Preliminary and Accelerated Stability Study of *Melissa Officinalis* Syrup Cultivated in the South of Tocantins

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Abstract

Melissa officinalis L. (Lamiaceae) is known as a true lemon balm and is popularly used in traditional medicine as a tranquilizer. The present research aimed to propose a source of syrup based on the tincture of M. officinalis and perform preliminary and accelerated stability tests, and microbiological evaluation. The leaves of M. officinalis were collected and after botanical identification, the raw material processing step was followed to obtain the dye. The tincture obtained was used to prepare the syrup based on M. Officinalis. The syrup was used as steps of physical-chemical and microbiological quality control. It was found that the M. Officinalis syrup, in the analysis of pH, organoleptic characteristics, density, volume, and microbiological assay, is by the consulted literature. The observed results indicate that the syrup of M. Officinalis is within the defined parameters, which guarantees the patient the efficacy and safety of the herbal medicine.

Keywords: herbal medicine; physicochemical stability; medicinal plants; microbiological tests.

1. Introduction

Melissa (Melissa officinalis L.), known as true lemon balm, is part of the Lamiaceae family, is native to Asia and Europe, and was introduced to Brazil more than a century ago and is now cultivated throughout the country [1].

This species is part of the list of medicinal species regulated by the National Health Surveillance Agency; and according to Anvisa Resolution No. 89, Melissa officinalis is listed as a simplified registration herbal medicine, and can be used as a sedative, carminative, antiviral, and antispasmodic [2].

In 2006, through the Decree of the Presidency of the Republic nº. 5.813, of June 22, the Ministry of Health (MS) published the National Policy of Medicinal Plants and Herbal Medicines (BRASIL, 2006) [3]. To ensure the safety of the population, one of Anvisa's actions is the registration of medicines, a step in which they are evaluated for their safety, quality, and efficacy and then released for use by the population [4].

Quality control of herbal medicines is of utmost importance so that the quality, efficacy, and safety of the medicines are proven and the population that acquires herbal medicine safely and effectively [5].

One of the quality control steps is the evaluation of the microbiological quality of herbal medicines, which is based on microbial standards described in official documents and regulatory norms. In this way, the maximum limits for the presence of microorganisms in the product and the absence of pathogens are stipulated. Microbiological studies are fundamental, mainly because of the safety, efficacy, and acceptability of these products. Errors in preventive and control actions in the manufacturing development may lead to compromised performance due to the breakdown of stability, the alteration of physical (color and appearance) and chemical characteristics, inactivating of the active ingredient [6;7].

An herbal drug must go through all stages of research: proposition, creation, and development, including stability testing, to ensure activity throughout its shelf life [8].
Thus, the present research aimed to propose a syrup formulation based on *Melissa officinalis* tincture and carry out preliminary and accelerated stability tests, and also to develop the microbiological evaluation of the phytotherapeutic obtained.

**2. Materials and Methods**

**2.1. Cultivation and Collection of Melissa officinalis**

*Melissa officinalis* was cultivated and collected in the city of Gurupi-TO, specifically at 11°43'45.6"S 49°03'18.0"W (Figure 1) by planting the seeds in Styrofoam trays, then transplanted into pots containing black earth + tanned manure, and after reaching the adult stage of the plant species it was possible to harvest and start the laboratory experiments.

![Figure 1: The geographic location of the City of Gurupi, Tocantins State, 2021.](image)

**2.2. Identification of the plant material**

Duplicate specimens of each species were prepared and identified by the Professor of Botany, Rodney Vianna, where they are deposited in the Herbarium of the Center for Environmental Studies of the Federal University of Tocantins (Porto Nacional Campus). The species *M. Officinalis* is deposited in the herbarium under the code number HTO-12117.

