Article

Physicochemical properties of wild *Rubus* fruits with nutraceutical and nutritional potential

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Abstract

Rubus species are commonly exploited for fresh consumption, regional gastronomy and traditional herbalism, these benefits are attributed to the presence and action of their polyphenolic metabolites such as flavonoids and anthocyanins, which are known for their antidiabetic, anticancer, etc. In Mexico, around 15 wild species of *Rubus* are reported. However, population growth has invaded its territory, compromising its growth and development. Therefore, in order to rescue, study and incorporate Mexican wild materials into genetic improvement programs, the objective of this work was to evaluate the physicochemical composition and antioxidant activity in fruits of three wild species (*Rubus adenotrichus*, *Rubus pringlei* and *Rubus glaucus* Beth), compared against a commercial variety (Tupy). To accomplish this, fruits were collected in the state of Michoacán. Physicochemical parameters were evaluated and polyphenols, flavonoids, anthocyanins and antioxidant capacity (CA) were quantified in ethanolic extracts. In the results, the length of the fruit varied from 1.7-2.23 cm and the unit weight of 1.1-3.1 g, the ratio of total soluble solids and titratable acidity of 8.32-14.76. The total polyphenols reported data of 285.06-592.61 mg EAG/100 g PF, total flavonoids of 93.13-36.4 mg EQ/100 g PF and anthocyanins of 18.43-4.32 mg L^{-1}. The CPA of 65.70-25.15 µM ET/g PF. This study showed that wild species comply with physicochemical and nutraceutical characteristics appreciated to be incorporated in breeding programs or the pharmaceutical industry.

Keywords: antioxidant capacity, flavonoids, polyphenols.

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Introduction

The *Rubus* genus has 12 subgenres and an estimated 750 to 1 000 species (Tatjana *et al*., 2010; Moreno *et al*., 2018). As representative members of this genus, there are raspberries and blackberries, known for their edible fruits, which are classified as polydrupes, since they are aggregates of small fleshy fruits called drupels and each with seed, gathered around a common axis. In immature stages the drupels are usually green and as they mature, they turn reddish, in the case of raspberries or until they reach an intense and bright purple in the blackberries (Hummer *et al*., 2017).

They mainly develop in cold and temperate zones of America, Europe, North Africa and northwest Asia, considering China the place where the greatest diversity of *Rubus* species is concentrated in the world (Graham *et al*., 2011). In general, blackberries are considered as species with ample capacity to adapt to environmental conditions, which is why some are classified as invasive grass, promoting their elimination and threatening the loss of wild species.

In addition to this, the current implications of climate change influence wild specimens, which represent the loss of a genetic resource with high potential to be used within programs for the development of cultivars better adapted to changes in their environment (Graham *et al*., 2011), being the Mexican wild species a little investigated resource for commercial exploitation.

In the last thirty years the production of blackberry and raspberry have had a considerable growth, since in 1990 the extension cultivated in North America was 4 385 ha, contributing 75% the Pacific Northwest, a decade later, Central America became the largest production area in the world and its production was mostly destined to market fresh and export to retail markets in the United States and Europe, (Clark *et al*., 2011), eleven years later, around 20 035 ha of blackberries were calculated commercially grown throughout the world, in addition to 8 000 ha of fruit harvested under wild conditions for a total production of 140 292 t.

For 2014, more than 25 000 cultivated hectares were estimated in the world, considering Mexico to the greater extension; as well as, with the largest production of blackberries with a total of 248 517 t, followed by Colombia with 110 453 t and Italy with 107 479 t. Due to the above, the continuous search for improved species with attractive characteristics for the producer and consumer has arisen, example of this, are the varieties ‘Tupy’ and ‘Brazos’; however, in countries like Colombia and like almost all the regions of the world where *Rubus* is native, they have developed prosperous industries based on their local species (Ayala *et al*., 2013), but not in the case of Mexico.

