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Original Article

Rubus adenotrichus fruit extracts phytochemical characterization and antioxidant power evaluation for dermocosmetic formulations

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Abstract

There are more than 700 species of the genus *Rubus*, popularly known as Mora (tropical highland blackberry). In Costa Rica, the species *Rubus adenotrichus* has been characterized by its high content of antioxidant substances, becoming one of the most cultivated species and that may have characteristics to be used in cosmetics or medicine. 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. Ripe fruits of *Rubus adenotrichus* were fractionated with solvents of low, medium and high polarity, followed by a phytochemical screening according to the World Health Organization Guidelines for the Control of Quality of Products of Natural Origin. The antioxidant properties were evaluated by means of the H-ORAC and DPPH test; the concentration of total phenols by Folin-Ciocalteau and antocinanins by differential pH was determined. Finally, the physicochemical properties of the aqueous extract, such as pH, specific gravity, Brix grades, conductivity, and osmolarity was determined and an absorption spectrum from 260 to 700 nm was obtained. Phenolic compounds were found as condensed and non-condensed tannins, anthocyanins, flavonoids, terpenes, and alkaloids as major phytochemical groups, a high antioxidant power measured in H-ORAC 311 ± 7.63 µg/mol Trolox Equivalent/g of dry fruit, and EC 50 118.46 mg/L for the aqueous extract. The total phenols found was 20.85 ± 0.27 mg/g of dry sample of gallic acid equivalents which makes the *Rubus adenotrichus* fruit an excellent component for bacteriostatic, anti-aging, anti-wrinkle, nourishing and moisturizing formulations, and the astringent effect also allows its application to small superficial wounds on the skin. Likewise, the acidic pH of 3.55 ± 0.1 is beneficial for maintaining the cutaneous acid mantle thereby favoring the normal flora of the skin, but may be a problem for the formulation of carbomer-based gels or the incorporation of preservatives. The aqueous extract is hyperosmotic (559.66 ± 3.21 mOs/kg) and has a high electrical conductivity (285 ± 2 µS/cm) due to the presence of electrolytes and a considerable amount of sugars, according to Brix degrees (7.543° ± 0.005°). Finally, the 4% w/w aqueous extract shows an absorption of ultraviolet radiation of 25% in the wavelengths from 260 to 400nm, by which it also could be useful for formulating compositions for sun protection.

Keywords: *Rubus adenotrichus*, blackberry, antioxidant, phytochemical screening, cosmetic

INTRODUCTION

There are many plants with the presence of phenolic compounds, such as tannins, which have been determined to have antioxidant properties. One of the plants that has been better characterized in the last years, both phytotechnically and pharmacognostically, is *Rubus adenotrichus*, whose fruit is known as mora (blackberry). The genus *Rubus* comprises more than 700 species (Tomczyk & Gudej, 2005; Martínez-Cruz, *et al.*, 2013).

The blackberry (*Rubus adenotrichus*), presents three varieties identified as wine red spine, wine white spine and wine without spines, having a semi-erect growth and decumbent stems (inclined to the ground) with lengths of up to three meters, multiples of sexual form, that is to say by seed, or asexual by divisions of the crown, aerial layer, subterranean layer, stakes of subterranean stem and culture *in vitro* (Orozco-Rodríguez, *et al.*, 2011).

The flowers are hermaphroditic, fruits are small, formed

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by numerous drupes (polidrupas) and their seeds present a hard and impermeable cover. Its geographical distribution is from Mexico to Ecuador, especially at heights above 2000 meters above sea level. Among the common names in Spanish are mora, common mora, zarzamora and mora vinera. In Costa Rica it is cultivated in the south of the country in San Vito, and in the area "de los Santos" in the cantons of Tarrazú, Dota and Leon Cortes. In addition, it is cultivated in the high zones of Pérez Zeledón and in the canton Del Guarco in the province of Cartago (Castro, J; Cerdas, M., 2005; Orozco-Rodríguez, *et al.*, 2011).

Figure 1: *Rubus adenotrichus* fruits and flowers.
From (García-Saucedo, P., *et al.*, 2016)

Rubus adenotrichus is part of the wild flora of Costa Rica, commonly used in jellies, juices, wine making and soft drinks, in addition to being used in confectionery. When fresh, the taste is often sweet and sour; the fruits of *Rubus* species contain phenolic compounds, including anthocyanins. Several *Rubus* genotypes of Costa Rican and Mexican origin have been reported with antioxidant and anti-inflammatory activity; (Montoya-Castro, O., *et al.*, 2010; Zielinski, *et al.*, 2015) report phenolic compounds with antioxidant activity for *Rubus adenotrichus*.

