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Physical-chemical characterization of the medicinal plant and extraction solution based on Clarisia racemosa for the development of an antimicrobial herbal medicine

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ARTIGO ORIGINAL

Abstract

Clarisia racemosa, popularly known as "Guariúba", is a species of the Moraceae family, in Brazilian soils, this species can be found mainly in the Amazon rainforest. Currently, its commercialization is focused on the use of its wood in civil construction. Its chemical composition and therapeutic properties are still little studied in the literature. A study carried out previously, the *C. racemosa* species showed antimicrobial potential against gram-positive bacteria, especially *Staphylococcus aureus*. In this context, the aim of this work was to analyze the physicochemical characteristics of the pulverized plant drug obtained from the stem of *C. racemosa* and its ethanolic extract, for the future development of antimocrobial herbal medicines. In this context, the aim of this work was to analyze the physicochemical characteristics of the pulverized plant drug obtained from the stem of *C. racemosa* and its ethanolic extract, for the future development of antimocrobial herbal medicines. The ethanolic extract was obtained by maceration with 70% ethanol as the extracting solvent, after which pH, alcohol content, dry residue, density and phytochemical profile tests were carried out using thin layer chromatography. In the characterization of the plant drug, the powder was classified as semi-fine. The results of the ash content, foreign matter and moisture analysis were 0.82%, 0.002% and 7.13%, respectively. The ethanolic extract had the following values: density of 0.87g/mL, alcohol content of 71%, pH 5.93 and dry residue of 1.05%. Phytochemical prospecting revealed the presence of characteristic bands for cinnamic derivatives, condensed tannins and terpenes. In this way, this study evaluated the quality control parameters of the pulverized plant drug from the stem of *C. racemosa*, where the results were within the required parameters. The physicochemical tests contributed to the characterization of the plant drug and the extractive solution of *C. racemosa*, as well as to the future standardization of the dry extract, which met the standards required by the Brazilian Pharmacopoeia.

Keywords: Moraceae. *Clarisia racemosa*. Plant drug. Medicinal plants.

Caracterización físico-química de la planta medicinal y solución de extracción basada en Clarisia racemosa para el desarrollo de un medicamento herbal antimicrobiano

RESUMEN

Clarisia racemosa, conocida popularmente como «Guariúba», es una especie de la familia Moraceae que, en suelo brasileño, se encuentra principalmente en la selva amazónica. Actualmente, su comercialización se centra en el uso de su madera en la construcción civil. Su composición química y sus propiedades terapéuticas aún están poco estudiadas en la literatura. En un estudio realizado anteriormente, la especie *C. racemosa* mostró potencial antimicrobiano contra bacterias gram-positivas, especialmente *Staphylococcus aureus*. En este contexto, el objetivo de este trabajo fue analizar las características fisicoquímicas del medicamento vegetal pulverizado obtenido del tallo de *C. racemosa* y su extracto etanólico, para el futuro desarrollo de medicamentos herbales antimicrobianos. En este contexto, el objetivo de este trabajo fue analizar las características fisicoquímicas del medicamento vegetal pulverizado obtenido del tallo de *C. racemosa* y su extracto etanólico, para el futuro desarrollo de medicamentos herbales antimicrobianos. El extracto etanólico se obtuvo por maceración con etanol al 70 % como disolvente de extracción, tras lo cual se realizaron pruebas de pH, contenido de alcohol, residuo seco, densidad y perfil fitoquímico mediante cromatografía en capa fina. En la caracterización del medicamento vegetal, el polvo se clasificó como semifino. Los resultados del análisis del contenido de cenizas, materias extrañas y humedad fueron 0,82 %, 0,002 % y 7,13 %, respectivamente. El extracto etanólico presentó los siguientes valores: densidad de 0,87 g/mL, contenido de alcohol del 71 %, pH de 5,93 y residuo seco del 1,05 %. La prospección fitoquímica reveló la presencia de bandas características de derivados cinámicos, taninos condensados y terpenos. De esta manera, este estudio evaluó los parámetros de control de calidad del medicamento vegetal pulverizado del tallo de *C. racemosa*, cuyos resultados se encontraban dentro de los parámetros requeridos. Las pruebas fisicoquímicas contribuyeron a la caracterización del medicamento vegetal y la solución extractiva de *C. racemosa*, así como a la futura estandarización del extracto seco, que cumplía con los estándares exigidos por la Farmacopea Brasileña.

Palabras clave: Moraceae. *Clarisia racemosa*. Medicamento vegetal. Plantas medicinales.

