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Variability of phenolic compounds at different phenological stages in two populations of *Valeriana carnosa* Sm. (Valerianoideae, Caprifoliaceae) in Patagonia

[Variabilidad de compuestos fenólicos en diferentes estadios fenológicos en dos poblaciones de *Valeriana carnosa* Sm. (Valerianoideae, Caprifoliaceae) en Patagonia]

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Abstract: This study analysed the total content of phenolic compounds in roots and rhizomes extracts from two populations of *Valeriana carnosa* Sm. (Caprifoliaceae) at three phenological stages. Total phenolic content was determined through the Folin-Ciocalteu method, which ranged between individuals from 3.56 to 11.68 mg GAE/g of dry sample. Antioxidant activity was determined using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), which showed a significant positive correlation between antioxidant activity and total phenolic content. We tentatively identified 18 phenolic compounds by HPLC-MS, mostly phenolic acids, one of which was present only in the Hoya population at the vegetative stage and one only in the Piltri population at the flowering and fruiting stages. Phenolic compounds in subterranean organs of *V. carnosa* vary qualitatively (between populations/stages) and quantitatively at intra- and inter-population level at different phenological stages. In both populations, on average a higher phenolic content and antioxidant activity were recorded at the flowering and fruiting stages.

Keywords: Antioxidant activity; HPLC-MS; Phenols.

RESUMEN: En este estudio se analizó el contenido total de compuestos fenólicos en extractos de raíces y rizomas provenientes de dos poblaciones de *Valeriana carnosa* Sm. (Caprifoliaceae) en tres estadios fenológicos. El contenido total de fenoles se determinó sobre muestras secas, mediante el método de Folin-Ciocalteu, que varió de 3.56-11.68 mg GAE/g entre individuos. Se determinó la actividad antioxidante utilizando el radical estable 2,2-difenil-1-picrilhidrazilo (DPPH) y se observó una correlación positiva significativa entre la actividad antioxidante y el contenido total de fenoles. Identificamos tentativamente 18 compuestos fenólicos mediante HPLC-MS, principalmente ácidos fenólicos, dos de los cuales estuvieron presentes en la población Hoya en la etapa vegetativa y en la población Piltri en las etapas de floración y fructificación. Los compuestos fenólicos en órganos subterráneos de *V. carnosa* varían cualitativamente (entre poblaciones/estadios) y cuantitativamente a nivel intra- e inter-poblacional en diferentes estadios fenológicos. En ambas poblaciones, en promedio se registró un mayor contenido de fenoles y actividad antioxidante en las etapas de floración y fructificación.

Palabras clave: **A**ctividad antioxidante; Fenoles; HPLC-MS.

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INTRODUCTION

The genus *Valeriana* L. includes about 250 species distributed worldwide, except in Australia and New Zealand, with South America being an important centre of diversification (Eriksen, 1989; Backlund & Pyck, 1998; Hidalgo *et al*., 2004; Bell & Donoghue, 2005).

In Argentina, there are 50 species of the genus, 20 of which are in Patagonia (Borsini, 1944; Kutschker, 2011; Nagahama *et al*., 2016). This Andean Region has a rich diversity, and aboriginal groups and rural societies have used these plants extensively since ancient times for curing various human ailments. There is ethnobotanical evidence of medicinal use of at least five *Valeriana* species (Estomba *et al*., 2005; Molares & Ladio, 2008; Barboza *et al*., 2009). *Valeriana carnosa* Sm., commonly known as "Ñamkulawen" or "the remedy that cures the seven illnesses", is considered a sacred plant by regional ethnic groups. Its roots and rhizomes are traditionally used through decoction to treat different health conditions. Its subterranean organs have been cited as a remedy for liver, respiratory, circulatory, urinary and digestive disorders, as well as having analgesic, anti-inflammatory, antitumoral, anti-depressive and wound-healing properties (Estomba *et al*., 2005; Molares & Ladio, 2008; Richeri *et al*., 2013).

Traditionally, *V. carnosa* is harvested for medicinal purposes preferably in autumn. Richeri *et al*. (2013) suggest that people from the rural areas collect *V. carnosa*, like other species for medicinal use in the autumn/winter seasons while they are moving livestock, keeping the plant material in paper bags and later drying it and storing medicine for the whole year.

