Industrial Crops & Products 112 (2018) 705–715

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

Biological activity of *Hyptis* Jacq. (Lamiaceae) is determined by the environment

Kátia Pereira dos Santos*, Martha Dalila Sedano-Partida, Wilton Ricardo Sala-Carvalho, Beatriz Ortega San Juan Loureiro, Cíntia Luíza da Silva-Luz, Claudia Maria Furlan

Departamento de Botânica, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, 277, CEP 05508-090, São Paulo, SP, Brazil

ARTICLE INFO

Keywords:
Phytogeographic domain
phenolic compounds
antioxidant assays
abiotic microenvironment

ABSTRACT

The evolution of plant special metabolites is currently viewed within a phylogenetic perspective: the biosynthetic machinery needed to produce plant defense must be well-conserved and this origin should be monophyletic. But some questions thus arise: does a species occurring in different biogeographic domains present different levels of special metabolites? And if so, will it also represent a difference in its biological activity? For this study, seven Hyptis (Lamiaceae) species was collected and extracted by maceration in 70% ethanol, analyzed by highperformance liquid chromatography coupled with a diode array detector (HPLC-DAD) and performing antioxidants assays: DPPH (2,2-Diphenyl-1- picrylhydrazyl) and ABTS (2,20-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) radical scavenging activity, Iron (III) reduction to iron (II) and oxygen radical absorbance capacity (ORAC). Phenolic classes observed for Hyptis spp. were: phenolic acids, flavonoids, chlorogenic acid derivatives, and cinnamic acid derivatives. Results point out to a tendency of Hyptis populations growing at Cerrado domain present lower levels of phenolic compounds when compared to populations growing at Atlantic Forest. Furthermore, the abiotic microenvironment seems to exert a stronger influence regarding the phenolic composition and consequently the antioxidant activity of a plant extract than the phytogeographic domain.

1. Introduction

Lamiaceae has a cosmopolitan distribution that includes about 240 genera and 7500 species (Harley, 2012). Plants of this family are usually herbaceous and shrub, being trees less frequent. Lamiaceae species are well known for their aromatic constituents, and their essential oils are of economic interest for medicinal, cosmetic and food use.

Traditionally, Lamiaceae is composed by aromatic herbs such as rosemary (*Rosmarinus officinalis* L.), sage (*Salvia officinalis* L.), thyme (*Thymus vulgaris* L.) and lavender (*Lavendula angustifolia* Mill.), all native to the Mediterranean region and cultivated worldwide, and balm (*Melissa officinalis* L.) and spearmint (*Mentha spicata* L.), both common in Britain and other European countries. These species have been used in folk medicine for exhaustion, weakness, depression, memory enhancement, circulation improvement and strengthening fragile blood vessels. Researchers have found that these plants are a source of compounds possessing high antioxidant (Zheng and Wang, 2001) anti-inflammatory (al-Sereiti et al., 1999), anti-allergy (Ito et al., 1998) and anti-depression activity (Takeda et al., 2002). These activities appear to be related to the contents of phenolic compounds in each species (Li et al., 2008a,b; Tripathi et al., 2007; Wren, 1988).

Hyptis Jacq. occurs in tropical and subtropical regions, from North America to the Caribbean and South to Argentina. Phytochemical, biological and pharmacological interest of *Hyptis* occurred in 1952 when *Hyptis suaveolens* (L.) Poit, first *Hyptis* species chemically studied and being its essential oil used for the treatment of various infections (Nayak and Guha, 1952). Since then, the interest for *Hyptis* species has been reported due to their uses in traditional medicine, showing diverse medicinal properties and also being used as condiments (*Hyptis oblongifolia* Benth. and *Hyptis pectinata* (L.) Kuntze) (Pereda-Miranda et al., 1993; Pereda-Miranda and Delgado, 1990), insect repellents (*Hyptis spicigera* Lam.) (Pereda-Miranda and Delgado, 1990; Kini et al., 1993), and fungicide (*Hyptis ovalifolia* Pohl.) (Souza et al., 2003).

Regarding *Hyptis* uses in folk medicine, several species were reported as effective for the treatment of influenza and constipation (*Hyptis fruticosa* Salzm. ex Benth); respiratory diseases (*Hyptis macrostachys* Benth); stomach and intestinal disorders and as bactericidal (*Hyptis martiusii* Benth); colic and liver diseases (*Hyptis pectinata*); nasal and atrial disorders (*Hyptis umbrosa* Salzm. ex Benth) and to combat

* Corresponding author.
E-mail address: katiapsp@usp.br (K.P. dos Santos).

https://doi.org/10.1016/j.indcrop.2017.12.065
Received 26 September 2017; Received in revised form 16 November 2017; Accepted 25 December 2017
0926-6690/ © 2018 Elsevier B.V. All rights reserved.

fever (*Hyptis suaveolens)* (Judd et al., 2002, Agra et al., 2008; Coutinho et al., 2008).

From the chemical point of view, *Hyptis* presents a wide variety of chemical constituents, including triterpenes and flavonoids (Pereda-Miranda and Delgado, 1990), diterpenes with a labdane-like skeleton (Ohsaki et al., 2005), and some species also presenting diterpenes with a abietane-like skeleton (Cavalcanti et al., 2008; Chukwujekwu et al., 2005; da Cruz Araújo et al., 2004; Urones et al., 1998). As well as, lignans and α-pyran derivatives were already described for the genus (Deng et al., 2009; Pereda-Miranda et al., 1993).

Studies have validated the pharmacological activity of leaf aqueous extracts from *H. suaveolens* (Santos et al., 2007), as well as its organic extracts (hexane, chloroform or ethyl acetate) and aqueous extract from leaves from *H. pectinata* (Bispo et al., 2001; Lisboa et al., 2006). Results showed analgesic, anti-nematode and acute toxicity effects in *in vivo* assays. In relation to the essential oil, antinociceptive activities (mediated by peripheral and central pain pathways) were described for *Hyptis fruticosa* (Menezes et al., 2007) and *Hyptis pectinata* (Arrigoni-Blank et al., 2008).

Hyptis belongs to the subtribe Hyptidinae. Currently, the circumscription of the genus was deeply modified (Harley and Pastore, 2012). Pastore et al. (2011) demonstrated the monophyly for nine genera belonging to Hyptidinae, except *Hyptis*, which turned out to be paraphyletic. Thus, Harley and Pastore (2012) redefined the genera circumscription in Hyptidinae, proposing 144 new combinations and 23 new synonymies. Currently, 19 genera are recognized for this subtribe, and *Hyptis* was greatly reduced, but appear as monophyletic.

Due to this new circumscription proposed by Harley and Pastore (2012), some phytochemically important species, such as *H. suaveolens*, *H. oblongifolia, H. pectinata, H. spicigera, H. ovalifolia, H. macrostachys, H. fruticosa, H. martiusii, H. umbrosa* and more, were placed into other genera. As well as, species from *Peltodon* now belongs to *Hyptis* sect. *Peltodon*. All these changes resulted in the monophyly of *Hyptis*.

Brazil is the main diversity center for *Hyptis* (Harley et al., 2010; Harley, 2012), with the occurrence of species in different vegetation formation, especially the Atlantic Rain Forest and Cerrado. According to Harley (2012), within Lamiaceae, *Hyptis* is the genus with the largest number of species occurring in Brazil, being 69% of them, endemic.

Atlantic Forest is, in fact, a complex of varied formations covering wet forests, araucaria forests and coastal forests. The Atlantic Rain Forest has a rainfall index with mean annual values varying between 1800 and 3600 mm. Temperature and precipitation vary according to the altitude, *e.g* every 100 m, the temperature can decrease by 0.6 degrees and the precipitation increases up to 200 mm. The average annual temperature on the coast is 22° C, but it can reach values below zero at the top of Agulhas Negras mountain (Itatiaia-RJ) which is 2800 m high. The average annual precipitation at sea level is 1600 mm. In this phytogeographical domain, it is estimated that about 20,000 species of angiosperms occur. In addition, it has a high degree of endemism and is considered one of the top five biodiversity hotspots on the planet, remaining only 7% of its original coverage (Santos and Brandimarte, 2014; Myers et al., 2000).

Cerrado (tropical savanna) is the most important phytogeographical domain in Central Brazil and is characterized by different landscapes with varied vegetation, soil, climate, and topography. As Atlantic Rain Forest, Cerrado is considered one of the 25 biodiversity hotspots in the world. It is a tropical biome with well-defined seasons: the dry season during winter and the rainy season in the summer. The average annual temperature is 25 °C, and it can reach 40 °C in a short period of few days. Annual precipitation is around 1200 to 1800 mm. The main feature of Cerrado is its poor soil, which determines the vegetation physiognomy. Also, in addition to the deficiency of soil several minerals, there is a high concentration of aluminum, a toxic element for most plants. But despite this arid and poor soil appearance, Cerrado, like the Atlantic Rain Forest, has a rich biodiversity, is considered the most diverse savanna biome on the planet and presenting more than 10,000 plant species (Santos and Brandimarte, 2014, Klink and Machado, 2005, Myers et al., 2000).

Currently, it is known that many of the special metabolites are directly involved in the mechanisms that allow the adaptation of plants to their habitat (Santos, 2004; Miranda et al., 2013). In addition, the intensities of biological activity may vary depending on the influence of abiotic factors. For example, edaphoclimatic factors may affect the production of especial metabolites (Gobbo-neto and Lopes, 2007).

According to Kutchan (2001), special metabolites represent a chemical interface between plants and the environment around them and because of that, their synthesis is often affected by environmental conditions such as altitude, availability of water and macro and micronutrients in the soil, relative air temperature and soil pH. Despite the recognized influence of environmental factors on plant development, there are few studies that show the relationships and physiological adaptations of plants to the environment.

The evolution of plant special metabolites is currently viewed within a phylogenetic perspective (Agrawal, 2007): the biosynthetic machinery needed to produce plant defense must be well-conserved and this origin should be monophyletic. But some questions thus arise: does a species occurring in different biogeographic domains present different levels of special metabolites? And if so, will it also represent a difference in its biological activity? To study this relation, *Hyptis*, a Lamiaceae genus with several reports regarding its use in traditional medicine, was selected in order to answer the above questions. *Hyptis* presents a great diversity of species, several occurring in both biogeographic domains of Cerrado and Atlantic Rain Forest.

The aim of this study was to compare the biological activity of extracts from *Hyptis* spp. sect. *Peltodon* occurring in two biogeographic domains, in order to understand and relate the production of special metabolites with the variation across environments: Cerrado and Atlantic Rain Forest. These results could help to optimize the economic uses of a plant species.

2. Materials and methods

2.1. Plant material

Hyptis species are distributed throughout the Brazilian territory. For this study were selected seven *Hyptis* species: *H. radicans* (Pohl) Harley & J.F.B. Pastore, *H. campestris* Harley & J.F.B. Pastore, *H. meridionalis* Harley & J.F.B. Pastore and *H. comaroides* (Briq.) Harley & J.F.B. Pastore, from the sect. *Peltodon*; *H. lacustris* A.St.-Hil. ex Benth, *H. lappulacea* Mart. ex Benth. and *H. multibracteata* Benth., from the sect. *Capitata* (Fig. 1).

