



Justicia pectoralis Jacq., Acanthaceae: preparation and characterisation of the plant drug including chromatographic analysis by HPLC-PDA

Francisco N. Fonseca, Aline Holanda Silva, Luzia K. A. M. Leal*

¹Programa de Pós-graduação em Ciências Farmacêuticas, Setor de Farmacognosia, Laboratório de Tecnologia de Produtos Naturais, Departamento de Farmácia, Faculdade de Farmácia, Odontologia e Enfermagem, Universidade Federal do Ceará, 60430-370 Fortaleza-CE, Brazil.

RESUMO: *Justicia pectoralis* Jacq., Acanthaceae, é uma erva conhecida popularmente no Nordeste como chambá e, utilizada tradicionalmente no tratamento de doenças do trato respiratório, como a asma, tosse e bronquite. Essa espécie encontra-se na Relação Nacional de Plantas Medicinais de Interesse para o SUS. O objetivo do presente estudo foi elaborar protocolo para a preparação da droga vegetal a partir de *J. pectoralis* e realizar a sua caracterização visando seu emprego como matéria-prima farmacêutica. A parte aérea de *J. pectoralis*, após secagem em estufa com circulação e renovação de ar (35 °C) durante diferentes períodos de tempo (1-5 dias) mostrou a partir de 24 h de secagem um teor de umidade abaixo do valor máximo permitido para drogas vegetais. O pó da droga vegetal foi classificado como pó moderadamente grosso e, caracterizado quanto aos teores de cinzas totais, extrativos solúveis em água e etanol. Análise do extrato hidroalcoólico (etanol 20%) de *J. pectoralis* por Cromatografia Líquida de Alta Eficiência (CLAE-DAD) determinou um teor de cumarina e umbeliferona de 16,2 e 0,81 mg/g da droga vegetal, respectivamente. As condições de preparação da droga vegetal e os parâmetros de controle de qualidade determinados para *J. pectoralis* no presente estudo são de interesse no desenvolvimento de fitoterápicos que empreguem esse matéria-prima ativa.

Unitermos: *Justicia pectoralis*, cumarina, umbeliferona, CLAE-DAD, controle de qualidade, matéria-prima vegetal.

ABSTRACT: *Justicia pectoralis* Jacq., Acanthaceae, is a herb popularly known in Brazilian northeast as “chambá” and used in folk medicine for the treatment of respiratory tract conditions such as asthma, cough and bronchitis. This species is included in the National Register of Plants of Interest to the National Health System. The aim of the present study was to develop a protocol for the preparation of the plant drug from *J. pectoralis* and to characterise the plant drug for its use as a pharmaceutical raw material. The aerial parts of *J. pectoralis*, after drying chamber with forced air circulation (35 °C) for different periods of time (1-5 days), presented after one day a moisture content below the maximum allowed for plant drugs. The powder of the plant drug was classified as moderately coarse, and the total ashes content and the water- or ethanol-soluble extractives were determined. Analysis of hydroalcoholic (ethanol 20%) extract of *J. pectoralis* by high performance liquid chromatography-photo diode array (HPLC-PDA) determined the content of coumarin and umbelliferone (16.2 and 0.81 mg/g plant drug, respectively). The preparation conditions of the plant drug and the quality control parameters established for *J. pectoralis* in this study are of interest for the development of phytomedicines which use this active raw material.

Keywords: *Justicia pectoralis*, coumarin, umbelliferone, HPLC-PDA, quality control, plant raw material.

INTRODUCTION

The quality of a phytomedicine is determined by the quality of the plant drug, the intermediate product and by the properties of the final product, taking into consideration the requirements of good practice in the manufacture of pharmaceutical products of plant origin (Kroll & Cordes, 2006; WHO, 2005). The quality control of these products employs pharmacognostic methods, physico-chemical control and microbiological monitoring (Kroll & Cordes, 2006; Gaedcke, 2004; WHO, 1998). In this context, various analytical methods have been employed in the analysis of chemical markers, including thin layer chromatography, high performance liquid chromatography (HPLC) and gas chromatography (Angelova et al., 2008).

