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# PHYTOCHEMICAL PROSPECTION OF THE HEXANIC EXTRACT OF POLYGONUM ACRE (POLYGONACEAE) USED IN TRADITIONAL MEDICINE OF QUILOMBOLA COMMUNITIES OF REGIÃO DOS LAGOS - RJ

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**Abstract:** Plants have been used throughout the history of humanity for various purposes, from religious rituals to the treatment of diseases. Furthermore, concern began not only with the use, but with knowledge of the identity of these substances, contributing to the development of various sectors, such as medicine, pharmaceutical industry, cosmetics industry and food industry. This study aims to relate the traditional use of the plant species Polygonum acre with its phytochemical potential and biological activity. The species used is P. acre, popularly known as Catainha or Erva-de-bicho. P. acre is popularly used in the form of tea, ointment, sitz bath and tablets, due to its anti-inflammatory action. Previous studies have identified groups of secondary metabolites in the aqueous and hydroalcoholic extracts of P. acre, such as tannins, coumarins, flavonoids, saponins, sterols and triterpenes. In this study, a sample of the plant species was subjected to hexane extraction. To identify the presence of groups of secondary metabolites, phytochemical prospecting tests, Thin Layer Chromatography (LCD) and Gas Chromatography with Mass Spectrometry (GC/MS) were carried out. The results of the phytochemical tests were negative for the presence of secondary metabolites: tannins, flavonoids, triterpenes, saponin heterosides and alkaloids in the hexane extract, and positive for phytosterols. While CCD detected the presence of alkaloids and GC/MS identified esters, carboxylic acid and a specific phytosteroid, known as stigma-4-en-3-one. The results reflect the complexity involved in phytochemical studies and the challenges of investigating the chemical composition of a plant species.
Keywords: Gas Chromatography, Phytochemical prospecting, Polygonum acre.

## INTRODUCTION

The use of plants for food has always existed, throughout the development of humanity, where men sought resources in nature to improve their quality of life. In addition to food, plants gradually became the raw material for the manufacture of fabrics, objects and tools. From the observation of the characteristics and aspects of plants such as the power of regeneration and adaptation to different seasons of the year, this possibly contributed to their use for healing rituals (BRAGA, 2011).

The use of medicinal plants as medicine is very old and dates back to the beginnings of civilization. There are records, in China, of the cultivation of medicinal plants as early as 3,000 BC. In addition, there are records of cultivation by the Egyptian, Assyrian and Hebrew people in 2,300 BC. With the plantations, vermifuges, purgatives, cosmetics, diuretics and liquid products and gums for embalming mummies. Descriptions of plants and their effects are found in books from Egyptian temples, in the Book of the Dead in the form of recipes for embalming corpses and in the Book of the Living with descriptions of properties and use of plants for the treatment of diseases (NOLLA et al., 2005 apud COAN; MATIAS, 2013).

According to Akerele (1993), in many areas, especially tropical regions, the abundance of medicinal plants makes it possible to have safe and effective products to prevent, treat illnesses, and combat pests through traditional medicine. It is estimated that 80% of the world's population preferably uses traditional medicine in primary health care, with most traditional therapies involving the use of fresh plants or products manufactured from their extracts or active principles (WHS, 2006 apud QUADROS et al., 2015).

Brazil has a high variety of biomes, with an enormous wealth of both flora and fauna, housing the greatest biodiversity on the planet. Furthermore, many of the species are endemic, and several plant species are of global economic importance. The country is also home to a rich socio-biodiversity, represented by more than 200 indigenous peoples and several communities, such as: quilombolas, caiçaras and rubber tappers. These people carry an invaluable collection of traditional knowledge (BRAZIL, [201-]).

The Atlantic forest biome presents a great diversity of plants with biological action that has not yet been identified and that need to be studied and valued for their preservation in this biome. Contributing in this way, to technological innovation and the introduction of products that have less impact on socio-environmental aspects. To achieve this, it is necessary to intensify studies and research on plant species that can be used for medicinal purposes (QUADROS et al., 2015).

The valorization of ethnoknowledge about the therapeutic properties of plants and traditional knowledge proved to support technical-scientific knowledge. Information on the use of natural resources accumulated by traditional populations has offered scientists models of sustainable use of these same resources. Combining popular information about medicinal plants with chemical/pharmacological studies, this approach allows the formulation of hypotheses regarding the pharmacological activity and the active substance responsible for the reported therapeutic actions, as well as screening in order to validate their use (QUADROS et al., 2015).

The use of plants in the treatment of diseases is the result of an accumulation of centuries-old knowledge, which has been passed from generation to generation, until reaching the most sophisticated forms of medicine production, which today form traditional medicine (GIRALDI; HANAZAKI, 2010; MACHADO; VARGAS, 2018 apud NASCIMENTO, 2021).

In recent years there has been an increase in the consumption of medicinal plants by the world population. For reasons possibly guided by the emergence of new diseases without adequate treatment, the false propagation that natural products do not cause harm, difficulties in accessing health services, and the high cost of allopathic medicines (SILVA et al., 2009).

Phytotherapy is not always a choice between the popular and the conventional, but often because it is the only source of medicine found in several locations. Therefore, people who live in more remote areas often have little access to medicines, thus making use of plants with medicinal uses, and favoring the construction of the tradition of this practice (NIEHUES et al., 2011).

The consumption of medicinal plants in Brazil is characterized by empirical use and the lack of adequate proof of pharmacological actions. Factors such as: intoxication, allergic reactions and ineffective treatment may be relevant to the inappropriate use of these plants. Other attributions to the inappropriate use of medicinal plants are due to errors in identifying species, cultivation methods, storage and preparation. The use of certain plants considered medicinal can pose health risks when the individual handles and consumes them inappropriately, especially if they involve potentially toxic species (SILVA et al., 2009).

Continuing the phytochemical and ethnopharmacological research project with medicinal plants, developed at the Instituto Federal Fluminense Campus Cabo Frio, this work seeks to contribute with phytochemical data on the species Polygonum acre, used in traditional medicine by Quilombola communities of Região dos Lagos, RJ, Brazil.

## LITERATURE REVIEW

### PHYTOCHEMISTRY AND ETHNOPHARMACOLOGY IN BRAZIL

Plants have several chemical substances that are present in several species. Variations in these compounds are subject to the influence of climate, soil composition and the growing season, among others (CUNHA, 2014 apud SOARES, 2016). Knowledge of the chemical constituents of different parts of the plant favors their sustainable use and contributes to their preservation. Furthermore, the secondary metabolites have biological activity, also offering benefits to human health (FINÊNCIO; MININEL, 2019).

Phytochemical prospecting is a preliminary study capable of detecting the presence of certain classes of secondary metabolites. As this is a basic study for the investigation of medicinal plants, it is important to guide the next steps to be carried out in the production of herbal medicines or medicines of plant origin (SOARES et al., 2016).

