Infarma CIÊNCIAS FARMACÊUTICAS
e-ISSN: 2318-9312
Vol.: 38, ano 2026
e202608
Original

Preservation of cosmetics against *Burkholderia cepacia* complex using essential oils and multifunctional components

Preservação de cosméticos contra o complexo Burkholderia cepacia utilizando óleos essenciais e componentes multifuncionais.

doi: 10.14450/infarma.e202608
Recebido em: 10/1/2026
Aceito em: 18/6/2026

Ana Carolina Furian da Silva[1]; Thais Leite de Barros;
Larissa Pavanello[2]; Gabriela de Moraes Saqui;
Sebastião Donizetti Gonçalves; Gislaine Ricci Leonardi;
Karina Cogo-Müller
[1]Faculty of Pharmaceutical Sciences, Universidade Estadual de Campinas
[2]Faculdade de Odontologia de Piracicaba, Universidade Estadual de Campinas
e-mail: karinacm@unicamp.br

ABSTRACT

Objective: The increase in cosmetics contamination has raised safety concerns regarding preservatives. *Burkholderia cepacia* contamination can pose health risks, especially for sensitive or immunocompromised users. This study assessed antimicrobial efficacy of multifunctional compounds and essential oils (EO) compared to standard preservatives. **Methodology:** Caprylyl glycol (CG), ethylhexylglycerin (ETX), cinnamon and clove EO, methylparaben (MP), propylparaben (PP), phenoxyethanol (PE), benzyl alcohol (BA), potassium sorbate (PS), sodium benzoate (SB), and MCI/MI were tested against *Burkholderia cepacia* strains and other pathogens. **Results:** Parabens and MCI/MI showed robust antimicrobial activity. CG and cinnamon/clove EO individually demonstrated effective antimicrobial properties (0.093 mg/mL to 8 mg/mL). Combinations like CG + PE showed additive effects (FICI 0.5 to 1.0), while others were indifferent (FICI 1.0 to 4.0). Emulsion formulations with PE (0.3%), CG (0.3%), and cinnamon EO (0.15%) maintained microbiological stability over 7 days, with no impact on physical-chemical properties. **Conclusion:** CG combinations effectively preserved cosmetics, offering a viable alternative to traditional preservatives.

Keywords: *Burkholderia cepacia* complex; formulation/stability; essential oils; microbiology; safety testing.

RESUMO

Objetivo: O aumento da contaminação de cosméticos tem gerado preocupações quanto à segurança dos conservantes. A contaminação por *Burkholderia cepacia* pode representar riscos à saúde, especialmente para usuários sensíveis ou imunocomprometidos. Este estudo avaliou a eficácia antimicrobiana de compostos multifuncionais e óleos essenciais (OE) em comparação com conservantes convencionais. **Metodologia:** Caprilil glicol (CG), etilhexilglicerina (ETX), óleos essenciais de canela e cravo, metilparabeno (MP), propilparabeno (PP), fenoxietanol (PE), álcool benzílico (BA), sorbato de potássio (PS), benzoato de sódio (SB) e MCI/MI foram testados contra cepas de *Burkholderia cepacia* e outros patógenos. **Re-**

sultados: Parabenos e MCI/MI apresentaram atividade antimicrobiana robusta. CG e os óleos essenciais de canela e cravo, individualmente, demonstraram propriedades antimicrobianas eficazes (0,093 mg/mL a 8 mg/mL). Combinações como CG + PE apresentaram efeitos aditivos (FICI 0,5 a 1,0), enquanto outras foram indiferentes (FICI 1,0 a 4,0). Formulações em emulsão contendo PE (0,3%), CG (0,3%) e óleo essencial de canela (0,15%) mantiveram estabilidade microbiológica por 7 dias, sem impacto nas propriedades físico-químicas. **Conclusão:** Combinações contendo CG preservaram eficazmente os cosméticos, oferecendo uma alternativa viável aos conservantes tradicionais.

Palavras-chave: Complexo *Burkholderia cepacia*; formulação/estabilidade; óleos essenciais; microbiologia; testes de segurança.

INTRODUCTION

Contamination of cosmetic and pharmaceutical products poses risks to product quality and consumers (1,2). In annual reports of non-sterile pharmaceutical product recall issued by the Food and Drug Administration (FDA), the genus *Burkholderia* was cited as the most common microbial contaminant in several years over the past decade (1-6). Due to this alarming scenario, in 2019, the United States Pharmacopeia (USP) included tests to determine the absence of species of the *Burkholderia cepacia* complex (*Bcc*), especially in categories of non-sterile products (7). The complex is known to cause opportunistic infections, especially in immunocompromised patients and patients with chronic lung diseases such as cystic fibrosis (8,9).

Literature has studies that show the susceptibility of the *Bcc* genus to major preservatives dimethoxydimethylhydantoin (DMDMH), methylisothiazolinone/chloromethylisothiazolinone (MCI/MI), methylparaben (MP), phenoxyethanol (PE), among others (10,11). Despite their importance, traditional preservatives should not be seen as the only options for microbial inhibition in formulations, given the high probability of causing allergy conditions, such as dermatitis (12-15). Carcinogenic risks have also been associated with parabens (16,17).

Alternatively, the demand for preservative-free cosmetics or preservatives of natural origin has increased (18,19). Natural product options consist of multifunctional additives, such as emollients, surfactants, fragrance ingredients, and essential oils with antimicrobial activity, which can be used alone or combined with anpreservativen order to enhance preservative action. These products include caprylyl glycol (CG) and ethylhexylglycerin (ETX) (19-22). CG, an ingredient with emollient and humectant characteristics, has already demonstrated antimicrobial activity against *S. aureus, P. aeruginosa, E. coli,* and *C. albicans* at 0.5% concentration in cosmetic formulations (23-25). ETX, another ingredient used for the same purpose in formulations, has antimicrobial activity at 0.1% to 1% against *S. aureus, P. aeruginosa, P. fluorescens, P. putida, E. coli, S. epidermidis, E. gergoviae, A. niger, Penicillium funiculosum,* and *C. albicans* (26). Some essential oils have already demonstrated activity against *Bcc* strains, such as those extracted from lavender, lemongrass, marjoram, peppermint, tea-tree, rosewood, cinnamon, and clove (27,28).

Despite the susceptibility of this genus, resistance to chlorhexidine, benzalkonium chloride and alkyl diaminoethylglycine has been reported, which increased in biofilms when compared to planktonic cells (6,29,30). Resistance of *B. cepacia* to several antibiotic classes, including quinolones, aminoglycosides, and β-lactam agents has also been mentioned (31,32). Multifunctional compounds and preservatives were tested *in vitro* alone and combined with other substances. After the test, antimicrobial agents with the most promising results were incorporated into a non-ionic formulation that was subjected to microbiological and physical-chemical tests. Thus, this study aims to assess the efficacy of new combinations of antimicrobial agents incorporated into non-ionic formulations to offer significant contributions to the development of more effective and safer alternatives to cosmetic preservatives.

MATERIALS AND METHODS

STRAINS AND CULTIVATION CONDITIONS

The following species of the *Bcc* complex were used in this study: 1- Standard strains: *B. cepacia*

ATCC 25416 NCTC 10743, *B. cenocepacia* ATCC BAA-245, *B. multivorans* ATCC BAA-247; 2- Clinically isolated strains named clinical *B. cenocepacia* 1, clinical *B. cepacia* 2, and clinical *B. cepacia* 3. Clinical strains were acquired from the Department of Pathology of the School of Medical Sciences at Universidade Estadual de Campinas (UNICAMP) and identified by MALDI-TOF mass spectrometry. Frozen samples of *Bcc* strains were stored in Brain Heart Infusion Broth (BHI, Difco Co., Detroit, MI, USA).

Standard strains of *Escherichia coli* ATCC 9739, *Pseudomonas aeruginosa* ATCC 9027, and *Staphylococcus aureus* ATCC 6538 were also tested. These species were grown in Tryptic Soy Broth (TSB, Difco Co., Detroit, MI, USA) with 20% glycerol at -80°C. For the experiments, *Bcc* strains were grown in BHI agar medium complemented with 1% defibrinated sheep blood and the other genera were grown in Tryptic Soy Agar medium (TSA, Difco Co., Detroit, MI, USA) and incubated aerobically at 37ºC.

The fungal strain tested in this study was *Candida albicans* ATCC 10231, which was stored in Sabouraud broth (SbB, Sabouraud 2% Dextrose, Millipore, Darmstadt, DE) with 20% glycerol at -80°C. For cultivation, Sabouraud Dextrose Agar (SBA, Sabouraud 4% Dextrose agar, Millipore, Darmstadt, DE) was used and incubated aerobically at 32°C (33).

Access to the genetic heritage was authorized by SISGEN no. AF9DE1B.

