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Original Article

Selected Essential Oils as Natural Ingredients in Cosmetic Emulsions: Development, Stability Testing and Antimicrobial Activity

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ABSTRACT

Background: Essential oils are currently the subject of intensive scientific study, and their potential as active medicinal compounds or natural preservatives has attracted the attention of the cosmetic and pharmaceutical industries. **Objectives:** The objectives of the present study were: to develop a cosmetic emulsion based on different concentrations of synthetic preservative and essential oils, applied as natural preservatives; to evaluate and subsequently compare their stability and antimicrobial activity. **Materials and Methods:** Various samples were prepared using different concentrations of methylparaben, and essential oils as well. The fresh samples were evaluated immediately after preparation related to organoleptic and physicochemical testing. The behavior of the samples related to light tests, accelerated tests, and microbiological stability tests as well were performed for four weeks of storage. **Results:** During the accelerated stability test, formulations loaded with 2% essential oils were not stable as was noted a variation in the color and liquefaction in the second week of storage at 40°C; their rheological behavior showed a significant decrease in the viscosity, varying from 4890±34.7 to 3720±37.9. For all the samples, the pH levels were within the physiological skin pH range, it varied from 6.01±0.33 to 6.92±0.19. The self-preserving activity of all the cosmetic emulsions was satisfactory. **Conclusion:** Cosmetic emulsions loaded with essential oils, in the concentration of 1% showed promising stability, physico-chemical characteristics, and self-preserving properties, in different storage conditions. The formulations loaded with essential oils provide a novel alternative for the preservation of "paraben-free" cosmetic products.

Keywords: Cosmetic emulsion, Essential oils, Methylparaben, Formulation, Stability testing, Self-preserving activity.

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INTRODUCTION

Modern cosmetics, due to their complex compositions, watery formulations, and direct contact with bacterial skin flora, provide excellent habitats for microbe growth. Furthermore, bacterial contamination modifies the physical and chemical properties of cosmetics, resulting in phase separation, discoloration, and odor release.[1] The use of preservatives is required due to the high danger of contamination resulting in a risk to the health of consumers. Antimicrobial compounds are added to cosmetics to protect them from microbial contamination caused by raw materials, production processes, and consumer use. The derivates of paraben are the most extensively used preservatives in cosmetic preparation.[2]

In December 2005, the cosmetic ingredient review restarted the safety evaluation for parabens.[3] The finding of parabens in breast cancer cells and surrounding tissues triggered a discussion regarding the safety of parabens and suggested a connection between breast cancer and parabens.[4-6] Based on a variety of research, the European Commission set a maximum of 0.4 percent of paraben in cosmetic products.[7] Although most other countries still permit the use of parabens business has started to respond to the negative public perception of parabens by labeling their products as "paraben-free".[8] Additionally, it has been reported that some bacterial strains are resistant to conventional. All of these issues have directed the search for new preservation systems.

Nowadays the attention has been focused on essential oils that demonstrate antimicrobial properties and can easily react as

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natural preservatives, such as tea tree (*Melaleuca alternifolia*), thyme (*Thymus vulgaris*), lemongrass (*Cymbopogon citratus*), oregano (*Origanum vulgare*), rosemary (*Rosmarinus officinalis*), or lavender (*Lavandula officinalis*).[9-11] Essential oils, when utilized properly, in a concentration of 0.5–2.5 percent have great antibacterial potential.[12,13] Despite their natural origins, essential oils can cause allergic reactions; to minimize the risk of those effects the recommended concentration is 1-2%.[14]

Tea tree oil (TTO), is a volatile essential oil extracted from *Melaleuca alternifolia*. TTO.[15] In the early 1990s, many papers describe TTO's antibacterial activity; most bacteria are susceptible to TTO at concentrations of 1% or less, although it has been discovered that some organisms, including cutaneous *Staphylococci, Enterococcus faecalis,* and *Pseudomonas aeruginosa* have MICs of more than 2%.[16-19]

Lemongrass essential oil (LEO) derives from the plant *Cymbopogon flexuosus*.[20,21] The bactericidal effects of LEO are well established.[22-24] It has been reported that LEO causes bacterial biofilms to dissolve, preventing further bacterial growth and proliferation.[25] Additionally, LEO components can destroy bacterial membranes and kill them by weakening the connections between lipid bilayers.[26,27]

The objectives of the present study are: to develop a cosmetic emulsion based on different concentrations of synthetic preservative and essential oils, applied as natural preservatives; to evaluate and subsequently, compare their stability and antimicrobial activity.

