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CHEMICAL SCIENCES

Lychnophora pinaster in endangered *campos rupestres*: phenolic compounds and population ecogeography

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Abstract: *Lychnophora pinaster*, known as arnica-mineira, is endemic to *campos rupestres*, at risk of extinction. The present study aimed to characterize the ecogeography and phenolic constituents of 11 *L. pinaster* populations collected in the mesoregions North, Jequitinhonha, Metropolitan of Belo Horizonte and Campos das Vertentes in the state of Minas Gerais, Brazil. Phenolic constituents were identified and quantified by Ultra-high performance liquid chromatography- mass spectrometry. *Lychnophora pinaster* occurs in sites at high altitude (700 and 1498 m), annual rainfall of up to 1455 m, soils with low fertility and predominantly loamy texture. Therefore, it can be considered tolerant to acidic soils, with low availability of nutrient. The most abundant substances in all populations were vitexin (18 – 1345 ng/g) and chlorogenic acid (60 – 767 ng/g). The 11 populations formed four groups in relation to the phenolic constituents, with group 1 consisting of the populations of the North Mesoregion (GM, OD) and Jetiquinhonha (DIMa), group 2, the Metropolitan of Belo Horizonte (SRM, NLSC, SM, RPS, CTRA), group 3, the North Mesoregion (ODMa and DI), and group 4, the Campos das Vertentes (CC). Among the populations, only those from the Metropolitan of Belo Horizonte showed correlation of soil properties with phenolic constituents.

Key words: arnica-mineira, Asteraceae, flavonoids, medicinal plants, soil.

INTRODUCTION

Brazil is one of the mega-diverse countries of the world and it is the most species-rich country (Forzza et al. 2012). This is due to the presence of several centers of plant diversity with a plethora of environmental factors (Davis et al. 1997, Oliveira- Filho & Fontes 2000), but diversity is a result of historical processes over time, the interaction between evolutionary processes and environmental factors (Wiens et al. 2006, Antonelli et al. 2018). One of such hotspots of plant diversity is *campos rupestres* vegetation, which has been elevated to a formal bioregion at province level (Colli-Silva et al. 2019), such as Cerrado, Caatinga, and Pampa. This formalization is a recognition of its high biological importance for the South American biota, due its high biodiversity and endemism (Giulietti et al. 1997, Echternacht et al. 2011, Colli-Silva et al. 2019).

Campos rupestres is a fire-prone vegetation, composed mainly of grassland or shrublands intermixed with grasslands on rocky soils (Silveira et al. 2016, Mucina 2018). This vegetation occurs from 700 m to 2033 m and is considered a zonal vegetation, limited by the types of soils, quartzitic, sandstone, or ironstone (Alves

& Kolbeck 2010, Silveira et al. 2005). C*ampos rupestres* is an endangered vegetation due to anthropogenic activities (Fernandes et al. 2014, Pougy et al. 2015). The climate of *campos rupestres* corresponds to dry winters and rainy summers, diversified soils but, extremely-impoverished soils (Silveira et al. 2016, Oliveira et al. 2015).

Lychnophora Mart. is a typical and endemic genus found in *campos rupestres* with 39 species (Semir et al. 2011, 2014, 2020), some of which are endangered. *Lychnophora pinaster* Mart. [Synonyms: *Lychnophora affinis* Gardner, *Lychnophora trichocarpha* (Spreng.) Spreng.] is endemic to *campos rupestres* of the State of Minas Gerais, popularly known as arnica and arnica-mineira, it is at risk of extinction by habitat degradation due to mining activities, urban expansion, fires and indiscriminate use for medicinal purposes (CNCFlora 2012).

The aerial parts are widely used in folk medicine in the form of hydroalcoholic extract (topical use), in cases of contusions, swelling, bruising and trauma (Silveira et al. 2005, Rodrigues & Carvalho 2001), in all regions of occurrence.

Studies in the literature on *L. pinaster* trypanocidal potential, antibacterial action against *Staphylococcus aureus*, and anti-inflammatory to this species (Silveira et al. 2005, Abreu et al. 2011, 2013, Müller et al. 2019). The substances associated with biological properties of the species include 15-deoxy-goiazensolide, caffeic acid, isochlorogenic acid, vitexin, isovitexin and *E*-lychnoforic acid, with trypanocidal action (Silveira et al. 2005); 15-deoxy-goiazensolide as antibacterial (Keles et al. 2010); α-amyrine, quercetin, stigmasterol, sitosterol, friedelin, chlorogenic acid, cinnamic acid, caffeic acid, rutin and lupeol as anti-inflammatory agents (Abreu et al. 2013, Müller et al. 2019).

The production of specialized metabolites in plants is genetically and epigenetically controlled (Trapp & Croteau 2001), but environmental factors such as light (intensity and photoperiod), latitude, temperature (minimum, maximum and average), soil (chemical and physical properties) can also influence. In response to abiotic factors, plants show adaptations in primary and specialized metabolism, mainly to avoid, tolerate or even resist environmental stress (Souza & Lüttge 2015). Environmental factors such as seasonality, rainfall, temperature, altitude, soil, among others, have correlations with each other and do not work in isolation, and may influence jointly the specialized (Gobbo-Neto & Lopes 2007).