H. radicans is commonly found in the Central-West (Mato Grosso do Sul), Southeast (Minas Gerais, Rio de Janeiro, São Paulo) and Southern (Paraná, Santa Catarina) regions in the Cerrado and Atlantic Forest phytogeographical domains and in different types of vegetation such as: anthropic areas, altitude fields, clean field, rupestrian field, ombrophilous and mixed ombrophilous forest (Harley et al., 2015a). *H. campestris* is distributed in the North (Rondônia, Tocantins), Northeast (Bahia, Maranhão), Midwest (Goiás, Mato Grosso do Sul, Mato Grosso), Southeast (Minas Gerais, São Paulo), and South (Paraná) of Brazil, among the phytogeographical areas of Amazonia, Caatinga, Cerrado, Atlantic Forest and in vegetation types such as anthropic areas, altitude field and palm grove (Harley et al., 2015b). *H. meridionalis* and *H. comaroides* have restricted distribution. *H. meridionalis* occurs in the Southeastern (São Paulo) and Southern (Paraná) regions, in the phytogeographic domain of Cerrado and Atlantic Forest and vegetation types as altitude field and clean field (Harley et al., 2015c). *H. comaroides* are found in the Central-West (Mato Grosso do Sul, Mato Grosso) and Southern (Paraná, Rio Grande do Sul, Santa Catarina) regions, in the phytogeographical domains of Cerrado, Atlantic Forest, and Pampa, in vegetation types as altitude field and clean field (Harley et al., 2015d). *H. lacustris* is distributed in the North (Amazonas, Rondônia),

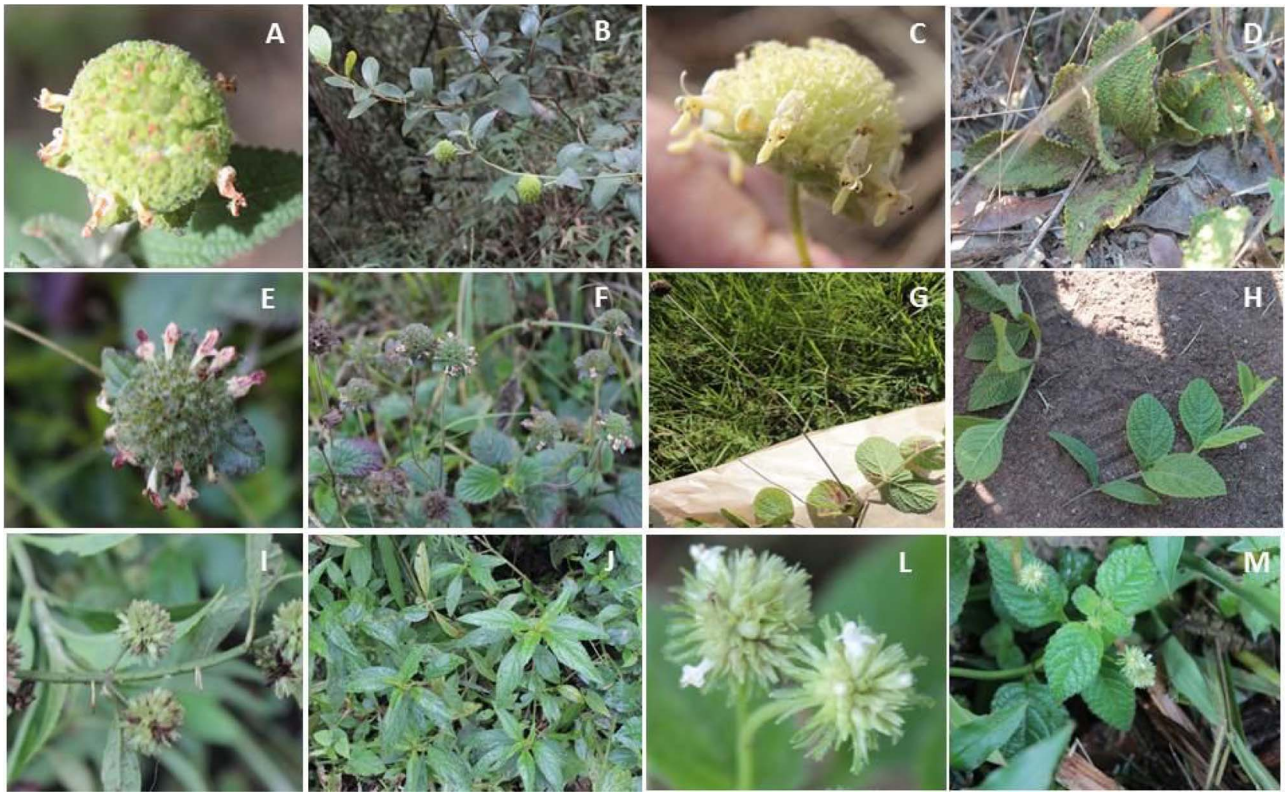

Fig. 1. *Hyptis* species used in this study. A, B – *H. campestris*; C, D – *H. meridionalis;* E, F – *H. radicans;* G, H – *H. comaroides;* I, J – *H. lacustris;* L, M – *H. multibracteata* (Photos: Claudia Furlan).

Central-West (Distrito Federal, Mato Grosso), Southeastern (Rio de Janeiro, São Paulo), and Southern (Paraná, Rio Grande do Sul, Santa Catarina) of Brazil, in the phytogeographical domains of Amazonia, Cerrado, and Atlantic Forest with vegetation of meadow field, ciliary forest, ombrophilous forest, and Restinga (Harley et al., 2015e). *H. lappulacea* occurs in the Southern (Paraná, Rio Grande do Sul, Santa Catarina) and Southeast (Espírito Santo, Minas Gerais, Rio de Janeiro, São Paulo) regions in phytogeographical domains of Atlantic Forest with a type of vegetation meadow field, the field of altitude, ombrophilous and mixed ombrophilous forest, and Restinga (Harley et al., 2015f). Finally, *H. multibracteata* can be found in the Northeast (Bahia), Southeastern (Espírito Santo, Minas Gerais, Rio de Janeiro, São Paulo), and South (Santa Catarina) in the phytogeographical domain of Atlantic Forest and in the type of vegetation as ombrophilous forest and Restinga (Harley et al., 2015g).

For each species, leaves, stems, and flowers were collected from a population. *H. lacustris*, *H. lappulacea*, *H. multibracteata* and some individuals of *H. radicans* were collected in the Biological Reserve Alto da Serra of Paranapiacaba, in the municipality of Santo André (SP), Mogi das Cruzes (SP), and Campina Grande do Sul (PR), an Atlantic Forest area. Populations of *H. radicans* were also collected in the Cerrado phytogeographic domain in Avaré (SP) and Mogi Guaçú (SP). Although *H. campestris* distribution is reported for the two phytogeographic domains, this species was found only in Cerrado areas, in the cities of Pirassununga (SP), Buriti, (MT), and Rio da Casca (MT). *H. meridionalis*, as well as *H. radicans*, was found and collected in both phytogeographic domains in the cities of Balsa Nova (PR, Atlantic Rain Forest) and Jaguariaíva (PR, Cerrado). *H. comaroides* was found and collected only in the phytogeographic domain of Atlantic Forest in the city of Curitiba (PR).

An exemplary sample of each species was deposited at the Herbarium of University of São Paulo (SPF) (Table 1).

2.2. Phytochemical screening

Plant material was collected, and oven dried at 40 °C for one week. Dried material (10 g) was ground and extracted by maceration in 70% ethanol for seven days at room temperature and solvent exchange every two days. The crude hydroethanolic extract (EB) was reduced under pressure and lyophilized.

Samples were taken up in 2 mg mL^{-1} DMSO and analyzed by high-performance liquid chromatography coupled with a diode array detector (HPLC-DAD) (LC1260 – Agilent Technologies) using a Zorbax C18 (150 × 4.6 mm, 3.5 µm) at 40° C, flow cell of 50 mm. Solvent gradient used was 0.1% acetic acid (AcOH) and acetonitrile (CH_3CN) starting with 10% CH_3CN (0–6 min), increasing from 10% to 15% (6–7 min), isocratic for 15 min in 15% (7–22 min), increasing from 15% to 50% (22–32 min), ranging from 50% to 100% (21–42 min), isocratic for 8 min in 100% (42–50 min). Solvent flow was 1.0 mL min^{-1}; injection volume 3 µL, and detection at 352 nm and 280 nm. Authentic samples of cinnamic acid derivatives, flavones, and flavonols *O* and *C*-glycosides were used for the preliminary identification of the constituents by their UV–vis absorption spectra and retention times. For the quantification of the constituents, areas in the chromatogram (HPLC-DAD) were compared with calibration curves obtained using authentic samples of *p*-coumaric acid (1.5 a 300 µg mL^{-1}) and luteolin (1.5 a 300 µg mL^{-1}). Results are expressed as mg g^{-1} of dry extract equivalent to the standard used.

2.3. Antioxidant assays

2.3.1. DPPH radical scavenging activity assay

DPPH radical scavenging activity assayfollowed the protocol described by Furlan et al. (2015). A 0.20 mM DPPH (2,2-Diphenyl-1-picrylhydrazyl) solution was mixed to the extracts diluted in 10% DMSO at concentrations of 15.62–500 µg mL^{-1}. Results were calculated using Trolox (6–200 µg mL^{-1}) as a positive control. 10% DMSO was used as a

Table 1
Hyptis spp. used in this study and placed in sections according to Harley and Pastore (2012).

Species	Section	Phytogeographic domain	Collection date	Municipality, State	Voucher
H. campestris Harley & J.F.B. Pastore	*Peltodon*	BC	03/11/2015	Pirassununga, SP	Santos 8
		BC	04/18/2015	Buriti, MT	Santos 31
		BC	04/20/2015	Rio da Casca, MT	Santos 39
H. comaroides (Briq.) Harley & J.F.B. Pastore	*Peltodon*	AF	01/27/2015	Curitiba, PR	Santos 21
H. lacustris A.St.-Hil. ex Benth.	*Capitatae*	AF	07/23/2014	Santo André, SP	Silva-Luz 296
H. lappulacea Mart. ex Benth.	*Capitatae*	AF	03/30/2012	Santo André, SP	Lombello 80
H. meridionalis Harley & J.F.B. Pastore	*Peltodon*	AF	05/21/2015	Balsa Nova, PR	Santos 45
		BC	05/22/2015	Jaguariaíva, PR	Santos 46
H. multibracteata Benth.	*Capitatae*	AF	07/23/2014	Santo André, SP	Silva-Luz 294
H. radicans (Pohl) Harley & J.F.B. Pastore	*Peltodon*	AF (dry season)	07/23/2014	Santo André, SP	Silva-Luz 295
		AF (humid season)	11/27/2014	Santo André, SP	Silva-Luz 295
		AF	12/10/2014	Mogi das Cruzes, SP	Santos 3
		AF	01/26/2015	Campina Grande do Sul, PR	Santos 15
		BC	11/21/2014	Avaré, SP	Silva-Luz 308
		BC	01/08/2015	Mogi Guaçu, SP	Santos 14

negative control. For each 20 µL of sample 200 µL of the DPPH solution was added and the absorbance was evaluated after 20 min of reaction (515 nm). Percentage of inhibition was calculated as [*Ac* − *As*/*Ac* × 100], where *Ac* is the absorbance of the negative control, *As* is the absorbance of the test samples.

2.3.2. ABTS radical scavenging activity assay

ABTS radical scavenging activity assay was evaluated according to the method described by Santos et al. (2016). First, a 7 mM ABTS (2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) solution in ultrapure water and a 2.6 mM potassium persulfate solution were prepared. For the formation of the ABTS radical, those solutions were mixed in a ratio of 1:1 and maintained in the dark at room temperature for 12–16 h. At the time of the test, 1 mL of the ABTS radical solution was diluted in 30 mL of methanol. To each 20 µL of samples (at concentrations of 15.62–500 µg mL^{-1}) were added 280 µL of the diluted ABTS radical solution. The mixture was incubated for 2 h at room temperature in the dark. The absorbance was detected at 734 nm. Results were calculated using Trolox (6–200 µg mL^{-1}) as a positive control. 10% DMSO was used as a negative control. Percentage of inhibition was calculated as [*Ac* − *As*/*Ac* × 100], where *Ac* is the absorbance of the negative control, *As* is the absorbance of the test samples.

