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Antioxidant activity and chemical composition of oleoresin from leaves and flowers of *Brunfelsia uniflora*

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ABSTRACT. In this study, the temperature and pressure of supercritical CO_2 extraction were evaluated to obtain oleoresin of *Brunfelsia uniflora* leaves and flowers. The oleoresin compounds were identified by gas chromatography-mass spectrometry. The antioxidant activity was evaluated by three different methods. The highest oleoresin yields were 3.32% at 40°C and 200 bar for the leaves, and 1.03% at 60°C and 200 bar for the flowers. The main extracted compounds from leaves were phytol varying from 11.95 to 36.42% and α-tocopherol from 15.53 to 43.10%, and from flowers were geranyl linalool from 11.05 to 21.42%

and α-amyrin from 9.66 to 22.12%. Oleoresin obtained at 60°C and 150 bar from leaves presented high antioxidant activity by DPPH (IC_{50} 1.90 mg/mL) and by FRAP (1.8 μmol Fe^{2+}/mg). β-carotene/linoleic acid co-oxidation oleoresin from leaves at 0.25 mg/mL presented higher antioxidant activity than Trolox. The total phenolic content of the oleoresin from leaves ranged from 66.20 to 83.33 μg/mg and from flowers it was just up to 12.46 μg/mg. The extraction conditions affected yield, chemical composition, and antioxidant activity of oleoresin from leaves and flowers. This is the first report on the antioxidant activity of *B. uniflora* oleoresin from leaves and flowers and provides subsidies for potential applications in chemical, pharmaceutical, and food industries.

Key words: Antioxidant activity; Oleoresin; Leaves; Flowers

INTRODUCTION

Several synthetic antioxidants such as butylated hydroxyanisole and butylated hydroxytoluene have been utilized in food, medication, and cosmetic industries against free radicals. However, synthetic antioxidants have been associated with possible risks to health like toxicity and carcinogenicity (Hoelzl et al., 2005). Natural antioxidants from plant extracts and essential oils are an alternative in search of new molecules to substitute or potentialize synthetic antioxidants.

Brunfelsia uniflora (Pohl.) D. Don - Solanaceae - is a shrubby plant found in several regions of Brazil, Bolivia, Peru, Ecuador, Colombia, and Venezuela. It leaves are utilized in popular medicine as anti-rheumatic, anti-syphilitic, diuretic, emetic, and laxative (Schultes, 1979). Phytochemical analyses of *Brunfelsia* spp indicated the presence of alkaloids (Ruppelt et al., 1991), flavonoids (Brunner et al., 2000), steroidal saponins, fatty acids, cyclopropenoid (Martins et al., 2009), benzenoids, terpenes, lactones, lipids (Castioni and Kapetanidis, 1996), coumarins, sapogenins (Ichiki et al., 1994), manacein, manacin, aesculetin, scopoletin (Taylor, 2003), and caffeic acid (chlorogenic acid) (Martins et al., 2009).

Some studies on *Brunfelsia* spp are related to extraction of essential oil by hydrodistillation (Castioni and Kapetanidis, 1996) or by alcoholic extraction (Raj and Radhamany, 2010). The traditional methods to obtain essential oils from plants are a steam distillation, hydrodistillation, and extraction with organic solvents. The first two methods have the disadvantage of destabilizing thermolabile compounds and the last leaves solvent residues in the essential oil with high environmental cost (Arranz et al., 2015). Supercritical CO_2 extraction allows a high-mass transfer rate under low-temperature conditions, eliminates steps in the process, such as extract concentration, and avoids the use of organic solvents. Moreover, CO_2 keeps the antioxidant capacity of the oleoresin, is odorless, non-toxic, non-inflammable, can be easily removed from the final product without leaving residues, and is easily found in the raw state and at low cost, which makes it an alternative to obtaining industrial biocompounds (Sovilj, 2010).

It was not found in the literature reports on *B. uniflora* oleoresin obtained by supercritical CO_2. Thus, considering the unexploited potential of this plant, this study aimed to evaluate different conditions of supercritical CO_2 extraction on yield, chemical composition, and antioxidant activity of oleoresin from *B. uniflora* leaves and flowers.

MATERIAL AND METHODS

Biological material

Cultivation of *B. uniflora* was in the Medicinal Garden of Paranaense University located in the coordinates 23°46'10.3"S 53°16'38.8"W and altitude of 410 m. The exsiccate was registered in the Educational Herbarium of Paranaense University under the registration number 2855. The leaves were collected from June 1, 2014 to June 1, 2015, and the flowers from August 1, 2014 to February 1, 2015, from 7 to 9 am. The average rainfall and temperature for the period were recorded for the winter, spring, summer, and fall. The leaves and flowers were dried at 28°C, ground, and used for supercritical CO_2 extraction.

