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RESEARCH ARTICLE OPEN ACCESS

EXTRACTION AND CHARACTERIZATION OF LATEX FROM THE *Euphorbia Tirucalli*

Marcelo Paulo Stracke[1], Bruna Teresinha Klassen Tusset[2], Antonio Vanderlei dos Santos[3] and Rozelaine de Fatima Franzin[4]

[1]Doutor e Professor do Mestrado do PPEnCT da Universidade Regional Integrada do Alto Uruguai e das Missões – URI Santo Ângelo. CEP: 98.802-470, Av. Universidade das Missões, 464, Santo Ângelo - RS, Brasil; [2]Mestranda do PPGEnCT da Universidade Regional Integrada do Alto Uruguai e das Missões – URI Santo Ângelo. CEP: 98.802-470, Av. Universidade das Missões, 464, Santo Ângelo - RS, Brasil; [3]Doutor e Professor do Mestrado do PPEnCT da Universidade Regional Integrada do Alto Uruguai e das Missões – URI Santo Ângelo. CEP: 98.802-470, Av. Universidade das Missões, 464, Santo Ângelo - RS, Brasil; [4]Doutora e Professora do Mestrado do PPEnCT da Universidade Regional Integrada do Alto Uruguai e das Missões – URI Santo Ângelo. CEP: 98.802-470, Av. Universidade das Missões, 464, Santo Ângelo - RS, Brasil

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Corresponding author:
Marcelo Paulo Stracke,

ABSTRACT

Brazil is the country with the greatest plant biodiversity of the planet, however, much of this biological variety does not have a caracterisc knowledge of plants. With the evolution of technology, it is possible to carry out analyzes and studies to identify and characterize these plant compounds. Among these plants, Euphobia Tirucalli, popularly known as aveloz, has been researched to identify their benefits and harms, for this, there is some equipment with the function of characterizing these compounds, such as the Gas Chromatograph coupled with the Mass Spectrometer (GC-MS). The present work has as objective to identify and to characterize the latex and the branch of the plant, being carried out extractions, as liquid-liquid and solid-liquid and also the solubilization. In the liquid-liquid extraction the compounds 4- (allyloxy) -2-methyl-2-pentanol, lanosterol and 4H-Piran-4-one, 2,3-dihydro-3,5-dihydroxy- 6-methyl. In solid-liquid extraction, 4- (allyloxy) -2-methyl-2-pentanol. In solubilization, tetracosane, heneicosane, hexatriacontane, lanosterol, tetratetracontane, beta-karyophylene, dotriacontane, 1-iodine, lupeol and butylated hydroxytoluene. In addition, an analysis was performed on the Scanning Electron Microscope (SEM) equipment, indicating that most of the hydrocarbon compounds are present in the plant branch. Finally, by performing analyzes on the GC-MS equipment, it was possible to identify different compounds with the solvents hexane, dichloromethane and ethyl acetate.

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INTRODUCTION

Natural resources have been used since the primitive era in various areas such as food, medicines, income sources, among others. Thus, with the evolution of technology, the use of these resources in a safer and more efficient way has been extensively researched, through studies of their properties and components. Brazil is the country with the largest plant biodiversity on the planet, it has around 120 thousand species of plants, equivalent to 25% of the known species, and a good part is not yet familiar. Thus, each year with the discoveries of new plants, several researches are carried out in order to characterize these vegetables. With these studies, chemistry has been developing equipment capable of performing a better definition of results for the characterization of plants, in addition, with the potential to assist the chemist in the synthetic production of these components.

The hazelnut plant known scientifically as Euphobia Tirucalli, is originally from Africa, but can be found in northeastern Brazil, grown in a semi-arid tropical climate. It has a height of up to 10 meters, its branches are light brown colored wood, and the new branches are green colored, it has a pencil-like characteristic. This plant is widely used for ornaments such as hedges or a protective barrier, in its branches a white viscous liquid, popularly known as latex, is found. This viscous liquid is characterized as being toxic and caustic, and can cause burns, ulcers and dermatitis when in contact with the skin. The Avelós plant has the scientific name Euphobia Tirucalli, also known scientifically as Arthrothamus tirucalli (L.) Klotzsch e Garcke, Euphorbia geayi Constantin e Gallaud, Euphorbia laro Drake, Euphorbia media N.E. Br., Euphorbia rhipsaloides Lem., Euphorbia scoparia N.E. Br., Euphorbia rhipsaloides Willd., Euphorbia suareziana Croizat, Euphoria tirucalli var. rhipsaloides (Willd.) Chev. In addition to being popularly known as Avelós, it can also be found

as blind-eye, green coral, labyrinth, casserole, oil plant, pencil-tree, almeidinha, São-Sebastião tree, coral-tree- de-são-sebastiao, crown-of-christ, devil's finger, dog-tooth, thorn-of-christ, thorn-of-jew, thorn-italian, cage, devil's twig, kills warts, stick-on-stick and naked dog. This plant belongs to the Euphorbiaceae family (LORENZI, 2002). This plant can be found in Africa and northeastern Brazil, grown in a semi-arid tropical climate. This plant has a description similar to the cactus, it can reach a height of 10 meters, its trunk and its branches are woody with a light brown color, the young branches have a green color in a cylindrical shape, which recalls the pencil's characteristic as one of their popular names (pencil-tree). Its leaves are tiny and usually fall very early and rarely have flowers that are small and yellow-green in color, easily multiply with a simple piece of branch on the ground (ITF, 2008). Due to the structure of the hazelnut plant, in some countries it is cultivated for ornamental purposes, such as: hedges to separate agricultural borders and a protective barrier against fires. In its branches it spills a viscous white liquid, also known as milk or lactescent sap, popularly characterized by the name Latex. Next, figures of the hazelnut plant will be presented. In figures 1 and 2, the approximate and adult forms of the hazelnut plant can be observed respectively.

Figure 1. Approximate branches of *Euphorbia tirucalli*

Figure 2. Adult form of *Euphorbia tirucalli*

According to ITF 2008, the chemical compounds found in the Euphorbia Tirucalli plant are: 4-deoxyporbolic ester, beta-sitosterol, casuariine, chorilagine, cycloeufordenol, gallic acid, glycosides, euforbine, eufol, euforcinol, cyclotyrucanenol, ellagic acids, euphrene, hentriacontane, hentriacontanal, ingenol, isoeuforal, caempferol, pedunculagin, phenols, formic esters, proteases, putranjivaine A and B, sapogenin acetates, succinic acid, taraxasterol, teraxerin, tirucalol and tirucalin A and B (ITF, 2008). According to Caseiro, 2006, it is said that the active principles present in the plant are: 12-0-0 (22) (4E) -octadienol-4-deoxifolia-13-acetate, 3, 3-di-0-methyl- ellagic, beta-sitosterol, citric acid, ellagic acid, eufol, euphoria, glucose, hentriacontanol, isoeuforal, kaempferol, malic acid, resin, sapogenin-acetates, succinic acid, taraxasferol, taraxerin and tirucalol, cited by Karen's dissertation (AVELAR, 2013). However, Avelar carried out an analysis by gas chromatography coupled with mass spectrometry of the biomass oil from the biomass powder of the avelós plant, being identified the following active principles: cyclopentenone, furanomethanol, methylcyclopentenone, furanyl ethanone, phenol, dimethylcyclopentenone, pyridinacarbonitrile, methylmethylphenocarbonol, methylpyronitrile, methyl pyridinecarbonitrile, methoxyphenol, ethylphenol, creosolG, dianhydro-glucopyranose, methenamine, benzenepropanonitrile, picolinamide, ethylmethylpyrazine, dihydroindenone, isobenzeno-furanone, hydroxybenzeneacetonitrile and methyltophenylethanone, with 11 different compounds being identified, which are subject to a total of 31 different components, which are subject to a total of 31 components. According to Machado, he performed an analysis by gas chromatography coupled with mass spectrometry of liquid-liquid extraction, using different fractions of the n-hexane solvent, and subsequently the macerated sample of the hazelnut plant was subjected to a water / ethanol mixture of 30/70% respectively and after evaporating the ethanol. In view of this, the compounds identified by CG / MS were: dodecane hydrocarbon, tetradecanoic acid, phytol, 2-methyl-tricosan, lanosterol, cyclotyrucanenol and lepeol (MACHADO, 2007).

