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Antioxidant activity and solar protection factor of two *Vismia* species colleted from Venezuelan Andean

Actividad antioxidante y factor de protección solar de dos especies de *Vismia* recolectadas de los Andes venezolanos

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Resumen

*La exposición prolongada a la radiación ultravioleta del tipo **UVA** y **UVB** puede ocasionar envejecimiento prematuro y la aparición de cáncer de piel. Como medida de prevención se establece la utilización de fotoprotectores de administración tópica que contengan compuestos químicos aromáticos oxigenados obtenidos de fuentes naturales. Las plantas del género Vismia (Hypericaceae) se distribuyen principalmente en la zona tropical de Centroamérica y Suramérica y han sido usadas en la medicina tradicional para aliviar algunas enfermedades. El presente estudio tiene como objetivo evaluar la capacidad secuestrante de los radicales libres, el contenido de fenoles y flavonoides totales, el factor de protección solar y el perfil fitoquimico cualitativo de los extractos metanólicos obtenidos de Vismia baccifera (**VB**) y Vismia macrophylla (**VM**). La actividad secuestrante de los radicales libres, el contenido de fenoles y flavonoides totatles se evaluó para determinar la actividad antioxidante de los extractos bajo investigación. El tamizaje fitoquímico permitió identificar en los extractos metanólicos de **VB** y **VM** la presencia de compuestos polioxigenados del tipo; antraquinona, xantona, antrona y flavonoides. El estudio de la actividad antioxidante con el ensayo de **DPPH** y el contenido de fenoles totales mostró valores similares para ambas especies, **IC_{50}** de 5,90 µg mL^{-1} (**VB**) y 5.65 µg mL^{-1} (**VM**); 375.32 and 381.23 **mg Eq ÁG/g Ext**, respectivamente. Sin embargo, se observó para **VB** una mayor cantidad de flavonoides con 267,07 **mg Eq Q/g Ext**. Por otro lado, el estudio In Vitro para determinar el factor de protección solar estableció 25,3 para **VB** y 20,7 en **VM**, siendo los mismos considerados de capacidad media según lo señalado por la FDA y COLIPA. Los resultados obtenidos se consideran un aporte al estudio del genero Vismia.*

Palabras clave: Radiación ultravioleta, *Vismia baccifera*, *Vismia macrophylla*, factor de protección solar, actividad antioxidante

Abstract

*Long lasting exposure to **UVA** and **UVB** radiation might lead to premature aging and the appearance of skin cancer. The use of photoprotectors that contain oxygenated aromatic chemical compounds obtained from natural sources has been established. Species of Vismia genus (Hypericaceae), distributed mainly in the tropical zone of Central and South America, has been used in traditional medicine to alleviate several ailments. Present investigation aims to evaluate the free radical scavenging capacity, total phenolic, flavonoids content, solar protect factor and the qualitative phytochemical screening of methanol extracts obtained from Vismia baccifera (**VB**) and Vismia macrophylla (**VM**). Free radical scavenging activity, total phenolic and total flavonoid content assays were performed in order to determine antioxidant activity on the extracts under investigation. Methanol extracts of **VB** and **VM** showed by means of phytochemical screening the presence of polyoxygenated compounds like anthraquinones, xanthones, anthrones and flavonoids. The antioxidant activity study carried out with **DPPH** and total phenol content assays showed similar values for both species, **IC_{50}** 5.90 µg mL^{-1} (**VB**) and 5.65 µg mL^{-1} (**VM**); 375.32 and 381.23 **mg Eq ÁG/g Ext**, respectively. However, **VB** showed a higher amount of flavonoids with 267.07 **mg Eq Q/g Ext**. On the other hand, the In Vitro study to determine the solar protection factor displayed values of 25.3 for **VB** and 20.7 for **VM**, being these considered as medium protection capacity according to FDA and COLIPA data. Results obtained are considered as contribution to Vismia genus activity investigation.*

Keywords: Ultraviolet radiation, *Vismia baccifera*, *Vismia macrophylla*, solar protection factor, antioxidant activity

1. Introducción

Long exposure to UVA and UVB radiation is considered as health risk due to its potentially harmful environmental effects on the skin. In this regard, recreational and occupational activities outdoors without the proper protective measures may cause photosensitivity, photodermatosis, genetic mutations and immunosuppressions, which are associated with premature aging and the appearance of skin cancer. Nowadays, there is an interest for the search on plants of aromatic chemical compounds with possible photoprotective effect that might be added to cosmetic preparations for topical administration that act by absorbing, filtering, dispersing and reflecting solar radiation (Gilaberte et.al., 2003, Azcona, 2003).