In Brazilian northeast, in particular in the state of Ceará, researchers have been engaged in the systematic study of species belonging to the project 'Living Pharmacies' ('*Farmácias Vivas*'; Matos, 2000), including *Justicia pectoralis*, which is also found in the National Register of Plants of Interest to the National Health System (Ministério da Saúde, 2008). Two varieties of *Justicia pectoralis* have been described, *stenophylla* and *pectoralis*, found in Brazil and the Caribbean, respectively (Oliveira & Andrade, 2000; Trueba et al., 2001). *Justicia pectoralis* Jacq. var. *stenophylla* Leonard, Acanthaceae, is a sub-erect herb cultivated in Brazilian northeast, popularly known as "chambá", "anador" and "trevo-cumaru". The aerial parts of "chambá" are used in the form of artisanal preparations or as a pharmaceutical formulation (syrup) indicated for the treatment of respiratory diseases, such as cough, bronchitis and asthma (Matos, 2000).

Phytochemical studies on *J. pectoralis* revealed the presence of various compounds including coumarin (1,2-benzopyrone - CM), umbelliferone (7-hydroxycoumarin - UMB), ortho-methoxylated glycosylflavones and justicidin B (Leal et al., 2000; Joseph et al., 1988). The pre-clinical toxicological assessment of the hydroalcoholic extract of "chambá" showed that the plant possesses low toxicity. In addition, the extract and chemical constituents, CM and UMB, obtained from the plant exhibits antioxidant, antiinflammatory, antinociceptive and muscle relaxant activities (Trueba et al., 2001; Lino et al., 1997; Leal et al., 2000), as well as central effects described more recently in rats (Venâncio, 2010; Pereira et al., 2009). Preliminary clinical assessment (Nobre et al., 2006) of the efficacy of the "chambá" syrup in the treatment of asthmatic patients with mild to moderate asthma, reported that after one week of treatment the patients presented reduced obstruction of the airways, with increases in forced expiratory volume, forced vital capacity and maximum expiratory flow.

Coumarin is a heterocyclic, aromatic, organic compound found in numerous plant species with

pharmacological potential such as *Amburana cearensis*, *Mikania glomerata* and *Hybanthus ipecacuanha* (Leal et al., 2000; Rocha et al., 2008). Due to its capacity to potentiate the proteolytic activity of macrophages, CM has been indicated for the treatment of patients with lymphoedema (Clodius & Piller, 1978), while coumarinic derivatives, such as UMB, possess numerous specific pharmacological properties including antiinflammatory (Vasconcelos et al., 2009), spasmolytic (Lino et al., 1997), hypoglycaemic and hypolipidemic activities (Ramesh & Pugalendi, 2005). Thus, considering the pharmacological potential of "chambá" and the lack of specifications for the quality control of this plant raw material, which is a pre-requisite for the production and registration of phytomedicines (Anvisa, 2010), the objective of the present study was to prepare and characterise the plant drug obtained from the aerial parts of *J. pectoralis*, with the determination of quality control parameters, including determination of the levels of active principles /chemical markers, coumarin and umbelliferone, in the plant by HPLC.

MATERIAL AND METHODS

Plant material

Aerial parts of *Justicia pectoralis* Jacq. var. *stenophylla*, Acanthaceae, were collected from the Medicinal Plants Garden of the Phytotherapy Research Group, Health Secretariat of the State of Ceará, Brazil. Exsiccates (numbers 16071 and 16079) of the species were deposited in the Herbário Prisco Bezerra at the Universidade Federal do Ceará.

Preparation of the plant drug

The aerial parts of *J. pectoralis* were dried in a drying chamber with circulation and continuous renewal of air for 6, 12, 24, 48, 72, 96 and 120 h at a temperature of 35±5 °C. After drying, the material obtained was processed in a silager.

