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Biological activity and flavonoid profile of five species of the *Bursera* genus

Actividad biológica y perfil de flavonoides de cinco especies del género *Bursera*

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Abstract

Background: The genus *Bursera* Jacq. ex. L. concentrates its diversity in Mexico. Among the secondary metabolites that can be isolated from organic its extracts are flavonoids and lignans.
Research question: Do the biological activity of the hydroalcoholic extracts from five *Bursera* species studied depend on their flavonoid content?
Study site: Oaxaca, Mexico.
Methods: The extracts of *B. aptera* Ramírez, *B. fagaroides* (Kunth) Engl., *B. schlechtendalii* Engl., *B. galeottiana* Engl., *B. morelensis* Ramírez were analysed by High Resolution Liquid Chromatography (HPLC) to determine their flavonoid composition. The antiradical DPPH, anti-inflammatory, antibacterial and antifungal activities of the extracts were determined.
Results: Phlorizin and quercetin were conserved in both stems and leaves of the five species studied. The phloretin was present only in the leaves of *B. aptera*.The highest antibacterial activity was observed against *Staphylococcus epidermidis* and *Salmonella typhimurium* by the extracts of leaves of *B. aptera* (MIC 0.002 mg/mL) and the stems of *B. fagaroides* (MIC 0.015 mg/mL). The extract of stems of *B. morelensis* against *Fusarium boothii* presented the highest antifungal effect (lower than 50 µg/mL). The anti-inflammatory activity of *B. fagaroides* was 51.24 % edema inhibition (1 mg/ear) compare to 78.76 % of indomethacin (0.358 mg/ear).
Conclusion: The presence of flavonoids in five species studied suggests the richness of these compounds in *Bursera* genus. The biological activities studied indicated the potential medicinal use of these species and the role that the flavonoids play in plant physiology.
Key words: Antibacterial, antifungal, anti-inflammatory, DPPH, leaves, stems.

Resumen

Antecedentes: el género *Bursera* Jacq. ex. L. concentra su diversidad en México. Los metabolitos secundarios que pueden aislarse de sus extractos orgánicos son los flavonoides y los lignanos.
Pregunta de investigación: ¿La actividad biológica de los extractos de las cinco especies de *Bursera* estudiadas depende de su contenido de flavonoides?
Sitio de estudio: Oaxaca, México.
Métodos: Los extractos hidroalcohólicos de *B. aptera* Ramírez, *B. fagaroides* (Kunth) Engl., *B. schlechtendalii* Engl., *B. galeottiana* Engl., *B. morelensis* Ramírez, se analizaron por HPLC para determinar su composición de flavonoides y se reporta su actividad antiradicalaria al DPPH, antiinflamatoria, antibacteriana y antifúngica.
Resultados: La florizina y la quercetina se conservaron tanto en tallos como en hojas de las cinco especies estudiadas. La floretina está presente solo en las hojas de *B. aptera*. La mayor actividad antibacteriana se observó contra *Staphylococcus epidermidis* y *Salmonella typhimurium* por los extractos de hojas de *B. aptera* y tallos de *B. fagaroides*. El extracto de tallos de *B. morelensis* contra *Fusarium boothii* presentó el mayor efecto antifúngico. La actividad antiinflamatoria de *B. fagaroides* fue 51.24% de inhibición del edema (1 mg/oreja) en comparación con el 78.76% de la indometacina (0.358 mg /oreja).
Conclusión: La presencia de flavonoides en las cinco especies estudiadas sugiere la riqueza de estos compuestos en el género *Bursera*. Las actividades biológicas estudiadas indicaron el posible uso medicinal de estas especies y el papel que juegan los flavonoides en la fisiología de las plantas.
Palabras clave: antibacteriano, antifúngico, antiinflamatorio, DPPH, hojas, tallos.

The genus *Bursera* plays an ecological role in the tropical deciduous forests of Mexico, where its diversity is greatest. Among the secondary metabolites isolated from this genus are polyphenolic compounds (Marcotullio *et al.* 2018).

