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Formulation and stability evaluation of a cosmetics emulsion loaded with different concentrations of synthetic and natural preservative

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Abstract

Essential oils are currently the topic of extensive scientific research, and their potential as active pharmacological chemicals or natural preservatives has attracted the attention of the cosmetic and pharmaceutical industries. Modern cosmetics, with their complex compositions, watery formulations, and direct contact with bacterial skin flora, provide excellent habitat for microbe growth. The use of preservatives is required due to the significant danger of contamination and, as a result, a risk to customers' health. The aim of the study was to formulate and subsequently evaluate the stability of a cosmetic emulsion based on different concentrations of synthetic and natural preservatives. We have performed a cosmetic emulsion base with triethanolamine as an emulsifier. Four samples were prepared using the formulation base and a synthetic preservative, methylparaben, in concentrations 0.2% and 0.3%, and a natural preservative, lemongrass essential oil, in concentrations, 1%, and 2%. The samples were stored for 4 weeks in room conditions. They were evaluated immediately after preparation and for four weeks related to smell, color, odor, consistency, homogeneity, pH, phase separation, viscosity changes and microbial growth. After two weeks, there was a phase separation in two formulations with methylparaben. After four weeks, other formulations were stable in odor, appearance, color, homogeneity. The essential oils didn't significantly affect the pH values of the formulation; it was in the recommended range. Adding the essential oil affected the viscosity of the formulation base, and the more the concentration of essential oil was more the viscosity of the formulation lowered. The emulsions formulated with lemongrass essential oil had no microbial and fungal growth, so the self-preserving effect was demonstrated. We

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concluded that cosmetic emulsion entrapped with lemongrass essential oil in a concentration of 1% showed promising stability, physicochemical characteristics and self-preserving effect in room storage conditions.

Keywords: Cosmetic emulsion, Methylparaben, Lemongrass essential oil, Stability, Self-preservation

1 Introduction

Emulsions have a high potential for the pharmaceutical industry, agricultural, food products, paints and cosmetics (Waqas et al., 2014). Cosmetics emulsions are colloidal systems made up of two liquid phases: oil and water phase, with one distributed in the other (Schramm, 2006, Kale and Deore, 2017). Oil-in-water (O/W) emulsions consist of an oil phase dispersed in a continuous aqueous phase as tiny droplets (Colucci et al., 2020) Consumers prefer this type of emulsion, but it poses the greatest danger of contamination due to the large volume of water in its formulation, which serves as a perfect growth factor for bacteria.

Nowadays, contamination of cosmetic items poses a health concern to consumers. According to the European Commission's (EC) Rapid Alert System (RAPEX), 62 cosmetics goods were recalled by the market between 2008 and 2014 owing to microbial contamination. 14 nations were notified of the recalled products, with the number of recalls increasing in 2013 and 2014 (Neza and Centini, 2016).

The quality and purity of ingredients (especially the quality of water), hygiene of the technological process (organization of the production, cleanliness of the devices used), physicochemical properties of the final product, type of packaging and packaging materials, or the presence of microbial inhibitors, i.e., preservatives, are all factors that influence the microbiological purity of cosmetics (including emulsions). Preservatives are added to cosmetics to keep them microbiologically pure during manufacturing, packing, storage, and, most importantly, during usage. They have an antibacterial effect, and when added to cosmetics, they prevent bacteria, mold, and fungi from growing, extending the shelf life. A list of 55 chemicals allowed for use as preservatives in cosmetics may be found in Annex V of EU Directive 1223/2009 (Sikora, 2019). Despite the fact that preservatives are often employed in low concentrations, they are one of the leading causes of allergies in consumers (Wong et al., 2000).

Methylparaben is the major component of the preservative system in most cosmetics products on the market, and its antibacterial action has been well documented (European Commission, 2014). Although the various benefits of methylparaben as a preservative, several researches suggest that this ingredient in cosmetic formulas may represent some health hazards (Okamoto et al., 2008; Handa et al., 2006).