Vismia genus belongs to Vismieae tribe, Hypericaceae family and it is formed by approximately 57 species distributed in the neotropic zone from South America, Central America and some regions of Mexico and United States. Although, there are reports of species in tropical areas of Africa, as well (Rojas et.al, 2017). These species are described as shrubs and trees from 3 to 15 m of height and are characterized by segregate an orange colour latex through a cut (Álvarez et.al., 2008). This latex has been used in traditional medicine as ointment for the treatment of dermatitis, syphilis, herpes, scabies and eczema. Furthermore, the infusion of bark and roots are used to treat intestinal parasites, high fever and volemia control (Buitrago et.al., 2015, Tamokou et.al., 2009).

Phytochemical studies have revealed the presence of oxygenated compounds such as anthrones, anthraquinones, bianthrones, xanthones, benzophenones and lignans while in minor proportions terpenes, flavonoids, sterols, among others have also been isolated (Buitrago et.al., 2015, Noungoue et.al., 2009). Regarding biological activities, several investigations have reported antimicrobial, antioxidant, noniceptive, anti-inflammatory and cytotoxic activity, among others, for different Vismia species (Buitrago et.al., 2016, 2015, Vizcaya et.al., 2014, Silvestre et.al., 2012, Tala et.al., 2011, Tamokou et. al., 2009, Kuete et.al., 2007). The inhibition for the Human Immunodeficiency Virus (**HIV**) of the cleavage of ribonucleic acid in viral deoxyribonucleic acid shown by the extracts of *Vismia* mexicana and *Vismia baccifera* and the cytotoxic activity of methanol extracts of *Vismia baccifera* and *Vismia macrophylla* against tumour cell lines **HeLa**, **MCF-7**, **PC3** y **SKBr3** have also been evaluated (Rojas et.al., 2017, Ferreira-Nobre et.al., 2015, Gómez-Cansino et.al., 2015, Gomes et.al., 2009).

The aim of this investigation is to determine qualitatively the presence of secondary metabolites in methanol extracts of *Vismia baccifera* and *Vismia macrophylla* and to evaluate in vitro the free radicals scavenging capacity and the photoprotective effect against solar radiations, measured by spectrophotometric methods.

2. Marco Teórico

2.1. Plant material:

V. macrophylla Kunth was collected from Michelena, Táchira State, at 1200 masl (7º56'30" N - 72º14'33" W) and *V. baccifera* L. Triana & Planch was harvested from La Hechicera, Mérida State at 1800 masl (8º37'41" N - 71º09'34" W). Both species were collected in April 2019, during rainy season and flowering stage. Botanical identifi¬cation was carried out by Dr. Pablo Meléndez, MERF Herbarium, Faculty of Pharmacy and Bioanalysis, University of Los Andes, Mérida, Venezuela. Voucher specimens were depos¬ited under the following codes: **VM-JR39** and **VB-JR25**.

2.2. Extraction:

Leaves of both species were placed into an oven at 45°C for three days. Dry material was ground in a mill and then weighed, *V. macrophylla* (2080 g **VM**) and *V. baccifera* (1280 g **VB**). Room temperature methanol extraction (4 L, Sigma-Aldrich, anhydrous 99.8%) was carried out with both species under investigation for 5 days, changing the solvent for fresh and leaving the extraction for another 5 days with same plant material to achieve an exhaustive extraction. Extracts were concentrated to dryness by using a rotaevaporator under reduced pressure; weights of concen¬trated dried extracts were also measured (150 g **VM** and 120 g **VB**).

2.3. Reagents and equipment:

Reagents used in this investigation were of analytic grade and these were purchased from the following commercial trade: methanol, sulfuric acid, sodium chloride, ferric chloride, sodium carbonate, sodium nitrite and aluminum chloride from Merck (Darmstadt, Germany); hydrochloric acid, ammonium hydroxide and sodium hydroxide from Riedel-de Haën (Seelze, Germany) and 2,2-Diphenyl-1-picrylhydrazyl (**DPPH),** Folin-Ciocalteu 2 M, galic acid and quercetin from Sigma-Aldrich (St. Louis-MO, USA). Ultra-pure water (18 MΩ.cm resistivity) was used to prepare these solutions and these were applied through a Milli-Q plus system (Water. Millipore, Milford MA, USA). Optic density measures of solutions were carried out in a UV-vis spectrophotometer Genesys with quartz cell of 1 cm (Thermo Fischer Scientific, Madrid) and rotaevaporator 51111 (Heidolph Instruments GmbH & Co., Germany).

