**Fronteiras: Journal of Social, Technological and Environmental Science**
http://periodicos.unievangelica.edu.br/fronteiras/
ISSN 2238-8869

*Article*

# Standardized *Rosmarinus officinalis* L. extract as an Antimicrobial Preservative of Cream Gel Formulations

**Gilberto Pereira Degani Filho[1], Mayk Teles de Oliveira[2], Virgínia Farias Alves[3] Maria Teresa Freitas Bara[4], Ieda Maria Sapateiro Torres[5], Danielle Guimarães Almeida Diniz[6]**

[1] Mestre em Ciências Farmacêuticas. Universidade Federal de Goiás (UFG), Brasil. ORCID: 0000-0003-4113-2993. E-mail: gilbertodegani@live.com
[2] Doutor em Ciências Farmacêuticas. Universidade Federal de Goiás (UFG), Brasil. ORCID:0000-0002-8094-3804. E-mail: mayk.teles@hotmail.com
[3] Doutora em Biociências Aplicadas à Farmácia. Universidade de São Paulo (USP), Brasil. Docente Universidade Federal de Goiás (UFG), ORCID: 0000-0002-5556-3711. E-mail: valves.ufg@gmail.com
[4] Doutora em Biologia Molecular. Universidade de Brasília (UnB), Brasil, Docente Universidade Federal de Goiás (UFG), ORCID: 0000-0003-4942-8721. E-mail: mtbara@gmail.com
[5] Doutora em Ciências da Saúde. Universidade de Brasília (UnB), Brasil, Docente Universidade Federal de Goiás (UFG), ORCID: 0000-0001-8407-627X. E-mail: ieda_sapateiro@ufg.br
[6] Doutora em Ciências da Saúde. Universidade de Brasília (UnB), Brasil, Docente Universidade Federal de Goiás (UFG), ORCID: 0000-0003-1867-3521. E-mail: danielle_diniz@ufg.br

**ABSTRACT**
The demand for cosmetic products free of synthetic preservatives is relevant due to the adverse reactions caused by these compounds. For this reason, there is considerable interest from industries in the development of phytocosmetics, and the use of plant extracts is a promising alternative to assist in the microbiological quality of these products. We hypothesized that *Rosmarinus officinalis* L. could inhibit the microorganisms growth in cosmetic formulations and provide chemical stability to it. Extract from leaves of *R. officinalis* was obtained and characterized by physicochemical parameters. The antimicrobial activity of the extract was analyzed by broth microdilution test for *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus brasiliensis*. The stability and preservative effectiveness test (challenge test) of two cream gel formulations containing the extract of *R. officinalis* at 0.2 and 0.4% and of a control formulation containing 0.2% methylparaben were evaluated. They were characterized by the organoleptic and physicochemical parameters. The extract was standardized and showed 56.96% solids, 6.12% polyphenols, 2.49% flavonoids, 2.91% tannins and 1.81% rosmarinic acid. The minimum inhibitory concentration was 71.2 μg/mL for bacteria, 35.6 μg/mL for yeast and more than 1130 μg/mL for mold. The formulations containing the extract remained physicochemical stable at room temperature, although it exhibited a slight modification of the organoleptic parameters at 90 days when stored at 40ºC. In addition, the formulations containing the extract showed antimicrobial efficacy, being able to inhibit the growth of tested bacteria and yeast and reducing the fungus growth, at both analyzed concentrations. Therefore, it presented results in accordance with established criteria. The results allows us to suggest the protection against microbial contamination and the maintenance of physicochemical stability when stored at room temperature. These data indicate the potential of *R. officinalis* extract as an antimicrobial preservative for the cream gel, given the conditions studied.
**Keywords:** herbal medicine; emulsions; antimicrobial activitity; plant-derived preservative.

**RESUMO**
A demanda por produtos cosméticos livres de conservantes sintéticos é relevante devido às reações adversas causadas por esses compostos. Por esse motivo, há considerável interesse das indústrias no desenvolvimento de fitocosméticos e o uso de extratos vegetais é uma alternativa promissora para auxiliar na qualidade microbiológica desses produtos. Nossa hipótese é que *Rosmarinus officinalis* poderia inibir o crescimento de microrganismos em formulações cosméticas e fornecer estabilidade química a elas. Extrato das folhas de *R. officinalis* foi obtido e caracterizado por parâmetros físico-químicos. A atividade antimicrobiana do extrato foi analisada

Submissão: 23/05/2025 Aceite: 01/07/2026 Publicação: 14/11/2026

v.15, n.3, 2026 • p. 42-52. • DOI http://dx.doi.org/10.21664/2238-8869.2026v15i5.8010

© 2021 by the authors. Esta revista oferece acesso livre imediato ao seu conteúdo, seguindo o princípio de que disponibilizar gratuitamente o conhecimento científico ao público proporciona maior democratização mundial do conhecimento. Este manuscrito é distribuído nos termos da licença Creative Commons – Atribuição - NãoComercial 4.0 Internacional (https://creativecommons.org/licenses/by-nc/4.0/legalcode), que permite reproduzir e compartilhar o material licenciado, no todo ou em parte, somente para fim não comercial; e produzir, reproduzir, e compartilhar material adaptado somente para fim não comercial.

pelo teste de microdiluição em caldo para *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* e *Aspergillus brasiliensis*. Foram avaliados o teste de estabilidade e eficácia conservante (teste de desafio) de duas formulações de gel creme contendo o extrato de *R. officinalis* a 0,2 e 0,4% e, de uma formulação controle contendo 0,2% de metilparabeno. Estas foram caracterizados quanto a parâmetros organolépticos e físico-químicos. O extrato foi padronizado e apresentou 56,96% de sólidos, 6,12% de polifenóis, 2,49% de flavonoides, 2,91% de taninos e 1,81% de ácido rosmarínico. A concentração inibitória mínima foi de 71,2 μg/mL para bactérias, 35,6 μg/mL para leveduras e mais de 1130 μg/mL para fungos. As formulações contendo o extrato permaneceram físico-quimicamente estáveis, embora exibiram uma ligeira modificação dos parâmetros organolépticos aos 90 dias quando armazenadas à 40ºC. Além disso, as formulações contendo o extrato mostraram eficácia antimicrobiana, sendo capazes de inibir o crescimento das bactérias e leveduras testadas e reduzir o crescimento do fungo, em ambas as concentrações analisadas. Portanto, apresentaram resultados de acordo com critérios estabelecidos. Os dados obtidos permitem sugerir a proteção contra contaminação microbiana e a manutenção da estabilidade físico-química quando armazenadas em temperatura ambiente e o potencial do extrato de *R. officinalis* como conservante antimicrobiano para o gel creme, nas condições experimentais estudadas.
Palavras-chave: Planta medicinal; emulsões; atividade antimicrobiana; conservante vegetal.

