19

Quality Control of Herbal Medicines with Spectrophotometry and Chemometric Techniques – Application to *Baccharis* L. Species Belonging to Sect – Caulopterae DC. (Asteraceae)

María Victoria Rodriguez[1,2], María Laura Martínez[1], Adriana Cortadi[1], María Noel Campagna[1], Osvaldo Di Sapio[1], Marcos Derita[2], Susana Zacchino[2] and Martha Gattuso[1]

[1]*Farmacobotánica, Área Biología Vegetal, Departamento Cs. Biológicas*

[2]*Farmacognosia, Área Farmacognosia, Departamento de Química Orgánica*

Facultad de Ciencias Bioquímicas y Farmacéuticas

Universidad Nacional de Rosario

Argentina

1. Introduction

Medicinal plants constitute a rich cultural and biological heritage in many countries, which could be very useful in meeting the therapeutic needs of the population (Rodriguez, 2010a). Traditional herbal medicines have been widely used for many years in many eastern countries (Liang et al., 2004). However, little work has been done to validate and standardize these products properly in order to match phytotherapy to chemotherapy which currently receives almost unconditional support from formal systems of health care. For several years now activities have been undertaken to systematize the identification, validation, production and use of medicinal plants, for both primary health care as well as a semi-industrial or industrial process, which implies their transformation into safe, reliable and stable phytopharmaceutical products. Therefore it is suggested that medicinal plants and their derived products would be a viable option for national development as an agricultural and therapeutic alternative, but standardization and industrialization, involving sustained yields, a quality control system and honest and reliable marketing would be needed for widespread implementation and official support. Consequently, on account of the above, education and research should be in agreement if any advance is to be made in this area (Rodriguez, 2010a). However, the necessary criteria for data quality, safety and efficacy of traditional medicine that would support its use in the world do not exist. Appropriate, accepted research methodology for evaluating traditional medicine is also lacking (Liang et al., 2004).

Most countries have developed organisms for controlling the quality of herbal remedies destined for the internal market or for export. The officially recognized drugs are subject to

testing and specifications of identity, purity and contents of the principal active ingredients or markers for each plant drug, in order to guarantee the conservation of the species and the specifications of microbiological purity. Currently the quality requisites of the different national pharmacopoeias vary between countries, however the International Federation of Pharmaceutical Manufacturer Associations, have taken a first step towards the global implementation of organized criteria (International Federation of Pharmaceutical Manufacturer Associations [IFPMA], 1997). The World Health Organization has conducted a review of the legal status of traditional medicine and complementary or "alternative" therapies in 123 countries (World Health Organization [WHO], 2001). Moreover, some countries and organizations have developed, or are developing, their own monographs, for example, the Commission E of the German Ministry of Health, WHO and European Scientific Cooperative on Phytotherapy, China, Brazil and Argentina, among others. These monographs recognise the quality standards applicable to drugs and herbal remedies in the pharmaceutical market. (Blumenthal, 1998; European Scientific Cooperative on Phytotherapy [ESCOP], 2003; Keller, 1991; WHO, 1991, 1999, 2002).

In Argentina there is great interest in controlling the Quality, Security and Efficacy of herbal medicines. In order to achieve this ambitious objective the following steps must be taken: a- Registration of the products, b- Verification of good practices of production and quality control, of the crude drug and its products, and c- Pharmacovigilance. ANMAT (National Administration of Drug, Food and Medical Technology) is the organism responsible for authorizing all activities related to medicines through its technical organism, the INAME (National Institute of Drug). The products based on herbal medicine and aromatics are considered as Phytotherapeutic Drugs in Resolution Nº 144/98 and the Supplementary Provisions (**Provision 2671/99; Provision 2672/99; Provision 2673/99)** that are now in effect. Provision 1788/2000 contains a list of 109 herbal medicines that can not be authorised for the production of herbal medicine on account of the contradictions or toxic effects reported in their traditional use.

It is very important that plant material is of the highest quality as it is used as a medicine. The aforementioned decrees aim to guarantee the quality of the raw material, the intermediate and finished products and a series of tests have been established among which, as a minimum requisite, are botanical identity, purity (physical-organoleptic and microbiological - for health) and their activity or composition (methodological analysis), taking into account the Good Practices of Agricultural Production and the Good Practices of Manufacturing (Rodriguez, 2010a).

1.1 Spectrophotometry and chemical fingerprints of herbal medicines

In general one or two markers or pharmacologically active compounds of herbal components or herbal blends were used to evaluate the quality and authenticity of herbal medicines in the identification of a single herb or preparation of herbal medicine and to evaluate the quantitative composition of a herbal product. However, this type of determination does not give a complete picture of the herbal products because multiple ingredients are usually responsible for its therapeutic effects. These therapeutic effects may work synergistically and change their activity on being separated into their active parts. So various analytical techniques can be applied for this type of registration and the complete herbal product can be considered as the "active compound" (Liang et al., 2004).

The concept of phytoequivalence was developed in Germany to ensure the consistency of herbal products (Tyler, 1999). According to this concept, a chemical profile, such as a spectrophotometric fingerprint for a herbal product should be constructed and compared with the profile of a clinically tested reference product. Therefore, a spectrophotometric fingerprint of a herbal medicine is a spectrophotometric pattern of an extract of some chemical components that are pharmalogically active and/or with chemical characteristics. It is suggested that with the help of spectrophotometric fingerprints obtained, the authentication and identification of herbal medicines would be appropriate, even when the amount and/or concentration of some typical chemical ingredients are not exactly the same for different samples of these herbal medicines. In this way, we are broadly considering multiple ingredients in the extracts of herbal medicines rather than one or two marker ingredients for evaluating the quality of the herbal products.

In general various analytical techniques can be used to obtain fingerprints of herbal medicines of which chromatography is most often used on account of its great efficiency in separating the different components of an extract. For the above mentioned reasons these chromatographic techniques have a high cost, especially if they are to be used for routine quality control. A simple alternative technique such as UV/Visible spectrophotometry, coupled to chemometric methods was carried out by Lonni et al. (2005) for taxonomic purposes. Authors were able to separate populations of three species of *Baccharis* according to their UV / Visible spectra. Here, we propose to apply the same methodology as part of quality control of herbal medicines.

Figure 1 shows the spectrophotometric profiles of ethanol extracts of the aerial parts of *Baccharis gaudichaudiana* DC. obtained from five areas in Misiones province, Argentina (MI1, MI2, MI3, MI4, MI5) (Rodriguez, 2010b). Here, of course, some differences can be observed in the profiles; however phytoequivalence for *B. gaudichaudiana* can be seen.

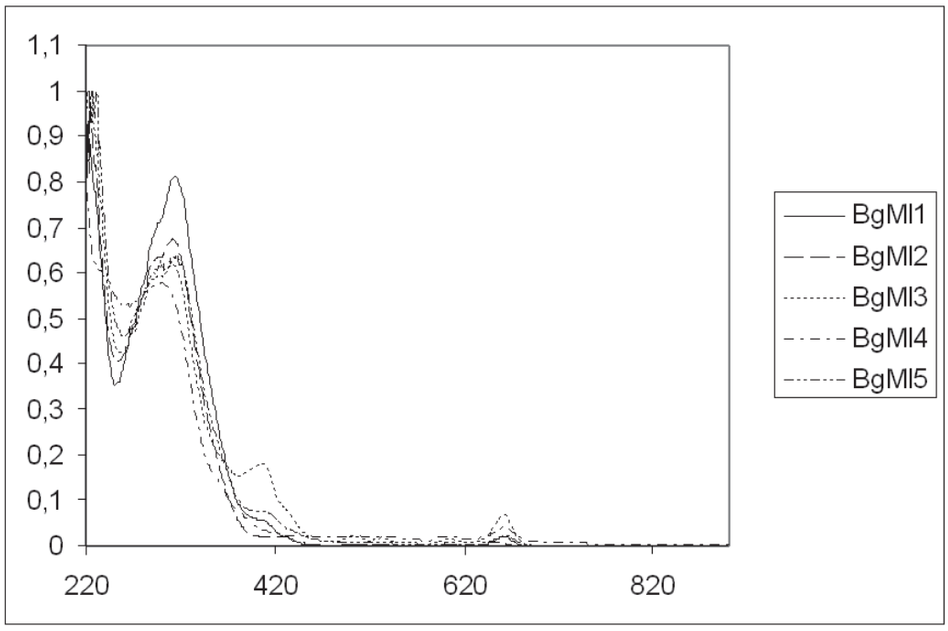


Fig. 1. Absorption UV/Visible spectra of *Baccharis gaudichaudiana* populations (Bg). MI, Misiones. Numbers indicate when there is more than one population of the same species.

1.2 Chemometric methods and quality control

The chemometric methods consist of a number of statistical, mathematical and graphic techniques that analyze many variables simultaneously (Lonni, 2005). The method used in this study is as follows:

Principal components analysis (PCA).

This method is based on the transformation of a group of original quatitative variables into another group of unrelated independent variables, known as principal components. The components have to be interpreted independently of one another, as they contain part of the variance that is not expressed in any other principal component (Pla, 1986; López & Hidalgo, 1994).