Determination of desiccation loss: method of drying on an infrared balance

Samples of the plant drug (3 g) were heated (105 °C) on a balance coupled to a system for drying by infrared irradiation. This procedure was repeated three times with three distinct samples and the results obtained for percent loss of mass were expressed as the mean and coefficient of variation.

Granulometric analysis by sieving

The granulometric characterization of the powder obtained from the aerial parts of *J. pectoralis* (100g) was

carried out by using a shaker coupled with sieves (mesh sizes: 0.125; 0.18; 0.25; 0.35; 0.71 and 0.20 mm). After 20 min under shaking the fractions were removed and the weight recorded. The results were expressed as the percentage of fractions retained. This procedure was repeated three times with three distinct samples (F. Bras. IV, 1988). The mean size of the particles was determined by the arithmetic method (Allen et al., 2007). The results were expressed as the mean and the coefficient of variation.

Determination of total ash content

Exactly three grams of pulverized plant drug were transferred to a porcelain crucible, previously baked and weighed. The sample was incinerated in a muffle furnace at a temperature of 450 °C. Following incineration and cooling of the material in a desiccator, total ash content was calculated as a function of dried plant drug (F. Bras. IV, 2000). This procedure was repeated three times with three distinct samples and the results are expressed as the mean and coefficient of variation.

Determination of the water extractable content

Four grams of the triturated plant drug were macerated in 100 mL of distilled water for 24 h, with the material being stirred during the first 6 h. The macerate was filtered and 25 mL were transferred to a previously weighed container; after drying in an oven (105 °C for 6 h) the extractable content was determined as a function of the weight of plant material (WHO, 1998). This procedure was repeated three times with three distinct samples and the results are expressed as the mean and coefficient of variation.

Determination of the ethanol extractable content

A filter paper cartridge, previously weighed, was filled with 2 g of plant drug and subjected to extraction in a Soxhlet system. Potassium hydroxide (200 mg) were added to the extraction solvent (ethanol) and the extraction was realized during for 5 h. Next, the residue from the cartridge was dried in an oven at 105 °C for 30 min. The extract content was calculated as a function of the weight of the plant drug (F. Bras. IV, 2000). This procedure was repeated three times with three distinct samples and the results are expressed as the mean and coefficient of variation.

Quantitative analysis of chemical markers in the hydroalcoholic extract of "chambá": coumarin and umbelliferone

The hydroalcoholic extract of "chambá" was

produced according to a method described previously by Fonseca (2009), involving 2³ factorial planning with center points, where the influence of three variables (time of maceration, drug:solvent ratio and content of ethanol in water) on production of the extract was examined, employing as the outcome the concentration of CM and UMB determined by high performance liquid chromatography-photo diode array (HPLC-PDA). In the present study 100 g of pulverized plant drug were subjected to percolation after macerating for 24 h at room temperature. Following filtration 1500 mL of the extract were collected, with a solid residue content of 1.51±0.03% (m/v). The detection and simultaneous quantification of CM and UMB in the extract was achieved on an Alliance HPLC-PDA system (Waters, USA), employing a method validated previously (Fonseca et al., 2008; Fonseca, 2009), according to the criteria proposed by the National Agency for Sanitary Surveillance (Anvisa, 2003). To this end, aliquots of the extract were diluted 1:5 in the mobile phase and filtered through a 0.45 µm filter unit (Millipore, USA). The analyses were performed under the following conditions: C18 column, mobile phase (A, AcN:MeOH:THF; B, H₂PO₄:Et₃N, pH 3), elution gradient, injection volume of 20 µL, flow rate 1.8 mL/min and λ= 323 nm.

Statistical analysis

The data were analyzed with the aid of the program Graph Pad Prism 4.0 (USA). The results were expressed as mean±standard deviation and coefficient of variation. The means were compared using Student's *t*-test. Differences were considered statistically significant when *p*<0.05.