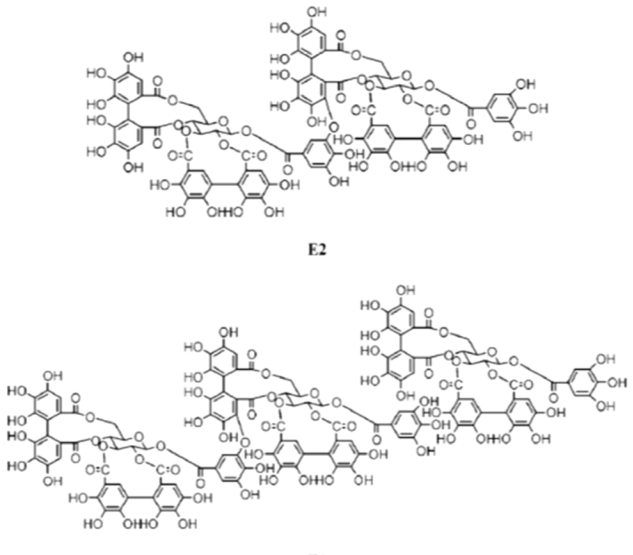


Figure 2: Ellagitannins detected in *Rubus adenotrichus*, (E1) Lambertianin C, y (E2) Sanguiin H-6. From (Mertz, C., *et al.*, 2007)

Nieves del Socorro Martínez-Cruz *et al.* (2011) describes the presence of flavonoids by analyzing the aqueous extract of lyophilized fruits by means of ultraviolet and differential pH analysis. In addition, the presence of anthocyanins was determined, with a concentration of 12.3 mg/g of dry fruit, in terms of cyanidin-3-O-glucoside, the mean effective concentration (EC 50) with DPPH was determined at 148 µg/mL for the aqueous extract of the lyophilized fruit. The total phenol content has been determined between 29.3 and 49.5 mg/g of lyophilized dried fruit extract; ellagitannins such as lambertianin C, and sanguiin H-6 were also found. (Martínez-Cruz, N., *et al.*, 2011).

In general, there are three main phenolic compounds which represent practically 90% of those reported in the literature. These are lambertianin C, sanguiin H-6 and cyanidin-3-O-glucoside. The presence, at approximately 515 nm of an absorption peak for cyanidin-3-O-glucoside in acidic medium and in the absence of practically other anthocyanins, allows the use of this characteristic as a reliable marker of the plant and the content of phenols together, with the determination of total phenols (Mertz, C., *et al.*, 2007).

Another advantage of using this species of blackberry is that there is a standardization of its cultivation in Copey, Dota (Costa Rica), which has been verified and optimized for years by the University of Costa Rica, which allows a greater stability of the compounds that have been found in this variety (Castro, J; Cerdas, M., 2005; Cozzano, 2007; Montoya-Castro, *et al.*, 2010).

It was reported by Ó. Acosta-Montoya *et al.* (2010), that anthocyanins increase with the degree of maturation, especially in cyanidin-3-O-glucoside – approximately 800% in fresh fruit with a slight decrease of ellagitannins. A slight decrease in total phenolic compounds from 77 µmol/g of fresh fruit to 69.5 µmol/g of fresh fruit was determined. Despite this decrease, it is shown that at higher maturity, it has a higher antioxidant power measured on the H-ORAC scale. Trolox equivalent values of 38.29 and 222 µmol/g were found in the H-ORAC for fresh and dry fruits, respectively, with lower maturation, whereas for fresh and dry fruits, respectively, with higher maturation, the value was 64 and 432 µmol/g, with a significant difference ($P > 0.05$) equivalent to approximately 167% and 194% higher antioxidant efficiency per gram of fresh and dry fruit, respectively (Acosta-Montoya, *et al.*, 2010).

MATERIALS AND METHODS

Full-grown fruits (Stage 3 according to the maturity scale of Ó Acosta-Montoya *et al.*, 2010) were obtained directly from the farm in Copey, Dota, for a total of 2 kg plant material.

Preparation of extracts and phytochemical screening

Aqueous extract: The fruits were separated into three parts; one part was processed in situ. 10 g of fresh fruits were liquefied with 100 g of distilled water (phase a). This extract was stored in amber bottles at -20°C for analysis.

Ethanol Extract: The second part was frozen and 300 g of fresh fruits were lyophilized for 48 h at -20°C and vacuum, the lyophilized fruits were liquefied and the sample was deposited in a flask. 100 mL of acidified ethanol (0.01 mol/L in HCl) was added to 0.5 g of the lyophilizate (step b), then stirred for 15 min at room temperature in a vacuum trap, pre-bubbling the ethanol with nitrogen for 1 min, protect-

ed from light. The solvent with the extract was recovered by vacuum filtering, repeated in triplicate. The extracts were concentrated by a rotary evaporator at 40°C to 50 mL. The ethanolic extract was brought to a volumetric flask and measured up to 100 mL with acidified ethanol, centrifuged for 30 min at 10000 rpm and the supernatant was filtered through a 0.45 µm pore membrane and cooled in an amber flask at -20°C until analysis.

