

Original Article

Seasonal effect on phenolic content and antioxidant activity of young, mature and senescent leaves from *Anredera cordifolia* (Ten.) Steenis (Basellaceae)

Efeito sazonal no conteúdo de fenólicos e ação antioxidante de folhas jovens, maduras e senescentes de *Anredera cordifolia* (Ten.) Steenis (Basellaceae)

T. M. Alba^a , E. Tessaro^b  and A. M. Sobottka^{a,b*} 

^aUniversidade de Passo Fundo – UPF, Instituto de Ciências Biológicas, Programa de Pós-Graduação em Ciências Ambientais, Passo Fundo, RS, Brasil

^bUniversidade de Passo Fundo – UPF, Instituto de Ciências Biológicas, Curso de Farmácia, Passo Fundo, RS, Brasil

Abstract

Anredera cordifolia (Ten.) Steenis is a vine species native to Brazil that is considered an unconventional food plant and a medicinal species whose phenolic compounds exert antioxidant activity. Since the production of metabolites is determined by environmental factors and leaf maturity, it is important to track these changes in order to determine the best time to harvest. This study aimed to verify whether leaf phenology and seasonality cause variations in the amount of phenolic compounds and in the antioxidant activity of this species. The leaves were collected in different seasons between September 2018 and April 2019, and separated according to maturity: young, mature, and senescent. Daily atmospheric temperature and rainfall data were used to characterize the collection period. The total phenolic content (TPC), determined by Folin-Ciocalteu method, was significantly higher in the young leaves collected in winter, a season of lower temperatures. These leaves showed 54.4 mg of gallic acid equivalents per 100 g of dry matter (mg GAE 100 g⁻¹ DM). Other results averaged 25.6 mg GAE 100 g⁻¹ DM. The highest antioxidant activity, assessed via the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, regardless of leaf phenology, was observed in leaves harvested in autumn (73.7%) and winter (71.1%), seasons with lower rainfall. Leaves harvested in summer and spring had lower antioxidant action rates (54.3 e 37.5%, respectively). There was no significant correlation between the total phenolic content and antioxidant activity. Thus, the phenolic composition of *A. cordifolia*, and consequently its activity on free radicals, varies seasonally in response to temperature and rainfall, and may or may not interact with the age of the leaves.

Keywords: DPPH, medicinal plants, seasonality, secondary metabolites.

Resumo

Anredera cordifolia (Ten.) Steenis é uma trepadeira nativa do Brasil considerada uma planta alimentícia não convencional e uma espécie medicinal, cujos compostos fenólicos exercem ação antioxidante. Como a produção de metabólitos é determinada por fatores ambientais e pela maturidade das folhas, é importante acompanhar essas mudanças a fim de determinar a época de colheita. Neste estudo objetivou-se verificar se a fenologia foliar e a sazonalidade ocasionam variação na quantidade de compostos fenólicos e na ação antioxidante dessa espécie. As folhas foram coletadas em diferentes estações, entre setembro de 2018 e abril de 2019, e separadas conforme a maturidade: jovens, maduras e senescentes. Os dados diários de temperatura atmosférica e pluviosidade foram usados para caracterizar o período de coleta. O conteúdo de fenólicos totais (TPC), determinado pelo método Folin-Ciocalteu, foi significativamente maior nas folhas jovens coletadas no inverno, período caracterizado por temperaturas mais baixas. Estas folhas apresentaram 54,4 mg equivalentes de ácido gálico por 100 g de matéria seca (mg GAE 100 g⁻¹ DM). A média dos outros resultados foi de 25,6 mg GAE 100 g⁻¹ DM. A maior ação antioxidante, avaliada pelo método do radical livre 2,2-difenil-1-picrilhidrazil (DPPH), foi observada em folhas colhidas no outono (73,7%) e inverno (71,1%), independentemente da fenologia foliar. Nestes períodos a pluviosidade foi menor. Folhas colhidas no verão e na primavera apresentaram menores percentuais de ação antioxidante (54,3 e 37,5%, respectivamente). Não houve correlação significativa entre o conteúdo de fenólicos totais e a atividade antioxidante. Conclui-se que a composição fenólica de *A. cordifolia* e, conseqüentemente, sua ação sobre radicais livres, varia sazonalmente em resposta à temperatura e pluviosidade, podendo ou não interagir com a idade das folhas.

Palavras-chave: DPPH, plantas medicinais, sazonalidade, metabólitos secundários.