RESULTS

The fresh plant (moisture: 81.7±0.36 %), dried in an oven with circulation and renewal of the air within different periods (6 to 120 h) revealed a moisture content that varied between 52.4 and 9.0%, with this value remaining constant at 9.9% from 24 h onwards. Analysis of the plant drug produced at room temperature, in turn, revealed a moisture content of 16.7% after 5 days of drying (Table 1).

The plant drug was further characterized through chromatographic analysis, performed by HPLC-PDA, of the hydroethanolic extract produced from the plant drug prepared either in an oven (drying time: 24 h) or at room temperature (Figure 2). The CM content (16.19 mg/g of drug) of the extract produced from the plant drug prepared in an oven was significantly higher (around five times, *p*<0.0001; Student's *t*-test) than that of the plant drug prepared at room temperature (2.39 mg/g of drug). On the other hand, the UMB content determined in the extracts mentioned did not differ significantly (Table 2).

Table 1. Moisture content of the plant drug from *Justicia pectoralis* prepared in an oven with circulation and continuous renewal of air, determined at different time points.

| Method of drying | Moisture (%) | |
|------------------|--------------|--------|
| Oven time (h) | X | CV (%) |
| 0 | 81 | 0,44 |
| 6 | 52,9 | 1,18 |
| 12 | 20,4 | 2,57 |
| 24 | 9,9 | 10,7 |
| 48 | 9,6 | 1,52 |
| 72 | 9,5 | 3,35 |
| 96 | 9,3 | 2,64 |
| 120 | 9,0 | 1,37 |
| Room (120 h) | 16,7 | 3,08 |

Plant material was dried at a temperature of 35±5 °C. The analyses were carried out in triplicate. X: mean value and CV: coefficient of variation.

Trituration of the plant drug enabled the material to be reduced to particles of a small size, with a mean diameter of 0.590 mm (Table 2), characterized as a moderately course powder (WHO, 1998). Figure 1 shows the granulometric distribution of the plant drug, where most of the particles were retained by screens with a mesh opening of 0.71 mm. The total ash content of the dried aerial parts of *J. pectoralis* was found to be 12.3%, while the mean content of substances extractable in water was found to be lower than that of substances extractable in ethanol (Table 2).

Table 2. Physical and chemical analysis of the ground aerial parts of *Justicia pectoralis*.

| Parameters | Plant drug | |
|---------------------------------|-------------|--------|
| | X | CV (%) |
| Mean diameter of particles | 0.590 mm | 2.54 |
| Total ashes | 12.3 % | 1.36 |
| Contents extractable in water | 0.2 % | 4.08 |
| Contents extractable in ethanol | 12.8 % | 4.94 |
| Coumarin (mg/g) | | |
| Oven drying | 16.19 mg/g* | 2.49 |
| Room temperature drying | 2.39 mg/g | 3.01 |
| Umbelliferone (mg/g) | | |
| Oven drying | 0.81 mg/g | 4.49 |
| Room temperature drying | 0.78 | 5.59 |

Plant material was dried at a temperature of 35±5 °C. The results represent the mean of three determinations. X: mean value; CV: coefficient of variation. * = vs drying at room temperature ($p < 0.05$, Student's t-test)

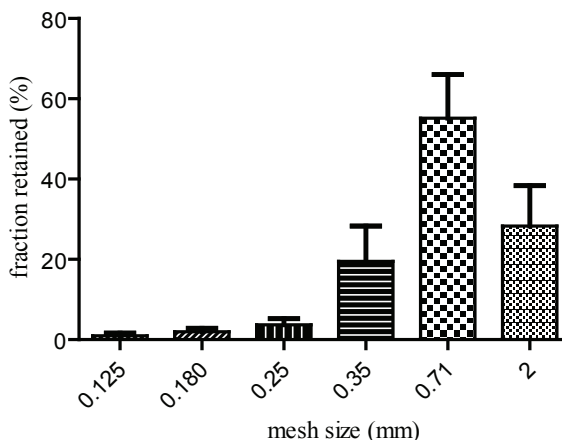


Figure 1. Granulometric distribution of particles of the ground, dried aerial parts of *J. pectoralis*. Screens with mesh sizes of 2.0-0.125 mm were employed. The results obtained from three separate determinations were expressed as the mean± standard deviation.