The criterion of Cliff in 1987 was adopted for selecting the number of components that should be used for the analysis, which states that the eigenvalues of acceptable components should explain 70% of the total variance (López & Hidalgo, 1994).

Proportion of variance explained: in general statistical programs provide information of the eigenvectors and, in some cases, the correlation between the original variables and the principal components. However, these correlations can be calculated from the eigenvectors in the following formula (Pla, 1986):

$$r(jk) = l(jk) \times (\lambda(\kappa))\ 1/2/s(ij) \quad (1)$$

where,

$r(jk)$= correlation between the original variable $x(j)$ and the k-esim component.

$l(jk)$ = j-esim element of the k-esim eigenvector.

$\lambda(\kappa)$= k-esim eigenvalue.

$s(ij)$ = variances of the correlation matrix.

In most studies it is important to determine the degree of discrimination of the variables so that those with the most and least variation can be identified.

Using PCA it is possible to determine the degree of discrimination, quantifying the proportion of variance explained by each original variable of the selected components; to do this it is necessary to add the squares of the correlation formed by each original variable with the selected components. This is possible as the components are not correlated (Pla, 1986). In the case of a variable in series: $rx1^2 + rx2^2 + rx3^2$ = proportion of the variance explained, having selected three components. It should to be taken into account that the variables that explain a larger proportion of variance are the most discrimatory and therefore they are more important.

The UV/Visible spectra of populations of two species, *Baccharis articulata* (Lam.) Pers. and *B. trimera* can be seen in Figure 2. In this figure it is difficult to distinguish which spectrum corresponds to which species, but the resolution is greater when PCA is applied (Figure 3) and different coordinates can be seen for the corresponding populations of one species or another.

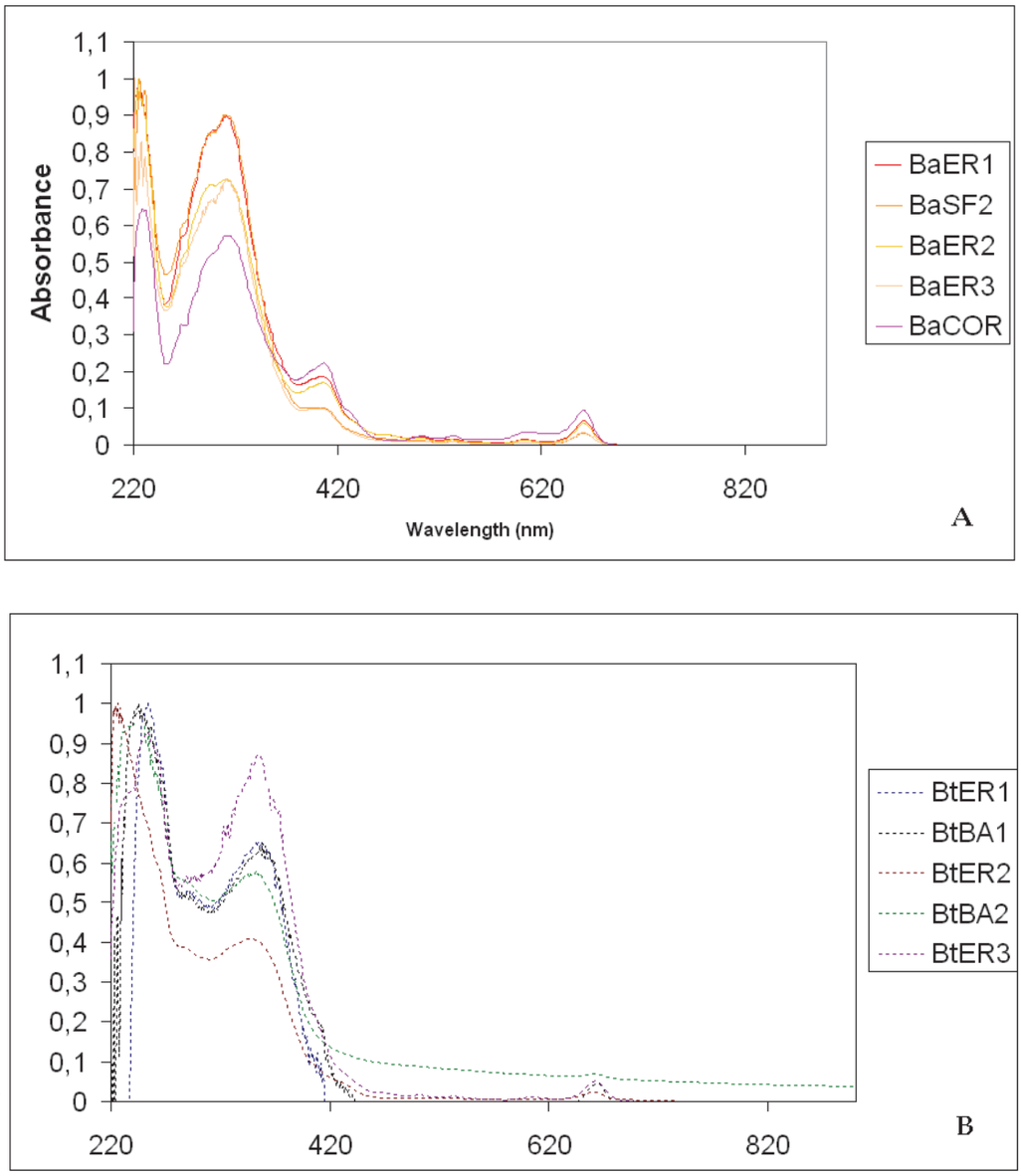


Fig. 2. Absorption UV/Visible spectra. **A**, *Baccharis articulata* (Ba); **B**, *Baccharis trimera* (Bt) populations. BA, Buenos Aires; COR, Corrientes; ER, Entre Ríos; SF, Santa Fe. Numbers indicate when there is more than one population of the same species

2. An example of differentiation of *Baccharis* L. species belonging to sect – Caulopterae DC. (Asteraceae) using UV/Visible spectrophotometry data and multivariate analysis

Baccharis L. is an exclusive American genus comprising approximately 500 species. Its distribution area covers the whole of South America and continues northwards up to the south of USA including the Atlantic coast up to Massachusetts, although its presence is greater in the intertropical and subtropical zones (Cuatrecasas, 1967; Müller, 2006). Several

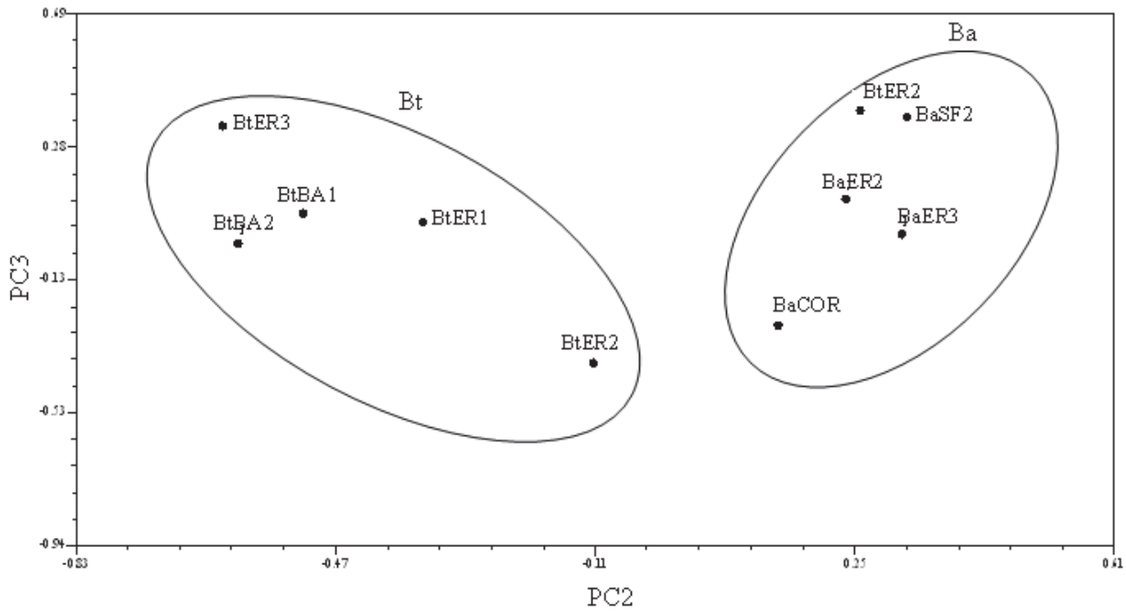


Fig. 3. Two-dimensional model derived from PCA of 700 quantitative variables of 10 *Baccharis* populations. Ba, *B. articulata*; Bt, *B. trimera*. BA, Buenos Aires; COR, Corrientes; ER, Entre Rios; SF, Santa Fe populations. Numbers indicate when there is more than one population of the same species.

authors have contributed to the infrageneric classification of *Baccharis* in general and regional floras (Ariza Espinar, 1973; Baker, 1882-1884; Barroso, 1976; Cuatrecasas, 1967; De Candolle, 1836; Giuliano, 2001; Heering, 1904; Lessing, 1831; Weddell, 1855-1856) and it was De Candolle (1836) who was the first to subdivide the genus in eight sections, mainly based on leaf morphology. More recently, Giuliano (2001) grouped 96 Argentine *Baccharis* species into 15 sections, among which the sect. Caulopterae DC. is characterized by the presence of species with alate stems. Two of nine Argentine species from this sect., i.e., *Baccharis articulata* (Lam.) Pers. and *Baccharis crispa* Spreng. are included in the National Argentine Pharmacopeia Ed. VI (1978), and a third one, *Baccharis trimera* (Less.) DC. in the Brazilian Pharmacopeia Ed. IV (2002) with the common name of "Carquejas". The nine species of this sect. are traditionally used in infusions or decoctions, as hepatic, colagogue, diuretic, ulcer healing and external antiseptics. They are also used in herbal remedies and phytotherapy and in the preparation of spirits and soft drinks (Correa, 1985; Gupta, 1995; Hieronymus, 1882; Martínez Crovetto, 1981; Sorarú & Bandoni, 1978; Toursarkissian, 1980). Beneficial effects of these species can be attributed in part to their high content of flavonoids. The chemistry of the flavonoids is predictive of their free radical scavenging activity, which confers the antioxidant activity (Harborne & Williams, 1992; Rice-Evans et al., 1995).