In Mexico, around 15 wild species distributed in the national territory are reported (Segura *et al*., 2009). Within the state of Michoacán, different species of wild blackberries and raspberries have been found, mainly in the municipalities of the Purepecha plateau, where their fruits are collected to be marketed in regional markets for fresh consumption, although they are commonly incorporated into regional gastronomy, either in the preparation of tamales or refreshing and fermented beverages.

On the other hand, its leaves and stems have been used in traditional herbal medicine to cure certain conditions such as flu, nausea during pregnancy, menstrual discomforts and facilitate delivery and fruit is considered a mild laxative if eaten in large quantities (Hummer *et al*., 2010). These health benefits are attributed to the presence and action of their polyphenolic metabolites such as flavonoids and anthocyanins (Cuevas *et al*., 2010), which are commonly known for their antidiabetic, anticancer, antimicrobial, anti-inflammatory and their outstanding antioxidant capacity (Azofeifa *et al*., 2013).

Therefore, in order to rescue, study and incorporate the Mexican wild materials into genetic improvement programs, the objective of this work was to evaluate the physicochemical composition and antioxidant activity in fruits of three wild species of the Rubus genus, compared against the commercial variety Tupy.

Materials and methods

Biological material

The fruits of blackberries and wild raspberries were collected in the municipalities of Uruapan and Nahuatzen in the state of Michoacán, Mexico. The collection sites were referenced by geographic coordinates for each species, which were identified by molecular biology in the Molecular Biology Laboratory of the Technological Institute of Tlajomulco, Jalisco, Mexico.

The species were recognized as *Rubus adenotrichus* (1 815 masl, 19° 26’ 48’’ north latitude, 102° 4’ 38’’ west longitude), *Rubus pringlei* (2 769 masl, 19° 40’ 30’’ north latitude; 101° 50’ 8’’ west longitude), *Rubus glaucus* Beth (2 769 masl, 19 ° 40' 29 " north latitude, 101° 50’ 9’’ west longitude) and the commercial variety Tupy (1 210 masl; 19° 39’ 16’’ north latitude; 101 91’ 50’’ west longitude). The fruits were selected in maturity of consumption according to their coloration. At the time of collection, the size of the fruit was recorded by means of a Vernier (standard analogue Truper), number of drupillas and weight in grams with a digital scale (Sartorius, model BL210S).

Physicochemical proximal composition

The physicochemical parameters were evaluated in fresh fruit, through the application of official analysis standards for: percentage of humidity (AOAC 934,06/2007), titratable acidity expressed in citric acid (AOAC 942,15/2007), pH by potentiometry (AOAC 981, 12/2002), total soluble solids (°Brix) with a 0-30 scale refractometer (Atago, master-BX/S28M model); likewise, the reducing sugars were evaluated according to the 3,5-dinitrosalicylic acid (DNS) method (Amid *et al*., 2014), which consisted in the preparation of the DNS reagent by dissolving 75 g of sodium tartrate and potassium tetrahydrate in distilled water , adding 50 mL of 2 M sodium hydroxide and 75 mL of hot distilled water, finally 0.25 g of 3,5-dinitrosalicylic acid was added, the reaction was carried out with 100 µL of the extract, adding 1 mL of the reagent DNS and incubating for 10 min. The absorbance was determined at 570 nm and the results were expressed in grams of fructose per 100 g of sample.

Quantification of polyphenols, flavonoids, total anthocyanins and antioxidant capacity

The extraction was made by maceration from 1 g of fresh fruit with 9 mL of 80% ethanol, for 48 h at 4 °C in the absence of light. The mixture was centrifuged at 13 000 rpm for 10 min, the supernatant was recovered and used for the quantification of total polyphenols (PT), total flavonoids (FT), total anthocyanins (ANT) and antioxidant capacity (CA).

The PT were quantified by what was reported by Zielinsli and Kozolwaska, (2000). 50 µL of sample was taken, mixed with 200 µL of distilled water and 250 µL of Folin-Ciocalteu reagent at 1 N, after three minutes, 500 µL of 7.5% Na_2CO_3 was added. It was incubated for 15 min and the absorbance was recorded at 760 nm. The results were expressed in gallic acid equivalents per hundred grams of fresh weight (mg EAG/100 g PF). A calibration curve was made with gallic acid at different concentrations 0.01-0.50 mg mL^{-1} (ten data points R^2= 0.998).

