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ORIGINAL ARTICLE

New Phytopharmaceutical Formulations: Development and Characterization of Tablets Containing the Aerial Part of the Plant Pulverized and the Soft Extract from *Bidens pilosa* Standardized on Rutin

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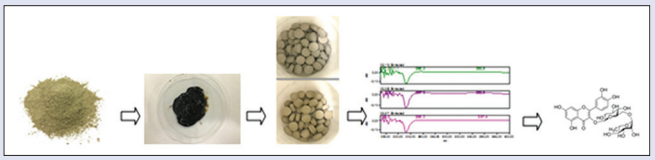
ABSTRACT

Background: *Bidens pilosa,* popularly known as black jack, has high interest as a medicinal plant. A large part of its biological activities are attributed to their constitution in predominance by secondary metabolites such as the flavonoids, like rutin. **Objective:** The aim of the present study was to obtain oral solid dosage forms (tablets) containing the aerial part of the plant pulverized and soft extract of *B. pilosa* standardized on rutin, as well as the validation of an analytical method for determination of the rutin content of the powder, soft extract and of the tablets. **Materials and Methods:** The liquid extract from *B. pilosa* was obtained by percolation and concentrated until it became a soft extract. An analytical method, by high-performance liquid chromatography, was validated for the quantification of rutin in the soft extract. Tablets containing the aerial part of the plant pulverized and the soft extract of *B. pilosa* quantified on rutin was obtained and characterized. After, the method for quantification of rutin on the tablets was covalidated. **Results:** The rutin content in the raw material was 0.71% (m/m), while in the soft extract was 7.29% (m/m). The dissolution efficiency of the tablets was significantly higher, for both formulations in the pH 6.8 medium compared to medium with pH 1.2. Pilot tablets PH and SE presented results above of that recommended after 120 days. **Conclusion:** Pilot tablets SE, with soft extract, presented higher content of rutin than pilot tablets PH, with powder of the aerial part from raw material. This new phytopharmaceuticals formulation proved to be promising to be a strategy to carry out the active principles of *B. pilosa*.

Key words: *Bidens pilosa,* extract standardization, phytopharmaceutical formulation, rutin, tablets

SUMMARY

- Method validation for quantification of rutin on soft extract from *Bidens pilosa*
- New tablets formulation using *Bidens pilosa* soft extract and aerial part of the plant pulverized as active principle
- Characterization of the tablets



Abbreviations used: HPLC: High-performance liquid chromatography; PDA: Photodiode array; k: Capacity factor; RS: Peak resolution; TF: Tailing factor; N: Theoretical plates; LOD: Limit of detection; LOQ: Limit of quantification; RSD: Relative standard deviation.

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INTRODUCTION

Bidens pilosa Linné (*Asteraceae*), popularly known in Brazil as black jack, is an annual herbaceous plant, considered as a weed, originated in South America, which is widely distributed throughout the world mainly in tropical and subtropical regions,[1] requiring minimal agricultural techniques.[2]

This specie has high interest as a medicinal plant.[3] As for the ethnobotanical studies, *B. pilosa* has been used for the treatment of several diseases such as hepatitis, jaundice, fever, sore throat, infections and inflammations.[4-6] In Brazil, *B. pilosa* was included in the List of Medicinal Plants of interest to the Unified Health System (RENISUS), indicated for the treatment of jaundice.[7]

Among its biological effects evidenced in scientific studies are: antioxidant;[8,9] hepatoprotector;[10,11] anti-hyperglycemic;[12] antihypertensive;[13] analgesic; and anti-inflammatory.[14] A large part of these activities are attributed to their constitution in predominance by secondary metabolites such as the flavonoids[15] described as chemotaxonomical markers of the family *Asteraceae*,[16] produced by the plant in response to ultraviolet (UV) radiation. Cortés-rojas *et al.* found that the highest concentration of this class of secondary matabolites was available in the aerial part of the plant.[17] The presence of the flavonoid rutin (quercetin 3-O rutinoside) [Figure 1] in *B. pilosa* was reported by several authors, related to different biological activities.[17-20]

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Oral pharmaceutical dosage forms (capsules and gelatin capsules) using rutin of synthetic origin are available on the market.[21] However, there has been an interest for the use of this compound of natural origin as an alternative to compose formulations, considering that the products derived from medicinal plants are complex matrices with several activities that result in a better activity, in general. The synergism between the compounds and greater bioavailability generated as a consequence may justify their use.[22] To guarantee the quality, safety and efficacy in the development of products of plant origin, standardization is one of the most important steps that include the selection of the starting plant material, the extraction conditions, and the quantification of secondary metabolites or bioactive compounds in the final product.[23]

Because of the its biological effects, several techniques for the extraction and concentration of flavonoids of *B. pilosa* and optimization of these processes are reported in the literature,[24] such as the obtaining of dry extract by spray dryer[25] an expensive technique that requires sophisticated equipment.[26]

The aim of the present study was to obtain oral solid dosage forms (tablets) containing the aerial part of the plant pulverized and soft extract of *B. pilosa* quantified on rutin, in order to denote the relevance of the extraction process to the final product, as well as the validation of an analytical method for determination of the rutin content of the powder, soft extract, and of the tablets.

