Orbital: The Electronic Journal of Chemistry
journal homepage: www.orbital.ufms.br
e-ISSN 1984-6428

| Vol 11 | | No. 5 | | July-September 2019 |

FULL PAPER

Chemical Composition and Evaluation of Antitumoral Activity of Leaf and Root Essential Oils of *Conyza canadensis* (Asteraceae)

Lucas W. Aragão[a], Shaline S. L. Fernandes[a], Viviane Mallmann[a], Janaína Thomasi Facco[a], Maria de Fátima Cepa Matos[b], Marcia Regina Pereira Cabral[c], João Ernesto de Carvalho[d], Ana L. T. G. Ruiz[d], Claudia Andréa Lima Cardoso[a], Rogério Cesar de Lara da Silva[a], Ademir dos Anjos[a], and Euclésio Simionatto[a,*]

[a]Universidade Estadual de Mato Grosso do Sul, 79950-000 Naviraí – MS, Brazil.
[b]Departamento de Farmácia-Bioquímica, Universidade Federal de Mato Grosso do Sul, 79070-900 Campo Grande-MS, Brazil.
[c]Departamento de Química, Universidade Estadual de Maringá, 87020-900 Maringá-PR, Brazil.
[d]Universidade Estadual de Campinas, 13081-970 Campinas – SP, Brazil.

Article history: Received: 13 January 2019; revised: 20 August 2019; accepted: 20 September 2019. Available online: 30 September 2019. DOI: http://dx.doi.org/10.17807/orbital.v11i5.1376

Abstract:

The leaf and root oils of *Conyza canadensis* were studied for chemical composition and antitumor activity. The results showed that there is a significant variation in the composition of the oils obtained from different parts. The main components in the leaf oil were limonene, caryophyllene oxide and spathulenol. In the root oil, the major component was the acetylenic ester lachnophyllum methyl ester. It was observed that according to the collection time (6 a.m. and 4 p.m.), significant variations in the content of the main components of this essential oil of leaves can occur. Limonene, spathulenol and caryophyllene oxide presented a distribution of 61% / 5.4% / 12.5% and 38% / 10.7% / 22.3% in oils obtained from plants collected at 6 a.m. and 4 p.m., respectively. The evaluation of antitumor activity of the oils showed that leaf oil had a more pronounced potential of inhibition, and this oil was distinguished by the activity against neoplastic cell lines K562 (leukemia) and NCI-ADR / RES (ovary with multidrug resistance phenotype) with TGI values of 16.8 and 19.0 $\mu g.mL^{-1}$, respectively. Comparing the leaf oils and their tumor cell inhibition potentials, it was noted that this activity is higher in the oil with higher contents of monoterpene limonene.

Keywords: *Conyza Canadensis*; essential oil; antitumoral activity; diurnal variation

1. Introduction

Asteraceae is one of the largest families of plants comprising about 1,600 genera and 23,000 species [1]. In Brazil, the family is represented by approximately 180 genera and 1,900 species, in distributed in areas with different vegetative aspects and occurring in a large part of the territory [2]. The distribution of secondary metabolites, such as terpenoids and flavonoids, can be considered in the chemotaxonomic evaluations of the Asteraceae family, however, this family is also notable for the presence of acetylenic compounds, being this one of the few botanical families able to biosynthesize these types of products [3].

The species *Conyza canadensis*, in Brazil popularly known as "buva", is a species belonging to the family Asteraceae, found in temperate zones of the northern hemisphere [4] and subtropical regions of the southern hemisphere, but is uncommon in tropical regions. It can be found in vacant lots, road margins, pastures, native fields and annual crops [4, 5]. Considered an adapted weed, it survives in various soil types and conditions. It is difficult to control this species, mainly by means of chemical methods, and

*Corresponding author. E-mail: eusimionatto@yahoo.com.br

consequently there are reports on the resistance of this plant to some herbicides, and the management practices of the species represent a challenge in the agricultural environment, mainly referring to the cultivation of soybean where this species affects the yield of grains [6].

