v. 3, n. 5, 2023

Journal of
Agricultural
Sciences
Research

INFLUENCE OF SEASONALITY AND DIAMETER CLASS ON RUTIN CONTENT IN *PIPTADENIA GONOACANTHA* (MART.) J. F. MACBR. (FABACEAE)

Cristiane Aparecida de Faria
Universidade Federal de Viçosa.
Departamento de Medicina e Enfermagem.
Viçosa – MG
http://lattes.cnpq.br/2440565632763511
https://orcid.org/0000-0002-8788-2514

Marina Bonifácio Fávaro
Universidade Federal de Viçosa.
Departamento de Engenharia Florestal.
Viçosa – MG
http://lattes.cnpq.br/1586227836242813
https://orcid.org/0000-0001-5112-6218

Gisele Carvalho Mendes
Universidade Federal de Viçosa.
Departamento de Medicina e Enfermagem.
Viçosa – MG
http://lattes.cnpq.br/0381650311768865
https://orcid.org/0000-0001-6823-0783

Marcelo Barcellos da Rosa
Universidade Federal de Santa Maria.
Departamento de Química.
Santa Maria – Rio Grande do Sul
http://lattes.cnpq.br/0308293154958870
https://orcid.org/0000-0001-5959-0381

Marilane de Oliveira Fani Amaro
Universidade Federal de Viçosa.
Departamento de Medicina e Enfermagem.
Viçosa – MG
http://lattes.cnpq.br/8475779445932134
https://orcid.org/0000-0002-9495-0861

Luciana Moreira Lima
Universidade Federal de Viçosa.
Departamento de Medicina e Enfermagem.
Viçosa – MG
http://lattes.cnpq.br/2013048264104100
https://orcid.org/0000-0001-5349-1577

Haroldo Nogueira de Paiva
Universidade Federal de Viçosa.
Departamento de Engenharia Florestal.
Viçosa, MG, Brasil
Viçosa – MG
http://lattes.cnpq.br/8319339345020079
https://orcid.org/0000-0002-8891-5373

Camilo Amaro de Carvalho
Universidade Federal de Viçosa.
Departamento de Medicina e Enfermagem.
Viçosa – MG
http://lattes.cnpq.br/2890862161339721
https://orcid.org/0000-0001-9539-8653

Abstract: Seasonality is related to the variations that occur in the distinct seasons. It affects the concentration of secondary metabolites in plants, as rutin content. This study assessed the influence of seasonality and diameter at breast height (dbh) class on rutin content in *Piptadenia gonoacantha* extracts. Leaflets harvested throughout the four seasons from trees in distinct dbh classes were dried and grinded. Hydroalcoholic extracts (80%) were prepared by maceration, filtrated, concentrated via rotary evaporation. Samples and standards were evaluated in a spectrophotometer (420 nm). The highest rutin contents were registered in the extracts from *P. gonoacantha* leaflets collected in the summer (average 117.4 μg mL^{-1}), while the lowest contents were detected in the extracts made with samples from winter harvesting (average 24.3 μg mL^{-1}). The average rutin contents in the extracts prepared with leaflets sourced during autumn and spring were not statistically different: 93.4 and 94.1 μg mL^{-1}, respectively. Rutin content increased in samples from spring, autumn and winter harvesting as trunk diameter augmented; no significant differences were seen among the dbh classes regarding the samples collected in the summer. Thus, harvest time and trunk diameter influenced the content of rutin in *P. gonoacantha* leaflets, which impacts on the quality and efficacy of products made from these extracts.

Keywords: Phenolic Compounds; Climate; Quality Control.

INTRODUÇÃO

The study of medicinal plants plays an essential role in the exploitation and discovery of their natural resources. Their therapeutic action is due to the secondary metabolites, whose contents and production undergo variations in amount or even in the nature of the active constituents, depending on characteristics as harvest time and developmental stage (JAMSHIDI-KIA LORIGOOINI, AMINI-KHOEI, 2018; LI et al., 2020). Seasonality, in turn, relates to the variations which arise from the different seasons of the year, and influences the content of the secondary metabolites (MARTINS, 2015).

Piptadenia gonoacantha (Mart.) J. F. Macbr. is an arboreal species of the family Fabaceae which is native from the Atlantic Forest in southern and southeastern Brazil. It is popularly known as alligator stick and typically used to treat inflammatory disorders (CARVALHO et al., 2004). Early studies with *P. gonoacantha* have demonstrated anti-inflammatory, antinociceptive, wound healing and antibacterial activities, as well as the presence of flavonoid compounds, thus indicating a potential for the development of pharmaceutical preparations (CARVALHO, 2004; CARVALHO et al., 2010; CARVALHO et al., 2014a; CARVALHO et al., 2014b; FRANCO et al., 2021; PAIVA et al., 2021).

