

ANTIOXIDANT ACTIVITY AND CYTOTOXIC EFFECT OF CHILEAN *Buddleja globosa* (MATICO) AND *Ribes magellanicum* (ZARZAPARRILLA) FLOWER EXTRACTS

Pedro Zamorano-Aguilar^{1,✉}, Marcela Morales², Yudrum Rivillas³, Juan López³, Benjamín A. Rojano²

¹ Graduate School, Faculty of Agricultural Science, Universidad Austral de Chile, Av. Carlos Ibañez del Campo S/N Valdivia, Chile
Postal: 5090000, Región de Los Ríos, Valdivia, Chile

² Grupo Química de los Productos Naturales y los Alimentos, Facultad de Ciencias, Universidad Nacional de Colombia,
Calle 59a N° 63-20 Medellín, Colombia

³ Grupo Biotecnología Animal, Facultad de Ciencias, Universidad Nacional de Colombia, Calle 59a N° 63-20 Medellín, Colombia

ABSTRACT

The native Chilean shrubs *Buddleja globosa* (matico) and *Ribes magellanicum* (zarzaparrilla) are used widely at a rural level, due to their medicinal properties. Nevertheless, little is known about their secondary metabolites and cytotoxic effect. The aim of this study was to evaluate the content of different compounds like catechin, epicatechin, p-coumaric acid and the antioxidant capacity by ABTS, ORAC, FRAP and DPPH methods. In addition, the cytotoxic activity of both extracts was evaluated against Chinese hamster ovary (CHO-K1) cell lines by MTT and neutral red ASSAYS. The results suggest that the most abundant constituent in *Buddleja globosa* and *Ribes magellanicum* were catechin (682.43 mg/100 g DW) and epicatechin (3362.08 mg/100 g DW) respectively; while the ORAC methodology showed an elevated antioxidant activity for matico (134147.31 μmol Trolox Eq/100 g DW). On the other hand, both extracts at the assayed concentrations affect the membrane stability and cellular metabolic capacity of the CHO-K1 cell lines. These findings provide a direction for further researches, and suggest the matico and zarzaparrilla flower extracts as promising sources of antioxidants, and as research objects through the analyze of their metabolic behavior and antitumoral potential.

Key words: matico, zarzaparrilla, antioxidant capacity, cytotoxic activity, bioactive compounds, polyphenols

INTRODUCTION

Several compounds with biological activity used in traditional and popular medicine systems are molecules extracted from plants and vegetal and are prepared as infusions or beverages [Gurib-Fakim 2006]. According to Lucini et al. [2016], the known health benefits associated with herbal infusions are partially associated to their antioxidant capacity. Bioactive compounds that have shown the ability to limit or

prevent oxidative damage such polyphenols, carotenoids and vitamins [Prior et al. 2003] are highly associated with the reduction of cardiovascular diseases [Pounis et al. 2013], various types of cancer, immune and neurodegenerative disorders [Marszalek et al. 2017], type II diabetes and in general in all diseases in which oxidative stress has an important role [Li et al. 2009].

✉ pedro.zamorano.a@gmail.com

Among importance bioactive compounds are flavonoids, secondary metabolites that have been recognized to have medicinal properties and beneficial impact on health, e.g. antioxidant activity, digestive stimulation action, hypolipidemic and antimutagenic potential [Aaby et al. 2004]. p-Coumaric acid, which has demonstrate chemoprotectant [Torres y Torres and Rosazza 2011] and hepatoprotective [Calil Brondani et al. 2017] properties. And, flavanols like catechin and epicatechin are acknowledged for their antibacterial and antiviral capacity; anti-inflammatory activity and cancer preventives for humans [Kumar and Pandey 2013].

Buddleja globosa (belonging to the family of Scrophulariaceae) is a perennial bush that have simple and opposite leaves of 5–20 cm long and 1.5–4.5 cm of wide. It is native to central and southern Chile and from some parts of South America such Argentina, Peru and Bolivia [Houghton 1984]. In Chile the shrub is known as matico and grows wild in hills and ravines. Its distribution extends from the region of Coquimbo to the province of Chiloé [Vogel et al. 2005]. Several studies report the presence of saponins, sesquiterpenes, triterpenoids, diterpenes and flavonoids such apigenin, quercetin, and hidroxiluteolin in leaves and flowers of matico [Houghton 2003]; that contribute to their gastroprotective effect [Placencia et al. 2002], analgesic and anti-inflammatory activity [Backhouse et al. 2002].

On the other hand, *Ribes magellanicum* (commonly known as zarzaparrilla) is a deciduous and erect shrub that belongs to the Grossulariaceae family. Its leaves are persistent ovate, orbicular (3–10 cm wide), with three lobes; and its distribution extends from Valdivia to the Magallanes and Chilean Antartica region [Muñoz et al. 1986].

