Physicochemical characterization of the powder from *Eugenia stipitata* McVaugh ssp. sororia McVaugh (Myrtaceae) leaf

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Abstract:
Brazilian biodiversity is recognized as one of the most expressive in the terrestrial biosphere. The *Eugenia stipitata* plant, known as araçá-boi, belongs to the Myrtaceae family composed of more than 130 genera and 4.000 species of green shrubs and trees throughout the year. However, for the plant to be used in medicinal form, there is a need to establish the parameters for the quality control of plant raw material and dry extract. In this sense, the present work aimed at contributing to the physical and chemical study of *E. stipitata* leaves. The plant was collected in the municipality of Porto Velho-RO, thus making a desiccantion that was deposited in a herbarium. The physical-chemical procedures were performed at the Organic Chemistry Laboratory of the Federal Institute of Science and Technology - IFRO, Campus Porto Velho-RO. Qualitative tests for the identification of the main classes of secondary metabolites were based on specific methodologies of Phytochemistry. Later, the extractive solution of the leaves of *E. stipitata* passed through the relative density, determining the potential of ionic hydrogen (pH). In addition, total ash and the granulometry of the vegetable raw material were determined. The data obtained indicate the presence of groups of substances normally associated with different biological activities and which may justify the medicinal use of the species. In addition, these groups agree with those observed in other species representing the Myrtaceae family. Therefore, future studies are needed that can contribute to obtain relevant information about the standardization and quality control of the raw material of this species.
Keywords: Araçá-boi; Quality control; Physical and chemical assays.

Caracterização físico-química do pó das folhas da *Eugenia stipitata* McVaugh ssp. sororia McVaugh (Myrtaceae)

Resumo:
A biodiversidade brasileira é reconhecida como uma das mais expressivas da biosfera terrestre. A planta *Eugenia stipitata*, conhecida como araçá-boi, pertence à família Myrtaceae composta por mais de 130 gêneros e 4.000 espécies de arbustos e árvores verdes durante todo o ano. Entretanto, para que a planta possa ser utilizada na forma medicinal, há a necessidade de estabelecer os parâmetros do controle de qualidade da matéria-prima vegetal e do extrato seco. Nesse sentido, o presente trabalho teve como objetivo contribuir para o estudo físico e químico das folhas de *E. stipitata*. A planta foi coletada no município de Porto Velho-RO, confeccionando assim uma exsicata que foi depositada em herbário. Os procedimentos físico-químicos foram realizados no Laboratório de Química Orgânica do

Instituto Federal de Ciência e Tecnologia – IFRO, Campus Porto Velho-RO. Os testes qualitativos para a identificação das principais classes de metabólitos secundários foram baseados em metodologias específicas de Fitoquímica. Posteriormente, a solução extrativa das folhas de *E. stipitata* passou pela densidade relativa, determinando o potencial de hidrogênio iônico (pH). Além disso, determinaram-se cinzas totais e a granulometria da matéria-prima vegetal. Os dados obtidos indicam a presença de grupos de substâncias normalmente associados a diferentes atividades biológicas e que poderão justificar o uso medicinal da espécie. Além disso, estes grupos concordam com os observados em outras espécies representantes da família Myrtaceae. Portanto, são necessários estudos futuros que possam contribuir para a obtenção de informações relevantes acerca da padronização e controle de qualidade da matéria-prima dessa espécie.
Palavras-chave: Araçá-boi; Controle de qualidade; Ensaios Químico e Físico.

