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

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

**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. 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. In literature, its hepatoprotective potential, antiulcerogenic, analgesic, anti-inflammatory, immunomodulator, antioxidant, hypoglycemic and smooth musculature relaxant effect is described. 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.

The variety of therapeutic 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 caffeoilquinic acid.

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 40°C. 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 Paulinia-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. 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.

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: acetic 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.

**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. 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

Table 1. Chromatographic and Reagent Systems Used in the Phytochemical Prospection of Dry Baccharis trimera

<table>
<thead>
<tr>
<th>Class of Metabolites</th>
<th>Elution System</th>
<th>Developer</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>AcOEt - HCOOH - AcOH - H₂O³</td>
<td>Dragendorff</td>
<td>16</td>
</tr>
<tr>
<td>Coumarin</td>
<td>Ether - Toluene - AcOH 10%²</td>
<td>UV (365nm)</td>
<td>16</td>
</tr>
<tr>
<td>Cinnamic Derivatives</td>
<td>AcOEt - HCOOH - AcOH - H₂O¹</td>
<td>NEU</td>
<td>16,17</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>AcOEt - HCOOH - AcOH - H₂O¹</td>
<td>NEU</td>
<td>16,17</td>
</tr>
<tr>
<td>Hydrolysable tannin</td>
<td>n-BuOH - H₂O - AcOH³</td>
<td>NEU</td>
<td>18</td>
</tr>
<tr>
<td>Triterpenes/Steroids</td>
<td>Toluene – AcOEt⁵</td>
<td>LB</td>
<td>19</td>
</tr>
</tbody>
</table>

Legend: ¹ 100:11:12:7 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, H₂O= water, UV= ultraviolet, NEU = NEU Reagent (methanolic solution of diphenylboriloxitetilamine 1%), n-BuOH = n-butanol, LB = Lieberman and Burchard Reagent.

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 antiarthritis activity, associated with anti-inflammatory and analgesic activities also described by other authors.⁸ 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 (Lioplop) under a pressure of 24 mm 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 °C.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-caffefoylquinic acid (5-CQA) and the dicafffeoylquinic 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. 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.

Determination of superficial area and porosity of lyophilized extract

The lyophilized extract was weighed (200 mg) and previously treated at 100°C 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) 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.

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.

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: \( U\% = \left( \frac{ww - dw}{dw} \right) \times 100 \). \textsuperscript{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 quercetin has it at 369 nm (Figure 1).

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) \textsuperscript{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 \textsuperscript{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 \textsuperscript{28,29}. The test of afrogenicity was positive, wherever there occurred abundance, persistence and height of the compatible foam with the method used \textsuperscript{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.

**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).

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
anlytes 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 $F_{cal}$ (with 0.95 as a confidence
interval) demonstrated to be lower than the values
of $F_{tab}$ which consists to say that any of type of
agitation can be used to obtain the extract solution.

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%.

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

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

**TABLE 3. PRECISION OF THE HPLC QUANTITATION METHOD.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Intra-day Precision</th>
<th>Inter-day Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$F_{cal}$</td>
<td>$F_{tab}$</td>
</tr>
<tr>
<td>5-CQA</td>
<td>0.1835</td>
<td>18.5128</td>
</tr>
<tr>
<td>3,4-diCQA</td>
<td>0.1835</td>
<td>18.5128</td>
</tr>
<tr>
<td>3,5-diCQA</td>
<td>0.3993</td>
<td>18.5128</td>
</tr>
<tr>
<td>4,5-diCQA</td>
<td>3.9602</td>
<td>18.5128</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Properties</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk density</td>
<td>0.24 g.mL⁻¹</td>
</tr>
<tr>
<td>Tapped density</td>
<td>0.38 g.mL⁻¹</td>
</tr>
<tr>
<td>Hausner Ratio</td>
<td>1.60</td>
</tr>
<tr>
<td>Compressibility Index</td>
<td>37.36</td>
</tr>
<tr>
<td>Angle of repose</td>
<td>27.81°</td>
</tr>
<tr>
<td>Flow time</td>
<td>Infinite</td>
</tr>
</tbody>
</table>

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) by applying the analyzed sample. The results obtained affirm that the analyzed product possesses mesopores, since the defined superficial area was 61.82 m².g⁻¹, with a pore volume and size of 0.055 cm³.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.

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.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Volume used</th>
<th>Described term</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>2.0 mL</td>
<td>Not very soluble</td>
</tr>
<tr>
<td>NaOH 0.1M</td>
<td>0.5 mL</td>
<td>Slightly soluble +++</td>
</tr>
<tr>
<td>Sodium lauryl sulphate 1%</td>
<td>1.0 mL</td>
<td>Slightly soluble</td>
</tr>
<tr>
<td>HCl 0.1M</td>
<td>&gt; 100 mL</td>
<td>Practically insoluble or insoluble</td>
</tr>
<tr>
<td>Methanol</td>
<td>75 mL</td>
<td>Very low solubility</td>
</tr>
<tr>
<td>Ethanol</td>
<td>75 mL</td>
<td>Very low solubility</td>
</tr>
</tbody>
</table>

+++ Close to soluble

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.

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