Currently, the morphoanatomical studies of these species in sect. Caulopterae only provide incomplete information which makes it difficult to differentiate each one properly in the non-flowering condition when the size of capitulum of each one varies (Giuliano, 2000; Müller, 2006). This fact has lead to the misuse of the same common name for botanically diverse species, which surely have different chemical compositions and therefore different pharmacological properties (Abdel-Malek et al., 1996; Desmarchelier et al., 1997; De Oliveira

et al., 2003; Gene et al., 1992, 1996; Lapa et al., 1992; Palacios et al., 1983; Stoicke & Leng-Peschlow, 1987). Also the high frequency of errors committed during the collection of these species for medicinal purposes is understandable due to the coexistence of these entities in certain habitats. Hence, considering that numerical methods have been shown to be useful for multi-component metabolic classification studies, we decided to carry out population studies including 53 samples of these nine sect. Caulopterae species, combining their spectrophotometric profiles with multivariate analysis. These nine species belonging to sect. Caulopterae are: *Baccharis articulata* (Lam.) Pers., *B. crispa* Spreng., *B. gaudichaudiana* DC., *B. microcephala* (Less.) DC., *B. phyteumoides* (Less.) DC., *B. penningtonii* Heering., *B. sagittalis* (Less.) DC., *B. triangularis* Hauman and *Baccharis trimera* (Less) DC. Of these nine species, only three are official and its use is permitted but all are indiscriminately collected for medicinal purposes due to their similar morphotypes as they have alate stem.

2.1 Material and methods

Fifty three samples of nine *Baccharis* species were collected from wild materials in different locations in Argentina. All samples were botanically identified by our group and voucher specimens were deposited at the herbarium of the National University of Rosario, Argentina (Table 1).

The aerial parts of the dried plants (5 g) were macerated (24 h, 3x) with absolute ethyl alcohol. The ethanolic extract was filtered and concentrated in a rotary evaporator at a temperature lower than 100 ºC. Thirty mg of dry extract were mix in 3 ml dichloromethane (DCM) and left for 1 h and then filtered with common filter paper. A dilution of 50 µl of this solution in 950 µl of methanol was prepared and filtered twice with a 0.45 µm Millipore filter (Lonni et al., 2003, 2005).

Spectrophotometric analyses were carried out using a Biochrom Model Libra S12 UV/Visible Spectrophotometer, equipped with tungsten halogen and deuterium arc light sources with a single solid state silicon photodiode detector, and operating software.

TLC analyses were carried out using silica gel 60 F254, Merck; mobile phase, DCM: Hexane:

MeOH (4:2:1). Chromatograms were evaluated under UV light at 254 and 365 nm to detect the presence of flavonoids. TLC was additionally sprayed with a diphenylborinic acid ethanolamine/polyethylene glycol reagent. Apigenin, chlorogenic acid, genkwanin, luteolin, quercetin and rutin were used as markers (purchased from Extrasynthèse, France).

HPLC analyses were carried out using a Spectra Physics Model SP8800 ternary pump chromatograph with Spectra 100 UV/Visible detector, having as chromatographic conditions, methanol eluent, 1 ml min^{-1} flow, Luna C18 phenomenex (250 x 4.6 mm, 5 µm particle size). The injection volume was 100 µl and elution was monitored al 254 nm. Apigenin, genkwanin and luteolin were used as markers (purchased from Extrasynthèse, France).

TLC and HPLC analysis were applied in order to complement the studies carried out by PCA of the spectrophotometric data and to find potential markers of the species that could not be characterized by the previous method.

Sample	Voucher	Date	Sample	Voucher	Date
BaCO1	1570	Apr-05	BmFO	1656	Feb-06
BaCO2	1607	Feb-06	BmMI	1657	Feb-06
BaCO3	1617	Mar-06	BphySF1	1888	Feb-07
BaCO4	1618	Mar-06	BphySF2	1939	Mar-08
BaCO5	1619	Mar-06	BpER	1594	Jan-06
BaCO6	1620	Mar-06	BpSF1	1887	Feb-07
BaCO7	1621	Mar-06	BpSF2	1938	Mar-08
BaCOR	1903	Mar-07	BsRN1	1906	Mar-07
BaER1	1927	Apr-07	BsRN2	1953	Nov-08
BaER2	1928	Aug-07	BtrBA[a]	SI Burkart[b]	Nov-1972
BaER3	1929	Apr-07	BtrCHU[a]	SI Dacnik[b]	Feb-1969
BaSF1	1930	Aug-07	BtrLP[a]	3262	Dec-1975
BaSF2	1916	Jan-07	BtrSL	1907	Mar-07
BaSF3	1917	Jan-07	BtBA1	1955	Aug-05
BcCO1	1543	Mar-05	BtBA2	1956	Mar-05
BcCO2	1623	Mar-06	BtBA3	1668	May-06
BcCO3	1624	Mar-06	BtCOR1	1539	Mar-05
BcME1	1590	Jan-06	BtCOR2	1553	Mar-05
BcME2	1591	Jan-06	BtCOR3	1574	Jul-05
BcME3	1909	Jan-07	BtCOR4	1954	Feb-06
BcME4	1910	Jan-07	BtER1	1535	Mar-05
BgMI1	1564	Oct-05	BtER2	1537	Mar-05
BgMI2	1566	Oct-05	BtER 3	1542	Dec-04
BgMI3	1655	Feb-06	BtER4	1583	Feb-06
BgMI4	1569	Mar-05	BtER5	1645	Mar-06
BgMI5	1654	Feb-06	BtER6	1926	Apr-07
BmCOR	1572	Oct-05			

Table 1. Collection data of analysed samples of *Baccharis* species. The abbreviation mean: species: Ba, *B. articulata*; Bc, *B. crispa*; Bg, *B. gaudichaudiana*; Bm, *B. microcephala*; Bp, *B. penningtonii*; Bphy, *B. phyteumoides*; Bs, *B. sagittalis*; Btr, *B. triangularis*; Bt, *B. trimera*; provinces: BA, Buenos Aires; CHU, Chubut; CO, Córdoba; COR, Corrientes; ER, Entre Ríos; FO, Formosa; LP, La Pampa; MI, Misiones; RN, Río Negro; SF, Santa Fe; SL, San Luis. Numbers indicate when there is more than one population of the same species. [a] Material extracted from Herbarium; [b] Names of collectors, numbers not provided by the Herbarium.

2.1.1 Statistical analysis

Principal components analysis (PCA) was applied to the population study. The analysis was performed using the NTSYS-pc 2.11w (Numerical Taxonomy and Multivariate Analysis System) designed by Rohlf (1998).

Basic data matrix was prepared considering 700 absorbance values (quantitative variable) of each analysed sample (53 extracts in total).

Before PCA the data were pre-processed with normalization to unit area technique (Beebe et al., 1998).

2.2 Results and discussion

2.2.1 Spectrophotometric analysis

Seven hundred absorbance values were utilised as quantitative variables for population analysis. Samples were collected in different provinces and seasons, mainly taking into account the quantitative variability of secondary metabolites during the year (Table 1). The principal component analysis showed that the first nine components explain almost 98.81% of the total variability. The second (PC2) and the third (PC3) principal components gathered relevant information for classifying species. Figure 4, shows a two dimension plot of PC2 vs. PC3 using all the variables. Samples could be classified in five large groups containing the species *B. crispa*, *B. microcephala*, *B. phyteumoides*, *B. triangularis* and *B. trimera*. PC2 clearly separates *B. microcephala* and *B. trimera* populations from *B. crispa* and *B. phyteumoides* populations. Moreover, samples corresponding to *B. triangularis* species were separated from those belonging to *B. microcephala*, *B. trimera*, *B. crispa* and *B. phyteumoides* populations by PC2. While PC3 separates *B. microcephala* samples from the rest of the species, it also separates *B. crispa* samples from *B. phyteumoides*, *B. trimera* and *B. triangularis* and between samples of the two latter species. However, PC3 did not completely distinguish *B. phyteumoides* from *B. trimera* and *B. triangularis* samples.

