Indian Journal of Traditional Knowledge
Vol 21(2), April 2022, pp 373-382

Seasonal monitoring of the antioxidant activity of *Erythroxylum suberosum* A. St.-Hil. leaves: Correlation with hyperoside and isoquercitrin contents

Nathalia de Almeida Morais[a], Diegue Henrique Nascimento Martins[b], Christopher William Fagg[c], Luiz Alberto Simeoni[a], Pérola Oliveira Magalhães[a], Dâmaris Silveira[a] & Yris Maria Fonseca-Bazzo[a,]*

[a]Faculty of Health Sciences, University of Brasilia, Darcy Ribeiro University Campus, Asa Norte, Brasília, Federal District, CEP 70910-900, Brazil

[b]Department of Pharmacy, União Pioneira de Integração Social - UPIS, Planaltina, Federal District, CEP 70390-125, Brazil

[c]Faculty of Ceilândia, University of Brasilia, Ceilândia, Federal District, CEP 72220-275, Brazil

E-mail: yrisfonseca@unb.br

Received: 03 February 2020; revised 16 November 2021

This study evaluated the seasonal effects of two flavonoids on antioxidant activity and chromatographic profiles by thin layer chromatography and high-performance liquid chromatography (HPLC) in *Erythroxylum suberosum* A. St.-Hil, a species used by the Brazilian indigenous community. These variables were observed from August 2013 to May 2014 in correlation with climatic variables, such as temperature, rainfall index and global radiation. The chromatographic profiles were found to be similar in the aqueous and ethanol extracts, and flavonoid hyperoside and isoquercitrin were identified and quantified. In the inhibition of the DPPH$^{\bullet}$ radical, the most active was the aqueous extract from the 2nd collection (IC_{50}: 4.45 µg/mL). For the phosphomolybdenum complex reduction method, the ethanol extract from the 1st collection was the most active (206.39 µg/mL equivalent ascorbic acid). Regarding the environmental correlations, it was observed that a higher global radiation index had a strong influence on the concentrations of hyperoside and contributed to the antioxidant activity. On the other hand, higher temperatures contributed to a higher isoquercitrin content in the aqueous extracts. These results indicate that August is the best month for the collection of *Erythroxylum suberosum* A. St.-Hil. leaves which have the highest isoquercitrin and hyperoside content and, thus, a high antioxidant activity.

Keywords: Antioxidant activity, Chromatographic profile, Environmental correlation, *Erythroxylum suberosum*, Seasonal variation

IPC Code: Int Cl.[22]: A61P 17/18, A61P 39/06, A61K 36/00

The genus *Erythroxylum* has approximately 230-240 pantropically distributed species[1]. Brazil is one of the main centers of diversity, with 127 species, of which 82 are endemic[2]. *Erythroxylum* genus became better known during the nineteenth century, after the discovery of the pharmacological activities of *Erythroxylum coca* Lam. (coca) leaves, which are chemically characterized by the presence of tropane alkaloids, in particular cocaine[3,4].

There is evidence of the medicinal use of coca dating back at least 8,000 years in South America. Furthermore, several Andean and Amazonian communities have traditionally consumed coca. In the traditional cultures of South America, coca has a broad social, cultural, and medicinal significance. Literature on these communities has reported on the use of coca as a medicine to relieve oral pain, digestive ailments, hunger, altitude sickness, muscle and skeletal pain, sadness, and sexual impotence[4]. More specifically, *Erythroxylum subracemosum* Turcz. is used in respiratory diseases, *Erythroxylum catuaba* A. J. Silva is used as an aphrodisiac in Brazil and *Erythroxylum argentinum* O. E. Schulz is used in the treatment of sinusitis[5]. In traditional medicine, Kayapó (a Brazilian indigenous community) uses *E. suberosum* as an analgesic and an anti-rheumatic remedy, as well as for poor digestion [6].

Erythroxylum suberosum A. St. Hil. is commonly known as "azougue-do-campo," "cabelo-de-negro," "galinha-choca," "mercúrio do campo" and "sessenta e dois"[2,7]. Similarly, synonyms for *E. suberosum* include *Erythroxylum areolatum* L., *E. areolatum* Vell., and *E. testaceum* Peyr[8]. It occurs in all Brazilian biomes, including the Amazonian, Caatinga, Atlantic Forest, Pantanal, and Cerrado biomes[8] and

*Corresponding author

has also been collected in Bolivia, Brazil, French Guiana, Paraguay and Venezuela[2].

Despite its traditional use, little scientific research has been conducted on the pharmacological effects of *E. suberosum*. This species has been reported to show antibacterial activity against *Staphylococcus aureus* and antifungal activity against *Cryptococcus neoformans*, *Candida krusei*, *Candida parapsilosis*, *Candida albicans*, and *Candida glabrata*[9]. Other studies have described the cytotoxicity activity of aqueous and ethanol extracts from the leaves of *E. suberosum* against head and neck carcinoma cells (FaDU, SCC-9 and SCC-25) and keratinocyte cells (HaCat) with or without combination with radiotherapy[10]. Additionally, the extracts of *E. suberosum* leaves have been reported to show antioxidant activity via DPPH radical scavenging, Fe^{+3} reduction, and cyclic voltammetry methods[11], as well as phosphomolybdenum complex reduction[12].

The phytochemical screening of the leaves of *E. suberosum* suggested the presence of alkaloids, coumarins, flavonoids, anthocyanins, condensed tannins, and triterpenes/steroids[11]. Rutin, ombuin-3-rutinoside and isoquercitrin were isolated from leaves[12,13], whereas the diterpenes 7-oxo-16-hydroxy-abiet-15(17)-en-19-al, 16-hydroxyabiet-15(17)-en-7-one, 7α,16-dihydroxy-abiet-15(17)-en-19-al, *ent*-12α-hydroxy-kaur-16-en-19-al and methyl *ent*-7α,15β-dihydroxy-kaur-16-en-19-oatewere isolated from branches[13]. Rutin and isoquercitrin are considered promising natural therapeutic agents, and several pharmacological activities[14], such as analgesic, anti-inflammatory and antioxidant, have been attributed to these compounds[15].