Phytochemical studies of *C. canadensis* revealed the presence of C10 acetylenes, sesquiterpene hydrocarbons, flavonoids, sterols, triterpenes and sphingolipids [7, 8]. The essential oils of young and mature herbs collected in Washington State (USA) were previously analyzed and 25 constituents including monoterpenes, sesquiterpenes and acetylenes were identified and the predominance of limonene (>60.0%) was confirmed [9]. In a sample of aerial parts collected in France, limonene was the major component (76.0%) among the 18 compounds identified in the oil [10]. The analysis of essential oil obtained from *C. canadensis* in Japan, led to the detection of 47 volatile components, of which 91.0% were terpenoids, the main constituent being limonene (31.2%). In oils obtained from aerial parts and roots of plants collected in Hungary, the presence of limonene (78%) and the acetylene *2Z, 8Z*-matricaria ester (88%), were detected, respectively [7, 11].

C. canadensis have been used throughout the world as traditional or official herbal medicines for the treatment of gastrointestinal symptoms, more commonly diarrhea and dysentery, and as a diuretic agent. In Chinese folk medicine, the species *Conyza canadensis* has also been applied for the treatment of sores, pumps and pains caused by arthritis. Volatile oil is also cited in applications against bronchitis and cystitis [12].

Considering that the essential oils of *Conyza canadensis* presented variations according to the region of collection, in the present work an evaluation of the oils obtained from roots and leaves of populations of *Conyza canadensis* that were developed in the central-western region of Brazil was carried out. The antitumor activity of these oils was also evaluated against eight cell lines.

2. Results and Discussion

Leaf and root oils of adult plant populations of *Conyza canadensis* collected at different times of the day (6 a.m. and 4 p.m.) were analyzed. A comparative study was performed on yield, variation of chemical composition during collection times and antiproliferative activity. Analysis of yield data indicates substantial differences in oil content according to the time of collection of the plant (Table 1). In the analysis of leaf oils, it was observed that the highest yields were obtained with samples of plants collected in the morning (6 a.m.), reaching 1.2%. With roots, the oil yield was 0.6% and a reduction of this oil yield was also observed when the plants were collected in the afternoon.

Table 1. Yield of the extractions (in %) of the volatile oils of *Conyza canadensis*, considering the part of the plant and the time of collection.

Time of collection	Leaves	roots
6 a.m.	1.2±0.3	0.6±0.2
4 p.m.	0.7±0.2	0.4±0.1

As reported in the literature, volatile oils may exhibit variations which affect the yield and composition [13]. The yield of essential oil from leaves of *Cistus monspeliensis* (Cistaceae) showed seasonal and diurnal variation [14]. Differences in yield and composition of essential oils under the influence of harvest time have already been reported for several plants [15-17], similar to the oil of *C. canadensis* investigated in this work**.** The *Myrcia sylvatica* species also showed significant variation in oil yield considering the collection time, with yields of 0.8% (material collected 6 a.m.) and 1.2% (material collected 9 p.m.) [18].

Table 2 shows the components and their concentration in essential oils of leaves and roots, considering the different times of collection of material.

Concerning the analysis of the oils of the leaves of *Conyza canadensis*, significant variations in the chemical composition were observed considering the period of collection (morning and afternoon). The compounds that showed more than 1.0% content in the qualitative analyzes of the oils were considered significant for the variation observations. It was identified 18 compounds in oils obtained from plants collected at both times. These compounds were

