Agrícola Los Esteros ®

Palo Negro

Leptocarpha rivularis

Traditional herb of southern Chile

Naturally caffeine-free

¿Quiénes somos?

Agricola Los Esteros is a family-run firm dedicated to producing a natural and effective tool in the fight against cancer and diabetes: Leptocarpha rivularis.

The plant known as palo negro in Chile contains leptocarpin, a molecule which has demonstrated potential as an anticancer agent in a number of scientific studies. The herb has long been used by Native Americans within the Mapuche nation located in southern Chile as an antifungal, anti-inflammatory and antimicrobial. Our family became convinced of the plant's utility when we saw how it helped one of our own.

The Leptocarpha rivularis utilized in our products is harvested under strict Chilean food-safety standards and processed on lines which are exclusively dedicated to packaging this particular plant.

Product line is exported under HS tariff codes 2106.90.99 and 1211.90.99

What is *Palo Negro*?

Palo Negro is the common name of *Leptocarpha rivularis*, a bush which is indigenous to the Bio Bio and Osorno regions of Chile. It normally grows in mountain ranges, next to waterways and prefers semi-shaded areas. It is comprised black branches and bright yellow flowers.

It is traditionally consumed to:

- Prevent and treat cancer
- Prevent and treat type II diabetes
- Reduce cholesterol
- Protect healthy cells

It is also consumed as a natural chemosensitizer (i.e., it makes chemotherapy more effective), is a good liver cleanser, an effective anti-inflammatory, and has three times more antioxidants than Goji berries. Best of all, *L. rivularis* also has no reported side effects.

What is Leptocarpin?

Leptocarpin, a molecule first identified by Humberto Dölz during his 1995 analysis of *Leptocarpha rivularis*, has been determined to inhibit the synthesis of proteins in HeLa cancer cells. It achieves this at no cost to nucleic acid synthesis in healthy cells. Several studies indicate that sesquiterpene lactones such as leptocarpin exhibit anticancer, antifungal, anti-inflammatory, antimicrobial and antioxidant properties.[1] Leptocarpin, in particular, is increasingly viewed as a driver of chemosensitivity (susceptibility of cancer cells to chemotherapy).[2] To date, no short or medium term side effects have been identified.

Leptocarpin has also been widely described as hypoglycemiant, which possesses the ability to lower blood sugar and decrease cholesterol levels.[3]

[1] Inter alia, https://www.ncbi.nlm.nih.gov/pubmed/26562779.
[2] Mena, C. (2008) Efecto apoptótico y quimiosensibilizador de leptocarpina en distintos modelos celulares de leucemias. Tesis de Grado para optar al Título de Químico Farmacéutico. Universidad Austral de Chile.
[3] Álvarez, C. (2005). Hypoglycemic effect of the infusion of *Leptocarpha rivularis* in type II alloxan diabetic Sprague-Dawley rats by. Universidad Austral de Chile.

Products

w: 6,7"

h: 8,8"

Doypack

Contains loose, chopped and lightly ground *L. rivularis*. The container features and easy-open, re-sealable top. The infusion may be prepared using the brewer of your choice.

Grind: 3-5mm
Format: 20-day supply
Contents: 125 g
Resealable: Yes
Barcode: Yes
Easy-open top: Yes
Duration: 24 months from packaging date.
Label language: Adaptable to market requirements.
Ministry of Health approval: Yes
Recyclable packaging: Yes
Nutritional information: Yes
Consumption information: Yes

Nutrition Facts

60 servings per container

Serving size	**1 Tbsp (2g)**
Amount Per Serving	
Calories	**0**
	% Daily Value*
Total Fat 0g	**0%**
Saturated Fat 0.02g	**0%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 0mg	**0%**
Total Carbohydrate 0g	**0%**
Dietary Fiber 1g	**4%**
Total Sugars 0g	
Includes 0g Added Sugars	**0%**
Protein 0g	**0%**
Vitamin D 0.2mcg	0%
Calcium 9.23mg	0%
Iron 0.099mg	0%
Potassium 26.32mg	0%

* The % Daily Value (DV) tells you how much a nutrient in a serving of food contributes to a daily diet. 2,000 calories a day is used for general nutrition advice.

Products

Box with infusion bags

Box contains 20 individual bags of 2 g each. Format contains no individual protective envelopes, threads or labels, in an effort to keep superfluous packaging to a minimum.

Grind: 3-5mm
Format: 7-day supply
Contents: 20 sachets in sealed liner
Individual wrappers: No
Thread, label: No
Barcode: Yes
Security Seal: yes
Barcode: Yes
Safety seal: Yes
Duration: 24 months from packaging date.
Label language: Adaptable to market requirements
Ministry of Health approval: Yes
Recyclable packaging: Yes
Nutritional information: Yes
Consumption information: Yes
Shipping dimensions: 50 x 30 x 30 cm (60 units per box)

Nutrition Facts

20 servings per container

Serving size	**1 Bag (2g)**
Amount Per Serving	
Calories	**0**
	% Daily Value*
Total Fat 0g	**0%**
Saturated Fat 0.02g	**0%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 0mg	**0%**
Total Carbohydrate 0g	**0%**
Dietary Fiber 1g	**4%**
Total Sugars 0g	
Includes 0g Added Sugars	**0%**
Protein 0g	**0%**
Vitamin D 0.2mcg	0%
Calcium 9.23mg	0%
Iron 0.099mg	0%
Potassium 26.32mg	0%

*The % Daily Value (DV) tells you how much a nutrient in a serving of food contributes to a daily diet. 2,000 calories a day is used for general nutrition advice.

Products

Supplement

L. rivularis enriched with Vitamin C and E within vegetable capsules. Packaged using child-safe lid, easy-open exterior seal and interior safety seal.