Caracterización fisicoquímica de *Eugenia stipitata* McVaugh ssp. sororia McVaugh (Myrtaceae)

Resumen:
La biodiversidad brasileña es reconocida como una de las más expresivas en la biosfera terrestre. La planta *Eugenia stipitata*, conocida como araçá-boi, pertenece a la familia Myrtaceae compuesta por más de 130 géneros y 4.000 especies de arbustos y árboles verdes durante todo el año. Sin embargo, para que la planta se use en forma medicinal, es necesario establecer los parámetros para el control de calidad de la materia prima vegetal y el extracto seco. En este sentido, el presente trabajo tuvo como objetivo contribuir al estudio físico y químico de las hojas de *E. stipitata*. La planta fue recolectada en la ciudad de Porto Velho-RO, haciendo así un desecante que fue depositado en un herbario. Los procedimientos físico-químicos se realizaron en el Laboratorio de Química Orgánica del Instituto Federal de Ciencia y Tecnología - IFRO, Campus Porto Velho-RO. Las pruebas cualitativas para la identificación de las principales clases de metabolitos secundarios se basaron en metodologías específicas de Fitoquímica. Más tarde, la solución extractiva de las hojas de *E. stipitata* pasó a través de la densidad relativa, determinando el potencial de hidrógeno iónico (pH). Además, se determinaron las cenizas totales y la granulometría de la materia prima vegetal. Los datos obtenidos indican la presencia de grupos de sustancias normalmente asociadas con diferentes actividades biológicas y que pueden justificar el uso medicinal de la especie. Además, estos grupos están de acuerdo con los observados en otras especies que representan la familia Myrtaceae. Por lo tanto, se necesitan estudios futuros que puedan contribuir a obtener información relevante sobre la estandarización y el control de calidad de la materia prima de esta especie.
Palabras clave: Araçá-boi; Control de calidad; Pruebas químicas y físicas.

INTRODUCTION

The Brazilian biodiversity is recognized as one the most expressive of the Earth biosphere, besides having a relevant role for the welfare and health of human beings by providing basic products and ecosystem services. With over 55,000 plant species described, which corresponds to 22% of the number of plant species in the Earth globe, this rich biodiversity is accompanied by a long acceptance in the use of medicinal plants and the traditional knowledge associated to them. Approximately 48% of medicines used are

originated either directly or indirectly from natural products, especially medicinal plants (ALHO, 2012).

Medicinal plants are the main raw materials for the production of phytotherapeutic agents and their use in Brazil has been consolidated in the last years. In order for medicinal plants to be used in the manufacture of medicines, various quality requirements must be fulfilled, in which the quality control sector is responsible for carrying out the necessary analyses.

Thus, for a good use of medicinal plants, you necessarily need the guidance of experts and professionals involved in health care to avoid adverse effects that may arise from self-medication. In this way, good administration and effectiveness in the treatment in all directions can be guaranteed. The activity is well controlled and organized, it brings many benefits to the population, avoiding intoxication through chemical substances (XAVIER *et al*., 2021).

Of the great biodiversity of plants, the family Myrtaceae has a pantropical and subtropical distribution, including roughly 130 genera and 4000 species, and represents one the largest botanical families of the Brazilian flora, with 26 genera and approximately 1,000 species in Brazil (SOUZA e LORENZI, 2014).

Araçá-boi (*Eugenia stipitata* McVaugh ssp. sororia McVaugh, Myrtaceae) is a fruit tree native to the Western Amazon, with potential for the fruit juice and flavor industries. Although seldom planted in the Brazilian Amazon because of its acidity, it is frequently cultivated in the Peruvian Amazon (FALCÃO *et al*., 2000).

The species is a shrub with approximately three meters in height, which produces fruits throughout the year; the fruits are globose berries of yellow coloration, with a thick pulp. Its economic importance is related to a high vitamin C content, proteins, carbohydrates, fibers and minerals (SANTOS *et al*., 2017). Leaves and fruits of *Eugenia* are considered to be excitatory, anti-fever, aromatic, antirheumatic and antidysenteric (QUEIROZ *et al*., 2015).

Of the great diversity of medicinal plants in the Amazon, araçá-boi was selected for this study due to the multitude of uses associated with this plant and the lack of scientific investigations on its biological activities. Considering the need for the pharmacognostic

standardization of this species, this study was carried out with the aim of obtaining the quality control parameters of the plant drug originated from its leaves.