DISCUSSION

The present work investigated the protocol for preparation, specifically the drying time, of the plant drug of *J. pectoralis*; the latter was also characterized with a view to its use as pharmaceutical raw material.

A plant drug corresponds to a medicinal plant, or its parts, containing the substances, or classes of substances, responsible for a therapeutic effect, following the processes of collection, stabilization, when necessary, and drying; the material may be in its whole form, scored, triturated or pulverized (Anvisa, 2010). When considering plant drugs, the moisture content is a parameter that can interfere considerably in the stability of the active constituents present in the plant, since excessive water in the plant raw material favours the action of enzymes whose activity may lead to the degradation of chemical constituents, as well as enabling the development of fungi and bacteria. Toxigenic fungi, such as those of the *Aspergillus* genus, are xerophilic, which means that they develop even in conditions of low humidity. For this reason the maximum moisture content established in several pharmacopoeias, including the Brazilian, is in the range 8-14 %, with few exceptions given in monographs (Farias, 2007; Yamamoto et al., 2004). In the present work drying the plant in an oven for at least 24 h resulted in the raw material having a moisture content below the maximum recommended limit of 8-14 % (F. Bras. IV, 2000).

In the analysis of the plant drug prepared at room temperature, performed since it is one of the methods most widely employed by Public Phytotherapy Programmes in Brazilian northeast, we observed that the mean moisture content was above 14%. In addition, the plant drug presented a distinct colour (dark green - black) when related to that produced in the oven. While drying at room

temperature took place in a room with a dehumidifier, it is possible that even under these conditions the atmosphere could be saturated with water vapour, so that it could favour the occurrence of fermentative processes in the plant with consequent changes in its organoleptic characteristics.

The size of the particles of a pulverized plant drug directly influences the efficiency of an extraction process; for example, methods that involve filtration of a very fine powder (particle size below 0.125 mm) can compromise the extraction (Prista et al., 1996, Sharapin, 2000; List & Schmidt, 2000). In this context, the moderately coarse powder of "chambá" is advantageous, and the use of powders of this nature is recommended for the majority of plant drugs (Sharapin, 2000).

The measurement of ashes is intended to establish the quantity of non-volatile inorganic impurities. The ash content comprises both the physiological ashes, derived from the plant tissues, as well as non-physiological ashes (F. Bras. IV, 2000; WHO, 1998). Elevated ash contents in aerial material of interest (leaves, inflorescences and flowers) may indicate external material adhered to the surface of the plant, such as sand and silica. In the present work, both the total ashes content and the content of extractable in water or ethanol determined were lower, in relation to the values reported by Govín et al. (2003) for the aerial parts of *J. pectoralis* var. *pectoralis*, cultivated in Cuba. These differences are possibly related to intrinsic

variations among the varieties (*J. stenophylla* and *J. pectoralis*), as well as differences in the metabolism of the plant, which is subject to change according to the habitat, the pattern of rainfall, sunlight, and soil, in other words the climatic-edaphic conditions (Evans, 1996; Blank et al., 2007).