Contents: 90 capsules (#2, using HPMC material)
Format: 30-day supply
Container: 100ml, PET1
Lid: Child-safe
Inner seal: Yes
Easy-open outer seal: Yes
Barcode: Yes
Duration: 2 years from packaging date
Language: Adaptable to market requirements.
Health Resolution: Yes
Recyclable material: Yes
Nutritional information: Yes

Supplement Facts

Serving Size 1 Capsule
Servings Per Container 90

Amount per Serving		% Daily Value
Vitamin C	23 mg	38%
Vitamin E	3 IU	10%
Palo Negro (Leptocarpha Rivularis)	113 mg	*

* Daily Value not established

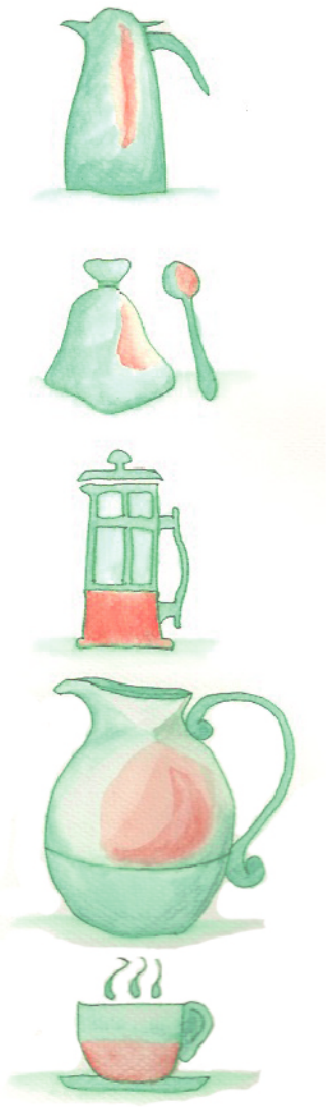
Other Ingredients: HPMC Capsule (Hydroxypropyl methylcellulose)

How should one consume *L. rivularis*?

Doypack
Infusion / Herbal tea:
Place one heaping tablespoon (6g) of Palo Negro into container and add one liter of hot water (90°C). Cover, allow to brew for at least 10 minutes. Strain and drink hot, warm or cold as desired throughout the day.

Infusion bags
Place infusion bag in container and add 330ml of hot water (90°C). Cover, allow to brew for at least 10 minutes. Consume hot, warm or cold three times daily.

Capsules
Consume one capsule three times daily with plenty of liquid.



Certifications and Trademarks

Gluten free and Soy free

Our products are naturally free of gluten and soy. Our production lines are 100% dedicated to packaging *Leptocarpha rivularis*, thereby ensuring our products are free of common allergens (Laboratory Analysis Nº 6187-1/1).

Vegan

Our products are certified by Fundación Vegetarianos Hoy, Chile is the most exacting NGO when it comes to certifying that products comply with vegan standards and practices.

Todo Kosher

Our products are certified kosher by Todo Kosher.

Producto de Chile

The "Producto de Chile" is a symbol of everything our firm represents: a nation which spans a truly extreme spectrum of landscapes, where the modern meets the traditional; a people dedicated to progress and producing goods they can be proud of.

PEFC - Responsible Forest Management

Our cardboard boxes are produced from trees harvested within sustainably-managed forests (Permit Nº PEFC/24-31-5900).

We have implemented BPM and BPA and will soon receive our ISO 22000 certification.
We also expect to be certified as an organic food producer by 2020.

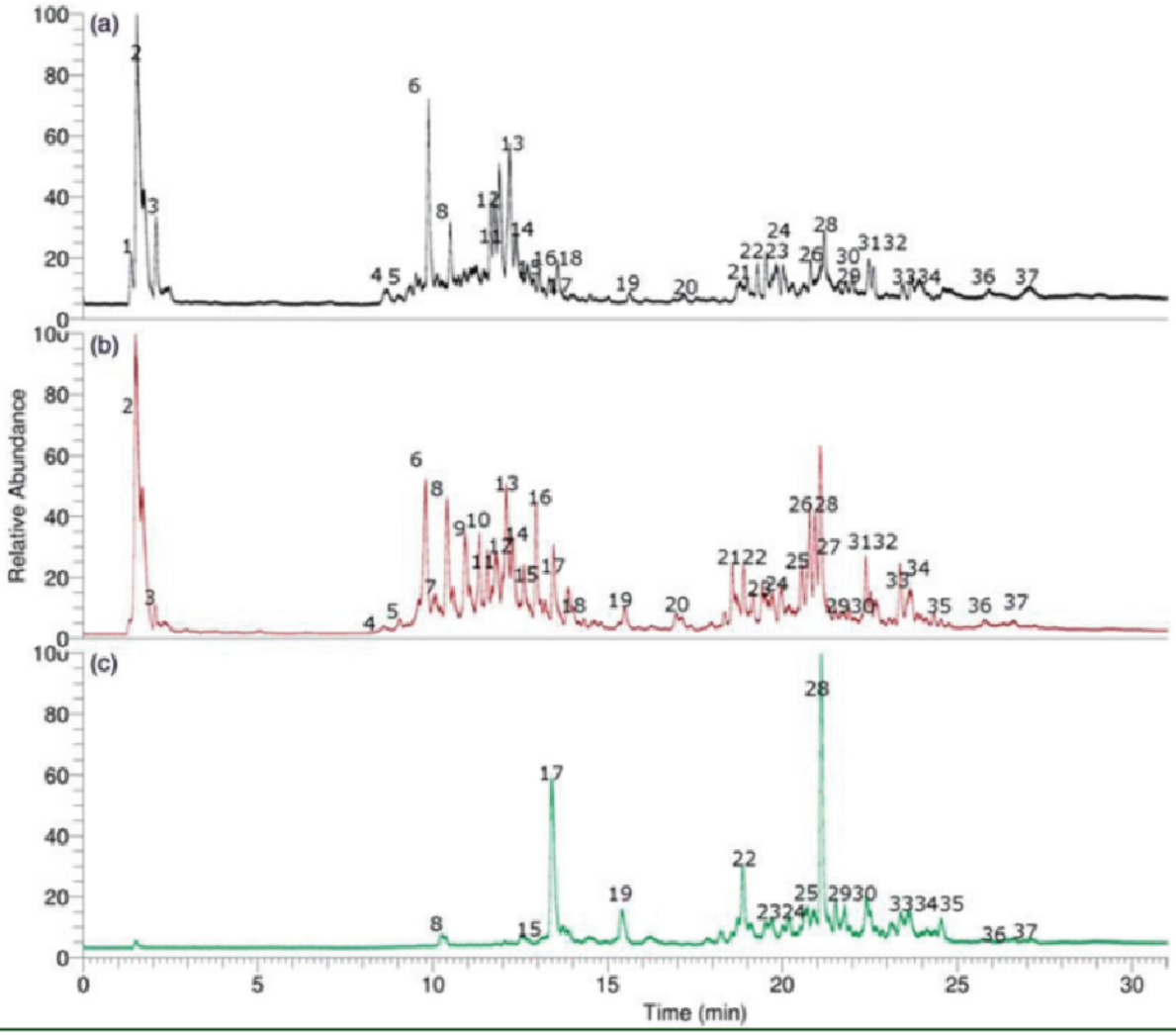
What does *Leptocarpha rivularis* contain?

