

Determination of the chemical profile extracts obtained from *Kalanchoe pinnata* (Lam.) Pers native of municipality Tabatinga-AM

Determinação do perfil químico de extratos obtidos de *Kalanchoe pinnata* (Lam .) Pers nativa do município de Tabatinga-AM

Determinación del perfil químico de los extractos obtenidos de *Kalanchoe pinnata* Lam .) Pers oriunda del municipio de Tabatinga-AM

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Abstract

Kalanchoe pinnata formerly known as *Bryophyllum pinnatum*, is a herbaceous species native to tropical regions such as Africa. In Brazil, it was successfully introduced and propagated, being commonly used in communities far from large capitals. The juice of the leaves is usually used in the treatment of inflammatory diseases, gastric ulcers, burns, diarrhea, vomiting, insect bites and body aches. In the literature, compounds such as anthocyanins and flavonoids are reported that have different biological activities such as antimicrobial, antioxidant, cytotoxic, antitumor, antiparasitic, antiallergic and hepatoprotective. Based on this information and considering that there are few studies on the chemical compounds of *K. pinnata* in Amazonas, especially in regions farther from the capital, such as in the municipality of Tabatinga. The present work aimed to determine the chemical profile of the fractions of the crude extract of *K. pinnata* native to the municipality of Tabatinga - AM. For this, plant material was collected from a local producer in the municipality of Tabatinga. Then the leaves were subjected to an extraction by infusion. Soon after, the crude extract obtained was fractionated with hexane, chloroform and ethyl acetate. The resulting fractions were analyzed by High Performance Liquid Chromatography (HPLC) at wavelength $\lambda=254$ nm and, using the information available in the literature, it was possible to establish a relationship between the compound quercetin 3- *O* - α -L- arabinopyranosyl - (1 \rightarrow 2)- α -L- rhamnopyranoside, as responsible for the chromatographic peaks observed in the hexane, chloroform, and ethyl acetate fractions. Thus, the present study will serve as a basis for future work on the characterization of chemical compounds present in *K. pinnata* from the municipality of Tabatinga.

Keywords: Chemical profile; *Kalanchoe pinnata*; Tabatinga; Chromatography.

Resumo

Kalanchoe pinnata anteriormente conhecido como *Bryophyllum pinnatum*, é uma espécie herbácea nativa de regiões tropicais como a África. No Brasil, foi introduzida e propagada com sucesso, sendo comumente utilizada em comunidades distantes das grandes capitais. O suco das folhas costuma ser usado no tratamento de doenças inflamatórias, úlceras gástricas, queimaduras, diarreias, vômitos, picadas de insetos e dores no corpo. Na literatura, são relatados compostos como antocianinas e flavonóides que possuem diferentes atividades biológicas como antimicrobiana, antioxidante, citotóxica, antitumoral, antiparasitária, antialérgica e hepatoprotetora. Com base nessas informações e considerando que existem poucos estudos sobre os compostos químicos de *K. pinnata* no Amazonas, principalmente em regiões mais distantes da capital, como no município de Tabatinga. O presente trabalho teve como objetivo determinar o perfil químico das frações do extrato bruto de *K. pinnata* nativa do município de Tabatinga - AM. Para isso, foi coletado material vegetal de um produtor local do município de Tabatinga. Em seguida, as folhas foram submetidas a uma extração por infusão. Logo após, o extrato bruto obtido foi fracionado com hexano, clorofórmio e acetato de etila. As frações resultantes foram analisadas por Cromatografia Líquida de Alta Eficiência (HPLC) no comprimento de onda $\lambda=254$ nm e, utilizando as informações disponíveis na literatura, foi possível estabelecer uma relação entre o composto quercetina 3-O - α -L- arabinopiranosil - (1 \rightarrow 2)- α -L-ramnopiranosídeo, como responsável pelos picos cromatográficos observados nas frações hexano, clorofórmio e acetato de etila. Assim, o presente estudo servirá de base para futuros trabalhos de caracterização de compostos químicos presentes em *K. pinnata* do município de Tabatinga.

Palavras-chave: Perfil químico; *Kalanchoe pinnata*; Tabatinga; Cromatografia.

