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Bioactive phenolic compounds and organic acids in the decoction of fruits and leaves of *Schinus areira* L.

[Compuestos fenólicos bioactivos y ácidos orgánicos en la decocción de frutos y hojas de *Schinus areira* L.]

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Abstract: Leaf and fruit decoctions of *Schinus areira* L. from northwest Argentina were investigated here. Phenolic compounds and organic acids were analyzed by HPLC. Antioxidant capacity and α-glucosidase inhibition were determined by using *in vitro* tests. The general toxicity was assessed against *Artemia salina* nauplii. Hyperoside and 3 O-caffeoylquinic acid in leaf decoctions; gallic acid and catechin in fruit decoction were the major phenolic compounds. Malic and citric acids were the main organic acid quantified in the leaf and fruit decoctions, respectively. Fruit decoction had a relatively important content of shikimic acid, precursor of Tamiflu. Leaf decoction presents a greater richness in bioactive compounds with antiradical activity against DPPH●, O_2●- and ●NO radicals. *S. areira* leaves and fruits had α-glucosidase inhibitory activity comparable to hyperoside and acarbose. Fruit decoction was not eco-toxic; leaf decoction showed significant eco-toxic activity and could be chosen for the search of other bioactive compounds with pharmacological activity.

Keywords: *Schinus areira*; Decoction; Phenolics; Organic acids; Bioactivity

Resumen: Se investigaron decocciones de hojas y frutos de *Schinus areira* L. del noroeste de Argentina. Compuestos fenólicos y ácidos orgánicos se analizaron mediante HPLC. Capacidad antioxidante e inhibición de α-glucosidasa se determinaron *in vitro*. Se evaluó toxicidad general con *Artemia salina*. Los principales compuestos fenólicos fueron hiperósido y ácido 3 O-cafeoilquínico en hojas y ácido gálico y catequina en frutos. Los principales ácidos orgánicos cuantificados fueron málico en hojas y cítrico en frutos. Ácido shikímico, precursor del Tamiflu está presente en decocción de frutos con un contenido relativamente importante. La de hojas presenta una mayor riqueza en compuestos bioactivos con actividad antirradicalaria frente a DPPH●, O_2●- y ●NO. Las hojas y frutos de *S. areira* tenían una actividad inhibidora de la α-glucosidasa comparable a la de hiperósido y acarbosa. La decocción de frutas no fue eco-tóxica, pero sí la de hojas que podría ser fuente de compuestos bioactivos con actividad farmacológica.

Palabras clave: *Schinus areira*; Decocción; Compuestos fenólicos; Ácidos orgánicos; Bioactividad.

INTRODUCTION

Into the genus *Schinus*, the popular names molle, aguaribay, aroeira (Brazil), falso pimentero (Spain), pirú (Mexico) and pepper tree (USA) make reference both to *Schinus areira* L. and to *Schinus molle* L. (Bigliani *et al*., 2012; Celaya *et al*., 2014; Murray & Murray, 2017). These two aromatic species are taxonomically similar but have differences between them related to essential oil composition (Viturro *et al*., 2010), and they also have different original geographical distribution, which for *S. areira*, syn. *S. molle* L. var. areira (L.) DC., native from South America, is Peru, Bolivia, North of Chile, Paraguay and in Argentina it is found in the pre-puna zone between 1800 and 2300 masl and in the Chaco-Serrano district. (Martinez-Crovetto, 1963). *S. molle* has more limited distribution, being found only and naturally in northeastern Argentina, Uruguay, Paraguay and southern Brazil (Viturro *et al*., 2010). Different parts of *S. areira* are extensively used in folk medicine (Bigliani *et al*., 2012). Furthermore, molle fruits are called "pimienta rosa" (pink pepper) and are used as a substitute of black pepper due to their pungent smell in the preparations of foods and drinks (Murray & Murray, 2017).

Molle is a rich source of essential oils (Celaya *et al*., 2014). Regarding the chemical composition of the polar extracts of molle, different research have previously reported the presence of flavonoids and phenolic acids in *S. areira* and *S. molle* (Graziano *et al*., 1967; Rahman *et al*., 1974; Wannan *et al*., 1985; Ibrahim & Haggag, 2013; Celaya *et al*., 2016). Celaya *et al.* (2016), investigated previously the phenolic composition and biological activity of *S. areira* leaves from northwest Argentina; quercetin-3-*O*-galactoside, kaempferol-3-*O*-rutinoside and 3-*O*-caffeoylquinic acid were the main phenolics in aqueous ethanol (70:30) extracts obtained from leaves by ultrasound assisted extraction; aqueous ethanol extracts showed to be really active as antioxidants and antimicrobials. Rebolledo *et al*. (2020) combine a phytochemical screening with an investigation of the antioxidant capacity of *S. areira* L. extracts from Chile.

The use of hot water extractions of *S. areira* leaves as antiseptic and for the treatment of foot edema in adults has been reported (Bandoni *et al*., 1972; Quiroga *et al*., 2001). The aim of this study was to evaluate the recovery of organic acids and phenolic compounds in the decoction of *S. areira* (fruit and leaves); in addition to this, the antioxidant capacity (against $DPPH^{\bullet}$, $O_2^{\bullet-}$ and $^{\bullet}NO$ radicals), the α-glucosidase inhibitory activity and the eco-toxic activity were assessed in the same extracts by *in vitro* tests.