The UHPLC/ESI/MS method for the identification of organic compounds is the first step in most of the screening techniques utilized to identify active metabolites in naturally-occurring sources. This technique was used to identify all of the compounds present in *L. rivularis.*

LEPTOCARPHA RIVULARIS COMPOUNDS

Four sesquiterpenes (peaks 34, 25, 28 and 29), ten flavonoids (peaks 10-13, 18, 20, 26, 24, 35 and 37), nine oxilipins (peaks 22, 23, 27, 30, 31, 32 and 36), two organic acids (peaks 1 and 2) and 11 phenolic acids (peaks 3-9, 14, 15, 17 and 33) were identified within *L. rivularis.*[4]
The results confirm that this plant is a rich source of phenolic compounds (**) that may be responsible for its bioactivity and potential medicinal uses.

L. rivularis infusion and its active components may serve as raw material for the isolation of effective AChE inhibitors, thus constituting a promising potential tool against Alzheimer's disease and related diseases.
The plan also possesses a great deal of potential in terms of dietary supplements due to its polyphenolic compounds.



(**) The total polyphenol content of the infusion was 230.76 ± 2.5mmol GAE / kg dry weight, while the antioxidant activity was 176.51 ± 28.84; 195.28 ± 4.83; and 223.92 ± 2.95 mmol ET / kg dry weight, for the DPPH, ABTS and FRAP tests, respectively.

[4] A Jiménez-González et al, 2018. UHPLC-ESI-ORBITRAP-MS analysis of the native Mapuche medicinal plant palo negro (*Leptocarpha rivularis* DC. – Asteraceae) and evaluation of its antioxidant and cholinesterase inhibitory properties, Journal of Enzyme Inhibition and Medicinal Chemistry, 33:1, 936-944.

Table 1. Identification of metabolites by UHPLC-PDA-OT-MS in three extracts of leaves of *L. rivularis*.

Peak #	t_R (min.)	UV max	Tentative identification	Elemental composition $[M-H]^-$	Theoretical mass (*m/z*)	Measured mass (*m/z*)	Accuracy (δppm)	MS^n ions (*m/z*)	Extract	References
1	1.47	210–272	Gluconic acid	$C_6H_{11}O_7^-$	195.05067	195.04993	3.82		A, B	35
2	3.87	–	Citric acid*	$C_6H_7O_7^-$	191.01959	191.01863	5.04		A, B	36
3	5.21	210–272	Chebulic acid	$C_{14}H_{11}O_{11}^-$	355.02959	355.03070	3.13	191.01933	A, B	38
4	7.55	172	Gallic acid*	$C_7H_6O_5^-$	169.01425	169.01639	3.81		A, B	54
5	9.79	207–309	Protocatechuic acid 4-*O*-glucoside	$C_{13}H_{15}O_9^-$	315.07106	315.07214	3.44		A, B	40
6	10.35	236–329	1,6 Dicaffeoyl-glucose	$C_{24}H_{23}O_{14}^-$	535.10913	535.10823	1.68		A, B	41
7	11.31	236–329	Chlorogenic acid (3-*O*-caffeoyl quinic acid)*	$C_{16}H_{17}O_9^-$	353.08781	353.08792	0.31	707.18102 $[2M-H]^-$, 191.05579 (quinic acid)	A, B	26,34,43
8	11.45	236–329	Cryptochlorogenic acid, (4-*O*-caffeoyl quinic acid)	$C_{16}H_{17}O_9^-$	353.08781	353.08795	0.39	707.18103 $[2M-H]^-$, 179.03465 (caffeic acid)	A, B	43
9	11.82	236–329	*Neo*-Chlorogenic acid, (5-*O*-caffeoyl quinic acid)	$C_{16}H_{17}O_9^-$	353.08781	353.08797	0.39	707.18105 $[2M-H]^-$, 179.03465 (caffeic acid)	A, B	43
10	12.01	254–361	Kaempferol 3-*O*-glucose	$C_{21}H_{19}O_{11}^-$	447.09351	447.09329	2.94	153.01877	A, B	46
11	12.28	275–324	Isorhoifolin (apigenin 7-*O*-rutinose)	$C_{26}H_{27}O_{16}^-$	577.15594	577.15652	−0.99	255.02986 (apigenin)	A, B	36
12	12.45	255–355	Quercetin 3-*O*-glucose	$C_{21}H_{19}O_{12}^-$	463.08838	463.08838	2.75	301.03538 (quercetin)	A, B	47
13	12.86	255–355	Lonicerin (luteolin-7-*O*-neohesperidose)	$C_{26}H_{27}O_{16}^-$	593.15070	593.15010	1.01	285.04035 (luteolin)	A, B	36
14	13.28	236–329	3,4-Di-caffeoyl-quinic acid	$C_{25}H_{23}O_{12}^-$	515.11840	515.11932	1.78	353.08789 (caffeoyl quinic acid)	A, B	43
15	13.48	236–329	Isochlorogenic acid A; 3,5-Dicaffeoylquinic acid	$C_{25}H_{23}O_{12}^-$	515.11840	515.11957	2.26	353.08786 (caffeoyl quinic acid)	A, B, C	43
16	13.98	254–361	Luteolin-3-*O*-rhamnose	$C_{21}H_{19}O_{10}^-$	431.09727	431.09854	2.94	285.04083 (luteolin), 255.02951	A, B	48
17	14.34	236–329	Caffeic acid*	$C_9H_7O_4^-$	179.03458	179.03389	3.86	135.04445	A, B, C	44
18	14.87	255–355	3′,5′Di-*O*-methyl-myricetin	$C_{17}H_{13}O_8^-$	345.06171	345.06049	3.51	315.01486 (dehydrogenated myricetin)	A	49
19	15.50	215	9,10,12-Trihydroxy-octadecadienoic acid	$C_{18}H_{31}O_5^-$	327.21660	327.21790	3.95		A, B, C	53
20	16.98	255–355	3, 3Di-*O*-methyl-myricetin	$C_{17}H_{13}O_8^-$	345.06171	345.06180	3.77	315.01486 (dehydrogenated myricetin)	A, B	49
21	18.57	210	9,10,12-Trihydroxyoctadecaenoic acid	$C_{18}H_{31}O_5^-$	329.23225	329.23358	4.04		A, B	27,46,52,55
22	19.25	235	11-Hydroxy-12-oxooctadeca-9,15-dienoic acid	$C_{18}H_{29}O_4^-$	309.20758	309.20604	4.99		A, B, C	12,34,37
23	19.43	235	11-Hydroxy-12-oxooctadeca-7, 9,15-trienoic acid	$C_{18}H_{27}O_4^-$	307.19183	307.19029	4.71		A, B, C	12,34,37
24	19.58	265–424	7-*O*-Methyl-8- prenyl-luteolin	$C_{21}H_{19}O_6^-$	367.11874	367.11761	3.07	285.04083 (luteolin),	A, B, C	50
25	19.85	225	Leptocarpin	$C_{15}H_{19}O_3^-$	361.16566	361.16577	0.30		A, B, C	1–3
26	19.98	266–419	8-Prenyl-kaempferol	$C_{20}H_{17}O_5^-$	337.10837	337.10842	0.14	217.05029, 134.03362	A, B	50
27	21.12	232	9-Hydroxy-octadecatrienoic acid	$C_{18}H_{29}O_3^-$	293.21112	293.21237	4.26		B	12,34,37
28	21.24	225	Leptocarpin dehydrated derivative	$C_{15}H_{19}O_3^-$	247.13287	247.13390	3.90		A, B, C	1–3
29	22.39	225	Leptocarpin dehydrated derivative	$C_{15}H_{19}O_3^-$	247.13397	247.13374	3.52		A, B, C	1–3
30	22.31	225	9-Hydroxy-octadecatetraenoic acid	$C_{18}H_{27}O_3^-$	291.19684	291.19814	−4.5		A, B, C	12,34,37
31	22.56	215	9-Hydroxy-octadecadienoic acid	$C_{18}H_{31}O_3^-$	295.22806	295.22677	4.35		A, B, C	12,34,37
32	22.78	225	8-Methoxy-13-hydroxy-9,11-octadecadienoic acid	$C_{19}H_{33}O_4^-$	325.23880	325.23734	3.15		A, B	12,34,37
33	23.36	246–310	Rosmarinic acid *	$C_{18}H_{15}O_8^-$	359.07769	359.07614	3.47		A, B, C	45
34	23.87	–	Ilicic acid	$C_{15}H_{23}O_3^-$	251.16527	251.16518	4.00		A, B, C	37
35	24.7		Diosmetin (4′-*O*-methyl-luteolin)*	$C_{16}H_{11}O_6^-$	299.05618	299.05501	3.90	285.04083 (luteolin)	A, B, C	51
36	26.32	212	Dihydroxyoctadecadienoic acid	$C_{18}H_{31}O_4^-$	311.22302	311.22169	4.29		A, B, C	12,34,37
37	27.27	254–354	Isorhamnetin*	$C_{15}H_7O_8^-$	315.01398	315.01354	1.37		A, B, C	26

