IJPSR (2014), Vol. 5, Issue 12 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 18 May, 2014; received in revised form, 19 July, 2014; accepted, 18 August, 2014; published 01 December, 2014

CHARACTERIZATION AND STANDARDIZATION OF THE HERBAL DRUG *BACCHARIS TRIMERA* (LESS.) DC AND ITS LYOPHILIZED EXTRACT

P. A. Ferreira [1], F. L. A. Santos [1], L. D. S. Alves [1], L. R. M. Ferraz [1], T. A. Rosa [1], R. M. F. Silva [1], G. M. A. Presmich [1], Rolim, L. A. [2], M. S. Silva [3], M. B. S. Maia [4] and P. J. Rolim-Neto [*1]

Department of Pharmaceutical Sciences [1], Department of Physiology and Pharmacology [4], Federal University of Pernambuco, Brazil.
Pharmaceutical Sciences Collegiate [2], Federal University of Vale do Sao Francisco, Brazil.
Department of Pharmaceutical Sciences [3], Federal University of Paraiba, Brazil.

Keywords:

Asteraceae, *Baccharis trimera*, caffeic acids, physicochemical characterization

Correspondence to Author:

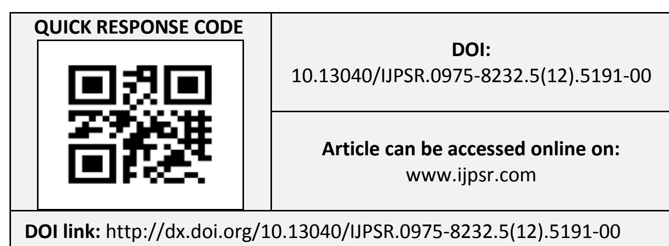
P. J. Rolim-Neto

Department of Pharmaceutical Sciences, Federal University of Pernambuco. Professor Arthur de Sa Street, s/n, Cidade Universitaria, 50740-521 Recife - PE, Brazil.

E-mail: pedro.rolim@pq.cnpq.br

ABSTRACT: *Baccharis trimera* is used in traditional medicine in South American countries for treatment of diseases, such as digestive disturbances, rheumatism, diabetes and inflammatory processes. Given its pharmacological potential, the aim was to characterize the vegetal drug *B. trimera* and attain and standardize its lyophilized extract to use as raw vegetal material in the development of phytotherapeutic medicine. Chemical identification and prospecting, granulometric determination, loss by desiccation, total ash and caffeic acid through High-Performance Liquid Chromatography were carried out. The lyophilized extract obtained was submitted to assays for determining caffeic acids, rheology, hygroscopicity, solubility, superficial area, porosity, particle size through laser granulometry and the thermogravimetric curve. The assays demonstrated that the sample is within the described specifications in literature and, therefore, is adequate to obtain the liquid extract and later dry and standardized in relation to the content of caffeic acids. The lyophilized extract was fine powder, demonstrating the necessity for addition of excipients that make possible the use of the input for the attainment of solid pharmaceutical forms. It was possible to infer characteristics of the vegetal drug and the dry extract obtained through the lyophilization process that will have to be considered in later studies of formularization.

INTRODUCTION: Medicinal plants are used as home remedies, over-the-counter medicines and as raw materials for the pharmaceutical industry. They possess the advantage of being well accepted by patients due to their traditional use and for being acquired at a lesser cost in relation to allopathic medicines, which make them more accessible to the needy population.



QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.5(12).5191-00

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(12).5191-00

Moreover, it is a lucrative sector that moves billions of dollars, representing a substantial proportion of the global medicine market [1].

In this overview, diverse organizations have stimulated the development of phytotherapeutics, through the achievements of research that comprise all aspects of technological development from botanical identification up to the assurance of the efficacy, safety and quality of the final pharmaceutical form. Thus, the development of a standardized phytotherapeutical adds a technological value in the final pharmaceutical forms that contain medicinal plants, such as *Baccharis trimera* [2, 3].

Baccharis trimera (Less) DC (Asteraceae) is a medicinal plant found mainly in Brazil, Argentina, Uruguay and Paraguay. It is used in traditional medicine in the treatment of digestive disturbances, liver and renal illnesses, rheumatism, diabetes and inflammatory processes [4, 5]. In literature, its hepatoprotective potential [6], antiulcerogenic [7], analgesic, anti-inflammatory [8], immunomodulator [9], antioxidant [10], hypoglycemic [11] and smooth musculature relaxant is described [12]. Additionally, the antiarthritic effect of the *B. trimera* aqueous extract prepared by infusion was demonstrated *in vivo* collagen-induced arthritis model. The results demonstrated a drastic reduction in the development of the illness with reduction in the migration, activation and proliferation of lymphocytes at an injury site, attributed to flavonoids present in the extract [13].

The variety of therapeutical actions in *B. trimera* extracts can be justified by the presence of composites of different polarities, amongst them mainly diterpene lactones, sesquiterpenes, flavonoids, saponins, polyphenols and cafeoilquinic acid [14].

Given the above, the present work had as an objective to carry out the characterization of the vegetal drug, as well as the attainment and standardization of lyophilized extract for its later use as vegetal raw material in the development of phytotherapeutic medicine based on *B. trimera.*

EXPERIMENTAL:

Vegetal Material

The aerial parts of *B. trimera* were collected on the morning of January 20th, 2011, in the city of Campinas, São Paulo, Brazil, at the following geographic coordinates: latitude 22° 47' 29.29"S and longitude 47° 6' 31.80"W. The fresh material was sprinkled with 70% ethyl alcohol, and after that dried in a circulating air kiln for five days at the temperature of 40ºC. After drying, the material was triturated in a cutting mill, with a 30 mesh.

