

Phenolic compounds, antioxidant properties and antifungal activity of jarilla (*Barkleyanthus salicifolius* [Kunth] H. Rob & Brettell)

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ABSTRACT

Plant biodiversity provides a natural source of several compounds with biological activity, such as antioxidant and antifungal properties; such effects are related with the concentrations of phenols and flavonoids and different organs of the plants. In this research, aqueous (W) and 70% ethanol (Et70) extracts from flowers (F), leaves (L), stems (S) and roots (R) of *Barkleyanthus salicifolius* (Kunth) H. Rob. & Brettell were used to test antioxidant and antifungal activities and also the total concentration of phenols and flavonoids were analyzed; and types of phenolic acids and flavonoids were determined for each plant organ extract by HPLC. The Et70 enhance the extraction process of phenols and flavonoids, also showed higher antifungal and antioxidant activities. The obtained extract of the Et70 × F interaction showed the higher concentration of total phenols (57.90 mg GAE), flavonoids (91.03 mg QE) and antioxidant activity (285.07 Mm Trolox) per gram of dry extract. In addition, this extract shows an antifungal inhibition interval of 66.17% (*Fusarium oxysporum*) to 92.89% (*Colletotrichum gloeosporioides*). The phenol ferulic acid (35.57 mg g⁻¹) and the flavonoid naringenina (206.60 mg g⁻¹) were the compounds with the highest values founded in Et70-R, both of them compounds had been tested against fungal pathogens. Data indicate that antifungal and antioxidant activities are in function of phenolic acids and flavonoids concentration, as well as solvent used to extraction. Therefore, *B. salicifolius* species growing on wild form have the ability of accumulate several compounds with biological activity.

Key words: *Barkleyanthus salicifolius*, flavonoids, HPLC, phenolic acids, phytopathogens, willow ragwort.

INTRODUCTION

Phytochemical compounds from medicinal plants have a wide range of biological activity, that include the antibiotic, antioxidant and anti-inflammatory activities (Canadanovic-Brunet et al., 2009). Polyphenols, phenolic acids and flavonoids are powerful antioxidants and its antibiotic, antiviral, anticarcinogen, anti-inflammatory and vasodilator effects have been described (Ghasemzadeh et al., 2011). On the other hand, phenolic compounds are usually related to defense responses in plants; through different mechanisms, as preformed inhibitors or as response-synthesized compounds against pathogen

attack (Pusztahelyi et al., 2015). Also due to higher efficiency and low toxicity for humans, phenolic acids and flavonoids have been proposed as potential natural fungicides in order to control fungal phytopathogens, which are a threat for agriculture because damage to crops during developmental and postharvest phases causing high losses of fruits and vegetables (Zabka and Pavela, 2013).

Nowadays, phytopathogenic fungi are controlled by synthetic fungicides; however, their use is more restricted due to the negative impact over environment and human health; in addition, the fast development of fungal resistance to synthetic fungicides makes necessary to seek novelty and safe sources of molecules with antifungal and antioxidant activity: wild plants have a great potential because they accumulate thousands of secondary metabolites (Wianowska et al., 2016).

Over the last years, flavonoids have been used against fungal pathogen of plants, since some of them have the ability to inhibit the germination of fungal spores, meanwhile other cause deformation and cellular lysis (Pusztahelyi et al., 2015). Flavonoids such as quercetin and phenolic acids such as ferulic acid have shown antifungal activity against some phytopathogens as *Alternaria alternata*, *Rhizoctonia solani*, *Fusarium oxysporum*, *Botrytis cinerea* and *Phytophthora infestans* (Wianowska et al., 2016).

Barkleyanthus salicifolius (Kunth) H. Rob & Brettell (ex *Senecio salignus*) belonging to *Asteraceae* family, commonly named jara, jaktin, jara-tokstini, buralillo, jarilla, barilla or willow ragwort, is a common bush of 1.5 m high, it grows alone or associated to genera of *Poaceae* spp., between pines forest and hillsides at an altitude between 1200 and 3000 m throughout the north, west and center of Mexico. Domínguez et al. (2005) reported that the antioxidant activity is higher in methanolic extracts of flowers than leaves. *Barkleyanthus salicifolius* is used as anti-inflammatory, antirheumatic, antimigraine, nephroprotective and hepatoprotective, properties that could be related with presence of terpenoids, alkaloids and flavonoids (Pérez-Ochoa et al., 2016).