*Identified by spiking experiments with an authentic compound. MS^n: Daughter ions.
A: Ethanolic extract; B: aqueous extract; C: ethyl acetate extract.

Anti-inflammatory

The sesquiterpene lactones are secondary metaboli-
The sesquiterpene lactones, which are almost exclusively found in species within the Asteraceae family, possess powerful anti-inflammatory properties. Research on genus Vernonia plants has resulted in the isolation of triterpenoids, steroidal glycosides and flavonoids which also possess anti-inflammatory properties.[13]
The phenolic compounds isolated within these plants are also widely valued for their anti-inflammatory properties. Therefore, Asteraceae extracts have been proposed as possible alternatives for the prevention and/or treatment of chronic inflammatory diseases.

Antioxidant

The antioxidant capacity measured by the DPPH method is 176.51 ± 28.84 mmol GAE/kg, while in the FRAP assay the infusion exerted a value of 223.92 ± 2.95 mmol TE/kg.[14]

Eleven antioxidants were identified as phenolic acids (peaks 3-9, 14, 15, 17 and 33) and ten flavonoids (peaks 10-13, 18, 20, 26, 24, 35 and 37) that may correlate to observed antioxidant capacity.

The nine oxilipins detected (peaks 22, 23, 27, 30, 31, 32 and 36), as well as the two organic acids (peaks 1 and 2) may contribute to antioxidant activity.

Furthermore, the total phenolic content measured spectroscopically (320.49 ± 3.58 mmol GAE/kg) of the infusion – the traditional form of usage – was greater than that of the hydroalcoholic extract (80:20 ratio of water to ethanol) of this plant (3.7 ± 0.02 mg GAE/g or 21.76 mmol GAE/kg).

[13] Campos, C. (2017) Actividad antioxidante y antiinflamatoria de extractos de Palo Negro (*Leptocarpha rivularis*) obtenidos por extracción supercrítica. Universidad de la Frontera, Chile.

[14] A Jiménez-González et al, 2018 UHPLC-ESI-ORBITRAP-MS analysis of the native Mapuche medicinal plant palo negro. (Leptocarpha rivularis DC. – Asteraceae) and evaluation of its antioxidant and cholinesterase inhibitory properties, Journal of Enzyme Inhibition and Medicinal Chemistry, 33:1, 936-944, DOI:10.1080/14756366.2018.1466880

Inmune System

EVALUATION OF PHAGOCYTIC ACTIVITY IN MACROPHAGES STIMULATED WITH PALO NEGRO EXTRACT

Test description: Expose primary cultures of fish macrophages (S. salar) to a 1:10 dilution of Palo Negro extract and measure increased phagocytosis.

Conclusion:
Regarding the analysis of the phagocytic activity in macrophages, it could be seen that in all treatments there were fluorescent beads consumed by macrophages, around 10,000 to 25,000 beads, however a greater difference in the amount of population that consumed these beads, where Palo Negro shows the highest value 1.2% v / s 0.3% of the macrophage population that was not treated and used as a negative control. This would indicate that the Palo Negro extract could be responsible for this substantial increase in the phagocytic population.