*e-mail: sobottka@upf.br

Received: 12 July, 2021 – Accepted: 08 Dec, 2021



This is an Open Access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

1. Introduction

Anredera cordifolia (Ten.) Steenis (Basellaceae) is a non-endemic vine native to Brazil that grows in the phytogeographic domains of the Cerrado, the Atlantic Forest, and the Pampas, and is popularly known as “bertalha, trepadeira-mimosa, bertalha-coração, and folha-gorda” (Souza and Lorenzi, 2019). This species has a wide tropical distribution, and can be found throughout India, America, Australia, China, Malaysia, Pacific Islands, and South Africa. In Indonesia and other neighboring regions, it is known as “binahong”, and Australians call it “madeira vine”. *Anredera cordifolia* can often be found in forest edges and in vacant lots in urban areas. In Brazil, mainly in the southern region, it is considered an unconventional food plant and a medicinal herb. In the state of Rio Grande do Sul, its fresh leaves are used to treat burns, wounds, onychomycosis, and also against insect bites, skin lesions and circulatory system disorders (Alba et al., 2020). Its leaves and aerial tubes are eaten in varied forms. The tubers can be eaten either cooked or fried (Kinupp and Lorenzi, 2014), and the leaves have high nutritional value for bread-making, serving as an excellent source of proteins and fibers (Martinevski et al., 2013). This species has also been used as a medicinal plant throughout the world, mainly to treat inflammatory skin diseases, wounds, fungi, and other types of infections. Many *in vitro* and *in vivo* pharmacological studies have been conducted with this species. Regarding the chemical composition, phytochemical screening evidenced the presence of alkaloids, flavonoids, saponins, steroids, and terpenoids in *A. cordifolia* extracts. Flavonoids as vitexin, isovitexin, morin, myricetin, and 3,5,3',4'-tetrahydroxyflavon, in addition to saponins such as ursolic acid were isolated. The probable presence of p-coumaric acid, lupeol, and β -sitosterol was researched (Alba et al., 2020).

The pharmacological properties of plants are directly related to the compounds they produce, especially those originating from secondary metabolism. Plant secondary metabolites are chemical bioactive compounds commonly synthesized as a defense against physiological and environmental stimulators, and are known to play a critical role in the adaptation of plants to their environment (Ramakrishna and Ravishankar, 2011). In addition to plant protection, these compounds have extensive applicability in human health.

Because of its many health benefits, phenolic compounds are one of the most researched secondary metabolite groups. The major treatment targets for phenolic compounds include cardiovascular diseases, cancer, obesity, diabetes, and infectious diseases (Rasouli et al., 2017). They also have antioxidant and anti-inflammatory properties that could have preventive and therapeutic effects for cardiovascular and neurodegenerative diseases (Cory et al., 2018).

Since several pathophysiological conditions can be related to an excessive amount of free radicals in the body causing oxidative stress, the interest in studies on antioxidant substances is rapidly increasing. Free radicals are atoms or molecules containing one or more unpaired electrons in their external orbitals, which makes them

extremely reactive (Meo and Venditti, 2020). Phenolic compounds have the ability to reduce the reactive oxygen species that cause oxidative stress (Osorio-Tobón, 2020), and they can be produced by plants under stressful conditions. Studies have highlighted the effect of environmental conditions on the phytochemical profile (Lin et al., 2020), and on the phenolic content and antioxidant activity of several plant species (Ben Ahmed et al., 2017; Dalmagro et al., 2018; Hrichi et al., 2020; Ko et al., 2018; Ribeiro et al., 2020).

Considering that the content of secondary metabolites in plant extracts can vary depending on leaf age and environmental conditions, this study tested the hypothesis that young, mature, and senescent leaves of *A. cordifolia*, harvested in periods with distinct patterns of air temperature and rainfall, vary in their phenolic composition and antioxidant activity. Through this analysis, we can establish the most suitable conditions for obtaining extracts with higher phenolic content and antioxidant activity from the species.

2. Material and Methods

2.1. Plant materials

The samples were collected in Sarandi, a municipality located in northern Rio Grande do Sul, Brazil (27°58'11.90" S; 52°54'29.78" W), between September 2018 and April 2019. The municipality is located at an altitude of 433 m and has a humid subtropical climate, with hot summers and monthly precipitation (Wrege et al., 2012). The leaves came from an *A. cordifolia* specimen that grew freely in a vacant lot, surrounded by sparse herbaceous vegetation and a few trees. The area where the species was harvested was chosen due to its remote location, with few passersby and animals, and its proximity to the residence of one of the authors. This preserved the area from any external intervention capable of compromising the experiment or directly interfering in the specimens under study. The plant was growing in the shade. The material was identified in the RSPF Herbarium, at the University of Passo Fundo (state of Rio Grande do Sul, Brazil), and a voucher specimen was deposited under number 14413.

The leaves were collected in the mornings, stored in plastic bags, and then separated into three categories, according to a visual indication of maturity: young, mature, and senescent (Figure 1). The young leaves, which were collected near the apical bud at the apex of the branches, were narrower and light green in color; the mature (adult) leaves were retrieved from the intermediate and basal part of the branches, and had a dark green color and leathery thickness; the senescent leaves were those that showed signs of aging, such as yellowish color and apical necrosis, in addition to signs of fungal spots. Each batch was dried separately in a hot air circulation oven at 45 °C.

2.2. Characterization of collection times

The daily data on maximum temperature, minimum temperature and rainfall for the period in which the



Figure 1. *Anredera cordifolia* leaves separated according to leaf age: young (A), mature (B), and senescent (C). Scale bars, 1 cm.

collections were made were obtained from the Cooperativa Trifíclica Sarandi Ltda, located in the municipality of Sarandi. To define the weather pattern, the average maximum and minimum temperatures were considered, as well as the total and average rainfall in the 45 days immediately before the day of collection.

2.3. Reagents and equipment

All solvents and reagents used were analytical grade. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical was obtained from Sigma-Aldrich and the Folin-Ciocalteu reagent from Merck. Gallic acid from Sigma-Aldrich was used as the standard. The samples were analyzed through a visible spectrophotometer (Kasuki, II-227).

2.4. Preparation of samples

About 1 g of dried and ground leaves, precisely weighed, were extracted by shaking for 20 minutes with 50 mL of methanol:water (1:1). The extract obtained was filtered into a 50.0 mL volumetric flask and made up to volume using the extraction solvent. Each solution thus obtained was considered a stock solution, and for each collection period, solutions were obtained from the young, mature, and senescent leaves, totaling 12 stock solutions that were used for TPC analysis.