As regards its chemical composition, Ruiz et al. [2015] reported in the fruit of zarzaparrilla the presence of Caffeoylquinic acid, feruloylquinic and coumaroylquinic acids, quercetin hexoside, rutinose and acetyl derivatives; kaempferol-acetylhexoside and rutinose derivatives, myricetin and kaempferol. In Europe the chemical profiling has been extensively described, mainly for the fruit of *Ribes nigrum* and *Ribes rubrum* (black and red currants respectively) [Jiménez-Aspee et al. 2016]; which present high contents of p-coumaric, ferulic and caffeic acid [Määttä et al. 2003]. However, no information was found regard-

ing phenolic compounds, antioxidant and biological activity from the flower of zarzaparrilla.

In Chile, both bushes are used widely at a rural and popular level. In the case of matico the stalks and leaves are applied directly such infusion or poultice for the treatment of wounds, ulcers and dysentery [Houghton 1984]. Moreover, zarzaparrilla is gaining importance, in particular its fruit, which can be consumed fresh, in preserves and in syrups, due to their high in vitro antioxidant capacity and significant cytoprotective effect against oxidative stress [Jiménez-Aspee et al. 2016].

According to this, and from the base that no background exists about the chemical characteristics of the flowers of these shrubs, the aim of this research was to determinate the content of secondary metabolites and their antioxidant capacity. In addition to evaluating its biological activity through their biological effect against Chinese hamster ovary (CHO-K1) cell line.

MATERIAL AND METHODS

Vegetal material and preparation of the sample.

The plant material was collected in the commune of Fresia (Chile). After being stored in perforated polypropylene bags were lyophilized and taken for the respective analyzes. For the antioxidant and cytotoxic assays; 2.0 g of the lyophilized samples were placing in 200 ml of boiling water for 10 min. In the case of the antioxidants assays, the supernatant was cooled to room temperature and analyzed directly. For the biological activity tests, the cooled supernatant was filtered using 0.20 µm pore-size nylon filter. Among assays, the extracts were stored at 4°C.

Chemical assays and antioxidant capacity. Reactive solvents were obtained from Sigma-Aldrich® (St. Louis, USA) and Merck (Germany). Ultraviolet-visible measurements were performed on a Multiskan Spectrum spectrophotometer (Thermo Scientific). The decrease in fluorescence intensity measured in the ORAC assay was performed on a Perkin-Elmer LS-55 (U.K.) Spectrofluorimeter. The chromatographic assays were carried out on a Shimadzu® series Prominence® UFLC liquid chromatograph.

Glucose and fructose were determined through high pressure liquid chromatography (HPLC), following a modified version of the protocol of Eyéghé-Bickong et al. [2012]. Reducing sugars (DNS colorimetric

method) was performed as describes by Najmus and Whitney [2011]. Total phenols were done by the colorimetric method of Folin-Ciocalteu designed by Singleton and Rossi [1965]. The determination of ascorbic acid was performed through HPLC, following the method described by Kelebek et al. [2009]. As a mobile phase 0.1% formic acid in purified water was used, by isocratic elution system, at a temperature of 35°C and flow of 0.8 mL/min. Detection was performed at 245 nm. The identification and quantification was done with calibration curves elaborated with different concentrations of ascorbic acid. Confirmation and quantification of (+)-catechin and (–)-epicatechin were analyzed by liquid chromatography (HPLC-DAD) using a Shimadzu LC-20AD/T HPLC equipped with a SPD-6AUV detector (Kyoto, Japan) and a Pinnacle (II) C-18 column (5 µm) 250 × 4.6 mm (Restek®, Bellefonte, USA) with an autoinjector and a photodiode array detector (PDA). The mobile phase was methanol (A) acidified water (0.1% formic acid) (B), with gradient elution of 0.01 min 60% A was used; 5–12 min 80% A; 13–14 min 60% A. Flow rate of mobile phase was 1.0 mL/min [Oliveiro et al. 2009]. The same equipment was used for the determination of the p-coumaric acid. In this case, the phenolic acid was adopted as the standard for its identification at 320 nm. The mobile phase was a mixture of acetonitrile, acidified water (phosphoric acid at pH = 2.5) (40 : 60) v/v, supplied at a rate of 0.8 mL/min [Kelebek et al. 2009]. Total flavonoids were determined by colorimetric method proposed by Marinova et al. [2005]. On the other hand, the measurement of the antioxidant capacity through FRAP (ferric reducing/antioxidant power assay) were expressed as AEAC (ascorbic acid equivalent antioxidant capacity: mg ascorbic acid per 100 g DW). According to described by Benzie and Strain [1996], the absorbance was determined at 593 nm. In brief, 50 µL of sample were added to 900 µL of pH 3.4 acetate buffer, TPTZ, FeCl₃ in a 10 : 1 : 1 ratio; after 30 min of reaction the absorbance was determined. The radical scavenging activity against the stable radical DPPH was measured using the methods proposed by Brand-Williams et al. [1995], with certain modifications. The method is based in the reaction of 10 µL of sample with 990 µL of DPPH solution for 30 min at room temperature. The absorbance decrease, associated with a reduction