Flavonoids are phenolic compounds isolated from a broad range of plants. There are approximately 8,000 known compounds with wide-ranging structural variety, classified as follows: anthocyanins, chalcones, flavanones, flavones, flavonols and isoflavonols (Panche *et al.* 2016). Many studies have analysed flavonoids as antioxidant, antibacterial and anti-inflammatory agents (Kumar & Pandey 2013). Flavonoids are not the only substances with these properties. It has been reported that the antioxidant properties of the essential oil from *B. graveolens* may be associated with its main constituents, limonene (34.9 %) and *α*-terpineol (13.4 %) which are terpenes (Fon-Fay *et al.* 2019). Furthermore, it has been demonstrated that the essential oil of *B. morelensis* includes terpenes, which contribute to its antimicrobial activity (Canales-Martínez *et al.* 2017).

Furthermore, in *B. simaruba,* the flavonoids luteolin, apigenin and kaempferol have been identified, these being three flavonoids with recognized strong antioxidant properties (Bah *et al.* 2014). In a recent work, six flavonoids have been reported in organic polar extracts of thirteen *Bursera* species, quercetin, rutin, naringenin, naringin, apigenin and apiin (Guevara-Fefer *et al.* 2017), the aerial parts of *B. grandifolia* have a high phenolic content and free radical scavenging activities (Ruiz-Terán *et al.* 2008). It has known that flavonoids participate in the defense mechanism of the plants protecting them against herbivorous organisms, phytopathogenic insects, bacteria and fungus pathogenic (Marcotullio *et al.* 2018). In addition, there are not enough studies regarding to the antimicrobial, antifungal properties of these species and majority of the works were made on barks and resins, and fewer on leaves, fruits or seeds. Based on the above findings, the aim of this work was to determine the flavonoid profile of hydroalcoholic extracts from the leaves and stems of the five mentioned species, the flavonoids found are shown in Figure 1. Their antibacterial, antifungal, antiradical DPPH and anti-inflammatory activities were also evaluated.

Material and methods

Plant material. The plant material was collected of adult organisms in Oaxaca state, Mexico in September 2015, rainy season and taxonomically determined by Rosa Linda

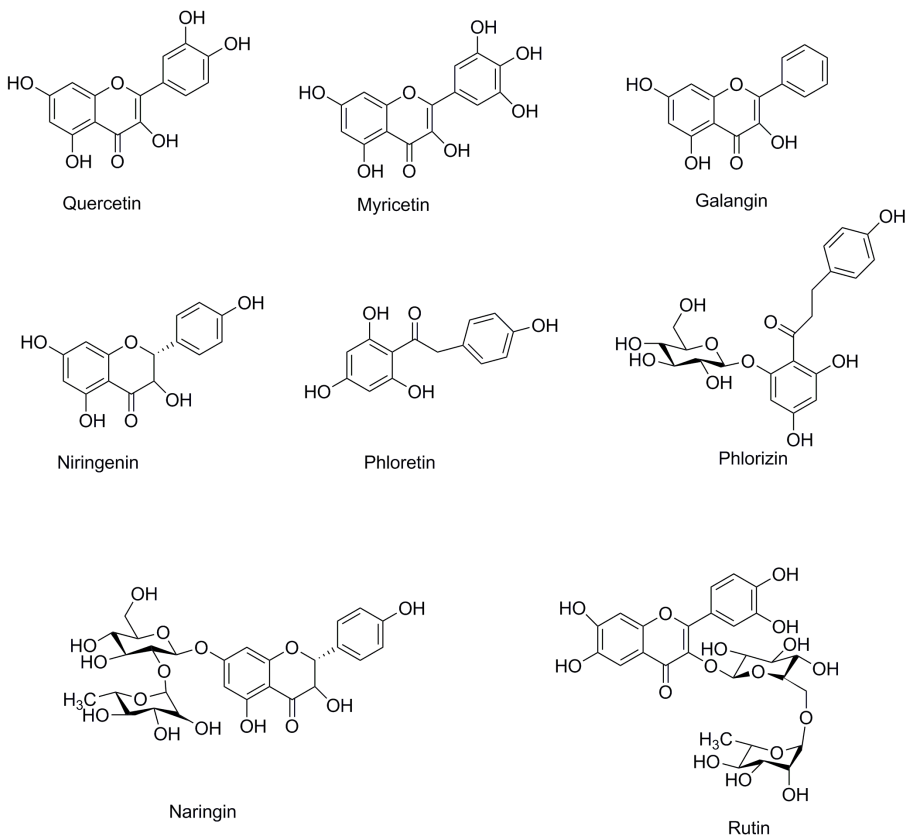

Figure 1. Chemical structures of flavonoids identified from *B. aptera*, *B. fagaroides*, *B. schlechtendalii, B. galeottiana*, *B. morelensis.* The chemical structures were drawn using ChemBioDraw 13.0 Ultra.

