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Original Article

Influence of abiotic environmental factors on the main constituents of the volatile oils of *Tithonia diversifolia*

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ABSTRACT

Tithonia diversifolia (Hemsl.) A. Gray, Asteraceae, commonly known as Mexican sunflower, is a wide distributed invasive species encountered around the world. We proposed herein to establish the relationship between different abiotic environmental factors and the variation in the production of volatile compounds in *T. diversifolia*, during a period of one year. Samples of leaf and inflorescence volatile oils obtained from individuals located at two different regions of Brazil were analyzed by GC–MS and the data were submitted to chemometric analysis. Based on the main constituents, the analysis allowed us to classify the volatile oils into two chemotypes, according to their geographical origin. The influence of soil nutrients, mainly Ca and P, was also observed in the composition of the volatile oils. Climate also seems to affect the constituents of the volatile oils, mainly the contents of leaf sesquiterpenes of individuals growing in areas with higher average temperatures and solar radiation levels. We can therefore highlight that the appropriate multivariate statistical analysis allowed us to propose for the first time the existence of chemotypes for the volatile oils of *T. diversifolia*, as well as reporting the main abiotic environmental factors related to the accumulation of the discriminant compounds in these oils.

Introduction

The production and accumulation of metabolites in individuals of a same species growing at different regions may be influenced by environmental factors, so that such substances appear to play a role as a chemical interface between the plant and the surrounding environment (Ramakrishna and Ravishankar, 2011; Gutbrodt et al., 2012; Pavarini et al., 2012). This variation in composition and production of certain classes of secondary metabolites according to different environmental conditions can be used for characterization of different groups or populations of one species. In this sense, one or more metabolites may be pointed as chemical markers for specimens of a specified geographical region, season of the year and/or phenological phase, based on qualitative and quantitative analysis of their chemical constituents (Silva et al., 2006; Telascrea et al., 2007; Nehme et al., 2008; Jones et al., 2013; Vilela et al., 2013).

In plants that produce volatile oils (a complex mixture of volatile compounds, usually derived from the biosynthetic pathways of terpenoids or phenylpropanoids) (Figueiredo et al., 2008), the interaction with the environment is often related to variations in the production of these oils (composition and concentration of its constituents) (Telascrea et al., 2007; Lukas et al., 2009; Rahimmalek et al., 2009; Stashenko et al., 2010; Vilela et al., 2013).

Tithonia diversifolia (Hemsl.) A. Gray, commonly known as Mexican sunflower, is a plant species that produces volatile oil largely spread around the world. This species is a perennial herb from the family Asteraceae, tribe Heliantheae, native from both Mexico and Central America, and it can be encountered mainly in the both tropical and sub-tropical areas of Americas, Africa and Asia (Moronkola et al., 2007; Sánchez-Mendoza et al., 2011; Chagas-Paula et al., 2012). *T. diversifolia* is described as an invasive weed in different ecosystems, mainly in Africa and China (Ayeni et al., 1997; Sun et al., 2007; Muoghalu, 2008), which is related to problems of ecological imbalance in areas infested with this plant because it is able to adapt to different climate and soil conditions (Muoghalu and Chuba, 2005; Muoghalu, 2008).

The chemical constituents of *T. diversifolia* are well known and many of its secondary metabolites are described in literature. Most of the compounds isolated from *T. diversifolia* extracts belong to the classes of the sesquiterpene lactones, flavonoids and caffeoylquinic acid derivatives (Chagas-Paula et al., 2012; Zhao et al., 2012). A recent publication by our research group describes the relationship between the LC–MS-based metabolic profile of *T. diversifolia* and some abiotic environmental factors (soil nutrients and climate

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factors) for individuals grown in two distinct regions of Brazil during a period of 24 months. The results demonstrate a clear and direct influence of the environmental conditions on the production and accumulation of some classes of non-volatile secondary metabolites, especially terpenes and phenolics, in four different plant tissues (leaves, stems, roots and inflorescences) (Sampaio et al., 2016).

The composition of the volatile oils from the leaves and inflorescences of specimens of *T. diversifolia* collected in Nigeria, Cameroon, South Africa and Vietnam are described in literature. The oils obtained from Nigerian samples are characterized by the major presence of hydrocarbon mono and sesquiterpenes, especially α-pinene and β-caryophyllene (Moronkola et al., 2007), while the oils from the Cameroon samples are characterized by the major components *cis*-β-ocimene (leaf) (Lamaty et al., 1991) and α-pinene (inflorescence) (Menut et al., 1992). The monoterpene α-pinene is the major constituent of the volatile oils of leaves and inflorescences obtained from South Africa (Lawal et al., 2012), as well as of leaf samples from Vietnam (Dai et al., 2015).

A recently published review article describes some differences for the chemical constituents of volatile oil samples of *T. diversifolia* originated from different regions of Africa, leading the authors to infer that the observed variability for the volatile oil composition can be related to environmental and climatic factors as well as geographical distribution (Ajao and Moteetee, 2017). Nevertheless, to the best of our knowledge, so far there is not any report describing the effect of the seasonality, phenological phase or the abiotic environmental factors on the composition of the volatile oils of *T. diversifolia* from different regions.

