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PHYSICAL, CHEMICAL AND PHYSICO-CHEMICAL CONTROL OF *HELIOTROPIUM INDICUM* LINN., BORAGINACEAE, POWDER AND TINCTURE

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ABSTRACT

The quality control of herbal drugs and their extracts is essential when they are used as raw material for the development of herbal medicines. In this work, several techniques (pharmacopoeial and non-pharmacopoeial) were performed, aiming at establishing quality control parameters of the leaves of *Heliotropium indicum* L. (Boraginaceae), a shrub popularly known as stinker (fedegoso) in Brazilian northern and northeastern regions. The results determined the physicochemical characteristics of the drug and the tincture of the leaves. They also showed that the values obtained are in accordance with the data found in the literature for plant material. Preliminary phytochemical analysis of the leaves demonstrated the presence of phenols, tannins and alkaloids. The chromatographic profile showed the presence of a common substance in the hexanic and alkaloidal fractions.

INTRODUCTION: The plants have long represented the only source of therapeutical agent to humans. Along with chemistry development, plants have become the primary source of substances for drug development [1]. Therefore, many companies are looking to incorporate new ingredients to the existing products and to those who still could be developed in future. So, there's a growing search for substances present in plant species which may function as an alternative therapy, and can be as effective as synthetic drugs [2].

Aiming at the market needs and the lack of quality control specifications, this paper emphasizes the study of *Heliotropium indicum* L. (Boraginaceae), a plant popularly known as stinker (fedegoso), which occurs especially in Brazilian northern and northeastern regions. It is a subshrub up to one meter high, widely distributed in Brazililan states. Its leaves are displayed alternate, opposite in the same individual; pedunculate inflorescences; white to purple corolla,mitriforme fruit and ellipsoid, pale and smooth seeds [4]. The leaves,when macerated in water are used in the Amazon region for topical treatment of hemorrhoids, skin disorders and burns [5, 6]. It is also reported that *H. indicum* L. leaves are also used on inflamed areas of the body [7]. These properties are mainly due to the leaves chemical composition, which is represented by pyrrolizidine alkaloids and were

isolated from the whole plant [8]. To these compounds antimicrobial activities were attributed [9]. Other classes of secondary metabolites were found such as polyphenols and tannins, triterpenes and flavonoid s [7, 10, 11]. This study aimed at developing parameters for quality control of the powder and the tincture of *H. indicum* L. leaves

MATERIAL AND METHODS:

Plant Material: The study was conducted with *Heliotropium indicum* L. leaves collected during their maturation in May 2008 in Sao Miguel do Guama – in the State of Para, located at 1°37'36 " S, 47°29'00" W. The voucher specimen is deposited in the Museu Emílio Goeldi Herbarium, Belem-PA, under the registration No. 145574 MG. The harvested fresh leaves were dried in a circulating air oven at a temperature of 40°C until weight stabilization.

Loss on drying of fresh leaves: Exactly 200 g of fresh vegetable drug were placed in a circulating air oven for drying at a temperature of 40°C. This material was daily weighed until constant weight. The results were expressed as loss on drying and / or water percentage by the mean of three determinations according to the Brazilian Pharmacopeia [12].

Grinding of Plant Material: After drying, the leaves were grinded in a knife mill. This process aimed at reducing the particle sizes in order to increase their contact with the extraction liquid .

Determination of Particle Size Distribution: According to the Brazilian Pharmacopeia [12] and with the objective of standardizing the size of the powder obtained from dried leaves of *H. indicum* L., exactly 10 g of ground leaf powder was subjected to a series of sieves with mesh size opening of 0.125mm, 0.18 mm, 0.25 mm, 0.355 mm, 0.710 mm, 1.7 mm, using vibratory sifters for thirty minutes. After this process, the fractions were collected from the sieves and the collector and quantified as to their proportions. Particle size of the powder was assessed by quantifying the percentage of powder retention in each sieve.

Physico and Chemical Characteristics:

Determination of loss on drying of the leaf powder: Samples of 3 g of leaf powder, accurately weighed, were subjected to a temperature of 105°C for 2 hours followed by cooling in a dessicator and weighed. This procedure was repeated until obtaining constant weight. Values are expressed as percentage (w / w), as average of three determinations [12].

Determination of total ash content: Accurately 3g of powdered plant were transferred to porcelain crucibles, which were previously calcined, cooled, and tared under the conditions employed throughout the analysis itself. The samples were charred in a muffle furnace at 450°C for 2 hours. After cooling in a desiccator for 30 minutes the samples were weighed on an analytical balance. The results were expressed as percentage by weight of ash in the dried drug (w/w %) and representing the average of three determinations according to the Brazilian Pharmacopoei a [12].

