PAPER

PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY OF WILD GRAPE (*VITIS TILIIFOLIA*)

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ABSTRACT

Vitis tiliifolia is a tropical grape with a deep purple colour and a high content of pigments. Total polyphenols content in the skin and pulp was 400.35 and 171.26 mg GAE/g dry sample of *Vitis tiliifolia*, respectively, which coincides with DPPH radical scavenging for skin (91.39%) and in the pulp (19.57%). The predominant individual phenolic compounds found in the skin were quercetin-3-glucoside (39.86 μg/g), rutin (37.01 μg/g) and *trans*-resveratrol (32.88 μg/g). The DPPH radical scavenging and reducing power revealed a high antioxidant activity. This study demonstrates that wild grape can thus be utilised as a novel functional resource.

Keywords: *Vitis*, wild grapes, anthocyanins, food composition, polyphenols

1. INTRODUCTION

Consumers have focused increased attention on functional foods, especially those containing antioxidants, which decrease reactive oxygen species (ROS) (MANACH *et al.*, 2005). It is reported that some fruits such as grapes can dramatically increase the balance between the production and manifestation of ROS and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage is interrupted (STAGOS *et al.*, 2006). At the same time, grape extracts and wine have been recognised to contain polyphenol compounds that have beneficial effects on human health. It is known that grapes are anti-mutagenic, antineoplastic, reduce human low-density lipoprotein (LDL) oxidation and allergic inflammation, decrease cardiovascular diseases (LEKAKIS *et al.*, 2005), exhibit antimicrobial (JAYAPRAKASHA *et al.*, 2001), antihypertensives (SOARES DE MOURA *et al.*, 2002), and antiulcer activities (CUEVAS *et al.*, 2011). On the one hand, anthocyanins are a type of polyphenol, and it is reported that they present strong antioxidant activity, inhibit the growth of cancerous cells and inflammation, and act as vasoprotectors and anti-obesity agents, in addition to having effects on diabetes and cardiovascular disease prevention, as well as the improvement of visual and brain functions (TSUDA, 2012). On the other hand, resveratrol (3,5,4-trihydroxy-*trans*-stilbene) is a natural polyphenolic that acts as a defense mechanism against deleterious microorganisms. These compounds are present in several fruits as grapes, and their manufactured products, especially red wine. Anthocyanins are primarily located in the skin and have pharmacological benefits. It has been known to exert its protective effect against cardiovascular disease, ischemia-reperfusion injury and diabetes mellitus through the modulation of adipocyte/fibroblast biology, platelet activation, blood vessel function, oxidative stress, inflammation, serum glucose maintenance, cardiomyocyte biology, the maintenance of cell structure, and serum lipid activity, cause body fat loss, and confer protection against disease or injury (TSUDA, 2012).

There are many types of grapes that have been widely studied. However, *Vitis tiliifolia* is a wild grape resource that has not yet been adequately recognised by researchers and winemakers. It is a small to very large climbing shrub with thick, woody stems that can be 10-35 m long and up to 20 cm in diameter, which commonly grows in wet to dry forest or thickets, often in pine-oak forest; It grows regularly around 1700 meters above sea level. *Vitis tiliifolia* grows in the southern states of Mexico and the Antilles to Colombia (FERNANDEZ, 2009). In Mexico, it is located in the states of Chiapas, Colima, Guerrero, Hidalgo, Nuevo Leon, Oaxaca, Querétaro, San Luis Potosí, Tabasco and Veracruz, where it is known by different names such as: wild grape, Gunhi, loobabi-chuli, uvilla, xocomecatl, tecamate and others, according to the region and growing area (ARELLANO *et al.*, 2003). Flowers are seen from May to June and fruits are harvested from August to November (IBARRA and SINACA, 1996). The fruits have been used as raw materials for juice and wine (ARELLANO *et al*., 2003). Fresh fruit is commonly used to make vinegar and soft drinks (FERNANDEZ, 2009), while the root and leaves are used empirically against haemorrhoids. Therefore, these products may be useful as a source of potentially functional ingredients providing the opportunity to develop innovative added value products. However, the further application of this wild grape requires the evaluation of their composition and there is little information on the physicochemical and antioxidant properties of *Vitis tiliifolia*. Therefore, the aim of this work was to investigate the physicochemical properties and antioxidant activity in the pulp and skin of *Vitis tiliifolia* fruit to provide sufficient experimental evidence for the antioxidant activity and potential for further development and utilisation of this species.

2. MATERIALS AND METHODS

2.1. Chemicals

2,2'-Diphenyl-1-picrylhydrazyl (DPPH), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), gallic acid, *trans*-resveratrol, Folin-Ciocalteu reagent and 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 4-hydroxybenzoic acid, (+) catechin, vanillin acid, scopolin, chlorogenic acid, caffeic acid, (-) epicatechin, vanillin, 4-coumaric acid, quercetin 3-glucoside, ferulic acid and *trans*-cinnamic acid were purchased from Extrasynthese (Lyon, France). The rest of the standards were bought at Sigma-Aldrich (USA). The solvents used for the extraction was analytical grade and MS grade for the ultra-high performance liquid chromatography (UPLC) procedures and other standards were also purchased from Sigma-Aldrich (USA). All the stock solutions, samples, solvents and reagents were filtered through 0.20 μm PTFE membrane filters (Phenomenex, USA) before separation or injection in the instrument.

