Revista Brasileira de Farmacognosia xxx (2017) xxx–xxx

Brazilian Journal of Pharmacognosy
REVISTA BRASILEIRA DE FARMACOGNOSIA
www.elsevier.com/locate/bjp

Original Article

Multivariate statistical analysis of morpho-anatomical data of nine sect. *Caulopterae* species (*Baccharis* – Asteraceae) used in folk medicine

Q1 María L. Martínez, Gabriel R. Bettucci, Matías D. Ferretti, María N. Campagna, Nazarena Ansaldi, Adriana A. Cortadi, María V. Rodriguez *

Área Biología Vegetal, Facultad de Ciencias Bioquímicas y Farmacéuticas, Consejo Nacional de Investigaciones Científicas y Técnicas, Universidad Nacional de Rosario, Rosario, Argentina

ARTICLE INFO

Article history:
Received 20 March 2018
Accepted 7 May 2018
Available online xxx

Keywords:
Anatomy
Baccharis
Carquejas
Herbal medicines
Multivariate analysis
Sect. *Caulopterae*

ABSTRACT

Baccharis species belonging to sect. *Caulopterae* are difficult to identify. Most countries are controlling the quality of herbal medicines destined for the internal market or export. "Carquejas" are used arbitrarily for the same medicinal purposes and only three species of sect. *Caulopterae* are official herbal medicines. In the present study, a morpho-anatomical and statistical analysis was performed with nine species of sect. *Caulopterae*: *Baccharis articulata*, *B. crispa*, *B. gaudichaudiana*, *B. microcephala*, *B. penningtonii*, *B. phyteumoides*, *B. sagittalis*, *B. triangularis* and *B. trimera*, emphasizing the importance of anatomy as a taxonomic tool. A total of 114 populations of these nine species were examined. The first three principal components of morphoanatomical data provided relevant information to classify the species (75.04% of the total variability). The most discriminatory variable in this issue was the stomatal index (1.0530). We determined the qualitative and quantitative variables in order to differentiate the species by using principal components analysis and ANOVA tests. Stomata type, uniseriate trichome type and presence/absence of collenchyma in the wing margin are the qualitative variables that should be analyzed. Regarding quantitative variables, the epidermal ones in superficial view are more important and discriminatory than those of alate stem cross section and they must be considered for proper quality control of the species of this work.

Introduction

The genus *Baccharis* L. consists of over 500 species, with a geographical distribution extending from Canada to Southern Argentina and Chile (Fielding, 2001). In this vast area, the genus occupies a large variety of habitats and is an important element in many vegetation communities (Giuliano, 2001). Some species of this genus are popularly known as "carquejas" and they are morphoanatomically very similar to each other. In popular medicine they are used for their digestive, hepatoprotective and anti-inflammatory properties. The beneficial effects of these species can be attributed at least in part to their antioxidant properties and free radical scavengers (Hieronymus, 1882; Sorarú and Bandoni, 1978; Toursarkissian, 1980; Martínez Crovetto, 1981; Correa, 1985). Giuliano (2001) subdivided the 96 Argentine *Baccharis* species into 15 sect., sect. *Caulopterae* DC. being characterized by the presence of species with alate stems. The species with alate stems are collected and used arbitrarily for the same therapeutic purposes, because they can be easily confused (Ariza Espinar, 1973; Lonni et al., 2005; Simões-Pires et al., 2005; Müller, 2006). Only three of these nine species are official herbal medicines, *Baccharis articulata* (Lam.) Pers. and *Baccharis crispa* Spreng. are included in the National Argentine Pharmacopeia Ed. VI (1978) and *Baccharis trimera* (Less.) DC. in the Brazilian Pharmacopeia Ed. V (2010). There is literature supporting the medicinal use of seven of these species (Stoicke and Leng-Peschlow, 1987; Gamberini et al., 1991; Gené et al., 1992, 1996; Lapa et al., 1992; Fullas et al., 1994; Brandão Torres et al., 2000; De Oliveira et al., 2003, 2012; Oliveira et al., 2005; Guo et al., 2006; Petenatti et al., 2007; Paul et al., 2009; Cifuente et al., 2010; Biondo et al., 2011). The identification of herbal medicines as part of the quality control is not obvious. Minimal morphological differences are described, which are often difficult to determine within the limits of species variability. These problems highlight the need for unequivocal parameters of identification and tests for the verification of its quality.