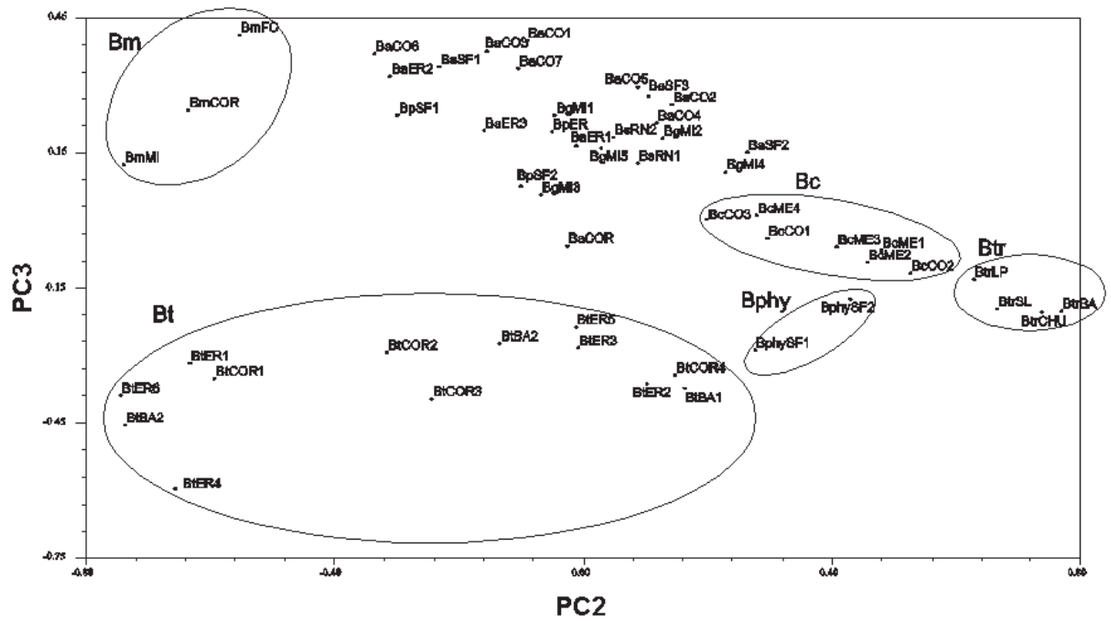


Fig. 4. Two dimensional model of PC2 *vs.* PC3 (15.63 and 7.80 %, respectively) derived from PCA of 700 quantitative variables of 53 *Baccharis* populations. Ba, *B. articulata*; Bc, *B. crispa*; Bg, *B. gaudichaudiana*; Bm, *B. microcephala*; Bp, *B. penningtonii*; Bphy, *B. phyteumoides*; Bs, *B. sagittalis*; Btr, *B. triangularis*; Bt, *B. trimera*; BA, Buenos Aires; CHU, Chubut; CO, Córdoba; COR, Corrientes; ER, Entre Ríos; FO, Formosa; LP, La Pampa; MI, Misiones; RN, Río Negro; SF, Santa Fe; SL, San Luis.

Figure 5, shows a two dimensional plot of PC7 vs. PC9 using all the variables. Here, the seventh (PC7) and ninth (PC9) principal components enable the *B. penningtonii* samples to be separated from the rest of the species.

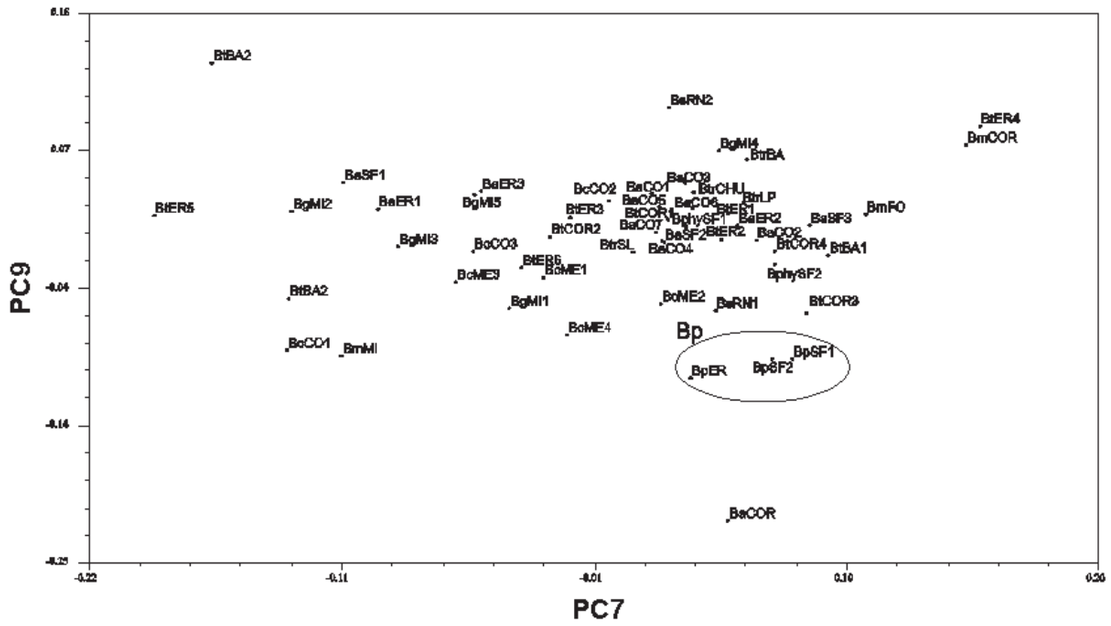


Fig. 5. Two dimensional model of PC7 *vs.* PC9 (0.62 and 0.36 %, respectively) derived from PCA of 700 quantitative variables of 53 *Baccharis* populations. Ba, *B. articulata*; Bc, *B. crispa*; Bg, *B. gaudichaudiana*; Bm, *B. microcephala*; Bp, *B. penningtonii*; Bphy, *B. phyteumoides*; Bs, *B. sagittalis*; Btr, *B. triangularis*; Bt, *B. trimera*; BA, Buenos Aires; CHU, Chubut; CO, Córdoba; COR, Corrientes; ER, Entre Ríos; FO, Formosa; LP, La Pampa; MI, Misiones; RN, Río Negro; SF, Santa Fe; SL, San Luis.

Spectrophotometric PCA data allowed the distinction of six out of the nine species examined in this study. However the three species *B. articulata*, *B. gaudichaudiana* and *B. sagittalis* could not be separated which is shown in the figure 6, where it is seen that the average spectra of the species are very similar.

As can be observed in Figure 4, the *B. microcephala* and *B. trimera* samples have PC2 scores with opposite signs to those of the *B. crispa*, *B. phyteumoides* and *B. triangularis* samples. This contrast can be explained with the help of Figure 7 A; C. Figure 7 A shows a graph of the loading values (eigenvalues) on PC2 vs. λ. Positive values are situated in a region between 200 and 230 nm. It is possible to verify from Figure 7 C that the analytical signals for *B. crispa*, *B. phyteumoides* and *B. triangularis* in this region are more intense than those of *B. microcephala* and *B. trimera*, and that the *B. crispa*, *B. phyteumoides* and *B. triangularis* samples have positive scores.

Figure 7 A also shows that three regions present negative values between: 225 and 275, 325 and 375 and 375 and 450 nm. In Figure 7 C, one can verify that in these intervals the most intense analytical signals belong to samples of the *B. microcephala* and *B. trimera* species. For this reason the *B. microcephala* and *B. trimera* species samples have negative PC2 scores.

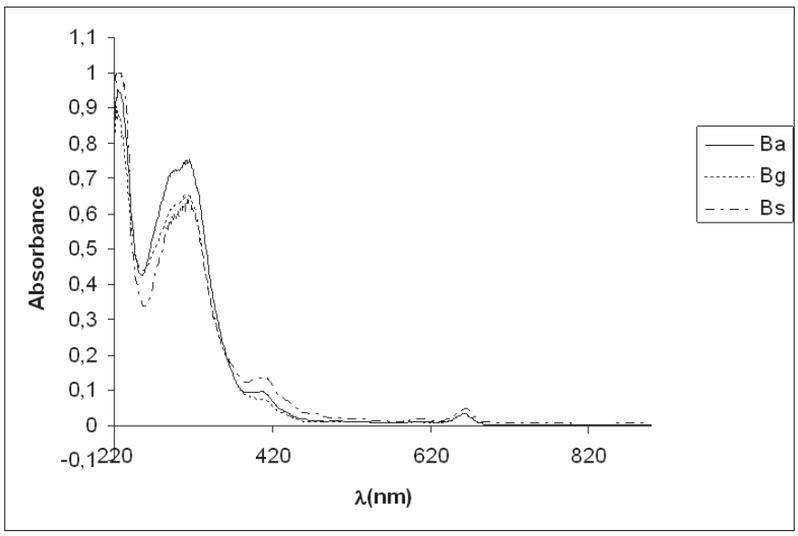


Fig. 6. Representative UV-Visible spectra of *B. articulata* (Ba), *B. gaudichaudiana* (Bg), *B. sagittalis* (Bs).