MATERIALS AND METHODS

Chemicals and standards

The rutin standard (>97%) was purchased from Sigma-Aldrich (St Louis, MO, USA). Ultrapure water was obtained by a Milli-Q system (Millipore, Bedford, MA, USA). High-performance liquid chromatography (HPLC) grade acetonitrile and methanol solvents were purchased from J. T. Baker (Philpsbur, NJ, USA). Formic acid was supplied by Sharlau (Sharlab, Spain).

Herbal material

The aerial part of *B. pilosa* was commercially acquired from the company Santosflora® (Brazil) in the pulverized form (fine powder granulometry) with absence of foreign material, total ash of 9.12% and moisture content of 7.10%.

Obtaining of *Bidens pilosa* soft extract

Initially, 1 kg of the aerial part powder of the plant material of *B. pilosa* was put in contact with ethanol/water extraction solution 50/50 (m/m) for 24 h under stirring (dynamic maceration). Subsequently, the crude extract was submitted to percolation method, using a stainless-steel percolator (10 L capacity). The resulting extract was concentrated in a pneumatic concentrator until solids content more than 70%, being characterized as soft extract.[27] The soft extract obtained was then stored at −20°C.

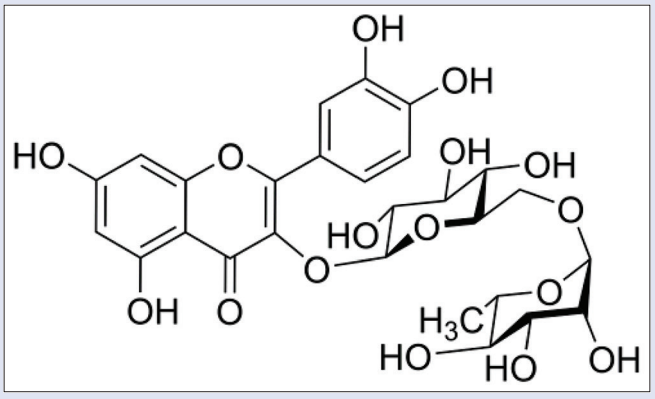

Figure 1: Rutin structure

Determination of the chromatographic profile by thin-layer chromatography

The determination of the chromatographic profile of the samples was performed according to a methodology described by Wagner and Bladt. Ethyl acetate (Neon, Brazil), formic acid (Acros Organics-98%, Belgium), glacial acetic acid (Synth, Brazil) and distilled water (100: 11: 11: 26) were used as mobile phase.[28] An aluminum impregnated with silica gel 60 F_{254} (Merck) chromatographic plate (10 cm × 10 cm) was used as stationary phase. The developer employed was a 1% 2-aminoethyl diphenylborinate (NP) metanolic solution (p/v) (Sigma-Aldrich, St Louis, MO, USA).

The plant material and the soft extract were submitted to reflux method in a water bath at 60°C for 10 min. The sample/solvent ratios were: 1 g of the *B. pilosa* plant material to 10 mL of methanol and 40 mg of the soft extract to 10 mL of methanol. The resulting material was filtered and concentrated to about 25% of the initial volume and applied to the chromatographic plate as well as the standard of rutin (0.5 mg/mL in methanol). The chromatographic plate was then placed in contact with the mobile phase in a sealed chromatographic vessel. After elution and drying of the plate at room temperature, the chromatographic plate was visualized in a UV chamber under 365 nm UV light (Solab SL-204 UV chamber). The value of the retention factor (R_f) of the samples bands was compared with the R_f value of the standard band.

Validation of the analytical method by high-performance liquid chromatography (diode-array detector) for quantification of rutin marker in the soft extract of *Bidens pilosa*

The analytical method used for the quantification of the rutin was adapted using a high performance liquid chromatograph Waters HPLC Alliance® e2695 (Milford, Massachusets, USA) with photodiode array detector (PDA 2998). The treatment of data was performed using *Empower 2.0*®. A gradient elution was performed on a RP18 column (Agilent Zorbax Eclipse Plus model, 4.6 mm × 250 mm and 5 µm) and pre-column Phenomenex RP18 (30 mm x 4 mm); the selected mobile phase is described in Table 1 and the method was maintained under at 30°C; the flow rate was 1.0 mL/min; injection volume of 10 µL; fixed wavelength of 320 nm and run time of 36 min. All samples were previously filtered on a 0.45 µm membrane (Millipore, Merck, Darmstadt, Germany) before being injected.