The time of year in which a plant drug is collected, combined with plant age and habitat characteristics (e.g. circadian rhythm, solar radiation, rainfall index, temperature, altitude and nutrients present in the soil), may contribute to changes in secondary metabolic compounds[16]. These variables, in turn, are essential factors because the active constituents present in plants are not constant during the year[17]. There are several reports of seasonal variations influencing the contents of practically all classes of secondary metabolites[18]. Thus, the quality and therapeutic value of natural products can be influenced by environmental variables. Studies on seasonal variations are necessary to improve the quality of these products. Therefore, this study evaluated the influence of seasonal variation on the chemical composition and biological activities of aqueous and ethanol crude extracts obtained from *Erythroxylum suberosum* leaves growing in the Cerrado biome.

Materials and Methods

Plant material

Erythroxylum suberosum A. St.-Hil. leaves were collected in the Cerrado vegetation of the University of Brasília (UnB) Darcy Ribeiro campus during August (1st collection) and November (2nd collection) of 2013, February (3rd collection) and May (4th collection) of 2014. The leaves were identified by Dr Christopher Fagg and voucher specimens were deposited in the UB herbarium (Fagg CW 2192).

This project was registered at SisGen (National System for the Management of Genetic Heritage and Associated Traditional Knowledge) under the number A215A9A.

The drying process was monitored using an infrared moisture analyzer. After the residual moisture on the plant material reached approximately 8.5% (w/w), the leaves were powdered using a knife mill (Marconi®).

Extraction

Aqueous extracts (EA) were obtained by infusion at a ratio of 1:10 (leaves/water), filtered, and frozen at –20°C. The extract was then lyophilized and stored at –20°C.

Ethanol extracts (EE) were obtained by maceration. The leaves were macerated with hexane (1:10) for seven days. After maceration with hexane, the solvent was removed, and ethanol was added at the same ratio and extraction time. The extracts were then concentrated in a rotary evaporator and stored at –20°C.

Extract yields were calculated by considering the final product weight of the initial dried plant material weight. Extracts EA and EE were assigned by numbers 1 to 4, related to each of the collected samples (e.g. EE1/EA1 = 1st collection (August 2013), EE2/EA2 = 2nd collection (November 2013), EE3/EA3 = 3rd collection (February 2014), and EE4/EA4 = 4th collection (May 2014).

Seasonal study

Climatic data

The climatic data included global radiation, temperature and rainfall index, which were kindly provided by the Agroclimatology Laboratory of Fazenda Água Limpa-UnB. These data were statistically tested to verify the existence of linear

correlations between the levels of hyperoside and isoquercitrin and the antioxidant activity of the *E. suberosum* leaf extracts.

Antioxidant activity

Antioxidant potential by reduction of DPPH• radical

Antioxidant activity was evaluated by DPPH radical scavenging, as previously described[19]. Briefly, 100 µL of the sample was added to a mixture of 1 mL of sodium acetate buffer (pH 5.5; 100 mM) and 1 mL of ethanol (95%). Then, 500 µL of DPPH• alcoholic solution (250 µM) was added to the mixture. After 15 min, the absorbance of the solutions was measured at 517 nm using a spectrophotometer. The following reference standards were used: ascorbic acid (Sigma-Aldrich®), rutin (Sigma-Aldrich®) and butylhydroxytoluene (BHT). The inhibition potential was determined using the control sample response. IC_{50} was determined using a linear regression of the data[20].

Antioxidant potential by inhibition of the phosphomolybdenum complex

The antioxidant activity of *E. suberosum* crude extracts was evaluated using the phosphomolybdenum method[21]. The antioxidant activity was expressed in ascorbic acid equivalents using the equation obtained by performing a linear regression of the data acquired with the ascorbic acid standard (Sigma-Aldrich®). The assay was performed by adding 1 mL of reagent solution (28 mM phosphate solution, 4 mM molybdate, and 0.6 M sulfuric acid) to 0.1 mL of sample/standard. The negative control consisted of 0.1 mL of solvent and reagent solution (1 mL). After 90 min of reaction in a water bath at 95°C, the absorbance was determined using a spectrophotometer at 695 nm.

Chromatographic profile by thin layer chromatography

Phenolic compounds were detected by thin layer chromatography (TLC), as previously described[22], with some modifications. The mobile phase was composed of ethyl acetate, acetic acid and formic acid (100: 10: 10). The NP/PEG reagent [diphenylboryloxyethylamine (1% in methanol) and polyethylene glycol 4000 (5% in ethanol)] was used to identify spots under ultraviolet light (365 nm). Hyperoside, quercetin, isoquercitrin, isovitexin, kaempferol, and rutin were used to compare retention factors.

Chromatographic profile by high-performance liquid chromatography

A liquid chromatograph (HPLC) (LaChrom Elite®; Hitachi) coupled to a diode array detector (L2455 DAD detector; Hitachi, Japan) was used. The detector was adjusted to collect data in the range of 220–400 nm and the chromatogram was extracted at 354 nm. The mobile phase consisted of 1% phosphoric acid solution and acetonitrile, with a flow rate of 0.6 mL/min. The column was a reverse phase C18 (150×4.6 mm, 5 mm) (PurospherStar®; Merck, Germany), coupled to a pre-column of the same characteristics (4×4; 5 mm particle size) (Merck, Germany). Data were acquired using the Agilent EZChrom Elite® software (version 3.3.2, SP1 Scientific Software, Inc.)[23].

