Revista Brasileira de Farmacognosia
Brazilian Journal of Pharmacognosy
23(1): 28-35, Jan./Feb. 2013

Bioactive compounds in *Bidens pilosa* L. populations: a key step in the standardization of phytopharmaceutical preparations

Diego F. Cortés-Rojas,[1] Daniela A. Chagas-Paula,[2] Fernando B. Da Costa,[2] Claudia R. F. Souza,[1] Wanderley P. Oliveira[*,1]

[1]Laboratório de Processos Farmacêuticos, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Brazil,
[2]Laboratório de Farmacognosia, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Brazil.

Article

Received 26 Apr 2012
Accepted 25 Jun 2012
Available online 23 Aug 2012

Keywords:
antioxidant activity
Bidens pilosa
extraction methods
HPLC fingerprint
phenolics
phytopharmaceutical preparation

ISSN 0102-695X
DOI: 10.1590/S0102-695X2012005000100

Abstract: The total flavonoid content (TFC), total polyphenols content (TPC), and *in vitro* antioxidant activity (AA) of six *Bidens pilosa* L., Asteraceae, populations harvested from different localities were evaluated in this work. The plants were separated in roots, stems, and leaves/flowers, and the influence of extraction methods was investigated. A reversed-phase high-performance liquid chromatography method (HPLC) was developed and employed to obtain characteristic HPLC fingerprints of the bioactive compounds present in the extractive solutions, which were correlated with the TFC, TPC, and AA. Extractive solutions of leaves/flowers presented a higher AA when compared with those obtained from other parts of the plant (IC50 of 35.35±0.10 µg/mL). The stem extracts presented the lowest AA (IC50 117.2±1.96 µg/mL). A direct correlation of AA with TFC and TPC was evidenced. The highest AA was obtained by dynamic maceration and was statistically different from the AA presented by the extractive solutions obtained by other extraction methods. The results of this work evidenced differences that can be found at different stages of development of phytopharmaceutical preparations from *B. pilosa* and highlighted the importance of using the concentration of marker compounds as well as HPLC fingerprints as quality control parameters.

Introduction

Plant-derived products have been used as therapeutic alternatives in the health-care systems of several countries. Medicinal plants are important sources of bioactive compounds, as their easy access is one of the main reasons that justifies their widespread application. The World Health Organization (WHO) has published several guidelines that aim at improving the quality, effectiveness, and safety of the herbal medicinal preparations (WHO, 2002).

Considering quality as one of the main factors related to consumer acceptance and sustainability in the market, it is important to strictly control and standardize all the stages involved in the production of phytopharmaceutical preparations, such as the selection of the starting plant material, the extraction conditions, and the quantification of secondary metabolites or bioactive compounds in the final product. Bioactive compounds in the pharmaceutical dosage form would undergo physical and chemical transformations during processing and storage, which might result in loss of the pharmacological activity (Bott et al., 2010). In order to guarantee the quality of phytomedicines, the Brazilian Health Surveillance Agency (Anvisa) established technical and scientific guidelines that should be followed before a product is registered by producer companies (Anvisa, 2010a).

Plants, similar to all life species, are affected by the interaction with the environment where they grow and live. Therefore, secondary metabolites are produced for the adaptation of the plants to particular conditions. The concentration of secondary metabolites can also vary from one part of the plant to another depending on the stimulus that triggers their production (Harborne & Williams, 2000).

Bidens pilosa L., Asteraceae, is a medicinal plant native of South America that nowadays is disseminated all over the world, mainly in tropical and subtropical regions (Oliveira et al., 2004). The pharmacological activities of this plant are generally associated with the presence of flavonoids and polyacetylenes (glycosylated or not), which are abundant in *B. pilosa* (Ubillas et al., 2000; Wang et al., 2010). Important biological activities

reported for this plant include antidiabetic (Chien et al. 2009), antitumoral (Kviecinski et al., 2008), antimicrobial (Rojas et al., 2006; Deba et al., 2008), hepatoprotective (Yuan, et al., 2008; Kviecinski et al., 2011), and antioxidant (Chiang et al., 2004; Krishnaiah et al., 2011). Leaves infusion of *B. pilosa* was recently included in a resolution of medicinal plants promulgated by Anvisa for the treatment of jaundice (Anvisa, 2010b). The polyacetylene phenyl-1,3,5-heptatryine, isolated from the *B. pilosa* leaves, presented promising anticancer and antimalarial activities (Kumari et al., 2009). In the Amazon region, *B. pilosa* roots are used to treat malaria (Brandão et al., 1997; Oliveira et al., 2004).