Supercritical CO_2 extraction

The oleoresin extraction from *B. uniflora* leaves and flowers were carried out in a supercritical fluid extractor using CO_2 as a solvent. Particulate material (10 g) was utilized for each extraction. The solvent was gradually pressurized (10-bar pressure intervals) using a syringe pump until reaching the desired pressure. The temperature in the extractor was kept constant using a thermostatic bath. After 20 min of stabilization, the expansion valve was open and the solvent was inserted into extractor at a 3 mL/min flow rate of solvent with a syringe pump. During the extraction, oleoresin was collected in an amber glass flask and its mass was recorded every 10 min until sample depletion. The extraction conditions were temperature (T) of 40, 50, and 60°C and pressure (P) of 150, 175, and 200 bar. These conditions were selected to obtain different specific gravity and solubilities for the solvent, observing limitations of the syringe pump. The values for specific gravity were calculated according to Linstrom and Mallard (2001). The solubilities were calculated from the linear part of the extraction curve dividing the extracted oleoresin mass by the utilized solvent mass. The extraction yield was obtained dividing the oleoresin mass obtained by the plant mass displayed in the extractor bed and multiplied by 100.

Oleoresin characterization by gas chromatography-mass spectrometry (GC-MS) analysis

The analysis of oleoresin from leaves and flowers were carried out in a gas chromatograph (Agilent 7890 B) coupled to the mass spectrum (Agilent 5977 A) equipped with an Agilent HP-5MS capillary column (30 m x 0.250 mm x 0.25 µm). The analyzed conditions were: injector temperature of 250°C, injection volume 1 µL at a ratio of 1:30 (splitless mode), an initial column temperature of 60°C and heated gradually to 285°C with a 4.3°C/min rate. The carrier gas (helium) flow was set at 1 mL/min. The temperatures of the transfer line, ion source, and quadrupole were 250, 200, and 150°C, respectively. The mass spectra were obtained in a range of 40-500 (m/z) provided through scan mode with a solvent delay time of 3 min and the compounds were identified based on a comparison of their retention indices obtained using various n-alkanes (C8-C25). Also, their electron ionization-mass spectra were compared with the NIST 11.0 library spectra and according to Adams (2007).

Antioxidant activity by β-carotene/linoleic acid co-oxidation (BCLA)

The antioxidant capacity of oleoresin from leaves and flowers was evaluated by a BCLA according to Mattos et al. (2009). A 1-mL solution (20 mg β-carotene in 1 mL chloroform) was mixed with 40 μL linoleic acid and 530 μL polysorbate-40 emulsifier. The chloroform was removed in a rotary evaporator at 50°C and 450 mL ultrapure water (previously saturated with oxygen for 30 min) was added under vigorous agitation. Aliquots (5 mL) of this emulsion were transferred to a series of assay tubes containing 1 mL oleoresin from leaves or flowers at the concentrations of 0.25, 0.50, 0.75, and 1.00 mg/mL. The tubes were submerged in water at 50°C for 120 min, and the absorbance was measured at 470 nm after 0, 15, 30, 45, 60, 75, 90, 105, and 120 min. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was utilized as standard reference (control). The antioxidant activity was expressed as oxidation inhibition percentage.

Antioxidant activity by sequestration of (2,2-diphenyl-1-picrylhydrazyl) (DPPH) free radicals

The antioxidant capacity was evaluated utilizing the sequestration of DPPH-free radicals according to Rufino et al. (2007). A volume of 0.1 mL oleoresin from leaves or flowers at concentrations of 0.25, 0.50, 0.75, and 1.00 mg/mL was mixed with 3.9 mL DPPH methanolic solution (60 μM). Methanol (0.1 mL) was used as a negative control. After 30 min, readings of sample absorbance were done at 515 nm in a UV/VIS spectrophotometer. The total antioxidant capacity of oleoresin was calculated utilizing a quercetin standard solution (60 μM) as 100% reference (Molyneux, 2004). The sample concentration needed to reduce 50% of the free radicals (IC_{50}) was determined from a correlation between the absorbance versus sample concentration. The absorbance of the half concentration of the radical (30 μM) in DPPH solution was obtained by a correlation between the absorbance and concentration of a DPPH solution at 10.0, 20.0, 30.0, 40.0, 50.0, and 60.0 μM.

Antioxidant activity of ferric ion reducing antioxidant power (FRAP)

For the FRAP, 25 mL acetate buffer (0.3 M), 2.5 mL TPTZ (2,4,6-Tris (2-piridil)-triazine) aqueous solution (10 mM) and 2.5 mL ferrous chloride aqueous solution (20 mM) were mixed according to Rufino et al. (2006). Oleoresin (90 μL) from leaves or flowers were mixed with 270 μL ultrapure water and 2.7 mL FRAP reagent for the antioxidant activity reaction. The mixture was vigorously homogenized and kept at 37°C for 30 min. The absorbance variance was read at 595 nm and the percentage of antioxidant activity was calculated with the standard curve of ferrous sulfate (2000 μM).

