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EVALUATION OF FLAVONOID CONTENTS AND ANTIBACTERIAL ACTIVITY OF FIVE BOLIVIAN *BACCHARIS* SPECIES

EVALUACIÓN DEL CONTENIDO DE FLAVONOIDES Y LA ACTIVIDAD ANTIBACTERIANA DE CINCO ESPECIES DE *BACCHARIS* DE BOLIVIA

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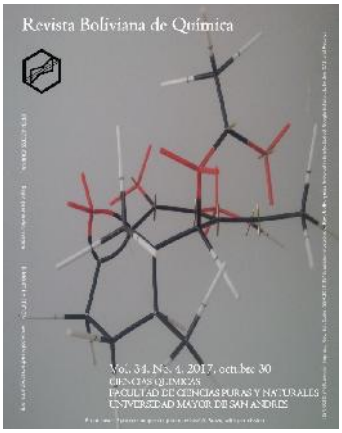

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Keywords: Flavonoids, antibacterial activity, Baccharis latifolia, Baccharis papillosa, Baccharis boliviensis, Baccharis tola, Baccharis pentlandii.

ABSTRACT

Five Bolivian *Baccharis* species (*Baccharis latifolia, B. papillosa, B. tola, B. pentlandii* and *B. boliviensis*) used in the folk medicine were analyzed by UV/Vis spectroscopy and HPLC to evaluate the flavonoid contents. First, using aluminium chloride colorimetric method, the total flavonoids (TF) contents respect of Luteolin was determined, showing that *B. latifolia* (8,03 mg TF eq Lu/g of leaves) presents the major quantity of total flavonoids in their leaves. Furthermore, our studies indicate that the method used for extraction gives extracts with high concentration of flavonoids between 53,06 and 85,86 mg TF eq Lu/g of EE (Ethanolic Extract) and that this concentration is increased in the last Sephadex LH-20 fractions, giving contents between 260,43 and 397,12 mg TF eq Lu/g of EFS (Enriched Fraction by Sephadex). On the other hand, the HPLC profiles of those extracts showed that the *B. latifolia* extract is the most complex; while the simplest is the *B. pentlandii* extract. Finally, the antibacterial activity was evaluated by agar well diffusion method, against nine bacteria ATCC and one bacterium clinical isolate, determining that all the EE have activity against *Staphylococcus aureus* (ATCC 25923 sensible) and *S. aureus* (ATCC 29213 resistant), but the major activity was observed in *B. tola* EFS (65,2 % of inhibition against *S. aureus* ATCC 25923 sensible).

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RESUMEN

Cinco *Baccharis* de Bolivia usadas en medicina tradicional (*B. latifolia, B. papillosa, B. tola, B. pentlandii* y *B. boliviensis*) fueron analizadas por espectroscopia UV/Vis y HPLC para determinar el contenido de flavonoides. Primero, usando el método colorimétrico de cloruro de aluminio, el contenido de flavonoides totales fue determinado respecto a la Luteolina, mostrando que *B. latifolia* (8,03 mg FT eq Lu/g de hojas) presenta la mayor cantidad de flavonoides totales en sus hojas. Además, nuestros estudios indicaron que el método de extracción utilizado da extractos con alta concentración de flavonoides entre 53,06 y 85,86 mg FT eq Lu/g de EE (Extracto Etanólico) y que esta concentración se incrementa en las últimas fracciones de Sephadex LH-20, dando contenidos entre 260,43 y 397,12 mg FT eq Lu/g de EFS (Fracción Enriquecida por Sephadex). Por otra parte, los perfiles de HPLC mostraron que el extracto de *B. latifolia* es el más complejo mientras que el extracto más simple es el de *B. pentlandii*. Finalmente, la actividad antibacteriana fue evaluada, por el método de difusión en agar, contra nueve bacterias ATCC y una bacteria aislada clínicamente, determinando que todos los EE tienen actividad contra *Staphylococcus aureus* (ATCC 25923 sensible) y *S. aureus* (ATCC 29213 resistente), pero la mayor actividad se observó en el EFS de *B. tola* (65,2% de inhibición frente a *S. aureus* ATCC 25923 sensible).

INTRODUCTION

Bolivia has a high plant biodiversity and a high cultural diversity with many ethnic groups that possess an extensive knowledge in traditional medicine, whose main expression is in the use of plants. Herbal medicines are an important element of indigenous medical system in Bolivia as well as in other countries of South America. According to Gimenez & Ibish [1] about 3000 Bolivian medicinal plants are known, identified and stored in various herbal institutions [2].

Baccharis is the largest genus in the family Compositae, with over 500 species distributed throughout American continent, mainly in the warm temperate [3]. In particular, in Bolivia many species of *Baccharis* genus grow in the highland region (3000-4000 m.a.s.l) where most of them are used as herbal medicines [4, 5].