Methanolic extract: 100 mL of acidified methanol (0.01 mol/L in HCl) was added to 0.5 g of lyophilisate (phase c), then stirred for 15 min at room temperature in a vacuum trap, pre-bubbling extract with nitrogen for 1 min, protecting it from light. The solvent with the extract was recovered by vacuum filtering, repeated in triplicate. The extracts were concentrated by a rotary evaporator at 40°C to 50 mL, the methanolic extract was brought to a volumetric flask and and measured up to 100 mL with methanol. 10 mL (c1 phase) was taken and separated for tannin analysis, amino acids, and flavonoids. The remaining 90 mL were again placed on a rotary evaporator at 40°C to dryness. 150 mL of aqueous solution of 0.01 mol/L HCl was added at 40°C and three washes were performed with 5 mL of aqueous solution of HCl 0.01 mol/L at 40°C. The extract and the washes were transferred to a 250 mL Erlenmeyer flask and the Liebermann-Burchard and Borntrager analysis was performed. The acid soluble part (c4 phase) was alkalinized to pH 9,0 with 0.1 mol/L ammonia solution, the alkaline extract was placed in a 250 mL separatory funnel, and the alkaline phase was extracted with three 30 mL portions of chloroform (c5 phase). The remaining aqueous phase (c6 phase) was saturated with sodium sulfate; the chloroform phase (c5) was washed with three 5 mL portions of 5°C cold water. From the organic phase (c5), 10 mL were taken and placed on the rotary evaporator at 40°C to dryness, 2 mL of chloroform (c5.1 phase) was added to perform the Liebermann-Burchard test. The remaining phase (c5.2) was taken to dryness and 10 mL of HCl was added to perform the Dragendorff, Mayer and Wagner test. The remaining aqueous phase was saturated with anhydrous sodium sulfate (c6). Three portions of 50 mL of a mixture of chloroform/ethanol (3:2) by weight were added, then placed in a separatory funnel and separated from the aqueous phase (c7 phase), to perform Shinoda and Rosenheim test. The chloroform/ethanol mixture (c.8 phase) was washed with saturated aqueous sodium sulfate solution, cooled in an amber flask at -20°C until tested for Shinoda, Rosenheim, Liebermann-Burchard, Dragendorff and Mayer.

Chloroformic Extract: From the third part, a sample of 10 g of fresh fruits was liquefied with 100 g of distilled water. The aqueous extract was alkalinized with 0.01 mol/L NaOH solution until reaching a pH of 12 (d phase), then placed in a 250 mL separatory funnel, extracting with three 30 mL portions of chloroform. The remaining aqueous phase was separated for analysis (d1 phase). The d1 phase was refrigerated in an amber flask at -20°C until its analysis. The chloroform extract (d2 phase) was refrigerated in an amber flask at -20°C until analysis. If the test could not be performed in the organic phase, 5 mL of the sample were dried and reconstituted with 0.1% w/w HCl in methanol.

Hexanic Extract: From the third part, a sample of 10 g of fresh fruits was liquefied with 100 g of distilled water. The aqueous extract was alkalinized with 0.01 mol/L NaOH solution to a pH of 12 (e phase), which was placed in a 250 mL separatory funnel, extracting with three 30 mL portions of hexane. The remaining aqueous phase was separated for analysis (phase e1), cooled in an amber flask at -20°C until analysis. The hexane extract (e2 phase) was refrigerated in an amber bottle at -20°C until its analysis. If the organic phase test could not be performed, 5 mL of the sample were dried and reconstituted with 0.1% w/w HCl in methanol.

Petroleum Ether Extract: From the third part, a sample of 10 g of fresh fruits was liquefied with 100 g of distilled water. The aqueous extract was alkalinized with 0.01 mol L NaOH solution until reaching a pH of 12 (f phase), which was placed in a 250 mL separatory funnel, extracting with three 30 mL portions of ethyl ether. The remaining aqueous phase was separated (f1 phase), cooling it in an amber flask at -20°C until its analysis. The ethereal extract (f2 phase) was refrigerated in an amber flask at -20°C until its analysis. If the organic phase could not be carried out, 5 mL of the sample are dried and reconstituted with 0.1% w/w HCl in methanol.

Phytochemical screening and reagents were performed according to Solís, *et al.*, (2003) and Anonymous (2007).

Determination of total phenols

It was carried out with the aqueous extract (Martínez-Cruz N. S., *et al.*, 2011).

Folin-Ciocalteu method

Calibration curve (2.50–40.0 mg/L): Aliquots of 0.25; 0.50; 1.00; 2.00 and 4.00 mL of gallic acid stock solution 500.0 mg/L were measured and placed in volumetric flasks of 50.0 mL. They are filled to volume with distilled water.

Reading the calibration curve and the aqueous samples: One milliliter of each standard or sample, 1 mL of diluted Folin-Ciocalteu reagent and 2 mL of 0.35 mol/L NaOH solution were mixed in a 10 mL test tube. It was shaken for 5 s and kept in the dark for 3 min. It was stirred for 5 s and the absorbance was measured at 760 nm using water as a blank. The result was expressed as mg of gallic acid equivalents (GAE) per gram of fresh fruit (Acosta-Montoya, *et al.*, 2010).

Antioxidant power determination

Aqueous extract: DPPH (2,2-diphenyl-1-picrylhydrazyl) test: a solution of 0.1 mM in methanol was prepared immediately before use and protected from light to prevent degradation.

Gallic Acid Pattern Curve (2.50-40.0 mg/L)

Curve Sample *Rubus Adenotrichus* (2.50-40.0 mg/L): lyophilized 50 mg *Rubus Adenotrichus* fruits were accurately weighed and placed in a 100.0 mL volumetric flask. Distilled water was used to dissolve. Aliquots of 0.25; 0.50; 1.00; 2.00 and 4.00 mL of the above solution were placed in volumetric flasks of 50.0 mL. Distilled water was used to dissolve. The supernatant liquid was centrifuged and used for analysis.

Dissolution of DPPH: 4-5 mg of DPPH was accurately weighed and placed in a 100.0 mL amber graduated flask. 50 mL of methanol was added and stirred for 10 min to dis-

solve, then made up to volume with methanol.