Studies involving the chemical characterization of non-volatile constituents of *L. pinaster* are restricted to the municipalities of Moeda and Itabirito, belonging to the Metropolitan Mesoregion of Belo Horizonte. To date, there are no studies involving the ecogeographic characteristics of the regions of occurrence of *L. pinaster*, whose information is relevant for understanding the adaptation of the species to the soil, climate and geographic factors, which can help in the development of species conservation strategies.

Thus, in view of the degradation of *campos rupestres*, the risk of extinction of *L. pinaster* and its medicinal potential, the objective of this study was to characterize the ecogeography and phenolic constituents of 11 *Lychnophora pinaster* populations collected in the mesoregions North, Jequitinhonha, Metropolitan of Belo Horizonte and Campos das Vertentes in the state of Minas Gerais, Brazil.

MATERIALS AND METHODS

Sampling

For the location of the natural populations of *L. pinaster*, we consulted publications (Semir 1991, Semir et al. 2011, 2014), materials deposited in herbaria (IAC, UEC, HUFU, RB, according to Thiers 2020), and the expert in the genus *Lychnophora*, PhD. João Semir. After selecting the potential areas for the study, field activity was carried out from June to July 2017 in the North, Metropolitan, Jequitinhonha and Campo das Vertentes mesoregions in the State of Minas Gerais, Brazil. Eleven populations distributed in the municipalities of Grão Mogol (GM), Olhos D'Água (OD, ODMa), Diamantina (DI, DIMa), Moeda (SM), Caeté (CTRA), Raposos (RPS), Nova Lima (NLSC, SRM) and Carrancas (CC) were visited for the collection of plant material and soil.

For analysis of the chemical composition, branches of eleven individuals (vegetative stage) were collected for each *L. pinaster* population. Samples of plant at the reproductive stage were also collected (leaves, inflorescences, and flowers or fruits) per population, for voucher preparation and deposit in the Herbarium UEC of the State University of Campinas. The species identification was confirmed by PhD. Marcelo Monge (Federal University of Uberlândia) and PhD. João Semir (State University of Campinas).

Information on latitude, longitude and altitude was collected for each population, using a Garmin GPS map 60CSX device. In addition, characteristics of the environment were observed, such as vegetation type (IBGE 1993, 2019) and the annual average rainfall and temperature from the database available at the Center for Weather Forecasting and Climate Studies of the National Institute for Space Research (CPTEC/INPE 2017). From the geographical position of sites of species occurrence, a map of occurrence of *L. pinaster* populations was constructed. Spatial data were processed in ARCMAP 2018 (Geographic Image Processing System), version 10.6. The collection was authorized by the Brazilian Ministry of the Environment (ICMBio: 22772-6; SisGen/MMA: A01F803) and the State Forestry Institute of Minas Gerais (authorization 005/2017 and 006/2017).

Physical and chemical characterization of the soil

Soil samples were collected from the 0-20 cm layer, approximately; about five samples were carried out per site to make a composite sample (Brasil 2002). Chemical characterization of soils followed the methodology of Raij et al. 2001. The soils were classified according to the Brazilian Soil Classification System (Santos et al. 2006).

Preparation of the extract and analysis of the phenolic constituents of *L. pinaster* with UHPLC-MS

Leaves of each individual from *L. pinaster* populations were manually separated from the branches, and lyophilized. The extracts were prepared by maceration, in a 2 mL Eppendorf tube, from 60 mg leaves crushed in liquid nitrogen and 2 mL 70% hydroethanolic solution. The extractive solution was kept in an ultrasonic bath for 20 minutes and centrifuged for 2 minutes at 10.000g, then filtered through filter paper and stored in vials (2 mL).

Chromatographic analysis was performed on a UHPLC Acquity equipment coupled to the TQD Acquity mass spectrometer (Micromass-Waters Manchester, England), with electrospray ionization source (ESI) in the negative mode and a C_{18} BEH Waters Acquity (2.1 mm x 50 mm x 1.7 µm) column. Elutions were performed at a flow rate of 200 µL/min, mobile phase A - purified

water (Milli-Q) with 0.1% formic acid and phase B - methanol (chromatographic grade) starting with 20% B, ramping to 60% B in 7 min, then to 100% at 8 min, held until 8.5 min, then returning to the initial condition and equilibrating until 10 min. Aliquots of 10 µL of each sample were injected in duplicate. The conditions of the mass spectrometer were: capillary voltage 3.00 kV, source temperature 150 °C and desolvation temperature 300 °C.

Commercial standards for chlorogenic acid (Sigma-Aldrich, ≥ 95%), *p*-coumaric acid (Sigma-Aldrich, 98%), quercetin (Sigma-Aldrich, 98%), vitexin (Sigma-Aldrich, 95%), isovitexin (Sigma-Aldrich, ≥ 98%), rutin (Sigma-Aldrich, 95%) were used for the identification and quantification of substances. These were quantified from calibration curves constructed by the external calibration method with a stock solution of commercial standards (1 mg/mL), by successive dilutions to concentrations of 10; 100; 1.000; 5.000; 10.000 and 50.000 ng.mL^{-1}. The results were expressed as ng/g dry leaf. A total, of 110 extracts were analyzed, eleven per *L. pinaster* population.