Table 1: Gradient elution of the method developed by high-performance liquid chromatography

Time (min)	Acetonitrile (%)	Methanol (%)	Formic acid 1% (%)
0	7	7	86
15	7	7	86
20	15	15	70
30	15	15	70
31	7	7	86
36	7	7	86

Validation method

The validation method was performed according to Brazilian legislation (RDC nº 166/2017)[29] and International Conference on Harmonization (ICH) of Technical requirements on Pharmaceuticals for human use.[29] The chromatographic parameters analyzed were: selectivity, linearity, limits of detection and quantification (LOD and LOQ), precision (repeatability and intermediate precision), accuracy, robustness and matrix effect.

System suitability was analyzed prior to the validation. The parameters analyzed were taling factor, resolution (R), capacity factor (k') and number of theoretical plates (N).

Selectivity

The selectivity was analyzed by comparing the chromatograms of the samples, standards, and diluent (methanol) (to verify the presence of possible interfering peaks). The UV spectral similarities of rutin peaks in the standard solution and in the sample solution were also compared at 320 nm.

Linearity and range

The linearity was determined using standards analytical curves at six concentration levels of rutin: 0.008; 0.032; 0.056; 0.08; 0.12; and 0.16 mg/mL diluted in methanol. Each point was analyzed in triplicate. The resulting data was plotted as peak area versus concentrations of the chemical marker (by *Microsoft Excel* 2013) and evaluated by linear regression analyses. The linear range was determined by the correlation coefficient (r). The linear equations were used to quantify rutin in the samples. In addition, the significance of the angular coefficient was evaluated, and the homoscedasticity of the data was investigated. In the statistical tests, a level of significance of 5% was considered.

Limits of detection and quantification

The LOD and LOQ were calculated according to Equations 1 and 2, respectively, considering the standard deviation (SD) of the intercept with the y (σ) axis and the slope of the analytical curves.

$$LOD = \frac{3,3x\sigma}{IC} \quad (1)$$

$$LOQ = \frac{10x\sigma}{IC} \quad (2)$$

Precision

Precision was evaluated by repeatability and intermediate precision, which were demonstrated by relative SD (RSD). For repeatability, six replicates of the 100% *B. pilosa* soft extract sample were prepared within the linear range of the method. Intermediate precision was performed in the same way, by a different analyst on different days.

Accuracy

The accuracy of the method was evaluated by adding the standard solution at a known concentration (100 µg/mL) in the sample solutions of the soft extract at known concentrations (*B. pilosa* soft extract + rutin standard), in triplicate. The linear range of the analytical method was analyzed with three concentrations: low (378 µg/mL), medium (755 µg/mL), and high (1133 µg/mL), in triplicate. The accuracy was reported by the recovery rate, in percentage, of the known concentration standard added in the sample and the concentration of the standard before addition according Equation 3.

$$Recovery = \frac{\left[\left(sample + rutin\ standard\right) - \left(\text{sample without standard}\right)\right]}{(rutin\ standard)} \times 100 \quad (3)$$

Robustness

The robustness of the method was evaluated changing the following conditions: column lot (column 1– Lot: b11225; column 2-Lot: b12003); modification of the mobile phase [Table 2] and temperature during elutions (32°C and 28°C). The content of rutin in the soft extract obtained from the original analytical method and from modified conditions was compared to verify the robustness, then the results were evaluated by the RSD calculation.

Matrix effect

The effect of the matrix components on the analytical response was determined by comparing the angular coefficients of the standard of rutin calibration curves (0.0216, 0.0297, 0.0378, 0.0459, and 0.054 mg/mL) and with the soft extract sample solutions (0.625, 0.521, 0.416, 0.312, and 0.208 mg/mL) fortificated with the standard rutin solution at a concentration of 0.108 mg/mL.

The proof of the absence of matrix effect was demonstrated by the parallelism and confirmed by the *t*-test, comparing the T calculated with the T critical values considering the level of significance of 5%. The *t*-test was performed by the statistical software Past 3.0. (Copyright Hammer & Harper, University of Oslo)

Tablets obtaining

The granule used to produce the pilot formulations were obtained through manual wet granulation method. The herbal material powder and the extract from *B. pilosa* were added to the adjuvant microcrystalline cellulose, lactose monohydrate and the homogenized in a solid mixer homogenizer (V-mixer, Tecnal, TE 200/05, Brazil) for 20 min. Then, the binder solution (PVP-K30 diluted in 96% ethanol) was added, and the mixture was kneaded.

The mixture was dried in a circulating air oven (Marconi, Brazil) at 50°C for 24 h. The granule was then sized into a 2.0 mm mesh sieve, and magnesium stearate and magnesium silicate were added and homogenized. Just the pilot formulation containing soft extract was added with silicon dioxide. The proportions of the constituents of the pilot formulations are described in Table 3.

