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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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Citation: Celaya LS, Molina AC, González MA, Villa WC, Silva LR, Viturro CI. Bioactive phenolic compounds and organic acids in the decoction of fruits and leaves of *Schinus areira* L. Bol Latinoam Carib<u>e Plant Med Aromat</u> **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•, O2• 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•, O2•- 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; Celava 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 (Celava 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[•], $O_2^{\bullet^-}$ and •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 (Figure No. 1), was dried at room temperature one week, powdered and stored at -20°C until required.

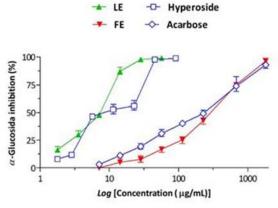


Figure No. 1 α-Glucosidase inhibition of LE and FE

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 μ m 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 at60 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-Ocaffeoylquinic 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 H₂SO₄, followed by filtration and analysis by HPLC. Elution was performed in isocratic mode with H₂SO₄ (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[•], $O_2^{\bullet-}$ (superoxide) and •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 (IC₅₀) was calculated through regression from the percentages of inhibition. With DPPH[•], the antioxidant Trolox was used as reference compound.

a-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. IC₅₀ 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 LC₅₀ (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).

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	$1794.3 \pm 7.3 (2.3\%)$	nq				
3	Protocatechuic acid	nq	$72.5 \pm 0.2 \ (4.4\%)$				
4	3-O-Caffeoylquinic acid	$12980.0 \pm 97.2 \ (16.6\%)$	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%)	$67.3 \pm 0.3 (4.1\%)$				
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%)	$55.7 \pm 0.4 (3.4\%)$				
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-3-0-rutinoside	1777.5 ± 3.1 (2.3%)	$53.6 \pm 0.2 (3.2\%)$				
14	Quercetin-3-O-arabinoside	1675.1. ± 2.9 (2.1%)	$37.6 \pm 0.2 \ (2.3\%)$				
15	Quercetin-3-O-rhamnoside	2501.2 ± 6.2 (3.2%)	$21.6 \pm 0.5 (1.3\%)$				
16	Kaempferol-3-0-rutinoside	$1959.6 \pm 4.9 (2.5\%)$	$8.3 \pm 0.1 \ (0.5\%)$				
17	<i>p</i> -Coumaric acid derivative	$585.7 \pm 1.2 \ (0.7\%)$	$13.7 \pm 0.1 \ (0.8\%)$				
	Σ	77964.7	1646.8				

Table No. 1Phenolic compounds quantified in LE and FE

^(a) Yield (μ g/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). evidence confirms Current 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).

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)	$73.0 \pm 4.0 \ (0.1\%)$	$150.5 \pm 8.8 \ (0.3\%)$			
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	$4252.3 \pm 760.2 (7.7\%)$	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	$80.0 \pm 2.3 \ (0.1\%)$	$282.5 \pm 28.0 \ (0.5\%)$			
	Σ	55577.8	56391.5			
	(a) XZ :-1-1/)			

Table No. 2

^(a)Yield, μ g/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 IC₅₀ 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 \pm 2.3 mg GAE/g of dry extract and 28.1 \pm 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 EC₅₀ = 475.6 μ g/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).

a-Glucosidase inhibitory activity

The α -glucosidase inhibitory activity of LE and FE was performed here. A concentration-dependent potential was observed in all assays (Fig.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).

Hyperoside was used as control for the α -glucosidase assay and the IC₅₀ 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 IC₅₀ (μ g/mL) values found in the antioxidant activity and α -glucosidase assays for LE and FE

In witro accan	Sample ^(a)					
In vitro assay	LE	FE	Trolox	Acarbose	Hyperoside	 Literature data
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). LC₅₀ is indicative of eco-toxicity level of plant extracts against *A. salina*. Extracts with LC₅₀ values lower than 1000 µg/mL are considered actives; with LC₅₀ values less than 250 µg/mL are considered significantly active (Meyer *et al.*, 1982). Finally, LC₅₀ 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 LC₅₀ value>1000 µg/mL. This result supports the traditional use of molle fruit in food and drinks. LE showed significant eco-toxic activity with LC₅₀ = 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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