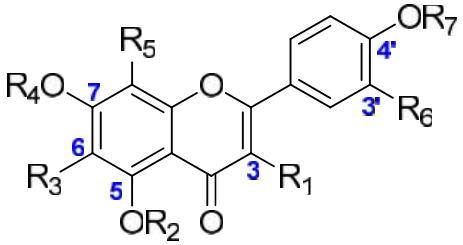

Table 1. *Flavones and flavonols reported in Bolivian Baccharis*

No.	R_1	R_2	R_3	R_4	R_5	R_6	R_7	Name	Source
1	OMe	H	H	H	H	OH	H	Quercetin 3-methyl ether	*B. papillosa* [23]
2	OMe	H	H	H	H	H	Me	Ermanine	*B. papillosa* [23]
3	OMe	H	H	H	H	H	H	Isokaempferide	*B. papillosa* [23]
4	H	H	OMe	Me	OMe	OMe	H	8-Methoxycirsi-lineol.	*B. pentlandii* [18]
5	H	H	OMe	Me	OMe	OH	H	Sideritiflavone	*B. pentlandii* [18]
6	H	H	OMe	Me	OMe	H	H	Xanthomicrol	*B. pentlandii* [18]; *B. boliviensis* [17]
7	H	H	OH	H	H	OH	H	6-Hydroxyluteolin	*B. boliviensis* [17]
8	OH	H	H	Me	H	OMe	Me	Quercetin 3',4',7-trimethyl ether	*B. latifolia* [22]
9	OH	H	H	Me	H	OMe	H	Rhamnazine	*B. latifolia* [22]
10	OH	H	H	Me	H	OH	H	Rhamnetin	*B. latifolia* [22]
11	OH	H	H	Me	H	H	H	Kaempferol 4',7-dimethyl ether	*B. latifolia* [22]
12	H	Me	H	H	H	H	Me	Apigenin 4',5-dimethyl ether	*B. latifolia* [22]
13	H	H	H	Me	H	OH	H	Apigenin 4',7-dimethyl ether	*B. latifolia* [22]
14	H	H	H	Me	H	OMe	Me	Gonzalitosin	*B. latifolia* [22]
15	H	H	H	H	H	H	Me	Acacetin	*B. latifolia* [22]
16	H	H	H	H	H	OH	H	Luteolin	*B. latifolia* [22]

The phytochemical research in *Baccharis* genus determined mainly diterpenoids and phenolic compounds as major components [3]. Some of those compounds and several *Baccharis* extracts were pharmacologically investigated for diverse properties as antioxidant [6, 7, 8], anti-inflammatory [9, 10] or antimicrobial [11, 12]. Moreover, several Bolivian *Baccharis* species have been investigated by our group and other research groups determining some antioxidant, anti-inflammatory and antimicrobial properties [13, 14, 15, 16], as well as flavonoids and cinnamic acid derivatives as major components [17, 18, 19, 20, 21, 22, 23].

Based on that, for this study, we selected five Bolivian *Baccharis* species (*B. latifolia, B. tola, B. boliviensis, B. papillosa* and *B. pentlandii*) widely distributed in the La Paz Valley, whose leaves are used in the folk Bolivian medicine for the treatment of rheumatism, liver diseases, infectious problems, wounds and ulcers [14, 5]. Four of them, were previously studied by our research group reporting sixteen flavonoids: *B. papillosa* (**1**-**3**) [23], *B. pentlandii* (**4**-**7**) [18], *B. latifolia* (**8**-**16**) [22] and *B. boliviensis* (**6**, **7**) [17] See Table 1. These flavonoids could be in part responsible of their antioxidant, antimicrobial and anti-inflammatory properties [24, 25, 26, 27, 28, 29].

As part of our research of Bolivian *Baccharis* species, the present paper was undertaken in order to quantify the total flavonoid contents in the leaves; as well as to investigate the flavonoid contents in EtOH extracts, both qualitatively and quantitatively by UV/Vis spectroscopy and HPLC, because this extraction method is related to their traditional and industrial uses [4]. Finally, we determine the antibacterial activity to contribute in the scientific support of these EtOH extracts widely used in the Bolivian folk medicine.

EXPERIMENTAL

Plant material

Aerial parts of five *Baccharis* species (Asteraceae): *Baccharis latifolia* (Ruiz & Pav.) Pers; *Baccharis boliviensis* (Wedd.) Cabrera; *Baccharis papillosa* subsp. *papillosa* Rusby; *Baccharis tola* subsp. *santelicis* (Phil.) Joch. Müll; *Baccharis pentlandii* DC syn. *Baccharis densiflora*; were collected on March, 2014 in Cota Cota (3600 m.a.s.l), located on the outskirts of the city of La Paz, Bolivia. The plants were identified by Esther Valenzuela at the JBLP (*Jardín Botánico del Herbario Nacional de Bolivia*) where the voucher specimens are kept.