Statistical analysis

Soil properties and chemical composition of phenolic compounds data were subjected to principal component analysis (PCA) and hierarchical group analysis (HCA) and partial least squares-discriminant analysis (PLS-DA) using the MetaboAnalyst online platform (Xia et al. 2015). Data were previously normalized by autoscaling. The Euclidean distance was used for HCA.

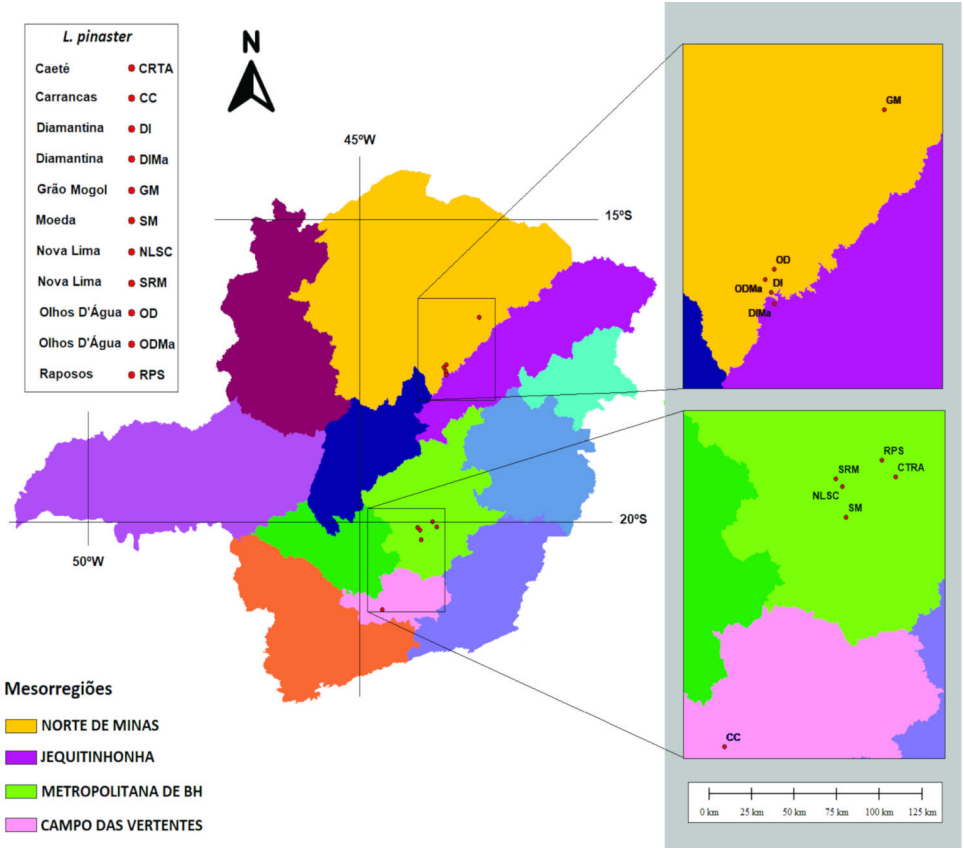

Figure 1. Map showing the sampling sites in the mesoregions of the State of Minas Gerais, Brazil.

RESULTS AND DISCUSSION

Ecogeographic analysis

The sampling sites of *L. pinaster* populations and the respective mesoregions of occurrence are illustrated in Figure 1. With the exception of Campos das Vertentes, the other mesoregions are located in the three floristic districts of *campos rupestres* in the northern part of the Espinhaço range. The district of Grão Mogol is in the northern part, in Diamantina, and close to Serra do Cipó, the district of Diamantina Plateau, and near the municipality of Belo Horizonte, the district of the iron quadrangle (Colli-Silva et al. 2019).

The northern mesoregion (municipality of Diamantina: DI; Olhos D 'Água: OD, ODMa) and Jequitinhonha (DIMa) are located on quartzite soils. The populations from the Metropolitan Mesoregion of Belo Horizonte, whereas the populations sampled in the municipalities of Caeté (CTRA), Raposos (RPS) and Nova Lima (NLSC, SRM), occur in a ferruginous rupestrian field, with development on canga, while the population of Serra da Moeda (SM), on quartzite soils. The mesoregion Campos das Vertentes (municipality of Carrancas: CC) is located in an area classified as a *campos rupestres* of altitude, with soil of quartzite origin (Coura 2006).

As shown in Table I, the altitude of the sampling sites ranged from 700 m to 1580 m. The populations of the North (GM, DI, ODMa, OD) and Jetiquinhonha (DIMa) mesoregions are at lower altitudes (700 to 859 m) than those of the Campos das Vertentes (CC:1307 m) and Metropolitan of Belo Horizonte (NLSC, SRM, RPS, CTRA, SM: 1366 to 1497 m). The lowest altitude observed was for the ODMa population (700 m) collected in the municipality of Olhos D'Água, and the highest for SRM (1498 m) occurring in the municipality of Nova Lima.

Table I. Location and characterization of regions of occurrence of natural populations of *Lychnophora pinaster*, State of Minas Gerais, Brazil.