MATERIALS AND METHODS

Reagents

Stearic acid, cetyl alcohol, paraffin oil, glycerin, triethanolamine (TEA) and methylparaben (MPa) were of analytical degree, purchased from Sigma Aldrich. Lemongrass essential oil, Lot 170678 was purchased from Flora, Salute and Benessere (Italy). Tea tree essential oil, Lot 21092 was purchased from Argital (Italy).

Materials

Digital pH meter HANNA checker (Romania), Water bath (China), Viscosimeter (NDJ 1), Electrical balance (G and G, Germany), Analytical balance (Ohaus Corporation, USA), Centrifuge machine (TDL 80-2B, Hinotech lab), vortex shaker (China), Mechanical mixer (IKA, Germany), Refrigerator (Hisense, Italia), Electro-Thermal Incubator, Microscope (Micros, Austria).

Data processing

Statistical procedures were performed using the statistical program SPSS version 21.0 with a 5% level of significance. The data are presented as means and standard deviations.

Cosmetic emulsion preparation

All the ingredients were carefully selected and used for the emulsion formulations according to the rules provided by The European Cosmetics Legislation.[28] The formulation of the cosmetic emulsion with triethanolamine was carried out according to Sikora E, Monograph 2019.[12]

As shown in Figure 1, both oily and aqueous phases, were heated and prepared at the same temperature, up to 70 ± 2°C. The aqueous phase was then added to the oil phase drop by drop with continuous stirring at 2000 rpm for around 15 min until the aqueous phase was completely added. After the aqueous phase was entirely added, the mixer's speed was then dropped to 1200 rpm for 7 min of homogenization and to 600 rpm for 5 other minutes. According to Sarkič *et al.*[29] 2018 essential oils, TTO and LEO were added when the temperature was down at 34-35°C.[2]

Stability evaluation

The behavior of all formulations was studied after 24 hr of samples` stabilization; the visual examination was performed to evaluate the organoleptic properties whereas microscopic analysis, mechanical vibration, centrifugation, and density were performed as physico-chemical testing.[30]

Additionally, pH, viscosity measurement, and visual examination were monitored in daylight room conditions, at 25°C and 40°C (accelerated tests) for four consecutive weeks. Microbiological stability tests were performed at the end of four weeks.[30]

RESULTS AND DISCUSSION

Preliminary cosmetic emulsion formulation

A natural moisturizing emulsion for skin application was chosen to be prepared as it provides a better application property, especially higher water absorption.

In the preliminary stages of these studies, an extemporarily cosmetic emulsion was formulated in many trials, until it showed

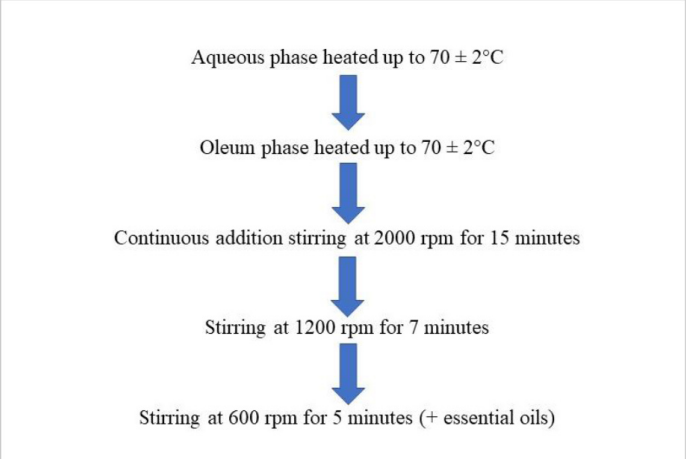

Figure 1: Schematic diagram for the preparation of the cosmetic emulsion.

no visible signs of physical instability such as creaming, cracking, sedimentation, phase inversion, and/or leaking of the cream base from the container. Different concentrations of acid stearic (4-7%), cetyl alcohol (1-3%), paraffin oil (3-10%), and glycerin (1-6%) were tried to formulate an emulsion with the desired characteristics. Meanwhile, many trials were carried out with the various concentrations of the emulsifier triethanolamine; stearate of triethanolamine is produced extempore with the combination between stearic acid and triethanolamine (0.2-1.2%).[31] Physical instability was assessed immediately after formulation and again after twenty-four hours of storage at room temperature of 15°C). Any formulation that showed evidence of physical instability immediately and/or after twenty-four hours of storage at room temperature (15°C) was considered unsuitable and undesirable and was not considered for further investigation. A cosmetic emulsion should be easy to spread and absorb, leaving the skin with a pleasant "non-greasy" sensation.

