BJPS

Brazilian Journal of
Pharmaceutical Sciences

http://dx.doi.org/10.1590/s2175-97902020000118394

Article

Tacinga inamoena vegetal drug characterization using phytochemistry, pharmacopoeial methods and thermoanalytical techniques

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Tacinga inamoena (K. Schum.) N.P. Taylor & Stuppy, also known as quipá, is a native cactus of the Caatinga used in traditional medicine to treat urethral infections and inflammation. This study aimed to determine the physicochemical characteristics of vegetal drug obtained from the roots of *T. inamoena*. Analytical techniques and phytochemical tests were used, such as thermal analysis, qualitative and semiquantitative determination of secondary metabolites and spectroscopy at the infrared region. The powder of the vegetal drug met the parameters established by the Brazilian Pharmacopoeia, except for compressibility, which was low. On the thermogravimetric curve, three events related to the mass loss were verified, which correlate with the vegetal drug quality control and play a part in their standardization. The qualitative screening suggested the presence of alkaloids, flavonoids and terpenes. The infrared spectrum reinforced the presence of hydroxyl, carbonyl, and ether groups. In the semiquantitative screening, a concentration for total polyphenols of 65 mg equivalent to gallic acid g^{-1} to the crude ethanol extract (CEE) was obtained. On the correlation of flavonoid content to seasonality, a concentration was obtained of 3.3 mg equivalent to quercetin g^{-1} to the CEE obtained during the drought period and of 10.6 mg equivalent to quercetin g^{-1} to the CCE obtained during the rainy season. In *T. inamoena,* the presence of important classes of secondary metabolites, which are associated with the pharmacognostic characterization, aids the authentication and quality control of vegetal drugs of importance in traditional Brazilian medicine.

Keywords: Cactaceae. Quality control. Herbal medicine. Secondary metabolites. Thermogravimetry.

INTRODUCTION

Tacinga inamoena (K. Schum.) N.P. Taylor & Stuppy is a cactus also known as quipá, cumbeba, or gogóia. It is a native of the northeast region in Brazil and is spread throughout almost all of the semiarid region. It is used in the countryside for animal feed and as a popular medicine to treat urethral diseases, asthma and inflammation, as well as to combat worms (Castro & Cavalcante, 2011; Zappi, Taylor, & Machado, 2012; Menezes, Taylor, & Loiola, 2013). In Brazil's semiarid areas, a growing interest has been observed in studies of the region's species (Arrais *et al*., 2014), particularly for their potentially abundant curative properties; yet there is been little study regarding their molecules' bioactive powers to this date (Arrais *et al*., 2014; Ribeiro *et al*., 2014).

Physicochemical analysis is the preliminary step for achieving a quality standard necessary for vegetal material and, thereby, assigning a high quality to new herbal medicines' constitutions (Souza-Moreira, Salgado, & Pietro, 2010; Peña Muniz *et al*., 2015).

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The milling of the raw plant material is a critical step in obtaining vegetal drugs, extracts, and herbal medicines, and therefore, granulometry powder control is an important step of the production process (Correia *et al*., 2016). Among the analyses performed to ensure vegetal drugs' quality are quantitative and qualitative analyses of purity, integrity, and active contents (USP, 2012). Such analytical methods, especially thermal analysis (DSC, DTA, and TG), are the best methods for studying drugs, extracts, and active substances derived from medicinal plants (Fernandes *et al*., 2016). Only a small number of studies have been conducted on the thermal characterization of these products, although these active ingredients have been in use for a long time for the production of herbal medicines (Cartaxo-Furtado *et al*., 2017).

Thus, the aim of this study was to determine the physicochemical and phytochemical characteristics of the vegetal drug obtained from the roots of *T. inamoena*.

MATERIAL AND METHODS

Raw material and extract production

Roots from *T. inamoena* were collected at the Canhoto Farm (latitude: 07º 28' 47", longitude: 38º 02' 32"), in Nova Olinda, Paraíba. The specimen voucher from the vegetal material was deposited at the Herbarium of the Federal University of Paraíba under protocol JPB 61263. The plant material was dried in a circulating air oven at 40 ± 1 °C and pulverized in a knife miller with a particle size of 10 mesh.

The dry and pulverized material (322 g) was exhaustively extracted with ethanol (EtOH) and concentrated under vacuum conditions at an average temperature of 50 ºC, yielding 23 g of the crude ethanol extract (CEE).