The identification of the vegetal material was carried out by the Pluridisciplinary Center for Chemical, Biological and Agricultural Research of the University of Campinas (UNICAMP) and therefore the deposited exsiccate was found in the herbarium of the same institution, located in the city of Paulinea-SP, under number 1286.

Chemical identification of *B. trimera*

The assay for the identification of the chemical marker (3-*O*-methylquercetine) was based on High-Performance Liquid Chromatography (HPLC) method [15]. The sample used was a *B. trimera* aqueous extract obtained by infusion of 3.3% (w/v) of vegetal drug in distilled boiling water for 45 min, with later filtration and storage in an amber glass bottle [13].

Analysis was performed in a Shimadzu® high efficiency chromatograph with a controlling system (Shimadzu SCL - 20VP). The following analytical conditions were used: Shimadzu® reverse column phase C18 (250 x 4.6 mm) 5 μm particle size; mobile phase system A (water: ascetic acid 95.5:4.5, v/v) and B (100% acetonitrile), with a flow of 1.5 $mL.min^{-1}$ and injection volume of 10 μL; λ=325 nm. The elution gradient system used for analyses of the samples was: 0.01-15 min (15-60% B); 15.01-18 min (60-100% B); 18.01-21 min (100% B); 21.01-24 min (100-15% B).

Phytochemical Prospecting

The sample was prepared as described at chemical identification test. The infusion was partitioned without interruption in ethyl acetate and n-butanol. Aliquots of these fractions (about 15μL) underwent phytochemical prospecting via thin layer chromatography (TLC) using silica gel plates for chromatography (Macherey-Nagel®). The reagents used in the characterization of the main secondary metabolite groups and representative standards of these groups are described in **Table 1**.

Additionally, for the identification of saponines the froth test was undertaken which consists of vigorous agitation of the solutions obtained for 1 min, followed by stasis. The presence of abundant foam and its persistent presence after 15 min of stasis was the criterion used to confirm the presence of saponosides [20].

Determination of granulometric distribution of the vegetal drug

The dry vegetal raw material sample (25g) were submitted to vibration in agitator (Bertel®) for 30 min in sieves with mesh openings of 850, 600, 425,

250, 150 and 90 µm [21]. The fractions retained in the sieves and the collector was weighed. The results were expressed by the average of three determinations and the data were analyzed by graphical method, creating a histogram distribution and retention curves and going on to the determination of the average diameter of particles.

TABLE 1. CHROMATOGRAPHIC AND REAGENT SYSTEMS USED IN THE PHYTOCHEMICAL PROSPECTION OF DRY *BACCHARIS TRIMERA*.

Class of Metabolites	Elution System	Developer	References
Alkaloids	AcOEt - HCOOH - AcOH - H_2O^1	Dragendorff	16
Coumarin	Ether - Toluene - AcOH 10%[2]	UV (365nm)	16
Cinnamic Derivatives	AcOEt - HCOOH - AcOH - H_2O^1	NEU	16,17
Flavonoids	AcOEt - HCOOH - AcOH - H_2O^1	NEU	16,17
Hydrolysable tannin	n-BuOH - H_2O - AcOH[3]	NEU	18
Triterpenes/Steroids	Toluene – AcOEt[4]	LB	19

Legend: [1] 100:11:11:27 v/v; [2] 50:50:50 v/v; [3] 40:50:10 v/v; [4] 90:10 v/v; AcOEt= Ethyl acetate, HCOOH = Formic Acid, H_2O= water, UV= ultraviolet, NEU = NEU Reagent (methanolic solution of diphenylboriloxietilamine 1%), n-BuOH = n-butanol, LB = Lieberman and Burchard Reagent.

Determination of the loss by desiccation and the total ash content of the vegetal drug

The analysis was carried out in triplicate using three weighing bottles, previously desiccated for 30 min in the same test conditions. One gram (1 g) of the vegetal drug was added to them. The weighing bottles with the samples were taken to the kiln at a temperature of 105ºC for a period of 2h and later cooled to room temperature in a desiccator and weighed. The procedure was repeated until reaching a constant weight.

The quantification of the non-volatile *B. trimera* residue powder was obtained in triplicate through determination of total ash [21], and the crucibles previously calcinated in a muffle furnace and then cooled and weighed. It were added 3 g of the dry drug powder to them, and the samples carbonized on a Bunsen burner and later incinerated in a muffle furnace at 500°C until constant weight.

Determination of the vegetal drug caffeic acids

The determination of caffeic acids was carried out according to a monograph of the same sort [21], ascertained by the quantification of chlorogenic acid in the sample via HLPC (Shimadzu®). *B. trimera* sample solution was prepared at a concentration of 2.4 $mg.mL^{-1}$. For determination of the caffeic acid concentration, a calibration curve with standard chlorogenic acid (Sigma Aldrich, 99% purity) was constructed from a stock solution of 1.12 $mg.mL^{-1}$ to concentrations of 336, 224, 112, 84, 56, 28 and 11.2$\mu g.mL^{-1}$. The result of the determination was expressed in grams of chlorogenic acid per 100 g of the drug (%), considering the loss by desiccation.

Attainment and control of the fluid extract

The attainment of *B. trimera* extract was carried out in accordance with Coelho *et al.* (2004) [13], aiming at evident antiarthritic activity, associated with anti-inflammatory and analgesic activities also described by other authors [8, 9]. An aqueous infusion of the vegetal drug was made at 3.3% (w/v). As a quality control of this stage of the process the pH and the density of the fluid extract was evaluated.