Based on the results obtained, the best combination for the emulsion preparation is shown in Table 1.

Afterward, the characteristics of cosmetic emulsion were checked by visual inspection. The freshly prepared basic emulsion was white, smooth, and uniform, the formulations loaded with methylparaben were in light yellow, whereas the formulations loaded with tea tree essential oil and lemongrass essential oil was in pink-white, and yellowish-white colors. The smell of the essential oils is noticeable in the formulations. After application on the skin, the created emulsion was easy to scoop, homogenous, formed a cone, and left a fine fatty coating.

Microscopic analysis

Microscopic analysis of the generated emulsion's globule size is a direct method to guarantee and predict emulsion stability over time. Microscopic analysis was performed with an optical microscope (Micros Austria) during the emulsion's development and storage temperatures, to observe any changes in globule size over time. Observations were carried out at 100X magnification after producing a very small smear on a glass slide.

Mechanical vibration test

Phase separation from the vibration may result in emulsion instability. Vibration during transportation in combination with the temperature change can reduce the stability of formulations, causing emulsion phase separation, suspension solidification, and viscosity changes.[30] The cosmetic formulations were physically stable as none of them showed phase separation after being subjected to the vibration test.

Centrifugation test

The centrifugation test performed using a centrifuge machine (TDL 80-2B, Hinotech Lab) at 3000 rpm for 30 min at 25°C, is an additional test for determining the formulation instability. This process causes stress in the sample, simulating an increase in gravity and causing more particle movement, which predicts future instabilities. Precipitation, phase separation, caking, and coalescence are only a few examples of these changes.[30] At the end of the centrifugation time, the formulations were assessed visually: there was no phase separation in all the formulations. According to our findings, we can conclude that all formulations had good stability, and enrollment of MPa, TTO and LEO as well did not affect the stability of the basic emulsion.

Density

The density of a cosmetic emulsion shows the formulation`s apparent density or the ratio of mass to the volume that a substance occupies. This measurement might indicate the incorporation of air or the loss of volatile compounds in liquids or semi-solids. It is crucial to avoid an overflow or an apparent lack of product in the recipient that contains it because the stated weight may be within

Table 1: Composition of the prepared cosmetic emulsions.

Phase	Ingredients	Function	Percentage by weight (%)						
			S_1	S_2	S_3	S_4	S_5	S_6	S_7
Oily phase	Stearic Acid	Emollient	4.5	4.5	4.5	4.5	4.5	4.5	4.5
	Cetyl Alcohol	Thickening Agents	1.5	1.5	1.5	1.5	1.5	1.5	1.5
	Paraffin Oil	Emollient	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Watery phase	Glycerin	Humectant	3.0	3.0	3.0	3.0	3.0	3.0	3.0
	Triethanolamine	Emulsifier	q.s	q.s	q.s	q.s	q.s	q.s	q.s
	Distilled Water	Solvent	q.s	q.s	q.s	q.s	q.s	q.s	q.s
	Methylparaben	Synthetic Preservative	-	0.2	0.3	-	-	-	-
	Tea Tree Essential Oil	Natural Preservative	-	-	-	1	2	-	-
	Lemongrass Essential Oil		-	-	-	-	-	1	2

S_1, basic/control formulation, without methylparaben/essential oils; S_2, formulation with methylparaben 0.2%; S_3, formulation with methylparaben 0.3%; S_4, formulation with tea tree essential oil 1%; S_5, formulation with tea tree essential oil 2%; S_6, formulation with lemongrass essential oil 1%; S_7, formulation with lemongrass essential oil 2%.

the required limits, but the customer may have the impression that some of the product is missing.[30]

The results of the density, calculated as a ratio between the mass and volume of the formulation, are shown in Table 2; the basic formulation S1, presented a density value of 1.1 g/mL, whereas the other values were similar to the basic one and ranged from 1.08 to 1.11 g/ml. As a result, we can state that the preservatives added to the cosmetic formulations (methylparaben, tea tree oil, and lemongrass oil) did not affect the density of the basic formulation. Each value is the mean of three observations ± standard deviation.