Statistical analysis

The results are presented as means and standard deviations (SD). The data were subjected to parametric distribution, and one-way analysis of variance (ANOVA) followed by Tukey's post-test were used with a 95% confidence interval ($p<0.05$).

Pearson's linear correlations were evaluated using the classical approach, with the following criteria: weak correlation $|r| < 0.3$, moderate correlation $0.3 \leq |r| < 0.6$, and strong correlation $|r| \geq 0.6$ (significant correlation, $p<0.05$)[24].

Results

Extract yields

The EA yields of *E. suberosum* ranged from 5.58% to 8.16% and EE from 12.91% to 15.12%. The best overall yield was EE of *E. suberosum*, with EE1 being the extract with the highest yield (15.12%). In EA, the highest yield was obtained with EA1 (8.16%). Thus, maceration with ethanol proved to be the best extraction process for obtaining the highest yields of crude extracts from the leaves of the analyzed species.

Seasonal study

Climatic data

The obtained weather variable data characterized a seasonal tropical climate, typical of the Cerrado. Annual precipitation (rainfall) presented an average of 1,558 mm, which usually occurs from October to March, followed by a dry period from May to September (Table 1)[25].

A variation occurred in the minimum monthly average temperature (9.43ºC to 16.17ºC) when compared with the maximum monthly average (27.17ºC to 28.62ºC). The maximum temperatures varied slightly during the months of 2013 (26.43ºC to 29.90ºC) (Table 1).

Table 1 — Environmental variables during the collection months of *E. suberosum* A.St.-Hil. leaves.

Collection months	Global radiation (MJm^{-2d-1})	Rainfall index (mm)	Temperature max (°C)	Temperature min (°C)
Aug/13	18.66	0.00	28.63	9.40
Nov/13	16.83	6.90	27.93	27.18
Feb/14	17.01	4.70	28.16	27.62
May/14	15.84	0.40	27.18	11.60

Global radiation, maximum temperature, minimum temperature, and rainfall index are presented in Table 1. The data represent the monthly average and refer to the collection of *E. suberosum* leaves.

Antioxidant activity by DPPH• radical scavenging

All extracts showed antioxidant activity according to the results of the DPPH radical scavenging assay. The most active extracts were EA2 (IC_{50}=4.45 µg/mL) and EE1 (IC_{50}=5.08 µg/mL), while BHT, ascorbic acid and rutin, used as reference, showed IC_{50} = 6.90 µg/mL, 1.06 µg/mL and 3.74 µg/mL, respectively (Fig. 1).

Antioxidant activity by inhibition of the phosphomolybdenum complex

The antioxidant activity of *E. suberosum* extracts, by the reduction of the phosphomolybdenum complex, was expressed as equivalents of ascorbic acid (AA). The equation, obtained by the linear regression analysis of ascorbic acid absorbance versus the concentration in the reaction medium, was $y = 3.663x–0.04300$ ($r = 0.99$), and was used to determine the equivalence of ascorbic acid (EAA) for the extracts.

The most active extracts were EE1 (206.39 µg/mL EAA) and EA1 (186.28 µg/mL EAA). Moreover, EE1 presented significantly higher activity than the other extracts (Fig. 2).

Correlation of antioxidant activity with climatic variables

The level of correlation between the antioxidant activity of each extract, obtained from plant material collected at different periods and the environmental variables, was established using Pearson's linear correlation.

For the antioxidant activity, evaluated by the phosphomolybdenum assay, strong and negative correlations were observed between the minimum temperature and antioxidant activity of EA (r=–0.7513, p=0.1243) and EE (r=–0.7747, p=0.1126), as well as a strong and positive correlation between maximum temperature and antioxidant activity of EA (r=0.6766, p=0.1617) and EE (r=0.7368, p=0.1316). Another strong and positive correlation was established between global radiation and EE (r=0.9050, p=0.0475). In relation to global radiation, EA was found to be positively correlated (r=0.8675, p=0.0662).