There is no consensus about the compounds responsible for the therapeutic action of valerians and there is particular interest in discovering the secondary metabolites responsible for the sedative effect associated with these plants. Some authors suggest chemical species such as valerenic acid (Stoll *et al*., 1957; Hendriks *et al*., 1985), valepotriates (Houghton, 1999; Mills & Bone, 2000; Maurmann *et al*., 2008) or phenolic compounds (Cuadra & Fajardo, 2002; Marder *et al*., 2003; Fernández *et al*., 2006; Lacher *et al*., 2007), among others, as responsible for the biological activity.

Phenolic compounds are particularly characterized by at least one phenol group in their structure, which is an aromatic ring attached to one or more hydroxyl groups. One of its main characteristics is the antioxidant activity, capturing free radicals and thus neutralizing dangerous reactive oxygen species and other ions (Shahidi *et al*., 1992).

The properties attributed to this large chemical family are a protective effect against cardiovascular diseases, with some of them having moderate vasodilator activity (Piccinelli *et al*., 2004), and a potential anticancer effect especially in the phenolic acids (Russell & Duthie, 2011).

The first aim of this work was tentatively identify phenolic compounds by HPLC-MS and analyze the phenolic contents and the antioxidant activities of ethanolic extracts of *V. carnosa* obtained from subterranean organs, in two populations at three phenological stages.

MATERIALS AND METHODS

Plants were collected in May and November of 2016 and January of 2017 from two populations about 150 km apart, on Piltriquitrón hill (Piltri; 41° 58´ 23´´S; 71° 28´ 44´´W), 1185 m a.s.l., Rio Negro province, and on La Hoya hill (Hoya; 42° 51´ 22´´S; 71° 17´ 10´´W), 848 m a.s.l., Chubut province. Piltriquitrón population is located into the Subantartic phytogeographic province, where the climate in general, is temperate to cold and humid, with snow during the winter and frosts all year round (Cabrera, 1971). Hoya population is located in an ecotone zone, between the Subantartic and Patagonica phytogeographic provinces (Cabrera, 1971), in general the climate is dry and cold with strong winds and strong frosts and snow during the winter.

Roots and rhizome from 30 plants of 40-50 cm diameter were collected, with five specimens for each phenological stage and population: Hoya population vegetative stage (H_{Ve}), Hoya population flowering stage (H_{Fl}), Hoya population fruiting stage (H_{Fr}), Piltri population vegetative stage (P_{Ve}), Piltri population flowering stage (P_{Fl}) and Piltri population fruiting stage (P_{Fr}). The stages considered were the vegetative (May), flowering (November) and fruiting (January) stages.

The material was shade-dried for 45 days and milled to a fine texture in a grinding machine. 1g of the powdered plant material was macerated with 10 mL of ethanol at room temperature for 7 days (root ethanolic extract). Each extract was filtered using Whatman's No. 1 filter paper to eliminate residues.

Determination of total phenols

Total phenolic content was determined by the Folin-Ciocalteu method, according to Chaisri & Laoprom (2017) as described below: 2 mL of distilled water was mixed with 20 µL of extract solution, followed by the addition of 0.2 mL of Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis USA) and 0.8 mL of Na_2CO_3. After 20 min of incubation at room temperature, the absorbance was measured at 765nm by spectrophotometer. All measurements were made in triplicate and the average value was used for quantification. A calibration curve was made with gallic acid to express the total phenolic content in mg equivalents of gallic acid per g of dry material (mg GAE/g).

DPPH Radical Scavenging Assay

Antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method according to Alam *et al*. (2013) and Gastaldi *et al*. (2016).

We mixed 3.9 mL of DPPH ethanol solution (15 mg DPPH/500 mL ethanol) at concentration of 30 mg/L, with 50 µL of root and rhizome ethanolic extracts. A cuvette containing only DPPH ethanol solution was used as control. The absorbance was measured at 517 nm using a spectrophotometer. All measurements were made in triplicate and the average values were used to estimate antioxidant activity. The reduced DPPH percentage of each cuvette was calculated according to the following equation:

% inhibition of DPPH = [(Abs control - Abs samples) ÷ Abs control] × 100

where "Abs control" is the absorbance of DPPH solution without extract and "Abs samples" is the sample absorbance with DPPH solution. Thus a higher % inhibition of DPPH indicates greater antioxidant activity.