Attainment of lyophilized extract

The attainment of *B. trimera* dry extract was attained from the fluid extract, previously described, which was frozen in an ultrafreezer at -90° ± 5°C for 24 h, and later lyophilized (Liotop®) under a pressure of 24 µmm of Hg; 220v ± 2 VCA vacuum for 96 h. The product was placed in a hermetically sealed vial and stored in a glass vacuum desiccator.

Thermal characterization of vegetal drug and lyophilized extract

The drug thermogravimetry curves (TG) were obtained by means of a Shimadzu® thermobalance, model DTG 60H, in a nitrogen atmosphere at 50 $mL.min^{-1}$ flow, with sample masses around 6 mg (± 0.5), packed in an alumina crucible, at the heating rate of 10 $^oC.min^{-1}$, up to 700°C. The thermoanalytical data were analyzed by means of

Shimadzu® TA-60WS® (Thermal Analysis) version 2.20 software.

Determination of caffeic acids via HPLC in the lyophilized extract

The determination of the lyophilized extract proceeded with the same adapted chromatographic conditions for the vegetal drug, with modification in the preparation of the sample. This solution, prepared from the lyophilized extract, was obtained in the concentration of 1 $mg.mL^{-1}$ of water.

The HPLC method was validated for the quantitative analysis of 5-o-caffeoylquinic acid (5-CQA) and the dicaffeoylquinic acids (3, 4-diCQA, 3,5-diCQA and 4,5-diCQA) in agreement with ICH guidelines, using the following analytical parameters: accuracy, linearity, precision (repeatability), limit of detection (LD) and limit of quantitation (LQ), robustness. Linearity was evaluated by the calculation of a regression line using the Least Squares Method. Precision was assessed by analyzing the average point of the extractive solution six times in the same day and by analyzing the same extract two times on two different days, performed by two different analysts (inter-day or intermediate precision).

The comparison between the means and the differences between them was evaluated by ANOVA one-way treatment. Accuracy was tested by determining the recovery of 5-CQA, 3, 4-diCQA, 3, 5-diCQA and 4, 5-diCQA at three different concentrations and by the calculation of the relative standard deviation (%RSD) of the recovery. The same was also performed for the extract contaminated with the standard substance of the vegetal material.

The values were considered acceptable when the RSD values were lower than 15%, in the case of raw vegetal material [21]. The selectivity was determined by checking peak purity of all the peaks, using a DAD detector. LD e LQ were calculated dividing the standard deviation of the linear coefficients of the three calibration curves for testing the linearity, by means of obtained slopes of the curves multiplied by three and ten, respectively. Robustness was evaluated by the kind of agitation upon obtainment of the extract solution. Three different types of agitation were applied: manual shaking, magnetic stirring and sonication. To assess whether there are differences between the types of agitation, the comparison between means was performed using ANOVA *One-Way*. All treatments were performed using the confidence interval of 95%.

Determination of rheology of *B. trimera* lyophilized extract

Physical characteristics like bulk density, tapped density, Hausner ratio, compressibility Index, angle of repose, flow time were carried out as per the standard methods [22].

Determinaion of superficial area and porosity of lyophilized extract

The lyophilized extract was weighed (200 mg) and previously treated at 100ºC for 5 h, in a kiln, for optimization of the adsorption process. Later, the sample was degasified for 48 h at 110°C to remove any adsorbed material in the interior of the pores and at the surface of the material. The analyses were carried out thereby obtaining adsorption and desorption isotherms, applying the appropriate models for the adjustment of the experimental points.

The adsorption/desorption isotherm was obtained by gradual physical adsorption of nitrogen at 77 K on the material, and subsequent desorption. The application of the Brunauer-Emmett-Teller (BET) (1938) [23] model on the appropriate portion of the curve supplied the superficial area value (SBET).

For the determination of porosity (size of pore and total volume of pores), the Barret-Joyner-Halenda (BJH) method was used. For the accomplishment of these assays, a Micromeritics® ASAP 2440 Superficial Area and Pore Size Analyzer was used, loaded with its own software to determine the superficial area and porosity [24].

Determination of particle size by laser granulometry of lyophilized extract

The samples were dispersed in isopropyl alcohol in a 1:2 ($mg.mL^{-1}$) ratio. This dispersion was agitated in the ultrasonic bath (Unique®, model USC-1400A), for 3 min before being analyzed. Microtac® S3500 particle size analyzer was used. Each analysis was carried out in triplicate.

Determination of solubility of lyophilized extract

For evaluation of solubility, about 10 mg of lyophilized extract was weighed, transferring it to an erlenmeyer. Slowly, the tested solvents (distilled water, methanol, ethanol, HCl and NaOH sodium sulphate 0.1 M and 1% sodium lauryl sulphate solution) were added until complete visual solubilization.

When there was no solubilization, even after the final 50 mL volume of solvent, the samples were submitted to sonication for 15 min. After persistent non-solubilization, a 25 mL quantity was added until complete solubilization, thereafter submitting the samples to sonication. At the end of adding a total of 100 mL, the assay was finalized [21].

Determination of hygroscopicity of lyophilized extract

Samples of 0.5 g were placed in weighing bottles and kept in a desiccator, in triplicate, which ensures the RH saturation constant at room temperature. Then the samples were subjected to exposure to different conditions of relative humidity: 28, 74 and 95% using silica gel and saline solutions of sodium chloride and zinc sulphate, respectively, for the environment saturation. Such conditions have been confirmed through a J. Prolab® model HS 122 digital thermohygrometer.