2.3.3. Iron (III) reduction to iron (II) (FRAP)

Iron (III) reduction to iron (II) (FRAP) was evaluated according to the method described by Furlan et al. (2015) using 25 µL of each extract (at concentrations of 15.62–500 µg mL^{-1}), 10 µL of ultrapure water and 265 µL of the FRAP reagent (using TPTZ reagent, 2,4,6-Tris(2-pyridyl)-s-triazine). The mixture was incubated for 30 min at 37 °C and the absorbance measured at 595 nm. Results were calculated using Trolox (6–200 µg mL^{-1}) as a positive control. 10% DMSO was used as a negative control. Percentage of inhibition was calculated as [Abs_{sample}/$Abs_{positivecontrol}$ × 100], where Abs_{sample} is the absorbance of the test samples, $Abs_{positivecontrol}$ is the absorbance of a maximum concentration of the positive control.

2.3.4. Oxygen radical absorbance capacity (ORAC)

Oxygen radical absorbance capacity (ORAC) was evaluated according to the method described by Santos et al. (2016), using the fluorescent response at 485 nm excitation wavelength and 520 nm emission. 25 µL of each sample in phosphate buffer (at concentrations of 0.15–15.62 µg mL^{-1}) were mixed to 150 µL of fluorescein solution (48 nM). Microplates were incubated at 37 °C for 20 min. After addition of 25 µL of 75 mM AAPH (2,2′-Azobis(2-methylpropionamidine) dihydrochloride), the fluorescence was measured at intervals of 2 min for 120 min. The antioxidant capacity was based on the calculation of the area under the curve (AUC), using the formula (AUC) = 1 + f1/f0 + f2/f0 + f3/f0 + … fi/f0, (AUC) = 1 + f1/f0 + f2/f0 + f3/f0 + … fi/f0, where f_0 is the initial fluorescence reading at 0 min, and f_1 is the fluorescence reading at time 1. The net AUC was obtained by subtracting the AUC of the negative control from the AUC of the sample. ORAC values were calculated using the linear regression equation of Trolox (6.25–50 mM) as a positive control.

For all antioxidant assays, samples were analyzed in triplicates and the antioxidant potential of each sample was expressed as milligram per gram of dry extract equivalents to Trolox (mg g^{-1} TE). Percentages of antioxidant activity of samples in their different concentrations were used to calculated the effective concentration of each sample to achieve 50% of the antioxidant activity (EC_{50} µg mL^{-1}).

3. Results and discussion

3.1. Phytochemical screening

Phenolic classes observed for the seven *Hyptis* spp studied were: phenolic acids, flavonoids, chlorogenic acid derivatives, and cinnamic acid derivatives. As an abundant constituent in the subfamily Nepetoideae, rosmarinic acid was quantified separately. These phenolic classes were stablished according to their UV–vis spectral features (Markham, 1982) and detected by HPLC-DAD in hydroethanolic extracts of *Hyptis* spp (Table 2).

Results showed *H. campestris* collected in Buriti, MT (BC), *H. comaroides* collected in Curitiba, PR (AF), and *H. campestris* collected in Rio da Casca, MT (BC) as the species presenting the highest contents of total phenolic compounds: 123.70 mg ρ-CE g^{-1}, 99.62 mg ρ-CE g^{-1} and 98.97 mg ρ-CE g^{-1}, respectively. Species presenting the lowest values were *H. multibracteata* and *H. lacustris*, both from *Capitatae* sect. and occurring in the Atlantic Rain Forest (14.53 mg ρ-CE g^{-1} and 16.19 mg ρ-CE g^{-1}, respectively).

Regarding phenolic acid contents, *H. campestris* (Rio da Casca, MT, BC) presented the highest value of these compounds, 5.94 mg ρ-CE g^{-1}, while *H. radicans* (Santo André, SP, AF) collected in the humid season, presented the lowest value, 1.06 mg LE g^{-1}. *H. lacustris* (Santo André, AF) did not present any constituent presenting UV–vis absorption spectra similar to phenolic acids.

H. campestris (Buriti, MT, BC) presented the highest value of flavonoids contents: 114.43 mg LE g^{-1}, while *H. multibracteata* (Santo André, SP, AF) presented the lowest content of flavonoids (0.47 mg LE g^{-1}).

For contents of chlorogenic acid derivatives, *H. radicans* collected in Santo André, SP (AF) in the humid season presented the highest value, 9.66 mg ρ-CE g^{-1}, while *H. multibracteata* also collected in Santo André (AF) showed the lowest content of chlorogenic acid derivatives, 2.64 mg ρ-CE g^{-1}. *H. meridionalis* collected in both phytogeographic

Table 2
Contents of phenolic compounds of the hydroethanolic extracts *H. radicans* (Hrad), *H. multibracteata* (Hmul), *H. campestris* (Hcam), *H. meridionalis* (Hmer), *H. comaroides* (Hcom), *H. lacustris* (Hlac) analyzed by CLAE-DAD (280 nm).

Specie	City	Phenolic acids[a]	Flavonoids[b]	Chlorogenic acid derivatives[a]	Cinnamic acid derivatives[a]	Rosmarinic acid[a]	Total phenolic compounds
H. campestris	Pirassununga, SP (BC)	5.21	42.74	6.23	1.75	5.03	60.96
	Buriti, MT (BC)	2.51	114.43	4.48	0.66	1.62	123.70
	Rio da Casca, MT (BC)	5.94	83.95	3.01	3.63	2.44	98.97
H. comaroides	Curitiba, PR (AF)	4.13	72.47	0.00	3.34	19.68	99.62
H. lacustris	Santo André, SP (AF)	0.00	3.67	5.71	2.98	3.83	16.19
H. lappulacea	Santo André, SP (AF)	5.48	28.89	3.76	5.61	13.83	57.57
H. meridionalis	Balsa Nova, PR (AF)	3.28	76.91	0.00	3.69	12.64	96.52
	Jaguariaíva, PR (BC)	3.39	50.14	0.00	3.07	11.15	67.75
H. multibracteata	Santo André, SP (AF)	4.52	0.47	2.64	5.26	1.64	14.53
H. radicans	Santo André, SP (AF, humid season)	1.06	50.24	9.66	4.48	12.13	77.57
	Santo André, SP (AF, dry season)	2.11	38.34	5.69	2.43	16.26	64.83
	Mogi das Cruzes, SP (AF)	2.18	43.54	8.28	5.67	14.71	74.38
	Campina Grande do Sul, PR (AF)	2.04	40.10	0.00	4.83	7.02	53.99
	Avaré, SP (BC)	1.11	37.18	4.07	3.91	3.43	49.70
	Mogi Guaçú, SP (BC)	1.30	32.21	3.04	3.74	8.08	48.37

[a] milligrams equivalents of ρ-coumaric acid per gram of dry mass (mg ρ-CE g^1).
[b] milligrams equivalents of luteolin per gram of dry mass (mg LE g^1).

domains BC and AF and *H. comaroides* collected in Curitiba (PR, AF) did not present chlorogenic acid derivatives. However, *H. radicans* collected in Campina Grande do Sul (PR, AF), differ from individuals collected in the other 6 sites where this species was collected and did not present chlorogenic acid derivatives detected by the UV–vis absorption spectra.

Regarding contents of cinnamic acid derivatives, *H. radicans* collected in Mogi das Cruzes, SP (AF) presented the highest value, 5.67 mg ρ-CE g^{-1} and *H. campestris* collected in Buriti, MT (BC) presented the lowest value of cinnamic acid derivatives, 0.66 mg ρ-CE g^{-1}.

H. comaroides collected in Curitiba (PR, AF) presented the highest value of rosmarinic acid, 19.68 mg ρ-CE g^{-1} while *H. campestris* (Buriti, MT, BC) presented the lowest value, 1.62 mg CE g^{-1}.

These results point out to a tendency of *Hyptis* populations growing at Cerrado domain present lower levels of phenolic compounds when compared with populations growing at Atlantic Forest. *H. radicans* for example, individuals collected in Avaré and Mogi Guaçú, both areas of Brazilian Cerrado (SP) domain, showed similar contents of phenolic compounds. Individuals of *H. radicans* from Atlantic forest domain seem to present higher levels of these substances, especially when collected in the same state (SP) as Mogi das Cruzes and Paranapiacaba. *H. radicans* collected in Campina Grande (PR), despite this area be included in the Atlantic Forest domain, phenolic contents of this population were closer to the individuals from Brazilian Cerrado. *H. meridionalis* showed similar results to *H. radicans* presenting higher contents of phenolic compounds when collected in the Atlantic Forest than in Brazilian Cerrado.

A very interesting result was the different phenolic content of *H. radicans* when collected in the same site but in different seasons of the year. For samples collected in the humid season (summer) it was observed a tendency for higher levels of flavonoids, chlorogenic acid derivatives, and cinnamic acid derivatives when compared with samples collected in the dry season (winter). However, for phenolic acids and rosmarinic acid, contents of these substances tend to increase when the plant was collected in the dry season. Seasonality is one of the most important factors influencing the synthesis of special metabolites (Gobbo-neto and Lopes, 2007). Several studies already reported seasonal variations in the content of practically all classes of special metabolites including phenolic acids (Zidorn and Stuppner, 2001; Grace et al., 1998) and flavonoids (Atkinson and Blakeman, 1982; Brooks and Feeny, 2004; Clark and Clark, 1990; Gobbo-neto and Lopes, 2007; Jalal et al., 1982; Lobstein et al., 1991; Menkovic et al., 2000; Wilt and Miller, 1992).

H. campestris, although all samples were collected in Brazilian Cerrado domain, individuals collected in the state of Mato Grosso presented higher content of phenolic compounds than those collected in São Paulo.

H. comaroides was collected in Curitiba, PR (AF) and presented similar content of phenolic compounds to *H. meridionalis* collected in Atlantic forest and *H. campestris* collected in Brazilian Cerrado domain. As well as, *H. lappulacea* presented contents of phenolic compounds that resembled those of *H. radicans* collected in Brazilian Cerrado domain.

One of the most widely special metabolites distributed in nature is phenolic compounds. More than 8000 substances of this group have already been described in plants. This large and complex group is part of a variety of vegetables, fruits, and industrialized products. They may be pigments, which give the colored appearance to foods, or products usually derived from plant defense reactions against environmental stress. These compounds are involved in the antioxidant activity acting as metal chelators and/or scavenging reactive oxygen species (ROS), forming stable intermediate structures, and thus limiting free radical initiation or propagation (Brand-Williams et al., 1995; Chen et al., 2016; Moon and Shibamoto, 2009; Zheng and Wang, 2001).

It is very common find studies reporting the presence of phenolic derivatives in Lamiaceae species, especially cinnamic and chlorogenic acid derivatives but also phenolic acids. As examples, it was reported for *Rosmarinus officinalis* flavonoids and phenolic acids as major chemical constituents; *Lavandula angustifolia* presents caffeic acid and rosmarinic acid; for *Stachys officinalis* (L.) Trev. (Bruconic) were reported caffeic acid, rosmarinic and chlorogenic acid; *Orthosiphon spicatus* Bak. (Orthosifon) presents rosmarinic and caffeic acids, as well as glycolic and benzoic acids (Cunha et al., 2012). In *Hypenia salzmannii* (Benth.) Harley, some phenolic derivatives already reported were *p*-methoxycinnamic acid (Messana and Ferrari, 1990), some glycosylated phenylpropanoids, chlorogenic acid, protocatechuic acid, hydroquinone and thymohydroquinone (Pedersen, 2000). *Hyptis* spp here studied presented similar chemical composition regarding phenolic constitution corroborating previous studies with Lamiaceae species.

