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Obtainment and characterization of raw material of *Brosimum gaudichaudii* Trecul (Moraceae)

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ABSTRACT

Brosimum gaudichaudii Trecul (Moraceae) is popularly known as mamacadela. Studies have shown the presence of psoralen and bergapten in roots. It is widely used in the treatment of depigmentation of the skin known as vitiligo and psoriasis. These compounds can be obtained by chemical synthesis, However it is an expensive process, the compound obtaining. Thus occurs through the extraction of species *B. gaudichaudii* were evaluated quality parameters of raw material, such as particle size distribution, moisture content, swelling index, Investigation and quantification of the presence of the Psoralen and bergapten and Optimization of the extraction method of psoralen and bergapten. For the hydroalcoholic extract was evaluated; pH, relative density, viscosity, solid content, investigation and quantification of the presence of the psoralen and bergapten.

Keywords: Furanocoumarins extraction, medicinal plants, vitiligo.

INTRODUCTION

*Brosimum gaudichaudii*Trecul (Moraceae) is popularly known as mamacadela. Studies have shown the presence of psoralen and bergapteno in roots that are widely used in the treatment of depigmentation of the skin known as vitiligo and psoriasis [1-6].These compounds can be obtained by chemical synthesis, however it is an expensive process, thus obtaining the compound occurs through the extraction of species *B.gaudichaudii*[6-8]. The objective of this research was to determine parameters concerning the characterization and quality control of *B.gaudichaudii* roots powder and development of a standardized hydroalcoholic extract.

MATERIAL AND METHODS

Plant material

Roots of *B.gaudichaudii* were collected from the Jussara-Goiás - Brazil (13 °43'08.04 "S and 50 °31'44.98" W. The elevationis 332m). The species was identified by Dr. José Realino de Paula by comparing the characteristics described in botanical literature[6-7] . The voucher specimen have been deposited in the Herbarium UFG (n 45,517). The roots were dried at 40°C in forced ventilation and fed into a mill knife.

Characterization of the plant material

Determination of particle size distribution

This test was performed according to the Brazilian Pharmacopeia, 5th edition. It was also utilize a sieve shaker (Bertel) with sieves 710, 355, 300, 250, 150, 106-mesh opening size.[9]

Determination of moisture content

This assay was realized in Karl Fischer titration using instrument Titrando 851®-Metrohm.

Intumescence index

The intumescence index of this plant material was determined according the Brazilian Pharmacopeia, 5th edition.[9]

Investigation of the presence of the Psoralen and Bergapten

The presence of psoralen and bergapten was assessed by thin layer chromatography (TLC). It was used silica gel chromatography plates (60 F254 G - 0.25 mm-Merck®). 50 µL extract and standards solutions (Sigma-Aldrich®5mg/mL) were applied to the origin of the plate. The mobile phase was dichloromethane: diethyl ether acetic acid 10%(v/v) (05:01:05 v/v). The furanocoumarins stains were viewed under UV light at 365 nm and the retention factor (Rf) of the sample and standard were calculated and compared.

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Optimization of the extraction method

Optimization of extraction of water-alcohol furanocoumarins (psoralen and bergapten) was performed using 1 g of plant drug extracted in 100 mL of solvent mixture ethanol: water in proportions relative to ethanol 40, 50, 60, 70, 80 and 90%. The alcoholic extracts of each were made in triplicate, carried ultrasound for 30 minutes, filtered and analyzed by HPLC.

Preparation of plant extract

The powder of *B.gaudichaudii* was extracted by percolation (flow: 0,5mL.min^{-1}), water-alcohol solution (30-70 v/v). The extract was concentrated on rotavapor (Buchi® – model R-220 SE).

Characterization of the extract

Determination of pH

Determination of pH was carried out in pH meter (Tecnal®–model TEC 3-MP), previously calibrated with buffer solutions.

Determination of relative density

This assay was realized according the methodology of picnometer described in Brazilian Pharmacopeia, 5th edition.[9]

Determination of viscosity

The viscosity was measured using a viscometer (Brookfield® – model DV-III).

Determination of solid content

The solid content was determined using a balance with halogen lamp (Ohaus® – model MB45).

Characterization of the extract by HPLC

Analyses were performed using a chromatographic system from Waters® ,model HPLC- Alliance, separation module e2695, diode array detector e2998 (PDA) and data processing system Enpower 3. The chromatographic separations were conducted in column Luna 5µm,C8, 250X4.6 mm (Phenomenex®).

The mobile phase used was a mixture of acetonitrile and ultrapure water (45:55v/v) under isocratic flow of 0.6 mL.min.$^{-1}$ for 30 min. Detection wavelength was 244 nm to Psoralen and 220 nm to Bergapten. The analytical method was validated according to guideline 899/2003 from National Agency for Sanitary Surveillance of Brazil (ANVISA)[10].