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INTRODUCTION

The use of herbal medicines has gradually increased over the years and, due to their medicinal effects, their use has become an important therapeutic resource worldwide (Leal; Tellis, 2016). Faced with a growing resistance of microorganisms to current synthetic drugs, the search for medicinal plant-based products has become a good source of new antimicrobial agents (Amparo *et al*., 2018).

In this scenario, there has been a surge of interest in studying the active principles of natural substances and their applications (Simões *et al*., 2016). In this respect, Brazil, as a country with a vast reserve of plant species, contributes to the advancement and development of new plant-based products (Assis; Morelli-Amaral; Pimenta, 2015).

Many Brazilian states have a large part of the planet's biodiversity and with the aim of ensuring that the entire population has access to natural and herbal products in a safe and rational way, the National Policy for Medicinal Plants and Herbal Medicines was created in 2006 (Macedo, 2016). This policy, implemented by the Ministry of Health, is present in around 930 Brazilian municipalities (Brasil, 2016).

Among the species of Brazilian flora is *Clarisia racemosa*, which was first described by Ruiz & Pav in 1794 (Lanjouw, 1936). It is popularly known as “guariúba” and belongs to the Moraceae family. The plant, which reaches a height of around 40m, is very common in the state of Amazonas, but is also found in other Brazilian states, such as Pernambuco, southern Bahia, Minas Gerais and Espírito Santo (Loureiro; Lisboa, 1979).

This species is widely used in the commercialization of valuable wood, in the diet of animals that eat its fruit and in the fight against dermatological diseases (Melo, 2015; Santos, 2008). As a result of its exploitation, its presence can be considered an indicator of the environmental quality of the flora of São José da Coroa Grande, in Pernambuco (Lucena, 2009). Due to the exploitation and removal of trees from their native area, the genetic conservation of *C. racemosa* is very important (Santos, 2008).

Despite Brazil's abundant biodiversity, as well as the emergence of new techniques and methodologies for studying plant species, few studies have been

published on the *C. racemosa* species. Information on its therapeutic properties, as well as its bioactive compounds, is still quite limited in the literature.

In a pharmacological study carried out by our research group, it was observed that the *C. racemosa* species has antimicrobial potential against gram-positive bacteria, especially *Staphylococcus aureus*, where its minimum inhibitory concentration and minimum bactericidal concentration was 15.62 µg/mL..

In this context, studying the physicochemical characterization of the plant drug based on *C. racemosa* is very important to know the characteristics of the raw material used, in order to promote the future technological development of an antimicrobial herbal medicine with proven quality.

METHODOLOGY

2.1 Collecting and Obtaining the Plant Drug

The stem of the Clarisia racemosa species from the Forest Management Unit (Chain of Custody) was sent to INPA (National Institute for Amazonian Research) for identification by the company Mil Madeiras Preciosa Ltda, which is located in the municipality of Itacoatiara (Latitude 3°03'00.6“S and Longitude 58°43'04.1”W), in the metropolitan region of Manaus, in the state of Amazonas. The company has five production lines, where in each area a single species is cut each week.

The residue of the botanical material collected was transported to the Institute of Exact Sciences and Technology (ICET) located at UFAM (Federal University of Amazonas). The material was laid out in thin layers and then dried in a circulating air oven at a temperature of 45ºC controlled by a thermostat to ensure homogeneity. The final drying process was defined when the constant weight of the wood waste was obtained using an analytical scale. These residues were then ground in a knife mill, weighed and subsequently identified for storage in plastic bags.

2.2 Characterization of the Plant Drug

2.2.1 Particle size determination

For particle size determination, the Brazilian Pharmacopoeia, 5th ed. (Brazil, 2010) was adapted. 25g of the plant drug was weighed and six sieves with different openings were selected, where the sieve with the largest opening was placed on top of the others with the smallest opening. The mesh openings chosen were 850, 600, 425, 250, 150 and 75 µm.

The pulverized plant drug was evenly distributed on top of the sieve with the largest opening and then the sieve and collector were placed on top of a vibrational apparatus (Bertel®, model 2499) for 15 minutes. After this process, the fractions retained in each sieve and in the collector were weighed on a Shimadzu® analytical balance.

The percentage of the plant drug retained in each sieve was calculated using the formula below:

$$\% \text{ retained by the sieve} = (P1/P2) \times 100$$

Where: P1 = Weight of the sample retained in each sieve (in grams); P2 = Sum of the weights retained in each sieve and in the collector (in grams); 100 = Percentage factor.

