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Fases fenológicas da jabuticaba (*Plinia cauliflora*) e composição química do óleo essencial das folhas e atividade antioxidante

Phenological stage of jaboticaba tree (*Plinia cauliflora*) in the chemical composition of the essential oil of the leaves and antioxidant activity

Fases fenológicas del árbol de jaboticaba (*Plinia cauliflora*) en la composición química del aceite esencial de las hojas y actividad antioxidante

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Resumo

Plantas como *Plinia cauliflora*, uma árvore nativa do Brasil, podem ser uma fonte natural de antioxidantes mais seguros, porém não há estudos sobre os efeitos das fases de desenvolvimento da planta sobre os óleos essenciais (OE) ou a atividade antioxidante. Objetivo: avaliar a atividade antioxidante, a composição química e a produção de óleo essencial das folhas de *P. cauliflora* nas fases fenológicas vegetativa, floração e frutificação. Metodologia: a composição química foi determinada por cromatografia gasosa acoplada à espectrometria de massa e a atividade antioxidante por três métodos *in vitro*. Resultados: o rendimento de óleo essencial das folhas foi o mesmo para as fases vegetativa, floração e frutificação. Os compostos de óleo essencial apresentam maior diversidade química nas fases de floração e frutificação. A maior atividade antioxidante foi obtida pelo sistema de co-oxidação β-caroteno/ácido linoléico. O óleo da fase vegetativa protege 40,6% de β-caroteno, enquanto os óleos das fases de floração e frutificação protegem apenas 27,5% e 14,5% de β-caroteno, respectivamente. A análise dos principais compostos evidenciou que a classe predominante foram dos sesquiterpenos de hidrocarbonetos com 67,0% (vegetativo), 66,1% (floração) e 59,4% (frutificação). Os principais compostos do OE nas fases vegetativa, floração e frutificação foram biciclogermacreno (17,0, 14,5 e 11,0%), germacreno D (16,9, 15,9 e 12,4%) e trans-cariofileno (9,2, 7,8 e 9,2%), respectivamente. Na fase vegetativa, destacaram-se o trans-2-hexenal (8,8%) e o óxido de cariofileno (5,4%). Conclusão: as folhas de *P. cauliflora* são desperdiçadas após a poda e o conhecimento de seu óleo essencial pode agregar valor à produção de jabuticaba no Brasil.

Palavras-chave: α-copaeno; Jaboticabeira; Myrtaceae; Radical livre; Espatulenol.

Abstract

Plants as *Plinia cauliflora*, a native Brazilian tree, could be a natural sources of safer antioxidants however there is no studies about the effects of the plant development stage on the essential oils (EO) or antioxidant activity. Objective: to evaluate the antioxidant activity, chemical composition and yield of essential oil from *P. cauliflora* leaves at phenological phases vegetative, flowering and fruiting. Methodology: the chemical composition was

determined by gas chromatography coupled to mass spectrometry, and the antioxidant activity by three in vitro methods. Results: the leaf essential oil yield was the same for the vegetative, flowering and fruiting phases. The essential oil compounds have greater chemical diversity at flowering and fruiting phases. The higher antioxidant activity was obtained by β-carotene/ linoleic acid co-oxidation system. The oil from the vegetative phase protects 40.6% of β-carotene whereas the oils from the flowering and fruiting phases protect only 27.5% and 14.5% β-carotene, respectively. The analysis of the major compounds made evident that the predominant class were hydrocarbon sesquiterpenes with 67.0% (vegetative), 66.1% (flowering), and 59.4% (fruiting). The major compounds of EOs in the vegetative, flowering and fruiting phases were bicyclogermacrene (17.0, 14.5, and 11.0%), germacrene D (16.9, 15.9, and 12.4%) and trans-caryophyllene (9.2, 7.8, and 9.2%), respectively. In the vegetative phase, trans-2-hexenal (8.8%) and caryophyllene oxide (5.4%) stood out. Conclusion: *P. cauliflora* leaves are wasted after pruning and know about their essential oil could add value to jabuticaba production in Brazil.

Keywords: α-copaene; Jaboticabeira; Myrtaceae; Free radical; Spathulenol.

Resumen

Las plantas como *Plinia cauliflora*, un árbol nativo de Brasil, pueden ser una fuente natural más segura de antioxidantes, pero no hay estudios sobre los efectos de las fases de desarrollo de la planta en los aceites esenciales (AE) o las actividades antioxidantes. Objetivo: evaluar la actividad antioxidante, la composición química y el rendimiento del aceites esenciales de las hojas de *P. cauliflora* en las fases fenológicas vegetativas, de floración y fructificación. Metodología: la composición química se determinó por cromatografía de gases acoplada a espectrometría de masas y la actividad antioxidante por tres métodos in vitro. Resultados: el rendimiento del aceite esencial de las hojas fue el mismo para las fases vegetativa, de floración y fructificación. Los compuestos de aceites esenciales tienen una mayor diversidad química en las fases de floración y fructificación. La mayor actividad antioxidante se obtuvo mediante el sistema de cooxidación de β-caroteno / ácido linoleico. El aceite de la fase vegetativa protege el 40.6% del β-caroteno, mientras que los aceites de las fases de floración y fructificación protegen solo el 27.5% y el 14.5% del β-caroteno, respectivamente. El análisis de los principales compuestos mostró que la clase predominante fueron los sesquiterpenos de hidrocarburos con 67.0% (vegetativo), 66.1% (floración) y 59.4% (fructificación). Los principales compuestos de aceite esencial en las fases vegetativa, de floración y fructificación fueron biciclogermacreno (17.0, 14.5 y 11.0%), germacreno D

(16.9, 15.9 y 12.4%) y transcariofileno (9.2, 7.8 y 9.2%), respectivamente. En la fase vegetativa, se destacaron trans-2-hexenal (8,8%) y óxido de cariofileno (5,4%). Conclusión: las hojas de *P. cauliflora* se desperdician después de la poda y el obtention de su aceite esencial puede agregar valor a la producción de jabuticaba en Brasil.

Palabras clave: α-copaeno; Jaboticabeira; Myrtaceae; Radicales libres; Spatulenol.

1. Introduction

The oxidative stress occurs when there is a metabolic unbalance between the production of free radicals and antioxidants (Halliwell & Gutteridge, 2015) and may cause damages to the organism, such as neoplasia, atherosclerosis and neurodegenerative diseases (Shahidi & Ambigaipalan, 2015). In addition, oxidation is a major cause of the quality deterioration of food, cosmetics, and medicines. It causes unpleasant odors and tastes, reduces shelf life and changes the texture and color of the product (Shahidi & Ambigaipalan, 2015). Therefore, antioxidants have become an indispensable group of additives to control oxidative reactions and extend shelf life.

The industry uses synthetic antioxidants as butylated hydroxytoluene (BHT), butylated hydroxyanisol (BHA), propyl gallate (PG) and tert-butyl hydroquinone (TBHQ) but they have been associated with possible risks to health (Oyetayo, 2009). The plants are natural sources of antioxidant compounds and an alternative to synthetic antioxidants. The European Union, Japan, and China have authorized the use of food additives in foodstuffs such as rosemary extracts, encouraging the use of natural products with antioxidant activity in the food industry (Carocho et al., 2018).