SUBSTANCES USED IN THE STUDY

Standard preservatives tested in this study were: phenoxyethanol (PE - ProServ Química, São Paulo, Brazil), methylparaben (MP - ProServ Química), propylparaben (PP - ProServ Química), benzyl alcohol (BA - ProServ Química), sodium benzoate (SB - 31%), and potassium sorbate (PS - 15.4%, ProServ Química), as well as a mixture of isothiazolinones (methylchloroisothiazolinone (MCI - 0.375%) and methylisothiazolinone (MI - 125%) (ProServ Química). Test preservatives were multifunctional compounds caprylyl glycol (CG - Engenharia das Essências, São Paulo, Brazil) and ethylhexylglycerin (ETX - ProServ Química). Regarding essential oils, the activities of oils from the leaves of *Cinnamomum zeylanicum Blume* (cinnamon) (Laszlo, Belo Horizonte, Brazil) and *Syzygium aromaticum* (clove) (Laszlo, Belo Horizonte, Brazil) were assessed.

MINIMUM INHIBITORY CONCENTRATION (MIC), MINIMUM BACTERICIDAL CONCENTRATION (MBC), AND MINIMUM FUNGICIDAL CONCENTRATION (MFC) ASSAYS

Individual MICs of essential oils, preservatives, and multifunctional compounds were determined by the broth microdilution method in 96-well microplates (33,34). Preservative diluents were alcohol 30% (Dinâmica, São Paulo, Brazil), sterile distilled water and polysorbate 80 (Dinâmica, São Paulo, Brazil). The experiment was conducted in duplicate (Table I). Diluents, samples, culture media, and microorganisms were evaluated as the control group.

Colonies of each bacterial strain grown for 24h and each fungal strain grown for 48h were used to prepare the microbial suspension in BHI broth for *Bcc* strains, TSB for *E. coli, S. aureus*, and *P. aeruginosa*, and 0.9% saline solution for *C. albicans*. Absorbance of microbial suspensions was measured using a UV-vis spectrophotometer (Biodrop, Biochrom Ltd, United Kingdom) at a wavelength of 540nm. Then, serial dilutions (2x) of the substances were performed in 100µL Mueller Hinton broth (MHB, Difco Co., Detroit, MI, USA) for bacteria and RPMI 1640 medium (Sigma Aldrich, Missouri, USA) for *C. albicans*. Finally, 100µL microbial suspension was added to all wells of the plate (final concentration of 5×10^5 CFU/mL for bacteria and $5x10^2$ CFU/mL for yeast). The 96-well plates were incubated at 37°C and examined after 24h (bacteria) and 48h (yeast). After incubation, the plates were stained with resazurin dye (Inlab, São Paulo, Brazil) at 0.01%. The lowest concentration of the compound that did not present turbidity and maintained the blue color was considered the MIC.

To determine MBC and MFC, an aliquot of 10µL from wells of 8xMIC, 4xMIC, 2xMIC, and MIC concentrations were grown in a Petri dish containing TSA (bacteria) and SBA (yeast) media and incubated at 37°C for 24h (bacteria) and 48h (yeast). The plate with the lowest concentration without microbial growth was considered the MBC and MFC (35,36).

ANTIMICROBIAL ASSOCIATION ASSAY

The microdilution association method was used to assess possible effects of preservative interactions (37,38). This assay assessed dilutions with different concentrations of ETX, CG, cinnamon EO, and PE, as well as the antimicrobial activity. For preservative selection, the compounds presenting the lowest MIC and in recommended concentration range were selected (39,4). For the experiment, 96-well plates containing MHB (bacteria) and RPMI 1640 (yeast) were used. Serial dilutions (2×) using two antimicrobial agents were prepared in different directions (vertical and horizontal). Bacterial and fungal suspensions were prepared as in MIC assays to produce an inoculum with a final concentration of around 5×10^5 CFU/mL (bacteria) and $5x10^2$ CFU/mL (yeast). The plates were incubated for 24h at 37°C (bacteria) and 48 h at 37°C (yeast). The results were analyzed using the FICI (Fractional Inhibitory Concentration Index). FICI values <0.5 represent synergism, FICI values of 0.5 to 1.0 represent an additive effect, FICI values of 1.0 to 4.0 were indifferent, and FICI >4.0 were classified as antagonism (37). The experiment was conducted in duplicate.

FORMULATION OF NON-IONIC EMULSIONS

Knowing that emulsions contain between 60% and 80% water, susceptibility to microbial contamination becomes a concern for the cosmetic industry (41). Also, emulsions are widely used by consumers on different parts of the body and, as they are not rinsable, they are absorbed by the skin.

Five non-ionic emulsions were formulated: Control emulsion without preservative; Emulsion 1 with PE as standard preservative; Emulsion 2 with a multifunctional agent and an essential oil (CG + cinnamon EO); Emulsion 3 with a standard preservative and an essential oil (PE + cinnamon EO), and Emulsion 4 with a multifunctional agent and a standard preservative (CG + PE). Table II shows the compositions of the emulsions. For the development of the emulsions, the ingredients were divided into phases. The oil phase (B) and the aqueous phase (A) were preheated to 70°C. Then, phase B was added to phase A slowly, under constant stirring (42). After cooling, preservatives (C) were added to the emulsions.

Table I. Technical information of substances used in the study

CATEGORY	SUBSTANCES	INCI NAME	CAS NUMBER	TEST CONCENTRATION RANGE (mg/mL)	DILUENT
Preservative	Phenoxyethanol	*Phenoxyethanol*	22-99-6	64 to 0.0078	Sterile distilled water
	Methylparaben	*Methylparaben*	99-76-3	32 to 0.00048	Alcohol 30%
	Propylparaben	*Propylparaben*	94-13-3	32 to 0.00048	Alcohol 30%
	MCI/MI	*Methylchloroisothiazolinone* and *Methylisothiazolinone*	55965-84-9	4 to 0.00000048	Sterile distilled water
	Benzyl alcohol	*Benzyl alcohol*	100-51-6	24 to 0.011	Sterile distilled water
	Sodium benzoate / Potassium sorbate	*Sodium benzoate/ Potassium Sorbate*	532-32-1/24634-61-5	57 to 0.0029	Sterile distilled water
Multifunctional compounds	Caprylyl glycol	*Caprylyl glycol*	1117-86-8	16 to 0.0039	Sterile distilled water
	Ethylhexylglycerin	*Ethylhexylglycerin*	70445-33-9	32 to 0.015	Sterile distilled water
Essential oils	*Cinnamomum zeylanicum Blume* (cinnamon)	*Cinnamomum Zeylanicum Blume*	84649-98-9	24 to 0.00036	Polysorbate 80 (10%)
	Syzygium aromaticum (clove)	*Syzygium aromaticum*	84961-50-2	24 to 0.00036	Polysorbate 80 (10%)

Table II. Composition of formulations divided into phase A (aqueous phase), B (oil phase), and C (thermolabile compounds).

INGREDIENTS	ACTION	CONTROL EMULSION (%)	EMULSION 1 (%)	EMULSION 2 (%)	EMULSION 3 (%)	EMULSION 4 (%)
Water (A)	Solvent	83.4	83	83	83	82.5
Propylene glycol (A)	Humectant	5	5	5	5	5
Cetearyl alcohol and Polysorbate 60 (B)	Thickening and emulsifying agent	10	10	10	10	10
Caprylic triglycerides (B)	Emollient	1.5	1.5	1.5	1.5	1.5
Butylhydroxytoluene (BHT) (B)	Antioxidant	0.05	0.05	0.05	0.05	0.05
PE (C)	Preservative	-	0.4	-	0.3	0.3
CG (C)	Preservative	-	-	0.3	-	0.3
Cinnamon EO (C)	Preservative	-	-	0.15	0.15	-

PRESERVATIVE CHALLENGE TEST

The preservative efficacy of the associations incorporated into non-ionic emulsions was assessed according to criteria of the Personal Care Products Council (PCPC) and Chapter 51 of the United States Pharmacopeia (USP), in duplicate (43,44). The method and preservative inactivating substances were previously validated. Ten grams of each experimental group were placed in polyethylene bottles and stored at room temperature (20°C to 25°C) throughout the test. The samples were contaminated with a microbial suspension at a concentration of $1x10^6$ CFU/mL (bacteria) and $1x10^5$ CFU/mL (yeast). Then, the samples were homogenized and diluted in a neutralizing agent (Table III), obtaining concentrations of 10x, 100x, and 1,000x. Of each dilution, 1.0 mL was pipetted into TSA or SBA plates using the spread-plate technique. The plates were incubated at 32.5°C ± 2.5°C for 24h (bacteria) and 48h (yeast). After this period, the colonies were counted to determine the CFU/mL, considering 20 to 300 CFU as an acceptable range. The emulsions were evaluated using the following intervals: 0, 7, 14, and 28 days.

Table III. Culture media used in the preservative challenge test.