Apparatus, Chemicals and Culture media

UV absorption spectra were performed in an UV/Vis Thermo Scientific spectrometer, Genesys 10S, using methanol from Sigma-Aldrich as solvent. HPLC chromatograms were obtained in Agilent 1100 Series equipment with a quaternary pump, a diode array detector DAD and a RP-Silica C18 250 * 4.6 mm E10174 column. All solvents used were HPLC grade and the ultra-pure water was obtained by ultrafiltration equipment Sartorius Stedim brand. The extractions and fractionations were performed with commercial solvents previously purified by distillation. The preliminary phytochemical analysis was performed using Sigma-Aldrich reagents. The standard compound for determination of total flavonoid content, luteolin was acquired from Sigma – Aldrich as well as the aluminum chloride and potassium acetate. All solutions were stored in a dark flask and refrigerated until use. The antibacterial assays were carried out using Mueller Hinton agar and Mueller Hinton broth purchased from BBL™ trademark of Becton, Dickinson and Company. Dimethyl Sulfoxide (DMSO) was purchased from Sigma-Aldrich Coorporation.

Preparation of EtOH extracts (EE)

The collected plant materials were cleaned and air dried at room temperature, then the leaves were separated from the stems to proceed to a manual grinding for maceration.

The dried leaves of the five *Baccharis* species (*B. latifolia, B. tola, B. boliviensis, B. papillosa* and *B. pentlandii*) were extracted by maceration 1:15 (w/v) for 15 min at room temperature, with EtOH 96º (distilled). The extracts were filtered and the solvents were evaporated using a rotatory evaporator (Heidolph). The dried crude extracts were stored at room temperature until use.

Preparation of enriched fractions in flavonoids and cinnamic acid derivatives (EFS)

A portion of EtOH crude extract (100 mg) was fractionated on Sephadex LH-20 employing MeOH as solvent. The fractions were controlled by TLC stained with H_2SO_4, $FeCl_3$ and UV lamp at 312 and 360 nm, selecting the fractions with yellows spots (H_2SO_4), brown spots ($FeCl_3$) and spots with highest intensities under UV lamp. So, the last fractions were selected and joined together to give the fraction enriched in flavonoids and derivatives of cinnamic acid, called EFS (Enriched Fraction by Sephadex)

Preliminary phytochemical analysis

Sterols and triterpenes were identified by the Liebermann-Burchard reaction. Phenolic compounds were qualitatively determined by examining the redox reaction between the ethanolic extracts (diluted 10 times) and a solution of ferric chloride (300 µM). Flavonoids were identified by Shinoda's reaction, alkaloids by the Dragendorf reagent and saponins by shaking (2 min) the ethanolic extract (diluted 10 times) and observing the formation of stable foam [30].

Determination of total flavonoid content

The total flavonoid (TF) content of leaves, EE and EFS were determined using the colorimetric method of aluminum chloride [31] modified, using Luteolin as standard compound. According to this modification, 0.5 ml of the sample was mixed with 1.5 ml of EtOH 96º (distilled). This was followed by the addition of 2.8 ml distilled water, 0.1 ml of 10% $AlCl_3$ (w/v) solution, and 0.1 ml of 1 mol/L solution of potassium acetate. The solution was incubated for 30 - 40 min and then subjected to spectral analysis in the range of 200 to 500 nm. The samples turn to pink whose absorbance was measured at 406 nm.

The calibration curve was made by preparing solutions at 30, 60, 90, 120 and 150 ppm of standard compound and recording the absorbance at λ_{max} = 406 nm, where the Luteolin showed a good linearity (Y= 0,0823X, R^2=0,9988). The TF respect of Luteolin was calculated using the calibration equation.

Statistical analysis

The results were recorded after repeating the experiments three times. The experimental results were expressed as mean ± standard deviation (SD) of (3n) measurements. The statistical analysis of the data were carried out using Kruskal-Wallis t-test and the results were considered significant when $p<0.05$.

Chromatography

The HPLC method employed in this work was based in an established method by Angela San Martin for analysis of *B. latifolia* extracts described below.

The samples were prepared dissolving 20 mg of the EFS in 1 ml of MeOH and then, filtered with a 0.45 µm membrane filter. The column was operated at 40°C and the injection volume was 25 µl. The UV spectra were scanned between 200 and 600 nm and the wavelengths of 315 nm and 370 nm were chosen for cinnamic derivatives and flavonoids detection, respectively. The mobile phase components consisted of A= 0.1% aq. H_3PO_4 and B= CH3CN. Linear gradient elution was performed at a flow rate of 0.6 ml/min as follows: Initial, 0 min, 25% of B; 5 min, 28% of B; 10 min, 30% of B; 17 min, 32.8% of B; 20 min, 36% of B; 25 min, 42% of B; 33 min, 44.8% of B; 40 min, 53.5% of B and after 60 min, 100% of B.