Extract preparation: The preparation of the hydro-alcoholic extract (tincture) was performed using the method of cold maceration of the leaf powder in ethanol 70 °GL, using the ratio 1:5 (w/v) of solute/solvent, with agitation for 10 days according to the Brazilian Pharmacopoeia procedure [13]. Soon after, the filtration was performed on a fine quality filter paper. The organic solvent was evaporated by using rotary evaporator at 40°C. After the evaporation of ethanol, the extract was lyophilized and stored in a closed amber bottle under refrigeration.

Determination of pH and density of the tincture: ThepH of the herbal tincture was determined in a previously calibrated potentiometer. The bulk density was determined by the pycnometer method. Determinations followed procedures established by the Brazilian Pharmacopeia [12] and the results were calculated by the averages of three determinations.

Determination of Total Dry Residue: Samples of 1 mL of the tincture were transferred to porcelain capsules, evaporated to dryness in water bath and cooled to dry at 105°C. The percentage of dry weight was calculated by the average of individual determinations [14].

Preliminary Phytochemical Analysis: This analysis aimed at establishing the characterization of different classes of secondary metabolites through chemical reactions, following the methods proposed by Barbosa [15]. A qualitative investigation was performed to detect the presence of saponins, reducing sugars, polysaccharides, phenols and tannins, proteins and aminoacids, total flavonoids, cardiac glycosides, catechins, benzoquinones derivatives, naphthoquinones and fenantroquinones, sesquiterpene lactones, alkaloids, purines, steroids and triterpenoids, azulenes, carotenoids, and depsides, depsidones, coumarin derivatives and anthraquinones.

Preparation of the alkaloidal fraction: The alkaloidal fraction was obtained by liquid-liquid partition in a sequence acidic and basic and which treatment consisted of 2.61 g of extract with 50 mL of 5% HCl. The acid filtrate was treated with *n*-hexane aliquots (5x10 mL), resulting in an acid hexane fraction (HF). The resulting aqueous solution was alkalized with NH_4OH (up to pH 10). Then the alkaline aqueous solution was treated with aliquots of $CHCl_3$ (5x10 mL) until obtaining a negative reaction to Dragendorff reagent. The alkaloidal fraction (AF) obtained was washed with distilled water, filtered on anhydrous sodium sulfate, concentrated in a rotary evaporator at low pressure at room temperature.

Chromatographic profiles by TLC: The profiles of the alkaloidal fraction were determined on chromatographic silica gel (Merck ®), using as eluents $CHCl_3/CH_3OH$ (90:10) under a NH_4OH atmosphere. The chromatograms were visualized under visible light, ultraviolet light (254 nm) and after the application of the Dragendorff reagent, followed by 5% HCl.

Chromatographic profiles by HPLC: Analyses of the alkaloidal fraction (3 mg / mL) and the extract of *H. indicum* L. (20 mg/ mL) were carried out by high performance liquid chromatography (Merck Hitachi D-7000 LaChrom®) with diode array ultraviolet detection (UV). An Agilent column LiChrospher 100®, RP8 (5 µm-250 x 3.0 mm) was used, maintained at a temperature of 26°C, in which 20 µL of sample was applied.

The mobile phase consisted of acetonitrile and methanol in a linear gradient from 80% acetonitrile and 20% methanol to 99% acetonitrile and 1% methanol at a rate of 0.75 mL /min for 18 min. The detection was from 200 to 350 nm.

RESULTS AND DISCUSSION: The pharmacognostic profile *H. indicum* L. was determined according to the requirements of Resolution RDC No.48/2004 of the National Health Surveillance Agency [16]. Although there's a lack of quality control studies it intends to contribute with the precepts of quality for the proposed herbal drug [16].

The leaves were kept in air-circulating oven at a temperature of 40°C for four days, when they reached constant weight. The result of the loss on drying of the leaves was 98.79% (**Table 1**). This result is important because it prevents the material from remaining humid due an inefficient drying, which also causes the growth of pathogenic microorganisms.

Determination of particle size distribution: After drying and grinding of the plant material, particle size distribution of *H. indicum* L. powder was determined. The powder was classified as thick. The particles passed entirety through the 1.7 mm mesh size opening sieve and less than 40% passed through the 0.355mm sieve [12] (**Figure 1**). It is an important parameter for choosing the appropriate extraction process and the solvent used, since it has a significant influence on its efficiency as different particle sizes can affect the filtration [17].