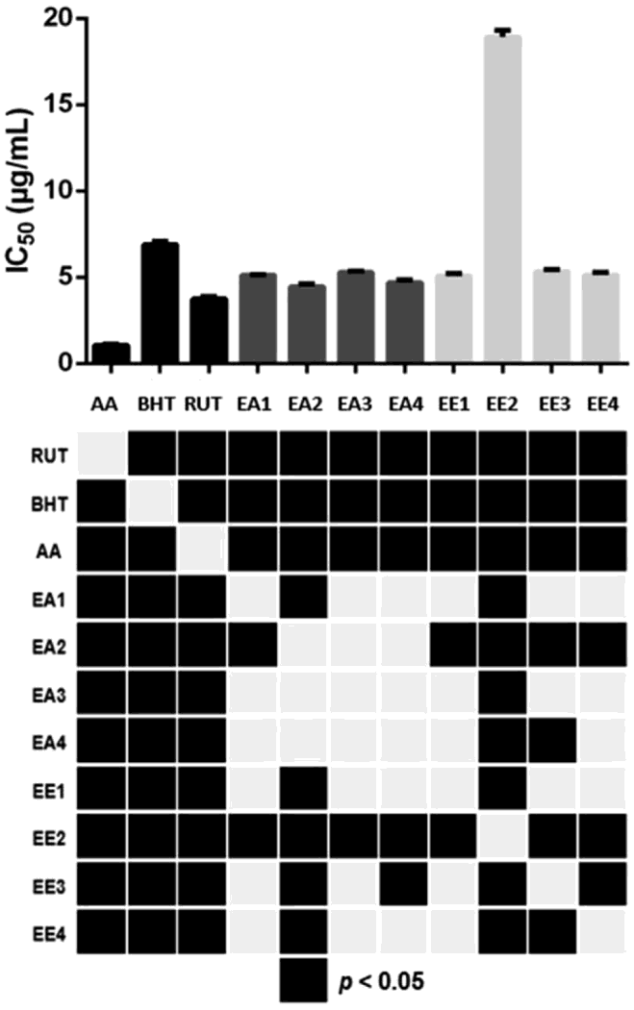

Fig. 1 — Antioxidant activity by reduction of DPPH radical for the aqueous and ethanolic extracts of *Erythroxylum suberosum* A. St.-Hil. leaves. Comparison test by ANOVA-Tukey for the IC_{50} of the extracts. Samples with $p<0.05$ were considered significantly different. Data represent the triplicate means ± standard deviation (SD). Standards: BHT = butylhydroxytoluene; AA = ascorbic acid. Extracts: EA = aqueous extract; EE = ethanolic extract. The number in the front of each acronym EA and EE refers to the collection period, where EA1 = 1st collection, and so on.

For the antioxidant activity by the DPPH radical scavenging assay, a positive correlation was observed between the rainfall index and antioxidant activity of EE (r=0.7845, p=0.1078) and between the minimum temperature and antioxidant activity of EE (r=0.6452, p=0.1773).

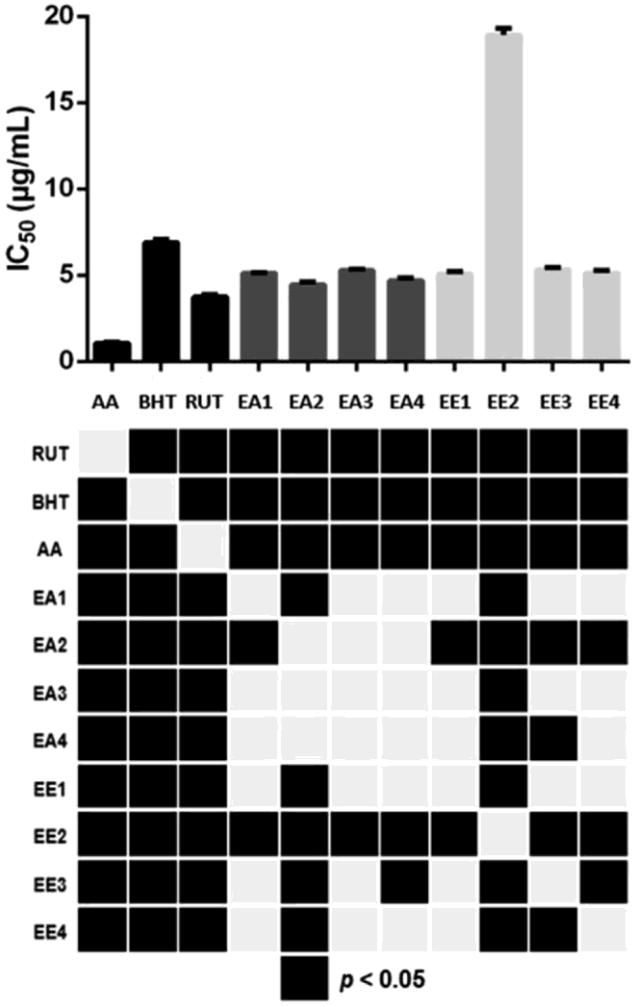

Fig. 2 — Antioxidant activity by the reduction of the phosphomolybdenum complex for the aqueous and ethanolic extracts of *Erythroxylum suberosum* A. St.-Hil. leaves. Comparison test by ANOVA-Tukey for the IC_{50} of the extracts. Data represent the triplicates means ± SD. The number in the front of each acronym EA and EE refers to the collection period, where EE1 = 1st collection, and so on. EA = aqueous extract; EE = ethanolic extract.

Chromatographic profile by thin layer chromatography

The chromatographic profiles of aqueous and ethanol extracts from the *E. suberosum* leaves by TLC were similar to those of aqueous and ethanol extracts in the presence of four spots. Using a mixed point technique, the presence of hyperoside (Rf = 0.38) and quercitrin (Rf = 0.64) was confirmed.

Chromatographic profile by high-performance liquid chromatography

HPLC analysis showed a similar chromatographic profile for both aqueous (EA) and ethanol (EE) extracts, with four major peaks. Based on the retention time and UV spectra, in comparison with authentic samples, it was possible to identify hyperoside (peak 2, RT=28.82 min) and isoquercitrin (peak 3, RT=30.38 min) (Fig. 3). Based on the UV spectra, peak 1 referred to a cinnamic acid derivative and peak 4 to a quercetin derivative.

Analytical curves were established to quantify hyperoside and isoquercitrin in the extracts (Table 2) by using equations from a linear regression of the data obtained with the standard substances: y=238000x–6677 (r=0.9998) for hyperoside and y=114300x–554200 (r=0.9986) for isoquercitrin.

Regarding the hyperoside content, the extract with the highest content was EA1 (41.8 µg/mL), while extract EE3 presented the lowest content (19.0 µg/mL). EA1 also presented the highest content of isoquercitrin content (18.0 µg/mL), while the lowest content was presented by EE1 (10.9 µg/mL).

Correlation of the hyperoside and isoquercitrin content with antioxidant activity

The correlation between the antioxidant activity of each extract and the hyperoside and isoquercitrin content was determined using Pearson's correlation.

A statistically significant positive and strong correlation was observed between antioxidant activity by the phosphomolybdenum assay and the hyperoside content (r=0.9700, p=0.0150) in the EA. Another significant correlation was observed between the DPPH$^{\bullet}$ reducing activity and hyperoside content of EE (r=0.9692, p=0.0154).