Resumen

Kalanchoe pinnata, anteriormente conocida como *Bryophyllum pinnatum*, es una especie herbácea originaria de regiones tropicales como África. En Brasil, se introdujo y propagó con éxito, siendo de uso común en comunidades alejadas de las grandes capitales. El jugo de las hojas se usa a menudo en el tratamiento de enfermedades inflamatorias, úlceras gástricas, quemaduras, diarrea, vómitos, picaduras de insectos y dolores corporales. En la literatura se reportan compuestos como las antocianinas y los flavonoides que poseen diferentes actividades biológicas como antimicrobiana, antioxidante, citotóxica, antitumoral, antiparasitaria, antialérgica y hepatoprotectora. Con base en esta información y considerando que existen pocos estudios sobre los compuestos químicos de *K. pinnata* en Amazonas, especialmente en regiones más alejadas de la capital, como en el municipio de Tabatinga. El presente trabajo tuvo como objetivo determinar el perfil químico de las fracciones del extracto crudo de *K. pinnata* originaria del municipio de Tabatinga - AM. Para ello se recolectó material vegetal de un productor local del municipio de Tabatinga. Luego, las hojas fueron sometidas a una extracción por infusión. Poco después, el extracto bruto obtenido se fraccionó con hexano, cloroformo y acetato de etilo. Las fracciones resultantes fueron analizadas por Cromatografía Líquida de Alta Resolución (HPLC) a longitud de onda $\lambda=254$ nm y, utilizando la información disponible en la literatura, fue posible establecer una relación entre el compuesto quercetina 3-O - α -L- arabinopiranosil - (1 \rightarrow 2)- α -L-ramnopiranosídeo, como responsable de los picos cromatográficos observados en las fracciones de hexano, cloroformo y acetato de etilo. Así, el presente estudio servirá de base para futuras caracterizaciones de compuestos químicos presentes en *K. pinnata* en el municipio de Tabatinga.

Palabras clave: Perfil químico; *Kalanchoe pinnata*; Tabatinga; Cromatografía.

1. Introduction

1.1 Amazon and its biodiversity

The so-called Brazilian Amazon encompasses the states of Acre, Amapá, Amazonas, Mato Grosso, Pará, Roraima, Rondônia and Tocantins. Together, these states comprise 60% of the national territory, which is the equivalent of 5,000,000 km², 11,000 km of international borders with 12,000,000 hectares of floodplains and 25,000 km of navigable rivers (Higuchi, 2004; Benchimol, 2010).

Regarding the flora of the Brazilian Amazon, the totality of species remains undetermined, however, it is estimated that about 40,000 plant species can be found in the region, 2,500 of which are large and more than 30,000 are medium and small plants. It is worth mentioning that approximately 2,000 species of plants in the region are used as food, in folk medicine and in the production of oils, greases, waxes, among other products that benefit the population (Wright, 2002; Embrapa, 2014).

In the midst of this immense biodiversity, the Amazon has an unexplored potential when it comes to the study of medicinal plants, especially in regions further away from the capital. Medicinal plants are often fundamental in the prevention and treatment of primary diseases in communities, as they are easily accessible and readily available. It is worth mentioning that studies involving these organisms are relevant since science can contribute to the valorization of products or even encourage the

commercialization of these plants (Zucchi, et al., 2012; Batista, et al., 2019).

According to the literature, in 2012 the trade in medicinal plants was estimated at US\$ 2.2 billion. In 2017, the indication was that the market for plant extracts and natural medicines would move more than US\$ 100 billion dollars. In addition, the works are relevant because they allow the expansion of knowledge about the bioactive compounds present in these organisms, encourage the preservation of the flora and the rational use of natural resources (Zucchi, Oliveira Júnior, et al., 2012; Ahn, 2017).

1.2 *Kalanchoe pinnata* (Lam .) Pers.

Belonging to the Crassulaceae family , the genus *Kalanchoe* , according to the literature, has approximately 125 species distributed in tropical and subtropical areas, the vast majority being native to Africa. Among the species, *Kalanchoe pinnata* stands out for being widely used in the treatment of inflammatory diseases, gastric ulcers, burns, diarrhea, vomiting, insect bites and body aches, from the juice of the plant. In the academic field, this organism is the subject of studies because, according to the reports found in the literature, there are compounds that have biological activities such as antimicrobial, antioxidant, cytotoxic, antitumor, antiparasitic, antiallergic, hepatoprotector, among others (Almeida et al., 2000; Costa., 2008; El Abdellaqui, et al., 2010).

It is worth mentioning that *K. pinnata* was formerly known as *Bryophyllum pinnatum* and popularly as saião, leaf-da-fortuna, courama, coirama, leaf-of-coast, leaf-of-pirarucu, pirarucu, roda-da-fortuna, leaf thick (Almeida, Silva, et al., 2000; Smith, 2004). It is a perennial herbaceous organism, with little branching that can reach up to 1.5 m in height with the presence of simple, alternate, opposite, succulent, oval leaves with a crenate margin with a maximum length of 20 cm. Its seedlings grow on the margins of its leaves and its development occurs preferentially in regions with a hot and humid climate. Thus, this organism can be found in certain parts of Asia, Australia, the West Indies, the Galapagos Islands and in other countries with tropical and subtropical climates (Lorenzi & Matos, 2008; Kamboj & Saluja, 2009; Snafi, 2013).