Furthermore, among phenolic compounds, despite rosmarinic acid, cinnamic and chlorogenic acid derivatives, all described as major phenolic constituents in *Hyptis*, lignans, such as brevipolides are also considered as very frequent substances among *Hyptis* spp. (Falcão and Menezes, 2003).

A study considering the leaf surface constituents of several species of Lamiaceae revealed the presence of two characteristic phenolic compounds in many of them. These compounds, called nepetoidins A and B were considered as taxonomic markers (Grayer et al., 2003) due to their presence in the great majority of species investigated and belonging to subfamily Nepetoideae. Some of these species studied are representatives of known genera of herbs used in food, such as mint, rosemary, sage, thyme, and basil. Nepetoidins are esters of caffeic acid present in Nepetoideae, thus distinguishing this subfamily from other within Lamiaceae. Nepetoidin B showed higher antioxidant activity than gallic, rosmarinic, and caffeic acids and showed activity as an insect phagostimulant. In addition, both nepotoidins presented antifungal activity (Grayer et al., 2003).

Returning to our first question, if a species occurring in different biogeographic domains presents different levels of special metabolites, considering *H. radicans* and *H meridionalis*, species collected in Atlantic Forest and Brazilian Cerrado, the present results point out to a tendency of higher levels of phenolic compounds in individuals growing in the Atlantic Forest domain than in Brazilian Cerrado (Table 2).

3.2. Antioxidant assays

Antioxidant activity of samples and standards (Trolox and rosmarinic acid) using DPPH·, ABTS·, FRAP and ORAC antioxidant assays, are presented in Tables 3 and 4, respectively.

According to the results, was observed that the extracts with higher activity of DPPH· quenching were from *H. lappulacea* (408.23 mg TE g^{-1}) and *H. radicans* (350.14 mg TE g^{-1}), both collected in Paranapiacaba (SP, AF); *H. radicans* during the dry season. *H. radicans* collected in Paranapiacaba (SP, AF) in the humid season (117.58 mg TE g^{-1}) presented the lowest results.

For the ABTS· assay, sample extracts behaved differently. *H. comaroides* collected in Curitiba (PR, AF) showed the highest antioxidant activity (581.46 mg TE g^{-1}) followed by *H. meridionalis* collected in Balsa Nova (PR, AF) (572.32 mg TE g^{-1}). *H. multibracteata* collected in Paranapiacaba (SP, AF) (247. 51 mg TE g^{-1}) presented the lowest results.

The two species that were most effective in DPPH· quenching, *H. lappulacea* and *H. radicans* from Paranapiacaba, and *H. comaroides*, presenting the higher antioxidant activity according to the ABTS radical assay, were also the ones that presented the highest levels of cinnamic acid derivatives and/or rosmarinic acid (Table 2).

Phenolic acids are usually divided into two main groups: benzoic acid derivatives, containing seven carbon atoms (C_6C_1), and cinnamic acid derivatives, comprising nine carbon atoms (C_6C_3). These compounds exist predominantly as hydroxybenzoic and hydroxycinnamic acids and may occur either in their free or conjugated forms (Teixeira et al., 2013). Recent data supports their beneficial application as preventive and/or therapeutic agents against oxidative stress, which is related to several diseases, such as atherosclerosis, inflammatory injury, and cancer (Fresco et al., 2006; Razzaghi-Asl et al., 2013). Antioxidant efficacy of hydroxycinnamic acids seems to be dependent on their structural features and related to the presence of hydroxyl function(s) in the aromatic structure (Razzaghi-Asl et al., 2013; Rice-Evans et al., 1996).

Chlorogenic acids (CGA) are esters of hydroxycinnamic acids and quinic acid. The most common CGA is formed by esterification of caffeic acid to quinic acid at the position 5 (Clifford et al., 2003; Razzaghi-Asl et al., 2013). Chlorogenic acid derivatives possess a variety of biological activities ranging from antifungal (Bowlers and Miller, 1994; Ma et al., 2007), antiviral (Jassim and Naji, 2003; Wang et al., 2009), and neuroprotective (Li et al., 2008a,b) to antidiabetic (Paynter et al., 2005; Karthikesan et al., 2010) and cholesterol-lowering effects (Razzaghi-Asl et al., 2013; Rodriguez de Sotillo and Hadley, 2002).

Rosmarinic acid is an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid commonly found in species from Boraginaceae,

Table 4
Effective concentration to achieve 50% of the antioxidant activity (EC_{50}) of *Hyptis* spp in the assays DPPH·, ABTS·, FRAP and ORAC.

Species	Municipality	DPPH·	ABTS·	FRAP	ORAC
H. campestris	Pirassununga, SP (BC)	78.92	21.93	15.69	2.03
	Buriti, MT (BC)	75.87	17.89	14.68	1.27
	Rio da Casca, MT (BC)	72.75	17.85	19.47	1.31
H. comaroides	Curitiba, PR (AF)	28.66	8.84	5.88	0.96
H. lacustris	Santo André, SP (AF)	24.79	9.03	5.35	1.49
H. lappulacea	Santo André, SP (AF)	12.33	6.13	8.83	0.56
H. meridionalis	Balsa Nova, PR (AF)	30.47	8.94	7.53	0.84
	Jaguariaíva, PR (BC)	37.30	10.73	8.93	1.26
H. multibracteata	Santo André, SP (AF)	72.19	23.17	17.60	30.09
H. radicans	Santo André, SP (AF, humid season)	48.11	11.91	10.61	1.32
	Santo André, SP (AF, dry season)	37.61	6.01	6.01	2.68
	Mogi das Cruzes, SP (AF)	42.11	13.95	9.59	1.53
	Campina Grande do Sul, PR (AF)	50.48	15.57	12.60	1.21
	Avaré, SP (BC)	70.86	19.39	20.69	2.28
	Mogi Guaçú, SP (BC)	61.58	16.86	13.49	2.18
Rosmarinic acid	–	7.51	2.22	0.89	0.12
Trolox	–	9.40	4.33	3.08	1.58

EC_{50} are expressed as μg mL^{-1}.

Table 3
Antioxidant capacity of crude extracts of *Hyptis* spp in the assays DPPH·, ABTS·, FRAP and ORAC.

Specie	City	DPPH·[a]		ABTS·[a]		FRAP[a]		ORAC[b]	
H. campestris	Pirassununga, SP (BC)	126.34	± 4.16	290.49	± 30.90	408.52	± 4.53	4516.61	± 1953.17
	Buriti, MT (BC)	138.74	± 2.66	329.22	± 22.89	441.80	± 5.46	5127.41	± 504.76
	Rio da Casca, MT (BC)	131.23	± 4.81	284.55	± 8.60	210.92	± 12.07	6540.55	± 557.36
H. comaroides	Curitiba, PR (AF)	326.31	± 7.54	581.46	± 84.26	718.72	± 13.30	6454.88	± 1350.31
H. lacustris	Santo André, SP (AF)	265.63	± 29.55	395.79	± 4.57	366.96	± 9.59	3960.67	± 299.07
H. lappulacea	Santo André, SP (AF)	408.23	± 76.83	488.23	± 13.64	399.85	± 90.33	16302.77	± 8359.17
H. meridionalis	Balsa Nova, PR (AF)	318.24	± 4.63	572.32	± 40.08	433.03	± 12.61	9259.46	± 1947.61
	Jaguariaíva, PR (BC)	266.28	± 4.03	467.78	± 25.86	388.08	± 17.48	4915.47	± 220.01
H. multibracteata	Santo André, SP (AF)	214.35	± 4.99	247.51	± 11.10	357.59	± 6.27	906.05	± 286.03
H. radicans	Santo André, SP (AF, humid season)	117.58	± 6.55	442.66	± 26.59	288.30	± 9.64	5822.48	± 1161.22
	Santo André, SP (AF, dry season)	350.14	± 13.67	495.54	± 7.47	852.76	± 14.11	2648.77	± 370.70
	Mogi das Cruzes, SP (AF)	204.59	± 3.98	444.67	± 25.85	325.98	± 8.10	3617.65	± 425.80
	Campina Grande do Sul, PR (AF)	174.55	± 7.50	388.65	± 21.81	336.10	± 20.21	7312.93	± 1175.95
	Avaré, SP (BC)	121.15	± 3.30	297.62	± 23.52	149.15	± 5.49	2896.41	± 162.65
	Mogi Guaçú, SP (BC)	139.60	± 6.08	354.39	± 20.96	235.07	± 8.23	3050.25	± 124.35

[a] DPPH, ABTS, and FRAP are expressed as milligrams of Trolox equivalents/gram of dry extract (mg TE g^1).
[b] ORAC activities are expressed as μMol of Trolox equivalents/μgram of dry extract (μM TE g^1).

Lamiaceae (especially from the Nepetoideae subfamily), and less frequently in some other families (Petersen and Simmonds, 2003). The presence of rosmarinic acid confers an antioxidant action, reducing numerous deleterious events to the organism, such as the formation of reactive oxygen species, lipid peroxidation and DNA fragmentation (Izzo and Capasso, 2007, Ji and Zhang, 2008).

Hyptis spp. analyzed in the present study presented rosmaric acid as one of their phenolic constituents. Due to this fact, contents of rosmarinic acid were quantified separately from other cinnamic and chlorogenic acid derivatives. Furthermore, these results showed interspecific and intraspecific variation for rosmarinic acid contents among studied species (Table 2).

Sevgi et al. (2015) evaluated ten cinnamic acid derivatives for their antioxidant activity through four different methodologies: β-carotene bleaching, DPPH free radical scavenging, reducing power, and chelating effect. According to the authors, the cinnamic derivative that presented the highest antioxidant activity in all methodologies was rosmarinic acid.

According to the literature, in *in vivo* assays, polyphenols such as rosmarinic acid and flavonoids exert cytoprotection effects by increasing the production of endogenous prostaglandins, reducing histamine secretion, inhibiting the development of *Helicobacter pylori*, and reducing the formation of oxygen free radicals (Alimi et al., 2011; Awaad et al., 2013; Yesilada et al., 2014). These molecules can increase the activity of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and glutathione reductase, chelate iron and copper ions, and inhibit the Fenton reaction. Moreover, they can interfere with electron transport and oxy-reduction reactions, as well as reducing lipid radicals (Harborne and Williams, 2000; Zheng and Wang, 2001). These biological activities are mainly correlated, as already mentioned, with the presence of electron donating hydroxyl groups and double bonds (De Lira Mota et al., 2009), as observed for rutin and quercetin, two flavonols (Pietta, 2000).

H. radicans collected in Paranapiacaba (SP, AF) in the dry season presented the highest efficiency on reducing iron (852.76 mg TE g^{-1}) when tested by FRAP assay, followed by *H. comaroides* collected in Curitiba (PR, AF) (718.72 mg TE g^{-1}). *H. radicans* collected in Avaré (SP, AF) (149.15 mg TE g^{-1}) presented the lowest results. *H. comaroides* (Curitiba, PR, AF) and *H. radicans* (Santo André, SP, AF, dry season) presented the highest amounts of rosmarinic acid, suggesting the importance of this compound for iron-reducing activity.

Evaluating the protective power of the sample against lipid peroxidation using ORAC assay, *H. lappulacea* (Paranapiacaba, SP, AF) presented the highest antioxidant activity (16302.77 μM TE g^{-1}), followed by *H. meridionalis* collected in Balsa Nova (PR, AF) (9259.46 μM TE g^{-1}). *H. multibracteata* collected in Paranapiacaba (SP, AF) (906.05 μM TE g^{-1}) presented the lowest results.