Population	Municipality	Latitude	Longitude	Altitude (m)	Rainfall (mm)	*T. M (C°) Max.	Min.	Exsiccate (Code)
GM	Grão Mogol	16°55'39.5"S	042°89'17.1"W	859	969	29.0	11.2	UEC 208038
OD	Olhos D' Água	17°40'39.9"S	043°57'15.1"W	704	1387	27.3	15.0	UEC 208032
DI	Diamantina	18°14'54.9"S	046°36'11.5"W	819	1498	24.0	15.0	UEC 208027
ODMa	Olhos D' Água	17°40'37.7"S	043°57'01" W	700	1387	27,3	15.0	UEC 208037
DIMa	Diamantina	18°14'56.2"S	046°36'17.2"W	796	1498	24.0	15,0	UEC 208026
CTRA	Caeté	19°52'51.6"S	043°40'11.2"W	1497	1456	27.0	16.0	UEC 208036
SM	Moeda	20°19'58.6"S	044°03'10.8"W	1366	1345	27.2	14.5	UEC 208030
NLSC	Nova Lima	19°59'09.8"S	043°58'59.7"W	1450	1320	27.0	14.0	UEC 208028
SRM	Nova Lima	19°59'08.8"S	044°01'18.8"W	1498	1320	27.0	14.0	UEC 208029
RPS	Raposos	19°96'39.2"S	043°80'47.4"W	969	1377	29.5	15.2	UEC 208031
CC	Carrancas	21°28'11.6"S	044°38'07.6"W	1307	1580	25.0	12.5	UEC 208033

***Average temperature.**
North Mesoregion: municipality of Olhos D'Água (OD and ODMa), Diamantina (DI) Grão Mogol (GM); Jequitinhonha: municipality of Diamantina (DIMa); Metropolitan of Belo Horizonte: municipality of Moeda (SM), Caeté (CTRA), Raposo (RPS), Nova Lima (SRM and NLSC); Campos das Vertentes: municipality of Carrancas (CC).

The average annual rainfall in mesoregions where *L. pinaster* populations were sampled ranged from 969 m (North mesoregion, GM population, municipality of Grão Mogol) to 1580 mm (Campos das Vertentes, CC population, municipality of Carrancas). The average annual temperature varied between the minimum of 11.2 °C (North mesoregion, GM population, municipality of Grão Mogol) and the maximum of 29.5 ° C (Metropolitan of Belo Horizonte, RPS population, municipality of Raposos).

The environments where *L. pinaster* occurs are subject to strong anthropogenic pressures, such as fires and mineral extraction, in addition to urban sprawl (CNCFlora 2012). This last event was observed in the expedition to collect the OD population in the municipality of Olhos D'água, where the species was located on a site close to houses and roads. During the expedition, we observed that the populations of the municipality of Diamantina (DIMa and DI) showed signs of fires, but the physiological process of regrowth was not observed.

Physical and chemical characterization of soils in *L. pinaster* occurrence sites

Results of soil analysis of the *L. pinaster* sampling sites are listed in Table II. The soils were predominantly acidic, with high levels of aluminum (Al^{+3}), low base saturation and poor in organic matter. Regarding nutrients, low levels of macronutrients such as P, K, Mg and high concentrations of micronutrients, such as Fe and Zn were observed. Soil texture is predominantly loamy, also known as medium texture, which has good drainage and good water holding capacity (Brady & Weil 2013, Centeno et al. 2017).

The soil of the CTRA population belonging to the Metropolitan Mesoregion of Belo Horizonte showed slightly acidic pH values (6), absence of aluminum (Al^{+3}) and base saturation of 78%. These conditions are ideal for nutrient

Table II. Physical and chemical properties of soils in the regions of occurrence of natural populations of *Lychnophora pinaster*, State of Minas Gerais, Brazil.

Population	pH	OM	P_{resin}	Al^{+3}	H+Al	K	Ca	Mg	SB	CEC	V%	S	B	Cu	Fe	Mn	Zn	Physical analysis
		mg/dm³	g/dm³	---------- $mmol_c/dm^3$ ----------								------- ----- mg/dm³ ----------						
GM	3,8	33	1	10	32	0.9	9	1	11	43	25	4	0.23	0.2	124	0.4	0.1	sandy loam
OD	4.1	25	2	7	27	1.4	8	1	10	37	27	3	0.22	1.0	66	16.0	0.2	loamy
ODMa	4.0	26	2	17	60	0.9	8	2	11	71	15	3	0.22	0.6	178	3.3	0.2	loamy
DI	4.2	19	2	9	29	0.8	12	2	14	43	33	6	0.20	0.2	58	0.4	0.1	loamy
DIMa	4.0	16	2	9	25	0.7	6	1	7	32	22	4	0.21	0.3	68	0.3	0.1	silty loam
SRM	3.6	66	14	17	216	1.8	21	4	27	243	11	25	0.74	0.5	332	9.0	3.7	loamy
SM	4.1	49	12	4	106	2.9	31	6	40	145	27	12	0.56	0.6	204	51.2	5.6	loamy
CTRA	6.0	49	5	0	26	1.1	60	31	91	117	78	11	0.26	0.4	74	39.3	1.4	sandy loam
NLSC	3.5	74	7	16	233	1.8	17	2	21	254	8	19	0.42	0.5	338	3.4	2.9	clay loam
RPS	4.1	44	2	6	60	1.5	23	3	28	88	32	8	0.26	1.8	324	36.1	1.0	loamy
CC	4.2	26	1	7	28	1.9	14	3	19	48	41	6	0.26	0.4	119	3.1	0.4	loamy sand