In Brazil, this species can be found, preferentially, along the coast between the states of Bahia and São Paulo. Because it is easy to propagate, this plant can be found in the State of Amazonas and its leaves are widely used in inland cities, such as Tabatinga, for the treatment of topical wounds, stomach pain, fever remedy and as a vermifuge (Lisboa et al., 2006; Pedroza, et al., 2017).

1.3 Chemical compounds of *Kalanchoe pinnata* (Lam .) Pers.

Chemical investigations on *K. pinnata* reveal the presence of compounds from different classes such as organic acids, alkaloids, fatty acids, steroids, bufadienolides, saponins, terpenes, tannins, flavones and flavonoids (Wu, et al., 2006). Among these, the most commonly found compound in *K. pinnata* are the flavonoids derived from quercetin (Ichikawa et al., 1986; Costa, Jossang, et al., 1994; Muzitano, 2006; El Abdellaqui, et al., 2010; Sobreira, 2013) and kaempferol (Muzitano, 2006; Tatsimo, Tamakou, et al., 2012).

According to reports found in the literature, these compounds are obtained from methodologies that involve the preparation of ethanolic, hydroalcoholic or aqueous extracts followed by biological tests. As an example of this, there is the study carried out by PEREZ et al., (1999) who used the ethanolic extract of *Kalanchoe* sp. to reduce gastric lesions caused by indomethacin. Using the aqueous extract of *K. pinnata* Sousa and collaborators (2005), Afzal and collaborators (2012) found an anti-inflammatory potential, as there was a reduction in edema in rats, caused by chemical and physical stimuli.

To evaluate the antioxidant potential, Chibli (2013) used the ethanolic extract of *K. pinnata*, and determined, through the DPPH method, that the EC50 is at least 16.43 µg/ mL. The study also revealed that the ethyl acetate fraction had an EC50 of 9.44 µg/ mL, a value close to that observed for rutin, used as a positive control, which has an EC50 of 8.66 µg/ mL. In a study carried out by de Souza and collaborators (2013), only the aqueous extract of *K. pinnata* was evaluated, and an IC50 of 98.13

µg/ mL was found. These results are justified by the presence of flavonoids in the crude extract and its fractions.

The literature reports that extracts of *K. pinnata* present compounds with antimicrobial activity. Biswas and collaborators (2011) observed the juice of *K. pinnata* was used for the treatment of diarrhea, in this way when elaborating the ethanolic extract of *K. pinnata* and testing it against the pathogenic bacterium *Escherichia coli*, which is responsible by infections of the gastrointestinal tract, the ability to inhibit the growth of the pathogen was observed.

In another study, Okwu and Nnamdi, (2011) isolated and identified for the first time the alkaloid ethamino-7-Hex-1-in-5-one phenanthrene from the ethanolic extract of *K. pinnata*. This compound was able to inhibit the growth of the pathogens *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* and *Aspergillus niger*.

1.4 High performance liquid chromatography (HPLC) as tool for identifying organic compounds

Chromatography is an analytical method used effectively in the separation, identification and determination of chemical components of complex samples such as biological fluids, natural products, river sediments, among others (Skoog, et al., 2010).

Obtaining this profile is considered one of the best strategies for analyzing secondary metabolites and their derivatives. However, the metabolome of an organism such as a plant is highly complex due to the diversity of chemical structures generated by metabolism, so it is necessary to use complementary approaches in order to differentiate and obtain the largest number of compounds. Even with the great advance of new techniques in liquid chromatography, the identification and resolution of chromatographic peaks is still a difficult task (Klitgaard, et al., 2014; Wehrens, et al., 2013). Therefore, the present study aimed to determine the chemical profile of the fractions of the crude extract of *K. pinnata* native to the municipality of Tabatinga - AM.

2. Methodology

2.1 Obtaining plant material, elaboration of the extract and fractionation

The plant material was obtained from a local producer in the city of Tabatinga. The plant was properly stored in paper bags and transported to the Multidisciplinary Laboratory of Chemistry of the Centro de Estudos Superiores de Tabatinga, Universidade do Estado do Amazonas, where it was washed and placed to dry in an oven at 70 °C. When dry, the weight of 260 grams was recorded on a semi-analytical scale (Shimadzu). After that, the material was stored in glass jars to be transported to the MBT Bioorganic Laboratory for preparation of the extract according to the methodology established by Rodrigues and collaborators (2016), Oliveira and collaborators (2016) with some adaptations. After heating 2 liters of distilled water to a temperature of 100 °C, 260 g of the leaves were infused. Upon reaching room temperature, the experiment was filtered and then the water was removed with the aid of a hot plate. When dry, the sample was resuspended in 500 mL of distilled water and centrifuged to remove suspended solids. After this process, the sample was fractionated by liquid-liquid extraction in a 1:1 proportion with the solvents hexane, chloroform and ethyl acetate. Using a rotary evaporator, the solvent was removed from the fractions and the excess water was removed in a desiccator. Finally, 1 mg of each fraction was weighed for liquid chromatography analysis.