2.2. Samples

The samples of *Vitis tiliifolia* were collected at "Cafetal" ranch, located in the Veracruz state, situated at 19° 37' 0.4 " north latitude and 96° 50' 2.7" west longitude, at an elevation of 734 meters above mean sea level. Ten kg of grapes were harvested during the month of August of the years 2015 and 2016 with the optimum stage of maturity and with a concentration of soluble solids between 12 and 14 °brix. Samples were washed, drained and subsequently, skins, seeds and pulp were directly obtained by manual separation. One part of the samples was frozen at -40°C for the analysis of the composition and physicochemical properties of the pulp and skin and the other part was subjected to lyophilization for the preparation of the extracts.

2.3. Determination of some basic physicochemical parameters

Total nitrogen was determined by the micro-Kjeldahl method and protein was calculated as nitrogen·6.25. Oil was extracted for 24 h with diethyl ether in a Soxhlet system. Ash was determined by incineration in a furnace at 550°C and weight, moisture, titratable acids, reducing sugars and total dietary fibre and pH were determined following the AOAC (2000) methods. The water activity was measured at 25ºC using Aqualab 4 TE (Decagon 142 Devices, Pullman, WA, USA) and °Brix were measured with a hand refractometer. The colour was measured with a colorimeter (ColorFlex V1-72 SNHCX 1115 s/n: Cx1115 Hunter Lab, USA) using parameters a^o (yellow-red), b^o (blue-green) and L^o (intensity and brilliance) on the scale of the system CIE Lab (International Commission on Illumination, Vienna). Browning index was determined according to the method reported by BUERA *et al.* (1986). Equations 1 and 2 were used to calculate hue angle (H°) and Chroma, respectively.

$$Hue\ angle = \tan^{-1}\frac{b}{\bar{a}} \qquad \text{Eq. (1)}$$

$$Chroma = \sqrt{a^2 + b^2} \qquad \text{Eq. (2)}$$

2.4. Chemical compounds and antioxidant analysis

2.4.1. Extraction

The dry sample pulp and skin (approximately 10 g each one) were mechanically homogenised with 10 mL of acidified methanol/0.1% HCl in a manual blender and sonicated in an Ultrasonic bath (Branson model 2510) for 30 min and agitated in a horizontal shaker at room temperature (24°C) for 1.5 h. Then, the sample was centrifuged (Hettich, Mod. Universal 32R) at 2200 g for 15 min. The supernatant was removed and the residue was re-extracted twice with 10 mL of a mixture of methanol: HCl 0.1 v/v according to CHIOU *et al.,* (2014). The three supernatants were pooled and brought to a final volume of 100 mL with the same solvent used in the last two extractions. This concentration was considered by quantification of the components present in the sample. This extract was prepared in triplicate and used for the analysis of individual phenolic compounds, total phenolics, monomeric anthocyanins, and antioxidant activity.
The identification and quantitation of individual phenolic compounds, it was established by Ultra High Performance Liquid Chromatography (Agilent 1290 series) and dynamic multiple reaction monitoring (dMRM) following the protocol conditions of DURAND-HULAK *et al.* (2015). The chromatographic analysis were carried out on a ZORBAX SB-C18 column (1.8 μm, 2.1 × 50 mm) (Agilent Technologies) with the column temperature at 40°C. The mobile phase consisted of (A) water containing 0.1% formic acid and (B) acetonitrile containing 0.1% formic acid. The gradient conditions of the mobile phase were: 0 min 1% B, 0.1-40 min linear gradient 1-40% B, 40.1-42 min linear gradient 40-90% B, 42.1-44 min isocratic 90% B isocratic, 44.1-46 min linear gradient 90-1 %B, 46.1-47 min 1% B isocratic (total run time 47 min). The flow rate was 0.1 mL/min, and 5 μL of sample injection volume. dMRM were obtained on an Agilent 6460 Triplequadropole (QqQ) mass spectrometer. The ESI source was operated in positive and negative ionization modes, desolvation temperature of 300°C, Cone gas (N_2) flow of 5 L/min, nebulizer 45 psi, sheath gas temperature 250°C, sheath gas flow of 11 L/min, capillary voltage (positive and negative) 3,500 V, nozzle voltage (positive and negative) 500 V. For quantitation of each phenolic compound a calibration curve in a concentration range of 0.3 to 30 μM was prepared (R^2 values ≥ 0.97 were considered for the linearity range) and quantities were established by using MassHunter Workstation Software version B.06.00 (Agilent Technologies) (Table 1). The results were expressed as μg/g of sample (dry weight).