in the DPPH concentration, was measured at 517 nm. The results were expressed in µmol trolox equivalents per 100 g DW. The antioxidant activity measured through the ABTS assay was determined the method reported by Re et al. [1999] with some modifications. 10 µL of sample was added to 990 µL of a solution of ABTS•+. The ability of the samples to trap the cationic radical ABTS•+ was evaluated by means of decrease in absorbance read after 30 min of reaction at a wavelength of 732 nm. The absorbance value was compared with the reference curve constructed with Trolox as the primary standard, and the results were expressed as TEAC values (µmol Trolox per 100 g DW).

For the oxygen radical absorbance capacity (ORAC) assay, the method described by Prior et al. [2005] and Romero et al. [2010] was used. 30 µL of the sample was added to 21 µL of 1 × 10⁻² M fluorescein in PBS (75 mM); 2.9 µL PBS (75 mM); and 50 µL of 0.6 M AAPH in PBS (75 mM). The temperature was controlled at 37°C and the pH was maintained at 7.4. The readings were made at an excitation λ 493 nm and excitation slit 10 nm; and an emission λ 515 nm with an emission slit 15 nm, with 1% attenuator and no attenuator plate. The protective effect of the antioxidant is calculated using the differences in areas under the decay curve of fluorescein between the blank (reaction in the absence of the sample) and the sample. It was compared against the curve of the primary Trolox® standard. The results were expressed as TEAC, µmol equivalents of Trolox per 100 g DW according to equation 1.

$$\text{ORAC} = \frac{\text{AUC} - \text{AUC}^\circ}{\text{AUC}_{\text{Trolox}} - \text{AUC}^\circ} f[\text{Trolox}] \quad (1)$$

Where AUC is the area under the curve of the sample, AUC[°] area under the curve for the control, AUC_{Trolox} area under the curve for the Trolox, *f* is the dilution factor of the extracts.

Assessment of cytotoxic activity. Chinese hamster ovary (CHO-K1) cell line was used for the cytotoxic determination and was purchased from ATCC (American Type Culture Collection). The extracts were applied at different concentrations. 3-(4,5-Dimethylthiazolyl-2)-2, 5-diphenyl-tetrazolium bromide (MTT) assay was measured using the methods proposed by Mosmann [1983], with certain modifications.

6000 viable cells were seeded in a 96-wellplate and treated for 24 h with different treatments, guaranteeing the presence of this during a cell cycle. Thereafter, 10 μ L of MTT solution (5 mg/mL) were added to each well and incubated for 3½ h. Later, the formazan crystals were dissolved by using 100 μ L of acidified isopropanol. Detection was performed at 570 nm using a Multiskan Spectrum spectrophotometer. On the other hand, neutral red was based on the protocol described by Borenfreund and Puerner [1985]. In this methodology, afterwards than cells were seeded and exposed to the different treatment, the medium was discarded; each well of the plate was washed with PBS and neutral red solution (0.1 mg/mL) was added. After one hour of incubation, neutral red was retired and was washed with PBS. Later, the neutral red remnant was dissolved with 400 μ L of acidified ethanol. The lecture was determined at 540 nm.

Statistical analysis. All the experiments were performed in triplicate. The regressions were calculated with a 95% significance level ($p < 0.05$), using the Statgraphics Plus program version 5.0 (Statistical Graphics Corp., Rockville, MD).

RESULTS AND DISCUSSION

The flowers intervene in the ecological interactions between the plants and its environment, because par-

ticipate in their diversification, mimicry, camouflage and defense [Jersáková et al. 2012]. Extracts and infusions of endemic plants played an important role in the life of rural and local people [Ladio et al. 2007]. According to Houghton [1984], especially in South America the peasants and indigenous groups use the structures of several vegetal species taking advantage of their biological properties. In this context we look for highlight the flowers of these endemic bushes, through its antioxidant activity and cytotoxic effect athwart the cellular viability of CHO-K1 cell line.