responsible for 97.3% and 89.4% of the total composition of the oils obtained from plants collected at 6 a.m. and 4 p.m., respectively. The major component was the monoterpene limonene, already identified as predominant in the oil of leaves of *C. canadensis* collected in regions of Seattle (USA) and Szeged (Hungary) [6, 7]. The concentration of this monoterpene varied significantly according to the collection times, with 61% and 38% being observed in oils obtained from plants collected at 6 a.m. and 4 p.m., respectively. The main sesquiterpenes of leaf oils were spathulenol and caryophyllene oxide. Significant variations related to collection schedules were observed with these oxygenated sesquiterpenes, where spathulenol/caryophyllene oxide contents of 5.4%/12.5% and 10.7%/22.3% were detected in plants collected at 6 a.m. and 4 p.m., respectively. The sum of the concentrations of the main components (limonene, spathulenol and caryophyllene oxide) in oil from leaves of *C. canadensis* represents 78.9% and 71.0% of the composition of oils obtained from plants collected at different times. In the study with the species *Cistus monspeliensis*, considering different collection times, the relative composition of the classes of compounds varied significantly considering the schedules of 6 p.m. and 12:00 a.m., occurring variation of 0-7.69% and 11.76-42.82% for monoterpenes and sesquiterpenes, respectively [14]. The essential oil of leaves of *Virola surinamensis* also presented diurnal variation in the levels of elemycin and monoterpenes [15]. In the essential oil of the species *Myrcia sylvatica* there were variations in the levels of α-pinene and β-pinene according to the collection period (12 a.m. and 6 p.m.) during rainy season [18]. The composition of the essential oil of *Lippia origanoides* also has significative influence in the different collection times, and these differences are more accentuated for the compounds cymene, 1,8-cineol, γ–terpinene, carvacrol, methyl cinnamate, caryophyllene and nerolidol [19].

Table 2. Composition of leaf and root oils (in %, obtained by peak-area) of *Conyza canadensis*, considering the time of collection of plants.

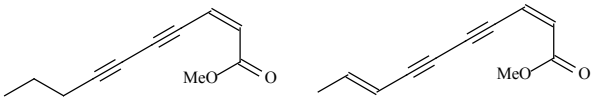
Compounds[a,b]	IR	leaves[d,e]		roots[d,e]	
		6 a.m [f]	*4 p.m.*[g]	*6 a.m.*[h]	*4 p.m.*[h]
β-Pinene	979	1.4 ± 0.3	0.3±0.0		
Limonene	1028	61.0±0.9	38.0±0.6		
Linalool	1134	1.2±0.2	0.8±0.1		
Limonene oxide	1139	0.9±0,1	0.6±0.2		
Carveol	1220	1.2±0.4	0.9±0.3		
Carvone	1245	1.9±0.3	1.2±0.4		
Isopulegol acetate	1309	1.1±0.2	0.3±0.0		
Myrtenil acetate	1323	1.1±0.3	0.4±0.0		
Ni	1334	0.4±0.1	0.1±0.0		
δ-Elemene	1341	0.4±0.1	-		
Carveol acetate	1365	1.7±0.4	0.7±0.2		
β-Patchoulene	1380	1.9±0.5	4.4±0.5		
Ni	1412	-	0.4±0.1		
γ-Elemene	1437	0.7±0.1	1.7±0.3		
Ni	1450	-	0.7±0.3		
Ni	1477	-	0.9±0.2		
Ni	1495	-	0.7±0.1		
[c]Lachnophyllum methyl ester	1527	-	-	93.7±1.2	91.6±1.0
Matricaria methyl ester	1547	-	-	5.2±0.5	6.7±0.8
Ni	1555	0.4±0.0	0.6±0.0		
Nerolidol	1565	1.4±0.3	0.6±0.2		
Spathulenol	1581	5.4±0.2	10.7±0.5		
Caryophyllene oxide	1586	12.5±0.6	22.3±0.6		
Guaiol	1599	0.9±0.3	2.0±0.4		
Ni	1613	0.8±0.3	2.4±0.3		
Ni	1638	-	1.2±0.1		
Ni	1645	-	0.4±0.1		
α–Cadinol	1658	1.4±0.4	2.5±0.3		
2,3-Dihydrofarnesol	1681	1.2±0.3	2.0±0.2		
Total identified		97.3	89.4	98.9	98.3

[a]Components listed in order of elution in column ZB-5. [b]Identification: retention indexes, mass spectra; [c]Identification also by NMR. [d]Component concentrations were calculated from peak areas under GC-FID analysis. [e]Results expressed as mean ± standard deviation ([f]of five extractions, [g]four extractions, [h]three extractions).