MATERIAL AND METHODS

The selection of the plant material used was carried out using a bibliographic survey of the species found in the region, in which healthy leaves of *E. stipitata,* collected in the city of Porto Velho, state of Rondônia (GC: S 08º 47 221' W 063º 53 136') were used. An exsiccated sample was made and deposited at the Municipal Botanical Museum of Curitiba under number MBM384025. Physicochemical analyses were carried out at the Organic Chemistry Laboratory of the Federal Institute of Science and Technology – IFRO, Porto Velho Campus.

PHYTOCHEMICAL SCREENING

The qualitative tests for the identification of major secondary metabolite groups were performed as recommended by Matos (2009). For the identification of saponins, 2 mg of dry ethanolic extract was diluted in 5 mL of distilled water. Thereafter, the solution was vigorously shaken for two minutes; for phenols and tannins, 2 mg of dried extract was diluted in 5 mL of distilled water, followed by addition of two drops of a 1% $FeCl_3$ ethanol solution; for flavonoids, 2 mg of dried extract was diluted in 10 mL of methanol, with subsequent filtration and addition of five drops of concentrated HCl and magnesium ribbons; for alkaloids, 2 mg of dried extract was diluted in 5 mL of a 5% HCl solution. After the filtration, a few drops of Bouchardat reagent were added; for triterpenes, 2 mg of dried extract was diluted in 10 mL of chloroform, with subsequent filtration in activated carbon.

The filtered part was transferred to a clean dry test tube and 1mL of acetic anhydride was added, under gentle shaking. Subsequently, a few drops of concentrated H_2SO_4 were added, and the solution was shaken.

SAMPLE PROCESSING

After collection, 580 g of fresh plant material was washed with water, sprayed with ethyl alcohol (70%) and oven-dried in an incubator (Odontobras 1.4) for seven days, at room temperature, between 42 and 45º C. After removing the plant material from the incubator, the material was ground in a cutting Mill, obtaining a dried powder. The tests performed aimed to determine the pH, relative density, loss on drying, total ash, powder granulometry and dry residue in dried extracts (BRASIL, 2010).

OBTAINMENT OF THE EXTRACTIVE SOLUTION

The extractive solution was made using 75g of the plant drug, diluting in 500 mL of ethanol (96% pa). The extract was macerated for 48h, under constant stirring and at room temperature, followed by common filtration and after that, vacuum filtration. The solvents used were selected on the basis of their degrees of toxicity and/or risk when handling them, besides the efficiency in the extraction process.

CHARACTERIZATION OF THE EXTRACTIVE SOLUTION

Relative density

A 5 % ethanol (p/v) solution of the sample was prepared and stored in an amber bottle for the pH determination. The sample was then transferred to a pycnometer (100 mL). The procedure consisted of weighing an empty pycnometer; weighing a pycnometer with distilled water; and weighing a pycnometer with the sample. Temperature was adjusted to 20° C, and thereafter, weighings were performed. The sample weight was obtained by the difference between the weight of the pycnometer with samples minus the weight of the empty pycnometer.

Determination of the pH in the sample solution

The determination of the potential of hydrogen (pH) of the extracts was performed through potentiometric pH determination (BRASIL, 2010), using a pH meter (trademark Orion, model 420A), previously calibrated with buffer solutions (pH=4.0 and pH=7.0) with 10 mL of samples.

Determination of loss on drying

With the substance made into powder, 2g of it was transferred to a flat weighing bottle, which has been previously dried for 30 minutes. After cooling in the desiccator, the weighing bottle was sealed, containing the sample. The weighing bottle was gently shaken to distribute the sample as uniformly as possible, at 5 mm height. The sample was oven-dried at 105 ºC for 2 hours. Thereafter, the sample was allowed to cool to room temperature in a desiccator. This process was repeated until a constant weight was obtained (BRASIL, 2010).

CHARACTERIZATION OF THE PLANT RAW MATERIAL (PRM)

Determination of total ash

Samples of powdered plant material (3g) were weighed and uniformly distributed in crucibles, which have been previously calcined and incinerated in a muffle furnace, gradually increasing the heat to 600 ° C until it becomes free from carbon. The crucibles were then cooled in desiccators and weighed (BRASIL, 2010). After that, the ash percentage was calculated in relation to the air-dried drug.

