Quim. Nova, Vol. 38, No. 10, 1289-1292, 2015 http://dx.doi.org/10.5935/0100-4042.20150158

Artigo

CONSTITUENTS OF ESSENTIAL OIL AND HYDROLATE OF LEAVES OF *Campomanesia viatoris* LANDRUM

Iara Lisboa de Matos[a], Samísia Maria Fernandes Machado[a], Adauto Ribeiro de Souza[b], Emmanoel Vilaça Costa[c,d], Angelita Nepel[e], Andersson Barison[e] e Péricles Barreto Alves[a,*]

[a]Departamento de Química, Universidade Federal de Sergipe, Jardim Rosa Elze, 49100-000 São Cristóvão – SE, Brasil

[b]Departamento de Biologia, Universidade Federal de Sergipe, Jardim Rosa Elze, 49100-000 São Cristóvão – SE, Brasil

[c]Departamento de Química, Universidade Federal de Sergipe, Campus Professor Alberto Carvalho, 49500-000 Itabaiana – SE, Brasil

[d]Departamento de Química, Universidade Federal do Amazonas, Av. Gal. Rodrigo Otávio Jordão Ramos, 6200, Campus Sen. Arthur Virgilio Filho, 69077-000 Manaus – AM, Brasil

[e]Centro de RMN, Universidade Federal do Paraná, Centro Politécnico, Jardim das Américas, CP 19081, 81530-900 Curitiba – PR, Brasil

Recebido em 20/05/2015; aceito em 01/09/2015; publicado na web em 20/10/2015

The chemical composition of the essential oil and hydrolates of *Campomanesia viatoris* Landrum were investigated by gas chromatography/mass spectrometry (GC/MS) and a GC flame ionization detector (GC-FID). The major constituents were tasmanone (70.50, essential oil; 74.73%, hydrolate), flavesone (12.77, essential oil; 12.24%, hydrolate) and agglomerone (6.79, essential oil; 10.84%, hydrolate). Tasmonone was isolated and its structure was characterized by spectrometric analysis, specifically 1D and 2D nuclear magnetic resonance (NMR) and mass spectrometry (MS). These findings supports the quimiotaxonomic relationship with *Campomanesia* and *Eucalyptus* genera.

Keywords: β-triketones; tasmanone; Campomanesia viatoris.

INTRODUCTION

Campomanesia Ruiz&Pav. is considered one of the most well--defined genera of Myrtaceae comprising of about 40 species distributed in South America, most of them endemic to Brazil. Species are trees or shrubs and occur mainly in areas of forest, savannah, scrub and dunes. The fruits of *Campomanesia* species popularly known as 'gabiroba', or "guaviriba" or "guaviroba" or "guavira" are used to make liqueurs, juices and sweets.[1]

Several species of *Campomanesia* have been reported for uses in folk medicine in infusion form. *Campomanesia xanthocarpa* O. Berg., popularly known as 'gabiroba', or "guaviriba" or "guaviroba" or "guavira" is used to treat ulcers, diarrhea and inflammation.[2,3] However, Souza-Moreira *et al.*[3] evaluated the antimicrobial and antidiarrheal activity of *C. xanthocarpa* fruits and concluded that it is inactive, in opposed to the popular use of the plant.

Previous studies have shown that *Campomanesia* species produce essential oils rich in terpenes. Pascoal *et al.*[4] investigated the essential oil from fresh leaves of *Campomanesia guaviroba* (DC). Kiaersk., and reported a predominance of monoterpenes (71.1%). The major constituents identified were mirtenal (27.0%), myrtenol (24.7%) and *trans*-pinocarveol (15.7%).