2.2 Determination of the chemical profile of extracts by HPLC

Analytical High Performance Liquid Chromatography (HPLC) was performed on a Shimadzu HPLC system (LC-6 AD pump; DGU-20A5 degasser; SPD-20AV UV detector; CBM-20A modular communication) (Columbia, MD, USA) equipped with the Luna C18 column (250 × 10 mm, 5 µm) (Phenomenex – Torrance, CA, USA). All solvents used in the chromatography were HPLC grade and purchased from JT Baker (Phillipsburg, NJ, USA), and the water was purified using a Mili-Q system (Millipore, Bedford, MA, USA). Elution was performed with a gradient of 20-100% B over 25 minutes at a flow rate of 1 ml

/min. UV spectra were recorded in the range of 254 nm.

3. Results and Discussion

3.1 Yield of fractions obtained from *K. pinnata* extract

After obtaining the crude extract, the yield obtained was 19.15 g, approximately 7.36% of the dry plant material. This amount was resuspended in water and fractionated with various organic solvents. The yield of the fractions obtained is described in Table 1.

Table 1: Yields of fractions obtained from the crude extract of *K. pinnata*.

Work code	Fraction	Dry weight (g)
1	Aqueous	3.08
2	hexane	0.55
3	Chloroform	1.58
4	Acetate	0.72

Source: Authors.

After obtaining the yield, 1 mg of each fraction was weighed for HPLC analysis. When comparing the yields of the fractions obtained in the present study, with those observed in the work carried out by Ferreira et al. (2014) on *K. pinnata*, certain similarities are found since, starting from 2.52 kg of crushed fresh inflorescences, infused with distilled water at 50 °C, 90.2 g (3.82%) of lyophilized extract were obtained. The initial extract was then precipitated in ethanol and then partitioned in ethyl acetate and water solvents, yielding the ethyl acetate fractions with a yield of 5.58 g (6.2%) and aqueous with 12.35 g (13, 7 %). These results show that, although the material used in the authors' research and in the present study integrate different parts of the plant, and consequently present different morphological characteristics, when similar extraction processes are used, it is possible that the yield is similar. Corroborating the studies, Muzitano (2006) using 6.76 kg of fresh leaves, being extracted by infusion with distilled water at 50 °C, obtained the crude extract, being partitioned with CH₂Cl₂ and NaOH. The residual aqueous phase was partitioned into EtOAc, yielding 423.2 mg (0.006%), 113.0 mg (0.001%) and 1.67 g (0.02%), respectively. In this way, the extraction method is efficient because it is a simple methodology to obtain the extract, in addition to simulating the way of preparation by the population, which constantly consumes the plant in the form of tea. It is worth mentioning that, after obtaining the extracts, there is the possibility of prospecting their chemical components and evaluating their biological potential (Silva, 2019).

Tatsimo (2012) used the whole dry plant, with 1.50 kg of plant material, applying the extraction technique by percolation with methanol, at room temperature, reducing the material to 148 g, this generating the yield fractions of 38.03 g (25.69%) for hexane and 34.03 g (22.99%) for ethyl acetate. In contrast, Lanna and coauthors (2019), Almeida (2019) and Sobreira (2013) used the maceration-type extraction technique. Sobreira (2013), in turn, used ethanol and water, where they compared the hydroethanolic extract (incubated for 7 days) of leaves harvested at two times of the year. The initial extract was dissolved in distilled water and passed through liquid-liquid partition, generating the following fractions, with their respective yields: chloroform with 1.1 g and 4.4 g (5.5 % and 5.6 %), acetate ethyl with 0.74 g and 2.4 g (3.7% and 4%), and aqueous with 13.6 g and 41.6 g (68% and 69.3%). Concluding that, there is little difference between the fractions from samples with weights and collected in different climatic conditions. Similarly, Chibli (2013) and Soares (2017) used the technique by steeping in ethanol and methanol in dry leaves, being kept in contact with the solvent for periods of 24 hours and two weeks. The initial extract was partitioned liquid-liquid in solvents, including ethyl acetate and hexane, obtaining the hexane fractions with 9.11 g and 7.6 g and