Considering the wide distribution and adaptive capacity of the Mexican sunflower as an invasive species and the lack of studies covering this subject, as well as the medicinal properties of this plant (Ajao and Moteetee, 2017; Chagas-Paula et al., 2012), we proposed herein to carry out a comparative study with samples of volatile oils from leaves and flowers of *T. diversifolia* obtained from specimens located in two different regions of Brazil and collected in different seasons throughout a year. Our approach involves comparing the data obtained by chemical analysis of these oils with the environmental data (climate and soil) from both regions, with the aiming to observe the existence or the absence of a seasonal pattern and also a relationship with the environmental factors in the production of volatile oils in *T. diversifolia*.

Materials and methods

Plant material

The volatile oils from *Tithonia diversifolia* (Hemsl.) A. Gray, Asteraceae, used to perform this study were obtained from six adult specimens of the plant, located in two different states of Brazil, in order to observe the effect of the different environments on the volatile oil production in *T. diversifolia*. Three adult specimens from one cultivated population of the species located at Campus of Ribeirão Preto of the University of São Paulo (USP-RP), municipality of Ribeirão Preto, state of São Paulo, and other three specimens from cultivation area located at the Santo Antonio Farm, municipality of Pires do Rio, state of Goiás, were selected for the study.

Leaves and inflorescences were collected by BL Sampaio at dawn, before the sunrise, from the six specimens located at the states of São Paulo and Goiás (three in each state), in the months of January, May, June, July, August and December of the year of 2013, thus contemplating a complete reproductive cycle of the plant. Voucher specimens for each sampled population were deposited and identified at Herbarium SPF, Institute of Biosciences, University of São Paulo, under the responsibility of the curation of Dr. Renato de Mello Silva. Voucher numbers for each specimen were assigned as Sampaio #01 (samples from Pires do Rio-GO) and Sampaio #02 (samples from Ribeirão Preto-SP).

The samples from Goiás, immediately after the harvesting, were frozen in dry ice in order to avoid any alteration in composition of the volatile oils. The samples were then transported to the Laboratory of Pharmacognosy of the School of Pharmaceutical Sciences of Ribeirão Preto of the University of São Paulo (FCFRP-USP). Samples from São Paulo were collected and immediately taken to the Laboratory of Pharmacognosy (FCFRP-USP), where the extraction of the volatile oils of all samples was performed.

Extraction of volatile oils

The volatile oils of *T. diversifolia* were obtained from fresh leaves and inflorescences by the process of hydrodistillation performed in a Clavenger apparatus as described by Brazilian Pharmacopoeia modified (Anvisa, 2010), and after the extraction, the oils were storage in microtubes and kept in a freezer at a temperature of $-20\,^{\circ}$C.

Analysis of the volatile oils by GC–MS

The volatiles oils from *T. diversifolia* were analyzed by gas chromatography coupled to mass spectrometry in an instrument model Gas Chromatograph Mass Spectrometer – Shimadzu® – QP 2010, using a DB-5MS column ($30\,m \times 0.25\,mm \times 0.25\,\mu m$) Agilent®, employing the following method for the chromatographic analysis: oven temperature of $60\,^{\circ}$C; injection temperature of $250\,^{\circ}$C; split injection mode; gas flow rate of 1.30 ml/min; carrier gas – nitrogen (N_2); heating ramp from 60 to $240\,^{\circ}$C; heating increase rate of $3\,^{\circ}$C/min; and the ionization source was electron impact (EI).

The Retention Indexes (RI) for the components of the volatile oils were calculated by the equation of Dool and Kratz (1963), using the retention times of the components of the oils and of the homologous series of alkanes (C_9–C_{25}), and the calculated indexes were then used to the identification of these components by comparing the obtained RI and mass spectra with those available in literature and in the following libraries: Flavours and Fragrances of Natural and Synthetic Compounds (FFNSC 1.3), Wiley® Mass Spectral Library, National Institute of Standards and Technology (NIST Webbook) and The Pherobase online.

Environmental data analysis

The environmental data used to carry out this study were divided into climate and soil data. The climate data consisted of rainfall, humidity, temperature and level of solar radiation, provided by the National Institute of Meteorology (Instituto Nacional de Meteorologia – Inmet). Regarding soil data, from December 2012, three collections were performed every six months, until December 2013, following the standard procedure for soil analysis recommended by the Agronomic Institute of Campinas (Instituto Agronômico de Campinas, IAC). Soil samples were placed in labelled plastic bags (four replicates for each geographical locations) and a 200 g aliquot was separated and sent for soil analysis of macro and micronutrients, including sulphur and aluminium composition (Silva, 2009).

Data processing and multivariate analysis

The data obtained after the analysis of the volatile oils by GC–MS (chemical data) and the climate and soil data (environmental data) were prior pre-treated in MS Excel© and used for further multivariate statistical analysis by the software CANOCO 4.5