Plinia cauliflora (DC.) Kausel [*Sinonímia Eugenia cauliflora* DC., *Eugenia jaboticaba* (Vell.) Kiaersk., *Myrcia jaboticaba* (Vell.) Baill., *Myrciaria cauliflora* (Mart.) O. Berg, *Myrciaria jaboticaba* (Vell.) O. Berg, *Myrtus cauliflora* Mart., *Myrtus jaboticaba* Vell., and *Plinia jaboticaba* (Vell.) Kausel, popularly known as jabuticabeira, belonging to the Myrtaceae family, is found in Argentina and Paraguay, and cultivated in Florida (USA), Central and South Americas (Lorenzi, 2002). Also, it is native to Brazil, and it presents widespread all over the country, mainly in the Atlantic rainforest and cultivated in São Paulo, Rio de Janeiro, Minas Gerais, and Espírito Santo regions. It is a medium-sized tree with up to 15 m high, single leaves with up to 7 cm long, and yellowish white flowers. The fruits are cultivated and appreciated *in natura* by consumers or eaten as jelly, ice cream, yogurt, liqueur and fermented beverages, juices, jams, and vinegar (Lima et al., 2008).

Apel et al. (2006) evaluated the chemical composition of the essential oil from *Plinia* spp. leaves, including *P. cauliflora*; however, there are no reports on its biological activity. Moraes et al. (2019) reported high antioxidant and anticancer activity of hydroalcoholic extract of *Plinia peruviana* (Poir.) Govaerts leaves a different species from the same genus. Also, *Myrtus communis*, a separate genus from the same Myrtaceae family of *P. cauliflora*, had variation of leaf essential oil components at different vegetative, flowering, and fruiting phases (Wannes et al., 2009). So, there are no studies on the essential oils from *P. cauliflora* leaves regarding the chemical composition and biological activity in different phenological phases. Thus, this study aimed to evaluate the antioxidant activity, chemical composition, and yield of the essential oil from *P. cauliflora* leaves collected in the vegetative, flowering and fruiting phases. *P. cauliflora* leaves are generally considered waste, especially after pruning, the research about their essential oil is a way to add a value of fruit tree production in Brazil.

2 Material and methods

2.1 Biological material

An adult sample (40 years old) of *P. cauliflora* was used for harvesting, located in Francisco Beltrão at the coordinates 26°04'15" S and 53°02'31" W, and altitude of 609 m. The leaf harvest occurred in three phenological phases of the plant: vegetative (May, June, and October), flowering (July and August), and fruiting (August and September) in 2016. The leaves were dried at ambient temperature (~30 °C) in the shade. The Western Paraná State University did the botanic identification of the plant, and an exsiccate was deposited in the Herbarium of the University under the registration number UNOP 3001. The research was registered in the National System of Genetic Heritage Management and Associated Traditional Knowledge (SISGEN) under the number A0BE13A.

2.2 Analysis of the soil and *P. cauliflora* leaves

The land was collected at the layer of 0-20 cm of depth at different points resulting in a compound sample, with approximately 200 g of soil. From this compound sample, the analyses of pH, macronutrients, micronutrients, base saturation (V %) and granulometry were done according to Veloso et al. (2006) and Silva (2009). The soil classification was according to Embrapa (2013). The soil analysis for calcium, magnesium, and aluminum was extracted

with 1 M KCl, phosphorus and potassium were extracted with Mehlich-1 method, hydrogen+aluminum (H+Al) was determined by SMP (Shoemaker, Mac Lean, and Pratt) and carbon by Walkley and Black methods, cation exchange capacity (CEC) and base saturation (V%) were determined according to Silva (2009). The leaf analysis was done from five plant leaves in the vegetative, flowering, and fruiting phases according to Silva (2009).

2.3 Obtaining the essential oil

The essential oil was extracted using 200 g of dry leaves from each plant phenological phases by hydrodistillation (2 L of distilled water) for 4 h in a modified Clevenger device (Duarte et al., 2010). The essential oil was removed from the equipment with *n*-hexane, filtered in anhydrous sodium sulfate (Na_2SO_4), stored in an amber flask and kept at - 4 ºC until complete evaporation of the solvent to calculate yield (Omolo et al., 2004). The essential oil yield (dry basis) was calculated in triplicate by the essential oil mass obtained (g) divided by the mass of dried leaves (g), multiplied by 100 and expressed in percentage.

2.4 Identification of chemical compounds

A sample of 1 µL dichloromethane (HPLC degree) with essential oil (20 mg/mL) was used for each analysis. The chemical identification carried out by gas chromatographer (Agilent 7890B) coupled to a mass spectrometer (GC-MS) (Agilent 5977A MSD), and an HP5-MS UI Agilent fused silica capillary column (30 × 250 × 0.25 µm; Agilent Technologies), with initial oven temperature from 80 °C (1 min), followed by an increase to 185 °C at 2 °C/min, and maintained for 1 min. Then there was an increase to 275 °C at 9 °C/min, and the temperature was kept for 2 min. Finally, the temperature was increased to 300 °C at 25 °C/min and maintained for 1 min. Helium was utilized as the carrier gas at the linear speed of 1 mL/min up to 300 °C, and pressure release of 56 kPa. The injector temperature was 280 ºC; the injection volume was 1 µL, and the injection occurred in split mode (2:1). The warmth of the transfer line, ion source and quadrupole were 280, 230, and 150 ºC, respectively. The EM detection system was utilized in "scan" mode, at the mass/charge rate/load (*m/z*) of 40-600, with "solvent delay" of 3 min. The compounds were identified by comparing the mass spectra found in NIST 11.0 libraries and by comparing the retention index (RI) obtained by a homologous series of *n*-alkane standard (C_7-C_{28}) (Adams, 2017).

2.4.1 Analysis of the major components

The multivariate exploratory analysis was applied to determine the principal component analysis (PCA) to separate the group of major chemical compounds and the chemical classes found in the essential oil. The analysis result was represented by a biplot graph (Moita Neto & Moita, 1998). The percentage in number and the area were presented for each significant chemical compound and their respective chemical classes. They were identified in the samples of the essential oil obtained in the three phenological phases (vegetative, flowering and fruiting). These data were transformed in orthogonal latent variables named major components, which are linear combinations of original variables made up of self-values of the data covariance matrix (Hair et al., 2009).

Kaiser's criterion was utilized to select the major components. A self-value preserves relevant information when it is higher than the unit. This analysis was done in two ways: the former containing only data referring to the chemical composition of significant compounds obtained in each of the three phenological phases, and in the latter, the data were analyzed by grouping the chemical classes to each the compounds belonged (Ferré, 1995, Camacho et al., 2010). Both analyses were done in Statistica 7 program.

2.5 Antioxidant activity

2.5.1 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) method

The essential oil sequestration capability of free radicals by 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) was done according to Rufino et al. (2007). The essential oil was prepared in methanol at 1.00, 0.75, 0.50, and 0.25 mg/mL, and from this mixture 0.1 mL was added to 3.9 mL of DPPH$^{\bullet}$ methanolic solution (60 µM). The negative control had 0.1 mL of methanol in DPPH$^{\bullet}$ solution (60 µM). The mixture was kept in the dark at ambient temperature for 30 min. The absorbance reduction was measured at 515 nm in a UV/VIS spectrophotometer. The total antioxidant capacity was calculated utilizing a standard quercetin solution (60 µM) with a 100.0% reference. From the correlation between absorbance and concentration of the antioxidant sample, the inhibitory concentration to reduce 50.0% of the free radicals (IC_{50}) was determined.

