

Article

Antifungal and Safety Assessment of a Natural Topical Formulation Based on Lamiaceae Essential Oil and Sulfurous Natural Mineral Waters Against *Malassezia furfur*

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Abstract

Pityriasis versicolor (PV) is a condition of the skin caused by the yeast *Malassezia* that manifests itself through scaly, well-demarcated patches with a dark, depigmented or erythematous coloration. Recurrence is common, and few treatment alternatives are available in the market. In this study, we aim to develop a body lotion for the improvement of PV skin with natural ingredients, such as essential oils and natural mineral waters. The antimicrobial activity of the selected ingredients was evaluated by the broth microdilution test, the volatile assay and the checkerboard assay. In addition, the cytotoxicity to HaCaT was assessed by the MTT assay. Finally, the most promising ingredients were combined in a formulation for topical application, and its efficacy and safety profile was assessed in vitro. *Origanum vulgare* essential oil was found to be the most active against *M. furfur* (MIC 0.625% v/v) and synergic with São Pedro do Sul natural mineral water (SPS NMW), while also being biocompatible with keratinocytes (EC₅₀ < 0.06% v/v). The body lotion that was formulated with the most natural ingredients possible (>95%) and showed great activity against *M. furfur*, being able to reduce its growth by 100%; in addition, it was found to be a non-irritant in 3D reconstructed skin models.

Keywords: body lotion; circular economy; cosmeceuticals; dermatology; natural products; *Origanum vulgare*; pityriasis versicolor; SPS NMW

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1. Introduction

Pityriasis versicolor (PV), also known as tinea versicolor, is a superficial skin infection caused by fungi of the genus *Malassezia* [1,2]. According to current taxonomy, the genus *Malassezia* belongs to the phylum *Basidiomycota*, class *Malasseziomycetes*, order *Malasseziales* and family *Malasseziaceae*, and it currently comprises 18 recognized species [2]. These yeasts are part of the normal human skin microbiota, colonizing predominantly sebaceous areas due to their lipid dependence [2]. PV is mainly associated with species belonging to the *Malassezia furfur* species complex, particularly *M. furfur*, *M. globosa* and *M. sympodialis*. Under physiological conditions, *Malassezia* spp. persist on the skin as

commensal yeasts; however, under specific host and environmental conditions, they can undergo a morphological transition from a saprophytic yeast form to a pathogenic hyphal or pseudohyphal form, which is considered a hallmark of PV development [1,2]. Some predisposing factors for the conversion to the pathogenic form are a hot and humid environment, hyperhidrosis, the application of oily creams or lotions, the use of a mask, excessive sebum excretion, immunological factors and genetic predisposition [1,2]. Recent studies also point to an association with oxidative stress [3]. PV manifests itself through scaly, well-demarcated, scattered or confluent patches that are dark in color, depigmented or erythematous. The lesions can be distributed according to the lipophilic nature of the fungus and its preference for areas rich in sebum. They appear mainly on the upper trunk but can also extend to the neck and abdomen [1]. There are parts of the body where the presence of PV is atypical, for example, the armpits, the genital area, the thighs and the scalp [1,3,4]. Typically, the lesions are asymptomatic, but there may be a slight itching that worsens in conditions of greater humidity and heat. Another issue is that hyper- or hypopigmentation associated with the lesion may persist even after treatment [1,4].

Although PV is benign in origin, it can lead to social stigma, low self-esteem and fear of contagion. Due to its chronic and recurrent nature and the factors previously mentioned, people with PV have a low quality of life [3]. PV is difficult to treat and is recalcitrant, recurring often. The first line of treatment is a topical therapy. The most common topical treatments are lotions, creams or shampoos with antifungals, such as ketoconazole and terbinafine, or with other compounds such as selenium sulfide and zinc pyrithione [5]. Systemic therapy is recommended in cases of severe and recurrent PV. In these cases, oral antifungals such as itraconazole, fluconazole, and more recently, pramiconazole, are administered [5].

Natural products are increasingly sought by the population, and some of the most relevant of these products are essential oils. Essential oils are liquid, volatile, clear or colored mixtures composed of numerous aromatic compounds, mainly terpenes, terpenoids and phenylpropanoids. Their chemical composition varies not only among species but also within the same species depending on the geographical origin, the environmental conditions, the plant developmental stage and the extraction procedures [6–8]. Owing to their complex composition, essential oils exhibit multiple mechanisms of antifungal action [9–11]. Their activity is largely attributed to lipophilic terpenes and terpenoids, which interact with fungal cell membranes by disrupting membrane integrity and permeability, ultimately leading to cell death [12,13]. In addition, essential oils may interfere with ergosterol biosynthesis, mitochondrial function and efflux pump activity, thereby impairing fungal growth and survival [12,13]. Several studies report the effect of different essential oils on inhibiting the growth of the fungus *Malassezia furfur* [13]. Through those results, it is possible to conclude that essential oils have a promising future in the treatment of *M. furfur* infections and that combinations of different oils can lead to synergistic activities. Another class of natural products that are highly sought after for topical dermatological application are natural mineral waters. Several studies have shown that these waters have beneficial properties for the skin, such as repairing the skin barrier, antimicrobial, antioxidant, anti-inflammatory and immunomodulatory effects, moisturizing properties and regulating the aging process [14–16]. When used in cosmetics, natural mineral waters soothe the skin and exhibit moisturizing and anti-irritation properties [17,18].

The main objective of this research was to evaluate the anti-*Malassezia* activity of natural ingredients, namely essential oils obtained from aromatic plants belonging to the Lamiaceae family and São Pedro do Sul sulfurous natural mineral water (SPS NMW), to include these in the preparation of a body lotion based on natural products, and to assess its efficacy and safety for the prospective control of symptoms in pityriasis versicolor. This study is a pilot experiment that needs to be investigated in humans.

2. Materials and Methods

2.1. Natural Ingredients Used in Test and Phytochemical Profile of Selected Essential Oils

Natural mineral water was collected at São Pedro do Sul Thermal Centre in a sterile bottle (VWR, Radnor, PA, USA), following appropriate water collection procedures [19]. After collection, the bottle was sealed and transported to the lab, where it was maintained refrigerated until use (5 °C). According to the Institute of Hydrology of Lisbon, São Pedro do Sul natural mineral water (SPS NMW) is classified as a sulfurous/bicarbonate/fluoridated/sodic NMW (SBFS NMW) [19,20].