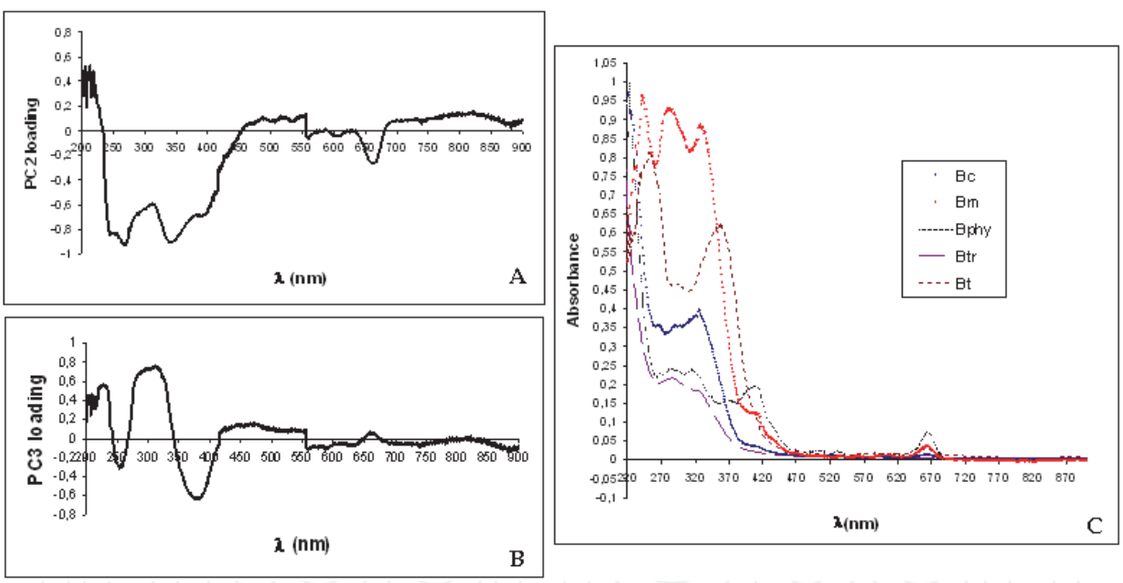


Fig. 7. A-B, PC loadings graph. **A**, PC2 (explains 15.63 % of the total data variance); **B**, PC3 (explains 7.80 % of the total data variance). C, Representative UV-Visible spectra of *B. crispa (Bc)*, *B. microcephala (Bm)*, *B. phyteumoides (Bphy)*, *B. triangularis (Btr)*, *B. trimera (Bt)*.

Figure 7 B (PC3 eigenvalues) shows two regions, one with positive values (270 to 350 nm) and one with negative values (350-420 nm). From Figure 7 C it is possible to verify that in the first region the analytical signals for *B. microcephala* are more intense than those of *B. trimera*, *B. crispa*, *B. phyteumoides* and *B. triangularis*, and that the *B. microcephala* samples have positive PC3 scores. The rest of the species have negative PC3 scores. In Figure 7 B, the region with negative values (270 to 350 nm) matches the more intense analytical signals for *B. crispa*, *B. phyteumoides* and *B. triangularis* in Figure 7 C.

B. penningtonii species samples have negative PC9 scores; PC9 separates *B. penningtonii* samples from the rest as seen in Figure 5. Figure 8 A shows a graph of the loading values (eigenvalues) on PC9 vs. λ; it also shows that two regions present negative values between: 200 and 250 and 320 and 370 nm and it can be observed that the most intense analytical signals for *B. penningtonii* species samples are in these regions (Figure 8 B).

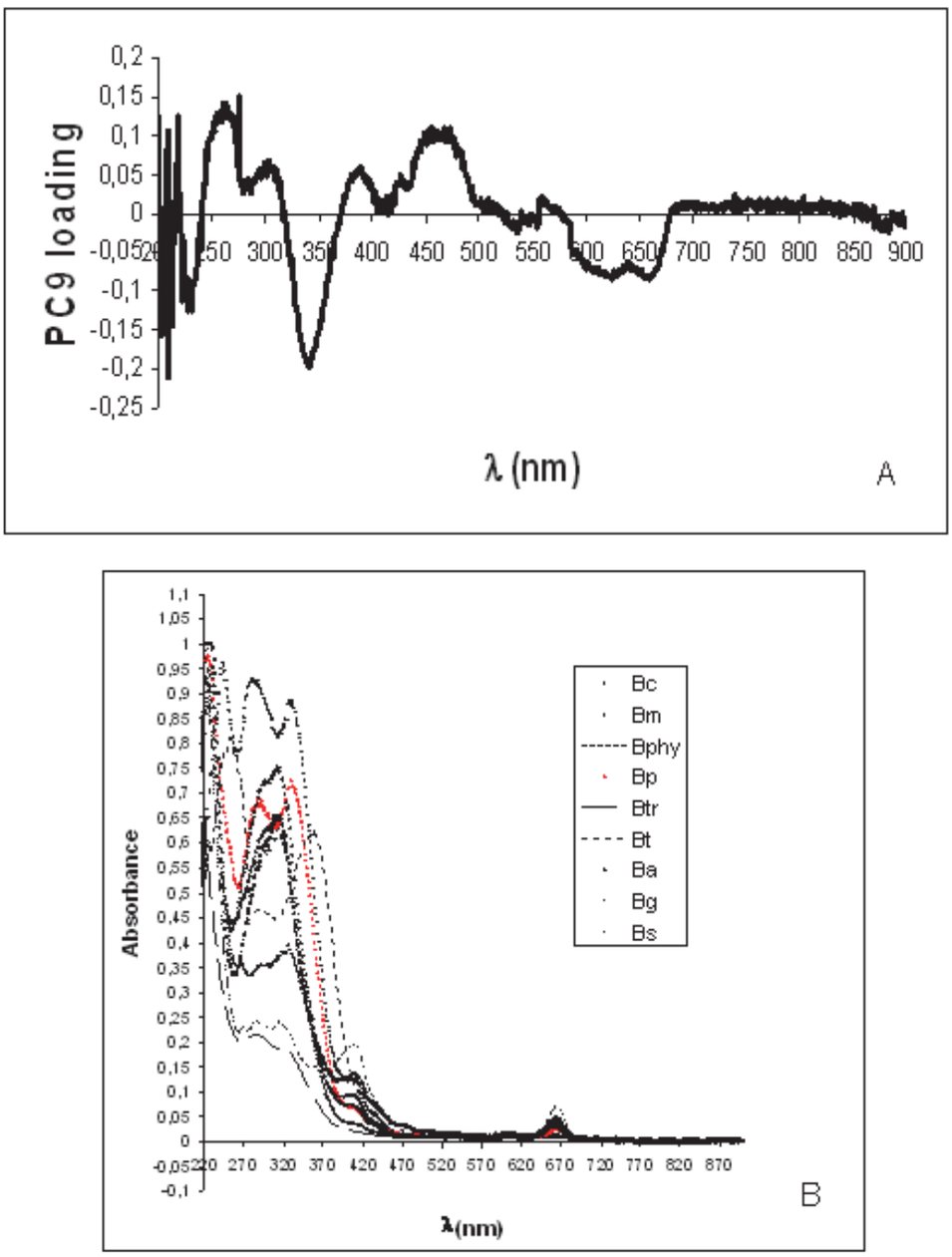


Fig. 8. A, PC loadings graph. PC9 (explains 0.36 % of the total data variance);
B, Representative UV-Visible spectra of *B. articulata* (Ba), *B. crispa* (Bc), *B. gaudichaudiana* (Bg), *B. microcephala* (Bm), *B. phyteumoides* (Bphy), *B. penningtonii* (Bp), *B. sagittalis* (Bs), *B. triangularis* (Btr), *B. trimera* (Bt).

These results suggest that the substances responsible for the discrimination between species are those which have peaks around 225, 250, 300, 325, 350, 375 and 425 nm. Considering that the ethanol extracts used in this study might possess mainly flavonoids and that the region studied is where they present their main absorption bands (Greenham et al., 2003), it is likely that the components responsible for the spectra in this work would be flavonoids. It is worth taking into account that previous studies on the phytochemistry of *Baccharis* spp. have shown the presence of flavonoids, mainly flavanones and flavones (Coelho et al., 2004; Gene et al., 1996; Torres et al., 2000). From species of this genus 298 flavonoids were isolated. Among them 24 were flavanones and 85 were flavones and among them 48% are oxygenated in the C-3 (Gonzaga Verdi et al., 2005). They were described as good chemotaxonomic markers for the lower hierarchical levels of the Asteraceae family (Emerciano et al., 2001). In contrast to other characterization studies in which it is necessary to isolate and identify chemical substances, this study uses the complete ultraviolet-visible spectra measured between 200 nm to 900 nm, avoiding the isolation, purification and characterization of chemical compounds (Lonni et al., 2005). This methodology does not distinguish the spectra of individual components in the extract. Instead, the full spectrum ranges are analyzed as a whole. Absorption spectra of figure 7 C show that the qualitative composition of flavonoids is different in the different species.