2.2.2 Moisture determination (desiccation loss)

Moisture was determined according to the methodology described in the Brazilian Pharmacopoeia, 5th ed. In triplicate, approximately 2g of the plant drug was weighed out on previously desiccated filter weighers. They were then placed in an oven at a temperature of approximately 105ºC for 2 hours to dry. The filter weighers were then cooled in a desiccator for subsequent weighing. This operation was repeated until a constant weight was obtained.

2.2.3 Determination of total ash content

To determine the total ash content, around 3g of the plant drug was weighed and transferred to a crucible which had been previously desiccated at 200ºC for 30 min. The plant drug was distributed in the crucible and placed in a muffle furnace (Quimis®) for incineration, where a temperature gradient was used (30 min at 200ºC, 60 min at

400ºC and 90 min at 600ºC), until white or grayish ash was obtained.

After 3 hours in the muffle furnace, the crucible was placed in the desiccator to cool and then weighed to calculate the total ash, using the percentage of ash in relation to the air-dried drug. This procedure was carried out in triplicate, following the methodology of the Brazilian Pharmacopoeia (Brazil, 2010).

2.2.4 Determination of foreign particles

The methodology was adapted from the Brazilian Pharmacopoeia, 5th edition (Brazil, 2010). To carry out this procedure, around 50g of the plant drug was weighed in triplicate, spread out on a flat surface and separated by quartering. The foreign elements were separated from the material using tweezers, retained in a beaker and then weighed.

2.2.5 Thermogravimetry

The TG curves of the plant drug C. racemosa were obtained using a simultaneous thermobalance (TG/DTA) of the Shimadzu® equipment, model DTG-60H at a heating rate of 10ºC.min-1, in a temperature range from 25ºC to 600ºC, under an ultrapure nitrogen flow of 100mL.min-1. A mass of approximately 5 ± 0.1mg of the material was used and transferred to a platinum crucible. The calcium oxalate standard was used to calibrate the equipment. The curves obtained were processed using TA 60 software. This procedure was carried out following the methodology of Silva (2019).

2.3 Ethanolic Extract Obtained and Characterized

2.3.1 Determination of ethanolic extract

The extract was made by maceration, where ± 50g of the pulverized plant drug was weighed and extracted with 500mL of the extracting solvent, 70% ethanol, in a ratio of 1:10 (m/v) for approximately 7 days, in a closed glass container, at room temperature and protected from light. The material was then filtered to obtain the ethanolic extract.

2.3.2 Determination of alcohol content

To determine the alcohol content, the reading was taken using an Incoterm® Gay Lussac and Cartier 20ºC alcoholmeter, in which the solution was transferred to a beaker and the alcoholmeter was immersed, thus measuring the alcohol content in the *C. racemosa* solution using the Gay Lussac and Cartier scale. The procedure was carried out following the methodology of the National Formulary of the Brazilian Pharmacopoeia 2nd ed.

2.3.3 Determining pH

The pH was read using a pH meter (POT®-003), previously calibrated with a buffer solution. This procedure was carried out in triplicate. The pH was determined by directly immersing the electrode in the samples (Brasil, 2010).

2.3.4 Density determination

The methodology used was based on the Brazilian Pharmacopoeia 5th edition (BRASIL, 2010). A previously calibrated pycnometer with a volume of 25 mL was used (the empty pycnometer was weighed and then the weight of the pycnometer was obtained with distilled water). The ethanolic extract of *C. racemosa* was then transferred to the pycnometer and weighed. The weight of the sample in grams was obtained from the difference in mass between the full and empty pycnometer. The whole procedure was carried out in triplicate.

2.3.5 Determination of dry residue

The procedure was carried out in triplicate, using previously weighed filters, where 2mL of the ethanolic extract based on *C. racemosa* was weighed and then evaporated in a water bath until dry. With the oven (Ethik Technology®) already stabilized at 100-105ºC, the sample was placed for 3 hours to obtain the dry residue. Finally, the material was placed in a desiccator for 30 minutes to cool and then the filters were weighed. The dry residue was calculated as a percentage of the mass (Brasil, 2010).

2.3.6 Phytochemical prospecting

The characterization of the phytochemical profile of the ethanolic extract of *C. racemosa* was carried out by thin layer chromatography (DLC), following the methodology of Wagner and Bladt (1996), at the Center for the Analytical and Technological Development of Herbal Medicines (NUDATEF/UFPE).

