocarp extracts.

The phenols absorb in the ultraviolet region. In the case of flavonoid type phenols, there are 2 distinctive absorption bands, the band of the aromatic ring A with a maximum absorption in the range 240-285 nm (benzoyl band) and another band of ring B with maximum absorption in the 300-550 nm range (cinamoil band).

Each of the phase extracts (a, b, c, d and e) was dissolved 1 in 100 in the extraction medium and the absorbance was measured in a UV-visible spectrophotometer from 200 nm to 800 nm with a resolution of 1 nm (Berrocal, Fonseca, Vargas, & Madrigal, 2012; Convención de la Farmacopea de los Estados Unidos de América, 2016).

Quantification of Total Carotenoids by Uv-Visible Spectroscopy
The test was performed only on hexane extracts of pejibaye seed and its mesocarp. The determination of total carotenoids was performed as follows (the previous procedure was used) (Jatunov et al., 2010): 100 grams of fresh fruits were peeled off, the seeds and the mesocarp were separated, then chopped and ground in a hand mill and placed in a tray at 72°C in a convection oven. The sample was reground in a food processor; 5 grams of mesocarp were taken, and 5 grams of seed were independently placed in an erlermeyer with 50 mL of 5% KOH solution in methanol in a nitrogen atmosphere for 24 hours at room temperature and protected from light. The carotenoids are extracted by adding 150 mL of pure acetone and stirring for 15 minutes. The extract is concentrated to 50 mL in a rotary evaporator at 40°C, the sample is placed in a separatory funnel and 30 mL portions of a mixture of ethyl ether / Hexane 1:1 until the color of the aqueous (lower) phase disappears; the colored upper phase is collected.

The extracts were dried in a rotary evaporator at 40°C and placed in a 50 mL volumetric flask, then diluted to 50 mL with hexane, placed in a an amber flask protected from light, in a nitrogen atmosphere at -20°C. 5 mL of the solution were taken and measured at 450 nm. White hexane is used.

$$\text{Carotenoids } \left(\frac{\mu g}{g}\right) = \frac{\text{Absorption x volume(mL) x } 10^6}{A^{1\%}_{1cm} \text{ x 100 x sample weight (g)}}$$

Where Absorption is the absorbance of the sample, volume of the flask 50 mL, A1%1cm is the absorption coefficient in this case for total carotenoids 2500 100mL/gcm is used. Do this in triplicate (Jatunov, Quesada, Díaz, & Murillo, 2010; Rodriguez Amaya, 2001).

For the caratenoid fat content, 0.1 gram of either the seed or mesocarp fat must be weighed separately in a beaker and then add exactly 0.1 grams in 10 mL of acetone and the measurement is performed at 450 nm (Rodriguez Amaya, 2001).

RESULTS
The results obtained are summarized below:

Table III: Results phytochemical screening for aqueous, ethanolic, ethereal and hexanic extracts from *Bactris gasipaes* seed.