Flavonoids are phenolic compounds that are largely studied and recognized for their health benefits. Over 8.000 flavonoids have been identified thus far. They are among the most ubiquitous groups of secondary metabolites, being mostly found within the surfaces or cells of multiple plant organs and tissues (MUTHA et al., 2021). Rutin is one of the most important flavonoids; it is widespread in the plant kingdom, being present in considerable amounts in *P. gonoacantha* leaves (ARAÚJO, 2012; MACÊDO et al., 2017).

Numerous investigations have demonstrated the physiological effect and the pharmacological properties of rutin by revealing its efficacy in reducing the risk of chronic diseases and evidencing its protective effect. Researches indicate a myriad of health benefits of this flavonoid, such as anticancer, antidiabetic, neuroprotective, antihypertensive and cholesterol-lowering effects, besides reducing the risk of cardiovascular diseases and improving hearing and vision. Its remarkable functionality indicates the promising potential of rutin to be used in drug formulation (CHUA, 2013).

Current trends in the extraction process have focused on sustainable extraction from natural sources aimed at optimization. In order to contribute towards a better understanding of the species, this research aims at quantifying the content of rutin in *P. gonoacantha* extracts prepared with leaflets collected from trees in different diameter classes throughout the four seasons.

MATERIAL AND METHODS

PLANT MATERIAL

The plant material was harvested in a 44.11-ha forest fragment in the Technology Center of Viçosa – MG (CENTEV) situated at a mean altitude of 721 m (SOUZA et al., 2014) within the geographic coordinates 42° 51' W and 20° 42' S (TORRES et al., 2013). It was comprised of leaves and leaflets of alligator stick (*P. gonoacantha*), which were sourced from three trees within each of the following diameter at breast height (dbh) classes: 1 (5-10 cm), 2 (10-15 cm), 3 (15-20 cm), 4 (20-25 cm) and 5 (> 25 cm). The specimens were identified and marked, and sampling was performed along the four seasons: spring, summer, autumn and winter. The obtained material was identified and the exsiccate was deposited in the Herbarium of the Federal University of Viçosa (UFV) under the registration number 35530.

After sampling, the leaves and leaflets were dried in an oven with air circulation at 60°C for three days. Subsequently, the leaflets were manually separated from the petiole and chopped in a knife mill.

PREPARATION OF EXTRACTS

The use of ethanol 80% (v/v) as solvent was based on a previous study assessing extracts of *P. gonoacantha* (CARVALHO, 2014b). The extracts were prepared from the powdered leaflets of *P. gonoacantha* in a ratio of 1:5 (25 g powder: 125 mL of a 80% v/v solution of ethanol in water with 0.3% citric acid), which resulted in a concentration of 20% of dry extract (m/v).

Each extract was subjected to the maceration process for 48h at room temperature. When this period had elapsed, the extract was filtered through a filter paper and the filtrate was stored at 2-8°C in amber glass in the absence of light. The residue retained on the filter paper was then extracted twice using the same extracting solution. At the end of the process, the filtrates were collected in amber glass and kept away from light until solvent removal. The extracts were concentrated using a rotary evaporator (Buchi®) at 60°C with the aid of a vacuum pump (Prismatec®). Lastly, the samples were properly identified and refrigerated at 2-8°C in amber glass protected from light (RIBEIRO, 2018).

QUANTIFICATION OF RUTIN

The rutin standard was prepared in 100.0 mL volumetric flasks with methanolic solution of aluminium chloride at 5%. Each flask received 0.6 mL of glacial acetic acid, 10.0 mL of methanolic solution of pyridine at 20% and 2.5 mL of aluminium chloride reagent in methanol 50.0 mg L^{-1}. Distilled water was then added to give the final volume of 100.0 mL. This solution was diluted in aluminium chloride reagent at 5% in methanol. After 30 minutes at room temperature, reading was performed in spectrophotometer at 420 nm using the methanolic solution of aluminium chloride at 5% as the white solution (PEIXOTO SOBRINHO, *et al*., 2008).

The same methanolic solution of aluminium chloride used to build the curve with the rutin standard was added to the extracts. After 30 minutes at room temperature, reading was carried out in spectrophotometer at 420 nm using quartz cuvettes. Rutin content in the extracts, expressed as rutin equivalent, was obtained using the linear regression equation given by the standard curve of rutin.