Microorganisms employed

The microbial strains studied were *Escherichia coli* ATCC 25922 (sensible), *Escherichia coli* ATCC 35218 (resistant), *Staphylococcus aureus* ATCC 25923 (sensible), *Staphylococcus aureus* ATCC 29213 (resistant), *Listeria monocytogenes* ATCC 7644, *Pseudomona aeruginosa* ATCC 27853 (sensible), *Klebsiella pneumoniae* ATCC 70063 (resistant), *Bacillus subtilis* ATCC 6636, *Shigella flexneri* ATCC 12022 and *Salmonella typhi* (clinical isolate) and were obtained from INLASA (*Instituto Nacional de Laboratorios en Salud*) of Bolivia.

Antibacterial assay

Fresh pure bacteria suspensions were obtained from overnight cultures in Muller Hinton Broth cultivated at 37°C for 24 h. The bacterial suspensions were adjusted to an inoculum size 10^8 cells/mL for inoculation of the agar plates.

After the medium Mueller Hinton Agar (25 mL approximately) was solidified in the plates, the test strain (50 *u*L) was inoculated into the media. Care was taken to ensure proper homogenization. The suspensions were spread on the medium. Four ditches were made in the plates with the help of a cup-borer. For the *in vitro* studies, extracts and fractions were dissolved in 200 µL of dimethyl sulfoxide (DMSO) and water 1:1. One ditch was used with 200 *u*L of dimethyl sulfoxide (DMSO) and water 1:1 as solvent control, and another one was used with 200 *u*L of gentamicin as antibacterial control. The test samples, the DMSO and water 1:1 and the gentamicin were introduced in each ditch and the plates were incubated at 37°C for 24 h. Microbial growth was determined by measuring the diameter of the zone of inhibition in millimeters (IH) and the percent of inhibition (%I) was calculated comparing the extract zone inhibition respect of the positive control zone inhibition using the follow equation. All tests were performed in triplicate.

$$\%I = [(IH_{sample} - IH_{negative\ control})/(IH_{positive\ control} - IH_{negative\ control}) \times 100$$

RESULTS AND DISCUSSION

Preliminary phytochemical screening

The preliminary phytochemical screening of the five Bolivian *Baccharis* species reveals high amounts of flavonoids and a clear presence of phenols. The results are shown in Table 2.

Table 2. *Preliminary phytochemical screening of EtOH extracts from 5 Bolivian Baccharis.*

Trial/ Metabolite	*B. latifolia*	*B. papillosa*	*B. boliviensis*	*B. pentlandii*	*B. tola*
Dragendorff/ Alkaloids	±	±	±	+	±
Lierberman Buchard / Triterpenes/ Sterols	+++	+++	++	++	+++
Borntrager/Quinones	-	-	±	++	+++
$FeCl_3$/ Phenols	+++	++	+++	++	+++
Foam / Saponins	-	±	-	-	±
Shinoda/ Flavonoids	+++	+++	+++	+++	+++

-: Absent, ±: Doubt presence, +: Traces, ++: Presence, +++: High amounts

Evaluation of flavonoid contents

The present study was conducted to obtain and evaluate extracts and fractions with high concentration of flavonoids, because several, of this type of compound, have been reported by their anti-inflammatory, antioxidant and/ or antimicrobial activities, properties close related to their traditional uses in the Bolivian folk medicine.

Our previous studies in these species suggested that a fast extraction (15 min) by maceration with EtOH of the leaves produce extracts with high concentration of flavonoids and acid cinnamic derivatives [4]. In addition, it is known that these compounds normally are concentrated in the last fractions of a Molecular Exclusion Chromatography Sephadex LH-20, because of their lower molecular weight respect of the other secondary metabolites.

Based on that and the preliminary phytochemical screening, evaluation of the total flavonoid contents (TF) was done in the leaves, EtOH extracts and the last fractions of Sephadex LH-20. For this, the colorimetric method was selected using $AlCl_3$. The calibration curve was prepared with Luteolin instead of Quercetin, like in other similar studies, because its λ_{max} after the reaction with $AlCl_3$ is more similar to those of EtOH *Baccharis* extracts. So the TF contents is expressed in mg of luteolin equivalents per g of leaves or extracts. See Table 3 and Figure 1.

Table 3. *Quantification of total flavonoids in mg of Luteolin equivalent per 1 g of leaves, EE and EFS, from five Bolivian Baccharis* species

Sample	Leaves (mg TF/g)	EE (mg TF/g)	EFS (mg TF/g)
Baccharis latifolia	8,03 ± 0,31	85,86 ± 3,35	397,12 ± 8,89
Baccharis papillosa	4,22 ± 0,24	68,45 ± 5,90	296,68 ± 2,99
Baccharis boliviensis	6,23 ± 0,17	78,57 ± 2,45	338,80 ± 12,98
Baccharis tola	7,69 ± 0,47	82,83 ± 4,48	349,33 ± 5,84
Baccharis pentlandii	2,63 ± 0,11	53,06 ± 6,32	260,43 ± 8,20

Values are expressed as mean ± SE mg of Luteolin equivalent per g or 100 g of dry sample

The results showed that *B. latifolia* is the species with the major quantity of flavonoids followed by *B. tola* and *B. boliviensis*. The TF contents in extracts showed the same relation demonstrating that the flavonoid content is highly increased by Sephadex LH-20 fractionation.