STRAINS	CULTURE MEDIUM FOR INOCULUM PREPARATION	NEUTRALIZING CULTURE MEDIA	RECOVERY MEDIUM WITH AGAR
B. cepacia ATCC 25416	BHI[1] broth	DE[3] broth	BHI[1] agar + 0.7% soy lecithin + 2% tween 80
Clinical B. cenocepacia	BHI[1] broth	DE[3] broth	BHI[1] agar + 0.7% soy lecithin + 2% tween 80
S. aureus ATCC 6538	TSB[2]	DE[3] broth	BHI[1] agar + 0.7% soy lecithin + 2% tween 80
E. coli ATCC 9739	TSB[2]	DE[3] broth	BHI[1] agar + 0.7% soy lecithin + 2% tween 80
P. aeruginosa ATCC 9027	TSB[2]	DE[3] broth	BHI[1] agar + 0.7% soy lecithin + 2% tween 80
C. albicans ATCC 10231	0.9% saline solution	SbB + 0.7% soy lecithin + 2% tween 80	SBA[4] + 0.7% soy lecithin + 2% tween 80

[1]Brain Heart Infusion; [2]Trypticase Soy Broth; [3]Dey Engley broth; [4]Sabouraud Dextrose Agar.

As a criterion for preservative system efficacy, according to the PCPC method, the bacterial count must be reduced to 3log or 99.9% after 7 days. For the USP, this reduction is expected on day 14. Yeast and mold counts should be reduced by 90.0% on day 7 according to the PCPC method. The USP specifies that after 7, 14, and 28 days, yeast and mold levels must remain at or below the initial concentrations for the preservative system to be considered efficient. With both methods, no growth is expected after 28 days (43,44).

ACCELERATED STABILITY TEST

Emulsion stability was preliminarily evaluated under four different conditions (45). The samples were submitted to the following conditions: 40 ± 20°C, 2-5 ± 20°C, ambient light, protected from light at room temperature (20 to 25°C). Samples were evaluated in intervals of 0, 7, 15, 30, and 45 days, with analysis of organoleptic characteristics (phase separation, precipitation, color, and odor) and physical-chemical characteristics (pH) in relation to baseline. Control emulsion (without preservative) was stored at room temperature, protected from light. The experiment was conducted in duplicate.

RESULTS

MINIMUM INHIBITORY CONCENTRATION (MIC), MINIMUM BACTERICIDAL CONCENTRATION (MBC), AND MINIMUM FUNGICIDAL CONCENTRATION (MFC) ASSAYS

The antimicrobial activity of conventional preservatives, when evaluated separately, was similar for *Bcc, S. aureus, P. aeruginosa,* and *C. albicans* strains, with efficacy decreasing from the highest to the lowest in the following order: MCI/MI > PP > MP > PE > BA > SB/PS (Table IV). Regarding *E. coli* and *Bcc*, MP showed a lower MIC when compared to PP. For multifunctional compounds, CG showed the lowest MIC for all microorganisms (1 to 8 mg/mL), followed by ETX (2 to 32 mg/mL). Cinnamon EO presented the lowest MIC values (0.093 to 3 mg/mL) when compared to clove EO, except for *C. albicans* (12 mg/mL).

MIC results between ATCC and clinical *Burkholderia* strains were similar.

The results for MBC of standard preservatives were similar to the MIC results (Table V). For *C. albicans*, PP had stronger fungicidal capacity than MCI/MI. Among the multifunctional compounds, CG showed the highest bactericidal potential (1 to 16 mg/mL) followed by ETX (4 to 64 mg/mL). Cinnamon EO presented the lowest biocidal concentrations when compared to clove essential oil, except for *P. aeruginosa* and *C. albicans*.

For comparison, the Brazilian National Health Surveillance Agency (ANVISA) issued through RDC 528/2021 a list of substances with preservative action for personal hygiene products, cosmetics, and perfumes with maximum concentrations, as did the European Union with Regulation no. 1223/2009. Table VI shows the percentages established by legislation and their equivalent MIC found experimentally (39,40).

Table IV. Minimum inhibitory concentration (MIC) provided in mg/mL.

Strains/Preservatives	PE[1]	MP[2]	PP[3]	MCI/MI[4]	BA[5]	SB/PS[6]	ETX[7]	CG[8]	Cinnamon EO[9]	Clove EO[10]
B. cepacia ATCC 25416	2	0.5	0.5	0.0003	3	5.5/2.7	4	2	3	6
B. cenocepacia ATCC BAA-245	2	0.5	0.5	0.0002	3	5/2.5	4	2	3	6
B. multivorans ATCC BAA-247	2	0.5	0.5	0.0003	3	5.5/2.7	2	2	1.5	6
Clinical B. cenocepacia	4	0.5	0.5	0.0003	1.5	8.6/4.3	8	1	1.5	6
Clinical B. cepacia 2	4	0.5	0.5	0.0003	1.5	8.6/4.3	8	1	1.5	3
Clinical B. cepacia 3	4	0.2	0.5	0.00018	3	8.6/4.3	8	1	1.5	3
S. aureus ATCC 6538	2	2	1	0.5	24	2.2/0.7	32	8	0.093	3
E. coli ATCC 9739	4	2	2.8	0.031	12	18/6	4	2	0.3	1.5
P. aeruginosa ATCC 9027	16	4	4	0.5	4	28.8/9.6	16	4	2	0.3
C. albicans ATCC 10231	4	4	1	1	8	28.8/9.6	8	2	12	0.18

Legends: [1]PE: phenoxyethanol; [2]MCI/MI: methylchloroisothiazolinone/methylisothiazolinone; [3]MP: methylparaben; [4]PP: propylparaben; [5]BA: benzyl alcohol; [6]SB/PS: sodium benzoate/potassium sorbate; [7]ETX: ethylhexylglycerin; [8]CG: caprylylglycol; [9,10]EO: essential oil.

Table V. Minimum bactericidal concentration (MBC) and minimum fungicidal (MFC)concentration provided in mg/mL.

Strains/ Preservatives	PE[1]	MP[2]	PP[3]	MCI/MI[4]	BA[5]	SB/PS[6]	ETX[7]	CC[8]	Cinnamon EO[9]	Clove EO[10]
B. cepacia ATCC 25416	4	2	0.5	0.0003	6	11/5.5	16	2	3	6
B. cenocepacia ATCC BAA-245	4	2	1	0.0004	6	20/10	16	4	3	6
B. multivorans ATCC BAA-247	8	1	1	0.0012	6	44/22	8	2	1.5	6
Clinical B. cenocepacia	4	0.5	0.5	0.0003	1,5	8.6/4.3	8	1	1.5	6
Clinical B. cepacia 1	4	0.5	0.5	0.0003	1,5	8.6/4.3	8	1	1.5	3
Clinical B. cepacia 2	4	0.2	0.5	0.00018	3	8.6/4.3	8	1	1.5	3
S. aureus ATCC 6538	8	8	2	1	24	4.5/1.5	32	16	0.093	3
E. coli ATCC 9739	16	16	2.8	0.031	12	18/6	4	4	0.7	1.5
P. aeruginosa ATCC 9027	64	16	32	4	4	57.6/19.2	64	8	24	1.5
C. albicans ATCC 10231	8	4	1	2	8	57.6/19.2	16	4	12	0.18

Legends: [1]PE: phenoxyethanol; [2]MCI/MI: methylchloroisothiazolinone/methylisothiazolinone; [3]MP: methylparaben; [4]PP: propylparaben; [5]BA: benzyl alcohol; [6]SB/PS: sodium benzoate/potassium sorbate; [7]ETX: ethylhexylglycerin; [8]CG: caprylylglycol; [9,10]EO: essential oil.

Table VI. MIC results in percentage and maximum concentrations for preservatives by current legislations.

Preservatives/ Strains	Maximum MIC results (%)					Maximum allowed amount (%)	
	***Bcc* strains**	***S. aureus ATCC 6538***	***E. coli ATCC 8739***	***P. aeruginosa ATCC 9027***	***C. albicans ATCC 10231***	**Brazil (RDC 528/2021)**	**Europe (EC no. 1223/2009)**
Methylparaben	0.05%	0.17%	0.17%	0.33%	0.33%	0.8%	0.4%
Propylparaben	0.05%	0.095%	0.26%	0.38%	0.095%	0.8%	0.4%
Benzyl alcohol	0.3%	2.31%	1.15%	0.38%	0.77%	1%	0.001%
Phenoxyethanol	0.4%	0.18%	0.36%	1.44%	0.36%	1%	1%
Potassium sorbate/Sodium benzoate	0.7%	0.10%	0.87%	1.4%	1.4%	0.5%	0.6%
MCI/MI	0.00031%	0.049%	0.030%	0.049%	0.097%	0.0015%	0.0015%

ANTIMICROBIAL ASSOCIATION ASSAY

When associated, the compounds showed inhibitory concentrations that were similar to isolated compounds. FICI values were 0.75 to 4.0 (Table VII).