STATISTICAL ANALYSIS

The data were analyzed using GraphPad Prism 6.01 (GRAPHPAD SOFTWARE Inc., San Diego, CA, USA). Statistically significant differences between treatments were assessed via analysis of variance (ANOVA) followed by multiple comparisons. The significance threshold was set at 0.05.

RESULTS

A calibration curve of the rutin standard was built and evaluated through spectrophotometry, and linearity was shown at 420 nm for the investigated concentrations (62.5 - 7.81 μg mL^{-1}). The linear regression equation obtained for the calibration curve was $y = 24.725x + 0.0284$, where y corresponds to absorbance (nm) and x to content (μg mL^{-1}) expressed as rutin equivalent (figure 1).

The equation was used to calculate the rutin equivalent. Results are presented in table 1.

The highest rutin contents were observed in the extracts from *P. gonoacantha* leaflets collected in the summer, with an average of 117.4 μg mL^{-1} ($p<0.05$). The average rutin contents in the extracts made with leaflets sourced during autumn and spring were not statistically different ($p>0.05$): 93.4 and 94.1 μg mL^{-1}, respectively. The average rutin contents in the extracts made with plant samples from winter harvesting was 24.3 μg mL^{-1}, which was 79.3% lower than that found in the extracts made with leaflets collected in the summer (figure 2).

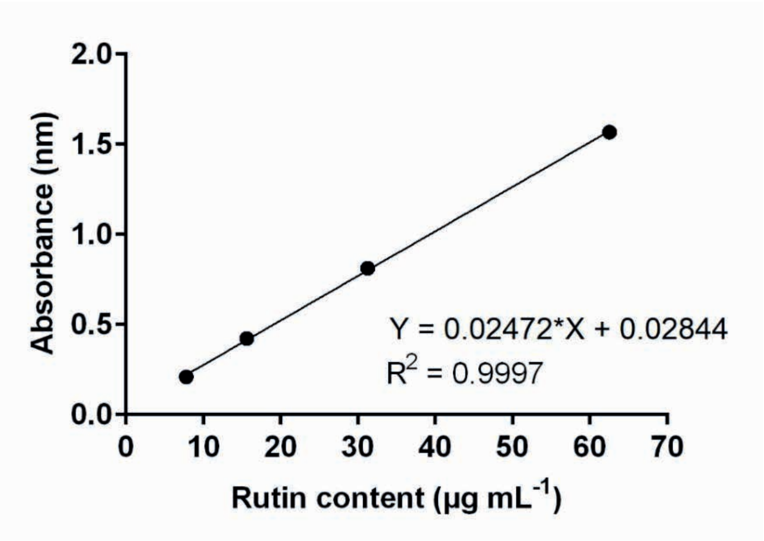

Figure 1 – Calibration curve constructed with the rutin standard at 420 nm.

		Tree (rutin content in μg mL^{-1})		
Season	***dbh** class**	**1**	**2**	**3**
Spring	**1**	83.2	90.5	86.4
	2	80.9	93.0	86.95
	3	104.4	101.8	103.1
	4	92.8	94.8	93.8
	5	97.9	102.4	100.15
Summer	**1**	118.9	108.4	120.8
	2	118.4	103.5	120.9
	3	121.8	120.9	112.8
	4	118.8	119.0	118.7
	5	119.7	119.6	118.9
Autumn	**1**	81.9	77.2	85.8
	2	85.8	70.1	79.1
	3	93.3	79.1	113.4
	4	104.9	104.7	90.0
	5	119.5	111.1	104.5
Winter	**1**	16.6	24.5	16.9
	2	17.6	22.1	18.1
	3	38.1	33.8	20.8
	4	30.4	27.2	23.6
	5	17.4	31.0	26.3

*dbh – diameter at breast height.

Tabela 1 – Rutin content, expressed as rutin equivalent, in the extracts obtained from the leaflets of *Piptadenia gonoacantha* per diameter class and season.

Tree diameter also proved to influence the content of rutin extracted from the leaflets (figure 3). Such data suggest a positive correlation between plant diameter and rutin content.

The average rutin contents found for classes 1 to 5 were, respectively: 75.9, 74.7, 87.0, 84.9 and 89.0 μg mL^{-1}. A greater rutin content was registered for the trees in the > 25 cm dbh class, followed by those in the 15-20 cm and 20-25 cm dbh classes, respectively.