In the oils obtained from the roots of *C. canadensis* a chromatographic profile with low diversity of components was observed. The presence of a major compound with a content that reached 93.7% in the oil obtained from plants collected at 6 a.m. and 91.6% when obtained from oil of plants collected at 4 p.m. was observed through the chromatographic analysis. To confirm the structure of this compound, ^{1}H and ^{13}C nuclear magnetic resonance analyzes were performed. It was observed the presence of 11 carbon signals, indicating that it was a compound with carbon skeleton not common in essential oils, in which mononoterpene compounds (10 carbons) and sesquiterpenes (15 carbons) are predominated. This observation was confirmed by mass spectrometry which indicated that the molecular mass of the compound is 176, which is also different from the expected ranges for mono- and sesquiterpenes. In the ^{13}C NMR spectra were observed the presence of four signals with chemical shifts characteristic of acetilenes (δ 65.0, 70.08, 86.5 and 90.0) and two alkene signals (δ 130.08 and 122.05). In the ^{1}H NMR spectrum, the signals indicate that the acetylenic carbons were characterized as internal parts of the molecule (absence of signals of acetylenic hydrogens). In the DEPT-135 spectral data, the presence of two methylene carbon signals (δ 21.60 and 21.75) was verified. The DEPT-135 spectrum showed the absence of $–CH_2$ groups in the region of unsaturated carbons, also proving that the unsaturated carbons (δ 130.08 and 122.05) form part of an internal double bond.

The presence of a methyl ester was also observed through the ^{13}C NMR spectrum with chemical shift in δ 51.25 ppm and with the signal for methyl hydrogens in δ 3.78 ppm in ^{1}H-NMR spectrum. Thus, by linking the information obtained with literature data [20], it was identified that the major compound of the volatile oil of the roots of *C. canadensis* is lachnophyllum methyl ester (1) (Figure 1). In addition to this acetylenic compound, it was also identified the matricaria methyl ester (2), which showed a variation of 5.2-6.7% during collection times.

Comparative data between this work and studies already described for *C. canadensis* species demonstrate that the main compounds identified have already been listed as components of the oils of this species. In relation to the composition of the oil from the roots, we noticed a significative change when compared to studies realized with this plant in other regions. Studies have shown the high content of acetylenic esters in the oil of the roots of this species as the matricaria methyl ester identified as the major component in plants collected in Hungary [21]. However, none of the studies with *C. canadensis* oil carried out to date have described the lachnophyllum methyl ester as the major component, being detected as a trace component or absent from volatile oils of *C. canadensis* roots [7].



1 2

Figure 1. Structures of acetylenic compounds identified in the essential oil of *C. canadensis* roots.

The oils were evaluated for antitumor potential against eight cell lines. Root oil did not present significant activity, and the best result was found against the 786-0 line (kidney), with a TGI of 30.5 $\mu g.mL^{-1}$. On the other hand, leaf oil obtained from samples collected at 6 a.m. showed a more pronounced inhibitory activity, especially against K562 (leukemia) and NCI-ADR/RES (ovary with multidrug resistance phenotype) with TGI values of 16.8 and 19.0 $\mu g.mL^{-1}$, respectively. The oil obtained from plants collected at 4 p.m. also showed more pronounced activity against the K562 and NCI-ADR/RES lines, however, with lower TGI values being 27.3 and 32.2 $\mu g.mL^{-1}$, respectively. The antitumoral activity was separated into 4 categories: inactive, weak activity, moderate activity and potent activity [32]. The results obtained with the oil of *C. Canadensis* (collected at 6 a.m.) against K562 and NCI-ADR/RES cells indicate a weak activity, whereas for the other cells the samples are considered inactive.

This more pronounced activity of leaf oil can be attributed to the presence of monoterpene limonene, which is already reported as responsible for antitumor activities against certain cell lines. This monoterpene inhibits the formation and development of different types of tumors, such as mammary carcinomas, lung neoplasms,

pancreatic tumors, liver cancer, pulmonary adenomas and tumors of the stomach [22-25]. Limonene and some of its derivatives have been the object of research related to its anticancer properties [26-28]. Side effects in the kidneys, caused by the use of the chemotherapeutic doxorubicin were attenuated through the combined use with limonene, indicating that this terpene can be used in combination with doxorubicin [26].