**2.3. Obtaining the tincture from M. Officinalis**

A tincture is a form of preparation in which the active principles of medicinal plants are extracted. According to the form of herbal medicines of the Ministry of Health, we used 20 grams of fresh leaves of *M. Officinalis* (Figure 2) were washed, subjected to the drying process, and crushed. Then, 100ml of 70% alcohol was added and stored in a well-sealed amber glass bottle in a cool, dry place away from light for 7 days [9].
2.4. Preparation of simple syrup

To obtain the phytotherapeutic syrup, the simple syrup was first prepared according to the Phytotherapeutic Form of the Brazilian Pharmacopoeia, 1st edition. In a 500ml beaker, 50g of crystallized sugar were dissolved with the aid of a glass rod and gradually 50ml of distilled water [9] was added and subjected to a water bath at a temperature of up to 80ºC. After cooling, an equal volume of distilled water was added.

2.5. Preparation of the herbal formulation based on M. Officinalis

After obtaining the tincture, it was submitted to simple filtration with the aid of a separation funnel, having as a product the vehicle itself to be added to the simple syrup. Thus, the formula proposed in Table 1 was used to manufacture the phytotherapeutic. The syrup was homogenized and solubilized. After this, its preparation was bottled in a sterilized amber glass flask container. The sample was left at room temperature and cool place [9].

Table 1: Syrup based on Melissa’s officinalis

<table>
<thead>
<tr>
<th>Components</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melissa officinalis tincture</td>
<td>20%</td>
</tr>
<tr>
<td>Simple syrup q.s.p</td>
<td>100ml</td>
</tr>
</tbody>
</table>

2.6. Stability studies

The stability study was monitored for 0-3 months (preliminary and accelerated stability). The formulation was evaluated concerning physicochemical parameters (determination of pH, density, volume, and, organoleptic characteristics) and microbiological parameters [10].

2.6.1 PH Determination

The pH determination was performed on a digital parameter. The reading was made by introducing the electrode
into the sample, proceeding with three successive readings, and obtaining the average of the readings as a result, at times 0, 1, 2, and 3 months [10].

2.6.2 **Volume**

The volume was determined by weighing the individual capped glass bottle containing the formulation. After removing the contents, it was washed, dried, and weighed again. The weight of the contents was calculated by the difference between the weight of the full vial and the empty vial [10]. The volume was calculated using equation 1.

Equation 1: \( V = \frac{m}{p} \)

**Being,**

\( m = \) weight of content (g)

\( p = \) mass density of the product (g/mL)

2.6.3 **Density**

The determination of the relative density of the syrups was performed in triplicate (test and control formulation) using a glass pycnometer with a capacity of 50 ml, previously cleaned and dried, at times 0, 1, 2, and 3 months [10]. Density was calculated using equation 2.

Equation 2: \( d = \frac{(M_2 - M_0)}{(M_1 - M_0)} \)

**Being,**

\( M_0: \) empty pycnometer

\( M_1: \) pycnometer with water

\( M_2: \) pycnometer with the sample

2.6.4 **Organoleptic characteristics**

The organoleptic characteristics of the syrup containing the activity were evaluated at times 0, 1, 2, and 3 months. The samples were analyzed comparing times 1, 2, and 3 months with time 0.

2.7. **Obtaining the tincture from M. Officinalis**

2.7.1. **Sample Preparation**

The *Melissa officinalis* herbal syrup was analyzed and had been prepared 7 days before. The procedure was
performed through microbiological tests to verify whether the syrup presented microbiological contamination or not. [11]

2.7.2. Sample Preparation

Cromolyn Agar is a non-selective culture medium, whose objective is the isolation, differentiation, direct identification, and counting of pathogens present in samples. There was direct identification of Escherichia coli, Staphylococcus saprophyticus, Enterococcus spp, as well as the groups KESC (Klebsiella spp., Enterobacter spp., Serratia spp. and Citrobacter spp.), and PPM (Proteus spp., Providencia spp. and Morganella spp.). In addition, other gram-negative bacilli were isolated, whether fermenting or not (Pseudomonas spp. Edwardsiella spp., Hafnia spp., and others). The gram-positive cocci isolated were Staphylococcus spp., Streptococcus spp. etc. and yeasts such as Candida spp. Its formulation is Chromopeptone 16.1 g/L, Heart Peptone (bovine or porcine) 5.0 g/L, Agar base 18.0 g/L, chromogenic substrate mixture 1.5 g/L, ultra-purified H2O 1L, and pH 7.0 ±0.2 at 25°C [12].