Investigations regarding the essential oil from fresh leaves of *C. adamantium* (Camb) O. Berg. showed the predominance of sesquiterpenes.[5] However, different study about the chemical composition of the essential oil from the leaves of *C. adamantium* during the reproductive (flowering and fruiting) and vegetative stages, showed different results. The monoterpenes limonene and α-pinene were found as the major components of the leaves in the flowering stage, and the sesquiterpenes bicyclogermacrene, germacrene D and globulol as the major constituents of the vegetative and fruiting stages.[6] On the other hand, the essential oil from flowers of *C. adamantium* presented ledol (20.9%), globulol (9.3%) and *epi*-α-muurolol (5.0%) as the major compounds, revealing the predominance of sesquiterpenes (86.5%).[7]

The analysis of the chemical composition of the essential oil from the leaves of *C. pubescens* (Mart. Ex DC) O. Berg allowed the identification of 61 compounds with predominance of monoterpenes. However, the essential oil of the flowers and fruits of *C. pubescens* is predominantly represented by sesquiterpenes, which can highlight the ledol (19.8%), globulol (9.2%), α-cadinol (7.3%) for the flowers and criptomeridiol (14.2%), spathulenol (6.7%) and globulol (6.2%) for the fruits.[8]

Campomanesia viatoris Landrum is a species found in Brazil from Bahia to Ceará. Recent studies report the presence of this specie in Atlantic forest remainders in Sergipe, Brazil.[1] This work aims to investigate the chemical composition and cytotoxic activity of the essential oil from *C. viatoris*. This is the first report of the chemical composition of essential oil of the leaves and the hydrolate of *C. viatoris*.

EXPERIMENTAL

Botanical material

Leaves of *C. viatoris* Landrum were collected from the month of December 2013 in municipality of São Cristóvão, Sergipe, Brazil (geographical coordinates: lat: -11.014722 long: -37.206389 WGS84). The species was identified and voucher specimen was deposited in the Herbarium of Sergipe Federal University (ASE 16859).

Essential oil

Dried (continuous air flow at 45 °C for 48 h) and powdered leaves (200 g) of *C. viatoris* were submitted to essential oil extraction with 1.3 L distilled water on a Clevenger type apparatus for 3 h. Time start was clocked from the condensation of the steam in the Clevenger. The essential oil was dried with anhydrous sodium sulfate and yields was

*e-mail: periclesbalves@gmail.com

expressed as a percentage (oil mass/dry leaves mass). The essential oils were stored in amber glass flasks under refrigeration for further analysis. The extraction of essential oils were performed in triplicate.

Liquid–liquid extraction of hydrolate

The hydrolates were collected during the hydrodistillation of the leaves of *C. viatoris*. The hydrolates (500 mL) were subjected to extraction with, ethyl acetate (3 x 30 mL). Then the organic fraction was separated, dried with anhydrous sodium sulfate, and the solvent removed under reduced pressure. The hydrolates were stored in amber glass flasks under refrigeration for later chromatographic analysis. The experiment performed in triplicate.

Gas Chromatography / Mass Spectrometry (GC-FID/ MS) Analysis

The GC-FID/MS analysis of the essential oils were performed on a Shimadzu GC-2010 Plus and on a Shimadzu GCMS-QP2010 Ultra, equipped with an Shimadzu autoinjector AOC-20i using the following conditions: fused silica capillary column Restek Rtx®-5MS (30 m × 0.25 mm i.d. x 0.25 mm thick film) consisting of 5%-diphenyl-95%--dimethylpolysiloxane. Helium (99.999%) was used as the carrier gas with a constant flow rate of 1.2 mL min^{-1} and injection volume of 1.0 µL of a solution at a concentration of 10 mg mL^{-1} (split ratio of 1:30). The injector temperature was 250 °C and the ion source temperature was 200 °C. Data from Mass Spectrometry (MS) and Flame Ionization Detector (FID) were simultaneously recorded using a system of separation of detector; the flow split ratio was 4:1 (MS:FID). A tube restrictor 0.62 m x 0.15 mm i.d. (capillary column) was used to connect the splitter to the detector; a tube restrictor 0.74 m x 0.22 mm i.d. was used to connect the splitter to the detector FID. FID temperature was adjusted to 250 °C. The oven temperature was programmed to be kept at 50 °C for 1.5 min, with an increase of 4 °C min^{-1} up to 300 °C, in which the temperature was maintained for 5 min. The impact ionization was performed using electron energy of 70 eV with a scan rate of 0.3 s scan, and detected in the range of 40-350 Da. The quantification of each constituent was estimated by normalizing the peak area generated in FID (%). The relative proportions of the compounds were determined from the GC peak areas and are arranged in order of elution. The analysis carried out in triplicate standard deviation for each chemical constituent was calculated and shown in the Table 1.