Physicochemical characterization

Granulometry

The granulometry test followed the methodology described in the Brazilian Pharmacopoeia (Brazil, 2010). The procedure used 25 grams of powder from *T. inamoema* roots, submitted to a series of sieves with different-sized mesh openings (710, 355, 180, 150, 75, and 38 µm) under vibration for 20 minutes. The particle sizes were analyzed in triplicate and evaluated through the powder-retention percentage quantification in each sieve according to equation (1):

$$\% \text{ retained by sieve} = P_1/P_2 \times 100 \quad (1)$$

where,
P_1 = weight of the sample retained by each sieve (in grams)
P_2 = sum of the weight retained in each sieve and in the collector (in grams).

Density determination

For density determination, 10 grams of powder from *T. inamoema* were packed in a graduated cylinder of 50 mL (Lagos, Pereira, & Bertol, 2012), with the measured initial volume, and the apparent density (ad) was calculated (equation 2). The compacted density (cd) (equation 3) was measured after the graduated cylinder was subjected to successive beat movements vertically at a height of 20 cm up to 500 times. From the apparent and compacted densities, the Hausner Factor (HF) and the compressibility index (CI) were determined (USP, 2007), according to equations (4) and (5), respectively.

$$ad = \frac{\text{Am}}{Av} \quad (2)$$

$$cd = \frac{\text{Am}}{Cv} \quad (3)$$

$$\text{HF} = \frac{cd}{ad} \quad (4)$$

$$\text{CI} = \frac{cd - ad}{ad} \quad (5)$$

where,
Am = apparent mass,
Av = apparent volume,
Cv = compacted volume.

Ash contents

A total of 9 grams of root powder obtained from *T. inamoema* was spread in equal proportions in three porcelain containers previously calcined, cooled, and weighed. The samples were carbonized in a muffle

furnace and incinerated at 450 ºC for 2 hours. After cooling in desiccators, the samples were weighed using an analytical balance, and the procedure was repeated until a constant weight was obtained (Brazil, 2010). The ash percentage, obtained in triplicate, was calculated in relation to the dried powder, according to equation 6:

$$\% \, Ashes = \frac{P_2 - P_1}{P_3} \times 100 \quad (6)$$

where,
P_1 = crucible weight after calcination and cooling
P_2 = crucible weight of the sample after calcination and cooling
P_3 = initial weight of the sample

Moisture content determination

The test was conducted in triplicate and the obtained values were evaluated in terms of weight percentage of the sample quantity (equation 7), according to Brazil (2010):

$$\% \, loss = \frac{P_u - P_s}{P_a} \times 100 \quad (7)$$

P_a = sample weight (g).
P_u = weight of the filter containing the sample before desiccation (g).
P_s = weight of the filter containing the sample after desiccation (g).

Phytochemical prospecting

The CEE was subjected to phytochemical screening (Table II) following the methodologies described by Matos (2009).

Quantification of polyphenols

The total polyphenol content of plant extracts was measured by the Folin-Ciocalteu reagent method, as described by Chaves *et al.* (2013). The extracts were dissolved in distilled water to obtain a final concentration of 200 μg mL^{-1}. From each solution, a 1 mL aliquot was added to 1 mL of 1 mol L^{-1} Folin-Ciocalteu reagent (Sigma-Aldrich®). This mixture remained undisturbed for 2 min before the addition of 2 mL of 20% (w:v) Na_2CO_3 solution and then left undisturbed for 10 min. Thereafter, the reading was performed using a Shimadzu® spectrophotometer at 757 nm. The calibration curve was obtained with a solution of gallic acid (Sigma-Aldrich®) from which dilutions were made at concentrations between 1 and 40 μg mL^{-1}. The test was conducted in triplicate.

Quantification of flavonoids

The total flavonoid content was determined by the $AlCl_3$ method (Meda *et al*., 2005; Chaves *et al*., 2016). The extracts were diluted with methanol (1000 μg mL^{-1}). The same volume of 2% (w:v) $AlCl_3$ solution in methanol was added to each test solution. This mixture remained undisturbed for 10 min before the UV spectrophotometric reading at 415 nm wavelength. The total flavonoids was determined by the calibration curve using quercetin (Sigma-Aldrich®) as a standard at concentrations between 2 and 30 μg mL^{-1}. The test was conducted in triplicate.