* Corresponding author.
E-mail: mrodrigu@fbioyf.unr.edu.ar (M.V. Rodriguez).

https://doi.org/10.1016/j.bjp.2018.05.002

The anatomy of *Baccharis* L. genus belonging to Argentina and Bolivia has been studied by Ariza Espinar (1973), Hadad et al. (2013) and Müller (2006) among others. The species with the highest number of anatomical studies are *B. articulata* and *B. crispa* (Ariza Espinar, 1973; Cortadi et al., 1999; Barboza et al., 2001; Budel et al., 2003a; Müller, 2006). The anatomical structure of *B. trimera* has also been studied exhaustively (Cortadi et al., 1999; Budel et al., 2003a; Müller, 2006; Budel and Duarte, 2009). Regarding these three species, Gianello et al. (2000) contributed with quantitative micrographic data to differentiate the raw drug. Rodriguez et al. (2008) provided new micrographic characters such as the number and size of the schizogenous secreting structures in the wing and stem, finding differences between *B. crispa* and *B. trimera*. Petenatti et al. (2007) determined quantitative micrographic characters of *Baccharis sagittalis* (Less.) DC. and *Baccharis triangularis* Hauman species. The species *B. sagittalis* was also studied anatomically by Müller (2006). Freire et al. (2007) studied the epidermis of 38 medicinal species of *Baccharis*, including *B. articulata*, *B. crispa*, *Baccharis gaudichaudiana* DC., *Baccharis microcephala* (Less.) DC. and *B. trimera*, also analyzed in the present work (qualitative and quantitative anatomical variables revision of *Baccharis* species with alate stems are listed in Box 1S and Table 1S (Supplementary Material). However, the information from these studies is inconclusive about the proper differentiation of the nine species of the *Caulopterae* sect.

Numerical methods consist of a number of statistical, mathematical and graphic techniques that analyze many variables simultaneously and are useful for taxonomical purposes (Lonni et al., 2005; Rodriguez et al., 2010). Therefore, the objective of this research is to combine chemometric methods with morpho-anatomical data to identify *Baccharis* species belonging to sect. *Caulopterae*. This work will therefore provide qualitative and quantitative differential micrographic characters of these species contributing with their effective quality control.

Materials and methods

Plant material

The *Baccharis* species (sect. *Caulopterae*), Asteraceae, with alate stems included in the study were: *B. articulata* (Lam.) Pers., *B. crispa* Spreng., *B. gaudichaudiana* DC., *B. microcephala* (Less.) DC., *Baccharis penningtonii* Heering, *Baccharis phyteumoides* (Less.) DC., *B. sagittalis* (Less.) DC., *B. triangularis* Hauman and *B. trimera* (Less.) DC. and samples of each species from different regions of Argentina were examined (114 populations). Specimens from the following herbaria: UNR, SI, CTES, BAF and LP (abbreviations according to Holmgren et al., 1990), or fresh material collected by the authors and checked by Dr MA Gattuso and Dr SJ Gattuso during collecting campaigns were examined. All materials were collected with flowers and/or fruits to enable identification and stored in the UNR herbarium. The superscript numbers indicate the plant material used to obtain the quantitative micrographic variables (1) and the plant material used for microscopic and macroscopic examination (2) (Voucher specimens and locations are detailed in Box 2S and Fig. 1S, Supplementary Material).

Morphoanatomy

The fresh material was fixed in F.A.A. (70° ethanol, glacial acetic acid, formaldehyde and water 50:5:30:15). The herbarium material was hydrated in boiling water with added drops of detergent. Zeiss MC 80 Axiolab light microscope equipped with a photographic camera and Nikon Alphaphot YS light microscope with polarized light and a Nikon Type 104 stereoscopic drawing tube were used for microscopic examination.