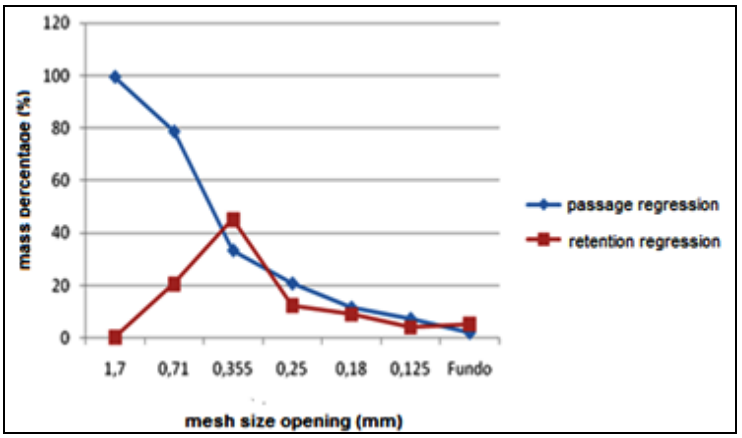

FIG. 1: DETERMINATION OF PARTICLE SIZE DISTRIBUTIONS OF *HELIOTROPIUM INDICUM* L. LEAF POWDER

Physico and Chemical Characteristics: Determination of loss on drying of the leaves of H. indicum was performed at 105°C, and showed a value of 12.88% (Table1), indicating the percentage of residual moisture in plant drugs. This value is within the range established by the Brazilian Pharmacopeia 12 (maximum of 14% for herbal drugs), which points out for a good preservation and drying of vegetable raw material18. The importance of this result is the fact that it ensures the microbiological and chemical stability of the plant drug. Moisture levels above the standards favor the proliferation of fungi and bacteria and a possible degradation of chemicals by hydrolysis processes even in a short period [14]. So, the leaves of the species examined showed satisfactory results for the loss on drying assay.

As for the total ash content, the value found was 17.14% (**Table 1**), which corresponds to the amount of non-volatile residual substance resulting from the incineration process. In the case of herbal drugs, the main objective of this type of assessment is to check non-volatile inorganic impurities present in the analyzed material [12].

The herbal tincture of H. indicum L. presented neutral feature (pH 7.81) and density of 0.895 g/cm^3 (**Table 1**). The pH value represents an important data concerning the stability of intermediate formulations and the choice of the adjuvant used in the final formulation [14]. The density of the tincture remained within the limit recommended for herbal tinctures (0.87 and 0.98 g/cm^3) [19]. The percentage of dry residue of the tincture was expressed at 1.44% (w/ v). This assay involves the quantification of substances extracted from the plant by removing the extraction solvent [20].

TABLE 1: PHYSICAL, PHYSICO-CHEMICAL AND CHEMICAL ANALYSIS OF THE POWDER AND THE TINCTURE OF *H. INDICUM* L. LEAVES

Parameters	Results obtained
Loss on drying	12,88% ± 0,046%
Total ash content	17,14% ± 0,726%
pH	7,81
Aparent density	0,895 g/cm^3
Dry residue	1, 44%
Preliminary phytochemical screening	Reducing sugars, phenols, catechists tanines, carotenoids, proazulenes, alkaloids

Phytochemical Analysis: Medicinal plants produce different chemicals and do it in different proportions, depending on the habitat and climatic-soil conditions. So, some chemical compounds are quite specific for a particular plant and therefore can be a parameter for its characterization and identification [21]. Data from the preliminary phytochemical analysis performed with the leaves of *H. indicum* L. revealed the presence of phenols, tannins, carotenoids and alkaloids (the later ones only in alkaloidal fraction) (**Table 1**).

These results corroborate the earlier classes of secondary metabolites reported in the literature for this species [7, 22]. The herbal tincture showed no reaction for alkaloids, but the alkaloidal fraction suggested a red- orange colored precipitate when reacted with Dragendorff reagent. TLC analysis showed the presence of a substance in the acid- hexane fraction with the same value of the retention factor of the alkaloidal fraction (Rf = 0.84) (analyzed in the same chromatographic conditions).

Since this common substance in the fractions reacts with Dragendorff reagent when applied to CCD, and the fact that the Boraginaceae family possesses species rich in alkaloids, it is suggested that this substance is an alkaloid.

Chromatographic profiles by HPLC: According to the literature, the leaves of H. indicum L. are characterized by the presence of alkaloids [11, 22]. This information justifies the characterization by HPLC of the tincture and the alkaloidal fraction for this class of metabolites.

The analysis of the chromatogram of the extract of *H. indicum* L. (**Figure 2A**) showed a peak with a retention time of 1.60 min and purity of 0.9607, which was also reproduced both in the alkaloidal and the hexanic fractions with Rt =1.73 min and purity of 0.9621and 0.914, respectively (**Figure 2B and 2C**).