Identification of essential oil constituents

The chemical constituents were identified by comparison of mass spectra and retention indices (RI) with those in the literature.[9] Experimental RI was obtained from Van den Dool and Kratz[10] equation in relation to a homologous series of *n*-alkanes ($C_8 - C_{22}$). The libraries WILEY8, NIST107, NIST21 were also used to assist in the identification of compounds.

Isolation of tasmanone

The isolation of the compound was performed by preparative thin layer chromatography (TLC) over glass supports of 20 × 20 cm, using 30 g of silica gel 60, and 80 mL of water. After water evaporation at ambient temperature, TLC plates were activated in an oven at approximately 110 °C for 60 min. Then, 250 mg of essential oil was applied to TLC that were eluted (two times) with hexane–ethyl acetate (9.5:0.5 *v/v*). The recovery of the isolated compounds was performed using a mixture of dichloromethane and ethyl acetate 20%.

Nuclear Magnetic Resonance (NMR)

1D and 2D NMR data were acquired at 303 K in $CDCl_3$ on a Bruker AVANCE III 400 NMR spectrometer operating at 9.4 Tesla, observing ^1H and ^{13}C at 400.13 and 100.61 MHz, respectively. The spectrometer was equipped with either a 5 mm multinuclear direct detection probe (1D NMR experiments) or a 5 mm multinuclear inverse detection probe (1D NOE and 2D NMR experiments), both with *z*-gradient. One-bond and long-range ^1H-^{13}C correlations from HSQC and HMBC NMR experiments were optimized for average coupling constants of $^1J_{(C,H)}$ and $^{LR}J_{(C,H)}$ of 140 and 8 Hz, respectively. All ^1H and ^{13}C NMR chemical shifts (δ) are reported in ppm relative to the TMS signal at 0.00 ppm, as internal reference, and the coupling constants (*J*) in Hz.

RESULTS AND DISCUSSION

The essential oils of *C. viatoris* were obtained in triplicate with medium yields of 0.97±0.01%. The GC–FID and GC–MS analyze permitted to identify sixteen compounds, representing 99.98% from essential oil (Figure 1). High amounts of ketones were observed in the composition of essential oil, representing 93.43% of the total composition of the compounds identified.

The major components of the essential oil of *C. viatoris* were tasmanone (70.50±1.40%), flavesone (12.77±0.74%) and agglomerone (6.79±0.21%) (Table 1, Figure 2). Compounds with structures of ketones are found in several species of Myrtaceae, especially in *Eucalyptus* and *Lepstopermum.*[11]

The chemical compositions of the hydrolates from leaves of *C. viatoris* were also analyzed. In this case, only the presences of triketones were observed in the samples of hydrolates, corresponding to