2.5.2 β-carotene/linoleic acid method

The antioxidant activity of the essential oil was evaluated using the method based on *β*-carotene/linoleic acid (BCLA) co-oxidation system (Rufino et al., 2006a), where the protocol was adjusted to use 96-well microplates. A 1.0 mL solution (20 mg β-carotene in 1 mL chloroform) was mixed to 40 µL linoleic acid and 530 µL polysorbate-40 emulsifier. Chloroform was removed in a rotary evaporator at 50 °C, and 450 mL of ultra-purified water (previously saturated with oxygen for 30 min) was added under vigorous agitation until reaching absorbance of 0.7 at 470 nm. Aliquots (280 µL) of this emulsion were transferred to microplates with 96 wells. In each well, 20 µL of ethanolic solution with essential oil at 1.00, 0.75, 0.50, or 0.25 mg/mL was poured. The reaction was kept at 40 °C for 120 min, and the absorbance was measured at 470 nm in a spectrophotometer (Spectra Max Plus^{384} microplate reader) every 5 min from zero time until 120 min. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was utilized as a reference at 200 µg/mL. The results were expressed in absorbance reduction along the reaction time. The bleaching rate of β-carotene was calculated according to Eq. (1).

$$\text{Eq. (1) } R = \ln (a/b)/t$$

Where R = bleaching rate of *β*-carotene in the mixture; ln = natural log; a = absorbance at zero time; b= absorbance at t time (t = 0, 5, 10 ... 120 min).

The antioxidant activity was calculated according to the percentage inhibition to the control, using Eq. (2).

$$\text{Eq. (2) Antioxidant activity} = [(R_{control}-R_{sample})/R_{control}] \times 100$$

where $R_{control}$ and R_{sample} were the bleaching rates of *β*-carotene in the mixture without the antioxidant ($R_{control}$) and with essential oil (R_{sample}), respectively (Velioglu et al., 1998).

2.5.3 Ferric reducing antioxidant power (FRAP)

For the ferric reducing antioxidant power (FRAP), 25 mL of acetate buffer (0.3 M), 2.5 mL of TPTZ (2,4,6-Tris (2-piridil)-triazine) aqueous solution (10 mM) and 2.5 mL of ferrous chloride aqueous solution (20 mM) were mixed according to Rufino et al. (2006b).

Essential oil (90 µL), previously prepared in methanol at 1.00, 0.75, 0.50, and 0.25 mg/mL, was mixed to 2.7 mL of FRAP reagent antioxidant reaction. The mixture was vigorously homogenized and kept at 37 °C for 30 min. The absorbance variance was read at 595 nm. A standard curve of ferrous sulfate (0–2000 µM) was used to calculate the antioxidant activity. The antioxidant activity was expressed in µM of ferrous sulfate per mg of sample.

2.6 Statistical analysis

The results were submitted to analysis of variance (ANOVA), and the differences among the arithmetical averages were determined by Duncan's test ($p \leq 0.05$) utilizing IBM SPSS Statistics 22.0 software. All these tests were done in triplicates.

3. Results

The hydrodistillation of *P. cauliflora* dried leaves from three phenological phases provided a yellow-greenish oil. The average yield of the essential oil in dry basis was equal to ($p \leq 0.05$) of 0.043 ± 0.007% from the vegetative, 0.050 ± 0.003% flowering, and 0.046 ± 0.003% fruiting phases, indicating that there is no significant difference in the oil yield in the three evaluated periods.

The cultivation soil of *P. cauliflora* was classified as Red Distroferric Latosol (Embrapa, 2013), with granulometric composition of 24.6 ± 0.7% of sand, 23.3 ± 0.7% of silt, and 52.1 ± 1.4% of clay, and low/medium acidity with pH of 5.6 ± 0.3. The soil macronutrient concentration was 92.6 ± 67.9 mg/dm³ of phosphorus, 1.0 ± 0.4 $cmol_c/dm^3$of potassium, 12.2 ± 1.4 $cmol_c/dm^3$ of calcium, 2.7 ± 0.5 $cmol_c/dm^3$ magnesium, and 78.4 ± 0.6% of soil fertilization by base saturation (V%) (Table I). The soil micronutrient concentration is 30.3 ± 0.5 mg/dm³ of iron, 88.6 ± 26.4 mg/dm³ of magnesium, and 17.3 ± 7.5 mg/dm³ of zinc (Table I).

Table I - Analysis of *Plinia cauliflora* cultivation soil.

	pH AND MACRONUTRIENTS								
Phase	pH*	cmolc/dm^3					mg/dm^3	g/dm^3	%
	$CaCl_2$	**Ca**	**Mg**	**K**	**Al**	**H+Al**	**P**	**C**	**V**
Vegetative	5.8	11.46	2.37	0.70	0.00	3.97	154.73	21.75	78.54
Floring	5.3	11.34	3.34	1.43	0.00	4.61	20.12	45.88	77.75
Fruting	5.7	13.86	2.43	0.93	0.00	4.61	102.90	33.75	78.88

	MICRONUTRIENTS				GRANULOMETRY			
Phase	mg/dm^3				%			
	Cu	**Fe**	**Mn**	**Zn**	**Sand**	**Silt**	**Clay**	**Classification**
Vegetative	5.80	30.69	63.73	16.93	25.00	23.75	51.25	RdfL
Floring	6.68	30.46	85.82	10.02	25.00	23.75	51.25	RdfL
Fruiting	5.43	29.71	116.30	24.94	23.75	22.50	53.75	RdfL

* pH: hydrogenionic potential; $CaCl_2$: calcium chloride; Ca: calcium; Mg: magnesium; K: potassium; Al: aluminum; H+Al: hydrogen+aluminum; P: phosphorus; C: carbono; V: base saturation; Cu: copper; Fe: iron; Mn: manganese; Zn: zinc; RdfL: Red distroferric Latosol. Source: Authors.

P. cauliflora leaves had phosphorus content of 0.9 ± 0.5 g/kg, potassium of 7.7 ± 3.1 g/kg, and nitrogen of 19.1 ± 4.1 g/kg. The most considerable amount of phosphorus found in *P. cauliflora* leaves was verified in the fruiting phase whereas nitrogen was in the vegetative stage. The highest micronutrient concentration of *P. cauliflora* leaves was iron, zinc, and copper in the fruiting, and manganese and boron in the vegetative stage (Table II).

Table II - Analysis of macronutrients and micronutrients of *Plinia cauliflora* leaves (dry basis) in the vegetative, flowering and fruiting phases.

	MACRONUTRIENTS					
Phase	g/kg					
	P	**N**	**K**	**Ca**	**Mg**	**S**
Vegetative	0.58	22.92	5.64	13.04	1.71	2.04
Flowering	0.79	14.82	6.17	13.51	2.30	1.44
Fruiting	1.47	19.47	11.27	6.23	2.67	2.03