Light test

Once the oxygen and ultraviolet radiation are combined, free radicals are produced and the oxidation-reduction reactions are initiated. This may result in the breakdown of the formulation ingredients and a significant change in the product`s color and odor. Products that are sensitive to light action must be stored away from light, in opaque or dark containers, and antioxidant compounds must be added to the formulation to delay the oxidation process.[30]

The cosmetic formulations were placed in clear glass packaging and exposed to intense natural light for four weeks using a daylight source with a photoperiodicity of 10 hr of light and 14 hr of dark. At the end of the exposure period: there was no change of color and odor in all formulations, and there was no phase separation or liquefaction as well. As a result, all the formulations visually remained stable during the test.

Determination of pH in light testing

Human skin pH normally ranges from 4.5 to 6.0, with an average pH of 5.5.[32] At the end of the testing period, the pH of all the samples was found to be between 6.38±0.01 to 6.88±0.02. Although this pH is higher than the range, it is typical of emulsions containing TEA stearate as an emulsifier. The pH measurements are presented in Figure 2. From the measurements performed we note that the pH of the samples is close to the pH of human skin, so the formulations are appropriate for preventing irritation when applied to the skin. It is noted that the pH of the fresh formulations loaded with synthetic and natural preservatives showed a slight increase compared to the basic one, while it showed some variations during the four weeks storage period. Although, non-significant alterations in pH were observed for any of the formulations because they did not exceed the pH range of the skin.

Table 2: Density data for all the cosmetic emulsions elaborated.

Emulsion code	Density g/mL
Sample S_1	1.1 ± 0.03
Sample S_2	1.11± 0.02
Sample S_3	1.08 ± 0.03
Sample S_4	1 ± 0.01
Sample S_5	1.09 ± 0.02
Sample S_6	1,08 ± 0.02
Sample S_7	1.1 ± 0.03

S_1, basic/control formulation, without methylparaben/essential oils; S_2, formulation with methylparaben 0.2%; S_3, formulation with methylparaben 0.3%; S_4, formulation with tea tree essential oil 1%; S_5, formulation with tea tree essential oil 2%; S_6, formulation with lemongrass essential oil 1%; S_7, formulation with lemongrass essential oil 2%.

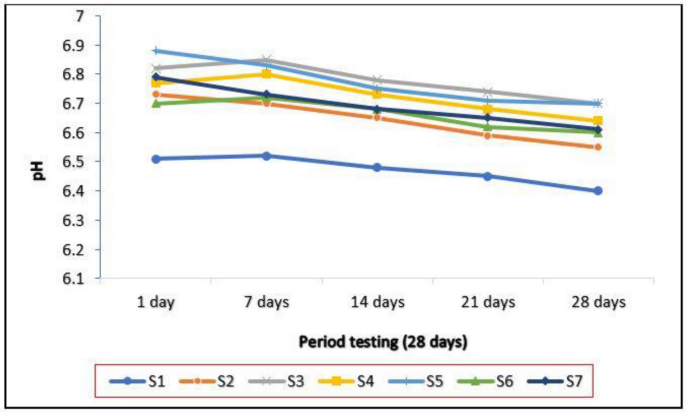

Figure 2: Variation of pH over time (S_1, basic/control formulation, without methylparaben/essential oils; S_2, formulation with methylpzaraben 0.2%; S_3, formulation with methylparaben 0.3%; S_4, formulation with tea tree essential oil 1%; S_5, formulation with tea tree essential oil 2%; S_6, formulation with lemongrass essential oil 1%; S_7, formulation with lemongrass essential oil 2%)

It is essential to maintain and protect the normal/physiological skin pH. Cosmetics, because of their widespread use, may be able to preserve skin health by regulating skin pH levels, so it is important to carefully assess the pH of any topically applied application as well as its buffering capacity and ensure that it is closer to the usual range.[33]

A previous study regarding the formulation and evaluation of moisturizing cream entrapped *Aegle marmelos* leaves extract, using TEA as emulsifier was conducted by Maske S. *et al* 2018: the variation of pH was found to be 6.31-6.53.[34] Maru A. *et al*, worked on the formulation and evaluation of a moisturizing cream containing sunflower wax, using TEA than borax as an emulsifier, the value of pH was in the range 7.20±0.185 to 7.24±0.158.[35]

Determination of viscosity in light testing

The viscosity value of all the formulations was calculated as the average of three triplicates; it was found to be in the range of 4772 ± 18.91 to 4839 ± 13.27 cps (m Pa.s) at 30 rpm as shown graphically in Figure 3.