Phytochemical and pharmacological analyses of *B. pilosa* employing roots (Brandão et al., 1997; Oliveira et al., 2004), leaves (Yuan et al., 2008; Kumari et al., 2009), or the whole aerial parts (Ubillas et al., 2000; Chiang et al., 2004; Kviecinski et al., 2011) have also been published. However, a direct comparison of a specific activity of the different parts of the plant and a comparison of different extraction methods for this plant have not yet been reported.

The aim of this work was, therefore, to investigate the effect of the growing places (rural and urban locations) on the concentration of bioactive substances in the plant material and the effect of the extraction conditions based on the solid content (SC), total flavonoid content (TFC), total polyphenol content (TPC), and antioxidant activity (AA) of the extractive solutions obtained. The results presented herein can guide further development of phytopharmaceutical preparations of *B. pilosa.*

Materials and Methods

Chemicals

Rutin, quercetin, 2,2-diphenyl-1-pycrylhydrazyl (DPPH), gallic acid, and hyperoside were acquired from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA); ferric chloride ($FeCl_3.6H_2O$) and ethanol were acquired from LabSynth (Diadema, SP, Brazil). Pure 4,5-*O*-dicaffeoylquinic acid isolated from *Lychnophora ericoides* Mart. (Asteraceae) was kindly donated by Prof. Dr. L. Gobbo-Neto from FCFRP-USP, Brazil.

Plant material

Individuals of *Bidens pilosa* L., Asteraceae, with approximately 1.5 m height in the flowering phenophase were collected in urban and rural locations by Prof. Dr. W. P. Oliveira (FCFRP/USP). Chart 1 shows the coordinates where the samples of *B. pilosa* were collected. A voucher specimen (collection no. SPFR 12751) of the sample 2 was identified by Prof. Dr. M. Groppo and deposited in the herbarium (SPFR) of the Biology Department of the University of São Paulo, Campus Ribeirão Preto, Ribeirão Preto, SP, Brazil. The other samples were identified by a visual comparison analyzing organ morphology (shape, size) and its distribution. A combination of chromatographic fingerprint (HPLC) and online UV spectrum detection via a diode array detector of extractive solutions prepared from the samples was also performed in order to confirm the identity of the plant samples (Springfield et al., 2005; USP, 2006).

Samples were cleaned and separated in roots, stems, and leaves/flowers. Afterwards, the plant material was dried in an air-forced air circulation stove at 37 °C for 48 h (Nova Ética B.O.D 411D, Vargem Grande Paulista, SP, Brazil) and powdered with a knife mill (Marconi, model MA-680, Piracicaba, SP, Brazil), using a 20-mesh sieve.

Extract preparation

Extractive solutions were prepared at two

Chart 1. Collection places of *Bidens pilosa* populations analyzed.

Sample	Collection place	Coordinates (GPS)*	Collection date	Collection time (h)
1	Monte Alegre do Sul - SP, Experimental Farm APTA, sector 1	S 22º42.599 W 040.40.551	Nov 20 2009	15:00
2	Monte Alegre do Sul - SP, Experimental Farm APTA, sector 2	S 22º41.57.91'5 W 46º40'32.70.0	Nov 20 2009	15:30
3	Ribeirão Preto, Irajá neighborhood. Cav. Torquatro Rizzi street 1645.	S 21º12,576' W 047º48,100'	Nov 13 2009	18:00
4	Ribeirão Preto, close to USP campus. Lucien Lison street close to Fritz Koberle square.	S 21º10,401' W 047º50,735'	Nov 23 2009	16:00
5	Ribeirão Preto, Jardim Canadá neighborhood. Primo Zambianchi street 120.	S 21º13,076' W 047º48,768'	Nov 29 2009	15:30
6	Ribeirão Preto, alto da Boa Vista neighborhood. Av. João Fiúsa corner with São José street.	S 21º11,775' W 047º49,243'	Nov 29 2009	15:00

* Coordinates obtained with a Garmin GPS model eTrex Vista HCx (Olathe, KS, United States of America).

different conditions in order to evaluate the influence of specific variables of the process.

Extraction condition 1

The extraction condition was fixed as follows: dynamic maceration carried out in jacketed glass containers coupled to a thermostatic water bath set at 45 °C, ethanol 70% (v/v) as extraction solvent, and a plant-to-solvent ratio of 1/10 (w/v). The extraction mixture was maintained under agitation for 30 min by using a mechanical stirrer set at 200 rpm. Then, the extraction mixture was filtered in a laboratory filtration system, consisting of a Büchner funnel (number 4 - diameter of 150 mm) coupled to a 1 L Büchner flask, connected to a vacuum pump (Prismatec, Mod 131, São Paulo, Brazil). A filter paper (80 g/m^2, 14 µm pore diameter, and a thickness of 205 µm - J. Prolab, S. José dos Pinhais, PR, Brazil) was used as filter media, and the vacuum filtration pressure was set at 500 mmHg (Bott et al., 2010). The extracts obtained under this condition were employed to evaluate the influence of the plant material and a part of the plant in the SC, TFC, TPC, and AA.