## Introduction

Cosmetic products do not require sterility, which allows for a limited presence of microbial load, but which does not compromise their final quality, ensuring consumer safety (Brazil 2024).

To provide the microbiological stability of cosmetic products, the incorporation of a preservative in the formulation is essential, since the presence of water allows the development of a variety of microorganisms (Fang et al. 2016). The ideal preservative should have a good antimicrobial efficacy (Yazar et al. 2011), be compatible with the other components of the formulation (Svedman et al. 2012), present physicochemical stability over a wide range of pH and temperature (Halla et al. 2018) and can not cause hypersensitivity reactions (Kerdudo et al., 2016).

Scientific researches involving plant-derived products, such as extracts and essential oils that have antimicrobial activity has been investigated to be applied in cosmetic products (Ostrosky et al. 2011; Cho et al. 2020; Martelli et al. 2021). In this context, the cosmetic industry is looking for non-toxic and effective preservatives and also for new natural, sustainable and effective preservatives for their formulations to minimize toxicity and expected side effects (Halla et al. 2018; Carvalho et al. 2024, Rubio et al, 2024).

Among these plants with potential to be used as natural preservatives we have *Rosmarinus officinalis* L., commonly known as rosemary, *Lamiaceae* family. Bioactivity investigations have shown that the *R. officinalis* extract possess antimicrobial activity (Zhang et al. 2014; Vaz et al. 2022; Vaz et al. 2023; Soliman et al. 2024; Ali et al. 2025). Phytochemical studies revealed the presence of several phenolics in *R. officinalis* including carnosol, carnosic acid and rosmarinic acid, flavonoids such as rosmaricine and essential oils such as α-pinene, camphor and eucalyptol (Aumeeruddy-Elalfi et al. 2016; Soliman et al. 2024; Mir et al. 2025). Phenolics as caffeic, p-coumaric, ferulic, gallic, gentisic, protocatechuic and vanillic acids, and quercetin, luteolin and naringenin were identified by Liquid Chromatography tandem High Resolution Mass Spectrometry (LC-HRMS) in rosemary (Vaz et al. 2022). Studies have correlated the bioactivity of natural products with the properties of phenolics (Khan 2018; Tocci et al. 2018; Chiorcea-Paquim et al. 2020).

Although *R. officinalis* extract is well known for its antimicrobial properties, there are no studies in the consulted literature regarding their incorporation as a preservative in cosmetics formulations. Therefore, the aim of this study was to evaluate the preservative efficacy of cream gel formulations containing standardized *R. officinalis* extract through challenge test and to evaluate its stabilities.

## Materials and Methods

### *Reagents used*

Aquagel 35 S® (Polyacrylamide & C13-14 Isoparaffin & Laureth-7) from All Chemistry® (São Paulo, SP, Brazil), methylparaben from Viapharma® (São Paulo, SP, Brazil), EDTA from Pharmanostra® (Goiânia, GO, Brazil).

The culture media were: Tryptic soy agar and broth, Müller Hinton agar, Sabouraud-dextrose agar and broth from Kasvi® (São Paulo, SP, Brazil), RPMI agar from Himedia® (India), and TAT (Tryptone-Azolectin-Tween) broth from Acumedia® (USA). All other reagents used were analytical grade.

### *Obtaining and characterization of Rosmarinus officinalis L. extract*

*R. officinalis* leaves were cultivated and collected in the Technical Assistance and Rural Extension Company (EMATER) (16º 36´ 19´´S, 49º 15´ 48´´W, 710 m altitude) situated in Goiânia-Go, in October 2016. A voucher

specimen was deposited at the UFG Herbarium under number UFG-50.506 and identified morphologically by Profa. Dr. Maria Teresa F. Bara of the Faculty of Pharmacy of the Federal University of Goiás (FF-UFG).

The *R. officinalis* leaves were dried at 25ºC and protected from direct exposure to sunlight. Subsequently, the extract was obtained by static maceration for 24 hours of the dried leaves, followed by 10 cycles of percolation using 80% (v/v) ethanol as the extraction solvent in both processes. Extract was concentrated in a rotary evaporator (Büchi® – R-220 SE, Switzerland) at 40ºC, 70 rpm, 70 mbar.

*R. officinalis* extract was characterized in terms of pH, solids content, relative density and viscosity (Brazil 2024). Spectrophotometric techniques were performed to quantify polyphenols and total tannins (Mole & Waterman, 1987) and the flavonoids content (Rolim et al., 2005). The content of rosmarinic acid was determined by HPLC, using the model e2695 (Waters®), equipped with a quaternary pump and diode array detector (Waters®), using the method proposed by Canelas & Costa (2007) with adaptations. The chromatographic column used was the XDB C-18 (5 µm, 150 x 4.6 mm, Agilent Technologies®). The mobile phase was solvent A - 25% (v / v) of acetonitrile grade HPLC (Tedia® High Purity Solvents, United States) and solvent B - 75% (ultrapure water / acetonitrile / phosphoric acid 94: 5: 1% v/v/v pH 1.8) under an isocratic flow rate of 0.5 mL / min. The detection wavelength was 329 nm, with a scan from 191 to 400 nm.