In addition, Machado carried out an analysis by high-performance liquid chromatography (HPLC), with the same procedure being performed, however, in liquid-liquid extraction different solvents (dichloromethane, ethyl ether and ethyl acetate) were used. With the analysis, the following components were identified, according to the solvent, for dichloromethane: linoleic acid, pentadecanoic acid and ethyl citrate; for ethyl ether: stearic acid, rhinoleic acid and diisobutyl phthalate; for ethyl acetate: α-linoleic acid and 2- (2butylcyclopropyl) -9 (MACHADO, 2007). In the gas chromatography analysis coupled with a mass spectrometer carried out by Martins 2018, of the hexanic extract of the Avelós plant, he presented the following compounds: undecanoic acid, hexadecanoic acid, linoleic acid, stearic acid, phytol, methyl 9-octadecenoate, octacosane, eicosano , lupenone and lanosterol, however, 4 components were not possible to be identified. With the results found by the authors mentioned above, it is understood that depending on the solvent, different components are identified, which means that the solvents have solubility with only some compounds found in the extraction of latex from the hazelnut plant. The ester compound is formed by the chemical reaction between an acid and an alcohol that result in the production of an ester and water, its general formula RCO2R 'or RCOOR'. In this case, the compound in question is the formic esters, it is considered a natural organic compound, generally found in plants of the Euphorbiacea family, by characterizing the ITF some of the compounds classified in this category can promote the development of tumors (ITF, 2008; LORENZI, H; ABREU MATOS, FJ, 2002).

Phenols are compounds that form aromatic rings through carbon bonds that have a connection with one or more radicals of the hydroxyl group (OH), usually found in solid form. They are considered as acidic compounds that allow an interaction with organic bases forming acids and water. The acetate can be characterized as an organic salt or as a combined base, it depends on its function in a chemical reaction. In this case, its bond is involved with sapogenin, it is a compound that belongs to the saponin family of natural products, when in contact with water it forms a colloidal solution, sapogenin is produced by hydrolysis, being only soluble in organic solvents. The latex found in the branches of the Euphorbia tirucalli plant is considered toxic and caustic, because in contact with

the skin it can cause burns, ulcers and dermatitis; in contact with the eyes can cause temporary blindness; by internal consumption it can cause bleeding and stomach ulcers, in minimal and diluted quantities it can cause nausea, vomiting, diarrhea and ulceration in the mouth and throat (ITF, 2008). Although this plant is considered dangerous for consumption, in many places it is used as a medicinal plant, in Brazil it is used externally and diluted in snake bites, benign and malignant tumors and to cauterize abscesses and warts. As much as latex can bring some health benefits, it has also shown harm, due to the fact that it is rich in terpenes that are compounded formic esters that are clinically proven to cause tumors. One of the compounds found is ester-4-deoxyporbolic acid, documented as a promoter of infection by the Epstein-Barr virus (EBV), because it causes the inability of the T cells that are responsible for killing EBV, it is a biological agent linked to development of some types of cancer such as Burkitt's lymphoma and nasopharyngeal carcinoma (ITF, 2008). Analyzing the active principles of the plant, some of them are scientifically proven to be beneficial for health. These have a preventive activity against some types of cancer, antitumor, antimutagenic, antibacterial, laxative, antiseptic, disinfectant, anti-inflammatory and anti-streptococcal (COSTA, 2011). Because it is beneficial in some aspects of health, modern medicine has been using latex for several years, in some countries doctors advise their patients to take the latex diluted as 3 drops in 200 ml of water, usually after meals daytime. According to Lorenzi's ideas, the active ingredients mentioned above are not yet proven by chemical analysis, so research on this subject is still taking place in order to prove these components (LORENZI, H., ABREU MATOS, F.J., 2002). Between August 1998 and April 1999, a therapeutic clinical study was carried out with 60 patients with some type of cancer (there is no cancer specification), at first they underwent conventional treatments such as surgery, chemotherapy and radiotherapy, after, they underwent a treatment with the hazelnut latex diluted in water for 30 days. This research resulted in 44 patients who showed to have some benefit with this treatment, some with complete regression of the disease, but the rest of the patients (16) did not show improvement in their disease, considering that in some cases it resulted in an incidence of deaths (VARRICCHIO, 2000).

In addition, some studies carried out in order to increase the percentage of positive T lymphocytes for the IFN-γ TNF-α cytokines and T-CD4 lymphocytes, which are responsible for distinguishing infected or tumor cells and attacking them without stimulation, being applied to the extraction of raw latex from the hazelnut plant on leukocytes of peripheral blood in vitro. It has been shown to be promising for the use of the gross extraction of the diluted hazelnut plant to combat tumors and infections by intracellular pathogens (AVELAR, 2010). In addition, an evaluation of the anticarcinogenic effect of the latex of hazelnuts was carried out by testing for tumor clones in drosophila melanogaster that were pretreated with mitomycin C, after which the aqueous extract of the hazelnut latex was applied. This analysis demonstrated that latex decreases the concentration of tumor cells, thus, according to the literature, the substances present in latex, ingenane and esters of phorbol, which increase cellular immunity and favor apoptosis (ALVES, 2012). Currently, the latex found in the hazelnut plant has been used in certain medicinal treatments for some diseases, but the benefits and harms of the plant's components are still being investigated. According to research already carried out, it was discovered that latex has components that pose health risks due to the fact that it is rich in terpenes that are compounds of formic esters. However, with some research carried out with people who consumed the latex in a diluted form in water for the cure of cancer, there was evidence that some of the components present in the latex help to cure cancer, but it is not yet defined what these are compounds. Thus, the following work suggests a method for identifying the possible components found in the plant's latex, as a way of proving and describing which elements are found in the material sample. The results will be compared between research by different authors that will be properly cited and referenced during the development of this work. It is noteworthy, therefore, that these analyzes and research were carried out with different methodology, as a result of this, some research / analysis failed to identify which was the element present in the substance. In view of this, a liquid-liquid and solid-liquid extraction will be carried out, followed by a simple distillation for sample preparation, with a qualitative and quantitative analysis by gas chromatography coupled with the mass spectrometry detector (CG-MS).

METHODOLOGY

In this chapter, topics and methods that will be carried out for the quantitative and qualitative analysis of the latex of the Avelós plant will be presented in topics. The methodology was based on articles and dissertations, being characterized as an experimental research.

Sample Collection: The sample collection will be carried out roughly, from November to March, in the region of the missions, as the climate is tropical semi-arid, time of plant development. For liquid-liquid extraction, 125 branches of the plant will be collected, a cut will be made and 125 drops of latex will be collected which will be stored in a closed beaker and placed in the refrigerator to preserve the sample. For solid-liquid extraction, branches approximately 10 cm above the main stem will be cut and collected and cut again into pieces of 10 cm.