2.4.2. Anthocyanins Profile

Anthocyanins were identifying according to LIANG *et al*. (2008). Twenty grams of dry methanol pulp and skin extracts were dissolved in 1 mL of MeOH with 0.1% of formic acid (LCMS grade, SIGMA). The samples were filtered in PTFE filters and 1 μL injected in a UPLC-MS system (Acquiti Class-I coupled to mass spectrometer Synapt G2 Si, Waters™) for high resolution mass analysis. The mobile phases were water (A) and acetonitrile (B), both with 0.1% of formic acid. The elution gradient was: at T= 0 minutes, 1% of B, then in 13 minutes changes from 1 to 80% of B. Isocratic in 80% of B for 1 minute and finally change in 1 minute from 80 to 1% of B and remains for 5 minutes. The flow rate of the mobile phase was 0.3 mL/min and the column oven temperature was 40°C. The mass spectrometer was operated in positive mode, with capillary, sampling cone and source offset voltages of 3, 40 and 80 kV, respectively. The source and desolvation temperatures were 100 and 450°C, respectively. The gas flows of desolvation was 600 L/h and the nebulizer pressure was 6.5 Bar. The data were analyzed with the Waters Masslynx

software v4.1 and the mass spectra compared with the public databases Metlin and Massbank and analyzed with the Masslynx tool named Massfragment (v4.1).

Table 1. Protocol used in the analysis of the compounds was a dynamic MRM (Multiple Reaction Monitoring).

Reference compounds	Precursor ion	Product ion	Retention time	Collision energy	Polarity	R^2	Linearity range (μM)
Gallic acid	168.9	125	1.5	10	Negative	0.996	0.3 - 24
4-hydroxybenzoic acid	137.02	93.03	9.4	10	Negative	0.997	0.3 - 24
(+)-Catechin	291.1	139.03	11.3	10	Positive	0.971	0.3 - 12
Vanillic acid	169.04	151.04	12	10	Positive	0.998	0.3 - 12
Scopolin	355.1	193	12.2	20	Positive	0.998	0.3 - 12
Chlorogenic acid	353.08	191.05	12.3	10	Negative	0.998	0.3 - 12
Caffeic acid	179	135	12.5	10	Negative	0.999	0.3 - 12
(-)-Epicatechin	291.1	139.1	14.6	10	Positive	0.998	0.3 - 12
Vanillin	153	93	15.3	10	Positive	0.998	0.3 - 12
4-Coumaric acid	163.05	119	16.4	10	Negative	0.996	0.3 - 12
Quercetin 3,4-di-O-glucoside	627.15	303.04	17.7	10	Positive	0.997	0.3 - 12
Scopoletin	193.04	133.02	18.6	10	Positive	0.995	0.3 - 12
Ferulic acid	193.1	133.9	18.8	5	Negative	0.998	0.3 - 12
Rutin	611.16	465.1	20.4	10	Positive	0.994	0.03 - 12
Quercetin 3-D-galactoside	465.1	303.04	20.6	10	Positive	0.999	0.3 - 12
Quercetin 3-glucoside	465.2	303.04	20.9	10	Positive	0.987	0.03 - 12
Luteolin 7-O-glucoside	449.1	287.05	21.3	10	Positive	0.993	0.3 - 12
Kaemperol 3-O-glucoside	449.1	287.05	23.3	10	Positive	0.986	0.03 - 12
2,4-Dimethoxy-6-methylbenzoic acid	197.08	79.05	23.4	10	Positive	0.992	0.3 - 12
Trans-resveratrol	229.08	135.04	25.9	10	Positive	0.999	0.3 - 24
Trans-Cinnamic acid	147.01	103.05	28.7	10	Negative	0.999	1.5 - 24
Quercetin	303.05	153.1	29.4	35	Positive	0.990	0.03 - 12
Piperine	286.14	201.05	43.8	10	Positive	0.981	0.03 - 12

The retention time variation allowed for the search of the compounds was 2 min in each case. The fragmentor voltage was 100 V and the cell accelerator voltage was 7 V for each compound. It was made a calibration curve for each compound in a concentration range of 0.03 to 30 μM.

2.4.3. Total phenolic content

Total phenolic content was estimated using the Folin-Ciocalteu method (SINGLETON AND ROSSI, 1965). Briefly, the grape extracts were mixed with Folin-Ciocalteu reagent, and sodium carbonate solution (10%) was added. The mixture was allowed to react at room temperature in the dark for 120 min, and then the absorbance was measured at 765 nm in a UV/VIS spectrophotometer (JENWAY, model 6305, Japan). The result was then referred to a calibration curve obtained with a similarly prepared set of different Gallic acid concentrations, and was expressed as mg of Gallic acid equivalent (GAE) per g of dry sample (R^2=0.980).

2.4.4. Total flavonoid content

Each grape extract was analysed for total flavonoid content according to a previously reported colorimetric method with modifications (VELURI *et al.*, 2006). Specifically, 10 mg of lyophilised grape extract or 1 mL of quercetin standard (Sigma, St. Louis, MO) was mixed with 0.3 mL of 0.7 mol/L sodium nitrite ($NaNO_2$), 0.3 mL of 0.8 mol/L aluminium chloride ($AlCl_3$), and 2 mL of 1 mol/L sodium hydroxide (NaOH). All samples were analysed in duplicate and compared against a blank at an absorbance of 510 nm in a UV/VIS spectrophotometer (JENWAY, model 6305, Japan). Results were expressed as milligram quercetin equivalent per gram of dry sample (mg/g).