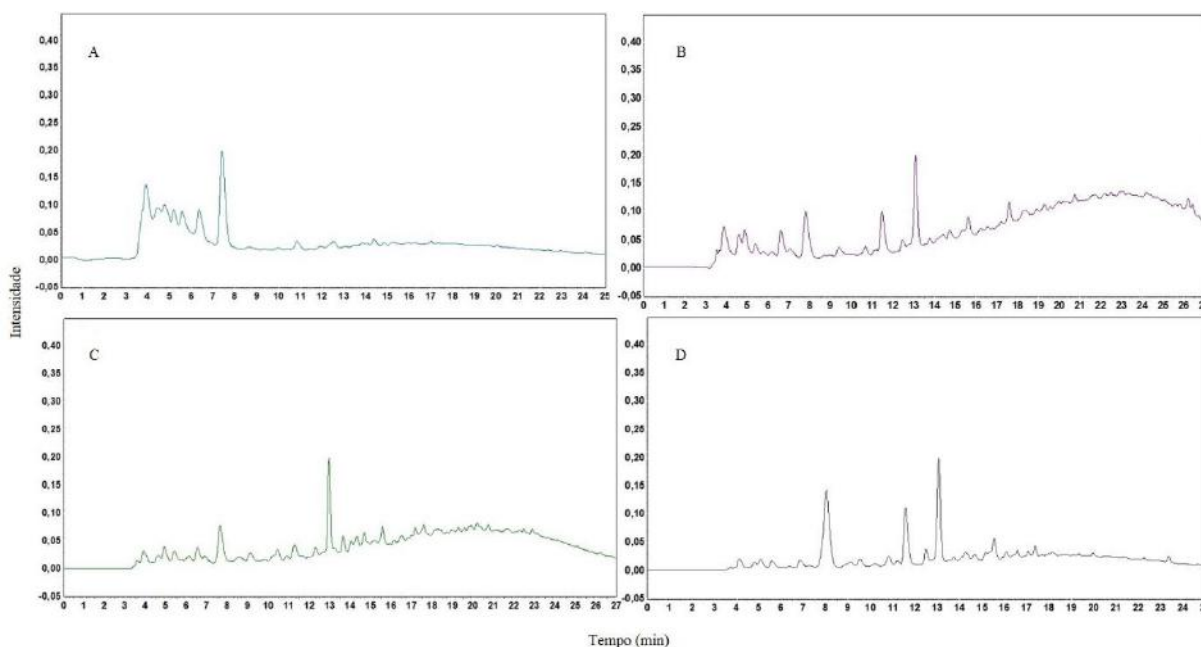
in ethyl acetate 4.37 g and 8.2 g, giving a yield of 22.8%, 49.35%, 10.9% and 53.25%, respectively. In this way, the analysis by Soares (2017) deserves to be highlighted, as it achieved a notably higher yield.

Comparing the methodologies, the present study presented results close to those observed in other studies. The differences in yields can be explained by the use of different extraction techniques and solvents with different degrees of polarity, as well as the temperature and application time (Oliveira et al., 2016) influence the production of extracts and fractions with different levels of concentration and weight (Marques, 2005; Brum et al., 2009).

3.2 HPLC analysis of *K. pinnata* fractions

When performing the HPLC analyses, the chemical profiles of the *K. pinnata* fractions shown in Figure 1 were determined.

Figure 1: Perfil químico das frações de *K. pinnata* em $\lambda=254$ nm. Em A: fração aquosa, B: fração em hexano, C: fração em clorofórmio e D: fração em acetato de etila.



Source: Authors.

As can be seen in the figure above, there is a variety of metabolites in all fractions that occur between 4 and 16 minutes. The fractions in chloroform and ethyl acetate show similarities with respect to the chromatographic profile at the retention times of 13 minutes. It is worth noting that the fraction in hexane presents a major compound in 13 minutes.

The literature points out that several chemical compounds absorb at a wavelength of 254 nm, such as flavonoids and anthocyanins.

In a study carried out by Araújo (2017), it was possible to detect in the aqueous extract, by means of chromatographic techniques, the compounds pautelin - *O* - hexoside- *O* -hexoside, kaempferol, quercetin - *O* - hexoside, quercetin - *O* -deoxyhexoside- *O* - pentoside, kaempferol- *O* -deoxyhexoside- *O* - pentoside, eupafoline - *O* -deoxyhexoside- *O* - pentoside, and patuletin-di- *O* -acetyl-deoxyhexoside. While Sobreira (2013) verified the presence of quercetin 3 - *O* - α -L-arabinopyranosyl-(1 \rightarrow 2) - α -L-rhamnopyranoside, also identified by Coutinho et al (2021), Cruz et al (2008) and Muzitano and collaborators (2011). The authors also verified the presence of quercitrin and kaempferol 3-*O*- α -L-arabinopyranosyl (1 \rightarrow 2) α -L-rhamnopyranoside, which have antioxidant and anti-inflammatory properties (Araújo et al., 2018), and 4',5-trihydroxy-3',8-

dimethoxyflavone 7-β-D-glucopyranoside; compounds attributed to the glycolized quercetin, patuletin, eupafoline and kaempferol derivatives groups, all with gastroprotective activity (Sobreira, 2013).