Sample Preparation: For liquid-liquid extraction, the collected sample will be mixed with a mixture of 70:30 ethanol: water in beakers, after which the sample will be subjected to rotary evaporation to eliminate ethanol (MACHADO, 2010). For the solid-liquid extraction, 20 g of the branch of the plant will be collected to be crushed, which will be mixed with 100 mL of the mixture of 70:30 ethanol: water, which will be left to rest for 6 days, being carried out daily. (AVELAR, 2013).

Choice of Solvent: The choice of the solvent that will be reacted with the sample is very important and depends on the type of analysis to be carried out, in this case, the equipment in question is the gas chromatograph coupled with mass spectrometry, so you must choose a solvent with polarity growing. This same solvent will be used in liquid-liquid extraction in order to separate the organic phase from the inorganic phase, for the equipment in question only the organic phase will be analyzed and in the solid-liquid extraction the liquid phase of the sample will be analyzed. For this experiment, due to the fact that the sample has unknown compounds, extractions with different solvents will be performed in order to compare the results obtained. Therefore, for liquid-liquid and solid-liquid extraction, hexane solvents with polar characteristics, dichloromethane with apolar characteristics and ethyl acetate with apolar characteristics will be used. (MARTINS, 2018; MACHADO, 2007).

Extraction: The extractions will be carried out between February and April, these were based on the experiments of Machado 2007, Martins 2018 and Avelar 2013, with some changes being made.

Liquid-Liquid Extraction: Liquid-liquid extraction is a procedure used in aqueous samples with the purpose of separating, purifying and concentrating specific substances, this process is based on the physical properties of the substance, in this case its solubility. In this type of extraction, two types of solvents are required, which must be immiscible with each other, for these analyzes a simple extraction will be performed, using a phase separation funnel. After the preparation of the aqueous solution (sample), it will be placed inside the separating funnel (which has a valve with a tube for the solvent outlet), then 100 ml of solvent will be added, using solvents of increasing polarity: hexane (apolar), dichloromethane (polar) and ethyl acetate (apolar), shake and open the valve to collect the organic phase of the solution into a beaker. The organic phase of the aqueous solution (sample) will be reserved for use in the chromatographic analysis after simple distillation.

Solid-Liquid Extraction: The solid-liquid extraction is used for the separation and purification of one or more components of a solid sample, using a liquid extractor solvent. The solvent used must have a

solubility with the components. For this operation it is necessary to have a solid sample and a solvent, it will be performed in the soxhlet equipment. In this equipment, the sample will be placed in a cartridge, while 150 mL of the solvent added to the 200 mL flask on heating, when it reaches the boiling point, the solvent rises through the device's arm, being condensed when it reaches the refrigerator, the drops fall over the cartridge, that when filled, the reflux happens and the process starts again. The different solvents used will be methanol, ethanol and ethyl acetate, this procedure will be carried out for 3 hours, with 3g of sample.

Simple distillation: The samples obtained in the extractions will be subjected to a simple distillation, a process of separation and purification of miscible liquids in order to separate the substances of the samples according to their volatility. The samples are placed in a round flask that will be inserted on a heating blanket. The sample will be heated up to approximately 150 ° C, where the substances will be evaporated when they reach their boiling point, using a thermometer for monitoring. This steam will pass through the condensation process in which the glassware has 2 inlets, one for water and the other for steam and two outlets, one for the sample and the other for water and the liquid with volatile impurities will be collected in an Erlenmeyer flask.

Gas chromatography coupled with a mass spectrometer (CG-MS): The liquid sample collected in the distillation will be diluted in 1: 100, 1:50 and 1:25 with the same solvent used for the extraction, and first it will be submitted to an analysis in the gas chromatography equipment coupled with a mass spectrometer, model QP - 2020, using the GCMSsolutions software. This equipment has a capillary column, model SH-Rtx-5MS, 5% diphenyl, 95% dimethyl polysiloxane of dimensions 30 mx 0.25 mm x 0.25 in which it has a temperature range of 330/350 ° C, the equipment in question was acquired from the company shimadzu. The sample will be injected by a microsyringe suitable for the 1.0 µm equipment in split mode with a ratio of 1: 100 at a temperature of 280 ° C, the oven at a temperature of 70 ° C (2 min); 230 ° C (10 ° C / min); 230 ° C (17 min), the carrier gas used will be helium, the detector is the mass spectrometer equipment at temperature 280 ° C with EI at 70 eV, with scanning mode of 0.5 sec / scan, with range mass of 40 - 500 daltons, with filament disconnected at 4 minutes and with transfer line at 280 ° C. The analysis will be carried out in the chemistry laboratory of the URI-campus of Santo Ângelo between the months of February to April (AVELAR, 2013).

RESULTS AND DISCUSSIONS

To carry out the experiment described above, the solvents used were first read, these being hexane, dichloromethane and ethyl acetate in the gas chromatograph equipment coupled with a mass spectrometer, to check the present molecules, as there may be some contaminant and thus, can be discarded if it appears in the samples to be analyzed. To choose the solvent, he decided to carry out the experiment with a nonpolar one, with hexane and polar being dichloromethane, however, ethyl acetate is one of the recommended solvents to carry out experiments on the CG-MS equipment, this being nonpolar, as well , three different solvents were used for comparison. According to the description of the methodology, it is suggested to perform a simple distillation to make sure that all the molecules would be eliminated from the column so as not to contaminate, but ended up not generating results, so this idea ended up being disregarded. Distillation is generally used to purify the sample, however, the solvents chosen for carrying out the experiments have a boiling temperature below 100 ° C, however, the equipment begins its analysis at a temperature of 70 to 280 ° C, with that, in the analysis of the sample collected from the distillation, only the solvent was detected, which is understood that the sample remained in the flask, thus not being favorable for the possible characterization of the sample components. Next, the graphs obtained on the CG-MS equipment for each of the solvents will be presented: hexane, dichloromethane and ethyl acetate, considering that the x-axis represents the time in minutes and the y-axis represents the peak intensity in all graphs.

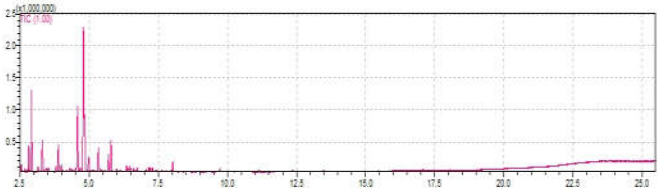

Figure 3: Spectrum obtained from the hexane solvent

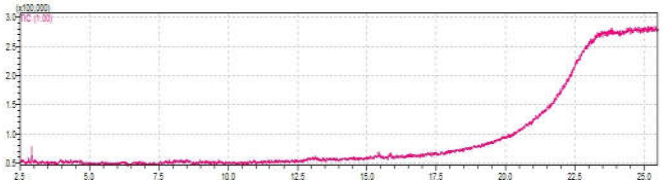

Figure 4: Spectrum obtained for the solvent Dichloromethane

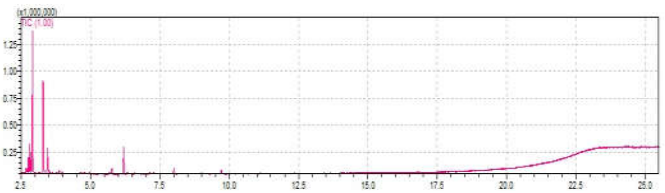

Figure 5: Spectrum obtained for the ethyl acetate solvent

Analyzing the graphs in Figures 3, 4 and 5, it can be seen that the hexane and ethyl acetate solvents that are nonpolar have some contaminants, therefore, in the results obtained for the samples, these graphs will be used as a comparison to be discarded, comparing with the component peaks present in the graphs of the solvent samples. However, the dichloromethane that has the characteristic of being polar is clean of contaminants. To perform the extractions proposed previously, it was necessary to prepare 2 different samples, the first being a solution only of the latex found in the branches of the plant and the second corresponding to a mixture with the branches of the crushed plant. In the preparation of the first sample, in a 200 mL conical flask, 125 drops of latex were added, with each drop containing one drop, with 100 mL of a solution of water and ethanol (30:70), which was left standing 2 days, however, for its use in the experiment, it used rotavapor to evaporate the ethanol. For the second sample, the branch of the aveloz plant was used, with the aid of a scale, 20.0070 g of the branch were weighed, then it was crushed and placed in a 200 mL conical flask with 100 mL of a water solution and ethanol (30:70), which remained at rest for 6 days. The two samples prepared were stirred daily and a picture of the samples will be presented below.