2.4.5. Total monomeric anthocyanin content

The total monomeric anthocyanin (TMA) content was estimated using the pH differential method (WROLSTAD, 2001). Here, 10 mg of grape extract was diluted with buffers at pH 1.0 and 4.5 to obtain the same dilution. Absorbance was measured in a UV/VIS spectrophotometer (JENWAY, model 6305, Japan) at 510 and 700 nm in both pH 1.0 and 4.5 buffers. The TMA content (expressed in terms of cyanidin-3-glucoside) was calculated using the following formula:

$$A = (A_{510} - A_{700})_{pH\ 1.0} - (A_{510} - A_{700})_{pH\ 4.5} \quad (3)$$

$$TMA\ content = (Ax\ MWx\ DFxVx1000)/(\varepsilon\ x\ 1\ x\ M) \quad (4)$$

Where *MW* is the molecular weight of cianindin-3-glucoside (449 g mol^{-1}), *DF* is the dilution factor, *V* is the extract volume, ε is the molar extinction coefficient of cyanindin-3-glucoside (29,600), and *M* is the mass of *Vitis tiliifolia* extracted.

2.4.6. Condensed tannins determination

The determination of condensed tannins was performed according to the method described by PORTER *et al.* (1986). The dry extract of the pulp or skin (200 mg) and ten mL of aqueous acetone (70%) were added and suspended in an ultrasonic water bath, then the content was centrifuged for 10 min at approximately 3000g at 4°C, then 0.50mL of the supernatant was diluted with 70% acetone, 3.0 mL of butanol-HCl reagent and 0.1 mL of the ferric reagent. This sample was boiled for 60 min and measure the absorbance at 550 nm was obtained in the cool sample.

2.4.7. Determination of ascorbic acid content.

Ascorbic acid contents were carried out by using a colorimetric method. The extract (10 mg) was mixed with metaphosphoric acid (10 mL, 1.0%) for 45 min at R.T. and filtered through Whatman No. 1 filter paper. The filtrate (1.0 mL) was mixed with 2,6-dichlorophenolindophenol (9.0 mL) and the absorbance was measured within 30 min against a blank at 515 nm in a UV/VIS spectrophotometer (JENWAY, model 6305, Japan). Ascorbic acid contents were calculated on the basis of the calibration curve of authentic ascorbic acid (R^2= 0.927). The results were expressed as l g of ascorbic acid of extract.

2.4.8. DPPH free radical scavenging capacity

DPPH free radical-scavenging capacity was estimated using the method of GÜDER AND KORKMAZ (2012). Briefly, grape extracts and DPPH methanol solution were mixed and kept in the dark for 30 min. The absorbance of the reaction mixture was measured at 517 nm in a UV/VIS spectrophotometer (JENWAY, model 6305, Japan). The calibration curve was made with standard solutions of Gallic acid in the range 1-100 mg mL (R^2=0.935).

2.4.9. Reducing power

The determination of the reducing power was made according to the method of JAYAPRAKASHA *et al.* (2001). 0.125 mL of the sample in methanol (1 mg/mL), 1.25 mL of phosphate buffer (200 mM, pH 6.6) and 1.25 mL of potassium ferricyanide (1%) were added. The mixture was incubated at 50°C for 20 minutes. Then, 1.25 mL of 10% trichloracetic acid was added to the mixture, which was centrifuged at 650 *g* for 10 minutes. An aliquot of 2.5 mL was taken, 2.5 mL of distilled water and 0.5 mL of ferric chloride were added, and the absorbance measured at 700 nm in UV/VIS spectrophotometer (JENWAY, model 6305, Japan). Solutions of Trolox (6-hydroxy-2.5.7.8-tetramethyl-chroman-2- carboxylic acid) in a range of concentrations were used for calibration of the FRAP assay. The values were expressed as mg of Trolox/L of seed extracts. All determinations were performed in triplicate.

2.5. Statistical analysis

Data were subjected to ANOVA and Tukey tests (Statistica 7.0 software) at a 0.05 level of significance. Five samples ($n=3$) of pulp and skin were analysed.

3. RESULTS AND DISCUSSIONS

3.1. General composition and physicochemical properties

The grapes of *Vitis tiliifolia* are round and dark violet; with an average weight around 0.15 g, their dimensions were: length 3.74 mm and width 3.14 mm. Physicochemical parameters of the pulp and skin in *Vitis tiliifolia* fruits are described in Table 2. Pulp and skin showed moisture content of 84.88 and 82.00%, respectively. Reducing sugars, ash and total dietary fibre were the major components in the pulp and skin of the grape. Proteins content varied from 0.45 to 0.95 % in the pulp and skin, respectively; both values were lower to those reported for red and white grape (BRAVO and SAURA-CALIXTO, 1998). The grape had a weight, brix, titratable acidity percentage and dimensions lower than those reported for other species, but a similar pH to those reported by JIANG-FEI *et al.* (2012) for four varieties of grapes, three red varieties (Junzi #1, Junzi #2 and Liantang) and one white variety (Baiyu). These differences are due to these properties being influenced by cultivar, ripening stage and environmental factors (CORDENUNSI *et al.*, 2002).
Fruit colour is a tool that is commonly used by winemakers as a selection parameter to define the optimal moment for harvesting during the wine production process. However, this parameter generally is estimated visually and there is not enough information about relations among fruit colour, different harvest dates and some chemical parameters of the *Vitis tiliifolia* fruit (OBREQUE-SLIER *et al.*, 2012). In this work, colour parameters showed significant differences ($p<0.05$) for the pulp (L*=6.23, a*=17.13 and b*=2.28) and skin (L*=38.42, a*=10.28 and b*=4.74). Hue value of the skin (H=24.71°) was located in the first

quadrant of the colour plane which corresponds to a red-violet colour. Parameter a*and b* assumed a positive values, indicating a characteristic violet colour. These colour parameters has been associated with the colour of anthocyanins present in grapes. All colour parameters of this fruit exhibited significant differences with respect to the other grape varieties reported (PÉREZ-MAGARIÑO and GONZÁLEZ-SAN JOSÉ, 2003), but similar values to those reported for fruits such as chagalapoli (JOAQUÍN-CRUZ *et al.*, 2015).