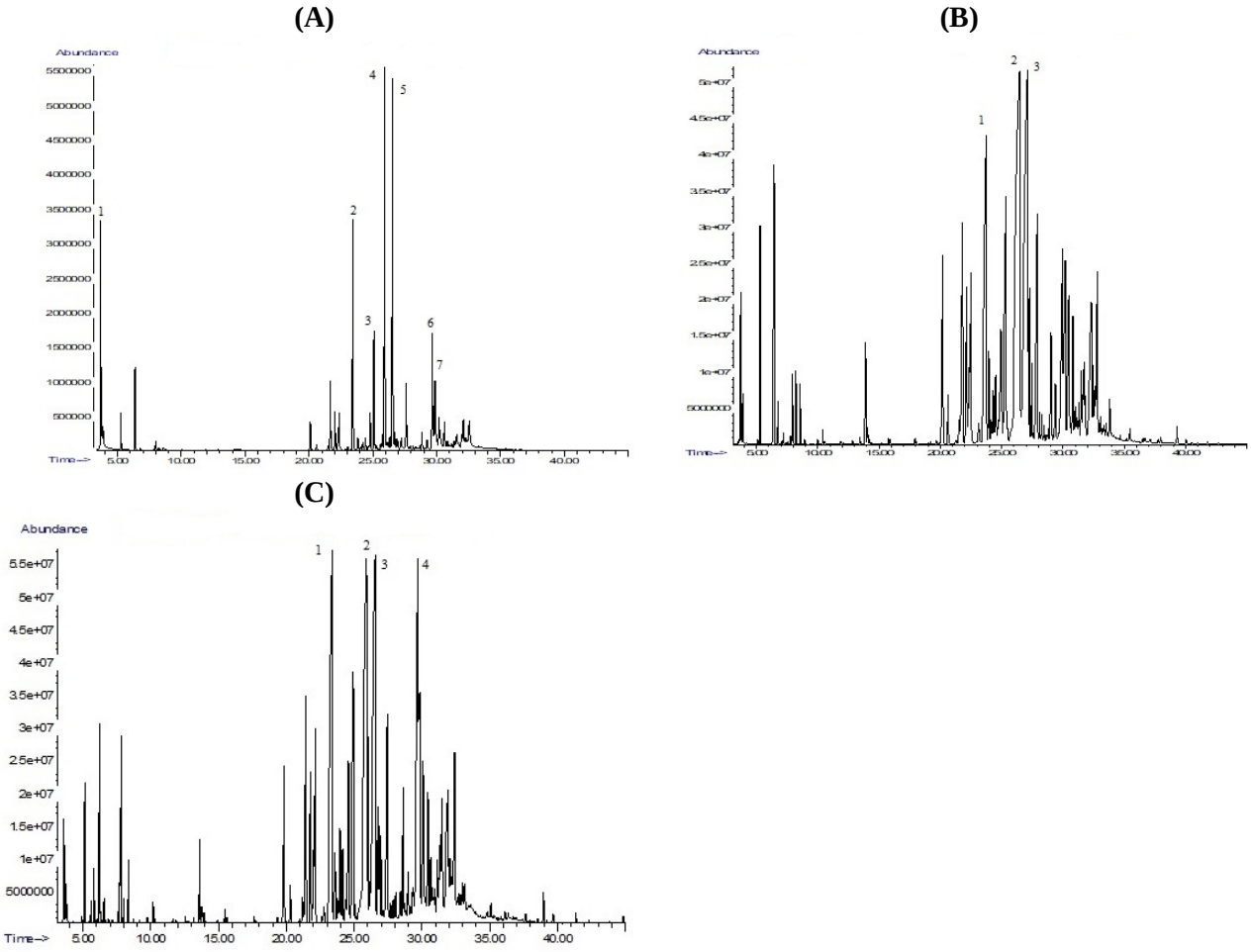
	MICRONUTRIENTS				
Period	mg/kg				
	Fe	**Mn**	**Zn**	**B**	**Cu**
Vegetative	263.32	535.00	11.20	66.46	10.58
Flowering	134.00	316.00	6.00	63.50	8.50
Fruiting	643.17	268.94	21.05	33.93	14.42

Subtitles: P: phosphorus; N: nitrogen; K: potassium; Ca: calcium; Mg: magnesium; S: sulfur; Fe: iron; Mn: manganese; Zn: zinc; B: boron; Cu: copper. Source: Authors.

The essential oil from *P. cauliflora* leaves from the vegetative phase had 35 identified compounds, from the flowering stage 80 compounds were identified, and fruiting one, 99 compounds were identified, with a greater diversity of compounds in the essential oil from leaves in the flowering and fruiting phases (Table III). The predominant chemical class was hydrocarbon sesquiterpenes in the leaves from the vegetative stage (67.0%), from flowering (66.1%) and fruiting (59.4%). The oxygenated sesquiterpenes had an increase in the essential oil of leaves from the fruiting phase (30.0%) compared to the flowering stage (23.6%) and the vegetative one (18.9%) (Table III and Figure 1).

The major compounds of the essential oil of leaves from the vegetative phase were germacrene D (16.8%), bicyclogermacrene (17.0%), trans-caryophyllene (9.2%), trans-2-hexenal (8.8%), spathulenol (5.8%), caryophyllene oxide (5.4%) and allo-aromadendrene (5.1%) (Figure 1a). From the flowering phase, they were bicyclogermacrene (14.4%), germacrene D (15.8%) and trans-caryophyllene (7.8%) (Figure 1b); and from the fruiting stage, they were germacrene D (12.4%), bicyclogermacrene (11.0%), trans-caryophyllene (9.1%) and spathulenol (6.7%) (Figure 1c) (Table III).

Figure 1a. Essential oil from *Plinia cauliflora* leaves (A) from the vegetative stage were (1) trans-2-hexenal (8.8%), (2) trans-caryophyllene (9.2%), (3) allo-aromadendrene (5.1%), (4) germacrene D (16.8%), (5) bicyclogermacrene (17.0%), (6) spathulenol (5.8%) and (7) caryophyllene oxide (5.4%); (B) from the flowering stage were (1) trans-caryophyllene (7.8%), (2) germacrene D (15.8%) and (3) bicyclogermacrene (14.4%); (C) from the fruiting stage were (1) trans-caryophyllene (9.1%), (2) germacrene D (12.4%), (3) bicyclogermacrene (11.0%) and (4) spathulenol (6.7%).



Source: Authors.

Table III - Phytochemical composition of the essential oil from *Plinia cauliflora* leaves from the vegetative, flowering and fruiting phases.

			Relative area (%)			
Peak	**[a]Compound**	**[b]Calculated RI**	**Vegetative**	**Flowering**	**Fruiting**	**Identification methods**
1	*Trans*-2-hexenal	800	8.83	-	-	a.b.c
2	Ethylbenzene	825	-	-	0.29	a.b.c
3	*Cis*-3-hexenol	826	1.00	1.35	0.33	a.b.c
4	α-thujene	926	-	0.03	0.06	a.b.c
5	α-pinene	936	1.09	1.43	0.71	a.b.c
6	Camphene	955	-	-	0.14	a.b.c
7	o-ethyl toluene	963	-	-	0.40	a.b.c
8	β-pinene	970	2.60	2.58	0.03	a.b.c
9	Myrcene	979	-	0.47	1.22	a.b.c
10	δ-2-carene	990	-	-	0.18	a.b.c
11	α- phellandrene	1001	-	0.11	0.04	a.b.c
12	*Trans*-2, trans-4-heptadienal	1004	-	-	0.04	a.b.c
13	n.i	1013	-	-	0.04	a.b.c
14	α-terpinene	1017	-	0.02	0.02	a.b.c
15	*p*-cymene	1027	-	0.05	-	a.b.c
16	Limonene	1026	-	0.62	0.07	a.b.c
17	1,8 cineole	1033	0.33	-	1.52	a.b.c
18	*Cis*-β-ocimene	1039	-	0.41	0.17	a.b.c
19	*Trans*-β-ocimene	1049	-	0.33	0.40	a.b.c
20	γ-terpinene	1054	-	0.04	0.03	a.b.c
21	Terpinolene	1088	-	0.03	0.08	a.b.c
22	*Cis*-linalool oxide	1074	-	-	0.03	a.b.c
23	Linalool	1099	-	0.35	0.14	a.b.c
24	Nonanal	1103	-	0.02	0.03	a.b.c
25	*Trans*-pinocarveol	1139	-	0.03	0.09	a.b.c
26	*Cis*-3-hexenyl isobutanoate	1146	-	0.02	0.03	a.b.c
27	Pinocarvone	1163	-	0.03	0.05	a.b.c
28	β-phellandren-8-ol	1168	-	-	0.03	a.b.c
29	Terpinen-4-ol	1177	-	0.05	0.05	a.b.c
30	*Cis*-3-hexenyl butanoate	1187	-	0.85	0.52	a.b.c
31	α-terpinol	1190	-	0.15	0.19	a.b.c
32	Myrtenal	1195	-	0.03	0.15	a.b.c
33	β-cyclocitral	1220	-	0.02	0.04	a.b.c
34	*Cis*-3-hexenyl *trans* -2-butenoate	1233	-	0.04	0.09	a.b.c
35	*Cis*-3-hexenyl-3-methyl butanoate	1237	-	0.05	0.05	a.b.c
36	*Cis*-3-hexenyl valerate	1284	-	0.06	0.09	a.b.c
37	*Cis*-3-hexenyl tiglate	1326	-	0.02	0.06	a.b.c