The content of metabolites is shown in Table 1, which for total phenols has been quantified as total gallic acid equivalent polyphenols. Also, reducing sugars, flavonoids, and bioactive compounds such as ascorbic acid, catechin, epicatechin and p-coumaric acid were determined by HPLC technique. The HPLC-PDA chromatograms of catechin and epicatechin are illustrated in Figure 1. The antioxidant and reducing activity of matico and zarzaparrilla flower extracts was characterized by different assays (ABTS, FRAP, DPPH, ORAC). The results of these analyzes can be seen in Table 2. In addition, cytotoxicity tests were performed through MTT and neutral red assays. The interaction between the extracts with MTT is shown in Figure 2. On the other hand, in the case of matico the tested concentrations were in the range between 10–500 μ g/ml (Fig. 3). Likewise, the concentrations

Table 1. The content of bioactive compounds and reducing sugars quantified in 100 g DW of *Buddleja globosa* (matico) and *Ribes magellanicum* (zarzaparrilla) flowers

Compound	<i>Buddleja globosa</i>	<i>Ribes magellanicum</i>
Total Phenols (mg gallic ac. Eq.)	6336.7 \pm 224.3*	5535.3 \pm 319.5*
Flavonoids (mg catechin Eq.)	3331 \pm 36.5*	1486.8 \pm 24*
p-coumaric acid (mg)	ND	478.6
Catechin (mg)	682.4	695.4
Epicatechin (mg)	304.6	3362.1
Ascorbic acid (mg)	ND	116.6
Fructose (mg)	16.4	26.4
Glucose (mg)	15.2	9.1
DNS (mg glucose Eq.)	6237.2 \pm 55.6*	6965.4 \pm 260.5*

* The mean \pm standard deviation (n = 3)
ND: no detected, DNS: 3,5-dinitroxylic acid

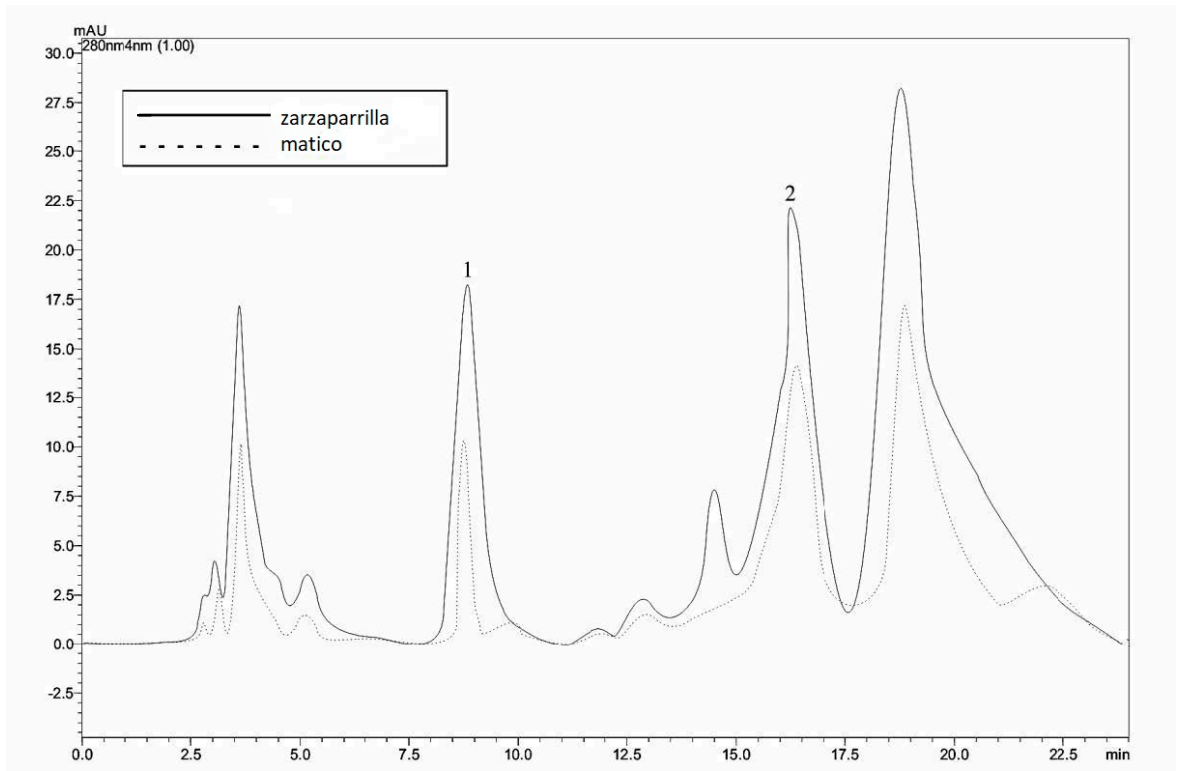


Fig. 1. HPLC-PDA chromatograms at 280 nm of (1) catechin, (2) epicatechin from matico (*Buddleja globosa*) and zarzaparrilla (*Ribes magellanicum*) flowers collected in the commune of Fresia, X region of Chile