Samples were analyzed at 0, 4, 6, 8, 10, 12, 14 days and the percentage of water absorbed (U%) was calculated in regards to the weight in grams of dry weight (dw) and wet weight (ww) by the expression: U%= [(ww-dw)/dw] x100 [25, 26].

RESULTS AND DISCUSSION:

Chemical identification of *B. trimera*

Beginning from the analysis performed, the chromatogram **(Figure 1),** in reference to the aqueous extract, was obtained. From this it is possible to visualize the peaks referring to the quercetin and 3-*O*-methylquercetine, previously analyzed, in retention times (T_R) of 14.7 and 15.1 min, respectively.

The scanning spectra obtained by DAD detector of the markers indicated that 3-*O*-methylquercetine has a characteristic absorption at 355 nm, whereas quercetine has it at 369 nm **(Figure 1).**

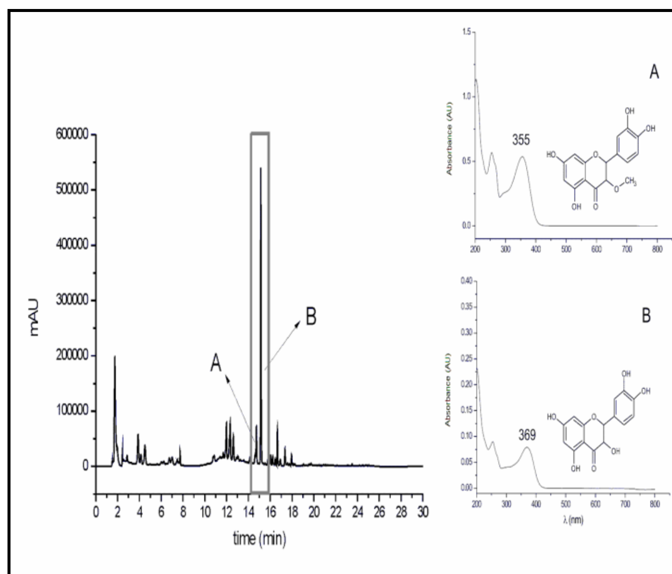


FIGURE 1. CHROMATOGRAM AND ABSORPTION SPECTRA OF THE AQUEOUS EXTRACT OF *B. TRIMERA* INDICATED THE COMPOUNDS 3-O-METHYLQUERCETINE (A) AND QUERCETINE (B).

The results obtained disclosed the presence of flavonoids, saponins, triterpenes, mono, di and sesquiterpenes and cinnamic derivatives. Were negative for coumarins, leucoanthocyanidins, proanthocyanidins, gallic tannins, and alkaloids. Taschetto (2010) [27], using an aqueous infusion, did not observe the presence of alkaloids. A fact justified by the time of the collection. As to the extraction method some authors observed the presence of such a metabolyte only in organic solvents of low polarity like chloroform, n-hexane and n-butanol [28].

The analyses that presented discrepancy, as compared to the literature, in relation to the absence of tannins and coumarins, it can be justified by the extraction method and by the solvent used, since the extraction methodologies used were percolation, turbolization and maceration, in which non-polar organic solvents, like ethanol, n-hexane, n-butanol, and chloroform were used [28, 29]. The test of afrogenicity was positive, wherever there occurred abundance, persistence and height of the compatible foam with the method used [30].

Determination of the granulometric distribution of the vegetal drug

The results obtained of granulometric distribution of the aerial parts of *B. trimera* demonstrate that the

particles of vegetal material were predominantly retained in the 250µm sieve representing 31.9% of the material. The average size of particles determined from the intersection point of the retention and passage curves was 239.5µm ± 9.7 (CV% 4.0). Following pharmacopoeic criteria, the results of the granulometric determination assay classified the powder as semi-fine. This determination is of extreme relevance, since the state of division of the vegetal drug is one of the main factors that interfere with the efficiency of the extraction process.

Determination of loss by desiccation and the total ash content of vegetal drug

The results of the tests were 8.5% of loss by desiccation and 5.5% of content of total ashes with 1.23 and 0.67 of coefficient variation, respectively.

The loss by desiccation is within the established limit by Brazilian Pharmacopeia, 12%, indicating good conservation and an efficient drying of the vegetal raw material. This determination is important for the microbiological quality control, therefore an excess of water in the vegetal drug favors the growth of fungi and bacteria, which can lead to hydrolysis of its constituents [21, 31].

The result of determination of total ashes also has shown to be within the limit established in the monograph about *B. trimera* described in the Brazilian Pharmacopeia which is 8%, indicating that it does not possess excess earth and/or sand.

Determination of caffeic acid of vegetal drug

The vegetal chromatographic profile is represented in **Figure 2.** The caffeic acid content was 0.99 g in 100 g of vegetal drug. On the basis of described relative retention times for the method in the Brazilian Pharmacopeia 5th edition (2010) [21], it was possible to confirm the chromatogram times obtained, that is: chlorogenic acid = 1 (T_R = 20.7 min), 3,4-diCQA = 1.45 (T_R = 31.5 min); 3,5-diCQA = 1.51 (T_R = 32.8 min) and 4,5-diCQA = 1.54 (33.6 T_R = min). Some works cite the pharmacological importance of these caffeic acids, including antioxidant [32], hepatoprotection [33], antibacterial [34], anti-inflammatory [35], and antiviral [36] protections attributed to these quinic acid derivatives, justifying the use of these as *B. trimera* pharmacologic markers.