38	Bicycloelemene	1328	-	0.05	-	a.b.c
39	δ-elemene	1338	1.37	2.26	1.68	a.b.c
40	α-cubebene	1342	-	0.42	0.02	a.b.c
41	α-longipinene	1349	-	-	0.35	a.b.c
42	Cyclosativene	1367	-	0.07	0.07	a.b.c
43	α-ylangene	1373	-	0.32	0.32	a.b.c
44	α-copaene	1376	2.94	3.01	2.54	a.b.c
45	β-bourbonene	1387	1.77	1.92	1.46	a.b.c
46	β-cubebene	1389	0.49	-	-	a.b.c
47	β-elemene	1391	1.70	2.89	2.80	a.b.c
48	n.i	1403	-	-	0.11	a.b.c
49	α-gurjunene	1410	-	0.32	0.24	a.b.c
50	*Trans*-caryophyllene	1418	9.23	7.83	9.16	a.b.c
51	α-ionone	1428	0.56	-	0.75	a.b.c
52	Calarene	1433	-	0.78	0.28	a.b.c
53	δ-gurjunene	1436	-	0.28	-	a.b.c
54	Aromadendrene	1441	-	0.59	0.94	a.b.c
55	Guaia-3,7-diene	1443	0.50	-	0.75	a.b.c
56	α-humulene	1453	1.61	0.83	0.37	a.b.c
57	α-patchoulene	1453	-	2.25	1.86	a.b.c
58	*Allo*-aromadendrene	1460	5.14	4.75	3.52	a.b.c
59	α -amorphene	1476	0.70	-	0.23	a.b.c
60	Germacrene D	1485	16.85	15.85	12.39	a.b.c
61	β-selinene	1490	0,63	-	-	a.b.c
62	Bicyclogermacrene	1495	17.02	14.46	10.99	a.b.c
63	α-muurolene	1498	0.84	-	-	a.b.c
64	Eremophilene	1502	0.47	-	-	a.b.c
65	*Trans-trans*-α-farnesene	1507	-	-	1.17	a.b.c
66	Germacrene A	1510	-	-	0.95	a.b.c
67	α-amorphene	1513	0.6	-	-	a.b.c
68	γ-cadinene	1514	-	1.19	0.69	a.b.c
69	α-elemene	1519	-	0.70	-	a.b.c
70	δ-cadinene	1523	3.25	3.32	2.82	a.b.c
71	*Trans*-cadina-1,4-diene	1532	-	0.22	0.25	a.b.c
72	α-cadinene	1537	-	0.23	0.21	a.b.c
73	α−calacorene	1542	-	0.18	0.37	a.b.c
74	Widdrene	1551	-	-	0.44	a.b.c
75	Elemol	1554	-	0.24	-	a.b.c
76	Germacrene B	1559	0.67	1.16	1.65	a.b.c
77	δ-gurjunene	1566	0.61	-	-	a.b.c
78	Palustrol	1566	-	0.76	0.56	a.b.c
79	n.i	1569	-	-	0.18	a.b.c
80	*Trans*-3-hexenyl benzoate	1575	-	-	0.60	a.b.c
81	Spathulenol	1576	5.77	3.39	6.66	a.b.c
82	Caryophyllene oxide	1581	5.42	0.50	-	a.b.c
83	Globulol	1589	-	2.87	2.88	a.b.c

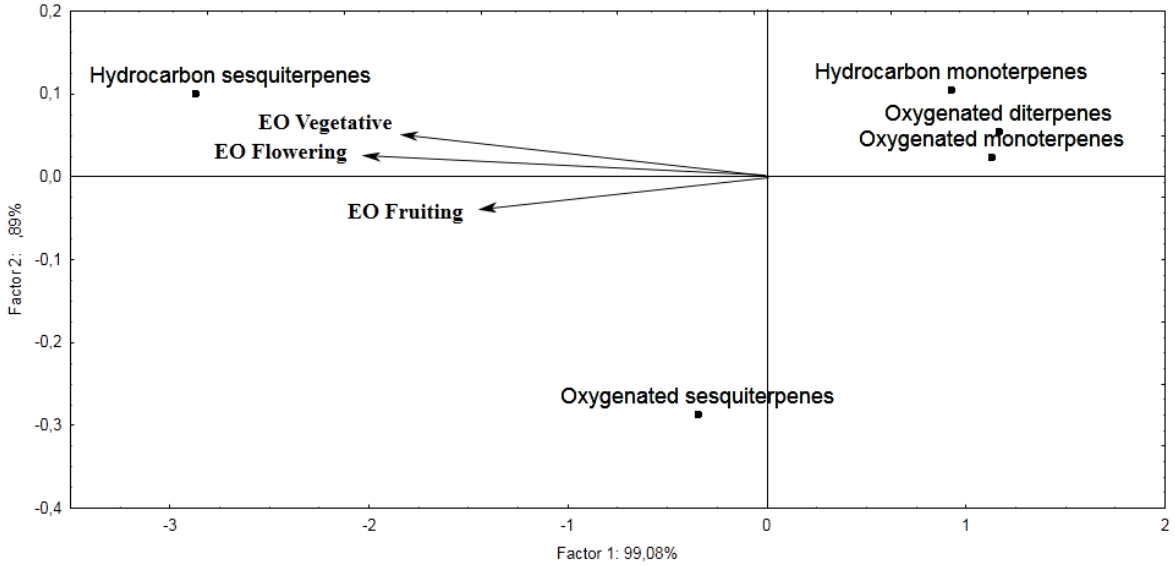
84	Viridiflorol	1596	1.39	2.63	2.28	a.b.c
85	Capnellane-8-one	1597	0.70	-	0.24	a.b.c
86	Ledol	1602	1.17	-	1.50	a.b.c
87	β-oplopenone	1608	-	1.40	0.84	a.b.c
88	Caryophyllenol-II	1611	-	0.51	0.53	a.b.c
89	Caryophylla-3,8(13)-dien-5β-ol	1614	-	0.35	-	a.b.c
90	Valerenol	1616	-	-	0.44	a.b.c
91	Fonenol	1619	-	0.38	0.65	a.b.c
92	n.i	1625	-	0.68	-	a.b.c
93	n.i	1627	0.52	-	-	a.b.c
94	Caryophylla-4(12),8(13)-dien-5β-ol	1631	-	1.65	2.68	a.b.c
95	Isospathulenol	1637	0.37	-	-	a.b.c
96	Torreyol	1643	-	3.05	3.32	a.b.c
97	Khusilal	1647	-	-	0.71	a.b.c
98	T-cadinol	1641	1.46	-	-	a.b.c
99	β-eudesmol	1651	-	0.71	3.19	a.b.c
100	T-muurolol	1654	1.67	-	-	a.b.c
101	γ-cadinol	1655	-	0.72	-	a.b.c
102	Vulgarone B	1662	-	-	0.50	a.b.c
103	α-copaene-8-ol	1671	-	2.36	1.04	a.b.c
104	Cedrelanol	1668	0.37	-	-	a.b.c
105	Aristol-1(10)-en-9-ol	1672	0.03	-	-	a.b.c
106	Cadalene	1675	-	0.20	-	a.b.c
107	Caryophylla-3,8(13)-dien-5β-ol	1678	-	0.28	0.50	a.b.c
108	Ledene oxide-(I)	1684	-	-	0.49	a.b.c
109	Shyobunol	1688	-	0.76	0.21	a.b.c
110	Germacrone	1699	-	-	0.16	a.b.c
111	n.i	1705	-	-	0.08	a.b.c
112	Vulgarol B	1711	-	0.11	-	a.b.c
113	Vulgarol A	1714	-	-	0.10	a.b.c
114	n.i	1717	-	-	0.20	a.b.c
115	Calarene epoxide	1724	-	-	0.09	a.b.c
116	n.i	1732	-	-	0.15	a.b.c
117	Valerenal	1736	-	-	0.04	a.b.c
118	Mintsulfide	1740	-	0.14	0.07	a.b.c
119	*Trans*-2, *trans*-6-Farnesol	1747	-	-	0.02	a.b.c
120	n.i	1754	-	-	0.12	a.b.c
121	n.i	1762	-	-	0.14	a.b.c
122	n.i	1764	-	0.04	-	a.b.c
123	β-costol	1768	-	0.10	0.08	a.b.c
124	α-costol	1774	-	0.05	0.02	a.b.c
125	2-propenyl ionone	1793	-	-	0.03	a.b.c
126	n.i	1798	-	0.03	0.06	a.b.c
127	(E,E)-farnesyl acetone	1804	-	0.06	0.30	a.b.c