MATERIAL AND METHODS

Plant samples

Leaves and fruit of *S. areira* were collected at full fruit mature stage in Tilcara, Jujuy Province (Argentina). *S. areira* specimen selected for this study was Tg2 (one specimen of the β-phellandrene chemotype of essential oil from fruits (Celaya *et al*., 2016). Professor Gustavo Giberti (University of Buenos Aires, Argentina) identified the plant material. Voucher specimens have been deposited in Herbarium BAF (Buenos Aires Farmacobotánica, University of Buenos Aires, Argentina) and in Herbarium PRONOA-UNJu (Faculty of Engineering, National University of Jujuy, Argentina). The plant material, was dried at room temperature one week, powdered and stored at -20ºC until required.

Decoction of leaves and fruits

Decoction extracts were prepared according to Celaya *et al.* (2017). The yields obtained in the decoctions were 24.2% and 33.5% (from starting dry material) for leaf extracts (LE) and fruit extracts (FE), respectively. The resulting decoctions were filtered through a Büchner funnel, frozen and lyophilized. The lyophilized extracts were kept in a desiccator, in the dark, until analysis.

HPLC-DAD analysis of phenolic compounds

LE and FE were re-dissolved in methanol (30 and 90 mg/mL for LE and FE, respectively) and filtered through a 0.45 µm PTFE membrane. The phenolic compounds were analyzed using a previously described procedure, with a Spherisorb ODS2 (Waters, Milford, MA) column (Cabana *et al*., 2013; Celaya *et al*., 2016). Twenty microliters of each extract were analyzed on an analytical HPLC unit (Gilson), using a Spherisorb ODS2 (25.0 × 0.46 cm; 5 µm, particle size; Waters, Milford, MA) column. The solvent system used was a gradient of water:formic acid (19:1) (A) and methanol (B), starting with 5% methanol and installing a gradient to obtain 15% B at 3 min, 25% B at 13 min, 30% B at 25 min,35% B at 35 min, 45% B at 39 min, 45% B at 42 min, 50% B at 44 min,55% B at 47 min, 70% B at 50 min, 75% B at 56 min and 80% B at 60 min, at a solvent flow rate of 0.9 mL/min. Detection was achieved with a Gilson diode array detector (DAD). Spectral data from peaks were accumulated in the

range 200–400 nm, and chromatograms were recorded at 280 (tannins), 320 (phenolic acids) and 350 nm (flavonoids). The data were processed on Unipoint System software (Gilson Medical Electronics, Villiers-le-Bel, France). The compounds in each sample were identified by comparing their retention times and UV -spectra, with those of authentic standards from Extrasynthèse (Genay, France) and with the library of spectra previously compiled by the authors in addition to the literature data. Quantification of the phenolic compounds was achieved by measuring the peak area recorded in the chromatograms relative to external standards. 3-*O*-caffeoylquinic acid and others phenolic acid derivatives were quantified as 5-*O*-caffeoylquinic acid, quercetin derivatives were quantified as quercetin-3-*O*-glucoside. This procedure was performed in triplicate.

HPLC-UV analysis of organic acids

The separation and quantification of organic acids was carried out according to the procedure described by Silva *et al*. (2013), in an analytical HPLC unit (Gilson), using an ion exclusion Nucleogel Ion300 OA (300 × 7.7 mm; Macherey-Nagel, Düren, Germany) column. Dried extracts (50 mg of each extract) were dissolved in 1 mL of 0.01 N H_2SO_4, followed by filtration and analysis by HPLC. Elution was performed in isocratic mode with H_2SO_4 (0.01 N), at a flow rate of 0.2 mL/min. Detection was achieved with a UV detector set at 214 nm. The standard compounds used for the assay were from Sigma-Aldrich (St. Louis, MO, USA). Organic acids quantification was achieved by measuring the peak area recorded in the chromatograms relative to external standards. This procedure was performed in triplicate.

Total phenolic contents and antioxidant activity

The total phenolic content (mg of gallic acid equivalents per g of dry extract) was determined in LE and FE according to the Folin-Ciocalteau method (Celaya *et al.*, 2016). The total phenols were calculated as a gallic acid equivalent (GAE) from a calibration curve of gallic acid standard solutions.

The scavenging activity against DPPH$^{\bullet}$, $O_2^{\bullet-}$ (superoxide) and $^{\bullet}$NO (nitric oxide) radicals was evaluated according to the literature (Silva *et al.*, 2013). The concentration of extract that reduces 50% of the free-radical concentration (IC_{50}) was calculated through regression from the percentages of inhibition. With DPPH$^{\bullet}$, the antioxidant Trolox was used as reference compound.

α-Glucosidase inhibitory activity

α-Glucosidase inhibitory activity was assessed according to the method described by Celaya *et al*. (2017). Quercetin-3-*O*-galactoside (hyperoside) and acarbose were used as reference compounds for the assay. IC_{50} values represent the concentrations that caused 50% activity loss.