2.4. Phytochemical screening:

Methanol extracts of **VM** and **VB** were evaluated to determine the presence of chemical constituents using standard procedures. A blank sample containing only the reagents, according to the test conducted, was prepared in

order to compare to the main reaction. Every procedure is described below:

a) Testing for alkaloids (Tiwari et.al., 2011): Each extract (10 mg) was dissolved in 2 mL of 5% hydrochloric acid; after mixing and filtering by gravity, three aliquots were taken. Drops of Dragendorff reagent were added to each. A red-orange precipitate indicated the presence of such metabolites.

b) Testing for coumarins (Trease & Evans, 2002): A 10 mg amount of each sample were added to 0.5 mL of ethanol along with two drops of ammonium hydroxide 8.23 M. If examination under UV light at a wavelength of 365 nm shows the presence of blue or green fluorescence, it might be indicative of a positive result.

c) Testing for glycosides (Shyamala-Gowri, 2010): A 10 mg amount of each extract were dissolved in 1 mL of distilled water followed by five drops of aqueous sodium hydroxide 2.5 M. A yellow colour indicated a positive reaction

d) Testing for cardiotonic glycosides (Tiwari et.al., 2011): Keller-Killiani reaction: A 10 mg amount of each extract were dissolved in Keller's reagent and five drops of sulfuric acid 18 M. The occurrence of a brown ring between the two phases formed is indicative of deoxy glycosides.

e) Testing for flavonoids (Trease & Evans, 2002): Shinoda test: A volumen of 10 drops of diluted extract in methanol was added with 1 mL of methanol and three drops of hydrochloric acid 12 M. Formation of a red color indicated the presence of aurones and chalcones. Pew's test: A volume of 1 mL of each diluted extract in methanol was added with 2 mg of zinc powder and 5 drops of 5 N hydrochloric acid. The presence of red, pink or coffee colour indicated the existence of flavonones, and dihydrochalcones. Test with 10% sodium hydroxide: A volume of 1 mL of diluted extract in methanol was added with three drops of sodium hydroxide 10%. Formation of yellow, coffee or purple coloration indicated the presence of xanthones, flavones, chalcones and anthocyanins.

f) Testing for steroids and/or triterpenoids (Tiwari et.al., 2011): Salkowski test: A volume of 10 drops of the extract dissolved in methanol was mixed with 2 mL of chloroform and 1 mL of sulfuric acid 18 M were added to until double phase formation. The presence of a reddish-brown color in the middle layer was indicative of steroidal ring. Rosenthaler test: A volume of 2 mL of the extract dissolved in methanol was added with 3 drops of Rosenthaler reagent and 2 drops of sulfuric acid 18 M. Formation of violet color in the middle layer was indicative of terpenoids. Lieberman-Bouchard test: A volume of 2 mL of the extract dissolved in methanol was mixed with 1 mL of anhydrous acetic acid and 3 drops of sulfuric acid 18 M. After five min a blue color middle layer was indicative of sterols, but a pink, red or violet color revealed the presence of terpenoids.

g) Testing for quinones and anthraquinones (Tiwari et.al., 2011): Borntraguer test: An amount of 10 mg of each extract were dissolved in 3 mL of distilled water and filtrated by gravity. After filtration, three mL of 5% potassium hydroxide solution were added to each one. The mixture was heated to boiling for three min. Alkaline solution was allowed to cool down and then extracted with 3 mL of chloroform. The organic phase was separated and shaken with 2 mL of 5% potassium hydroxide solution. Occurrence of red color in alkaline phase indicated the presence of quinones. Test with ammonium hydroxide: One drop of concentrated ammonium hydroxide was added to 10 mg of each extract, previously dissolved in methanol. After 2 min, formation of red colour indicated the presence of anthraquinone. Test with sulfuric acid: One drop of concentrated sulfuric acid was added to 10 mg of each extract dissolved in methanol. Formation of red colour indicated the presence of quinones.