On the other hand, HPLC chromatograms were carried out to analyze the complexity of the samples for the five *Baccharis.* The EFS chromatograms were analyzed at two wavelengths because of the chemical antecedents for this species [17, 18, 22, 23]: At λ= 370 nm to analyze mainly the flavonoids that showed the typical two bands in UV spectroscopy, as example Luteolin (Figure 2) and at λ= 315 nm to analyze cinnamic acid derivatives whose showed one broad band around 315 nm as can be seen for Drupanine (Figure 2).

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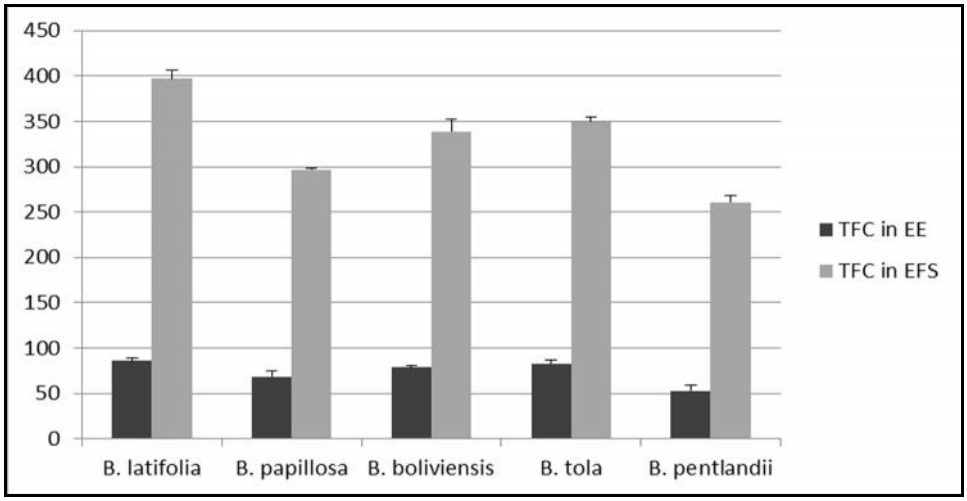

Figure 1. *Quantification of TFC (Total Flavonoids Contents) in the 5 Bolivian Baccharis expressed in mg of Luteolin equivalent per g of EtOH Extracts (EE) and Enriched Fraction by Sephadex LH-20 (EFS).*

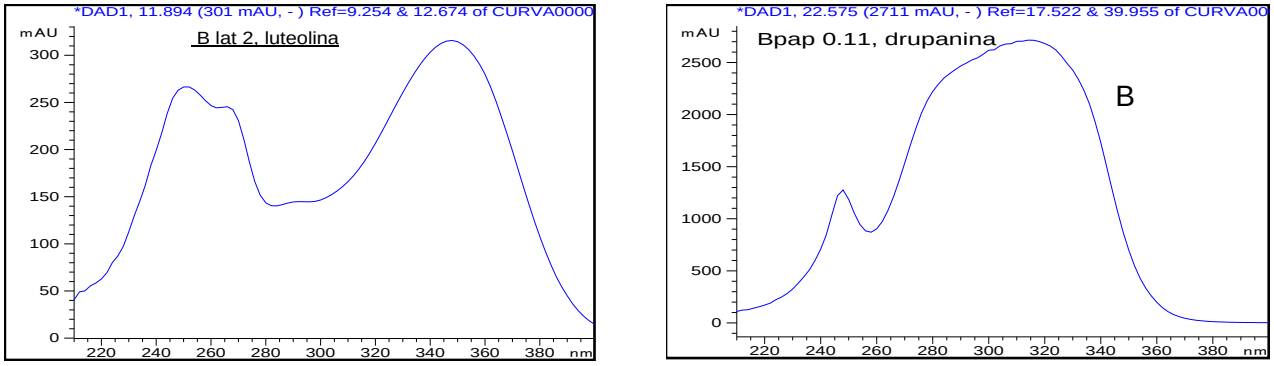

Figure 2. *UV spectra of a flavonoid Luteolin* ***(16)*** *from B. latifolia (A) and a cinnamic acid derivative Drupanine from B. papillosa (B).*

The used HPLC method was established taking into account the *B. latifolia* extract, because of its complexity. Figure 3 showed the HPLC chromatograms of the EFS at 370 nm, where mainly the flavonoids are shown. The analysis of UV spectra from each signal in the chromatograms led to assign each peak to a flavonoid (two typical bands) or cinnamic derivatives (one broad band). So, the flavonoids in the chromatograms of Figure 3 were numbered observing more complexity in the follow order: *Baccharis latifolia* > *Baccharis tola* ≥ *Baccharis boliviensis,* > *Bacchari papillosa* > *Baccharis pentlandii*.