Total phenolic content (TPC)

The TPC was determined by the colorimetric method (Albarici et al., 2009). Folin-Ciocalteau reagent (2.5 mL) diluted at 10% in ultrapure water, 2.0 mL Ca_2CO_3 at 0.75 g/L, and 500 μL oleoresin from leaves or flowers at the concentration of 1.0 mg/mL was kept at 50°C for 15 min and analyzed at 760 nm in a UV/VIS spectrophotometer. A standard curve with a gallic acid solution from 10 to 100 μg/mL was utilized as standard.

Data analysis

All analyses were carried out at least in triplicates. The results were submitted to analysis of variance (ANOVA) and the differences among means were determined by the Scott-Knott test ($P \leq 0.05$).

RESULTS

The average rainfall during the collection of biological material was 151 mm in the winter, 144 mm in the spring, 186 mm in the summer, and 156 mm in the fall. The minimum and maximum temperatures were 15°-25°C in the winter, 19°-30°C in the spring, 21°-31°C in the summer, and 18°-27°C in the fall.

The particulate fraction of 850 µm was the highest mass yield in sieving of dried and ground leaves or flowers. This granulometric fraction was selected for the supercritical CO_2 extraction. Oleoresin extraction was obtained under five extraction conditions, and specific gravity and solubility values were calculated (Table 1). The extraction yield for oleoresin from leaves or flowers is shown in Figures 1 and 2, respectively. The extraction depletion time of oleoresin from leaves was 190 min and the one from flowers was 120 min. The highest yield of oleoresin from leaves was 3.32% obtained at T = 40°C and P = 200 bar (Figure 1), whereas the one from flowers was 1.03% obtained at T = 60°C and P = 200 bar (Figure 2).

Table 1. Experimental conditions of supercritical carbon dioxide extraction assays.

Code	Temperature (°C)	Pressure (bar)	CO_2 specific gravity (kg/m^3)[a]	Solubility (g oleoresin/kg CO_2)[b]
T40P150	40	150	780.87	0.62
T40P200	40	200	840.67	1.18
T50P175	50	175	749.84	1.05
T60P150	60	150	604.61	0.29
T60P200	60	200	724.11	0.94

[a]Carbon dioxide densities were obtained from Linstrom and Mallard (2001); [b]the solubility was calculated from the linear part of the extraction curve (extracted oleoresin mass/utilized solvent mass).

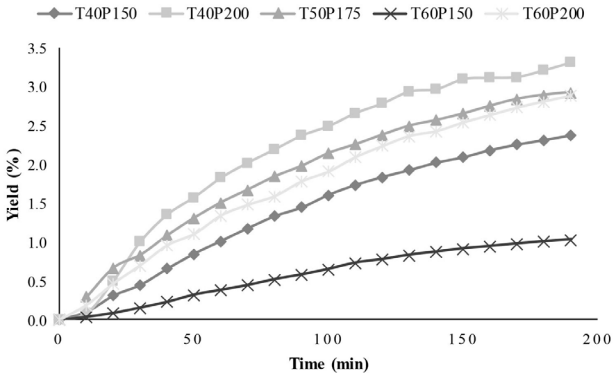

Figure 1. Yield of oleoresin extraction from *Brunfelsia uniflora* leaves obtained by supercritical carbon dioxide extraction under different conditions of temperature and pressure. Extraction conditions: T40P150 - temperature of 40°C and pressure of 150 bar; T40P200 - temperature of 40°C and pressure of 200 bar; T50P175 - temperature of 50°C and pressure of 175 bar; T60P150 - temperature of 60°C and pressure of 150 bar; T60P200 - temperature of 60°C and pressure of 200 bar.

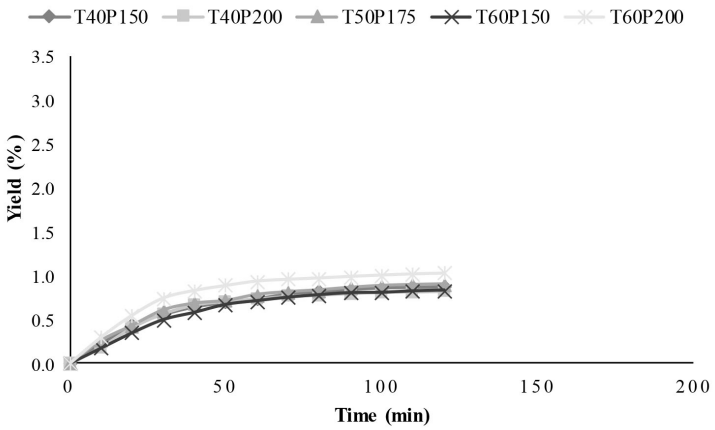

Figure 2. Yield of oleoresin extraction from *Brunfelsia uniflora* flowers obtained by supercritical carbon dioxide extraction under different conditions of temperature and pressure. Extraction conditions: T40P150 - temperature of 40°C and pressure of 150 bar; T40P200 - temperature of 40°C and pressure of 200 bar; T50P175 - temperature of 50°C and pressure of 175 bar; T60P150 - temperature of 60°C and pressure of 150 bar; T60P200 - temperature of 60°C and pressure of 200 bar.