### *Antimicrobial activity of R. officinalis extract*

The *R. officinalis* extract was submitted to the broth microdilution test to determine the Minimum Inhibitory Concentration (MIC), under aerobic conditions, against *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Candida albicans* ATCC 10231 and *Aspergillus brasiliensis* ATCC 16404 from National Institute for Quality Control in Health (Instituto Nacional de Controle de Qualidade em Saúde - INCQS, Brazil). In the broth microdilution test, 0.04 g of the extract and 100 µL of Müller Hinton broth were used, which was then incubated at 35ºC for 24 hours for bacteria, at 25ºC for 24 hours for yeast and at 25ºC for 72 hours for mold (CLSI 2008a; CLSI 2008b; CLSI 2015).

### *Cosmetic formulations*

The cream gel formulations consisted of 5% (w/w) Aquagel 35 S®, a self-emulsifying, 0.1% (w/w) ethylenediamine tetra acetic acid (EDTA) and purified water. The preservatives used on each formulation were 0.2% (w/w) of *R. officinalis* extract (F1), 0.4% (w/w) of *R. officinalis* extract (F2) and 0.2% (w/w) of methylparaben 0.2% (F3) was used as control.

### *Stability evaluation*

For preliminary stability tests, 10 g of each formulation were centrifuged at 3000 rpm and 25ºC for 30 minutes and evaluated the organoleptic parameters (appearance, color and odor) the and physical parameters (viscosity, pH and conductivity) in the beginning of the test and after 6 cycles of 24 hours at 45ºC and 6 cycles of 24 hours at -5ºC alternately, totalizing 12 days (Brazil 2004).

The accelerated stability test was performed with the formulations stored at temperatures of 40ºC, 25ºC and 5ºC for a period of 90 days, evaluating the same parameters as the preliminary tests of stability in the formulation at times 0 and 24 hours, 7, 15, 30, 60 and 90 days after preparation (Brazil 2004).

The results obtained were submitted to analysis of variance (one-way ANOVA) with a confidence level of 95% ($p < 0.05$), using GraphPad Prism 7.0 program.

### *Challenge Test*

*Neutralizing Medium Efficacy*

Approximately $10^3$ CFU of *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Candida albicans* ATCC 10231, and *Aspergillus brasiliensis* ATCC 16404 of the standardized suspension was inoculated separately in 9 mL of the neutralizer medium (TAT) in the presence of 1 g of the formulation containing 0.4% (w/w) of *R. officinalis* extract, and 1 mL of each prepared samples were transferred to Petri dishes where 20 mL of Tryptic Soy Agar (for bacteria) and Sabouraud-Dextrose Agar (for fungi) were added, melted and cooled to about 45°C, homogenized and incubated respectively at 32.5 ±2.5ºC during 48 hours, and at 22.5 ± 2.5ºC during 7 days, being performed the count of CFUs (Brazil 2024).

*Preservative system effectiveness test*

Approximately $10^8$ CFU of the calibrated inoculum of *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Candida albicans* ATCC 10231, and *Aspergillus brasiliensis* ATCC 16404 were added separately, to 100 g of each cream gel formulation in sterile containers. The formulations were properly homogenized and incubated at 25°C. At 2, 4, 6, 24, 48, 168, 336 and 672 h after inoculation, 10 g of these formulations were aseptically removed and diluted in 90 mL of the neutralizer medium (TAT). Then, serial decimal dilutions were performed in 0.85% sterile saline solution and 1 mL of each dilution was transferred, in duplicate, to Petri dishes, in which were added, approximately 20 mL of Tryptic soy agar (for bacteria) and 20 mL of Sabouraud-dextrose agar (for fungi). Finally, the plates were homogenized and incubated respectively at 32,5 ± 2,5ºC (for bacteria) during 72 hours and at 22,5 ± 2,5ºC (for fungi) during 7 days. The CFUs count performed (Brazil, 2024).

## Results and Discussion

### *Obtaining and characterization of R. officinalis L. extract*

The plant extract preparation requires prior knowledge of the class of substances to be extracted and the selectivity of the solvent among other parameters (Ibarra et al. 2010). In this sense, considering the extraction procedures used, we present the data related to the standardization of the *R. officinalis* extract obtained (Table 1).

Table 1 - Characterization of *R. officinalis* extract

| pH | Solids content | Relative density (g/mL) | Viscosity (cP) | Polyphenol content | Tannin content | Flavonoid content | Rosmarinic acid content |
|---|---|---|---|---|---|---|---|
| 4.70 ± 0.01 | 56.96% ± 0.12 | 1.08 ± 0.01 | 92.0 ± 0.17 | 6.12% ±0.002 | 2.91% ± 0.008 | 2.49% ± 0.01 | 1.81% ± 0.02 |

Source: The authors

The pH value found corresponds to an acid pH, since the plant has several phenolic compounds (Vaz et al. 2022) that lead to this characteristic. Furthemore, it is in accordance with Garcia et al. (2017), who determined the pH value 4.64 for the liquid extract of *R. officinalis a*nd similar to Couto et al. (2012), which was pH 5.1.

The total solids content found classifies the extract produced in this study as a fluid extract (Brazil 2024), and this parameter is related to the extracted metabolites, which confer its pharmacological activities (Garcia et al. 2017). The relative density found for the fluid extract was 1.08 g/mL ± 0.01 and corroborates with Couto et al. (2012) who found 0.964 g/mL ± 0.002. The viscosity was 92 ± 0.17 CP, and this parameter is decisive for the consistency and fluidity suitable for future incorporation in semi-solid formulations. Furthermore, it is a parameter that interferes with the stability of cosmetic products, as well as with density and pH (Brazil 2004). In addition, it is related to the solids content, since the increase in the amount of solids in the extract leads to a proportional increase in its density and viscosity (Oliveira et al. 2010).

The total polyphenols content was determined using the tannic acid standard curve (y=11.474x-0.0233; $R^2$=0.9997) was 6.12% ± 0.002, which is close to what was reported by Garcia et al. (2017), who found polyphenol content of 7.76% ± 0.05 in the extract of *R. officinalis* leaves. Some biological activities of rosemary have been attributed to this class of metabolites (Tavassoli & Djomeh 2011).