Phenolic compounds content and antioxidant activity of extracts of *B. salicifolius* have been reported and the plant is used in folk medicine to control gastrointestinal disorders; however; there are few data about the kind and quantity of phenolic and flavonoids compounds present at organ level in *B. salicifolius*; data could open the door to study the secondary metabolites of *B. salicifolius* to control different diseases caused by fungi at crops. Therefore, the aim of this work was to determine and identify phenolic and flavonoids compounds and their antioxidant activity in ethanolic (70%) and aqueous extracts from different organs of *B. salicifolius* and evaluate the effects against fungal phytopathogens.

MATERIALS AND METHODS

Collected plant material

Roots, stems, leaves and flowers of *Barkleyanthus salicifolius* (Kunth) H. Rob & Brettell were collected during dry season on January 2018, at Zitacuaro (19°25'44" N, 100°22'23" W; 1942 m a.s.l.), Michoacan, Mexico, where the accumulated precipitation was 917 mm³ and the annual average temperature was 17.6 °C. The weather of area is classified as Cwb according to Köppen (García, 2004). The plant was identified at the herbarium of the Universidad Autónoma del Estado de Hidalgo (collector: Hernández-Fuentes, A.D.; identification number 06).

Preparation of the extracts

A sample of 100 g dried roots (R), stems (S), leaves (L) or flowers (F) were mixed inside a 2 L-glass bottle with 1000 mL solvent, deionized water (W) or 70% ethanol solution (Et70). Mixtures were store in the dark at 20 °C for 15 d. Ethanolic extracts were vacuum evaporated in a rotary steamer at 45 °C (V-700, Büchi, Flawil, Switzerland) to concentrate samples. Later, samples were frozen and freeze drying at -40 °C and 5 mPa (Freezone 12, Labconco, Kansas City, Missouri, USA) to remove completely the solvent. Aqueous extract was directly frozen and freeze drying under previously described conditions. Samples were store in the dark at 4 °C.

Determination of total phenols content

Total phenols content (TPC) was determined using the Folin-Ciocalteu method, described by Waterman and Mole (1994). Briefly, 1 g dried sample of roots, stems, leaves or flowers was mixed with 10 mL 70% ethanol and later shaken in a vortex (IKA, Staufen, Germany) during 30 min and later was centrifuged at 10 000 × g (ST 16R, Thermo Fisher Scientific,

Waltham, Massachusetts, USA) for 10 min at 5 °C and the supernatant was recovered. After, 5 mL Folin-Ciocalteu reactant diluted 1:10 with distilled and deionized water were added to supernatant and left to rest for 7 min; later 4 mL sodium carbonate anhydrous (7.5% w/v) was added, the reaction was carried on for 2 h. Finally, the absorbance was measured at 760 nm in a UV/Vis spectrophotometer (6715, Jenway, Cole-Parmer, Staffordshire, UK). The results were expressed as milligrams of gallic acid equivalents per gram of dried extract (mg GAE g⁻¹ DE).

Determination of total flavonoid content

Total flavonoid content (TFC) was determined according to the method described by Chang et al. (2002) where 2 mL supernatant were mixed with 2 mL distilled water and 0.15 mL NaNO₂ (5% w/v); after 5 min, 0.15 mL AlCl₃·6H₂O (10% w/v) solution was added and the mix was store at room temperature for another 5 min; later, 1 mL NaOH (1 M) solution was added. The mix was left for 15 min in darkness; finally, absorbance was measured at 415 nm. The results were expressed as milligrams of quercetin equivalents per gram of dried extract (mg QE g⁻¹ DE).

