FORMULATION AND CHARACTERIZATION OF TOPICAL PHARMACEUTICAL FORM WITH ORIGANO OIL

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RESEARCH ARTICLE

Abstract

In our country, there is a rich and long history of using plants to treat various desease. Due to its high content of flavonoids, phenolic acids, carvacrol, thymol, pinene, limonene and other compounds, the plant Origanum vulgare L. has a very high antioxidant potential and possesses antiviral, antibacterial, antifungal, choleretic and cholagogic properties.

Among the therapeutic effects of oregano essential oil, the antifungal action of the plant has not been fully researched. Therefore, we aim to highlight the antifungal effect of oregano oil obtained by hydro-distillation from the leaves of the plant, by including it as an active substance in a pharmaceutical preparation for vaginal application. Thus, the purpose of this study was to formulate topical pharmaceutical form with oregano essential oil using hydrophilic excipients and to demonstrate that the obtained preparation meets the quality requirements specified in the Romanian Pharmacopoeia 10th edition.

Keywords: *Origanum vulgare L.*, antifungal, essential oil, topical pharmaceutical form
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INTRODUCTION

Origanum vulgare L. is part of the genus *Origanum*, family Lamiaceae (Bojor, 2018; Pallag, 2015). This plant, also known as wild marjoram, oregano, forest basil, or arigan, grows in Europe, the Mediterranean region, and southern and central of Asia (Bojor, 2018; Şahin et al., 2004; Lombrea et al., 2020). The volatile oil of the plant *Origanum vulgare L*. has been shown to have a high antimicrobial effect *in vitro* (Alzoreky & Nakahara K., 2003) as well as antioxidant properties (Teixeira et al., 2013; Busatta et al., 2007). Also has been used in traditional medicine for its properties such as stomachic, carminative, expectorant and emmenagogue (Oniga et al., 2018). It has been used in the form of tinctures, teas, and ointments for respiratory and digestive disorders, wounds, indigestion, diarrhea, cough, bronchitis, pruritus, headaches, and depression (Licina et al., 2018; Veenstra & Johnson, 2019; Fikry et al., 2019; Bahmani et al., 2018). Oregano is also used in perfumery and as a flavoring for alcoholic beverages (Papp et al., 2011; Polat et al., 2012; Sharifi et al., 2020; Kaurinovic et al., 2011).

According to studies, the main active compounds responsible for the antimicrobial effect of the volatile oil are: thymol, carvacrol, α-thujene, sabinene, γ-terpinene, p-cymene, p-cymen-8-ol (Ruberto, 2002), geraniol, linalool, linalyl acetate, myrcene, camphene, camphor, borneol, bornyl acetate, β-caryophyllene, β-bisabolene (Lukas et al., 2015; Leyva-López et al., 2017; Teixeira et al., 2013; Stesevic et al., 2018), p-hydroxybenzoic acid, vanillic acid, syringic acid, gallic acid, p-coumaric acid, caffeic acid, ferulic acid, and sinapic acid (Lukas et al., 2015).

Considering the fact that the resistance of pathogenic microorganisms to known antifungals has increased in recent years due to their excessive use, which is not always justified

(Constantinescu & Hațieganu-Buruiană , 1986), in this study we attempted to formulate a natural pharmaceutical product based on oregano oil to prevent and treat vaginal conditions caused by *Candida albicans*.

MATERIAL AND METHOD

BOTANICAL ANALYSIS OF THE *ORIGANUM VULGARE L.*

According to the Romanian Pharmacopoeia, 10th edition, the macroscopic examination of the plant product was conducted using a magnifying glass, while the microscopic examination was performed on vegetative organs (stem and leaf) by preparing cross-sectional slides that were clarified and stained using optical microscopy OPTIKA B 380 (Italia).

The identification and differentiation of the structure and characteristic elements (such as surface hairs and secretory structures) were made possible by the differential staining of cell membranes using chemical dyes: the cellulose membrane was stained in red, while the lignified membrane was stained in yellow.