In addition, Figure 4 showed the HPLC chromatogram of EFS at 315 nm, where mainly cinnamic acid derivatives appear, which were numbered with a 0 previous the number to distinguish these numbers of the numbers for flavonoids. The analysis of cinnamic acid derivatives showed clearly more quantity of this type of compounds for *B. papillosa* followed by *B. tola* and *B. boliviensis.*

In conclusion, the evaluation of flavonoid contents in EE and EFS for *B. latifolia*, *B. papillosa*, *B. tola*, *B. pentlandii* and *B. boliviensis* showed that *B. latifolia* present the highest concentration and the largest number of flavonoids. On the other hand, the simplest extract is that of *B. pentlandii* which presents only 3 flavonoids already identified by our group [18]. In addition, we determined that the extraction process also gives a good proportion of cinnamic acid derivatives, analyzed at 315 nm, and that the species with the largest number of this type of compounds is *B. papillosa.*

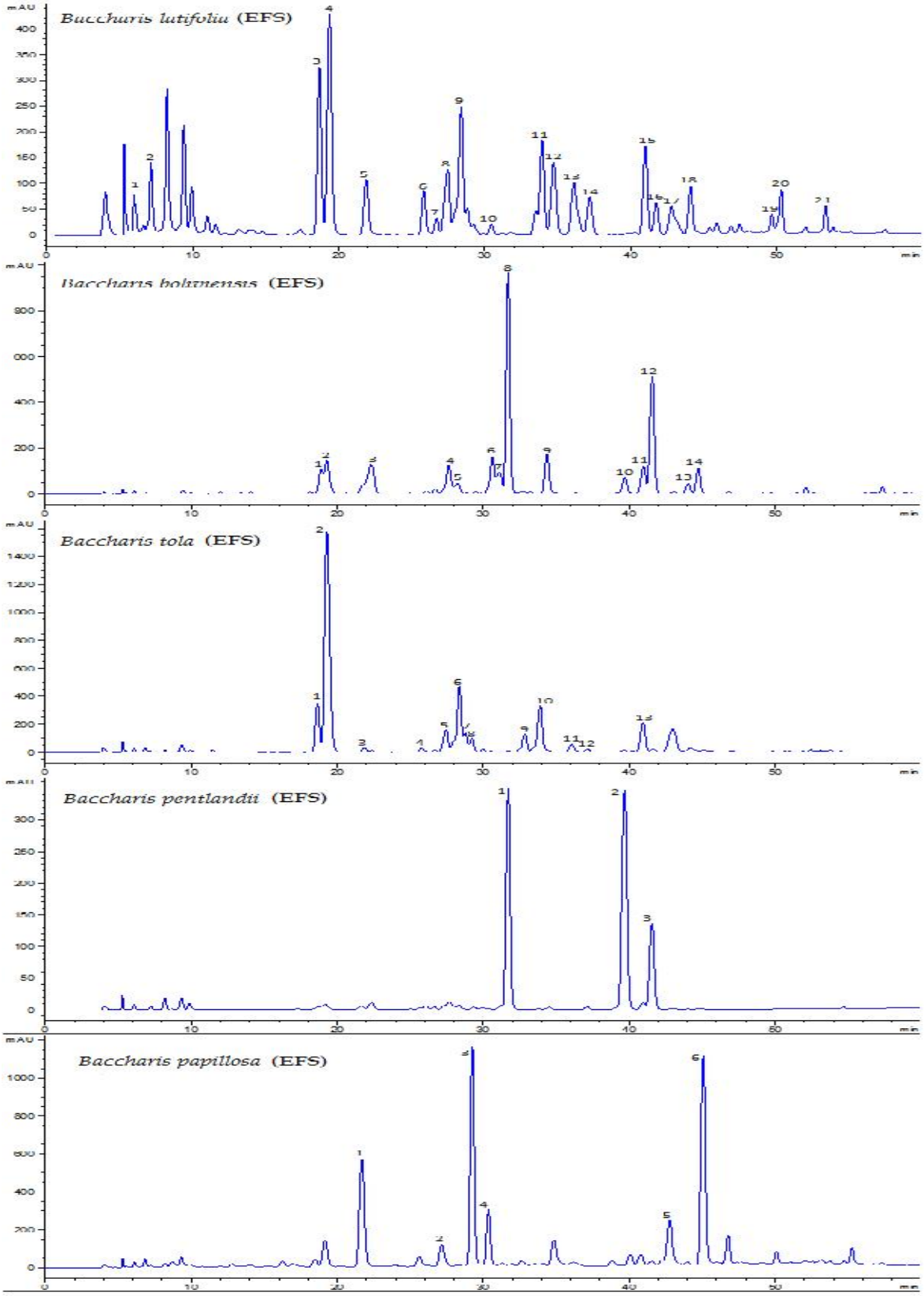

Figure 3. *Chromatograms at 370 nm of fractions enriched by Sephadex LH-20 (EFS) for the five Bolivian Baccharis, showing with numbers the signals for flavonoids.*