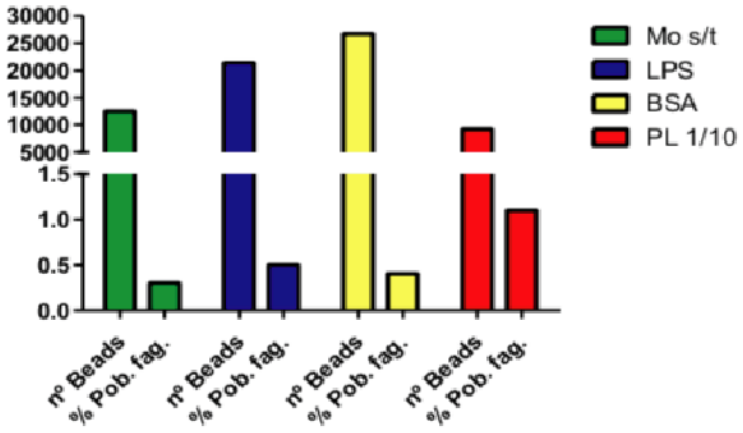
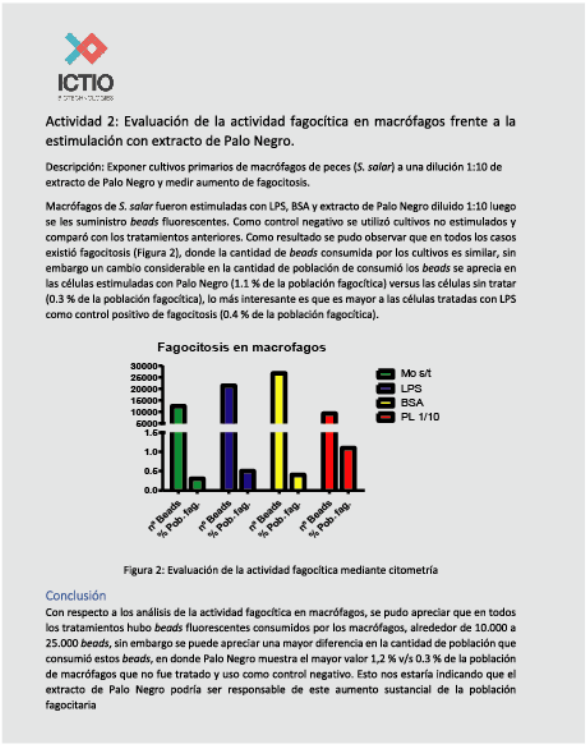


Fig.- Evaluation of phagocytic activity by cytometry

"Free radicals are produced during normal cellular metabolism and consequence from the metabolism of definite medicines or xenobiotics. Oxidative stress is a major contributing aspect to the high mortality rates associated with dysregulation of immune and lead to several diseases and the immune system is mainly sensitive to oxidative stress. An adequate intake of vitamins and antioxidant can notably improve confident immune system." (*)

(*)Hajian S. Positive effect of antioxidants on immune system. Immunopathol Persa. 2015;1(1):e02.



ICTIO

Actividad 2: Evaluación de la actividad fagocítica en macrófagos frente a la estimulación con extracto de Palo Negro.

Descripción: Exponer cultivos primarios de macrófagos de peces (*S. salar*) a una dilución 1:10 de extracto de Palo Negro y medir aumento de fagocitosis.

Macrófagos de *S. salar* fueron estimuladas con LPS, BSA y extracto de Palo Negro diluido 1:10 luego se les suministro *beads* fluorescentes. Como control negativo se utilizó cultivos no estimulados y comparó con los tratamientos anteriores. Como resultado se pudo observar que en todos los casos existió fagocitosis (Figura 2), donde la cantidad de *beads* consumida por los cultivos es similar, sin embargo un cambio considerable en la cantidad de población de consumió los *beads* se aprecia en las células estimuladas con Palo Negro (1.1 % de la población fagocítica) versus las células sin tratar (0.3 % de la población fagocítica), lo más interesante es que es mayor a las células tratadas con LPS como control positivo de fagocitosis (0.4 % de la población fagocítica).

Fagocitosis en macrofagos

Figura 2: Evaluación de la actividad fagocítica mediante citometría

Conclusión

Con respecto a los análisis de la actividad fagocítica en macrófagos, se pudo apreciar que en todos los tratamientos hubo *beads* fluorescentes consumidos por los macrófagos, alrededor de 10.000 a 25.000 *beads*, sin embargo se puede apreciar una mayor diferencia en la cantidad de población que consumió estos *beads*, en donde Palo Negro muestra el mayor valor 1,2 % v/s 0.3 % de la población de macrófagos que no fue tratado y uso como control negativo. Esto nos estaría indicando que el extracto de Palo Negro podría ser responsable de este aumento sustancial de la población fagocitaria

Multiple drug resistance Modulation

Multiple drug resistance (MDR) can severely curtail the treatment of a variety of diseases, including cancer, infections, epilepsy and schizophrenia. Research suggests that leptocarpin modulates the activity of MDR proteins by functional interaction with multidrug resistance associated protein (MRP).[11]

Vitamins C and E*

VITAMIN C

Vitamin C, known as ascorbic acid, is a water-soluble nutrient found in certain foods. It acts as an antioxidant, helping to protect cells against damage caused by free radicals. Free radicals are compounds that are formed when the body converts the food we consume into energy. Humans are exposed to free radicals in the environment from cigarette smoke, air pollution and ultraviolet solar radiation.

VITAMIN E

Vitamin E is a fat-soluble nutrient present in many foods. In the body, it acts as an antioxidant, helping to protect cells against damage caused by free radicals.
On the other hand, the body needs vitamin E to stimulate the immune system so that it can fight bacteria and viruses. It helps dilate the blood vessels and prevent the formation of blood clots. In addition, cells use vitamin E to interact with each other and fulfill a variety of important functions.

- STRENGTHENS THE ANTIOXIDANTS PROPERTIES
- CONTRIBUTES TO TISSUE REGENERATION
- FACILITATES THE ABSORPTION OF IRON
- STIMULATES THE IMMUNE SYSTEM
- HELPS TO AVOID THE FORMATION OF CLOTS

[11] M González-Oyarzún et al. Leptocarpina modula la actividad de MRP1 en células de oligodendrogliomas. Institute of Biochemistry, Universidad Austral de Chile.

(*)https://ods.od.nih.gov/factsheets/VitaminC-DatosEnEspanol/

Diabetes

Scientific evidence regarding the efficacy of leptocarpin in terms of reducing blood sugar levels is fairly strong. *L. rivularis* was used to perform preclinical research and the preliminary results are truly promising.

HYPOGLYCEMIC EFFECT

The hypoglycemic effects of leptocarpin have been detected during the initial phases of treatment, as can be seen in Figure 1 which illustrates results within all four control and treatment groups involving alloxan diabetic rats. It is also important to note that the treatment control group showed **no signs of hypoglycemia.**[5]

The consumption of *L. rivularis* modified responses to high doses of glucose (2g/kg) within the alloxan diabetic treatment group, resulting in improved tolerance to same (Figure 2). This may be due to restoration of subjects' peripheral response to insulin, to suppression of intestinal uptake of glucose as per Bajaj 1999, and/or to increased production of insulin by beta cells. It is interesting to note that the diabetes control group (DC) experienced results which deviated significantly from other groups.