The following essential oils were purchased commercially from companies that produced the plants in biological productions: *Origanum vulgare* (F.J. Campos, Lisboa, Portugal), *Thymus mastichina* (Centro de Biotecnologia de Plantas da Beira Interior, Instituto Politécnico de Castelo Branco, Castelo Branco, Portugal), and *Thymus zygis* (Biover, Nazareth-De Pinte, Belgium). The phytochemical profile of each essential oil was determined by analysis with gas chromatography–mass spectrometry that was carried out commercially (Centro de Biotecnologia de Plantas da Beira Interior, Instituto Politécnico de Castelo Branco, Castelo Branco, Portugal) according to the protocol described previously [21]. Volatile constituents were characterized by gas chromatography–mass spectrometry (GC–MS) using an HP-5MS fused-silica capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness; Agilent J&W GC Columns, Santa Clara, CA, USA). Helium (X505_BIP) was employed as the carrier gas at a constant flow rate of 1.0 mL/min. Prior to analysis, *Origanum vulgare* and *Thymus zygis* essential oils were diluted in n-pentane (VWR, Radnor, PA, USA) and 1.0 µL of each sample was injected using a split ratio of 1:80. *Thymus mastichina* essential oil was diluted in pentane and analyzed under identical injection conditions, except that a split ratio of 1:50 was applied. The GC oven temperature program was initiated at 45 °C, followed by a linear increase of 3 °C/min to 175 °C. The temperature was subsequently raised at 15 °C/min to a final temperature of 300 °C and maintained for 10 min. The injector and transfer line temperatures were set at 220 °C and 250 °C, respectively. Compound identification was performed by comparing the acquired mass spectra with those available in the NIST 17 spectral library, together with the evaluation of calculated retention indices against published Kovats retention indices. Whenever possible, compound identities were further confirmed by comparison with experimentally determined retention indices obtained from a homologous series of n-alkanes analyzed under identical chromatographic conditions, as well as with authentic reference standards. The relative abundance of each identified constituent was calculated from the integrated chromatographic peak areas and expressed both as the percentage of the total chromatographic area corresponding to all identified compounds and as the percentage relative to the predominant constituent in each essential oil.

2.2. Antimicrobial Susceptibility Testing

2.2.1. Broth Microdilution Method

The antifungal activity of essential oils against *M. furfur* was determined using the broth macrodilution method, with slight modifications [22]. All experiments were performed with *M. furfur* DSM 6170 strain, which was preserved in Brain Heart Infusion (BHI, VWR, Radnor, PA, USA) broth supplemented with 20% glycerol (VWR, Radnor, PA, USA) at −80 °C. The strain was subcultured twice in Sabouroud Dextrose Agar (SDA, VWR, Radnor, PA, USA) supplemented with 0.5% olive oil (Azeite Gallo Clássico, Abrantes, Portugal) and 0.5% Tween 80 (VWR, Radnor, PA, USA). The culture media used was RPMI-1640 medium (Sigma-Aldrich, St. Louis, MO, USA), supplemented with 2.5 g/L of olive oil, 2.5 g/L of Tween 80 and 10 g/L of glucose (VWR, Radnor, PA, USA). The pH of the solution was adjusted to pH 7. A calibrated suspension of *M. furfur* cells of

approximately 5×10^5 CFU/mL was used. Essential oils were initially solubilized with DMSO (1:2); serial dilutions of DMSO starting at 10% (v/v) in culture media (final concentration in contact with *M. furfur* cells starting at 5% v/v) were tested in parallel to ensure that the growth of *M. furfur* would not be affected by the surfactant. The concentrations of essential oils in test (% v/v) were 0.078, 0.156, 0.3125, 0.625, 1.25, and 2.5. The tubes were incubated at 37 °C without agitation for 24 h. After this time, an aqueous solution of 0.015% (v/v) resazurin (ChemCruz, Dallas, TX, USA) was added to each mixture; the assay was re-incubated for another 24 h at 37 °C. The minimum inhibitory concentration (MIC) was defined as the lowest concentration when resazurin conversion from blue to pink was not observed, indicative of low cellular viability. The antimicrobial activity of SPS NMW was tested using the microdilution broth assay, as described above; the concentrations in test (% v/v) were 1.5, 3.125, 6.25, 12.5, 25, and 50.

2.2.2. Antifungal Activity of the Volatile Fraction

In parallel, the volatile fraction of essential oils was tested using the following procedure, adapted from previous studies [23]: a calibrated suspension of 1×10^6 CFU/mL *M. furfur* DSM 6170 strain was prepared in 0.85% NaCl (VWR, Radnor, PA, USA) solution, which was spread on the top of SDA supplemented with 0.5% olive oil and 0.5% Tween 80. The Petri dishes were carefully inverted. Immediately after this, a drop of 5 µL of each essential oil was spotted in the center of the lid of the Petri dish. The plates were incubated, inverted, at 37 °C for 24 h. The inhibition of the volatile fractions of the essential oils was assessed by the measurement of an halo in the center of the streaked culture (mm).

2.2.3. Assessment of Synergistic Effect of São Pedro do Sul Natural Mineral Water and Essential Oils

The possible synergistic effect of São Pedro do Sul natural mineral water (SPS NMW) with each essential oil (*Origanum vulgare* EO, *Thymus mastichina* EO and *Thymus zygis* EO) was assessed for *M. furfur* DSM 6170 strain using RPMI-1640 medium (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 2.5 g/L of olive oil, 2.5 g/L of Tween 80 and 10 g/L of glucose, as previously described [24]. The interaction between SPS NMW and each essential oil was assessed, based on the fractional inhibitory concentration index (FICI), which is calculated with the following formula:

$$FICI = \frac{\text{MIC SP NMW when in combination}}{\text{MIC SP NMW}} + \frac{\text{MIC EO when in combination}}{\text{MIC EO}}$$

Synergistic, indifferent, and antagonist interactions were defined by FICI values of <0.5, 0.5 to 4, and >4, respectively.

2.3. Assessment of Cytotoxicity of Selected Essential Oils

Cytotoxicity of the essential oils was assessed using human keratinocytes as previously described [21]. HaCaT cell line CVCL_0038 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% heat-inactivated FBS (Life Technologies, San Francisco, CA, USA), 35.9 mM sodium bicarbonate, 25 mM glucose, 100 U/mL penicillin, and 100 µg/mL streptomycin (Sigma-Aldrich, St. Louis, MO, USA). Cells were maintained at 37 °C in a humidified atmosphere with 5% CO₂, and sub-culturing was performed following the manufacturer's recommendations. The cytotoxicity activity of HaCaT cells was assessed using the 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay [25]. Keratinocytes (1×10^4 cells) were seeded in 96-well plates and, after 24 h, were exposed to essential oils for an additional 24 h period. Afterwards, the medium was removed and 100 µL of an MTT solution (at a final concentration of 1 mg/mL, Alfa Aesar, Ward Hill, MA, USA) prepared in incomplete culture medium was added to each well. The MTT-containing medium was discarded after a 4-hour incubation at 37 °C,

and propane-2-ol (VWR, Radnor, PA, USA) was added to dissolve the formazan crystals. Absorbance was measured at 570 nm and 630 nm using the xMark™ Microplate Absorbance Spectrophotometer (Bio-Rad, Hercules, CA, USA). The controls used in the experiment were exposure with culture medium only, exposure with the maximum amount of DMSO tested for the solubilization of the essential oils (0.25%, *v/v*) and exposure with a toxic concentration of SDS (Sodium Dodecyl Sulfate 2% *v/v*, VWR, Radnor, PA, USA). During the statistical analysis, we used one-way ANOVA followed by Dunnett's test for multiple comparisons. The results were considered statistically significant when the *p*-value was less than 0.05. All statistical analyses were carried out using GraphPad Prism 8.0.1.