GC-MS analysis under different extraction conditions of oleoresin from leaves resulted in the identification of 23 compounds and the major compounds were phytol and tocopherol found under all extraction conditions (Table 2). The increase in temperature and pressure extraction conditions increased phytol extraction in detriment of tocopherol. The extraction conditions of average temperature and pressure resulted in oleoresin with equal concentrations of phytol and alpha-tocopherol.

Table 2. Chemical composition of *Brunfelsia uniflora* leaf oleoresin obtained by supercritical carbon dioxide extraction under different conditions.

Peak	[A]Compound	[a]RI	Area (%)					Identification method
			T40P150	T40P200	T50P175	T60P150	T60P200	
1	Butanoic acid	782	t	t	8.13	t	t	a.b.
2	Farnesol	1759	t	10.89	t	2.39	1.85	a.b.
3	2-hexyl, 1-decanol	1770	t	0.61	t	t	t	a.b.
4	Octadecane	1779	4.14	t	t	3.31	2.14	a.b.
5	Farnesyl acetate (2E,6E)	1879	t	1.39	14.74	9.23	6.94	a.b.
6	*n*-hexadecanoic acid	1982	4.14	7.96	t	2.10	5.10	a.b.
7	Phytol	1991	11.95	21.55	19.18	24.60	36.42	a.b.
8	9,12,15-octadecatrienoic acid	2102	t	6.06	t	t	7.79	a.b.
10	Linoleic acid	2133	t	t	t	t	3.31	a.b.
11	Oleic acid	2159	t	t	t	t	t	a.b.
12	Octadecanoic acid	2160	t	1.11	t	t	t	a.b.
13	Geranylgeraniol	2197	t	t	t	1.90	t	a.b.
14	*Trans*-geranylgeraniol	2200	t	3.04	2.38	t	t	a.b.
15	Hexacosane	2606	t	t	t	t	t	a.b.
16	Heptacosane	2717	11.18	5.54	12.46	11.81	8.42	a.b.
17	Octacosane	2809	2.23	1.08	3.34	2.89	1.32	a.b.
18	Campestrol	2854	t	t	t	t	t	a.b.
19	Nonacosane	2912	17.48	6.36	17.51	15.77	9.25	a.b.
20	β-sitosterol	3180	t	2.49	t	t	t	a.b.
21	Squalane	3198	9.92	1.87	1.94	1.73	1.50	a.b.
22	α-α-tocopherol	3216	43.10	27.92	15.53	24.27	15.96	a.b.
23	Stigmasterol	3356	t	2.12	4.78	t	t	a.b.
	Total identified		**100.00**	**99.99**	**99.99**	**100.00**	**100.00**	

[A]Compounds listed in elution order in HP-5MS column; [a]identification based on retention index (RI); [b]identification based on comparison of mass spectrum; RI: retention index; t: traces; T: temperature in °C; P: pressure in bar.

GC-MS analysis under different extraction conditions of oleoresin from flowers resulted in the identification of 30 compounds and the major compounds were geranyl linalool (11.05 to 21.42%), tetracosane (9.35 to 24.23%), and alpha-amyrin (9.66 to 22.12%) found under all extraction conditions (Table 3). The increase in temperature extraction condition did not affect the extracted amount of geranyl linalool, but the pressure increase from 150 to 200 bar almost doubled the extraction of geranyl linalool. On the other hand, the increase in temperature to 60°C and the pressure at 150 bar increased tetracosane. As higher was the pressure and temperature higher was the alpha-amyrin concentration in the oleoresin.

Table 3. Chemical composition of *Brunfelsiauniflora*flower oleoresin obtained by supercritical carbon dioxideextraction under different conditions.