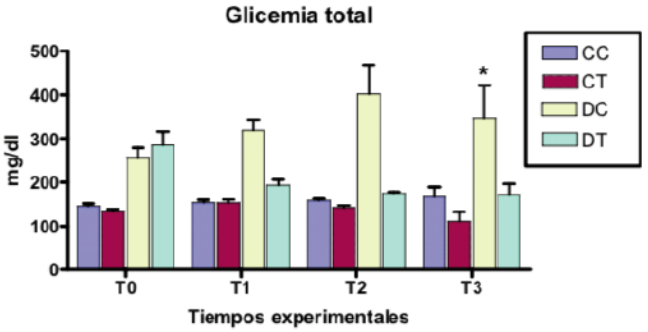


Fig. 1.-Plasma glucose of control and treatment groups, by treatment stage ± Average standard deviation

CC: Control Control; CT: Treatment Control; DC: Diabetic Control; DT: Diabetic Treatment

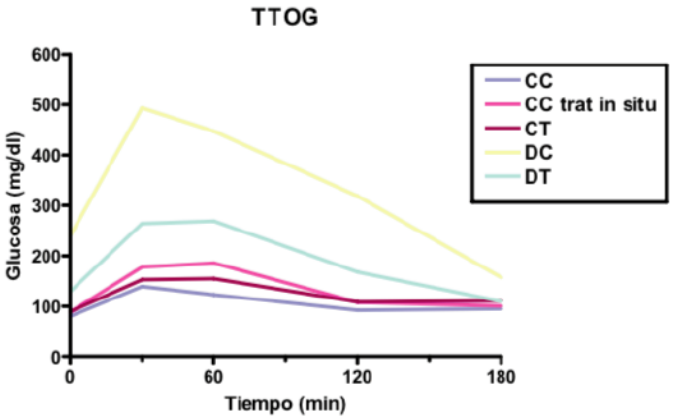


Fig 2.- Oral glucose tolerance test (OGTT)

[5] Contreras, C. Efecto hipoglicemiante de la infusión de *Leptocarpha rivularis* en ratas Sprague Dawley diabéticas tipo II por inducción con aloxano, Universidad Austral de Chile, 2005.

EFFECT ON DIABETIC NEPHROPATHY

Alloxan diabetic rats were treated with *L. rivularis* for 10 weeks.[6] Kidney slices from untreated rats showed a relative increase in thickness of glomerular basal membrane, capillary wall, mesangial matrix and PAS positive material (i.e., vis-à-vis treated and healthy control rats). Findings were corroborated by clinical signs of increased GFR, proteinuria and no evidence of clinical parameters values (total protein, albumin, serum creatinine and urea).

The results show that the hypoglycemic effect of *L. rivularis* contributes to the control of the diabetic state, minimizing the evolution of associated secondary pathologies.

HISTOPATHOLOGICAL EVALUATION OF THE PANCREAS[7]

The effect of leptocarpin on the pancreas of adult male Sprague-Dawley alloxan diabetic rats was studied over a period of ten weeks. Results included a decrease in the number and size of Langerhans islets (75% in treated diabetic rats), in addition to a decrease in pancreatic fibrosis and depletion of endocrine cells; no hypertrophy of beta cells observed.

Treated rats demonstrated an increased in volume and distribution of insulin in the islets of Langerhans. No morphological changes in insulin observed within treated, healthy control group.

[6] Álvarez, C et al. Effect of *Leptocarpha rivularis* administration in diabetic nephropathy rats, Biological Research, 39 (Suppl. B), 1-145.
[7] Álvarez, C. et al. Histopathological evaluation of the effect of the infusion of *Leptocarpha rivularis* in pancreas of alloxan diabetic rats.

Cancer

Studies carried out (Martinez et al 1995) have determined that leptocarpin inhibits the synthesis of proteins in HeLa cancer cells, without affecting the synthesis of nucleic acids. The literature also states that leptocarpin has cytotoxic, apoptotic and chemosensitizing activity on a fairly wide variety of leukemia and tumor cells. In addition, it has consistently failed to generate any observable side effects.

At the preclinical level, leptocarpin has antitumor activity characterized by "low toxicity, and high specificity". Leptocarpin is highly efficient in terms of its ability to inhibit in vivo and in vitro growth of a wide variety of cancer cells:

Tumor lymphocytes (EL-4)
Myeloma (NSO-2)
Cervico-uterine cancer (HeLa)
Melanoma (A-375)
Ovarian cancer (CHO)
Human laryngeal carcinoma epithelial cells (HEP-2)
Retinal epithelial cells (ITO)
Mastocytoma (P-815)
Macrophages (J774.2)
Hybridoma producing antiperoxidase antibodies (4FIO-67)
Liver human adenocarcinoma cell line (SK Pet-1)
Lung cancer (T 84)
Pancreatic adenocarcinoma: (NP-9)
Pancreatic adenocarcinoma: (NP-18)
Histiocytic lymphoma: (U937)
Myeloblastic leukemia: (HL60)

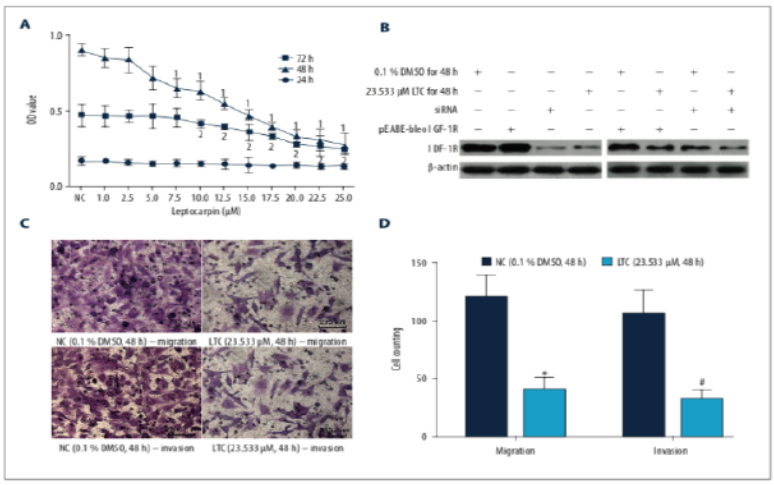
APOPTOTIC AND CHEMOSENSITIZING EFFECT OF LEPTOCARPIN

Leptocarpin showed a cytotoxic and antiproliferative effect in the hematological cell lines HL60 (promyelocytic leukemia) and U937 (monocytic leukemia), with IC50 values of 31.7 µM and 22.8 µ M respectively, via the trypan blue exclusion method; and IC50 values of 22.7 µM and 19.8 µM using the MTT method.[8] Remaining cell viability reached 19% and 9% respectively for HL60 and U937 cells, which indicates a better cytotoxic effect vis-à-vis HeLa cells, where a 34% cell survival is obtained when exposed to 30 µM leptocarpin for 24 hours, under similar conditions (Martinez et al, 2006).
In a first step towards determining the mechanism involved in the decrease in cell viability by leptocarpin, the variation of caspase-3 activity with and without its specific inhibitor was tested. Activation of caspases plays a key role in the execution of the cellular apoptosis process; mainly caspase-3, an effector caspase that is the highest caspase activated in response to different apoptotic stimuli (Jow et al, 2004). Caspase-3 showed a substantial increase in its activity in response to leptocarpin, in all the assays performed on cell lines HL60 and U937, as well as in cells from patients with chronic myeloid leukemia. In all three cell types, statistically significant differences at different concentrations were observed.
The results obtained with regard to the expression of multidrug resistance proteins open the hope of a possible therapeutic use of leptocarpin as an adjunct to chemotherapy, due to its sensitizing effect, associated with or without another drug, as the inhibitory potential of the expression of multidrug resistance proteins is as effective, or more effective, than the cytotoxic effect alone.