For the antioxidant activity assay, the samples were prepared from about 1 g of the leaves, precisely weighed, and extracted by shaking with 5 mL of methanol for 20 minutes. The solution was filtered and the volume made up to 5.0 mL using methanol to obtain sample (1), with 200 mg mL⁻¹ of dry matter. Sample (1) was then diluted to obtain three other samples: (2) 120 mg mL⁻¹; (3) 80 mg mL⁻¹; (4) 40 mg mL⁻¹. These samples were obtained for the young, mature, and senescent leaves, at all four concentrations and four collection times, totaling 48 samples.

2.5. Total phenolic content

The total phenolic content (TPC) was determined by the modified Folin-Ciocalteu method (Sousa et al., 2007). A 1.0 mL portion of each stock solution was transferred to 10.0 mL volumetric flasks. Subsequently, 500 µL of Folin-Ciocalteu reagent and 2 mL of 14% aqueous sodium carbonate solution were added, and the volume was completed with distilled water. After incubation for 2 hours at room temperature, absorbance was measured at 750 nm against a blank that was prepared with all reagents except the sample. The assay was performed

in triplicate. The results were derived from a calibration curve ($y = 0.107x + 0.0754$; $R^2 = 0.9995$) of standard gallic acid and expressed as mg of gallic acid equivalents (GAE) 100 g⁻¹ of dry matter (DM).

2.6. DPPH radical scavenging assay

The evaluation of the free radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was performed according Sousa et al. (2007), with modifications. Initially, dilutions of 35, 30, 25, 20, 15, 10, 5, and 1 µg mL⁻¹ from a stock methanolic solution of DPPH at the concentration of 40 µg mL⁻¹ (0.1 mM) were obtained (in triplicate), and absorbances were read at 516 nm, using methanol as blank. The calibration curve of DPPH was built using the absorbance results.

For the analysis, aliquots of 0.3 mL of each sample, at four different concentrations, and 2.7 mL of the DPPH stock solution (0.1 mM) were used. Methanol (0.3 mL) and DPPH 0.1 mM (2.7 mL) were mixed and used as control. Each sample generated one blank that consisted of 0.3 mL of sample and 2.7 mL of methanol. All solutions were stored away from any light sources, and the absorbances were read at 515 nm, 30 min after addition of DPPH. The assays were carried out in triplicate.

For each sample, the percentage of antioxidant activity (% AA) was calculated through the Equation 1:

$$\% AA = \left\{ \frac{[Abs_{control} - (Abs_{sample} - Abs_{blank})] \times 100}{Abs_{control}} \right\} \quad (1)$$

The percentage of remaining DPPH was also estimated by the following equation (Equation 2):

$$\% DPPH = \frac{[DPPH]_{30}}{[DPPH]_0} \times 100 \quad (2)$$

$[DPPH]_{30}$: the concentration of DPPH after reaction with the samples

$[DPPH]_0$: the initial concentration of DPPH (40 µg mL⁻¹)
Based on an exponential curve graph between the percentage of remaining DPPH and the concentration of the sample, the effective concentration of the antioxidant (mg mL⁻¹) necessary to reduce the initial DPPH concentration by 50% (EC50), within 30 min, was obtained. Therefore, the lower EC50, the higher antioxidant capacity.

2.7. Statistical analysis

Results of the daily rainfall in the 45 days before each collection were submitted to analysis of variance (ANOVA). The results of the daily maximum and minimum temperatures were also submitted to analysis of variance, followed by comparison of means by Tukey's test at 5% significance level.

The results of TPC and the effective sample concentration necessary to decrease the initial concentration of DPPH by 50% (EC50) were submitted to analysis of variance in a 4 (collection season) x 3 (leaf maturity) two-tailed model, with comparison of means by Tukey's test at 5% significance level. Subsequently, Pearson's correlation analysis was performed to verify a possible association between the chemical and biological results and the environmental variables. The results were expressed as mean \pm standard deviation (SD). Statistical analyses were performed using IBM SPSS version 20.0 software.

3. Results

3.1. Rainfall and atmospheric temperature

Total and average rainfall and temperature data (maximum and minimum) in the 45-day period prior to each collection are shown in Table 1. Average rainfall did not vary significantly in any of the seasons ($p = 0.502$); however, the total rainfall in the analyzed period was lower in autumn and winter. During winter, both maximum and minimum temperature averages were also recorded significantly lower than the other seasons.

3.2. Effect of seasonality on total phenolic content

The results of TPC showed that seasonality influences the production of these compounds in young, mature, and senescent leaves of *A. cordifolia*. The highest level of phenolic compounds was found in the young leaves collected during winter (54.4 ± 7.36 mg GAE 100 g⁻¹ DM) (Figure 2).

Figure 2A shows the TPC results in each season and the corresponding total precipitation, comparing young, mature, and senescent leaves. In those collected during winter and summer, young leaves (winter 54.4 ± 7.36 ; summer 35.2 ± 2.14) showed significantly higher TPC results than mature (winter 32.9 ± 2.23 ; summer 26.3 ± 1.73) and

senescent leaves (winter 27.5 ± 1.51 ; summer 23.8 ± 6.47). In those collected during autumn, TPC results did not vary by leaf age, showing no significant differences between young (25.3 ± 5.10), mature (19.2 ± 0.97), and senescent (18.9 ± 1.30) leaves. Spring was the only season in which mature leaves showed higher TPC (29.2 ± 0.11). This result did not differ significantly from senescent leaves (22.3 ± 1.87), which in turn did not differ from young leaves (21.5 ± 1.78).