The ethanolic extract of C. racemosa, previously prepared and stored in an eppendorf, was used as a sample. All the standards used were previously prepared at a concentration of 1mg.mL-1 in methanol. The sample and standards were applied to silica gel 60 - F254 chromatography plates. The samples were applied at a distance of 0.5cm from the origin and end of the plate. The samples and standards were then placed in a vat and eluted to saturation with the corresponding mobile phase for 15 minutes at room temperature. Finally, the plates were observed under ultraviolet light at 254nm and 365nm and visible light to detect the presence of possible metabolites.

The chromatographic bands of the sample observed on the plates were compared with the bands of the corresponding standards. To do this, the Retention Factor (Rf) was calculated for each sample and standard used, following the equation below.

$$Rf = dc / ds$$

Where: dc = distance traveled by the sample; ds = distance traveled by the elution system.

The systems and developers used in the phytochemical prospecting can be seen in Table 1. The classes of secondary metabolites tested, as well as the standards used, can be seen in Table 2.

RESULTS AND DISCUSSION

3.1 Characterization of the Plant Drug

3.1.1 Powder granulometry

The pulverized plant drug was subjected to particle size analysis in order to obtain data on the average size of the *C. racemosa* powder. The results obtained are described below (Table 3 and Figure 1).

The particle size distribution analysis showed that the *C. racemosa* plant drug was retained in greater quantities on the 425µm and 250µm sieves. According to the Brazilian Pharmacopoeia, a powder is classified as semi-fine when all its particles pass through the sieve with a nominal mesh opening of 355µm and a maximum of 40% through the sieve with a nominal mesh opening of 180µm (BRASIL, 2010). As the 355µm mesh is one of the meshes in which the powder is retained the most, it is recommended that the powder be of the semi-fine type.

Another type of particle size classification has been observed for different species of the Moraceae family. According to Noronha (2014), who analyzed the average particle size of *Ficus pumila L*., it was found that the plant drug obtained from the stem of this species is classified as a moderately coarse powder. Studies carried out by Guizzo and colleagues (2015) show that *Morus nigra L.* leaves are also classified as a coarse powder. It can therefore be seen that the *C. racemosa* species differs in the average size of the powders compared to other species in its family.

The particle size of powders is an important index for determining the contact surface that a plant drug has available to interact with an extracting solvent to obtain liquid solutions, such as tinctures or extracts (Correia; Macedo, 2011)..

3.1.2 Moisture determination (desiccation loss)

The value obtained as a percentage of loss due to desiccation of the *C. racemosa* stem was 7.13% ± 0.11. According to the Brazilian Pharmacopoeia, the maximum moisture limit for plant drugs is between 8-14%. Therefore, the plant drug is within the pharmacopoeial limits (Brasil, 2010).

The *Morus nigra L*. and *Morus alba L*. species, also belonging to the Moraceae family, studied by Guizzo *et al*. (2015) and Pereira *et al*. (2011), respectively, showed water loss values close to those found in *C. racemosa*, where the first species obtained a value of 7% and the second between 8% and 12%, corroborating the data obtained in this study.

It is worth noting that this method is extremely important for determining the amount of volatile substance present in a raw material, since changes in moisture content can influence the quality of the final product (Oliveira; Hellmeister; Tomazello,

2005).

3.1.3 Total ash content

With regard to the ash content, it was possible to observe that in 3g of the pulverized plant drug sample, around 0.0248g was inorganic matter, which corresponds to 0.82% ± 0.00736 of total ash. This result is within the range recommended by the Brazilian Pharmacopoeia, which allows a maximum of 8% (Brasil, 2010).

Silva *et al*. (2009) obtained results very close to those of this study. They analyzed the ash content of various wood species and obtained total ash values of between 1.8% and 0.3%, with *C. racemosa* being among these species.

Determining the total ash content allows the quantification of non-volatile inorganic residues present as a constituent or contaminant in a vegetable (Couto *et al*., 2009).

3.1.4 Determination of foreign matter

According to the Brazilian Pharmacopoeia, plant drugs must be free of foreign matter such as fungi, insects and other contaminants of animal origin (Brazil, 2010). The result obtained in the determination of foreign material in a 50g sample of the plant drug was 0.002% ± 0.0009, and it was possible to see a relatively low amount of particles present with the naked eye.

Studies carried out by Pereira *et al*. (2011) showed a small amount (around 0.02%) of foreign matter in the plant drug *M. alba* (a species belonging to the Moraceae family). The plant drug is within the pharmacopoeial parameters, which set a maximum limit of 2% for foreign matter. This value shows that the material underwent good quality controls when it was selected and processed (Fonseca, 2005).

In order to guarantee the production of a good herbal product, it is of fundamental importance that the plant material is free of impurities, guaranteeing the quality of the final product (Toledo *et al*., 2003).