Root and rhizome ethanolic extract analyses

Phenolic compounds profile determination was performed by liquid chromatography and tandem mass spectrometry (LC-UV-MS) (Simirgiotis *et al*., 2012; Kaliora *et al*., 2014; Simirgiotis *et al.*, 2015) as described below. 10 µL of ethanolic extracts were filtered and injected. Reference standards of caffeic acid, chlorogenic acid and gallic acid (Sigma-Aldrich, St. Louis, USA) were solubilized in methanol, filtered and injected. The chromatographic equipment was an Ultimate 3000 RSLC Dionex model from Thermo Scientific with a UV-Vis detector model VWD-3400 RS and mass detector TSQ Quantum Access Max. The separation was performed on a C18 Hypersil-GOLD column (50 x 2.1 mm; 1.9 um particle size) kept at 30° C, at a flow rate of 0.20 mL/min for 50 minutes. Gradient elution: solvent (A) H_2O (containing 2.0% AcOH), solvent (B) MeOH; 85%-60% from A to 30 minutes, 60%-25% to 40 minutes, 25%-15% to 45 minutes, ending isocratic to 50 minutes. The analysis was monitored at 254, 280, 330 and 365 nm by ESI in the positive mode at a probe temperature of 360° C, probe voltage of 4.5 kV. The tentative identification is proposed based on retention time (RT), UV spectral maxima and MS fragmentation (m/z), reference standards, database and bibliographic data.

Statistical analysis

Total phenolic compound and antioxidant activity among populations/stages were analysed by non-parametric analysis of variance (Kruskal–Wallis). Pairwise comparison was carried out for an *a posteriori* test of each pair of means with a critical value of $p \leq 0.05$ (Conover, 1999). Pearson correlation analysis was performed between the antioxidant activity and total phenolic content variables. All data were analysed using the Infostat v. 2010 program (Grupo InfoStat, Córdoba, Argentina; Di Rienzo *et al*., 2010).

RESULTS

Determination of total phenols

A high variation in the content of phenols was observed among specimens. Although no statistically significant difference was found between the values of Piltri and Hoya, the following trends of values in the different stages can be appreciated.

Vegetative stage

As shown in Appendix No. 1, a higher content of phenolic compounds was observed in the Hoya population, with an intra-population variation of between 5.27 mg GAE/g and 10.57 mg GAE/g for individuals H_{Ve5} and H_{Ve2}, respectively. In the Piltri population, a lower content of phenolic compounds

was observed on average, with values between 3.56 mg GAE/g and 11.68 mg GAE/g for individuals P_{Ve2} and P_{Ve5}, respectively (Figure No. 1; Appendix No. 1).

Flowering stage

In November, higher phenolic contents were observed in the Piltri population. The values varied from 5.19 mg GAE/g to 10.74 mg GAE/g for P_{Fl5} and P_{Fl1}, respectively. For the Hoya population, the variation in phenolic content was 5.53 mg GAE/g and 9.55 mg GAE/g for H_{Fl5} and H_{Fl1} respectively (Figure No. 1; Appendix No. 1).

Fruiting stage

At this stage, a higher phenolic content was observed in the Hoya population (7.67 to 10.66 mg GAE/g for H_{Fr1} and H_{Fr3}, respectively). For the Piltri population, values varied between 4.59 and 10.15 GAE/g for P_{Fr2} and P_{Fr3} respectively (Figure No. 1; Appendix No. 1).

DPPH Radical Scavenging Assay

A high variation was observed among specimens. Only Piltri population in vegetative stage (P_{ve}) showed statistically significant difference (Fig. 2). However, the following trends of values in the different stages can be observed.

Vegetative stage

On average, higher antioxidant activity was registered in the Hoya population, with 67.24% inhibition of DPPH. In this population, the highest value was greater than 80%, corresponding to the H_{Ve4} sample (Figure No. 2; Appendix No. 2).

Flowering stage

Both populations showed similar antioxidant activity. The P_{Fl5} sample of the Piltri population showed 47% reduced DPPH, while values for the rest of the individuals in this population were higher than 80%. For the Hoya population, the H_{Fl5} sample presented the lowest antioxidant activity, with 56% inhibition of DPPH, while the others exceeded 70% (Figure No. 2; Appendix No. 2).

Fruiting stage

During January, the antioxidant activity of the Hoya population was seen to be slightly higher than that the Piltri population. On the other hand, all the extracts of individuals from the Piltri population showed more than 80% inhibition of DPPH, with the exception of P_{Fr2}, which presented only 40%. The Hoya population, with the exception of H_{Fr4}, showed values higher than 80% (Figure No. 2; Appendix No. 2).