Table 3. Total growth inhibition values (TGI, in µg/mL) of the essential oils of *Conyza canadensis*, against tumor cells.

	TGI (µg/mL)			
	CCF1	**CCF2**	**CCR**	**Doxo**
U251 (glioma)	30.6	73.4	49.0	0.057
UACC-62	57.1	73.0	42.2	*
MCF-7	46.6	93.6	50.0	0.56
NCI/ADR/RES	19.0	32.2	63.6	0.24
786-0	81.4	85.1	30.5	1.5
NCI-H460	214.4	44.1	42.1	*
NCI-H460	93.0	155.7	69.0	**
OVCAR-03	42.2	77.3	95.0	*
HT29	72.4	77.4	47.6	0.35
K562	16.8	27.3	78.4	0.18
HaCat	67.1	84.7	79.1	0.049

Oils: CCF1 (plant oils collected at 6 a.m.); CCF2 (plant oils collected at 4 p.m.); CCR (oil obtained from the roots). Human tumor lines: U251 (glioma); UACC-62 (melanoma); MCF-7 (breast); NCI-ADR / RES (ovary with multiple drug resistance phenotype); 786-0 (kidney); NCI-H460 (lung, non-small cell type); PC-3 (prostate); OVCAR-03 (ovary); HT29 (colon); K562 (leukemia). Human non-tumor line: HaCat (immortalized keratinocytes). * <0.025 µg / mL; **> 25 µg / mL.

3. Material and Methods

3.1. General information

Analytical grade n-hexane, ethyl acetate and Na_2SO_4 were obtained from commercial source (VETEC) and used as received. The NMR spectra were obtained on a Bruker DPX-300 spectrometer, and the samples were solubilized in $CDCl_3$, using as internal reference standard the TMS.

3.2. Plant material

The collection was carried out in February 2015 in a rural area in the municipality of Naviraí, Mato Grosso do Sul, in the center-west region of Brazil (coordinates 27º 35 '52,87 "S and 48º 31' 24,90" W). The plants were collected (leaves and roots) in two hours of the day, 6 a.m. and 4 p.m., totalizing 10 kg of plant material. After collecting the plants, the leaves (8.5 kg) and roots (1.5 kg) were carefully separated, conditioned under refrigeration and submitted to extraction to obtain the essential oil. The species was identified by Dr. Arnildo Pott of UFMS, Campo Grande, MS, Brazil. An voucher specimen is deposited in the Herbarium of Campo Grande-MS, under the number 21748.

3.3. Obtaining the essential oil

All vegetal material was subjected to the extraction of the essential oil using a hydrodistillation system in Clevenger modified apparatus for 4 hours, with capacity of 5 liters. Fifteen extractions were carried out, using in each of these extractions approximately 900g of leaves and 250g of roots, to obtain the respectives volatile oils. Nine extractions were performed (five with material collected at 6 a.m. and four with material collected at 4 p.m.) to obtain leaf oil. Six extractions (three extractions with material collected at 6 a.m. and three extractions with material collected at 4 p.m.) were carried out to obtain the oil from the roots. The oil yield was calculated considering the average of these extractions.

3.4. Chemical analysis of oils

The chemical analyzes of the essential oils were performed by GC/FID and CG-MS. Samples from each extraction (9 for leaves and 6 for roots)