Five parabens were added to the list of prohibited compounds in cosmetic products in an amendment published in the Official Journal on April 9, 2014: isopropylparaben, isobutylparaben, phenylparaben, benzylparaben, and pentylparaben. Furthermore, other than the esters listed above, hydroxybenzoic acid and its salts and esters are restricted to a maximum acid content of 0.4 percent for a single ester and 0.8 percent for mixes of esters (Andersen, 2008). As a result, there is an increasing demand for preservative-free cosmetics. The use of chemicals (e.g., herbal extracts and essential oils) that are not preservatives but have antibacterial action is an alternative technique to handle the problem of microbiological purity in cosmetics.

Natural preservatives appear to be a viable and practical concept because plant-derived chemicals are widely available and environmentally beneficial. Essential oils such as tea tree (*Melaleuca alternifolia*), thyme (*Thymus vulgaris*), lemongrass (*Cymbopogon citratus*), oregano (*Origanum vulgare*), rosemary (*Rosmarinus officinalis*), calamint (*Calamintha officinalis*), or lavender (*Lavendula officinalis*) have received a lot of attention recently because they have antimicrobial properties and have been suggested as natural preservatives (Dreger and Wielgus, 2013). Essential oils, properly selected and used at a concentration of 0.5–2.5%, demonstrate a high antimicrobial potential (Adaszyńska and Swarcewicz, 2012, Sikora, 2011). Several studies demonstrate that: tea tree, lemongrass, oregano, and clove are the most promising choices, having MICs (minimal inhibition concentrations) of less than 1% against a wide spectrum of gram-positive and gram-negative bacteria (with the exception of *Pseudomonas aeruginosa*) (Dreger and Wielgus, 2013).

The aim of the study was to formulate and subsequently evaluate the stability of a cosmetic emulsion based on different concentrations of synthetic and natural preservatives.

2 Materials and Methods

2. 1. Materials and apparatus

All the following ingredients used for the cosmetics emulsions formulation: stearic acid, cetyl alcohol, paraffin oil, glycerin, triethanolamine (TEA) and methylparaben were analytical degree, purchased from Sigma Aldrich, 100% pure lemongrass essential oil (LEO) (*Cymbopogon flexuosus*), was purchased from Flora, Salute & Benessere (Lorenzana, Italy) Lotto nr. 170678.

Digital pH meter HANNA checker (Romania), Water bath (China), Viscosimeter (NDJ 1), Electrical balance (G & G, Germany), Analytical balance (Ohaus corporation, USA), Centrifuge machine (TDL 80-2B, Hinotech lab), Mechanical mixer (IKA, Germany), Refrigerator (Hisense, Italia), Electro Thermal Incubator, Microscope (Micros, Austria), DIP-SLIDES (cosmetics bacteria & yeasts/ microbial test kits, United Kingdom) and SPSS version 21.0, were used.

Staphylococcus aureus ATCC 6538 and *Candida albicans* ATCC 10231 (purchased from Profarma, Albania) were used for microbial quality control studies.

2. 2. Preparation of the samples

Selection of raw materials used in skin care emulsion composition and their content (%), as well, were performed according to Sicora E, Monograph 2019 and IFSCC Monograph Nr. 4, 1997. The cosmetics emulsion base formulation containing TEA stearate as an emulsifier agent was conducted in many trials until the final formulation was in the desired consistency and appearance. The preservatives ingredient added in the sample's preparation were a synthetic preservative agent, methylparaben and a natural one, lemongrass essential oil: both of them were added in different concentrations. According to, Mukarram et al., 2022, lemongrass essential oil possess a great antimicrobial and antifungal properties. All the formulations were prepared in the laboratory according to the composition summarized in table 1.

Table 1. Composition of the prepared cosmetic emulsions: E1, emulsion base, without methylparaben/essential oil; E2, emulsion with methylparaben 0.2%; E3, emulsion with methylparaben 0.3%; E4, emulsion with essential oil 1% and E5, emulsion with essential oil 2%