h) Testing for tannins (Shyamala-Gowri, 2010): 100 mg of each extract was dissolved in 10 mL of methanol, each methanol extract was mixed with 25 mL of distilled water and allowed to boil for 15 min. Once the mixture is fresh, 0.2 mL of 10% sodium chloride solution were added and then filtered by gravity. The filtrate was divided into four equal portions in test tubes. A volume of 5 drops of 1% jelly solution were added to the first portion, five drops of gelatin-salt solution (1% jelly + 10% salt) were added to the second, four drops of 10% ferric chloride solution were added to the third, and 3 drops of 1% potassium ferricyanide solution were added to the fourth portion. Precipitation observed after addition of either second or third reagent was indicative of tannins. Those samples showing grayish colour after addition of third reagent indicated the presence of tannins with catechol or pyrogallol nuclei. Samples turning to blue colour after the fourth reagent revealed the presence of phenolic compounds.

i) Testing for phenols (Shyamala-Gowri, 2010): An amount of 10 mg of each extract were dissolved in 1 mL of methanol, then 2 mL of distilled water was added followed by 4 drops of ferric chloride aqueous solution 10% w/v. Formation of a blue or green color indicated the presence of phenol.

j) Testing for saponins (Trease & Evans, 2002): Foam height test (without sodium bicarbonate: One mL of distilled water was added to 10 drops of the extract dissolved in methanol (20 mg mL^{-1}) in a test-tube, shaken vigorously to froth, then allowed to stand for 30 min. Saponin content was measured as follows: no froth (absence); froth less than 3 mm high (low); froth 6 mm high (moderate) and froth greater than 8 mm high (abundant). Foam test (with sodium bicarbonate): One mL of distilled water and 1 drop of sodium bicarbonate saturated solution were added to 5 drops of the extract dissolved in methanol (20 mg.mL^{-1}) in a test-tube and shaken vigorously during three min. Formation of honeycomb shaped foam indicated the presence of saponins.

2.5. Antioxidant activity:

Free radical scavenging activity, total phenolic and total flavonoid content assays were performed in order to determine antioxidant activity on the extracts under investigation.

a) 2,2-diphenyl-1-picryl-hydrazyl (**DPPH**) radical scavenging method (Goupy y col., 2009): Different concentrations ranging between 2 to 200 µg mL^{-1} of each extract were added to 2.8 mL of DPPH solution at 6 x 10^{-2} mM diluted in methanol. The mixture was left in the dark at room temperature for 30 min; then, the absorbance at 517 nm was measured using an UV-visible spectrophotometer (Perkin Elmer, Lambda 11).

DPPH test was accomplished by triplicate; ascorbic acid at concentration between 0.4 to 2.0 µg mL^{-1} (Y= 36.808X-0.2649; r = 0.9964) was used as positive control while methanol was measured as negative reaction. The capability to scavenge the DPPH radical was calculated by using the following equation:

$$\textbf{DPPH scavenging effect}\ (\%) = \frac{\mathbf{A_1 - A_2}}{\mathbf{A_1}}\ \mathbf{x\ 100}\ (\mathbf{1})$$

Where: $\mathbf{A_1}$ = absorbance of control reaction (**DPPH** + methanol), $\mathbf{A_2}$ = absorbance in the presence of sample (**DPPH** + sample).

The $\mathbf{IC_{50}}$ value was defined as the concentration (µg mL^{-1}) necessary to decrease the initial DPPH amount by 50%.

b) Total Phenolic Content (Folin-Ciocalteu method) (Škerget et.al., 2005): A volume of 0.5 mL of each extract previously diluted in methanol was added to 2.5 mL of Folin-Ciocalteu reagent and left at room temperature for three min. Then, 2 mL of sodium carbonate 0.71 M were added to the mixture and left in the dark for another 5 min. Absorbance was measured at 760 nm using an UV-visible spectrophotometer (Perkin Elmer, Lambda 11). Gallic acid was used as positive control at concentration ranging between 2 to 16 µg mL^{-1} (Y= 0.0632X+0.0024; r = 0.9989), while methanol was measured as negative reaction. Total phenolic content was determined as milligrams of Gallic acid equivalents per gram of dry extract (**mg Eq AG / g Ext**).

c) Total Flavonoid Content (Kim et. al., 2003): A mixture of 0.3 mL of 0.72 M sodium nitrite and 0.3 mL of 0.75 M aluminum chloride were added to each sample previously diluted in methanol. The mixture was left at room temperature for 5 min. Then, 2 mL of 1 M sodium hydroxide solution was also added. Distilled water was incorporated to each sample to complete a total volume of 10 mL. After mixing each sample by using a vortex, the absorbance at 510 nm was measured using an UV-visible spectrophotometer (Perkin Elmer, Lambda 11). Quercetin was used as positive control at concentration ranging between 10 to 25 µg mL^{-1} (Y = 0.0135X-0.0021; r = 0.9987). Flavonoid content was determined as milligrams of quercetin equivalent per gram of dry extract (**mg Eq Q / g Ext**).