Table 2. Composition of pulp and skin of *Vitis tiliifolia* fresh fruit.

	Pulp	**skin**
Moisture (%)	84.88±0.14^{b}	82.00±0.96^{a}
Brix (°)	12.7±0.17^{b}	8.16±0.28^{a}
pH	3.20±0.10^{a}	3.30±0.50^{a}
Titratable acid (%)	3.00±0.20^{a}	3.50±0.20^{b}
Reducing sugars (%)	15.82±0.16^{b}	4.41±0.31^{a}
Protein (%)	0.45±0.10^{a}	0.95 ±0.05^{b}
Oil	0.37±0.10^{a}	0.50 ±0.10^{b}
Ash	0.28 ±0.01^{a}	0.85± 0.07^{b}
Total Dietary Fiber	0.73±0.25^{a}	1.53±0.10^{b}
a_w	0.98±0.06^{b}	0.64±0.05^{a}
Color parameters		
L	6.23±1.69^{a}	38.42±0.04^{b}
a	17.13±0. 57^{b}	10.28±0.16^{a}
b	2.28±0.26^{a}	4.74±0.09^{b}
Hue angle (°)	82.37±1.12^{b}	24.71± 0.74$_{a}$
Chroma	17.28±0.52^{b}	11.31±0.12^{a}
Browning index	81.63±7.94^{b}	26.3±1.91^{a}

Results are expressed as the mean (n=3)±SD. Note: Diameter of single grape was calculated on the basis of the mean of random 100 grapes. Values of other parameters are mean±SD values of three replicates. Means followed by different letters in column are significantly different by Tukey's test 5%.

3.2. Chemical compounds and antioxidant properties

The antioxidant and functional properties of the different types of grapes depend to a great extent on the bioactive compounds it possesses. So that, in order to determine the compounds that may be responsible for the high antioxidant activity, we investigated the chemical constituents in pulp and skin of the grape (Table 3). Twelve compounds were identified and quantified in the skin, and only two were found in the pulp (vanillin and quercetin-3-D-galactoside). The most abundant compounds identified in skin were: quercetin-3-glucoside (39.86 μg/g dry sample), rutin (37.01 μg/g dry sample) and *trans*-resveratrol (32.88 μg/g dry sample). The majority of these compounds contain double bonds in their aromatic ring structure, reported to be responsible for electron delocalisation, which is attributed to their radical scavenging activity (RICE-EVANS *et al.*, 1996). These compounds may contribute to the antioxidant activity of this grape. Moreover, it has been reported that the bioactivity of the grape is strongly correlated with the composition and the presence of polyphenol compounds (BURIN *et al.*, 2014), which form an important group of secondary metabolites that is abundant and play an important

role in the quality and nutritional value of grapes. Some of these polyphenol are synthesised in the skin of the fruit (JEANDET *et al.,* 1991) and their concentration depend of several factors such as climate, geographical area of cultivation, growing conditions and storage conditions (GEROGIANNAKI-CHRISTOPOULOU *et al.*, 2006).

Table 3. Phenolic compounds (μg/g dry sample) presents in pulp and skin from *Vitis tiliifolia* grape.

Compounds	Pulp	Skin
4-hydroxybenzoic acid	-	0.16±0.06
(+)-Catechin	-	8.29±0.35
Vanillic acid	-	12.60±0.18
Caffeic acid	-	3.68±0.15
(-)-Epicatechin	-	5.17±0.06
Vanillin	0.004±0.00	0.33±0.01
4-Coumaric acid	-	3.37±0.21
Rutin	-	37.01±0.13
Quercetin-3-D-galactoside	1.86±0.82	13.91±0.29
Quercetin-3-glucoside	-	39.86 ±1.36
Trans-resveratrol	-	32.88±0.72
Quercetin	-	22.08±0.67

Data are expressed as means±SD (*n=3*).

Anthocyanins were tentatively identified based on their mass spectra fingerprint (exact mass values and fragmentation pattern) in high resolution compared with public metabolomics databases (Table 4). Overall, the *V. tiliifolia* skin presented higher level of anthocyanins compared to the pulp (Fig. 1). In total, five and seven anthocyanins were identified in pulp and skin, respectively. The most abundant anthocyanin tentatively detected in the skin was malvidin 3-glucoside, while that in pulp was malvidin 3,5-diglucoside. The result that malvidin derivatives were the major anthocyanins agreed with the data reported by LIANG *et al*. (2008).