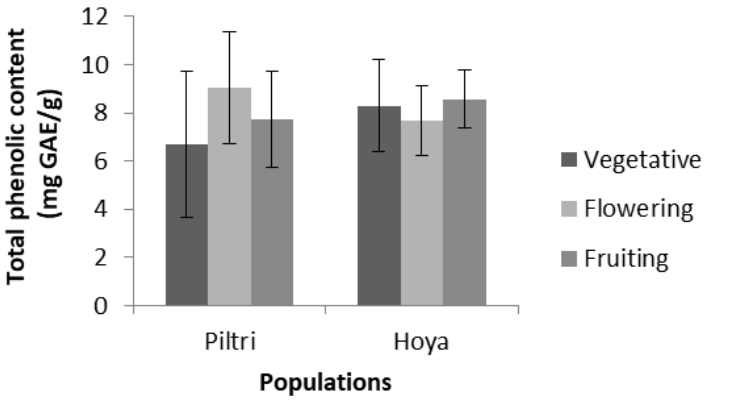

Figure No. 1
Total phenolic content of both populations at each phenological stage analysed.
The lines above the bars refer to standard deviation.

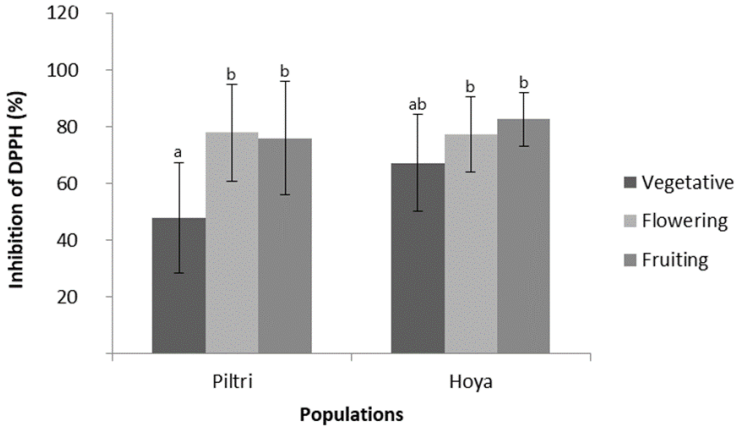

Figure No. 2
Antioxidant activity of both populations at each phenological stage analysed.
The lines above the bars indicate the standard deviation. Different letters means significant differences.

HPLC-MS analyses

In total eighteen compounds were tentative identified in *V. carnosa* ethanolic extracts. Sixteen were found in both populations, for the three phenological stages. In contrast, the compound chlorogenic acid isomer conjugate was present only in the sample H_{Ve} (Hoya, vegetative stage) and syringic acid was observed only in the samples P_{Fl} and P_{Fr}, corresponding to the Piltri flowering and fruiting stages, respectively (Table No. 1). Figure No. 3 shows representatives HPLC-MS chromatograms at 280 nm, the fingerprint is similar for the different populations and phenological stages.

Table No. 1
Summary of the assigned compounds in each of the samples analysed by HPLC-MS: H_{Ve} (Hoya, vegetative stage), H_{Fl} (Hoya, flowering stage), H_{Fr} (Hoya, fruiting stage), P_{Ve} (Piltri, vegetative stage), P_{Fl} (piltri, flowering stage), P_{Fr} (Piltri, fruiting stage). Full circles represent presence of the compound, empty circles its absence. The standards used are in red. RT: retention time, m/z: molecular ion, λ max : absorbance maximum value, others: others absorbance value

N° of compound	RT	m/z	λ_{max}(nm)	others	Tentative structure	H_{Ve}	H_{Fl}	H_{Fr}	P_{Ve}	P_{Fl}	P_{Fr}
1	2.2	355	330	280/254	Chlorogenic acid	●	●	●	●	●	●
2	5.2	451	254	330/365	Cyanidin glucoside	●	●	●	●	●	●
3	5.8	579	254	330	Teucrol succinoyl glucoside	●	●	●	●	●	●
4	6.6	195	330	280/254	Ferulic acid	●	●	●	●	●	●
5	8.4	435	280	254	Pelargonidin glucoside	●	●	●	●	●	●
6	13.4	517	330	280/254	3,4-Dicaffeoylquinic acid	●	●	●	●	●	●
7	13.8	403	330	280/254	Ferulic acid glucoside	●	●	●	●	●	●
8	19.0	553	330/254	280	Chlorogenic acid-isomer conjugate	●	○	○	○	○	○
9	19.6	539	330	280/254	Isomer of lithospermic acid	●	●	●	●	●	●
10	23.3	545	330	254	Diferuloylquinic acid isomer	●	●	●	●	●	●
11	25.4	199	330	254	Syringic acid	○	○	○	○	●	●
12	28.6	465	330	254/280	Monomethyl anthranol	●	●	●	●	●	●
13	30.4	165	330	280/254	(o-/p-) Coumaric acid	●	●	●	●	●	●
14	31.1	369	330	254	Feruloylquinic acid	●	●	●	●	●	●
15	32.5	153	254	330	p-Hydroxy-phenylacetic acid	●	●	●	●	●	●
16	43.9	149	254	280	Cinnamic acid	●	●	●	●	●	●
17	45.4	361	254	280/330	Rosmarinic acid	●	●	●	●	●	●
18	47.3	171	254	280	Gallic acid	●	●	●	●	●	●