2.4. Formulation with the Most Promising Ingredients

A body lotion was developed as an oil-in-water (O/W) emulsion using the ingredients listed in Table 1. The formulation was designed following a minimalist approach, selecting ingredients with specific technological functions while incorporating *Origanum vulgare* essential oil (F.J. Campos, Portugal) (0.63% *w/w*) as the core ingredient. In addition, the selection of raw materials considered naturality criteria, and the formulation naturality index was calculated according to ISO 16128 [26].

Table 1. Ingredients used in the development of the body lotion, organized according to the formulation phase, including their International Nomenclature of Cosmetic Ingredients (INCI) names, concentration (%) and technological functions.

Phase	Ingredient	INCI	%	Function	Supplier/Country
A	Natural mineral water	Water/Aqua	60.85	Solvent	Termas São Pedro do Sul, Portugal
	Glicerina vegetal EP E422	Glycerin	5.00	Humectant	Escuder, Barcelona, Spain
	Goma xantan	Xanthan gum	0.30	Thickening and stabilizing agent	Acofarma, Barcelona, Spain
	Microcare SBCT	Sodium benzoate, potassium sorbate, cetrimonium chloride and aqua	1.25	Preservative	Thor, Düsseldorf, Germany
B	Dermofeel GSC SG	Glyceryl stearate citrate	2.84	Co-emulsifier	Evonik, Essen, Germany
	Cera blanca	Cera alba	6.00	Consistency agent	Acofarma, Barcelona, Spain
	Tegin pellets	Glyceryl stearate SE	2.00	Emulsifier	Evonik, Essen, Germany
	Cetiol SB-45	<i>Butyrospermum parkii</i> (Shea) butter	8.00	Emollient	Escuder, Barcelona, Spain
	Myritol 318	Capric/Caprylic triglycerides	7.00	Emollient	Escuder, Barcelona, Spain
	Grape seed oil	Vinifera (Grape) seed oil	6.00	Emollient	EFP Biotek, Figueira da Foz, Portugal
	Advantiox 70 OC	Tocopherol and <i>Helianthus annuus</i> seed oil	0.05	Antioxidant	AOM, Valencia, Spain
	Óleo essencial de oregãos	<i>Origanum vulgare</i> essential oil	0.63	Core ingredient	F.J. Campos, Lisboa, Portugal

Acido citrico anhydro	Citric acid	0.08	pH adjuster	Acofarma, Barcelona, Spain
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The ingredients were separated into oily (Phase B) and aqueous (Phase A) phases. Both phases were heated to 80 °C in a thermostatic bath, and subsequently, the aqueous phase was mixed with a helical stirrer (Heidolph RZR 2041, Heidolph Instruments GmbH & Co., Schwabach, Germany). After homogenization, the oily phase was added under agitation, which allowed the formation of the emulsion. *Origanum vulgare* essential oil was added after the formation of the emulsion and homogenized under stirring. Finally, citric acid was used to adjust the pH of the emulsion to 4.5.

A placebo formulation was also prepared using the same composition and manufacturing procedure described above, except that the *Origanum vulgare* essential oil was omitted. To maintain the overall composition at 100% (w/w), the corresponding percentage of essential oil was replaced with an equivalent amount of grape seed oil. This placebo formulation was used for the preliminary efficacy assessment to determine whether the observed antimicrobial effects could be attributed to the core ingredient (*Origanum vulgare* essential oil) rather than to the formulation matrix itself.

2.5. Screening Stability Tests and Safety of the Formulation

For screening stability, the body lotion was submitted to centrifugation at 3000 rpm for 30 min (Micro Star 17/17R, VWR, Radnor, PA, USA) and to a temperature cycling test, alternating heating and refrigerating conditions, 4 ± 2 °C for 24 h and 40 ± 2 °C for 24 h, for a total of 4 weeks. The appearance of the emulsion (homogeneity, color, apparent viscosity) was evaluated before and after centrifugation. Organoleptic characteristics (color, odor, texture) and pH were evaluated before and after the cycling test.

The formulation was developed according to the safety requirements established by EC Regulation No. 1223/2009 [27] and SCCS Notes of Guidance (12th revision) [28]. The selection and concentration of each ingredient were defined considering the complete quantitative composition of the formulation, the toxicological profiles of the individual ingredients, the information provided in the corresponding Safety Data Sheets (SDSs), the available toxicological literature, the ingredient restrictions established by the European cosmetic regulatory framework, and the intended conditions of use of the final product. The product was intended for topical application to the body once daily by adults. *Origanum vulgare* essential oil was characterized by GC–MS and found to contain naturally occurring fragrance allergens, namely D-limonene and linalool (Supplementary Table S1), which were considered during formulation development to ensure compliance with labeling requirements established by Regulation (EC) No. 1223/2009.

2.6. Rheological Characterization

For prototype rheological characterization, a cone-plate rheometer (Brookfield DV3 T, Brookfield, New York, NY, USA) was used, and measurements were performed using 0.5 mL of the formulation [29] at room temperature (25 ± 2 °C) and for 1 min (>5 cone revolutions). The cone spindle used was the CPA-52Z (Brookfield, New York, NY, USA) with 3° cone angle and 1.2 cm radius. Measurements were performed using ascending and descending shear rate ramps ranging from 60 to 100 s^{−1} to evaluate the flow behavior and to investigate the potential thixotropic characteristics. Measurements were performed in duplicate.

2.7. Modified Challenge Test to Assess the Efficacy of the Formulation

The efficacy of the formulation against *M. furfur* DSM 6170 was assessed with a modified challenge test, as previously described [30]. Briefly, a 1 mL suspension of

approximately 1×10^5 CFU/mL of *M. furfur* DSM 6170 in PBS 1× (VWR, Radnor, PA, USA) was mixed with 10 g of the formulation. The mixture was incubated for a total of 28 days at 37 °C. At selected time-points, an aliquot was removed, serially diluted in PBS 1× and plated in Sab Dextrose Agar (SDA) supplemented with 0.5% olive oil and 0.5% Tween 80. After incubation at 37 °C, the colonies were counted to assess the number of CFU/mL in the formulation.

2.8. Challenge Test to Assess Formulation Preservatives' Efficacy and Stability

The cosmetic's preservation efficacy was performed through a challenge test (CT) according to Ph. Eur. 10.0, chapter 5.1.3 [31], to assess the ability of the cosmetic formulation to inhibit microbial growth over time. The following strains were tested: *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Candida albicans* ATCC 10231 and *Aspergillus brasiliensis* ATCC 16404. The cosmetics were inoculated with tested microorganisms for 7, 14, and 28 days. Before the start of the CT, the microbiological quality (total aerobes at 32.5 °C and fungi-yeasts at 22.5 °C) of the products under study was verified, following the standards ISO 21149:2017 [32] and ISO 16212:2017 [33], respectively, to ensure that it did not invalidate the test results.

2.9. In Vitro Tests to Assess Safety of the Formulation

The skin irritation test was performed in a 3D model of reconstructed human epidermis (RhE, Episkin, Lyon, France), as suggested by OECD [34,35]. Briefly, the test consists of exposing RhE to the formulation for 24 h. Cellular viability is then assayed by assessing the conversion of MTT, in comparison with a control (treatment with water). Irritant cosmetics are identified by their ability to decrease cell viability below-defined threshold levels (below or equal to 50%). If the viability exceeds 50%, the cosmetic is classified as a non-irritant. The test was performed in triplicate. The acceptance criteria are established for the observed standard deviation (SD) for the percentage of cell viability. The SD should be less or equal to 18%.