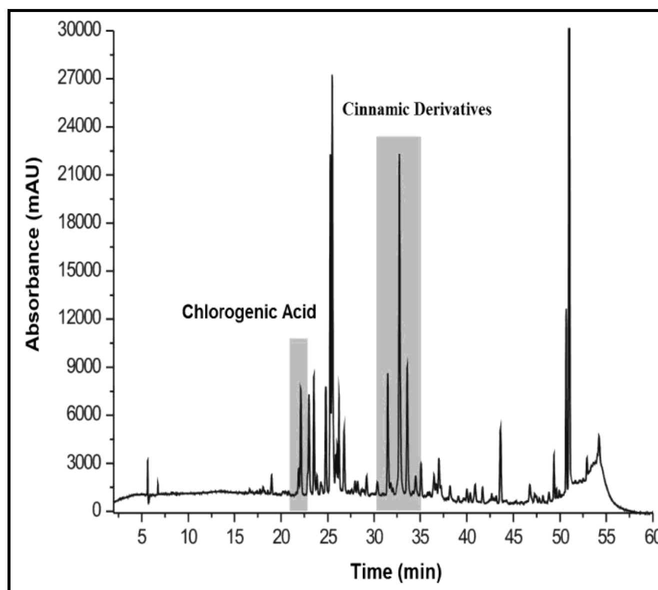


FIGURE 2. CHROMATOGRAPHIC PROFILE FOR CAFFEIC ACIDS FROM *BACCHARIS TRIMERA* SAMPLE.

Attainment of fluid and lyophilized extract and control

The attainment of the fluid extract for infusion made possible the later attainment of the dry extract to be used as raw material for solid pharmaceutical forms. In this sense the control of this stage makes it extremely relevant. The extraction allowed for the attainment of 3 L of fluid extract with a pH of 5.38 and a density of 1.06g.mL^{-1}.

From the process of drying via lyophilization of the fluid extract, it was possible to get a dry extract of 9%. Similar output was found in the work of Coelho *et al.* (2004) [13], who used the same procedure for the attainment of lyophilized extract that was later tested in relation to its antiarthritic activity.

Thermal characterization of the vegetal drug and lyophilized extract

TG curve of *B. trimera* vegetal drug presents the loss of water in the 27 to 100ºC (Δm = 7.6%) range as a first event. Thermal decomposition is initiated around 230ºC with the percentage of loss of total mass at 64% until the final temperature of 800ºC. The thermal analysis of the *B. trimera* lyophilized extract demonstrated a differentiated thermal profile. Initially the loss of water event was not observed until 100ºC, showing the efficiency of the drying process. Thermal decomposition presented

two deteriorating events, the first one between 160 and 181°C and the second in the range of 251 and 312°C, evidenced by slopes in the TG curve. The first deterioration event was earlier if compared to the beginning of the deterioration of the vegetal drug **(Figure 3).**

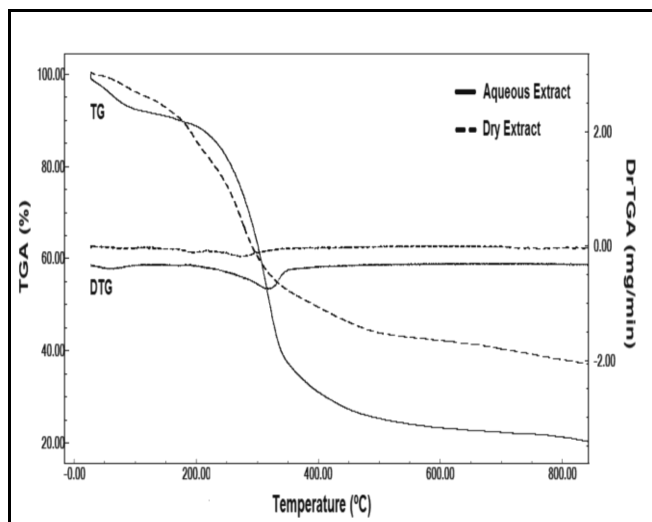


FIGURE 3. TG AND DTG CURVES OF THE VEGETAL DRUG AND *B. TRIMERA* LYOPHILIZED EXTRACT.

This result is of extreme importance for the planning of the productive process of solid pharmaceutical forms from this extract, therefore, the necessity of temperature control during the storage process and production is evident.

Determination of caffeic acids of the lyophilized extract

All compounds showed an adequate linearity for quantification. The following linear equations and the coefficient of determination (R^2) were demonstrated on **Table 2.** The repeatability, based on six samples of each residue was analyzed via HPLC and the relative standard deviation of the contents of each constituent ranged between 3.11 and 3.73%.

The values of overall intra- and inter-day time variations (time precision) of the four major analytes of *B. trimera* lyophilized extracts were expressed on **Table 3**. The results obtained for the parameter robustness for different types of agitation, were statistically analyzed by the one-way ANOVA to assess the differences between the averages. All data were evaluated separately and thus the values of Fcal (with 0.95 as a confidence interval) demonstrated to be lower than the values of Ftab which consists to say that any of type of agitation can be used to obtain the extract solution.

TABLE 2. RESULTS OF LINEARITY, LIMIT OF DETECTION AND QUANTITATION TESTS.

Compound	Linear Equation	R^2	LD ($mg.mL^{-1}$)	LQ ($mg.mL^{-1}$)
5-CQA	f(x) = 223028.88 x – 44767.86	0.996	0.30	1.00
3,4-diCQA	f(x) = 265491.29 x – 5612.43	0.998	0.61	2.05
3,5-diCQA	f(x) = 612543.77 x – 24339.46	0.994	0.65	2.19
4,5-diCQA	f(x) = 243624.90 x – 18684.20	0.992	0.63	2.10

TABLE 3. PRECISION OF THE HPLC QUANTITATION METHOD.

