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Research Article

Essential Oil Constituents and Yields from Leaves of *Blepharocalyx salicifolius* (Kunt) O. Berg and *Myracrodruon urundeuva* (Allemão) Collected during Daytime

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The purpose of this study was to evaluate the essential oil composition and yield from leaves of two Brazilian species (*Myracrodruon urundeuva* and *Blepharocalyx salicifolius*) harvested during daytime. Essential oils were obtained by steam distillation and had their yield determined. *Blepharocalyx salicifolius* presented yields of 0.049% (9 a.m.), 0.045% (1 p.m.), and 0.069% (5 p.m.). For *Myracrodruon urundeuva*, we found 0.13% (9 a.m.), 0.11% (1 p.m.), and 0.08% (5 p.m.). Finally, compound identification and quantification were carried out by GC-MS and GC-FID techniques, respectively. Thirteen major compounds were identified for *Blepharocalyx salicifolius*, representing 91.6% of the EOs, of which *p*-cymene (25.9%) was detected as amajor component. Nine major compounds were identified for *Myracrodruon urundeuva*, representing 90.3% of the EOs, whereas β-myrcene showed the greatest concentration (66.4%).

1. Introduction

Essential oils (EOs) are products of the secondary metabolism of plants, defined as the volatile lipid soluble portion of plant fluids containing odiferous compounds of vegetable plant matter [1]. Essential oils are obtained from plant material: flowers, buds, twigs, bark, herbs, wood, fruits, and roots [2]. They are widely used on pharmaceutics, cosmetics, food industry, and popular medicine, as well as aromatherapy and pesticide industries. EOs are composed mainly of isoprene units in addition to alcohol, ester, aldehyde, ketone, carboxylic acid, and alkane [1].

Qualitative and quantitative variation in essential oil composition have been reported between different daytime and seasons of collection [3–5]. EOs may be affected by several factors including nutrition, solar radiation, temperature, humidity, location, and genetics as well as the daytime upon which harvesting is made.

The Brazilian savanna (Cerrado) consists of a rich flora that comprises several species [6]. *Blepharocalyx* is a common genus of the family Myrtaceae, widely spread in many countries of Latin America, namely, Brazil, Argentina, Uruguay, and Paraguay. It is commonly used for medicinal purposes in view of its antidiarrheal and digestive properties. Moreover, it is used to treat urethritis and cystitis [7], cough, bronchitis, rheumatism, arthritis, and sinusitis. It is also used as hypotensive, astringent, antibacterial, and antispasmodic [8]. Recent reports investigating chemical composition of *Blepharocalyx salicifolius* EOs by GC-MS analysis have already found several constituents, such as 1,8-cineole, linalool, β-caryophyllene [9], β-pinene, limonene [10, 11], myrcene, α-pinene [11], spathulenol, and pinene [10].

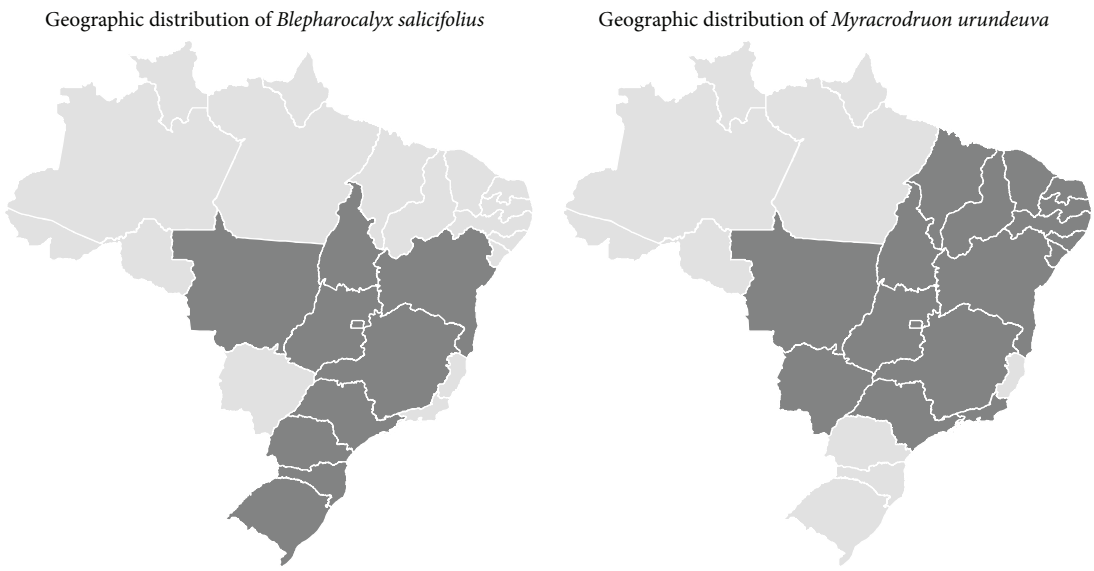

Figure 1: Geographic distribution of *Blepharocalyx salicifolius* and *Myracrodruon urundeuva*.