Phase	Ingredient	Function	Percentage by weight				
			E1	E2	E3	E4	E5
	stearic acid	emollient	4.5	4.5	4.5	4.5	4.5
A	cetyl alcohol	consistency/thickening agents	1.5	1.5	1.5	1.5	1.5
	paraffin oil	emollient	4.0	4.0	4.0	4.0	4.0
	glycerin	humectants	3.0	3.0	3.0	3.0	3.0
B	triethanolamine	emulsifier	q.s	q.s	q.s	q.s	q.s
	distilled water	diluent/solvent	q.s	q.s	q.s	q.s	q.s
C_1	methylparaben	preservative *synthetic	-	0.2	0.3	-	-
C_2	lemongrass essential oil	preservative *natural	-	-	-	1	2

A, Oil phase; B, water phase; C1, preservative in solid phase; C2, heat-sensitive preservative and fragrance

Preparation of the emulsion base, E1: oil phase composed of stearic acid, cetyl alcohol and paraffin oil was heated up to 70 ± 1°C. At the same time, an aqueous phase composed of glycerin, water and triethanolamine was also heated to the same temperature. The aqueous phase was then added to the oil phase drop by drop with continuous stirring at 1700 rpm with the use of a mechanical mixer for around 13 minutes until the aqueous phase was completely added. After the aqueous phase was entirely added, the mixer's speed was dropped to 1000 rpm for 5 minutes of homogenization and then to 500 rpm for 5 other minutes (figure 1).

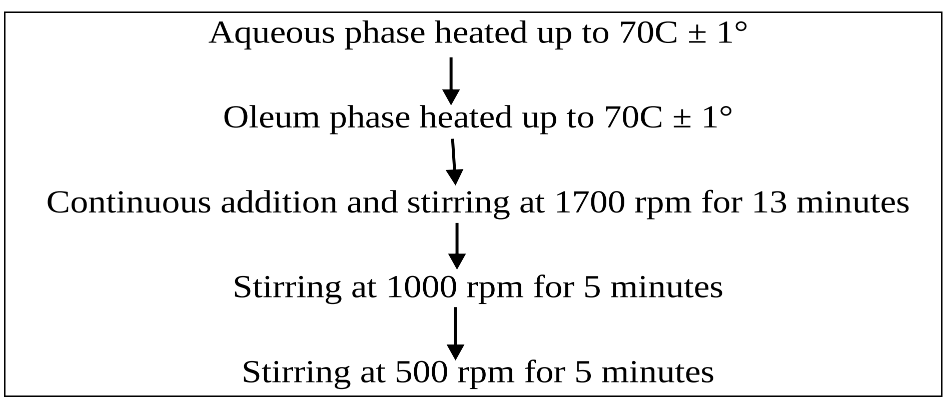

Figure 1. Preparation of the cosmetic emulsion base

The preservative, methylparaben was added in the water phase, before mixing (E2 & E3). Lemongrass essential oil was added when the temperature downs to 35°C (E4 & E5). The temperature at which essential oils are applied to composition has a big impact on the quantity of essential oils that end up in the environment. According to a study, essential oils should be used in cosmetic formulations at temperatures below 40°C (Sarkic and Stappen, 2018).

2. 3. Stability evaluation

Evaluation of cosmetics stability is necessary to ensure the quality, safety, and efficacy of products with the potential for commercial success.

The behavior of all formulations was studied further for 4 weeks, towards natural light in room conditions (they were exposed to daylight source with photoperiodicity 10 hours of light and 14 hours of dark). The samples were evaluated for four weeks with respect to smell, color, odor, consistency, homogeneity, pH, phase separation, viscosity changes and microbial growth. The fresh emulsions were tested one day after the preparation, in order for the samples to be stabilized.

2. 3. 1. Microscopic analyses

Cosmetics emulsions were analyzed under a microscope to confirm the dispersity scale of the oily phase in water one. A drop of each emulsion was placed on the glass slide and covered by a glass cover.

2. 3. 2. Physical analyses

Emulsions were tested both organoleptically (appearance, odor and color) and physically (consistency and homogeneity and phase separation,) through the naked eye.

2. 3. 3. pH tests

A digital pH-Meter was used to determine the pH of freshly made emulsions and emulsions that had been stored at room conditions for four weeks. The apparatus was standardized using pH 4.0 and 7.0 standard buffers before use, and the pH was determined at 20±3°C. The pH tests were repeated for multiple emulsions after 24 hours, 7 days, 14 days, 21 days and 28 days of preparation. The samples were analyzed in triplicate and the average value was determined.