Therefore, the substance that appears in the alkaloidal fraction may also be present in the hexanic fraction, which had already been observed by TLC analysis.

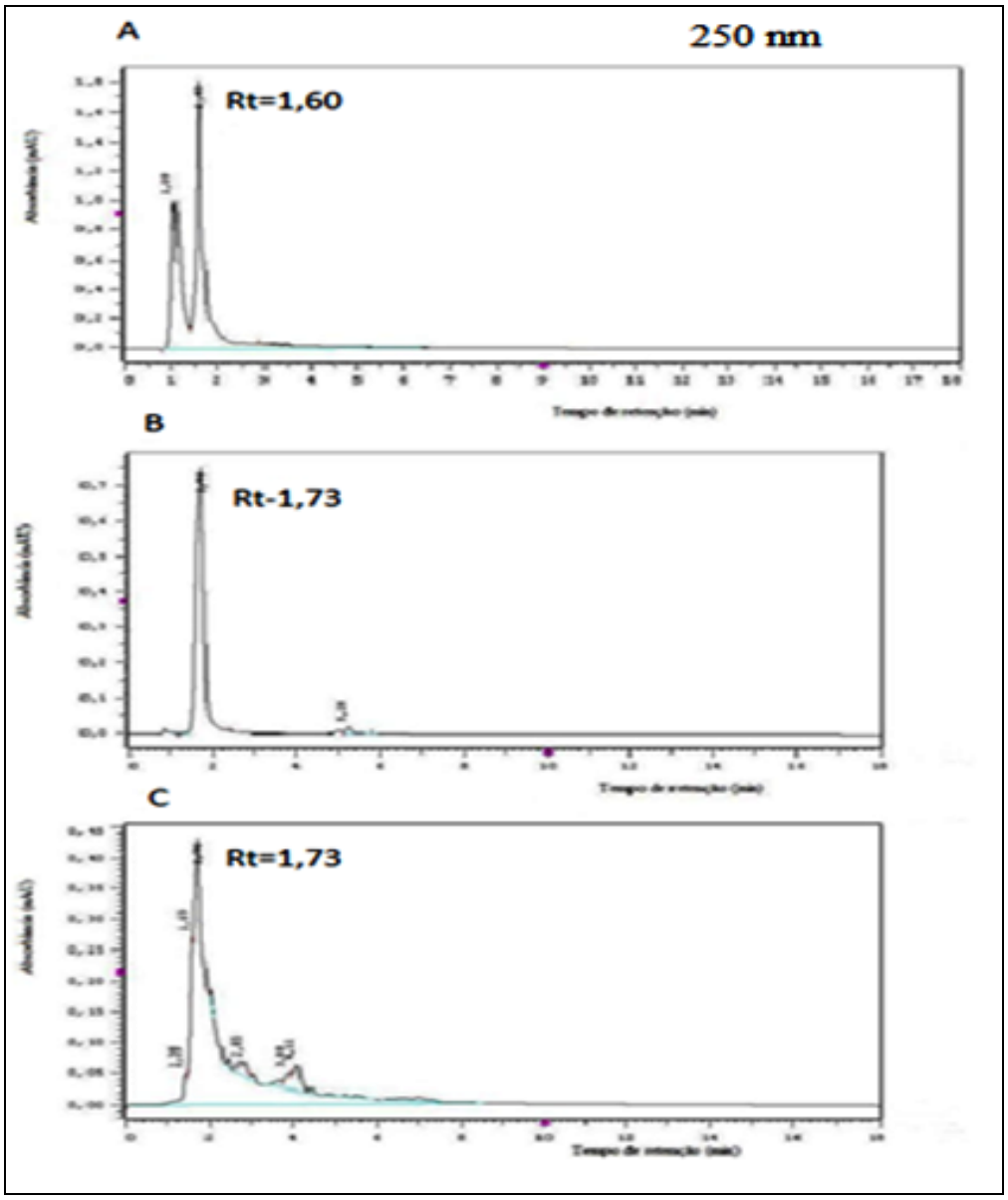

FIG. 2: CHROMATOGRAPHIC PROFILES BY HPLC- DAD/UV OF H. INDICUM L. BRUTE EXTRACT

CHROMATOGRAMES: A- BRUTE EXTRACT (1, 60 MIN); B- ALKALOIDAL FRACTION (1, 73 MIN), C- HEXANIC FRACTION (1, 73 MIN)

CONCLUSION: The powdered plant material of H. indicum presented satisfactory physical, physico-chemical and chemical characteristics. Many of the results are in accordance with the preset parameters in the literature for drugs and plant extracts. So, the methods used were suitable to evaluate the quality of the plant drug, and all steps used in this study are important parameters for quality control of the leaves of *Heliotropium indicum* L.

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