As expected, EC_{50} (Table 4) observed for DPPH assay points out for *H. lappulacea* (EC_{50} 12.33 μg mL^{-1}) as the most active sample. In the ABTS assay, the most active extract was from *H. radicans* (Paranapiacaba, SP, AF, dry season), with EC_{50} of 6.01 μg mL^{-1}. In the assay that evaluates the reduction of iron (FRAP), the most active extract was from *H. lacustris* (EC_{50} 5.35 μg mL^{-1}); and finally, in the ORAC assay, the most active extract was from *H. lappulacea* (EC_{50} 0.56 μg mL^{-1}). EC_{50} is currently the most important result used in biological activities, it allows to obtain a dose-response curve using different concentrations of a tested sample. It also allows comparing different samples with standards compounds, for example, Trolox, rosmarinic acid, or another commercial antioxidant.

Phenolic compounds in plants cover a range of chemical structures, from simple molecules to those with a high degree of polymerization, such as tannins and lignins. Gallic acid and cinnamic acid derivatives, such as caffeic, synaptic and ferulic acid, have proven activity in inhibiting lipid peroxidation (Soares, 2002).

Phenolic compounds have, in their structure, several benzene groups, having, in general, a few hydroxyl groups substituents (Ángel and González, 1999). The hydrogen atoms of the adjacent hydroxyl group (ortho-diphenyl), located at various positions of rings A, B and C, the double bonds of the benzene rings and the carbonyl double bond (−C═O) of some flavonoid molecules guarantee to these compounds their high antioxidant activity (Hrazdina et al., 1970; Rice-Evans et al., 1996; Silva et al., 2002).

Antioxidant activity of phenolic acids and polyphenols is also related to their ability to homolytically release hydrogen from the O−H bond and to form stable radicals either by chemical resonance or by hyperconjugation. The hydroxylation and/or methoxylation pattern influences the antioxidant potential of these molecules and can also retard oxidative reactions in biological systems. Although, phenolic acids have been reported as less efficient in radical scavenging than other phenolic classes (Farhoosh et al., 2016; Hsieh et al., 2005), but some structural variations as the introduction of different electron-donating/withdrawing groups to the various positions of the phenolic ring, can promote higher antioxidant activity (Shahidi and Wanasundara, 1992, Farhoosh et al., 2016).

The antioxidant activity of phenolic compounds suggests that diseases caused by oxidative reactions in biological systems could be delayed by the ingestion of natural antioxidants found in plants, such as using rosemary as food flavor (Simões et al., 2001; Quideau et al., 2011). It is known that both natural and synthetic antioxidants are extremely effective in controlling the deleterious effects caused by an excess of free radicals (Holst and Williamson, 2008; Kapravelou et al., 2015; Martins et al., 2016; Valko et al., 2007; Yeh and Yen, 2006). Furthermore, it is believed that several diseases such as cancer, arthritis, atherosclerosis, diabetes, malaria, AIDS and heart disease, have an important component of oxidative stress, in their genesis. It is also known that reactive oxygen species (ROS) are directly related to processes responsible for body aging (Brenna and Pagliarini, 2001; Yildirim et al., 2001). And these are just some of the reasons why studies like this are necessary.

To obtain an overview and summary of results, we correlate the chemical composition of the different *Hyptis* extracts to their antioxidant activities. Multivariate analysis by the principal component analysis method (PCA) (Fig. 2), showed chemical composition as the determinant variable for the separation of samples along axis 1. Samples on the positive side of this axis are those with the highest levels of flavonoids, cinnamic acid derivatives, phenolic acid derivatives, and rosmarinic acid contents; these samples are the ones that presented the highest antioxidant potential in all antioxidant assays tested. *H. lappulaceae* and *H. comaroides* (both collected in Atlantic rain Forest) were placed in the end of the positive side of axis 1, showing their superior antioxidant power.

On the negative side of axis 1 were placed samples presenting higher levels of chlorogenic acid derivatives, as *H. radicans* collected in Brazilian Cerrado, *H. campestris*, *H. mulitbracteata* and *H. lacustris*, also presenting lower antioxidant activity (Fig. 2). PCA suggests extracts having higher contents of flavonoids, cinnamic acid derivatives, and rosmarinic acid as more efficient as antioxidant than those presenting higher contents of chlorogenic acid derivatives.

To analyze the influence of the phytogeographic domain on chemical composition and consequently on antioxidant activity, a comparison was made with populations of the species collected in both domains (Brazilian Cerrado and Atlantic Forest): *H. radicans* and *H. meridionalis*.

Regarding chemical composition, *H. meridonalis* collected in Brazilian Cerrado showed a tendency to present lower levels of rosmarinic acid, flavonoids, and cinnamic acid derivatives when compared with individuals collected in Atlantic Forest.

The same tendency was observed for *H. radicans*, higher levels of rosmarinic acid, flavonoids, and cinnamic acids derivatives in samples from Atlantic Forest than in those collected in Brazilian Cerrado. Furthermore, *H. radicans* was also sampled at Atlantic Forest during two periods of the year, dry and rainy seasons. Although samples collected

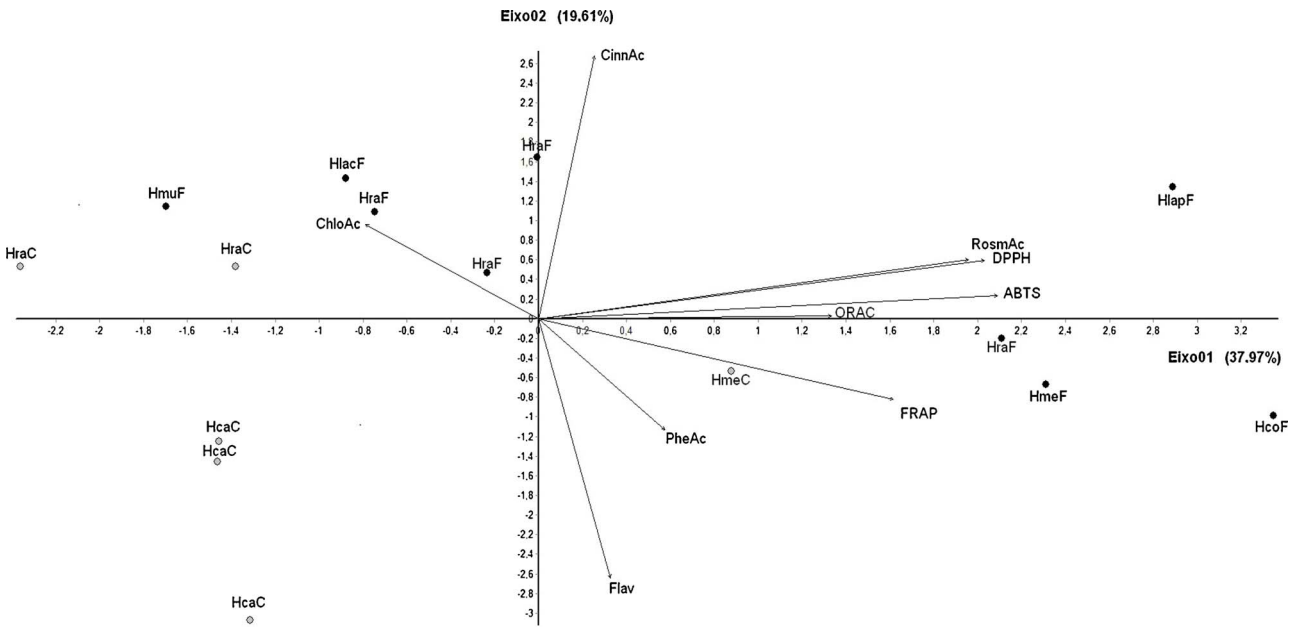

Fig. 2. Principal component analysis of 9 variables related to chemical composition and antioxidant activity of *Hyptis* spp extracts separated by phytogeographic domain: HraC: *H. radicans* collected in Brazilian Cerrado; HmuF: *H. multibracteata* collected in Atlantic Forest; HcaC: *H. campestris* collected in Brasilian Cerrado; HraF: *H. radicans* collected in Atlantic Forest; HlacF: *H. lacustris* collected in Atlantic Forest; HmeC: *H. meridionalis* collected in Brazilian Cerrado; HmeF: *H. meridionalis* collected in Atlantic Forest, HlapF: *H. lappulacea* collected in Atlantic Forest and HcoF: *H. comaroides* collected in Atlantic Forest; ChloAc: chlorogenic acid derivatives; CinnAc: cinnamic acid derivatives; Flav: flavonoids; PheAc: phenolic acid derivatives, RosmAc: rosmarinic acid.

in the rainy season showed a tendency of presenting higher contents of flavonoids, chlorogenic, and cinnamic acid derivatives, was observed less antioxidant activity of these extracts.

When comparing populations of *H. radicans* collected in different sites at Atlantic Rain Forest, it was observed a tendency of lower contents of all classes of phenolic compounds analyzed in samples from Campina Grande do Sul (Southern of Brazil) when compared with samples collected in Mogi das Cruzes (Southeast of Brazil)

Studies that evaluate the influence of abiotic factors on the chemical composition of plant species are scarce in the literature. Southwell and Bourke (2001), quantified two flavonoids constituents in *Hypericum perforatum* L. (St. John's Wort) in dry and humid seasons and verified an increase in the content of these constituents in the humid season. Differently, when *Hypericum brasiliensi* Choisy was submitted to water stress, it presented an increase in the contents of phenolic compounds as quercetin, rutin, 1,5-dihydroxyxanthone, and isouliginosin B (Nacif de Abreu and Mazzafera, 2005).

Global solar irradiation and temperature have a high inter-seasonal variation in the Southern region of Brazil. It is in the South region that the lowest values of global irradiation are observed in Brazil, notably on the north coast of the state of Santa Catarina, Paraná coast, and south coast of São Paulo (Pereira et al., 2006). Studies have indicated a positive correlation between solar radiation intensity and concentration and/or composition of phenolic substances such as flavonoids (Markham et al., 1998; Tattini et al., 2004), tannins (Dudt and Shure, 1994), and anthocyanins (Jeong et al., 2004). However, according to Gobbo-neto and Lopes (2007) in their last review, studies have already shown conflicting results regarding contents of phenolic metabolites and the influence of environmental factors. It seems very difficult to establish a clear correlation between the concentration of phenolic compounds and, for example, osmotic stress (Dement and Mooney, 1974; Cooper-Driver et al., 1977; Dustin and Cooper-Driver, 1992; Horner, 1990; Tattini et al., 2004; Guinn and Eidenbock, 1982; Gershenzon, 1984; Mattson and Haack, 1987). Drought short-term effects seem to increase the production of special metabolites, while in the long run an opposite effect was observed (Waterman and Mole, 1989; Waterman and Mole, 1994; Gobbo-neto and Lopes, 2007; Horner, 1990; Mattson and Haack, 1987; Medina et al., 1984). In general, for abiotic stress it seems that special metabolites production is dependent on the degree of stress, meaning duration and intensity of stress are important to determine the plant response.

Regarding our second question, results showed different contents of phenolic compounds when considering populations of *H. radicans* and *H meridionalis* growing in Atlantic Forest and Brazilian Cerrado. As expected, populations of both species presenting high levels of phenolic compounds (Atlantic Forest) also presented higher antioxidant activity.

Martins et al. (2016), in a review, emphasized the importance of studies like this and commented that the antioxidant potential of plant extracts is one of the most relevant subjects within the scientific community. However, *in vitro* assays are the most common warning of the importance of continuing *in vivo* research (Dai and Mumper, 2010; Larrosa et al., 2010; Rubió et al., 2013).