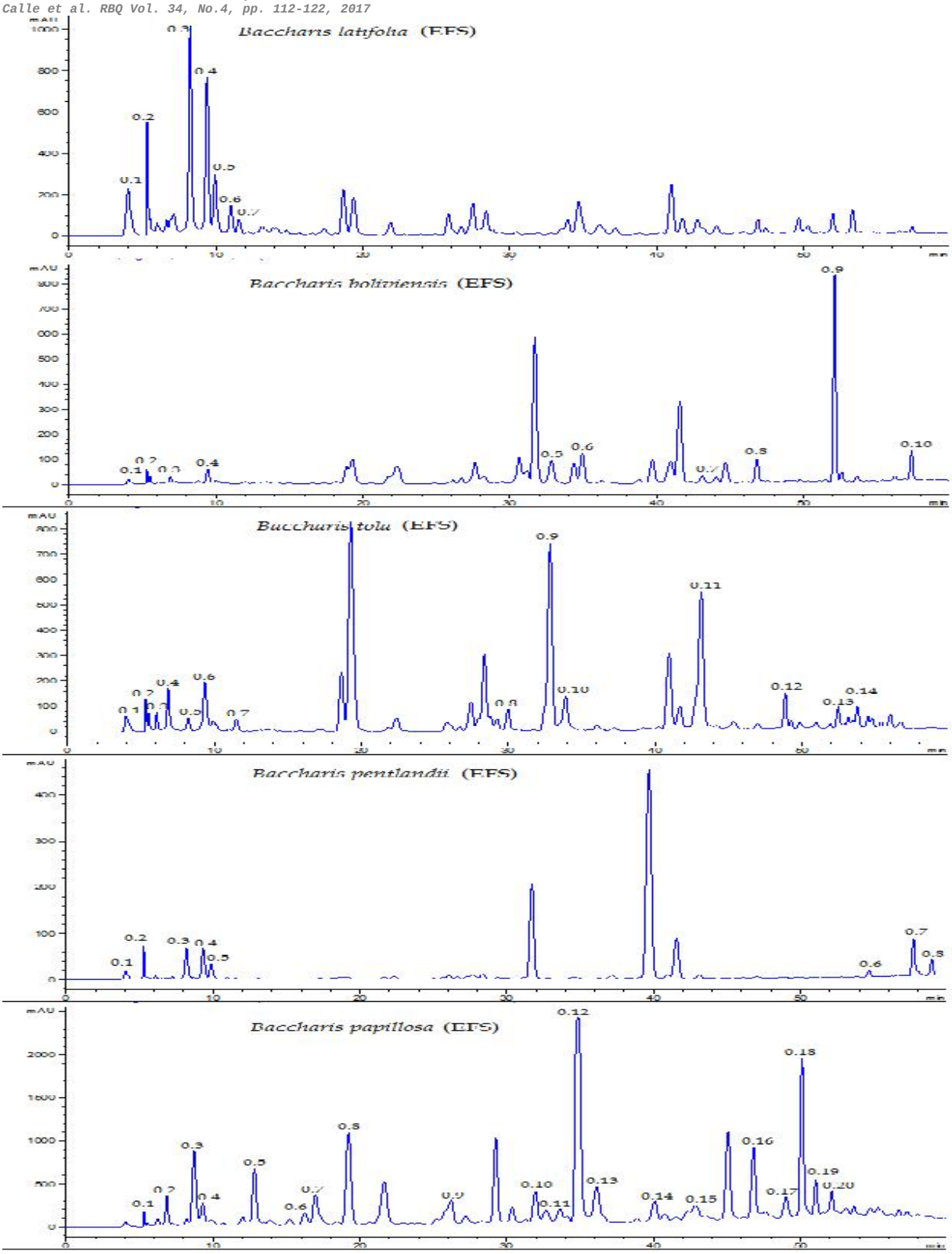

Figure 4. *Chromatograms at 315 nm of enriched fractions by Sephadex LH-20 (EFS) for the five Bolivian Baccharis, showing with numbers the signals for cinnamic acid derivatives.*