Table 2: Alterations on the mobile phase to evaluate the robustness

	Time (min)	Acetonitrile (%)	Methanol (%)	Formic acid 1% (%)
Alteration 1	0	9	9	82
	15	9	9	82
	20	15	15	70
	30	15	15	70
	31	9	9	82
	36	9	9	82
Alteration 2	0	5	5	90
	15	5	5	90
	20	15	15	70
	30	15	15	70
	31	5	5	90
	36	5	5	90

Table 3: Proportion of the ingredients used in the production of the granule

Ingredients	Proportion (%)	
	Formulation PH	Formulation SE
Aerial part of the plant pulverized from *B. pilosa*	30	-
Soft extract from *B. pilosa*	-	27.09
Microcrystalline cellulose	40	36.12
Lactose monohydrate	18	16.25
PVP - K30	6	13.54
Magnesium stearate	3	3.15
Magnesium silicate	3	3.15
Silicon dioxide	-	0.7

Not contain (-). Formulation PH: Formulation with powdered herbal; Formulation SE: Formulation with soft extract; *B. pilosa: Bidens pilosa*; PVP: Polyvinylpyrrolidone

Formulation PH: Formulation with powdered herbal; formulation SE: Formulation with soft extract.

The obtained granule were compressed in a compression machine with a pair of biconvex punches (Lemaq, Monopress LM-1, Brazil) of 8.00 mm diameter with yield of 50 tablets/min.

Co-validation of the analytical method for quantification of rutin in the tablets

The method was co-validated evaluating the analytical parameters of linearity, selectivity, precision and system suitability according to the Brazilian legislation[29] and ICH.[30]

Tablets characterization

Determinations of the flow properties of the granule

The flow properties of the obtained granule (formulations PH and SE) were determined by the analysis of the Carr's index (CI%) and Hausner's ratio (HR) calculated according to Equations 4 and 5, respectively. Apparent density (aρ,) and compacted density (cρ) in g/cm^3 were considered in the calculation. Also, angle of repose (α) was evaluated using an Erweka® equipment (model GTB).[31]

$$CI\% = c\rho - \left(\frac{a\rho}{c\rho}\right) \times 100 \quad (4)$$

$$HR = \frac{c\rho}{a\rho} \quad (5)$$

Water activity

The water activity (Wa) of the tablets was measured on an Aqualab Wa measurer (4 Tev, Brazil) at 25°C ± 0.1°C.[32]

Average weight determination

Twenty individual units of the tablets (Wn) were weighed. The mean weight (Mw) was calculated by dividing the sum of the weight of all the units by 20. The variation of the weight of each tablet (Vw) was calculated in relation to the average weight, in % (m/m) (Equation 6).[27]

$$w = \left(\frac{Wn - Mw}{Mw}\right) \times 100 \quad (6)$$

Content uniformity test

The test for content uniformity was determined by using 10 units of tablets of each formulation. The acceptance value (AV) were calculated by the Equation (7), considering the average content (Ca) and the SD.[27]

$$AV = |M\text{-}Ca| + k.\ SD \quad (7)$$

The reference value "M," can be 98.5–101.5, depending on the Ca of the units. "*k*" *is* the acceptability constant, equal to 2.4 for 10 units tested in the first step, so the equation for this work will be $AV = |M\text{-}Ca| + 2.4.\ SD$.[27]

Mechanical resistance

The mechanical resistance of the tablets was evaluated throw the friability and hardness tests.[27]

The friability test was conducted in a friability tester (New Ethics, model 300). For this purpose, 20 units of the tablets were weighed previously and subjected to rotate 100 times. Then any loose dust from the tablets was removed and the tablets were weighed again. Differences in the weights of the units before and after the test were calculated, expressed in % (m/m).[27]

The hardness test was performed in a durometer (New Ethics 298 DGP). The result was expressed in Newtons (N). Ten units were tested.[27]

Disintegration test

Six units of tablets of each formulation were tested. The dosage units were placed in each of the six tubes of the basket and discs were added. The distilled water maintained at 37°C ± 1°C was used as the immersion fluid. At the end of the time all of the dosage units have disintegrated.[27]

Dissolution profile

The dissolution profile of the tablets was carried out using a Hanson Vision Elite 8 equipment, with USP apparatus 2 (paddle), at 100 rpm and 900 mL of the medium.[27]

The medium of dissolution to simulate physiological conditions were: buffer pH 1.2 (acid medium, simulating the stomach) and pH 6.8 (neutral medium, simulating the first portion of the duodenum), previously degassed in a single ultrasonic bath for 20 min and kept at 37°C ± 1°C in the dissolution vessel. All buffers were prepared according to the second supplement of USP 35-NF 30.[33]

Aliquots of 3 mL were collected at 15, 30, 45, 60, 90, and 120 min and were filtrated through 0.45 µm membrane (Millipore, Merck, Darmstadt, Germany) before to injection. The tests were performed in triplicate. Subsequently, the determination of rutin content in the samples was performed by HPLC. A dissolution profile curve was made (percent dissolved on each point vs. the collection time. The tests were carried out in order to ensure Sink conditions[34] to obtain more accurate results and to guarantee that the dissolution is not limited to the solubility characteristics.[35] This condition was verified dissolving the tablets (pilot tablets 1 and 2) in to 20% of the medium capacity (900 mL), corresponding to 180 mL, under stirring on a shaker table for 60 min. Subsequently, the relation between the maximum concentration of the marker obtained using 900 mL of medium and the concentration in 180 mL of medium was calculated.