Total polyphenols concentration found in the skin (400.35 mg GAE/g dry sample) was higher than in the pulp (171.26 mg GAE/g dry sample) (Table 5). This difference in the total polyphenol concentration between pulp and skin might be attributed to the different inherent components present in each part of the grape. The total soluble polyphenolic content of our grape was higher than those of other fruits, such as apple, melon, peach, pear, prune and strawberry (ISHIWATA *et al.,* 2004) and similar to those reported in previous works with other varieties of grapes grown in various parts of the world (BURIN *et al.*, 2014), but lower than reported by the red grape variety (APOSTOULO *et al.*, 2013). These differences probably depend on the variety of grape and are influenced by climatic and geographical factors, cultural practices, and the stage of ripeness (BURIN *et al.*, 2014). By other hand, flavonoids are secondary metabolites presents in plants and fruit such as grapes, which possess biological activities and have an impact on human health. The flavonoid content of the pulp (17.22 mg QE/g dry sample) was lower than quantified in the skin (282.57 mg QE/g dry sample) of the grape.

Table 4. Anthocyanins Profile by Ultra high resolution Liquid chromatography and high-resolution mass spectrometry (UPLC-HRMS-ESI-QTOF).

RT (min)	Mass detected (m/z)	Formula	Fragments (m/z)	Tentative identification	Formula	Ion type	Mass calculated	Error (ppm)
Pulp								
2.36	655.187	$C_{29}H_{35}O_{17}$	493.1341, 331.0816, 287.0543	Malvidin 3,5-diglucoside	$C_{29}H_{35}O_{17}$	$[M]^+$	655.1874	-0.6
2.74	479.1181	$C_{22}H_{23}O_{12}$	317.0662	Petunidin-3-O-β-glucoside	$C_{22}H_{23}O_{12}$	$[M]^+$	479.119	-1.9
3.09	493.1341	$C_{23}H_{25}O_{12}$	331.0809, 287.0541	Malvidin 3-O-glucoside	$C_{23}H_{25}O_{12}$	$[M]^+$	493.1346	-1
3.77	757.1971	$C_{36}H_{37}O_{18}$	449.1087, 287.0550	Cyanidin 3-O-(6-O-p-coumaroyl)glucoside-5-O-glucoside	$C_{36}H_{37}O_{18}$	$[M+H]^+$	757.198	-1.2
4.02	801.2236	$C_{38}H_{41}O_{19}$	639.1685, 493.1356, 331.0816, 287.0551	Malvidin 3-O-(6-O-(4-O-caffeoyl-alpha-rhamnopyranosyl)-beta-glucopyranoside)	$C_{38}H_{41}O_{19}$	$[M+H]^+$	801.2242	-0.7
Skin								
2.36	655.1872	$C_{29}H_{35}O_{17}$	493.1346, 331.0818, 287.0546	Malvidin 3,5-diglucoside	$C_{29}H_{35}O_{17}$	$[M]^+$	655.1874	-0.3
2.69	479.1183	$C_{22}H_{23}O_{12}$	317.0652	Petunidin-3-O-glucoside	$C_{22}H_{23}O_{12}$	$[M]^+$	479.119	-1.5
3.02	493.1347	$C_{23}H_{25}O_{12}$	331.0818, 287.0551	Malvidin 3-O-glucoside	$C_{23}H_{25}O_{12}$	$[M]^+$	493.1346	0.2
3.73	757.1961	$C_{36}H_{37}O_{18}$	287.0551	Cyanidin 3-O-(6-O-p-coumaroyl)glucoside-5-O-glucoside	$C_{36}H_{37}O_{18}$	$[M+H]^+$	757.198	-2.5
4.01	801.2236	$C_{38}H_{41}O_{19}$	639.1699, 493.1339, 331.0815, 287.0556	Malvidin 3-O-(6-O-(4-O-caffeoyl-alpha-rhamnopyranosyl)-beta-glucopyranoside)	$C_{38}H_{41}O_{19}$	$[M+H]^+$	801.2242	-0.5
4.18	463.1234	$C_{22}H_{23}O_{11}$	301.0706	Peonidin 3-O-glucoside	$C_{22}H_{23}O_{11}$	$[M+H]^+$	463.124	-0.6
4.73	639.1705	$C_{32}H_{31}O_{14}$	331.0813	Malvidin 3-(6''-p-coumarylglucoside)	$C_{32}H_{31}O_{14}$	$[M+H]^+$	639.1714	-1.4