When comparing diameter class and season (figure 4), there was a tendency towards higher average rutin content as the diameter class increased in winter, autumn and spring. In summer, nonetheless, there was no significant difference in average rutin contents among the five dbh classes. Rutin contents were greater in the summer in classes 1, 2, 4 and 5 ($p<0.05$), while no differences were seen between spring and autumn in these four classes ($p>0.05$). In class 3, there were no significant differences in rutin contents between summer, spring and autumn ($p>0.05$). Significantly lower rutin contents were detected in winter ($p<0.05$) in all dbh classes.

DISCUSSION

The current study found the highest rutin contents in the leaflets of *P. gonoacantha* sampled in the summer. This season is characterized by higher temperatures and rapid changes in the daily weather patterns, with intensive rain showers over a short period (CPTEC/INPE, 2021).

Scientific evidence shows that the quality of phenolic compounds in plant tissues may be affected by factors as genotype, developmental stage, environment, harvest time, processing and storage conditions, and the analytical method (VAGIRI et al., 2020; BUJOR et al., 2018). In keeping with such data, Isah (2019) states that cellular and developmental physiological processes of plants are influenced by environmental factors; the plants, in turn, produce an assortment of secondary metabolites to counteract the consequences of these changes. Seasonality is reported as a contributing factor towards the production of secondary metabolites (GOBBO-NETO, LOPES, 2006).

The central parts of Brazil, including the Southeast region, are characterized by a six-month wet season, from October to March, and a six-month dry season, from April to September (RAO *et al.,* 2015). Therefore, the region where the samples were collected for this assessment has a greater water availability in summer and spring than in autumn and winter.

In line with the findings of this investigation, research data points to a greater content of phenolic compounds and flavonoids in the rainy season compared to the dry season (ZANELLA, 2017). The highest precipitation values in the *P. gonoacantha* sampling area are seen during the summer, and the leaflets sourced in this season presented the highest rutin contents. Likewise, a study developed with *Hypericum perforatum* L. (St. John's wort) revealed a significant increase in the content of flavonoids when water availability is improved (GRAY *et al.,* 2003).

Martins (2015) suggested that the production of these compounds may be related to the climate variability along the year. The author stated that there may be an alteration in the composition of the metabolites produced by a plant according to the harvest time, since the presence of flavonoids tends to increase in the leaves as the temperature rises, as observed in the current analysis. In opposition to the present findings, however, Martins (2015) found a lower availability of flavonoids in leaves collected during periods of heavier rainfall.

A greater content of total phenolic acids and flavonoids was seen in apical leaves of

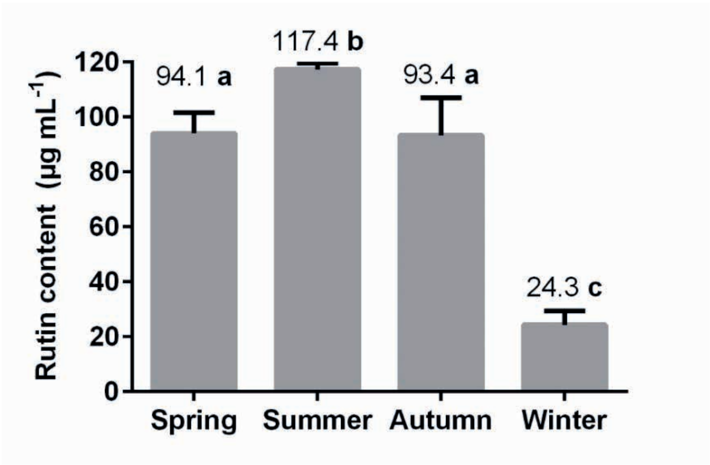

Figure 2 – Rutin content in *Piptadenia gonoacantha* leaflets per season. Statistical analysis was performed using analysis of variance (ANOVA) followed by multiple comparisons with a significance level of 5%. Distinct letters indicate statistically significant difference between the analyzed groups.

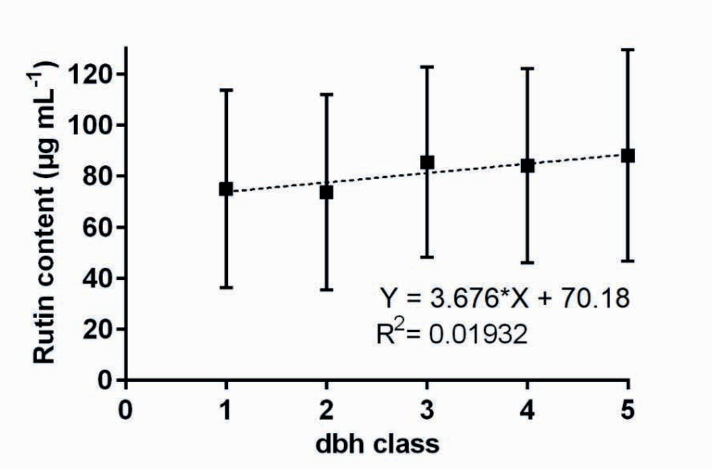

Figure 3 – Correlation between rutin content and trunk diameter of *Piptadenia gonoacantha* according to the following diameter at breast height (dbh) classes: 1 = 5-10 cm, 2 = 10-15 cm, 3 = 15-20 cm, 4 = 20-25 cm and 5 = > 25 cm.