2. 3. 4. Viscosity tests

A viscosimeter NDJ 1 was used for rheological studies. The sample (70-100 g) was placed in a beaker and was allowed to equilibrate for 5 min before measuring the dial reading using the appropriate rotor at 30 rpm, at 20±2°C. Direct multiplication of the dial readings with factors given in the viscometer catalog gave the viscosity in centipoises. The average of three triplicates was computed.

2. 3. 5. Contamination tests

The microbial stability of formulations was evaluated through contamination test using dip-slides kits for cosmetic contamination test. The plates were taken out after the incubation time (24-48 hours at 30-35°C) and examined for microbial growth by comparing them to the positive control of two bacteria: Staphylococcus aureus, ATCC 6538 and Candida albicans, ATCC 10231.

2. 4. Statistical analysis

The statistical program SPSS version 21.0 according to two-way ANOVA test defining a 5%

level of significance was used for all statistical operations. The data is presented in the form of means and standard deviations.

3 Results and Discussions

After application on the skin, the created emulsion was easy to scoop, homogenous, formed a cone, and left a fine fatty coating (figure 2).

Figure 2. Cosmetics emulsion with TEA (base), methylparaben and lemongrass essential oil

3. 1. Optical microscopy analyses

Cosmetic emulsions were analyzed under an optical microscope, the homogeneity and the dispersity scale are presented in figures 3 and 4.

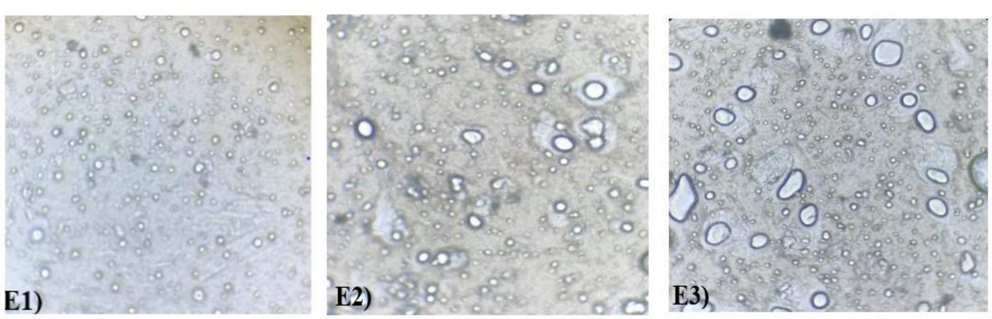

Figure 3. Microscopic view of the fresh emulsions E1, E2 and E3

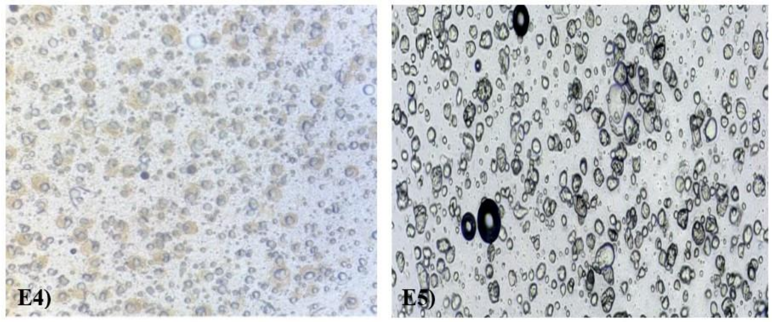

Figure 4. Microscopic view of the fresh emulsions, E4 and E5

TEA stearate, as an emulsifier, is the triethanolamine salt of stearic acid. It is used in cosmetic formulations as a surfactant-cleansing agent or a surfactant-emulsifying agent in many leave-on and rinse-off formulations: baby bath, eye make-up, fragrance, hair, make up, nails, personal cleanliness, shaving, skin and suntan preparations (Andersen, 1995). TEA stearate is a cream-colored wax-like solid witch gives stable emulsions with a higher dispersion rate. The emulsion formulated with acid stearic and TEA is in white color and very smooth.

3. 2. Organoleptic and physical characterization

These tests were conducted out over a four-week period of storage, under room conditions, at regular intervals, every week (Table 2, 3, 4, 5).