were diluted 1% (m / v) in hexane. For the root oil in addition to the above analyzes, the NMR (^{1}H and ^{13}C) spectra were also obtained, using $CDCl_3$ as solvent. *GC/FID Analysis:* Sample analyses (in triplicate) was performed on a HP5890 SERIE II Gas Chromatograph system series equipped with flame ionization detector (FID) using a fused silica capillary column (DB-5; 30 m × 0.25 mm, film thickness 0.2 μm). Oven temperature was programmed from 50 to 250 °C at a rate of 4 °C min^{-1}, with injector at temperature 230 °C and detector temperature 250 °C. The split ratio was (1:20). The volume injected was 2.0 μL. A C_7-C_{21} n-alkanes mixture diluted in *n*-hexane was prepared for determination of the temperature programmed retention indexes. Samples diluted in n-hexane were analyzed. Internal standards (n-alkanes) were then added to each sample to aid in the standardization of retention times and the samples were analyzed again. Then, retention indexes (RI) for all compounds were determined. The identification of the chemical constituents was based on comparison of their retention indexes (RI) and mass spectra with those obtained from authentic samples and/or the Wiley and NBS/NIST libraries and those published by Adams (2001) [29]. The quantitative data regarding the volatile constituents were obtained by peak-area normalization using GC/FID operated under similar conditions to the GC–MS. For quantification, were considered compounds that the concentration was equal or superior than 0.1%. Percentage values were the mean of three injections of the sample. *Gas chromatography/mass spectrometry analysis:* The analyses were performed using a gas chromatograph (GC-2010 Plus, Shimadzu, Kyoto, Japan) equipped with a mass spectrometer detector (GC-MS Ultra 2010), employing a fused silica DB-5 capillary column (30 m length x 0.25 mm inside diameter x 0.25 mm film thickness). The carrier gas was helium (99.999%) at a flow rate of 1.0 mL/min, and 1 μL sample volume was injected in split mode (ratio 1:20). The initial oven temperature was 50 ºC, followed by a ramp to 280 °C at a rate of 3 °C/min for 10 min. The injector, detector, and transfer line temperatures were kept at 280 °C. The detector was operated in scanning mode using an electron ionization voltage of 70 eV and a mass range of 45-600 m/z. The identification of the compounds was performed using the calculated retention indexes employing a mixture of linear alkanes (C_8-C_{30}, Sigma Aldrich, purity ≥90%) as reference. The sample retention indexes and mass spectra were compared to literature values (Adams, 2007) [29] and the mass spectra obtained for the samples also were compared to the NIST21 and WILEY229 databases.

3.5. Antiproliferative assay

Cancer cells lines U251 (glioma) MCF-7 (breast), NCI-ADR/RES (drug resistant ovarian), 786-0 (kidney), NCI-460 (lung), OVCAR-3 (ovarian), HT-29 (colon), K562 (leukemia) and PC-3 (prostate) obtained from the Frederick MA, National Cancer Institute/USA, were grown in RPMI 1640 medium (Sigma Chemical Co., St. Louis, MO, USA), supplemented with 5% fetal bovine serum (Gibco, EUA) and maintained in a humidified atmosphere at 37 ºC in 5% CO_2. The medium was changed every 2 days until the cells reached confluence, at which point they were subcultured. The essential oils from of *C. canadensis* were evaluated for their cytotoxic activities against cells using a Sulforhodamine B (SRB) assay. The SRB method was used as described previously [30, 31]. The microtiter plates containing cells were pre-incubated for 24h at 37 ºC to allow stabilizations prior to addition (100 μL) of the crude oil. The plates were incubated with the samples (oils and reference) for 48h at 37 ºC and 5% CO_2 at four concentrations (0.25, 2.5, 25, and 250 $\mu g.mL^{-1}$) each in triplicate wells. Doxorubicin was used as the positive control at concentrations of 0.025, 0.25, 2.5, and 25.0 $\mu g\ mL^{-1}$. The samples were initially solubilized in dimethylsufoxide (DMSO) (Sigma). The final concentration of DMSO (0.25% at the higher sample concentration) did not affect the cell viability. The stock solution was diluted with complete medium containing 50.0 $\mu g\ mL^{-1}$ of gentamicin (Schering-Plough). The plates were air-dried and protein-bound dye was solubilized and the resulting optical density was read in a multiwell plate reader at 540nm. Antiproliferative activity is expressed as the concentration that causes the total growth inhibition (TGI). Growth was determined from non-linear regression analysis using the ORIGIN 8.0 (OriginLab Corporation). These results presented here refer to a representative experiment since all assays were run in triplicate and the average standard error was always <5%.