In an analysis of the hexane fraction, Chibli (2013) found an ultraviolet spectrum with characteristic bands of the flavonoids quercetin at T_R : 8.83 minutes. He also identified the chemical constituents pentadecanal, hexadecanoic acid ethyl ester, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, 9,12-octadecadienoic acid ethyl ester, 9, 12,15-octadecatrienoic acid, octadecanoic acid ethyl ester, trans-squalene, α-tocopherol, 2-methyl-7-phenylindole, 3-[4-(1-methyl-ethyl)-phenyl]-1-phenyl-2-propen-1-one, 2-acetoxymethyl-3-(methoxycarbonyl)-biphenylene, 1-methyl-2-phenylindole, and β-amyryn, the latter being the major constituent detected.

In a study of the ethyl acetate fraction, Gonçalves (2017) identified the compounds myricetin 3-*O*-α-L-arabinopyranosyl-(1→2)-α-L-rhamnopyranoside, myricetin 3-*O*-rhamnopyranoside, meamsetin 3-*O*-pentosyl rhamnopyranoside, quercetin 3-*O*-α-L-arabinopyranosyl-(1→2)-α-L-rhamnopyranoside, 4',5-trihydroxy-3',8'-dimethoxyflavone 7-β-D-glucopyranoside, and kaempferol-3-*O*-α-L-arabinopyranosyl-(1→2)-α-L-rhamnopyranoside, and quercetin 3-*O*-α-L-arabinopyranosyl-(1→2)-α-L-rhamnopyranoside, verified also by Sobreira et al. (2017), the authors also identified quercitrin with a high peak at R_t : 8.5 min, all of them showed antiulcer activity. While Chibli (2013) identified the flavonoid Kanpherol at R_t : 15.54 min.

4. Final Considerations

The extraction method used in the present study proved to be efficient to obtain the extract and its fractions with yields in line with those observed in the literature. After analyzing the data obtained by HPLC, a good separation of the compounds was observed, with major peaks that increase the chance of identification or isolation of the compounds in further studies.

Several methodologies were found to obtain extracts from the leaves of *K. pinnata*, with aqueous extracts and ethyl acetate being the most used, containing mostly compounds of the flavonoid class. Comparative studies show that these derivatives are detectable in the 254 nm range. In the present study, the retention time of 13 minutes stands out for presenting a large peak in the hexane, chloroform, and ethyl acetate fractions, indicating that it may be the compound quercetin 3-*O*-α-L-arabinopyranosyl-(1→2)-α-L-rhamnopyranoside because the same was obtained in several studies found in the literature.

Therefore, in the future, the fractions will be analyzed using chromatographic techniques, such as HPLC-MS/MS, to characterize the chemical compounds present in the fractions. When it is not possible to identify the substance by HPLC-MS/MS, the compound will be isolated and sent to NMR for the determination of its structure.

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References

Ahn, K. (2017). The worldwide trend of using botanical drugs and strategies for developing global drugs. *BMB Reports*.