Antioxidant activity

Antioxidant activity by 2,2'-diphenyl-1-picrylhydrazyl (DPPH) method was determined according to Brand-Williams et al. (1995). Sample (500 µL) was taken from the supernatant obtained of extractions and was mixed with 2500 µL DPPH[•] solution 6 × 10⁻⁵ M previously prepared. The mix was store for 1 h in darkness and at room temperature, later the absorbance was measured at 517 nm. The results are reported as µM of Trolox per gram of dried extract (µM T g⁻¹ DE).

On the other hand, antioxidant activity by 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) method was measured according to Re et al. (1999). The ABTS^{•+} was prepared as follows: ABTS (7 mM) was mixed with 10 mL potassium persulfate (2.45 mM) and shaken for 16 h protected from light. Later, when radical was obtained, a sample (100 µL) of supernatant (phenols and flavonoid extractions) was mixed with 3 mL ABTS^{•+} (stabilized reagent) and shook for 15 s, later the samples were stored for 6 min in darkness. After, absorbance was measured at 734 nm. The results were expressed as µM of Trolox per gram of dried extract (µM T g⁻¹ DE).

Antifungal activity

Phytopathogenic microorganisms used to assay the antifungal activity of the extracts were: the fungi *Sclerotium rolfsii*, *Colletotrichum gloeosporioides* (provided by laboratory of Phytopathology of Colegio de Posgraduados campus Montecillos, Estado de Mexico, Mexico), *Fusarium oxysporum* and *Rhizoctonia solani* and the oomycete *Phytophthora capsici* (provided by Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias [INIFAP], experimental field of the Bajío, Guanajuato, Mexico).

The strains were preserved on potato dextrose agar (PDA) (BD Bioxon, Becton Dickinson, Mexico) and stored at 4 °C. A 5 mm diameter disc of each phytopathogen was inoculated on PDA plates (90 × 15 mm) and incubated at 28 °C for 8 d at microbiological incubator (FE-133D, Felisa, Mexico).

Quantitative determination was carried out according to Meléndez-González et al. (2015). The media for antifungal activity assays were prepared by sterilizing a 2X of PDA solution that was diluted to 1X with sterile water; previously each extract was dissolved on sterile water and added to final solution obtaining 250 µg mL⁻¹ each extract in the media. Temperature was maintained at 40-45 °C during the process to keep a liquid medium and minimize the effects of high temperatures on the active compounds of the extracts; the prepared media were 10 for each phytopathogen: 8 for aqueous and ethanolic extracts of roots, stems, leaves and flowers; 1 negative control (medium without extract) and 1 positive control (250 µg mL⁻¹ captan; 2-(trichloromethylsulfanyl)-3a,4,7,7a-tetrahydroisoindole-1,3-dione). Four replicates of each treatment were used during the assay. Radial growth was recorded after 7 d of incubation. Percentage inhibition was calculated according to the following equation:

$$\%inh = [(A_c - A_p)/A_c] \times 100$$

where %inh is percentage of radial growth inhibition, A_c is fungal or oomycete growth on negative control, and A_p is fungal or oomycete growth on medium with extract or captan.

Identification of phenolic compounds

Phenolic acids and flavonoids were determined according to Aguiñiga-Sánchez et al. (2017). Analytes of the extracts were identified by high performance liquid chromatography (HPLC Agilent 1100, Agilent Technologies, Santa Clara, California,

USA). Phenolic acids were determined using a Nucleosil 100 SA column (Macherey-Nagel, Düren, Germany): 125 × 4.0 mm id and particle size 5 µm; with gradient elution, using H₂O at pH 2.5 with trifluoroacetic acid (TFA) as solution A and acetonitrile (ACN) as solution B, as follows: 10 min, 85% A; 20 min, 65% A; 23 min, 65% A. The injection volume was 20 µL, with a flow of 1 mL min⁻¹ at 22 °C; detection was carried out at 280 nm at an analysis time of 25 min. Standards used were caffeic, gallic, chlorogenic, vanillic, *p*-hydroxybenzoic, *p*-coumaric, ferulic and syringic acids (Sigma-Aldrich, St. Louis, Missouri, USA) and were injected at the same conditions. Regarding to flavonoids, the analysis was carried out using a column Hypersil ODS (Agilent): 125 × 40 mm id, particle size 5 µm, with gradient elution, using as solution A and B under the same conditions used for the phenolic acids; only with different gradients (10 min, 65% A; 20 min, 65% A; 25 min, 65% A) and temperature flow (25 °C). Detection was carried out at an analysis time of 25 min. Standards used were rutin, phloridzin, myricetin, quercetin, naringenin, phloretin and galangin (Sigma Aldrich) and were injected at the same conditions. Results are expressed as µg per gram of dried extract (µg g⁻¹ DE).