Medina Lemos. Collection data is presented in table 1, voucher specimens were deposited in the herbariums of the Faculty of Sciences, UNAM. The extracts were prepared from air-dried leaves and stems of the plants (150 g each), using ethanol (70 %) in proportion 1:5 and maceration at room temperature for 24 h. The solvent was removed with a rotavapor by reduced pressure distillation.

Table 1. Collection data of the studied species of *Bursera*.

Species	Code	Voucher #
***Bursera aptera* Ramírez**	Bap	FCME162074
***Bursera schlechtendalii* Engl.**	Bsc	FCME162073
***Bursera fagaroides* (Kunth) Engl.**	Bfa	FCME162071
***Bursera galeottiana* Engl.**	Bga	FCME162075
***Bursera morelensis* Ramírez**	Bmo	FCME162072

Flavonoid profiling. The extracts were analysed by high-performance liquid chromatography (HPLC) on a Hypersil ODS (125 × 4.0 mm) Hewlett Packard column eluted with a gradient of (A) H_2O adjusted at pH 2.5 with trifluoroacetic acid and (B) acetonitrile; 0-10 min, in the following mixtures, A:B 85:15; for 20 min, and A:B 65:35 for 25 min. The following parameters were used: flow at 1 mL/min at 30 °C, detection wavelengths, 254, 316 and 365 nm; injection volume, 20 µL and analysis time, 25 min. The analysis of naringin was performed in a Nucleosil 100 A column 125 × 4.0 mm i. d., 5 µm C-18. The flow of the mobile phase remained at 1.3 mL/min and consisted of (A) acetonitrile, (B) water adjusted at pH 3.0 con H_3PO_4 with the following gradient: 0.10 min, 12 % A, 88 % B; 10 min, 18 % A, 82 % B; 15 min, 18 % A, 82 % B; 30 min, 45 % A, 55 % B and 32 min, 100 % A, at a temperature of 30 °C. The injection volume of the sample was 20 µL. The equipment was calibrated to a wavelength of 350 nm. The analysis time for this method was 32 minutes. Identification was done by comparison with retention time of standards. Quantification was based on the calibration curves of the reference compounds, obtained under the same chromatographic conditions. The standards used were rutin, phlorizin, myricetin, quercetin, naringenin, naringin, phloretin and galangin.

Antibacterial activity. Bacterial strains used: *Bacillus subtilis* (Ehrenberg 1835) Cohn (ATCC6633), *Escherichia coli* (Escherich) (ATCC11229), *Staphylococcus epidermidis* (Winslow & Winslow) Evans (ATCC12228), *Pseudomonas aeruginosa* (Schroeter) (ATCC27853), *Staphylococcus aureus* Rosenbach (ATCC 6538), *Salmonella typhimurium* (Kauffmann & Edwards) Le Minor & Popoff (ATCC14028), *Salmonella typhi* (Schroeter) Warren and Scott (ATCC6539) and *Staphylococcus aureus* ATCC 29213 (donated by the Microbiology Laboratory at the Department of Biology of the Chemistry Faculty, UNAM).

Bacterial strains were grown to exponential phase in Mueller-Hinton broth (Merck) at 37 °C for 18 h and adjusted to a final density of 10^8 CFU/mL by diluting fresh cultures and comparing with the 0.5 McFarland turbidity standard (Kiehlbauch *et al*. 2000).