Compound	Intra-day Precision		Inter-day Precision	
	F *cal*	F *tab*	F *cal*	F *tab*
5-CQA	0.1835	18.5128	1.1710	18.5128
3,4-diCQA	0.1835	18.5128	1.1710	18.5128
3,5-diCQA	0.3993	18.5128	5.9895	18.5128
4,5-diCQA	3.9602	18.5128	0.4032	18.5128

The described validated method was used to determine the caffeic acids lyophilized extract. In 100 mg of the sample, it were quantified 1.3033 mg of 5-CQA, 1.55µg of 3, 4-diCQA, 1.37 mg of 3,5-diCQA and 1.29 mg of 4,5-diCQA, with standard deviation below to 2%.

Determination of the rheologic properties of the lyophilized extract:

The dry *B. trimera* extract via lyophilization exhibited an excellent flow if evaluated with the result obtained for the angle of repose **(Table 4),** however the flow time of the powder showed as infinite, a fact that it is related to the well aerated powder and with a large surface area as in this case, resulting in a large variation between the apparent and compacted density of the dry extract. The Hausner and Carr rates obtained **(Table 4)** demonstrate that the *B. trimera* powder needs excipients as dilutants and lubricants that increase its flow properties when employed in the development of pharmaceutical preparations.

TABLE 4. RESULTS OF RHEOLOGICAL PROPERTIES OF *B. TRIMERA* DRY EXTRACT.

Properties	Average
Bulk density	0.24 $g.mL^{-1}$
Tapped density	0.38 $g.mL^{-1}$
Hausner Ratio	1.60
Compressibility Index	37.36
Angle of repose	27.81°
Flow time	Infinite

Determination of the superficial area and porosity of the lyophilized extract

The analyzed sample followed the BET isotherm showing linearity according to the model proposed for the calculation of the superficial area. The range of application of the BET isotherm is valid for the determination of micropores (< 20 Å) and mesopores (between 20 Å and 500 Å) [24], by applying the analyzed sample. The results obtained affirm that the analyzed product possesses mesopores, since the defined superficial area was 61.82 $m^2.g^{-1}$, with a pore volume and size of 0.055 $cm^3.g^{-1}$ e 35.48 Å, respectively. Considering the wetting properties parameter of the particles, in virtue of the size of the pores and the raised surface area, the dry extract obtained possesses adjusted characteristics to be used as vegetal input in the attainment of solid pharmaceutical forms.

Determination of particle size by laser granulometry

The analysis data of the granulometric distribution showed that the sample presented an average particle diameter of 188.6µm classified as a fine powder in accordance with **Figure 4**.

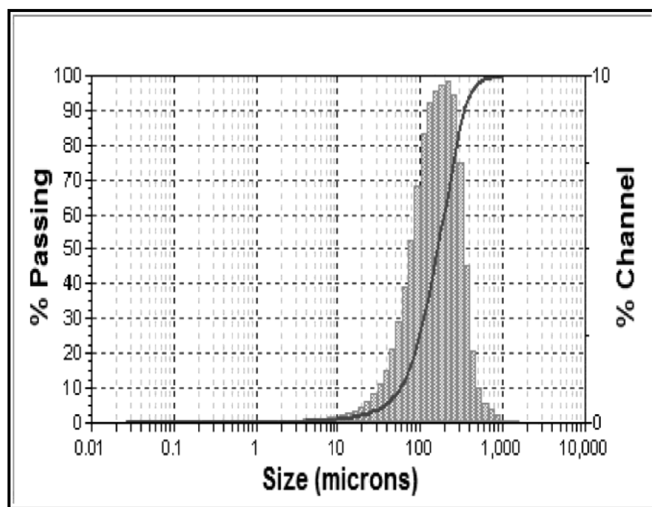


FIGURE 4. DISTRIBUTION OF *B. TRIMERA* DRY EXTRACT PARTICLE SIZE OBTAINED BY LYOPHILIZATION.

Soon, in this case, it is foreseen that the particles will have greater propensity to adsorption of humidity and, therefore, can result in the formation of agglomerated particles [37].

Determination of the solubility of the lyophilized extract

According to the study of the dry extract solubility compared to methanol and ethanol, the vegetal input showed very little solubility and/or practically insolubility, due to the type of solvent used for development of the fluid extract.

The solubility analyses are valid at the temperature of 25ºC, so the term, not very soluble in water, can be justified. The use of a tensioactive, sodium lauryl sulphate, favored the dry extract for an increment in solubility, due to the increase of the wetting properties generated by the break in superficial tension of water. The dry extract of showed better solubility in NaOH 0.1M than in the other solvents tested. The ability to be justified by the predominance of substances of an acid character, for example, the polyphenols, the same thing was not observed in the HCl 0.1M aqueous solution. The results obtained are described in **Table 5**.

TABLE 5. SOLUBILITY OF *B. TRIMERA* DRY EXTRACT IN DIFFERENT SOLVENTS.

Solvent	Volume used	Described term
Water	2.0 mL	Not very soluble
NaOH 0.1M	0.5 mL	Slightly soluble +++
Sodium lauryl sulphate 1%	1.0 mL	Slightly soluble
HCl 0.1M	> 100 mL	Practically insoluble or insoluble
Methanol	75 mL	Very low solubility
Ethanol	75 mL	Very low solubility

+++ Close to soluble [21]

Hygroscopicity

The humidity sorption curves of lyophilized extract of *B. trimera* versus time are shown in **Figure 5**. It was observed, after 14 days, an increase of approximately 1.8 and 3.0% in weight of the samples kept under the conditions of 74 and 95% RH. The results ensures adequate security on the physiochemical stability of the dried extract against humidity, since, even in extreme conditions of RH,

the weight of the samples did not present major changes.