3.1.5 Thermogravimetry

In the analysis of the thermogravimetric curve, three events are observed (Figure 2). In the first event, which occurs in the temperature range between 39°C and 75°C, there is a mass loss of 7.063%, which can be evidenced by a loss of water.

In the second event, there is a considerable increase in mass loss, with a total of approximately 60%, showing the decomposition of volatile substances in the sample, in a temperature range that starts at 334ºC and goes up to 376ºC. In the third event, which occurs at a temperature of 387ºC to 419ºC, the mass loss is around 9.44%. This event is characterized by the breakdown of more complex groups (such as hydrocarbons and carbonyls). In total, the mass loss of the plant drug was 76% (Peixoto, 2018).

According to an analysis carried out by Rocha (2012) with the species Brosimum gaudichaudii, which belongs to the same family as C. racemosa, the dry extract of the plant showed a 100% loss of mass before the temperature of 550ºC, where the entire sample would have been degraded.

The thermal behavior of the plant drug *C. racemosa* is described in Table 4.

3.2 Obtaining and characterizing the ethanolic extract

3.2.1 Obtaining the ethanolic extract

With the maceration process, it was possible to obtain the ethanolic extract based on *C. racemosa*, with a yield of approximately 64.25%.

3.2.2 Alcohol content determination

The alcohol content of the extract obtained was approximately 71% according to the table of actual strength of spirits in the Brazilian Pharmacopoeia National Formulary. The value of the alcohol content is only accurate when the solution in which the content is determined has a temperature of 20ºC (Brazil, 2012).

As the temperature of the ethanolic extract of *C. racemosa* was higher than 20ºC, it was necessary to correct the temperature. A high percentage of alcohol content was already expected, due to the use of a high concentration of ethanol.

Cunha *et al*. (2016) determined the alcohol content of 70% ethyl alcohol samples

according to the real strength table for spirits (Brazil, 2012). It was possible to observe an alteration of approximately 5% of the samples analyzed in relation to the permitted ethyl alcohol content (70% w/w).

Thus, the result of the alcohol content of the ethanolic extract of *C. racemosa* may have been higher than that of the extracting solvent applied (70% ethanol), due to variations in the actual value of the alcohol content of the solvent used.

3.2.3 Determining pH

Hydrogen potential (pH) refers to the acidity or basicity of a solution and can vary on a scale from 0 to 14. The pH result for the alcoholic extract of *C. racemosa* was 5.93, indicating that the extract is acidic. Coutinho (2012) determined the pH value for the extract of the fruit of *M. nigra*, which belongs to the *C. racemosa* family, to be 3.76.

Determining pH is an index that regulates many microbiological reactions. The acidic pH of a solution (below 4.5) is important to prevent the proliferation of microorganisms (Santos, 2017).

3.2.4 Density determination

The density of the ethanolic extract of *C. racemosa* was 0.87g.mL-1 . According to Silveira and colleagues (2013), the basic density of *C. racemosa* wood was 0.665g/cm3. Considering that the density of alcohol (0.79 g/cm3) is lower than that of water (1.0g/cm3) and that the extractive solution is alcohol-based, the relative density is close to that found in alcohol. Therefore, the density value obtained is adequate, since there is no previous value established for this species in pharmacopoeial parameters.

3.2.5 Determination of dry residue

The value in percentage of the dry residue of the ethanolic extract based on *C. racemosa* was 1.05%, with an initial mass of ± 1.72g of the extract and a final mass of approximately 0.0182g after drying. No data on the amount of total solids of this species was found in the literature, but a study of a species from the Moraceae family, carried out by Balestrin *et al*. (2008), calculated the dry residue content in the ethanolic extract of Dorstenia multiformis, which was 7.22%.

Determining the dry residue of an extractive solution expresses the solvent's capacity to extract solutes present in the plant drug (Soares, 1997). Evaluating this method is essential for obtaining a dry extract with good physicochemical characteristics, as well as having an influence on the yield of spray-dried products (Vasconcelos et al., 2005).

3.2.6 Phytochemical prospecting

The phytochemical profile was carried out in order to detect the chemical compounds of *Clarisia racemosa*. According to the results of the thin layer chromatography shown below (Table 5), the ethanolic extract showed characteristic bands for cinnamic derivatives, condensed tannins and terpenes. It was not possible to observe the presence of characteristic bands for flavonoids when compared to the rutin standard, alkaloids and hydrolyzed tannins. However, some compounds could not be identified.