North Mesoregion: municipality of Olhos D'Água (OD and ODMa), Diamantina (DI) Grão Mogol (GM); Jequitinhonha: municipality of Diamantina (DIMa); Metropolitan of Belo Horizonte: municipality of Moeda (SM), Caeté (CTRA), Raposo (RPS), Nova Lima (SRM and NLSC); Campos das Vertentes: municipality of Carrancas (CC). OM: Organic Matter; P_{resin}: resin phosphorus; Al^{+3}: exchangeable aluminum; H+Al; potential acidity; K: potassium; Ca: calcium, Mg: magnesium; SB: Sum of bases; CEC: Cation Exchange Capacity; V%: Base saturation; S: sulfur; B: boron; Cu: copper; Fe: iron; Mn: manganese; Zn: zinc.

absorption, according to criteria used in agronomy for cultivated plants (Ribeiro et al. 1999). However, in relation to native plants, the response to physical and chemical conditions of the soil is different, once adaptability of these species to soils with adverse conditions and low fertility is recorded (Haridasan 2000) In the case of *L. pinaster*, Oliveira-Júnior et al. (2005) evaluated the effect of liming and fertilization, concluding that it is a species with low nutritional requirements, except for the micronutrients zinc (Zn) and manganese (Mn), and highlight that although it is not an accumulating species, it is tolerant to aluminum (Al^{+3}).

The soils in the regions of occurrence of *L. pinaster* populations were classified according to the Brazilian Soil Classification System (Santos et al. 2018), as shown in Figure 2. Populations GM, OD, ODMa, DI, DIMa are found on Litolic Neosol, which are shallow soils, normally found in more sloping reliefs, with low percentages of phosphorus, as observed in the present study, with phosphorus ranging from 1 to 2 mg/dm^3 for these populations. The soils of the other populations were classified as Haplic Cambisol, which presents variable fertility, sloping or mountainous relief, shallow soils with, rocky masses. In the regions where these populations occur, there are some variations in relation to the activity of the clay and the presence of iron, being divided into Haplic Cambisol PV Dystrophic (NLSC, SM, SRM, RPS), Haplic Cambisol Perférrico (CTRA) and Haplic Cambisol AVL Dystrophic (CC) (Santos et al. 2018).

Phenolic constituents identified in leaves of *Lychnophora pinaster* populations

In these *L. pinaster* populations in the State of Minas Gerais, chlorogenic and *p*-coumaric acids, as well as flavonoids (quercetin, vitexin and rutin) were identified and quantified (Table III).

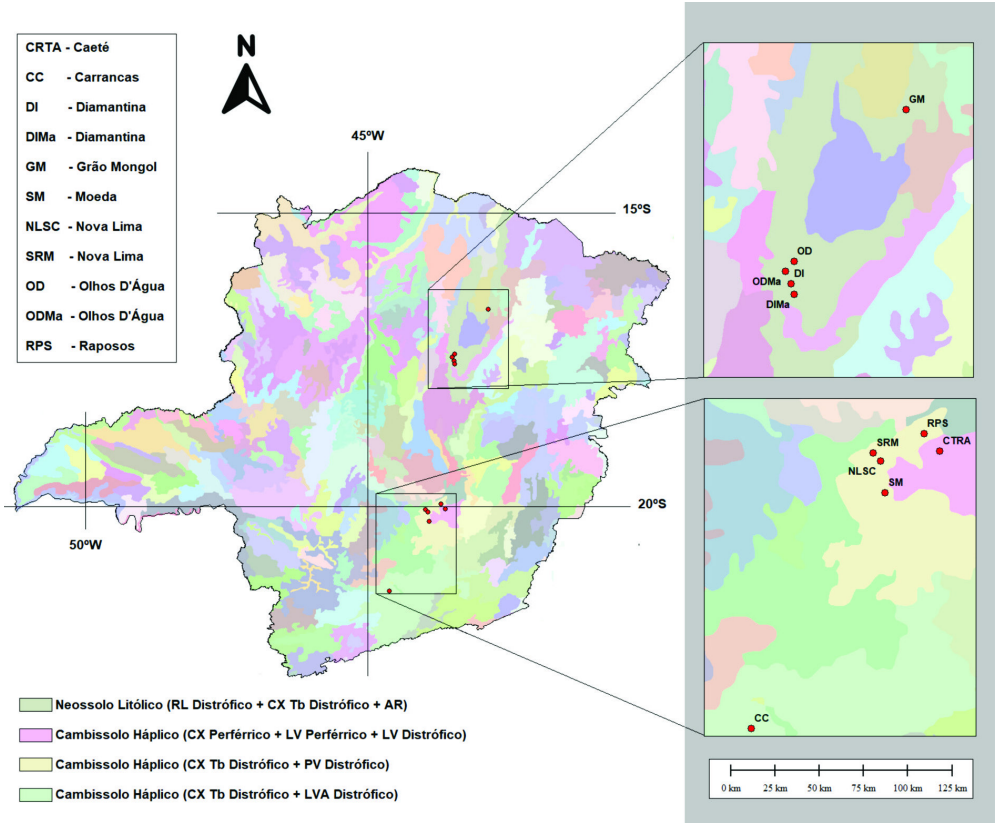

Figure 2. Classification map of soils in the regions of occurrence of natural populations of *L. pinaster* in the State of Minas Gerais, Brazil.