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 were mixed. It was shaken for 5 s and kept in the dark for 30 min. It was shaken for 5 s and the absorbance was measured at 517 nm using water as a blank. The radical removal activity was reported as ECS0 (mean effective concentration required to reduce 50% of the DPPH moiety) (Acosta-Montoya, *et al*., 2010; Martínez-Cruz N. S. *et al*., 2011).

The inhibition stock was calculated by the equation:

% Inhibition = (1 – (Sample Absorbance/DPPH Absorbance) x 100

Percent inhibition versus pattern or sample concentration was plotted.

The C50 was calculated by using the % inhibition equation by the following calculation:

$$C50 = (50\text{-}B)/A$$

where B is the intercept of the percent inhibition vs. concentration curve and A is the slope of the percent inhibition versus concentration curve.

ORAC assay: This test measures 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 radical (ROO). The method measures the ability of antioxidants in the sample to protect the protein from oxidative damage and allows determination of the equivalent moles of Trolox in a range of 5 to 200 ug/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 (Acosta-Montoya, *et al*., 2010; Gancel, Feneuil, Acosta, & Vaillant, 2011).

Calibration curve: Trolox 5 mg were exactly weighed and taken to a 10 mL volumetric flask, completely solubilized in the phosphate buffer solution and removed. A solution was obtained at 2000 µM. Dilutions were made for the Trolox curve by taking 5, 12.5, 25, 50, 75 and 100 µL of the above solution and bringing to volume of 1 mL with distilled water. 150 µL of fluorescein, 25 µL of the respective dilution of Trolox were added in sequence to each well. In parallel, a test blank was prepared containing 150 µL of fluorescein and 25 µL of phosphate buffer solution, pre-incubated for 30 min at 37°C. 25 µl of 250 mM AAPH solution was added to each well. Fluorescence intensity was measured every 2 min for 2 h with excitation and emission wavelengths of 485 and 520 nm, respectively.

Measurement of samples

Preparation of solid samples: Make a 1 in 100 dilution in methanol with 50% w/w NaOH with reflux for 30 minutes and extract with ether, dry the extract and reconstitute with ethanol. A 5 mL aliquot is filtered through a nylon membrane (0.45 um), stored at 4°C and protected from light. Dilutions in water of 1:10 and/or 1: 100 are then made.

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

Reagents

Sodium Phosphate/Biphosphate Buffering Solution 10 mM

Fluorescein 1 µM: A 1 mM fluorescein solution was initially prepared. For this, 3.76 mg fluorescein should be weighed and filled with the Phosphate Buffering solution in a 10 mL volumetric flask (if necessary, heat to a temperature not greater than 50°C). Then make the dilution at 1 µM.

Solution of AAPH (2,2'-azobis (2-amidino-propane) 250 mM: 678 mg was weighed and brought to volume with phosphate buffer solution in a 10 mL volumetric balloon (Acosta-Montoya, et al., 2010, Gancel, et al., 2011, Tiwari, *et al*., 2013, Thangaraj, 2016).

Specific Gravity Determination: For aqueous extract only, it was determined following the usual procedure described in Berrocal, *et al*., 2012, Anonymous, 2016.

pH Determination: For aqueous extract only, it was determined following the usual procedure described in Berrocal, *et al*., 2012, Anonymous, 2016.

Conductivity Determination: For aqueous extract only, it was determined following the usual procedure described in Berrocal, *et al*., 2012, Anonymous, 2016.

Determination of Osmolarity: For aqueous extract only, it was determined following the usual procedure described in Berrocal, *et al*., 2012, Anonymous, 2016.

Brix Degree Refraction Index Determination: For aqueous extract only, it was determined following the usual procedure described in Berrocal, *et al*., 2012, Anonymous, 2016.

UV-Visible Spectroscopy: Phenols absorb in the ultraviolet region. In the case of flavonoid phenols, two characteristic absorption bands were present, the band of the aromatic A ring with a maximum absorption in the range 240-285 nm (benzoyl band) and another of the B ring with maximum absorption in the range 300-550 nm (cinamoil band) (Acosta-Montoya *et al*., 2010; Martínez-Cruz NS, *et al*., 2011). Blackberry fruits 100 g were liquefied, then filtered juice 2 g was taken and dissolved in 100 g of distilled water. The absorbance was measured in a UV-Visible spectrophotometer from 260 nm to 650 nm with a resolution of 1 nm (Acosta-Montoya, *et al*., 2010; Martínez-Cruz N. S. *et al*., 2011).

Results

Table 1: *Rubus Adenotrichus* Phytochemical screening of aqueous, ethanolic, ethereal, chloroformic and hexane extracts