Experimental design and statistical analysis

Data were analyzed using a two-factorial ANOVA ($P \leq 0.05$) as follows: the solvent with two levels (water and 70% ethanol) and plant organs with four levels (root, stems, leaves and flowers). The ANOVA was followed by comparison of means by Tukey method with $P \leq 0.05$ for each one of response variables analyzed.

RESULTS AND DISCUSSION

Solvents, phenolic compounds and antioxidant activity

Table 1 shows the antioxidant activity by inhibition of ABTS^{•+} and DPPH[•] radicals; the higher activity was for Et70 extracts of jarilla, also higher phenolic compounds were showed; similar results were reported in other research (Ahmed et al., 2011); some authors reported that a binary system solvent has a better performance than a mono-solvent, water or pure ethanol (Do et al., 2014). Also, both types of solvents used for the extraction of antioxidant compounds recorded a higher antioxidant activity in the ABTS^{•+} assay compared to DPPH.

Table 1. Antioxidant activity and total phenols and flavonoids content of leaves, stems, roots and flowers of *Barkleyanthus salicifolius*.

Treatment	ABTS ^{•+} radical scavenging activity	DPPH [•] radical scavenging activity	Total phenols content	Total flavonoids content
	———— µM Trolox g ⁻¹ DE ————		mg GAE g ⁻¹ DE	mg QE g ⁻¹ DE
Extraction solvent effect				
Et70	192.43a	75.35a	31.92a	31.99a
Water (W)	56.29b	25.50b	3.67b	2.25b
Plant organ effect				
Flower (F)	208.97a	69.94a	31.98a	48.21a
Leaf (L)	108.73b	43.61b	11.39c	6.82c
Root (R)	92.57c	42.30c	17.22b	9.15b
Stem (S)	87.13d	39.87d	10.60d	4.31d
Extraction solvent × Plant organ interaction effect				
Et70 × F	285.07a	83.58a	57.90a	91.03a
Et70 × L	169.33b	75.38b	19.20c	10.51c
Et70 × R	161.77c	72.70c	32.28b	18.05b
Et70 × S	153.53d	69.77d	18.31d	8.35d
W × F	132.88e	56.30e	6.06e	5.39e
W × L	48.14f	23.84f	4.49f	3.12f
W × R	23.37g	11.90g	2.17g	0.26g
W × S	20.74h	9.97h	1.99g	0.25g

Values are mean of three replicates. Same letters in each column are significantly equal (Tukey, $p \leq 0.05$).
 ABTS: 2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid); DPPH: 2,2'-diphenyl-1-picrylhydrazyl;
 Et70: 70% ethanol; QE: quercetin equivalents; GAE: gallic acid equivalents; DE: dried extract.

The antioxidant activity by ABTS^{•+} and DPPH[•] as well as total content of phenols and flavonoids were significantly different for each plant organ (Table 1); flowers showed the highest antioxidant activity and total content of phenols and flavonoids, meanwhile the lowest values were for stems. There are reports of cell damage caused to reproductive tissues by different types of abiotic stress, this damage has effects in the number and quality of the seeds (De Storme and Geelen, 2014); and the increase of the reactive oxygen species (ROS) is associated to stress damage like light, cold and drought (Smith and Zhao, 2016).