- Almeida, A. P., da Silva, S. A., Souza, M. L., Lima, L. M., Rossi-Bergmann, B., de Moraes, V. L., & Costa, S. S. (2000). Isolation and chemical analysis of a fatty acid fraction of *Kalanchoe pinnata* with a potent lymphocyte suppressive activity. *Planta Medica*, 66(2), 134-137.
- Almeida, Arimar Chagas de. (2019). *Atividade antimalárica da Kalanchoe pinnata sobre o Plasmodium berghei em modelo experimental in vivo*. Dissertação de Mestrado em Biociências. Universidade Federal do Oeste do Pará – UFOPA.
- Araújo, E. R. D. (2017). *Kalanchoe brasilienses Cambess e Kalanchoe pinnata (Lamarck) Persoon: caracterização química, avaliação gastroprotetora e anti-inflamatória tópica*. Dissertação de mestrado em Ciências Farmacêuticas. Universidade Federal do Rio Grande do Norte.
- Araújo, E. R. D., Guerra, G. C. Bernardo, Araújo, D. F. de S., De Araújo, A. A., Fernandes, J. M., Júnior, R. F. De A., Da Silva, V. C., De Carvalho, T. G., Ferreira, L. De S., & Zucolotto, S. M. (2018). Gastroprotective and Antioxidant Activity of *Kalanchoe brasiliensis* and *Kalanchoe pinnata* Leaf Juices against Indomethacin and Ethanol- Induced Gastric Lesions in Rats. *International Journal of Molecular Sciences*, 19(5), 1265.
- Batista, L. A., Brandão, E. G., Rosas, L. V., Pinto, M. N., Pantoja, T. d., De Araújo, T. M., & Lima, R. A. (2019). Levantamento de plantas medicinais utilizadas contra parasitoses e verminoses intestinais no município de Atalaia do Norte-AM. *Biota amazônia*.
- Benchimol, S. (2010). *Amazônia: um pouco antes e além-depois*. Universidade Federal do Amazonas. (2 ed.).
- Biswas, S. K., Chowdhury, A., Das, J., Karmakar, U. K., & Shill, M. C. (2011). Assessment of cytotoxicity and antibacterial activities of ethanolic extracts of *Kalanchoe Pinnata* Linn. (Family: Crassulaceae) Leaves and Steams. *International Journal of Pharmaceutical Sciences and Research*.
- Brum, A. A. S., De Arruda, L. F., & Regitano-D'Arce, M. A. B. (2009). Extraction methods and quality of the lipid fraction of vegetable and animal samples. *Química Nova*, 32(4), 849-854.
- Chibli, L. A. (2013). Caracterização química e atividades biológicas de *Bryophyllum pinnatum* (Lam.) Oken.
- Costa, S. S., Jossang, A., Bodo, B., Souza, M. L., & Moraes, V. L. (1994). Patuletin acetylramnosides from *Kalanchoe brasiliensis* as inhibitors of human lymphocyte proliferative activity. *Journal of Natural Products*, 1503-1510.
- Costa, S. S., Muzitano, M. F., Camargo, L. M., & Coutinho, M. A. (2008). Therapeutic Potential of *Kalanchoe* Species: Flavonoides and other Secondary Metabolites. *Natural Product Communications*, 2151-2164.
- Coutinho, M. A. S., Casanova, L. M., Dos Santos L. B., Leal, D., Palmero, C., Toma, H. K., Dos Santos, E. P., Nasciutti, L. E., & Costa, S. S. (2021). Wound healing cream formulated with *Kalanchoe pinnata* major flavonoid is as effective as the aqueous leaf extract cream in a rat model of excisional wound. *Natural Product Research*, 35(24), 6034-6039.
- Cruz, E. A., Da Silva, S. A. G., Muzitano, M. F., Silva, P. M. R., Costa, S. S., & Rossi-Bergmann, B. (2008). Immunomodulatory pretreatment with *Kalanchoe pinnata* extract and its quercitrin flavonoid effectively protects mice against fatal anaphylactic shock. *International immunopharmacology*, 8(12), 1616-1621.
- Do Nascimento, A. L., & Yara, R.. (2015). Isolamento e caracterização de produtos bioativos extraídos de ulomoides dermestoides. *Conic XXIII*. pp. 4.
- El Abdellaoui, S., Destandau, E., Toribio, A., Elfakir, C., Lafosse, M., Renimel, I., Landemarre, L. (2010). Bioactive molecules in *Kalanchoe pinnata* leaves: extraction, purification, and identification. *Analytical and Bioanalytical Chemistry*, pp. 1329-1338.
- Embrapa. (2014). *embrapa.br*. Acesso em 23 de 01 de 2022, disponível em Contando Ciência: <https://www.embrapa.br/contando-ciencia/bioma-amazonia>
- Ferreira, R. T., Coutinho, M. A. S., Malvar, D. do C., Costa, E. A., Florentino, I. F., Costa, S. S., & Vanderlinde, F. A. (2014). Mechanisms underlying the antinociceptive, antiedematogenic, and anti-inflammatory activity of the main flavonoid from *Kalanchoe pinnata*. *Evidence-Based Complementary and Alternative Medicine* (v.2).
- Gonçalves, F. S. M. (2017). Mecanismos de ação relacionados à atividade antiúlcera de *Kalanchoe pinnata* (Lam.) Pers. (Crassulaceae). Universidade de São Paulo.
- Higuchi, M. I. (2004). A floresta amazônica e suas múltiplas dimensões: uma proposta de educação ambiental. INPA / CNPq.
- Ichikawa, M., Ogura, M., & Lima, T. (1986). Antiallergic flavone glycoside from *Kalanchoe pinnatum*.
- Kamboj, A., & Saluja, A. K. (2009). *Bryophyllum pinnatum* (Lam.) kurz: phytochemical and pharmacological profile: A review. *Pharmacognosy Review*, pp. 364-375.
- Klitgaard, A., Iversen, A., Andersen, M. R., Larsen, T. O., & Frisvad, J. C. (2014). Aggressive dereplication using UHPLC–DAD–QTOF: screening extracts for up to 3000 funga secondary metabolites. *Anal Bioanal Chem*, pp. 1933-1943.
- Lanna, E. G., moreira, A. M. S., bittencourt, V. C. S., Sousa, O. V., & Denadai, A. M. L. (2019). Avaliação da atividade antioxidante de frações de *Bryophyllum pinnatum* (Lam.) Oken incorporadas em β -ciclodextrina. *Revista Virtual de Química*, 11(4).
- Lisboa, M. S., Ferreira, S. M., & Silva, M. S. (2006). Uso de plantas medicinais para tratar úlceras e gastrites pela comunidade do povoado vila do capim, Município de Arapiraca-AL, Nordeste do Brasil. *Setienbulus Série Ciências Biológicas (Etnobiologia)*.
- Lorenzi, H., & Matos, F. J. (2008). *Plantas medicinais no Brasil: nativas exóticas cultivadas*. (2a ed.), Instituto Plantarum de Estudos da Flora.
- Marques, L. C. (2005). Preparação de extratos vegetais. *Jornal Brasileiro de Fitomedicina*, 3(2), 74-76.
- Muzitano, Michelle F., Bergonzic, M. C., De Melo, G. O., Lage, C. L. S., Bilia, A. R., Vincieric, F. F., Rossi-Bergmann, B., & Costa, S. S. (2011). Influence of cultivation conditions, season of collection and extraction method on the content of antileishmanial flavonoids from *Kalanchoe pinnata*. *Journal of Ethnopharmacology*, 133(1), 132-137.