FORMULATION OF TOPICAL PHARMACEUTICAL FORM WITH ORIGANO OIL

The topical pharmaceutical form with oregano essential oil were obtained by the melt and pour method. In the first phase, the suppository base was prepared: 10.5 g of jelly was soaked with a part of water (10 mL), and in the other part of water (23 mL) the surfactant 3.5 g was dissolved, warm. The two mixtures were combined in a container, after which the container was placed on the water bath. Glycerine 52.5 g was added to the mixture formed, which had previously been heated to the same temperature, and mixed until homogeneous. In the second phase, 0.5 g of oregano oil was incorporated into the suppository base, after which we poured the obtained composition into the mold. The forms were then covered with paraffin oil. The preparation formula is shown for 100g suppository.

QUALITY CONTROL OF TOPICAL PHARMACEUTICAL FORM WITH ORIGANO OIL

According to the Romanian Pharmacopoeia 10th Edition, suppositories must have a homogeneous appearance and retain their shape and consistency at room temperature. In the longitudinal section, examined with a magnifying glass (4.5 x) it must not present agglomerations of particles, crystals or air bubbles.

To determine the average mass, we weighed all 20 suppositories on the analytical balance type ABT 220-5DNM from Kern and Sohn GmbH (Balingen, Germany), and then weighed them individually.

To determine how the suppositories dissolve in water, we put 1 suppository in 50 mL of water, at a temperature of 37ºC, shaking the bottle every 5 minutes.

To determine the disintegration of suppositories *in vitro* using water and acidic environment, we inserted suppositories of the cylindrical baskets of the dissolution test apparatus equipped with rotating blades, (Electrolab TDT-08L Dissolution Tester), with a capacity of 500 mL. Solution with a neutral pH (distilled water) or an HCl solution with an acidic pH, similar to the vaginal pH, previously heated to 37ºC, were chosen as dissolution media, after which we placed them in the lower part of the basket setting the speed of rotation of the blades of the device to 37 rpm. Four samples of 5 mL of each dissolution medium were taken at 4 different time intervals (15min, 30min, 45min, 60min). After sampling, the samples were titrated with a 0.1N NaOH solution to determine the total acid content of each sample.

The acidic content was calculated using the equation (1):

$$I_A = \frac{M_{NaOH} \cdot V_{NaOH}}{V_{proba}} \quad (1)$$

Where: M_{NaOH} – molecular mass of NaOH; V_{NaOH} – consumed volume of NaOH; V_{proba} – sample volume; I_A- the number of mg of NaOH required to neutralize the fatty acids contained in one gram of the sample.

RESULTS AND DISCUSSIONS

BOTANICAL ANALYSIS OF THE *ORIGANUM VULGARE L.*

Dried plant product of *Origanum vulgare L.* was purchased from the company Health Embassy (Tewkesbury Rd, Cheltenham), United Kingdom. Following the macroscopic examination carried out with a magnifying glass, we were able to highlight the tector brushes and secretory glands of the plant, as can be seen in figure 1. To carry out the microscopic examination, we made a cross-section through a tetramuchy stem, as can be seen in figure 2. At the microscope, we used both the x 10 and the x 40 objective and we observed from the outside to the inside that the epidermis is unistratified formed from cells

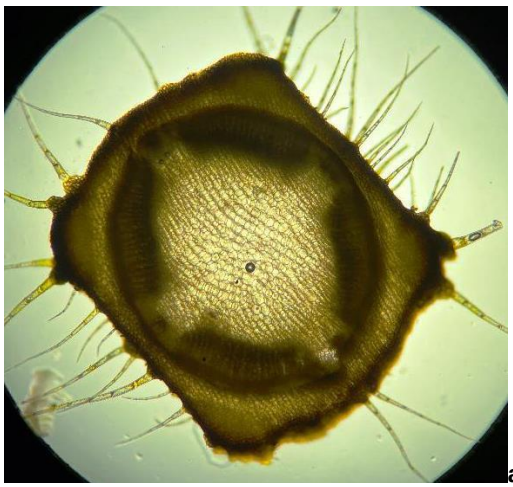
closely united to each other and that the external walls are slightly convex.