On the other hand, leaves were the second organ with the highest antioxidant activity (Table 1). The photosynthesis process requires luminous energy and often this energy exceeds the amount manageable by the photosystems and the photodamage is triggered; however, plants have several mechanisms to protect the photosynthetic system, since the repair of the proteins involved, such as D1 (Kato and Sakamoto, 2009), until to the synthesis of compounds to dissipate excess energy and neutralize ROS generated during the photosynthesis process (Varela et al., 2016). Therefore, compounds such as phenols and flavonoids could be involved in the response protection of the photosynthetic apparatus of plants.

Roots showed a total content of phenols and flavonoids only lower than flowers but higher than leaves and stems (Table 1); similar data were found in another works (Bazdar et al., 2018). Roots are the first tissue that detects a condition of water deficit and induces signs of stress (Michel et al., 2012; Gutiérrez-Tlahque et al., 2019); the plant material was collected during cold-dry season, this environment could induce the accumulation of secondary metabolites, including phenolic compounds, which may play an important role in resistance to abiotic stress (Varela et al., 2016).

Interactions among the solvent extraction and plant organ of *B. salicifolius* are showed in Table 1. Organ hydroalcoholic extracts showed higher phenols and flavonoid content as well as higher antioxidant activity by ABTS^{•+} and DPPH[•] assays than organ aqueous extracts. The highest antioxidant activity was found in the Et70 × F interaction, meanwhile in the interaction W × S the lowest antioxidant activity values were recorded, these results follow a similar pattern as reported by Bazdar et al. (2018) at the plant *Prangos ferulacea* relative to the total content of phenols and flavonoids and the ability to inactivate the DPPH[•] radical; some studies had showed that increasing the percentages of water in solvents (alcohol, methanol, acetone) the concentrations of phenols and total flavonoids increase as well as the antioxidant activity (Anwar and Przybylski, 2012; Do et al., 2014), probably because the hydroalcoholic extracts contain polar and non-polar compounds.

The concentration of phenols and total flavonoids in Et70-R extracts is only below the concentration of the Et70×F interaction; meanwhile, in antioxidant activity the values are below the Et70 × L interaction that have less phenols and flavonoids, that is, there is no relation between concentration of phenols and flavonoids and antioxidant activity. The synthesis of secondary metabolites in plant systems is conditioned to the environment where they are growing to help them to survive, in addition, the tissues between genera and plant species can interact differently with solvents used for the extraction of phenolic compounds and even affect the chemical structure of these compounds, resulting in variations in antioxidant activity (Bystrická et al., 2010).

Effects of solvent and plant organ on antifungal activity

Antifungal activity in relation to the solvent was assessed against phytopathogenic fungi *Sclerotium rolfsii*, *Colletotrichum gloeosporioides*, *F. oxysporum*, *Rhizoctonia solani* and the oomycete *Phytophthora capsici* (Table 2). The highest antifungal activity was developed by ethanolic extracts which could be related to the content of phenolic compounds which is the highest in ethanolic extracts. Differences in inhibition values of different treatments could be due to interactions of phenolic compounds with membrane proteins of different phytopathogens and to the mechanisms that microorganisms begin in response to the presence of phenols and flavonoids (Rodríguez-Pedroso et al., 2012; Pusztahelyi et al., 2015).

When factor organ of the plant was analyzed (Table 2), extracts of flower showed the highest antifungal activity followed by leaf, root and stem for fungi *F. oxysporum*, *S. rolfsii* and *R. solani*; the stem showed the lowest antifungal activity except for *P. capsici* and *C. gloeosporioides* where the lowest antifungal activity was of root extracts; however, nonsignificant difference in growth inhibition of *P. capsici* between leaf and stem extracts were observed; according to Rhouma et al. (2009) these secondary metabolites are preformed antibiotics and further synthesized from remote precursors in response to pathogen attack.

Table 2. Antifungal activity from extracts of *Barkleyanthus salicifolius* expressed as mycelial growth inhibition percentage.