The total tannin content found in this extract (2.91% ± 0.008) was determined by the tannic acid standard curve (y=0.2069x-0.0467; $R^2$=0.9921). According to Garcia et al. (2017), the total tannin content present in the concentrated extract of *R. officinalis* leaves also obtained by percolation with 80% ethyl alcohol (v/v) were 1.37% ± 0.003. This difference may be due to the final stage of extract concentration, since we obtained a fluid extract.

The total flavonoids content (2.49% ± 0.01) was performed using the rutin standard curve (y=31.066x-0.033; $R^2$=0.9992). Garcia et al. (2017) found value of 3.82% ± 0.02 in the concentrated extract of *R. officinalis* leaves.

The rosmarinic acid content (1.81± 0,06%) in *R. officinalis* extract was determined through the standard curve (y=59.748x+323.179; $R^2$=0.9999). This value corroborates data from Garcia et al. (2017), which was 1.87% ± 0.02. Rosmarinic acid was used as a chemical marker for standardization. Others phytochemical parameters obtained in this characterization are important for the standardization of the extract, which are tools

to achieve reproducibility and contribute to its performance in relation to biological activities (Oliveira et al. 2010).

### *Antimicrobial activity of R. officinalis extract*

The antimicrobial activity of *R. officinalis* extract was considered a good inhibitory activity since the MIC value was less than 100 µg/mL (Ayres et al. 2008; Souza et al. 2014). According to these authors, an inhibitory activity is considered moderate in the range of 100-500 µg/mL, weak inhibitory activity, between 500-1000 µg/mL and absence of activity is considered when values are >1000 µg/mL (Table 2).

Table 2 - Minimum Inhibitory Concentration (MIC - µg/mL) of *R. officinalis* extract.

| Tested microorganisms | MIC | Antimicrobian activity |
|---|---|---|
| *S. aureus* ATCC 6538 | 71.2 | Good activity |
| *E. coli* ATCC 8739 | 71.2 | Good activity |
| *P. aeruginosa* ATCC 9027 | 71.2 | Good activity |
| *C. albicans* ATCC 10231 | 35.6 | Good activity |
| *A. brasiliensis* ATCC 16404 | >1139 | Inactive |

Source: The authors

Oluwatuyi et al. (2004) found a MIC of 60 µg/mL for the methanolic extract of *R. officinalis* against a clinical isolate of *S. aureus* by the broth microdilution test. It is noted that this value was close to that determined in this study against the *S. aureus* strain ATCC 6538. Abkhoo & Jahani (2016) found that the ethanolic extract of *R. officinalis* showed MIC of 25, 50 and 100 ppm, respectively against strains of *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* from the Standard Bacterium Laboratory of the Veterinary Department, Islamic Azad University. Ostrosky et al. (2008) reported that variations in the determination of the MIC of plant extracts can be attributed to the extraction and standardization processes, the amount of extract tested and also due to the microorganism and strain used in the tests.

Khan (2018) related that the antimicrobial activity of a plant extract may be associated with synergistic interactions between the phenolics present in the phytocomplex. Tocci et al. (2018) and Chiorcea-Paquim et al. (2020) correlated the bioactivity of several plants based on the properties of phenolics. These data corroborate those found in our study, since in the characterization of the standardized *R. officinalis* extract we quantified 6.12% polyphenols, 2.49% flavonoids, 2.91% tannins and 1.81% rosmarinic acid.

The literature reports antimicrobial activity for rosmarinic acid (Kostic et al. 2015; Alagawany et al. 2017). Based on Khan (2018), Tocci et al. (2018) and Chiorcea-Paquim et al. (2020), we suggest that the preservative activity of the cream gel studied may be due to the synergism between the phenolics of the obtained rosemary extract.

### *Stability Evaluation*

We used Aquagel 35 that is a rheology modifier, stabilizer, thickener and emulsifier. It swells fast and allows you to create gel or cream immediately without neutralization or heat. This product maintains viscosity under UV radiation and resists shearing for a long time. Applications include creams, lotions, creamy gels, make-up foundation, sunscreen cream, formulas containing polar solvents, alcohol, polyol and formulas containing silicone or AHA (manufacturer information).

The cream gel formulations with *R. officinalis* extract obtained showed a viscous appearance, slightly yellowish color and a slight aroma of rosemary, and the formulation with methylparaben presented a viscous appearance, white and odorless color.

The results of the centrifugation test showed that all formulations analyzed were stable in appearance, color and odor, without precipitation and phase separation. The same occurred during the organoleptic evaluation of the formulations submitted to the preliminary stability study, which means that appearance, color, and odor remained stable during 12 days under extreme temperature variation.

No significant changes ($p>0.05$) in pH, viscosity and conductivity were also observed during the preliminary stability study in both temperature conditions (-5 and 45ºC - Table 3).

Table 3 – Physicochemical parameters of cream gel formulations submitted to preliminary stability study.

| Formulations | Initial pH | Final pH | Initial Viscosity (cP) | Final Viscosity (cP) | Initial Conductivity (μS/cm) | Final Conductivity (μS/cm) |
|---|---|---|---|---|---|---|
| **F1** | 4.78 | 4.90 | 8889.0 | 8890.0 | 235.4 | 238.2 |
| **F2** | 4.69 | 4.91 | 8835.2 | 8864.3 | 235.7 | 237.1 |
| **F3** | 4.76 | 4.89 | 8880.0 | 8865.7 | 243.7 | 253.5 |
| ***p* value** | 0.97 | | 0.23 | | 0.10 | |

F1 - Formulation 1 (0.2% extract); F2 - Formulation 2 (0.4% extract); F3 - Formulation 3 (0.2% methylparaben). Source: The authors

Since there was no change in the stability of the formulations during the preliminary stability test, the accelerated stability test was carried out. A slight modification of the organoleptic characteristics was observed only in formulations containing the *R. officinalis* extract stored at 40ºC in 90 days of analysis. The pHs found in the analyzed formulations are compatible with the cutaneous pH, which ranges from 4.0 to 6.0 (Ali & Yosipovitch 2013). The pH is evaluated in cosmetic formulations because it can influence on the solubility of the substances, chemical stability and physiological compatibility with the organic tissues, where it will be applied, with the intention of minimizing irritation (Ali & Yosipovitch 2013).