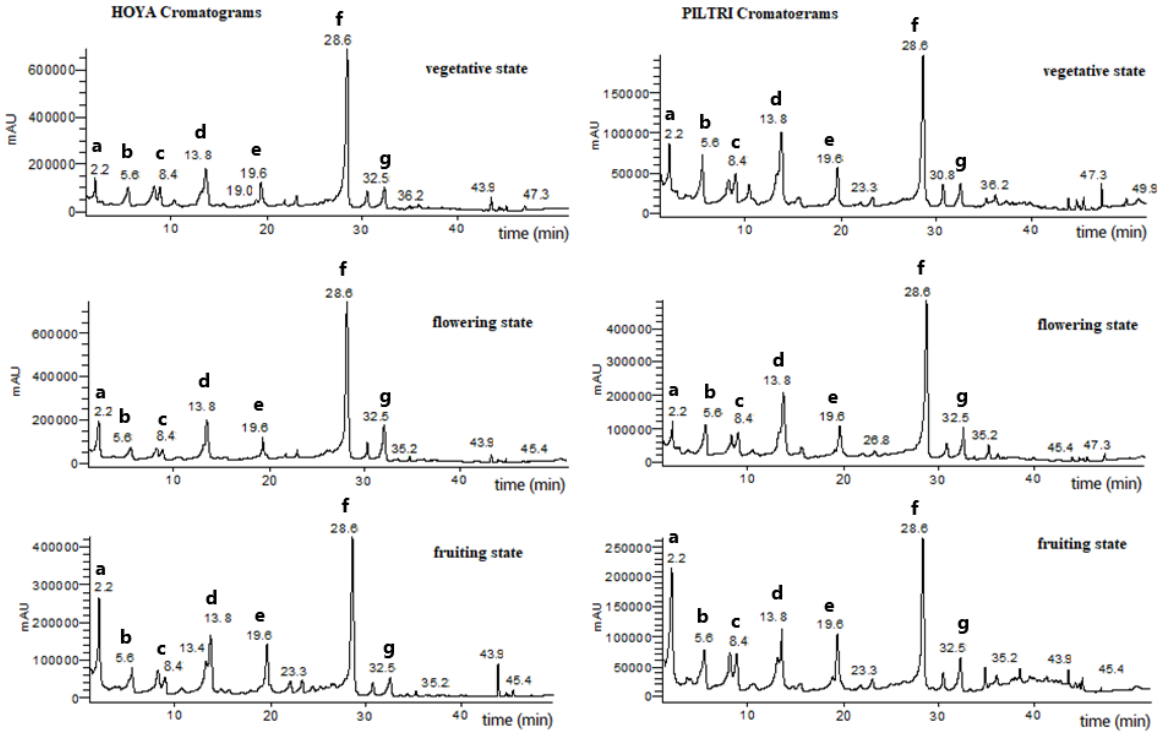

Figure No. 3

Shows representatives HPLC-MS chromatograms at 280 nm, the fingerprint is similar for the different populations and phenological stages. Major compounds identified. Note: a=Chlorogenic acid; b= Teucrol succinoyl glucoside; c= Pelargonidin glucoside; d= Ferulic acid glucoside; e=Isomer of lithospermic acid; f= Monomethyl anthranol; g= p-Hydroxy-phenylacetic acid