A mild change in viscosity was observed for formulations containing essential oils compared to the base formulation and formulations with MPa, so we can conclude that the consistency of the emulsions is affected by the addition of essential oils.

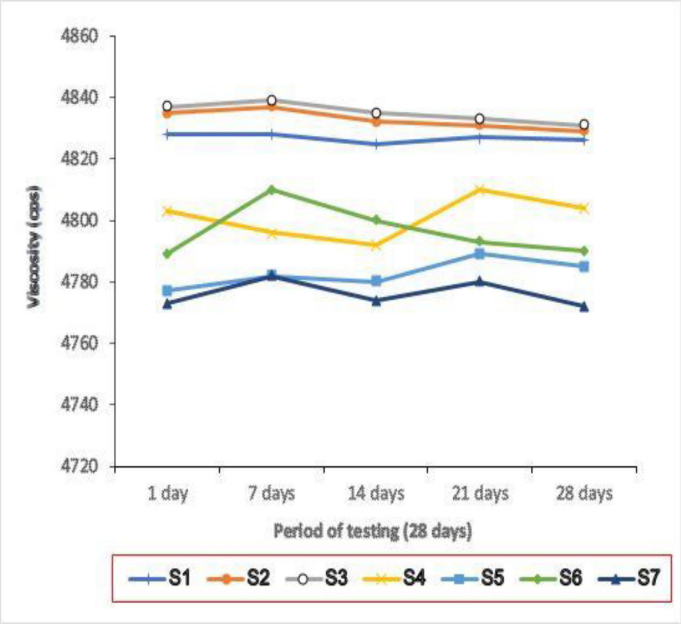

Figure 3: Variation of viscosity over time.
(S_1, basic/control formulation, without methylparaben/essential oils; S_2, formulation with methylparaben 0.2%; S_3, formulation with methylparaben 0.3%; S_4, formulation with tea tree essential oil 1%; S_5, formulation with tea tree essential oil 2%; S_6, formulation with lemongrass essential oil 1%; S_7, formulation with lemongrass essential oil 2%)

The change in viscosity of different samples was insignificant at different intervals of time.

The flow properties of emulsions are highly dependent on their viscosity. The physico-chemical characteristics of a formulation as well as the temperature conditions to which it is exposed determine its viscosity. The evaluation of the viscosity is crucial for the determination of the suitable consistency and fluidity, as well as the indication of the product's performance over time.[36-38]

Temperature and storage time have an impact on the prepared emulsion`s viscosity and liquefaction, resulting in decreased viscosity and increased liquefaction. According to Stoke's rule, the viscosity of the outer phase has an inverse relationship to the rate of sedimentation. The more the viscosity of the formulation decreases as a result of this component, the more liquefaction is visible.[39]

According to Maru A. *et al.*, the variation in viscosity for the moisturizing cream was 1256 ± 6.24 to 4897 ± 7.52.[35]

Accelerated stability

The formulations were stored for four weeks in an incubator, at temperatures of 25 ± 2°C and 40 ± 2°C; their physicochemical stability (organoleptic properties, pH, and viscosity) was

Table 3: Results of quality control of formulations.

Sample	Duration	1 week	2 weeks	3 weeks	4 weeks
		25±2°C 40±2°C	25±2°C 40±2°C	25±2°C 40±2°C	25±2°C 40±2°C
Sample S_1	Colour	White	White	White	White
	Odor	No Smell	No Smell	No Smell	No Smell
	Liquefaction	-	-	-	-
Sample S_2	Colour	Light Yellow	Light Yellow	Light Yellow	Light Yellow
	Odor	No Smell	No Smell	No Smell	No Smell
	Liquefaction	-	-	-	-
Sample S_3	Colour	Light Yellow	Light Yellow	Light Yellow	Light Yellow
	Odor	No Smell	No Smell	No Smell	No Smell
	Liquefaction	-	-	-	-
Sample S_4	Colour	Pink White	Pink White	Pink White	Pink White
	Odor	Agreeable	Agreeable	Agreeable	Agreeable
	Liquefaction	-	-	-	-
Sample S_5	Colour	Pink White	Pink White	Pink White	Pink White
	Odor	Sharp	Sharp	Sharp	Sharp
	Liquefaction	-	- +	- +	- +
Sample S_6	Colour	Yellowish-White	Yellowish-White	Yellowish-White	Yellowish-White
	Odor	Agreeable	Agreeable	Agreeable	Agreeable
	Liquefaction	-	-	-	-
Sample S_7	Colour	Yellowish-White	Yellowish-White +	Yellowish-White +	Yellowish-White +
	Odor	Sharp	Sharp	Sharp	Sharp
	Liquefaction	-	- +	- +	- +