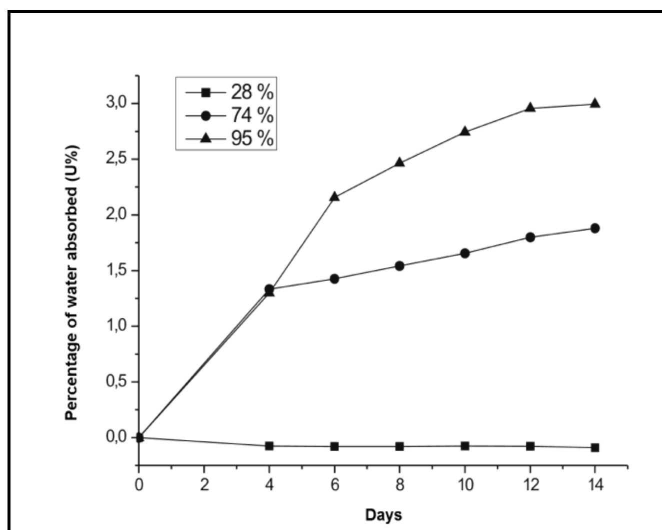


FIGURE 5. THE HUMIDITY SORPTION CURVES OF LYOPHILIZED EXTRACT OF *B. TRIMERA*.

As to the 28% RH condition, for being silica gel, there was a reduction in weight of the samples, which is explained by its ability of water sorption. Approximately 0.09% of water was adsorbed by the silica gel at the end of the experiment. This result ensures the storage of the dried extract in a desiccator containing silica gel, because after 14 days the loss of water by solids had not yet reached a constant level.

In conclusion, it was showed that it is possible to perform technological development in the form of herbal-based pharmaceutical solid based on *B. trimera*, which has shown significant pharmacological properties. Therefore parameters analyzed in this work should be considered in future studies of formulation.

ACKNOWLEDGEMENTS: This work was supported by Science and Technology Support Foundation of the state of Pernambuco (FACEPE), National Counsel of Technological and Scientific Development (CNPq) and Northeast Center for Strategic Technologies (Cetene).

REFERENCES:

1. Booker A, Johnston D, Heinrich M: Value chains of herbal medicines—Research needs and key challenges in the context of ethnopharmacology. Journal of Ethnopharmacology 2012; 140:624-633.
2. Klein T, Longhini R, Bruschi ML, Mello JCP: Fitoterápicos: um mercado promissory. Journal of Basic and applied Pharmaceutical Sciences 2009; 30:241-248.
3. Cordell GA, Colvard MD: Natural Products and Traditional Medicine: Turning on a Paradigm. Journal of Natural Products 2012; 75:514−515.
4. Abad MJ, Bermejo M: *Baccharis* (Compositae): A review update. Arkivoc 2007; 7:76–96.
5. Trojan-Rodrigues M, Alves TLS, Soares GLG, and Ritter MR: Plants used as antidiabetics in popular medicine in Rio Grande do Sul, Southern Brazil. Journal of Ethnopharmacology 2012; 139:155– 163.
6. Soicke H, Leng-Peschlow A: Characterisation of flavonoids from *Baccharis trimera* and their antihepatotoxic properties. Planta Medica 1987; 53:37–39.
7. Biondo TMA, Tanaea MM, Coletta ED, Lima-Landmana MTR, Lapa AJ, Souccar C: Antisecretory actions of *Baccharis trimera* (Less.) DC aqueous extract and isolated compounds: Analysis of underlying mechanisms. Journal of Ethnopharmacology 2011; 136:368–373.
8. Gene RM, Cartarana C, Adzet T, Marin E, Parella T, Canigueral S: Anti-inflamatory and analgesic activity of *Baccharis trimera:* identification of its active constituents. Planta Medica 1996; 62:232-235.
9. Paul EL, Lunardelli A, Caberlon E, Oliveira CB, Santos RCV, Biolchi V, Bastos CMA, Moreira KB, Nunes FB, Gosmann G, Oliveira JR: Anti-inflammatory and Immunomodulatory Effects of *Baccharis trimera* Aqueous Extract on Induced Pleurisy in Rats and lymphoproliferation *in vitro.* Inflammation 2009; 32:419-425.
10. Oliveira CB, Comunello LN, Lunardelli A, Amaral RH, Pires MGS, Silva G, Manfredini V, Vargas CR, Gnoatto SCB, Oliveira JR, Gosmann G: Phenolic enriched extract of *Baccharis trimera* presents anti-inflammatory and antioxidant activities. Molecules 2012; 17:1113-1123.
11. Oliveira ACP, Endringer DC, Amorim LAS, Brandão MGL, Coelho MM: Effect of the extracts and fractions of *Baccharis trimera* and *Syzygium cumini* on glycaemia of diabetic and non-diabetic mice. Journal of Ethnopharmacology 2005; 102:465–469.
12. Torres LMB, Gamberini MT, Roque NF, Landman MTL, Souccar C, Lapa AJ: Diterpene from *Baccharis trimera* with a relaxant effect on rat vascular smooth muscle. Phytochemistry 2000; 55: 617-619.
13. Coelho MPG, Reis PA, Gava VB, Marques PR, Gayer CR, Laranja GAT, Felzenswalb I, Sabino KCC: Antiarthritic effect and subacute toxicological evaluation of *Baccharis genistelloides* aqueous extract, Toxicology Letters 2004; 154:69-80.
14. Oliveira RN, Rehder VLG, Oliveira ASS, Júnior IM, Carvalho JE, Ruiz ALTG, Jeraldo VLS, Linhares AX, Allegretti SM: *Schistosoma mansoni*: In vitro schistosomicidal activity of essential oil of *Baccharis trimera* (less) DC. Experimental Parasitology 2012; 32:135-43.
15. Borgo J, Xavier CAG, Moura DJ, Richter MF, Suyenaga ES: Influência dos processos de secagem sobre o teor de flavonoides e na atividade antioxidante dos extratos de *Baccharis articulata* (Lam.) Pers., Asteraceae. Brazilian Journal of Pharmacognosy 2010; 20:12-17.
16. Wagner H, Bladt S: Plant Drug Analysis: A Thin Layer Chromatography Atlas. Springer, Second Edition 2001.
17. Neu R: A New Reagent for differentiating and determining flavones on paper chromatogram. Naturwissenschaften 1956; 43:82.