Assays were carried out by triplicate and values are expressed as means of standard deviation (± SD). Data was subjected to two-way variance analysis (ANOVA) and significant difference between means was determined by LSD Fisher test ($p < 0.05$) using Statistical Package R Core Team (2015).

2.6. In Vitro Solar Protection Factor Determination (SPF):

This study was carried out according to the method described by Mansur et.al. (1986). This method is based on measuring the absorption of solar filters through a spectrophotometric analysis on a diluted solution at a concentration of 0.2 mg mL^{-1}. This low-cost, fast and simple procedure allows for a correlation with the *In Vivo* assay.

The standard solutions of **VB** and **VM** were prepared as follows: 100 mg of each dry methanol extract were weighted separately and transferred to 100 mL volumetric flasks. This extract was diluted with 50 mL of ethanol, filtrate to eliminate suspended particles and then more ethanol was added to obtain a 1000 µg mL^{-1} concentration for each extract. From these solutions, 5 mL aliquots were taken and transferred to 25 mL volumetric flaks and completed with ethanol until reach the required volume. Absorbances were measured within the range of 290 nm to 320 nm (**UVB**) at 5 nm intervals using a 1 cm optical path quartz cell. **SPF** was determined applying the following equation:

$$\mathbf{SPF} = \mathbf{CF} \times \boldsymbol{\Sigma}_{\mathbf{290}}^{\mathbf{320}} \times \mathbf{EE}_{(\lambda)} \times \mathbf{I}_{(\lambda)} \times \mathbf{Abs}_{(\lambda)} \quad (2)$$

Where: **SPF**= Solar Protector Factor, **CF**= 10 (correction factor), $\mathbf{EE}_{(\lambda)}$= wavelength λ,radiation eritemogenic effect $\mathbf{I}_{(\lambda)}$= wavelength λ sun intensity, $\mathbf{Abs}_{(\lambda)}$= wavelength λ absorbance. The product obtained of $\mathbf{EE}_{(\lambda)}$ and $\mathbf{I}_{(\lambda)}$ are constants established by Sayre y col. (1979), and these are described in Table 1.

Table 1.-Function of the normalized product used in the calculation of the **SPF**

Wavelength (nm)	**290**	**295**	**300**	**305**	**310**	**315**	**320**	**Total**
$\mathbf{EE}_{(\lambda)} \times \mathbf{I}_{(\lambda)}$	0.0150	0.0817	0.2874	0.3278	0.1864	0.0839	0.0180	1.000

3. Results and Discussion

Phytochemical screening of methanolic extracts of **VB** and **VM** was carried out to identified the presence of different secondary metabolites such as: alkaloids, coumarins, phenols, flavonoids, glycosides, quinones, saponins, tannins, steroids and triterpenes. Results (Table 2) show abundant presence of anthraquinones and glycosides for both species while anthrones, tannins and quinones were observed moderately. Flavones showed abundant presence in **VB** but moderate for **VM**. Xanthones were only observed in **VB**, while, flavonols in **VM**. Regarding phenols and saponins, these were detected in low to moderate concentrations for both species analyzed. Furthermore, Lieberman and Rosenthaler tests revealed triterpenes for **VB** and **VM**, while Salkoswky test showed abundant steroids for **VB** and moderate for **VM**. Alkaloids, coumarins and mucilages were not detected in these assays**.**

Table 2.-Phytochemical screening of *Vismia baccifera* and *Vismia macrophylla* methanolic extracts.