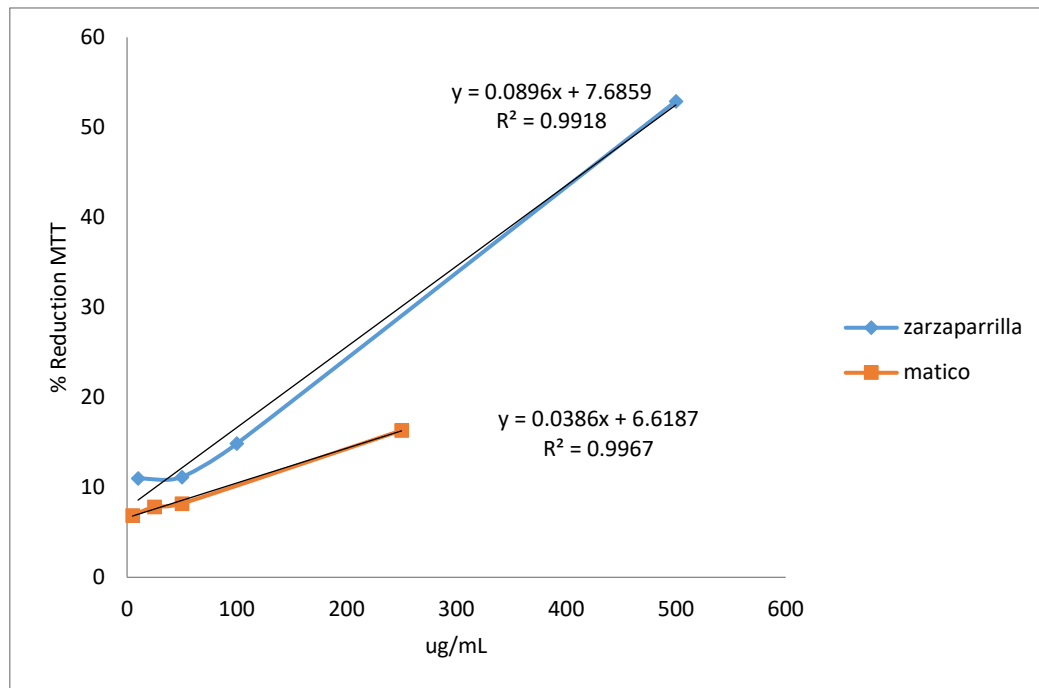


Fig. 2. Interaction between matico (*Buddleja globosa*) and zarzaparrilla (*Ribes magellanicum*) extracts with MTT

Table 2. Antioxidant activity quantified in 100 g DW of *Buddleja globosa* (matico) and *Ribes magellanicum* (zarzaparrilla) flowers

Methodology	<i>Buddleja globosa</i>	<i>Ribes magellanicum</i>
FRAP (mg ascorbic acid)	7492.1 ±312.7	8384.1 ±91.1
ABTS (µmol Trolox Eq.)	10475.7 ±1105.3	33488.9 ±3692.6
DPPH (µmol Trolox Eq.)	25836.6 ±155.8	19450.5 ±1525.2
ORAC-H (µmol Trolox Eq.)	134147.3 ±5792.5	104336.7 ±7408.8

The results of each analysis are presented as the mean ±standard deviation (n = 3)

FRAP: ferric reducing/antioxidant power assay; ABTS: 2,2'-azinobis-(3-ethylbenzthiazolin-6-sulfonic acid; DPPH: 2,2-diphenyl-1-picryl-hydrazyl-hydrate; ORAC-H: oxygen radical absorbance capacity assay

corresponding to 5, 25, 50 and 250 µg/mL obtained from zarzaparrilla and its effect are shown in Figure 4.

Bioactive compounds and reducing sugars. Comparing to another vegetal species with a known antioxidant potential; the total polyphenol content of both extracts (Tab. 1) are the order of the fruit of *Anacardium occidentale* (5286.5 mg gallic acid/100 g DW), and higher than those for *Eugenia uniflora* (3957.2 mg gallic acid/100 g DW) [Silva et al. 2014] and *Camellia chrysantha* (2351.1 mg gallic acid/100 g DW respectively) [Song et al. 2011]. Additionally, the concentration of flavonoids accounted for zarzaparrilla and matico are superior to those calculated for *Vitex doniana* (1960 µg catechin eq/g DW) and for *Ficus capensis* (flavonoids was not detected) [Muanda et al. 2011].