Test	Compounds	Phase a	Phase b	Phase d1	Phase d2	Phase e1	Phase e2	Phase f1	Phase f2
Gelatin	Tannins	+	+	-	-	-	NA	NA	NA
Gelatin-Salt	Tannins	+	+	-	-	-	NA	NA	NA
$FeCl^3$	Tannins (gallic and catechinic)	+	+	+	-	-	NA	NA	NA
Formaldehyde - HCl	Condensed Tannins	+	+	+	-	+	-	-	-
Dragendorff	Alkaloids	-	+	+	-	+	+	-	-
Mayer	Alkaloids	-	+	+	-	+	+	-	-
Wagner	Alkaloids	-	+	+	-	+	-	-	-
Enfriamiento	Mucilages	-	-	-	-	-	NA	-	-
Nihidrina	Amino acids	-	-	-	-	-	-	-	-
Liebermann-Burchard	Steroids Terpenes	-	-	+	-	-	-	-	-
Salkowski	Terpenes	+	-	+	-	-	+	+	+
Tortelli-Jaffe	Terpenes	-	-	-	-	-	-	-	-
Ammonium IV Molybdate	Terpenes	+	+	+	-	+	-	+	+
Perchloric Acid	Terpenes	-	+	-	-	+	-	+	-
Shinoda	Flavonoids	+	+	-	-	+	-	+	-
Pews	Flavonoids	+	+	+	-	+	-	+	-
Borntrager	Quinones	-	-	-	-	-	-		
Rosenheim	Anthocyanins	+	+	+	NA	+	NA	+	NA
Espuma	Saponins	-	-	-	-	+	-	-	-
Rosenthaler	Saponins Steroids	-	-	+	-	+	-	+	-
Fheling	Carbohydrates	+	+	-	-	+	-	-	-
Molish	Carbohydrates	-	+	-	-	+	-	+	-
Benedict	Carbohydrates	+	+	+	-	+	-	+	-
Tollens	Carbohydrates	-	+	+	-	+	-	-	-
Baljet	Lactones Coumarines	-	+	+	-	+	-	-	-
Hidroxamato Férrico	Sesquiterpenic Lactones Esters	-	-	-	-	-	-	-	-
Kedde	Cardiotonic glycosides		-	-	-	-	-	-	-
Guignard	Cyanogenic glycosides		-	-	-	-	-	-	-
Sudan	Fat	-	-	-	-	+	-	-	-
Carr-Price	Carotenes	-	-	-	-	-	-	-	
Emmerie-Engel	Tocopherols	-	-	-	-	-	-	-	-

Nomenclature; NA: not applicable, - Negative test + Positive test.

Table 2: *Rubus Adenotrichus* methanolic extract Phytochemical screening

Test	Compounds	Phase C1	Phase C3	Phase C5.1	Phase C5.2	Phase C7	Phase C8
Ninhydrin	Amino acids	-	NA	NA	NA	NA	NA
Shinoda	Flavonoids	+	NA	NA	NA	-	-
Gelatina	Tannins	+	NA	NA	NA	NA	NA
Gelatina-sal	Tannins	+	NA	NA	NA	NA	NA
$FeCl^3$	Tannins	+	NA	NA	NA	NA	NA
Borntrager	Quinones	+	-	NA	NA	NA	NA
Liebermann-Burchard	Steroids Terpenes	+	-	-	NA	NA	-
Dragendorf	Alkaloids	+	NA	NA	+	NA	+
Mayer	Alkaloids	+	NA	NA	+	NA	+
Wagner	Alkaloids	+	NA	NA	+	NA	+
Rosenheim	Alkaloids	+	NA	NA	-	-	-

Nomenclature; NA: not applicable, - Negative test + Positive test.

Table 3. Differential pH Anthocyanins Quantification

Sample	Cyanidin 3-O-Glucoside equivalent quantity/ mg g^{-1}
1	9.85
2	10.52
3	10.85
Mean	10.51
Standard Deviation	0.66

Figure 3. Absorbance versus concentration of Radical DPPH for Gallic acid and *Rubus adenotrichus* lyophilized fruits.

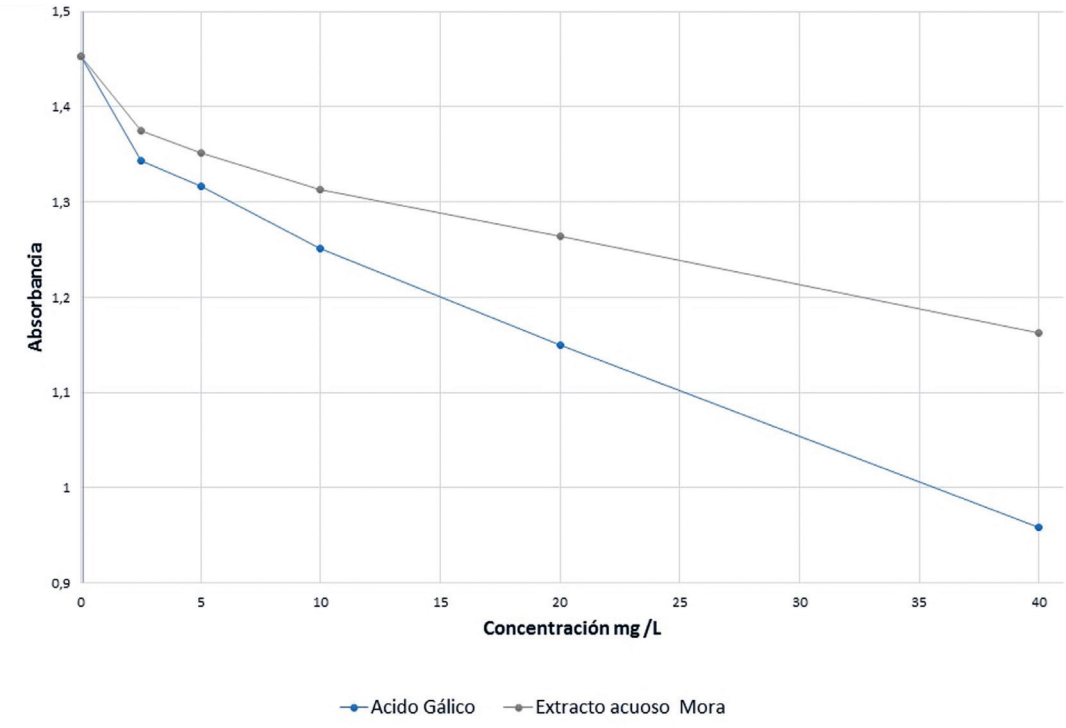


Figure 4. Percent Inhibition Curve by DPPH quantification in Gallic acid aqueous solution.