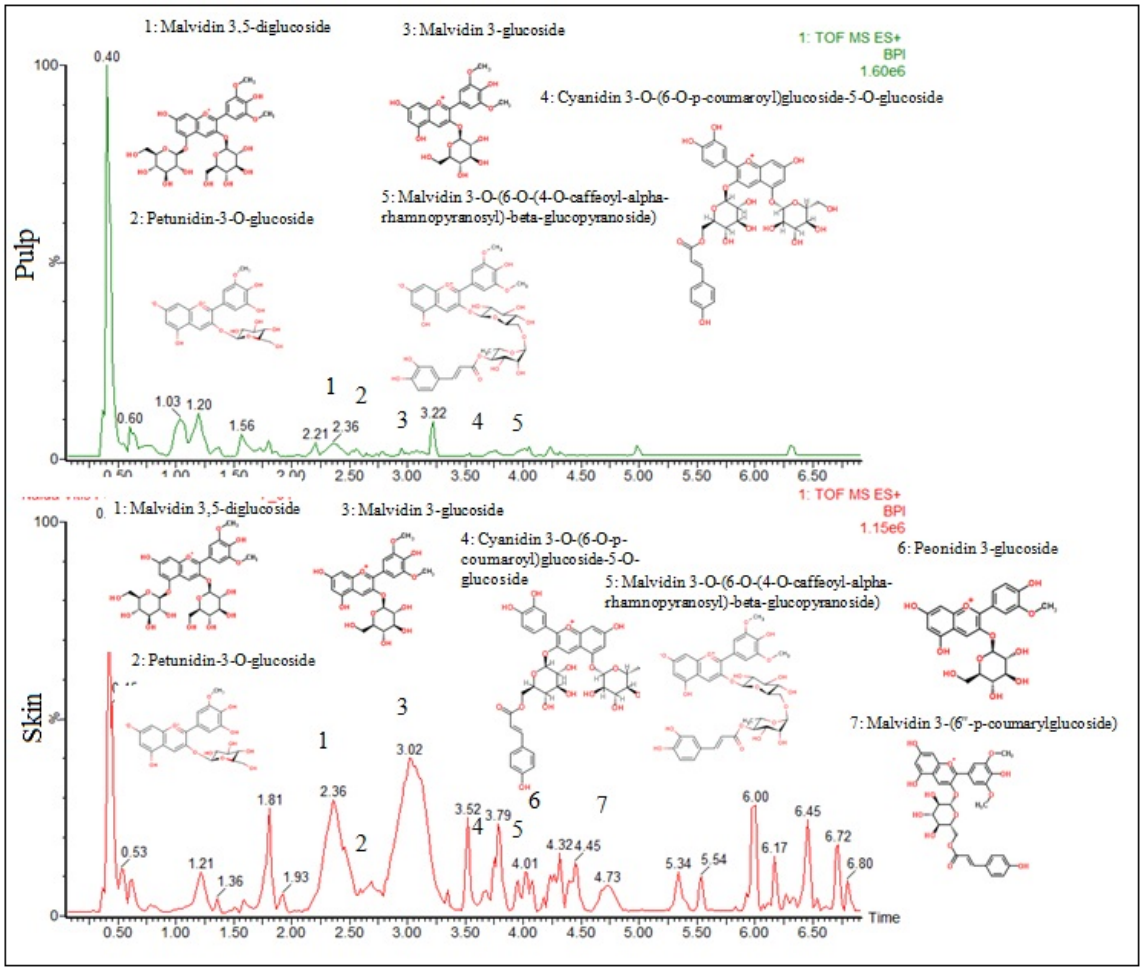

Figure 1. Chromatograms and structure of anthocyanins tentatively identified based on their mass spectra fingerprint (exact mass values and fragmentation pattern).

These data were lower than those reported in other varieties of grapes (GÜDER *et al.*, 2014). Similarly, the total monomeric anthocyanins content was significantly higher ($p < 0.05$) in skin (188.11 mg Cy3/g dry sample) than in pulp (150.93 mg Cy3/g dry sample), and these values were similar to the values of anthocyanins reported for other varieties of grapes (DE PASCUAL-TERESA *et al.*, 2010).

Vitis tiliifolia skin had a high ratio of anthocyanins/total polyphenols close at 0.5, whereas pulp had a ratio close 1.0 indicating than more than half of the polyphenols present in pulp and skin are anthocyanins. A high proportion of the total polyphenols content presents in the grape correspond to anthocyanins, which are considered important groups of plant pigments that contribute to the coloration and sensorial attributes and diverse biological properties; therefore, these are considered secondary metabolites with potential nutritional value, as chronic diseases can be reduced by the regular consumption of anthocyanins in the diet. Anthocyanins are regarded as important nutraceuticals due to their antioxidant activity (KALLITHRAKA *et al.*, 2005). At the same time, total tannins were analysed in the pulp and skin, with a higher concentration found in the skin (188.37 mg Leucocyanidin/g dry sample) than in the pulp (60.26 mg Leucocyanidin/g dry sample). The result in total tannins concentration was consistent with other research that has reported a higher concentration of tannins in the skin and seeds of grapes, playing a

relevant role to define the sensory characteristics of red wines, contributing to bitterness and astringency, in addition to providing antioxidant and antibacterial activity (FIGUEROA-ESPINOZA *et al.*, 2015). By last, in the present work, the pulp and skin from *Vitis tiliifolia* presented a content of ascorbic acid of 130.88 and 5.75 mg AA/g dry sample, respectively, which could contribute to the recommended dairy dietary intakes (0.04-0.09 g/day) suggested by the United Kingdom Food Standards Agency or the United States National Academy of Science (DEL BUBBA *et al.*, 2009). Ascorbic acid is a good reducing agent present in grape juices that is associate with the biosynthesis of tartaric acid (DEBOLT *et al.*, 2006). In plants, ascorbic acid as vitamin C provides protection against free radicals generated during photosynthesis and respiration processes, and is also involved in cell growth; in addition, it is a co-factor of several enzymes participating in the synthesis of anthocyanidins and several secondary metabolites (BRAVO and SAURA-CALIXTO, 1998).

Table 5. Antioxidant activity of dry pulp and skin of *Vitis tiliifolia*.