Quantification of tannins

The content of condensed tannins was verified using the method described by Makkar and Becker (1993) and Chaves *et al*., (2016), wherein 0.25 mL of the sample (1000 μg mL^{-1}) was added to 1.5 mL vanillin (Sigma-Aldrich®) dissolved in methanol (4% w:v) and, subsequently, in 0.75 mL of concentrated HCl (37%). After the HCl addition, the contents of the tube were shaken in a water bath at 30 °C before being read on a spectrophotometer at a 500 nm wavelength. Catechin (Sigma-Aldrich®) was used as a standard at concentrations between 10 and 100 μg mL^{-1}. The test was conducted in triplicate.

Analytical techniques

Differential Thermal Analysis (DTA)

The DTA curves were obtained with a Q600 SDT (TA Instruments®) simultaneous analyzer using aluminum crucibles containing 2.0 ± 0.1 mg of the sample under a nitrogen atmosphere (flow of 50.0 mL min^{-1}). The experiments were conducted between temperatures of 30 and 400 °C min^{-1} by heating at 10 °C min^{-1}. Indium (mp 156.6 °C) was used as the standard for equipment calibration.

Thermogravimetry (TG)

The TG curves were obtained using a simultaneous thermal analyzer, model Q600 (TA Instruments®). The mass of the samples was 8.00 ± 0.05 mg, weighed on an analytical balance and packed in aluminum crucibles. The sample was uniformly distributed on the bottom of the crucible. The experiments were programmed at a temperature range from 30 to 900 °C under a dynamic atmosphere of nitrogen (50 mL min^{-1}) using a heating rate of 10 °C min^{-1}.

Fourier-transform infrared spectroscopy (FT-IR)

FT-IR spectra of vegetal extracts were recorded on a Perkin-Elmer model 1600 apparatus using KBr stressed discs in the range of 4000–500 cm^{-1}.

RESULTS AND DISCUSSION

According to the pharmacopoeia, the powder resulting from the vegetal drug was classified as semi-thin. The granulometric distribution of vegetal drugs represents a parameter for the choice of the extractive process and, consequently, obtaining the chemical constituents of pharmaceutical interest, as well as the aspects inherent to the pulverized vegetal material, represent a critical stage in the production of phytotherapeutic drugs (Alves *et al*., 2010).

The average of the obtained value of the matter lost by desiccation in *T. inamoena* was 10.11%, according the established limits from the Brazilian Pharmacopoeia this result that can vary from 8 to 14% (Brazil, 2010). values within acceptable limits implies a positive results in regard to microbiological control and hydrolysis reactions, because both cause chemical deterioration (Couto *et al*., 2009).

The Hausner Factor was calculated based on the brute and compaction density and corresponded to 1.43 (Table I), classifying the vegetal material as cohesive. Thereby, the powder of the roots obtained from *T. inamoena* presented poor flow properties, as corroborated by the to the compressibility index (43.47%), because IC values > 38%, are considered very flow poor. Consequently, this powder will present difficulties on the compression process, a relevant point of the medicine's production network in a solid form (USP, 2007; Garcia, Pereira, & Dias, 2012; Lagos, Pereira, & Bertol, 2012).

TABLE I - Physicochemical properties of the vegetal drug from *T. inamoena*

Physicochemical properties	Values
Compaction density (g mL^{-1})	0.33
Brute density (g mL^{-1})	0.23
Compressibility (mL)	10.5
Compressibility index (%)	43.47
Hausner ratio	1.43
Ashes content (%)	11.06
Desiccation loss (%)	10.11

For the total amount of ash test, a percentage of 11.06% of inorganic matter was observed, below 14%, according to the established limits from the Brazilian Pharmacopoeia (Brazil, 2010), thereby indicating that samples did not contain excess soil and/or sand.

In the qualitative screening, it was possible to detect the presence of nitrogenous composed as alkaloids, beyond steroids/terpenes and flavonoids (Table II). The presence of those compounds provides a larger valuation of the vegetal species due to the therapeutic potentiality related to those metabolites, among other activities (Souza *et al*., 2014; Zhao *et al*., 2016; Ding *et al*., 2017).