Eloff's microplate method (1998) was used to determine the minimal inhibitory concentration (MIC) values for plant hydroalcoholic extracts (70 % EtOH) with antibacterial activity. Plant extracts were dissolved at 10 mg/mL with the bacterial culture medium. All extracts were initially tested at 3.84 mg/mL in 96-well microplates and serially diluted two-fold to 1.87×10^{-3} mg/mL.

The antibiotic ampicillin (Sigma-Aldrich), was included as reference in each assay, and a standard antibiotic solution was made in an appropriate solvent as recommended by CLSI guidelines, dilutions of 0.05 to 5 mg/mL were used. Extract-free solution was used as a blank control. The microplates were incubated at 37 °C for 24 h. As an indicator of bacterial growth, 40 µl of 0.02 mg/mL 2,3,5-triphenyl-2H-tetrazolium chloride (TTC) (Sigma) dissolved in water were added to the wells and incubated at 37 °C for 30 min. MIC values were defined as the lowest extract concentration that prevents visible bacterial growth after 24 h of incubation at 37 °C. The colourless tetrazolium salt acts as an electron acceptor and is reduced to a red-coloured formazan product by biologically active organisms. Where bacterial growth was inhibited, the solution in the well remained clear after incubation with TTC. All the experiments were conducted in triplicate.

Antifungal screening. The fungal strain *Colletotrichum musae* (Berk. & Curt.) von Arx was obtained from plantain in Monterrey, Nuevo León, Mexico and the strain of *Fusarium boothii* O´Donnell, T. Aoki, Kistler & Geiser was isolated from wheat. Both were kept for 7 days in PDA (Potato dextrose agar), light/dark 12 h, 22 ºC. The fungal culture was obtained by making a suspension of the conidia of both species (20×10^4 conidia/mL) in sterile distilled water + 0.1% Tween 20. This procedure was performed at the time of inoculation of the 96-well plates.

For screening purposes, the hydroalcoholic extracts of leaf and stem were dissolved with DMSO at a concentration of 1,000 mg/mL. Subsequently, the corresponding dilutions were made in a PDA medium to reach the following final concentrations: 480, 240, 60, 30, 15 µg/mL in 96-well plates. Three repetitions were performed at each concentration. The following controls were used: PDA, PDA + DMSO 0.3% and the broad-spectrum contact fungicide Captan 50 (Bayer) at the same concentrations as

the extracts. Finally, 50 µL of the dilutions of the extracts in PDA medium and 50 µL of the conidia suspension were added per well until reaching a final concentration of 10×10^4 conidia/mL, with a final volume per well of 200 µL. Before inoculating the microplate, the optical density was read at 490 nm using a microplate reader (Synergy HT). After the plate was inoculated, this procedure was repeated every 24 h for 72 h. The microplates were incubated under light/dark 12 h at 22 °C (Raposo *et al.* (1995).

The difference in optical density is used to calculate the biomass of the fungus at a certain time. From these data, it is possible to calculate growth inhibition as Equation 1:

$$\%IC = \frac{[(At_x - At_0)nottreated) - [(At_x - At_0)treated]}{(At_x - At_0)nottreated} \times 100 \quad (1)$$

Where:
% IC - Percentage inhibition of growth
At_x - absorbance at a given time
At_0 - zero-time absorbance

The results of the inhibition percentages were analysed statistically by means of a two-way ANOVA. All analyses were performed in SPSS Statistics 21.0. The differences between means were calculated with Tukey's post hoc test ($p \leq 0.05$). The IC_{50} was calculated for each extract (leaf and stem) of each species at 72 h, using a Probit regression. The results were analysed statistically by means of a one-way ANOVA. The differences between the means were calculated with the Ryan-Einot-Gabriel-Welch posthoc test, based on a test of ($p \leq 0.1$). To establish the assumptions of each respective ANOVA, the normal distribution was verified via the Shapiro-Wilk test ($p \leq 0.05$) and the homogeneity of variances with the Levene test ($p \leq 0.05$).