GRANULOMETRIC ANALYSIS

The pulverized plant material usually represents an essential input for the obtainment of intermediary preparations and the yield of the extraction process regarding

the chemical constituents of interest in the drug; it is directly related to the pulverization degree of the material (SILVA-JÚNIOR, 2006).

The granulometry was determined with tamises operated by a mechanical device, which reproduces the horizontal-vertical movements of manual operation, through uniform mechanical action. For the analysis, six tamises were prepared. 25 g of the sample was weighed, followed by transference of the sample to a series of sieves of mesh sizes 8, 10, 30, 40, 50 and 100 µm, with a uniform distribution of the powder. The sieve assembly was then sealed. The apparatus was on for about 15 minutes, with proper vibration. Subsequently, all sample retained in the upper surface of each mesh was removed for a waterproof paper, and the powder was weighed using a suitable brush. Subsequently, the powder retained in the collector was also weighed. The results were expressed as percentages of material retained in each sieve.

RESULTS AND DISCUSSION

Phytochemical screening

The data obtained (Table 1) indicate the presence of groups of compounds usually associated with different biological activities and that might justify the medicinal use of this species. Moreover, these groups agree with those observed in other Myrtaceae, since previous investigations carried out in our laboratory (COUTO *et al.,* 2009) observed the presence of these same classes of secondary metabolites in the leaves of *E. dysenterica* DC.

Table 1 - Results obtained in the phytochemical screening of the powder from *E. stipitata* leaves

Classes of secondary metabolites	Presence / Absence
Saponins	Absence
Fenols/tannins	Presence
Flavonoids	Absence
Alkaloids	Presence
Steroids/triterpenes	Presence

Source: Autors.

Quality control

The carrying out of quality control analyses for drugs and/or plant extracts is of great importance to know their chemical characteristics. The sector named Quality Control is responsible for the analyses carried out in raw materials, in-process products, and final products. Such sector must perform samplings and analyses, ensuring authenticity and reliability in the results obtained in order to release them. The physicochemical analyses, together with the microbiological analyses, are of great importance to confirm the authenticity and quality of raw materials.

The Brazilian Pharmacopeia is the official pharmaceutical code of Brazil, and is currently in its 5th edition. Its use is mandatory for those who manufacture, manipulate, fractionate and control pharmaceutical products. It describes the quality standards of raw materials, medicines and the methods used for their analyses. Through these analyses, one can quantify the chemical markers of a plant, and then assess if the results obtained are within the pre-established specifications (SOUZA e MOREIRA, 2010).

Potential of hydrogen

The potential of hydrogen is related to microbial growth, according to the 5th edition of the Brazilian Pharmacopeia (BRASIL, 2010), with reference values ranging from 4.0 to 7.0. Most of the bacteria are neutrophiles, growing in a pH between 6.0 and 8.0. However, there

are groups of bacteria which also grow in acidic pH's. The survival of these microorganisms in a wide pH range requires the secretion of different enzymes, making the environmental pH adequate for their growth.

Plant tissues differ in relation to their pH values, which may influence colonization by microorganisms in these different environments. The pH of the extract was calculated by the average of three determinations, which resulted in a pH of 6.62 (Table 2), agreeing with the standard reference value.

Table 2 - pH determination in the sample solution

Sample	Tissues	pH
1	21 C	6.29
2	20.6 C	6.13
3	20.3 C	6.06
Average		6.16

Source: Autors.

Relative density

The relative density is an important physical property which can be used to distinguish between a pure and an impure material (or alloys of this metal). This parameter can also be used in the identification and quality control of a certain industrial product; it is also related to the concentration of solutions. The extract density was calculated as percents by the average of three determinations, according to the equation: $(m_2 - m) / (m_1 - m)$, wherein m_2 represents the pycnometer mass plus the sample; m_1 represents the pycnometer mass plus water and m is the mass of the empty pycnometer. The result was 0.82%.