CYTOTOXIC ACTIVITY OF LEPTOCARPINE IN LINES OF UNRELATED HUMAN CELLS [9]

Leptocarpin has been tested for its cytotoxic effect on five human cancer cell lines: breast adenocarcinoma (MCF-7), pancreatic adenocacinoma (NP-9 and NP-1 8), histiocytic lymphoma (U937) and myeloblastic leukemia (HL-60). Leptocarpin caused cytotoxicity as evaluated by trypan blue exclusion, via a cell counter, and MTT reduction. In microscopic examinations, cells treated with leptocarpin exhibi-

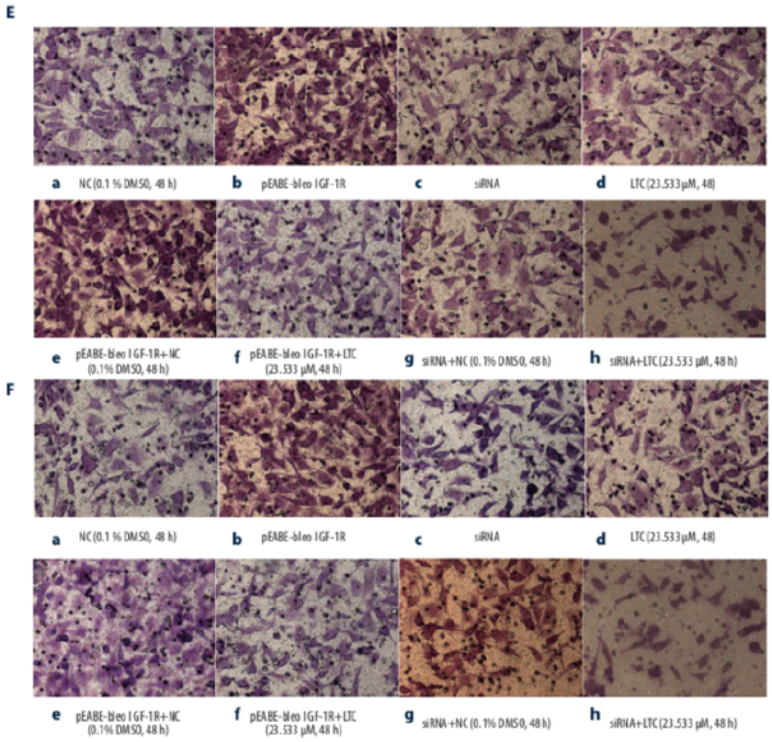
Effect of LTC on IGF-1R and MG63 cell proliferation, migration, and invasion. (A) Effect of 0.1% DMSO or LTC on MG63 cells proliferation at 24, 48 and 72 h (1 compared to NC (72 h), P<0.05; 2 compared to NC (48 h), P<0.05). (B) Effect of LTC on IGF- 1R expression in MG63 cells (b-actin: internal reference). (C) Effect of LTC on MG63 cell migration and invasion. (D) Effect of LTC on MG63 cell migration and invasion rates (1 compared to migration in NC, P<0.05; 2 compared to migration in NC, P<0.05).



ted morphological characteristics typical of apoptosis, such as cell contraction and the formation of apoptotic bodies. Fluorescent cytoplasmic and nuclear staining revealed distinctive chromatin condensation and nuclear fragmentation. DNA scaling was also observed; the apoptotic percentage tracked parallel to cytotoxic parameters.
In conclusion, leptocarpin was shown to be active in human tumor cells of different origin through induction of apoptosis. Cytotoxic activity was much higher than that observed in normal blood cells, suggesting a potentially broad-spectrum antitumor role.

LEPTOCARPINE SUPPRESSES THE PROLIFERATION, MIGRATION AND INVASION OF HUMAN OSTEOSARCOMA[10]

Leptocarpin suppressed the proliferation, migration and invasion of osteosarcoma. IGF-1R is one of the targets in leptocarpin's suppression of osteosarcoma, which provides clinical treatments for osteosarcoma via a new pharmacological and molecular objective.



(E) Effect of Leptocarpine on the cellular migration of MG63.
(F) Effect of Leptocarpine on the cellular invasion of MG63.

[8] Mena, C. (2008) Efecto apoptótico y quimiosensibilizador de leptocarpina en distintos modelos celulares de leucemias. Universidad Austral de Chile.
[9] J Cárcamo JG et al. Cytotoxic activity of Leptocarpin on unrelated human cancer cell lines. Institute of Biochemistry, Universidad Austral de Chile.
[10] Li, C Sun. Leptocarpin suppresses proliferation, migration, and invasion of human osteosarcoma by targeting type-1 insulin-like growth factor receptor (IGF-1R). Med Sci Monit, 2017; 23: 4132-4140.

Cholesterol Control

HYPOCHOLESTEROLEMIC EFFECT[12]

The evaluation of results involving a group of alloxan diabetic rats revealed that daily doses of *L. rivularis* lowered overall cholesterol levels over time (Figure 3).

The lipid profile also addressed HDL cholesterol (Figure 4), LDL cholesterol (Figure 5), triglycerides (Figure 6) and atherogenic index values, with an eye to analyzing cholesterol and fatty acid metabolism.

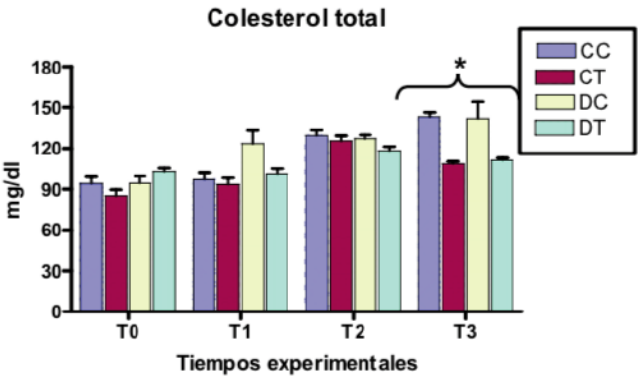


Fig 3.-. Concentrations of total cholesterol, according to treatment time (± average SD).