Table 2 shows the organoleptic and physical parameters of the formulation`s stability after the first two weeks, there are no changes.

Table 2. Organoleptic and physical parameters of cosmetics emulsions after the first two weeks

Emulsion code	Appearance	Odor	Color	Consistency	Homogeneity	Phase separation
E1	S	N	W	V	H	-
E2	S	N	Y	V	H	-
E3	S	N	Y	V	H	-
E4	S	SS	YW	V	H	-
E5	S	SS	YW	V	H	-

S: Smooth (uniform, without lumps and impurities), N: Noticeable, SS: Strong smell, W: White, Y: Yellow, YW= Yellowish White, V: Viscous, H- homogeneous, -: No phase separation

The freshly prepared base was white and uniformed, whereas the formulations E2 and E3, were yellow (due to the presence of methylparaben); E4 and E5 were yellowish-white (due to the presence of yellow lemongrass essential oil). The samples E4 and E5 had a strong odor (similar to the lemon) due to the odor of the essential oil, but it is a pleasant and acceptable aroma. There was no change in the parameters of the five formulations, up to the observation period of 14 days.

Table 3 shows the organoleptic and physical parameters of the formulation`s stability after the third week.

Table 3. Organoleptic and physical parameters of cosmetics emulsions after the third week

Emulsion code	Appearance	Odor	Color	Consistency	Homogeneity	Phase separation
E1	S	N	W	V	H	-
E2	S+	N	Y	V	H+	+
E3	S+	N	Y	V	H+	+
E4	S	SS	YW	V	H	-
E5	S	SS	YW	V+	H	-

S: Smooth (uniform, without lumps and impurities), S+: change in appearance, N: Noticeable, SS: Strong smell, W: White, Y: Yellow, YW= Yellowish White, V: Viscous, V+: change in viscosity, H-homogeneous, H+-change in homogeneity, -: No phase separation, +: Phase separation

The samples E2 and E3 were found to be unstable at room conditions as shown in Table 2 and these samples were not selected for further "*in vitro*" study. Samples E2 and E3 had a crystallization which leads to phase separation and emulsion instability. This phenomenon may be due to any possible interaction of methylparaben with any of the components or to the presence of any insoluble crystal of the methylparaben in the aqueous medium (even its concentrations are within use and solubility limitation).

Another alternative of formulation`s instability is the pH value: acidic preservatives (acids, phenol derivatives, parabens) are active only in an acidic environment (in non-dissociated forms), but they dissociate and lose their activity in formulations with an alkaline pH (Sikora, 2019).

Table 4 shows the organoleptic and physical parameters of the formulation`s stability after the fourth week.

Table 4. Organoleptic and physical parameters of cosmetics emulsions after the fourth week

Emulsion code	Appearance	Odor	Color	Consistency	Homogeneity	Phase separation
E1	S	N	W	V	H	-
E4	S	SS	LY	V	H	-
E5	S	SS	LY	V	H	-

S: Smooth (uniform, without lumps and impurities), N: Noticeable, SS: Strong smell, W: White, YW= Yellowish White, V: Viscous, H-homogeneous, -: No phase separation

The stability of the emulsion base, E1, and the two formulations, E4 and E5, throughout the observation period may be attributed to different contributing factors. The ingredients of the non-aqueous portion are cetyl alcohol (fatty alcohol) and paraffin oil (hydrocarbons), which are fatty constituents and contribute in the stability of the system. TEA stearate is an excellent emulsifier that is formed "ex tempore" and produces a highly stable emulsion. Glycerin is a humectant and helps to maintain the water in the formulation. Lemongrass essential oil is added to the formulation: it affects the viscosity of the formulation but not in the phase separation. The major constituents of LEO are citral (mixture of geranial and neral) (26.1%), isoneral, isogeranial, geraniol, geranyl acetate (2.27%), and citronellol giving to the essential oil the antimicrobial, antifungal, antiviral and antioxidant effects (Hanaa et al., 2012). This could help preserve the formulation from degradation by organisms that produce chemicals that cause color changes in the formulation during storage.