To achieve this, important steps are followed that will lead to phytochemical research. Among the steps to conduct a phytochemical investigation, the first is developed from the search for traditional knowledge, in order to indicate therapeutic potential in the choice of the plant to be studied, the production or obtaining of the plant, the preparation of the plant drug, obtaining the extract, phytochemical prospecting, chemical determination of the structure of the active ingredient and obtaining the medicine (SOARES et al., 2016).

Therefore, phytochemical analysis gains more importance when there are few chemical studies with the species of popular interest, to verify the quality of the plant drug and provide useful data for understanding the biodiversity and phylogenetics of plants (BESSA et al., 2013).

Natural products are composed of chemical substances produced by a living organism found in nature, having pharmacological or biological activity favoring the discovery of drugs. It can be extracted from tissues of land plants, marine organisms or microorganisms. A crude extract from any of these sources usually contains new, structurally diverse chemical compounds. (LAHLOU, 2013).

For Rodrigues (2018, p. 11), products of natural origin have:

> [...] enormous structural and functional chemical variability due to the metabolic flexibility necessary to adapt these products to different situations of environmental stress (caused by various factors such as chemical, physical and biological agents), consequently, they constitute a renewable source of specialized metabolites, that is, verifies There is an accumulation of secondary metabolites by living organisms. These metabolites play a determining role in adapting to environmental conditions, since organisms are forced to activate certain biochemical and genetic mechanisms in order to survive.

Knowledge about natural products often represents the only therapeutic resource in many communities. Studies involving its use demonstrate a great dimension and awareness through the empirical application of herbal medicine among the population. In Brazil, this alternative therapeutic practice serves to treat many illnesses, as the discovery of new medicines based on natural products has been emphasized by the pharmaceutical industry (SOUSA et al., 2011; GEARY et al., 2012 apud MACHADO et al., 2014).

In 2022, ANVISA reinforced the seriousness of the use of irregular natural products. ANVISA requires that every natural product to be sold in capsules in Brazil must have its composition previously evaluated, so that the risks related to its use are known and avoided. Furthermore, the use of the word

'Natural' is prohibited, to avoid the idea that the use of the medicine is completely risk-free (BRAZIL, 2022).

The use of medicinal plants is the oldest known medication, passed down from generation to generation. However, plants have active ingredients that act on specific cells and organs, or on the entire organism. Some of these principles are harmful to human health. Therefore, it is necessary to know the information about the plant, such as its origin and use (BRAZIL, 2015).

The use of traditional medicine in Quilombola communities is very common, and this practice is attributed to the centuries-old influences of indigenous and African cultures and knowledge. The Quilombos' relationship with their land and the cultivation of medicinal plants known to them is evidence of the cultural connection with their origins, possessing a wide arsenal of knowledge about medicinal plants. Therefore, it is extremely important that they are consulted for the appropriate categorization of Brazilian medicinal plants, facilitating scientific research and drug development (SANDES et al., 2018).

According to an interview carried out by Carvalho (2019) with representatives of the Quilombola communities in the municipalities of cites: of Búzios and Cabo Frio, it was possible to observe a diversity of plant species reported to be used for medicinal purposes.

The Policy and the National Program of Medicinal Plants and Phytotherapeutics (PNMPF) proposed the expansion of therapeutic options and improvement of health care for users of the Unified Health System (SUS), thus guaranteeing the Brazilian population safe access and use rationale of medicinal plants and herbal medicines. The SUS offers around twelve herbal medicines that are included in the National List of Essential Medicines (Rename). In 2009, the List of Medicinal Plants of Interest to the SUS was drawn up, listing 71 species with therapeutic potential, with Polygonum acre ranking 53rd (BRASIL, 2017).

## FAMÍLIA POLYGONACEAE

Polygonaceae is a cosmopolitan family of herbs, vines, shrubs and small trees known for having simple leaves with ocher coated stipules, unilocular ovary and endospermic seeds (HUTCHINSON; DALZIEL, 1954; BRUMMITT, 1992 apud AYODELE; OLOWOKUDEJO, 2006).

Consisting of around 42 genera and approximately 1,100 species, Polygonaceae is distributed mainly in the temperate regions of the Northern Hemisphere (CIIALDELLA; BRANDBYGE, 2001 apud MELO, 2018). Its distribution is cosmopolitan, being distributed in tropical, subtropical and temperate regions. They are frequently observed in humid and flooded environments, along rivers and lake shores (BARROSO, 1978; ENGLER, 1964; SILVA-BRAMBILLA; MOSCHETA, 2001 apud, 2012).

Among the Polygonaceae family, there are seven native genera in Brazil, namely: Polygonum, Lapathifolium, Rumex, Coccoloba, Muehlenbeckia, Ruprechtia, Symmeria and Triplaris (MELO, 2018).

The maps below (Figure 1 and 2) show the geographic distributions of the Polygonaceae Family in the world and in Brazil:

| Popular name | Part used | Traditional Action/Purpose |
|---|---|---|
| Avocado | Leaf | Renal insufficiency |
| Pumpkin | Leaf, fruit, seed | Problems in dealing digestive/Vermifuge/Anti-inflammatory |
| Alfazema | Flor, leaf, stalk | Soothing/ Anti-spasmodic/ Analgesic/ Antidepressant |
| Blackberry | Leaf | Anti-inflammatory |
| Anador | Leaf | Respiratory diseases/ Healing/ Anti-bacterial |
| Arnica | Leaf | Anti-inflammatory/ Renal failure/ Lung diseases |
| Aroeira | Fruit, flower, seed, shell | Healing/ Anti-infective |
| Arrebenta-cavalo | Leaf | Anti-inflammatory |
| Arruda | Leaf | Abortive |
| Assa peixe | Leaf | Lung diseases |
| Cabelo de milho | Stigmata | Anti-inflammatory/ Renal failure/ Urinary infection |
| Cashew | Leaf, fruit | Healing |
| Cânfora | Leaf | Healing |
| Caninha-do-brejo | Leaf | Renal insufficiency |
| Capim-limão | Leaf | Soothing |
| Catainha/ Erva-de-bicho | Stalk, leaf, seeds | Anti-inflammatory |
| Onion | Bulb (stem) | Antioxidant/ tract problems digestive/respiratory diseases |
| Lemongrass | Leaf | Soothing |
| Erva-de-jacaré | Leaf, caule | Antiallergic/Healing/Diuretic/ Antipyretic |
| Erva-de-passarinho | Leaf | Lung diseases |
| Erva-tostão | Leaf, flower, stem, root | Kidney and liver failure/ Analgesic for menstrual pain |
| Espinheira santa | Seeds | Vermifuge |
| Hibisco | Leaf, flower | Diabetes/High Cholesterol |
| Macaé / Maria-Augusta | Leaf | Diarrhea |
| Noni | Leaf, fruit | Diabetes/kidney failure |
| Pau-brasil | Bark | Bronquitis |
| Pé-de-galinha | Leaf | Anti-inflammatory/ Analgesic |
| Penicillin | Stalk, leaf | Anti-infective |
| Picão | Leaf | Anemia |
| Pitanga | Leaf | Pneumonia/Cicatrizing |
| Poejo | Leaf | Cold |
| Quebra-pedra | Leaf | Renal insufficiency |
| Okra | Fruto | Healing/Diabetes |

Table 1 - Plants used for medicinal purposes mentioned in the interviews

Source: Carvalho (2019).


