Biochemical Systematics and Ecology 79 (2018) 21–29

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Seasonal and circadian study of the essential oil of *Myrcia sylvatica* (G. Mey) DC., a valuable aromatic species occurring in the Lower Amazon River region

Juliana Divina A. Raposo[a,d], Pablo Luis B. Figueiredo[d], Rosiele L. Santana[a], Antonio Q. da Silva Junior[a], Chieno Suemitsu[b], Rodrigo da Silva[c], Rosa Helena V. Mourão[a,c], José Guilherme S. Maia[c,d,*]

[a] Laboratório de Bioprospecção e Biologia Experimental, Universidade Federal do Oeste do Pará, 68035-110, Santarém, PA, Brazil
[b] Laboratório de Botânica, Universidade Federal do Oeste do Pará, 68035-110, Santarém, PA, Brazil
[c] Programa de Pós-Graduação em Recursos Naturais da Amazônia, Universidade Federal do Oeste do Pará, 68035-110 Santarém, PA, Brazil
[d] Programa de Pós-Graduação em Química, Universidade Federal do Pará, 66075-900, Belém, PA, Brazil

ARTICLE INFO

Keywords:
Myrcia sylvatica
Myrtaceae
Essential oil composition
Circadian and seasonal variation
Monoterpenes and sesquiterpenes

ABSTRACT

Myrcia sylvatica (G. Mey.) DC., Myrtaceae, is an aromatic species that occurs in savanna areas of the Lower Amazon River, Brazil. Its essential oil showed an excellent yield, presenting a moss green coloration and a woody and spicy scent. The seasonal and circadian study of the leaf oils and the analysis of oil composition of the fruits were performed by GC and GC-MS. The primary compounds identified in fruit oil were δ-cadinene, β-selinene, 1-*epi*-cubenol, cubenol, α-calacorene, β-pinene, and *trans*-muurola-3,5-diene. In the leaf oils, the main compounds found were β-selinene, 1-*epi*-cubenol, cadalene, mustakone, δ-cadinene, α-calacorene, *trans*-calamenene, cubenol and caryophyllene oxide. Analyses of PCA and HCA applied to the samples of leaf oils presented a quantitative variation in their compositions, attributed to the rainy and dry periods. Also, it was observed a significant influence on the volatile constituents of the oils in the rainy season, depending on the time of collection. Thus, it was confirmed that the seasonal variation in the oil composition from leaves of *M. sylvatica* should be due to the influence of the climatic parameters, during the plant collection.

1. Introduction

Myrtaceae Juss. comprises 132 genera and 5671 species of trees and shrubs, which are distributed mainly in tropical and subtropical regions of the world, particularly South America, Australia and Tropical Asia (Govaerts et al., 2008). It is one of the most prominent families in Brazil, represented by 23 genera and 1034 species, with occurrence in all regions of the country (Landrum and Kawasaki, 1997; Govaerts et al., 2008; Sobral et al., 2015). *Myrcia* De Candolle is one of the largest genera in the Americas, with more than 300 species distributed from Mexico to Southern Brazil, occurring in its different biomes, especially in savannas and secondary forests (Marchiori and Sobral, 1997; Lucas et al., 2011).

Myrcia sylvatica (G. Mey) DC. [syn. *M. ambigua* DC., *M. ambigua* var. *dives* O. Berg, *M. ambigua* var. *multiflora* O. Berg., *M. ambigua* var. *pauciflora* DC., *Myrtus lucida* L., *M. sylvatica* G. Mey] (Missouri Botanical Garden, Tropicos, accessed December 2017),. is a shrub or small tree 2–5 m high, known as “cumatê-folha-miúda”, “murta” and “vassourinha”, widely scattered in savanna and secondary forest areas of the Pará state, Brazil, where the infusion of its leaves is used in the control of dysentery and intestinal diseases (Silva et al., 2015). *M. sylvatica* oil showed antimicrobial activity against Gram-positive bacteria, such as *Enterococcus faecalis*, *Bacillus cereus*, *Staphylococcus aureus*, and *S. epidermidis*, confirming the medicinal use of the plant in intestinal diseases (Silva et al., 2016). Also, *M. sylvatica* was included among other *Myrcia* species, all known as “Pedra-ume-caá”, which has been used to treat diabetes (Yoshikawa et al., 1998; Matsuda et al., 2002; Ferreira et al., 2011).

Many species of *Myrcia* have been reported as essential oil producers, and some of them presenting very diversified biological activities (Stefanello et al., 2011; Cascaes et al., 2015). On the other hand, most of these reports do not consider the seasonal and intraspecific variations that can occur in different specimens of the same plant. In the case of *M. sylvatica* essential oil, some works have been published concerning the

* Corresponding author. Programa de Pós-Graduação em Química, Universidade Federal do Pará, 66075-900, Belém, PA, Brazil.
E-mail address: gmaia@ufpa.br (J.G.S. Maia).

https://doi.org/10.1016/j.bse.2018.04.017
Received 23 December 2017; Received in revised form 24 April 2018; Accepted 26 April 2018