Figure 2B presents the TPC results at each leaf age, comparing winter, spring, summer, and autumn. The TPC was higher in winter for all leaf ages, being significantly higher for young leaves. Mature and senescent leaves did not differ significantly between winter, spring, and summer, with the lowest TPC values observed in autumn.

3.3. Antioxidant activity

The DPPH radical scavenging activity of the samples, expressed in terms of EC50 values (mg mL⁻¹), in each collection season, is shown in Table 2. Young leaves collected in autumn, as well as mature and senescent leaves collected in winter, showed the lowest EC50 values, and thus the highest antioxidant activity.

From the analyses performed for each leaf age and in each season, with samples at concentrations of 40, 80, 120, and 200 mg mL⁻¹ dry matter, the percentage of antioxidant activity and remaining DPPH were calculated. Figure 3 presents the percentage of antioxidant activity for *A. cordifolia* leaves at the concentration of 200 mg mL⁻¹, regardless of phenology, and the total rainfall in each season. Hence, considering all leaf ages, leaves collected in autumn and winter showed the highest percentages of radical-scavenging activity (73.7% and 71.1%, respectively). In these two periods, total rainfall was much lower, which may be considered periods of water stress.

3.4. Correlation between TPC and antioxidant activity

The correlation between total phenolic content and DPPH scavenging activity was calculated and found to be $r = -0.152$ ($p = 0.384$), thus it was not significant.

4. Discussion

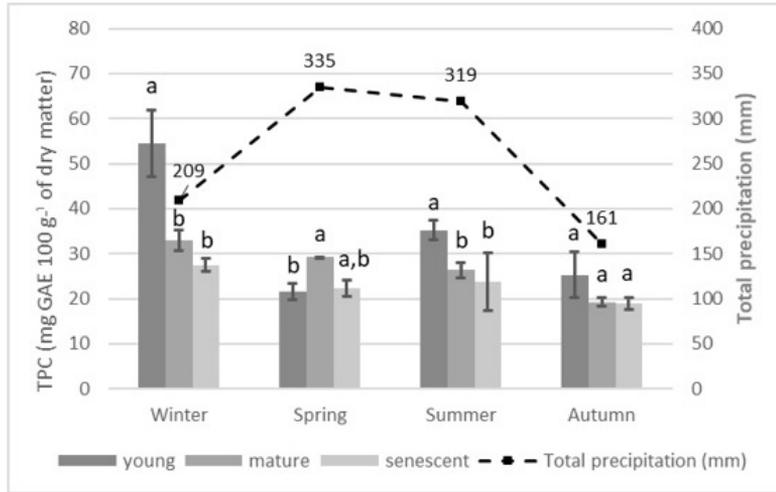
In plant-environment interactions, phenolic compounds play an important role. The amount of polyphenols can

Table 1. Characterization of the 45-day period immediately prior the collections regarding rainfall and atmospheric temperature in Sarandi, Rio Grande do Sul, Brazil.

Collection season	Day of collection	Rainfall (mm)		Temperature (°C)	
		Total	Mean \pm SD	Maximum (mean \pm SD)	Minimum (mean \pm SD)
Winter	14 Sept 2018	209	4.6 \pm 15.0 a*	19.5 \pm 4.4 c	8.2 \pm 3.5 c
Spring	5 Dec 2018	335	7.4 \pm 16.0 a	28.7 \pm 3.5 a,b	14.9 \pm 2.8 b
Summer	20 Jan 2019	314	7.0 \pm 14.1 a	29.6 \pm 3.2 a	18.9 \pm 3.1 a
Autumn	2 April 2019	161	3.6 \pm 10.0 a	27.0 \pm 3.2 b	16.3 \pm 2.5 b

N = 45; SD: standard deviation. *Values followed by the same letter do not differ according to Tukey's test at 5% probability.

2A



2B

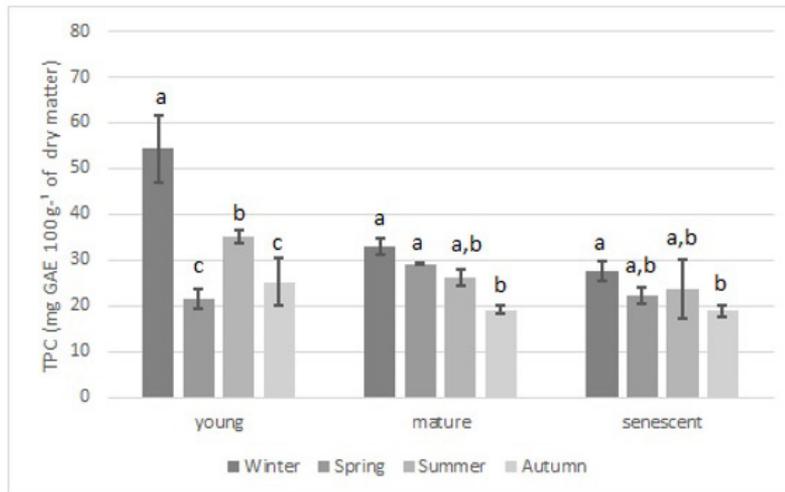


Figure 2. Total phenolic content (TPC) in young, mature and senescent *Anredera cordifolia* leaves, collected during winter, spring, summer and autumn in Sarandi, Rio Grande do Sul, Brazil. Results grouped by season, with the corresponding total precipitation (2A); results grouped by leaf age (2B). Each value represents the mean of three experiments. The lines in the bars refer to standard deviation. Different letters (within each group) indicate significant differences by ANOVA and Tukey's post test ($p < 0.05$).