3. Results

In an initial approach, three essential oils obtained from aromatic plants belonging to the Lamiaceae family were selected based on the previous results from the research group and the available literature. In addition, a sulfuric natural mineral water (São Pedro do Sul natural mineral water, SPS NMW), which is widely used for the treatment of dermatologic afflictions, was also selected as a possible natural ingredient. The four ingredients were tested for their antimicrobial activity against *M. furfur* DSM 6170, following two procedures. The results are presented in Table 2. We found that SPS NMW had no inhibitory effect on the growth of *M. furfur* DSM 6170, either in the vapor phase (no inhibitory halo) or in broth (MIC > 50% v/v). The vapor phase of the essential oils was also found to have no inhibitory effect (Table 2). On the other hand, the essential oils in broth showed some inhibitory effect against *M. furfur*. Specifically, the *O. vulgare* essential oil was found to be the most effective, being able to inhibit *M. furfur* growth at 0.625% (v/v). The ingredient used in the placebo formulation as a substitute for *O. vulgare* essential oil was found to be also non-effective in the inhibition of growth of *M. furfur* (MIC > 2.5% v/v, Table 2). Likewise, since the SPS NMW was found to be non-effective in the inhibition of *M. furfur* growth, we proceeded with the characterization of bioactivity with the essential oils only. Therefore, the cytotoxicity was assessed in a keratinocyte cell line for all the essential oils (Figure 1). We found that all the essential oils were biocompatible with the keratinocytes at concentrations below 0.06% (v/v).

Table 2. Results regarding antimicrobial susceptibility testing. Minimum inhibitory concentration (% *v/v*) of each ingredient (OE: essential oil; NMW: natural mineral water) and size of the inhibition halo (mm) against *M. furfur* DSM 6170. The major component of each essential oil, as determined by GC–MS analysis, is also shown. In addition, *Origanum vulgare* essential oil naturally contains fragrance allergens (e.g., D-limonene and linalool, Supplementary Table S1).

Ingredient	Main Component (%) / Chemical Class	MIC (% <i>v/v</i>)	Inhibition Halo (mm)
<i>Origanum vulgare</i> OE	Carvacrol (51%)	0.625	0
<i>Thymus mastichina</i> OE	Eucalyptol (73%)	1.25	0
<i>Thymus zygis</i> OE	Linalool (54%)	>2.5	0
Grape seed oil	-	>2.5	0
São Pedro do Sul NMW	SBFS	>50	0

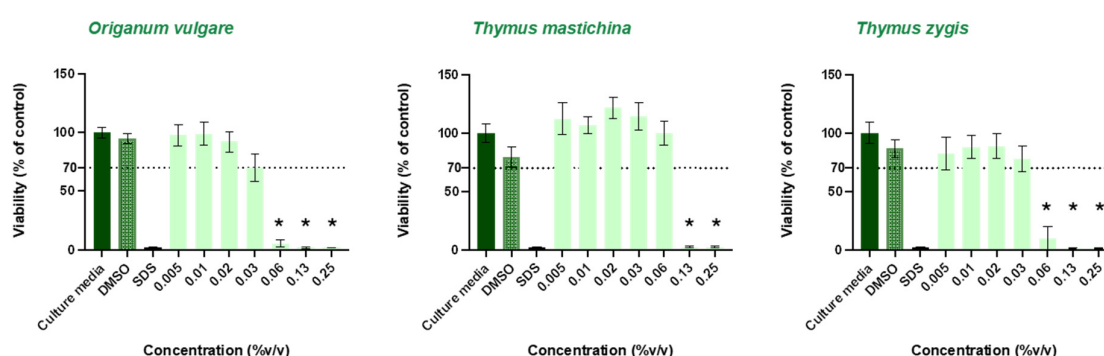


Figure 1. Keratinocytes' (HaCat cell line) cellular viability in the presence of essential oils (% *v/v*). Cell viability (% of control, with only culture media) was determined by MTT assay. If the effect on cellular viability was above 70% (represented as a dotted line), the corresponding concentration was considered noncytotoxic. The results regarding solvent control (DMSO) and positive control for cytotoxicity (SDS 2%) are also shown. Error bars represent the mean $\pm$ SD of two independent tests. * $p < 0.05$.

The interaction between the SPS NMW and each essential oil in the inhibition of *M. furfur* DSM 6170 growth was investigated with the checkerboard assay (Table 3). Interestingly, we found a synergistic effect against *M. furfur* in the combinations SPS NMW–OV EO and SPS NMW–TM EO. With the lower *FICI* observed (0.125), the combination between SPS NMW and OV EO was found to be the most relevant to proceed.

Table 3. Fractional inhibitory concentrations index (*FICI*) of São Pedro do Sul natural mineral water in combination with essential oils in the inhibition of growth of *M. furfur* DSM 6170.

Test	Individually	In combination	<i>FICI</i>	Result
MIC SPS NMW (% <i>v/v</i>)	>50	3.125	0.125	Synergy (≤ 0.5)
MIC OV EO (% <i>v/v</i>)	0.625	0.039		
MIC SPS NMW (% <i>v/v</i>)	>50	6.25	0.156	Synergy (≤ 0.5)
MIC TM EO (% <i>v/v</i>)	1.25	0.078		
MIC SPS NMW (% <i>v/v</i>)	>50	25	2.5	Indifferent (0.5–4)
MIC TZ EO (% <i>v/v</i>)	>2.5	2.5		

MIC: minimum inhibitory concentration; SPS NMW: São Pedro do Sul natural mineral water; OV EO: *Origanum vulgare* essential oil; TM EO: *Thymus mastichina* essential oil; TZ EO: *Thymus zygis* essential oil.

Due to its interesting inhibitory effect against *M. furfur*, *O. vulgare* essential oil was selected to be included in the formulation, as well as SPS NMW, among other ingredients. The body lotion was formulated respecting the criteria of minimalism and naturalness (>95% of natural ingredients, according with ISO 16128). It consisted of an easy-to-spread creamy emulsion with rapid absorption that was capable of providing a comforting effect, with a moisturizing and protective action provided by ingredients that mimic the skin's natural hydration factor and skin-replenishing lipids, thus restoring balance and improving the skin's barrier function, leaving the skin feeling hydrated and soft. The prototype was found to be stable (Table 4) and maintain its organoleptic characteristics (white color, characteristic odor from *Origanum vulgare* essential oil, creamy texture) and pH (4.5) over time.

Table 4. Body lotion preliminary stability tests. A positive result means the prototype is stable.

Test: Preliminary Stability	Result
Centrifugation 30 min at 3000 RPM	Positive. Homogeneous and without phase separation after centrifugation.
Cycle test 4 °C and 40 °C for 4 weeks	Positive. Maintains homogeneity and organoleptic characteristics at the end of the test.
pH 4.5 ± 0.01	Positive. Maintains pH over time.

The viscosity of semisolids is a key parameter for product performance. Based on the rheological characterization, the body lotion exhibited a non-Newtonian, shear-thinning (pseudoplastic) behavior, as evidenced by the decrease in viscosity with increasing shear rate (Figure 2). Furthermore, the slight differences observed between the ascending and descending shear rate ramps suggest the presence of thixotropic characteristics.