Extraction condition 2

With the aim of comparing the influence of the extraction method in the SC, TFC, TPC, and AA, four extracts from the same plant material (aerial parts) were prepared, using ethanol:water at a concentration of 62.7% (v/v) and a plant-to-solvent ratio of 1/10 (w/v). This extraction condition was based on an optimization study previously developed (Cortés-Rojas et al., 2011). Sample number 2 was used in this comparison due to its relatively higher concentration of bioactive compounds. The following extraction methods were tested:

• dynamic maceration: carried out in jacketed glass containers coupled to a thermostatic water bath set at 66.2 °C for 30 min, with the extraction mixture being maintained under agitation by a stirrer set at 200 rpm;

• solid-liquid extraction using a Soxhlet apparatus (Unividros, Ribeirão Preto, SP, Brazil), during 35 min, corresponding to five extraction cycles;

• microwave-assisted static maceration using a domestic microwave oven (Continental®, São Paulo, Brazil) 2450 MHz, using five cycles of 20 s with intervals of 6 min, at a potency of 50%;

• ultrasound-assisted static maceration: three cycles of 10 min each (Odontobras 2840DA, São Paulo, Brazil) with a frequency of 40 KHz and an agitation of 10 s between the cycles.

Characterization of the extractive solutions

The extractive solutions obtained were characterized through determination of SC, TFC, TPC, and AA as well as by the high-performance liquid chromatography (HPLC) fingerprints according to the methods described next.

Solids content

Oven drying method was employed for SC determination. Approximately 2 g of the extract were weighed in an analytical balance (Mettler Toledo AG204, Greifensee, Switzerland), placed in a Petri dish, and then dried in an air-circulating oven at 105±1 °C. The samples were reweighed every 8 h until constant mass was reached (Bott et al., 2010; Cortés-Rojas et al., 2011). SC was calculated as the percentage ratio of the weight of the dried residue by the original herbal extract weight. Results were expressed as the average of three determinations.

Total flavonoid content

TFC was quantified by a spectrophotometric method based on the absorbance displacement caused by the reaction with an $AlCl_3$ solution 0.5% (w/v) (Cortés-Rojas et al., 2011). Samples were diluted with 40% ethanol, and the absorbance was measured at 425 nm, using a spectrophotometer UV-vis HP 8453 (Agilent Technologies, Waldbronn, Germany) after 30 min of reaction. An analytic curve of quercetin was built, and TFC was expressed as mg of quercetin/g of extract (dried base). All samples were analyzed in triplicate.

Total polyphenol content

TPC in the extractive solutions was determined by the Folin–Denis method (Cortés-Rojas et al., 2011). Diluted extract solutions were mixed with the Folin-Denis reagent, and a saturated solution of $CaCO_3$ was added. Absorbance at 750 nm was measured exactly after 2 min in a HP 8453 spectrophotometer. An analytic curve of gallic acid was built, and the results were expressed as milligrams of gallic acid equivalents (GAE)/g of extract dried base. Triplicate tests were conducted for each sample.

Antioxidant activity

In vitro AA was evaluated through the DPPH free radical scavenging ability according to Georgetti et al. (2006). Aliquots of 30 µL of an appropriate plant extract dilution were added to a mixture of 1 mL of sodium acetate buffer pH 5.5 and 1 mL of absolute ethanol. Afterward, 0.5

mL of a 0.250 mM radical solution of DPPH in absolute ethanol was added, and its absorbance was measured at 517 nm after 20 min. A mixture of 1.5 mL of absolute ethanol and 1.0 mL of sodium acetate buffer was employed as blank. The DPPH radical solution in the reaction medium without the extract was considered the positive control. Results were expressed as IC50 (average of three determinations).

High-performance liquid chromatography coupled to a diode array detector (HPLC-DAD)

The chromatographic profile of different extractive solutions from *B. pilosa* was monitored by HPLC-DAD. Analyses were performed in a Shimadzu LC-20A series and an LC-6A double pump (Shimadzu Corporation, Kyoto, Japan) using a C-18 column (Shimadzu Shim-Pack CLC(M) 4.6 mm x 25 cm, 5µm, 100 Å) at 30 °C. The chromatograms were recorded at 254 nm. Gradient acetonitrile–acidified water with 1% acetic acid at pH 2.8 was used as mobile phase. Acetonitrile concentration was gradually increased as follows: 0-min, 10%; 5-7 min, 20%; 7-31 min, 31%; 32-44 min, 40%; 44-50 min, 100%; and 55-58 min, 10%. Samples were exactly weighted in an analytic balance and diluted at a concentration of 7 mg/mL. The diluted samples were filtered through a 0.45 µm Millipore membrane, and 10 µL were injected in the chromatograph. Previous works have reported the occurrence of rutin, 4,5-O-dicaffeoylquinic acid, and hyperoside in *B. pilosa* extracts (Brandão et al., 1997; Chiang et al., 2004; Wu et al., 2007; Wang et al., 2010; Kviecinski et al., 2011). The presence of these three compounds in the chromatograms was assessed by comparing their retention times, UV spectra, and spiking the samples with a known concentration of analytic standards.