RESULTS AND DISCUSSION

The results for characterization of raw material and hydroalcoholic extracts are described in Tables 1 and 2.The size analysis of ground plant material is an important parameter to be established, it represents a direct influence on the extraction efficiency of the process. The particle size analysis showed that less 40%(v/v) of roots powder passed through 355µm mesh, characterizing it as a coarse powder according to the Brazilian Pharmacopoeia 5th[8]. According List et al[11], which makes the drug spray particles larger than the classification of fine is more suitable for the extraction processes.

The values for volatile content is below the limit specified by Oliveira et. al [12] of 8-14% (v/v) for raw vegetables, excess moisture in the plant tissue is

Table 1: Evaluation of quality control parameters of B.gaudichaudii L. roots powder

Test	Results
Total ash content	5.87% ± 0.06(w/w)
Acid insoluble ash content	2.15% ± 0.07(w/w)
Particle sinze distribution	326,6µm
Moisture content	8.3 ± 0.09% (w/w)
Intumescence índex	6.06 ± 0.057 mL
Thin layer chromatography	$R_{fp=}$ 7,4 and $R_{fb=}$ 7,9
Hight perfomace cromatography	R_{fp}= 12,6 min and R_{fb}=16,2min
Quantification by HPLC	T_p =0,039±0,017(w/w) and T_b 0,18±0,005 %(w/w)

R_{fp} = retention time of psoralen
R_{fb} = retention time of psoralen
T_p=psoralen content
T_b=psoralen content

Table 2: Evaluation of quality control parameters of B.gaudichaudii L. hydroalcoholic extract.

Test	Results
pH	5.87% ± 0.06(w/w)
Density	0,930% ± 0.0002(w/w)
Viscosity	3,33± 0.021 mPas
Solid content	5,47%± 0,01(w/w)
Thin layer chromatography	$R_{fp=}$ 7,4 and $R_{fb=}$ 7,9
Hight perfomace cromatography	R_{fp}= 12,6 min and R_{fb}=16,2min
Quantification by HPLC	T_p =1,09%(w/w) and T_b 1,89%(w/w)

R_{fp} = retention time of psoralen
R_{fb} = retention time of psoralen
T_p=psoralen content
T_b=psoralen content

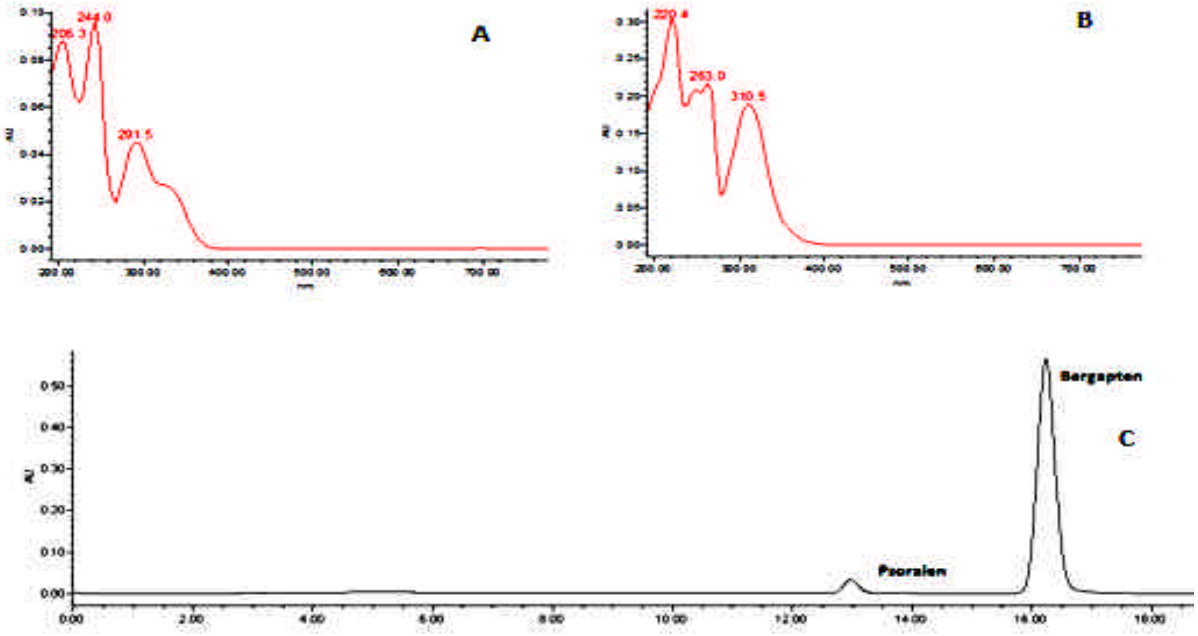

Figure 1: Spectrum of psoralen (A), Bergapten (B) and HPLC-PDA chromatograms of standard (C)