The prototype was further analyzed for its efficacy and safety. Regarding efficacy, the MIC of the formulation against *M. furfur* was determined for the formulation with essential oil and for the placebo, for comparison purposes. The results are displayed in Table 5. Additionally, the modified challenge test using a calibrated suspension of *M. furfur* revealed that the body lotion efficiently reduced the load of *M. furfur* over time (Figure 3).

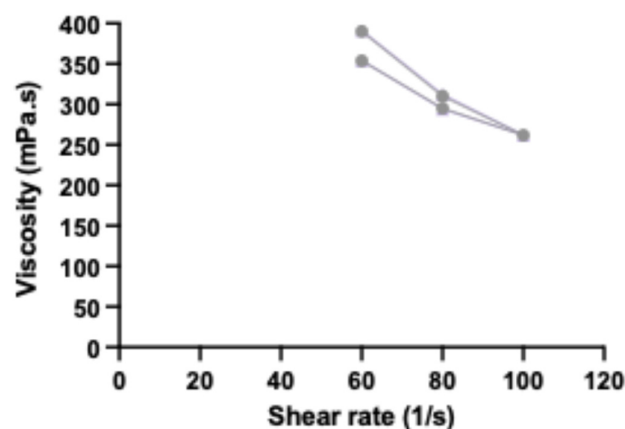
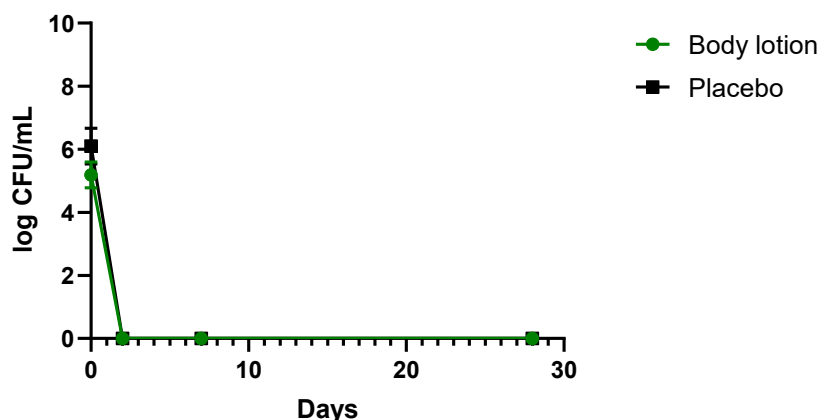


Figure 2. Viscosity of the *Origanum vulgare* body lotion under controlled temperature conditions at $T = 25 \pm 2$ °C. Measurements were performed in duplicate using ascending and descending shear rate ramps between 60 and 100 s^{-1} . Viscosity values are expressed in mPa.s.

Table 5. Results from preliminary test of antimicrobial activity of body lotion and the placebo.

Formulation	MIC (% <i>v/v</i>)
Placebo body lotion	50
Body lotion	25

**Figure 3.** Modified challenge test results. Efficacy of the *Origanum vulgare* body lotion (and the placebo formulation) on the reduction of *M. furfur* DSM 6170 growth depicted in log CFU/mL, over an incubation period of 28 days. Log CFU/mL were estimated at 2, 7 and 28 days after inoculation.

The safety of the formulation for use in humans was assessed by testing the preservative ability of the prototype with the challenge test (Figure 4) and the in vitro skin irritation potential (Table 6). Similar to the results obtained in the efficacy test, the challenge test revealed that the formulation had a relevant preservative ability, since it was able to efficiently reduce the growth of the bacteria *S. aureus* and *P. aeruginosa* as well as the fungi *C. albicans* and *A. brasiliensis*. Regarding the in vitro skin irritation test, it was found that the 3D skin sample maintained cellular viability after contact with the prototype. These two results taken together provide an indication that the prototype is safe for human use, although further testing is still needed.

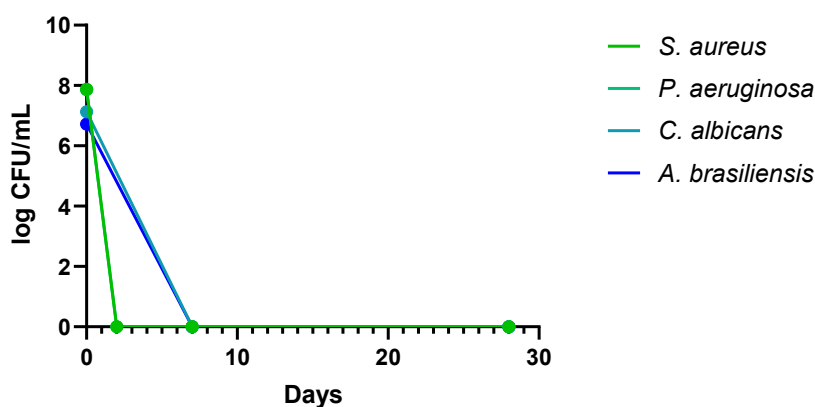
**Figure 4.** Challenge test results. Assessment of the preservative ability of *Origanum vulgare* body lotion by estimating the reduction of *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Candida albicans* ATCC 10231, and *Aspergillus brasiliensis* ATCC 16404 growth depicted in log CFU/mL, over an incubation period of 28 days. Log CFU/mL were estimated at 2, 7 and 28 days after inoculation.

Table 6. Results of the 3D reconstructed skin irritation test. Cellular viability of the tissue after contact with the formulation is shown, with the respective standard deviation (SD) value.

In Vitro Tissue Viability (SD)	Classification
102.1% (3.55%)	Non-irritant

4. Discussion

In this work, the general aim was to develop a prototype to alleviate the symptoms associated with pityriasis versicolor (PV). PV is characterized by the presence of depigmentation, and it is associated with a low self-esteem and a low quality of life. Although generally asymptomatic, PV is difficult to treat. Thus, the objective of PV treatment is “to eradicate the hyphal-shaped fungus and restore the commensal flora of the skin; eliminate clinical signs, such as scaly appearance and erythema, and symptoms, such as itching; reduce recurrences and increase the time between them; increase the quality of life and user satisfaction” [36].

With this aim in mind, and taking into account the growing interest of the general public in natural products, we screened essential oils for their activity against *Malassezia furfur*, representative of the *M. furfur* species complex and one of the species frequently associated with the onset of PV [1,2]. The essential oils tested in this work were the essential oils of *Origanum vulgare*, *Thymus mastichina* and *Thymus zygis*. We determined that the essential oil of *O. vulgare* has carvacrol as its main constituent. This essential oil has many valuable effects, such as anti-inflammatory, antifungal, antiparasitic, antimicrobial, anticancer, antioxidant, and other beneficial effects on the skin [37]. The main constituent of the *T. mastichina* essential oil is 1,8-cineole, better known as eucalyptol. This essential oil has been described to have antibacterial, antifungal, antioxidant, anticancer, antiviral, insecticidal, insect repellent, and anti-enzymatic activity [38]. Previous research has also revealed relevant anti-inflammatory activity [39]. The main active constituent of the *T. zygis* essential oil is linalool, an oxygenated monoterpene. This compound has antimicrobial, antifungal, antiparasitic, insecticidal and larvicidal activity as well as cytotoxic activity [40]. We found that the vapor phase of these essential oils had no inhibitory effect against *M. furfur*, but that when in contact with the fungal cells, the *Origanum vulgare* and *Thymus mastichina* essential oils were effective. These results support the choice of a topical formulation to keep the antifungal effect that was demonstrated in vitro. The superior ability of the *O. vulgare* essential oil to inhibit *M. furfur*'s growth is possibly due to the presence of a high amount (>50%) of carvacrol in its composition. Its antifungal action against *M. furfur* has already been described, and it probably occurs due to the interference of carvacrol in the synthesis of ergosterol, which is essential for the structure of the cell membrane [41].