On the other hand, raised contents of p-coumaric acid, catechin and epicatechin were determined (Tab. 1). These high levels are relevant data, because these bioactive compound exhibits chemoprotectant, anti-inflammatory activities [Torres y Torres and Rosazza 2001]; and, in the case of catechins, properties that allow them to prevent oxidative stress by chelating free ferrous ions [Prior et al. 2003]. Also, it is been reported that these metabolites actuate eliminating reactive oxygen species in human lung (A549) and colon adenocarcinoma (HT29-D4) cell lines [Bouzaieni et al., 2015].

In addition, the HPLC-PDA chromatograms of catechin and epicatechin showed a concentration of catechin of 34.3 mg catechin/L for matico and 36.2 mg catechin/L for zarzaparrilla. For epicatechin the levels obtained were 15.3 mg catechin/L (matico) and 175.18 mg catechin/L (zarzaparrilla) (Fig. 1).

Antioxidant capacity. The chemical complexity of vegetal species often is a mixture of many compounds with different functional groups, polarity and chemical behavior; could lead to scattered results [Sacchetti et al. 2005]. According to this, Frankel et al. [1994] propose that many antioxidant assays are available, and that the results obtained by the methods depend on the oxidant/antioxidant models employed and on lipophilic/hydrophilic balance. Consequently, is highly advisable approach with multiple assays, thus covering a wider range of possible chemical behaviors [Sacchetti et al. 2005]. Taking this into account, the antioxidant activity was quantified by DPPH, ABTS, FRAP and ORAC-H tests (Tab. 2).

FRAP and Folin-Ciocalteu methods quantify the activity of phenolic compounds, but also are capable of detecting other reducing substance such reducing sugars and organic acid (including ascorbic and citric acid) [Prior et al. 2005]. Therefore, the yields were complemented by techniques, which quantify only antioxidant metabolites. According to Prior et al. [2005] one of the most assured methodologies is DPPH; since it quantifies only antioxidant compounds, and also presents a high selectivity due to its high molecular impediment. By DPPH, the antioxidant activities described for *Laurel nobilis* (12905 µmol Trolox/100 g DW), *Mentha piperita* (3356.1 µmol Trolox/100 g DW) [Fernandes et al. 2006], *Juniperus communis* (10947.3 µmol Trolox/100 g DW) and *Cinchona ledgeriana* (6832.1 µmol Trolox/100 g DW) [Al-Mustafa and Al-Thunibat 2008] are largely overcome by both flowers extracts.

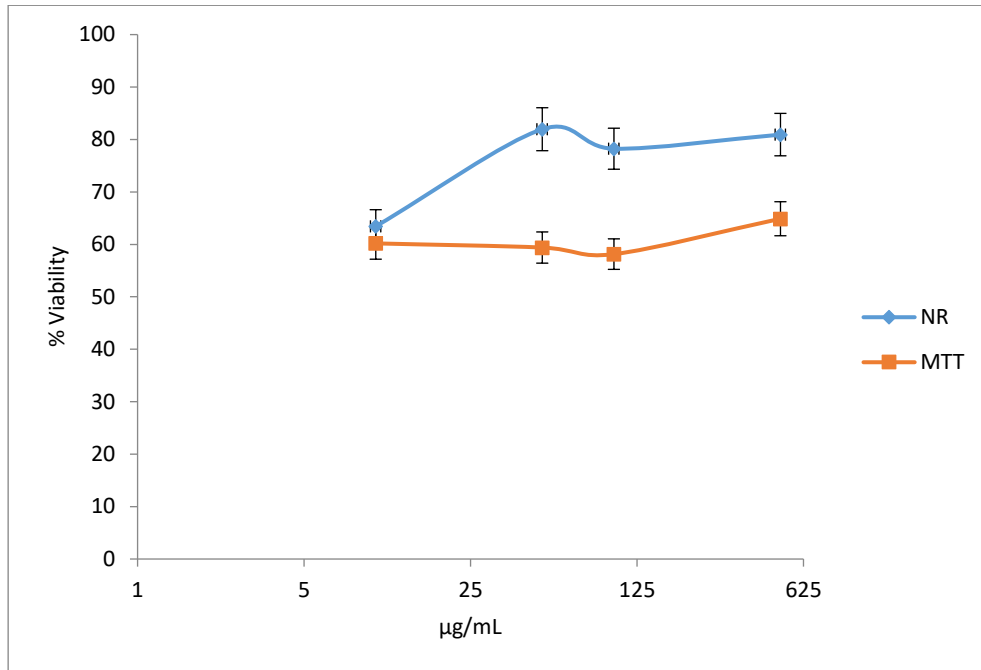


Fig. 3. Effect of matico (*Buddleja globosa*) flower extract on Chinese hamster ovary (CHO-K1) cell viability evaluated through MTT and neutral red techniques (NR). Data are the mean \pm standard deviation (n = 3)