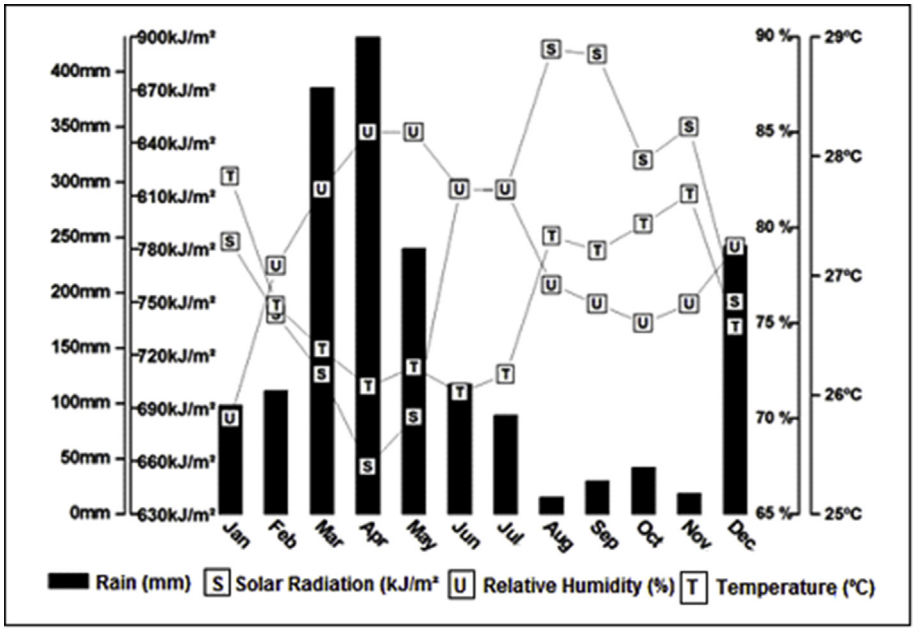

Fig. 1. Climatic parameters monitored during the seasonal and circadian study of *Myrcia sylvatica* oils.

diversity of its chemical composition in Brazil. From three specimens collected in the Tocantins state, the primary compound identified in their oils was spathulenol (Zoghbi et al., 2003), in the oil of a specimen harvested in the Maranhão state, the main constituent was (*E*)-caryophyllene (Rosa et al., 2016), and in the oil of another specimen sampled in Santarém, Pará state, the principal components were 1-*epi*-cubenol, *ar*-curcumene, cadalene, and β-selinene (Silva et al., 2016). The compositional variation observed for the oils of these specimens of *M. sylvatica* is attributed to the influence of the environmental conditions in the collection areas, as soil and climate, resulting in the appearance of different chemical types for the species. This fact could be due to the collection of a single sample, at various locations, times of the year and times of the day.

The seasonal and circadian study of *M. sylvatica* is considered of fundamental importance to complement its chemotaxonomic and ecological data, based on the economic potential of the plant to the Brazilian Amazon. So, this was the objective of the work carried out in the region of Santarém, Pará state, Brazil, where the plant has a wide occurrence.

2. Material and methods

2.1. Plant material and climatic data

The leaves and fruits of *M. sylvatica* were collected from a specimen located in the community of Alter do Chão, Santarém municipality, Pará state, the Lower Amazon River region, Brazil (coordinates 02°30′33.6″ S and 054°56 ′45.7″ W). For the seasonal study, the leaves were collected at 9 a.m., from January to December 2016. For the circadian study, the leaves were sampled at 6 a.m., 9 a.m., 12 p.m., 3 p.m., and 6 p.m., in March (rainy season) and September (dry season), 2016. The fruits were collected in April 2016, the only month of fruiting of the species, after its flowering period between February and March. Professor Chieno Suemitsu, from the Herbarium of Federal University of Western Pará (UFOPA), Santarém, PA, Brazil, identified the botanical material, deposited under the number HSTM 000098. Professor Marcos Sobral, a Myrtaceae specialist, confirmed the plant identification. Climatic parameters, like temperature, solar radiation, relative air humidity and rainfall precipitation, were measured at a weather station of UFOPA, installed in the collection area of the plant, from January to December 2016. The climatic equipment used were a Datalogger model CR1000 (Campbell, North Logan, Utah, USA), a Thermo-Hygrometer model HMP45C (Vaisala, Ventura, California, USA), a Pyranometer model LI200 (LI-COR, Lincoln, Nebraska, USA) and a Pluviometer model TR-525 (Texas electronics, Dallas, Texas, USA).

2.2. Isolation and composition of the oils

Air-dried leaves and fruits were ground and submitted to hydrodistillation using a Clevenger-type apparatus (3 h). The oils were dried over anhydrous sodium sulfate, and the yields were calculated from the plant dry weight. The moisture content of the samples was calculated using an infrared humidity measuring scale. The procedure was performed in duplicate.

The analysis of the oils was performed on a GCMS-QP2010 Ultra system (Shimadzu Corporation, Tokyo, Japan), equipped with an AOC-20i auto-injector and the GCMS-Solution software containing the NIST (Nist, 2011) and FFNSC 2 (Mondello, 2011) libraries. A Rxi-5ms (30 m × 0.25 mm; 0.25 µm film thickness) silica capillary column (Restek Corporation, Bellefonte, PA, USA) was used. The conditions of analysis were: injector temperature of 250 °C; Oven temperature programming of 60–240 °C (3 °C/min); Helium as carrier gas, adjusted to a linear velocity of 36.5 cm/s (1.0 ml/min); split mode injection for 1 µl of sample (oil 5 µl: hexane 500 µl); split ratio 1:20; ionization by electron impact at 70 eV; ionization source and transfer line temperatures of 200 and 250 °C, respectively. The mass spectra were obtained by automatic scanning every 0.3 s, with mass fragments in the range of 35–400 m/z. The retention index was calculated for all volatile components using a homologous series of C8-C20 n-alkanes (Sigma-Aldrich, USA), according to the linear equation of Van Den Dool and Kratz (1963). The quantitative data regarding the volatile constituents were obtained by peak-area normalization using a GC 6890 Plus Series, coupled to FID Detector, operated under similar conditions of the GC-MS system. The components were identified by comparing their retention indices and mass spectra (molecular mass and fragmentation pattern) with those existing in the GCMS-Solution system libraries, and also with the literature spectra (Adams, 2007).