General toxicity of decoction extracts

General toxicity (eco-toxicity) of LE and FE was determined against *A. salina* nauplii according to Barbosa *et al*. (2009), with minor modifications. Eggs from *A. salina* were hatched at 26-30°C in seawater (pH 8.0) in contact with a light source (70 watt). After 48 h three tubes with groups of 10 *Artemia* nauplii were prepared for each dose. Test solutions at appropriate amounts (10, 50, 100, 500 and 1000 µg/mL) were prepared in distilled deionized water, and transferred into 5 mL tubes. The control group consisted of only seawater and nauplii. All tubes were maintained under illumination. The lethal concentration fifty LC_{50} (95% confidence interval), was determined from the 24 hour counts using the probit analysis method. The assay was performed in triplicate.

RESULTS AND DISCUSSION

Phenolic compounds

Phenolic compounds are secondary metabolites produced in plants. These metabolites have attracted considerable attention due to their beneficial functional and nutritional effects including antioxidant activity and antidiabetic action (Celaya *et al*., 2017). The phenols/polyphenols have also actions on the cardiovascular system (Behl *et al*., 2020), on the age related eye-diseases (Bungau *et al*., 2019) and on many other diseases.

In the present study, the HPLC-DAD analysis of FE and LE showed that decoction of *S. areira* provides large amounts of phenolic compounds (Table No. 1). The analysis allowed the determination of 17 phenolic compounds, which comprise two hydroxybenzoic acids (**1** and **3**), five hydroxycinammic acids and derivatives (**2**, **4**, **6**, **9** and **17**) and ten flavonoids (**5**, **7, 8, 10**-**16**) (Table No. 1).

Table No. 1
Phenolic compounds quantified in LE and FE

	Compound	LE[(a)]	FE[(a)]
1	**Gallic acid**	nq	681.5 ± 0.2 (41.4%)
2	**Phenolic acid derivative 1**	1794.3 ± 7.3 (2.3%)	nq
3	**Protocatechuic acid**	nq	72.5 ± 0.2 (4.4%)
4	**3-*O*-Caffeoylquinic acid**	12980.0 ± 97.2 (16.6%)	nq
5	**Catechin**	nq	435.5 ± 0.2 (26.4%)
6	**5-*O*-Caffeoylquinic acid**	2773.5 ± 48.6 (3.6%)	nq
7	**Quercetin derivative 1**	6536.5 ± 21.1 (8.4%)	67.3 ± 0.3 (4.1%)
8	**Quercetin derivative 2**	1621.2 ± 9.0 (2.1%)	nq
9	**Phenolic acid derivative 2**	1816.7 ± 24.3 (2.3%)	nq
10	**Quercetin derivative 3**	1557.6 ± 5.3 (2.0%)	55.7 ± 0.4 (3.4%)
11	**Quercetin-3-*O*-galactoside**	38095.2 ± 52.3 (48.9%)	243.1 ± 0.1 (14.8%)
12	**Quercetin-3-*O*-glucoside**	2290.7 ± 9.1 (2.9%)	nq
13	**Quercetin-3-*O*-rutinoside**	1777.5 ± 3.1 (2.3%)	53.6 ± 0.2 (3.2%)
14	**Quercetin-3-*O*-arabinoside**	1675.1. ± 2.9 (2.1%)	37.6 ± 0.2 (2.3%)
15	**Quercetin-3-*O*-rhamnoside**	2501.2 ± 6.2 (3.2%)	21.6 ± 0.5 (1.3%)
16	**Kaempferol-3-*O*-rutinoside**	1959.6 ± 4.9 (2.5%)	8.3 ± 0.1 (0.5%)
17	***p*-Coumaric acid derivative**	585.7 ± 1.2 (0.7%)	13.7 ± 0.1 (0.8%)
	Σ	**77964.7**	**1646.8**

[(a)] Yield (µg/g of dry extract); nq, not quantified; Σ, sum of the determined phenolic compounds. Phenolic acid derivatives quantified as 5-*O*-caffeoylquinic acid, quercetin derivatives quantified as quercetin-3-*O*-glucoside.

The phenolic profile of both decoctions, LE and FE was different in quantitative and qualitative terms. Quantitatively, the sum of the identified compounds was 77.964,7 and 1646,8 µg/g in LE and FE, respectively. The decoction was really effective for the recovery of phenolic compounds. The major compound in LE was quercetin-3-*O*-galactoside (hyperoside) followed by 3-*O*-caffeoylquinic acid (Table No. 1). FE with a minor recovery, showed distinct phenolic profile; eleven compounds being determined; gallic acid was the most abundant phenolic in FE followed by catechin (Table No. 1). The hyperoside inhibits the proliferation of osteosarcoma and may stimulate osteoblastic differentiation in osteosarcoma cells (Zhang *et al.*, 2014). Current evidence confirms the pharmacological and therapeutic interventions of gallic acid in multiple health complications: gastrointestinal, neuropsychological, metabolic, and cardiovascular disorder (Kahkeshani *et al.*, 2019). Catechins and their chemical derivatives are effective antiviral agents (Ide *et al.*, 2016). Chlorogenic acid has many beneficial health, antioxidant, anti-inflammatory, anti-diabetic properties (Ahn *et al*., 2011) and it acts as anti-arthritic agent (Chauhan *et al*., 2012), and as neuroprotective (Mikami & Yamazawa, 2015)

The results obtained here are in agreement with the phenolic compounds identified previously in *S. areira* and *S. molle* leaves and twigs (Saleh *et al*., 1969; Domínguez *et al*., 1971; Marzouk *et al*., 2006; Ranilla *et al*., 2010; Ibrahim & Haggag, 2013; Celaya *et al*., 2016). In *S. areira* fruits, only quercetin derivatives, amentoflavone derivatives and colored peonidin glycosides and cyanidin glycosides were previously reported (Graziano *et al*., 1967; Wannan *et al*., 1985).