TABLE II - Qualitative screening and content metabolites present in the CEE from *T. inamoena*

Qualitative *screening*	Results
Bouchardat	+
Mayer	-
Dragendorff	+
Catechins	-
Steroids/Terpens	+

(continuing)

TABLE II - Qualitative screening and content metabolites present in the CEE from *T. inamoena*

Qualitative *screening*	**Results**
Tannin	-
Flavonoids	+
Polisaccharides	-
Saponines	-
Phenols compounds content	**Results**
Flavonoids (september)	3.3 mg g^{-1}
Flavonoids (february)	10.6 mg g^{-1}
Poliphenols (september)	65 mg g^{-1}

(-) negative; (+) positive

The total polyphenol analyses revealed the presence of 65 mg equivalent to gallic acid g^{-1} to the CEE, without a quantified value of tannins (Table II). Among the phenolic compounds belonging to the secondary metabolism of the vegetal matter are found structures such as phenolic acids, flavonoids, coumarins, water-soluble pigments, tannins, alkaloids, and conjugated terpenes that are related, mainly, to the protection of the vegetal species related to its resistance to microorganisms and pests (Rocha *et al*., 2011).

The flavonoid content presents a concentration of 3.3 mg equivalent quercetin g^{-1} to the CEE, corresponding to a drought period in the Paraíba (September 2014), while the values for the rainy season (February 2014) provided 10.6 mg equivalent quercetin g^{-1} to the CEE (Table II). Given this scenario, a greater variation of flavonoids was understood to be aligned with a higher rainfall index. It can be inferred that the higher amount of water availability predisposed the growth of flavonoid production, possibly because in this period, the xerophytes species demonstrate higher metabolic production. Budding, flowering, and fruiting occur during this stage, while in the drought period, the plant tends to discontinue several functions in order to guarantee the species' survival, conferring a level of tolerance to the plants related to water stress. This variation on the synthesis of secondary metabolites, affected by environmental conditions, represents a chemical interface between the plant and the surrounding ambient climate (Kutchan, 2001; Gobbo-Neto, Lopes, 2007; Santos, Reis, 2008; Chaves *et al*., 2013).

The TG curves demonstrated the occurrence of three events of mass loss (Figure 1, Table III). For the first, an endothermic stage was observed (Figure 1, Table IV), with a mass loss of 9.7%. This event can be related to the vegetal drug's dehydration and to the evaporation of volatile constituents (Santos *et al*., 2011; Brandão *et al*., 2016).

For the second event, there was a loss equivalent to 47.16% of the material mass, a significant loss that can be attributed to the carbohydrates' thermal decomposition and other organic compounds present (Santos *et al*., 2011; Costa *et al*., 2013; Brandão *et al*., 2016). In the third and last event, the mass loss was equivalent to 32.34% between the temperatures of 359.03 ºC and 532.49 ºC. This final loss can be attrbuted to the burning of the remains of organic matter (Costa *et al*., 2013).

The events described by the TG curves can also be observed when analyzing the DTA curves (Figure 1, Table IV), which confirms the occurrence of the three events related to the mass loss, those events being important to the standard of the vegetal drugs obtained from the roots of *T. inamoena*.

On the IR (Figure 2) spectrum, it was possible to view a broad band with medium intensity in 3300 cm^{-1} characteristic of a hydroxyl grouping (OH) and two bands with low intensity in 2900 and 2880 cm^{-1}, indicative of methyl and methylene groups with hybridization sp^3. A narrow band suggestive of carbonyl grouping can be observed at 1650 cm^{-1} and an intense band characteristic of CO grouping at 1000 cm^{-1} (Pavia *et al*., 2012). The indicative signals from those functional groups correlate with the phytochemical analysis, suggesting the presence of phenolic compounds.

The study indicates the presence of classes of metabolites with recognized health benefits, establishes a connection of flavonoid production associated with the medium in which *T. inamoena* is inserted, and determines parameters for the control of the quality of the vegetal drug. In these ways, it contributes to the knowledge of medicinal species and establishes data that may refer to the identity of the vegetal drug produced from *T. inamoena*.