Peak	[A]Compound	[a]RI	Area (%)					Identification method
			T40P150	T40P200	T50P175	T60P150	T60P200	
1	*Cis*-linalool oxide	1084	t	0.66	0.85	t	t	a.b.
2	*Trans*-linalool oxide	1187	2.04	1.88	2.95	t	t	a.b.
3	Pentadecane	1502	0.28	0.31	0.66	t	t	a.b.
4	(*E*)-nerolidol	1566	t	0.52	0.49	t	t	a.b.
5	(*2Z,6Z*)-farnesol	1688	t	0.40	0.62	t	t	a.b.
6	Tetradecanoic acid	1697	8.37	0.63	1.23	9.44	t	a.b.
7	Heptadecane	1704	t	0.64	0.52	t	t	a.b.
8	Octadecane	1796	0.84	0.75	1.87	t	t	a.b.
9	Nonadecane	1901	2.00	3.06	3.18	t	2.49	a.b.
10	Hexadecanoic acid	1950	1.70	2.85	2.47	1.02	2.12	a.b.
11	9,12,15-octadecatrien-1-ol	1965	t	t	1.35	t	t	a.b.
12	Hexadecanoic acid ethyl ester	1967	1.08	5.94	2.54	2.36	6.81	a.b.
13	(*E,E*)-geranyllinalool	1996	11.70	21.42	19.32	11.05	19.25	a.b.
14	Phytol	2015	t	0.98	0.90	0.83	1.57	a.b.
15	Linoleic acid	2140	2.28	3.72	3.06	3.40	2.30	a.b.
16	n.i	2163	t	0.30	0.15	t	0.19	a.b.
17	Octadecanoic acid	2167	t	1.42	1.08	1.02	2.00	a.b.
18	(*E,E*)-9,12-octadecadienoic acid, methyl ester	2172	t	1.43	2.23	2.97	1.68	a.b.
19	Eicosatrienoic acid	2182	3.19	3.67	4.06	t	3.75	a.b.
20	Docosane	2199	2.42	6.02	6.03	3.05	5.28	a.b.
21	Tetracosane	2401	16.69	10.71	9.35	24.23	10.62	a.b.
22	Docosanoic acid	2500	2.47	4.01	4.82	5.00	t	a.b.
23	Pentacosane	2517	14.26	4.14	3.42	7.35	10.78	a.b.
24	Nonacosane	2922	7.74	5.97	6.71	2.55	4.52	a.b.
25	*β*-sitosterol	3167	1.53	0.33	1.14	t	t	a.b.
26	*α*-amyrin	3176	13.23	11.01	9.66	19.55	22.12	a.b.
27	*α*-tocopherol	3201	2.79	3.88	4.35	3.41	4.53	a.b.
28	Dotriacontane	3217	1.47	1.02	1.19	1.84	t	a.b.
29	Stigmast-5-ene-3,beta,29-diol	3328	t	0.63	0.78	t	t	a.b.
30	Tetradecanoic acid, hexadecyl ester	3396	3.90	2.09	2.10	0.70	t	a.b.
	Total identified		99.90	99.80	98.93	99.77	99.82	

[A]Compounds listed in elution order in HP-5MS column; [a]identification based on retention index (RI); [b]identification based on comparison of mass spectrum; RI: retention index; t: traces; T: temperature in °C; P: pressure in bar; n.i.: non-identified compound.

The antioxidant activity of oleoresin by BCLA, under different extraction conditions, from leaves varied from 120.27 to 124.14% Trolox at 400 µM, and from flowers it was from 57.09 to 67.17% Trolox at 400 µM (Table 4). Figures 3 and 4 show the absorbance of the BCLA along the reaction time. The absorbance of negative control (without antioxidant) reduced from 0.7 to 0.3 after 60 min; for the oleoresin from leaves the absorbance was from 0.7 to 0.66 after 60 min; although the oleoresin from flowers decreased from 0.7 to 0.40 after 60 min. Therefore, oleoresin obtained from leaves did not allow the oxidation of fatty acid, avoiding the loss of β-carotene absorbance. At the lowest tested concentration (0.25 mg/mL), oleoresin from leaves was more efficient than the positive control Trolox (0.1 mg/mL). For

oleoresin from leaves, the absorbance after 120 min was around 0.65 whereas it was 0.52 (Figure 3) for the positive control Trolox. For oleoresin from flowers (Figure 4) the opposite occurred, with a quick reduction of absorbance, similar to the one observed for the negative control without antioxidants.

Table 4. Antioxidant activity of oleoresin (0.5 mg/mL) from *Brunfelsia uniflora* leaves extracted by supercritical carbon dioxide under different extraction conditions.

Extraction condition		BCLA (%)	DPPH (%)	IC_{50} (mg/mL)	FRAP (μmol Fe^{2+}/mg^{-1})	TPC (μg gallic acid/mg sample)
T (°C)	P (bar)					
40	150	120.94 ± 0.89[a]	47.08 ± 8.74[d]	5.64 ± 0.50[c]	0.92 ± 0.07[b]	68.89 ± 4.97[b]
40	200	122.51 ± 2.47[a]	47.61 ± 13.84[d]	6.75 ± 0.84[c]	0.83 ± 0.03[c]	68.02 ± 0.07[b]
50	175	120.27 ± 1.66[a]	117.45 ± 15.71[b]	3.91 ± 0.57[b]	0.75 ± 0.02[c]	80.07 ± 3.51[a]
60	150	121.90 ± 0.65[a]	165.07 ± 14.11[a]	1.90 ± 0.21[a]	1.80 ± 0.05[a]	66.20 ± 5.81[b]
60	200	124.14 ± 9.51[a]	91.53 ± 8.91[c]	3.26 ± 0.53[ba]	1.00 ± 0.01[b]	83.33 ± 0.07[a]

Values are the mean ± standard deviation. Values in the same column with different letters are significantly different by the Scott-Knott test ($P \leq 0.05$). T: temperature and P: pressure. The percentage for β-carotene/linoleic acid co-oxidation (BCLA) was calculated using trolox 400 μM and for sequestration of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was quercetin 60 μM. For DPPH the IC_{50} of the positive control quercetin was 0.06 ± 0.01 mg/mL. For ferric ion reducing antioxidant power (FRAP) the positive control trolox was 11.14 ± 0.07 μmol Fe^{2+}/mg. Total phenolic content (TPC).