Statistical analysis

Results were expressed as mean±SD. The levels of significance between the samples were compared by one-way analysis of variance (ANOVA) using Bonferroni as a post test for a comparison of means.

Results and Discussion

Flavonoid, polyphenol content, and antioxidant activity

Flavonoids and other phenolic compounds of *Bidens pilosa* L., Asteraceae, have been linked to its biological activities. Figure 1 shows the SC, TFC, TPC, and AA with regard to the collection place and a part of the plant analyzed (extracts obtained under extraction condition 1). Root extracts presented lower SC (Figure 1a) when compared with those from the leaves/flowers and stems. Based on this observation, the results of TFC, TPC, and AA were expressed in dried base for an easier comparison. Significant differences due to plant populations and a part of the plant used can be observed. Leaves/flowers were the part of the plant that presented the highest AA (lowest IC50). Root extracts presented a slightly higher TFC and TPC than the stems, evidencing a correlation between TFC and TPC with AA when comparing each part of the plant. This correlation was previosuly reported by Cortés-Rojas et al. (2011). Flavonoids are reported to protect plants against UV radiation light (Harborne & Williams, 2000), and the highest concentration observed in the leaves could be explained by this statement.

It is observed that samples 2 and 3 presented higher TFC, TPC, and AA when compared with the

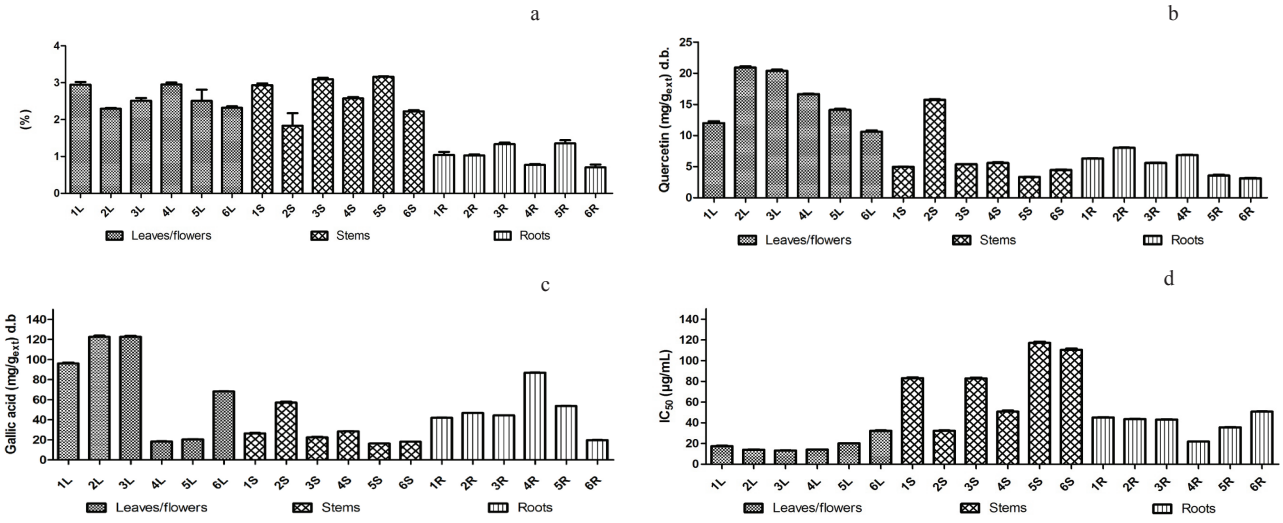

Figure 1. Averaged results (n=3) of solid content (a), total flavonoid content (b), total polyphenol content (c) and antioxidant activity (d) of the extracts of *Bidens pilosa* prepared with different plant materials. (L: leaves/flowers, S: steams, R: roots).

other plant populations. Extractive solutions obtained from populations 5 and 6 showed the lowest AA, also associated to lower TFC and TPC. By comparing the part of plants used, the leaves and flowers showed higher concentrations of TFC and TPF. As mentioned earlier, one of the main functions of the flavonoids is to protect plants from sun radiation and scavenge free radicals. Therefore, the highest concentration of flavonoids in the leaves/flowers could be expected as those are the parts of the plant that are more exposed to sun radiation. The results of the AA of the leaves/flowers showed a higher correlation with TFC than with TPC. This fact is clearly evidenced in samples 4L and 5L, where the TPC was remarkably low, but AA was not significantly affected. These results can suggest a more direct relationship of the AA with the flavonoid compounds than the other types of polyphenols present in the leaves/flowers.