Table 2. Effective concentration (mg mL^{-1}) of young, mature and senescent *A. cordifolia* leaves, collected during winter, spring, summer and autumn in Sarandi, Rio Grande do Sul, Brazil, necessary to reduce the initial DPPH concentration by 50% (EC₅₀), within 30 min.

Maturity of leaves	Young	Mature	Senescent
Collect season			
Winter	241.0 ± 12.1 c B	70.5 ± 10.1 a A	96.8 ± 22.5 a A
Spring	709.1 ± 56.3 d B	247.5 ± 6.4 c A	275.1 ± 45.4 c A
Summer	167.2 ± 8.5 b A	128.8 ± 6.8 b A	843.2 ± 8.9 d B
Autumn	56.8 ± 12.2 a A	170.1 ± 7.5 b B	170.9 ± 10.4 b B

Each value represents the mean ± SD of three experiments. Means followed by the same lowercase letter in the column, and uppercase letter on the line, don't differ by ANOVA and Tukey's post test ($p < 0.05$).

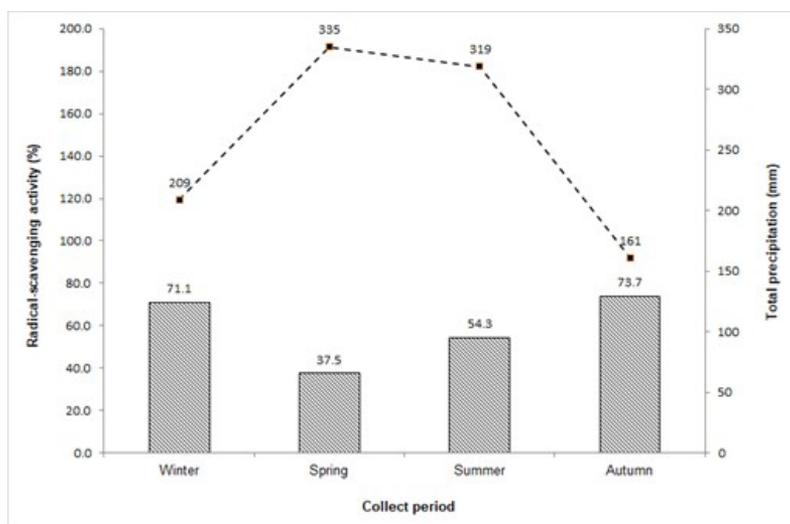


Figure 3. Radical scavenging activity (%) of *Anredera cordifolia* leaves, in a concentration of 200 mg mL⁻¹, and total precipitation (mm) during winter, spring, summer and autumn.

change according to phenological and developmental stages of the plants and the environmental conditions. In *A. cordifolia* leaves, the seasonal effect led to a variation in phenolic content and antioxidant activity. In accordance to recent studies that have demonstrated a higher phenolic content in plant species collected in winter, the TPC found in this study was higher in winter, differing significantly from the other seasons. This was a period of thermal stress, with significantly lower average temperatures. In extracts of *Convolvulus althaeoides* L. leaves, the phenol compounds were also predominant in winter (Hrichi et al., 2020).

Susanti (2019) determined the TPC and the antioxidant activity by DPPH of hexane, chloroform and methanol extracts of *A. cordifolia*, and obtained values of 8.54 ± 0.49, 17.30 ± 0.47 and 32.5 ± 1.11 mg GAE g⁻¹ of dry extract. The values of ES50 (EC50) extracts were 583.601 ± 2.533, 446.219 ± 2.268 and 237.683 ± 13.373 µg mL⁻¹. Our values varied from 18.9 ± 1.30 to 54.4 ± 7.36 mg GAE 100 g⁻¹ of dry matter for TPC and from 56.8 ± 12.2 to 843.2 ± 8.9 mg mL⁻¹ for EC50. Considering that there were different methods of obtained extracts, a comparison between these values might be difficult to make. In Susanti's research, each extract was concentrated and dried by using a rotary evaporator. In our research, due to the large number of samples tested, the obtained extracts were not concentrated in rotary evaporator. Therefore, they were expressed as mg of gallic acid equivalents (GAE) 100 g⁻¹ of dry matter (DM) for TPC and in terms of EC50 values (mg mL⁻¹) for antioxidant activity. The TPC values from our study can be compared with those of Kumar et al. (2020). The study of callus cultures of *Basella rubra* showed that the highest TPC value was 74 mg GAE 100 g⁻¹ of fresh weight. Our results can also be compared to some TPC values of *Basella alba* leaves, that varied from 779.58 to 29.14 mg GAE 100 g⁻¹ (Jayswal et al., 2021). Regarding the chemical composition, the isolation and identification of flavonoids in *A. cordifolia* supports our findings on the total phenolic content and, in turn, the antioxidant action of the plant.