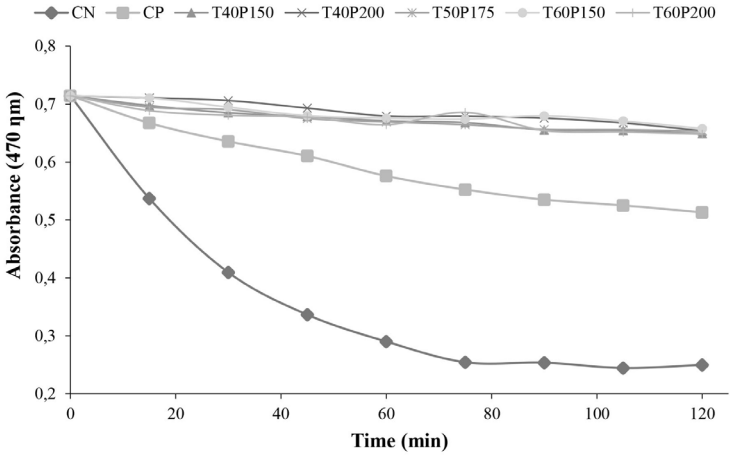

Figure 3. Absorbance along mixture time of β-carotene/linoleic acid co-oxidation (BCLA) with oleoresin extraction from *Brunfelsia uniflora* leaves obtained by supercritical carbon dioxide extraction under different extraction conditions. CN: mixture without antioxidant; CP: mixture with the presence of 0.1 mg/mL trolox; mixture added with 0.25 mg/mL oleoresin extracted from leaves according to the extraction conditions: T40P150 - temperature of 40°C and pressure of 150 bar; T40P200 - temperature of 40°C and pressure of 200 bar; T50P175 - temperature of 50°C and pressure of 175 bar; T60P150 - temperature of 60°C and pressure of 150 bar; T60P200 - temperature of 60°C and pressure of 200 bar.

Oleoresin from leaves presented greater antioxidant activity than oleoresin from flowers by the BCLA. However, there was no difference in the antioxidant activity under different leaf extraction conditions. The absorbance curves (Figure 3) were similar among the different extraction conditions, showing the absence of antioxidant activity variability with the extraction condition. The greater antioxidant activity of leaves can be related to the presence of great contents of phenolic compounds that varied from 83.33 to 66.20 μg gallic acid/mg and the flowers were up to 12.46 μg gallic acid/mg (Tables 4 and 5).

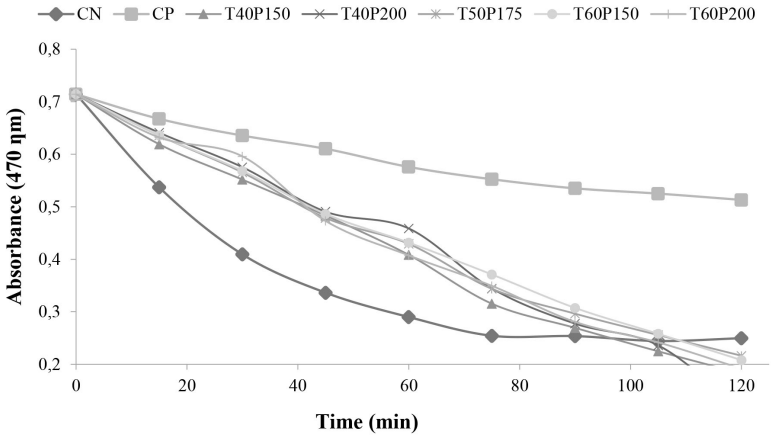

Figure 4. Absorbance along mixture time of β-carotene/linoleic acid co-oxidation (BCLA) with oleoresin extraction from *Brunfelsia uniflora* flowers obtained by supercritical carbon dioxide extraction under different extraction conditions. CN: mixture without antioxidant; CP: mixture with the presence of 0.1 mg/mL trolox; mixture added with 0.25 mg/mL oleoresin extracted from leaves according to the extraction conditions: T40P150 - temperature of 40°C and pressure of 150 bar; T40P200 - temperature of 40°C and pressure of 200 bar; T50P175 - temperature of 50°C and pressure of 175 bar; T60P150 - temperature of 60°C and pressure of 150 bar; T60P200 - temperature of 60°C and pressure of 200 bar.

Table 5. Antioxidant activity of oleoresin (0.5 mg/mL) from *Brunfelsia uniflora* flowers extracted by supercritical carbon dioxide under different extraction conditions.