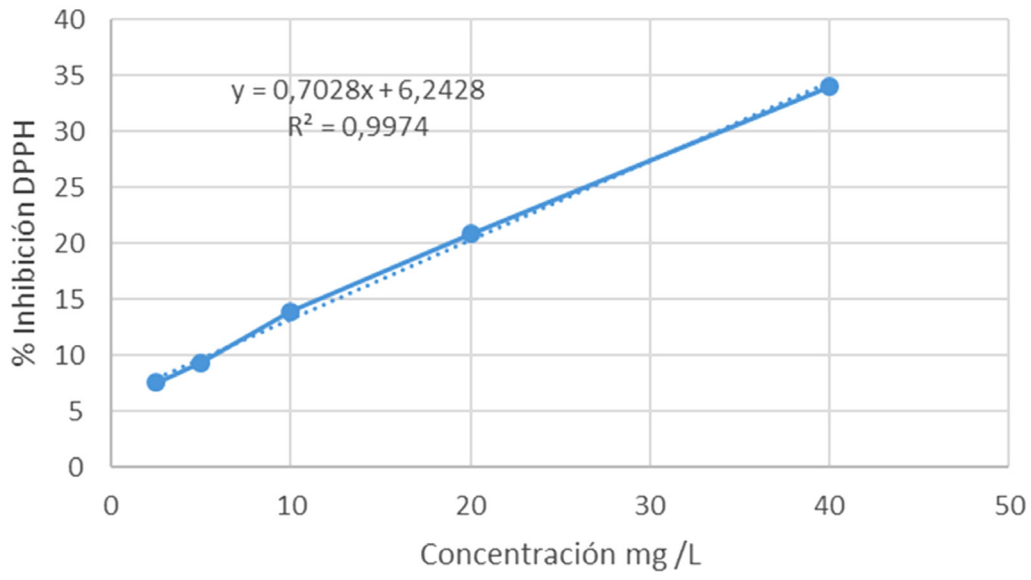


Figure 5. Percent Inhibition Curve by DPPH quantification in *Rubus Adenotrichus* aqueous extract.

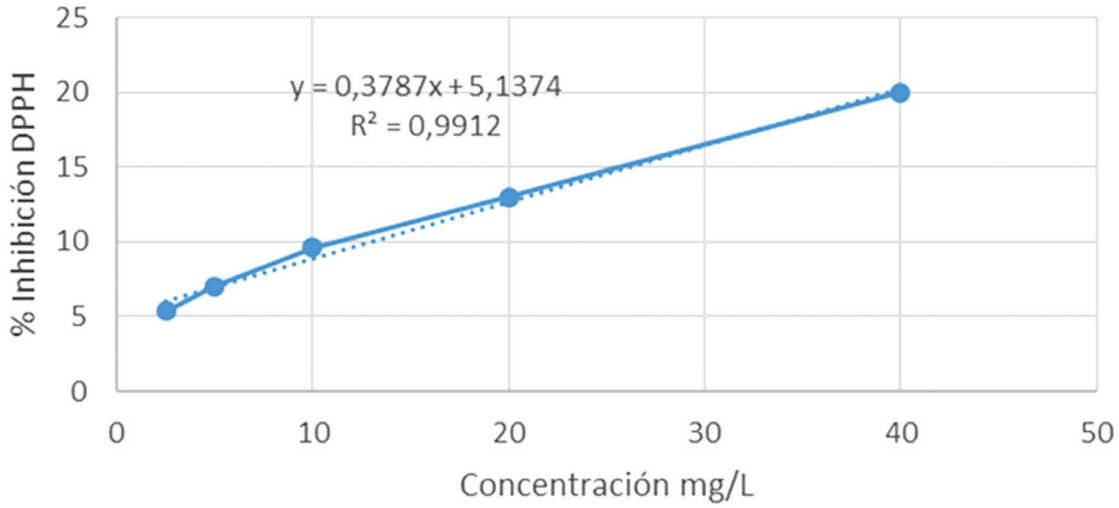


Figure 6. *Rubus Adenotrichus* lyophilized fruits aqueous extract Folin-Ciocalteau test.