In our study, we also included a natural mineral water that is widely used in thermal health treatments. A natural mineral water can be described as a water considered bacteriologically safe, with stable physicochemical particularities at the origin within the range of natural fluctuations, resulting in therapeutic properties or simply favorable health effects [21]. The natural mineral water of São Pedro do Sul (SPS NMW) emerges from the interior of the earth at a temperature of 68.7 °C, it has a pH of 8.82, making it an alkaline water. This water belongs to the group of sulfuric waters, with sulfur being the component that gives it its characteristic smell [18,19]. It is considered a weakly mineralized water, since it has a total mineralization between 200 and 1000 mg/L; its dominant ions are bicarbonate (HCO_3^-) and sodium (Na^+), and it has high amounts of silica and fluorine [19]. Sulfurous waters have several therapeutic indications, including in the respiratory system (rhinitis, pharyngitis), dermatology (seborrhea and acne, chronic eczema, psoriasis), rheumatic diseases and skeletal muscle (joint, tendon component,

neurological) and gynecological afflictions (postmenopausal trophy) [18]. In our study, we found that this ingredient did not have an inhibitory effect on the growth of *M. furfur*; however, when in combination with the *Origanum vulgare* EO or the *Thymus mastichina* EO, it was found to have a synergistic effect in the inhibition of *M. furfur* growth ($FICI < 0.5$). Therefore, it was added to the formulation due this particular ability and also due to its mineral and anti-irritating properties of the skin barrier [19], which may help with the tolerability of the formulation.

The body lotion developed contains a natural origin content exceeding 95%, according to ISO16128, and its minimalist formula still contains emollient and humectant ingredients that increase hydration and promote the replacement of lipids in the skin. In addition to the proven action of the *O. vulgare* essential oil, the formulation must contain ingredients that make it appealing to the consumer to apply [42,43]. Hence, developing a low-viscosity O/W emulsion was crucial to facilitate application while maintaining the creamy texture. The rheological characterization of the body lotion revealed it to be a non-Newtonian, shear-thinning (pseudoplastic) fluid, exhibiting characteristics suggestive of thixotropy. This means its viscosity decreases when a shear force is applied, making it easier to spread during the application, and gradually increases again afterwards, helping it to stay on the skin. The observed rheological behavior is mainly attributed to xanthan gum, which forms a structured polymeric network in the aqueous phase, together with the structural contribution of beeswax and shea butter in the oily phase. These characteristics provide a smooth application, stability, and a non-sticky finish, which are crucial for effective skincare performance [44,45]. The preliminary stability testing confirmed that the formulation maintained its organoleptic properties, such as its white color, its characteristic odor derived from the *Origanum vulgare* essential oil, its creamy texture, and its pH of 4.5 over time. These characteristics are crucial for ensuring the product's appeal, stability, and functionality, indicating that the prototype can effectively moisturize and protect the skin while being pleasant to use and remaining stable during storage [46]. A limitation on the formulation development is that the viscosity was not reassessed following the preliminary stability testing. Although no visible changes in the appearance or the texture were observed, a rheological evaluation after accelerated stability testing would provide a more comprehensive assessment of the formulation's physical stability. Nonetheless, confirming its preliminary stability and evaluating the capacity of the preservative system through the challenge test allows the stability of the product to be assessed. In addition, the prototype was developed taking into account the safety requirements established by EC Regulation No. 1223/2009 [47,48], and the skin irritation test supported the safety of the prototype for topical application [34]. The naturally occurring fragrance allergens identified in the *Origanum vulgare* essential oil, namely D-limonene and linalool, were considered during the formulation development and will be declared on the final product label whenever required by current cosmetic legislation. Overall, the developed body lotion may represent a promising supportive cosmetic approach for individuals prone to pityriasis versicolor, potentially contributing to skin comfort and the maintenance of skin homeostasis while complementing conventional therapeutic strategies.

Although still scarce, recent studies have emerged in the literature that emphasize the need for new topical products to alleviate the symptoms of this condition. Specifically and most interestingly, these studies also focused on the search for new active natural ingredients that could be introduced in greener formulations, and they were also found to be active against *M. furfur* and expected to be safe [49,50]. Regarding our prototype, it would be interesting to test its acceptability in a focus group or a pilot study involving humans, as previously carried out by others [51], or even to proceed with a clinical study.

5. Conclusions

In this study, we characterized natural ingredients, namely Lamiaceae essential oils and sulfurous natural mineral waters, in an effort to develop a body lotion for daily use to help control the recurrence of PV. Our prototype proved to be both effective in the inhibition of *M. furfur* growth and safe for use in 3D in vitro skin models while retaining the naturalness claim that is widely appreciated among cosmetic users. The interdisciplinary nature of this pharmaceutical and cosmeceutical research emphasizes the potential of combining natural ingredients in the development of supportive topical approaches for pityriasis versicolor. This study represents an initial proof-of-concept and further investigations are required, including long-term stability studies, formulation optimization, and clinical evaluation in humans to confirm its efficacy and its dermatological benefits.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/app16157724/s1>, Table S1: Composition of essential oils as determined by GC–MS analysis. R.T.: retention time.

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References

1. Theelen, B.; Cafarchia, C.; Gaitanis, G.; Bassukas, I.D.; Boekhout, T.; Dawson, T.L., Jr. *Malassezia* ecology, pathophysiology, and treatment. *Med. Mycol.* **2018**, *56*, S10–S25. <https://doi.org/10.1093/mmy/myx134>.
2. Leung, A.K.; Barankin, B.; Lam, J.M.; Leong, K.F.; Hon, K.L. Tinea versicolor: An updated review. *Drugs Context* **2022**, *11*, 2022-9-2.
3. Kurutas, E.B.; Ozturk, P. The evaluation of local oxidative/nitrosative stress in patients with pityriasis versicolor: A preliminary study. *Mycoses* **2016**, *59*, 720–725.
4. Saunte, D.M.L.; Gaitanis, G.; Hay, R.J. *Malassezia*-Associated Skin Diseases, the Use of Diagnostics and Treatment. *Front. Cell. Infect. Microbiol.* **2020**, *10*, 112.
5. Gupta, A.K.; Foley, K.A. Antifungal Treatment for Pityriasis Versicolor. *J. Fungi* **2015**, *1*, 13–296.
6. Nazzaro, F.; Fratianni, F.; Coppola, R.; De Feo, V. Essential Oils and Antifungal Activity. *Pharmaceuticals* **2017**, *10*, 86.
7. Burt, S. Essential oils: Their antibacterial properties and potential applications in foods—A review. *Int. J. Food Microbiol.* **2004**, *94*, 223–253.
8. Bakkali, F.; Averbeck, S.; Averbeck, D.; Idaomar, M. Biological effects of essential oils—A review. *Food Chem. Toxicol.* **2008**, *46*, 446–475.
9. Nazzaro, F.; Fratianni, F.; De Martino, L.; Coppola, R.; De Feo, V. Effect of essential oils on pathogenic bacteria. *Pharmaceuticals* **2013**, *6*, 1451–1474.