Extraction condition		BCLA (%)	DPPH (%)	IC_{50} (mg/mL)	FRAP (μmol Fe^{2+}/mg)	TPC (μg gallic acid/mg sample)
T (°C)	P (bar)					
40	150	57.09 ± 5.83[a]	≈0	-	-	7.67 ± 0.49[c]
40	200	62.34 ± 1.66[a]	≈0	-	-	11.00 ± 0.86[b]
50	175	62.22 ± 5.22[a]	≈0	-	-	10.75 ± 0.97[b]
60	150	67.17 ± 1.06[a]	≈0	-	-	12.32 ± 0.33[a]
60	200	63.37 ± 1.09[a]	≈0	-	-	12.46 ± 0.40[a]

Values are the means ± standard deviation. Values in the same column with different letters are significantly different by the Scott-Knott test ($P \leq 0.05$). T: temperature and P: pressure. The percentage for β-carotene/linoleic acid co-oxidation (BCLA) was calculated using trolox 400 μM and for sequestration of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was quercetin 60 μM. For DPPH the IC_{50} of the positive control quercetin was 0.06 ± 0.01 mg/mL. For ferric ion reducing antioxidant power (FRAP) the positive control trolox was 11.14 ± 0.07 μmol Fe^{2+}/mg. Total phenolic content (TPC). ≈0 value close to zero. - analysis not done due to low value of BCLA and DPPH.

The antioxidant activity of oleoresin by DPPH from leaves varied from 165.07 to 47.08% of quercetin at 60 μM (Table 4) whereas the antioxidant activity was almost inexistent in oleoresin from flowers (Table 5). Leaf extraction condition T = 60°C and P = 150 bar presented the smallest IC_{50} of 1.90 mg/mL, indicating the highest antioxidant activity for this condition. However, for DPPH, the antioxidant activity varied for the different extraction conditions. Oleoresin from leaves had a greater antioxidant activity under extraction condition of T = 60°C and P = 150 bar but presented smaller TCP (66.20 μg gallic acid/mg) (Table 4).

The antioxidant activity of oleoresin by FRAP from leaves varied from 1.8 to 0.75 μmol Fe^{2+}/mg (Table 4). The greatest activity was obtained under extraction condition of T = 60°C and P = 150 bar (Table 4), which is inferior to Trolox positive control (11.14 μmol Fe^{2+}/mg). FRAP corroborated the results obtained by DPPH in which the extraction condition of T

= 60°C and P = 150 bar had a greater antioxidant activity for leaves. For this condition, there was the smallest extraction yield of 1.03%. There was also equilibrium between the amount of phytol and tocopherol with 24.60 and 24.27%, respectively (Table 2). Possibly phytol and tocopherol synergistically acted in the increase of the antioxidant activity. However, oleoresin from leaves - extracted at 60°C and 150 bar - had high antioxidant activity and was still from 31 to 6 times smaller than the positive controls, quercetin for DPPH and Trolox for FRAP, respectively.

DISCUSSION

Oleoresin supercritical CO_2 extraction presents higher yield than the values reported by hydrodistillation. Castioni and Kapetanidis (1996) carried out hydrodistillation of *Brunfelsia grandiflora* aerial parts and obtained 0.07% yield. The highest yield obtained by supercritical CO_2 extraction in our study was 3.32%. Oleoresin yield of supercritical CO_2 extraction increased with pressure increase. According to Sovilj (2010), pressure increase raises CO_2-specific gravity because it reduces the distance between the molecules and increases oleoresin and CO_2 interaction as well as oleoresin solubility in carbon dioxide.

It was not found reports on the chemical composition of *B. uniflora* oleoresin. Bertrand et al. (2006) analyzed the volatile compounds of flowers from two other species of *Brunfelsia* genus by solid-phase microextraction and found monoterpenes (87%) for *Brunfelsia australis*, and as main compounds linalool (35.5%) and (*E*)-β-ocimene (41.8%). For *Brunfelsia pauciflora*, the predominant class was sesquiterpenes (83.4%), and the main compounds were γ-muurolene (32.9%) and α-copaene (31.2%). For the essential oil obtained by hydrodistillation (5 h), Castioni and Kapetanidis (1996) reported the presence of hydrocarbons, aldehydes, ketones, alcohols, and esters for *B. grandiflora*. Methyl salicylate was the main compound and almost a third of the identified compounds originated from terpenes, and some of them had traces of phytol. In our study, the main compounds of oleoresin from leaves were phytol and tocopherol, and from flowers was geranyl linalool.

Phytol is a diterpene component of the chlorophyll molecule, found in green leaves of several medicinal plants. Various therapeutic activities of phytol were reported as anticonvulsive (Costa et al., 2012), antispasmodic (Pongprayoon et al., 1992), anti-tuberculosis (Saikia et al., 2010), and anticancer (Lee et al., 1999). Some studies have shown promising pharmacological activities of phytol, but few have focused on an antioxidant activity (Santos et al., 2013). The antioxidant activity of phytol - an unsaturated alcohol with branched chain - can be related to the hydroxyl group (OH) present in its molecule and that reacts with a free radical, converting it into a less reactive species (Hoelzl et al., 2005). Phytol extraction is often difficult to obtain. In our study oleoresin with 11.95% phytol under the extraction condition of T = 40°C and P = 150 bar was obtained, but when the temperature and pressure increased to 60°C and 200 bar, the phytol obtained was three times higher (36.42%). Zekovic et al. (2009) analyzed laurel leaves (*Laurus nobilis*) by supercritical CO_2 extraction and obtained phytol only under the extraction condition of 40°C and 250 bar. Kamali et al. (2014) extracted phytol from *Biebersteinia multifida* by supercritical CO_2 under the condition of 60°C and 270 bar, where the maximum antioxidant activity occurred.