Figure 1 - Geographic distribution of the Polygonaceae family

Source: Brazilian Biodiversity Information System – SIBBr

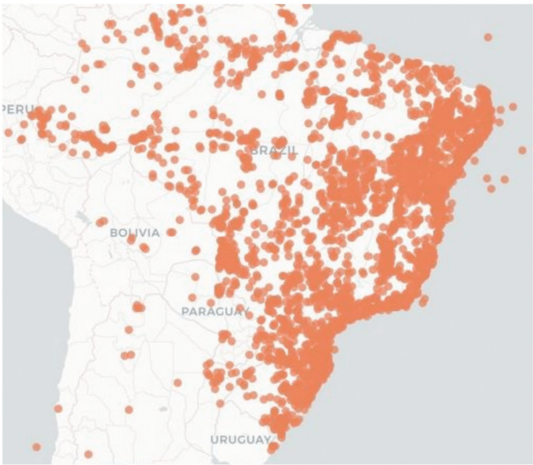


Figure 2 - Geographic distribution of the Polygonaceae family, in Brazil

Source: Brazilian Biodiversity Information System - SIBBr.

The Polygonaceae family has species used for various purposes, which stand out for their medicinal and toxic properties presented by species of the Polygonum genus. Some of the species belonging to this group are characterized by being crop invasive plants, in addition, others stand out for their timber, ornamental and landscaping importance (SILVA-BRAMBILLA; MOSCHETA, 2001 apud MENES, 2012).

## GÊNERO *POLYGONUM*

The genus Polygonum has approximately 200 species that are mostly distributed throughout North America, Central America, Asia and South America (CIIALDELLA; BRANDBYGE, 2001 apud MENES, 2012). Most of the species are aquatic, occurring on the edges of lakes, riverbanks and floodable areas (MELO, 2008). Consequently, due to the environment in which they are found, they are capable of developing in soil with good fertility, sandy or clayey and with a high content of organic matter (LIMA et al., 2001; BIESKI, 2005; LOPES et al., 2003 apud MENES, 2012).

Despite the several species of the genus in Brazil, only the species Polygonum bistorta has a monograph registered in the Brazilian Pharmacopoeia of 1926, and is not part of other subsequent editions (JACOME et al., 2004).

Several species of the genus Polygonum are in common use in traditional Chinese medicine due to their effects on various health disorders, e.g. antioxidant, anti-inflammatory, antibacterial and antifungal, anticancer, antiviral, lipid regulating, neuroprotective and estrogenic effect (DONG et al., 2014).

The use of thirty-four species of Polygonum have been reported for different traditional medicine uses in the eastern Himalayan region of India. The plant species are used as a laxative, febrifuge, emollient, tonic, diuretic, relief from gonorrhea, relief from menstrual and gynecological problems, relief from dysentery, wound healing agent, relief from jaundice, relief from hemorrhoids, relief from muscle pain, relief of heart problems (CHOUDHARY, 2011).

Many of the species belonging to the genus Polygonum have medicinal properties. The species of the genus present a great diversity of secondary metabolites, some being rich in flavonoids, terpenes or other classes of


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metabolites (XIAO et al., 2000; YAGI et al., 1994; FURUTA et al., 1986 apud PASTORI et al., 2015).

## POLYGONUM ACRE

Within the Polygonaceae family, the species Polygonum acre or Polygonum punctatum Ell stands out. This species is perennial and found in humid or flooded places, developing preferably in soil with good fertility, or clayey and with a good content of organic matter. It is a native Asian plant, and is found in Brazil in all states, mainly in Rio Grande do Sul, and in the South and Southeast states (LOPES et al., 2003).

Polygonum acre is popularly known by several names, such as: yerba-de-bicho, capiçoba, water pepper, pepper-do-brejo, cataia, potincoba, percicária-do-Brazil, petincobe, herb flea, cataria, capetiçoba, Acataia and Catalan (LOPES et al., 2003). The figures below (Figures 3 and 4) illustrate the aerial parts and inflorescences of P. acre, respectively:

Figure 3 - Aerial parts of *P. acre*

Source: BRAZIL (2014).

Figure 4 - Inflorescences of *P. acre*

Source: BRAZIL (2014).

Studies show that all parts of P. acre are used in folk medicine, mainly as an antidysenteric, antiseptic, stomachic, diuretic, antipyretic, antirheumatic, vermifuge and wound and ulcer healing agent. Furthermore, this species is also considered strongly emmenagogue and abortive, and is not recommended for pregnant women (NASCIMENTO, 2021).

P. acre has been reported to be used orally as an antihemorrhagic and expectorant. Topically, it is used as a disinfectant and in the treatment of hemorrhoids, in the form of a sitz bath (BRASIL, 2014).

The species Polygonum acre is cited by Brasil (2006) as one of the plants selected for further studies and evaluation, being referred to in the following classifications: diuretic and/or antilithiasis, hypotensive, anti-infectious, analgesic, antipyretic, anti-inflammatory and/or antispasmodic.

In the aqueous and hydroalcoholic extracts of P. acre, the presence of tannins, coumarins, flavonoids, leucoanthocyanins, saponins, sterols and triterpenes were identified (SIMOES, et al., 1989 apud MENES, 2012). Figure 5 shows some substances identified from P. acre.

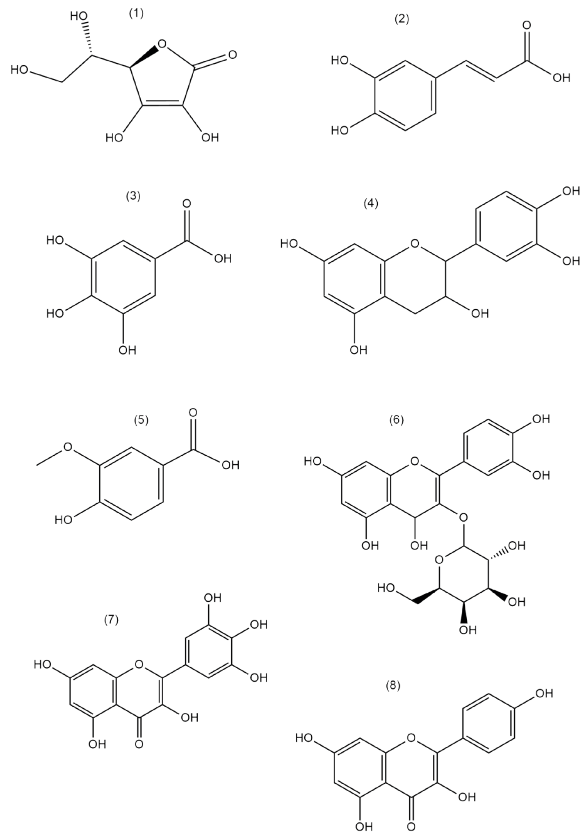

Figure 5 - Substances identified from P. acre. (1) ascorbic acid, (2) caffeic acid, (3) gallic acid, (4) catechin, (5) vanillic acid, (6) hyperoside, (7) myricetin and (8) kaempferol.