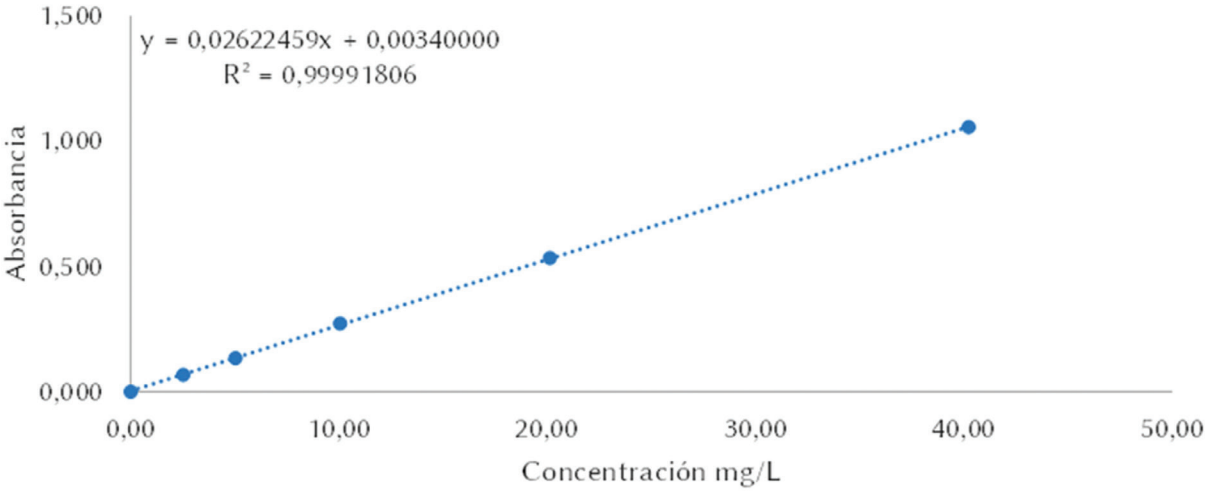


Table 4. *Rubus Adenotrichus* lyophilized fruits aqueous extract Folin-Ciocalteau total phenols quantification.

Sample	Blackberry fruit quantity/g	Amount Gallic acid equivalents/ mg g^{-1}
1	0.0505	21.17
2	0.051	20.7
3	0.0511	20.7
Mean	0.0508	20.85
Standard deviation	0.003	0.27

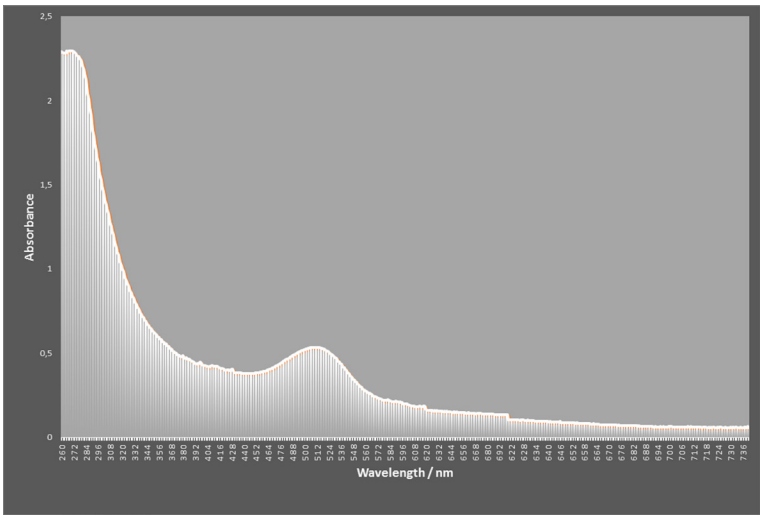
Table 5. *Rubus Adenotrichus* fruits quantification of H-ORAC Antioxidant Power.

Sample	Dry fruit Trolox equivalent / mg mol^{-1} g^{-1}
1	310
2	305
3	320
Mean	311
Standard deviation	7.63

Table 6. *Rubus Adenotrichus* lyophilized fruits aqueous extract Folin Ciocalteau total phenols quantification.

Sample	pH 25°C	Specific gravity 25°C	Brix Grade	Conductivity 25°C/µS cm^{-1}	Osmolarity 25°C /mOs Kg^{-1}
1	3.56	1.0224	7.54	287	561
2	3.55	1.0207	7.53	283	556
3	3.54	1.0196	7.54	285	562
Mean	3.55	10,209	7.543	285	559.66
Standard deviation	0.01	0.0014	0.005	2	3.21

Figure 7. *Rubus Adenotrichus* aqueous extract 4% w/w Uv-visible Absorption Spectrum.



DISCUSSION

Compounds commonly associated with dermocosmetic and therapeutic effects were found in fractions with different polarity. The presence of tannins, flavonoids, terpenes, carbohydrates, anthocyanins, already reported in the literature (Garcia-Saucedo, et al., 2016) is shown in the aqueous extract, according to the data in Table 1. These same results were confirmed by means of ethanolic and methanolic extract tests; no positive data were found for saponins, cyanogenic glycosides and cardiotonic glycosides, which are associated with the toxicity of the fruits containing them. In addition, Table 2 describes the results of the methanolic extract, where quinine and alkaloids were found, which had not previously been reported in the literature; the data in Tables 1 and 2 show the absence of free amino acids.

The screening of phytochemical groups in Tables 1 and 2 show highly antioxidant components such as flavonoids, anthocyanins, and tannins; the latter in their condensed and non-condensed form are related to an astringent, protein precipitating and bacteriostatic effect. In addition, sugars were found which are recognized as a source of skin moisturization due to their contribution to the transepidermal passage of water through the stratum corneum (Bronaugh & Maibach, 2005).