Organic acids

Organic acids are primary metabolites, which can be found in great amounts in all plants (Oliveira *et al.*, 2008). As phenolics, the organic acids may also have a protective role against various diseases due to their antioxidant properties (Silva *et al*., 2013; Guimarães *et al*., 2013). The HPLC–UV analysis of organic acids in LE and FE revealed a profile composed by oxalic, aconitic, citric, malic, malonic, quinic, shikimic, acetic and fumaric acid (Table No. 2). In the pharmaceutical industry, shikimic acid, obtained from star anise (*Illicium verum*), is used for the

production of the antiviral oseltamivir (tamiflu) (Bradley, 2005; Ohira *et al*., 2009).

The total amounts of organic acids in FE were higher than LE (Table No. 2). The sum of the identified organic acids was 55.6 and 56.4 mg/g in LE and FE, respectively. Significant differences were found between LE and FE. Citric acid (30% of the all determined compounds) was the major organic acid in FE (Table No. 2) followed by malic acid (17% of the determined compounds). Malic acid was the main organic acid in LE (54% of the determined compounds), followed by oxalic acid (19%) (Table No. 2).

Table No. 2
Contents of organic acids quantified in LE and FE

	Compound	LE[(a)]	FE[(a)]
1	**Oxalic acid**	10346.5 ± 87.2 (18.6%)	5839.7 ± 105.9 (10.4%)
2	**Aconitic acid [(b)]**	73.0 ± 4.0 (0.1%)	150.5 ± 8.8 (0.3%)
3	**Citric acid**	2591.0 ± 187.7 (4.7%)	16698.7 ± 1250.7 (29.6%)
4	**Malic acid**	29893.1 ± 1377.7 (53.8%)	9437.6 ± 223.7 (16.7%)
5	**Malonic acid**	nq	4665.3 ± 807.1 (8.3%)
6	**Quinic acid**	4252.3 ± 760.2 (7.7%)	7236.6 ± 302.5 (12.8%)
7	**Shikimic acid**	3093.7 ± 51.4 (5.6%)	5735.7 ± 14.7 (10.2%)
8	**Acetic acid**	5248.2 ± 1129.5 (9.4%)	6344.9 ± 601.3 (11.3%)
9	**Fumaric acid**	80.0 ± 2.3 (0.1%)	282.5 ± 28.0 (0.5%)
	Σ	**55577.8**	**56391.5**

[(a)]Yield, µg/g of dry extract. (b) aconitic acid (*cis* + *trans*)

Antioxidant activity and total phenols

The antioxidant activity of LE and FE was tested against DPPH•, superoxide and nitric oxide radicals (Table No. 3). DPPH• is a stable free radical, which provides a good indication of a sample's anti-radical potential. Superoxide and nitric oxide radicals are highly reactive molecules, constantly produced through numerous biological reactions (Phaniendra *et al*., 2015). Molle extracts are really actives as natural antioxidants. LE proved to be more active than FE against all radicals. For comparison purposes, the IC_{50} of Trolox against DPPH• was included in this study; the antioxidant activity of LE was comparable to that of Trolox (Table No. 3). In addition to scavenging activity, the recovery of total phenols assayed by the Folin-Ciocalteau method was 241.0 ± 2.3 mg GAE/g of dry extract and 28.1 ± 0.6 mg GAE/g of dry extract, for LE and FE, respectively. The obtained results are in agreement with previous reported data on total phenols and antioxidant activity of *S. areira* leaves, *S. molle* fruit and *S. molle* leaves and twigs (Ranilla *et al*., 2010; Barroso *et al*., 2011; Ibrahim & Haggag, 2013; Abir *et al*., 2016). The methanolic Soxhlet extract of *S. areira* fruit from Chile studied by Rebolledo *et al*. (2020) had a good polyphenol content of 195 mg GAE/g dry weight and antioxidant activity measured as DPPH• scavenging capacity with an EC_{50} = 475.6 µg/mL. Different plant parts differ in the composition of their pool of phytochemicals (Larrazabal *et al*., 2018). The biological activity of the plant extracts largely depends on the composition of these extracts (Li *et al*., 2013). The behavior observed in the scavenging activity may be attributed to complex mixtures of different kinds of compounds extracted from molle leaves and fruits (Guimarães *et al*., 2013); these compounds include flavonoids, phenolic acids and organic acids determined in the present study. The relationship between the antioxidant activity of different plants' compounds and their structure was determined by Glevitzky *et al*. (2019).