It has previously been observed that there is variability in the contents of several compounds in the different *Baccharis* species (Dresch et al., 2006; Gonzaga Verdi et al., 2005). With regards to the seasons, Borella et al. (2001) observed variations in the content of the total flavonoids, the largest number being found in a drug in the summer, which is an expected result due to the large number of functions attributed to them (Harborne & Williams, 2000). Our results are consistent with previous ones, showing different heights of peaks in the spectra in Figure 6, 7C and 8 B, even though these spectra were standardized. In a fertilization trial of *B. trimera,* in which the nutrient content of the soil was varied, no variation was observed in the contents of flavonoids (Borella et al., 2001). In our study we observed some changes in the content of flavonoids between collecting regions (Figure 4 and 5) but these did not prevent the grouping of various populations of the same species.

A routine step in multivariate data analysis is ordinarily to obtain a low-dimensional representation of the data. If two or three main components gives an accurate representation, a bi-or three-dimensional graph could be realized which mere observation is instructive. Clusters are usually easy to detect. After analysis of the eigenvalues (PC loading), more discriminant original variables are obtained. Then an ANOVA must be performing of each of these original variables between the OTUS (here species). The higher the eigenvalues, regardless of the sign will be more efficient in discriminating the OTUS. Variables that have negative eigenvalues (-) means that they are characterizing in the opposite direction in relation to the variables that have positive eigenvalues (+) and vice versa.

Thus, absorbance values of the wavelengths (225, 250, 300, 325, 350, 375 and 425 nm) obtained from the analysis of the eigenvalues were submitted to ANOVA and we have established the wavelengths that differ between pairs of species (Table 2). Thus we confirm that there are differences in the UV / Visible spectra not observable to the naked eye but are expressed as different clusters after a principal component analysis is applied.

	Ba	Bc	Bg	Bm	Bp	Bphy	Bs	Btr	Bt
Ba		300 325		250 325 350 375	250 350	300 325 350 425		225, 250, 300, 325 350	225, 250, 300, 325, 350, 375
Bc			300 325	250, 300, 325, 350 375	250 300 325 350	325 425	300 425	225 300 325 350	250 350 375
Bg				250 300 325 350	350	300 325 425		225, 250 300, 325 350	250 300 350 375
Bm					250 300	250 300 325 350	250 300 325 350	225, 250 300, 325 350, 375	300 325 375
Bp						250 300 325 350	250 350	225 250 300 325 350	250 300 325 375
Bphy							250 300 325	225 425	250, 300 325,350 375
Bs								225 300 325 425	250 300 350 375
Btr									225, 250 325, 350 375, 425
Bt									

Table 2. Wavelengths (nm) with statistically significant differences ($p < 0.05$) among the species. *B. articulata* (Ba), *B. crispa* (Bc), *B. gaudichaudiana* (Bg), *B. microcephala* (Bm), *B. penningtonii* (Bp), *B. phyteumoides* (Bphy), *B. sagittalis* (Bs), *B. triangularis* (Btr) and *B. trimera* (Bt)

2.2.2 TLC analysis

A TLC analysis was initiated of the dichloromethane extracts of *B. articulata*, *B. gaudichaudiana* and *B. sagittalis* used to perform UV-Visible spectra using the mobile phase DCM: Hexane: MeOH (4:2:1) in order to complement the studies carried out by PCA of the spectrophotometric data and to find potential markers of the species that could not be

characterized by the previous method. NP-PEG reagent under UV 365 nm and UV 254 nm were used to detect the polyphenolic compounds present in the extracts (Wagner & Bladt, 1996), in accordance with that published for the *Baccharis* genus on account of the high occurrence of these compounds in the genus (Bohm & Stuessy, 2001; Gonzaga Verdi et al., 2005). Different color bands will be detected with the NP-PEG reagent under UV 365 nm or quenching bands will be detected under UV 254 nm if these compounds are present in the extract. On the other hand, given that one of our objectives was the identification of the species in the state of a crude drug, it was very important to select appropriate components of easy access for chemical quality control. So apigenin, genkwanin and luteolin, which are compounds present in several *Baccharis* species, were selected as markers. Genkwanin has been reported in *B. articulata* (Gianello & Giordano, 1984) and apigenin in *B. gaudichaudiana* (Fullas et al., 1994). Luteolin has not yet been reported in any of the three species analyzed by TLC, but it has been found in the following species from the same section: *B. microcephala* (Bohlmann et al., 1985), *B. trimera* (Soicke & Leng-Peschlow, 1987) and *B. triangularis* (Pettenati et al., 2007). Our results are shown in Table 3. The TLC chromatograms showed differences for the three species studied (Figures 9A and 9B). The flavonoids apigenin and genkwanin were found in all three species, although the band that corresponds to genkwanin somewhat overlaps in *B. gaudichaudiana* and *B. sagittalis.* In the case of the other marker, we observed that the band corresponding to luteolin appeared in *B. gaudichaudiana* and *B. sagittalis*. There are at least two more bands at Rf 0,83 and 0,75 for *B. articulata* and there is another band for *B. gaudichaudiana* at Rf 0,58 and three more bands for *B. sagittalis* at Rf 0,33, 0,5 and 0,58 (Figure 9 A).

	Apigenin	Genkwanin	Luteolin	Band 0,83	Band 0,75	Band 0,58	Band 0,50	Band 0,33
B. articulata	X	X	-	X	X	-	-	-
B. gaudichaudiana	X	X	X	-	-	X	-	-
B. sagittalis	X	X	X	-	-	X	X	X

Table 3. Summary of the bands obtained by TLC for *B. articulata, B. gaudichaudiana* and *B. sagittalis.* Mobile phase: DCM: Hexane: MeOH (4:2:1). x indicates presence of the band, - indicates absence of the band.

2.2.3 HPLC analysis

HPLC analysis was carried out on the same extracts as used for the studies with UV-Visible spectrophotometry and for TLC in *B. articulata*, *B. gaudichaudiana* and *B. sagittalis.* The chromatophotographic profiles showed the main peaks with the following retention times for *B. articulata*: 2.32, 3.00, 3.22 and 3.30 min; for *B. gaudichaudiana*: 2.26, 3.00, 3.12 and 3.30 min and for *B. sagittalis*: 2.26, 2.40, 3.00, 3.12 and 3.30 min. The retention time for the apigenin marker was 3.00 min and for genkwanin was 3.30 min. These peaks appear in all three species studied. In *B. gaudichaudiana* and *B. sagittalis* there is also a peak at 3.12 min, and this retention time corresponds to the luteolin marker (Figure 10).

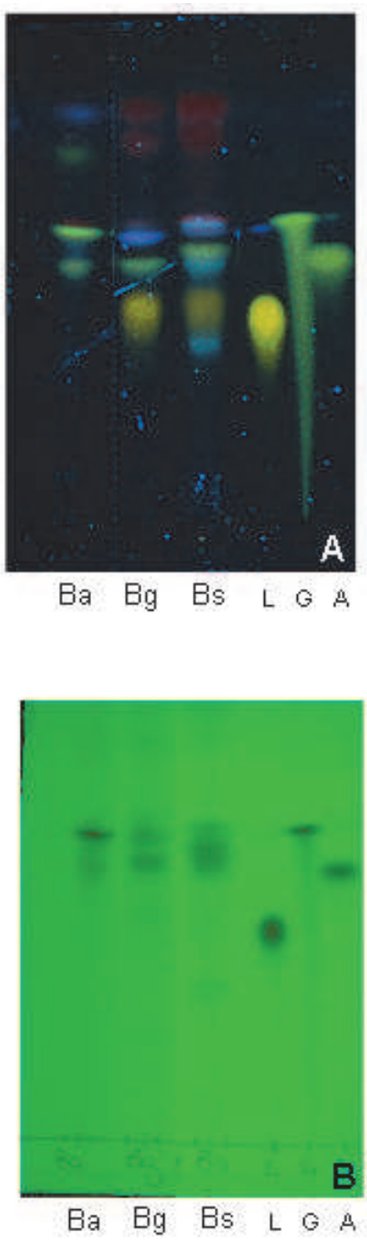


Fig. 9. TLC of *B. articulata (Ba), B. gaudichaudiana (Bg)* and *B. sagittalis (Bs)*. Chromatograms were sprayed with NP-PEG and observed under UV 365 (A) or under UV 254 without chemical treatment (B). A, apigenin; G genkwanin; L: luteolin. Mobile phase: DCM: Hexane:MeOH (4:2:1)

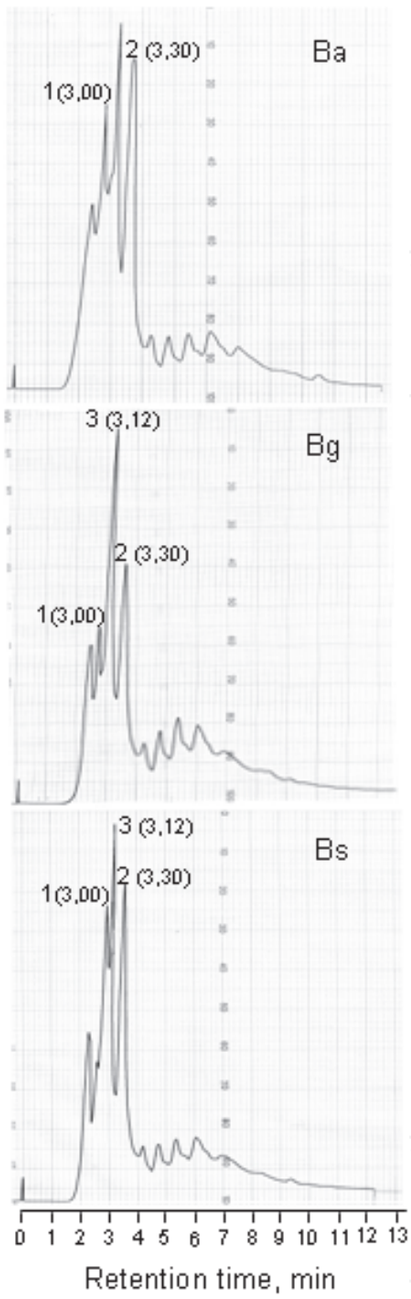


Fig. 10. Original chromatograms obtained by HPLC of *B. articulat*a (Ba), *B. gaudichaudiana* (Bg) and *B. sagittalis* (Bs),. Compounds: 1, apigenin; 2, genkwanin; 3, luteolin.