a)


b)

Figure 1 **Macroscopic analysis of the *Origanum vulgare L.* plant: a) highlight of the tector brushes and b) highlighting of the tector brushes and secretory structures located on the dorsal side of the leaves**

Filamentous pluricellular tector brushes are present on the epidermis and the angular collenchyma, characteristic of plants of the *Lamiaceae* family, is being developed in the four edges of the stem. Beneath the epidermis is the assimilating parenchyma tissue, with the role of carrying out photosynthesis.


a)

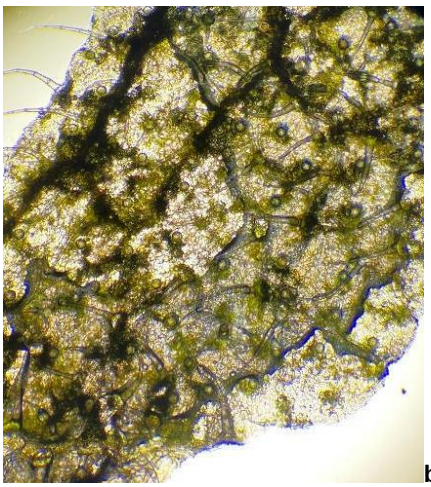

b)

Figure 2 **Cross-section through the: a) stem and b) leaf of *Origanum vulgare L.***

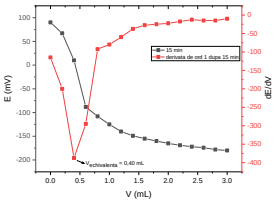
At the level of the leaf, we made sections by skinning, which, depending on the highlighted formation, we coloured with Congo red solution. *Origanum vulgare L.* presents leave with a bifacial structure, that is, only one side of the leaf is directly illuminated by the sun's rays and, consequently, this part is coloured in a darker green than the lower part. Under the upper epidermis, the palisade parenchyma is differentiated, consisting of photosynthesizing cells. We identify here the presence of large, polyhedral cells, less rich in chlorophyll, with a larger diameter than the surrounding cells, which contain a large amount of vacuole juice and which make up the aquifer tissue. From a histological point of view, the two sides of the limbus are different. On the surface of the epidermis, according to figure 2, different formations can be found: stomata, secretory structures and tector brushes. Tector bristles are outgrowths of epidermal cells, which elongate and divide perpendicularly to the epidermis. In *Origanum vulgare L.* they are multicellular, filamentous, unbranched. At the level of the leaves we find the presence of secretory structures of volatile oil of oregano. These structures have a circular shape of different sizes, arranged on the entire surface of the leaf blade.

TOPICAL PHARMACEUTICAL FORM WITH ORIGANO OIL QUALITY CONTROL

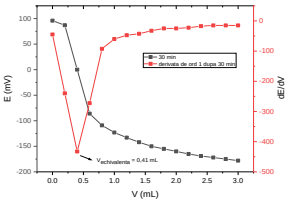
The prepared topical pharmaceutical form with *Origanum vulgare L.* presented a homogeneous appearance after sectioning, at room temperature they kept their shape and consistency, presented a yellowish-white colour, did not present agglomerations of

particles, crystals or air bubbles and presented an odour specific to the components used. After determining the mass uniformity, it was observed that the 20 vaginal suppository formulas presented a mass between 6.75-7.04 g, and the standard deviation was between +1.58% and -3.57%, which falls within the limits established by the Romanian Pharmacopoeia, 10th edition (±4%).