Anredera cordifolia leaves showed a higher capacity to sequester the DPPH radical in autumn and winter, regardless of leaf phenology, which is similar to *Sasa quelpaertensis* leaves that presented a high content of phenolic compounds as well as antioxidant activity during the same seasons (Ko et al., 2018). In the present study, the region where the plant material was collected does not have a dry season or a rainy season; precipitation occurs every month of the year. Thus, the average daily rainfall did not differ significantly in the assessed seasons, but the total rainfall was lower in autumn, followed by winter. These may therefore be considered seasons of water stress. In *papaya* leaves, the content and diversity of phenolic compounds and antioxidant activity increased under drought stress (Espadas et al., 2019). The dry season revealed the most potent antioxidant activity by DPPH test in *Secundatia floribunda* (Ribeiro et al., 2020). According to Albergaria et al. (2020), high concentrations of active substances were produced by plants grown under drought conditions.

In *A. cordifolia*, the thermal stress observed in the 45-day period before each collection caused an increase in phenolics in the younger leaves, so they were the ones whose properties were most affected. As the leaves aged, the phenolic content decreased. Young *A. cordifolia* leaves appear to have a more rapid metabolic response to thermal stress, which results in a greater accumulation of phenolics. Younger tissues have shown to have higher rates of secondary compound biosynthesis (Blum-Silva et al., 2015). Koukounaras et al. (2007) also found higher phenol content in younger leaves of *Eruca sativa* Mill. For *Ilex paraguariensis* leaves, there was a decrease in the production of phenolic compounds over time, being highest in the one-month-old leaves (Blum-Silva et al., 2015). *Ilex paraguariensis* leaves up to six months old, in turn, showed higher methylxanthine content (Esmelindro et al., 2004).

Bezerra et al. (2013) report an increase in total phenolic and rutin content in barley cultivars during periods of

higher rainfall and lower temperatures. However, our study observed that the period of lower total rainfall did not correspond to the period of higher phenolic production in *A. cordifolia*. Albergaria et al. (2020) carried out a systematic review on the effect of water supply on the contents of total phenolic compounds in medicinal plants, concluding that the idea that there is a widespread increase in phenolic compounds in response to water stress is most often incorrect. The authors also point out that most studies have been conducted in controlled conditions. In our case, *A. cordifolia* has been growing in a natural environment, and not under controlled conditions.

Although several studies have already demonstrated a significant correlation between phenolic content and DPPH reagent scavenging activity in plant species (Ghasemzadeh et al., 2010; Ko et al., 2018; Pandey et al., 2016; Ribeiro et al., 2020), in *A. cordifolia* leaves this was not the case. The interaction of an antioxidant with DPPH depends on its structural conformation (Brand-Williams et al., 1995), and phenolic compounds are known to be a very diverse group in terms of chemical structure. A limitation of this study is that the method used does not detect only compounds with favorable structural characteristics in terms of antioxidant activity. Therefore, other compounds may be involved in the reactions, which would justify the low correlation coefficient obtained in this research. Other studies have also found no correlation between phenolic concentration and antioxidant activity (Araújo et al., 2015; Gori et al., 2020).

5. Conclusion

The findings of this study confirmed that the metabolism and production of phenolic compounds in *A. cordifolia* leaves are affected by seasonal factors, such as precipitation and temperature. During winter and autumn, higher thermal and water stress were observed, respectively. As a defense mechanism in response to oxidative stress, plants can increase the synthesis of phenolic compounds, which have antioxidant properties. In the case of *A. cordifolia*, low temperatures led to an increase in phenolic biosynthesis. Within the period observed in this study, young leaves were the most affected. Considering that young tissues generally have higher rates of metabolites biosynthesis, a more intense response of young leaves to environmental stress was observed. Higher radical scavenging activity was observed during periods of lower rainfall, regardless of leaf age. Therefore, collection in periods of more stress, either thermal or water stress, favors the attainment of plant material richer in phenolic content and, consequently, with greater antioxidant potential.

Acknowledgements

The authors are grateful to Programa de Pós-Graduação em Ciências Ambientais at the Universidade de Passo Fundo and the Fundação Universidade de Passo Fundo for the scholarship, and to Renato Michel for statistical assistance.