No significant viscosity and conductivity variations were also evidenced by the analysis of variance ($p>0.05$) during the test. These parameters contribute to the quality control of the formulations, predicting instability signs such as coalescence, aggregation and modifications in spreadability (Masmoudi et al. 2005).

## *Challenge Test*

### *Neutralizing Medium Efficacy*

The data obtained show that there was a recovery of more than 65% of all the microorganisms inoculated in F2 formulation (Table 4), which suggest that the preservative system present in the formulation (extract of *R. officinalis* at 0.4%) was neutralized by the diluent (TAT broth supplemented with 0.4% polysorbate 80). A recovery above 50% for the microorganisms tested is recommended (Brazil 2024).

Table 4– Microbial recovery after the preservative system neutralization.

| Microorganisms | F2* |
|---|---|
| *Staphylococcus aureus* (ATCC 6538) | 91% |
| *Escherichia coli* (ATCC 8739) | 73% |
| *Pseudomonas aeruginosa* (ATCC 9027) | 65% |
| *Candida albicans* (ATCC 10231) | 86% |
| Aspergillus niger (ATCC 16404) | >90% |

*F2 - Formulation 2 (0.4% extract). Source: The authors

The importance of neutralizing the preservative system is to confirm that microorganisms have not been prevented from growing, leading to erroneous counts (Brazil 2024).

### *Preservative system effectiveness test*

The preservative system challenge test is a recomended procedure to verify the effectiveness of preservatives in a pharmaceutical product. Through this test, the product is exposed to specific types of bacteria and fungi, followed by microbial counting to assess microbiological stability and thus determine whether it is adequately preserved (Brazil 2024).

Cream gel formulations containing 0.2% or 0.4% of *R. officinalis* extract were able to inhibit the growth of *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Candida albicans* ATCC 10231, and *Aspergillus brasiliensis* ATCC 16404, being in compliance with the Antimicrobial Effectiveness Test criteria of the Brazilian Pharmacopoeia 7$^{th}$ edition (Figures 1 and 2).

For topical products analysis the criteria are, for bacteria: 14th day (336 hours): there should be a 2 log reduction in the number of CFU initially inoculated. 28th day (672 hours): there should be no increase in the count compared to the 14th day. Criteria for molds and yeasts: 14th and 28th days: there should be no increase in the number of CFU initially inoculated (Brazil 2024).

It was possible to observe through the preservative efficacy test that there was inhibition of the growth of bacteria and yeast through the reduction of 6 logarithmic cycles, in the formulations that have *R. officinalis* standardized extract as the preservative agent (F1 and F2) from the first week after inoculation (168 hours) (Figures 1 and 2). In these formulations, there was a reduction of approximately 3 logarithmic cycles of *A. niger* (Figures 1 and 2). For formulation containing 0.2% methylparaben (F3), a reduction of 6 logarithmic cycles occurred 24 hours after inoculation of bacteria and yeasts. For *A. niger* ATCC 16404, an inhibition of its growth of 6 logarithmic cycles was observed from the first week after inoculation (168 hours) (Figure 3). All this data is in accordance with the acceptance criteria (Brazil 2024).

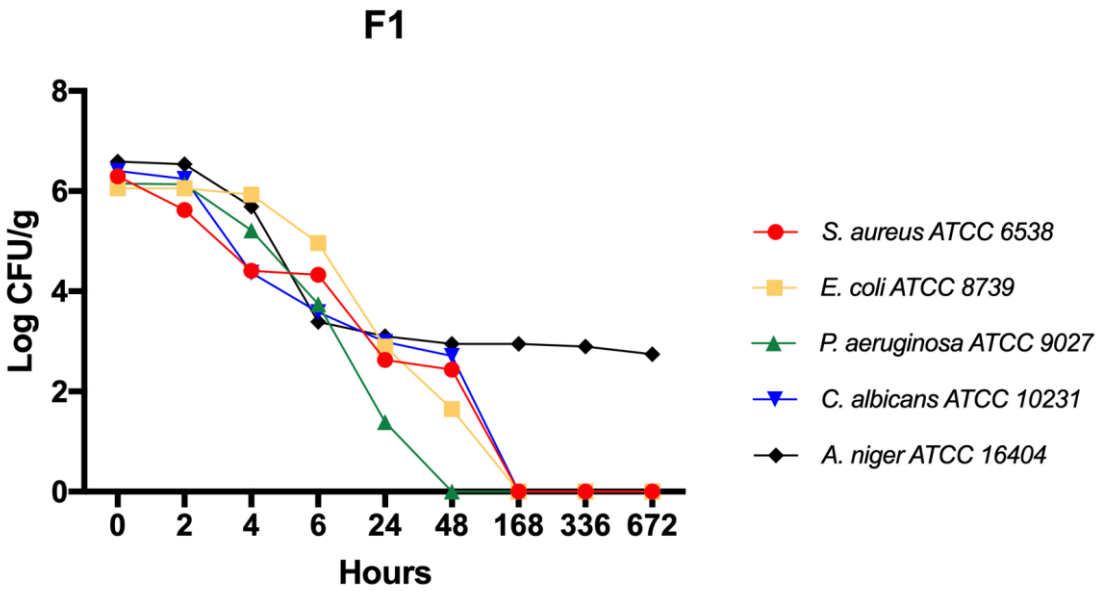



Figure 1. Survival curve log (Log CFU/g) of each microorganism in the preservative efficacy test in the formulation containing 0.2% of *R. officinalis* extract (F1). Source: The authors