3. 3. pH values

The average values of the pH for the samples E1, E4 and E5 are presented in table 5.

Table 5. pH and viscosity parameters of the cosmetics formulations

	pH (mean ± SD)			viscosity at 30 rpm (CPS) (mean ± SD)		
Period of time	E1	E4	E5	E1	E4	E5
1 day	6.81±0.01	6.89±0.02	7.02±0.01	5100±13.27	4595±12.34	3400±22.4
7 days	6.79±0.02	6.75±0.01	7.0 ±0.01	5100±15.43	4580±13.44	3350±18.55
14 days	6.83±0.02	6.78±0.03	7.05±0.01	5050±19.67	4550±12.67	3300±20.5
21 days	6.8±0.01	6.8±0.02	7.04±0.01	5030±21.89	4455±23.21	2800±16.69
28 days	6.85±0.02	6.82±0.02	7.08±0.02	5000±22.44	4400±18.94	2750±18.91

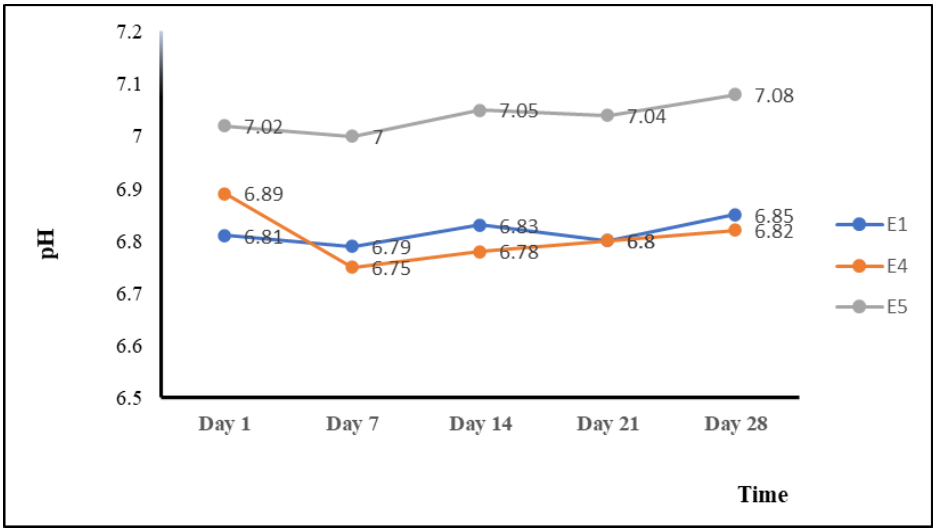

Figure 5. pH values of samples stored at room conditions

The pH of human skin normally ranges from 4.5 to 6.0, with 5.5 being the average pH (Akhtar et al., 2008). As a result, if the formulation is meant for skin application, the pH should be closer to this range. In the present study, the pH of the fresh formulations with 1% and 2% LEO showed an increase, while it decreased after the first week and then it showed some variations. No significant changes in pH were observed for any of the formulations. This result showed that the pH of the formulations E1, E4 and E5 was stable at temperature and light variation during the storage period. At the end of the study, the pH of the samples was found to be between 6.75±0.01 to 7.08±0.02 which is within the range (presented graphically in figure 5). Although neutral, this pH is typical of cosmetic preparations containing TEA stearate and acceptable by the ISI standards.

3. 4. Viscosity

Emulsion viscosity is an important element in their flow properties. The viscosity of an emulsion is a very useful tool for determining its quality and stability. The prepared emulsions are susceptible to temperature and storage period, resulting in decreased viscosity and increased liquefaction. The rate of sedimentation is inversely proportional to the external phase's viscosity, according to Stokes's law. As a result of this component, the more the viscosity of the formulation lowers, the more liquefaction is evident (Ghosh and Rousseau, 2011). Separation of phases, creaming and sedimentation, occurs in emulsions when the densities of the water and oil phases differ under the action of gravity, resulting in phase separation. Creaming is the upward movement of the scattered globules, whereas sedimentation is the downward movement of the dispersed phase. Furthermore, emulsions are thermodynamically unstable systems, and droplets in the dispersed phase combine to create larger droplets, causing the increase of the coalescence rate. One of the underlying causes of emulsion breakage is coalescence (Mao and Miao, 2015).