Test	Compounds	Phase									
		a	b	d1	d2	d3	d4	e1	e2	e3	e4
Gelatin	Tannins	+	+	+	+	NA	NA	-	-	NA	NA
Gelatin-Salt	Tannins	+	+	+	+	NA	NA	-	-	NA	NA
$FeCl_3$	Tannins gallics, catechinics	´+	´+	+	-	NA	NA	-	-	NA	NA
Formaldehyde/HCl	Condensed tannins	´+	´+	-	-	NA	NA	-	-	NA	NA
Dragendorff	Alkaloids	+	+	+	+	+	+	+	+	+	+
Mayer	Alkaloids	+	+	+	+	+	+	-	+	+	+
Wagner	Alkaloids	+	+	+	+	-	-	-	-	-	-
Cooling	Mucilages	+	+	+	+	NA	NA	-	-	NA	NA
Ninhydrin	Aminoacids	+	+	´+	-	-	-	-	-	-	-
Lieberman-Bhurchard	Steroids Terpenes	+	+	´+	-	NA	NA	+	-	+	+
Salkowski	Terpenes	-	-	-	-	NA	NA	+	-	-	-
Tortelli–Jaffe	Terpenes	-	-	-	-	NA	NA	-	-	-	-
Ammonium Molybdate IV	Terpenes	-	-	-	-	NA	NA	-	+	+	+
Perchloric acid	Terpenes	-	-	-	-	NA	NA	-	-	-	-
Shinoda	Flavonoids	+	+	+	+	NA	NA	-	-	-	-
Pew s	Flavonoids	´+	´+	´+	´+	NA	NA	+	+	-	-
Borntrager	Quinones	-	-	-	-	NA	NA	-	-	-	-
Rosenheim	Anthocyanins	-	-	-	-	NA	NA		-	-	-
Foam	Saponins	+	+	+	+	NA	NA	-	-	-	-
Rosenthaler	Saponins	+	+	+	+	NA	NA	-	-	-	-
Fheling	Carbohydrates	+	+	+	+	NA	NA	-	-	NA	NA
Molish	Carbohydrates	+	+	+	+	NA	NA	-	+	NA	NA
Benedict	Carbohydrates	+	+	+	+	NA	NA	-	+	NA	NA
Tollens	Carbohydrates	+	+	+	+	NA	NA	-	+	NA	NA
Baljet	Lactones Coumarins	-	-	-	-	NA	NA	-	+	NA	NA
Ferric Hydroxamate	Lactones Sesquiterpenes Esters	-	-	-	-	NA	NA	-	+	NA	NA
Kedde	Glycosides Cardiotonics	-	-	-	-	NA	NA	-	-	NA	NA
Guignard	Glycosides Cyanogenics	-	-	-	-	NA	NA	+	-	NA	NA
Sudan	Fats	+	+	NA	NA	+	+	+	NA	+	+
Carr-Price	Carotenes	+	+	NA	NA	+	+	+	NA	+	+
Emmerie-Engel	Tocopherols	+	+	NA	NA	+	+	+	NA	+	+

Nomenclature; NA: Not Applicable, - Negative Test, + Positive Test.

Table IV: Results phytochemical screening for acetone extracts from *Bactris gasipaes* seed.

Test	Compounds	Phase C1	Phase C3	Phase C5.1	Phase C5.2	Phase C7	Phase C8
Ninhydrin	Aminoacids	´+	**NA**	**NA**	**NA**	**NA**	**NA**
Shinoda	Flavonoids	´+	**NA**	**NA**	**NA**	-	-
Gelatin	Tannins	+	**NA**	**NA**	**NA**	**NA**	**NA**
Gelatin-salt	Tannins	+	**NA**	**NA**	**NA**	**NA**	**NA**
FeCl3	Tannins	´-	**NA**	**NA**	**NA**	**NA**	**NA**
Borntrager	Quinones	-	**NA**	**NA**	**NA**	**NA**	-
Lieberman-Bhurchard	Steroids Terpenes	+	**NA**	**NA**	**NA**	**NA**	+
Dragendorf	Alkaloids	+	**NA**	**NA**	+	**NA**	+
Mayer	Alkaloids	+	**NA**	**NA**	+	**NA**	+
Wagner	Alkaloids	+	**NA**	**NA**	+	**NA**	+
Rosenheim	Alkaloids	+	**NA**	**NA**	+	+	+
Pew s	Flavonoids	+	**NA**	**NA**	**NA**	**NA**	+

Nomenclature; NA: Not Applicable, - Negative Test, + Positive Test.

Table V: Quantification of the percentage of moisture of the *Bactris Gasipaes* seeds.

Sample	Seed Humidity %
Sample 1	**6.10**
Sample 2	**5.85**
Sample 3	**5.78**
Average	**5.91**
Standard deviation	**0.17**