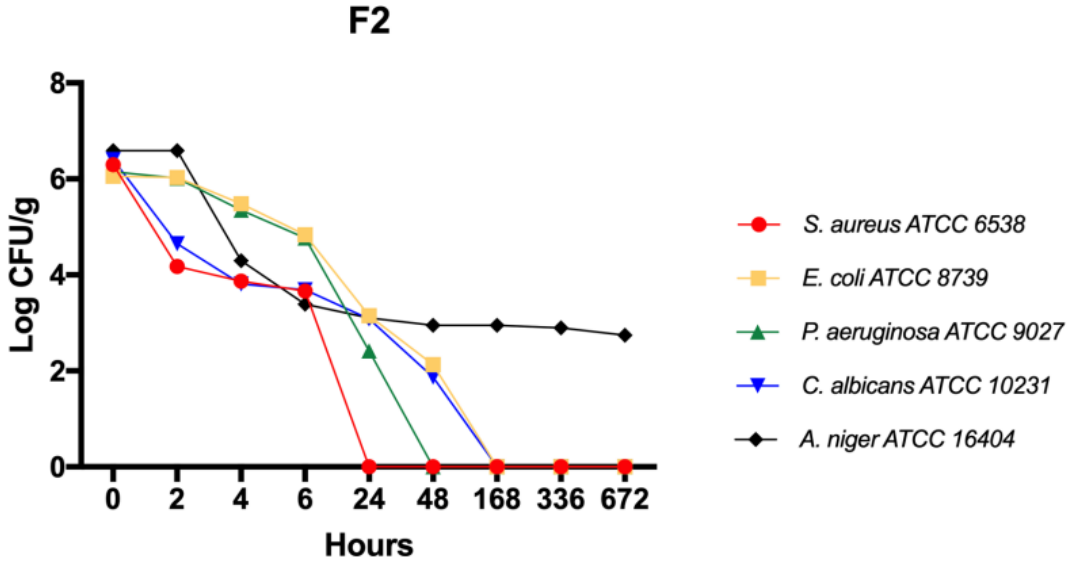



Figure 2. Survival curve log (Log CFU/g) of each microorganism in the preservative efficacy test in the formulation containing 0.4% of *R. officinalis* extract (F2). Source: The authors

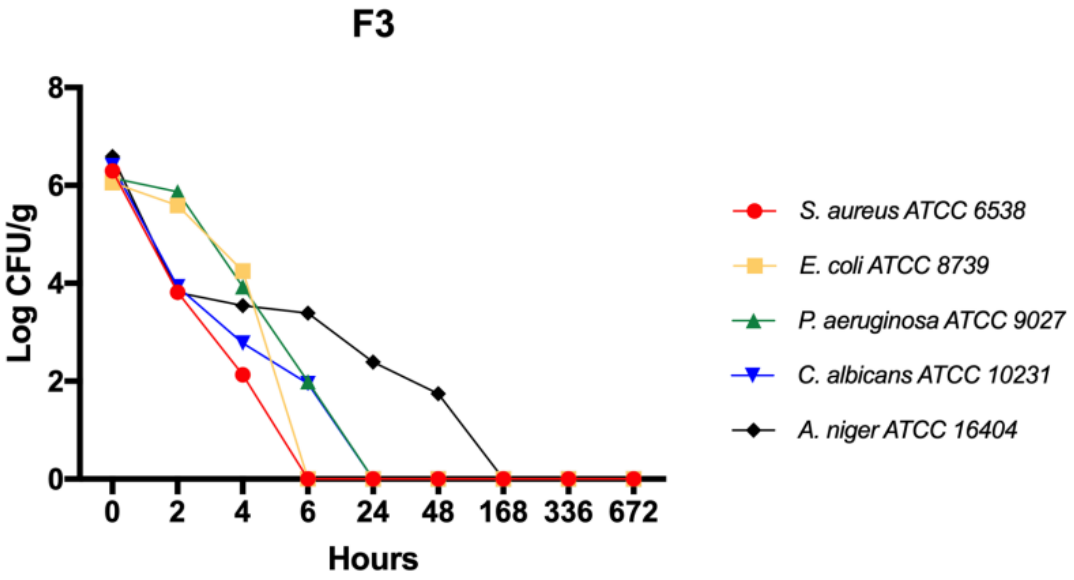



Figure 3. Survival curve log (Log CFU/g) of each microorganism in the preservative efficacy test in the formulation containing 0.2% of methylparaben (F3).
Source: The authors

Ostrosky et al. (2011) performed the preservative efficacy test on an emulsion containing Polawax® base and a Carbopol® gel, both prepared with 0.2% *Rubus rosaefolius* extract, which have phenolics, as a preservative agent against *S. aureus* ATCC 6538, *E. coli* ATCC 10536, *P. aeruginosa* ATCC 9027, *C. albicans* ATCC 10231 and *A. niger* ATCC 16404. The results showed that *R. rosaefolius* extract, at the studied concentration, reduced the bacterial inocula, meeting the criteria in all formulations, although it was not able to present effective preservative behavior against fungi.

Kočevar & Lunder (2018) investigated the efficacy of grapefruit (*Citrus* × *paradisi*) seed extract, which have phenolics, at 0.1 and 0.5% incorporated on a w/o cosmetic emulsion against *S. aureus* ATCC 6538, *P. aeruginosa* ATCC 9027, *C. albicans* ATCC 10231 and *A. niger* ATCC 16404. They found that the formulation containing 0.1% grapefruit seed extract was unable to reduce to growth of *C. albicans* ATCC 10231. However, the formulation containing 0.5% grapefruit seed extract reduce the growth of all challenged microorganisms.

We found no studies on the preservative efficacy of *R. officinalis* extract incorporated into cream gel formulations at concentrations of 0.2% and 0.4%. Our results allow us to suggest that the standardized rosemary extract obtained has potential for use as a preservative in the cosmetic studied, since it reduced the load of viable microorganisms in the formulations tested at both concentrations used, leading to protection against microbial contamination.

## Conclusion

Under the experimental conditions used it can be suggested that *Rosmarinus officinalis* standardized extract can be indicated as a preservative for the cream gel obtained, as it was able to inhibit the growth of the challenging microorganisms tested (bacteria and yeast) and reduced the growth of the fungus, in both analyzed concentrations. In addition to protection against microbial contamination, the cream gel formulations containing the extract remained physically and chemically stable at room temperature.

## References

Abkhoo J, Jahani S 2016. Antibacterial effects of aqueous and ethanolic extracts of medicinal plants against pathogenic strains. *Int J Infect* 4(2):e42624. doi:10.5812/iji.42624.