Some results from the test of CG + PE association showed an additive effect, with a reduction in MIC concentrations (½MIC and ¼MIC) for at least one of the compounds, for *S. aureus, E. coli,* and *Bcc*. The same result was observed for the CG + ETX association for *E. coli*, ETX + PE for *S. aureus*, *P. aeruginosa*, and *Bcc*, and PE + cinnamon EO for *P. aeruginosa* and *Bcc*. The remaining associations resulted in indifference, i.e., no increase was observed in the antimicrobial activity when using combined preservatives. In most cases, FICI values of 4.0 were obtained from associations with ETX. For this reason, associations without ETX were incorporated into the formulations.

PRESERVATIVE CHALLENGE TEST

Control emulsion (without preservative and previously contaminated) maintained microbial growth during the 28-day period (Figure 1). Standard control preservative (PE) was able to inhibit microbial growth on day 28. However, for *P. aeruginosa*, no reduction of 2logs was observed on day 14 (Figure 1).

Figure 1. Preservative challenge test in non-ionic emulsion for *B. cepacia* ATCC 25416, clinical *B. cenocepacia*, *S. aureus* 6538, *P. aeruginosa* 9027, *E. coli* 9739, and *C. albicans* 10231. a) Positive control (no preservative) and b) phenoxyethanol (0.4%). X-axis indicates the reading times in days (0, 7, 14, and 28 days) and Y-axis indicates CFU/mL values in Log.

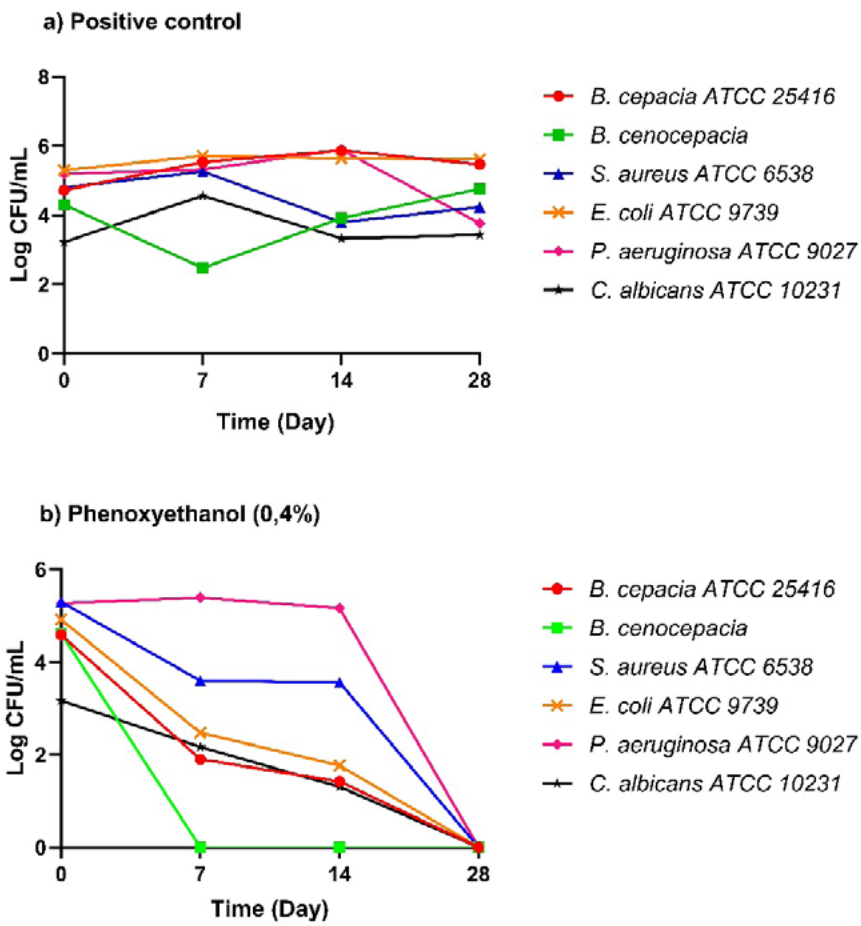
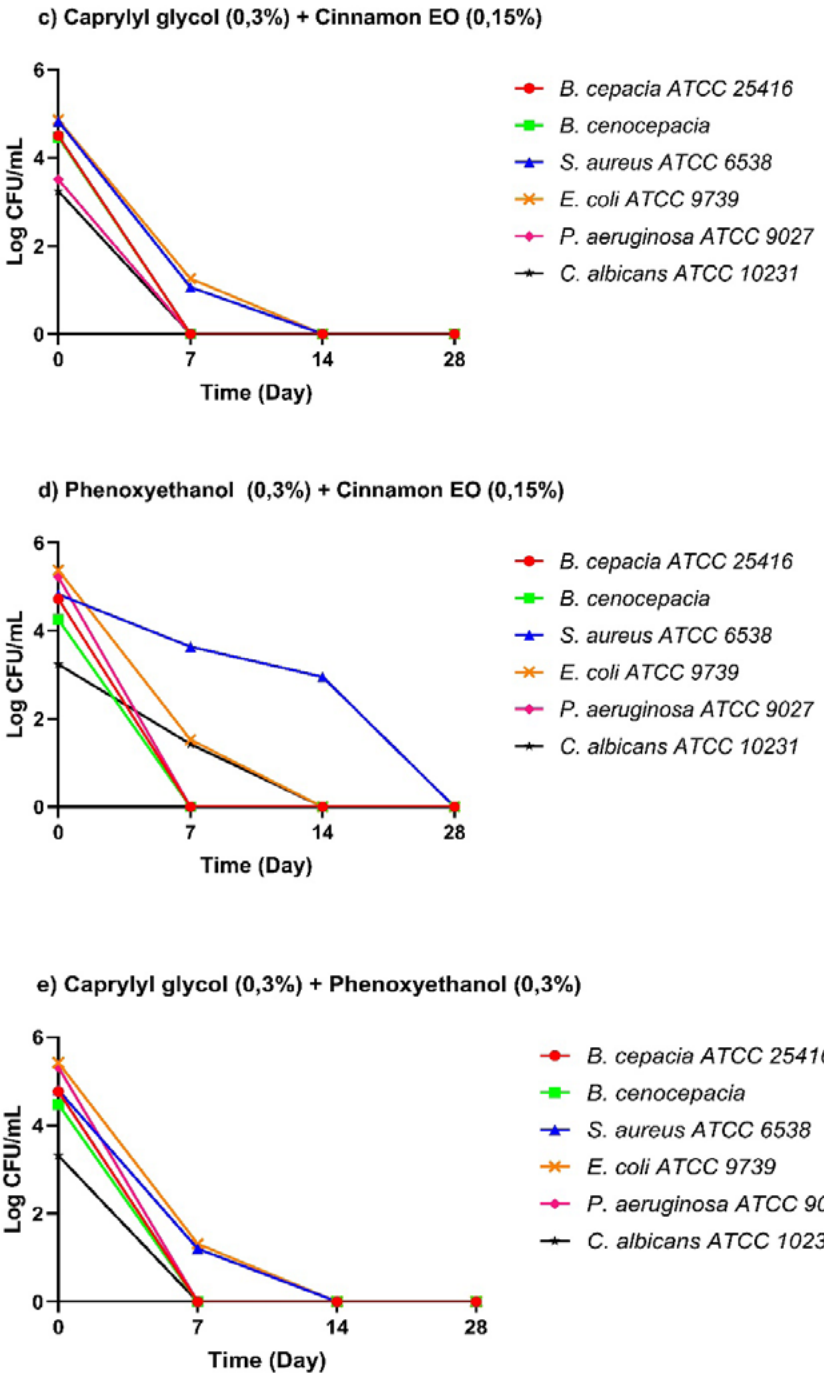


Table VII. FICI results for associations of caprylyl glycol (CG), phenoxyethanol (PE), ethylhexylglycerin (ETX), and EO (essential oil).

Strains/ Associations	CG + PE	CG + ETX	CG + Cinnamon EO	ETX + Cinnamon EO	ETX + PE	PE + Cinnamon EO
B. cepacia ATCC 25416	1.2	1.2	1.25	4.0	1.5	3.0
B. cenocepacia ATCC BAA-245	**1.0**	2.25	1.25	1.4	2.25	1.2
B. multivorans ATCC BAA-247	1.5	3.0	1.2	1.12	**1.0**	2.25
Clinical B. cenocepacia	**0.75**	4.0	2.0	4.0	1.25	1.0
Clinical B. cepacia 1	2.25	2.25	3.0	3.0	2.0	1.5
Clinical B. cepacia 2	1.25	2.25	2.0	2.25	2.0	1.5
S. aureus ATCC 6538	**1.0**	2.0	2.0	1.5	**0.75**	3.0
E. coli ATCC 9739	**1.0**	**1.0**	3.0	4.0	4.0	1.5
P. aeruginosa ATCC 9027	2.0	3.0	2.0	1.13	**1.0**	0.75
C. albicans ATCC 10231	4.0	1.5	1.25	1.5	2.0	4.0

The association of CG + cinnamon EO (Figure 2) interrupted microbial growth at day 7 for *Bcc*, *P. aeruginosa*, and *C. albicans*, and for *S. aureus* and *E. coli* at day 14. The same result was observed with CG + PE (Figure 2). The association PE+cinnamon EO (Figure 2) reduced microbial growth at day 7 for *B. cepacia* ATCC, clinical *B. cenocepacia*, and *P. aeruginosa*, and for *E. coli* and *C. albicans* at day 14. For *S. aureus* a reduction of 2logs was observed at day 14 in relation to the baseline and it fully reduced growth at day 28.