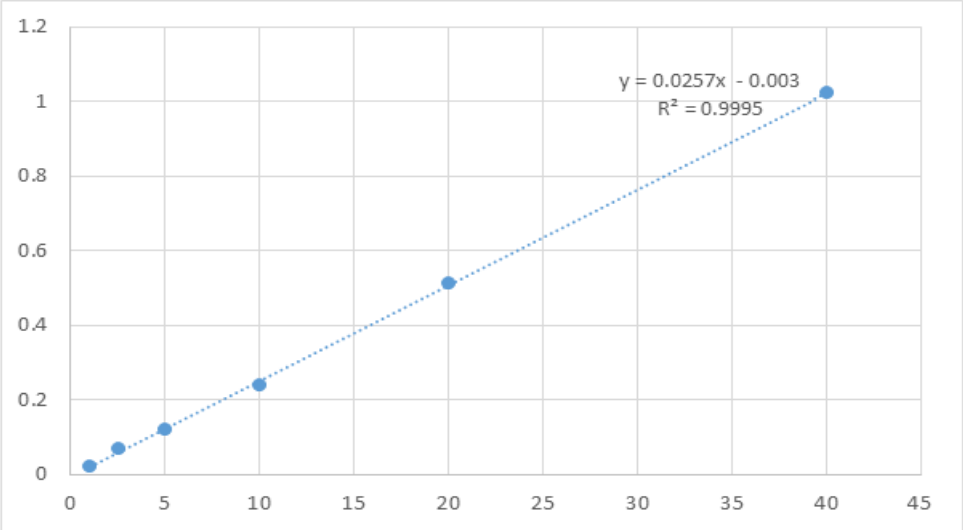
Table VI: Percentage Quantification of Ethereal and Hexanic *Bactris Gasipaes* Seed Extract.

Sample	Seed % Ethereal	Seed % Hexanic
Sample 1	7.02	7.20
Sample 2	6.56	6.75
Sample 3	6.20	6.43
Average	6.59	6.79
Standard deviation	0.41	0.38

Table VII: Quantification of Total Phenols Folin Ciocalteu *Bactris Gasipaes.*

Concentration mg/mL	Absorbance
1	0.023
2.5	0.072
5	0.122
10	0.24
20	0.515
40	1.024

Source: tests performed.



Slope	0.0257
Intercept	-0.003
Determination coeficient	0.9995

Figure 4: Calibration curve Quantification Folin Ciocalteu *Bactris gasipaes* seed and mesocarp extract. (pendiente=slope; intercepto=intercept).

Table VIII: Quantification of Total Phenols Folin Ciocalteu.

Sample	Sample Grams	mg/g amount equivalent to gallic acid
Sample 1 Seed	9.98	0.442
Sample 2 Seed	10.01	0.431
Sample 3 Seed	10.02	0.470
Average	10.00	0.447
Standard deviation	0.02	0.020

Source: tests performed.

Tabla IX: Datos Análisis de DPPH BHA y Aceite de Mesocarpo *Bactris gasipaes*.

Concentration mg/mL	Absorbance BHA	% Inhibition	Absorbance Bactris gasipaes seed Oil	% Inhibition Bactris gasipaes Seed Oil
0.000	1.454	0	1.457	0
0.400	0.814	44.02	1.023	29.79
0.600	0.753	48.21	0.967	33.63
0.800	0.689	52.61	0.839	42.42
1.000	0.597	58.94	0.734	49.62
1.200	0.523	64.03	0.627	56.97
C50 0.659 mg/mL	50		**C50 1.014 mg/mL**	50

Source: tests performed.

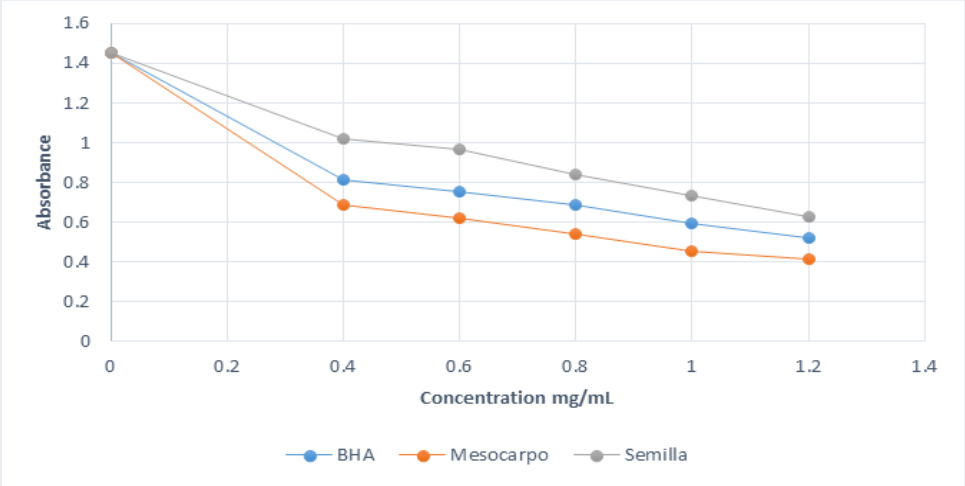


Figure 5: Absorbance curves versus radilcal concentration of DPPH for BHA and seed oil and mesocarp oil of *Bactris gasipaes*.