18. Xavier HS : *Lavandula stoechas* L. (Lamiaceae): Étude Botanique, Chimique et Pharmacodynamique. PhD thesis 1988, University of Montpellier I: Montpellier, France.
19. Harborne JB: Triterpenoids and steroids, in Phytochemical methods. Chapman and Hall, Rajkamal Electric Press, Third Edition 1998: 129-138.
20. Costa AF: Farmacognosia III: Farmacognosia experimental. Fundação Calouste Gulbenkian, Third Edition 2001.
21. Brazilian Pharmacopoeia Commission: Brazilian Pharmacopeia. Atheneu, Fifth Edition 2010.
22. United States Pharmacopeial Commission: USP 34, 2010.
23. Brunauer S, Emmett PH, Teller E: Adsorption of gases in multimolecular layers. Journal of the American Chemical Society 1938; 60:309-319.
24. Nery CGC, Pires MAS, Pianetti GA, Vianna-Soares CD: Caracterização do fármaco hipoglicemiante glibenclamida. Brazilian Journal of Pharmaceutical Sciences 2008; 44:61-73.
25. Lemos-Senna E, Petrovick PR, Ortega GG, Bassani VL: Preparation and characterization of spray-dried powders from *Achyrocline satureioides* (Lam.) DC extracts. Phytotherapy Research 1997; 11:123-127.
26. Souza KCB, Petrovick PR, Bassani VL, González OG: The adjuvants aerosil 200 and gelita-sol-P influence on the technological characteristics of spray-dried powders from *Passiflora edulis* var. *flavicarpa*. Drug Development and Industrial Pharmacy 2000; 26:331-336.
27. Taschetto APD: Avaliação da atividade antimicrobiana de extratos aquosos e etanólicos de *Baccharis trimera* e *Baccharis articulata* frente ao microorganismo *Helicobacter pylori*. MSc thesis 2010, Univates, Brazil.
28. Mendes FR, Carlini EA: Brazilian plants as possible adaptogens: ethnopharmacological surveys of books edited in Brazil. Journal of Ethnopharmacology 2007; 109:493-500.
29. Mello JCP, Petrovick PR: Quality control of *Baccharis trimera* (Less.) DC. (Ateraceae) hidroalcoholic extracts. Acta Farmaceutica Bonaerense 2000; 19:211-215.
30. Simões-Pires CA, Queiroz EF, Henriques AT, Hostettmann K: Isolation and on-line identification of antioxidant compounds from three Baccharis species by HPLC-UV-MS/MS with post-column derivatisation. Phytochemical Analysis 2005; 16:307-314.
31. Sharapin N: Fundamentos de tecnologia de produtos fitoterápicos. Convenio Andrés Bello, Santafé de Bogotá, 2000.
32. Chuda Y, Ono M, Ohnishi-kameyama M, Nagata T, Tsushida T: Structural identification of two antioxidant quinic acid derivatives from Garland (*Chrysanthemum coronarium* L.). Journal of Agricultural and Food Chemistry 1996; 44:2037–2039.
33. Basnet P, Matsushige K, Hase K, Kadota S, Namba T: Four di-O-caffeoyl quinic acid derivatives from propolis. Potent hepatoprotective activity in experimental liver injury model. Biological & Pharmaceutical Bulletin 1996; 19:1479–1484.
34. Sholz E, Heinrich M, Hunkler D: Caffeoylquinic acids and some biological activities of *Pluchea symphytifolia*. Planta Medica 1994; 60:360–364.
35. Gongora L, Giner RM, Mañez S, Recio MC, Schinella G, Rios JL: Effects of caffeoyl conjugates of isoprenyl hydroquinone glucoside and quinic acid on leukocyte function. Life Sciences 2002; 71:2995–3004.
36. Matsingou TC, Kapsokefalou M, Salifoglou A: In vitro anti-oxidant activity of black tea and Mediterranean herb infusions toward iron under simulated gastrointestinal conditions. Journal of Food Science 2000; 65:1060–1065.
37. Bellamy LJ, Nordon A, Littlejohn D: Effects of particle size and cohesive properties on mixing studied by non-contact NIR, International Journal of Pharmaceutics 2008; 361:87-91.

How to cite this article:
Ferreira P A, Santos FLA, Alves LDS, Ferraz LRM, Rosa TA, Silva RMF, Presmich GMA, Rolim, LA, Silva MS, Maia MBS and Rolim-Neto PJ: Characterization and Standardization of the Herbal Drug *Baccharis Trimera* (Less.) Dc and Its Lyophilized Extract. Int J Pharm Sci Res 2014; 5(12): 5191-00.doi: 10.13040/IJPSR.0975-8232.5 (12).5191-00.

All © 2014 are reserved by International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This Article can be downloaded to **ANDROID OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)