Also, the dissolution efficiency (DE%) was calculated for each dissolution profile. This value represents the relation between the area under the curve of the dissolution profile expressed as a percentage of the area of rectangle ATR described at 100% dissolution and abscissa in 120 min (Equation 8).[36]

$$DE\% = \left(\frac{AUC}{ATR}\right) \times 100 \quad (8)$$

The F variance test and ANOVA (both with 95% confidence interval, using Statistica 7) were used to compere the obtained results for DE% and the dissolved values for rutin on each dissolution media.

Accelerated stability study for tablets

The tablets (21 units) were tested. The samples were stored in a climatic chamber (Solab, SL-206) at 40°C ± 0.5°C, with 75% ± 0.5% relative humidity. The rutin content was quantified by HPLC after 0, 1, 2, 3 and 6 months. Curves of the content versus storage time under forced conditions of degradation were plotted.[37]

RESULTS AND DISCUSSION

The soft extract of *B. pilosa* obtained presented, after concentration, a solid content of 88.64% (m/m). The rutin content in the raw material was 0.71% (m/m), while in the soft extract was 7.29% (m/m), which shows that the extractive process was efficient. According to Azmin *et al.*, the extractive process can directly affect the quality of the final product.[38] The extraction process by percolation guarantees a more exhaustive extraction of compounds, this method was also chosen by Echeverry *et al.* to optimize the flavonoid extraction process from *Passiflora quadrangularis* leaves.[39]

Determination of the chromatographic profile by thin-layer chromatography

The thin layer chromatographic analysis of the samples, plant material and soft extract from *B. pilosa* at 365 nm showed the presence of characteristic yellow bands with the same R_f value (0.55) corresponding to the rutin substance [Figure 2] this result corroborates with the result verified by Bu *et al.* that also identified rutin in a sample of *B. pilosa* by thin-layer chromatography.[40]

Validation method

System suitability data [Table 4] are in agreement with FDA (1994), which ensures that the chromatographic method developed is suitable to separate and to quantify the rutin marker.[41]

Table 4: Results of the system suitability

Parameters	Results	FDA (1994)
Capacity factor (k')	9731	≥2
Rs	4453	≥2
TF	1328	≤2
Theoretical plates (N)	85,911	≥2000

FDA: Food and drug administration; TF: Tailing factor; Rs: Resolution

Figure 2: Chromatographic profile of rutin standard (R), plant material (PM) and soft extract (E) from *B. pilosa*

Selectivity

The Figure 3a-d provides the chromatograms and the (UV) spectra of the rutin in the analysis by HPLC-PDA of the soft extract from *B. pilosa* and the standard, as well as the chromatogram of the diluent (methanol). The chromatographic profiles of the standard and the soft extract and the similarity of the UV spectrum between the extract and the standard show the selectivity of the method. In addition, no interfering substances were observed in the retention time corresponding to that of the rutin. Due to the fact that this is an analysis of a complex matrix, selectivity is an important parameter that shows if the marker is indeed being detected unequivocally.

Linearity and range

The standard linear equation for rutin was: y = 11.129.961, 5063x − 11.055, 0189 (n = 6, r^2 = 0,9997, r = 0,9998). The