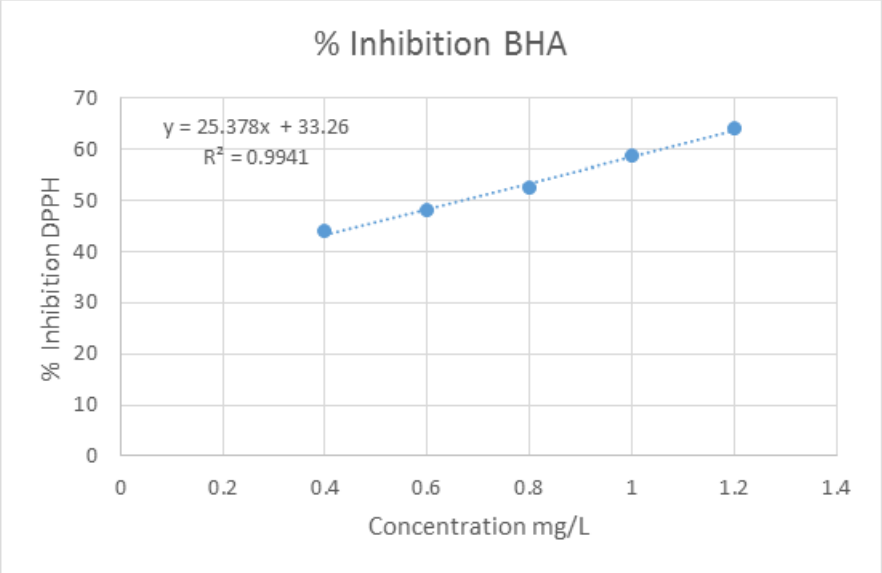


Figure 6: Percent inhibition curve Quantification DPPH acetone solution BHA.

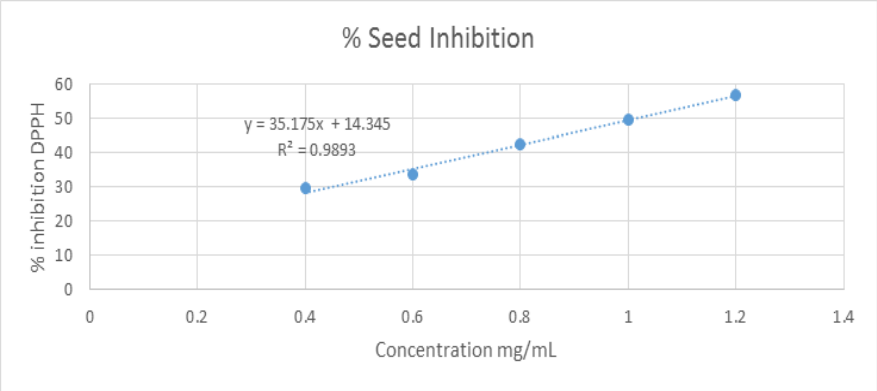


Figure 7: Inhibition Rate Curve DPPH quantification of *Bactris gasipaes* seed oil.

Table X: Quantification of Antioxidant Power H-ORAC *Bactris Gasipaes* Seed.

Sample	Quantity µg/mol Trolox Equivalent /g dry seed
Sample 1	12.63
Sample 2	13.72
Sample 3	12.89
Average	13.08
Standard deviation	0.57

Source: tests performed.

Table XI: Physicochemical properties aqueous extract of pejibaye seed *Bactris Gasipaes*.

Sample	pH 25°C	Specific Gravity 25°C	ºBrix	Conductividad mS 25°C	Osmolarity mOs/Kg 25°C
Sample 1	6.33	0.9973	3.46	1509	2173
Sample 2	6.35	0.9956	3.43	1534	2165
Sample 3	6.29	0.9971	3.42	1498	2184
Average	6.32	0.9996	3.43	1513	2174
Standard deviation	0.03	0.0010	0.02	18.48	9.54

Source: tests performed.