Figure 2. Challenge test of combined preservatives in non-ionic emulsion for *B. cepacia* ATCC 25416, clinical *B. cenocepacia*, *S. aureus* 6538, *P. aeruginosa* 9027, *E. coli* 9739, and *C. albicans* 10231. c) Caprylyl glycol 0.3%+Cinnamon EO 0.15%, d) Phenoxyethanol 0.3%+Cinnamon EO 0.15%, e) Caprylyl glycol 0.3%+Phenoxyethanol 0.3%. X-axis indicates the reading times in days (0, 7, 14, and 28 days) and Y-axis indicates CFU/mL values in Log.



ACCELERATED STABILITY TEST

The samples maintained their organoleptic characteristics during the experiment, including a milky white color, characteristic odor, and no phase separation. pH of the formulations ranged from 5.13 to 6.19, with the highest variations in formulations stored in an oven at 40°C and exposed to sunlight. Control formulation (without preservatives) showed pH of 5.83 to 6.0.

DISCUSSION

The microbiological quality of pharmaceutical and cosmetic products is a critical factor, as contamination can alter the physical and chemical characteristics, reducing product shelf life and potentially putting patient health at risk (1, 46). The genus *Burkholderia* has gained relevance in contamination of cosmetic and pharmaceutical products, especially water-based products (2,6). However, despite the relevance of this bacterial complex, there is little evidence of preservative efficacy against these microorganisms.

Antimicrobial activity results were lower for *Bcc* strains when compared to other microbial genera. Among traditional preservatives, MCI/MI presented the lowest concentrations for MIC/MBC, with antimicrobial activity of 0.00018 to 4 mg/mL, and presented higher efficacy against *Bcc* strains. In one study, wild-type *Bcc* strains were more sensitive to MCI/MI than non-*Bcc* strains, showing an antimicrobial concentration in the range of 0.49 to 1.3 mg/mL (11). Regarding PE and parabens, the concentrations for *Bcc* were also lower, 2 to 8 mg/mL and 0.2 to 2 mg/mL, respectively. The results agree with Rushton et al. (11) who reported 2.5 to 13 mg/mL and 1.0 to 5.8 mg/mL, respectively. Regarding BA, the results indicated stability at MIC/MBC values for clinical *Bcc* strains (1.5 to 3 mg/mL). In contrast, ATCC *Bcc* strains presented double values for MBC in relation to MIC, reaching 3 mg/mL and 6 mg/mL, respectively. For SB/PS, MIC and MBC values ranged from 2.7 to 5.5 and from 22 to 44 mg/mL respectively, showing lower efficacy compared to other preservatives.

Regarding multifunctional compounds, clinical *Bcc* strains showed lower values than ATCC *Bcc* strains. CG presented the lowest concentrations for MIC, MBC/MFC: 1 mg/mL for clinical *Bcc* and 2 to 4 mg/mL for ATCC *Bcc* strains. ETX results showed consistent values for MIC and MBC in relation to clinical *Burkholderia* strains (8 mg/mL). However, an increase in MBC values was observed in ATCC *Bcc* strains, ranging from 2 to 4 mg/mL for MIC and from 8 to 16 mg/mL for MBC. For essential oils, cinnamon EO presented the lowest values for MIC (1.5 to 3 mg/mL) when compared to clove EO (6 mg/mL) for *Bcc* strains.

In relation to the standard microorganisms used in microbiological quality control tests (*S. aureus, E. coli P. aeruginosa*, and *C. albicans*), different MIC ranges were obtained for the standard preservatives, with some variation between species. PE presented efficacious concentrations for *S. aureus* of 2 to 8 mg/mL, and for *C. albicans* of 4 to 8 mg/mL. For gram-negative bacteria *E. coli* and *P. aeruginosa*, higher MICs were observed of 4 and 16 mg/mL and MBC of 16 mg/mL and 64 mg/mL, respectively. This result differs from Lundov et al. (47), who found the following values for other strains of different origins using PE: 10 mg/mL for *S. aureus*, 4 mg/mL for *P. aeruginosa*, and 6 mg/mL for *C. albicans*. In Wang et al. (48), MP was evaluated for *S. aureus* and *E. coli* and the results were the same as our findings (1 to 4 mg/mL) for MIC. For *S. aureus* and *E. coli*, the values found for MCI/MI ranged from 0.012 to 0.1 mg/mL and for *C. albicans*, 0.005 mg/mL (48).

For BA, *S. aureus* presented MIC of 32 mg/mL and *E. coli*, 12 mg/mL for both MIC and MBC. The results of *S. aureus* for MBC were also high (24 mg/mL) for both compounds. The literature shows the efficacy of BA against bacteria such as *S. aureus*, *P. aeruginosa*, and *E. coli*, with MIC below 2 mg/mL, and lower efficacy against Gram-negative bacteria (49).

For PS/SB, *S. aureus* showed the best results for MIC/MBC (0.7–2.2 mg/mL and 1.5–4.5 mg/mL, respectively), followed by *E. coli* (6–18 mg/mL), *P. aeruginosa* and *C. albicans* (9.6–28.8 mg/mL and 19.2–57.6 mg/mL). In studies that evaluated these compounds separately, the MIC values for SB against *S. aureus*, *P. aeruginosa*, *E. coli*, *B. cepacia*, *C. albicans* and others were less than 0.01 mg/mL, and for PS, 5 to 50 mg/mL, which suggests the efficacy against these microorganisms is superior when these compounds are used alone when compared with combined uses (11, 50, 51).

CG presented MIC of 2 mg/mL and 4 mg/mL for *E. coli* and *C. albicans* and 4 mg/mL and 8 mg/mL for *P. aeruginosa*. However, in relation to the Gram-positive species *S. aureus*, CG presented higher MIC/MBC of 8 mg/mL and 16 mg/mL, respectively. These results suggest the promising potential of this compound as an alternative preservative. Also, when compared to ETX, it presented lower MIC and MBC values in general. Such promising use is also observed in a study that incorporated CG into an emulsion at 5 mg/mL, leading to inhibition of strains of *S. aureus*, *P. aeruginosa*, *E. coli*, and *C. albicans* in one day and *A. niger* in 28 days, at the preservative challenge test (24).

The highest MIC found for ETX were 32 mg/mL for *S. aureus* and 16 mg/mL for *P. aeruginosa*. For BA, *S. aureus* presented MIC of 32 mg/mL and *E. coli* showed 12 mg/mL for both MIC and MBC. The results of MBC for *S. aureus* were also high (24 mg/mL) for both compounds. The results obtained for ETX differ from the findings of Wang et al. (48), who reported concentrations of less than 3 mg/mL for wild-type strains *E. coli* and *S. aureus* isolated from the skin.

The antimicrobial efficacy values of cinnamon EO for *S. aureus* and *E. coli* were 0.093 to 0.7 mg/mL. For *P. aeruginosa* and *C. albicans*, higher values were obtained: 24 mg/mL for MBC and 12 mg/mL for MIC/MFC, respectively. In other studies, cinnamon EO showed MIC of 0.13 to 0.31 mg/mL for *E. coli* and 0.21 to 0.62 mg/mL for *S. aureus*; higher MIC was obtained for *P. aeruginosa* (0.55 to 300 mg/mL) and lower MIC for *C. albicans* (1 to 2 mg/mL). For MBC, 0.62 mg/mL for *S. aureus* and 1.25mg/mL for *E. coli* were reported (52-55).

The fungicidal activity of clove EO was superior when compared to cinnamon EO, with MIC/MFC values of 0.18 mg/mL for *C. albicans*. Hassan et al. (56) found, for clove EO, MIC of 1 mg/mL for *C. albicans* and *A. niger*. For the other bacteria, the values ranged from 0.3 to 3 mg/mL, lower for *P. aeruginosa*, with MIC of 0.3 mg/mL and MBC of 1.5 mg/mL. Recent studies with clove EO reported MIC values of 1.25 to 2.5 mg/mL for *E. coli*, 1.25 to 2.5 mg/mL for *S. aureus*, and 5 mg/mL for *P. aeruginosa*. For MBC, it maintained concentrations for *S. aureus* and *P. aeruginosa*, with lower efficacy against *E. coli* (1.25 to 10.9 mg/mL) (54,57,58).