Figure 6: Photo of the prepared samples, with the latex solution on the left side and the crushed branch solution on the right side

To perform the extractions with sample 2 (solution with the crushed branches), it was necessary to perform a filtration, as the solid and liquid were analyzed separately.

Extraction Liquid – Liquid: With the solutions already prepared, first the extractions were carried out with the first sample, in which 8 drops of the solution were added in 70 ml of solvent and 30 ml of water in a separating funnel, this was stirred and the organic phase was collected. was analyzed on the equipment, this procedure was performed for each solvent, hexane, dichloromethane and ethyl acetate. Next, the graphs obtained by the CG-MS equipment will be presented.

After performing the extraction procedure with the separating funnel, the samples were analyzed in the CG-MS equipment, then the graphs obtained with each of the solvents will be presented. The results obtained by the graphs shown in figures 7-12 correspond to liquid - liquid extraction experiments performed with sample 1, which would be the white colored solution prepared with the plant 's latex. With that, it can be concluded that the extractions carried out with this sample were not possible to obtain components, only with the dichloromethane solvente. With the dichloromethane solvent extraction, the CG-MS identified from its library a high probability of being the chemical compound Lanosterol that has the formula C30H50O.

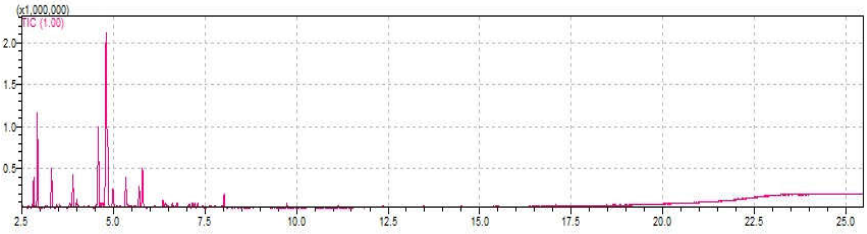

Figure 7: Spectrum obtained by CG-MS with hexane solvent

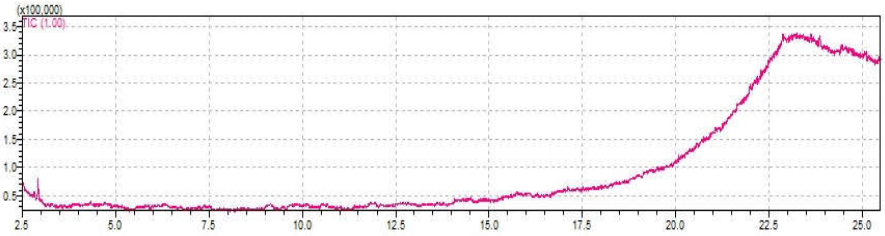

Figure 8. Spectrum obtained by CG-MS with Dichloromethane Solvent

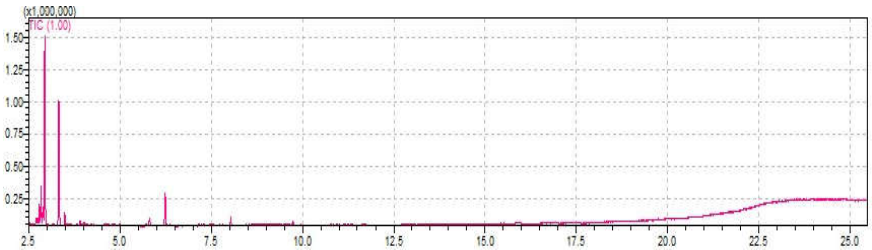

Figure 9. Spectrum obtained by CG-MS with ethyl acetate solvent

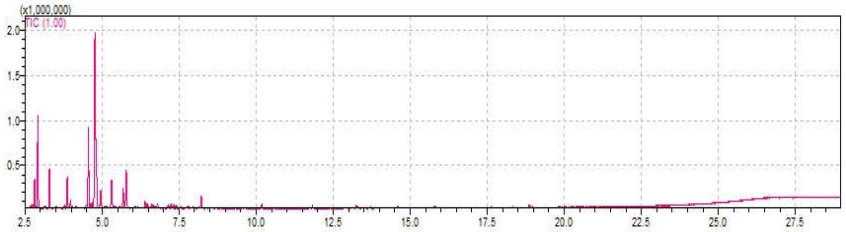

Figure 1. Spectrum obtained by CG-MS with hexane solvent

The graphs in figures 7-9 were compared to those of the pure solvent of hexane, dichloromethane and ethyl acetate, with figures 8, 9 and 10 being, respectively. Comparing the graphs, it can be seen that they are identical to those of the pure solvent , indicating that the sample was very diluted and that it ended up not obtaining peaks of the latex molecules. With this, the experiment was redone, but the volume of the solvent was reduced, using only 10 mL of solvent with 10 drops of the first solution. When the mixture was placed in the separating funnel, it was noticed that the solution of the ethyl acetate solvent did not obtain a visible separation, so 10 mL of water was added, thus making it possible to collect the organic part, however, for the solvents dichloromethane and hexane did not require this addition.

This compound is a tetracyclic triterpenoid, it is considered to be a lipid molecule of sterol, and can be found in solid form and being practically insoluble in water, but neutral. Lanosterol acts as a bacterial, vegetal, human metabolite, beer yeast (Saccharomyces cerevisiae) and mice, being found in the cytoplasm, membrane and endoplasmic reticulum cells. It has the function in the steroid biosynthesis, in the action of fluvastatin and in the action of ibandronate, with that, since 2015 studies have been carried out that the synthesis of lanosterol in the form of eye drops is capable of melting cataracts, which in humans is responsible focus of vision, in 2018 the development of this eye drop occurred, but studies are still being carried out for its approval.