Myracrodruon is a genus in the family Anacardiaceae, which presents tropical and subtropical distribution, comprising about 70 genera and 700 species [12]. The species *Myracrodruon urundeuva* is popularly used for treating skin disorders, ulcers, and respiratory and urinary tract problems [13–15]. Scientific studies showed anti-inflammatory, antiulcer, analgesic [16], antibacterial, antifungal [17], antidiarrheal [18], and antiviral against rotavirus [19] properties. It is a potential larvicidal agent for *Aedes aegypti* control [20]. The EOs obtained from leaves present nearly 16 constituents such as α-Pinene, γ-Terpinene, β-Caryophyllene [9], and δ-3-Carene [21].

Considering the scarcity of studies with regard to the chemistry of oils from Brazilian savanna species, this study aims at analyzing the chemical compounds from leaves of the Brazilian savanna species known due to their EOs production: *Blepharocalyx salicifolius* (Kunt) O. Berg and *Myracrodruon urundeuva* (Allemão).

2. Experimental

Leaves were collected from an experimental station at University of Brasilia (Brasilia, Brazil). *Blepharocalyx salicifolius* is spread in south, center, and southeast Brazil [8]. *Myracrodruon urundeuva* is spread in center, southeast, and northeast Brazil [22] (Figure 1).

Blepharocalyx salicifolius species samples were collected on March 24 and April 21, 2009, while *Myracrodruon urundeuva* samples were collected on May 23, 2009, at weather conditions presented in Table 1.

2.1. Extraction and Yield Calculation. Leaves were collected during daytime (9 a.m., 1 p.m., and 5 p.m.), resulting in 18 samples (3 samples × 3 periods × 2 species) of 250 g each. Dry leaves samples were extracted by steam distillation using a Linux D1 distiller for 90 minutes. The resulting liquid was stored in sealed amber vials and kept under refrigeration (~10°C/50% humidity). Ethyl acetate was used as solvent to separate both organic and aqueous phases. The former was dried with sodium sulfate and submitted to rotary evaporation at 25°C to yield pure essential oil.

Table 1: Weather conditions for collection months [23].

Weather condition	March	April	May
Max. temperature (°C)	27.0	26.2	25.4
Min. temperature (°C)	18.0	14.9	14.3
Humidity (%)	90–50	47	62

The yield was calculated using the equation $\mathrm{RO}_u = (M/B_m) \times 100$, where M is the mass of the extracted oil (g) and B_m is the initial plant biomass (g). Tukey's test was used with a 5% significance level.

2.2. GC-FID and GC-MS Analysis. Essential oil analysis was carried out in an Agilent 7890A gas chromatograph fitted with a HP-5 capillary column (5%-phenyl-95%-methylpolysiloxane, 30 m × 0.32 mm × 0.25 μm), using hydrogen as carrier gas (1.5 mL/min). Oven temperature was programmed from 60 to 240°C at 3°C/min. A 1% solution of the oil in dichloromethane was injected at 250°C in split mode (1 : 20). Results are expressed as normalized relative area, calculated from a FID (280°C) signal.

In order to identify the oil components, an Agilent 6890 gas chromatograph coupled to an Agilent 5973N mass selective detector was used, fitted with a HP5MS capillary column (5%-phenyl-95%-methylpolysiloxane, 30 m × 0.25 mm × 0.25 μm). Helium was used as carrier gas (1.0 mL/min). Both the injection procedure and oven temperature program were equivalent to the latter. The mass selective detector was operated in electron ionization mode (70 eV). Mass spectra obtained were compared with data from a Wiley 6th edition

library. Linear retention indices were calculated by injecting a series of *n*-alkanes in the same column and chromatographic conditions as above [24]. Retention indices were calculated using the equation of van den Dool and Kratz [24]. Positive identification was considered only when both mass spectrum and retention index were in good agreement with library data [25].

3. Results and Discussion

3.1. Extraction and Yield Calculation. *B. salicifolius* and *M. urundeuva* presented yields are shown in Figure 2. Yields obtained by steam distillation technique were inferior to those found in the literature. Mattos (1983) cited by Marques [26] found 0.17% and Castelo et al. [27] found 0.10% for *Blepharocalyx salicifolius*.

Through statistical analysis results, one can conclude that collection time has no role in EOs yield. However, a slight trend towards a higher yield is observed for *B. salicifolius* at 5 p.m. These results can be explained by the fact that the temperature and humidity variation along the day are not significant as to affect the production of EOs in these species, which, as most Cerrado species, are known for their ability to withstand long periods of drought. Besides, Castello et al. [28] found that during the raining season the production of essential oil can be lower than during the drought season. The same results were found for *M. urundeuva*, but a trend for higher yields was observed for 9 a.m. samples.