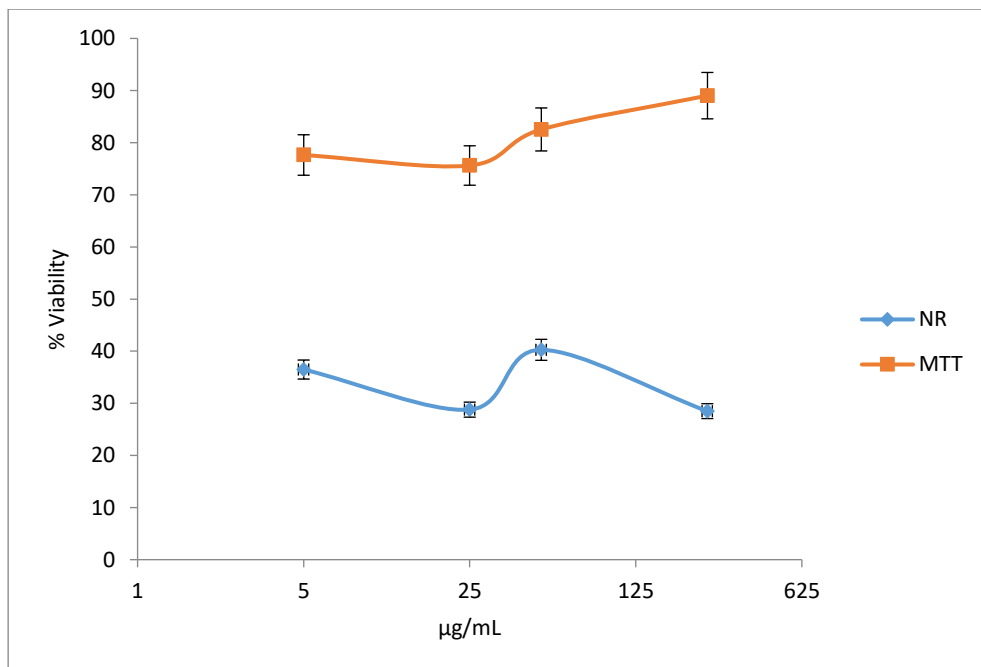


Fig. 4. Effect of zarzaparrilla (*Ribes magellanicum*) flower extract on Chinese hamster ovary (CHO-K1) cell viability evaluated through MTT and neutral red techniques (NR). Data are the mean \pm standard deviation (n = 3)

On the other hand, ABTS test is based on the quantification of the discoloration of the ABTS•+ radical, due to the interaction with hydrogen or electron donor species [Moon and Shibamoto 2009]. According to Lu et al. [2010], this methodology is mainly applied to antioxidants of an aqueous nature. Particularly, the content reported for zarzaparrilla (obtained from ABTS assay) are greater than those obtained for Chinese medicinal plants such *Ardisia japonica* (Horst) Bl. (16417 µmol Trolox/100 g DW), *Inula britannica* L. (9612 µmol Trolox/100 g DW) and *Mentha haplocalyx* Briq (8780 µmol Trolox/100 g DW) [Song et al. 2010].

Taking into account that ABTS can be dissolved easily in aqueous media [Arnao 2000]; while DPPH can only be dissolved in organic media [Wojdylo et al. 2007]; it can be observed that the molecular behavior is different in both bushes. The results suggest that the antioxidant compounds of zarzaparrilla shown a major solubility in aqueous media; while the nature of matico compounds are highly lipophilic and therefore more sensitive to the DPPH technique.

The ORAC technique measures the capacity of the polyphenols to neutralizing the peroxy radicals generated in situ through a HAT proton transfer mechanism [Prior et al. 2003]. In addition, supplies a controllable source of peroxy radicals and can be adapted to detect both hydrophilic and hydrophobic antioxidants by modifying the source of the radical and the solvent [Huang et al. 2005]. The concentration of both flowers are superior to those reported for *Centella asiatica* (69978 µmol Trolox/100 g DW), *Ruscus aculeatus* (40172 µmol Trolox/100 g DW), *Elymus repens* 13888 µmol Trolox/100 g DW [Wojcikowski et al. 2007] and Murta (*Ugni molinae*) (860–2380 µmol Trolox/100 g DW) [Peña-Cerda et al. 2017].