Journal of Agricultural Sciences Research ISSN 2764-0973 DOI 10.22533/at.ed.9733523260410

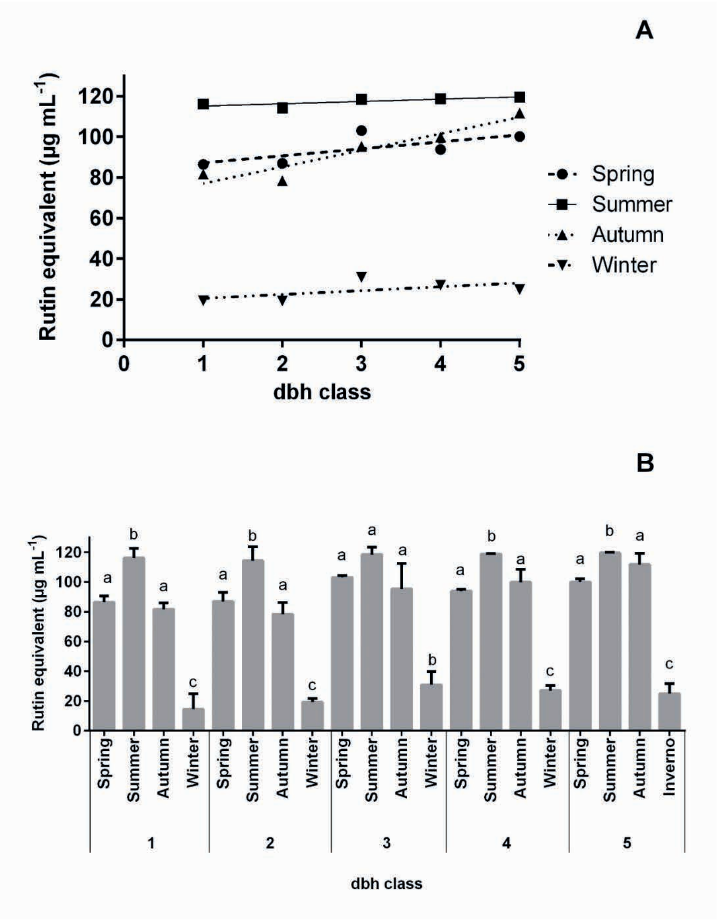

Figure 4 – Correlation between rutin equivalent (µg mL^{-1}) per season and trunk diameter of *Piptadenia gonoacantha* according to the following diameter at breast height (dbh) classes: 1 = 5-10 cm, 2 = 10-15 cm, 3 = 15-20 cm, 4 = 20-25 cm and 5 = > 25 cm. Distinct letters indicate statistically significant difference between the analyzed groups.

Mentha spicata L (spearmint), *Cymbopogon citratus* (DC) Stapf. (lemon grass) and *Aloysia citriodora* Palàu (lemon verbena) harvested in the hottest months of summer; no difference was identified for the samples of *Thymus x citriodorus* L (lemon thyme) (RITA, 2017). Such data ties well with the present results, which indicate a variation in rutin content according to season, with higher contents being detected in the plant samples from summer harvesting.

According to Vagiri *et al.* (2015), ontogenic age as well as sampling time affected the content of phenolic compounds, with higher levels being registered in the beginning of summer regardless the position of the leaves on the shoot; furthermore, some of the compounds isolated from apical leaves presented particularly elevated contents in the same harvest time. Similarly, Masa et al. (2016) verified that the production of compounds derived from secondary metabolism in *Cistus ladanifer* L. (gum rockrose) is dependent on season, with greater amounts being obtained in the summer. These researchers further identified a high variability in the content of these compounds within the plant and along the year; the young leaves showed higher levels in the summer than the mature leaves in the winter.

When solar radiation is of high intensity, plants tend to enhance the production of antioxidant substances, such as phenolic compounds and flavonoids, to protect themselves from the ultraviolet rays (TAIZ and ZEIGER, 2004). During the summer, the ultraviolet index reaches maximum levels in a large area of Brazil, including the Southeast region, because the solar radiation suffers less attenuation by atmospheric components as a result of the angle of inclination of the Earth as it revolves around the Sun (CORRÊA, 2015). This condition is likely to have influenced the rutin contents in *P. gonoacantha* extracts, which were greater in summer than in winter.