Regarding the isoquercitrin content, a significantly strong correlation was observed between the antioxidant activity by the phosphomolybdenum test and the isoquercitrin content of EA (r=0.9613, p=0.0194). A strong correlation was also found between the reducing activity of DPPH$^{\bullet}$ and the isoquercetin content of EE (r=0.7017, p=0.1491). In contrast, a strong negative correlation was observed between the antioxidant activity of the phosphomolybdenum assay and the isoquercitrin content of EE (r=–0.770, p=0.11147).

Correlation of hyperoside and isoquercitrin contents with environmental variables

Correlation coefficients between hyperoside levels and climatic variables were established. Strong negative correlations were observed between the rainfall index and hyperoside content of EA (r = –0.7156, p=0.1422) and between the minimum temperature and hyperoside content of EA (r = –0.8750, p=0.0625). In contrast, strong positive correlations were observed between the rainfall index

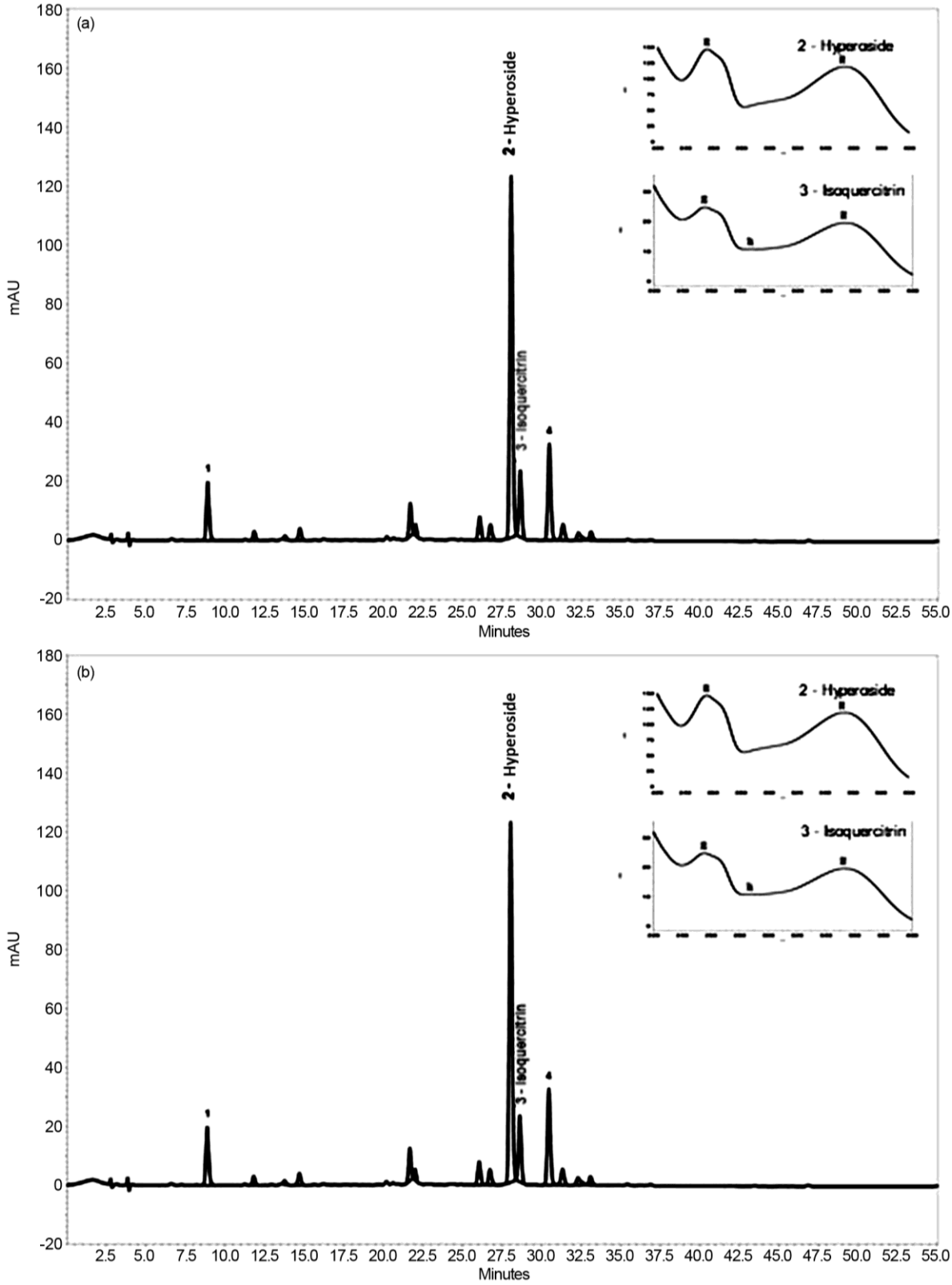

Fig. 3 — Chromatographic profile by HPLC-DAD of *Erythroxylum suberosum* A. St.-Hil. leaf extracts. Mobile phase consisting of 1% phosphoric acid and acetonitrile. Flow, 0.6 mL/min. The chromatogram was recorded at 354 nm. A: Chromatographic profile of aqueous extract. B: Chromatographic profile of ethanolic extract.

Table 2 — *Hyperoside and isoquercitrin contents in the* Erythroxylum suberosum *A. St. -Hil. leaf extracts.*

Extracts	Hyperoside content (μg/mL)	Isoquercitrin content (μg/mL)
EA1	41.75 ± 0.05	17.96 ± 0.01
EA2	23.42 ± 0.03	13.63 ± 0.01
EA3	23.25 ± 0.03	12.93 ± 0.01
EA4	26.56 ± 0.01	14.58 ± 0.01
EE1	19.89 ± 0.03	10.94 ± 0.01
EE2	25.47 ± 0.05	14.37 ± 0.01
EE3	18.96 ± 0.03	11.86 ± 0.01
EE4	20.62 ± 0.07	13.65 ± 0.02

The number in front of each acronym EA and EE refers to collection, where EE1 = 1st collection, and so on. EA=aqueous extract; EE = ethanolic extract. Data represent the mean ± standard error (n=3).

and hyperoside content of EE (r = 0.6260, p=0.1870), and between the global radiation and the EA hyperoside content (r = 0.8069, p=0.0966).