In our study, the viscosity values of all the formulations were found to be in the range of 2750±18.91to 5100±15.43 cps at 30 rpm as shown in table 5 and graphically in figure 6. All the formulations were showed pseudoplastic flow.

The viscosity of formulation E4 (LEO 1%) and E5 (LEO 2%), is lower than E1, the addition of essential oils affects the consistency of the emulsions. Sample E5 formulated with 2% LEO showed a significant difference in the viscosity after three weeks of storage.

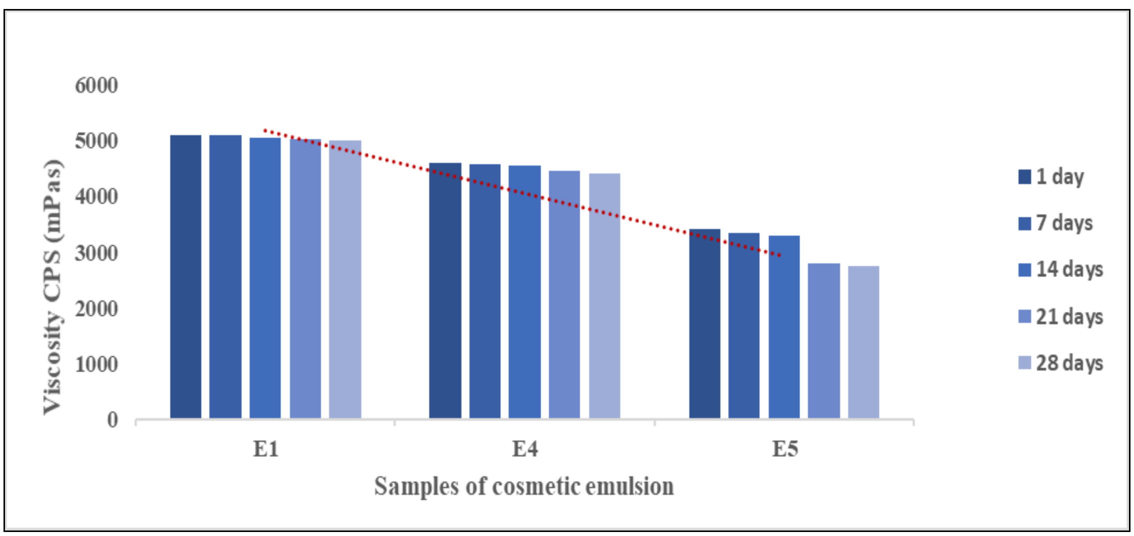

Figure 6. Viscosity values of samples kept at room conditions

3. 5. Contamination tests

As indicated in figure 7 and figure 8, no microbiological growth was seen in formulations including both natural preservative concentrations (1% and 2%). The obtained results had confirmed the microbial stability of our formulations.

Consumer safety and the cosmetics industry's reputation are both threatened by microbial contamination. As a result, it's critical to assess a product's microbiological stability to assure its safety.