Differences between yields may be explained considering whether younger or older leaves were collected, although extreme care was taken to guarantee uniform collection. Favorito [29] explained that young leaves have more trichomes, the morphologic structures that produce EOs. At the same time that leaf expands, the density of trichomes gets lower, resulting in a lower production of EOs [30]. Also, damage to the leaves, like the one caused by fungus, can damage glands that produce EOs.

3.2. GC-FID and GC-MS Analysis. Results from GC-FID and GC-MS analysis (Table 2) present *B. salicifolius* identified compounds with respective retention indices and relative peak area along with total area for each chemical class. Forty-one components were identified in essential oil leaves of *B. salicifolius*, comprising 90.3% of the EOs. The results found could be compared to those reported by Limberger et al. (2001) [9], obtained through hydrodistillation method (oil extraction) and GC-MS technique (chemical compounds identification), of *B. salicifolius* plants: 1,8-cineole (25.2%), linalool (20.4%), and β-caryophyllene (22.9%), as well as α-pinene (1.1%), β-pinene (0.2%), limonene (3.0%), terpinen-4-ol (1.2%), α-terpineol (2.0%), and caryophyllene oxide (1.3%). Castello et al. [28], using the same technique, found differences in chemical composition throughout the seasons and that *p*-cymene, α-pinene, α-terpineol, aromadendrene, globulol, and caryophyllene oxide are major compounds of *B. salicifolius* EOs. Tucker et al. [10] found limonene (5.26%), α-pinene (9.18%), spathulenol (5.30%), 1,8-cineole (1.32%), and linalool (0.14%). The compositions varied within the same species in view of differences in soil composition, climate, temperature, period of collection, and humidity. Geographic and genetic factors were indicated by Vitti and Brito [31].

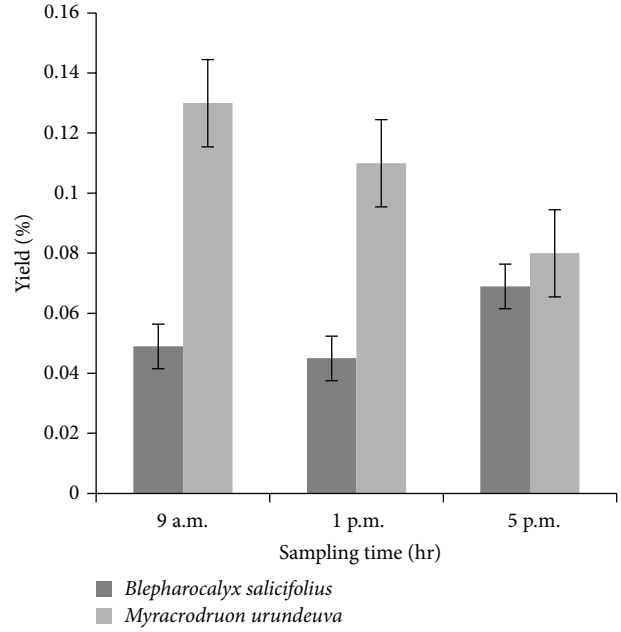

Figure 2: *Blepharocalyx salicifolius* and *Myracrodruon urundeuva* essential oils yields obtained from leaves harvested at day time.

The chemical analyses also revealed that the major components are monoterpenes: α-pinene (9.0), *p*-cymene (25.9), and γ-terpinene (12.5%), whereas for oxygenated monoterpenes, 1.8-cineole (2.7%) was the principal constituent. Caryophyllene oxide (9.6%), aromadendrene (4.6%), trans-calamenene (3.5%), and β-caryophyllene (3.2%) were the main compounds of sesquiterpenes. Spathulenol (2.0%) was the major constituent of oxygenated sesquiterpenes.

M. urundeuva EOs compounds, their respective retention indices, and relative peak area along with total area for each chemical class are shown in Table 3. Araújo et al. [32] studying the chemical composition of *M. urundeuva* in different seasons extracted the essential oils by hydrodistillation technique in a Clevenger apparatus and determined chemical composition by ^{1}H-NMR. The results revealed a unique constituent in major proportion (>90%) and five principal compounds: limonene, β-ocymene, δ-3-carene, α-pinene, and myrcene. Souza and Lorenzi [12] found m-pentadecadienylphenol in *M. urundeuva* studying the insecticidal activity against *Aedes aegypti*.

The monoterpene β-myrcene comprised 66.4% of the identified components, appearing as the main compound. Linalool (3.6%) was the main oxygenated monoterpene. *trans*-Caryophyllene (3.7%) was the main compound of sesquiterpenes, followed by δ-cadinene (3.6%), δ-selinene (2.9%), and 14-hydroxy-9-*epi*-caryophyllene (2.8%). Spathulenol (0.3%), caryophyllene oxide (1.0%), and *epi*-α-cadinol (1.0%) were the only representatives of oxygenated sesquiterpenes (2.3%), with minor proportion in the total identified components compared with major subclasses.