A strong negative correlation coefficient was observed between the rainfall index and isoquercitrin content of the aqueous extract (r = –0.7416, p=0.1272). Similarly, global radiation and isoquercitrin content showed a strong positive correlation (r = 0.7261, p=0.1370). A significantly strong negative correlation was also found between the minimum temperature and isoquercitrin content in the EA group (r = –0.9008, p=0.0496).

For the ethanol extract, strong negative correlations were observed between minimum temperature and isoquercitrin content (r =–0.7469, p=0.1265), and between global radiation and isoquercitrin content (r = –0.7786, p=0.1107).

Discussion

Seasonal study

Climatic data

Global radiation is electromagnetic radiation coming from the sun that reaches the Earth. Solar radiation is of great importance to life, as it is responsible for most of the biological and physiological processes of plant species. In addition, this radiation is directly responsible for the availability of energy for the soil-plant-atmosphere processes[26]. In the Cerrado biome, solar radiation is very intense, but can diminish due to a dense cloud cover during the rainy period (October to April). Commonly, October tends to be warmer than December or January. On the other hand, solar radiation is intense during the dry season (May to September), although it can be reduced by dry haze caused by bush fires[25]. The variations in temperature, radiation, and pluviometric index presented for these periods are characteristic of the Cerrado biome.

Antioxidant activity

No significant changes in antioxidant activity were observed in the majority of extracts over the seasons. Only EE2 showed a reduction of activity by approximately 3.5-fold when compared to the other aqueous and ethanol extracts (Fig. 1).

In this study, EE (IC_{50} = 5.08–18.93 μg/mL) was more active than *E. suberosum* ethanol extract (IC_{50} = 118 μg/mL), as previously reported[11]. Such divergences may be due to differences in the extractive process, the occurrence of variation in soil nutrients and even climatic influence in different regions. EA extracts were more active (IC_{50} = 4.45–5.28 μg/mL) than the aqueous extract of *Erythroxylum launifilium* (IC_{50} = 195.00 μg/mL)[27].

In comparison to previous results, the EE and EA extracts showed activity to reduction of the DPPH• radical similar to those from *Erythroxylum gonocladum* (IC_{50} = 8.81 μg/mL), *Erythroxylum suberosum* (IC_{50} = 5.72 μg/mL), and *Erythroxylum tortuosum* (IC_{50} = 6.55 μg/mL)[28].

Erythroxylum species have shown better antioxidant activity than other genera, such as C. *dicoccum* (Gaertn.) ethanolic extract and *Amischophacelus axillaris* (L.) ethyl acetate extract, which showed 54.21±1.477 and 51.59±0.147 of DPPH (%) inhibition at a concentration of 250 μg /mL, respectively[29]. Another example is *S. irio* L., which was found to inhibit approximately 60% of DPPH at a concentration of 1 mg/mL[30].

E. suberosum leaf extracts exhibited efficient antioxidant activity by inhibiting the phosphomolybdenum complex. These results are in agreement with those of Barros *et al.*[12] who reported antioxidant activity via the inhibition of the phosphomolybdenum complex of *E. suberosum* leaf extracts. These results indicate that all tested samples were quantitatively more efficient in reducing Mo(VI) to Mo(V) than the positive control BHT.

Correlation of antioxidant activity with climatic variables

The analyses showed that as the number of rainfall events increased, the antioxidant activities of both methods tended to decrease. Interestingly, as temperature and global radiation increased, the phosphomolybdenum-reducing activity tended to increase, while the DPPH• radical scavenging activity tended to decrease with increasing radiation. Although some correlations were not statistically

significant, they all showed a clear trend and have been highlighted accordingly.

HPLC: Correlation of the hyperoside and isoquercitrin content with antioxidant activity

The results showed that the antioxidant power of EA was correlated with isoquercitrin and hyperoside levels. Thus, the antioxidant activity of EA is largely attributed to these flavonoids. Other studies have confirmed the antioxidant potential of isoquercitrin and hyperoside compounds. Sukito and Tachibana (2014) reported the antioxidant activity of compounds isolated from *Camellia sasanqua*[31]. In addition, isoquercitrin was found to exhibit antioxidant activity and inhibit photoaging in CCD-986Sk fibroblast cells[32]. Another study showed that hyperoside could treat oxidative stress injury through the NF-κB/ Bax/Caspase 3, P62/LC3B and PI3K/Akt pathways[33].

On the other hand, for EE, a negative correlation was observed between the isoquercitrin and hyperoside content and antioxidant activity. Thus, the antioxidant activity of EE could not be attributed to these flavonoids. Similarly, no correlation between the total flavonoid content and antioxidant activity (DPPH assay) of *Bellis perennis* flowers was observed[34]. *Erythroxylum suberosum* contains other compounds with potential antioxidants, such as anthocyanins[35] and coumarins[36]. The antioxidant potential of *E. suberosum* may be due to the synergism of these compounds.

HPLC: Correlation of the hyperoside and isoquercitrin contents with environmental variables

Temperature variation, sun exposure, the amount of water and nutrients, and drying storage can significantly affect the secondary metabolite content and, consequently, the therapeutic value of medicinal plants[16]. The results presented here in showed strong negative correlations between rainfall and the hyperoside index of EA. The hyperoside content tended to decrease in aqueous extracts as the rain index increased. This process may be due to the leaching process suffered by the leaves during the rainy period[37].