DPPH radical scavenging activity. 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH) is a free radical that is widely used to test the free radical-scavenging ability of molecules. DPPH forms stable-radical intermediate species due to electron delocalization (Blois *et al.* 1958). Ethanolic solutions of DPPH have a deep violet colour and a strong absorption band at 517 nm. If DPPH reacts with a hydrogen-atom donor, the solution becomes colourless. The test was performed by mixing 50 µL (4 mg/mL) of *Bursera* extract with 150 µL of 133.33 µM DPPH ethanolic solutions in 96-well microplates. The final concentration of extract in the solution was adjusted to 1, 10, 100 y 1000 µg/mL, and the final concentration of DPPH in the solution was 100 µM. The plates were incubated at 37 °C for 30 min at a constant shaking speed. Subsequently, absorbance was measured at 517 nm using a microplate reader (Synergy HT). The decrease of absorbance was attributed to the amount (in %) of DPPH reduction.

The scavenging activity of each concentration was calculated as a percentage of the reduction of the DPPH concentration as follows (Equation 2):

$$\%\ Reduction = \frac{[(Abs\ DPPH - AbsSample)]}{Abs\ DPPH} \times 100 \quad (2)$$

Where:
Abs DPPH - absorbance of the DPPH in ethanol
Abs Sample - absorbance of each sample in the presence of each concentration of *Bursera* extracts.

The radical scavenging activity was expressed as IC_{50}, the concentration in µg/mL of the sample or positive control that reduces the absorbance of DPPH by 50 % compared to the negative control. This value was calculated by a linear model in Microsoft Excel 2010. The results were analysed with Student's *t*-test ($p \leq 0.05$) to determine the differences between the experimental and control groups. Quercetin and α-tocopherol were used as a positive control.

Anti-inflammatory activity: TPA-induced mouse-ear oedema assay. The ear oedema in mice assay was based on the method described by Merlos *et al.* in 1991. A group of six male CD1 mice (25-30 g) were anaesthetised with Sedalphorte® (63 mg/kg i.p.) and a solution of 12-O-tetradecanoylphorbol-13-acetate (TPA 0.25 mg/mL) dissolved in ethanol was topically applied to both sides (5 µL each) of the right ear. The left ear received only ethanol (10 µL). After 10 minutes of TPA-treatment, the doses of 1 mg of the test extracts, indomethacin (0.358 mg/ear) as a reference drug was applied to both sides (10 µL each) of the right ear. Control animals received only ethanol:acetone (1:1) as a negative control. Four hours later the mice were anaesthetised and killed by cervical dislocation. A 7-mm-diameter plug was removed from each ear. The swelling was assessed as the difference in weight between right and left ear plugs oedema inhibition (EI %) was calculated by the equation: The edema inhibition was measured as the weight difference between the two circular samples.

The % inhibition of oedema was calculated by Equation 3:

$$\%\ Inhibition = [(oedema\ A - oedema\ B)/oedema\ A] \times 100 \quad (3)$$

Where:

Oedema A - induced by TPA alone
oedema B - induced by TPA plus sample

The oedematous response was measured as the weight difference between the two circular samples. Data were analysed by ANOVA and Dunnet's test, to compare with the control.

Results

Flavonoid profile. The quantified flavonoids belong to the structural subgroups flavonol (Quercetin, rutin, myricetin, galangin), flavanone (naringenin, naringin) and chalcone (dihydro) (phloretin, phlorizin). The results are summarised in table 2. Content was generally higher in leaves than stems, with exception of galangin, which showed the highest content in stems of *B. aptera*. It is to be noted that this species contains all of the flavonoids evaluated. Phlorizin and quercetin were present in all the species, while phloretin was the least represented, found only in *B. aptera* followed by naringenin in *B. aptera* and *B. fagaroides*. The highest quantity detected corresponded to phlorizin in the leaf of *B. galeottiana*. According to a survey of the literature, this is the first report of the occurrence of phlorizin and its aglycone phloretin in the genus *Bursera*.

Antibacterial activity. Four *Bursera* species were active, mostly against the Gram-positive bacteria, but the Gram-negative *S. typhimurium,* was the most susceptible (Table 3). The leaf and stem extracts of *B. aptera* and *B. fagaroides* were active against both bacterial types. Leaf extract of *B. aptera* was the most effective, with an MIC value of 0.002 mg/mL. However, *B. schlechtendalii* was not effective at the concentrations tested.