128	n.i	1846	-	-	0.07	a.b.c
129	n.i	1868	-	0.04	-	a.b.c
130	2-nonadecanone	1921	-	0.04	0.10	a.b.c
131	Geranyl linalool isomer	1946	-	-	0.03	a.b.c
132	Phytol	2106	-	0.03	0.07	a.b.c
133	Isophytol	2103	-	0.22	0.03	a.b.c
134	Phytol Isomer	2111	-	0.26	0.87	a.b.c
135	Linoleic acid	2139	-	0.03	0.05	a.b.c
136	n.i	2822	-	0.04	0.03	a.b.c
Total identified			**99.15**	**98.87**	**98.52**	
Hydrocarbon monoterpenes			3.69	6.12	3.19	
Oxygenated monoterpenes			0.33	0.66	2.29	
Hydrocarbon sesquiterpenes			66.95	66.12	59.38	
Oxygenated sesquiterpenes			18.87	23.57	30.02	
Oxygenated diterpenes			0	0.55	1.03	
Not identified			0.52	0.83	1.18	
Others			9.31	1.85	2.61	

Subtitles: [a]Compounds listed according to the elution order in HP-5ms column; [b]retention index (RI) calculated utilizing *n*-alkanes C_7 to C_{28} in a capillary column (HP-5ms); [c]Identification based on the comparison with mass spectrum from NIST 11.0 library; Relative area (%): percentage of the area occupied by the compounds in the chromatogram. n.i. = not identified. t = traces. (-) = Absent. Source: Authors.

The projection of the classes of major compounds by PCA indicates that factor 1 represents 97.7% of the compound class's variability found in the essential oil from *P. cauliflora* leaves in each phenological phase (Figure 1). The essential oil from leaves obtained in the flowering stage had a vector with a smaller angle with the x-axis and, therefore, it contributed to a more significant effect on the variation of factor 1. The vector projection indicates greater distancing of the compound class variation among the essential oils from leaves obtained in the vegetative and flowering phases. The essential oil from *P. cauliflora* leaves in all phenological stages are positively correlated and with greater projection for hydrocarbon sesquiterpenes. There is a greater tendency to variation of compound classes of hydrocarbon sesquiterpenes to oxygenated sesquiterpenes of the essential oil from leaves in the vegetative phase into the fruiting phase through the flowering one (Figure 1). None of the essential oil from leaves in the phenological stages indicated a lack of correlation or negative correlation (Figure 2).

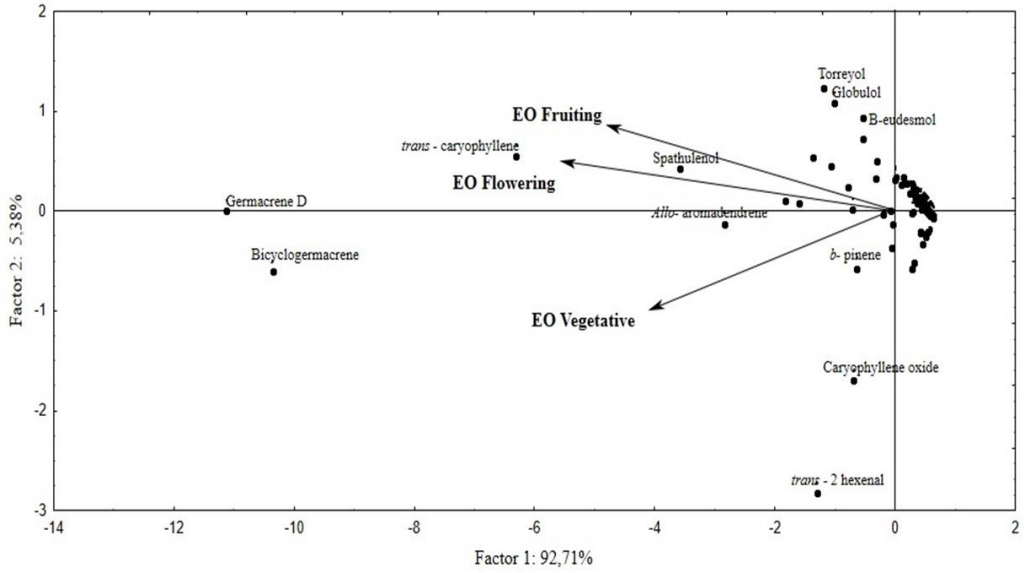
Figure 2 - Principal component analysis biplot of major compound classes of *Plinia cauliflora* leaf essential oil at vegetative, flowering, and fruiting phases.



Source: Authors.

The principal compound projection by PCA indicates that factor 1 represents 89.2% of the variability of significant compounds found in the essential oil from *P. cauliflora* leaves in each phenological phase (Figure 3). The vectors for the essential leaf oil from all phenological stages indicated positive projections for the compounds *trans*-caryophyllene, germacrene D and bicyclogermacrene, demonstrating that they are characteristic of the essential leaf oil regardless of the phenological stage. The essential leaf oil vector in the flowering phase indicated the most significant variation for factor 1, followed by the fruiting phase and after the vegetative one. The vector distancing of the essential leaf oil from the vegetative phase compared to the fruiting one indicates some distancing, but without characterizing a lack of correlation among them. The leaf essential oil vector in the vegetative phase showed projections for the compounds *trans*-2-hexenal and caryophyllene oxide, while the leaf essential oil from fruiting showed prediction for ß-eudesmol and torreyol, indicating that they are specific compounds for each one of these phenological phases, respectively (Figure 3).

Figure 3 - Principal component analysis biplot of major compounds of *Plinia cauliflora* leaf essential oil from vegetative, flowering, and fruiting phases.



Source: Authors.