Phytol and tocopherol reached up to 43.10% of oleoresin. These compounds are antioxidants because of their capacity to donate their hydrogens to free radicals, interrupting the propagation of chain oxidation reaction. Tocopherols are labile in the presence of

oxygen, light, and heat (Ramalho and Jorge, 2006). In our study, the increase in pressure and temperature extraction conditions reduced tocopherol content in oleoresin. Lucas et al. (2002) reported that the optimal temperature for tocopherol extraction is 40°C. This suggests that tocopherol is unstable at high temperatures. However, Nyam et al. (2010) obtained tocopherol by supercritical CO_2 extraction from watermelon (*Citrullus lanatus*) and hibiscus (*Hibiscus sabdarriffa*) seeds under temperature and pressure conditions ranging from 40° to 80°C and from 200 to 400 bar, respectively, and pointed out that temperature did not have any effect on watermelon seed extraction but had an effect on hibiscus seed extraction. Moreover, Xu et al. (2008) reported obtaining tocopherol by supercritical CO_2 extraction from *Hippophae thamnoides* berries under temperature and pressure extraction conditions from 40° to 60°C and from 200 to 400 bar, respectively, emphasizing the positive linear effects of temperature and pressure on tocopherol extraction. Under isobaric conditions, the increase of temperature reduces CO_2-specific gravity and increases solute vapor pressure (King et al., 2001). Both these conditions have opposing effects on the compound solubility. While a lower CO_2-specific gravity reduced solute solubility, a greater vapor pressure would increase tocopherol solubility. Therefore, the decrease of tocopherol extraction yield with an increase in temperature could be explained. Thus, it is possible that the temperature effect on CO_2-specific gravity, which can reduce tocopherol solubility, has been greater than the increase of tocopherol vapor pressure within the utilized experimental interval.

Oleoresin from leaves presented greater antioxidant activity than flowers. The TPC was five to seven times greater in leaves than in flowers. The low TPC for oleoresin from flowers is compatible with the smaller antioxidant activity of it. In oleoresin from flowers, the major compound was geranyl linalool. This compound has insecticide and antibacterial action (Lemaire et al., 1990; Jirovetz et al., 2007), but no reports on the antioxidant activity have been found.

Leaves and flowers are different vegetal tissues with different biological functions (Ferri, 1999), which define the substances produced. Leaves are specialized in light capture and gas exchange with the atmosphere for photosynthesis and respiration. The reactive oxygen species are produced during the metabolic functions of peroxisomes or are induced by environmental stimuli in chloroplasts and mitochondria (D'Autréaux and Toledano, 2007). Reactive oxygen species accumulation can cause damage to biomolecules like DNA, RNA, proteins, and cell membranes (Jaleel et al., 2009). To avoid these damages, a plant produces secondary antioxidant metabolites, mainly represented by dismutase superoxide enzyme, peroxidase, and catalase, whereas the non-enzymatic mechanism occurs through molecules as ascorbate, glutathione, tocopherols, carotenoids, phenolic compounds (Jaleel et al., 2009), and terpenes like phytol (Santos et al., 2013). Thus, because the leaves have higher metabolic activity than the flowers, they also concentrate more antioxidant compounds.

The greatest antioxidant activity and diversity of compounds in leaves are also described for other plants. Azevedo et al. (2014) verified that *Costus spicatus* leaf extracts present triterpenes, steroids, flavonoids, saponins, whereas the flower has tannins and alkaloids. Andrade et al. (2014) reported that *Smallanthus sonchifolius* leaves presented phenolic content ranging from 88 to 94% more than flowers using two different extraction methods (infusion and cooking); also IC_{50} for DPPH was from 2 to 28% lower for leaves than flowers, showing a greater leaf antioxidant potential. Fernando et al. (2013) described TPC and the total antioxidant capacity of different parts of *Withania somnifera* in which leaves had the greater antioxidant activity than other parts. No reports have been found on the antioxidant activity and TPC of *B. uniflora* oleoresin.

In conclusion, oleoresin from *B. uniflora* leaves obtained by supercritical CO_2 extraction has greater antioxidant activity and TPC than flowers. The extraction condition affects the yield, chemical composition, and antioxidant activity of oleoresin. The main compounds of oleoresin from leaves are phytol and tocopherol, and from flowers is geranyl linalool and alpha-amyrin. The increase in pressure and temperature increases phytol extraction and reduces tocopherol extraction. This report is the first of antioxidant activity of oleoresin in *B. uniflora* leaves and flowers and provides subsidies for potential applications in the chemical, pharmaceutical, and food industries.

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