It was observed that even when the coordinates of the collecting places did not vary that much (similar latitudes), remarkable differences were detected. These results might be explained by the highly dependent relationship between the production of secondary metabolites of the plant and the enviromental conditions such as sunlight, rain, insects, and predator atacks (Harborne & Williams, 2000), which differed for the samples studied. Andrade-Neto et al. (2004), in a previous research, demonstrated that the in vitro antimalarial activity of *B. pilosa* root extracts is highly affected by climatic and soil conditions. According to their results, plants collected in dry seasons (May to August in Minas Gerais state, Brazil) showed better antimalarial activity than those collected during the wet season (November to March). In future studies, populations from more extreme latitudes will be included and compared with the results presented herein.

Chromatographic profile of B. pilosa *extracts*

HPLC is a common technique employed for separation and quantification of compounds present in complex samples as plant extracts. HPLC-DAD fingerprint also offers an alternative to help in the correct identification of plant species and has been successfully employed for the authentication of crude drugs or their extracts (Springfield et al. 2005). The HPLC method developed for the *B. pilosa* extractive solutions allowed the separation of the main peaks in the extractive solutions, as presented in Figure 2. Three different profiles of the roots, stems, and leaves/flowers are observed for the plants collected in region 2, which was the sample that presented the highest TFC, TPC, and AA.

Rutin, hyperoside, and 4,5-*O*-dicaffeoylquinic acid were identified in the chromatograms and marked as peaks 1, 2, and 5 (Figure 2b). Peaks from 15 to 24 min correspond to compounds of medium polarity such as flavonoids and other phenolic compounds (Figure 2b). It is observed that peaks 1 and 7 are almost absent in the roots and that peak 3 indicates a lower concentration than in the other parts of the plant. With the exception of peak 8, which is more intense in the stems and roots, all the peaks are more intense in the leaves/flowers, which could be linked with the highest AA presented by the extractive solutions obtained from this part of the plant. The other region of the chromatogram, from 36 to 42 min (Figure 2c), corresponds to lower polarity compounds such as the polyacetylenes, which are very common in this species (Ubillas et al., 2000; Tobinaga et al., 2009; Wang et al., 2010). These compounds have typical absorption bands in their UV spectra (maximums at 256, 270, 305, 326, 348, 378 nm) that are attributed to the carbon-carbon triple bonds. In this region, it is observed that peaks corresponding to these compounds are more intense in the leaves/flowers, less intense in the stems, and almost absent in the roots. The cytotoxic and antimicrobial activity of polyacetylenes has been associated to UV radiation by a mechanism of photo activation in which the conjugated tripe bonds are involved (Wat et al., 1979; Ebermann et al., 1996). Polyacetylenes could be used for the plant as a defense against microorganisms and insects (Minto & Blacklock, 2008), and this fact would explain the more intense peaks corresponding to these compounds in the leaves/flowers. It is interesting to note that in some communities the traditional use of *B. pilosa* for jaundice treatment includes sun exposition after ingestion or a bath with the infusion of the plant.

The chromatographic profiles of the six extracts from the leaves collected at different places (obtained with extraction condition 1) are presented in Figure 3. The intensity of the peaks from one extract to another varies, but the general profiles are quite similar. Low intensity peaks in the flavonoids and polyphenols region (13 to 21 min) are observed for extract 6, which presented the lowest antioxidant activity. This extract also presented the most intense peak with the characteristic UV profile of polyacetylenes. A higher concentration of polyacetylenes in the extract would result in higher antimalarial and antitumor activities as reported by Kviecinski et al. (2010), which demonstrated that extracts rich in polyacetylenes, obtained by supercritical fluids, presented higher antitumor activity than extracts obtained by hydroethanolic maceration.

On the other hand, the chromatographic profiles of extracts 2, 3, and 4, which presented the highest antioxidant activity, also show more intense peaks in the region from 13 to 21 min (flavonoids and other polyphenols). The flavonoids found in *B. pilosa*

have been associated to liver protective activity (Yuan et al., 2008), and it is belived that in a crude extract they would exert a protective and antioxidant effect increasing the stability of polyacetylenes, which are labile compounds associated to antimalarial activity. Extracts with a higher concentration of flavonoids may present more intense hepatoprotective effects based on the study reported by Yuan et al., (2008), therefore proving the protective effects of total flavonoid content of *B. pilosa* on animal liver injury and liver fibrosis.