	Pulp	Skin
Total polyphenols (mg GAE/g dry sample)	171.26±7.90[a]	400.35±5.90[b]
Total monomeric anthocyanins (mg Cy3/g dry sample)	150.93±5.55[a]	188.11±3.15[b]
Total Flavonoids (mg QE/g dry sample)	17.22±2.40[a]	282.57 ±2.20[b]
Condensed Tannins (Leucocyanidin/g dry sample)	60.26±0.34[a]	188.37±0.20[b]
Ascorbic acid (mg AA/g dry sample)	130.88±9.60[a]	5.75±1.20[b]
DPPH radical scavenging activity (%)	19.57±2.13[a]	91.39±3.04[b]
FRAP (mg TE/g dry sample)	40.67±1.17[a]	7.24±1.80[b]

GAE: Gallic Acid Equivalents, Cy3: Cyanidin-3-glucoside, QE: Quercetin Equivalents, AA: Ascorbic Acid, TE: TROLOX Equivalents. Results are expressed as the mean (n=3)±SD. Means followed by different letters in column are significantly different by Tukey's test 0.05.

The presence of these compounds has been demonstrated confer antioxidant activity. One of the techniques used to evaluate this capacity is through the percentage inhibition of the DPPH radical and reducing power. DPPH is a stable free radical and the effect of antioxidants on DPPH scavenging is thought to be due to their hydrogen- or electron-donating abilities. In its radical form, DPPH radical absorbs at 517 nm, but this absorbance value decreases in the presence of an antioxidant or a radical species due to the reaction between antioxidant molecules and the DPPH radical (GÜDER and KORKMAZ, 2012). The radical scavenging activity of pulp and skin showed values from 19.57% and 91.39%, respectively, at a concentration of 10 mg/mL, showing that the skin was highly antioxidant than pulp, which is consistent with the concentration of some polyphenols compounds, such as flavonoids, tannins and anthocyanins. Therefore, the data obtained reveal that these compounds present in this grape act as free radical inhibitors that confer antioxidant activity. Similarly, the reducing power measured by the FRAP value was 40.67 and 7.24 mg TE/g of dry pulp and skin, respectively. The value of reducing power were lower than those reported by red globe grapes (TAGLIAZUCCHI *et al.*, 2010), but FRAP values were consistent with other antioxidant techniques evaluated. FRAP assay does not react fast with some antioxidants, such as glutathione, but some authors consider the FRAP assay to still be suitable for assessment of the antioxidant activity of fruit samples because only limited amounts of plant glutathione are absorbed by humans (SCHAFER and BUETTNER, 2001). On the other hand, it is reported that the antioxidant activity determined by this technique corresponds to approximately 55% of the bioavailability at

the end of digestion (TAGLIAZUCCHI *et al.*, 2010). Therefore, these reports are based on an estimate that approximately 13% of the total antioxidant is used by the human body. The reducing properties are generally associated with the presence of reductones, which also react with certain precursors of peroxide, thus preventing peroxide formation; a higher absorbance of the reaction mixture indicates greater reducing power (PIN-DER, 1998).

Table 6 shows the correlation analysis in the pulp and skin of *Vitis tiliifolia* grape. A linearly relation of DPPH radical scavenging activity with anthocyanins (R^2=0.728), polyphenols (R^2=0.878) and condensed tannins (R^2=0.680), suggesting a strong antioxidant effect of these mixtures of components from *Vitis tiliifolia* pulp. Instead, ascorbic acid had a positive correlation (R^2=0.850) with reducing power, and revealed a moderately strong relationship between ferric ion reducing power and ascorbic acid content. It is reported that in several fruits including grapes, over 80% of the FRAP value was from vitamin C contribution (GUO *et al.*, 2003).

The results on the antioxidant activity of *Vitis tiliifolia* pulp seems to be due to the presence of polyphenols and anthocyanins which may act in a similar fashion as reductones by donating the electrons and reacting with free radicals to convert them to more stable products and terminate the free radical chain reaction (JAYAPRASKASHA *et al.*, 2001), which may serve as significant evidence of their potential antioxidant activity. Instead, in the skin, the antioxidant activity is mainly due to the presence of polyphenols, anthocyanins, resveratrol, tannins and ascorbic acid.

Table 6. Correlation coefficient (R^2) between antioxidant activity and chemical components presents in *Vitis tiliifolia* pulp.

	DPPH	Reducing power (FRAP)
Total polyphenols	0.878	0.650
Total Flavonoids	0.650	0.500
Total Monomeric anthocyanins	0.728	0.567
Resveratrol	0.320	0.450
Condensed tannins	0.680	0.720
Ascorbic acid	0.576	0.850

Correlation was statistically significant at $p < 0.05$.

4. CONCLUSIONS

The results of the present study showed that skin from this wild grape has a higher concentration of polyphenols than pulp. The more abundant individual polyphenols in skin were quercetin-3-glucoside, rutin and *trans*-resveratrol. Instead, the pulp has a large amount of ascorbic acid. Malvidin 3-glucoside and malvidin 3,5-diglucoside were the most abundant anthocyanins identify in skin and pulp, respectively. All this compounds confer a strong antioxidant activity comparable to other grape varieties, and may explain in part the benefits for human health. In addition, the skin has an intense violet blue color that could be exploited to obtain pigments that can be used as food colorant, as food additives or as food supplements.

ACKNOWLEDGEMENTS

The authors' acknowledges the support to the project 124229 (L-IDEA), and the National Council of Science and Technology (CONACyT).

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Paper Received July 20, 2017 Accepted September 20, 2017