The antimicrobial activity of cinnamon is due to three main bioactive components in its essential oil: eugenol, cinnamaldehyde, and linalool (59,60). The essential oil from cinnamon bark or leaf has antimicrobial activity in planktonic cells and biofilm (55,61,62). Clove EO has a high content of eugenol, a bioactive with rich antimicrobial activity (63,64). In leaves, eugenol represents around 95% of the extracted oil (65) and in cloves, it is also the main component of the oil, varying from 70% to 85% (66,67).

Although the antimicrobial activity results of PE were higher than other preservatives, MCI/MI and parabens are associated with a higher risk of allergy (12-15). Carcinogenic risks have also been reported with parabens (16,17). Due to such reported risks, for tests with combined compounds, PE was selected as the standard preservative based on its potential use. Also, when compared to other preservatives, the efficacy range of PE was within the pre-established limits for use in formulations in RDC 528/2021 and European Union regulation no. 1223/2009. This result was not obtained for BA and PS/SB, as MIC was very close to or exceeded the limits (39,40).

In tests with combined compounds, the combination of CG and PE was the most promising association, as it presented predominantly additive results (FICI = 0.75 to 1.0) when compared to the other associations. These results are interesting and show that such association can reduce the minimum inhibitory concentration and, consequently, reduce the amount required for efficacy.

In preservative challenge experiments, PE at 0.4% effectively inhibited growth over 28 days. A reduction of 2Logs was observed at day 14 for *Bcc*, *S. aureus, E. coli*, and *C. albicans*. The results for PE were approved according to USP criteria (44). For the PCPC parameters, only the results for the clinical strain of *Bcc* were accepted (43). The test failed for *P. aeruginosa* according to USP and PCPC, as there was no Log reduction on day 7 and day 14 when compared to the baseline. In contrast, previous studies indicated that PE at 0.5% was able to inhibit the growth of *P. aeruginosa* (68,69). Combinations of CG, PE and/or cinnamon EO were able to eliminate bacterial growth for strains of *Bcc* and *C. albicans* up to day 7, and for strains of *S. aureus* and *E. coli* up to day 14. All associations

were approved according to USP and PCPC criteria. In previous studies, the addition of 0.3% (w/w) CG significantly increased the preservative efficacy when combined with PE or benzoic acid/dehydroacetic acid, leading to about 50% improvement in the antimicrobial activity of the preservatives in the challenge test (70,71).

Other studies investigated the preservative activity of the combination of 1,2-hexanediol, CG, and PE against strains of *E. coli, P. aeruginosa, S. aureus,* and *C. albican*s. Concentrations between 0.5% 1,2-hexanediol and 0.3% CG showed efficacy against *E. coli, P. aeruginosa,* and *S. aureus*, and such efficacy was even higher with the addition of 0.3% PE. Regarding *C. albicans*, the combination of glycols was less effective, but the mixture of 0.3% PE, 0.3% 1,2-hexanediol, and 0.3% 1,2-octanediol reduced and suppressed the growth. In the same study, CG associated with another diol with lower antimicrobial activity showed lower efficacy than other associations of diols with PE. This finding highlights that PE can enhance the effect of CG (72).

Regarding the association of PE and cinnamon EO and/or PE alone, the results were comparable, suggesting that cinnamon EO did not enhance the antimicrobial effect of PE. *S. aureus* was less susceptible to PE, as its growth stopped only on day 28, while *Bcc* was more susceptible, showing no growth on day 7. The inferior performance of PE in challenge tests when compared to CG can be explained by the likely incompatibility of PE with non-ionic surfactants, as reported in previous studies (73,74).

According to our findings, CG and cinnamon EO are a promising combination, as it presented similar results to the association of glycol with PE. Recent studies observed the antimicrobial activity of essential oils varies according to the formula base, as hydrophilic formulations are more effective than lipophilic formulations, given the affinity of EOs to lipophilic components (75-77). A clinical trial compared the preservative activity of essential oils, including cinnamon EO, plant extracts, and parabens in skin creams. Cinnamon EO at 2.5% inhibited the growth of bacteria, yeast, and fungi, providing better results when compared to methylparaben and plant extracts (18). However, it is important to consider the safety aspects associated with cinnamon EO. In a toxic study, cinnamon EO at 2% triggered contact dermatitis (78). Also, a cell viability assay showed cinnamon EO at 0.1mg/mL had no cytotoxic effect on human skin cells (79). Combinations of essential oil with other antimicrobial agents can reduce the amount of oil used and consequently the allergenic potential.

Glycols (1,2 diols), due to their amphiphilic character and medium molecular size, present viscosity modulating properties, which complement their antimicrobial properties (80). As a possible mechanism of action, it destabilizes and disrupts the microbial cell membrane (81). Studies show that combining CG with phenethyl alcohol, glyceryl caprylate and ETX can result in a synergistic antimicrobial effect (24,82). CG at 0.5% has demonstrated antimicrobial activity against *S. aureus*, *P. aeruginosa*, *E. coli*, and *C. albicans* in cosmetic formulations, as analyzed in previous studies (23-25).

Combinations with CG (PE and/or cinnamon EO) offer potential preservative applications, as they may stop the microbial growth of different pathogens, including *Bcc* bacteria, and reduce the concentration of chemical preservatives used in applications. Considering the problem of *Burkholderia* complex resistance to preservatives, CG showed efficacy that can be compared to that of standard preservatives. Also, CG can offer more benefits from an economic and environmental perspective when used as a preservative as it presents, in addition to antimicrobial activity, properties that improve the organoleptic characteristics of the formulation and, consequently, the acceptance of cosmetic products by the consumer. Our results highlight the importance of understanding the potential interactions between preservatives and other ingredients in cosmetic formulations to use proper amounts of preservative. The amount of preservative required to effectively control the growth of bacteria and fungi can be considerably reduced by using essential oils or combined multifunctional compounds, creating conditions that do not favor the development of unwanted toxicity reactions in end products.

CONCLUSION

As an innovative alternative to traditional preservatives to reduce the use of chemical products in cosmetics, the combination of CG and PE and CG and cinnamon EO show potential antimicrobial

efficacy against common microorganisms found in cosmetic products, including the genus *B. cepacia*. However, further studies are required to evaluate the efficacy of these combinations in different formulations and assess the safety of essential oils in formulations for topical use.

DECLARATIONS

ACKNOWLEDGEMENTS

We would like to thank teacher Angelica Zaninelli Schreiber for providing us with bacterial samples, FAPESP (grant number 2021/10967-2) for its financial support, and Espaço da Escrita – Pró-Reitoria de Pesquisa – UNICAMP – for the language services provided.

CONFLICT OF INTERESTS

Sebastião Donizetti Gonçalves is the owner of Proserv Química, which supplied raw materials and provided partial funding for this research. This potential conflict of interest has been duly disclosed and does not compromise the objectivity or integrity of the data presented in this manuscript.

FUNDING

This study received a scholarship and Research Grant from the São Paulo Research Foundation (grant number 2021/10967-2) and financial support from the company ProServ Quimica ltda.

Ethical considerations: Not applicable.

Informed Consent Statement: Not applicable.

REFERENCES

1. Dao H, Lakhani P, Police A, et al. Microbial Stability of Pharmaceutical and Cosmetic Products. AAPS PharmSciTech. 2018;19(1):60-78. DOI: 10.1208/s12249-017-0875-1
2. Michalek IM, John SM, Caetano Dos Santos FL. Microbiological contamination of cosmetic products - observations from Europe, 2005-2018. J Eur Acad Dermatol Venereol. 2019;33(11):2151-2157. DOI: 10.1111/jdv.15728
3. Jimenez L. Microbial diversity in pharmaceutical product recalls and environments. PDA J Pharm Sci Technol. 2007;61(5):383-399.
4. Sutton SW, Jimenez L. A review of reported recalls involving microbiological control 2004 – 2011 with emphasis on FDA considerations of "objectionable organisms". Am Pharm Rev, 2012 15:42–57
5. Ali M. Burkholderia cepacia in pharmaceutical industries. Int J Vaccines Vaccin. 2016;3(2):11–12. DOI: 10.15406/ijvv.2016.03.00064
6. Tavares M, Kozak M, Balola A, Sá-Correia I. Burkholderia cepacia Complex Bacteria: a Feared Contamination Risk in Water-Based Pharmaceutical Products. Clin Microbiol Rev. 2020;33(3):e00139-19. Published 2020 Apr 15. DOI: 10.1128/CMR.00139-19
7. United States Pharmacopeia (USP). Microbiological examination of nonsterile products – tests for Burkholderia cepacia complex. United States Pharmacopeia 2019. Rockville, MD.
8. Wiener-Well Y, Segonds C, Mazuz B, Kopuit P, Assous MV. Successful outbreak investigation of Burkholderia cepacia complex bacteremia in intensive care patients. Am J Infect Control. 2014;42(5):580-581. DOI: 10.1016/j.ajic.2013.12.015
9. Antony B, Cherian EV, Boloor R, Shenoy KV. A sporadic outbreak of Burkholderia cepacia complex bacteremia in pediatric intensive care unit of a tertiary care hospital in coastal Karnataka, South India. Indian J Pathol Microbiol. 2016;59(2):197-199. DOI: 10.4103/0377-4929.182010
10. Wen X, Xie X, Zhang S, Sun T, Liu J, Li W. Burkholderia Cepacia Complex in Personal Care Products: Molecular Epidemiology and Susceptibility to Preservatives. J Cosmet Sci. 2020;71(3):133-148.
11. Rushton, L; Sass, A; Baldwin, A; et al. Key role for efflux in the preservative susceptibility and adaptive resistance of Burkholderia cepacia complex bacteria. Antimicrob Agents Chemother 2013, 57:2972–2980. DOI: 10.1128/AAC.00140-13
12. Shaughnessy CN, Malajian D, Belsito DV. Cutaneous delayed-type hypersensitivity in patients with atopic dermatitis: reactivity to topical preservatives. J Am Acad Dermatol. 2014;70(1):102-107. DOI: 10.1016/j.jaad.2013.08.046
13. Beene KM, Scheman A, Severson D, Reeder MJ. Prevalence of Preservatives Across All Product Types in the Contact Allergen Management Program. Dermatitis. 2017;28(1):81-87. DOI: 10.1097/DER.0000000000000259