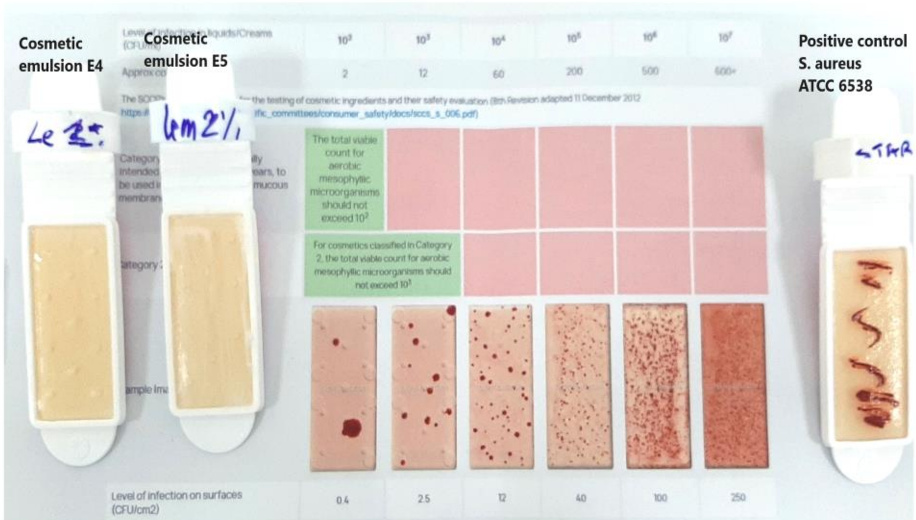

Figure 7. Bacterial stability evaluation of the various formulations

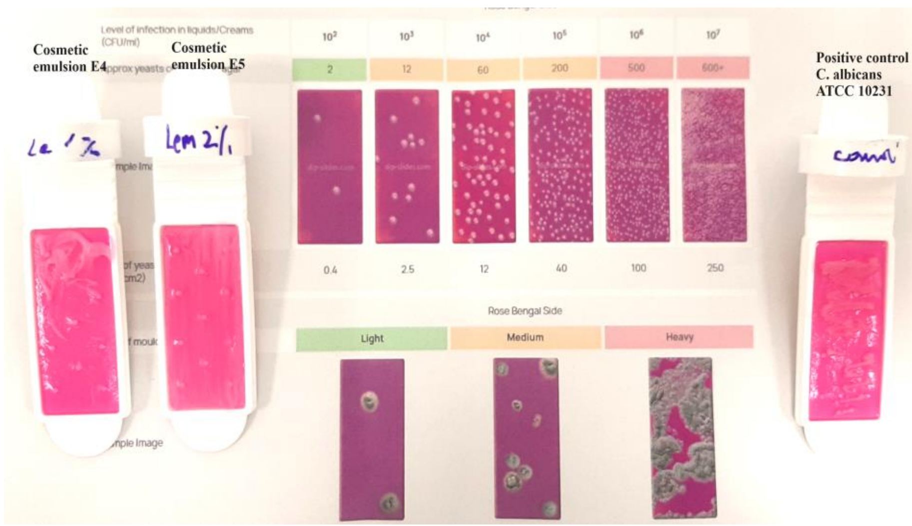

Figure 8. Yeast stability evaluation of the various formulations

Nowadays, cosmetics consumers have questioned the safety of chemical preservatives. Preservatives, usually, are used in small quantities and low concentrations as well. They may represent a risk to human health by triggering allergies and, in some circumstances, such as methylparaben, by creating free radicals when exposed to sunlight (Handa et al., 2006). Despite their reputation for safety, parabens have been linked to a variety of health issues, including breast cancer and estrogen-like characteristics (Kirchhof and de Gannes, 2013). As a result, increased demand for natural and preservative-free cosmetics was launched, the notion of replacing synthetic preservatives with natural antibacterial substances was born. Essential oils have strong antibacterial properties against germs and fungi, showing a great potential for the creation of stabilized micellar systems which is meaningful in regard to their utilization in cosmetic formulations (Edris and Malone, 2012).

4 Conclusion

In our study, we realized to formulate and optimized a cosmetic emulsion with triethanolamine stearate as an emulsifier. After two weeks, there was a phase separation in the two formulations with methylparaben. There was no change in appearance, homogeneity, color, odor and no phase separation, in the basic emulsion, E1, and formulation with different concentrations of essential oils, E4 and E5, for four weeks of storage in room conditions. Adding the essential oils didn't significantly affect the pH values of the basic formulation (the pH value was in the recommended range) but affected its viscosity; and the more the concentration of essential oil added the more the viscosity of the formulation lowered. The viscosity of the samples with the higher concentration of LEO significantly decreased in room condition, essential oil is affected by light and room temperature. The emulsions formulated with lemongrass essential oil had no microbial and fungal growth, so the self-preserving effect was demonstrated. From the current study, we concluded that cosmetic emulsion entrapped with lemongrass essential oil in a concentration of 1% showed promising stability, physicochemical characteristics and self-preserving effect in room storage conditions. Our research has been proved to be favorable in terms of future possible benefits of Lemongrass essential oil in dermo-cosmetic products.

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Conflict of interests

The authors declare that there are no competing interests.

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