Antifungal screening. The activity of the *Bursera* extracts on fungal growth is expressed as the half maximal inhibitory concentration (IC_{50}) as shown in Table 4. The most active extract against *F. boothii* had an IC_{50} value lower than 50 µg/mL and was obtained from stem of *B. morelensis*, on the other hand, the leaves of this species were among the least active, along with the stems of *B. galeottiana* with values above 2000 µg/mL. Contrastingly, these were the two most active against *C. musae*.

DPPH radical scavenging activity. The most active extracts were from *B. fagaroides* and *B. aptera* leaves, followed by *B. galeottiana* and *B. schlechtendalii* stems, with values

Table 2. Flavonoid concentration in hydroalcoholic extracts from five *Bursera* species.

Compound	RT (min), R^2 and linear regression+	Organ	Flavonoid concentration in *Bursera* species (µg/mg dry weight)				
			Bap	Bsc	Bfa	Bga	Bmo
Rutin	5.7; R^2 = 0.999	L	12.77	7.38	2.4	0	56.51
	y = 565.109x + 10.05	S	9.75	0	1.38	0.59	0
Phlorizin	6.7; R^2 = 0.997	L	173.18	47.43	22.47	528.11	368.87
	y = 77.675x + 9.29	S	55.65	1.15	2.86	9.95	0.48
Myricetin	7.9; R^2 = 0.976	L	65.29	25.16	0	23.44	8.41
	y = 151.67x + 25.34	S	0	7.86	14.03	0	0
Naringin*	11.2; R^2 = 0.999	L	37.55	28.28	23.74	9.67	2.56
	y = 165.92x + 5.28	S	12.33	4.09	8.81	0	4.2
Quercetin	11.9; R^2= 0.985	L	32.91	6.9	20.44	194.37	6.68
	y = 345.008x + 15.75	S	4.16	0.85	22.33	3.39	2.16
Phloretin	13.8; R^2 = 0.979	L	10.48	0	0	0	0
	y = 37.22x + 2.50	S	0	0	0	0	0
Naringenin	15.4; R^2 = 0.995	L	62.06	0	0	0	0
	y = 123.845x +9.019	S	0	0	3.04	0	0
Galangin	22.0; R^2 = 0.982	L	1.24	0	0	0	0
	y = 326.345x - 19.83	S	18.54	10.78	0	1.39	0

RT: retention time; R2: Correlation coefficient + y = area under the curve in milliunits of absorbance per minute (mUA min−1); x = mg flavonoid per milliliter (mg mL−1); Bap: *B. aptera*; Bsc: *B. schlechtendalii*; Bfa: *B. fagaroides*; Bga: *B. galeottiana*; Bmo: *B. morelensis*; L: leaves; S: stems. * This flavonoid was analysed using the H_3PO_4 method.

lower than α-tocopherol (IC_{50} 7.73 ± 0.51, 5.72 ± 1.02, 7.20 ± 0.61, 10.88 ± 0.73, 13.67 ± 0.45 µg/mL respectively). The results indicate that the reducing capacity of DPPH in *Bursera* depends on the species and the organ of the plant. Well, in the case of *B. galeottiana*, *B. schlechtendalii* and *B. morelensis* the stems are more active, while for *B. aptera* and *B. fagaroides* are the leaves (Table 5).

Table 3. Minimal inhibitory concentrations (MIC) values of hydroalcoholic extracts from leaves and stems of *Bursera* species against gram-positive and gram-negative strains.

Bacteria	MIC (mg/mL)										
	Bap		Bsc		Bfa		Bga		Bmo		Amp
	L	S	L	S	L	S	L	S	L	S	
B. subtilis (+)	--	3.84	--	--	3.84	3.84	--	3.84	--	3.84	0.16
S. epidermidis (+)	3.84	--	--	--	--	3.84	--	--	--	--	5.00
E. coli (-)	3.84	--	--	--	--	--	--	--	--	--	0.63
S. typhimurium (-)	0.002	0.004	--	--	0.12	0.015	--	--	--	--	2.50

Bap-*B. aptera*; Bsc-*B. schlechtendalii*; Bfa-*B. fagaroides*; Bga-*B. galeottiana*; Bmo-*B. morelensis*; Amp-ampicillin; L-leaves; S-stems; --, no inhibition at the tested concentrations. (+) Gram-positive; (-) Gram-negative. MIC values represent the average of three independent experiments.