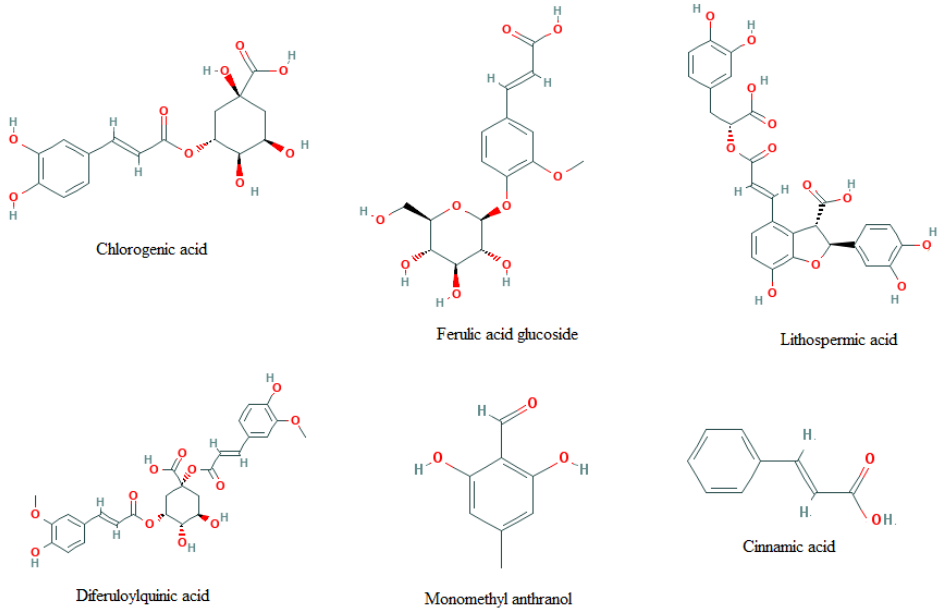

Figure No. 4
Structures of the major compounds identified

Statistical analysis

Piltri population presented less antioxidant activity in vegetative stage than in flowering and fruiting stages (p=0.0445). However, no significant difference was observed between the two populations in vegetative stage. The Pearson correlation analysis between antioxidant activity and total phenol content was found to be significant $p<0.001$ with a correlation coefficient of 0.77 (Figure No. 5).

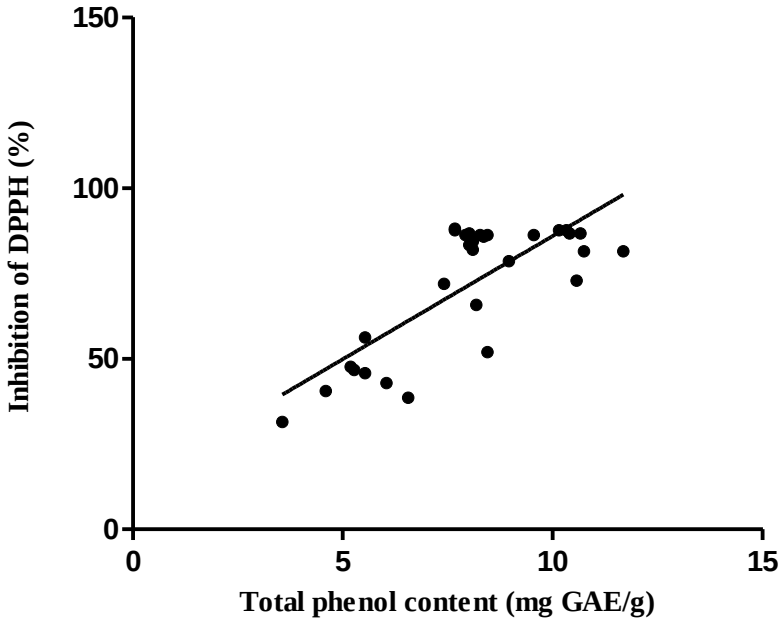

Figure No. 5
Correlation between total phenolic content and DPPH radical scavenging activity of the extracts. Pearson correlation coefficient, r = 0.77.

DISCUSSION

Among secondary metabolites, a chemical characteristic of phenols is their ability to neutralise free radicals, minimising oxidative stress in living beings.