4. Conclusion

For the same species, populations from Atlantic Forest presented higher levels of phenolic compounds when compared with populations from Brazilian Cerrado. Considering the main environmental difference between these two biomes, pluviosity, it seems that there is a tendency of higher production of phenolic compounds in rainy areas (mean annual precipitation: 1800–3600 mm and 1200–1800 mm, Atlantic Rain Forest and Brazilian Cerrado respectively). Furthermore, the abiotic microenvironment seems to exert a stronger influence regarding the phenolic composition and consequently the antioxidant activity of a plant extract than the phytogeographic domain.

Conflicts of interest

None.

Acknowledgements

The authors thank Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for financial support (FAPESP 2012/10079-0). KPS

thanks the Coordenacão de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for a Doctor's research grant. CMF are fellow researcher of Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

References

Ángel, M.H., González, E.A.P., 1999. Plantas que contienen polifenoles Antioxidantes dentro del estilo de vida. Rev. Cuba. Investig. Biomed. 18, 12–14.

Agra, M.F., Silva, K.N., Basílio, I.J.L.D., Freitas, P.F., Barbosa-Filho, J.M., 2008. Survey of medicinal plants used in the region Northeast of Brazil. Revista Brasileira de Farmacognosia 18 (3), 472–508.

Agrawal, A.A., 2007. Macroevolution of plant defense strategies. Trends Ecol. Evol. 22, 103–109. http://dx.doi.org/10.1016/j.tree.2006.10.012.

Alimi, H., Hfaiedh, N., Bouoni, Z., Sakly, M., Ben Rhouma, K., 2011. Evaluation of antioxidant and antiulcerogenic activities of *Opuntia ficus* indica f. inermis flowers extract in rats. Environ. Toxicol. Pharmacol. 32, 406–416. http://dx.doi.org/10.1016/j.etap.2011.08.007.

Arrigoni-Blank, M.F., Antoniolli, A.R., Caetano, L.C., Campos, D.A., Blank, A.F., Alves, P.B., 2008. Antinociceptive activity of the volatile oils of *Hyptis pectinata* L. Poit. (Lamiaceae) genotypes. Phytomedicine 15, 334–339. http://dx.doi.org/10.1016/j.phymed.2007.09.009.

Atkinson, P., Blakeman, J.P., 1982. Seasonal occurence of na antimicrobial flavanone, sakuranetin, associated with glands on leaves of *Ribes nigrum*. New Phytol. 92, 63–74. http://dx.doi.org/10.1111/j.1469-8137.1982. tb03363.x.

Awaad, A.S., Al-Jaber, N.A., Moses, J.E., El-Meligy, R.M., Zain, M.E., 2013. Antiulcerogenic activities of the extracts and isolated flavonoids of *Euphorbia cuneata* Vahl. Phyther. Res. 27, 126–130. http://dx.doi.org/10.1002/ptr.4872.

Bispo, M.D., Mourão, R.H.V., Franzotti, E.M., Bomfim, K.B.R., Arrigoni-Blank, M.d.F., Moreno, M.P.N., Marchioro, M., Antoniolli, A.R., 2001. Antinociceptive and antiedematogenic effects of the aqueous extract of Hyptis pectinata leaves in experimental animals. J. Ethnopharmacol. 76, 81–86. http://dx.doi.org/10.1016/S0378-8741(01)00172-6.

Bowlers, B.L., Miller, A.J., 1994. Caffeic acid activity against clostridium botulinum spores. J. Food Sci. 59 (4), 905–908.

Brand-Williams, W., Cuvelier, M.E., Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. LWT − Food Sci. Technol. 28, 25–30. http://dx.doi.org/10.1016/S0023-6438(95)80008-5.

Brenna, O.V., Pagliarini, E., 2001. Multivariate analysis of antioxidant power and polyphenolic composition in red wines. J. Agric. Food Chem. 49, 4841–4844.

Brooks, J.S., Feeny, P., 2004. Seasonal variation in *Daucus carota* leaf-surface and leaf-tissue chemical profiles. Biochem. Syst. Ecol. 32, 769–782. http://dx.doi.org/10.1016/j.bse.2004.01.004.

Cavalcanti, B.C., Moura, D.J., Rosa, R.M., Moraes, M.O., Araujo, E.C.C., Lima, M.A.S., Silveira, E.R., Saffi, J., Henriques, J.A.P., Pessoa, C., Costa-Lotufo, L.V., 2008. Genotoxic effects of tanshinones from *Hyptis martiusii* in V79 cell line. Food Chem. Toxicol. 46, 388–392. http://dx.doi.org/10.1016/j.fct.2007.08.009.

Chen, L., Teng, H., Xie, Z., Cao, H., Cheang, W.S., Skalicka-Woniak, K., Georgiev, M.I., Xiao, J., 2016. Modifications of dietary flavonoids towards improved bioactivity: an update on structure?activity relationship. Crit. Rev. Food Sci. Nutr. 8398, 1–15. http://dx.doi.org/10.1080/10408398.2016.1196334.

Chukwujekwu, J.C., Smith, P., Coombes, P.H., Mulholland, D.A., van Staden, J., 2005. Antiplasmodial diterpenoid from the leaves of *Hyptis suaveolens*. J. Ethnopharmacol. 102, 295–297. http://dx.doi.org/10.1016/j.jep.2005.08.018.

Clark, L.E., Clark, W.D., 1990. Seasonal variation in leaf exudate flavonoids of *Isocoma acradenia* (Asteraceae). Biochem. Syst. Ecol. 18, 145–148. http://dx.doi.org/10.1016/0305-1978(90)90049-L.

Clifford, M.N., Johnston, K.L., Knight, S., Kuhnert, N., 2003. Hierarchical scheme for LC-MS in identification of chlorogenic acids. J. Agric. Food Chem. 51, 2900–2911. http://dx.doi.org/10.1021/jf026187q.

Cooper-Driver, G., Finch, S., Swain, T., Bernays, E., 1977. Seasonal variation in secondary plant compounds in relation to the palatability of *Pteridium aquilinum*. Biochem. Syst. Ecol. 5, 177–183. http://dx.doi.org/10.1016/0305-1978(77)90002-3.

Coutinho, H.D.M., Costa, J.G.M., Siqueira-Júnior, J.P., Lima, E.O., 2008. In vitro antistaphylococcal activity of *Hyptis martiusii* Benth against methicillin-resistant *Staphylococcus aureus*: MRSA strains. Rev. Bras. Farmacogn. 18, 670–675. http://dx.doi.org/10.1590/S0102-695X2008000500005.

Cunha, A.P., Silva, A.P., Roque, O.R., 2012. Plantas E Produtos Vegetais Em Fitoterapia. Fundação Calouste Gulbenkian, Lisboa 731p.

Dai, J., Mumper, R.J., 2010. Plant Phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules 15, 7313–7352. http://dx.doi.org/10.3390/molecules15107313.

De Lira Mota, K.S., Dias, G.E.N., Pinto, M.E.F., Luiz-Ferreira, Â., Souza-Brito, A.R.M., Hiruma-Lima, C.A., Barbosa-Filho, J.M., Batista, L.M., 2009. Flavonoids with gastroprotective activity. Molecules 14, 979–1012. http://dx.doi.org/10.3390/molecules14030979.

Dement, W.A., Mooney, H.A., 1974. Seasonal variation in the production of tannins and cyanogenic glucosides in the chaparral shrub, *Heteromeles arbutifolia*. Oecologia 15 (1), 65–76.

Deng, Y., Balunas, M.J., Kim, J., Lantvit, D.D., Chin, Y., Chai, H., Sugiarso, S., Kardono, L.B.S., Fong, H.H.S., Pezzuto, J.M., Swanson, S.M., Carcache de Blanco, E.J., Kinghorn, A.D., 2009. Bioactive 5, 6-dihydro-α-pyrone derivatives from *Hyptis brevipes*. J. Nat. Prod. 72, 1165–1169. http://dx.doi.org/10.1021/np9001724.

Dudt, J.F., Shure, D.J., 1994. The influence of light and nutrients on foliar phenolics and insect herbivory. Ecology 75, 86–98.

Dustin, C.D., Cooper-Driver, G.A., 1992. Changes in phenolic production in the hay-scented fern (*Dennstaedtia punctilobula*) in relation to resource availability. Biochem. Syst. Ecol. 20, 99–106. http://dx.doi.org/10.1016/0305-1978(92)90096-V.

Falcão, D.Q., Menezes, F.S., 2003. Revisão etnofarmacológica, farmacológica e química do gênero Hyptis: the *Hyptis* genus: an ethnopharmacological and chemical review. Rev. Bras. Farmacogn. 84, 69–74.

Farhoosh, R., Johnny, S., Asnaashari, M., Molaahmadibahraseman, N., Sharif, A., 2016. Structure-antioxidant activity relationships of o-hydroxyl, o-methoxy, and alkyl ester derivatives of p-hydroxybenzoic acid. Food Chem. 194, 128–134. http://dx.doi.org/10.1016/j.foodchem.2015.08.003.

Fresco, P., Borges, F., Diniz, C., Marques, M.P.M., 2006. New insights on the anticancer properties of dietary polyphenols. Med. Res. Rev. 26, 747–766. http://dx.doi.org/10.1002/med.20060.

Furlan, C.M., Santos, K.P., Sedano-Partida, M.D., Motta, L.B., da Santos, D.Y.A.C., Salatino, M.L.F., Negri, G., Berry, P.E., van Ee, B.W., Salatino, A., 2015. Flavonoids and antioxidant potential of nine Argentinian species of *Croton* (Euphorbiaceae). Brazilian J. Bot. 38, 693–702. http://dx.doi.org/10.1007/s40415-014-0115-9.

Gershenzon, J., 1984. Changes in the levels of plant secondary metabolites under water and nutrient stress. Phytochemical Adaptations to Stress. Springer US, pp. 273–320.

Gobbo-neto, L., Lopes, N.P., 2007. Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários. Quim. Nova 30, 374–381.

Grace, S.C., Logan, B.A., Adams, W.W., 1998. Seasonal differences in foliar content of chlorogenic acid, a phenylpropanoid antioxidant, in *Mahonia repens*. Plant, Cell Environ. 21, 513–521. http://dx.doi.org/10.1046/j.1365-3040.1998.00282.x.

Grayer, R.J., Eckert, M.R., Veitch, N.C., Kite, G.C., Marin, P.D., Kokubun, T., Simmonds, M.S., Paton, A.J., 2003. The chemotaxonomic significance of two bioactive caffeic acid esters, nepetoidins A and B, in the Lamiaceae. Phytochemistry 64, 519–528. http://dx.doi.org/10.1016/S0031-9422(03)00192-4.

Guinn, G., Eidenbock, M.P., 1982. Catechin and condensed tannin contents of leaves and bolls of cotton in relation to irrigation and boll load. Crop Sci. 22 (3), 614–616.

Harborne, J.B., Williams, C.A., 2000. Advances in flavonoid research since 1992. Phytochemistry 55, 481–504. http://dx.doi.org/10.1016/S0031-9422(00)00235-1.

Harley, R., Pastore, J., 2012. A generic revision and new combinations in the Hyptidinae (Lamiaceae), based on molecular and morphological evidence. Phytotaxa 58, 1–55.

Harley, R., França, F., Santos, É.P., Santos, J.S. dos, 2010. Lamiaceae, in: Catálogo de Plantas E Fungos Do Brasil. Volume 2. Jardim Botânico do Rio de Janeiro, Rio de Janeiro, pp. 1130–1146.