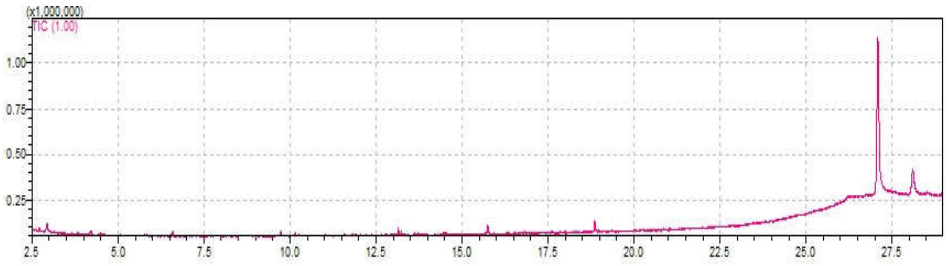

Figure 2. Spectrum obtained by CG-MS with Dichloromethane solvent

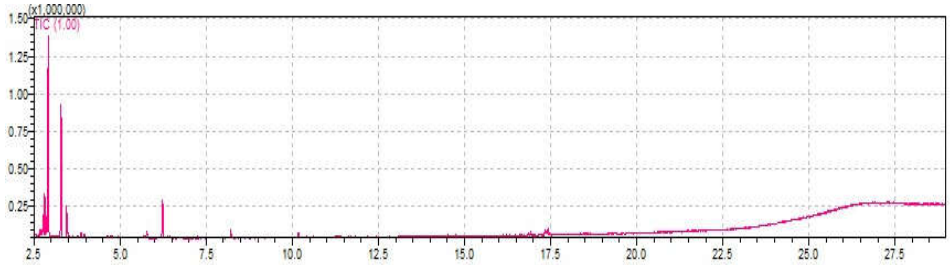

Figure 12. Spectrum obtained by CG-MS with ethyl acetate solvent

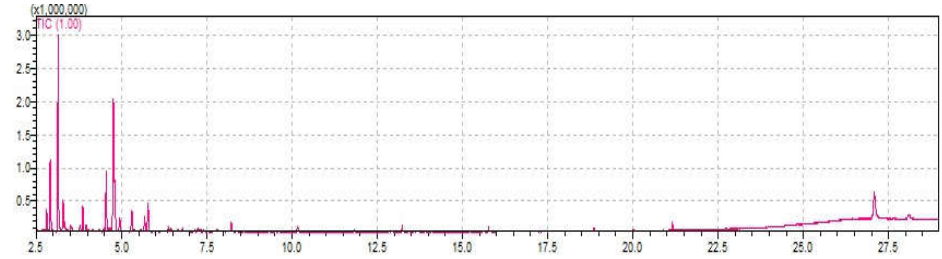

Figure 13. Spectrum obtained from the 1st filtration with hexane

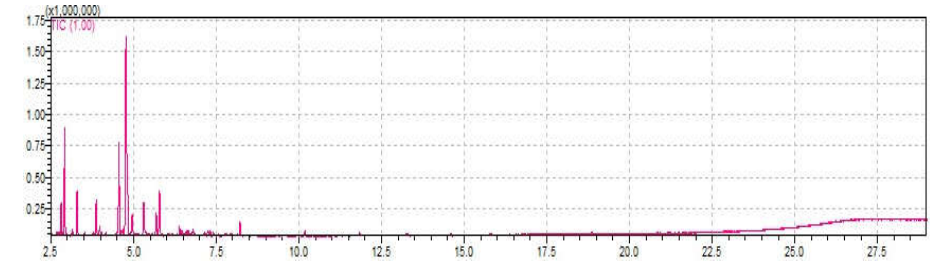

Figure 14. Spectrum obtained from the 3st filtration with hexane

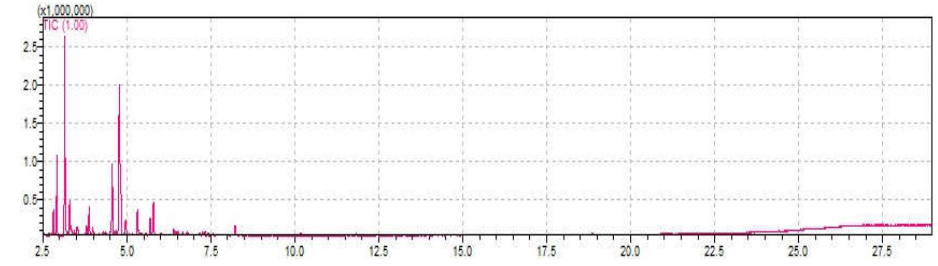

Figure 15. Spectrum obtained by CG-MS with the rotavapor sample

The sample 2 is a solution with the crushed branch mixed with 100 mL of water: ethanol respectively 30:70, with this, with a funnel, the sample was filtered and the procedure was repeated with 3 filtrations. The filtrations were reserved for liquid - liquid extractions, 10 mL of the solution and 20 mL of solvent were mixed in the separatory funnel. First, the extraction with the hexane solvent of the first and third filtration was analyzed in the CG-MS equipment to identify which has the largest number of components. Next, the graphs obtained by the equipment with the hexane solvent will be presented. Comparing Figures 13 and 14, it can be seen that in the liquid - liquid extraction of the first filtration a greater number of components was obtained, with the molecule with the highest intensity being in 3 minutes and 0.133 seconds, with a high probability of being the compound 4- (allyloxy) -2-methyl-2-pentanol (C9H18O2). It has a molecular mass of 158.24 g / mol, however, there is no proven information about this molecule regarding what it corresponds to and what it is used for. In addition, the tables constructed by the equipment were verified to verify, with this it was realized that this compound is present in both extractions, however, in the first filtration it is presented with greater intensity. It also identified a peak in time of 27 minutes and 0.077 seconds in the extraction with the first filtration, with a high probability of being the Lanosterol

molecule, being the same molecule found in the liquid - liquid extraction of the first sample with the dichloromethane solvent, but in a smaller intensity. Thus, it is understood that this molecule is found in the plant's latex, as it is the only component in common in the two samples, with this, 30 mL of the first filtration was collected and passed through the rotavapor to evaporate the ethanol and performed the extraction procedure. This sample was analyzed to check the influence of this evaporation, then the graph obtained by the equipment will be presented. Comparing with Figure 13, it is clear that they have in common the peak with a high probability of being the molecule 4- (allyloxy) -2-methyl-2-pentanol ($C_9H_{18}O_2$), however with an intensity of approximately 25000000, already in the liquid - liquid extraction of the 1st filtration corresponds to an intensity of approximately 30000000. With these results and considering that in Figure 13 more peaks were obtained, the analyzes carried out with the solvents dichloromethane and ethyl acetate used the 1st filtration that corresponds to having a greater number of molecules present. The procedure performed was the same, as the objective is to verify if the sample has different components that may interact with different solvents and thus identify them, the graphs obtained with these solvents will be presented below.

is C6H8O4, it has a molecular weight of 144.12 g / mol, there is not much information about this molecule and its function. Taking into account all the liquid-liquid extraction procedures carried out with the two samples, it can be seen that the results of the 1st filtration analysis with all solvents obtained the highest number of components dragged in the plant. With that, it was possible to verify that the component lanosterol is identified with all the solvents used, thus proving its existence in the plant.

Extraction solid–liquid: To carry out this extraction, the whole branch was used and the second sample, first, a comparison was made to check if there was a difference between the possible components if the whole branch was to be used or only the crushed one, for this, only if used the hexane solvent. For the extraction with the crushed branch, it used the composition of the second prepared sample that was filtered after the 6 days of rest. With the help of the analytical balance, 3.0052g of crushed branch and 3.0097g of whole branch were weighed, these samples were placed in a cartridge especially for use in this type of extraction, in the flat-bottom flask 150 mL of solvent, the reflux procedure took place for 3 hours, after which, the aqueous solution that remained in the flask was analyzed

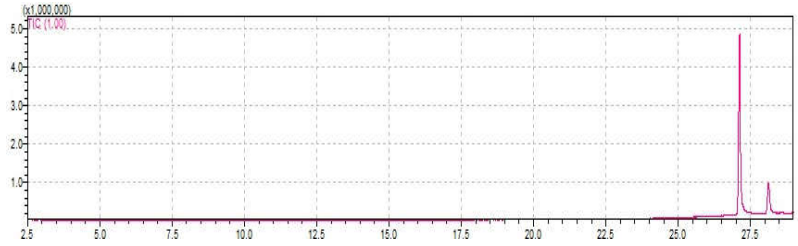

Figure 3. Spectrum obtained by CG-MS of the sample wiht dichloromethane

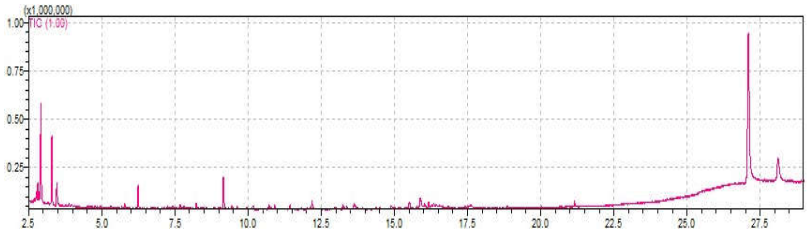

Figure 17. Spectrum obtained by CG-MS of the sample wiht ethyl acetate

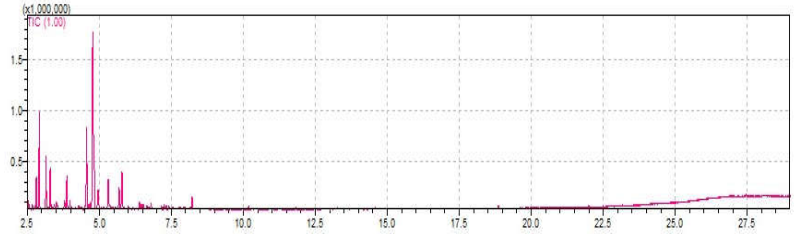

Figure 18. Graph obtained by the equipment for solid-liquid extraction from the entire branch

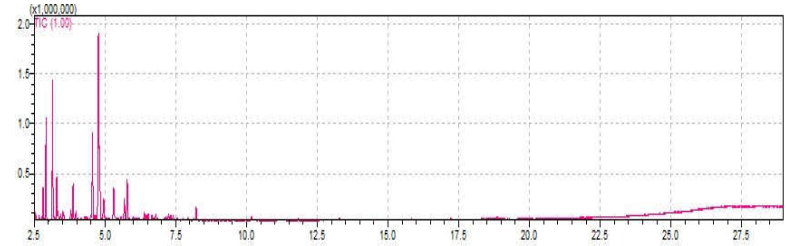

Figure 19. Graph obtained by the equipment for the solid-liquid extraction of the crushed branch

Comparing the graphs represented in figures 16 and 17, it was identified that the two peaks found in both dichloromethane and ethyl acetate are the same component, with a high probability of corresponding to the compound that lanosterol, in addition, it is noticed that the peaks obtained are at the same time. However, in ethyl acetate, an unknown peak was obtained in 9 minutes and 0.150 seconds, with a high probability of corresponding to the molecule (4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl), its formula

in the CG-MS equipment. Next, the graphs obtained by the equipment with the possible components found in the samples will be presented. Comparing the two graphs shown in figures 24 and 25 with the graph obtained from the pure hexane shown in figure 7, it can be seen that they are almost identical, however, in the extractions a different peak was obtained in the time of 3 minutes and 0.140 seconds. This peak has a high probability of being the molecule 4-(allyloxy) -2-methyl-2-pentanol ($C_9H_{18}O_2$), has a molecular mass of

158.24 g / mol, however, there is no proven information about this molecule. referring to what is corresponding and what is used, being the same molecule found in the liquid - liquid extraction of the 1st and 3rd filtration with hexane corresponding to Figures 19 and 20. In addition, it is possible to see from the graphs that this molecule found has a greater intensity in the extraction with the crushed branches, as they have greater ease of contact with the latex found within the branch. For this reason, for the solid-liquid extraction with the other solvents, the crushed branches were used, following the same procedure described above, the graphs obtained by the equipment with dichloromethane and ethyl acetate will be presented below. Comparing the graphs of the solid-liquid extraction with the graphs of pure solvents, with figure 9 being dichloromethane and figure 10 being ethyl acetate, it is clear that the graphics are identical, indicating that the branches did not have an affinity with these solvents in this type of extraction, even though ethyl acetate is of the same polarity as hexane.

samples in the CG-MS equipment, it was necessary to perform a filtration to collect only the liquid part, then the analysis was performed, then the graphs obtained by the equipment will be presented. The peak obtained in 23: 573 minutes, has a high probability of corresponding to the compound tetracosane ($C_{24}H_{50}$) is an alkane hydrocarbon, being considered a hydrocarbon lipid molecule, it is found as a solid and practically insoluble in water, but neutral. In the cell is located in the membrane, this chemical compound can be found in human saliva and also in some foods such as linden, sunflower, citrus and coconut, it has a function as a vegetable metabolite and as a volatile component of oil. The peak obtained in 24: 363 and 25: 127 minutes, has a high probability of corresponding to the compound heneicosane ($C_{21}H_{44}$) is an alkane hydrocarbon, is also considered a hydrocarbon lipid molecule, is found in solid form and practically insoluble in water , but neutral. This chemical compound has a waxy flavor that can be found in certain foods, such as oregano, pepper, sunflower and kohlrabi, has a

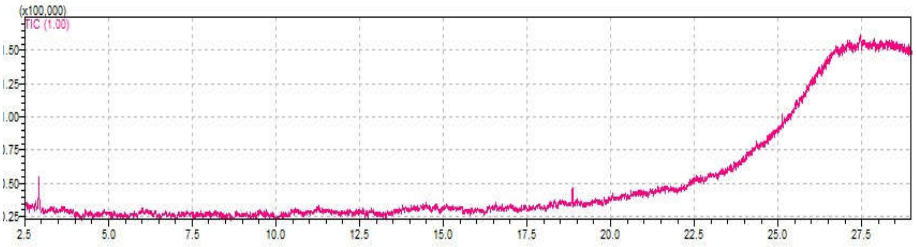

Figure 20. Graph obtained by extraction with dichloromethane solvent

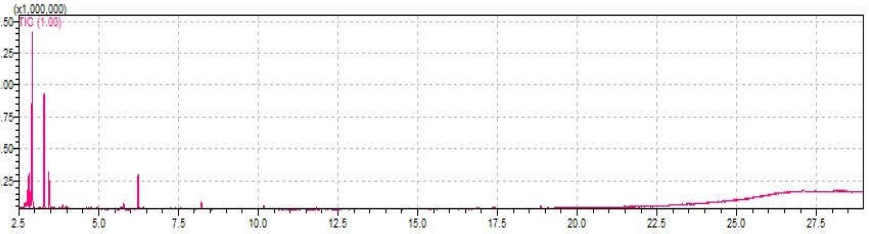

Figure 21. Graph obtained by extraction with ethyl acetate solvent

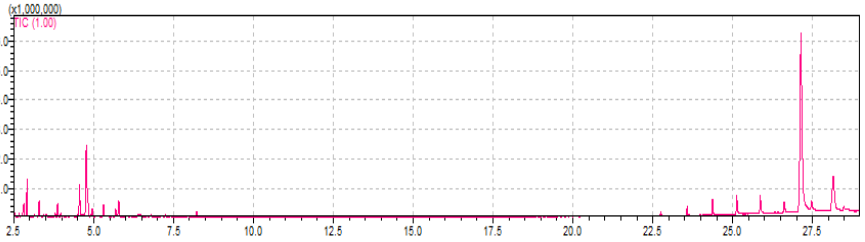

Figure 22. Graph obtained by the hexane solution

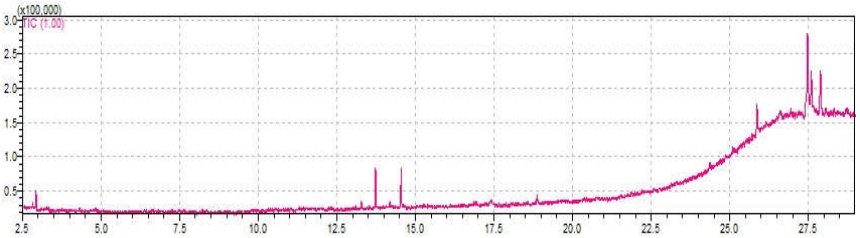

Figure 23. Graph obtained by solubilization with dichloromethane

As the results found with the extractions did not correspond to those expected, it was decided to make a change in the methodology, so, for solubilization, a solution was prepared in a 50 mL beaker, mixing 10 mL of solvent with 20 drops of latex, this The procedure was carried out with the solvents hexane, dichloromethane and ethyl acetate. The samples with a nonpolar characteristic showed a formation of a glue-like aqueous liquid in which the drops were joined, but with the polar solvent, the latex formed small balls with the appearance of a solid as a powder. For the possible analysis of the

function of pheromone, plant metabolite and a volatile component of the oil. The peaks obtained in 25: 860 minutesand 26: 610 minutes, have a high probability of corresponding to the chemical compound hexatriacontane ($C_{36}H_{74}$) is an alkane hydrocarbon, has a white wax-like format, however, it has a chemical property in the form of flakes bright whites. This compound does not have much information about its origin. The peaks obtained in 27: 133 and 28: 150 minutes have a high probability of corresponding to the chemical compound lanosterol, it is noticed that the peak in 27 minutes has the highest

intensity found in comparison with the other peaks obtained in the graph. This compound was found in the liquid-liquid extractions in the 1st and 3rd filtration with hexane, dichloromethane and ethyl acetate from the second sample, however, in the solubilization a greater intensity was obtained. The peak obtained in 27: 470 minutes, has a high probability of corresponding to the chemical compound tetratetracontane ($C_{44}H_{90}$) is an alkane, has a pure format, there is not much information about this molecule, but it is known to have a human metabolite function. Analyzing all the experiments carried out with the hexane solvent, the solubilization showed to have a greater drag of the components, as well as with peaks of greater intensity. Thus, it proved to be the best form of experiment to obtain a higher percentage of peaks. Next, the graph obtained by the CG-MS of the solubility experiment with dichloromethane will be analyzed, figure 29. The peaks obtained in 13: 727 and 14: 543 minutes, have a high probability of corresponding to the beta-karyophylene molecule ($C_{15}H_{24}$) is an organic compound of the sesquiterpenoid type, is considered an isoprenoid lipid molecule, can be found in solid form and also in yellow oily liquid form with an odor intermediate between the clove and turpentine odor, being practically insoluble in water, but neutral. In the cell it can be found in the membrane and in the cytoplasm, however it is detected mainly in feces, but being present in essential oils, mainly in clove oils. This chemical compound has a non-steroidal anti-inflammatory function, fragrance, metabolite and attractive to insects.

The peak obtained in 25: 863 minutes, has a high probability of corresponding to the chemical compound dotriacontane, 1-iodine-($C_{32}H_{65}I$), has a molecular mass of 576.8 g / mol, there is not much information of this chemical compound on its form , where it is found and its function. The peak found in 27: 470 minutes has a high probability of corresponding to the chemical compound hexatriacontane ($C_{36}H_{74}$), this molecule was also found in the solubilization of hexane in the near future. The peaks found in 27: 593 and 27: 883 minutes correspond to the chemical compound lupeol ($C_{30}H_{50}O$) is a pentacyclic triterpenoid, but derived from lupine hydride. This molecule can be found in the husks of lupine seeds, in the latex of fig and rubber trees, in many edible fruits and vegetables, has an anti-inflammatory and plant metabolite function, in addition, lupeol is being investigated as a potential treatment against acne. Analyzing all the graphs obtained in the experiments carried out with the dichloromethane solvent, it was noticed that the solubilization procedure resulted in a greater drag of plant components, thus indicating a greater affinity with the solvent in this process. Next, the graph obtained by solubilizing the latex with the ethyl acetate solvent will be presented, analyzing the peaks found, Figure 24.

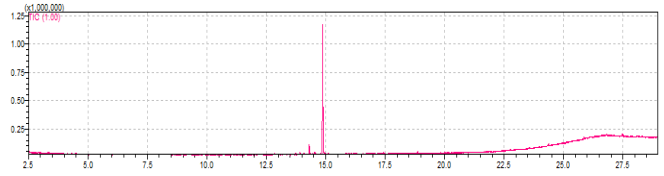

Figure 24. Graph obtained by the solution with ethyl acetate

The peak obtained in 14: 870 minutes, has a high probability of corresponding to the chemical compound butylated hydroxytoluene ($C_{15}H_{24}O$) is an organic compound of the phenylpropanes type, it can be found in the form of a white crystalline solid and practically insoluble in water, but neutral. In the cell, this compound can be found in the membrane, it can be detected in saliva, as well as in eukaryotes from yeast to humans, in addition, it is a compound with a mild flavor, camphor and mold found in garlic. This chemical compound inhibits the self-oxidation of saturated organic compounds, being used in food, cosmetics and industrial fluids to prevent oxidation and the formation of free radicals, however it is considered a toxic compound. Analyzing all the experiments carried out with the ethyl acetate solvent, it is clear that depending on the experiment carried out, it is able to identify different chemical components in different experiments. With this, it was only possible to identify in the liquid-liquid extraction experiment the compound 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- and in the solubilization the butylated hydroxytoluene. However, the highest intensity molecule was found in the solubilization experiment, so it is clear that the active components of the plant have little affinity with this solvent, due to the fact that only 2 chemical compounds are found.

In addition, performing a complete analysis and comparing all the different experiments carried out with the solvents hexane, dichloromethane and ethyl acetate, it can be said that the procedure that obtained a greater drag of the active components of the plant was in solubility. However, comparing the results obtained in this procedure, it is understood that the hexane solvent has a greater affinity, due to the fact that a greater number of chemical compounds is found, considering that the molecule of greater intensity was also found, this being, the lanosterol ($C_{30}H_{50}O$). The procedure carried out by Machado, corresponds to the same liquid-liquid extraction experiment, however, comparing the active principles found in the two experiments, it is possible to verify that the chemical compound lanosterol is present. However, this chemical compound was found with the hexane solvent in the experiments carried out by Machado in the liquid-liquid extraction, but in this work it was found with the dichloromethane solvent. However, the liquid-liquid extraction experiment carried out with sample 2, a solution prepared with the crushed branches of the plant, identified this same compound with the solvents dichloromethane and ethyl acetate, with this, it is understood that this compound it has an affinity with all solvents, because in the solubilization carried out with hexane, this peak was identified with an intensity of approximately 6000000, being the largest peak identified.

However, the experiment carried out by Machado identified a greater number of active compounds found in the plant, therefore, it should be considered that the solvents used in the experiments described in this work had some contaminants that may have caused difficulty in dragging certain components, also , the solvents used have a different purity percentage and thus, caused this difference between the results found. In addition, it was noticed that the active component mentioned by Caseiro and by ITF, eufol ($C_{30}H_{50}O$) has a molecular formula of lanosterol ($C_{30}H_{50}O$), however the molecular structure presents a small difference. With this, it must be considered that the gas chromatography equipment coupled with a mass spectrometer, has its own library for identifying the peaks, however each year new molecules are added, so, depending on the equipment library, the identified compounds can be different. Also, the results obtained by the experiments carried out, were compared with the active principles found by Martins, who only have one compound in common, that being lanosterol. In addition, Avelar carried out an analysis of the bio-oil of the powder of the mass of the hazelnut plant, in this experiment 31 peaks were obtained, but 11 were not identified, but the active ingredients identified are different than the compounds found in this work. However, it was possible to identify different compounds in which the experiments carried out by the mentioned authors did not find. In the liquid-liquid extraction with the hexane solvent, compounds 4- (allyloxy) -2-methyl-2-pentanol and lanosterol were identified, with dichloromethane lanosterol was identified and with ethyl acetate, 4H- Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl and lanosterol. In the solid-liquid extraction, with the hexane solvent the compounds 4- (allyloxy) -2-methyl-2-pentanol were identified, however with the dichloromethane and acetate no sample peak was obtained. On solubilization, with the hexane solvent, tetracosane, heneicosane, hexatriacontane, lanosterol and tetratetracontane were identified; beta-karyophylene, dotriacontane, 1-iodine, hexatriacontane and lupeol were identified with dichloromethane; butylated hydroxytoluene was identified with ethyl acetate. The experiments carried out with solid-liquid extraction ended up not being considered, since peaks of relevant compounds were not obtained, however in the extraction with the hexane solvent a peak was obtained, but with a small intensity, and the same compound was found in the extraction. liquid-liquid. It is concluded that in the solubilization experiment more results were obtained with the identification of different chemical compounds, and in this experiment the solvents had a direct connection with latex.

However, the objective of this experiment is to characterize and identify the active compounds of the plant with different solvents, to verify their affinity with hexane, dichloromethane and ethyl acetate. With the results obtained, in the liquid-liquid, solid-liquid and solubility extraction experiments, it was possible to verify that certain active compounds have a greater affinity, however the chemical compound lanosterol was the most identified compound in the experiments, except in the solid-liquid extraction. , indicating that this molecule has an affinity with all solvents, however, for its extraction it depends on the procedure performed, however, the solubility resulted in a greater drag of chemical compounds.

CONCLUSIONS

With the realization of the experiments described above and after making the necessary analyzes and considerations, it can be said that the general objective of this work was fulfilled. During the experiments, some methodological changes were made considering the results obtained to be possible to analyze and characterize the hazelnut plant extract in gas chromatography equipment coupled with a mass spectrometer. In the performance of liquid-liquid extractions, with the execution of the methodology described with sample 1, it ended up not obtaining results, with that, it was understood that the latex sample was very diluted. Then a change was made, this being the decrease of the solvent, however, only the solvent dichloromethane ended up obtaining a peak that corresponds to the molecule lanosterol. With this, it is noticed that in the liquid-liquid extraction carried out with sample 1 it is not favorable to obtain the active components of the plant, because these molecules end up being stored in the inorganic part that is together with the water. In addition, liquid-liquid extraction was performed with sample 2, in which the prepared solution had the crushed branch of the plant in which latex is found at the end of the branch. With this sample, after the extractions were carried out, the following compounds were identified, with hexane o 4- (allyloxy) -2-methyl-2-pentanol ($C_9H_{18}O_2$) and in ethyl acetate 4H-Pyran-4-one, 2, 3-dihydro-3,5-dihydroxy-6-methyl), its formula is ($C_6H_8O_4$). However, in this experiment a component was found in all solvents, this being the lanosterol ($C_{30}H_{50}O$) that is composed is a tetracyclic triterpenoid, it is considered to be a lipid sterol molecule, and can be found in solid form and being practically insoluble in water, but neutral. In the solid-liquid extractions carried out with both the whole and the crushed branch, it ended up not obtaining significant results, thus, it is understood that this procedure is not favorable for the dragging of chemical compounds. Thus, with the solubilization it was possible to identify some of the chemical compounds of the plant, it is clear that the direct contact between solvent and latex provided a greater drag of the active chemical compounds of the plant. However, with the hexane solvent, tetracosane, heneicosane, hexatriacontane, lanosterol and tetratetracontane were identified; beta-karyophylene, dotriacontane, 1-iodine, hexatriacontane and lupeol were identified with dichloromethane; butylated hydroxytoluene was identified with ethyl acetate. Comparing with the active compounds found by other authors, such as Avelar, Machado, Martins, Cicero and the book ITF, it is clear that the common component is lanosterol, which has a greater affinity with hexane.

With this in mind, one must take into account some errors that may have occurred during the experiment as well as the contaminants that were present in the pure solvents. It is concluded that the objective of analyzing and characterizing both the latex and the branch of the hazelnut plant was satisfactory, in addition, it was possible to identify that in each solvent it has an affinity with different chemical compounds. With the identification of the compounds by the CG-MS, it is understood that most of the compounds are hydrocarbons, with that an analysis was made in the scanning electron microscope (SEM). The results obtained were 57.58% carbon (C), 36.25% oxygen (O), 0.68% sodium (Na), 0.51% magnesium (Mg), 1.09% aluminum (Al), 0.22% sulfur (S) and 0.12% chlorine (Cl), thus proving that the plant has a large amount of hydrocarbon compounds.

REFERENCES

ALVES, E.M.; NEPOMUCENO, J.C. Avaliação do efeito anticarcinogênico do látex do Avelós (*Euphorbia Tirucalli*), por meio do teste para detecção de clones de tumor (warts) em Drosophila melanogaster. Artigo – Centro Universitario de Patos de Minas. Caiçaras, Patos de Minas – MG, 2012.

AVELAR, B.A DE. Detecção in vitro de citocinas intracitoplasmáticas (interferon gama, fator de necrose tumoral, interleucina 4 e interlucina 10) em leucócitos humanos tratados com extrato bruto diluído de *Euphorbia Tirucalli*. Dissertação de Mestrado – Universidade Federal dos Vales do Jequitinhonha e Mucuri. Diamantina – MG, 2010.

AVELAR, K.P.B DE. Estudo da influência da temperatura na degradação termoquímica da biomassa de avelós (euphorbia tirucalli Linn). Dissertação de Mestrado – Universidade Federal do Rio Grande do Norte. Natal – RN, 2013.

COSTA, L.S. Estudo do uso do Aveloz (*Euphorbia Tirucalli*) no Tratamento de Doenças Humanas: Uma Revisão. Trabalho de Conclusão de Curso – Universidade Estadual da Paraíba, Centro de Ciências Biológicas e da Saúde. Campina Grande, 2011.

ÍNDICE TERAPÊUTICO FITOTERÁPICO (ITF). Ervas Medicinais. 1ª Edição. Petrópolis, RJ: EPUB, 2008.

LORENZI, H.; ABREU MATOS, F.J. Plantas Medicinais no Brasil. Instituto de Estudos da Flora Ltda. São Paulo, 2002.

MACHADO, M.M. Perfil fitoquímico e avaliação dos principais efeitos biológicos e imonológicos *in vitro* da *Euphorbia Tirucalli L.*Dissertação de Mestrado – Universidade Federal de Santa Maria. Santa Maria – RS, 2007.

MARTINS, A.M DE. Avaliação das atividades citotóxica e antifúngica dos extratos orgânicos de euphorbia tirucalli linn. (aveloz). Dissertação Pós-Graduação – Universidade Federal de Pernambuco. Recife – PE, 2018.

MATOS, S.P.DE. Operações Unitárias: Fundamentos, Transformações e Aplicações dos Fenômenos Físicos e Químicos. 1° Edição. Érica -São Paulo, 2015.

VARRICCHIO, M.C.B.N.; PINTO, L.F.; ANDRADE, E.M.; PELLAGIO, S.S. Emprego do Avelós (*Euphorbia Tirucalli*) Dinamizado no Tratamento do Câncer. Revista Homeopatia Brasileira. Rio de Janeiro, 2000.

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