Many authors have correlated this antioxidant activity with total phenolic content, this is greater when more phenolic compounds the samples contain (Pennycooke *et al*., 2005; González *et al*., 2013; Thusoo *et al*., 2014). We therefore hypothesised that the antioxidant activity in ethanol extracts of *V. carnosa* may be mostly due for the presence of phenolic, and particularly hydroxycinnamic acids. In this study, we observed a significant positive correlation between antioxidant activity and total phenolic content. However, it is interesting to note that, among the individuals with higher phenol content, the individual P_{ve5} with the highest value did not show the greatest antioxidant activity. It is important to highlight that in Folin-Ciocalteu method has some interference compounds that could overestimate or underestimate the results (Lester *et al*., 2012; Ludwing *et al*., 2013; Muñoz-Bernal *et al*., 2017). The compounds that cause interferences could be sugars, ascorbic acid, aromatic amines and sulphur dioxide, among others. Thus, the presence of reducing nonphenolic compounds (fructuose, ascorbic acid, some proteins, among others) could has overestimated the value of phenol content in P_{ve5}. For this reason, some authors suggest this method should be seen as a measure of total antioxidant capacity rather than phenolic content (Everette *et al*., 2010; Ludwing *et al*., 2013).

The values obtained from the phenol quantification ranged from 3.56 to 11.68 mg GAE/g, similar to those reported for root ethanol extracts in other medicinally used species within the family Valerianaceae, such as *Nardostachys jatamansi* (Jones) DC (3.4 mg GAE/g) and *V. officinalis* (14.2 mg GAE/g) (Surveswaran *et al*., 2007). In *V.*

jatamansi, a very important species in the pharmacopoeia of India, the phenolic content of methanol root extracts, ranged between 6.83 and 27.54 mg GAE/g in different populations in Himalayan regions (Jugran *et al*., 2013). Unlike extracts obtained from subterranean organs of these species, the phenolic content of aerial tissue extracts (leaves) of *V. officinalis* is considerably lower, at 0.017 mg GAE/ g (Wojdylo *et al*., 2007).

The extracts from *V. carnosa* roots presented variable antioxidant activity, reducing DPPH with percentages that ranged between 48% and 82%. In the vegetative stage, there were no significant differences between the two populations in this variable, but the Piltri population presented significantly lower values in this stage than in, the other stages (p<0.0445). On average, we observed the highest antioxidant activity in the Hoya population, although this was not statistically significant. The values were higher in the climatologically dry periods, when plants had higher physiological activity (flowering and fruiting stages), and this may be related to the water deficit in these seasons or may be a response to attack by insects, which were observed on the specimens collected during the warmer months. Many plants synthesise metabolites such as terpenes, peptides, phenols and hormones as a defence strategy against stimuli caused by animals or insects (Schultz, 2002).

An important aspect to highlight is the phenolic content variability among individuals in each population (at intra-population level). This could be indicating a great genotypic variability within each population.

We tentatively identified eighteen phenolic compounds by HPLC-MS. Several of these compounds have been widely reported in plant extracts. Among those most studied, chlorogenic acid, ferulic acid and gallic acid have been reported with anti-inflammatory, antioxidant and anticancer properties (Graf, 1992; Martinez-Valverde *et al*., 2000; Yen & Tsai, 2002; Clifford *et al*., 2005; Stushnoff *et al*., 2008; Bah *et al*., 2014). Anti-inflammatory, antioxidant and antibacterial properties were also reported for cinnamic and rosmarinic acids (Petersen & Simmonds, 2003; Borges *et al*., 2015), other widely studied compounds found in these plant extracts. Kasetti *et al.* (2012) suggested cinnamic acid also as an antidiabetic active principle.

Most of the compounds were present in both populations, although it is important to mention two chemical compounds that were each present in only one population and each in a different phenological stage: chlorogenic acid conjugated isomer in H_{ve} and syringic acid in P_{Fl} and P_{Fr} (Table No. 1). Syringic acid has been reported to have anti-inflammatory (Fernández *et al*., 1998), antibacterial (Aziz *et al*., 1998) and hepatoprotective activity (Itoh *et al*., 2009).

Finally, it is interesting to notice that the traditional harvesting season of the plant appear not to be in line with the phenological stages at which antioxidant activity and phenolic content are highest on average, at least in the populations and individuals studied. However, we observed that the specimen with highest total phenolic content was collected in the vegetative stage (P_{ve5}).

CONCLUSIONS

This work analyses and compares the variability of phenolic content at different phenological stages in *V. carnosa* populations. The results demonstrated a higher content and antioxidant activity in the flowering and fruiting stages, at least in the studied populations. In addition, we observed qualitative and quantitative variability between individuals within each population and between populations. We tentatively identified 18 phenolic compounds, of which two (chlorogenic acid and syringic acid) were each specific for only one population and specific for some phenological stages.