4. Conclusions

The results obtained in this work demonstrate that the essential oil (volatile) of the leaves of *Conyza canadensis* can present significant changes in the yield and chemical composition, considering the collection time of the plants (morning or afternoon). As for the chemical composition, it was observed that populations of plants of this species produce an essential oil with predominance of monoterpene limonene, when obtained from the extraction of leaves. Considering the essential oil obtained from the extraction of roots, we can note the presence of the acetylenic ester lachnophylum ester. This is the first report of the predominance of this ester in the oil of *C. canadensis*, which has already been reported as a secondary component of the essential oil from this plant collected in other parts of the world. Some studies show the predominance of compound matricaria methyl ester in oils of this species, which is also an acetylenic ester and was found as a secondary compound in this study. These two esters (lachnophylum ester and matricaria methyl ester) present structural similarity, differing only by the presence / absence of a double bond. Due to the purity profile, facile isolation and structural characteristics of the main compound, the root oil of *C. canadensis* (rich in lachnophylum methyl ester, > 90%) can serve as a starting material to be exploited in structural modifications studies. It was also observed that the antitumor activity of the oils is very different and the oil with higher content of limonene showed more pronounced activity. The oil that presented the most marked activity was that obtained from leaves collected at 6 a.m,, which presented better TGI values against the cell lines K562 and NCI-ADR/RES, whereas for the other samples the results indicated an absence of activity. This oil also had the highest limonene content.

Acknowledgments

Capes, CNPq and Fundect.

References and Notes

[1] Andenberg, A. A.; Baldwin, B. G.; Bayer, R. G.; Breitwieser, J.; Jeffrey, C.; Dillon, M. O.; Eldeñas, P.; Funk, V.; Garcia-Jacas, N.; Hind, D. J. N.; Karis, P. O.; Lack, H. W.; Neson, G.; Nordenstam, B.; Oberprieler, C. H.; Panero, J. L.; Puttock, C.; Robinson, H.; Stuessy, T. F.; Susanna, A.; Urtubey, E.; Vogt, R.; Ward, J.; Watson, L. E.; *Compositae.* In: Kadereit, J. W.; Jeffrey C.; *Eds. Flowering Plants Eudicots Asterales, Vol. VIII. The Families and Genera of Vascular Plants,* Kubitzki, K. (Ed.). Springer – Verlag, 2007.

[2] Roque, N.; Bautista, H.; Asteraceae: Caracterização e morfologia floral. EDUFBA: Salvador, 2008.

[3] Christensen, L. P.; Lam, J. *Phytochemistry*, **1991**, *30*, 2453. [Crossref]

[4] Holm, L.; Doll, J.; Holm, E.; Pancho, J. V.; Herberger, J. P. *World weeds: natural histories and distribution.* John Wiley & Sons: Toronto, 1997.

[5] Kissmann, K. G.; Groth, D.; *Plantas infestantes e nocivas – Tomo II*, 2.ed., BASF: São Paulo: 1999.

[6] Lazaroto, C. A.; Fleck, N. G.; Vidal, R. A. *Cienc. Rural* **2008**, *38*, 852. [Crossref]

[7] Veres, K.; Csupor-Loffler, B.; Lazar, A.; Hohmann, J. *Scientific World Journal* **2012**, *Article ID 489646,* 5 pages [Crossref]

[8] Porto, R. S.; Rath, S.; Queiroz, S. C. N. *J. Braz. Chem. Soc.* **2017**, *28,* 913. [Crossref]

[9] Hrutfiord, B. F.; Hatheway, W. H.; Smith, D. B.; *Phytochemistry* **1988**, *27*, 1858. [Crossref]

[10] Curini, M.; Bianchi, A.; Epifano, F.; Bruni, R.; Torta, L.; Zambonelli, A. *Chem. Nat. Compd.* **2003**, *39*, 191. [Crossref]

[11] Miyazawa, M.; Yamamoto, K.; Kameoka, H.; *J. Essent. Oil Res.* **1992**, *4*, 227. [Crossref]