10. Hyldgaard, M.; Mygind, T.; Meyer, R.L. Essential oils in food preservation: Mode of action, synergies, and interactions with food matrix components. *Front. Microbiol.* **2012**, *3*, 12.
11. Pereira, A.G.; Perez-Vazquez, A.; Barciela, P.; Jorge, A.O.S.; Yuksek, E.N.; Prieto, M.A. Natural Antimicrobials from Plants Used as Food Preservatives. *Foods* **2026**, *15*, 1309. <https://doi.org/10.3390/foods15081309>.
12. Ahmad, A.; Khan, A.; Akhtar, F.; Yousuf, S.; Xess, I.; Khan, L.A.; Manzoor, N. Fungicidal activity of thymol and carvacrol by disrupting ergosterol biosynthesis and membrane integrity against *Candida*. *Microb. Pathog.* **2011**, *50*, 181–186.
13. D'Auria, F.; Tecca, M.; Strippoli, V.; Salvatore, G.; Battinelli, L.; Mazzanti, G. Antifungal Activity of Different Essential Oils against *Malassezia* Pathogenic Species. *J. Ethnopharmacol.* **2020**, *249*, 112376.
14. Oliveira, A.S.; Vaz, C.V.; Silva, A.; Ferreira, S.S.; Correia, S.; Ferreira, R.; Breitenfeld, L.; Martinez-de-Oliveira, J.; Palmeira-de-Oliveira, R.; Pereira, C.; et al. Chemical signature and antimicrobial activity of Central Portuguese Natural Mineral Waters against selected skin pathogens. *Environ. Geochem. Health* **2020**, *42*, 2039–2057.
15. Vaz, C.V.; Figueiredo, A.C.; Oliveira, A.S.; Palmeira-de-Oliveira, A.; Palmeira-de-Oliveira, R. Protective Role of Portuguese Natural Mineral Waters on Skin Aging: In Vitro Evaluation of Anti-Senescence and Anti-Oxidant Properties. *Int. J. Biometeorol.* **2022**, *66*, 2027–2038.
16. Silva, A.; Viegas, C.; Figueiredo, A.C.; Palmeira-de-Oliveira, A.; Martinez-de-Oliveira, J.; Palmeira-de-Oliveira, R. Anti-Inflammatory Potential of Portuguese Thermal Waters. *Sci. Rep.* **2020**, *10*, 22313. <https://doi.org/10.1038/s41598-020-79394-9>.
17. Figueiredo, A.C.; Rodrigues, M.; Mourelle, M.L.; Araujo, A.R.T.S. Thermal Spring Waters as an Active Ingredient in Cosmetic Formulations. *Cosmetics* **2023**, *10*, 27.
18. Ferreira, M.O.; Costa, P.C.; Bahia, M.F. Effect of São Pedro do Sul Thermal Water on Skin Irritation. *Int. J. Cosmet. Sci.* **2010**, *32*, 205–210.
19. Rice, E.W.; Baird, R.B.; Eaton, A.D.; Clesceri, L.S. (Eds.) *Standard Methods for the Examination of Water and Wastewater*, 22nd ed.; American Public Health Association: Washington, DC, USA; American Water Works Association: Denver, CO, USA; Water Environment Federation: Alexandria, VA, USA, 2012.
20. Cantista, P. O termalismo em Portugal. *An. Hidrol. Medica.* **2008**, *3*, 79–107.
21. Valente, J.V.; Gomes, C.P.; Oliveira, A.S.; Rolo, J.; Gaspar, C.; Caramelo, D.; Gonçalves, J.C.; Delgado, F.; Breitenfeld, L.; Duarte, A.P.; et al. Bioactivity of *Matricaria chamomilla*, *Echinacea purpurea*, *Thymbra capitata* and *Ocimum basilicum* Hydrolates and Essential Oils in View of Their Application in the Skin. *Molecules* **2026**, *31*, 1156.
22. Leong, C.; Buttafuoco, A.; Glatz, M.; Bosshard, P.P. Antifungal Susceptibility Testing of *Malassezia* spp. with an Optimized Colorimetric Broth Microdilution Method. *J. Clin. Microbiol.* **2017**, *55*, 1883–1893.
23. Fernandes, L.; Costa, R.; Silva, S.; Henriques, M.; Costa-de-Oliveira, S.; Rodrigues, M.E. Effect of Vapor-Phase Oregano Essential Oil on Resistant *Candida* Species Biofilms: Mechanisms of Action. *Microbiol Spectr.* **2023**, *11*, e0512422.
24. Oliveira, A.S.; Gaspar, C.A.; Palmeira-de-Oliveira, R.; Martinez-de-Oliveira, J.; Palmeira-de-Oliveira, A. Anti-*Candida* activity of fluoxetine alone and combined with fluconazole: A synergistic action against fluconazole-resistant strains. *Antimicrob Agents Chemother* **2014**, *58*, 4224–4426.
25. ISO 10993-5:2009; Biological Evaluation of Medical Devices—Part 5: Tests for In Vitro Cytotoxicity. International Organization for Standardization: Geneva, Switzerland, 2009. Available online: <https://www.iso.org/standard/36406.html> (accessed on 5 May 2025).
26. ISO 16128-2:2017; Cosmetics—Guidelines on Technical Definitions and Criteria for Natural and Organic Cosmetic Ingredientspart 2: Criteria for Ingredients and Products. International Organization for Standardization: Geneva, Switzerland, 2017. Available online: <https://www.iso.org/standard/87527.html> (accessed on 5 May 2025).
27. Regulation-1223/2009-EN-Cosmetic Products Regulation-EUR-Lex. of Document: 30/11/2009Date of Effect: 11/01/2010; Entry into Force Date Pub. + 20 See Art 40.1 Date of Effect: 01/12/2010; Partial Application See Art 40.2 Date of Effect: 11/01/2013; Partial application See Art 40.2 Date of Effect: 11/07/2013; Application See Art 40.2 Date of End of Validity: No end date. Available online: <https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32009R1223> (accessed on 5 May 2025).
28. Scientific Committee on Consumer Safety. *The SCCs Notes of Guidance for the Testing of Cosmetic Ingredients and Their Safety Evaluation, 12th Revision*; European Commission: Brussels, Belgium, 2023.
29. Machado, R.M.; Palmeira-de-Oliveira, A.; Breitenfeld, L.; Martinez-de-Oliveira, J.; Palmeira-de-Oliveira, R. Optimization and Application of In Vitro and Ex Vivo Models for Vaginal Semisolid Safety Evaluation. *J. Pharm. Sci.* **2019**, *108*, 3289–3301.
30. Tomás, M.; Rolo, J.; Gaspar, C.; Palmeira-de-Oliveira, A.; Simões, S.; Katz, D.F.; Martinez-de-Oliveira, J.; Palmeira-de-Oliveira, R. Sodium bicarbonate gels: A new promising strategy for the treatment of vulvovaginal candidosis. *Eur. J. Pharm. Sci.* **2021**, *157*, 105621.