Table IV - Antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH•), ferric reducing antioxidant power (FRAP), and β-carotene/linoleic acid (BCLA) method for the essential oil from *Plinia cauliflora* leaves in the vegetative, flowering and fruiting phases.

	DPPH• IC_{50} (mg/mL)	**FRAP (µmol ferrous sulfate mg^{-1})**	**BCLA (%)**
vegetative phase	105.99 ± 5.83^{c}	0.140 ± 0.005^{b}	40.51 ± 7.83^{b}
flowering phase	89.22 ± 3.31^{b}	0.117 ± 0.020^{b}	27.50 ± 7.16^{c}
fruiting phase	106.50 ± 8.29^{c}	0.026 ± 0.003^{c}	14.45 ± 6.34^{d}
Quercetin (positive control)	$0.015 \pm < 0.001^{a}$	-	-
Trolox (positive control)	-	11.14 ± 0.071^{a}	73.94 ± 1.17^{a}

Subtitle: IC_{50} = inhibitory concentration to reduce 50.0% of the free radicals; positive control = trolox (0.2 mg/mL) or quercetin (60 µM). Different letters in the column indicate significant differences by Duncan's test ($p \leq 0.05$). Source: Authors.

The antioxidant activity by DPPH• method, which inhibited 50.0% of free radical concentration (CI_{50}) of essential oil from *P. cauliflora* leaves in the flowering phase (89.22 ± 3.31 mg/mL) was higher ($p \leq 0.05$) that the ones in the vegetative stage (105.99 ± 5.83 mg/mL) and the fruiting one (106.50 ± 829 mg/mL) (Table IV). The greater antioxidant activity of the leaves from the flowering phase had CI_{50} 595 times greater than CI_{50} of

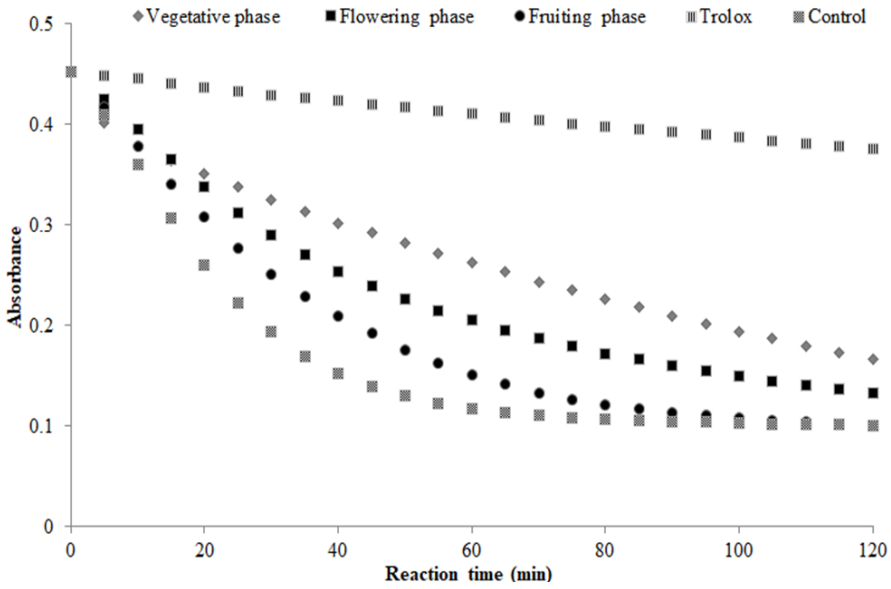
quercetin (positive control), indicating that the plant's essential oil in any phenological phase had inexpressive antioxidant activity.

The antioxidant activity by FRAP also showed inexpressive antioxidant activity of the leaf essential oil in the three phenological phases, ranging from 0.026 ± 0.003 to 0.140 ± 0.005 µmol of ferrous sulfate mg^{-1}, with equivalent values from 0.2 to 1.2% of the positive control trolox (Table IV).

The antioxidant activity by BCLA had antioxidant activity of the essential leaf oil from the three phenological phases ranging from 14.4 ± 6.3 to 40.5 ± 7.8%, with equivalent values from 19.5 to 54.8% of the positive control trolox (Table IV). Because they have a complex composition, different from the pure trolox compound, the leaves in the vegetative phase that had greater ($p \leq 0.05$) antioxidant activity have greater possibility of commercial use.

The absorbance of the negative control (without antioxidant) reduced from 0.45 to 0.10 after 60 min; however, for the system added with leaf essential oil in the vegetative phase, the absorbance changed from 0.45 to 0.16 after 60 min. In the leaves from the flowering stage, this reduction was from 0.45 to 0.13 after 60 min, and in the leaves from the fruiting stage, the decrease was from 0.45 to 0.10 in 60 min. Therefore, *P. cauliflora* essential oil obtained from leaves in the vegetative stage had greater antioxidant activity by BCLA method (Figure 4).

Figure 4 - Absorbance at 470 ηm along reaction time of *β*-carotene/linoleic acid (BCLA) co-oxidation of essential oil from *Plinia cauliflora* leaves in the vegetative, flowering, and fruiting phases (1.0 mg/mL); positive control = trolox; negative control = reaction without antioxidant.



Source: Authors.

4. Discussion

The average yield of the essential oil from *P. cauliflora* leaves (dry basis) did not differ in the three phenological phases, and it was similar to the one found by Apel et al. (2006) and lower than the one found by Duarte et al. (2010). According to the European Pharmacopoeia, the minimum essential oil yield is 2 mL/kg for the development of applications, and in our study, the return is lower than that (European Pharmacopoeia, 2013). However, *P. cauliflora* is a species that has been studied to produce fruit instead of leaves, and the essential oil yield can be improved with the selection of biological material related to that natural and genetic breeding.

In general, the concentration of macronutrients and micronutrients in the soil is high, indicating that there are needed nutrients for good plant development. There is no definition of a specific criterion for the cultivation of *P. cauliflora*, but in general, our results of the soil analysis showed good fertility due to the high values of base saturation. According to Sobral et al. (2015), soils are considered fertile when the base saturation values are higher than 70.0%.

In the fruiting period, the phosphorus concentration was higher in the leaves and probably related to the production of energy in the fruit development phase since that it is the component of adenosine mono, di and, triphosphate (ATP), besides forming nucleic acids, phospholipids and many coenzymes (Brasil & Nascimento, 2010). Thus, phosphorus can be accumulated in the leaves to supply the plant reproduction necessities with important maturation action and seed formation (López-Bucio et al., 2002). Similarly, in our study potassium concentration in the leaves was greater in the fruiting phase. Potassium is an essential metabolic activator and participates in photosynthesis (Figueiredo et al., 2008) and the hydric balance of vegetal cells keeping the internal cell pressure (Taiz & Zeiger, 2009). Thus, the increase in the potassium concentration in the leaves during the fruiting phase is possibly related to the increase in the plant's cellular metabolism for fruit formation.

Our results for *P. cauliflora* showed that the nitrogen concentration in the leaf was more significant in the vegetative phase. This mineral nutrient is responsible for the production of new cells and vegetative tissues, and it is fundamental for the plant growth and development of leaves (Natale & Marchal, 2002). Several studies examined the relationship between the content of leaf nitrogen and CO_2 assimilation since the substantial fraction of leaf nitrogen is associated with the photosynthetic system. Sinclair and Horie (1989) reported that the nitrogen concentration in the leaf reflects the foliar potential of CO_2 assimilation by the plant the production of vegetal biomass.