As a result, the results obtained for anthracene derivatives, coumarins and saponins were indeterminate. Pre-analytical errors may have caused this indeterminacy, making it necessary to repeat the test. The chromatographic plates for cinnamic derivatives, flavonoids, condensed tannins and terpenes were observed under UV light and can be seen below (Figure 3). The band in the first column refers to the sample and that in the second column to the standard used.

The Rf (retention factor) was also calculated for each sample and standard used (Table 6). Therefore, from the comparison between the Rf of the plant drug and the Rf value of the standards, it is possible to assume the presence of these compounds in the ethanolic extract based on *C. racemosa*.

The retention factor is a parameter that is used to identify compounds present in the analyzed sample (Alves *et al*., 2012). Determining this tool is important to reduce possible errors in identifying chemical compounds in chromatography (Von Muhlen, 2009). The results obtained in the phytochemical evaluation of *C. racemosa* differ from some data already in the literature.

In a study carried out by Silva *et al*. (2003) on certain timber species, including *C. racemosa*, phytochemical prospecting was carried out to detect certain classes of

metabolites. Phenolic, tannin, alkaloid and cyanogenic compounds were tested in the aqueous extract of *Clarisia racemosa*, where the presence of all the compounds was observed.

The quantification of alkaloids and certain phenols (flavones and flavonols) in *C. racemosa* was also observed in a study by Silva *et al*. (2009). A substance from the steroid class and another from the stilbene class were also detected in this plant species in work described by Gottlieb *et al.* (1974).

According to Gobbo-Neto and Lopes (2007), the production of secondary metabolites in plants can be affected by environmental conditions. Seasonality is considered to be one of the most important factors, since the amount of active ingredient is not constant and may differ at certain times of the year. Other factors such as age, plant development, temperature, altitude, among others, can also affect the final content of chemical compounds present in medicinal plants.

The secondary metabolites present in medicinal plants have therapeutic effects against various pathologies. They are therefore an important source of research in the production of herbal medicines (Rodrigues *et al*., 2016).

Studies in the literature show that certain secondary metabolites present in plant species have antimicrobial action against various bacteria. This activity can be attributed to the presence of tannins, flavonoids and terpenes (Bustamante, 2010; Flambó, 2013; Monteiro, 2005). Thus, the ethanolic extract of *C. racemosa* has secondary metabolites which can be attributed to its antimicrobial activity.

FINAL CONSIDERATIONS

This study evaluated the quality control parameters of the pulverized plant drug from the stem of Clarisia racemosa, where the results were within the parameters required by the official compendium. The physicochemical tests contributed to the characterization of the plant drug and the extractive solution of C. racemosa, as well as to the future standardization of the dry extract.

It is therefore necessary to obtain and characterize the dry extract in order to analyze the product's behavior in a future incorporation into the intended pharmaceutical form. This monograph allows these results to become a starting material for the development of a herbal medicine, since scientific data on this species is scarce in the literature.

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Table 1 *- Systems and developers used to analyze the phytochemical profile of C. racemosa. Legend: (A) toluene: ethyl acetate: diethylamine; (B) ethyl ether: ethyl acetate: saturated with 10% acetic acid; (C) n-propanol: ethyl acetate: water: glacial acetic acid; (D) ethyl acetate: formic acid: water; (E) chloroform: glacial acetic acid: methanol: water; (F) toluene: ethyl acetate.*

Metabolite Class	System	Revealing
Alkaloids	70:20:10 **(A)**	Dragendorff
Coumarins	50:50:50 **(B)**	KOH 10% + Δ
Anthracene derivatives	40:40:29:1 **(C)**	HNO3 + Δ + KOH 10%
Cinnamic derivatives	90:5:5 **(D)**	AlCl3 or diphenylboriloxyethylamine + polyethylene glycol
Flavonoids	90:5:5 **(D)**	AlCl3 or diphenylboriloxyethylamine + polyethylene glycol
Condensed tannins	90:5:5 **(D)**	Hydrochloric vanillin + Δ
Hydrolyzed tannins	90:5:5 **(D)**	FeCl3
Saponins	60:32:12:8 **(E)**	Anísaldeído sulfúrico
Terpenes and steroids	70:30 ou 90:10 ou 97:3 **(F)**	Lieberman-Burchard + Δ

Source: *Adapted from the methodology used at the Center for the Analytical and Technological Development of Herbal Medicines (NUDATEF/UFPE).*