Another observation regarding the hyperoside content in EA is that when the temperature decreases, the content of hyperoside tends to increase. In addition, when the overall radiation increased, the hyperoside content tended to increase. Thus, the increase in hyperoside content could be a defense mechanism against increased solar radiation[38].

Similarly, for EA, as the rainfall index increased, the isoquercitrin content decreased. Moreover, when the overall radiation increased, the isoquercitrin content also increased. In addition, an increase in isoquercitrin content was found to correlate with a decrease at lower temperatures.

The strong negative correlations found for EE showed that as the temperature decreased and the overall radiation increased, the isoquercitrin content also increased, most likely due to the defense process against solar radiation, similar to that observed for hyperoside.

Conclusion

The study of seasonality comparing the meteorological variables, secondary metabolites, and biological activity indicated changes in the composition of metabolites produced by a plant species over the year.

All of the extracts evaluated showed antioxidant activity in the two tested methodologies. Some extracts had similar activity to BHT, which was used as a reference. Therefore, the extract of *Erythroxylum suberosum* leaves seems to be a promising agent for the treatment of diseases related to oxidative stress, such as atherosclerosis and Alzheimer's disease.

It was possible to establish the level of correlation between the antioxidant activity and the hyperoside and isoquercitrin contents in the obtained extracts. The correlations related to EA indicated that the antioxidant activity could be largely attributed to these flavonoids. Correlations with EE indicate that the reducing activity of DPPH cannot be attributed principally to these flavonoids.

As the rainfall increased, the hyperoside and isoquercitrin contents tended to decrease, showing a correlation between the environmental variables and the content of these compounds. Moreover, as the temperature decreased and the overall radiation increased, the hyperoside and isoquercitrin contents tended to increase as well.

These results indicate that the time of year for the collection of the *E. suberosum* leaves with the highest isoquercitrin and hyperoside content and the highest antioxidant activity is August, wherein the best extractive solvent to obtain higher yields of these flavonoids is water, using an infusion process.

Acknowledgments

The authors are grateful to the Laboratory of Agroclimatology of the FAL - UnB, National Council

for Scientific and Technological Development (CNPq), Coordination for the Improvement of Higher Education Personnel (CAPES), Federal District Research Support Foundation (FAP-DF) and University of Brasília (UnB).This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) (finance code 001). We would like to thank Editage (www.editage.com) for English language editing.

Conflict of Interest

The authors declare that they do not have any conflicts of interest related to this manuscript.

Authors' Contributions

The acquisition, analysis, and interpretation of data were performed by NAM and DHNM. Identification and deposition in the herbarium of leaves was conducted by CWF. The drafting of the work and critical revision for important intellectual content were conducted by LAS and POM. The conception, design, and revision of the work were performed by DS and YMFB.

References

1 Plowman T & Hensold N, Names, types, and distribution of neotropical species of *Erythroxylum* (Erythroxylaceae), *Brittonia*, 56 (1) (2004) 1-53.

2 Loiola M I B, Agra M F, Baracho G S & Queiroz R T, Flora da Paraíba, Brasil: Erythroxylaceae Kunth, *Acta Bot Brasilica*, 21 (2) (2007) 473-487.

3 Plowman T, The ethnobotany of coca (*Erythroxylum* spp., Erythroxylaceae), *Adv Econ Bot*, 1 (1984) 62-111.

4 Restrepo D A, Saenz E, Jara-Muñoz O A, *et al.*, *Erythroxylum* in focus: An interdisciplinary review of an overlooked genus, *Molecules*, 24 (20) (2019) 3788.

5 Dantas C A G, Abreu L S, da Cunha H N, *et al.*, Dereplication of phenolic derivatives of three *Erythroxylum* species using liquid chromatography coupled with ESI-MSn and HRESIMS, *Phytochem Anal*, 32 (6) (2021) 1011-1026. doi:10.1002/pca.3043

6 Barbosa W L R & Pinto L N, Documentação e valorização da fitoterapia tradicional Kayapó nas aldeias A'Ukre e Pykanu-sudeste do Pará, *Rev Bras Farmacogn*, 13 (1) (2003) 47-49.

7 Brandão M G L, Pignal M, Romaniuc S, Grael C F F & Fagg C W, Useful Brazilian plants listed in the field books of the French naturalist Auguste de Saint-Hilaire (1779–1853), *J Ethnopharmacol*, 143 (2) (2012) 488-500. doi:10.1016/j.jep.2012.06.052

8 REFLORA, Erythroxylaceae, Accessed: January 16, 2020. [http://reflora.jbrj.gov.br]

9 Violante I M P, Hamerski L, Garcez WS, *et al.*, Antimicrobial activity of some medicinal plants from the cerrado of the central-western region of Brazil, *Braz J Microbiol*, 43 (4) (2012) 1302-1308.

10 Elias S T, Borges G A, Amorim D A, *et al.*, Radiation induced a supra-additive cytotoxic effect in head and neck carcinoma cell lines when combined with plant extracts from Brazilian Cerrado biome, *Clin Oral Investig*, 19 (3) (2015) 637-646.

11 Oliveira F D, Aguiar PN, Ribeiro G D, *et al.*, Antioxidant activity and phytochemical screening of extracts of *Erythroxylum suberosum* A. St.-Hil (Erythroxylaceae), *Res J Phytochem*, 9 (1) (2015) 68-78.

12 Barros I M C, Leite B H M, Leite C F M, *et al.*, Chemical composition and antioxidant activity of extracts from *Erythroxylum suberosum* A. St. Hil. Leaves, *J Appl Pharm Sci*, 7 (03) (2017) 088-094.