3. Conclusion

The growing tendency to use high quality, standardized plant extracts, with a guarantee of security and efficacy, is going to continue. Therefore, all efforts should be directed at researching the chemical-pharmalogical profiles of the extracts and combinations and in rationalizing their therapeutic applications.

In the present example, sect. Caulopterae species are very similar between them and only *B. articulata*, *B. crispa* and *B. trimera* are official in pharmacopeia. UV/Visible spectrophotometry coupled to PCA grouped populations of these three species in different areas in a two dimensional graph. Three additional species were grouped in different areas in the same graph too (*B. microcephala*, *B. phyteumoides* and *B. penningtonii*). Populations of species that fall outside the areas of official species are unfit for medicinal purposes. It should be noted that in the case of the official species *B. articulata*, additional techniques, such as TLC should be applied to distinguish between *B. articulata*, *B. gaudichaudiana* and *B. sagittalis*. According to this, the combination of these techniques could to be used for routine quality control of official herbal medicines.

4. Acknowledgment

The present work was supported by a grant from the Agencia Nacional de Promoción Científica y Tecnológica de Argentina, PICT 1494. Dr. M V Rodriguez and Dr. Marcos Derita are research workers of CONICET.

5. References

Abdel-Malek, S., Bastien, J.W., Mahler, W.F., Jia, Q., Reinecke, M.G., Robinson, W.E., Shu, Y.H. & Zalles-Asin, J. (1996). Drug leads from the kallawaya herbalists of Bolivia. 1. Background, rationale, protocol and anti-HIV activity. *Journal of Ethnopharmacology,* Vol 50, No. 4 (March 1996), pp. 157-166, ISSN 0378-8741.

Akaike, S., Sumino, M., Sekine, T., Seo, S., Kimura, N. & Ikegami, F. (2003). A New *ent*-Clerodane Diterpene from the Aerial Parts of *Baccharis gaudichaudiana*. *Chemical & Pharmaceutical Bulletin*, Vol 51, No. 2 (February 2003), pp.197-199, ISSN 0009-2363.

Ariza Espinar, L. (1973). Las especies de *Baccharis* (Compositae) de Argentina Central. *Boletín de la Academia Nacional de Ciencias,* Vol 50 (Septiembre 1973), pp.175-305, ISSN 0325-2051.

Baker, J.G. (1882-1884). Compositae III. Asteroideae, Inuloideae. IV. Heliathoideae, Anthemideae, Senecionideae, Cynaroideae, Ligulatae, Mutisiaceae, In: *Flora brasiliensis: enumeratio plantarum 6 (3),* C.F. Martius, A.W. Eichler, (Eds.), 1-100, F. Fleischer, Leipzig, Munich.

Barroso, G.M. (1976). Compositae, Subtribo Baccharidinae Hoffman. Estudo das espécies ocorrentes no Brasil. *Rodriguésia*, Vol 28, pp.3-273.

Borella, J.C., Fontoura, A., Menezes, Jr.A. & França, S.C. (2001). Influência da adubação mineral (N-P-K) e sazonalidade no rendimento e teor de flavonóides em indivíduos masculinos de *Baccharis trimera* Less. (Asteraceae) – Carqueja. *Revista Brasileira de Plantas Medicinais*,Vol 4, No.1 (Outubro 2001), 101-104, ISSN 1516-0572.

Bohlmann, F., Banerjee, S., Jalupovic, J., Grenz, M., Misra, L.N., Hirschmann, G.S., King, R.M. & Robinson, H. (1985). Clerodane and labdane diterpenoids from *Baccharis* species. *Phytochemistry*, Vol 24, No. 3 (October 1985), pp. 511-515, ISSN 0031-9422.

Bohm, B.A. & Stuessy, T.F. (2001) *Flavonoids of the Sunflower Family (Asteraceae)*. Springer, Wien, New York.

Blumenthal, M. (1998). *The complete german commission E monographs. Herbal guide to herbal medicines*, American Botanical council, Austin.

Coelho, M.G.P., Reis, P.A., Gava, V.B., Marques, P.R., Gayer, C.R., Laranja, G.A.T., Felzenswalb, I. & Sabino, K.C.C. (2004). Antiarthritic effect and subacute toxicological evaluation of *Baccharis genistelloides* aqueous extract. *Toxicology Letters,* Vol 154, No. 1-2 (December 2004), pp. 69-80, ISSN 0378-4274.

Correa, M.P. (1985). *Dicionário das plantas úteis do Brasil e das exóticas cultivadas*, IBDF, Río de Janeiro, Brasil.

Cuatrecasas, J. (1967). Revisión de las especies colombianas del género *Baccharis. Revista de la Academia Colombiana de Ciencias Exactas*, Vol 13, pp. 5-102.

De Candolle, A.P. (1836). Compositae: *Baccharis*. *Prodromus Systematis Naturalis Regni Vegetabilis*, Vol 5, pp. 398-429.

De Oliveira, S.Q., Dal-Pizzol, F., Gosmann, G., Guillaume, D. Moreira, J.C. & Schenckel, F.P. (2003). Antioxidant activity of *Baccharis articulata* extracts: isolation of a new compound with antioxidant activity. *Free Radical Research*, Vol 37, No. 5 (January 2003), pp.555-559, ISSN 1071-5762.

Desmarchelier, G., Bermúdez, M.J.N., Coussio, J., Ciccia, G. & Boveris, A. (1997). Antioxidant and prooxidant activities in aqueous extracts of argentine plants. *International Journal of Pharmacognosy*, Vol 35, No.2 (January 1997), 116-120, ISSN 1388-0209.

Dresch, A.P., Montanha, J.A., Matzenba, N.E. & Mentz, C.A. (2006). Controle De Qualidade De Espécies Do Gênero *Baccharis* L.(Asteraceae) Por CCD A Partir De Extratos Rápidos. *Infarma*, Vol 18, No. 11-12, pp. 37-40, ISSN 0104-0219.

Emerciano, V.P., Militão, J.S.L., Campos, C.C., Romoff, P., Kaplan, M.A.C., Zambon, M. & Brant, A.J.C. (2001). Flavonoids as chemotaxonomic markers for Asteraceae. Biochem. *Biochemical Systematic and Ecology*, Vol 29, No. 9 (October 2001), pp.947-957.

ESCOP (European Scientific Cooperative on Phytotherapy) (2003). *ESCOP Monographs*. *The scientific foundation for herbal medicinal products*, ESCOP, Exeter; Georg Thieme Verlag, Sttutgart; Thieme NewYork, New York.

Farmacopea Nacional Argentina VI ed. (1978). *Codex Medecamentarius Argentino.* Buenos Aires, Argentina.

Farmacopéia Brasileira IV Edição. (2002). Oficializada Governo Federal. Atheneu Editora S. A., Sao Paulo LTDA.

Fullas, F., Hussain, R.A., Chai, H., Pezzuto, J.M., Soejarto, D.D. & Kinghorn, A.D. (1994). Cytotoxic constituents of *Baccharis gaudichaudiana. Journal of Natural Products*, Vol 57, No. 6 (June 1996), pp.801-807, ISSN 0163-3864.

Gené, R.M., Marín, E. & Adzet, T. (1992). Anti-Inflammatory Effect of Aqueous Extracts of Three Species of the Genus *Baccharis*. *Planta Medica*, Vol 58 (January 1992), pp.565-566, ISSN 0032-0943.

Gené, R.M., Cartañá, C., Adzet, T., Marín, E., Parella, T. & Cañigueral, S. (1996). Anti-Inflammatory and Analgesic Activity of *Baccharis trimera*: Identification of its Active Constituents. *Planta Medica*, Vol 62, pp. 232-235, ISSN 0032-0943.

Gianello, J.C. & Giordano, O.S. (1984). Exámen químico en seis especies del género *Baccharis*. *Revista Latinamericana de Quimica*, Vol 15, pp.84-86, ISSN 0370-5943.

Giuliano, D.A. (2001). Clasificación Infragenérica de las especies argentinas de *Baccharis* (Asteraceae, Astereae). *Darwiniana,* Vol 39, No. 1-2, pp. 138-154, ISSN 0370-5943.