Mac Conkey Agar is a selective differential medium for isolating and differentiating enterobacteria and a range of other gram-negative rods from clinical samples. In this medium, it is possible to differentiate lactose fermenting bacteria, which form pink colonies, from lactose non-fermenting bacteria, which form colorless colonies. Its composition is a pancreatic hydrolysate of gelatin 17.0 g/L, peptic hydrolysate of animal tissue 1.5 g/L, pancreatic hydrolysate of casein 1.5 g/L, Lactose 10.0 g/L, Bile salts 1.5 g/L, Sodium chloride 5.0 g/L, Agar Base 15.0 g/L, Crystal violet 0.001 g/L, H2 O ultra-purified 1L and the pH 7.1 ±0.2 at 25°C [13].

2.7.3. Preparing plates for pathogenic bacteria analysis

The Melissa officinalis herbal syrup was incubated using the striation technique with a 10µl calibrating loop on Chromoclin US Agar (triple) and Mac Conkey Agar media, where they were placed in the oven at 37°C for 24hrs [10].

3. Results

3.1. PH Determination

The evaluation of the pH of the formulation helps to verify stability and allows the detection of problems related to the decomposition of the active substance, which can compromise its pharmacological action. [14] The results obtained are described in Table 2.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Time 0</th>
<th>Time 1</th>
<th>Time 2</th>
<th>Time 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active Syrup</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>5</td>
</tr>
</tbody>
</table>
3.2. Volume Determination

The values obtained in the volume analysis of M. officialis syrup are described in Table 3. It was analyzed in times 0, 1, 2, and 3.

Table 3: Results of the M. Officinalis syrup volume test.

<table>
<thead>
<tr>
<th>Volume (mL)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Time 0</td>
<td>130.31</td>
</tr>
<tr>
<td>Time 1</td>
<td>129.92</td>
</tr>
<tr>
<td>Time 2</td>
<td>129.34</td>
</tr>
<tr>
<td>Time 3</td>
<td>129.45</td>
</tr>
<tr>
<td>Average</td>
<td>129.75</td>
</tr>
</tbody>
</table>

3.3. Volume Determination

The density of the sample was evaluated at time 0 and at other times determined up to 3 months. The results are shown in Table 4.

Table 4: Results of density determination of M. Officinalis syrup.

<table>
<thead>
<tr>
<th>Time</th>
<th>Syrup (mean ± SD*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time 0</td>
<td>± 1.131</td>
</tr>
<tr>
<td>Time 1</td>
<td>± 1.133</td>
</tr>
<tr>
<td>Time 2</td>
<td>± 1.137</td>
</tr>
<tr>
<td>Time 3</td>
<td>± 1.134</td>
</tr>
</tbody>
</table>

3.4. Volume Determination

The organoleptic characteristics of the syrup were evaluated at 0, 1, 2, and 3 months. The results are described in Table 5.

Table 5: Results of the organoleptic characteristics of M. Officinalis syrup.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Time (months)</th>
<th>Color</th>
<th>Odor</th>
<th>Aspect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syrup</td>
<td>0</td>
<td>Dark Green</td>
<td>Strong</td>
<td>Clear</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Dark Green</td>
<td>Strong</td>
<td>Clear</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Dark Green</td>
<td>Strong</td>
<td>Clear</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Light Green</td>
<td>Strong</td>
<td>Clear</td>
</tr>
</tbody>
</table>
3.5. Volume Determination

After 24 hours incubated in the oven, it can be seen that there was no bacterial growth on both the Chromoclin US Agar medium (triplicate) as shown in Figure 3, and on the Mac Conkey Agar medium (Figure 4).