Alagawany M, El-Hack MEA, Farag MR, Gopi M, Karthik K, Malik YS, Dhama K 2017. Rosmarinic acid: modes of action, medicinal values and health benefits. Anim Health Res Rev 18(2):167-176. doi:10.1017/S1466252317000081.

Ali MK, Galut HS, Matar EETM, Mamand SF, Sabrie LS, Mala SF, Jghef MM 2025. Exploring the antibacterial and anti-biofilm properties of *Rosmarinus officinalis* extracts: a natural strategy against methicillin-resistant *Staphylococcus aureus*. *Open Vet J* 15(10): 5549-5561.doi: 10.5455/OVJ.2025.v15.i11.11.

Ali SM, Yosipovitch G 2013. Skin pH: from basic science to basic skin care. *Acta Derm Venereol* 93(3):261-267. doi:10.2340/00015555-1531.

Aumeeruddy-Elalfi Z, Gurib-Fakim A, Mahomoodally MF 2016. Chemical composition, antimicrobial and antibiotic potentiating activity of essential oils from 10 tropical medicinal plants from Mauritius. *J Herb Med* 6(2):88-95. doi:10.1016/j.hermed.2016.02.002.

Ayres MCC, Brandão MS, Vieira-Júnior GM, Menor JCAS, Silva HB, Soares MJS, et al 2008. Antibacterial activity of useful plants and chemical constituents of the roots of *Copernicia prunifera*. *Braz J Pharmacogn* 18(1):90-97. doi:10.1590/s0102-695x2008000100017.

Brazil 2004. Agência Nacional de Vigilância Sanitária. *Guia de Estabilidade de Produtos Cosméticos*, 1 ed., 52 p. Brasília:ANVISA.

Brazil 2024. Agência Nacional de Vigilância Sanitária. *Farmacopeia Brasileira*, 7ª ed., p.603-606. Brasília:ANVISA.

Canelas V, Costa CT 2007. Quantitative HPLC analysis of *Rosmarinic acid* in extracts of *Melissa officinalis* and spectrophotometric measurement of their antioxidant activities. *J Chem Educ* 84(9):1502-1504. doi:10.1021/ed084p1502.

Carvalho MJ, Pedrosa SS, Pintado M, Oliveira ALS, Madureira A 2024. New natural and sustainable cosmetic preservative based on sugarcane straw extract. *Molecules* 29(16): 3928-3944. doi: 10.3390/molecules29163928.

Chiorcea-Paquim AM, Enache TA, Gil ES, Oliveira-Brett AM 2020. Natural phenolic antioxidants electrochemistry: towards a new food science methodology. *Compr Rev Food Sci Food Saf* 19(4):1680-1726. doi: 10.1111/1541-4337.12566.

Cho TJ, Park SM, Yu H, Seo GH, Kim HW, Kim SA, Rhee MS 2020. Recent advances in the application of antibacterial complexes using essential oils. *Molecules* 25(7):1752, 2020. ~~doi:~~10.3390/molecules25071752.

CLSI (Clinical and Laboratory Standards Institute). M07-A10: Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. 2015.

CLSI (Clinical and Laboratory Standards Institute). M27-A3: Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts. 2008a.

CLSI (Clinical and Laboratory Standards Institute). M38-A2 Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi. 2008b.

Couto RO, Conceicao EC, Chaul LT, Oliveira EMS, Martins FS, Bara MTF, Rezende KR, Alves SF, Paula JR 2012. Spray-dried rosemary extracts: physicochemical and antioxidant properties. *Food Chem* 131(1):99-105. doi:10.1016/j.foodchem.2011.08.036.

Fang B, Yu M, Zhang W, Wang F 2016. A new alternative to cosmetics preservation and the effect of the particle size of the emulsion droplets on preservation efficacy. *Int J Cosmet Sci* ;38(5):496-503. doi:10.1111/ics.12317.

Garcia ML, Ferreira NLO, Arruda RL, Filippi MCC, Conceição ECD 2017. Standardized rosemary extract induces host plant defenses and suppresses rice leaf blast. *J Med Plants Stud* 5(5):39-45.

Halla N, Fernandes IP, Heleno SA, Costa P, Boucherit-Otmani Z, Boucherit K, Rodrigues AE, Ferreira ICFR, Barreiro MF 2018. Cosmetics Preservation: A review on present strategies. *Molecules* 23(7):1571. doi:10.3390/molecules23071571.

Ibarra A, Cases J, Bily A, He K, Bai N, Roller M, Coussaert A, Ripoll C 2010. Importance of extract standardization and in vitro/ex vivo assay selection for the evaluation of antioxidant activity of botanicals: a case study on three *Rosmarinus officinalis* L. extracts. *J Med Food* 13(5):1167-1175. doi:10.1089/jmf.2009.0259.

Kostic M, Zlatkovic B, Miladinovic B, Živanovic S, Mihajilov-Krstev T, Pavlovic D, Kitic D 2015. Rosmarinic acid levels, phenolic contents, antioxidant and antimicrobial activities of the extracts from *Salvia verbenaca* L. obtained with different solvents and procedures. J Food Biochem 39:199-208. doi:10.1111/jfbc.12121.

Kerdudo A, Burger P, Merck F, Dingas A, Rolland Y, Michel T, Fernadez X 2016. Development of a natural ingredient-natural preservative: a case study. *Comptes Rendus Chim* 19(9):1077-1089. doi:10.1016/j.crci.2016.06.004.

Khan RA 2018. Natural products chemistry: The emerging trends and prospective goals. *Saudi Pharm J* 26(5):739-753. 10.1016/j.jsps.2018.02.015.

Kočevar NG, Lunder M 2018. Preservative efficacy of selected antimicrobials of natural origin in a cosmetic emulsion. *Int J Cosmet Sci* 40(3):276-284. doi:10.1111/ics.12461.

Martelli EC, Camargo MCGD, Vieira J, Gandra RF 2021. Use of bioactive substances as natural preservatives in pharmaceutical forms: a review. *Braz J Health Review* 4(2):8120-8133. doi: 10.34119/bjhrv4n2-342.