Harley, R.M., 2012. Checklist and key of genera and species of the Lamiaceae of the Brazilian Amazon. Rodriguésia 63, 129–144. http://dx.doi.org/10.1590/S2175-78602012000100010.

Harley, R., França, F., Santos, E.P., Santos, J.S., Pastore, J.F., 2015a. Lamiaceae in Lista de Espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro. Disponivel em: < http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB133004 > . BFG. Growing knowledge: an overview of Seed Plant diversity in Brazil. Rodriguésia, v.66, n.4, p. 1085–1113. 2015. https://dx.doi.org/10.1590/2175-7860201566411.

Harley, R., França, F., Santos, E.P., Santos, J.S., Pastore, J.F., 2015b. Lamiaceae in Lista de Espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro. Disponivel em: < http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB133011 > . BFG. Growing knowledge: an overview of Seed Plant diversity in Brazil. Rodriguésia, v.66, n.4, p.1085–1113. 2015. https://dx.doi.org/10.1590/2175-7860201566411.

Harley, R., França, F., Santos, E.P., Santos, J.S., Pastore, J.F. 2015c. Lamiaceae in Lista de Espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro. Disponivel em: < http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB133007 > . BFG. Growing knowledge: an overview of Seed Plant diversity in Brazil. Rodriguésia, v.66, n.4, p. 1085–1113. 2015. https://dx.doi.org/10.1590/2175-7860201566411.

Harley, R., França, F., Santos, E.P., Santos, J.S., Pastore, J.F. 2015d. Lamiaceae in Lista de Espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro. Disponivel em: < http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB133002 > . BFG. Growing knowledge: an overview of Seed Plant diversity in Brazil. Rodriguésia, v.66, n.4, p. 1085–1113. 2015. https://dx.doi.org/10.1590/2175-7860201566411.

Harley, R., França, F., Santos, E.P., Santos, J.S.,Pastore, J.F. 2015e. Lamiaceae in Lista de Espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro. Disponivel em: < http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB8216 > . BFG. Growing knowledge: an overview of Seed Plant diversity in Brazil. Rodriguésia, v.66, n.4, p. 1085–1113. 2015. https://dx.doi.org/10.1590/2175-7860201566411.

Harley, R., França, F., Santos, E.P., Santos, J.S., Pastore, J.F. 2015f. Lamiaceae in Lista de Espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro. Disponivel em: < http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB8219 > . BFG. Growing knowledge: an overview of Seed Plant diversity in Brazil. Rodriguésia, v.66, n.4, p. 1085–1113. 2015. https://dx.doi.org/10.1590/2175-7860201566411.

Harley, R., França, F.,Santos, E.P.,Santos, J.S., Pastore, J.F. 2015g. Lamiaceae in Lista de Espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro. Disponivel em: < http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB8232 > . BFG. Growing knowledge: an overview of Seed Plant diversity in Brazil. Rodriguésia, v.66, n.4, p. 1085–1113. 2015. https://dx.doi.org/10.1590/2175-7860201566411.

Holst, B., Williamson, G., 2008. Nutrients and phytochemicals: from bioavailability to bioefficacy beyond antioxidants. Curr. Opin. Biotechnol. 19, 73–82. http://dx.doi.org/10.1016/j.copbio.2008.03.003.

Horner, J.D., 1990. Nonlinear effects of water deficits on foliar tannin concentration. Biochem. Syst. Ecol. 18, 211–213. http://dx.doi.org/10.1016/0305-1978(90)90062-K.

Hrazdina, G., Borzel, A.J., Robinson, W.B., 1970. Studies on the stability of the anthocyanidin-3,5diglucosides. Am. J. Enol. Vitic. 21 (4), 201–204.

Hsieh, C.L., Yen, G.C., Chen, H.Y., 2005. Antioxidant activities of phenolic acids on ultraviolet radiation-induced erythrocyte and low density lipoprotein oxidation. J.