14. Hollins LC, Hallock K, Disse M, et al. Occupationally Induced Allergic Contact Dermatitis to Methylchloroisothiazolinone/Methylisothiazolinone Among Water Bottle Plant Workers. Dermatitis. 2020;31(4):265-267. DOI: 10.1097/DER.0000000000000527

15. Sukakul T, Dahlin J, Pontén A, et al. Contact allergy to polyhexamethylene biguanide (polyaminopropyl biguanide). Contact Dermatitis. 2021;84(5):326-331. DOI: 10.1111/cod.13728

16. Darbre PD, Aljarrah A, Miller WR, Coldham NG, Sauer MJ, Pope GS. Concentrations of parabens in human breast tumours. J Appl Toxicol. 2004;24(1):5-13. DOI: 10.1002/jat.958

17. Dogan S, Tongur T, Erkaymaz T, et al. Traces of intact paraben molecules in endometrial carcinoma. Environ Sci Pollut Res Int. 2019;26(30):31158-31165. DOI: 10.1007/s11356-019-06228-1

18. Herman A. Comparison of antimicrobial activity of essential oils, plant extracts and methylparaben in cosmetic emulsions: 2 months study. Indian J Microbiol. 2014;54(3):361-364. DOI: 10.1007/s12088-014-0454-z

19. Alvarez-Rivera G, Llompart M, Lores M, Garcia–Jares C. Preservatives in Cosmetics. In: Analysis of Cosmetic Products. Elsevier, 2018, 2nd ed, pp. 175–224

20. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils-a review. Food Chem Toxicol. 2008;46(2):446-475. DOI: 10.1016/j.fct.2007.09.106

21. Kim SA, Rhee MS. Marked synergistic bactericidal effects and mode of action of medium-chain fatty acids in combination with organic acids against Escherichia coli O157:H7. Appl Environ Microbiol. 2013;79(21):6552-6560. DOI: 10.1128/AEM.02164-13

22. Cho TJ, Park SM, Yu H, et al. Recent Advances in the Application of Antibacterial Complexes Using Essential Oils. Molecules. 2020;25(7):1752. Published 2020 Apr 10. DOI: 10.3390/molecules25071752

23. Lawan, K; Kanlayavattanakul, M; Lourith, N. Antimicrobial efficacy of caprylyl glycol and ethylhexylglycerine in emulsion. J Health Res. 2009, 23(1): 1-3

24. Ziosi, P; Manfredini, S; Vandini, A; et al. Caprylyl glycol/phenethyl alcohol blend for alternative preservation of cosmetics. Cosmet Toilet 2013, 128:538–549.

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

26. Beilfuss W, Leschke M, Weber K. A new concept to boost the preservative efficacy of phenoxyethanol. 2005. SÖFW-journal 131:30–36

27. Ragno, R., Papa, R., Patsilinakos, A. et al. Essential oils against bacterial isolates from cystic fibrosis patients by means of antimicrobial and unsupervised machine learning approaches. Sci Rep 10, 2653 (2020). DOI: 10.1038/s41598-020-59553-8

28. Vasireddy L, Bingle LEH, Davies MS. Antimicrobial activity of essential oils against multidrug-resistant clinical isolates of the Burkholderia cepacia complex. PLoS One. 2018;13(8):e0201835. DOI: 10.1371/journal.pone.0201835

29. Jacobson M, Wray R, Kovach D, Henry D, Speert D, Matlow A. Sustained endemicity of Burkholderia cepacia complex in a pediatric institution, associated with contaminated ultrasound gel. Infect Control Hosp Epidemiol. 2006;27(4):362-366. DOI: 10.1086/503343

30. Ko S, An HS, Bang JH, Park SW. An outbreak of Burkholderia cepacia complex pseudobacteremia associated with intrinsically contaminated commercial 0.5% chlorhexidine solution. Am J Infect Control. 2015;43(3):266-268. DOI: 10.1016/j.ajic.2014.11.010

31. Uluer A, Marty F. Principles and practices of infectious disease, 8th ed, 2014.

32. Uwumiro F, Edigin E, Okpujie V. Atypical Burkholderia Cepacia Resistance to Ceftazidime/Avibactam and Co-trimoxazole: A Case of Open Wound Contamination and Persistent Bacteremia. Cureus. 2021;13(6):e15836. DOI: 10.7759/cureus.15836

33. Clinical and Laboratory Standards Institute (CLSI). Reference method for broth dilution antifungal susceptibility testing of yeasts—third edition: Approved standard M27-A3. CLSI Document M27-A3 and Supplement S., 2008.

34. Clinical and Laboratory Standards Institute (CLSI). Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious: Aproved guideline – Second Edition. CLSI document M45-A3, 2015

35. Cremers N, Belas A, Santos Costa S, Couto I, de Rooster H, Pomba C. In vitro antimicrobial efficacy of two medical grade honey formulations against common high-risk meticillin-resistant staphylococci and Pseudomonas spp. pathogens. Vet Dermatol. 2020;31(2):90-96. DOI: 10.1111/vde.12811

36. Murugesh J, Annigeri RG, Mangala GK, Mythily PH, Chandrakala J. Evaluation of the antifungal efficacy of

different concentrations of Curcuma longa on Candida albicans: An in vitro study. J Oral Maxillofac Pathol. 2019;23(2):305. DOI: 10.4103/jomfp.JOMFP_200_18

37. Odds FC. Synergy, antagonism, and what the chequerboard puts between them. J Antimicrob Chemother. 2003;52(1):1. DOI: 10.1093/jac/dkg301

38. Graziano TS, Cuzzullin MC, Franco GC, et al. Statins and Antimicrobial Effects: Simvastatin as a Potential Drug against Staphylococcus aureus Biofilm. PLoS One. 2015;10(5):e0128098. DOI: 10.1371/journal.pone.0128098

39. Agência Nacional De Vigilância Sanitária (Brasil). Resolução de Diretoria Colegiada - RDC nº 528, de 04 de agosto de 2021. Dispõe sobre a lista de substâncias de ação conservante permitidas para produtos de higiene pessoal, cosméticos e perfumes e internaliza a Resolução GMC MERCOSUL nº 35/20. Brasília, 2021

40. Union P. Regulation (EC) No 1223/2009 of the European Parliament and of the Council on cosmetic products. Official Journal of the European Union 2009, L342, 59–209.

41. Draelos ZD. The science behind skin care: Moisturizers. J Cosmet Dermatol. 2018;17(2):138-144. DOI: 10.1111/jocd.12490

42. Barzotto ILM, Oliveira SMM, Tavares B, Dallabrida S. Estabilidade de emulsões frente a diferentes técnicas de homogeneização e resfriamento. VA 2009, 10.

43. CTFA. Quality Assurance Guidelines. The Personal Care Products Council. In: Cosmetic T& FA (ed). Washington, 2007

44. United States Pharmacopeia. <51> Antimicrobial Effectiveness Testing. In: USP29-NF2. Rockville, 2012, MD, USA, p. 2499

45. Agência Nacional de Vigilância Sanitária (Brasil). Guia de Estabilidade de Produtos Cosméticos. 1st ed. Brasília: ANVISA, 2004.