Table 2: *Blepharocalyx salicifolius* EOs chemical compounds, retention indices, and relative peak area.

Peak	Identification	Retention index	Peak area (%)
1	α-Thujene	925	1.9
2	α-Pinene	932	9.0
3	β-Pinene	976	0.1
4	1-Felandrene	1005	0.1
5	δ-3-Carene	1010	0.9
6	α-Terpinene	1016	0.9
7	*p*-Cymene	1023	25.9
8	Limonene	1027	1.5
9	1,8-Cineole	1030	2.7
10	*cis*-Ocimene	1035	0.1
11	*trans*-Ocimene	1045	0.1
12	γ-Terpinene	1057	12.5
13	γ-Terpinolene	1087	3.3
14	Linalool	1099	0.3
20	4-Terpineol	1176	0.2
21	*p*-Cymen-8-ol	1184	0.2
22	α-Terpineol	1189	0.1
24	*p*-Menth-2-en-1,4-diol	1267	0.2
33	α-Cubebene	1348	0.1
36	α-Copaene	1376	0.3
37	α-Gurjunene	1408	0.5
38	β-Caryophyllene	1418	3.2
39	Aromadendrene	1437	4.6
41	α-Humulene	1452	0.6
45	*allo*-Aromadendrene	1459	0.6
48	β-Selinene	1484	0.3
50	δ-Selinene	1493	1.1
53	γ-Cadinene	1513	0.2
54	*trans*-Calamenene	1522	3.5
55	Cadina-1,4-diene	1531	0.6
59	Nerolidol	1563	0.4
63	Spathulenol	1576	2.0
64	Caryophyllene oxide + globulol	1582	9.6
65	Viridiflorol	1590	0.7
66	Cubeban-11-ol	1592	0.4
67	Rosifoliol + ledol	1601	0.8
68	Humulene epoxide II	1607	0.6
74	1-*epi*-Cubenol	1627	0.7
79	δ-Cadinol	1645	0.2
80	β-Eudesmol	1649	0.2
81	α-Cadinol	1653	0.4
Total			91.6
Group		Total peak area (%)	
Monoterpenes		56.1	
Oxygenated monoterpenes		3.5	
Sesquiterpenes		25.9	
Oxygenated sesquiterpenes		5.8	

Table 3: *Myracrodruon urundeuva* EOs chemical compounds, retention indices, and relative peak area.

Peak	Identification	Retention index	Peak area (%)
1	β-Myrcene	990	66.4
2	Limonene	1027	0.7
3	1,8-Cineol	1030	0.3
5	γ-Terpinene	1057	0.5
6	Linalool	1100	3.6
7	α-Copaene	1374	0.3
8	*trans*-Caryophyllene	1418	3.7
9	Aromadendrene	1437	0.4
10	α-Humulene	1452	0.3
13	β-Selinene	1485	1.3
15	δ-Selinene	1493	2.9
16	α-Muurolene	1499	0.4
18	γ-Cadinene	1513	0.8
19	δ-Cadinene	1522	3.6
22	Spathulenol	1576	0.3
24	Caryophyllene oxide	1582	1.0
25	*epi*-α-Cadinol	1640	1.0
	14-Hydroxy-9-*epi*-caryophyllene	1663	2.8
Total			90.3

Group	Total peak area (%)
Monoterpenes	67.5
Oxygenated monoterpenes	3.9
Sesquiterpenes	16.5
Oxygenated sesquiterpenes	2.3

4. Conclusions

Statistical analysis results suggest that collection at different times does not interfere in essential oils yield of *Blepharocalyx salicifolius* and *Myracrodruon urundeuva*. Although a slight trend of higher yields was observed for *B. salicifolius* samples collected at 17 p.m., for *M. urundeuva* samples, this trend was observed for 9 a.m. samples. *M. urundeuva* presented, in general, higher EOs yields than *B. salicifolius*.

Beyond environmental factors, like temperature, solar radiation intensity, soils, and genetics, experimental factors must be considered, such as matrix effect, solvent extraction and evaporation, and, of course, storage cares.

The GC-MS technique was primordial to identify 13 major compounds of *B. salicifolius* with *p*-cymene (25.9%) presenting the highest concentration. In *M. urundeuva*, 9 major compounds were identified with β-myrcene (66.4%) representing the main component.

Despite the subject relevance, there are few deep studies about extraction techniques and analysis of Cerrado species EOs. More studies regarding the circadian cycle for other species in Bioma Cerrado are needed for a full understanding of the complex equilibriums involved in the production of EOs.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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