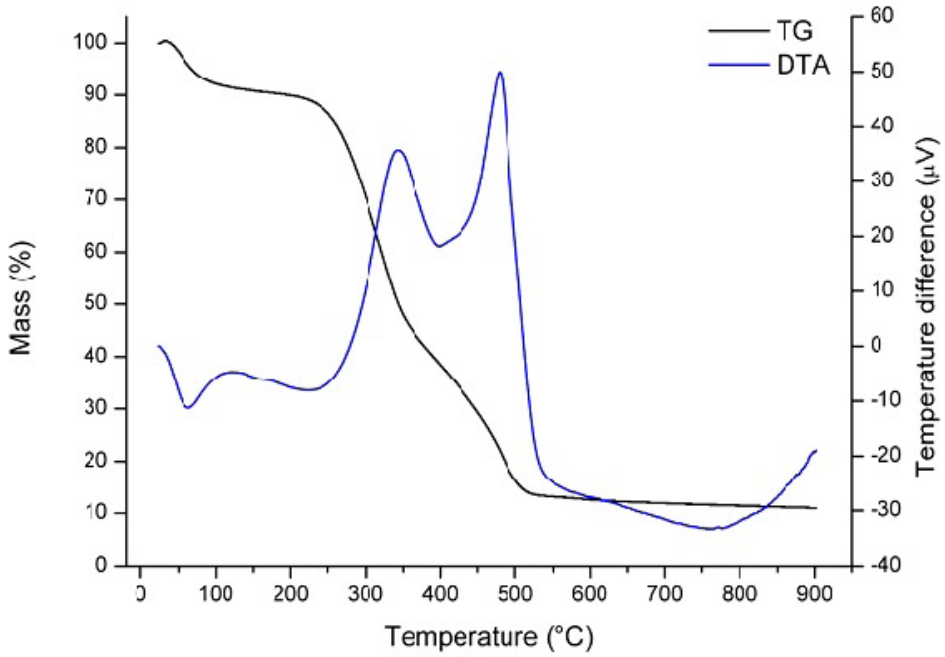

FIGURE 1 – Thermogravimetric curve and differential thermal analysis of vegetal drug from *T. inamoema*

TABLE III - Data of thermogravimetric curve of the roots from *T. inamoema,* with their respective losses of mass, in each temperature range (°C)

Events	Onset - Endset (°C)	Loss of mass (%)	Initial mass
1ª stage	33.59 – 160.58	9.7	7.11 mg
2ª stage	203.78 – 359.03	47.16	Residue
3ª stage	359.03 – 532.49	32.31	10.97 %

TABLE IV - Data of differential thermal analysis of the roots from *T. inamoema*

Events	Onset - Endset (°C)	ΔH (J g^{-1})	Peak (°C)
1ª stage	25.32 – 111.27	- 530.59	63.22
2ª stage	229.89 – 400.55	2440.72	342.83
3ª stage	400.16 – 539.46	2884.22	473.06

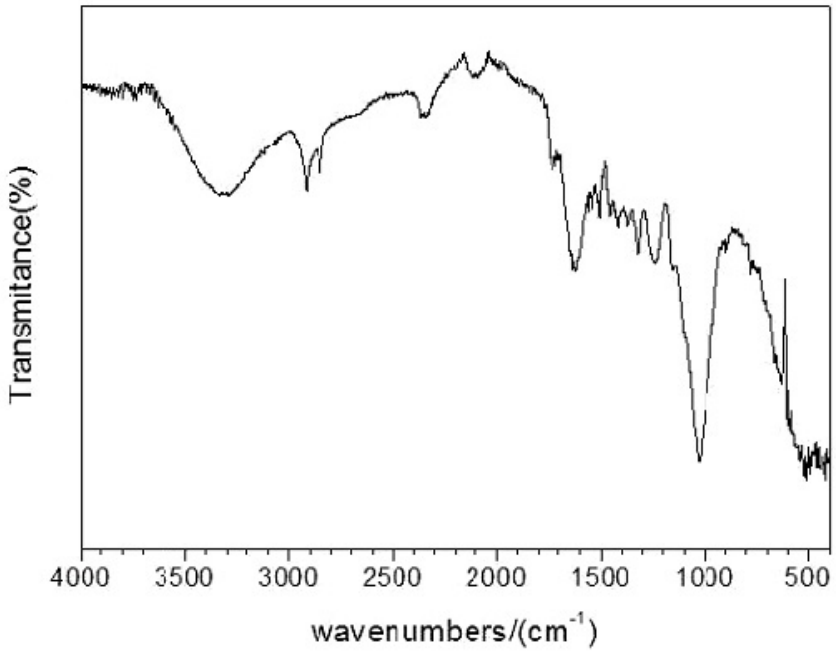

FIGURE 2 – Infrared spectrum of the vegetal drug from *T. inamoema.*

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Received for publication on 09th June 2018
Accepted for publication on 21st January 2019