Anti-inflammatory activity in the mouse ear oedema model. The anti-inflammatory activity of *Bursera* extracts are summarised in table 6. All extracts showed an efficacy lower than reference drugs, indomethacin (COX-1 inhibitor) and celecoxib (COX-2 inhibitor).

Discussion

The *Bursera* species that were studied (Table 1) are found in environments where stress due to excess UV light and temperature produces reactive oxygen species (ROS). The presence of flavonoids in the five species analysed enriches the knowledge of the chemistry of the genus, because the reported studies have focused mainly on terpenes and lignans. The detection of flavonoids, as well as antioxidant, antibacterial and antifungal activities, becomes important when we consider that flavonoids act as a defense system in plant tissues exposed to different stress types, both biotic and abiotic. The flavonoids are found in the nucleus of the cells of the mesophyll in the chloroplast where they have an important role in diminishing the effect of reactive species, such as hydrogen peroxide, hydroxyl radicals and oxygen singlets. In particular, the phlorizin is contained in other plant species, which belong mainly to the Rosaceae and Ericaceae families. Furthermore, dihydrochalcones are especially abundant in the species *M. domestica* (Rosaceae), in which the quantity of dihydrochalcones can be variable depending on the tissue but represent the majority (> 70 %) of the total phenolic compounds (Gosch *et al.* 2010). The other compounds quantified in this work (Table 2) are frequently found in plants with dietary and medicinal uses and have antimicrobial and anti-inflammatory properties (Cushnie & Lamb 2005, Cushnie & Lamb 2006, Li *et al.* 2016, Barreca *et al.* 2014).

The biological activities of species of the genus *Bursera* are broad, regarding to the antibacterial activity detected in the species studied is important not only in the research for compounds useful for medicine, but also for the importance of plants being able to synthesize flavonoids and phenolic compounds in response to microorganisms. Extracts from plants rich in flavonoids are reported to have antibacterial activity. These compounds include: apigenin, galangin glycosides, flavones, flavonols and chalcones which have potent antibacterial properties (Cushnie & Lamb 2006). The antibacterial mechanism of polyphenols may be related to their ability to deactivate enzymes of crucial importance for bacterial survival, towards to form complexes with proteins through hydrogen bonds with consequently formation of quinone, probable targets for quinone bindings on the bacterial cell are proteins of cell envelope and cell wall, surface-exposed adhesins and membrane-bound enzymes (Brudzynski & Maldonado-Alvarez 2015). It has been reported the use of *B. simaruba* bark for skin infections, as well as antibacterial, antifungal and cytotoxic activities (Canales-Martínez *et al.* 2017). The results indicate the

Table 4. Antifungal activity of the hydroalcoholic extracts of *Bursera* species.

Species	IC_{50} (µg/mL)									
	Bap		Bsc		Bfa		Bga		Bmo	
	L	S	L	S	L	S	L	S	L	S
F. boothii	231.42^{ab}	397.41^{ab}	341.10^{ab}	199.23^{ab}	310.35^{ab}	493.78^{ab}	--	16231.48^{c}	2272.01^{ab}	42.44^{a}
C. musae	--	984.36^{b}	966.32^{b}	--	--	--	550.16^{a}	322.59^{a}	306.39^{a}	396.48^{a}

Bap-*B. aptera*; Bsc-*B. schlechtendalii*; Bfa-*B. fagaroides*; Bga-*B. galeottiana*; Bmo-*B. morelensis*, L, leaves, S, stems. --: indicates no activity at concentrations tested. ANOVA test, $p \leq 0.1$ (REGW-F). ^{a,b,c}Equal letters indicate no significant differences.