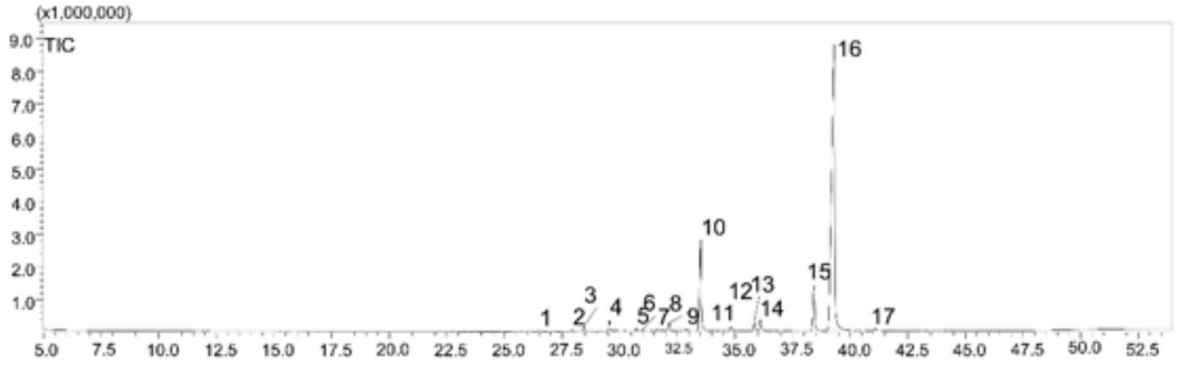

Figure 1. *Total Ion Chromatogram (TIC) of the essential oil from the leaves of C. viatoris*

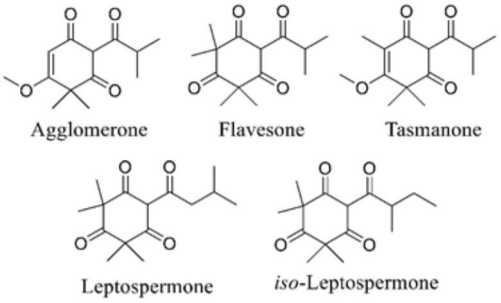

Figure 2. *Ketones identified in C. viatoris*

Table 1. Chemical composition of essential oil and hydrolate of leaves *C. viatoris*

Peak	Compounds	RI_{exp}	% oil ± Std. Dev.	% hydrolate ± Std. Dev.
1	δ-elemene	1347	0.19 ± 0.02	-
2	α-copaene	1388	0.20 ± 0.03	-
3	β-elemene	1403	0.70 ± 0.05	-
4	(*E*)-caryophyllene	1436	1.58 ± 0.18	-
5	α-humulene	1471	0.40 ± 0.07	-
6	9-*epi*-(*E*)-caryophyllene	1479	0.19 ± 0.03	-
7	germacrene D	1498	0.33 ± 0.02	-
8	biciclogermacrene	1514	1.29 ± 0.13	-
9	δ-cadinene	1538	0.39 ± 0.10	-
10	flavesone	1558	12.77 ± 0.74	12.24 ± 2.28
11	spathulenol	1599	0.66 ± 0.04	-
12	viridiflorol	1606	0.26 ± 0.06	-
13	*iso*-leptospermone	1633	1.61 ± 0.07	1.08 ± 0.17
14	leptospermone	1642	1.76 ± 0.10	1.11 ± 0.20
15	agglomerone	1721	6.79 ± 0.21	10.84 ± 1.64
16	tasmanone	1754	70.50 ± 1.40	74.73 ± 2.82
17	NI	1824	0.40 ± 0.08	-
Total			100.0	99.62

Note: RI_{exp}, retention indices on Restek Rtx®-5MS column calculated according to Van den Dool and Kratz.[10] Analysis carried out in triplicate. NI, not identified.

100% of the total composition (Figure 3). Thus, the sesquiterpenes were found only in the essential oil of *C. viatoris*.

In the hydrolate, such as essential oil, the major compounds identified were tasmanone (74.73±2.82), flavesone (12.24±2.28) and agglomerone (10.84±1.64). The agglomerone showed relative percentual highest in hydrolate than in essential oil. The high amount of ketones in hydrolate can be explained by the strong intermolecular interactions involving hydrogen bonding between the ketone groups of the molecules and water.