Secondary metabolites	**Tests**	**VB**	**VM**	**Secondary metabolites**	**Tests**	**VB**	**VM**
Quinones Anthraquinones	NH_4OH conc	+++	+++	**Tannins**	Jelly 1%	+	+
	H_2SO_4 conc	++	++		Jelly-NaCl	++	++
	Borntraguer	++	++		$K_3Fe(CN)_6$	+	+
Glycosides	$NaOH_{conc}$	+++	+++		$FeCl_3$ 10%	++	++
	KellerKilliani	-	-	**Saponins**	Foam height	++	+
Steroids	Lieberman Bouchard	++	+++		$NaHCO_3$	+	+
Terpenoids	Rosenthaler	+	+	**Alkaloids**	Dragendorff	-	-
	Salkowskí	++	+++	**Coumarins**	NH_4OH	-	-
Flavonoids	Shinoda	++	++	**Mucilages**	Cooling to 5ºC	-	-
	Pew´s	+++	++	**Phenols**	$FeCl_3$ 5%, NaCl 0,9%	+	++
	NaOH 10%	++	+++				

VB: *Vismia baccifera*, **VM**: *Vismia macrophylla*, abscence: (-), low: (+), moderate: (++), high: (+++).

According to literature, anthraquinones, xanthones, anthrones, bianthrones and other related compounds are commonly observed in *Vismia* species. Similarly, glycosides, flavonoids, phenols triterpenes and saponins, have been previously isolated from different *Vismia* species such as *V. parviflora, V. guianensis, V. laurentii, V. guaramirangae, V. japurensis, V. martiana, V. rubescens, V. baccifera, V. cayenensis* and *V. magnoliifolia* (Marin et.al., 2017, da Silva et.al., 2016, Hussain et.al., 2012) However, alkaloids, coumarins and mucilages were not detected either in **VB** or **VM**. These analyses confirm previous investigations, where none of these secondary metabolites has been reported (Buitrago et.al., 2016; Vizcaya et.al., 2012).

Antioxidant activity evaluation of **VB** and **VM** methanolic extracts was carried out following **DPPH** method using ascorbic acid as reference pattern to build up the calibration curve (Y= 35.906X-0,2589; r= 0.9976). Results revealed a high free radical scavenger capacity for both species at low doses ($\mathbf{IC_{50}}$ < 6 µg mL^{-1}). This behavior might be related to aromatic polyoxygenated compounds present in high concentrations in these extracts.

Student´s T statistical analysis (Figure 1), allowed to compare the **IC_{50}** values (**VB**: 5.90 µg mL^{-1} and **VM**; 5.65 µg mL^{-1}), with a confidence level of 95% (α=0.05). Results indicate that there is no significant difference in both species assayed regarding the free radicals scavenger capacity since the confidence intervals intersect (**VB**= 4.51 to 7.22 / **VM**=3.82 to 6.89).

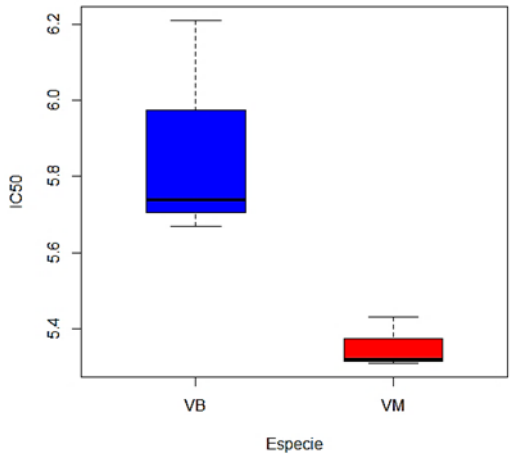

Fig 1.-Comparison of **IC_{50}** values of methanol extracts of **VB** and **VM**.

Likewise, total phenols were determined through the lineal regression equation obtained with galic acid (**AG**) used as calibration standard (Y=0.0742X–0.0021; r= 0.9969). Results were expressed as milligram equivalents for each gram of extract. In this regard, phenols concentration observed for **VB** and **VM** were established in 375.32 mg **Eq AG/g Ext** and 381.23 mg **Eq AG/g Ext**, respectively. Figure 2 obtained from Student T statistical assay (95%; α=0.05); shows a higher concentration of this kind of compound in **VM** extract.

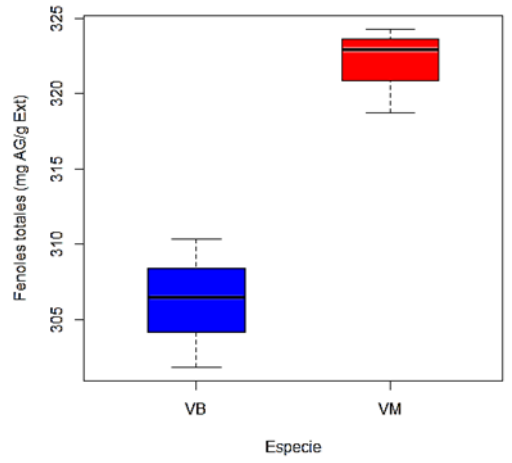

Fig 2- Comparison of Total Phenols Content for methanolic extracts of **VB** and **VM**.