Source: elaborated by the author (2023).

Sofiati (2009) highlights that even though there is an intense use of P. acre by the population, there are still few supporting studies that certify its use, demonstrating the urgency of more basic and clinical studies for this species.

In his studies, Pastori (2015), claims that the present studies are just the beginning of research and more studies are needed to establish the safe concentration for the use of Polygonum acre in popular medicine. Furthermore, the author warns that the use of this medicinal species by the population must be careful.

## METHODOLOGY

The methodology adopted in this research is based on the investigation of groups of secondary metabolites through techniques for identifying these groups and the chemical composition of plants. Phytochemical tests consist of applying specific chemical reagents that interact with different groups of secondary metabolites, resulting in visible changes, such as color changes, formation of bubbles or precipitates. These changes provide indicative evidence of the presence or absence of certain groups of secondary metabolites (CARRERA et al., 2014).

The flowchart below (Figure 6) was developed with the aim of providing a clear and general overview of the research steps to be followed.

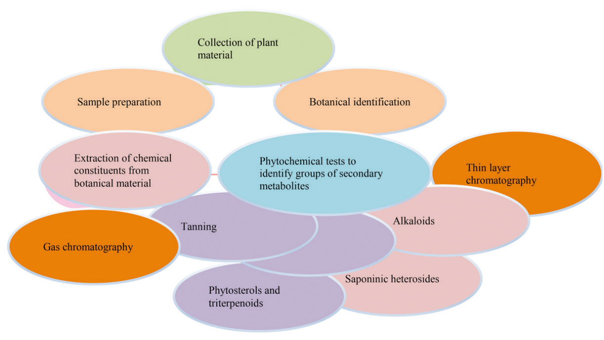


Figure 6 - Methodology steps

Source: elaborated by the author (2023).

## COLLECTION OF PLANT MATERIAL AND BOTANICAL IDENTIFICATION

The choice of the plant species Polygonum acre for the application of phytochemical and toxicological tests is due to its use in traditional medicine, as well as the fact that there are few published studies on its ethnopharmacological activity and to continue the work of Carvalho (2019).

A sample of the aerial part of the plant (Figure 7) was collected in the rural area of the city of Cachoeiro de Itapemirim/ES, in the district of Córrego dos Monos. The sample was collected in another state, due to the difficulty of finding this plant species in ``Região dos Lagos``, and this difficulty had already been found in research by Carvalho (2019) using unpublished data. With the help of a botanist professor at the Instituto Federal Fluminense, Cabo Frio campus, a botanical analysis and classification (Table 2) of the inflorescence of the plant material sample was carried out.

Figure 7 - *Polygonum acre*

Source: elaborated by the author (2023).

| | |
|---|---|
| **Family** | *Polygonaceae* |
| **Gender** | *Polygonum* |
| **Species** | *Polygonum acre* |

Table 2 - Botanical identification of the plant species

Source: elaborated by the author (2023).

## SAMPLE PREPARATION AND EXTRACTION OF CHEMICAL CONSTITUENTS FROM BOTANICAL MATERIAL

The collected sample was dried in the open air (Figure 8) and subsequently crushed manually. To prepare the crude extract of P. Acre, the sample, previously treated, was subjected to percolation of organic solvent (Hexane) for two weeks, removing the solvent after a completed week, replacing it with more solvent. The extract was concentrated and distilled in a rotary evaporator.

Figure 8 - Outdoor drying of the aerial part of *P. acre*

Source: elaborated by the author (2023).

## PHYTOCHEMICAL TESTS TO IDENTIFY GROUPS OF SECONDARY METABOLITES

The phytochemical tests carried out were based on the methodology used by Carvalho (2019) and adapted from the techniques cited by URCAMP (2021). The hexane extract of Polygonum acre was subjected to analysis of: tannins, flavonoids, phytosterols and triterpenoids, saponin heterosides and alkaloids (Table 3).

| Secondary Metabolite | Reagents | Positive result |
|---|---|---|
| Tannins | $FeCl_3$ 5% in ethanol | Precipitated, Hydrolysable Tannins – Blue; Condensed tannins – Green. |
| Flavonoids | Hydrochloric Acid and Magnesium Ribbon | Orange or red coloring |
| Phytosterols and triterpenoids | Dichloromethane, Acetic anhydride and Sulfuric acid. | Phytosterols – Green color; Triterpenoids – Dark red color. |
| Saponin heterosides | Dissolving in water and stirring. | Permanent foam formation. |
| Alkaloids | Hydrochloric acid, ninhydrin 2% w/v in 95° ethanol%. | Purple coloring for the presence of primary amine. |

Table 3 - Phytochemical tests to identify groups of secondary metabolites

Source: elaborated by the author (2023).

TANNIN ANALYSIS

To identify tannins in the sample, 100 mg of the extract was solubilized in 10 mL of distilled water and 5 mL of the solution was transferred to a test tube, then 3 drops of 5% FeCl3 in ethanol were added. It was observed whether there was the formation of a precipitate, which gives a positive character to the test. Based on the color of the precipitate, it is possible to classify it as hydrolysable tannins (blue) and condensed tannins (green).

FLAVONOID ANALYSIS

To identify the presence of flavonoids in the sample, 100 mg of the extract was solubilized in 10 mL of absolute ethanol and 3 mL of the solution was transferred to a test tube. Subsequently, 1 cm of magnesium tape was added and, carefully, 5 drops of hydrochloric acid were added. It was observed whether the red color appeared, thus giving a positive character to the test.

ANALYSIS OF PHYTOSTEROLS AND TRIPERPENOIDS

To identify the presence of phytosterols in the sample, the Liebermann-Burchard test was used. 25 mg of the extract was solubilized in 10 mL of dichloromethane and transferred to the test tube. Soon after, 1 mL of acetic anhydride was added, stirring gently, and 1 mL of concentrated sulfuric acid was carefully added along the sides of the tube. If the upper layer turns green, it shows the presence of steroids, while if a dark red color is formed, it indicates the presence of triterpenoids.