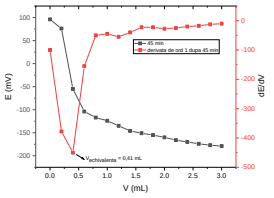
According to the sigmoidal curves and the first-order derivative obtained from the following potentiometric titration in an acid medium (figure 3) and in an aqueous medium (figure 4), at different time periods 15 minutes, 30 minutes, 45 minutes and 60 minutes, knowing the volume of the NaOH solution with which the sample was titrated was calculated the total amount of acids in the titrated sample. After the experiment, it was observed that the acid index, determined in an acidic environment 32 µg (15 minutes), 32.8 µg (30 minutes), 32.8 µg (5 minutes) and 33.6 µg (60 minutes). According to these results, an increase of 0.8 µg in the release of acids from oregano oil is observed after 30 minutes, an amount that remains constant after 45 minutes, so that after 60 minutes it increases to 1.6 µg. It should be noted that after 60 minutes the percentage increase is 5% compared to the sample taken after 15 minutes. But in an aqueous environment the acid index appears only at 30 minutes = 16.8 µg and at 45 minutes = 16.8 µg. This may be due to the fact that in the first 15 minutes the active compound is not released, and after 60 minutes the acidic character of the sample disappears which may be due to too high a dilution of the analysed sample.



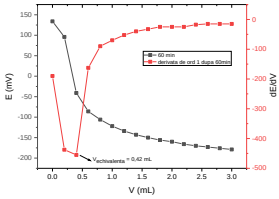
t = 15 min



t = 30 min

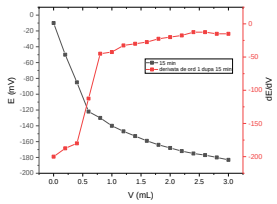


t = 45 min

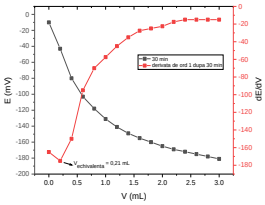


t = 60 min

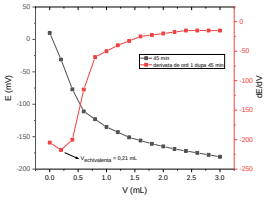
Figure 3 **Sigmoid curves and first-order derivative for potentiometric titrations in acidic medium at different time periods**



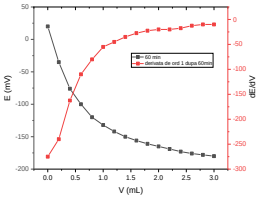
t = 15 min



t = 30 min



t = 45 min



t = 60 min

Figure 4 **Sigmoid curves and first-order derivative for potentiometric titrations in aqueous medium at different time periods**

According to the results obtained, the prepared topical pharmaceutical form with oregano oil disaggregated in an appropriate time, according to the provisions of the Romanian Pharmacopoeia, 10th edition, ensuring at the same time a good release of the active substance at the application site.

CONCLUSIONS

Following the microscopic study carried out on transversal sections, stained with Congo red, at the level of the vegetative organs of the plant *Origanum vulgare L.*, filamentous pluricellular tector brushes were identified, both at the level of the leaf and at the level of the stems, and secretory structures of volatile oil, on the entire surface of the leaf blade. The hot melt and pour method used in the preparation of topical pharmaceutical form with oil of oregano proved to be effective, and the hydrophilic excipients used allowed the successful incorporation of volatile oil of oregano at a concentration of 0.5% into the suppository base. Formed, due to the emulsifying properties of gelatine and solid surfactant. According to the results obtained following the organoleptic analysis, the suppositories with oregano oil prepared, at room temperature, presented a homogeneous appearance, a yellowish-white colour and a specific smell. They did not show agglomerations of particles, crystals or air bubbles. The vaginal suppository with oregano oil fell within the norms provided for in the Romanian Pharmacopoeia, 10th edition regarding the dissolution test because it dissolved after 45 minutes, in water, at a temperature of 37ºC. Also, the disaggregation of the ovules in the acidic environment, similar to the vaginal pH, occurred after 30 minutes. The acid index increased after 60 minutes by 5% in acidic medium compared to 15 minutes, and in aqueous medium it appeared only at 30 minutes and 45 minutes, suggesting that ova dissolve in acidic medium much better than in medium neutral. In other words, the obtained suppositories correspond to the quality conditions stipulated in the Romanian Pharmacopoeia, 10th edition, which suggests that this pharmaceutical preparation could be successfully applied as a natural complementary medication in the treatment of vaginal candidiasis.

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