Agric. Food Chem. 53, 6151–6155. http://dx.doi.org/10.1021/jf050707a.
Ito, H., Miyazaki, T., Ono, M., Sakurai, H., 1998. Antiallergic activities of rabdosiin and its related compounds: chemical and biochemical evaluations. Bioorg. Med. Chem. 6, 1051–1056. http://dx.doi.org/10.1016/S0968-0896(98)00063-7.
Izzo, A.A., Capasso, F., 2007. Herbal medicines to treat Alzheimer's disease. Pharmacol. Sci. 28 (2), 47–48.
Jalal, M.A.F., Read, D.J., Haslam, E., 1982. Phenolic composition and its seasonal variation in *Calluna vulgaris*. Phytochemistry 21, 1397–1401. http://dx.doi.org/10.1016/0031-9422(82)80150-7.
Jassim, S.A.A., Naji, M.A., 2003. Novel antiviral agents: a medicinal plant perspective. J. Appl. Microbiol. 95, 412–427. http://dx.doi.org/10.1046/j.1365-2672.2003.02026.x.
Jeong, S.T., Goto-Yamamoto, N., Kobayashi, S., Esaka, M., 2004. Effects of plant hormones and shading on the accumulation of anthocyanins and the expression of anthocyanin biosynthetic genes in grape berry skins. Plant Sci. 167, 247–252.
Ji, H.F., Zhang, H.Y., 2008. Multipotent natural agents to combat Alzheimer's disease. Functional spectrum and structural features. Acta Pharmacol. Sin. 29, 143–151. http://dx.doi.org/10.1111/j.1745-7254.2008.00752.x.
Judd, W., Campbell, C., Kellogg, E., Stevens, P., Donoghue, M., 2002. Plant Systematics: a Phylogenetic Approach, 2nd ed. Sinauer Associates Sunderland.
Kapravelou, G., Martínez, R., Andrade, A.M., López Chaves, C., López-Jurado, M., Aranda, P., Arrebola, F., Cañizares, F.J., Galisteo, M., Porres, J.M., 2015. Improvement of the antioxidant and hypolipidaemic effects of cowpea flours (*Vigna unguiculata*) by fermentation: results of *in vitro* and *in vivo* experiments. J. Sci. Food Agric. 95, 1207–1216. http://dx.doi.org/10.1002/jsfa.6809.
Karthikesan, K., Pari, L., Menon, V.P., 2010. Protective effect of tetrahydrocurcumin and chlorogenic acid against streptozotocin-nicotinamide generated oxidative stress induced diabetes. J. Funct. Foods 2, 134–142. http://dx.doi.org/10.1016/j.jff.2010.04.001.
Kini, F., Kam, B., Aycard, J.P., Gaydou, E.M., Bombarda, I., 1993. Chemical composition of the essential oil of *Hyptis spicigera* Lam. from Burkina Faso. J. Essent. Oil Res. 5 (2), 219–221.
Klink, C.A., Machado, R.B., 2005. Conservation of the Brazilian Cerrado. Conserv. Biol. 19, 707–713. http://dx.doi.org/10.1111/j.1523-1739.2005.00702.x.
Kutchan, T.M., 2001. Ecological arsenal and developmental dispatcher. the paradigm of secondary metabolism. Plant Physiol. 125, 58–60. http://dx.doi.org/10.1104/pp.125.1.58.
Larrosa, M., García-Conesa, M.T., Espín, J.C., Tomás-Barberán, F.A., 2010. Ellagitannins, ellagic acid and vascular health. Mol. Aspects Med. 31, 513–539. http://dx.doi.org/10.1016/j.mam.2010.09.005.
Li, H.-B., Wong, C.-C., Cheng, K.-W., Chen, F., 2008a. Antioxidant properties in vitro and total phenolic contents in methanol extracts from medicinal plants. LWT − Food Sci. Technol. 41, 385–390. http://dx.doi.org/10.1016/j.lwt.2007.03.011.
Li, Y., Shi, W., Li, Y., Zhou, Y., Hu, X., Song, C., Ma, H., Wang, C., Li, Y., 2008b. Neuroprotective effects of chlorogenic acid against apoptosis of PC12 cells induced by methylmercury. Environ. Toxicol. Pharmacol. 26, 13–21. http://dx.doi.org/10.1016/j.etap.2007.12.008.
Lisboa, A.C.C.D., Mello, I.C.M., Nunes, R.S., dos Santos, M.A., Antoniolli, A.R., Marçal, R.M., Cavalcanti, S.C., de, H., 2006. Antinociceptive effect of Hyptis pectinata leaves extracts. Fitoterapia 77, 439–442. http://dx.doi.org/10.1016/j.fitote.2006.06.001.
Lobstein, A., Rietsch-Jako, L., Haag-Berrurier, M., Anton, R., 1991. Seasonal variations of the flavonoid content from *Ginkgo biloba* leaves. Planta Med. 57 (05), 430–433.
Ma, C.M., Kully, M., Khan, J.K., Hattori, M., Daneshtalab, M., 2007. Synthesis of chlorogenic acid derivatives with promising antifungal activity. Bioorg. Med. Chem. 15, 6830–6833. http://dx.doi.org/10.1016/j.bmc.2007.07.038.
Markham, K.R., Tanner, G.J., Caasi-Lit, M., Whitecross, M.I., Nayudu, M., Mitchell, K.A., 1998. Possible protective role for 3', 4' − dihydroxyflavones Induced by enhanced UV-B in a UV-tolerant rice cultivar. Phytochemistry 49, 1913–1919. http://dx.doi.org/10.1016/S0031-9422(98)00438-5.
Markham, K., 1982. Techniques of Flavonoid Identification. Academic Press 350 p.
Martins, N., Barros, L., Ferreira, I.C.F.R., 2016. In vivo antioxidant activity of phenolic compounds: facts and gaps. Trends Food Sci. Technol. 48, 1–12. http://dx.doi.org/10.1016/j.tifs.2015.11.008.
Mattson, W.J., Haack, R.A., 1987. The role of drought stress in provoking outbreaks of phytophagous insects. Insect Outbreaks 365–407.
Medina, E., Olivares, E., Diaz, M., 1984. Water stress and light intensity effects on growth and nocturnal acid accumulation in a terrestrial CAM bromelias (*Bromelia humilis* Jacq.) under natural conditions. Oecologia 70, 441–446.
Menezes, I.A.C., Marques, M.S., Santos, T.C., Dias, K.S., Silva, A.B.L., Mello, I.C.M., Lisboa, A.C.C.D., Alves, P.B., Cavalcanti, S.C.H., Marçal, R.M., Antoniolli, A.R., 2007. Antinociceptive effect and acute toxicity of the essential oil of *Hyptis fruticosa* in mice. Fitoterapia 78, 192–195. http://dx.doi.org/10.1016/j.fitote.2006.11.020.
Menkovic, N., Savikin-Fodulovic, K., Savin, K., 2000. Chemical composition and seasonal variations in the amount of secondary compounds in *Gentiana lutea* leaves and flowers. Planta Med. 66, 178–180.
Messana, I., Ferrari, F., de Moraes e Souza, M.A., Gács-Baitz, E., 1990. (−)-Salzol, an isopimarane diterpene, and a chalcone from *Hyptis salzmanii*. Phytochemistry 29, 329–332. http://dx.doi.org/10.1016/0031-9422(90)89065-H.
Miranda, G.S., Santana, G.S., Machado, B.B., Coelho, F.P., Carvalho, C.A., 2013. Atividade antibacteriana in vitro de quatro espécies vegetais em diferentes graduações alcoólicas. Rev. Bras. Pl. Med. 15 (1), 104–111 Botucatu.
Moon, J., Shibamoto, T., 2009. Antioxidant assays for plant and food components antioxidant assays for plant and food componentes. J. Agric. Food Chem. 57, 1655–1666. http://dx.doi.org/10.1021/jf803537k.
Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B., Kent, J., 2000. Biodiversity hotspots for conservation priorities. Nature 403, 853–858. http://dx.doi.org/10.1038/35002501.
Nacif de Abreu, I., Mazzafera, P., 2005. Effect of water and temperature stress on the content of active constituents of *Hypericum brasiliense* Choisy. Plant Physiol. Biochem. 43, 241–248. http://dx.doi.org/10.1016/j.plaphy.2005.01.020.
Nayak, U.G., Guha, P.C., 1952. Essential oil from *Hyptis suaveolens*. J. Indian Chem. Soc. 29, 183–186.
Ohsaki, A., Kishimoto, Y., Isobe, T., Fukuyama, Y., 2005. New labdane diterpenoids from *Hyptis fasciculata*. Chem. Pharm. Bull. (Tokyo). 53, 1577–1579. http://dx.doi.org/10.1248/cpb.53.1577.
Pastore, J.F.B., Harley, R.M., Forest, F., Paton, A., van den Berg, C., 2011. Phylogeny of the subtribe Hyptidinae (Lamiaceae tribe Ocimeae) as inferred from nuclear and plastid DNA. Taxon 60, 1317–1329.
Paynter, N.P., Yeh, H.C., Voutilainen, S., Schmidt, M.I., Heiss, G., Folsom, A.R., Brancati, F.L., Linda Kao, W.H., 2005. Coffee and sweetened beverage consumption and the risk of type 2 *Diabetes mellitus*. Am. J. Epidemiol. 164 (11), 1075–1084.
Pedersen, J.A., 2000. Distribution and taxonomic implications of some phenolics in the family Lamiaceae determined by ESR spectroscopy. Biochem. Syst. Ecol. 28, 229–253.
Pereda-Miranda, R., Delgado, G., 1990. Triterpenoids and flavonoids from *Hyptis albida*. J. Nat. Prod. 53, 182–185. http://dx.doi.org/10.1021/np50067a028.
Pereda-Miranda, R., Hernández, L., Villavicencio, M.J., Novelo, M., Ibarra, P., Chai, H., Pezzuto, J.M., 1993. Structure and stereochemistry of pectinolides A-C, novel antimicrobial and cytotoxic 5, 6-Dihydro-α-pyrones from *Hyptis pectinata*. J. Nat. Prod. 56, 583–593. http://dx.doi.org/10.1021/np50094a019.
Pereira, E.B., Martins, F.R., Abreu, S.L., Rüther, R., 2006. Atlas Brasileiro De Energia Solar −São José Dos Campos. INPE.
Petersen, M., Simmonds, M.S.J., 2003. Rosmarinic acid. Phytochemistry 62, 121–125. http://dx.doi.org/10.1016/S0031-9422(02)00513-7.
Pietta, P.G., 2000. Flavonoids as antioxidants. J. Nat. Prod. 63, 1035–1042. http://dx.doi.org/10.1021/np9904509.
Quideau, S., Deffieux, D., Douat-Casassus, C., Pouységu, L., 2011. Plant polyphenols: chemical properties, biological activities, and synthesis. Angew. Chemie − Int. Ed. 50, 586–621. http://dx.doi.org/10.1002/anie.201000044.
Razzaghi-Asl, N., Garrido, J., Khazraei, H., Borges, F., Firuzi, O., 2013. Antioxidant properties of hydroxycinnamic acids: a review of structure-activity relationships. Curr. Med. Chem. 20, 4436–4450. http://dx.doi.org/10.2174/0929867311320990141.
Rice-Evans, C., Miller, N., Paganga, G., 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic. Biol. Med. 20, 933–956.
Rodriguez de Sotillo, D.V., Hadley, M., 2002. Chlorogenic acid modifies plasma and liver concentrations of: cholesterol, triacylglycerol, and minerals in Zucker rats. J. Nutr. Biochem. 13, 717–726. http://dx.doi.org/10.1016/S0955-2863(02)00231-0.
Rubió, L., Motilva, M.-J., Romero, M.-P., 2013. Recent advances in biologically active compounds in herbs and spices: a review of the most effective antioxidant and anti-inflammatory active principles. Crit. Rev. Food Sci. Nutr. 53, 943–953. http://dx.doi.org/10.1080/10408398.2011.574802.
Santos, D.Y.A.C., Brandimarte, A.L., 2014. Biomas e biodiversidade: biomas brasileiros (Cap. 5) em Diversidade Biológica, História da vida na Terra e Bioenergética. UNIVESP, São Paulo.
Santos, T.C., Marques, M.S., Menezes, I.A.C., Dias, K.S., Silva, A.B.L., Mello, I.C.M., Carvalho, A.C.S., Cavalcanti, S.C.H., Antoniolli, Â.R., Marçal, R.M., 2007. Antinociceptive effect and acute toxicity of the Hyptis suaveolens leaves aqueous extract on mice. Fitoterapia 78, 333–336. http://dx.doi.org/10.1016/j.fitote.2007.01.006.
Santos, K.P., Sedano-Partida, M.D., Motta, L.B., Cordeiro, I., Furlan, C.M., 2016. Antioxidant activity of flavonoids from *Croton sphaerogynus* Baill. Brazilian J. Bot. 39, 1021–1030. http://dx.doi.org/10.1007/s40415-016-0302-y.
Santos, R.I., 2004. Metabolismo básico e origem dos metabólitos secundários. In: in Simões, C.M.O., Schenkel, E.P., Gosmann, G., Mello, J.C.P., Mentz, L.A., Petrovik, P.R. (Eds.), Farmacognosia: Da Planta Ao Medicamento. Editora da UFSC, Porto Alegre pp. 403–434.
Sevgi, K., Tepe, B., Sarikurkcu, C., 2015. Antioxidant and DNA damage protection potentials of selected phenolic acids. Food Chem. Toxicol. 77, 12–21. http://dx.doi.org/10.1016/j.fct.2014.12.006.
Shahidi, F., Wanasundara, P.K.J.P.D., 1992. Phenolic antioxidants. Crit. Rev. Food Sci. Nutr. 32, 67–103.
Silva, M.M., Santos, M.R., Caroço, G., Rocha, R., Justino, G., Mira, L., 2002. Structure-antioxidant activity relationships of flavonoids: a re-examination. Free Radic. Res. 36 (11), 1219–1227.
Simões, C.M.O., Schenkel, E.P., Gosmann, G., Mello, J.C.P., Mentz, L.A., Petrovick, P.R., 2001. Farmacognosia: Da Planta Ao Medicamento. Ed. Universidade UFRGS/Ed. da UFSC, Porto Alegre/Florianópolis 833p.
Soares, S.E., 2002. Ácidos fenólicos como antioxidantes. Revista de nutrição.
Southwell, I.a., Bourke, C.a., 2001. Seasonal variation in hypericin content of *Hypericum perforatum* L. (St. john's wort). Phytochemistry 56, 437–441. http://dx.doi.org/10.1016/S0031-9422(00)00411-8.
Souza, L.K., Oliveira de, C.M., Ferri, P.H., Oliveira Júnior, J.G., de Souza Júnior, A.H., de Fernandes, O., et al., 2003. Antimicrobial activity of *Hyptis ovalifolia* towards dermatophytes. Mem. Inst. Oswaldo Cruz 98, 963–965. http://dx.doi.org/10.1590/S0074-02762003000700018.
Takeda, H., Tsuji, M., Matsumiya, T., Kubo, M., 2002. Identification of rosmarinic acid as a novel antidepressive substance in the leaves of *Perilla frutescens* Britton var. acuta Kudo (Perillae Herba). Nihon Shinkei Seishin Yakurigaku Zasshi 22 (1), 15–22.
Tattini M., Galardi C., Pinelli P., Massai R., Remorini D., Agati G., 2004. Differential accumulation of flavonoids and hydroxycinnamates in leaves of 547–561.
Teixeira, J., Gaspar, A., Garrido, E.M., Garrido, J., Borges, F., 2013. Hydroxycinnamic

acid antioxidants: an electrochemical overview. Biomed Res. Int. 2013, 1–11. http://dx.doi.org/10.1155/2013/251754.

Tripathi, R., Mohan, H., Kamat, J.P., 2007. Modulation of oxidative damage by natural products. Food Chem. 100, 81–90. http://dx.doi.org/10.1016/j.foodchem.2005.09.012.

Urones, J.G., Marcos, I.S., Diez, D., Cubilla, R.L., 1998. Tricyclic diterpenes from *Hyptis dilatata*. Phytochemistry 48, 1035–1038.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T.D., Mazur, M., Telser, J., 2007. Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol. 39, 44–84. http://dx.doi.org/10.1016/j.biocel.2006.07.001.

Wang, G.-F., Shi, L.-P., Ren, Y.-D., Liu, Q.-F., Liu, H.-F., Zhang, R.-J., Li, Z., Zhu, F.-H., He, P.-L., Tang, W., Tao, P.-Z., Li, C., Zhao, W.-M., Zuo, J.-P., 2009. Anti-hepatitis B virus activity of chlorogenic acid, quinic acid and caffeic acid in vivo and in vitro. Antiviral Res. 83, 186–190. http://dx.doi.org/10.1016/j.antiviral.2009.05.002.

Waterman, P.G., Mole, S., 1989. In: In: Bernays, E.A. (Ed.), Em Insect-plant Interactions 1 CRS Press, Boca Raton 4.

Waterman, P.G., Mole, S., 1994. Analysis of Phenolic Plant Metabolites, 1st ed. Blackwell Scientific Publications, Oxford cap. 3.

Wilt, F.M., Miller, G.C., 1992. Seasonal variation of coumarin and flavonoid concentrations in persistent leaves of wyoming big sagebrush (*Artemisia tridentata* ssp. *wyomingensis*: Asteraceae). Biochem. Syst. Ecol. 20, 53–67. http://dx.doi.org/10.1016/0305-1978(92)90072-L.

Wren, R.C., 1988. Potters New Cyclopaedia of Botanical Drugs and Preparations. The CW Daniel Company Ltda., England.

Yeh, C.-T., Yen, G.-C., 2006. Modulation of hepatic phase II phenol sulfotransferase and antioxidant status by phenolic acids in rats. J. Nutr. Biochem. 17, 561–569. http://dx.doi.org/10.1016/j.jnutbio.2005.10.008.

Yesilada, E., Gürbüz, İ., Toker, G., 2014. Anti-ulcerogenic activity and isolation of the active principles from *Sambucus ebulus* L. leaves. J. Ethnopharmacol. 153, 478–483. http://dx.doi.org/10.1016/j.jep.2014.03.004.

Yildirim, a., Mavi, a., Kara, a.a., 2001. Determination of antioxidant and antimicrobial activities of *Rumex crispus* L. extracts. J. Agric. Food Chem. 49, 4083–4089.

Zheng, W., Wang, S.Y., 2001. Antioxidant activity and phenolic compounds in selected herbs. J. Agric. Food Chem. 49, 5165–5170.

Zidorn, C., Stuppner, H., 2001. Evaluation of chemosystematic characters in the genus *Leontodon* (Asteraceae). Taxon 50, 115–133.

al-Sereiti, M.R., Abu-Amer, K.M., Sen, P., 1999. Pharmacology of rosemary (*Rosmarinus officinalis* Linn.) and its therapeutic potentials. Indian J. Exp. Biol. 37, 124–130.

da Cruz Araújo, E.C., Sousa Lima, M.A., Rocha Silveira, E., 2004. Spectral assignments of new diterpenes from *Hyptis martiusii* Benth. Magn. Reson. Chem. 42, 1049–1052. http://dx.doi.org/10.1002/mrc.1489.