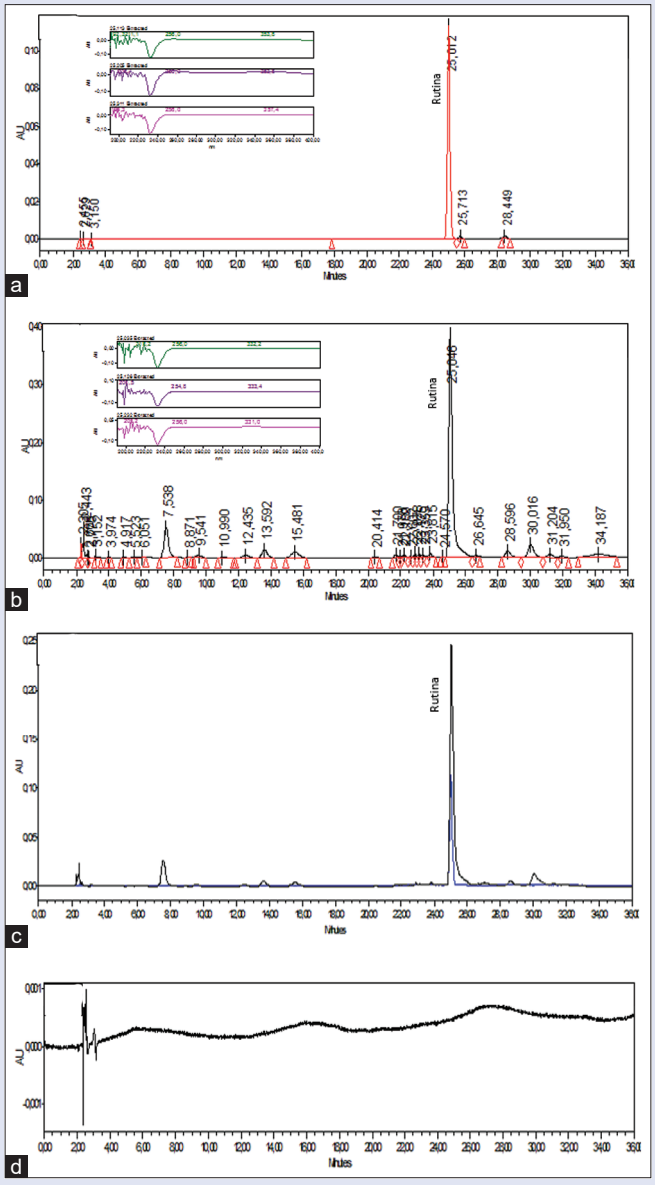

Figure 3: Chromatogram of the standard (a). Chromatogram of the soft extract from *B. pilosa* (b). Chromatogram overlap (blue) of the standard and chromatogram (black) (c) of the soft extract from *B. pilosa*; and methanol (d), followed by the ultraviolet spectrum of the rutin (320 nm)

homoscedasticity investigation by the Cochran C test showed that the C calculated (0.583) < C critical (0.616), so the null hypothesis was accepted and the data presented homoscedasticity. The significance of the angular coefficient by the ANOVA *F*-test was evaluated and indicated F calculated (12430.081) > F (0.05, 1, n-2), then the null hypothesis was rejected and "y" effectively varies in function of "x" and the method was considered linear. The linear coefficient was evaluated by Student's *t*-test and showed T calculated (−0.006) < T tabulated (3.182), so the null hypothesis was accepted and there is no evidence that the linear coefficient is statistically different from zero. The normality of the residual was analyzed by the Shapiro-Wilk test and it was concluded that there was a normal distribution with W calculated (1.05) > W tabulated (0.897), accepting the null hypothesis. According to the Guidelines for Statistical Treatment of Analytical Validation (ANVISA, 2017), the residual quantifies the distance between the real and the estimated values. When the error of the model is because just to the common variations of the analysis, the residual are expected to be independent of each other and present a normal distribution.[42] The independence was investigated by Durbin-Watson test and the results evidenced that the residual were independents, dU < dw < 4-dU; (1,23 < 1,64 < 4-1,23, respectively). All criteria presented to agree with Brazilian legislation.[29,42]

Limits of detection and quantification

The LOD and LOQ of rutin were: 0.00322 mg/mL and 0.00976 mg/mL, respectively. The limit of detection refers to the lowest amount of analyte (marker) that can be identified in the sample, while the limit of quantification refers to the lowest amount of analyte that can be measured in the sample.[43]

Precision

The results of RSD for the precision parameter by repeatability and intermediate precision were: 4.43% and 3.38%, respectively. The Brazilian legislation recommended that the RSD should be <5%,[29] therefore the method presented precision, showing agreement between the several responses obtained by detector.[44]

Accuracy

The average results obtained from recovery of rutin at the low level was 99.14% and RSD of 2.51%, at the mean level was 94.39% and RSD of 1.36% and at the high level was 95.33% and RSD of 1.61%. The recovery interval varied from 92.19% to 101.38%. The accuracy of an analytical method is the closeness of test results obtained by that method to the true value adopted as a reference.[45]

Robustness

The observed results of RSD of the areas (μv*S) of the peaks and the retention time of rutin (*R*t) from the original method proposed and the peak areas and retention time of the marker during variations in the method conditions were in accordance with Brazilian legislation, <5%, which suggests that the developed method was able to withstand to small modifications in the original method without a significant change in peak areas and retention time of the marker.[29]

Matrix effect

The absence of the matrix effect was confirmed by the parallelism between the concentration curves versus the analytical response of the rutin standard and the sample solution fortified with standard, according to Figure 4.

In addition, the T calculated (0.6557) by applying the *t*-test was lower than the T critical (2.306). According to the Guidelines for Statistical Treatment of Analytical Validation, when T calculated <T the angular coefficients are not statistically different, therefore, there is no significant matrix effect and the method is sufficiently selective.

The matrix effect is an important parameter to the analysis of complex matrices, since the aim of this test is to verify if components inherent to the matrix in which the marker is inserted do not interfere in the quantification of the concentration of this analyte.[29,46]

Covalidation of the analytical method for quantification of rutin in the tablets

Concerning to the linearity parameter of the method by the analyzes of the samples of the raw material powder and of the pilots tablets 1 and 2, the values of the correlation coefficients (r) of the average analytical curves were, respectively: 0.9999; 0.9999, and 0.9992, all above 0.99, as recommended. Also, all data were homocedastic and the angular coefficient was significantly different from zero. The results of DPR% between the levels of rutin in the samples by the parameter precision were: 4.71%; 3.59%, and 3.36%, up to 5%, as recommended.[29] The method was considered linear, precise and selective for rutin quantification in these samples.