Masmoudi H, Dréau Y Le, Piccerelle P, Kister J 2005. The evaluation of cosmetic and pharmaceutical emulsions aging process using classical techniques and a new method: FTIR. *Int J Pharm* 289(1-2):117-131. doi:10.1016/j.ijpharm.2004.10.020.

Mir H, Alachaher FZ, Guenzet A, Berzou S, Khelladi HM, Dida-Taleb N, Krouf D, Hammana I 2025. Composition, antioxidant and antibacterial properties of *Rosmarinus officinalis L.* essential oils from Mostaganem, Algeria: regional valorisation perspective. *Phyton-Ann Rei Bot* 65(2): 1-19. doi:10.3390/MOLECULES29091825.

Mole S, Waterman PG 1987. A critical analysis of techniques for measuring tannins in ecological studies - I. Techniques for chemically defining tannins. *Oecologia* 72:137-147. doi:10.1007/BF00385058.

Oliveira OW, Petrovick PR 2010. Spray drying of plant extracts: basic remarks and application. *Braz J Pharmacogn* 20(4):641-650. doi:10.1590/S0102-695X2010000400026.

Oluwatuyi M, Kaatz GW, Gibbons S 2004. Antibacterial and resistance modifying activity of *Rosmarinus officinalis*. *Phytochemistry* 65(24):3249-3254. doi:10.1016/j.phytochem.2004.10.009.

Ostrosky EA, Mizumoto MK, Lima ME, Kaneko TM, Nishikawa S, Freitas BR 2008. Methods for evaluation of the antimicrobial activity and determination of Minimum Inhibitory Concentration (MIC) of plant extracts. *Braz J Pharmacogn* 18(2):301-307. doi: 10.1590/S0102-695X2008000200026.

Ostrosky EA, Marcondes EMC, Nishikawa SO, Lopes PS, Varca GHC, Pinto TJA, T Consiglieri VO, Baby AR, Velasco MVR, Kaneko TM 2011. *Rubus rosaefolius* extract as a natural preservative candidate in topical formulations. *AAPS PharmSciTech* 12(2):732-737. doi:10.1208/s12249-011-9635-9.

Rolim A, Maciel CPM, Kaneko TM, Consiglieri VO, Salgado-Santos IMN, Velasco MVR 2005. Validation assay for total flavonoids, as rutin equivalents, from *Trichilia catigua* Adr. Juss (Meliaceae) and *Ptychopetalum olacoides* Bentham (*Olacaceae*) commercial extract. *J AOAC Int* 88(4):1015-1019. doi:10.1093/jaoac/88.4.1015.

Rubio L, Pita A, Garcia-Jares C, Lores M 2024. Natural extracts and essential oils as ingredients in cosmetics: search for potential phytomarkers and allergen survey. *Cosmetics* 11(3): 84. doi:10.3390/cosmetics11030084.

Soliman MM, Elsaba YM, Soliman MAS, Ahmed EZ 2024. Composition and antimicrobial activity of *Rosmarinus officinalis* L. and *Artemisia monosperma* L. leaf essential oils and methanolic extracts from plants grown in normal and saline habitats in Egypt. *Sci Rep* 14(1):7342. Doi: 10.1038/s41598-024-57301-w.

Souza AM, Armstrong L, Merino FJZ, Cogo LL, Monteiro CLB, Duarte MR, Miguel OG, Miguel MD 2014. *In vitro* effects of *Eugenia pyriformis* Cambess., Myrtaceae: Antimicrobial activity and synergistic interactions with Vancomycin and Fluconazole. *Afr J Pharm Pharmacol* 8(35):862-867. doi:10.5897/ajpp2014.4100.

Svedman C, Andersen EK, Brandão FM, Bruynzeel Derk DP, Diepgen TL, Frosch PJ, Rustemeyer T, Giménez-Arnau A, Gonçalo M, Goossens A, Johansen JD, Lahti A, Menné T, Seidenari S, Tosti A, Wahlberg JE, White IR, Wilkinson JD, Mowitz M, Bruze M 2012. Follow-up of the monitored levels of preservative sensitivity in Europe. Overview of the years 2001-2008. *Contact Dermat* 67(5):312-314. doi:10.1111/j.1600-0536.2012.02140.x.

Tavassoli S, Djomeh ZE 2011. Total phenols, antioxidant potential and antimicrobial activity of methanol extract of rosemary (*Rosmarinus officinalis* L.). *Glob Vet* 7(4):337-341.

Tocci N, Weil T, Perenzoni D, Narduzzi L, Madriñán S, Crockett S, Nürk NM, Cavalieri D, Mattivi F 2018. Phenolic profile, chemical relationship and antifungal activity of Andean *Hypericum* species. *Ind Crops Prod* 112:32-37. doi: 10.1016/j.indcrop.2017.12.022.

Vaz PDS, Souza WRN, Wendland A, Oliveira MG, Cirilo HNC, Cortes MVCB, Bara MTF 2023. Antibacterial activity of *Origanum vulgare* and *Rosmarinus officinalis* standardized extracts against *Curtobacterium* and *Xanthomonas*. *Front., J. Soc., Technol. Environ. Sci*. 12(1):110-122. doi: 10.21664/2238-8869.2023v12i1.

Vaz PDS, Souza WRN, Wendland A, Paz ATS; Cortes MVCB, Bara MTF 2022. Antifungal activity of *Origanum vulgare* and *Rosmarinus officinalis* phenolics-containing extracts against *Colletotrichum lindemuthianum* and in the suppression of anthracnose in common beans. *Res. Soc. Dev.* 11(9): e47911932145. doi: 10.33448/rsd-v11i9.32145.

Yazar K, Johnsson S, Lind ML, Boman A, Lidén C 2011. Preservatives and fragrances in selected consumer-available cosmetics and detergents. *Contact Dermatitis* 64(5):265–72. doi:10.1111/j.1600-0536.2010.01828.x.

Zhang Y, Adelakun TA, Qu L, Li X, Li J, Han L, Wang T 2014. New terpenoid glycosides obtained from *Rosmarinus officinalis* L. aerial parts. *Fitoterapia* 99(6):78-85. doi:10.1016/j.fitote.2014.09.004.