The FT were evaluated using the information reported by Chang *et al*. (2002), mixing 100 µL of the extract with 200 µL of 1 M potassium acetate, 200 µL of 10% aluminum nitrate and 1 mL of 80% ethanol, then incubated for 40 min and absorbance was read at 415 nm. The results were reported in quercetin equivalents per hundred grams of fresh weight (mg EQ/100 g PF). The calibration curve was calculated at different quercetin concentrations that included ten points between 0.001-0.01 mg mL^{-1} (R^2= 0.998).

For ANT, it was based on the differential pH method, according to Wrolstad *et al.* (2005). Two buffer solutions were prepared, one with pH 1 of potassium chloride at 0.025 M and the second with pH 4.5 of sodium acetate at 0.4 M. In both solutions the pH was adjusted with concentrated HCl. 20 µL of the extract was taken and it was completed to 1.5 mL with the respective solution. The absorbance of each solution was recorded at 520 and 700 nm. The concentration of total anthocyanins was determined by the following equation:

$$\text{Total anthocyanins mg g}^{-1} = \frac{\text{A x MW x DF x 1000}}{\sum \text{x 1}}$$

Where: MW= molecular weight of cyanidin-3-glucoside (449.2 g mol^{-1}); DF= dilution factor (75) and Σ= molar extinction coefficient (26 900) and the value of A was obtained with the equation: A= (Abs 520-Abs 700) pH 1 (Abs 520-Abs 700) pH 4.5. The results were expressed in mg of total anthocyanins per hundred grams of fresh weight (mg/100 g PF).

The CA was performed by the DPPH radical method (1,1-diphenyl-2-picrylhydrazyl), according to the reports by Brand *et al*. (1995). For this, 150 mM DPPH was dissolved in 80% methanol. An aliquot of 100 µL of the extract of each sample was taken and 900 µL of DPPH solution was added. The mixtures were incubated for 30 min and their absorbance was determined at 515 nm. The results were expressed as micromolar equivalent of trolox per gram of fresh weight (µmol ET/g PF). The calibration curve was calculated at different concentrations of trolox that included ten points between 1 to 1 500 µM mL^{-1} (R^2= 0.998).

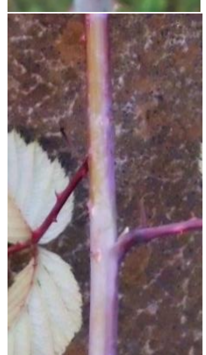
Statistical analysis

The statistical analyzes were carried out with the statistical package Statgraphics Centurion Version XV.II. The mean values and their standard deviations were reported. One-way analysis of variance was performed, multiple means were compared using the Tukey test ($p \leq 0.05$).

Results

In Table 1, the main distinctive morphological characteristics of each biological material used in the present study are shown, which are described below.

Table 1. Characteristics morphologies of the flower, stem and fruit of wild species of *Rubus*.

Species	Flower	Stem	Ripe fruit
R. adenotrichus			
R. glaucus Beth			
R. pringlei			
Tupy variety			

R. adenotrichus (blackberry): it has a suberect, angular circular stem, which is covered by a large number of glandular hairs of reddish coloration; shows a habit of climbing growth with separate stingers, its leaves are dentate with dark green beam, in addition its inflorescence is pyramidal with pale pink flowers, as a result clusters that produce 70 to 150 fruits.

R. glaucus Beth (blackberry): presents an erect and circular stem with numerous thorns and covered by a thin layer of whitish wax known as pruina, its leaves have leaflets of laminar and suorbicular form, its inflorescence is given in the form of a rosette whose flowers they present petals of whitish coloration, small and separated; its clusters show between 6 and 8 fruits.