Treatment	<i>Fusarium oxysporum</i>	<i>Phytophthora capsici</i>	<i>Colletotrichum gloeosporioides</i>	<i>Sclerotium rolfii</i>	<i>Rhizoctonia solani</i>
Extraction solvent effect					
Et70	29.05a	59.80a	61.77a	49.38a	46.23a
Water (W)	25.14b	47.68b	21.97b	22.28b	12.21b
Plant organ effect					
Flower (F)	58.35a	70.91a	53.53a	82.80a	60.69a
Leaf (L)	34.48b	47.40b	47.01b	42.45b	24.81b
Root (R)	11.41c	46.32c	30.24d	11.70c	21.49c
Stem (S)	4.12d	50.33b	36.75c	6.41d	9.88d
Extraction solvent × Plant organ interaction effect					
Et70 × F	66.17a	82.53a	92.89a	89.46a	83.64a
Et70 × L	59.69b	48.86cd	68.41b	82.03b	44.85b
Et70 × R	18.30d	50.09c	38.97d	13.47d	37.16c
Et70 × S	6.79ef	57.73b	46.81c	12.59d	19.28d
W × F	50.53c	59.30b	38.68d	60.13c	37.74c
W × L	9.28e	45.94de	0.97f	2.86e	4.78e
W × R	4.52ef	42.93ef	21.50e	9.89d	5.82e
W × S	1.47f	42.53f	26.71e	0.22e	0.50f

Means with same letters in each column are significantly equal according to Tukey ($p \leq 0.05$).

Et70: 70% Ethanol.

The analysis of the interaction Solvent × Plant organ on the inhibition of phytopathogenic fungi (Table 2) showed that the highest percentage of inhibition on *F. oxysporum*, *C. gloeosporioides*, *P. capsici*, *S. rolfii* and *R. solani* was for Et70 × F interaction, which also showed the highest antioxidant activity and content of phenolic compounds (Table 3), characteristics that have been positively related to antifungal activity similar to the reported by Jeyaseelan et al. (2012) in the plant *Lawsonia inermis*. The interactions W × S and W × R had the lowest inhibition percentage for fungi *F. oxysporum*, *P. capsici* and *C. gloeosporioides*; meanwhile the W × S interaction showed the lowest antifungal activity against *S. rolfii* and *R. solani*; the solvent used to extract secondary metabolites with antifungal properties is an important factor; for example, Eloff et al. (2017) used solvents with different polarities at the plant *Meliantus comosus* found that the best averages of minimum inhibitory concentrations for different fungi were into the range of 3.1 to 5.1 (polarities), where the ethanol is located.

Table 3. Phenolic acids and flavonoids content in the extracts of different organs of *Barkleyanthus salicifolius*.

	Et70 × F	W × F	Et70 × L	W × L	Et70 × R	W × R	Et70 × S	W × S
	µg g ⁻¹ dried extract							
Phenolic acids								
Gallic	41.07	9.43	29.91	12.90	184.08	11.87	64.46	50.65
Chlorogenic	252.22	1 285.61	6 890.16	161.24	8 468.95	271.33	2 567.92	176.33
Syringic	38.28	85.12	571.78	ND	631.95	56.08	35.68	218.18
Vanillic	29.11	112.83	1 349.98	109.06	203.43	26.93	469.96	25.47
<i>p</i> -Hydroxy-benzoic	797.51	ND	ND	52.68	ND	95.03	110.22	ND
Caffeic	38.73	84.08	1 725.68	3 273.00	43.20	ND	312.97	153.75
Ferulic	85.18	236.38	3 921.50	75.20	35 575.45	148.43	39.33	30.84
<i>p</i> -Coumaric	919.16	10.48	20.39	15.08	11 092.05	28.68	67.63	11.83
Flavonoids								
Rutin	1 076.82	1 699.67	1 672.69	310.59	827.11	369.41	267.75	155.88
Phloridzin	5 203.71	15 683.48	13 156.42	3 558.60	1562.20	7 715.31	181.84	1 661.24
Myricetin	85.51	825.59	4079.86	1 111.81	ND	4 172.46	ND	625.72
Quercetin	184.64	14.56	323.12	471.84	6773.83	1 683.58	386.65	102.21
Naringenin	12 482.31	279.71	79 802.34	10 656.87	206 593.55	12 480.03	5 323.10	15 246.81
Phloretin	3 464.51	26.12	140.27	5 137.70	2597.81	583.50	101.90	780.81
Galangin	2 765.02	121.67	5 345.72	1 311.47	17 710.56	157.50	304.82	309.09

Data are mean of two replicates.