Bignell, Dunlop and Brophy[12] detected the presence of tasmanone in the essential oil of several *Eucalyptus* species in Australia. However, the amounts varied between 0.1–0.4% lower than that observed in the essential oil of *C. viatoris*. High percentage of tasmanone was found in the essential oil from leaves of *Eucalyptus suberea* and *E. lateritic* with 93.6% and 37.0%, respectively.[13] Tasmanone was also reported by Tam *et al.*[14] in the essential oil from the leaves of *Baeckea frutescens* L. (Myrtaceae) with up to 24.3% of the total composition of the essential oil from the leaves. This compound was isolated and noted which did presented in the tautomers form. The agglomerone were also detected in the essential oils from the leaves of *B. frutescens* species, *Eucalyptus ovate*, *Eucalyptus grandis*, *Eucalyptus diversicolor* and *Eucalyptus botryoides*.[14,15]

The tasmanone was isolated of the essential of *C. viatovis* yielding of 90% according to GC-FID analysis. The identification was performed by comparison of its MS spectrum and ^{1}H and ^{13}C NMR spectra with those reported in the literature.[9,14] Based on the NMR analysis it was noted that the tasmanone is presented in two tautomers forms (Figure 4). The occurrence of the keto enol forms were observed from the presence in the ^{13}C-NMR spectra of two series of the three chemical shift (δ_C 190.6, 197.0 and 208.6; δ_C 185.4, 197.9 and 211.2), which are characteristic of carbonyl groups. ^{1}H-NMR spectra showed two signals in δ_H 19.16 and 18.44 indicating the presence of strong intramolecular hydrogen bond. The proportion of formation to the keto enol forms were 66:34. The 1D (^{1}H, DEPT 135 and ^{13}C NMR data) and 2D (COSY, HSQC and HMBC) at the support information indicated that tasmonone is in equilibrium in two forms of tautomers as depicted in the Figure 4. This observation can be explained by the formation of the intramolecular hydrogen bond between the hydrogen of the hydroxyl group and the carbonyl group giving origin a ring of six members that was also observed by Tam *et al.*[14]

Thus, the essential oil of *C. viatoris* is a source of triketones. Therefore, the investigation of the chemical composition of *C. viatoris* plant is important and should be continued.

CONCLUSION

According to the findings, the triketones, tasmanone, flavesone and agglomerone were the major constituents in the essential oil and

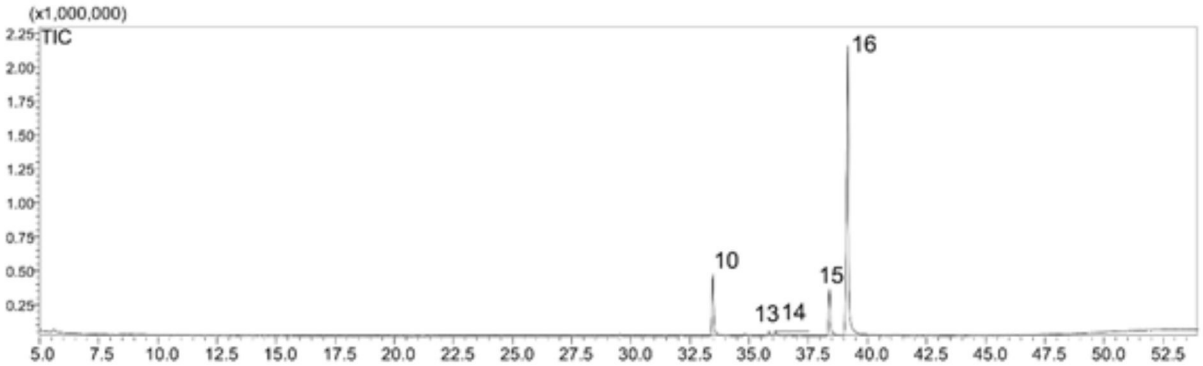

Figure 3. *Total Ion Chromatogram (TIC) of the hydrolate from the leaves of C. viatoris*

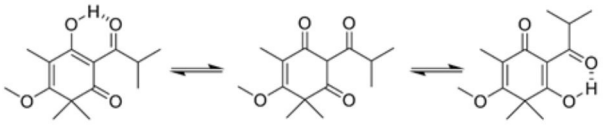

Figure 4. *Tasmanone tautomers*

hydrolate from the leaves of *C. viatoris*. These results are reported for the first time in this work the essential oil and hydrolate of *C. viatoris* are contribute for chemotaxonomic knowledge over *Campomanesia* species as well as the family Myrtaceae.