ANALYSIS OF SAPONIN HETEROSIDES

To detect the presence of saponin heterosides, 100 mg of the extract was solubilized in 10 mL of distilled water and transferred to a test tube, shaking it for a few seconds. It was observed whether foam formed for 15 minutes, giving the test a positive character.

ALKALOID ANALYSIS

To detect the presence of alkaloids, 100 mg of the extract was solubilized in ethanol and boiled. Then, 30 mL of hydrochloric acid was added and the solution was filtered. Transferring the solution to a test tube, 1 mL of 2% (w/v) ninhydrin solution in 95% ethanol was added, finally stirring. The formation of a purple color was observed, which indicates the presence of primary amine.

**ALKALOID ANALYSIS VIA THIN LAYER CHROMATOGRAPHY**

To identify alkaloids via chromatography, thin layer chromatography was performed. Initially, the extract was diluted with chloroform, and a spot was applied to the base of two chromatographic plates, eluting with chloroform and 5% ethanol. For development, Dragendorff was used, and for the other, sulfuric vanillin and heating.

**ANALYSIS BY GAS CHROMATOGRAPHY**

GC/MS parameters: Gas chromatography coupled to mass spectrometry analyzes were carried out using Agilent equipment, model 5975C mass coupled to a 7890 A gas chromatograph. The sample was inserted with a 10 μL syringe into an injector automatic model 7693A. A capillary column (HP-5 ms, 30 m × 250 μm × 0.25 μm (5% phenyl)-methylpolysiloxane film) was used with helium as carrier gas at a constant flow rate of 1.0 mL/min. The injected volume was 1 μL in 1:2 split mode. Mass detector analyzes were performed, applying the following parameters: interface temperature, and electron impact ionization (IE) mode at 70 eV.

## RESULTS AND DISCUSSION

### SAMPLE PREPARATION AND EXTRACTION OF CHEMICAL CONSTITUENTS FROM BOTANICAL MATERIAL

The collected plant went through the process of drying the aerial part, with the aim of preserving its chemical compounds and avoiding decomposition or the action of microorganisms that could degrade them. This process helps to preserve the sample during transport and storage of the plant until carrying out subsequent analyses. Furthermore, drying is an initial step towards extracting compounds from the plant. Removing the water present in the aerial part facilitates the extraction of the desired compounds, since the presence of water can interfere with solubility or the extraction process (TISCHER, 2014).

### TANNIN ANALYSIS

Tannins are complex phenolic substances that can be found abundantly in various parts of plants such as: roots, branches, leaves, flowers, fruits and seeds. They have medicinal applications, due to their astringent and hemostatic properties. These compounds interact with amines, amides and protein substances, forming a precipitate. Furthermore, they have the ability to react and precipitate in the presence of metallic salts, such as: lead, copper, zinc, chromium and iron (URCAMP, 2021).

The detection of this group of substances can be done through the complexation reaction between the phenolic hydroxyl group and ferric chloride, as illustrated in the scheme below. The complex formed has a yellow color. Therefore, any significant change in color indicates the presence of phenols (BARBOSA et al., 2020).

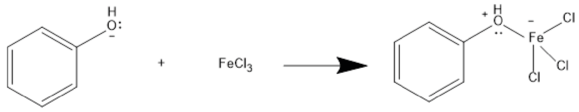


Figure 9 - Complexation reaction between phenolic compound and ferric chloride

Source: Barbosa *et al.* (2020).

### FLAVONOID ANALYSIS

Flavonoids are one of the most important and diverse groups of phenolic compounds found in products of natural origin. The group performs a variety of functions in plants, being responsible for protection against UV rays, insects, fungi, viruses and bacteria. Flavonoids have significant pharmacological properties, the main one being antioxidant action. (URCAMP, 2021).

The Shinoda or cyanidin reaction is one of the most used techniques to analyze the flavonoid group. This reaction is based on the reduction of flavonoid derivatives, as illustrated in the scheme below, yellow in color, into anthocyanins, with a reddish color (BARBOSA et al., 2020).

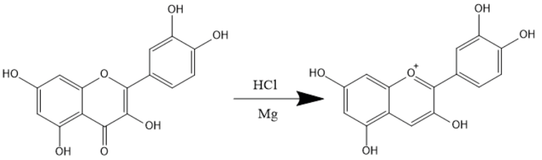


Figure 10 - Flanoid derivatives reduction reaction

Source: Barbosa *et al.* (2020).

### ANALYSIS OF PHYTOSTEROLS AND TRITERPENOIDS

The Liebermann-Burchard Test is a phytochemical test used to detect cholesterol and certain compounds, including phytosterols and triterpenoids, in samples. These compounds can be found in plants and have diverse applications in medicine and cosmetics. The Liebermann-Burchard reaction involves the formation of colored dyes when phytosterols or triterpenoids react with concentrated acetic acid and sulfuric acid. These compounds react in a specific way, producing characteristic colors that can be used to identify their presence. Therefore, the Liebermann-Burchard test can be used as a quick and qualitative method to determine the presence of these compounds (COSTA, 1994).

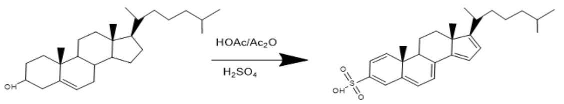


Figure 11 - Liebermann-Burchard reaction, steroid detection

Source: Queiroz (2009).

### ANALYSIS OF SAPONIN HETEROSIDES

Saponins are secondary metabolites that have surfactant properties, forming foam when agitated in water due to their ability to reduce surface tension. The detection of these substances is based on this persistent foaming property. Furthermore, saponins are known for their biological activities, including anti-inflammatory, antimicrobial and antioxidant actions. Laboratory and experimental studies on animals have revealed the potential of saponins as antitumor agents (MARCUZ et al., 2019).

### ALKALOID ANALYSIS

Alkaloids play an important role as protective chemical agents in plants. They are responsible for a variety of physiological effects in animals and may have antimicrobial properties. Some alkaloids act as insecticides and repellents for herbivores and are considered chemosystematic markers. They also exhibit antitumor, anesthetic, antimalarial and antibacterial actions. The presence of alkaloids has been observed in a wide range of investigated biological activities (OLIVEIRA et al., 2009).

The reaction between amino acids and ninhydrin is a method that consists of a colorimetric reaction between the amino and carboxyl functional groups of amino acids with the reagent ninhydrin (CHO). In this reaction, the amino group of amino acids undergoes oxidation by a weaker oxidizing agent, ninhydrin, forming ammonia, $CO_2$ and aldehyde (RCOH), obtained by the loss of a carbon atom of the original amino acid. Thus, an equivalent of ninhydrin serves as an oxidant of the amino acid, forming a ninhydrin in the reduced form. A second equivalent of the oxidized ninhydrin then reacts with the reduced ninhydrin and the ammonia formed, to form a purple complex (SPACKAM et al., 1958 apud OLIVEIRA, 2005).