Various chromatographic methods, including high performance liquid chromatography with a photo diode array detector (HPLC-PDA), have been used to separate, identify and quantify chemical markers in plant extracts, which in general have a complex chemical composition, besides the presence of interfering compounds (Wang & Yang, 2007). A previous study (Leal et al., 2000) carried out in our laboratory determined the preliminary phytochemical profile of "chambá", detecting the presence of coumarin among other constituents of the plant. In the present study, analysis of the extract of "chambá" by HPLC-PDA enabled the separation and quantification of the coumarins (1,2-benzopyrone and 7-hydroxycoumarin) present in the plant and showed that the extract produced from the plant drug prepared in an oven possessed a higher concentration of CM in relation to the extract produced from the plant drug prepared at room temperature. By contrast, no significant difference was observed in the UMB content. Similarly, Rocha et al. (2008) showed that the tincture of *Mikania glomerata* ("guaco") formulated from the plant drug produced

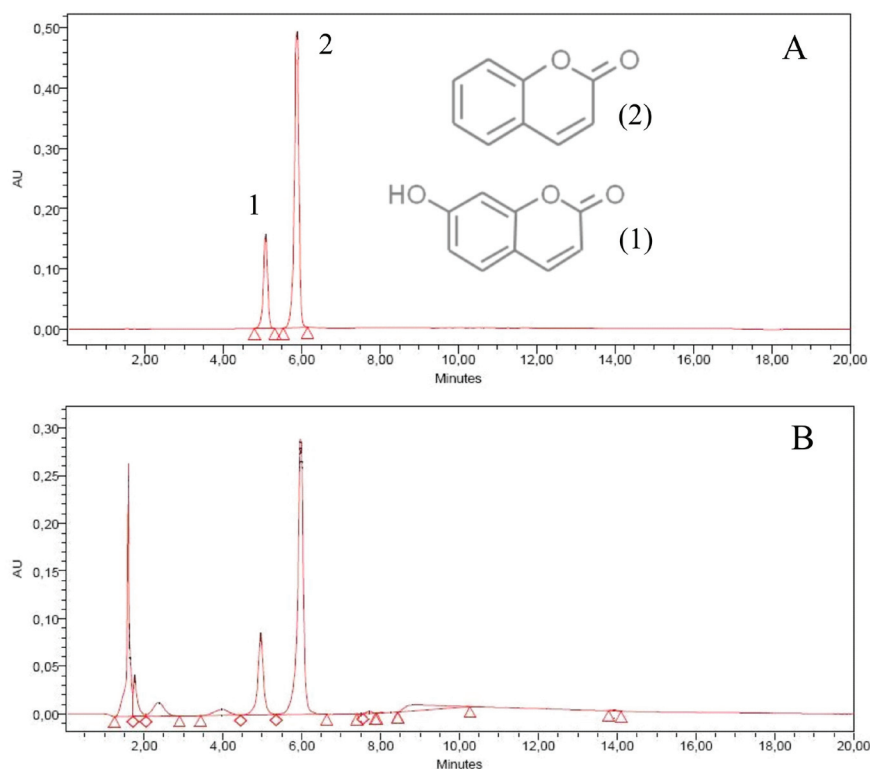


Figure 2. Chromatograms generated by the HPLC-PDA system for coumarin (CM) and umbelliferone (UMB) (A) and for the hydroethanolic extract of *Justicia pectoralis* (B). Conditions: C18 column, mobile phase (A, AcN:MeOH:THF; B, H₂PO₄:Et₃N, pH 3), elution gradient, injection volume of 20 μ L, flow rate 1.8 mL/min and $\lambda=323$ nm.

in an oven had a higher CM content in relation to that produced at room temperature; furthermore, Costa et al. (1998) noted a better visual quality in "guaco" leaves dried in an oven when compared to the leaves dried at room temperature. Given the evidence, the drying of "chambá" in an oven appears to have advantages related to the prevention of possible physical and chemical modifications of the plant (Figure 2).

The results obtained in the present study concerning the preparation and characterization of the plant drug produced from the aerial parts of *J. pectoralis* are highly relevant to the development of phytomedicines and can be recommended as safe parameters for the quality control of *J. pectoralis*, the active plant raw material with potential applications in the production of phytomedicines useful for the National Health Service.

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