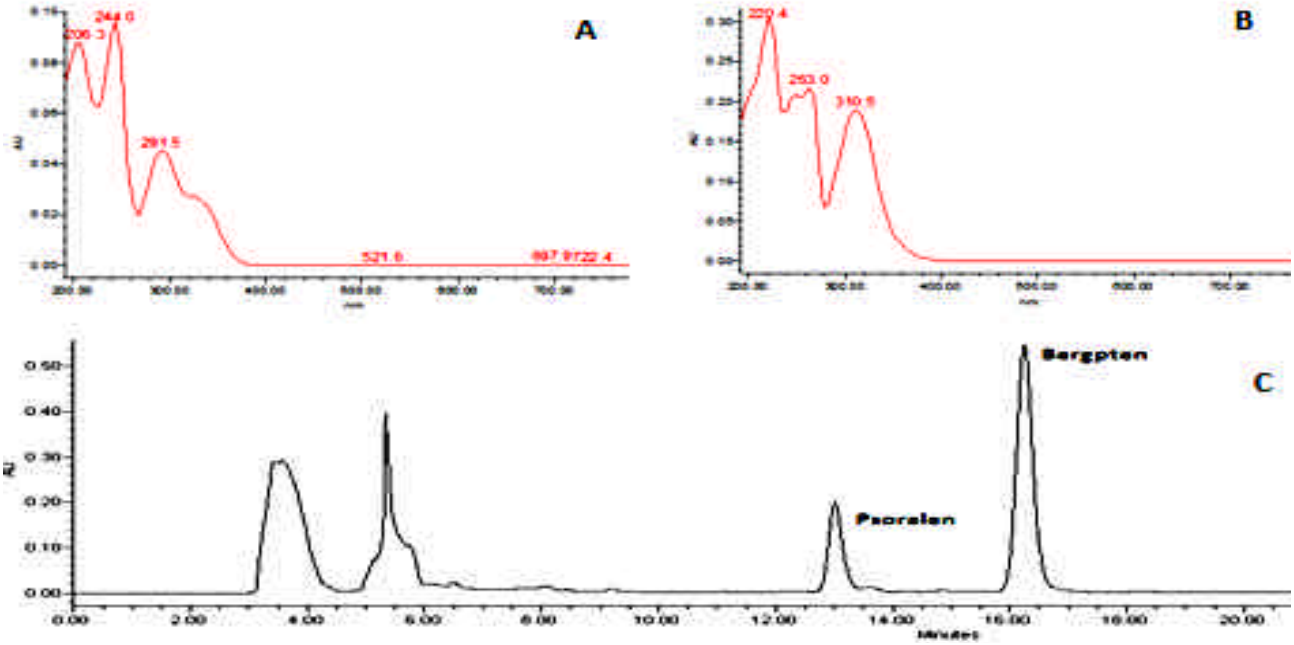

Figure 2: Spectrum of psoralen (A), Bergapten (B) and HPLC-PDA chromatograms of hydroalcoholic extracts (C)

Tabela 3: Extraction of Psoralen and bergapten in different proportions alcoholic.

Proportion of alcoholic	Psoralen content	Bergapten content
40%	0,037±0,0015	0.124±0,010
50%	0,036± 0,0025	0,144±0,014
60%	0,030±0,0032	0,125±0,014
70%	0,039±0,0017	0,186±0,042
80%	0,040±0,00173	0,174±0,01
90%	0,031±0,0036	0,130±0,017

linked to the microbiological stability of the drug, as an expression of their susceptibility the development of bacteria and fungi, and chemical stability, represented especially by hydrolysis processes[13].The moisture content can be reduced, provided that there is standardization in the parameters of planting, gathering, storage and pretreatment drug use. The loss on drying can provide data about the performance of extraction, since the drying affects the state of integrity of cellular structures by exposing them to more or less contact with solvents [14] . From the technological point of view and production, it is important to know quantitatively the content of water in the vegetable raw material, so that this amount is considered income in the calculations.

The intumescence index may indicate the presence of mucilage[15]. This parameter is important for predicting the extraction process, the information obtained in this test can predict the amount of solvent to be used in the extraction process and the dimension of the percolator.

Analysis by TLC revealed the presence of psoralen and bergapteno the raw material. It was possible using this technique to obtain the separation of two substances of interest, Figure 1 and 2.

The optimization study for the extraction process of furanocoumarins showed that the proportion of alcohol 70%(v/v) and 80%(v/v) extracted in greater quantity, Table 3. The values found in this study contradicts the findings of Celeghini et al [16], who worked with an alcohol concentration of 50%. The difference between the kind employed in both studies could be an explanation for this difference.

The analytical method used in the analysis of qualitative and quantitative furanocoumarins proved to be practical and able to separate the two substances of interest. However because it is different substances have been working with two different wavelengths, being 244 nm for the detection of psoralen and 220 nm for detecting bergapten. (Figure 1 and 2)

In Conclusion, the results show that it is possible to establish security parameters of quality raw material and to hydroalcoholic extract standardized. This work is most useful for the preparation of a monograph to describe the parameters of quality for products from *B. gaudichaudii*.

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