Table XII: Determination Pharmacodynamic indexes of Pejibaye seed *Bactris Gasipaes*.

Sample	Acidity index	Saponification Index ng KOH/g	Iodine Index cg I/g	Peroxide index meq/Kg	Refractive index
Sample 1 S	2.72	246.26	10.68	2.26	1.4440
Sample 2 S	2.66	239.45	10.48	2.28	1.4438
Sample 3 S	2.70	240.67	10.23	2.30	1.4442
Average S	2.69	242.13	10.46	2.28	1.4440
Standard	0.03	3.63	0.22	0.02	0.0002 **deviation S**

Nomenclature S= Seed. Source: tests performed.

Table XIII: Determination Composition fatty acids of pejibaye seed *Bactris Gasipaes*.

Compound	Mesocarp
% Solids	74 ± 1.9
% Fat in dry weight	30 ± 1.2
Caprilic	-
Capric	1.2 ± 0.5
Lauric	46.8 ± 1.7
Myristic	25.2 ± 1.3
Palmitic	8.3 ± 0.8
Palmitoleic	--
Stearic	3.2 ± 0.4
Oleic	12.1 ± 1.2
Linoleic	3.1 ± 0.6
Linolenic	--
Total saturated	84.8 ± 1.7
Total unsaturated	15.2 ± 1.7

Source: tests performed.

Table XIV: Determination of total carotenoids in fat *Bactris Gasipaes*.

Sample	Carotenoids ug /g seed
Sample 1	12.672
Sample 2	12.904
Sample 3	12.772
Average	12.783
Standard deviation	0.116

Source: tests performed.

Table XV: Determination of Total Carotenoids in Dry Matter *Bactris Gasipaes*.

Sample	Carotenoides ug /g mesocarpo
Sample 1	13.698
Sample 2	13.665
Sample 3	13.688
Average	13.684
Standard deviation	0.0164

Source: tests performed.
UV-Visible Spectroscopy

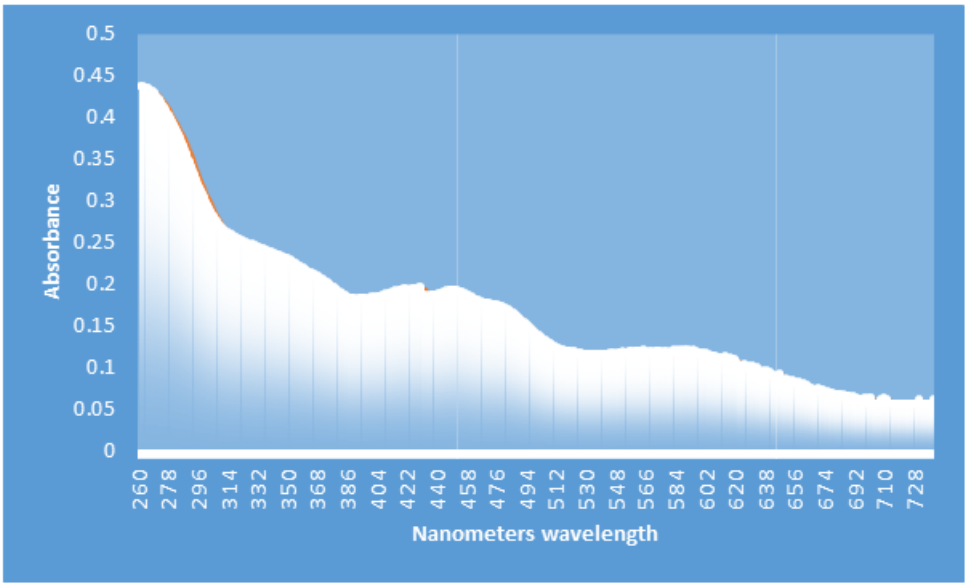


Figure 8: UV-Visible Absorption Spectrum 260 nm at 800 nm seed oil 2% in ethanol *Bactris gasipaes*.