References

- ALBA, T.M., PELEGRIN, C.M.G. and SOBOTTKA, A.M., 2020. Ethnobotany, ecology, pharmacology, and chemistry of *Anredera cordifolia* (Basellaceae): a review. *Rodriguésia*, vol. 71, pp. 01042019. <http://dx.doi.org/10.1590/2175-7860202071060>.
- ALBERGARIA, E.T., OLIVEIRA, A.F.M. and ALBUQUERQUE, U.P., 2020. The effect of water deficit stress on the composition of phenolic compounds in medicinal plants. *South African Journal of Botany*, vol. 131, pp. 12-17. <http://dx.doi.org/10.1016/j.sajb.2020.02.002>.
- ARAÚJO, T.A.S., CASTRO, V.T.N.A., SOLON, L.G.S., SILVA, G.A., ALMEIDA, M.G., COSTA, J.G.M., AMORIM, E.L.C. and ALBUQUERQUE, U.P., 2015. Does rainfall affect the antioxidant capacity and production of phenolic compounds of an important medicinal species? *Industrial Crops and Products*, vol. 76, pp. 550-556. <http://dx.doi.org/10.1016/j.indcrop.2015.07.008>.
- BEN AHMED, Z., YOUSFI, M., VIAENE, J., DEJAEGHER, B., DEMEYER, K., MANGELINGS, D. and VANDER HEYDEN, Y., 2017. Seasonal, gender and regional variations in total phenolic, flavonoid, and condensed tannins contents and in antioxidant properties from *Pistacia atlantica* ssp. leaves. *Pharmaceutical Biology*, vol. 55, no. 1, pp. 1185-1194. <http://dx.doi.org/10.1080/13880209.2017.1291690>. PMID:28245729.
- BEZERRA, A.S., NÖRNBERG, J.L., LIMA, F.O., ROSA, M.B. and CARVALHO, L.M., 2013. Parâmetros climáticos e variação de compostos fenólicos em cevada. *Ciência Rural*, vol. 43, no. 9, pp. 1546-1552. <http://dx.doi.org/10.1590/S0103-84782013000900003>.
- BLUM-SILVA, C.H., CHAVES, V.C., SCHENKEL, E.P., COELHO, G.C. and REGINATTO, F.H., 2015. The influence of leaf age on methylxanthines, total phenolic content, and free radical scavenging capacity of *Ilex paraguariensis* aqueous extracts. *Revista Brasileira de Farmacognosia*, vol. 25, no. 1, pp. 1-6. <http://dx.doi.org/10.1016/j.bjbp.2015.01.002>.
- BRAND-WILLIAMS, W., CUVELIER, M.E. and BERSET, C., 1995. Use of a free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft + Technologie*, vol. 28, no. 1, pp. 25-30. [http://dx.doi.org/10.1016/S0023-6438\(95\)80008-5](http://dx.doi.org/10.1016/S0023-6438(95)80008-5).
- CORY, H., PASSARELLI, S., SZETO, J., TAMEZ, M. and MATTEI, J., 2018. The role of polyphenols in human health and food systems: a mini-review. *Frontiers in Nutrition*, vol. 5, pp. 87. <http://dx.doi.org/10.3389/fnut.2018.00087>. PMID:30298133.
- DALMAGRO, A.P., CAMARGO, A., SILVA FILHO, H.H., VALCANAI, M.M., DE JESUS, P.C. and ZENI, A.L.B., 2018. Seasonal variation in the antioxidant phytochemicals production from the *Morus nigra* leaves. *Industrial Crops and Products*, vol. 123, pp. 323-330. <http://dx.doi.org/10.1016/j.indcrop.2018.06.085>.
- ESMELINDRO, Â.A., GIRARDI, J.S., MOSSI, A., JACQUES, R.A. and DARIVA, C., 2004. Influence of agronomic variables on the composition of mate tea leaves (*Ilex paraguariensis*) extracts obtained from CO₂ extraction at 30 degrees C and 175 bar. *Journal of Agricultural and Food Chemistry*, vol. 52, no. 7, pp. 1990-1995. <http://dx.doi.org/10.1021/jf035143u>. PMID:15053541.
- ESPADAS, J.L., CASTAÑO, E., MARINA, M.L., RODRÍGUEZ, L.C. and PLAZA, M., 2019. Phenolic compounds increase their concentration in *Carica papaya* leaves under drought stress. *Acta Physiologiae Plantarum*, vol. 41, no. 11, pp. 180. <http://dx.doi.org/10.1007/s11738-019-2972-0>.
- GHASEMZADEH, A., JAAFAR, H.Z.E. and RAHMAT, A., 2010. Antioxidant activities, total phenolics and flavonoids content in two varieties of malaysian young ginger (*Zingiber officinale* Roscoe). *Molecules*, vol. 15, no. 6, pp. 4324-4333. <http://dx.doi.org/10.3390/molecules15064324>. PMID:20657444.