31. European Directorate for the Quality of Medicines & HealthCare (EDQM). General Chapter 5.1.3: Efficacy of Antimicrobial Preservation. In *European Pharmacopoeia 10.0*; Council of Europe: Strasbourg, France, 2020.
32. *ISO 21149:2017*; Cosmetics—Microbiology—Enumeration and Detection of Aerobic Mesophilic Bacteria. International Organization for Standardization: Geneva, Switzerland, 2017. Available online: <https://www.iso.org/standard/72240.html> (accessed on 5 May 2025).
33. *ISO 16212:2017*; Cosmetics—Microbiology—Enumeration of Yeast and Mould. International Organization for Standardization: Geneva, Switzerland, 2017. Available online: <https://www.iso.org/standard/72241.html> (accessed on 5 May 2025).
34. Skin Irritation: SkinEthic Skin Irritation Test (SIT). 2024. Available online: https://joint-research-centre.ec.europa.eu/reference-measurement/european-union-reference-laboratories/eu-reference-laboratory-alternatives-animal-testing-eurl-ecvam/alternative-methods-toxicity-testing/validated-test-methods-health-effects/skin-irritation/skin-irritation-skinethic-skin_en (accessed on 5 May 2025).
35. Mini, C.A.; Ballester, G.; Munari, C.C.; Carrão, D.B.; Fonseca, B.C.; Albuquerque, N.C.; Marquete-Oliveira, F. Reliability and relevance of the ES®-RHE model for in vitro skin irritation test application. *Toxicol. Vitro* **2024**, *101*, 105932.
36. Bamford, J.T.; Gessert, C.E.; Renier, C.M.; Jackson, M.M.; Laabs, S.B.; Dahl, M.V. Interventions for the Treatment of Pityriasis Versicolor. *Cochrane Database Syst. Rev.* **2018**, *11*, CD010209.
37. Ortiz-Mendonza, N.; Martínez-Gordillo, M.J.; Martínez-Ambriz, E.; Basurto-Peña, F.A.; González-Trujano, M.E.; Aguirre-Hernández, E. Ethnobotanical, Phytochemical, and Pharmacological Properties of the Subfamily Nepetoideae (Lamiaceae) in Inflammatory Diseases. *Plants* **2023**, *12*, 3950.
38. Rodrigues, M.; Lopes, A.C.; Vaz, F.; Filipe, M.; Alves, G.; Ribeiro, M.P.; Coutinho, P.; Araujo, A.R.T.S. *Thymus mastichina*: Composition and Biological Properties with a Focus on Antimicrobial Activity. *Pharmaceuticals* **2020**, *13*, 479.
39. Oliveira, A.S.; Silva, C.; Viegas, C.; Figueiredo, A.C.; Palmeira-de-Oliveira, A.; Palmeira-de-Oliveira, R. *Thymus mastichina* (L.) L. and *Cistus ladanifer* L. for Skin Application: Chemical Characterization and In Vitro Bioactivity Assessment. *J. Ethnopharmacol.* **2023**, *302*, 115890.
40. Costa, A.; Silva, F.; Duarte, A.P. Biological Properties of *Thymus zygis* Essential Oil with Emphasis on Antimicrobial Activity and Food Application. *Food Chem.* **2022**, *393*, 133393.
41. Vinciguerra, V.; Rojas, F.; Tedesco, V.; Giusiano, G.; Angiolella, L. Chemical Characterization and Antifungal Activity of *Origanum vulgare*, *Thymus vulgaris* Essential Oils and Carvacrol against *Malassezia furfur*. *Nat. Prod. Res.* **2019**, *33*, 3672–3676.
42. Lapenna, S.; Gemen, R.; Wollgast, J.; Worth, A.; Maragkoudakis, P.; Caldeira, S. Assessing Herbal Products with Health Claims. *Crit. Rev. Food Sci. Nutr.* **2015**, *55*, 201–213.
43. Kumar, P.; Verma, A.; Ashique, S.; Bhowmick, M.; Mohanto, S.; Singh, A.; Gupta, M.; Gupta, A.; Haider, T. Unlocking the Role of Herbal Cosmeceutical in Anti-Ageing and Skin Ageing Associated Diseases. *Cutan. Ocul. Toxicol.* **2024**, *43*, 333–349.
44. Gore, E.; Picard, C.; Savary, G. Spreading behavior of cosmetic emulsions: Impact of the oil phase. *Biotribology* **2018**, *16*, 17–24.
45. Gallegos, C.; Franco, J.M. Rheology of Food, Cosmetics and Pharmaceuticals. *Curr. Opin. Colloid Interface Sci.* **1999**, *4*, 288–293.
46. *ISO/TR 18811:2018(en)*; Cosmetics Guidelines on the Stability Testing of Cosmetic Products. International Organization for Standardization: Geneva, Switzerland, 2018. Available online: <https://www.iso.org/obp/ui/en/#iso:std:iso:tr:18811:ed-1:v1:en> (accessed on 5 May 2025).
47. Halla, N.; Fernandes, I.P.; Heleno, S.A.; Costa, P.; Boucherit-Otmani, Z.; Boucherit, K.; Rodrigues, A.E.; Ferreira, I.C.F.R.; Barreiro, M.F. Cosmetics Preservation: A Review on Present Strategies. *Molecules* **2018**, *23*, 1571.
48. European Commission. Regulation (eu) 2017/745 of the European Parliament and of the Council of 5 April 2017 on Medical Devices, Amending Directive 2001/83/EC, Regulation (EC) No 178/2002 and Regulation (EC) No 1223/2009 and Repealing Council Directives 90/385/EEC and 93/42/EEC. 2017. Available online: <https://eur-lex.europa.eu/eli/reg/2017/745/oj/eng> (accessed on 5 May 2025).
49. Mirzaii, M.; Yaeghoobi, M.; Afzali, M.; Amirkhalili, N.; Mahmoodi, M.; Sajirani, E.B. Antifungal Activities of Quince Seed Mucilage Hydrogel Decorated with Essential Oils of *Nigella sativa*, *Citrus sinensis* and *Cinnamomum verum*. *Iran. J. Microbiol.* **2021**, *13*, 278–287.

50. Susano, P.; Silva, J.; Alves, C.; Martins, A.; Pinteus, S.; Gaspar, H.; Goetttert, M.I.; Pedrosa, R. *Saccorhiza polyschides*—A Source of Natural Active Ingredients for Greener Skincare Formulations. *Molecules* **2022**, *27*, 6678.
51. Campione, E.; Mazzilli, S.; Lanna, C.; Cosio, T.; Palumbo, V.; Cesaroni, G.; Lozzi, F.; Diluvio, L.; Bianchi, L. The Effectiveness of a New Topical Formulation Containing GSH-C4 and Hyaluronic Acid in Seborrheic Dermatitis: Preliminary Results of an Exploratory Pilot Study. *Clin. Cosmet. Investig. Dermatol.* **2019**, *12*, 13–19.

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