- Muzitano, Michelle F., Tinoco, L. W., Guette, C., Kaiser, C. R., Rossi-Bergmann, B., & Costa, S. S. (2006) The antileishmanial activity assessment of unusual flavonoids from *Kalanchoe pinnata*. *Phytochemistry*, 67(18), 2071-2077.
- Okwu, D. E., & Nnamdi, F. U. (2011). A novel antimicrobial phenanthrene alkaloid from *Bryophyllum pinnatum*. *Journal of Chemical and Pharmaceutical Research*, 27-33.
- Oliveira, V. B., Zuchetto, M., Oliveira, C. F., Paula, C. S., Duarte, A. S., Miguel, M. D., & Miguel, O. G. (2016). Efeito de diferentes técnicas extrativas no rendimento, atividade antioxidante, doseamentos totais e no perfil por clade de *dicksonia sellowiana* (presl.) Hook dicksoniaceae. *Revista Brasileira de Plantas Mediciniais*, 230-239.
- Pedroza, M. S., Carvalho, C. M., Sanchez, C. T., & Silva, I. O. (2017). Conhecimento etnobiológico sobre o uso de plantas medicinais e ensino de biologia: aproximações iniciais. *Latin American Journal of Science Education*.
- Rodrigues, F. A., Pimenta, V. C., Braga, K. D., & De Araújo, E. G. (2016). Obtenção de extratos de plantas do cerrado. *Enciclopédia biosfera*, 870.
- Silva, R. Y. A. (2019). Estudo dos efeitos de espécies vegetais da relação nacional de plantas medicinais de interesse ao SUS sobre o receptor PXR.
- Skoog, D. A., Holler, W. D., F. J., & Crouch, S. R. (2010). *Fundamentos de Química Analítica* (8a ed.). Cengage Learning.
- Smith, G. (2004). *Kalanchoe* species poisoning in pets. *Vet. Medic.*, 933-936.
- Snafi, A. S. (2013). The Chemical Constituents and Pharmacological Effects of *Bryophyllum calycinum*. A review. *International Journal of Pharma Sciences and Research*.
- Soares, A. M. da S. (2017). Propriedade anti-inflamatória de *Kalanchoe pinnata* pode estar associada à inibição nitrérgica e a ação antioxidante. Universidade Federal do Oeste do Pará.
- Sobreira, F. C. (2013). Avaliação da atividade antiúlcera de *Kalanchoe pinnata* (Lam.) Pers (Crassulaceae). Universidade de São Paulo.
- Sousa, P. C., Rocha, J. S., Pessoa, A. M., & Carvalho, J. C. (2005). Estudo preliminar da atividade anti-inflamatória de *Bryophyllum calycinum* Salisb. *Revista Brasileira de Farmacognosia*, 60-64.
- Tatsimo, S. J. N., Tamokou, J. De D., Havyarimana, L., Csupor, D., Forgo, P., Hohmann, J., Kuate, J., & Tane, P. (2012). Antimicrobial and antioxidant activity of kaempferol rhamnoside derivatives from *Bryophyllum pinnatum*. *BMC Research notes*, 5(1), 1-6.
- Wehrens, R., Carvalho, E., Masuero, D., de Juan, A., & Martens, S. (2013). High-throughput carotenoid profiling using multivariate curve resolution. *Anal Bioanal Chem*, 5075-5086.
- Wright, S. J. (2002). Plant diversity in tropical forests: a review of mechanisms of species coexistence. *Oecologia*, 130.
- Zucchi, M. R., Júnior-Oliveira, V. F., Gussoni, M. A., Silva, M. A., & Marques, N. E. (2013). Levantamento etnobotânico de plantas medicinais na cidade de Ipameri - GO. *Revista Brasileira de Plantas Mediciniais*.