Non-polar (chloroform, hexane and ethereal) fractions composition of *Rubus adenotrichus* fruits has not been reported in the literature. From the screening, terpenes, flavonoids, carbohydrates and steroids were found, as well as the absence of compounds normally associated with toxicity such as coumarins, cyanogenic or cardiotonic glycosides

(Evans, 2009). The terpenoid compounds are of high and low polarity and therefore, it is common to find them both in polar and non-polar fractions. Furthermore, in the methanolic extract (Table 2), alkaloid compounds were found, which are usually related to highly vary therapeutic and cosmetics actions (Evans, 2009); fatty or carotenoid-like compounds or tocopherols were not found in any of the fractions.

The main recognized effect of *Rubus adenotrichus* fruits reported in the literature is an antioxidant effect (Montoya-Castro, *et al*., 2010). Commonly this antioxidant effect is measured in Trolox equivalent units (known as H-ORAC). Table 5 shows an average of Trolox Equivalent 311 ± 7.63 µg/mol/g of dry fruit. This high antioxidant capacity is associated with multiple dermocosmetic effects – for example, an antiaging effect, protection against ultraviolet radiation, and an anti-wrinkle effect. Additionally, the protective effect against ultraviolet radiation is associated with the prevention of skin cancer, regenerative effects of damaged tissue and an antimicrobial effect.

Another way of measuring the antiradical effect is by DPPH test, which studies the percentage of inhibition of the free radical 2,2-diphenyl-1-picrylhydrazyl. This test studies a different mechanism of oxidation to H-ORAC and therefore, can be defined as complementary. In this case, Figure 3 shows the anti-radical inhibitory effect, with the Gallic acid pattern. Gallic acid is a recognized polyphenolic antioxidant, of the tannin type; Figure 3 shows a higher EC 50 equivalent of 118,46 mg/L extract compared to an EC50 62,26 mg/L for Gallic acid.

The antioxidant effect is associated with the presence of phenolic compounds; the Follin-Ciocalteau test determines the concentration of total phenols. 20.85 ± 0.27 mg/g of dry sample were found in the analyzed sample as Gallic acid equivalents (see Table 4). Anthocyanins, another main component, are associated with the purple color of the fruit and that its concentration increases with fruit maturation. In addition to its recognized antioxidant action, its resonant structure favors the absorption of ultraviolet radiation, especially in the UVB range. This photoprotective effect is very important and almost exclusively related to the presence of cyanidin 3-O-glucoside. In the sample analyzed (Table 4), an anthocyanin content of 10.51 ± 0.66 mg/g equivalent to cyanidin 3-O-glucoside was found.

Analysis of aqueous extract physicochemical properties not only allows for the characterization and reproduction of future extracts but also allows for the analysis of their compatibility to combine with excipients and to obtain suitable dermocosmetic formulations, which, for example, guarantee an antioxidant or sunscreen effect, as well to analyze if the extracts are compatible with the human skin. Table 6 summarizes the main parameters studied, showing that the aqueous extract has a very common acid character, close to pH 3.55, probably related to the ascorbic acid reported in the literature (Kalt, Forney, Martin, & Prior, 1999). The mean specific gravity was 1.0209 ± 0.0014, slightly higher than water at 25°C; this is related to dissolved compounds, especially sugars, tannins, and anthocyanins.

The high content of dissolved solids is verified with osmolarity, resulting in a hyperosmotic extract, according to the data of Table 6. The analyzed sample showed an osmolarity of 559.66 ± 3.21 mOs/kg, twice the osmolarity of biological fluids (Sinko, 2010). Similarly, Brix grades, which describe the dissolved solids (especially sugars) is 7.543° ± 0.005°. The conductivity of the extract is higher than water, which implies that it has a large amount of dissolved electrolytes; its value is 285 ± 2 µS/cm. This is important because, when formulating carbomer gels with this extract, they may be affected in their viscosity and stability, due to the presence of electrolytes in solution (Wilkinson, Moore, Rodriguez, & Rodriguez, 1990).

As shown in Figure 7, the 4% w/w lyophilized fruits aqueous extract shows an absorption in the range of UVB (260 nm to 320 nm) and UVA (320 nm to 400 nm) of about 25% of received radiation, which may be associated with a low to medium photoprotective effect; however, it can be increased proportionally to the content of ultraviolet radiation absorbing compounds, mainly anthocyanins and tannins.

CONCLUSIONS

The results of the studies performed for the mature fruits of *Rubus adenotrichus* show a high antioxidant power due to a H-ORAC 311 ± 7.63 µg/mol Trolox Equivalent /g dry fruit superior even to fruits such as blueberry and pomegranate, also known for their high antioxidant power, and EC50 of 118,46 mg/L for the aqueous extract. This effect is associated with the presence of hydrophilic compounds such as anthocyanins, flavonoids and tannins. The qualitative results of the phytochemical screening were verified. In addition, terpenoid compounds, alkaloids and carbohydrates were found, which makes *Rubus adenotrichus* fruit an excellent component for bacteriostatic, antiaging, antiwrinkling, skin nourishing and moisturizing formulations. It's astringent effect also allows its application to close small superficial wounds on the skin. Likewise, the acidic pH of 3.55 is beneficial to maintain the cutaneous acid mantle and thereby favors normal skin flora, but may be a problem for the formulation of carbomer-based gels or the incorporation of preservatives based on salts of weak acids.

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