An assessment conducted by Vázquez-León *et al.* (2017) with *Moringa oleifera* Lam. (drumstick tree) observed an elevation in contents of bioactive compounds showing antiradical activity with the increase in solar radiation, mean temperature, ultraviolet radiation and precipitation. The authors suggest that such environmental conditions stress the plants, whose antioxidant defense system is then activated to counteract the harmful effects.

Tree growth is the increase in dimensions with time. Diameter increment reaches its peak in the early- to mid-life of a tree, progressively reducing with size and age (BOWMAN *et al*., 2013). When using the tree-ring analysis approach to evaluate *P. gonoacantha* growth dynamics, Brandes *et al*. (2016) found that the radial growth rate is not constant; instead, it oscillates across lifespan, besides differing among the individuals. Nonetheless, it is generally assumed that the increase in diameter is related to the age of trees; therefore, trees of larger diameter tend to be older than those of smaller diameter, and vice-versa.

Shrubs of two, three and six years were sampled during flowering and analyses of their antioxidant, phenol and flavonoids contents showed the greatest results for the six-year-old plant (MORADI H, GHAVAM M, TAVILI, 2019). The composition of essential oils also varies according to plant age at harvest (FARIAS, 2018). This is in agreement with the findings of the current research regarding the detection of higher rutin contents in the extracts made with leaflets from trees with wider trunks, that is, older trees.

Different accumulation trends may also be seen in *Salix pyrolifolia* Ledeb (Balsam Willow) on account of the developmental and aging processes as well as the distinct phases of ontogeny; flavonoids predominate in the vegetative buds, with a significantly lower concentration in 1-year-old trees compared to older ones (LAVOLA, MAUKONEN, JULKUNEN-TIITTO, 2018). Such data is consistent with the lower rutin contents registered in *P. gonoacantha* extracts prepared with leaflets sourced from trees with a smaller diameter, which are most probably younger.

Results that contradict those disclosed herein may also be found in the scientific literature, thus evidencing the complexity and the variability regarding the concentration of active plant constituents. Szymborska-Sandhu et al. (2020), for instance, obtained higher quantities of flavonoids in 2-year-old *Melittis melissophyllum* L. (bastard balm) plants compared to 3- and 4-year-old plants; the researchers suggest that developmental biology may be accountable for this outcome.

CONCLUSION

The average rutin content was higher in the extracts from *P. gonoacantha* leaflets sampled in the summer, and no statistically significant difference was observed among the diameter classes in this season. The lowest amounts of rutin were detected in the extracts prepared with the leaflets collected in winter. Rutin content linearly increased in samples from spring, autumn and winter harvesting as trunk diameter augmented. Overall, the variations in rutin content in the analyzed material indicate that harvest time and trunk diameter influenced the amount of this constituent in *P. gonoacantha* leaflets.

As rutin is a biomarker, the season when the plant is harvested as well as the dbh may be used as parameters for an optimized collection of the material to be used in the production of *P. gonoacantha* extracts with respect to the desired composition of bioactive compounds destined for a specific purpose.

REFERENCIAS

ARAÚJO M. E. M. B. **Avaliação da atividade antioxidante e antiproliferativa da rutina e seus produtos obtidos por hidrolise enzimática** [Dissertação]. Bragança Paulista: Universidade São Francisco; 2012.

BOWMAN D. M. J. S; BRIENEN J. W. R; GLOOR E; PHILLIPS L. O; PRIOR L. D. **Detecting trends in tree growth: not so simple.** Tre in Plan Sci 2013; 18: 11-17, doi: 10.1016/j.tplants.2012.08.005.

BRANDES A. F. N; ALBUQUERQUE R. P; MORAES L. F. D; BARROS C. F. **Annual tree rings in *Piptadenia gonoacantha* (Mart.) J.F.Macbr. in a restoration experiment in the Atlantic Forest: potential for dendroecological research**. Acta Bot Bras 2016; 30: 383-388, doi: 10.1590/0102-33062016abb0101.

BUJOR O. C; GINIES C; POPA V.I; DUFOUR C. **Phenolic compounds and antioxidant activity of lingonberry (*Vaccinium vitis-idaea* L.) leaf, stem and fruit at different harvest periods**. Food Chem 2018; 252: 356-365, doi: 10.1016/j.foodchem.2018.01.052.