DISCUSSION

Bactris Gasipaes fruits have been examined for their food properties, although their seed is an important industrial waste and little has been studied; practically no studies have been carried out in the cosmetic field, hence the importance of the present investigation, since—due to a high fat content— [*Bactris Gasipaes*] can be an important source of raw material and cosmetic active ingredients. Its seed is characterized by its high content of sucrose and fats, but also contains carotenoids, which makes it a very interesting and alternative source of these compounds. The composition of the fatty acids of the seed is very different from that found in the mesocarp (Hammond, Pan, & Mora-Urpí, 1982), both fatty acids and carotenoids have been used in cosmetic formulations of antioxidant type, such as sun protection by the absorption that presents in the range of ultraviolet radiation. For the study, a fractionation with pure solvents of different polarities was realized by means of a process of drying and extraction by reflux. Table V shows the percentage of moisture obtained is on average 5.91% ± 0.17, Table VI shows the fat content extracted by means of ether and hexane, which corresponds to 6.59% ± 0.41, and 6.79% ± 0.38. The fractionation allows to obtain with more certainty the phytochemical groups contained in the seed samples; in addition, it allows to associate them to the cosmetic or medicinal applications.

In the different polar fractions were found compounds commonly associated with dermocosmetic and therapeutic effects. Being a waste of the industrialization of the mesocarp of *Bactris gasipaes*, can be a source active principles, or extracts with cosmetic properties; the presence of catechinic and condensed tannins, alkaloids, terpenoid, steroids, mucilages, saponins,

amino acids, flavonoids, carbohydrates, fats, carotenoids, tocopherols and the like in the aqueous extract are shown in Table III. Some already reported in the literature (Pizzani, 2008), the results were confirmed by the ethanol and acetone extract tests, and did not give positive tests for anthocyanins, cyanogenic glycosides, cardiotonic glycosides; the latter two are associated with a low toxicity of the seeds that they contain, in addition, it has been reported the use of seeds as part of the feed of animals like cows (Mora-Urpí, Weber, Clement, & C, 1997). Table IV describes the results of the acetone extract where compounds such as alkaloids, amino acids, flavonoids, terpenoid steroids, and tannins were described. These phytochemical groups were not previously reported in the literature, with the exception of fats, carbohydrates and amino acids. The data of Tables III and IV confirm the content of tocopherols and carotenoids though in a smaller amount than that reported in *Bactris Gasipaes* mesocarp (Jatunov, Quesada, Díaz, & Murillo, 2010).

The screening of phytochemical groups in Tables III and IV shows highly antioxidant components such as tocopherols, carotenoids, flavonoids and tannins. The latter in their form are related to an astringent effect, having the function of protein precipitant and bacteriostatic, and a high content of reducing sugars that are a source of skin moisturization, mainly sucrose, because it helps the tranpiphthermal passage of water through the stratum corneum (Bronaugh & Maibach, 2005). Data on the content of other compounds such as flavonoids and terpenes mainly of the steroid type—unlike extracts from other seeds such as Persea americana commonly referred to as avocado, Citrus bergamot commonly known as Bergamot orange and Casimiroa edulis commonly known as White sapote—does not present an aldehyde (benzaldehyde) odor; the extracts are practically odorless or present a mild sweet smell which implies the absence of volatile substances of the terpenoid type or derivatives of alcohols and aldehydes (Evans, 2009).

The composition of the nonpolar fractions (chloroform, hexane and ether) of the fruit mesocarp of *Bactris gasipaes* has not been reported in the literature; terpenoids, fats, alkaloids and steroids were found from the screening; terpenoid compounds are high and low polarity, therefore it is common to find them both in polar and nonpolar fractions. In the acetone extract Table IV, alkaloid compounds were found, which are usually related to very varied therapeutic and cosmetic actions (Evans, 2009). In all fractions were found fat compounds, tocopherols and carotenoids associated with sun protection products (Iwata & Shimada, 2013).