Tablets characterization

Flow properties of granule

The flow properties of powders and granule are critical for the pharmaceutical industry and may be a determining factor for the weight and content uniformity of tablets.[47]

The CI% and HR represent granule compressibility indexes, wherein ranges of values are analyzed and considered to determine the fluidity of granule.

The angle of repose determine whether the flow of the material is free. Materials with low angles of repose (<50) tend to be more fluid. The angle of repose <30° is the recommended.[48]

Table 5 shows the results of angles of repose, CI% and HR, as well as the flow classification.[31]

Table 5: Results of angles of repose, confidence interval % and Hausner's ratio to determine the flow of the granules

Formulation/ granules	Angle of repose (°)	CI %	HR	Classification USP (2006)
Tablets PH	11.3	5.94	1.06	Excellent flow
Tablets SE	18.5	10.20	1.12	Good flow

PH: Powdered herbal; SE: Soft extract; CI: Carr's index; HR: Hausner's ratio; USP: United States Pharmacopeia

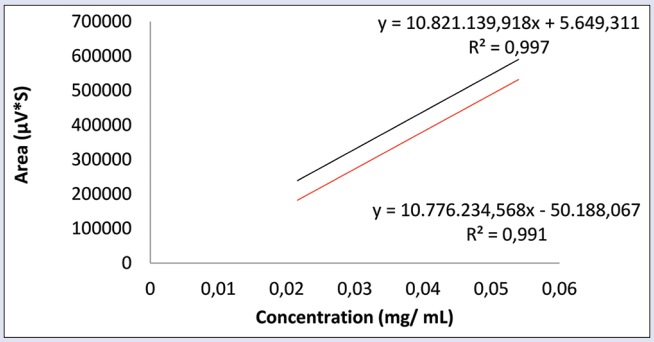

Figure 4: Parallelism test between curves (area vs. concentration) of the analytical response of rutin standard (red) and the soft extract solution fortified with standard (black)

Water activity

W_a represents the amount of free water in the product, that allows the development of micro-organisms that may be pathogenic and/or cause degradation of the product.[32] The Wa of solid oral dosage forms should be <0.750 at 25°C.[32] The formulations showed W_a within the recommended, which were: 0.436 for tablets PH and 0.440 for tablets SE.

Average weight determination

The mean weight of the pilot tablets PH and tablets SE were respectively: 225 mg (maximum variation of 1.50%) and 240 mg (maximum variation of 6.87%). The limit of variation allowed is ±7.50%, for each unit in relation to the average for tablets with an average weight above 80 mg and <250 mg.[27]

Content uniformity test

Rutin content, per formulation, was: 0.478 mg and 4.93 mg for tablets PH and tablets SE, respectively. The content uniformity test evaluate the individual contents in relation to the content of the active principle are within the established individual contents are within limits set with reference to the Ca of the sample.[27] The calculated AV, according to Brazilian legislation (2010), must be up to 15.0.[27] The results obtained for both formulations are in agreement with the recommended one, presenting AV of 14.10 for the pilot tablets 1; and 13.70 for the pilot tablets 2.

Mechanical resistance

The tablets PH had a hardness of 50.26 N and the tablets SE had a hardness of 136.3 N. Both formulations had a hardness higher than 30 N, as recommended. The friability results found were, for pilot tablets PH and tablets SE, respectively: 0.47% and 0.08%. Therefore, the observed values are in agreement with the acceptable one, that is up to 1.5%.[27]

Disintegration test

The disintegration time as specified by the Brazilian Pharmacopeia (2010), must be <30 min.[27] Just the tablets PH was within the recommended (27 min). The tablets SE presented disintegration time of 44 min. Such data may be the result of the possible agglutinating action of the soft extract associated with the highest percentage of binder adjuvant (PVP) in this formulation and also its hardness. According to Chime *et al.* the type and the concentration of binder in tablets has much influence on the disintegration time. The authors observed that in formulations with the same type of binder, but at higher concentrations, the disintegration time of the tablets increased to above that recommended.[49]

Dissolution profile

Throughout the dissolution test, the concentration of the medium must be up to 20% of saturation respecting Sink conditions. The Sink conditions results in the current study for the neutral medium tablets PH and tablets SE were: 12.3 and 11.7%; and for the acid medium for the pilot tablets 1 and 2 were: 4.0 and 14.2%. Figures 5 and 6 show the dissolution profiles in the acid and neutral media for the pilot tablets 1 and 2, respectively.