R. pringlei (raspberry): its main stem has a habit of erect, circular and reddish growth. Its leaves are pinnaticompuestas with 3-7 leaflets, obovate, sawed with roped base, show a green color by the beam and whitish velvety on the underside. The flowers have a corolla composed of 5 white petals and have numerous stamens and pistils giving rise to fruits of red color in the stage of maturity; its clusters generate between 2 and 5 fruits.

Variety Tupy: is a hybrid generated from the cross between the variety 'Comanche' and a selection of Uruguay. This variety presented stem with numerous short and sharp thorns, whose habit of growth is erect, its leaves are large and webbed, it has flowers with five whitish petals of 2 to 3 cm in diameter.

Physicochemical proximal composition

The results of the physicochemical analyzes carried out on the wild species of the genus *Rubus*, show variations in the length of the fruit ranging from 1.7 to 2.23 cm (Table 2). On the other hand, it was recorded from 34 to 89 in the number of drupillas, being the species *R. pringlei* the least amount and *Rubus adenotrichus* who reported the largest number of durpillas. Regarding the relationship between length and number of drupillas, it was observed that the greater the length of the fruit, the lower the number of drupillas. The unit weight varied between 1.1 and 3.1 g, with *R. pringlei* being the lowest weight and *R. glaucus* Beth with the statistically highest value.

Table 2. Proximal composition of mature fruits of blackberries (± standard deviation).

Parameter	*R. adenotrichus*	*R. glaucus Beth*	*R. pringlei*	Tupy variety
Length (cm)	2.23 ±0.15 bc	2.7 ± 0.26 b	1.7 ±0.15 c	4.6 ±0.36 a
No. of drupillas	89 ±3.6 b	70.3 ±2.08 b	34.6 ±4.5 d	64 ±4.58 c
Weight (g)	2.5 ±0.27 c	3.1 ±0.1 b	1.1 ±0.1 d	7.2 ±0.2 a
pH	3.1 ±0.08 b	3.2 ±0.02 b	3.3 ±0.02 a	3.3 ±0.01 a
Humedad (%)	75.1 ±0.04 d	86.89 ±0.1 b	82.6 ±0.02 c	87.7 ±0.03 a
Reducing sugars	1.34 ±0.02 b	1.55 ±0.1 a	1.48 ±0.2 a	1.56 ±0.15 a
Titratable acidity (% citric acid)	0.8 ±0.02 c	0.9 ±0.03 bc	1.05 ±0.12 ab	1.1 ±0.09 a
Total soluble solids (°Brix)	12.4 ±0.19 a	7.6 ±0.1 d	10.7 ±0.1 b	9.8 ±0.09 c
Soluble solids/titratable acidity ratio	14.76 ±0.41 a	8.32 ±0.14 c	10.24 ±1.08 bc	8.96 ±0.9 c

Average with different letters indicate significant differences between rows ($p \leq 0.05$).

Regarding the moisture content, it is highlight *R. glaucus* Beth, showing significant difference with respect to the other wild species. Although it is known that the high humidity in fruits and vegetables is a disadvantage because, as there is greater availability of free water, the shelf life is reduced, in the same way the concentration of sugars and therefore the sweetness of the product is reduced. As the wild to have lower percentages of moisture is expected a longer shelf life than the commercial variety Tupy.

The content of total soluble solids in the four species presented significant statistical differences; however, the highest values were observed for the wild species *R. adenotrichus* and *R. pringlei* with 12.4 ±0.19 and 10.7 ±0.10 °Brix, in contrast the Tupy variety reported 9.8 ±0.09 °Brix, while *Rubus glaucus* Beth obtained the lowest amount of soluble solids (7.6 ±0.1 °Brix) compared to the commercial variety.

The high content of total soluble solids is reflected in the sweetness of the fruit, characteristic qualified in fruits incorporated in the elaboration of wines and liquors (Coronel, 2008). Regarding the percentage of titratable acidity values of 0.8-1.1 were found, being the wild species those who showed statistically lower percentages of acidity, with respect to the commercial variety.