***α*-Glucosidase inhibitory activity**

The *α*-glucosidase inhibitory activity of LE and FE was performed here. A concentration-dependent potential was observed in all assays (Figure No. 1). LE was most active than FE (Table No. 3). *S. areira* possess a great potential to be used in the treatment of the diabetes Mellitus, since LE showed a considerable better inhibition than those obtained

previously with other plants used in the treatment of diabetes (Table No. 3) (Andrade-Cetto *et al*., 2008; Kazzem & Ashafa, 2015).

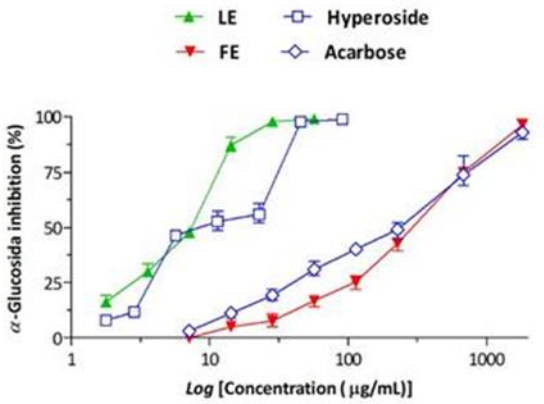

Figure No. 1
α-Glucosidase inhibition of LE and FE

Hyperoside was used as control for the *α*-glucosidase assay and the IC_{50} was comparable to that obtained with LE (Table No. 3). Acarbose is used as a medicine to treat type II diabetes by mechanism of *α*-glucosidase inhibition. Acarbose showed *α*-glucosidase inhibitory activity comparable to FE (Table No. 3, Figure No. 1).

The bioactivity determined in the present study may be attributed, at least partially, to the presence of higher amounts in specific compounds in molle (Bras *et al*., 2010; Ibrahim & Haggag, 2013). Bras *et al*. (2010) found a tendency of ethanolic extract from molle leaf, to lower the glucose level in mice. According to the results, inhibition of *α*-glucosidase may be one mechanism involved in the potential hypoglycaemic effect of *S. areira* (Thengyai *et al*., 2020). Quercetin derivatives and different phenolic acids have been described as inhibitors of *α*-glucosidase previously (Cho *et al*., 2003; Li *et al*., 2009; Wu *et al*., 2014; Djeridane *et al*., 2015; Oboh *et al*., 2016). We highlight the high amount in quercetin derivatives, caffeoylquinic acids and gallic acid found in the decoctions (Table No. 1).

Table No. 3
IC_{50} (µg/mL) values found in the antioxidant activity and *α*-glucosidase assays for LE and FE

***In vitro* assay**	**Sample [a]**					**Literature data**
	LE	**FE**	**Trolox**	**Acarbose**	**Hyperoside**	
DPPH•	24.1 ± 0.2	264.8 ± 6.6	27.0 ± 1.9	-	-	23-43 [(1)]
•NO	258.0 ± 12.0	1167.0 ± 68.3	-	-	-	442-483 [(1)]
$•O_2^-$	21.3 ± 0.5	85.6 ± 2.6	-	-	-	38-60 [(1)]
***α*-glucosidase**	7.4 ± 0.1	306.1 ± 10.9	-	272.4 ± 2.6	6.0 ± 0.2	7-109 [(2,3)]

[a]Values are expressed as mean ± standard deviation of three assays.
[(1)]Celaya *et al*., 2016
[(2,3)]Andrade-Cetto *et al*., 2008; Kazeem & Ashafa, 2015

Results of the general toxicity assay

The assay of eco-toxicity against *Artemia salina* nauplii, is used as general bioassay tool and indicates which extracts could be subjected to more elaborate bioassays in search of bioactive compounds with pharmacological activity (Apu *et al.*, 2013). LC_{50} is indicative of eco-toxicity level of plant extracts against *A. salina*. Extracts with LC_{50} values lower than 1000 µg/mL are considered actives; with LC_{50} values less than 250 µg/mL are considered significantly active (Meyer *et al*., 1982). Finally, LC_{50} values less than 100 µg/mL are indicative of the presence of potent cytotoxic compounds in the extracts (Peteros & Mylene, 2010).

In the general toxicity assay FE was not eco-toxic with LC_{50} value>1000 µg/mL. This result supports the traditional use of molle fruit in food and drinks. LE showed significant eco-toxic activity with LC_{50} = 187.1 ± 27.1 µg/mL. In a previous work, Ferrero *et al*. (2010) determined that the subchronic oral exposure to ethanolic extracts from molle (fruits and leaves) did not produce toxicity in mice. The LE eco-toxic value, suggests the presence of potent bioactive compounds in *S. areira* leaves. Therefore, leaves may be chosen for the search of potential cytotoxic agents in future investigations.

CONCLUSIONS

This study reports the chemical composition and biological activity potential of the decoction of leaves and fruit of *S. areira*, which are a rich source of bioactive compounds.

With respect to phenolic compounds and organic acids, leaf decoctions have more phenolic compounds compared to fruit. Fruit decoctions have more organic acids compared to leaves. As for the biological activity potential, the leaf extract proved to be really active as an antioxidant and inhibitor of α-glucosidase. *S. areira* can be considered as a plant matrix with great antidiabetic potential.

Leaf decoction proved to be active in the general toxicity test. Therefore, bioactive compounds with great pharmacological activity could be present in *S. areira* leaves.