Determination of loss on drying

The loss on drying consists of a relevant factor in quality control of drugs, since it refers to the moisture content and/or volatile substances present in the plant drug. It becomes an important factor when the plant is used as a raw material for the industrial production of drugs. This determination is important in the microbiological control because the excess water in the plant drug promotes the growth of fungi and bacteria, which might cause the hydrolysis of its constituents (SHARAPIN, 2000).

The loss on drying was determined according to the general methods (BRASIL, 2010), obtaining the value of 8.22% by the gravimetric method. After that, the difference in weight between the initial and final samples was calculated, thus determining the loss on drying. The results of the loss on drying fall within the thresholds for plant drugs, which range between 8 and 14% moisture for the dried drug.

Determination of the dry residue in dried extracts

The result was calculated in relation to 100 g of the drug, by the average of three determinations, which resulted in 59%. Dried extracts are solid preparations obtained by solvent evaporation, that, regardless of the drying method, must present at least 95% of dry residue, calculated as the mass percentage.

Determination of total ash

The determination of total ash allows for assessing the presence of non-volatile inorganic substances in organic substances, (i.e), to assess the presence of inorganic matter in the sample analyzed. The lower the values obtained for total ash, the lower the amount of non-volatile inorganic compounds in the sample (BRASIL, 2010). The present analysis was carried out with the dried powdered plant material.

The results obtained for *E. stipitata* were found to be 6.62%, in which the threshold for total ash is 8%. The combination of these physicochemical parameters is unique for every

species, constituting the identity of a species. In this study, the physicochemical parameters of the species were determined for the first time.

Granulometric analysis

The granulometric distribution of plant drugs determines the contact surface available for interacting with the solvent used in order to obtain the plant derivative. It is an important preliminary parameter for choosing the extraction process and a proper solvent, since it directly affects the efficiency of the extraction process (SANTOS, 2000; MIGLIATO *et al.*, 2007).

The granulometric repartition was performed according to the 5th edition of the Brazilian Pharmacopeia (BRASIL, 2010). The samples evaluated presented different particle size distributions, where the tamis presenting the greatest amount of retained material was that with a 600 µm mesh aperture, a total of 49.84% as the most abundant fraction. The fractions retained in the tamises and in the collector were weighed (Table 3). When comparing the results with the classification of the Brazilian Pharmacopeia (5th edition), we conclude that the powder of *E. stipitata* was considered to be moderately thick. There are no established standards for granulometric values of extracts from *E. stipitata,* since it is also an inedited determination for this species.

Table 3 - Fractions retained in the tamises

Mesh (ABNT/ASTM)	Aperture (µm)	Retained	Percentage
8	2.36 mm = 2.360	4.695	18.78
10	2.00 mm = 2.000	2.669	10.68
30	600 µm	12.461	49.84
40	425 µm	1.795	7.18
50	300 µm	1.151	4.6
100	150 µm	1.379	5.52

Source: Autors.

Due to their recognized properties in popular culture or scientific investigations carried out with them, Myrtaceae species are found in monographs of the IV Brazilian Pharmacopeia and/or the World Health Organization (WHO). Among these species, are: *Eucalyptus globulus* Labill; *Syzygium aromaticum* (L.) Merr. & Perry; *Psidium guajava* L.; *Eugenia uniflora* L. and *Melaleuca alternifolia* (Maiden & Betche) Cheel (FARMACOPÉIA BRASILEIRA IV, 2002; LORENZI e MATOS, 2002; WHO, 2002; COUTO *et al*., 2009).

CONCLUSION

Despite the investigations previously described in the literature on some species of Myrtaceae, no studies to date have standardized the physical and physicochemical parameters of *E. stipitata*. Thus, the results obtained represent a great contribution to future research on this species. The characterization performed with the plant material showed that the results obtained are within the pre-established standards, and that this raw material can be used to carry out further researches and specifications of a future pharmacopeia for *E. stipitata*.

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