In the fruits, as the maturation progresses, the organic acids are breathed or converted into sugars, decreasing their content (Seymour *et al*., 1993), so that their flavor is not only affected by the sugar content, but also by the presence of volatile compounds and organic acids, being citric, malic and tartaric acid the most abundant in fruits of blackberries and raspberries, constituting citric acid between 30-95% with respect to total organic acids (Petkovsek *et al*., 2012).

Some organic acids such as citric acid, have been widely incorporated in the food industry, because it acts as a regulator of acidity, provides firmness, enhances flavor, acts as a preservative and antioxidants, in addition to providing stability to lipids and antioxidants primary as phenolic acids (gallic and ferulic acid) and are capable of inactivating metal ions that act as pro-oxidants, such as iron and copper through the formation of chelates (Quitmann *et al*., 2014).

The ratio of total soluble solids and titratable acidity of *R. adenotrichus* reported 14.76 ± 0.41, was included as the highest value in wild fruits of *Rubus hirsutus* Thunb with ratios ranging from 31.31-11.23. Although the relationship obtained in the *Rubus glaucus* and *pringlei* species were statistically lower, with respect to *R. adenotrichus*, they are within the reported Lewers *et al*. (2010) in 21 commercial varieties of blackberries and raspberries (4.8-11.9). Lewers *et al*. (2010) make reference to a more pleasant flavor for strawberry with a relation around 10, in turn this could be achieved with high to moderate levels of total soluble solids and low titratable acidity, as in the case of the wild species of the present work.

A ratio greater than 12.5 is considered a characteristic appreciated in a sweet taste for the consumer. On the other hand, Vergara *et al*. (2016) make reference that the industry of the United States of America prefers good-colored blackberries with a high content of soluble solids and acidity, as well as looking for low numbers of seeds and pH, while, for consumers, the qualities, as sweetness, acidity, astringency, color, firmness and absence of seeds are important for both processed and fresh fruits.

These characteristics make it possible to consider wild species as candidates for incorporation into agronomic management programs that allow increasing the size of the fruit, as well as cross-species processes, since it has benefited the obtaining of large fruits with a moderate number of seeds (Clark *et al*., 2011).

Content of polyphenols, flavonoids, anthocyanins and antioxidant capacity

The registered values of total polyphenols for the materials studied, reported data ranging from 285.06 ±8.49 to 592.61 ±7.03 mg EAG/100 g PF. The wild species showed the highest values, being statistically different with respect to the commercial variety. The content of polyphenols found in this work exceeded that reported by Wang and Lin. (2000), in commercial cultivars of blackberries (204- 248 mg EAG/100 g PF) and raspberries (208-267 mg EAG/100 g PF) and agree with that reported by Ramune *et al*. (2012) for 19 improved varieties of raspberries (278.6- 714.7 mg EAG /100 g PF). Found in the wild species *Rubus hirsutus* Thunb 108. 23-269. 9 mg EAG/100 g PF.

Regarding the flavonoid content (Figure 1), it ranged from 93.13 ±1.7 to 36.4 ± .4 mg EQ/100 g PF, with the lowest number of wild species. On the other hand, in the content of anthocyanins stood out the wild species with respect to the commercial variety, whose quantities fluctuated between 18.43 ±1.05 to 4.32 ±0.24 mg L^{-1}, being *R. adenotrichus* and *R. glaucus* who reported the greatest amount of anthocyanins, without showing significant difference between them. The values of anthocyanins found in this study surpass that reported for *Rubus chamaemorus* (Koponen *et al*., 2007) and like that shown in *Rubus adentrichus* (Martínez *et al*., 2011).