existence of antibacterial compounds in the hydroalcoholic extracts of species studied (Table 3). However, the flavonoids detected in the extracts have records of antibacterial activity, and furthermore, the most effective species, *B. aptera*, contained all of those evaluated, these compounds might be only partially responsible for antibacterial activity (Cushnie & Lamb 2005, Cushnie & Lamb 2006, Barreca *et al*. 2014). This suggest that the leaf extract of *B. galeottiana*, which had the highest amount of phlorizin and quercetin, did not present antibacterial activity. In a recent work, it has found that the activity of phloretin was reduced by the addition of glucose to the chalcone structure (Barreca *et al*. 2014). Previously, it has been reported that *B. aptera* also has antifungal and antiprotozoal activities (Rodríguez-López 2014, Nieto-Yañez 2017). The authors found that podophyllotoxin was the most abundant (13.79 %) compound in methanolic bark extract, suggesting that it is responsible for the leishmanicidal activity, based on the relation between podophyllotoxin and its use in the treatment of skin infections. Furthermore, the flavonoids isolated in *Bursera* extracts (Table 4) have found to be effective antifungal agents against a wide range of fungus (Al Aboody & Mickymaray 2020).

Table 5. DPPH radical scavenging activity of hydroalcoholic extracts of *Bursera.*

Species	IC_{50} (µg/mL)	
	L	S
B. fagaroides	5.72 ± 1.02	15.41 ± 0.66
B. galeottiana	23.04 ± 3.17	7.20 ± 0.61
B. aptera	7.73 ± 0.51	32.89 ± 0.64
B. schlechtendalii	38.24 ± 1.35	10.88 ± 0.73
B. morelensis	73.48 ± 1.76	29.36 ± 0.74
Quercetin	3.68 ± 0.16	
α-tocopherol	13.67 ± 0.45	

L, leaves, S, stems. Average of three independent experiments ± standard deviation

On the other hand, there are many publications, which associate flavonoids with antioxidant activity (Panche *et al*. 2016); for example, hisperidine, rutin, quercetin, apigenin, naringenin and kaempferol have been reported for the bark *B. simaruba* (Bah *et al*. 2014). Which explains the reduction effect of the DPPH radical of the leaves and stems of the

Table 6. Anti-inflammatory activity of five *Bursera* species in the model of mouse ear oedema induced by TPA.

Species	Dose (1mg of extract/ear)			
	Oedema (mg)		Inhibition (%)	
	L	S	L	S
B. aptera	9.73 ± 0.5*	11.77 ± 1.80*	39.67*	27.07*
B. schlechtendalii	11.47 ± 0.35*	11.40 ± 0.7*	28.93*	29.34*
B. morelensis	11.97 ± 0.69	11.10 ± 1.97*	25.83	31.20*
B. fagaroides	7.87 ± 1.32*	12.13 ± 0.12	51.24*	24.79
B. galeottiana	13.60 ± 0.53	12.77 ± 1.52	15.70	20.87
Control	16.13 ± 0.35		0	
Indomethacin (0.358 mg/ear)	2.88 ± 0.73		78.76	
Celecoxib (methanol, 0.68 mg/ear)	4.34 ± 1.82		71.45	

L, leaves, S, stems. ANOVA and Dunnet's test ($p \leq 0.05$), which were used to compare with a control

five *Bursera* species in this study (Table 5). The antioxidant activity of other *Bursera* species have been associated with lignans (Nieto-Yañez 2017, Marcotullio *et al*. 2018) and phenols (Martinez-Elizalde *et al*. 2018)

Flavonoids such as hesperidin, apigenin, luteolin and quercetin, have anti-inflammatory and analgesic effects (Ferraz *et al*. 2020). In a previous work, anti-inflammatory effects have been reported with an average effective dose (ED_{50}) of *B. excelsa* (0.26 ± 0.01 mg/ear), *B. galeottiana* (0.23 ± 0.02 mg/ear) and *B. schlechtendalii* (0.25 ± 0.02 mg/ear) from bark using chloroform extracts (Acevedo *et al*. 2015). These reports suggested that the highest concentration of anti-inflammatory metabolites was found in the bark, however, our results indicated that the flavonoids were also found in stems and leaves (Table 6).

Finally, the presence of flavonoids in leaves and stems indicates the richness of these compounds in *Bursera* genus and, the antifungal and antibacterial, anti-radical and anti-inflammatory activities suggest the potential medicinal use of these species and the role that the flavonoids play in plant physiology.

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