46. Khan YH, Rasheed M, Alotaibi NH, et al. Role of Microbial Degradation on Drug Stability. In: Drug Stability and Chemical Kinetics. Springer Singapore, Singapore, 2020, pp 71–81

47. Lundov MD, Johansen JD, Zachariae C, Moesby L. Low-level efficacy of cosmetic preservatives. Int J Cosmet Sci. 2011;33(2):190-196. DOI: 10.1111/j.1468-2494.2010.00619.x

48. Wang Q, Cui S, Zhou L, et al. Effect of cosmetic chemical preservatives on resident flora isolated from healthy facial skin. J Cosmet Dermatol. 2019;18(2):652-658. DOI: 10.1111/jocd.12822

49. Bettini R, Cocconi D. Handbook of Pharmaceutical Excipients, Third Edition. Pharmaceutical Press, London, 2001

50. Stanojević D, Čomić L, Stefanović O. Synergy between Salvia officinalis L. and some preservatives. Open Life Sci. 2010; 5:491–495. DOI: 10.2478/s11535-010-0026-0

51. Dehghan P, Mohammadi A, Mohammadzadeh-Aghdash H, Ezzati Nazhad Dolatabadi J. Pharmacokinetic and toxicological aspects of potassium sorbate food additive and its constituents. Trends Food Sci Technol.2018; 80:123–130. DOI: 10.1016/j.tifs.2018.07.012

52. Teles AM, Rosa TDDS, Mouchrek AN, Abreu-Silva AL, Calabrese KDS, Almeida-Souza F. Cinnamomum zeylanicum, Origanum vulgare, and Curcuma longa Essential Oils: Chemical Composition, Antimicrobial and Antileishmanial Activity. Evid Based Complement Alternat Med. 2019;2019:2421695. Published 2019 Jan 15. DOI:10.1155/2019/2421695

53. Salma U, Sultana S, Saha SK, et al. In Vitro Antibacterial Activity of Ethanol Extracts of Cinnamomum zeylanicum against Pseudomonas aeruginosa. Mymensingh Med J. 2020;29(2):248-253.

54. Ramos AVG, Eninger MC, Amrein AM, et al. Effects of the combination of some essential oils with methylparaben and sodium metabisulfite against pathogenic foodborne bacteria J Food Process Preserv. 2022;46:e16459. DOI: 10.1111/jfpp.16459

55. Wijesinghe GK, Feiria SB, Maia FC, et al. In-vitro Antibacterial and Antibiofilm Activity of Cinnamomum verum Leaf Oil against Pseudomonas aeruginosa, Staphylococcus aureus and Klebsiella pneumoniae. An Acad Bras Cienc. 2021;93(1):e20201507. DOI: 10.1590/0001-3765202120201507

56. Hassan HA, Genaidy MM, Kamel MS, Abdelwahab SF. Synergistic antifungal activity of mixtures of clove, cumin and caraway essential oils and their major active components. J Herb Med. 2020; 24:100399. DOI: 10.1016/j.hermed.2020.100399

57. Selles SMA, Kouidri M, Belhamiti BT, Ait Amrane A. Chemical composition, in-vitro antibacterial and antioxidant activities of Syzygium aromaticum essential oil. Journal of Food Measurement and Characterization. 2020;14(4):2352-2358. DOI: 10.1007/s11694-020-00482-5

58. Al-Mijalli SH, El Hachlafi N, Abdallah EM, et al. Exploring the antibacterial mechanisms of chemically characterized essential oils from leaves and buds of Syzygium

aromaticum (L.) Merr. et Perry against Staphylococcus aureus and Pseudomonas aeruginosa. Ind Crops Prod 2023, 205:117561.

59. Hoque MM, Inatsu MB, Juneja V, Kawamoto S. Antimicrobial Activity of Cloves and Cinnamon Extracts against Food Borne Pathogens and Spoilage bacteria, and Inactivation of Listeria monocytogenes in Ground Chicken meat with their Essential oils. Rep Nat'l Food Res Inst, 2008; 72:9-21

60. Shahavi MH, Hosseini M, Jahanshahi M, et al. Evaluation of critical parameters for preparation of stable clove oil nanoemulsion. Arabian Journal of Chemistry, 2019; 12:3225–3230. DOI: 10.1016/j.arabjc.2015.08.024

61. Chouhan S, Sharma K, Guleria S. Antimicrobial Activity of Some Essential Oils-Present Status and Future Perspectives. Medicines (Basel). 2017;4(3):58. DOI:10.3390/medicines4030058

62. Park JB, Kang JH, Song KB. Antibacterial activities of a cinnamon essential oil with cetylpyridinium chloride emulsion against Escherichia coli O157:H7 and Salmonella Typhimurium in basil leaves. Food Sci Biotechnol. 2017;27(1):47-55. DOI: 10.1007/s10068-017-0241-9

63. Nascimento GGF, Locatelli J, Freitas PC, Silva GL. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Braz J Microbiol. 2000; 31:247-256. DOI: 10.1590/S1517-83822000000400003

64. Paoli S de, Giani TS, Presta GA, et al. Effects of clove (Caryophyllus aromaticus L.) on the labeling of blood constituents with technetium-99m and on the morphology of red blood cells. Braz Arch Biol Technol. 2007; 50:175–182. DOI: 10.1590/S1516-89132007000600022

65. Raina VK, Srivastava SK, Aggarwal KK, et al. Essential oil composition of Syzygium aromaticum leaf from Little Andaman, India. Flavour Fragr J. 2001; 16:334–336. DOI: 10.1002/ffj.1005

66. Brown PD, Morra MJ. Glucosinolate-containing plant tissues as bioherbicides. J Agric Food Chem. 1995; 43:3070–3074. DOI: 10.1021/jf00060a015

67. Ortiz EL. The Encyclopedia of Herbs, Spices, and Flavourings, 1992

68. Puschmann J, Herbig ME, Müller-Goymann CC. Correlation of antimicrobial effects of phenoxyethanol with its free concentration in the water phase of o/w-emulsion gels. Eur J Pharm Biopharm. 2018;131:152-161. DOI: 10.1016/j.ejpb.2018.08.007

69. Jeong J-J, Kim D-H. Effects of Cosmetics and Their Preservatives on the Growth and Composition of Human Skin Microbiota. J Soc Cosmet Sci Korea. 2015; 41:127–134. DOI: 10.15230/SCSK.2015.41.2.127

70. Janichen J. The quest for the ideal preserving system-reducing traditional preservatives in combination with Dermosoft Octiol. Eur Cosmet. 2004; 7/8: 10–16

71. Rigano L, Leporatti R. Systemic canstellations: with or without preservatives? SOFW J, 2003; 129;30-43

72. Choi EY. Effect of phenoxyethanol and alkane diol mixture on the antimicrobial activity and antiseptic ability in cosmetics. Kor J Aesthet Cosmetol. 2015; 13(2): 213-220

73. Sheskey PJ, Cook WG, Cable CG. Handbook of Pharmaceutical Excipients, 8th ed. Pharmaceutical Press, 2017

74. Schmitt P de O, Fischer AF, Silva RML da, Cruz AB. Compatibility and efficiency of preservatives in emulsive cosmetics containing high surfactant content. Braz J Pharm Sci. 2022; 58: e191088 https://DOI.org/10.1590/s2175-97902022e191088

75. Tomczykowa M, Wróblewska M, Winnicka K, et al. Novel Gel Formulations as Topical Carriers for the Essential Oil of Bidens tripartita for the Treatment of Candidiasis. Molecules. 2018;23(10):2517. DOI:10.3390/molecules23102517

76. Gemeda N, Tadele A, Lemma H, et al. Development, Characterization, and Evaluation of Novel Broad-Spectrum Antimicrobial Topical Formulations from Cymbopogon martini (Roxb.) W. Watson Essential Oil. Evid Based Complement Alternat Med. 2018;2018:9812093. DOI:10.1155/2018/9812093

77. Cunha C, Ribeiro HM, Rodrigues M, Araujo ARTS. Essential oils used in dermocosmetics: Review about its biological activities. J Cosmet Dermatol. 2022;21(2):513-529. DOI:10.1111/jocd.14652

78. de Groot AC. Fragrances and Essential Oils. Press Taylor and Francis Monographs in Contact Allergy, 2019, volum 2

79. Wanakhachornkrai O, Banglao W, Thongmee A, Sukplang P. Determination of antioxidant, anti-aging and cytotoxicity activity of the essential oils from cinnamomum zeylanicum. J Microbiol Biotechnol Food Sci. 2020; 10:436–440. DOI: 10.15414/jmbfs.2020.10.3.436-440

80. Halla N, Fernandes IP, Heleno SA, et al. Cosmetics Preservation: A Review on Present Strategies. Molecules. 2018;23(7):1571. Published 2018 Jun 28. DOI:10.3390/molecules23071571

81. Ibarra F, Johnson CH. Natural Preservation from Concepts in Nature. Cosmetics and toiletries. 2008; 81-90

82. Manning M, Orawski P. Food-grade esters used as personal care antimicrobials. Cosmetics and toiletries. 2005; 120: 63-68