Antibacterial study

Bacterial multidrug resistance represents a major hurdle in the treatment of infectious diseases. In this study, we tested a panel of bacterial strains. The EE for *B. latifolia, B. papillosa, B. tola, B. pentlandii* and *B. boliviensis* were assayed against nine bacteria ATCC and one bacterium clinical isolate, using Gentamicin as positive control and the solvent as negative control. The results at (10 mg/0,2 mL) of the EE showed good activity for all the extracts only against to *S. aureus* ATCC 25923 (sensible) and *S. aureus* ATCC 29213 (resistant) and they do not show antibacterial activity against: *S. flexneri* ATCC 12022, *L. monocytogenes* ATCC 7644, *E. coli* ATCC 35218 (resistant), *P. aeuruginosa* ATCC 27853 (sensible), *B. subtilis* ATCC 6633, *S. thyphi* (clinical isolated), *E. coli* ATCC 25922 (sensible) and *K. pneumoniae* ATCC 700603, as we can see in Table 4**.**

Table 4. *Antibacterial activity* of EE from five Bolivian Baccharis against nine bacteria ATCC and one bacterium clinical isolate*

BACTERIA	***B. latifolia*** **50 mg/mL**	***B. papillosa*** **50 mg/mL**	***B. boliviensis*** **50 mg/mL**	***B. pentlandii*** **50 mg/mL**	***B. tola*** **50 mg/mL**	**Gentamicin 0.8 mg/mL**	**Solvent control DMSO:Water (1:1) 0.2 mL**
S. aureus ATCC 25923 (sensible)	24*	15*	26*	21*	25*	40*	-
S. flexneri ATCC 12022	-	-	-			35	-
L. monocytogenes ATCC 7644	-	-	-			-	-
E. coli ATCC 35218 (resistant)	-	-	-			38	-
P. aeuruginosa ATCC 27853 (sensible)	-	-	-			43	-
B. subtilis ATCC 6633	-	-	-			40	-
S. typhi (clinical isolate)	-	-	-			40	-
E. coli ATCC 25922 (sensible)	-	-	-			40	-
S. aureus ATCC 29213 (resistant)	23	20	24	21	24	38	-
K. pneumoniae ATCC 700603 (resistant)	-	-	-			35	-

* Diameter of inhibition zone in mm

Finally, the inhibition of EFS was analyzed by agar-disc diffusion method against *S. aureus* (sensible). Figure 5 showed the inhibition zone for the five extracts compared with Gentamicine (positive control) and solvent (negative control). The evaluation showed that all the EFS analyzed are active against this bacterium, but the most active is the EFS of *B. tola* followed by the EFS of *B. boliviensis* (Table 5).

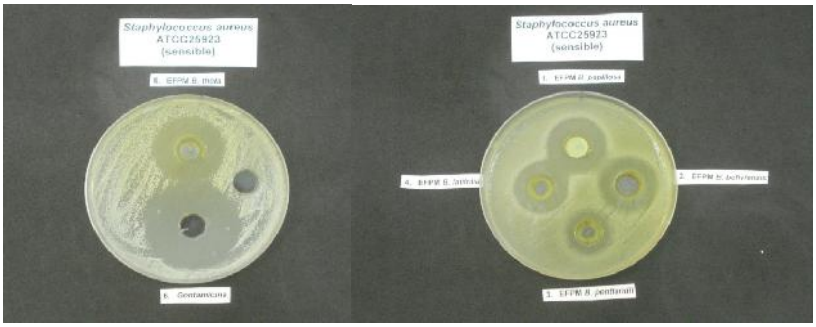

Figura 5. *Antibacterial evaluation against S. aureus (sensible) of:* ***A****) EFS of B. tola (above); Gentamicine, positive control (down); pure solvent, negative control (right);* ***B****) EFS of B. papillosa (above); EFS of B. latifolia (left); EFS of B. boliviensis (right); EFS of B. pentlandii (down).*

***Table 5**. Inhibition of S. aureus (ATCC 25923) sensible strain by EFS from five Bolivian Baccharis*

Sample	*B. latifolia*	*B. papillosa*	*B. boliviensis*	*B. pentlandii*	*B. tola*	*Gentamicine*	*Solvent*
% Inhibition	47,83	52,17%	60,87%	34,78%	65,22%	100,00%	0,00%
HI inhibition zone*	20	21	23	17	24	32	9†

* Diameter of inhibition zone in mm; †Diameter of hole

In conclusion, the antibacterial study showed that all the EE have activity against *S. aureus* ATCC 25923 (sensible) and *S. aureus* ATCC 29213 (resistant). In addition, the EFS from the five Bolivian *Baccharis* possessed activity *S. aureus* (sensible), contributing the study to the great potential of these medicinal plants used in Bolivian ethnomedicine. The antibacterial activity of flavonoids is being increasingly documented, other *Baccharis* species also showed good antibacterial activity and this activity could be related to their flavonoid and acid cinnamic derivatives [32, 33]. The EFS of *B. tola* was the most active against *S. aureus* (sensible), this species is widely distributed in the Bolivian Highlands and it does not have chemical studies, so it is recommendable to carry out more chemical and biological studies of this species.

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