In the tablets PH, in acid medium at 120 min the percentage of rutin marker dissolved did not reach 60% (55.9%), likewise the percentage of rutin dissolved in tablets SE was <10% (9.6%). This data is explained by the fact that rutin is poorly soluble at this pH and expressively more soluble in media with pH 6.8,[50] as observed in the current study in which the tablets PH reached 100% of rutin dissolved after 120 min and tablets SE reached the percentage of rutin dissolved >80% (82.4%) after 120 min. Furthermore, the disintegration process of the different formulations may have influenced the dissolution of the tablets making it slower in the case of the tablets SE in relation to the tablets PH. Gupta *et al.*(2009) correlated the slow dissolution of tablets with the longer disintegration time. The increase of the surface area caused by the disintegration process allow a faster rate of dissolution.[51]

The results DE% for the tablets PH and tablets SE in the acid medium were 28.86% and 3.87%, respectively. While in the neutral medium, the formulations had DE% of 70.96% for tablets PH and 52.23% for the tablets SE The DE was significantly higher ($P > 0.005$), for both formulations, in neutral pH medium denoting a greater percentage of rutin dissolved in this medium.

Accelerated stability studies for the tablets

The accelerated stability studies provide evidences of how the content of an active principle varies under the influence of time and environmental factors such as temperature and humidity, in addition, these studies enable the establishment of a shelf life for the product and recommendations about storage conditions.[52]

According to RE n° 1, July 29, 2005, the variation allowed compared to the time zero is ±10%.[37] Pilot tablets PH and SE presented results above of that recommended (18.35% and 19.34%, respectively) after 120 days, showing susceptibility to degradation under the assay conditions.

Data in the literature indicate that herbal extracts, due to their hygroscopicity, tend to be prone to chemical degradation.[53,54] Cortés-rojas *et al.* investigated the physico-chemical stability of a dry extract of *B. pilosa* and monitored the content of two flavonoids (rutin and hyperoside) and a polyacetylene and observed that flavonoids showed lower degradation compared to polyacetylene. They also concluded

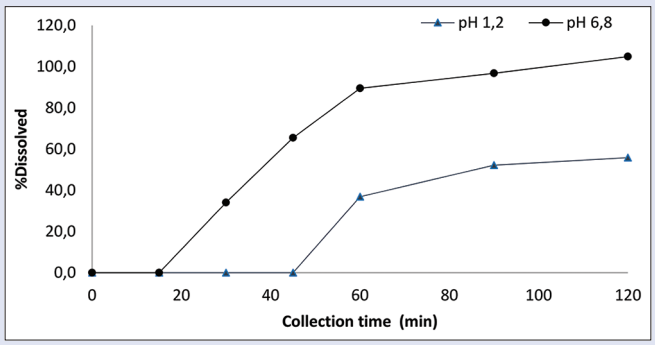

Figure 5: Dissolution profile of the tablets PH in the acid medium and neutral medium

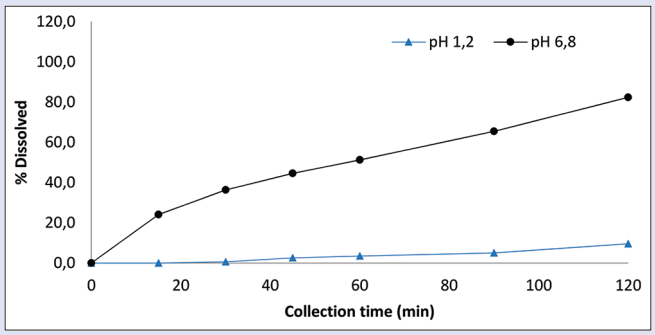

Figure 6: Dissolution profile of the tablets SE in the acid medium and neutral medium

Table 6: Results of the accelerated stability studies

Time (days)	Content variation (%)	
	Tablets PH	Tablets SE
30	4.51	4.46
60	6.20	6.44
90	8.74	9.58
120	18.35	19.34

PH: Powdered herbal; SE: Soft extract

that when exposed to storage at forced conditions of humidity (75%) and temperature (40°C) the stability of these extracts tended to decay significantly, unless the extract is stored in a properly sealed primary packaging that does not allow the degradation of the compounds. When stored in a refrigerator (at lower temperatures), no significant changes in the marker content were observed.[25]

The results verified in the current study [Table 6] suggest the need for studies that aim the increasing of the stability of the rutin marker in formulations containing herbal products from *B. pilosa*.

CONCLUSION

According to the results obtained for the validation parameters, the method for quantification of rutin by HPLC was linear, precise, selective, robust, and accurate and with absence of matrix effect. Pilot tablets SE, with soft extract, presented higher content of rutin than pilot tablets PH, with powder of the aerial part from raw material, demonstrating that the method to obtain the soft extract was efficient due to the extraction and concentration process. The tablets showed better dissolution in the medium with neutral pH (6.8) simulating the pH of the duodenum compared to the acid medium pH 1.2 simulating the pH of the stomach, which indicates the greater solubility and availability of this bioactive compound in the neutral medium of the first portion of the small intestine (a recognized region of active principals absorption). Drug dissolution is a prerequisite to drug absorption**.** The stability of rutin was affected after 120 days and studies are needed to increase the stability of the formulations. The use of soft extract from *B. pilosa* standardized on rutin in formulations has not been reported to date in the literature, but it presented as a promising active ingredient due to the fact it have flavonoids, such as rutin, with several beneficial biological activities proven in *in vitro* and *in vivo* studies.

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Conflicts of interest

There are no conflicts of interest.

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