Comparison of extraction methods

The results of different extraction processes are presented in Table 1. Higher antioxidant activity was observed in the extractive solution obtained by dynamic maceration, probably due to the effect of higher TFC and TPC. Maceration is the most used of all extraction methods (List & Schmidt, 1989), with its versatility to scale up being one of the main advantages. The optimization of factors such as temperature, solvent polarity, pH, particle size, and the type and intensity of the movement helps overcome the disadvantages of this non-exhaustive and occasionally time-consuming method.

The Soxhlet extraction showed similar results. In this method, the recirculation of the solvent caused by temperature improves the extraction by the modification of equilibrium concentration.

Ultrasound-assisted extraction increases the permeability of the cell walls, produces cavitations, and increases the mechanical stressing of the cells, accelerating the diffusion of bioactive compounds to the solvent. These effects depend on the frequency and capacity of the apparatus and the length of time for which it is applied (List & Schmidt, 1989). However, the process should be optimized individually for each drug. The microwave-assisted extraction causes similar effects to that of an ultrasound; however, higher energy is provided, causing faster temperature increases. A statistical analysis showed that there is a significant difference (p<0.05) between the AA of dynamic maceration and the other extraction methods, but there is no difference between the ultrasound- and microwave-assisted extractions.

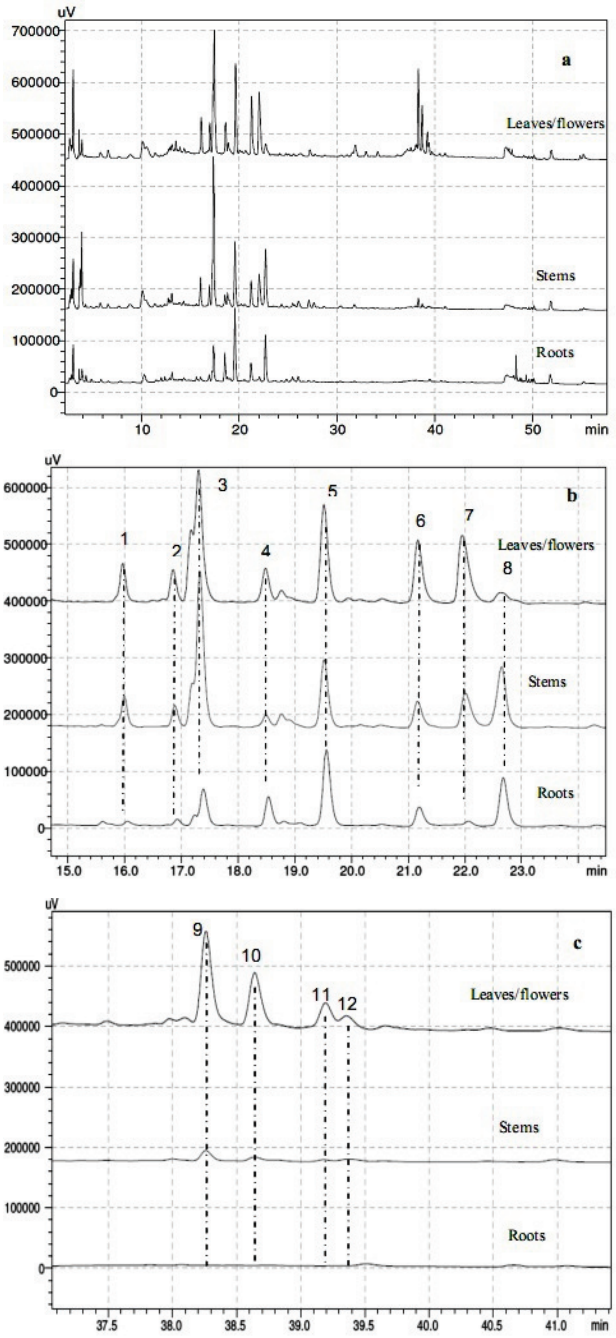

Figure 2. Characteristic chromatographic profiles of roots, stems and leaves/flowers of *Bidens pilosa* extracts obtained by dynamic maceration at optimized conditions (condition 2) (a). Expansion from 15 to 24 min (b) and from 36 to 42 min. (c). Standards: rutin (peak 1), hyperoside (peak 2) and 4,5-O-dicaffeoylquinic acid (peak 3).

Conclusions

The results presented herein showed strong evidences of correlation between the TFC and TPC with the AA. The leaves/flowers were the part of the plant presenting the highest AA, particularly for populations number 2 (rural sample) and 3 (urban sample), which presented an IC50 of 14.040±0.460 µg/mL and 13.350±0.997, respectively. HPLC profiles of the extractive solutions constitute a fingerprint of the plant and could be employed to confirm the authenticity as well as for quality control of the *B. pilosa* samples. Significant differences were observed in the chromatographic profiles of the extractive solutions obtained from the different parts of the plant, which could be associated with the antioxidant activity. With

Table 2. Comparison of different extraction methods for sample 2 (n=3).