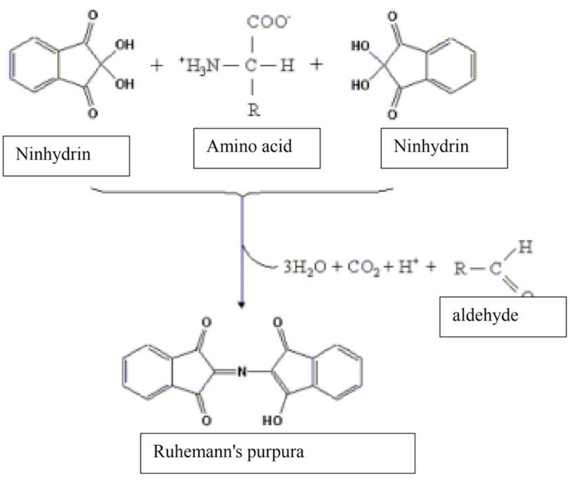


Figure 12 - Reaction between amino acids and ninhydrin

Source: UNESP (2014).

The first test performed was the phytochemical test with ninhydrin, which revealed the formation of a purple color, which would indicate the presence of a primary amine. The test had an inconclusive result, as there was no change in color. Therefore, we continued with the identification of alkaloids by thin layer chromatography. A mixture of chloroform and ethanol was used as eluent. Mixing chloroform and ethanol in chromatography offers advantages due to their complementary properties. Chloroform is a non-polar solvent, suitable for dissolving non-polar or moderately polar organic compounds. While, ethanol is a polar solvent, with greater affinity for polar compounds. This combination allows for the separation of different types of substances.

## PHYTOCHEMICAL TEST RESULTS

Table 4 contains the results obtained in the phytochemical tests, using the parameter physical changes expected in table 3, which would indicate a positive result for the presence of the groups of secondary metabolites tested.

| Chemical class | Result |
|---|---|
| Tannins | - |
| Flavonoids | - |
| Phytosterols | + |
| Triterpenes | - |
| Saponin heterosides | - |
| Alkaloids | - |

Table 4 - Result of phytochemical tests

Source: elaborated by the author (2023).

At this specific stage of the research, phytochemical tests were carried out to investigate the presence of groups of secondary metabolites in the hexane extract of the P. acre species. The results obtained did not present the physical changes expected in Table 3, which would indicate a positive result for the presence of the classes: tannins, flavonoids, triterpenes, saponin heterosides and alkaloids, which suggests the absence of these groups in the sample under study. Except for the phytosterol class, where a green color was formed (Figure 13).

The detection of compounds that have low concentrations in extracts can be masked during phytochemical tests due to the low sensitivity of the method. Thus, compounds that were not found in the test may be present in extracts obtained in different areas and seasons, but in low concentrations (MARTINS, 2012).

A broad assessment would be necessary to identify the factors responsible for negative results in tests where positive results were expected, already reported in the literature. However, this assessment was unfeasible due to the complexity of the correlations between the various environmental factors and their joint influence on secondary metabolism, in addition to the lack of time available to carry out tests and compare data (MORAIS, 2009).

The production of secondary metabolites in plants is influenced by age and stage of development. In general, younger tissues exhibit greater biosynthetic activity, leading to increased production of various compounds. Furthermore, the water factor plays a crucial role in the growth and development of plants, the amount of water available and water stress can affect several physiological processes, such as stomatal opening, photosynthesis and leaf growth, consequently impacting the secondary metabolism of plants. Nutrition is also important, since excess or deficiency of nutrients can directly affect the production of active ingredients. For example, nitrogen deficit can reduce the alkaloid content in certain plants, while others can show an increase in compounds such as morphine and atropine under this condition (MORAIS, 2009).

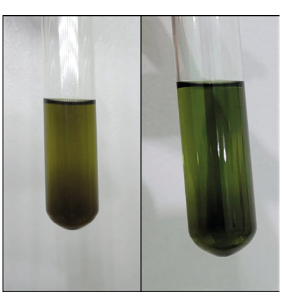

Figure 13 - Phytosterol test with green color, indicating a positive result

Source: elaborated by the author (2023).



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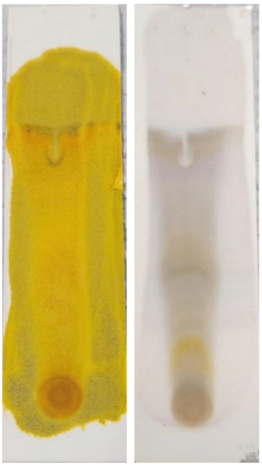
## RESULT OF ALKALOID ANALYSIS VIA THIN LAYER CHROMATOGRAPHY

The CCD test with Dragendorff reagent is a method used to detect the presence of alkaloids in a sample. This reagent is especially sensitive to alkaloid compounds and can produce characteristic colors that help in the preliminary identification of these compounds.

A CCD plate pre-coated with silica gel was used. A line was marked approximately 1 cm above the bottom of the plate, where the sample was applied with the aid of a capillary tube. Then, the CCD plate was placed in the development chamber with a small amount of solvent containing chloroform and 5% ethanol. The solvent traveled through the plate by capillary action, dragging the sample components.

After solvent migration, the plate was removed from the chamber, and after drying, it was checked under a UV lamp. Alkaloids often fluoresce under UV light. Then, the previously prepared Dragendorff reagent solution was sprayed under the CCD plate and waited a few minutes. After applying the reagent, spots on the CCD plate, which showed a characteristic color ranging from orange to red, were observed, indicating positive for alkaloids. It is important to remember that the Dragendorff test is a preliminary screening technique. The definitive identification of alkaloids requires the use of more advanced analysis techniques (DI STASI, 1996; CUNHA, 2005). The chromatographic plates had the following result with the developers: Dragendorff (I) and sulfuric vanillin/heating (II).



(I) (II)

Figure 14 - Result of chromatographic plates

Source: elaborated by the author (2023).

## GC/MS ANALYSIS RESULTS

The figure 15 lists the peaks of greatest interest from the point of view of secondary metabolites. The results were identified by comparison with the equipment's NIST library database, observing the highest similarity rates and visual inspection of the mass spectra obtained versus the equipment's library, and are indicated in figures 16 to 27.