CARVALHO C. A; SANTANA G. S; AMARO O. F. M; FRANCO A. J; PINTO R; ZATTI R. A; et al. **Antinociceptive and anti-inflammatory effects of hydroalcoholic extract of leaves of *Piptadenia gonoacantha* (Mart.) Macbr. in experi-mental animal models**. Ciê e Nat 2014; 36: 775–781, doi: 10.5902/2179460X13555. B

CARVALHO P. E. R. **Pau-Jacaré - *Piptadenia gonoacantha***. Cir Téc Embrap. 2004 dez; 91:12.

CARVALHO C. A; SANTANA G. S; AMARO M. O. F; LIMA L. M; PIRES F. B; PRÁ V. D; CARDOSO S; ROSA M. B; OLIVEIRA L. L. **Aspectos químicos e atividade antibacteriana de *Piptadenia gonoacantha* (FABACEAE)**. Ciência e Natura 2014; 36: 731-744, doi: 10.5902/2179460X13456. A

CARVALHO M. G; CARDOZOI M. A. R; CATUNDA JUNIOR F. E. A; CARVALHO A. G. **Chemical constituents of *Piptadenia gonoacantha* (Mart.) J.F. Macbr (pau jacaré).** Anais da Academia Brasileira de Ciências. Rio de Janeiro. v.82, n.3, pp. 561-567, 2010.

Centro de Previsão de Tempo e Estudos Climáticos/ Instituto Nacional de Pesquisas Espaciais (CPTEC/INPE). Estações do ano. http://clima1.cptec.inpe.br/estacoes/pt. Acesso em 17 de março de 2021.

CHUA L. S. **A review on plant-based rutin extraction methods and its pharmacological activities. J Ethnopharmacol 2013**; 150: 805-817. doi: 10.1016 / j.jep.2013.10.036

CORRÊA M. P. **Solar ultraviolet radiation: properties, characteristics and amounts observed in Brazil and South America**. A Bras Dermatol 2015; 90: 297-313, doi: 10.1590/abd1806-4841.20154089.

FARIAS J. P. **Influência da idade da planta da composição química e atividades biológicas do** óleo **essencial de erva-baleeira (*Varronia curassavica* Jacq.)** [Dissertação]. Parnaíba: Universidade Federal do Piauí, UFPI; 2018.

FRANCO A. F; PEREIRA C.G; SILVA K.V; ALMEIDA G. F. G; AMARO M. O. F; CALDEIRA E. A. C; et al. Antimicrobial activity of dermocosmetic formulations based on *Piptadenia gonoacantha*. Ciê. e Nat 2021; 43: 31, doi: 10.5902/2179460X43704.

GOBBO-NETO L; LOPES N. P. **Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários**. Quím Nova 2006; 30: 374-381, doi: 10.1590/S0100-40422007000200026.

GraphPad Prism Software Inc. Versão 6.01. [Programa de computador] San Diego: GraphPad Prism Software Inc. 2012.

GRAY D. E; PALLARDY S. G; GARRETT H. E; ROTTINGHAUS G. E. **Effect of acute drought stress and time of harvest on phytochemistry and dry weight of St. John's wort leaves and flowers**. Planta Med 2003; 69: 1024-1030, doi: 10.1055/s-2003-45150.

ISAH T. **Stress and defense responses in plant secondary metabolites production**. Biol Res 2019; 52: 25, doi: 10.1186/s40659-019-0246-3.

JAMSHIDI-KIA F; LORIGOOINI Z; AMINI-KHOEI H. **Medicinal plants: Past history and future perspective**. Jour Herb Pharm 2018, 7: 1 -7, doi: 10.15171/jhp.2018.01.

LAVOLA A; MAUKONEN M; JULKUNEN-TIITTO R. **Variability in the composition of phenolic compounds in winter-dormant *Salix pyrolifolia* in relation to plant part and age.** Phytochemistry 2018; 153: 102-110, doi: 10.1016/j.phytochem.2018.05.021.

LI Y; KONG D; FU Y; MICHAEL R. S; WU H. **The effect of developmental and environmental factors on secondary metabolites in medicinal plants. Plant Phys Bioche 2020**; 148: 80-89, doi: 10.1016/j.plaphy.2020.01.006.

MACÊDO I. S. V; CUNHA K. G; ALVES A. T. V; MARTINS R. M; SIMÕES M. O. S. **Atividade antioxidante da rutina: uma revisão**. BioFarm 2017; 3: 19, ISSN 1983-4209.

MARTINS D. H. N. **Avaliação da sazonalidade de compostos fenólicos e atividade antioxidante de folhas de *Erythroxylum daphnites*** [Dissertação]. Brasília: Universidade de Brasília; 2015.