**Statistically significant variation.*

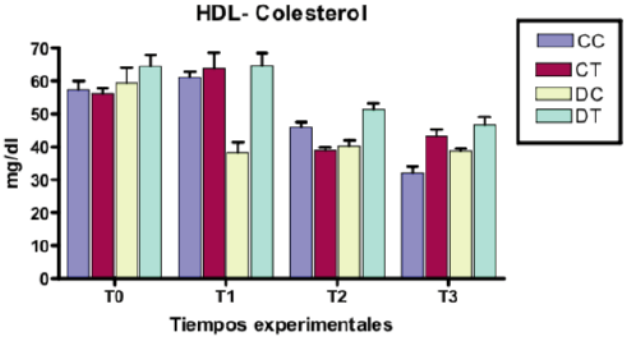
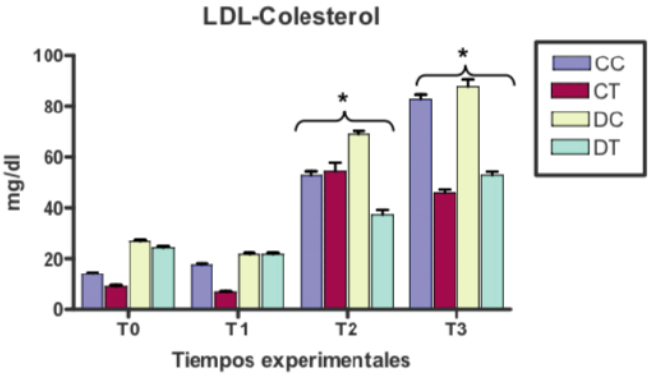


Fig 4.- Variations in HDL levels



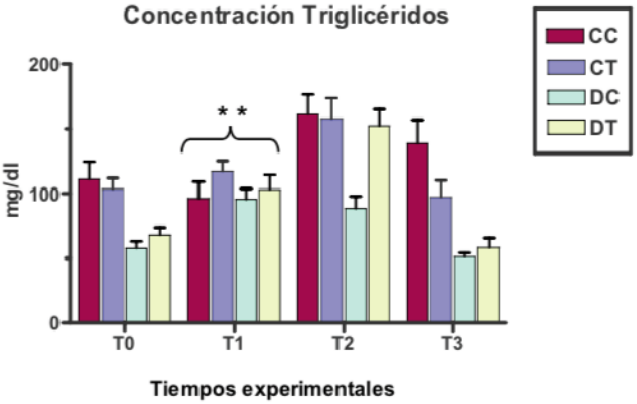
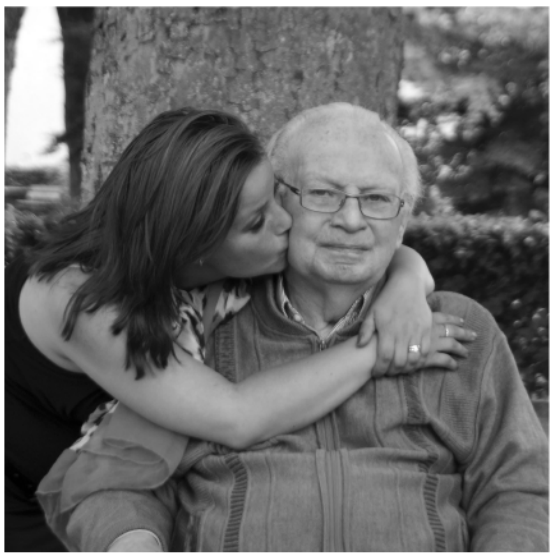


Fig 6.- Variation in triglyceride concentration, by post-induction time (Average ± SD)
** * Indicates no statistically significant variation.*

[12] Álvarez, C. (2005). Hypoglycemic effect of the infusion of *Leptocarpha rivularis* in type II alloxan diabetic Sprague-Dawley rats by. Universidad Austral de Chile.



Possible tool in treatment and prevention of Alzheimer's

The enzyme acetylcholinesterase (AChE) plays an important role in the activity of the central and peripheral nervous systems. It catalyzes the hydrolysis and inactivation of the neurotransmitter acetylcholine, producing choline and acetate.

The inhibitory activity of cholinesterase improves the cholinergic function of patients battling Alzheimer's disease (AD) by preserving acetylcholine levels. It has thus become the standard approach in the symptomatic treatment of AD. Compounds such as donepezil, galantamine and rivastigmine delay the degradation of acetylcholine released in the synaptic clefts and, as a result, reinforce cholinergic neurotransmission.
In this context, enzyme inhibitor assays have become, in recent years, very useful tools for evaluating the possible health benefits of medicinal herbs, fruits and related biological materials, as part of the research and development of functional foods or dietary supplements.
On the other hand, most of the usual assays involve relevant key enzymes in chronic neurodegenerative conditions such as AD (cholinesterases).

The effects of the extracts investigated in selected cholinesterases were evaluated by microtitre assays.
Leptocarpha rivularis with leptocarpin showed better cholinesterase activities, (2.12 ± 0.03 and 1.65 ± 0.06 GALAE per gram of extract).[15] The values are three times higher than those reported for leaves of the well-known Goji berry (L. barbarum) (1.02 ± 0.17 mg GALAE/g dry weight).
In addition, one of the components of this plant, chlorogenic acid, has been reported as a potent enzyme inhibitor in Goji leaves and blueberries.

[15] A Jiménez-González et al, 2018. UHPLC-ESI-ORBITRAP-MS analysis of the native Mapuche medicinal plant palo negro (*Leptocarpha rivularis* DC. – Asteraceae) and evaluation of its antioxidant and cholinesterase inhibitory properties, Journal of Enzyme Inhibition and Medicinal Chemistry, 33:1, 936-944.

WHY CHOOSE OUR PRODUCTS?

Our family grows our own L. rivularis which we package and distribute throughout Chile under Health Permit N° 161-448-8027 and N° 161-448-8023 (23 FEB 2017) SEREMI Los Ríos. This certification ensures that are processing complies with Chilean food safety regulations. As a result, we can confidently say that our products are ideal for individuals undergoing treatment for cancer and diabetes. All the evidence points to palo negro being an effective potentiator, as well as preventative measure. Our firm works closely with the University of Santiago (USACH) in its constant effort to review the literature available on this unique plant.
We have the ability to fill international orders and deliver goods throughout the world (FOB or CIF). Labeling can be prepared in any language which a market may require.

Please let us know if you are interested in selling or distributing our products.
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