- GORI, A., NASCIMENTO, L.B., FERRINI, F., CENTRITTO, M. and BRUNETTI, C., 2020. Seasonal and diurnal variation in leaf phenolics of three medicinal Mediterranean wild species: what is the best harvesting moment to obtain the richest and the most antioxidant extracts? *Molecules*, vol. 25, no. 4, pp. 956. <http://dx.doi.org/10.3390/molecules25040956>. PMID:32093396.
- HRICHI, S., CHAABANE-BANAOUES, R., GIUFFRIDA, D., MANGRAVITI, D., MAJDOUB, Y.O., RIGANO, F., MONDELLO, L., BABBA, H., MIGHRI, Z. and CACCIOLA, F., 2020. Effect of seasonal variation on the chemical composition and antioxidant and antifungal activities of *Convolvulus althaeoides* L. leaf extracts. *Arabian Journal of Chemistry*, vol. 13, no. 6, pp. 5651-5668. <http://dx.doi.org/10.1016/j.arabjc.2020.04.006>.
- JAYSWAL, V.B., PATEL, V.H. and DAVE, N.R., 2021. Effect of various solvents on the extraction of antioxidants from dried *Basella alba* leaves. *Applied Biological Research*, vol. 23, no. 2, pp. 129-135. <http://dx.doi.org/10.5958/0974-4517.2021.00018.5>.
- KINUPP, V.F. and LORENZI, H., 2014. *Plantas alimentícias não convencionais (PANC) no Brasil: guia de identificação, aspectos nutricionais e receitas ilustradas*. São Paulo: Instituto Plantarum de Estudos da Flora, 768 p.
- KO, H.C., LEE, J.Y., JANG, M.G., SONG, H. and KIM, S.-J., 2018. Seasonal variations in the phenolic compounds and antioxidant activity of *Sasa quepaertensis*. *Industrial Crops and Products*, vol. 122, pp. 506-512. <http://dx.doi.org/10.1016/j.indcrop.2018.06.031>.
- KOUKOUNARAS, A., SIOMOS, A.S. and SFAKIOTAKIS, E., 2007. Postharvest CO₂ and ethylene production and quality of rocket (*Eruca sativa* Mill.) leaves as affected by leaf age and storage temperature. *Postharvest Biology and Technology*, vol. 46, no. 2, pp. 167-173. <http://dx.doi.org/10.1016/j.postharvbio.2007.04.007>.
- KUMAR, S.S., ARYA, M., MAHADEVAPPA, P. and GIRIDHAR, P., 2020. Influence of photoperiod on growth, bioactive compounds and antioxidant activity in callus cultures of *Basella rubra* L. *Journal of Photochemistry and Photobiology B: Biology*, vol. 209, pp. 111937.
- LIN, Y., LOU, K., WU, G., WU, X., ZHOU, X., FENG, Y., ZHANG, H. and YU, P., 2020. Bioactive metabolites in of *Ginkgo biloba* leaves: variations by seasonal, meteorological and soil. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 80, no. 4, pp. 790-797. <http://dx.doi.org/10.1590/1519-6984.220519>. PMID:31800764.
- MARTINEVSKI, C.S., OLIVEIRA, V.R., RIOS, A. O., FLORES, S.H. and VENZKE, J.G., 2013. Utilização de Bertalha (*Anredera cordifolia* (Ten.) Steenis) e Ora-pro-nobis (*Pereskia aculeata* Mill.) na elaboração de pães. *Alimentos e Nutrição = Brazilian Journal of Food and Nutrition*, vol. 24, no. 3, pp. 1-6.
- MEO, S. and VENDITTI, P., 2020. Evolution of the knowledge of free radicals and other oxidants. *Oxidative Medicine and Cellular Longevity*, vol. 2020, pp. 9829176. <http://dx.doi.org/10.1155/2020/9829176>. PMID:32411336.
- OSORIO-TOBÓN, J.F., 2020. Recent advances and comparisons of conventional and alternative extraction techniques of phenolic compounds. *Journal of Food Science and Technology*, vol. 57, no. 12, pp. 4299-4315. <http://dx.doi.org/10.1007/s13197-020-04433-2>. PMID:33087945.
- PANDEY, M.M., KHATOON, S., RASTOGI, S. and RAWAT, A.K.S., 2016. Determination of flavonoids, polyphenols and antioxidant activity of *Tephrosia purpurea*: a seasonal study. *Journal of Integrative Medicine*, vol. 14, no. 6, pp. 447-455. [http://dx.doi.org/10.1016/S2095-4964\(16\)60276-5](http://dx.doi.org/10.1016/S2095-4964(16)60276-5). PMID:27854196.
- RAMAKRISHNA, R. and RAVISHANKAR, G.A., 2011. Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signaling & Behavior*, vol. 6, no. 11, pp. 1720-1731. <http://dx.doi.org/10.4161/psb.6.11.17613>. PMID:22041989.
- RASOULI, H., FARZAEI, M.H. and KHODARAHMI, R., 2017. Polyphenols and their benefits: a review. *International Journal of Food Properties*, vol. 20, no. S2, pp. 1700-1741. <http://dx.doi.org/10.1080/10942912.2017.1354017>.
- RIBEIRO, D.A., CAMILO, C.J., NONATO, C.F.A., RODRIGUES, F.F.G., MENEZES, I.R.A., RIBEIRO-FILHO, J., XIAO, J., SOUZA, M.M.A. and COSTA, J.G.M., 2020. Influence of seasonal variation on phenolic content and *in vitro* antioxidant activity of *Secondatia floribunda* A. DC. (Apocynaceae). *Food Chemistry*, vol. 315, pp. 126277. <http://dx.doi.org/10.1016/j.foodchem.2020.126277>. PMID:32004983.
- SOUSA, C.M. M., SILVA, H.R., VIEIRA-JR, G.M., AYRES, M.C.C., COSTA, C.L.S., ARAÚJO, D.S., CAVALCANTE, L.C.D., BARROS, E.D.S., ARAÚJO, P.B.M., BRANDÃO, M.S. and CHAVES, M.H., 2007. Fenóis totais e atividade antioxidante de cinco plantas medicinais. *Química Nova*, vol. 30, no. 2, pp. 351-355. <http://dx.doi.org/10.1590/S0100-40422007000200021>.
- SOUZA, V.C. and LORENZI, H., 2019. *Botânica Sistemática: guia ilustrado para identificação das famílias de Fanerógamas nativas e exóticas do Brasil*. 4th ed. São Paulo: Instituto Plantarum, 768 p.
- SUSANTI, H., 2019. Total phenolic content and antioxidant activities of binahong (*Anredera cordifolia*). *Indonesian Journal of Medicine and Health*, vol. 10, no. 2, pp. 171-175. <http://dx.doi.org/10.20885/IJKKI.Vol10.Iss2.art9>.
- WREGE, M.S., STEINMETZ, S., REISSER JÚNIOR, C. and ALMEIDA, I.R., 2012. *Atlas climático da região Sul do Brasil: estados do Paraná, Santa Catarina e Rio Grande do Sul*. 2nd ed. Brasília, DF: Embrapa, 333 p.