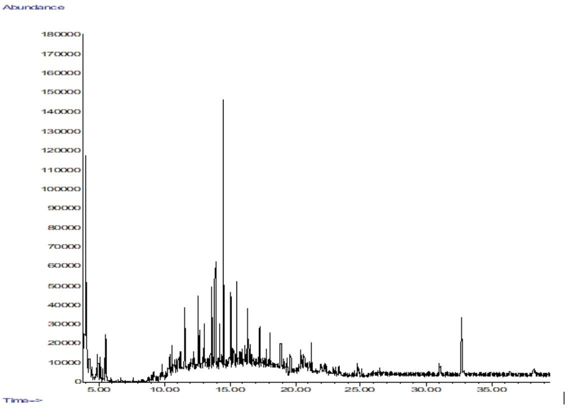



Figure 15 - Chromatogram obtained by GC/MS of the hexane extract of *P. acre*

Source: elaborated by the author (2023).

| Peak | R.T. First min | Max Last scan | scan | scan | PK Ty | Peak height | corr. area | corr. % max. | % of total |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 13.889 | 1053 | 1063 | 1068 | VV | 50643 | 895848 | 41.65% | 8.270% |
| 2 | 14.247 | 1098 | 1101 | 1104 | M3 | 20883 | 353616 | 16.44% | 3.265% |
| 3 | 14.552 | 1129 | 1133 | 1136 | VV | 130140 | 2067497 | 96.13% | 19.087% |
| 4 | 15.077 | 1183 | 1189 | 1197 | VV5 | 37021 | 812893 | 37.79% | 7.504% |
| 5 | 16.382 | 1324 | 1327 | 1329 | M2 | 19545 | 331782 | 15.43% | 3.063% |
| 6 | 32.712 | 3034 | 3054 | 3078 | PB8 | 29801 | 2043915 | 95.03% | 18.869% |

Table 5 - Signs referring to CG

Source: elaborated by the author (2023).

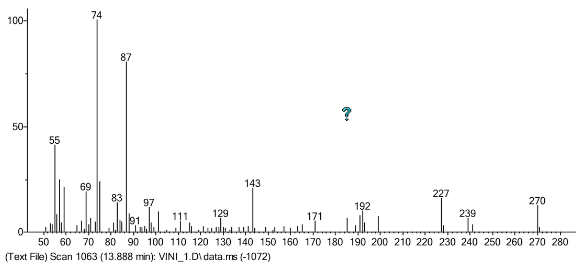



Figure 16 - Mass spectrum 70 eV – Peak 1

Source: elaborated by the author (2023).

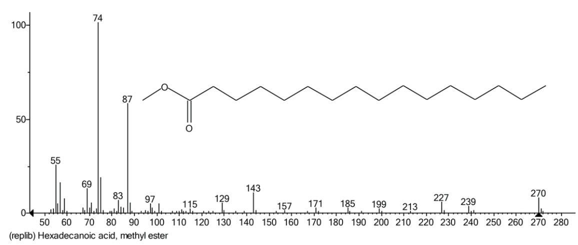



Figure 17 - Equipment NIST Library Database Reference for Hexadecanoic Acid Derivative Methyl Ester

Source: elaborated by the author (2023).

The peak with retention time 13.88 min compared to the instrument library data showed 41.65% correspondence to the methyl ester derived from hexadecanoic acid.

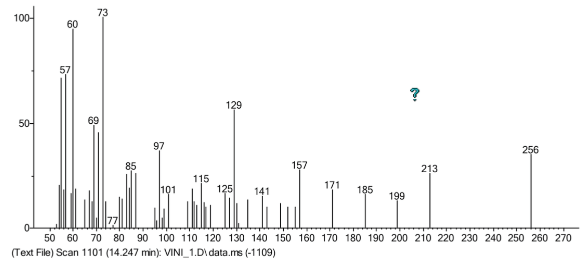



Figure 18 - Mass spectrum 70 eV – Peak 2

Source: elaborated by the author (2023).

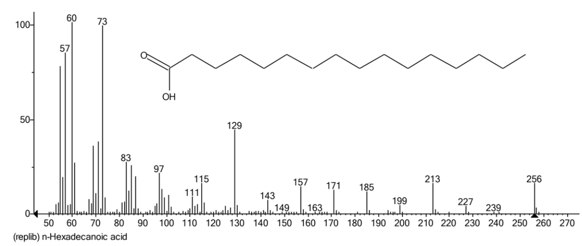



Figure 19 - Equipment NIST Library Database Reference for Hexadecanoic Acid

Source: elaborated by the author (2023).

The peak with retention time 14.24 min compared to the device library data showed a 16.44% match for hexadecanoic acid.

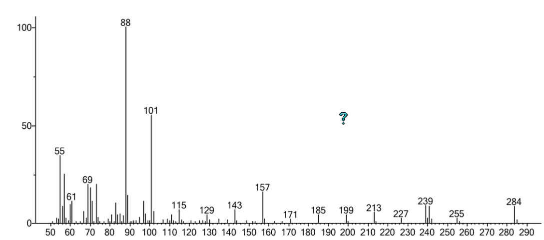



Figure 20 - Mass spectrum 70 eV – Peak 3

Source: elaborated by the author (2023).

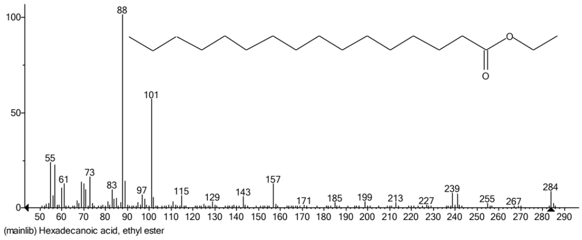



Figure 21 - Equipment NIST Library Database Reference ethyl ester derived from hexadecanoic acid

Source: elaborated by the author (2023).

The peak with retention time 14.55 min compared to the instrument library data showed 96.13% correspondence to the ethyl ester derived from hexadecanoic acid.

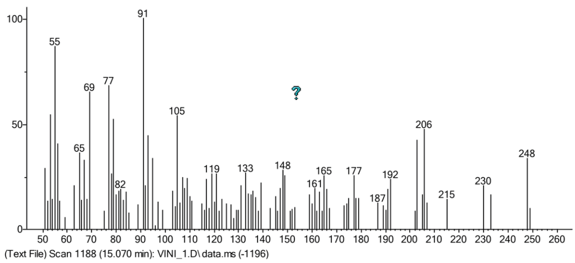



Figure 22 - Mass spectrum 70 eV – Peak 4

Source: elaborated by the author (2023).

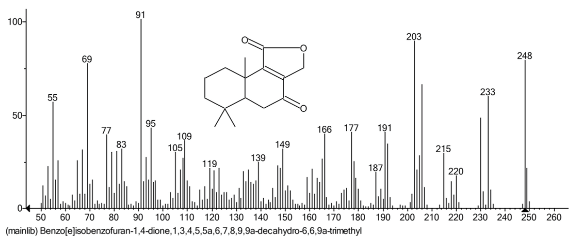



Figure 23 - Equipment NIST Library Database Reference for Isobenzofuran-1,4-dione

Source: elaborated by the author (2023).

The peak with retention time 15.07 min compared to the instrument library data showed 37.79% correspondence to isobenzofuran-1,4-dione.