Extraction method	Solid content (%)	Flavonoids (mg/g)*	Polyphenols (mg/g)*	DPPH (IC50 µg/mL)*
Dynamic maceration	1.75±0.7	21.988±0.127	69.637±0.237	17.805±0.704
Soxhlet	1.67±0.5	19.623±0.100	64.218±0.391	27.012±1.192
Ultrasound	1.74±0.2	16.482±0.180	57.098±1.394	36.408±2.352
Microwave	2.17±0.2	11.590±0.042	54.064±0.618	36.072±0.429

*dry base.

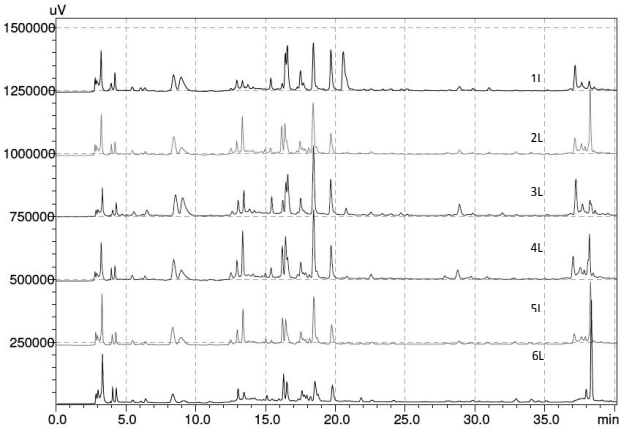

Figure 3. Characteristic chromatographic profiles of extracts from leaves (L) of *Bidens pilosa* collected in the six different locations.

regard to the extraction method, better results were obtained by dynamic maceration (IC50 17.805±0.704 µg/mL).

Acknowlegments

The authors acknowledge Dr. J. A. Azevedo Filho (Agronomic Institute of Campinas, Monte Alegre do Sul, SP, Brazil) for supplying the plant material; Prof. Dr. M. Groppo for the identification of the plant material; Prof. Dr. L. Gobbo-Neto for the donation of the 4,5-*O*-dicaffeoylquinic acid; FAPESP for the financial support and for the fellowship to the first author; and CNPq for the financial support.

References

Andrade-Neto VF, Brandão MGL, Oliveira FQ, Casali VWD, Njaine B, Zalis MG, Oliveira LA, Krettli AU 2004. Antimalarial activity of *Bidens pilosa* L. (Asteraceae) ethanol extracts from wild plants collected in various localities or plants cultivated in humus soil. *Phytother Res18*: 634-639.

Anvisa 2010a. Resolution RDC No. 14, 31st March 2010. Agência Nacional de Vigilância Sanitária.

Anvisa 2010b. Resolution RDC No 10, 9th March 2010, Agência Nacional de Vigilância Sanitária.

Bott RF, Labuza TP, Oliveira WP 2010. Stability testing of spray- and spouted bed-dried extracts of *Passiflora alata*. *Drying Technol 28*: 1255-1265.

Brandão MG, Krettli A, Soares LS, Nery CG, Marinuzzy H 1997. Antimalarial activity of extracts and fractions from *Bidens pilosa* correlated with the presence of acetylene and flavonoid compounds. *J Ethnopharmacol 57*: 131-138.

Chiang YM, Chuang D-Y, Wang, S-Y, Kuo Y-H, Tsai P-W, Shyur L-F 2004. Metabolite profiling and chemopreventive bioactivity of plant extracts from *Bidens pilosa*. *J Ethnopharmacol 95*: 409-419.

Chien S, Young P, Hsu Y, Chen C, Tien YJ, Shiu S, Li T, Yang C, Marimuthu P. Tsai LF, Yang W 2009. Anti-diabetic properties of three common *Bidens pilosa* variants in Taiwan. *Phytochemistry 70*: 1246-1254.

Cortés-Rojas DF, Souza CRF, Oliveira WP 2011. Optimisation of the extraction of phenolic compounds and antioxidant activity from aerial parts of *Bidens pilosa* L. using response surface methodology. *Int J Food Sci Tech 46*: 2420-2427.

Deba F, Xuan TD, Yasuda M, Tawata S 2008. Chemical composition and antioxidant, antibacterial and antifungal activities of the essential oils from *Bidens pilosa* Linn. var. Radiata. *Food Control 19*: 346-352.

Ebermann R, Alth G, Kreitner M, Kubin A 1996. Natural products derived from plants as potential drugs for the photodynamic destruction of tumor cells. *J Photoch Photobio B 36*: 95-97.

Georgetti SR, Casagrande R, Vicentini FTMD, Verri WA, Fonseca MJV 2006. Evaluation of the antioxidant activity of soybean extract by different *in vitro* methods and investigation of this activity after its incorporation in topical formulations. *Eur J Pharm Biopharm 64*: 99-106.