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REFERENCES

Abir K, Majdi H, Manef A, Same A. 2016. *Schinus molle*: chemical analysis, phenolic compounds and evaluation of its antioxidant activity. **J Chem Pharm Res** 8: 93 - 101.

Ahn EH, Kim DH, Shin MJ, Kwon SW, Kim YN, Kim D, Lim SS, Kim J, Park J, Eum WS, Hwang HS, Choi SY. 2011. Chlorogenic acid improves neuroprotective effect of PEP-1-ribosomal protein S3 against ischemic insult. **Exp Neurobiol** 4: 172 - 178. **https://doi.org/10.5607/en.2011.20.4.169**

Andrade-Cetto P, Becerra-Jimenez J, Cardenas-Vazquez R. 2008. Alfa-glucosidase-inhibiting activity of some Mexican plants used in the treatment of type 2 diabetes. **J Ethnopharmacol** 116: 27 - 32. **https://doi.org/10.1016/j.jep.2007.10.031**

Apu AS, Chowdhury FA, Khatun F, Jamaluddin ATM, Pathan AH, Pal A. 2013. Phytochemical screening and *in vitro* evaluation of pharmacological activities of *Aphanamixis polystachya* (Wall) Parker fruit extracts. **Trop J Pharm Res** 12: 111 - 116. **https://doi.org/10.4314/tjpr.v12i1.18**

Bandoni AL, Mendiondo ME, Rondina RVD, Coussio JD. 1972. Survey of argentine medicinal plants in folklore and phytochemical screening. **Lloydia** 35: 69 - 80.

Barbosa TP, Junior CGL, Silva FPL, Lopes HM, Figueiredo LRF, Sousa SC, Batista GN, da Silva TG, Silva TM, de Oliveira MR, Vasconcellos MLA. 2009. Improved synthesis of seven aromatic Baylise Hillman adducts (BHA): Evaluation against *Artemia salina* Leach. and *Leishmania chagasi*. **Eur J Med Chem** 44: 1726 - 1730. **https://doi.org/10.1016/j.ejmech.2008.03.016**

Barroso MST, Villanueva G, Lucas AM, Pere GP, Vargas RMF, Brun GW, Casse E. 2011. Supercritical fluid extraction of volatile and non-volatile compounds from *Schinus molle* L. **Braz J Chem Eng** 28: 305 - 312. **https://doi.org/10.1590/S0104-66322011000200015**

Behl T, Bungau S, Kumar K, Zengin G, Khan F, Kumar A, Kaur R, Venkatachalam T, Tit DM, Vesa CM, Barsan G, Mosteanu DE. 2020. Pleotropic effects of polyphenols in cardiovascular system. Biomed Pharmacother 130: 110714 **https://doi.org/10.1016/j.biopha.2020.110714**

Bigliani M, Rossetti V, Grondon E, Lo Presti S, Paglini P, Rivero V, Zunino M, Ponce A. 2012. Chemical compositions and properties of *Schinus areira* L. essential oil on airway inflammation and cardiovascular system of mice and rabbits. **Food Chem Toxicol** 50: 2282 - 2288. **https://doi.org/10.1016/j.fct.2012.04.025**

Bradley D. 2005. Star role for bacteria in controlling flu pandemic?. **Nat RevDrug Discov** 4: 945 - 946.

Bras C, Domínguez S, Codón S, Minetti A, Ferrero A. 2010. Consequences of subchronic exposure to ethanolic extract from fruits and leaves of *Schinus molle* var. *areira* L. in mice. **J Ethnopharmacol** 132: 321 - 327. **https://doi.org/10.1016/j.jep.2010.08.035**

Bungau S, Abdel-Daim MM, Tit DM, Ghanem E, Sato S, Maruyama-Inoue M, Yamane S, Kadonosono K. 2019. Health benefits of polyphenols and carotenoids in age-related eye diseases. **Oxid Med Cell Longev** 2019: 9783429 **https://doi.org/10.1155/2019/9783429**

Cabana R, Silva L, Valentão P, Viturro CI, Andrade PB. 2013. Effect of different extraction methodologies on the recovery of bioactive metabolites from *Satureja parvifolia* (Phil.) Epling (Lamiaceae). Ind Crop 48: 49 -

56. **https://doi.org/10.1016/j.indcrop.2013.04.003**

Celaya LS, Alabrudzińska MH, Molina AC, Viturro CI, Moreno S. 2014. The inhibition of methicillin-resistant *Staphylococcus aureus* by essential oils isolated from leaves and fruits of *Schinus areira* depending on their chemical compositions. **Acta Biochim Pol** 61: 41 - 46. **https://doi.org/10.18388/abp.2014_1921**

Celaya LS, Viturro CI, Silva LR, Moreno S. 2016. Natural antioxidants isolated from *Schinus* areira leaves by ultrasound-assisted extraction. Int J Food Stud 5: 1-14. **https://doi.org/10.7455/ijfs/5.2.2016.a5**

Celaya L, Viturro CI, Silva LR. 2017. Chemical composition and biological prospecting of essential oil and extracts of *Aphyllocladus spartioides* growing in Northwest Argentina. **Chem Biodiversity** 14: 1 - 10. e1600227. **https://doi.org/10.1002/cbdv.201600227**