Previous studies with aerial parts of *L. pinaster* occurring in the Metropolitan Mesoregion of Belo Horizonte identified the presence of chlorogenic acid and rutin (Müller et al. 2019), vitexin (Silveira et al. 2005, Müller et al. 2019) and quercetin (Abreu et al. 2013), as well as the anti-inflammatory activity of quercetin (Abreu et al. 2013), anti-inflammatory and anti-hyperuricemic activities of rutin and chlorogenic acid (Müller et al. 2019).

The populations of mesoregions North (OD, ODMa, DI, GM) and Jequitinhona (DIMa) showed the highest concentration of vitexin (692 to 1345 ng/g leaf) in comparison to the Metropolitan Mesoregion of Belo Horizonte (SRM, NLSC, SM, RPS, CTRA: 32 to 163 ng/g leaf) and Campos das Vertentes (CC: 18 ng/g leaf). The population of Grão Mogol (GM) had the highest concentration of vitexin (1345 ng/g leaf), and the Carrancas (CC) had the lowest (18 ng/g leaf).

Among the populations, ODMa (North mesoregion) had the lowest concentration of chlorogenic acid (60 ng/g leaf) and Carrancas (CC), mesoregion of Campos das Vertentes, the highest (767 ng/g leaf).

In three populations in the Metropolitan Mesoregion of Belo Horizonte, was not detected the *p*-coumaric acid (SRM, CTRA, SM), and the highest concentration was found in the ODMa population (35 ng/g leaf), North mesoregion. The highest concentration of quercetin (72 ng/g leaf) was found in the CC population, mesoregion of Campos das Vertentes.

Rutin was not detected in the populations of Carrancas (CC), Raposos (RPS), Caeté (CTRA) and Grão Mogol (GM), being more abundant in Diamantina (DIMa: 79 ng/g leaf), the latter belonging to the Jetiquinhonha mesoregion.

In the PCA biplot (Figure 3), the first two axes explained 99.6% total variance, in which PC1 accounted for 87.8%, and PC2 for 11.8%. The populations formed four groups, with group 1 consisting of the populations of the North Mesoregion (GM, OD) and Jetiquinhonha (DIMa), group 2, the Metropolitan Mesoregion of Belo Horizonte (SRM, NLSC, SM, RPS, CTRA), group 3, the North Mesoregion (ODMa and DI), and group 4, the Campos das Vertentes (CC) population.

The substances responsible for the groups were vitexin and chlorogenic acid. Groups 1 and 3 were formed by populations with a high concentration of vitexin and groups 2 and 4, of chlorogenic acid. The greater abundance of chlorogenic acid in the populations of

Table III. Phenolic constituents (ng/g) identified in leaves of populations of *Lychnophora pinaster*, State of Minas Gerais, Brazil.

			Population										
Component	tR (min)	ESI^- $[M - H]^-$ *m/z*	GM	OD	ODMa	DI	DIMa	NLSC	CTRA	RPS	SM	SRM	CC
Chlorogenic acid	1.75	353	417	553	60	187	555	224	360	295	361	324	767
p-coumaric acid	3.14	163	1	5	32	17	10	2	-	5	-	-	18
Vitexin	3.58	432	1345	1025	1180	829	692	49	32	44	163	34	18
Rutin	4.19	610	-	6	3	21	79	65	-	-	4	40	-
Quercetin	5.90	302	-	22	29	16	3	41	14	3	25	20	72

tR: retention time (minutes).
(-): component not detected for the population.
North Mesoregion: municipality of Olhos D'Água (OD and ODMa), Diamantina (DI) Grão Mogol (GM); Jequitinhonha: municipality of Diamantina (DIMa); Metropolitan of Belo Horizonte: municipality of Moeda (SM), Caeté (CTRA), Raposo (RPS), Nova Lima (SRM and NLSC); Campos das Vertentes: municipality of Carrancas (CC).

group 1 (417 to 555 ng/g leaf) contributed to its separation from group 3 (60 and 186 ng/g leaf), the same was observed for groups 2 and 4, where the CC population had the highet concentration of this substance (767 ng/g leaf).

The hierarchical group analysis (Figure 4) formed two large groups of populations, group 1 formed by the populations of the North Mesoregion (GM, OD, ODMa, DI) and Jetiquinhonha (DIMa), and group 2 by the populations of the Metropolitan Mesoregion of Belo Horizonte (SRM, NLSC, SM, RPS, CTRA) and Campos das Vertentes (CC). Two subgroups were formed in group 1, one composed of the populations ODMa, DI and GM and the other, of OD and DIMa, the last population belonging to the Jetiquinhonha Mesoregion and the others to the North Mesoregion.