13 Nascimento C J, Violante I M P, Garcez W S, Pott A & Garcez F R, Biologically active abietane and ent-kaurane diterpenoids and other constituents from *Erythroxylum suberosum*, *Phytochem Lett*, 5 (2) (2012) 401-406. doi: 10.1016/j.phytol.2012.03.013

14 Maalik A, Khan F A, Mumtaz A, *et al.*, Pharmacological applications of quercetin and its derivatives: a short review, *Trop J Pharm Res*, 13 (9) (2014) 1561-1566.

15 Lesjak M, Beara I, Simin N, *et al.*, Antioxidant and anti-inflammatory activities of quercetin and its derivatives, *J Funct Foods*, 40 (1) (2018) 68-75.

16 Gobbo-Neto L & Lopes N P, Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários, *Quím Nova*, 30 (2) (2007) 374-381.

17 Kouki M & Manetas Y, Resource availability affects differentially the levels of gallotannins and condensed tannins in *Ceratonia siliqua*, *Biochem Syst Ecol*, 30 (7) (2002) 631-639.

18 Monteiro J M, Albuquerque U P, Lins Neto E M F, Araújo E L, Albuquerque M M, *et al.*, The effects of seasonal climate changes in the Caatinga on tannin levels in *Myracrodruon urundeuva* (Engl.) Fr. All and *Anadenanthera colubrina* (Vell.) Brenan, *Rev Bras Farmacogn*, 16 (3) (2006) 338-344.

19 Blois M S, Antioxidant determinations by the use of a stable free radical, *Nature*, 181 (4617) (1958) 1199-1200.

20 Locatelli M, Gindro R, Travaglia F, Coïsson J-D, Rinaldi M, *et al*., Study of the DPPH-scavenging activity: Development of a free software for the correct interpretation of data, *Food Chem*, 114 (3) (2009) 889-897.

21 Prieto P, Pineda M & Aguilar M, Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E, *Anal Biochem*, 269 (2) (1999) 337-341.

22 Wagner H & Bladt S, *Plant drug analysis: a thin layer chromatography atlas* (2nd ed.), Springer Science & Business Media, 2001.

23 Leite C F M, Leite B H M, Barros I M C, *et al.*, Determination of rutin in *Erythroxylum suberosum* extract by liquid chromatography: applicability in standardization of herbs and stability studies, *B Latinoam Caribe Pl*, 13 (2) (2014) 135-143.

24 Callegari-Jacques S M, *Bioestatística: princípios e aplicações*, Artmed Editora, 2009.

25 Klein A L, *Eugen Warming e o cerrado brasileiro: um século depois*, Unesp, 2000.

26 Querino C A S, Moura M A L, Lyra R F F & Mariano G L, Avaliação e comparação de radiação solar global e albedo com ângulo zenital na região Amazônica, *Rev Bra Meteorol*, 21 (3a) (2006) 42-49.

27 Picot M C N, Subratty H A & Mahomoodally F M, Phytochemical profile and antioxidant properties of six medicinal plants traditionally used in the management of diabetes in Mauritius, *Pharmacologia*, 5 (1) (2014) 42-49.

28 Gusman G S, Campana P R V, Castro L C, Castilho R O, Teixeira M M, *et al.*, Evaluation of the effects of some Brazilian medicinal plants on the production of TNF-α and CCL2 by THP-1 cells, *eCAM*, ID 497123 (2015) 1-11.

29 Meghashree K, Latha K & Vagdevi H, Antioxidant and antitubercular activities of leaf extracts of *Canthium dicoccum* (Gaertn.) and *Amischophacelus axillaris* (L.), *Indian J Nat Prod Resour*, 11 (4) (2021) 244-249.

30 Hirigo T H, Gupta R K & Rani A, Phytochemicals and antioxidant activity of *Sisymbrium* irio L. seeds, *Indian J Tradit Know*, 20 (1) (2021) 26-32.

31 Sukito A & Tachibana S, Isolation of hyperoside and isoquercitrin from Camellia sasanqua as antioxidant agents, *Pak J Biol Sci*, 17 (8) (2014) 999-1006.

32 Lee E H, Park H J, Kim H H, Jung H Y, Kang I K, *et al.*, Isolated isoquercitrin from Green ball apple peel inhibits photoaging in CCD986Sk fibroblasts cells via modulation of the MMPs signaling, *J Cosmet Dermatol*, 20 (9) (2021) 2932-2939.

33 Feng Y, Wang D, Wang Q, *et al.*, Protective effects and mechanism of hyperoside in PC12 cells against oxidative stress injury induced by hydrogen peroxide, *Nat Prod Commun*, 16 (5) (2021) 1934578X211015126.

34 Siatka T & Kašparová M, Seasonal variation in total phenolic and flavonoid contents and DPPH scavenging activity of *Bellis perennis* L. flowers, *Molecules*, 15 (12) (2010) 9450-9461.

35 Tena N, Martín J & Asuero A G, State of the art of anthocyanins: Antioxidant activity, sources, bioavailability and therapeutic effect in human health, *Antioxidants*, 9 (5) (2020) 451.

36 Gerasimova E, Gazizullina E, Radosteva E & Ivanova A, Antioxidant and antiradical properties of some examples of flavonoids and coumarins—Potentiometric studies, *Chemosensors*, 9 (5) (2021) 112.

37 Nóbrega L H P, Lima G P, Martins G I & Meneghetti A M, Germinação de sementes e crescimento de plântulas de soja (*Glycine max* L. Merrill) sob cobertura vegetal, *Acta Sci Agron*, 31 (3) (2009) 461-465.

38 Treutter D, Significance of flavonoids in plant resistance and enhancement of their biosynthesis, *Plant Biol*, 7 (06) (2005) 581-591.