The aqueous extract of the *Bactris gasipaes* seed was described in Table II for its physicochemical properties, which are not described in the literature. These properties are important to understand the compatibility of these types of extracts with the excipients for the fabrication of different [medications]. [*Bactris gasipaes* seed presents] a low humidity of about 6% moisture, implying that most of the seed is formed of fibrous material and between 5% to 10% of fat, which is inferior compared to other seeds used in cosmetics such as Arachis hypogaea —commonly known as peanut—, Cocus nucifera—commonly known as Coconut—, Olea europaea —commonly known as Olive—, and Theobroma cacao—commonly known as Cacao tree— (Evans, 2009). However [the aqueous extract] appears to contain other antioxidant components such as tocopherols, and carotenoids, as well as others such as flavonoids, tannins, saponins, alkaloids, as shown in Table III and In Table IV. The summary of the physicochemical properties described in Table XI at pH 25°C was 6.32 ± 0.10, which states that the extract is slightly acidic mainly because of the non-acidic water soluble compounds and the free fatty acids which are not dissolved in the aqueous extracts in a significant amount. The specific gravity 25°C 1,0018 ± 0.0040 was very similar to the one of the water, nevertheless superior to it, which indicates the presence of soluble compounds like sucrose (Pizzani, 2008). Degrees Brix 3.43 ± 0,02 proves us the presence of soluble sugars and other substances in a relatively low or medium concentration. The conductivity 1513 ± 18.48 µS/cm 25°C; this high conductivity indicates the presence of many electrolytes or polyelectrolytes in solution, in addition, it shows a high osmolarity 2174 ± 9.54 mOs/Kg 25°C, this makes the extract hyperosmolar mainly due to soluble compounds (carbohydrates) and high electrolyte content. Osmolarity data should be important when studying stability especially in creams or emulsions of another type and in the decomposition of the fragrance.

The antioxidant effect associated with the presence of tocopherols—and especially carotenoids in the mesocarp (Jatunov, Quesada, Díaz, & Murillo, 2010) is commonly reported in this literature. This antioxidant effect is measured in Trolox Equivalent (TE) units with a method known as H-ORAC; nonetheless, no in-depth studies on the antioxidant capacity of the *Bactris gasipaes* seed extracts have been made. In Table X, an average of 13.08 ± 0.57 µg mol Trolox Equivalent /g dry seed was found. This high antioxidant capacity is associated with multiple dermocosmetic effects, for example an anti-aging effect, an ultraviolet radiation protection, an anti-wrinkle effect , in addition, the protective effect against ultraviolet radiation is also associated with the prevention of skin cancer, regrowth effects of damaged tissue, and an antimicrobial effect (Iwata & Shimada, 2013).

The antioxidant effect is also associated with the presence of phenolic compounds and the Follin Ciocalteu test determines the concentration of total phenols. In the analyzed sample, 0.447 ± 0.020 mg/ gram of dry sample of gallic acid equivalents were found (see Table VIII), which shows a low to medium concentration of these compounds. However it was found that there are condensed and non-condensed tannins, especially the

catechin type, as shown in Table III, since the sensitivity varies, showing a lower antioxidant power than other extracts, for example, fruits such as blackberry Rubus adenotrichus (Montoya-Castro, Vaillant, Cozzano, Mertz, & Perez, 2010), therefore the antioxidant action may be due to a combined action of the most lipophilic components, such as carotenoids and tocopherols, and unsaturated fats with compounds of polyphenolic type and flavonoids.

Table XII shows pharmacopoeial indices for the identification of oils and fats that verify the physical characteristics of the behavior of an opaque slightly yellow to viscous white semi-solid at 25°C acid index 2.69 ± 0.30, saponification index 242, 13 ± 3.63 mg KOH/g, iodine index 10.46 ± 0.22 cg I / g, peroxides index 2.28 ± 0.20 meq / kg, refractive index 1.4440 ± 0.0002, which demonstrates that the seed oil is mainly of the saturated type, and possesses medium to high molecular weight fatty acids.

Another way to measure the antiradical effect is by the DPPH test which studies the percentage inhibition of the free radical 2,2-diphenyl-1-picrylhydrazyl. This test studies a different oxidation mechanism to the H-ORAC, so they can be defined as complementary. There are several oxidation mechanisms, in this case figures 5, 6 and 7 show the antiradical inhibitory effect, with the pattern of butylated hydroxyanisole (BHA) —a recognized antioxidant used in emulsions and oils for their amphiphilic characteristics— which make it physicochemically similar to tocopherols and carotenoids. Figure 5 shows a lower oil EC50 equivalent of 1,014 mg/L compared to a BHA EC50 of 0.659 mg/L; as shown in Table VIII, the seed oil shows an antioxidant activity lower than BHA but higher than fats and oils of similar composition, due mainly to the presence of carotenoids and tocopherols, these compounds are associated with an anti-wrinkle and sunscreen effect (Baumann & Baumann, 2009).