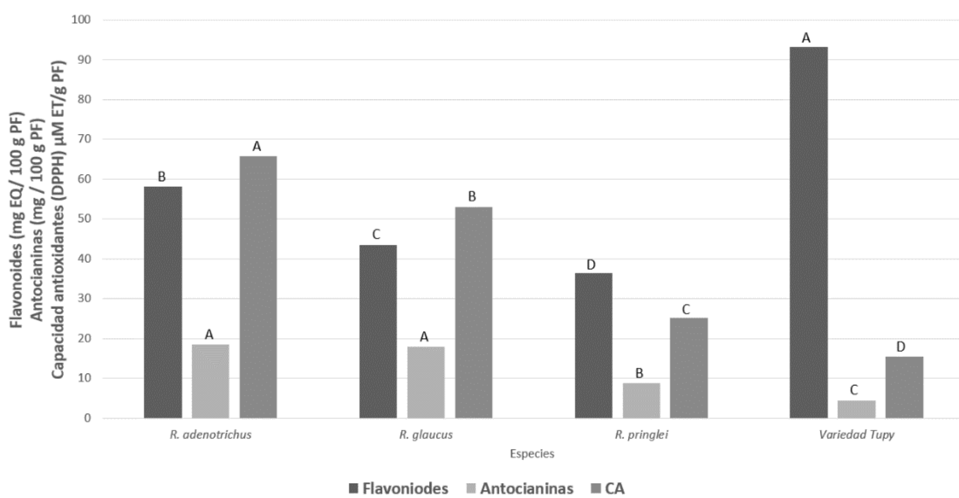


Figure 1. Concentration of flavonoids, total anthocyanins and antioxidant capacity (CA) in fruits of three wild species of the genus *Rubus* and the commercial Tupy variety (± standard deviation). Average with different letters indicate significant differences between rows ($p \leq 0.05$)

Although there have been diverse studies that quantify polyphenolic compounds such as flavonoids and anthocyanins in *Rubus* species, it is known that the synthesis of polyphenols is given by factors such as the conditions of cultivation, species and phenolic stage of the plant. Likewise, its obtaining can be conditioned by the process and the type of solvent used (Valencia *et al*., 2013). Therefore, the species analyzed in the present work may be suitable candidates for obtaining improved cultivars with high polyphenol content.

On the other hand, greater antioxidant potential was observed in wild species (65.7 ±2.31-25.15 ±2.65 μM ET/g PF) compared to the Tupy variety (15.35 ±1.01 μM ET/g PF), this agrees with that found in *Rubus fructicosus and Rubus ideaous* (25.3-35.5 μM ET/g PF) by Paredes *et al*. (2010), as mentioned by Cuevas *et al.* (2010), who reported that wild species of the genus *Rubus* have higher polyphenolic content and antioxidant potential than the commercial Tupy variety. These results demonstrate the importance of Mexican wild species as sources of polyphenols and their possible incorporation into antioxidant production programs with an industrial objective.

The antioxidant capacity of polyphenols is mainly related to structural characteristics, such as the presence of double bonds and *O*-diphenyl, hydroxyl or methoxy groups (Balasundram *et al*., 2006). The absence or substitution of some of these structural characteristics reduces or inhibits the antioxidant capacity. Skrovankova *et al*. (2015) mentioned that the antioxidant capacity of the blackberries is influenced by the concentration of the extract. However, Johnson *et al.* (2012), reported a close relationship between antioxidant activity and polyphenol content.

On the other hand, Hassimotto *et al*. (2008), showed that the elimination of radicals is influenced by the total of specific compounds such as anthocyanins. In counterpart, Silva *et al*. (2007) showed that the relationship between the capacity and the number of polyphenols could be given by the technique implemented for the antioxidant evaluation of the compound.

Conclusions

This study showed a high content of total soluble solids in the wild species being candidates to be incorporated in the elaboration of wines and liquors, in addition to the high content of polyphenols and antioxidant capacity that their extracts presented, quality valued in the pharmaceutical industry, they also showed that they comply with physicochemical characteristics appreciated for their fresh consumption. This highlights the importance of the rescue, utilization and more detailed studies of the Mexican wild species of the *Rubus* genus, which can be material for obtaining new commercial varieties, or to be introduced in programs for the production of polyphenolic metabolites for the food or pharmaceutical industry.

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