ND: Not detected; Et70: 70% ethanol; W: water; F: flower; L: leaf; R: root; S: stem.

Identification of phenolic compounds and flavonoids by HPLC

Table 3 shows the concentrations of phenolic compounds identified by HPLC among different treatments; some phenolic compounds analyzed have not been reported in *B. salicifolius* extracts. The highest concentrations of chlorogenic acid, belonging to the hydroxycinnamic acids group, were found in W-F, Et70-L, W-L, W-R and Et70-S extracts; while *p*-coumaric acid was most commonly presented in Et70-F and Et70-R extracts. The syringic acid was detected at high amounts in the Et70-R, Et70-L and W-T in reference to other extracts; meanwhile the *p*-hydroxybenzoic acid was determined at 7-fold concentration in Et70-F comparing to Et70-S extract, which is followed by the aqueous extracts of root and leaf.

Some natural phenolic acids have been shown antifungal activity and have been considered as biofungicides; chlorogenic acid is related to the inhibition of fungal pathogens relevant for horticultural crops (Martínez et al., 2017); also, antifungal activity of *p*-coumaric acid, caffeic acid, vanillic acid, gallic acid, ferulic acid and syringic acid have been showed against *F. oxysporum*, *Fusarium verticillioides*, *Penicillium brevicompactum*, *P. expansum*, *Aspergillus flavus* and *A. fumigatus* (Zabka and Pavela, 2013). Caffeic and gallic acids individually did not show effect on *Candida tropicalis* but had a synergistic effect in association with fluconazole (Lima et al., 2016). The activity could be a dynamic response in the accumulation of phenolic compounds by plants to stressful situations.

Flavonoids concentration of *B. salicifolius* for each plant organ, are shown in Table 3. A novelty research has been made because there are no previous reports in the literature regarding to these types of extracts. All compounds were detected in all eight extracts except myricetin, that was not detected in Et70-R and Et70-S. Flavone naringenin was the compound detected in highest amounts in Et70-R, then quercetin followed by phloretin in W-L, galangin in Et70-R, and phloridzin in Et70-F, among the highest concentrations detected. Flavonols as quercetin, myricetin, rutin, naringenin and galangin isolated from various sources showed inhibitory effect against *Candida albicans* at minimum inhibitory concentrations (MIC) within the range of 4 to 441 $\mu\text{g mL}^{-1}$ (Seleem et al., 2017); on the other hand, quercetin and rutin showed a synergistic effect in combination with amphotericin B against *Cryptococcus neoformans* (Oliveira et al., 2016).

The use of flavonoids extracted from wild plants with medicinal uses for the control of bacterial and fungal diseases has become very important, as they can be precursors of toxic substances for some pathogens (Rhouma et al., 2009). The mechanism by which flavonoids can inhibit microorganisms are the vital enzymatic pathways, such as cytochrome P450-dependent oxidases, in which flavonoids specifically block steroid hydroxylase enzymes (Arif et al., 2009); thus, the presence of these compounds and their characteristics allow to consider *B. salicifolius* extracts as an alternative for the biocontrol of plant pathogenic microorganisms.

CONCLUSIONS

The use of 70% ethanol allowed to extract higher concentration of phenolic acids and flavonoids than water from the *Barkleyanthus salicifolius* and favored antioxidant activity; the antifungal activity of the different extracts against five phytopathogens, showed differences between extracts of the organs and depending on the solvent used. The metabolites identified by HPLC had not been described previously in *B. salicifolius* extracts, but their antifungal activity has been tested individually and synergistically, either with other molecules of natural origin or with compounds used commercially to eradicate fungal infections of plants. These results show that wild plants used in traditional medicine remain an option as source of compounds with biological activity for the biocontrol of crop diseases and in other fields of human interest such as the pharmaceutical industry.

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