Chauhan PS, Satti NK, Sharma P, Sharma VK, Suri K A, Bani S. 2012. Differential effects of chlorogenic acid on various immunological parameters relevant to rheumatoid arthritis. **Phytother Res** 26: 1156 - 1165. **https://doi.org/10.1002/ptr.3684**

Cho EJ, Yokozawa T, Rhyu DY, Kim SC, Shibahara N, Park JC. 2003. Study on the inhibitory effects of Korean medicinal plants and their main compounds on the 1,1-diphenyl-2-picrylhydrazyl radical. **Phytomedicine** 10: 544 - 551. **https://doi.org/10.1078/094471103322331520**

Djeridane A, Hamdi A, Bensania W, Cheifa K, Lakhdari I, Yousfi M. 2015. The *in vitro* evaluation of antioxidative activity, α-glucosidase and α-amylase enzyme inhibitory of natural phenolic extracts. **Diabetes Metab Syndr** 9: 324 - 331. **https://doi.org/10.1016/j.dsx.2013.10.007**

Domínguez XA, Carmona FF, Venegas RB. 1971. Lignoceric acid and other compounds of *Schinus molle*. **Phytochemistry** 10: 1687.

Ferrero A, Minetti A, Bras C, Zanetti N. 2010. Acute and subacute toxicity evaluation of ethanolic extract from fruits of *Schinus molle* in rats. **J Ethnopharmacol** 113: 441 - 447. **https://doi.org/10.1016/j.jep.2007.06.019**

Glevitzky I., Dumitrel GA, Glevitzky M, Pasca B, Otrisal P, Bungau S, Cioca G, Pantis C, Popa M. 2019. Statistical analysis of the relationship between antioxidant activity and the structure of flavonoid compounds. **Rev Chim** 70: 3103 - 3107. **https://doi.org/10.37358/RC.19.9.7497**

Graziano MN, Segui M, Coussio JD. 1967. Estudio de los flavonoides presentes en plantas argentinas. **Anal Asoc Quim Argent** 55: 235 - 237.

Guimarães R, Barros L, Dueñas M, Calhelha RC, Carvalho AM, Santos-Buelga C, Queiroz MJ, Ferreira I. 2013. Infusion and decoction of wild German chamomile: bioactivity and characterization of organic acids and phenolic compounds. **Food Chem** 136: 947 - 954. **https://doi.org/10.1016/j.foodchem.2012.09.007**

Ibrahim MT, Haggag EG. 2013. Phenolics from *Schinus molle* stems and their anti-bacterial and anti-microalgal activity. **Life Sci** 10: 3.

Ide K, Kawasaki Y, Kawakami K, Yamada H. 2016. Anti-influenza virus effects of catechins: A molecular and clinical review. **Curr Med Chem** 23: 4773 - 4783. **https://doi.org/10.2174/0929867324666161123091010**

Kahkeshani N, Farzaei F, Fotouhi M, Alavi SS, Bahramsoltani R, Naseri R, Momtaz S, Abbasabadi Z, Rahimi R, Farzaei MH, Bishayee A. 2019. Pharmacological effects of gallic acid in health and diseases: A mechanistic review. **Iran J Basic Med Sci** 22: 225 - 237. **https://doi.org/10.22038/ijbms.2019.32806.7897**

Kazeem MI, Ashafa AOT. 2015. *In-vitro* antioxidant and antidiabetic potentials of *Dianthus basuticus* Burtt Davy whole plant extracts. **J Herb Med** 5: 158 - 164. **https://doi.org/10.1016/j.hermed.2015.06.003**

Larrazabal MJ, Palma J, Paredes A, Morales G, Mercado A. 2018. Effect of brewing conditions on pigments and total polyphenols content and biological activities of the *Acantholippia deserticola* (Phil.) infusion. **J Food** 16: 588 - 595. **https://doi.org/10.1080/19476337.2018.1441189**

Li S, Li SK, Gan RY, Song FL, Kuang L, Li HB. 2013. Antioxidant capacities and total phenolic contents of infusions from 223 medicinal plants. **Ind Crops Prod** 51: 289-298. **https://doi.org/10.1016/j.indcrop.2013.09.017**

Li YQ, Zhou FC, Gao F, Bian JS, Shan F. 2009. Comparative evaluation of quercetin, isoquercetin and rutin as inhibitors of alpha-glucosidase. **J Agric Food Chem** 57: 11463 - 11468. **https://doi.org/10.1021/jf903083h**

Martinez-Crovetto R. 1963. Estudio taxonómico y biométrico de *Schinus molle* y *Schinus areira* (Anarcadiaceae). **Bonplandia** 1: 225 - 244.