On the other hand, according to the values obtained from the equation (Y=0.0143X-0.0026; r= 0.9981) derived from the calibration curve with Quercetin (**Q**), used as pattern, allowed to established a higher concentration of total flavonoids content for **VB** (267.07 mg **Eq Q/g Ext**) compared to **VM** (195.94 mg **Eq Q/g Ext**). This data was checked with Student T test values (95%; α=0.05), (Table 3).

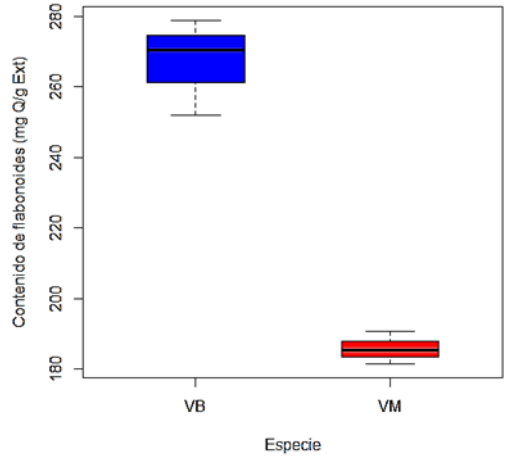

Fig 3- Comparison of Total Flavonoids Content of **VB** and **VM** methanol extracts.

Ethanol extract of *V. macrophylla* collected from Chocó, Colombia also showed antioxidant activity, researchers suggested a correlation between this activity and phenol compounds present in the extract (Gutiérrez et.al., 2011) Likewise, another investigation revealed that stem bark and flower extract of *Vismia* cauliflora have antioxidant activity by preventing oxidative burst in neutrophils and oxidative damage in erythrocytes (Ribeiro et.al., 2015). Results obtained in this investigation proved a relationship between the antioxidant activity, total phenol and flavonoids content of the extracts analyzed. Those samples with higher phenolic concentration showed higher antioxidant activity as well. Thus, these plant species might be considered as a promising source for medicine purposes.

Regarding the **SPF** assay carried out using the *In Vitro* spectrophotometric method; results revealed that sample collected from Mérida state showed a better photoprotective capacity (**VBFPS**= 25.3 ± 0.012) comparing to the species collected from Táchira state (**VMFPS**= 20.7 ± 0.024). These values are considered, according to the European Cosmetic and Perfumery Association (COLIPA) and Food and Drug Administration (FDA) with high degree of sun protection for **UVB** radiations.

The results observed might be associated to the presence, in both species, of moderated to high concentrations of chemical compounds such as quinones, anthraquinones, flavonoids and phenols that were determined through the

phytochemical screening. According to references consulted these compounds also possess antiinflammatory activity and have been used to treat erythema caused for the long exposure to solar radiation. Likewise, antioxidant activity might act to enhance the photoprotective activity that is important for skin health (Gilaberte et. al., 2003, Azcona, 2003, Nur et.al., 2018).

4. Conclusions

Results obtained in present investigation showed a relationship between phenols and flavonoids content with the antioxidant capacity of extracts assayed. In addition, the polyoxygenated secondary metabolites present in the extracts assayed possess a high **SPF** for the **UVB** radiations. This activity might be due to the presence of conjugated double bond present in these chemical structures that allows to absorb the radiation between UV wavelength of 280 nm to 315 nm. In this way, these compounds may block the transmission of solar radiation to the internal tissues avoiding the harmful effects of these.

Skin cancer and some other alterations related to ultraviolet radiation increases every day. In this regard, the use of different photoprotection methods are recommended in order to decrease the incidence of skin injuries. The combination of different photoprotective agents potentiate its efficacy and security and therefore, allows an optimal skin protection. In this regard, Vismia macrophylla and Vismia baccifera methanol extracts showed a high antioxidant activity and high levels of solar protection for the **UVB** radiations, thus, these species are considered as sources of these secondary metabolites with potential use as sun protectors.

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