The elevated antioxidant activity measured by the ORAC method suggest that the flowers extracts has a high hydrophilic antioxidants levels with the ability to neutralize radicals ROO• peroxides. The antioxidant activity indicates a greater potential that described for other species of recognized pharmaceutical capacity. In fact, the antioxidant capacity of matico and zarzaparrilla extracts measured is 91.7% and 49.1% higher than *Centella asiatica*, which has demonstrate antitumor effect against human breast cancer (MDA-MB-231) and human lung carcinoma

(A549) [Pittella et al. 2009]. On the other hand, matico and zarzaparrilla is a 113.9% and 66.4% superior to black tea (*Camellia sinensis*), specie with confirmed antiproliferative and genotoxic properties against human colon carcinoma cell line (HT-29) [Koňariková et al. 2015]. And, much greater to the extracts of *Paemus boldus* and *Ugni molinae* (Chilean endemic species) recognized for being as antioxidants, chemo-preventives, digestive stimulants, diuretics and gallbladder [Peña-Cerda et al. 2017, Soto et al. 2014, Simirgiotis and Schmeda-Hirschmann 2010, Avello et al. 2014].

Cytotoxic activity. Cytotoxicity tests were performed through MTT and neutral red assays. The interaction between the extracts with MTT is shown in Figure 2. The concentrations used for matico were 10, 50, 100 and 500 µg/ml (Fig. 3). Likewise, the concentrations corresponding to 5, 25, 50 and 250 µg/mL obtained from zarzaparrilla and its effect are shown in Fig. 4.

Measurement of mitochondrial metabolic rate using MTT has been widely applied, due to its high sensitivity and stability [Wang et al. 2010]. Nevertheless, studies have shown that its properties could be affected when MTT interacts with bioactive compounds (particularly antioxidants) that present a reducing potential and/or a mechanism of action which implies the transfer of hydrogen atoms [Wang et al. 2010]. Figure 2 shows that both extracts interact directly with MTT reducing it to formazan in a linear relation. In fact, our results indicate that the extract of zarzaparrilla reduces MTT 2.3 time more than matico. To reduce the error caused by this interaction and determinate only the cellular metabolic capacity, the effect exerted by the extracts was subtracted. Besides, neutral red assay was used; which is a sensitive technique and also presents a high selectivity due to its capacity to evaluate the cellular ability to form lysosomes [Borenfreund and Puerner 1985].

For matico, the Figure 3 reveals a constant tendency of a concentration greater than 50 µg/mL; with the difference that the cellular viability in neutral red oscillates nearby to 80%, while in MTT is close to 60%. It is possible indicate that (in the majority of concentrations assayed) the exerted effect against the normal cells is independent of the concentration applied. Through neutral red assay it is possible to observe that the dynamism and stability of the cytoplasmic

membrane is affected being reduced by 20% [Fields et al. 2017]. On the other hand, the MTT assay shows that the cellular metabolic capacity decrease 40%. It is worth mentioning that in both techniques, the cell viability was always over the 50%.

In relation to zarzaparrilla, in the Figure 4 is also observed a constant tendency for both techniques. However, the gap is wider than that reported for matico. The viability in neutral red method does not exceed the 40%, so it can be considered as a cytotoxic extract, because it modified the membrane structure and moreover interfered in the cellular division process. Besides, MTT test indicate a cellular viability that oscillate between 80 to 90%, which denote that the cells do not lose their reducing capacity, either in cytoplasm or mitochondria. Now that the membrane is not viable, it allows the transit of MTT, and its reaction with the NADH or NADPH cofactors that are presented in the cytoplasm [Berridge and Tan 1993].

According to this and to acquire a more complete knowledge on these plants, further studies need to be conducted over the biological properties of matico and zarzaparrilla; these extracts could be applied in human liver S9 fractions (HepG2) with the aim to studying their metabolic behavior and activation and/or against several types of cancer cell being able a future natural chemotherapy alternative in development.

CONCLUSIONS

In this research the antioxidant and cytotoxic activity of matico and zarzaparrilla were reported, highlighting its potential compared with other plants that are used in alternative medicine. Our results suggest that the flower extracts are a rich source of polyphenols and secondary metabolites, which directly participate in the high antioxidant capacity quantified by this study. On the other hand, its cytotoxic effect could be evaluated athwart several cell lines, so that it's necessary to evaluate a wide-range of concentrations than those used in this study, that allow reduce the amount of CHO-K1 cell damage. On the basis of the results obtained and its projections, is possible to consider them as (1) a rich source of antioxidants and bioactive compounds, and (2) as a research objects through the analysis of their behavior against divers normal and cancer cell lines and/or in human liver S9 frac-

tions (HepG2) with the purpose to obtain data about the metabolic behavior of these flower extracts and its antitumoral potential.

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