Marzouk MS, Moharram FA, Haggag EG, Ibrahim MT, Badary OA. 2006. Antioxidant flavonol glycosides from *Schinus molle*. **Phytother Res** 20: 200 - 205. **https://doi.org/10.1002/ptr.1834**

Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. 1982. Brine shrimp: a convenient general bioassay for active plant constituents. **Planta Med** 45: 31 - 34. **https://doi.org/10.1055/s-2007-971236**

Mikami Y, Yamazawa T. 2015. Chlorogenic acid, a polyphenol in coffee, protects neurons against glutamate neurotoxicity. **Life Sci** 139: 69 - 74. **https://doi.org/10.1016/j.lfs.2015.08.005**

Murray A, Murray M. 2017. Phytochemistry, traditional uses and bioactivity of the medicinal plant *Schinus areira* L. (Anacardiaceae): a review. **Nat Prod J** 7: 1 - 7. **https://doi.org/10.2174/2210315507666170117145728**

Oboh G, Ogunsuyi OB, Ogunbadejo MD, Adefegha SA. 2016. Influence of gallic acid on a-amylase and a-glucosidase inhibitory properties of acarbose. **J Food Drug Anal** 24: 627 - 634. **https://doi.org/10.1016/j.jfda.2016.03.003**

Ohira H, Torii N, Aida T, Watanabe M, Smith R. 2009. Rapid separation of shikimic acid from Chinese star anise (*Illicium verum* Hook. f.) with hot water extraction. **Sep Purif Tech J** 69: 102 - 108.

Oliveira AP, Pereira JA, Andrade PB, Valentão P, Seabra RM, Silva BM. 2008. Organic acids composition of *Cydonia oblonga* miller leaf. **Food Chem** 111: 393–399. **https://doi.org/10.1016/j.foodchem.2008.04.004**

Peteros NP, Mylene MU. 2010. Antioxidant and cytotoxic activities and phytochemical screening of four Philippine medicinal plants. **J Med Plant Res** 4: 407 - 414.

Phaniendra A, Jestadi DB, Periyasamy L. 2015. Free radicals: properties, sources, targets, and their implication in various diseases. **Indian J Clin Biochem** 30: 11 - 26. **https://doi:10.1007/s12291-014-0446-0**

Quiroga EN, Sampietro AR, Vattuone MA. 2001. Screening antifungal activites of selected medicinal plants. **J Ethnopharmacol** 74: 89 - 96. **https://doi.org/10.1016/S0378-8741(00)00350-0**

Rahman AU, Tomas MA, Frontera MA. 1974. Anthocyanins from fruits of *Schinus molle* var *areira* (Aguaribay). **Anal Asoc Quim Arg** 62: 169 - 170.

Ranilla LG, Kwon Y-I, Apostolidis E, Shetty K. 2010. Phenolic compounds, antioxidant activity and in vitro inhibitory potential against key enzymes relevant for hyperglycemia and hypertension of commonly used medicinal plants, herbs and spices in Latin America. **Bioresour Technol** 101: 4676 - 4689. **https://doi.org/10.1016/j.biortech.2010.01.093**

Rebolledo V, Otero MC, Delgado JM, Torres F, Herrera M, Ríos M, Cabañas M, Martinez JL, Rodríguez-Díaz M. 2021. Phytochemical profile and antioxidant activity of extracts of the peruvian peppertree *Schinus areira* L. from Chile. **Saudi J Biol Sci** 28: 1052 - 1062. **https://doi.org/10.1016/j.sjbs.2020.10.043**

Saleh N, El Sherbeiny AEA, El Sissi HI. 1969. Local plants as potential sources of tannins in Egypt. PART IV. (Aceraceae to Flacourtiaceae). **Qual Plant Mat Veg** 17: 384 - 394.

Silva LR, Pereira MJ, Azevedo J, Goncalves RF, Valentao P, de Pinho PG, Andrade PB. 2013. *Glycine max* (L.) Merr., *Vigna radiata* L. and *Medicago sativa* L. sprouts: A natural source of bioactive compounds. **Food Res Int** 50: 167 - 175. **https://doi.org/10.1016/j.foodres.2012.10.025**

Thengyai S, Thiantongin P, Sontimuang C, Ovatlarnporn C, Puttarak P. 2020. α-Glucosidase and α-amylase inhibitory activities of medicinal plants in Thai antidiabetic recipes and bioactive compounds from *Vitex glabrata* R. Br. Stem bark. J Herb Med 19: 100302. **https://doi.org/10.1016/j.hermed.2019.100302**

Viturro C, Bandoni A, Dellacassa E, Atti Serafini L, Elder H. 2010. **Problemática *Schinus* en Latinoamérica.** In Dellacassa E. Normalización de productos naturales obtenidos de especies de la flora aromática latinoamericana. Ed. Universitária da PUCRS, Porto Alegre, Brasil.

Wannan BS, Waterhouse JT, Gadek PA, Quinn CJ. 1985. Biflavonyls and the affinities of *Blepharocarya*. **Biochem Syst Ecol** 13: 105 - 108. **https://doi.org/10.1016/0305-1978(85)90066-3**

Wu C, Zhang X Zhang X, Luan H, Sun G, Sun X. 2014. The caffeoylquinic acid-rich *Pandanus tectorius* fruit extract increases insulin sensitivity and regulates hepatic glucose and lipid metabolism in diabetic db/db mice. **J Nutr Biochem** 25: 412 - 419. **https://doi.org/10.1016/j.jnutbio.2013.12.002**

Zhang N, Ying MD, Wu YP, Zhou ZH, Ye ZM, Li H, Lin DS. 2014. Hyperoside, a flavonoid compound, inhibits proliferation and stimulates osteogenic differentiation of human osteosarcoma cells. Plos One 9: e98973. **https://doi.org/10.1371/journal.pone.0098973**