According to traditional practices and ethnobotanical studies, the time of harvesting Ñamkulawen subterranean organs for medicinal use does not match with the seasons in which the plants have the highest content of phenolic compounds (at least in the two populations analysed). The time of collection could be related to the period of livestock movement (traditional practice) or it could be associated with a greater quantity of other chemical compounds. We therefore propose to study the dynamics of other secondary metabolites, such as valepotriates, valerenic acid and essential oils.

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Appendix No. 1

Total phenolic content of the plants in the analysed populations. $H_{Ve(n)}$, $H_{Fl(n)}$, $H_{Fr(n)}$ are the specimens of the Hoya population, while $P_{Ve(n)}$, $P_{Fl(n)}$, $P_{Fr(n)}$ are the specimens of the Piltri population.

Population	Stages	Specimen	mg GAE/g	Average mg GAE/g	Standard deviation
Hoya	Vegetative	H_{Ve1}	8.44	8.30	1.92
		H_{Ve2}	10.57		
		H_{Ve3}	8.95		
		H_{Ve4}	8.27		
		H_{Ve5}	5.27		
	Flowering	H_{Fl1}	9.55	7.65	1.44
		H_{Fl2}	7.67		
		H_{Fl3}	7.41		
		H_{Fl4}	8.09		
		H_{Fl5}	5.53		
	Fruiting	H_{Fr1}	7.67	8.57	1.19
		H_{Fr2}	8.35		
		H_{Fr3}	10.66		
		H_{Fr4}	8.18		
		H_{Fr5}	8.01		
Piltri	Vegetative	P_{Ve1}	5.53	6.68	3.02
		P_{Ve2}	3.56		
		P_{Ve3}	6.56		
		P_{Ve4}	6.04		
		P_{Ve5}	11.68		
	Flowering	P_{Fl1}	10.74	9.02	2.32
		P_{Fl2}	10.40		
		P_{Fl3}	10.32		
		P_{Fl4}	8.44		
		P_{Fl5}	5.19		
	Fruiting	P_{Fr1}	8.01	7.75	2.00
		P_{Fr2}	4.59		
		P_{Fr3}	10.15		
		P_{Fr4}	8.09		
		P_{Fr5}	7.92		

Appendix No. 2

Percentage reduced DPPH percentage of individuals at the study sites. $H_{Ve(n)}$, $H_{Fl(n)}$, $H_{Fr(n)}$ are the specimens of the Hoya population, while $P_{Ve(n)}$, $P_{Fl(n)}$, $P_{Fr(n)}$ are the specimens of the Piltri population.

Population	Stage	Specimen	Average	%reduced DPPH	Average % reduced DPPH	Standard deviation
Hoya	Vegetative	H_{Ve1}	0.34	**51.90**	67.24	**17.16**
		H_{Ve2}	0.19	**72.86**		
		H_{Ve3}	0.15	**78.57**		
		H_{Ve4}	0.10	**86.19**		
		H_{Ve5}	0.37	**46.67**		
	Flowering	H_{Fl1}	0.10	**86.19**	77.24	**13.31**
		H_{Fl2}	0.09	**87.62**		
		H_{Fl3}	0.20	**71.90**		
		H_{Fl4}	0.11	**84.29**		
		H_{Fl5}	0.31	**56.19**		
	Fruiting	H_{Fr1}	0.08	**88.10**	82.57	**9.46**
		H_{Fr2}	0.10	**85.71**		
		H_{Fr3}	0.09	**86.67**		
		H_{Fr4}	0.24	**65.71**		
		H_{Fr5}	0.09	**86.67**		
Piltri	Vegetative	P_{Ve1}	0.38	**45.71**	48.00	**19.45**
		P_{Ve2}	0.48	**31.43**		
		P_{Ve3}	0.43	**38.57**		
		P_{Ve4}	0.40	**42.86**		
		P_{Ve5}	0.13	**81.43**		
	Flowering	P_{Fl1}	0.13	**81.43**	77.90	**17.09**
		P_{Fl2}	0.09	**86.67**		
		P_{Fl3}	0.09	**87.62**		
		P_{Fl4}	0.10	**86.19**		
		P_{Fl5}	0.37	**47.62**		
	Fruiting	P_{Fr1}	0.12	**83.33**	75.90	**19.93**
		P_{Fr2}	0.42	**40.48**		
		P_{Fr3}	0.09	**87.62**		
		P_{Fr4}	0.13	**81.90**		
		P_{Fr5}	0.10	**86.19**		