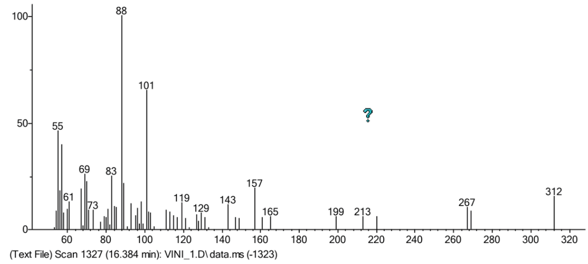



Figure 24 - Espectro de massas 70 eV – Pico 5

Source: elaborated by the author (2023).

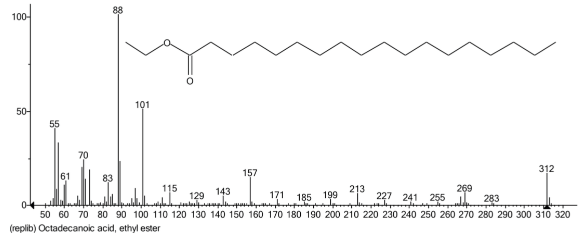



Figure 25 - Equipment NIST Library Database Reference for Octadecanoic Acid Derivative Ethyl Ester

Source: elaborated by the author (2023).

The peak with retention time 16.38 min compared to the device library data showed 15.43% correspondence to the ethyl ester derived from octadecanoic acid.

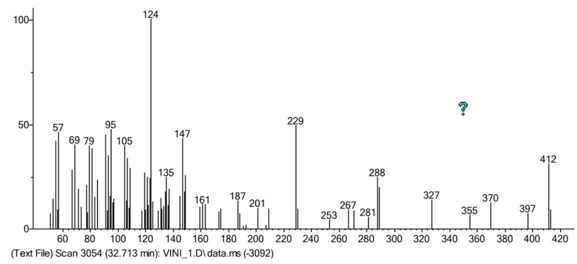



Figure 26 - Mass spectrum 70 eV – Peak 6

Source: elaborated by the author (2023).

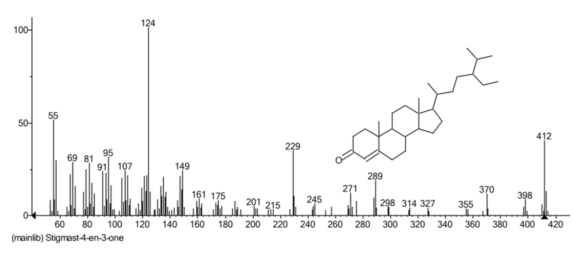



Figure 27 - Equipment NIST Library Database Reference for stigma-4-en-3-one

Source: elaborated by the author (2023).

The GC/MS analysis allowed the observation of the signal with retention time tR1 = 32.7 min (95.03%), and the mass spectrum obtained from each signal allowed assigning m/z = 412, suggesting the presence of stigma-4-en-3-one substance by comparison with the NIST library database of equipment and literature data (LIMA, 2016; SILVA, 2017). The proposals for the main fragmentations observed are found in Figure 28.

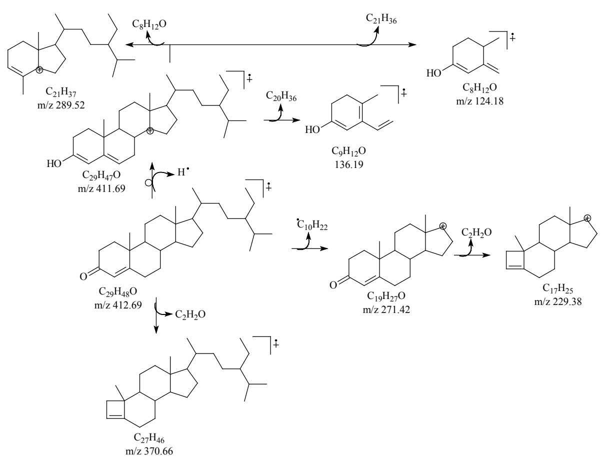



Figure 28 - Stigma Fragmentation Scheme -4-en-3-ona

Source: elaborated by the author (2023).

The substance stigma-4-en-3-one belongs to the class of phytosterols. This class has a biological function related to its ability to interfere with the fluidity of the plant membrane and its permeability to water. It also plays an important role in human health due to its potential benefits, especially in relation to cardiovascular health. Phytosterols are considered to have the ability to reduce the levels of total cholesterol, LDL-cholesterol and triacylglycerides in human blood serum. Therefore, with the aim of contributing to reducing the incidence of diseases, they are added to some foods (BREDA, 2010). Furthermore, its intake results in a potentially chemopreventive effect, mainly for breast cancer, colon cancer and prostate cancer and on oxidative stress, which is related to the development of atherosclerosis (FANI, 2018 apud SOUZA, 2019).

## FINAL CONSIDERATIONS

In this study, we sought to relate the traditional use of Polygonum acre with its potential pharmacological use and biological activity. To achieve this objective, specific objectives were outlined that included mapping groups of secondary metabolites in the hexane extract of Polygonum acre, carrying out phytochemical tests, in order to contribute to the ethnobotanical and ethnopharmacological knowledge of the ``Região dos Lagos``.

The results of the phytochemical tests carried out on the hexane extract revealed the presence of phytosterols in the sample, while for the other groups of secondary metabolites tested, a negative result was shown. The negative result can be attributed to several factors, including the complexity of the phytochemical tests and the development conditions of the plant species collected. It is important to highlight that the absence of positive responses does not necessarily rule out the presence of these metabolites, but may suggest the need to review testing methodologies or consider natural variations in the chemical composition of the plant. However, an analysis with extracts of different polarities is essential for a more comprehensive understanding of the chemical composition of the plant species.

Chromatographic analysis (CCD) detected the presence of alkaloids, as well as a mixture of other compounds in the sample. While GC/MS analysis, which is a high-resolution technique for separating compounds and identifying them, detected in the sample: esters, carboxylic acid and a specific phytosteroid, known as stigma-4-en-3-one.

In an extract produced using a low polarity solvent such as hexane, it is expected that non-polar substances will be extracted, such as fatty acids and phytosteroids. Therefore, the chemical classes and substances identified in the analyzes are aligned with the expectations for extraction from this type of solvent.

Phytochemical prospecting studies are essential due to their ability to identify and characterize chemical compounds present in plants. These studies play a crucial role in discovering new compounds with therapeutic potential, validating traditional uses of medicinal plants, understanding plant-insect interactions and ecological adaptations.

The results reflect the complexity involved in phytochemical studies, the challenges of investigating the chemical composition of a plant species. Furthermore, it highlighted alkaloids and phytosterols as possible groups of secondary metabolites present in the hexane extract of Polygonum acre.

Therefore, more tests are needed to better understand the composition of the species popularly used in Região dos Lagos. These results contribute to the ethnobotanical and ethnopharmacological understanding of the ``Região dos Lagos``and provide information for future research.

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