Table 2 *- Standards used to evaluate the phytochemical profile of C. racemosa*

Chemical compounds	Standard
Alkaloids	Atropine
Coumarins	Coumarin
Anthracene derivatives	Senoside A
Cinnamic derivatives	Caffeic acid
Flavonoids	Rutin
Saponins	Escin
Hydrolyzed tannins	Gallic acid
Condensed tannins	Catechin
Terpenes and steroids	*β-sitosterol*

Source: *Adapted from the methodology used at the Center for the Analytical and Technological Development of Herbal Medicines (NUDATEF/UFPE).*

Table 3 *- Particle size analysis of C. racemosa powder*

Opening the tamis (µm)	% retained

850	0,19
600	5,16
425	22,01
250	36,75
150	21,12
75	10,01
Coletor	4,73

Source: *Research Data*

Table 4 *- Thermal behavior of the plant drug C. racemosa*

Events	Temperature (ºC)	Loss of mass (%)
1	39 - 75	7,063
2	334 - 376	60,008
3	387 - 419	9,449

Source: *Research Data*

Table 5 *- Phytochemical Analysis of Plant Drugs. Indicators: (+) = presence; (-) = absence; (*) = undetermined result.*

Metabolites	Results
Flavonoids	-
Cinnamic derivatives	+
Saponins	*
Coumarins	*
Alkaloids	-
Anthracene derivatives	*
Condensed tannins	+
Hydrolysable tannins	-
Terpenes	+

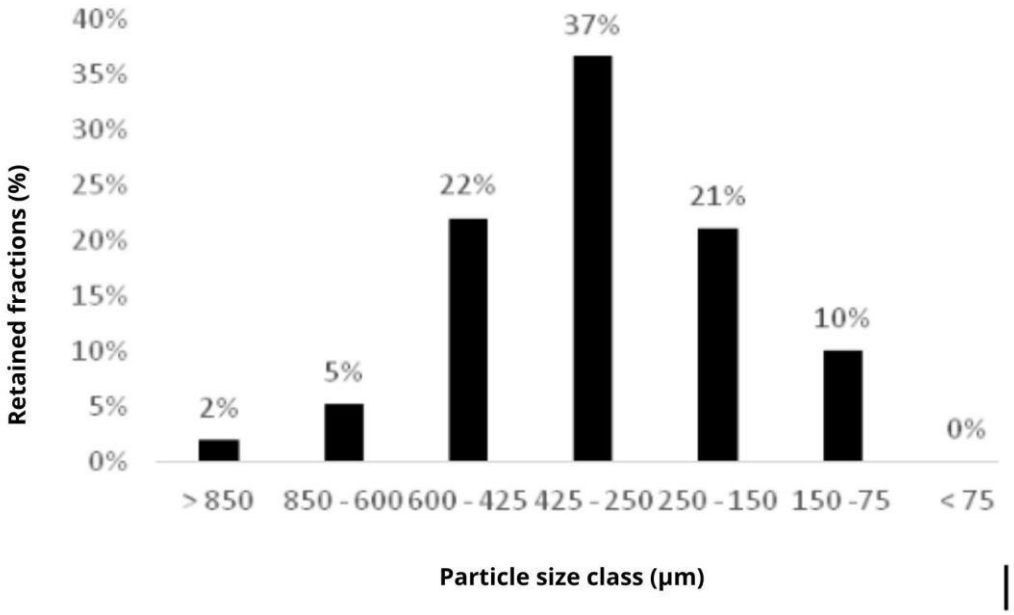
Source: *Research Data*

Table 6 *- Rf of Clarisa racemosa CCD plates*

Metabolites present in the plant drug	Sample	Standard
Cinnamic derivatives	0,79	0,81
Condensed Tannins	0,84	0,84
Terpenes	0,27	0,3