Table 1
Correlation between the original variables and the three first components in the characterization of nine *Baccharis* species.

Quantitative variables	Principal components (*R*)		
	*R*1	*R*2	*R*3
Wing width (mm)	0.5743	0.0930	0.6448
Stomatal density	0.1094	0.9155	−0.1835
Stomatal index	0.1129	0.8675	−0.2734
Stomatal length (μm)	0.1786	−0.9450	0.0767
Stomatal width (μm)	0.2514	−0.9091	−0.0126
Number of SSS in wing	0.9080	0.0106	0.1034
Number of SSS per wing (mm)	0.6259	−0.0925	−0.6017
Length of SSS in wing (μm)	0.8255	0.1731	0.0646
Width of SSS in wing (μm)	0.8569	0.0549	0.0796
Density of trichome tufts	−0.1480	−0.0963	−0.5808
Stem perimeter	−0.0399	0.2227	0.7848
Number of SSS in stem	0.8677	−0.0147	−0.0682
Number of SSS per stem mm	0.8033	−0.0416	−0.3870
Length of SSS in stem (μm)	0.7976	0.2437	0.2684
Width of SSS in stem (μm)	0.6987	−0.2138	−0.0964
$\sum^2$ = eigenvalue	5.6275	3.5261	2.1024

The wings were dehydrated with increasing concentrations of alcohol and coated with gold-palladium. Observations were made using a JEOL scanning electron microscope, model 35-CI.

(1) Surface view of epidermis

The stem wings were diaphanised according to Strittmatter's technique (1973) when KOH 10% was used to remove the resin layer.

(2) Cross-sections of winged stems

The material was dehydrated in increasing ethanol concentrations, then in ethanol/xylene and xylene and it was finally embedded in paraffin (Johansen, 1940). Cuts were performed manually with a Minot microtome, obtaining 12 μm thick sections. Diluted Safranine and Safranine-Fast green were used for staining (Strittmatter, 1979). The material was also dehydrated in increasing acetone concentrations, acetone/propylene oxide and propylene oxide, and embedded in Spurr's epoxy resin (Union Carbide International Co.). The stem segments were cut into 1 μm sections with an ultramicrotome equipped with a diamond knife. Toluidine Blue 1% and Acid Fuchsin 1% were used for staining (D'Ambrogio, 1986).

Crystals were observed using weak diluted acid and polarized light analysis (Johansen, 1940).

Both techniques (diaphanised and cross-sections) were used in order to obtain the quantitative micrographic variables (marked by superscript 1 in each sample tested).

Statistical analysis

Population analysis was performed by means of principal components analysis (PCA) using NTSYS-pc 2.11w (Numerical Taxonomy and Multivariate Analysis System) designed by Rohlf (1998). The aim of PCA is to reduce data dimensionality by transforming the original characteristic variables into others that are linear combinations of the first variables (Lonni et al., 2005).

The basic data matrix was prepared by considering fifteen micrographic quantitative variables (listed in Tables 1 and 2) of the alate stems as seen in the cross-section and diaphinased material (in total: 50 populations of nine species were studied).

Variables were grouped as follows: (1) surface view of epidermis: (a) stomatal length, (b) stomatal width, (c) stomatal index, (d) stomatal density, and (e) density of tufts; (2) cross section of winged stems: (a) wing width, (b) number of secreting schizogenous structures (SSS) in the wing, (c) number of SSS per mm stem, (d) SSS length in the wing, (e) SSS width in the wing, (f) stem perimeter,

Please cite this article in press as: Martínez, M.L., et al. Multivariate statistical analysis of morpho-anatomical data of nine sect. *Caulopterae* species (*Baccharis* – Asteraceae) used in folk medicine. Revista Brasileira de Farmacognosia (2017), https://doi.org/10.1016/j.bjp.2018.05.002