Group 2 shows the formation of a subgroup formed by the populations of the Metropolitan Mesoregion of Belo Horizonte (SRM, RPS, CTRA, SM, NLSC) and the second formed by the population of the municipality of Carrancas (CC), occurring in the mesoregion of Campos das Vertentes. These results demonstrate the profile of phenolic constituents in populations varied according to the geographical origin. The divergence in chemical composition can change the biological activity, a relevant fact, since the species is commonly used with the same medicinal purpose in all regions of the state of Minas Gerais.

Relationship between phenolic compounds and soil physical and chemical properties

Figure 5 presents in its axes the two most important principal components, summarizing 67.7% multivariate information that can be extracted from the soil × phenolic constituent data. The populations located in the extremes of the State were grouped, being they OD, DI, DIMa (Northern Minas), ODMa (Jequitinhonha) and CC (Campos das vertentes), while those in the central/Metropolitan region of Belo Horizonte (NLSC, SRM, SM, CTRA and RPS) were dispersed. The populations further north

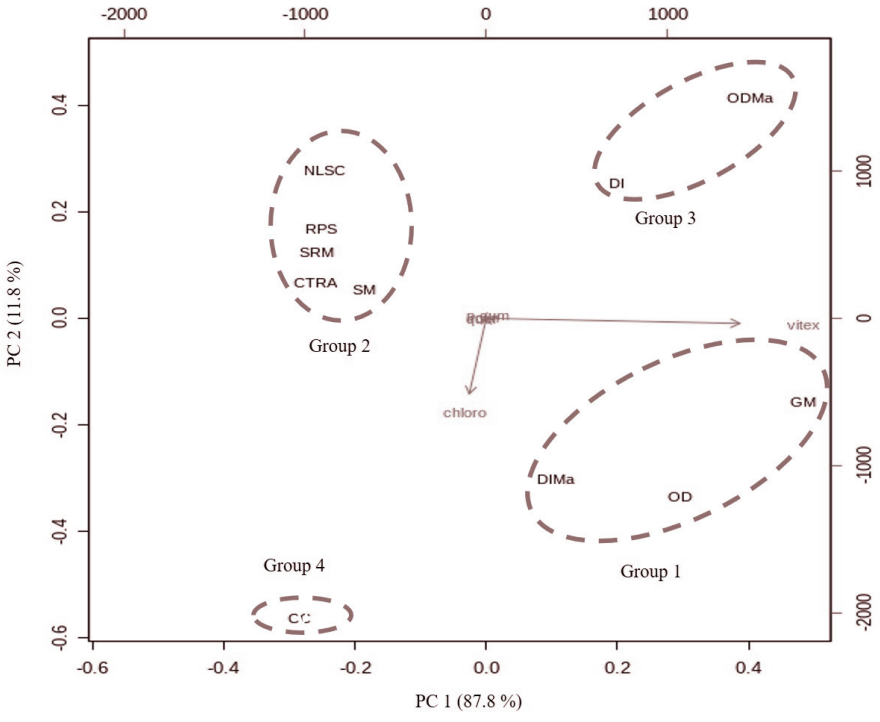

Figure 3. Biplot of principal component analysis (PCA) for samples and phenolic constituents from *L. pinaster* populations, state of Minas Gerais, Brazil.

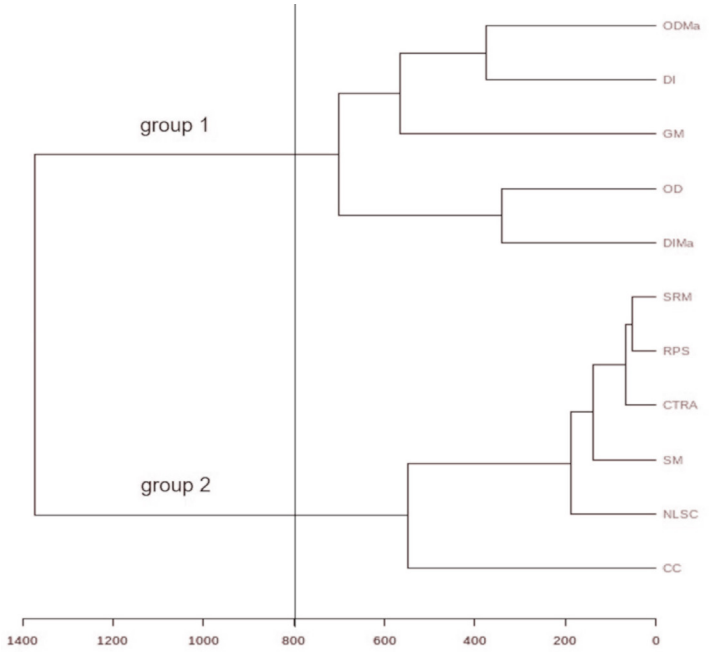

Figure 4. Hierarchical group analysis (HCA) of the 11 populations of *L. pinaster* occurring in the North, Jetiquinhonha, Metropolitan of Belo Horizonte and Campos das Vertentes mesoregions, state of Minas Gerais, Brazil.

(ODMa, OD, DIMa, DI and GM) and South (CC) showed low correlation with soil factors, the main characteristic of differentiation of these from the others, of chemical order, with higher percentages of phenolic compounds vitexin and *p*-coumaric acid.