The essential oil from *P. cauliflora* leaves was predominantly hydrocarbon sesquiterpenes. Duarte et al. (2010) also found hydrocarbon sesquiterpenes as significant components in the essential oil from *P. cauliflora* leaves cultivated in four different types of soil. Besides, Duarte et al. (2012) made a higher concentration of oxygenated sesquiterpenes evident in the essential oil from *P. cauliflora* fruits in the same cultivars evaluated by Duarte et al. (2010), and with enormous amounts of isomers of eudesmol type. In our study, 3.2% of β-eudesmol was found in the fruiting phase, 0.7% in the flowering and in the absence of this component in the vegetative stage. These isomers perform antifungal action (Kusuma et al., 2004) and defend the plant against insects (Marsaro et al., 2004). Therefore, the plant accumulates this compound to protect against pathogenic microorganisms to fruits.

In our study, it was possible to observe that the essential oil in the flowering and fruiting phases presented a greater diversity of chemical compounds. Similar results were described by Cecotti et al. (2012) which one evaluated the composition of essential oil of *Polygonum bistorta* L. (ambiguous synonym for *Bistorta officinalis* subsp. *officinalis* and infraspecific taxon of the current and accepted scientific name *Bistorta officinalis* Raf.)

obtained in the vegetative, flowering and fruiting phenological phases. For these authors, several compound classes were founded with a predominance of alcohol in the vegetative stage, terpenes, and saturated hydrocarbons of the linear chain in the flowering stage. At the same time saturated aliphatic acids and methyl esters were predominant in the fruiting stage. This variation in the diversity of compounds can be related to molecular markers found in the plant. Such markers can alter the route of secondary metabolites and the composition of secreted essential oils, which undergo alterations along the plant's reproductive cycle (Sousa et al., 2005). The greater diversity of compounds in the flowering and fruiting phases can still be related to the expansion of volatiles to attract pollinators and animals that can eat the fruits and distribute the seeds, expanding the plant reproduction extension.

In our study, *trans*-2 hexenal was found only in the vegetative phase. In plants with an oxidative cycle of lipids produces biologically active compounds such as aldehydes, ketols, and derivatives of epoxy-, hydroxyl- and divinyl-ether. These compounds are generated in response to several tensions like lesions. T*rans*-2-hexenal is produced in the lipids' oxidative cycle and released after the lesion of leaves and fruits (Hatanaka & Harada, 1973). T*rans*-2-hexenal also helps control the phytopathogen *Botrytis cinerea* Pers., responsible for the soft vegetal tissue degradation such as young leaves and fruits. For Fallik et al. (1998), the maintenance of the modified atmosphere with trans-2-hexenal vapor greater than 0.48 μmol/L inhibits the mycelial growth and may avoid post-harvest mold in strawberries. T*rans*-2 hexenal was also reported by Yang et al. (2015) at a concentration of 8.9% in the essential oil of *Forsythia koreana* (Rehder) Nakai; moreover, this essential oil had antioxidant action by $DPPH^{\bullet}$ and antimicrobial activity against *Salmonella enterica* (exKauffmann and Edwards) Le Minor and Popoff, *Escherichia coli* (Migula) Castellani and Chalmers, *Staphylococcus aureus* Rosenbach, and *Listeria monocytogenes* (Murray et al.) Pirie. Thus, *trans*-2-hexenal is likely to be produced during the foliar growth in the vegetative phase. In this phase, the lesions may occur naturally through foliar growth, and trans-2-hexenal can have a protective function against phytopathogenic fungi. Our study showed that in the vegetative stage, the essential oil had a greater concentration of the primary compounds bicyclogermacrene, germacrene D, and *trans*-caryophyllene. In the phase, two compounds stood out with high concentrations: *trans*-2 hexenal and caryophyllene acid. Similar data were found by Tavares et al. (2005) that analyzed the vegetative and flowering phases of the essential oil of three *Lippia alba* chemo types cultivated under the same conditions. For those authors, the qualitative analysis did not show variation in the necessary oil composition in relation to those developed under the same conditions. For these authors, the qualitative study did not show a

change in the essential oil composition compared to the significant compounds geranial, neral, carvon and linalool, but in the quantitative analysis, these compounds presented high concentration in the vegetative phase.

The compound caryophyllene oxide was also dominant in the essential oil from *P. cauliflora* leaves (Apel et al., 2006). According to Yang et al. (2000), caryophyllene oxide is utilized by the pharmaceutical, food and cosmetic industry as a preservative; besides that it is a significant compound in essential oil with antimicrobial activity (Dias et al., 2019), inhibiting action of acetylcholinesterase (Xiang et al., 2017) and antioxidant activity (Coté et al., 2017). The antioxidant action of caryophyllene oxide was evaluated from chemical compounds of the essential oil of *Tanacetum vulgare* L. α-pinene (IC_{50} of 3.4 µg/mL) and caryophyllene oxide (IC_{50} of 6.2 µg/mL) with antioxidant activity by DCFH-DA test (assay of dichloro-dihydro-fluorescein diacetate) in fibroblasts of human skin (Coté et al., 2017).

Germacrene D was the primary compound in the essential oil of *M. cauliflora* (*P. cauliflora*) (Duarte et al., 2010). Silva et al. (2017) reported the presence of this same compound in the essential oil of *Eugenia polystachya* Rich., a plant belonging to the Myrtaceae family. Silva et al. (2016) evaluated the chemical composition of the essential oil from *Endlicheria arenosa* Chanderb. (Lauraceae) leaves and the major compounds that stood out were bicyclogermacrene, germacrene D and β-caryophyllene. In our study, bicyclogermacrene was found in all evaluated periods with higher concentration in the vegetative phase. Bicyclogermacrene is a sesquiterpene commonly found in essential oils with antifungal activity (Silva et al., 2007). On the other hand, trans-caryophyllene is present in the primary compound of *Lippia chevalieri* Moldenke with antimicrobial activity (Mevy et al., 2007), anti-inflammatory activity in *Casearia sylvestris* Sw. and *Hyptis fruticosa* (current name *Eplingiella fruticosa* (Salzm. ex Benth.) Harley & J.F.B.Pastore) (Esteves et al., 2005; Menezes et al., 2007) and gastric anti-ulcer in *Casearia sylvestris* Sw. (Esteves et al., 2005). However, no reports have been found on *trans*-caryophyllene for the antioxidant activity.

In our study, the essential oil from *P. cauliflora* leaves presented intermediate antioxidant activity only by BCLA method, with more significant event in the leaves in the vegetative phase. This method was utilized to investigate the antioxidant activity of lipophilic substances since the technique works in an aqueous emulsion with linoleic acid and β-carotene that is discolored by radicals generated by spontaneous oxidation of the fatty acid. The quantification is based on the absorbance variation of β-carotene in increasing the antioxidant (Prieto et al., 2012). Thus, possibly the essential oil from the leaves in the vegetative phase presented higher antioxidant activity in the apolar lipid medium through the

greater concentration of apolar compounds such as hydrocarbon sesquiterpenes. According to Kulisic et al. (2004), compounds that present the CHO group in their structure have high antioxidant activity by BCLA method. The compound *trans*-2-hexenal found exclusively in the leaves in the vegetative phase has the formula $C_6H_{10}O$ and, therefore, can be related to the antioxidant activity of *P. cauliflora* essential oil.

Conclusion

The yield of the essential oil from jabuticabeira leaves is the same for the vegetative, flowering, and fruiting phases. The EO chemical composition is different for each plant stage. In the flowering and fruiting stages there is a greater diversity of chemical compounds and trans-2 hexenal was found only in the vegetative phase. The antioxidant activity of the essential oil is greater for the leaves in the vegetative phase mostly to β-carotene protection in a lipophilic mixture.

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