SUPPLEMENTARY MATERIAL

^{1}H and ^{13}C NMR spectra, MS of tasmanone was isolated of the essential of *C. viatovis* are available with free access at http://quimicanova.sbq.org.br in the form of a PDF file.

ACKNOWLEDGEMENTS

The authors are grateful to CNPq, CAPES, FAPITEC/SE, FINEP, UFS, and UFPR for financial support and fellowship.

REFERENCES

1. Oliveira, M. I. U.; Funch, L. S.; Landrum L. R.; *Acta Botânica Brasilica* **2012**, *26*, 512; Oliveira, M. I. U.; Funch L. S. and Landrum L. R.; *Sitientibus: Série Ciências Biológicas* **2012**, *12*, 91; Lima, D. F.; Goldenberg R. and Sobral M.; *Rodriguesia* **2011**, *62*, 683.
2. Markman, B. E. O.; Bacchi, E. M.; Kato, E. T. M.; *J. Ethnopharmacol.* **2004**, *94*, 55.
3. Souza-Moreira, T. M.; Salvagnini, L. E.; Santos, E.; Silva, V. Y. A.; Moreira, R. R. D.; Salgado, H. R. N.; Pietro, R. C. L. R.; *J. Med. Food* **2011**, *14*, 528.
4. Pascoal, A. C. R. F.; Lourenço, C. C.; Sodek, L.; Tamashiro, J. Y.; Franchi Jr., G. C.; Nowill, A. E.; Stefanello, M. E. A.; Salvador, M. J.; *J. Essent. Oil Res.* **2011**, *23*, 34.
5. Stefanello, M. E. A.; Cerve, A. C.; Wisniewski Jr, A.; Simionatto, E. L.; *J. Essent. Oil Res.* **2008**, *20*, 424.
6. Coutinho, I. D.; Cardoso, C. A. L.; Ré-Poppi, N.; Melo, A. M.; Vieira, M. C.; Honda, N. K.; Coelho, R. G.; *Braz. J. Pharm. Sci.* **2009**, *45*, 767.
7. Coutinho, I. D.; Ré-Poppi, N.; Cardoso, C. L.; *J. Essent. Oil Res.* **2008**, *20*, 405.
8. Silva, J. R. M.; Cardoso, C. A. L.; Re-Poppi, N.; *J. Essent. Oil Res.* **2009**, *21*, 315; Cardoso, C. A. L.; Re-Poppi, N.; *J. Essent. Oil Res.* **2009**, *21*, 433.
9. Adams, R. P.; *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy*, 4ª ed., Allured Publishing Corporation: Illinois, 2007.
10. Van Den Dool, H.; Kratz, P. D.; *J. Chromatogr.* **1963**, *11*, 463.
11. Hellyer, R. O.; *Aust. J. Chem.* **1968**, *21*, 2825.
12. Bignell, C. M.; Dunlop, P. J.; Brophy, J. J.; *Flavour and Fragrance J.* **1997**, *12*, 261.
13. Bignell, C. M.; Dunlop, P. J.; Brophy, J. J.; Fookes, C. J. R.; *Flavour and Fragrance J.* **1997**, *12*, 177.
14. Tam, N. T.; Thuam, D. T.; Bighelli, A.; Castola, V.; Muselli, A.; Richomme, P.; Casanova, J.; *Flavour and Fragrance J.* **2004**, *19*, 217.
15. Elaissi, A.; Medini, H.; Simmonds, M.; Lynen, F.; Farhat, F.; Chemli, R.; Harzallah-Skhiri, F.; Khouja, M. L.; *Chem. Biodiversity* **2011**, *8*, 362.