Populations of the central region of the state, located in the metropolitan mesoregion of Belo Horizonte, unlike the other regions, were strongly correlated with soil properties. Figure 5 showed that all factors evaluated have positive weights along the PC1. Despite the geographic proximity, the populations in this region differ from each other in terms of soil properties, which can be associated and responsible for the chemical variations observed.

Populations SRM, NLSC and SM presented the highest scores in the first principal component, mainly due to the concentration of the minerals Fe, Zn, S, K, P (P resin) and Al^{+3}, in addition, they stood out as regions of more acidic soil (H+Al), higher organic matter (OM) content and cation exchange capacity (CEC). According to the vectors, populations NLSC and SRM, geographically close, are chemically similar mainly as a function of rutin concentration and low concentration of p-coumaric acid. This last factor is also responsible, together with quercetin, for bringing the SM population closer to NLSC and SRM.

Due to the higher concentration of minerals Mn, Ca and Mg, present in the soil as cationic bases, which result in high correlation with sum of bases (SB), pH and base saturation (V%), the CTRA population presented a distinct behavior. In terms of soil fertility, the characteristics with greater weight for this population indicate that compared to the others it is under less stress, which justifies the low concentration of phenolic compounds observed in this population. The RPS population showed intermediate behavior between populations in chemical terms, with scores in both PC1 and PC2 close to the origin (point 0).

Discriminant analysis by partial least squares (PLS-DA), through the graph of variable importance in projection (VIP), in Figure 6, revealed that within the variation in PC1, the most important variables for the observed variation. In general, the soil properties were decisive for

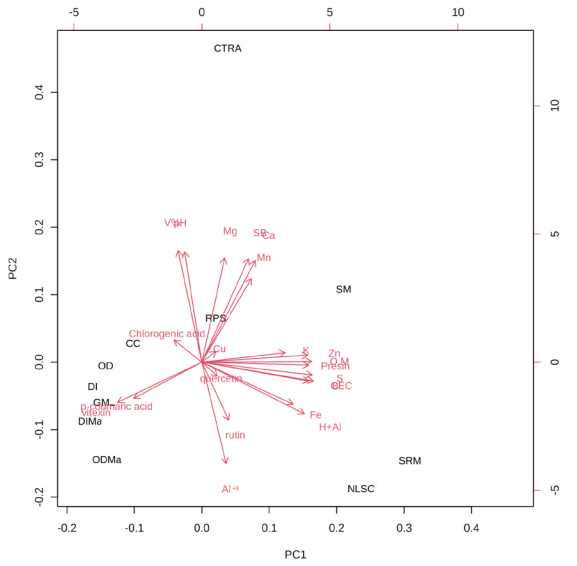

Figure 5. Biplot of principal component analysis (PCA) for soil and climate factors and phenolic constituents of *L. pinaster* population samples.

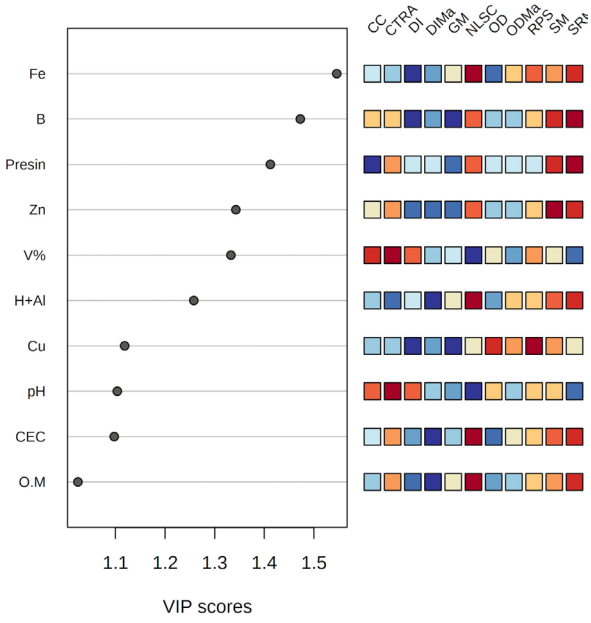

Figure 6. Importance of soil and climate variables in the projection according to the partial least squares analysis (PLS-DA).

the observed groupings, with greater relevance mainly for the minerals Fe, B, P and Zn.

CONCLUSION

Lychnophora. pinaster occurs in sites at high altitude (700 and 1498 m), with annual rainfall of up to 1455 mm with low fertility and predominantly loamy texture. The soils in the regions of occurrence of the species are divergent in relation to physical and chemical properties, being classified as Litholic Neosol (North and Jequitinhonha Mesoregions), Haplic Cambisol PV Dystrophic and Perferric (Metropolitan Mesoregion of Belo Horizonte), and Haplic Cambisol AVL Dystrophic (Campos das Vertentes).

The substances chlorogenic acid, *p*-coumaric acid, quercetin, vitexin and rutin were identified in the ethanol extract of the species. Populations from the North and Jetiqutinhonha Mesoregions contain the highest concentration of vitexin and the population from Campos das Vertentes, of chlorogenic acid. The physical and chemical properties of the soil affected the chemical composition of the species, in relation to the phenolic compounds, mainly from the populations of the Metropolitan Mesoregion of Belo Horizonte.

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**In memoriam*

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