MASA C. V; DÍAZ T. S; GALLEGO J. C. A; LOBÓN N. C. **Quantitative variation of flavonoids and diterpenes in leaves and stems of *Cistus ladanifer* L. at different ages**. Molecules 2016; 21: 275-289, doi: 10.3390/molecules21030275.

MORADI H; GHAVAM M; TAVILI A. **Study of antioxidant activity and some herbal compounds of *Dracocephalum kotschyi* Boiss. in different ages of growth**. Biotechnol Rep (Amst); 25: 8, doi: 10.1016/j.btre.2019.e00408.

MUTHA R. E; TATIYA A. U; SURANA S. J. **Flavonoids as natural phenolic compounds and their role in therapeutics: an overview**. Futur J Pharm Sci 2021; 7: 25, doi: 10.1186/s43094-020-00161-8.

PAIVA L. C; SILVA K. V; ALMEIDA G. F. G; CALDEIRA E. A. C; CRUZ C. E. S. G; SALGADO P. O; AMARO M. O. F; CARVALHO C. A. ***Piptadenia gonoacantha*-based natural dermocosmetic: a clinical trial**. Ciência e Natura, Santa Maria, v.43, Ed. Esp. UFV,e1, 2021.

PEIXOTO SOBRINHO T. J. S; SILVA C. H. T. P; NASCIMENTO J. E; MONTEIRO J. M; ALBUQUERQUE U. P; AMORIM E. L. C. **Validação de metodologia espectrofotométrica para quantificação dos flavonóides de *Bauhinia cheilantha* (Bongard) Steudel**. Rev Bras Cienc Farm 2008; 44: 683-689, doi: 10.1590/S1516-93322008000400015

RAO V. B; FRANCHITO S. H; SANTO C. M. E; GAN M. A. **An update on the rainfall characteristics of Brazil: Seasonal variations and trends in 1979-2011**. Inter J Clim 2015; 36: 291-302, doi: 10.1002/joc.4345.

RITA ÍAO. **Influência da época de colheita e estado fenológico na composição fenólica e propriedades bioativas de infusões de tomilho-limão, hortelã-vulgar, limonete e erva-príncipe** [Dissertação]. Bragança: Universidade de Salamanca; 2017.

SOUZA L. F. T; FERNANDES FILHO E. I; FARIA M. M. **Compartimentação geomorfológica e mapeamento digital de solos no município de Viçosa–MG**. Revista Geonorte. 2014; 5: 110 - 114.

SZYMBORSKA-SANDHU I; PRZYBYŁ J. L; KOSAKOWSKA O; BĄCZEK K; WĘGLARZ Z. **Chemical Diversity of bastard balm (*Melittis melisophyllum* L.) as affected by plant development**. Molecules 2020; 25: 2421-32, doi: 10.3390/molecules25102421.

TAIZ L; ZEIGER E. **Fisiologia vegetal**. Porto Alegre: Artmed; 2004.

TORRES C. M. M. E; JACOVINE L. A. G; SOARES C. P. B; OLIVEIRA NETO S. N; SANTOS R. D; CASTRO NETO F. **Quantificação de biomassa e estocagem de carbono em uma floresta estacional semidecidual, no Parque Tecnológico de Viçosa, MG**. Rev Árvore 2013; 37: .647-655. doi.org/10.1590/S0100-67622013000400008

VAGIRI M; CONNER S; STEWART D; ANDERSSON S. C; VERRALL S; JOHANSSON E; RUMPUNEN K. **Phenolic compounds in blackcurrant (*Ribes nigrum* L.) leaves relative to leaf position and harvest date**. Food Chem 2015; 172:135-42, doi: 10.1016/j.foodchem.2014.09.041.

VÁZQUEZ-LEÓN L. A; PÁRAMO-CALDERÓN D. E; ROBLES-OLVERA V. J; VALDÉS-RODRÍGUEZ O. A; PÉREZ-VÁZQUEZ A; GARCÍA-ALVARADO M. A; et al. **Variation in bioactive compounds and antiradical activity of *Moringa oleifera* leaves: influence of climatic factors, tree age, and soil parameters**. Eur Food Res Technol 2017; 243: 1593–1608. doi: 10.1007/s00217-017-2868-4.

ZANELLA D. F. P. **Potencial inseticida de *Azadirachta indica* e *Trichilia pallida* (Meliaceae) sobre *Aedes aegypti* (L.) (Dip.: Culicidae): Quantificação e perfil químico de metabólitos secundários** [Tese]. Campo Grande: Universidade Católica Dom Bosco; 2017.