The main components described in the mesocarp literature are the content of fats, sugars such as sucrose, and the presence of carotenoids (Pizzani, 2008), which give a yellow to orange color to the oil of this source. Table XIII shows the composition of seed oil which is mainly formed by saturated fats 84.8% ± 1.7 principally lauric, myristic and palmitic acids, therefore it has a composition similar to the Cocus nucifera seed oil, and a 15.2% ± 1.7 mainly composed of oleic and linoleic acids. The fat content in dry matter is about 30%.

These results coincide with previous studies on the characterization of seed oil (Hammond, Pan, & Mora-Urpí, 1982). Seed oil is a viscous semi-yellow viscous yellow to white. Due to the lauric acid content, it can also be a good surfactant and a good base for soaps, and have a use as a cosmetic raw material for emulsions, creams, soaps, among other products. The carotenoid content in the oil phase (see Table XIV) 12.783 ± 0.116 carotenoids µg/g mesocarp and dry matter (see Table XV) 13,684 ± 0,0164 carotenoids µg/g seed allows to establish an important antioxidant power for this type of oils which even allows to avoid self-ingrowth.

The neutral pH of the aqueous extract facilitates its incorporation into different cosmetic bases. Another important factor to consider in aqueous extracts is the high electrolyte content and high osmolarity. This is important since the formulation of carbomer gels can be affected in their viscosity and stability due to the presence of electrolytes in solution (Wilkinson, Moore, Rodriguez, & Rodriguez, 1990), which is why it is important to take on account (Baki & Alexander, 2015).

As shown in Figure 8, the 2% w/w *Bactris gasipaes* seed oil shows an absorption in the range 260 nm to 320 nm of medium type UVB radiation and 320 nm to 400 nm UVA of about 20% of the received radiation, which may be associated with a low to medium photoprotection effect; however this can be increased proportionally to the content of ultraviolet radiation absorbing compounds mainly anthocyanins and tannins, or increasing the content of tocopherols and carotenoids.

CONCLUSIONS

Results of the studies carried out for the seed extracts of *Bactris gasipaes* ripe fruits, show a higher antioxidant power than vegetable oils of similar composition used in cosmetics due to a H-ORAC 13.08 ± 0.57 µg mol Trolox Equivalent /g dry seed, and EC 50 1,014 mg/L for oil. This effect was associated with the presence of amphiphilic and/or lipophilic compounds, such as flavonoid polyphenols, tocopherols and carotenoids. Total phenols 0.447 ± 0.020 mg/gram dry sample of gallic acid equivalents, check the qualitative results of phytochemical screening and show a low presence of phenolic antioxidant compounds and a humidity of around 6% in the seed. In addition, terpenoid compounds, alkaloids, tannins, flavonoids, saponins, carbohydrates and fats were found. This makes *Bactris gasipaes* an excellent active for formulations, antioxidants, anti-aging, anti-wrinkle, skin nourishing and moisturizing, and emollient. It would also be interesting in future studies to evaluate their pharmacological or biological activity; in addition their oil also has characteristics suitable to be raw material in soaps, creams emulsions and ointments and other cosmetic forms that use oils with antioxidant action. Seed oil which is mainly composed of saturated fats 84.8% ± 1.7 mainly lauric acid, palmitic myristic, and 15.2% ± 1,7 mainly composed of oleic and linoleic acids. The practically neutral 6,32 ± 0,10 pH is beneficial for incorporation into different cosmetic bases such as gels, creams and emulsions. The aqueous extract is also hyperosmotic (2174 ± 9.54 mOs / kg) and has a high electrical conductivity (1513 ± 18.48 µS/cm) due to the presence of polyelectrolytes and a high concentration of sugars (mainly sucrose); this was verified according to the obtained Brix degrees (3.43° ± 0.02). Finally, the 2%

w/w seed oil shows an absorption of ultraviolet radiation of 20% in the wavelengths of between 260 nm to 400 nm, which could also be useful for formulating compositions for sun protection.

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