Gonzaga Verdi, L., Costa Brighente, I.M. & Pizzolatti, M.G. (2005). Gênero Baccharis (Asteraceae): Aspectos Químicos, Econômicos e Biológicos. Química Nova, Vol 28, No.1, pp. 85-94, ISSN 0100-4042.

Gupta, M.P. (1995). *270 Plantas medicinales iberoamericanas*. Programa Iberoamericano de Ciencia y Tecnología para el Desarrollo, CYTED. Convenio Andrés Bello Santafé de Bogotá, Colombia.

Greenham, J., Harborne, J.B. & Williams, C.A. (2003). Identification of Lipophilic Flavones and Flavonols by Comparative HPLC, TLC and UV Spectral Analysis. *Phytochemical Analysis*, Vol 14, No. 2 (March/April 2003) pp. 100-118, ISSN 1099-1565.

Harborne, J.B. & Williams, C.A. (2000). Advances in flavonoid research since 1992. *Phytochemistry*, Vol 55, No. 6 (November 2000), pp. 481-504, ISSN 0031-9422.

Heering, W.C. (1904). Die *Baccharis*-Arten des Hamburger Herbars. *Jahrbuch der Hamburgischen Wissenschaftlichen Anstalten*, Vol 21, pp. 1-46.

Hieronymus, J. (1882). Plantae Diaforicae, Florae Argentinae. Tomo VI. *Boletín de la Academia Nacional de Ciencias*, Vol 4, pp. 159-160, ISSN 0325-2051.

IFPMA (International Federation of Pharmaceutical Manufacturers Associations) (1997). Major steps towards global drugs regulations. Brussels.

Keller, K. (1991). Legal requirements for the use of phytopharmaceutical drugs in the Federal Republic of Germany. *Journal of Ethnopharmacology*, Vol 32, pp. 225-229, ISSN 0378-8741.

Lapa, A.J., Fischman, L.A. & Gamberini, M.T. (1992). Inhibitors of gastric secretion from Brazilian folk medicinal plants, In: *Natural Drugs and the Digestive Tract*, F. Capasso, N. Mascolo (Eds.), 63-68, EMSI, Roma.

Lessing, C.F. (1831). Synanthereae: Molins-Alatae. *Linnaea*, Vol 6, pp. 83-170.

Liang, Y., Peishan, X. & Chan, K. (2004) Quality control of herbal medicines. *Journal of Chromatography B*, Vol 812 (September 2004), pp. 53-70, ISSN 1570-0232.

Lonni, A.A.S.G., Scarminio, I.S., Silva, L.M.C. & Ferreira, D.T. (2003). Differentiation of Species of the Baccharis Genus by HPLC and Chemometric Methods. *Analytical Sciences*, Vol 19, pp. 1013-1017, ISSN 0910-6340.

Lonni, A.A.S.G., Scarminio, I.S., Silva, L.M.C. & Ferreira, D.T. (2005). Numerical Taxonomy Characterization of *Baccharis* Genus Species by Ultraviolet-Visible Spectrophotometry. *Analytical Sciences*, Vol 21, pp. 235-239, ISSN 0910-6340.

López, J.A. & Hidalgo, M.D. (1994) Análisis de Componentes Principales y Análisis Factorial, En: *Fundamentos de Estadística con Systat*, M. Ato, J.J. López, (Eds.), 457-503, Addison Wesley Iberoamericana.

Martínez Crovetto, R. (1981). *Plantas utilizadas en medicina en el NO de Corrientes*. Fund. Miguel Lillo, Miscelán, 69, Tucumán.

Müller, J. (2006). *Systematics of Baccharis (Compositae, Astereae) in Bolivia, including an overview of the genus.* (Systematics Botany Monographs v. 76). The American Society of Plant Taxonomists, ISBN 0912861762, Michigan.

Palacios, P., Gutkind, G., Randina, R.V.D., De Torres, R. & Coussio, J.D. (1983). Antimicrobial activity of *B.crispa* and *B. notosergila*. Genus Baccharis II. *Planta Medica*, Vol 49, pp. 128, ISSN 0032-0943.

Petenatti, E.M., Petenatti, M.E., Cifuente, D.A., Gianello, J.C., Giordano, O.S., Tonn, C.E. & Del Vitto, L.A. (2007). Medicamentos Herbarios en el Centro-Oeste Argentino. VI. Caracterización y Control de Calidad de dos Especies de "Carquejas": *Baccharis sagittalis* y *B. triangularis* (Asteraceae). *Latin American Journal of Pharmacy*, Vol 26 No. 2, pp. 201-208, ISSN 0326-2383.

Pla, L.E. (1986) *Análisis Multivariado: Método de Componentes Principales.* Secretaría de la Organización de Estados Americanos (OEA), Washington, D.C.

Rice- Evans, C.A., Miller, N.J. & Paganga, G. (1996). Structure-Antioxidant Activity Relationships of Flavonoids and Fenolic Acids. *Free Radical Biology and Medicine*, Vol 20, pp. 933-956, ISSN 0891-5849.

Rodriguez M.V. (2010a). Caracterización y Normalización de un recurso vegetal autóctono medicinal: especies del género *Baccharis* L. Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Argentina.

Rodriguez M.V., Martínez M. L., Cortadi A. A., Bandoni A., Giuliano D. A., Gattuso S. J. & Gattuso M. A. (2010b). Characterization of three sect. Caulopterae species (*Baccharis*-Asteraceae) inferred from morphoanatomy, polypeptide profiles and spectrophotometry data. *Plant Systematic and Evolution*, Vol 286, No. 3-4 (June 2010), pp. 175-190, ISSN 0378-2697.

Rohlf, F.J. (1998). On applications of geometric morphometrics to studies of ontogeny and phylogeny. *Systematic Biology*, Vol 47, pp. 147-158, ISSN 1063-5157.

Sorarú, S.B. & Bandoni, A.L. (1978). *Plantas de la Medicina Popular Argentina*. Albatros, Buenos Aires.

Stoicke, H. & Leng-Peschlow, E. (1987). Characterization of flavonoids from *Baccharis trimera* and their antihepatotoxic properties. *Planta Medica,* Vol 53, pp. 37-39, ISSN 0032-0943.

Torres, L.M.B., Gamberini, M.T., Roque, N.F., Lima-Landman, M.T., Souccar, C. & Lapa, A.J. (2000). Diterpene from *Baccharis trimera* with a Relaxant Effect on Rat Vascular Smooth Muscle. *Phytochemistry*, Vol 55, No. 6 (November 2000), pp. 617-619, ISSN 0031-9422.

Toursarkissian, M. (1980). *Plantas Medicinales de la Argentina*. Hemisferio Sur S. A., Buenos Aires.

Tyler, V.E. (1999). Phytomedicines: Back to the future. *Journal of Natural Products*, Vol 62, pp. 1589-1592, ISSN 0163-3864.

Wagner, H. & Bladt, S. (1996) *Plant Drug Analysis. A Thin Chromatography Atlas*. Springer-Verlag, Berlin.

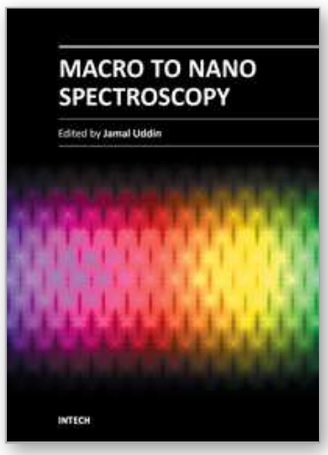
Weddell, H.A. (1855-1856). *Chloris andina*, vol. 1. P. Bertrand, Paris.

WHO (World Health Organization) (1991). Guidelines for the Assessment of herbal medicines. WHO/TRM/91.4, Ginebra.

WHO (World Health Organization) (1999). WHO monographs on selected medicinal plants. Vol 1, Ginebra.

WHO (World Health Organization) (2001). Legal status of traditional medicine and complementary alternative medicine: A world wide review. WHO/EDM/TRM/2001.2, Geneva.

WHO (World Health Organization) (2002). WHO monographs on selected medicinal plants. Vol 2, Ginebra.



Macro To Nano Spectroscopy

Edited by Dr. Jamal Uddin

ISBN 978-953-51-0664-7
Hard cover, 448 pages
Publisher InTech
Published online 29, June, 2012
Published in print edition June, 2012

In the last few decades, Spectroscopy and its application dramatically diverted science in the direction of brand new era. This book reports on recent progress in spectroscopic technologies, theory and applications of advanced spectroscopy. In this book, we (INTECH publisher, editor and authors) have invested a lot of effort to include 20 most advanced spectroscopy chapters. We would like to invite all spectroscopy scientists to read and share the knowledge and contents of this book. The textbook is written by international scientists with expertise in Chemistry, Biochemistry, Physics, Biology and Nanotechnology many of which are active in research. We hope that the textbook will enhance the knowledge of scientists in the complexities of some spectroscopic approaches; it will stimulate both professionals and students to dedicate part of their future research in understanding relevant mechanisms and applications of chemistry, physics and material sciences.

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen