Identification and pharmacognostic control of the species: *Mentha x villosa, Lippia alba, Cymbopogon citratus* and *Melissa officinalis*

Identificação e controle farmacognóstico das espécies: *Mentha x villosa, Lippia alba, Cymbopogon citratus* e *Melissa officinalis*

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ABSTRACT

The use of medicinal plants often symbolizes the only therapeutic resource of a community or group of people to establish quality of life and health, as they have bioactive compounds with therapeutic properties, there is a vast amount of plants spread throughout the territory, present in open markets, markets, and beyond the residential backyards. Popular observations on the use of medicinal plants are relevant to therapeutic action, even though we are not aware of all of its components. It emphasizes the care that must be taken with the identification of the desired species, the part that will be used, such as a flower, fruit, leaf, stem or root, as it can cause serious intoxication or poisoning. The main purpose of this study was to study the secondary metabolites of the species *Mentha x villosa, Lippia alba, Cymbopogon citratus* and *Melissa officinalis*, in addition to the quantification of their chemical and antimicrobial properties. The samples were acquired from desiccated leaves where tests were performed to determine moisture, total ash and acid-insoluble, showed the presence of cardiotonics, flavonoids, anthraquinones, saponins, alkaloids, tannins and antibacterial properties, as well as the quantification of the content of total flavonoids as a quality control reference for the species described above.

Keywords: secondary metabolites, medicinal plants, herbal medicines, active principles.

RESUMO
O uso das plantas medicinais simboliza muitas das vezes o único recurso terapêutico de uma comunidade ou grupo de pessoas para estabelecer a qualidade de vida e saúde, pois possuem compostos bioativos com propriedades terapêuticas, existe uma vasta quantidade de plantas espalhadas por todo território, presente em feiras livres, mercados, e além dos quintais residenciais. As observações populares sobre o uso das plantas medicinais são pertinentes à ação terapêutica, mesmo não tendo conhecimento de todos seus componentes. Salienta-se sobre os cuidados que se deve ter com identificação da espécie desejada, parte que vai ser utilizada, como flor, fruto, folha, caule ou raiz, pois pode provocar intoxicação ou envenenamento graves. O presente estudo teve como principal propósito o estudo dos metabólitos secundários das espécies *Mentha x villosa*, *Lippia alba*, *Cymbopogon citratus e Melissa officinalis*, além da quantificação de propriedades químicas e antimicrobianas que possuem. As amostras foram adquiridas a partir das folhas dessecadas onde foram realizados testes de determinação de umidade, cinzas totais e insolúveis em ácido, apresentou presença de cardiotônicos, flavonoides, antraquinonas, saponinas, alcaloides, taninos e propriedades antibacteriana, bem como a quantificação do teor de flavonoides totais como referência de controle de qualidade das espécies acima descritas.

Palavras-chave: metabólitos secundários, plantas medicinais, fitoterápicos, princípios ativos.

1 INTRODUCTION

The consumption of medicinal plants is part of the historical evolution of society, since the beginnings of humanity there are records of how the use of plant species for the treatment of the most varied diseases was carried out. The World Health Organization (WHO) maintains that approximately 80% of the population uses plants to treat primary health symptoms, which directly reflects on the poorest population, without access to conventional treatments, which certainly forces them to use medicinal plants as an alternative for the treatment of the primary symptoms of different pathologies (WHO, 2002). WHO considers traditional medicine (TM) to be the use of plants, minerals and animal parts (WHO, 2013).

In an attempt to regulate the use of medicinal plants and insert them in the Unified Health System (SUS), it was published through Decree n. 5,813, of June 22, 2006, the National Policy on Medicinal Plants and Herbal Medicines establishes guidelines and priority lines for the development of actions by the various partners around a single objective, dedicated to ensuring safe access and rational use of medicinal plants and herbal medicines in Brazil, as well as the development of technologies and innovations,

and the strengthening of production chains and arrangements, which refer to the sustainable use of the Brazilian biodiversity and the development of the productive complex (BRASIL, 2006).

In 2008, ordinance 2960 of December 9th was approved, approving the National Program of Medicinal Plants and Herbal Medicines and creating the National Committee of Medicinal Plants and Herbal Medicines. (BRASIL, 2008). 2016 marked the 10th anniversary of the SUS National Policy on Integrative and Complementary Practices, together with the PNPMF, jointly publishing the two policies with the objective of ensuring the safe use of medicinal and herbal plants, ensuring efficacy, quality and security (BRASIL, 2016).

Herbal medicine benefits from nature, to normalize impaired physiological functions, thus restore weakened immunity, promote detoxification and generate well-being. The increase in adverse reactions with herbal products has stood out in recent years, due to the search for natural therapies without adequate identification or preparation, even with low toxicity, can lead to serious problems since there are other risk factors such as contraindications or concomitant use of other medicines, thus one of the first attributes in the correct use of medicinal plants is botanical identification, as many species receive different nomenclature, which will certainly cause problems in identification. (MURRAY et al 2016; ZENI et al 2017).

The purpose of this study was to establish a practical methodology for botanical identification, through chemical reactions, of secondary metabolites, which can corroborate with quality control for the respective species and also analyze the antimicrobial properties. The importance of pharmacognostic studies is characterized by corroborating the safe use of the studied species, which were acquired in open markets in the city of Ji-Paraná, Rondônia, Brazil.

The species chosen were: *Mentha x villosa, Cymbopogon citratus, Lippia alba* and *Melissa officinalis*, which have similar characteristics and are sold in the form of powder or dried and cut into small pieces. Another determining factor for the choice was the popular use of species that resulted in a very significant increase in recent years. A brief description of the selected species follows.

Mentha x villosa - Lamiaceae, popularly known as mint or creeping mint, has the characteristic of being a creeping plant that is about 30 to 40 centimeters tall. It has oval leaves with small points, petiolate, with a strong and characteristic aroma, used in folk medicine as a giardicide and amebicide, as well as for stomach ache and colic. The

popular form of use is restricted to tinctures, juices and infusions (NASCIMENTO, 2013; BARACUHY et al. 2016).

The *Cymbopogon citratus* - *Poaceae*, popularly known as lemongrass, has a stalk-like stem, which generates large, compact clumps. The size varies from 1 to 2 meters in height and the leaves have acicular characteristics, measuring between 60 to 100 centimeters in length and 1.5 to 2 centimeters in width. The leaves, when injured, give off a strong characteristic odor, making it easily recognizable, where it is extracted an essential oil rich in citral. Its therapeutic indication is quite wide, such as: spasmolytic, analgesic, calming and gastric protective, used mainly in the form of infusions (NASCIMENTO, 2013; BARACUHY et al. 2016).

The *Lippia alba* - *Verbenaceae*, presents with popular names of false lemon balm or bush lemon balm, being a sub-shrub with a woody stem at the base and can reach up to 2 meters in height, has long, whitish and brittle branches, native to almost the entire Brazilian territory, its leaves are oval with a pointy apex, the flowers are colored. purplish pink and has a characteristic aroma. The therapeutic indication consists of calming, spasmolytic and analgesic properties, used in the form of infusions of leaves, flowers and branches (NASCIMENTO, 2013; BARACUHY et al. 2016).

Melissa officinalis - *Lamiaceae*, popularly known as veritable lemon balm, it has a perennial characteristic, in addition to being branched from the base, it can appear erect or with ascending branches, usually around 30 to 60 centimeters high, leaves with light green coloring, its flowers are initially small yellowish, then violet red. The therapeutic properties are based on carminative action, stimulant, tonic, sedative, anti-hysterism, used in the form of an infusion or fluid extract in 45% alcohol (GRANDI, 2014; BORTOLUZZI; SCHMITT; AZUR, 2020).

The species *Cymbopogon citratus*, *Lippia alba* and *Melissa officinalis* show similarity in the odor given off by the leaves, being quite difficult to identify them in the form of powders or scratches. Still, it can be highlighted that the species: *Mentha x villosa* and *Melissa officinalis*, despite presenting a difference in the exhaled odor, present similar morphological characteristics, which would certainly lead to confusion in the identification. Thus, a pharmacognostic study is necessary for the correct identification of the mentioned species, which are freely traded in cities.

2 MATERIALS AND METHODS

The material used was obtained from the leaves of *Mentha x villosa*, *Cymbopogon citratus*, *Lippia alba* and *Melissa officinalis*, which were acquired from the medicinal plant garden at College Estácio - Unijipa of Ji-Paraná, Rondônia, Brazil. The collected species were subjected to a drying process, in a wooden chamber with an incandescent lamp, until complete drying, in a closed environment, free from insects and particles. After drying, the drug was sent to the Estácio – Unijipa laboratory to start the pharmacognostic tests.

To carry out this research, a bibliographic survey was carried out using articles available in the databases: lilacs, web of science, to determine the secondary metabolites present in the species, facilitating their pharmacognostic identification. The execution of the tests was based on the methodologies described in the Brazilian Pharmacopoeia VI edition and on the website of the Brazilian Society of Pharmacognosy (SBF), on the education link.

2.1 PHYSICAL METHODS

The moisture content was determined according to the method described in the Brazilian Pharmacopoeia VI edition. This method is based on loss by drying in an oven and aims to determine the amount of volatile substances of any nature eliminated under the conditions specified in the monograph. (BRAZIL, 2019). The quantitative determination of the total ash was also carried out according to the method described in the Brazilian Pharmacopoeia VI edition, the sample was incinerated at a temperature of 450°C and then the percentage of ash in relation to the powder that was submitted to the drying process was calculated. After obtaining the total ash, it was heated for 5 minutes in 7% hydrochloric acid in a crucible covered with clock glass, transferring the residue to filter paper and then allocated to the original crucible and dried on a hot plate. incinerated in a muffle to constant weight obtaining the ratio of acid-insoluble ash. (BRAZIL, 2019).

The quantification of flavonoids present in the species was based on quercetin, carried out from the dried and ground plant, being submitted to an extraction process and the absorbance determined in a UV spectrophotometer. The result was given by applying the value obtained in absorbance measured at 425nm, in a 1,000cm thick cuvette. Calculation of content is based on the specific absorbance of quercetin, E1%1cm = 500.

2.2 CHEMICAL METHODS

In the determination of cardio tonic glycosides, the steroid nucleus identification reactions were carried out: Liebermann-Burchard reaction, pentagonal lactone ring identification reaction: Kedde reaction and 2-deoxysugars identification reaction: Keller-Kiliani reaction. In flavonoid compounds, Shinoda or Cyanidin reactions, aluminum chloride reaction, ferric chloride reaction, sodium hydroxide reaction was carried out, as well as in anthraquinones, possible Borntraeger reactions were analyzed, being a micro sublimation process.

In the saponin glycosides, a physical process was used that consists of stirring the solution to detect foam formation. In the chemical process, general reactions, were carried out the Rossol reaction, Micthell reaction, Rosenthalen reaction, reaction with Sulfo-vanillic reagent. In specific reactions, the Liebermann-Burchard reaction was carried out, reactions performed from chloroform solution. The general reaction with trichloroacetic acid and the specific Salkowski reaction was also carried out.

Analysis of alkaloids, the metabolite responsible for different antimicrobial activities, was carried out from the sprayed plant and after extraction with dilute sulfuric acid and chloroform, it was detected with neutral lead acetate. For the determination of tannins, general reactions are observed: reaction with 2% ferric chloride, reaction with neutral lead acetate. Specific reactions with lead acetate and glacial acetic acid and reaction with ferric chloride.

2.3 ANTIBACTERIAL ACTIVITY

The extracts were prepared from dried and pulverized leaves, submitted to an extraction process, by maceration for 7 days in hexane. After this period, gas filtration was performed and methanol was added to the pie, remaining in maceration for another 7 days and then filtration. Both the hexane and methanol extracts were evaporated to obtain a soft extract of the respective species and specific extracting solvents (RIBEIRO, 2009).

The development of the method consisted of appraising 0.1g of the soft extract of one of the obtained extracts and diluting it in dimethyl sulfoxide (DMSO). After the complete homogenization of the respective extracts, in the solvent used, dilutions of the following proportions were carried out: 1:1, 1:2, 1:4, 1:8, and 1:10, respectively, the proportions of the samples in the dilution are 100 mg, 50mg, 25mg, 12.5mg and 6.25mg.

Escherichia coli and *Staphylococcus aureus* bacterial strains were used, sown in CLED and MacConkey culture media. The results were presented in the determination of halos formed on the plates.

3 RESULTS AND DISCUSSIONS

3.1 DETERMINATION OF WATER, TOTAL ASH AND ACID-INSOLUBLE ASH

The determination of water is important in the analysis of plant drugs, loss by desiccation is a quality control factor for herbal medicines, as it refers to the content of moisture and/or volatile substances present in the plant drug.

In general, for good conservation, the plant drug must have a minimum moisture content. However, the presence of an excessive amount of water in plant drugs favors the development of microorganisms, insects, hydrolysis and consequent deterioration of the drug's constituents.

According to the Brazilian Pharmacopoeia, in its previous editions, the humidity limits for plant drugs are, in general, in the range of 8% to 14%**.**

Table 1: Results of quantitative tests for species.

Medicinal plant	Moisture	Total ash	Insoluble ashes
Mentha x villosa	6.66%	12.33%	2.33%
Lippia alba	6.66%	14.00%	2.00%
Cymbopogon citratus	3,33%	8,66%	2,33%
Melissa officinalis	10.66%	14.00%	2.66%

Source: research data.

In the tests performed, the results observed in table 1 were obtained, the leaves of the species *Mentha x villosa* (6.66% moisture), *Lippia alba* (6.66% moisture) and *Cymbopogon citratus* (3.33% moisture) presented values outside the reference standard of the Brazilian pharmacopoeia (between 8% and 14% moisture), only *Melissa officinalis* (10.66% moisture) obtained a result of moisture content within the reference standards.

In the determination of the total and acid-insoluble ash index, it was possible to observe, respectively 12.33% and 2.33% for *Mentha x villosa*, 14.00% and 2.00% for *Lippia alba*, 8.66% and 2.33% for *Cymbopogon citratus*, in *Melissa officinalis*, it obtained 14.00% and 2.66%. The total ash test was carried out by incinerating the vegetable drug, leaving an inorganic ash which, in the case of many drugs, varies within wide limits and therefore has little value for typification purposes. In other cases, such as labeling, benefits, purity, the content of total ash is important and can indicate the care that was taken in preparing the drug, that is, the verification of its purity.

In this verification, caution was taken so that the carbon burned at the lowest possible temperature (450°C), otherwise the alkali chlorides, volatile at high temperatures, are lost. The maximum limits of total ash, required in the monographs of the Brazilian Pharmacopoeia, in the 3rd edition (BRASIL, 1977), vary between 7% and 20% and in the 4th edition (BRASIL, 1996), between 5% and 15%, for plant drugs, where the part used is the leaves, and already in the 5th edition (BRASIL, 2010) and 6th edition (BRASIL, 2019) values for determination of total ash are not established. However, tests were carried out to determine total ash.

All species presented results indicating that the non-volatile residual substance contents are in accordance with the pharmacopoeia reference values. Highlighting the low percentage of inorganic materials, minimal impurity.

The determination of total ash aims to establish the amount of non-volatile residual substance in the specified incineration process. Total ash includes those derived from plant tissue (physiological ash) and foreign materials, especially sand and earth adhering to the drug surface (non-physiological ash), which makes this a methodology to verify the care taken in drug preparation.

The Brazilian Pharmacopoeia establishes, in the monographs for species, maximum contents of acid-insoluble ash, between 2.5% and 4% in the 3rd edition (BRASIL, 1977) and between 2.0% and 6.0% in the 4th edition (BRASIL , 1996) and in the 5th edition (BRASIL, 2010) and 6th edition (BRASIL, 2019) values of acid-insoluble ash are not established.

The values of total and acid-insoluble ash for the species studied in this work can be seen in Table 1. In the sheets of the cited species resulted in values within the reference standards, described in previous editions of the Brazilian Pharmacopoeia. therefore, risk of contamination.

3.2 QUANTIFICATION OF FLAVONOIDS

Flavonoids are substances found in most plant species, with antioxidant effects, being widely used in the recovery of inflammatory processes. Thus, it is important to quantify this metabolite for the safe and effective use of the studied species.

According to the results obtained in the quantification of flavonoids, for the species: *Mentha x villosa, Lippia alba, Cymbopogon citratus* and *Melissa officinalis*, by UV spectrophotometry, based on the quantification of quercetin, provided as a percentage (m/m) of each medicinal species studied. Thus, it can be seen that in the species *Mentha*

x villosa there was a percentage of 0.05% of flavonoids present, in the species *Lippia alba* the amount determined was 0.05%, for *Cymbopogon citratus* the amount of flavonoid was 0.009%, and in *Melissa officinalis*, the percentage was 0.05% (Table 2). Thus, it can be observed that the results, with the exception of the species *Cymbopogon citratus*, the others showed similar results for the quantification of flavonoids, even so, these amounts are significant for the studied species, popularly used by the population.

Table 2: Percentages of flavonoids found from the species.

Medicinal Species	**Percentage**
Mentha x villosa	0.05%
Lippia alba	0.05%
Cymbopogon citratus	0,009%
Melissa officinalis	0.05%

Source: research data.

3.3 DETERMINATION OF CARDIOTONIC GLYCOSIDES

The Liebermann-Burchard reaction showed positive results in the species *Mentha x villosa*, *Cymbopogon citratus* and *Melissa officinalis*, with a red border ring, indicating the presence of cardenolides and bufadienolides. This reaction is characteristic of steroid and triterpenoid compounds, as the reagent promotes dehydration and dehydrogenation of the fundamental nucleus, which results in derivatives with conjugated double bonds and, therefore, stained. For this reason, this characteristic is common in cardenolide and bufadienolide compounds. The results of the analysis for detection of cardio tonic glycosides demonstrate that the species had a negative result for the Kedde reaction, with no staining reaction. This reaction is used to differentiate cardenolides from bufadienolides. In the Keller-Kiliani reaction, the result was positive for *Mentha x villosa*, *Cymbopogon citratu*s and *Melissa officinalis*, stating that they have deoxysugar at the carbohydrate end. In the calculation of the results of Kedde's reaction, no change in color or ring formation was observed, thus being negative for the reaction (Table 3).

Table 3: Results of reactions indicating the presence or absence of cardiotonic glycosides from the species.

Medicinal Plant	Liebermann-Burchard reaction	Kedde's reaction	Keller-Kiliani reaction
Mentha x villosa	Red edge ring terpenic core (+)	No reaction (-)	Brownish red ring (+)
Lippia alba	Green edge ring	No reaction (-)	Green ring (acetic)
Cymbopogon citratus	Red edge ring terpenic Core (+)	No reaction (-)	Brownish red ring (+)
Melissa officinalis	Red edge ring terpenic Core (+)	Slightly yellowish (-)	Brownish red ring (+)

*(+): Positive, (-): Negative.
Source: research data.

3.4 DETERMINATION OF FLAVONOID GLYCOSIDES

In the qualitative determination of flavonoid glycosides, the Cyanidin test was used, as it is characteristic of the largest number of substances in this class, with a positive result only for *Lippia alba*, due to the occurrence of a red color in the sample. The study with ferric chloride and sodium hydroxide showed negative results, and an inconclusive result in the case of the reaction with aluminum chloride, as no change in the color of the reaction was observed (Table 4).

Table 4: Results of reactions indicating the presence or absence of flavonoid glycosides from the species.

Medicinal plant	Shinoda or Cyanidin Reaction	Reaction with ferric chloride	Reaction with sodium hydroxide	Reaction with aluminum chloride
Mentha x villosa	Green coloring (-)	Brown coloration (-)	Brown coloration (-)	Inconclusive reaction
Lippia alba	Red coloring (+)	Brown coloration (-)	Brown coloration (-)	Inconclusive reaction
Cymbopogon citratus	Green coloring (-)	Brown coloration (-)	Brown coloration (-)	Inconclusive reaction
Melissa officinalis	Green coloring (-)	Brown coloration (-)	Brown coloration (-)	Inconclusive reaction

*(+): Positive, (-): Negative.
Source: research data.

3.5 DETERMINATION OF ANTHRAQUINONE GLYCOSIDES

Anthraquinone derivatives, present in several plant species, are usually orange in color and confer laxative effects. In the tests carried out, mainly in the Borntraeger reaction, which should present a reddish-pink color for a positive reaction, no alteration was observed. Therefore, the result for the presence of anthraquinones was negative in all studied species (*Lippia alba*, *Mentha x villosa*, *Cymbopogon citratus* and *Melissa officinalis*). In the micro sublimation process, after heating in a plate under a metal ring,

it should have crystals for positive results, thus the result obtained was considered negative, confirming the result of the previous reaction, as there was no identification of crystals in any of the samples studied.

According to the results obtained for the reactions of anthraquinone glycosides in the species samples *Mentha x villosa, Lippia alba, Cymbopogon citratus* and *Melissa officinalis* were negative. Thus, it can be stated that the species studied do not have anthraquinonic glycosides.

3.6 DETERMINATION OF SAPONIN GLYCOSIDES

In the tests to verify the presence of saponins of the species *Mentha x villosa, Lippia alba, Cymbopogon citratus* and *Melissa officinalis*, physicochemical methods were used, which, after uninterrupted shaking of the tubes for 5 seconds, it was observed the formation of foam in all the tubes with the respective samples, corroborating the indication of the presence of saponin compounds. Even after leaving the extract to rest for 30 minutes, the foam formation remained stable, considering positive for the Rossol, Mitchell, Rosenthalen reaction, which showed a slight reddish-brown reaction (Table 5).

In the Sulfovanillic and trichloroacetic acid reactions, the results were negative for the species, as they did not show specific coloration (Table 5), whereas in the Salkowski tests the species *Mentha x villosa, Lippia alba, Cymbopogon citratus* and *Melissa officinalis* presented a triterpenoid nucleus and Liebermann-Burchard steroid nucleus due to the staining of the samples (Table 5).

Table 5: Results of reactions indicating the presence or absence of saponin glycosides from the species.

Medicinal plant	**stirring process**	**Rossol reaction**	**Micthell's reaction**	**Rosenthalen reaction**
Mentha x villosa	It presented foam for a time <30 min. (+)	Reddish brown (+)	Reddish brown (+)	Brown color (+)
Lippia alba	It presented foam for a time <30 min. (+)	Reddish brown (+)	Reddish brown (+)	Brown color (+)
Cymbopogon citratus	It presented foam for a time <30 min. (+)	Reddish brown (+)	Reddish brown (+)	Brown color (+)
Melissa officinalis	It presented foam for a time <30 min. (+)	Reddish brown (+)	Reddish brown (+)	Brown color (+)

*(+): Positive, (-): Negative.
Source: research data.

Table 5: Results of reactions indicating the presence or absence of saponin glycosides from the species. (conclusion)

Medicinal plant	Reaction with Sulfo-vanillic reagent	Liebermann-Burchard reaction	Reaction with trichloroacetic acid	Salkowski reaction
Mentha x villosa	Brown coloration (-)	Greenish coloration (Steroidal)	No noticeable coloration	Reddish-brown (triterpenoid) coloration
Lippia alba	Brown coloration (-)	Reddish-brown color (Steroidal)	No noticeable coloration	Reddish-brown (triterpenoid) coloration
Cymbopogon citratus	Brown coloration (-)	Reddish-brown color (Steroidal)	No noticeable coloration	Reddish-brown (triterpenoid) coloration
Melissa officinalis	Brown coloration (-)	Reddish-brown color (Steroidal)	No noticeable coloration	Reddish-brown (triterpenoid) coloration

*(+): Positive, (-): Negative.
Source: research data.

3.7 DETERMINATION OF ALKALOIDS

The result obtained in the reaction with neutral lead acetate was positive, as it showed formation of white precipitate in the samples of *Mentha x villosa, Lippia alba, Cymbopogon citratus* and *Melissa officinalis*, indicating the presence of alkaloids in the formation of insoluble complexes (precipitate) (Table 6).

Table 6: Results of reactions indicating the presence or absence of alkaloids from the species.

Medicinal plant	Reaction with neutral lead acetate
Mentha x villosa	White precipitate formation (+)
Lippia alba	White precipitate formation (+)
Cymbopogon citratus	White precipitate formation (+)
Melissa officinalis	White precipitate formation (+)

*(+): Positive, (-): Negative.
Source: research data.

3.8 DETERMINATION OF TANNINS

The reaction with ferric chloride showed characteristic coloring, green for condensed tannins or catechetical for the samples of *Mentha x villosa* and *Cymbopogon citratus*, and indicating the presence of hydrolysable or gallic tannins with blue coloration for the sample *Lippia alba* and *Melissa officinalis*. The test with neutral lead acetate showed a whitish precipitate confirming the presence of hydrolysable tannins in *Mentha x villosa, Lippia alba, Cymbopogon citratus*, and *Melissa officinalis*, as well as in the reaction with lead acetate and glacial acetic acid confirming the presence of hydrolyzed tannins (Table 7).

Table 7: Reaction results indicating the presence or absence of tannins from the species.

Medicinal plant	Reaction with 2% ferric chloride	Reaction with neutral lead acetate	Reaction with lead acetate and glacial acetic acid	Reaction with ferric chloride
Mentha x villosa	Green coloring (+)	White precipitate (+)	White precipitate (+)	Green coloring (condensates or catechism)
Lippia alba	Green coloring (+)	White precipitate (+)	White precipitate (+)	Blue coloring (hydrolysable or gallic)
Cymbopogon citratus	Green coloring (+)	White precipitate (+)	White precipitate (+)	Green coloring (condensates or catechism)
Melissa officinalis	Blue coloring (+)	White precipitate (+)	White precipitate (+)	Blue coloring (hydrolysable or gallic)

*(+): Positive, (-): Negative.
Source: research data.

3.9 ANTIBACTERIAL ACTIVITY

Dilutions from 100 mg (1/1), 50 mg (1/2), 25 mg (1/4), 12.5 mg (1/8) and 6.25 mg (1/10) were used in this analysis) of the DMSO extract. The results of formation of inhibition halos were compared with antibiotic discs, being Ciprofloxacin as broad spectrum and for gram-positive to Penicillin. According to the results, it is observed that the analysis was considerable, since in the sample of *Mentha x villosa* it generated in its concentrations from 1/4 to 1/10 halos with 1 and 2 mm effective for *Escherichia coli* and there was no sensitivity to *Staphylococcus aureus*, *Lippia alba* extract had halos between 3 and 2 mm at concentrations 1/1 and 1/2 respectively for *E. coli* and *S. aureus* showed halos between 1 and 2 mm in all dilutions, the *Cymbopogon citratus* sample generated 4 mm halo at 1/1 concentration for *E. coli* and for *S. aureus* varied the formation of halos between 1 and 2 mm at concentrations 1/2, 1/4, 1/8 and 1/10 the extract of the species *Melissa officinalis* obtained a halo of 1 mm at the concentration 1/1 and 1/2 and 2 mm at the concentration 1/4 for *E. coli* and 1 mm for *S. aureus* at the concentrations 1/1, 1/2 and 1/4 and 3 mm to 1/8.

Thus, it is observed that there was sensitivity in relation to extracts and bacteria used in the research. According to Ribeiro (2009), there is no consensus on acceptable levels for extracts obtained from medicinal plants, compared to conventional antibacterial, therefore, it is suggested to carry out new specific tests, which corroborate for new possible drugs and clarity in the effects here found.

4 CONCLUSIONS

The studies carried out in this work showed great importance in the pharmacognostic identification of the species: *Mentha x villosa, Lippia alba, Cymbopogon citratus* and *Melissa officinalis*, are cultivated in all regions of the country and widely used. These studies corroborate for greater safety and correct use of medicinal plants.

The plant species *Mentha x villosa* and *Melissa officinalis* are morphologically similar, which could certainly lead to errors in identification. And the species, *Lippia alba* and *Cymbopogon citratus*, are morphologically different, but present similar characteristic odors, which, in the dry and powdered form, would be difficult to identify.

It is concluded that the methods used in the identification and pharmacognostic control of the plant species *Mentha x villosa, Lippia alba, Cymbopogon citratus* and *Melissa officinalis* showed positive results, which corroborates the identification, safety and quality for their rational use and demonstrates simplicity and easiness in the execution of the proposed methodologies with low cost.

Despite presenting a quantitative test of flavonoids, which favors an indication, in addition to the identification, also of seasonal studies for the proper collection of the respective medicinal species. However, the need to carry out accurate controls, with state-of-the-art sophisticated equipment, on secondary metabolites in order to understand and identify the bioactive components that the leaves of medicinal plants will need for greater safety.

Thus, it can be concluded from this study that the pharmacognostic methods corroborate the identification of medicinal species, which, by virtue of the master pharmacies acquiring this raw material in the form of powder, being difficult to identify them, ensure identification and control for the use in magisterial prescriptions.

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ABBREVIATION

WHO: World Health Organization
TM: Traditional Medicine
SUS: Unified Health System
PNPMF: National Program of Medicinal Plants and Herbal Medicines
SBF: Brazilian Society of Pharmacognosy
DMSO: Dimethyl Sulfoxide
CLED: Cystine Lactose Eletrolyte Deficient
E. coli: Escherichia coli
S. aureus: Staphylococcus aureus