![Figure 3: chromoclin US Agar medium (triple)](image1)

![Figure 4: mac Conkey agar medium](image2)

4. Results

In the pH parameter, Almeida and collaborators [15] analyzed three samples of Acebrofilin, being reference, similar and generic. The pH varied little among the samples, being $4.3 \pm 0.2$ for the reference and similar drug and $4.4 \pm 0.4$ for the generic drug. [15] Santinho [16] verified a correlation between carcinogenic potential and pH in pediatric syrups based on isolated chemical substances. This reinforces the importance of conducting and encouraging studies with syrups based on plant extracts following the current legislation in Brazil, considering that it will be stimulating the cultivation of medicinal plants in Brazil, the valorization of the work of the small farmer, and traditional knowledge, as an example the syrup based on *M. Officinalis*.

The volume test applies to sterile and non-sterile liquid preparations, and also to liquid preparations obtained from powders for reconstitution. According to the parameters described in the Brazilian Pharmacopoeia, the average volume of the tested units must not be lower than the declared volume. In addition, none of the samples tested can have a volume less than 95.0% of the declared volume [10].

Reference [17] analyzed samples of *Mikania glomerata* syrup, commonly dispensed in Basic Health Units by the Unified Health System. They found that one-third of the analyzed samples had a volume below the 95% determined by the Brazilian Pharmacopoeia 6th edition [3]. Differently, the present study with *M. Officinalis* syrup showed results within the parameters described by the literature.

Sobreira and his colleagues [7] conducted a study to evaluate the legal aspects and the quality of the soursop (*Annona muricata* L.) based product obtained in different pharmacies in the municipality of Cuité, Parnaíba. In the samples studied they verified that the physicochemical parameters such as pH, density, and viscosity were in nonconformity from the legal point of view. Another study, conducted by Silva [18] and collaborators verified that the phytotherapeutic syrups industrialized from commercial pharmacies of Ourinhos (SP) in the parameter of density, presented of the 7 samples studied an average density value of 1.179 g/ml, which corroborates with
the present research made with the syrup of *M. Officinalis*. The analysis of organoleptic characteristics is performed through touch, smell, and taste and is considered one of the most relevant factors for the development of pharmaceutical forms [11]. Vilela and his colleagues [19] produced the oral spray pharmaceutical form from the extract of the plant *Echinacea purpurea* and observed that the physicochemical parameters were all in agreement with those described in the literature. They also suggested using the tincture form in the product, for probable complete solubilization and better patient acceptance and thus a new evaluation of the tests performed. According to Machado [20], the microbiological analysis has the purpose of ensuring product safety and efficacy. The existence of unintentional microorganisms in the sample acts directly on the stability of the solution and can change the chemical and physical characteristics of the product. The maximum limit of colony-forming units (CFU) authorized for bacteria and fungi is 10² CFU/mL for solutions [10]. In the samples under the analysis of *M. Officinalis* syrup bacteria and fungi development were absent.

The use of chemical preservatives is one of the main and most effective ways to ensure the microbiological quality of pharmaceutical preparations throughout shelf life and use. Sodium benzoate, benzoic acid, and parabens are examples of preservatives commonly used in non-sterile preparations and some sterile multi-dose preparations when referring to allopathic medicines [21].

With all these parameters analyzed with the *Melissa Officinalis* syrup, it is possible to conclude that the syrup displayed satisfactory results since the temperature and humidity conditions did not interfere with the microbiological safety of the syrup.

5. Final Considerations

According to the tests performed, it can be concluded that the results obtained for the syrup containing active substances from *Melissa officinalis*, in the analysis of pH, organoleptic characteristics, density, and microbiological analysis, are by the standards in force in Brazil for the manufacture of herbal medicines, because it remained stable within the parameters described, throughout the study period.

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