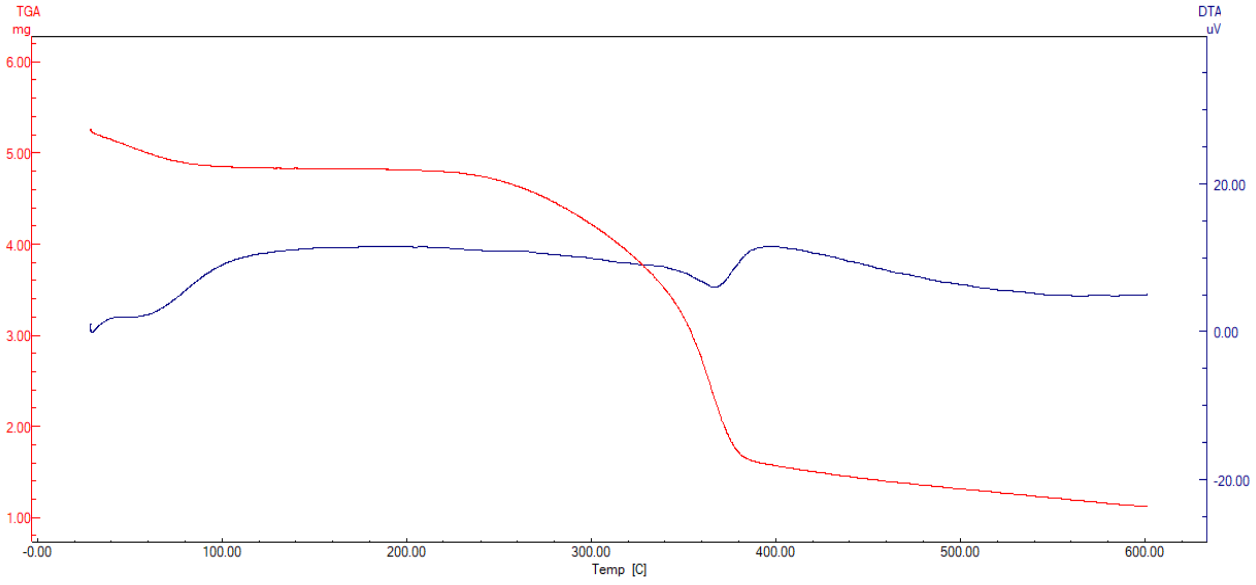
Source: *Research Data*

Fig. 1 *- Graph of the particle size profile of Clarisia racemosa.*



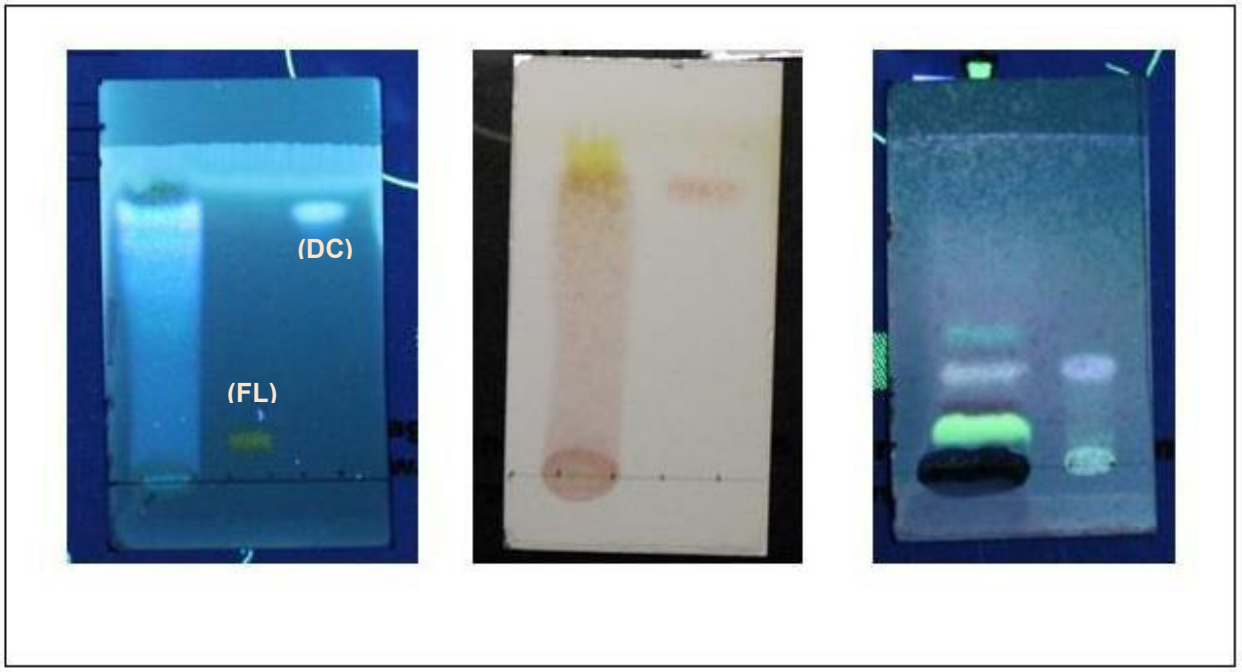
Source: Research Data

Fig. 2 *- Thermogravimetric curve of the plant drug Clarisia racemosa*.



Source: Research Data

***Fig. 3** - Chromatographic plates of the plant drug Clarisia racemosa. Legend: (FL) Flavonoids; (DC) Cinnamic Derivatives.*



Source: Research Data