Harborne JB, Williams CA 2000. Advances in flavonoid research since 1992. *Phytochemistry 55*: 481-504.

Krishnaiah D, Sarbatly R, Nithyanandam R 2011. A review of the antioxidant potential of medicinal plant species. *Food Bioprod Process 89*: 217-233

Kumari P, Misra K, Sisodia BS, Faridi U, Srivastava S, Luqman S, Darokar MP, Negi AS, Gupta MM, Singh SC, Kumar JKA 2009. A promising anticancer and antimalarial component from the leaves of *Bidens pilosa*. *Planta Med 75*: 59-61.

Kviecinski MR, Felipe KB, Schoenfelder T, Wiese LPL,

Rossi MH, Gonçalez E, Felicio JD, Filho DW, Pedrosa RC 2008. Study of the antitumor potential of *Bidens pilosa* (Asteraceae) used in Brazilian folk medicine. *J Ethnopharmacol 117*: 69-75.

Kviecinski MR, Benelli P, Felipe KB, Correia JFG, Pich CT, Ferreira SRS, Pedrosa RC 2010. SFE from *Bidens pilosa* Linné to obtain extracts rich in cytotoxic polyacetylenes with antitumor activity. *J Supercrit Fluid 56*: 243-248.

Kviecinski MR, Felipe KB, Correia JFG, Ferreira EA, Rossi MH, Gatti MFD, Filho DW, Pedrosa RC 2011. Brazilian *Bidens pilosa* Linné yields fraction containing quercetin-derived flavonoid with free radical scavenger activity and hepatoprotective effects. *Libyan J Med 6*: 5651.

List PH, Schmidt PC 1989. *Phytopharmaceutical Technology*. London: Heyden & Son Limited.

Minto RE, Blacklock BJ 2008. Biosynthesis and function of polyacetylenes and allied natural products. *Prog Lipid Res 47*: 233-306.

Oliveira FQ, Andrade-Neto V, Krettli, AU, Brandão MGL 2004. New evidences of antimalarial activity of *Bidens pilosa* roots extract correlated with polyacetylene and flavonoids. *J Ethnopharmacol 93*: 39-42.

Rojas JJ, Ochoa VJ, Ocampo SA, Muñoz JF 2006. Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: a possible alternative in the treatment of non-nosocomial infections. *BMC Complement Altern Med 6*: 1-6.

Springfield EP, Eagles PKF, Scott G. Quality assessment of South African herbal medicines by means of HPLC fingerprinting. *J Ethnopharmacology 101*: 75-83.

Tobinaga S, Sharma MK, Aalbersberg WGL, Watanabe K, Iguchi K, Narui K, Sasatsu M, Waki S 2009. Isolation and identification of a potent antimalarial and antibacterial polyacetylene from *Bidens pilosa*. *Planta Med 75*: 624-628.

Ubillas RP, Mendez CD, Jolad SD, Luo J, King SR, Carlson TJ, Fort DM 2000. Antihyperglycemic acetylenic glucosides from *Bidens pilosa*. *Planta Med 66*: 82-83.

USP 2006, United States Pharmacopoeia 29, NF 24, Identification of articles of botanical origin, p. 2603.

Wang R, Wu Q, Shi Y 2010. Polyacetylenes and flavonoids from the aerial parts of *Bidens pilosa*. *Planta Med 76*: 893-896.

Wat C, Biswas RK, Graham EA, Bohm L, Towers GHN 1979. Ultraviolet-mediated cytotoxic actiyity of phenylheptatriyke from *Bidens pilosa* L. *J Nat Prod 42*: 103-111.

WHO 2002 Traditional Medicine strategy 2002-2005. World Health Organization, http://whqlibdoc.who.int/hq/2002/who_edm_trm_2002.1.pdf. Accessed 5 Jul 2011.

Wu L, Chiang Y, Chuang H, Lo C, Yang K, Wang S, Shyur L 2007. A novel polyacetylene significantly inhibits angiogenesis and promotes apoptosis in human endothelial cells through activation of the CDK inhibitors and caspase-7. *Planta Med 73*: 655-61.

Yuan L, Chen F, Ling L, Dou P, Bo H, Zhong M, Xia L 2008. Protective effects of total flavonoids of *Bidens pilosa* L. (TFB) on animal liver injury and liver fibrosis. *J Ethnopharmacol 116*: 539-546.

***Correspondence**

Wanderley P. Oliveira
Laboratório de Processos Farmacêuticos, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo
Av. do Café s/n, Bloco Q, 14040-903
Ribeirão Preto-SP, Brazil
wpoliv@fcfrp.usp.br
Tel: +55 16 36024437