[12] Lateef, R.; Bhat, K. A.; Chandra, S.; Banday, J. A. *Am. J. Essent. Oil. Nat. Prod.* **2018**, *6*, 35. [Crossref]

[13] Gobbo-Neto, L.; Lopes, N. P. *Quim. Nova,* **2007,** *30*, 374. [Crossref]

[14] Angelopoulou, D.; Demetzos, C.; Perdetzoglou, D. *Biochem. Syst. Ecol* . **2002**, *30*, 1892. [Crossref]

[15] Lopes, N. P.; Kato, M. J.; Andrade, E. H. A.; Maia, J. G. S.; Yoshida, M. *Phytochemistry,* **1997**, *46*, 689. [Crossref]

[16] Duschatzky, C.; Bailac, P.; Carrasull, A.; Firpo, N.; Ponzi, M. *J. Essent. Oil Res.* **1999,** *11*, 104. [Crossref]

[17] Moudachirou, M.; Gbenou, J. D.; Chalchat, J. C.; Chabard, J. L.; Lartigue, C. *J. Essent. Oil Res.* **1999,** *11*, 109. [Crossref]

[18] Raposo, J. D. A.; Figueiredo, P. L. B.; Santana, R. L.; da Silva Junior, A. Q.; Suemitsu, C.; da Silva, R.; Mourão, R. H. V.; Maia, J. G. S.; *Biochem. Syst. Ecol.* **2018,** *79,* 21. [Crossref]

[19] Ribeiro, A. F.; Andrade, E. H. A.; Salimena, F. R. G.; Maia, J. G. S. *Biochem. Syst. Ecol.* **2014**, *55,* 249. [Crossref]

[20] Albuquerque, M. R. J. R.; Souza, E. B.; Lins, M. U. D. S.; Nogueira, N. A. P.; Lemos, T. L. G.; Silveira, E. R.; Pessoa, O. D. L. *Arkivoc* **2004**, *6*, 59.

[21] Hrutfiord, B. F.; Hatheway, W. H.; Smith, D. B. *Phytochemistry* **1986**, *27*, 1858. [Crossref]

[22] Wattenberg, L. W.; Coccia, J. B. *Carcinogenesis* **1991**, *12,* 115. [Crossref]

[23] Crowell, P. L.; Siar, A. A.; Burke, Y. D. *Adv. Exp. Med. Biol*. **1996**, *401*, 131.

[24] Vigushin, D. M.; Poon G. K.; Boddy, A.; English, J.; Halbert, G. W.; Pagonis, C.; Jarman, M.; Coombes, R. C. *Cancer Chemother. Pharmacol.* **1998,** *42,* 111. [Crossref]

[25] Bailey, H. H.; Levy, D.; Harris, L. S.; Schink, J. C.; Foss, F.; Beatty, P.; Wadler, S. *Gynecol. Oncol.* **2002**, *85*, 464. [Crossref]

[26] Vieira, A. J.; Beserra, F. P.; Souza, M. C.; Totti, B. M.; Rozza, A. L.; *Chem. Biol. Interact.* **2018**, *283*, 97. [Crossref]

[27] Chen, T. C.; Schonthal, A. H.; Hofman, F. H. Clin. *Cancer Res.* **2018**, *24*, 102.

[28] Chen, T. C.; da Fonseca, C. O.; Schonthal, A. H. *Am. J. Cancer Res.* **2015**, *5*, 1580.

[29] Adams, R. P. (2007). Identification of essential oils components by gas chromatography/Mass spectrometry. Allured Publishing Corporation, Carol Stream, USA.

[30] Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, 82, 1107. [Crossref]

[31] da Silva, C. E. L.; Minguzzi, S.; da Silva, R. C. L.; Matos, M. F. C.; Tofoli, D.; de Carvalho, J. E.; Ruiz, A. L. T. G.; da Costa, W. F. *J. Braz. Chem. Soc.* **2015**, *26,* 233. [Crossref]

[32] Fouche, G.; Khorombi, E.; Kolesnikova, N.; Maharaj, V. J.; Nthambeleni, R.; van der Merwe, M. *Pharmacol. Online* **2006**, *3,* 494.