The sample codes corresponding to the formulations containing: S_1- basic formulation without preservative, S_2- basic formulation loaded with 0.2% methylparaben, S_3-basic formulation loaded with 0.3% methylparaben, S_4- basic formulation loaded with 1% TTO, S_5-basic formulation loaded with 2% TTO, S_6- basic formulation loaded with 1% LEO, S_7- basic formulation loaded with 2% LEO.

Table 4: pH and viscosity variation of the formulations.

Temp	Sample	1 week		2 weeks		3 weeks		4 weeks	
		pH	Viscosity cps	pH	Viscosity cps	pH	Viscosity cps	pH	Viscosity cps
25°C	Sample S_1	6.55±0.34	4820±23.6	6.78±0.19	4890±34.7	6.81±0.41	4780±12.3	6.21±0.32	4785±36.5
	Sample S_2	6.71^{a}±0.23	4837^{a}±22.3	6.23^{a}±0.32	4810^{a}±45.1	6.32^{a}±0.23	4720^{a}±12.4	6.92^{a}±0.19	4745^{a}±39.8
	Sample S_3	6.99^{bg}±0.41	4839^{bg}±12.8	6.89^{bg}±0.29	4730^{bg}±20.4	6.66^{bg}±0.34	4745^{bg}±46.8	6.66^{bg}±0.26	4730^{bg}±52.1
	Sample S_4	6.21^{c}±0.12	4796^{c}±34.5	6.34^{c}±0.22	4720^{c}±34.5	6.78^{c}±0.33	4738^{c}±37.9	6.81^{c}±0.22	4650^{c}±42.2
	Sample S_5	6.79^{dh}±0.37	4782^{d*h}±47.7	6.89^{dh}±0.13	4725^{d*h}±56.1	6.73^{dh}±0.26	4695^{d*h}±50.1	6.68^{dh}±0.14	4670^{d*h}±37.1
	Sample S_6	6.64^{e}±0.25	4800^{e}±45.6	6.69^{e}±0.16	4720^{e}±23.4	6.72^{e}±0.12	4690^{e}±48.1	6.65^{e}±0.13	4675^{e}±28.3
	Sample S_7	6.92^{fk}±0.17	4782^{f*k*}±25.7	6.66^{fk}±0.23	4610^{f*k*}±32.1	6.18^{fk}±0.17	4585^{f*k*}±38.7	6.01^{fk}±0.33	4510^{f*k*}±29.5
40°C	Sample S_1	6.67±0.12	4785±31.2	6.88±0.12	4810±34.6	6.13±0.34	4725±23.4	6.87±0.36	4630±23.5
	Sample S_2	6.61^{a}±0.19	4790^{a}±23.4	6.25^{a}±0.34	4825^{a}±34.7	6.76^{a}±0.30	4740^{a}±33.2	6.21^{a}±0.42	4710^{a}±32.5
	Sample S_3	6.73^{bg}±0.25	4815^{bg}±35.4	6.65^{bg}±0.31	4785^{bg}±46.7	6.12^{bg}±0.10	4695^{bg}±45.2	6.12^{bg}±0.40	4710^{bg}±36.1
	Sample S_4	6.69^{c}±0.31	4780^{c}±23.3	6.65^{c}±0.27	4760^{c}±41.2	6.79^{c}±0.16	4695^{c}±43.1	6.58^{c}±0.37	4685^{c}±23.7
	Sample S_5	6.86^{dh}±0.42	4650^{d*h*}±12.5	6.32^{dh}±0.25	3890^{d*h*}±38.7	6.28^{dh}±0.15	3805^{d*h*}±36.7	6.37^{dh}±0.26	3735^{d*h*}±37.8
	Sample S_6	6.33^{e}±0.21	4610^{e*}±34.7	6.16^{e}±0.29	4725^{e*}±46.5	6.92^{e}±0.17	4565^{e*}±25.7	6.14^{e}±0.25	4420^{e*}±47.5
	Sample S_7	6.56^{fk}±0.24	4680^{f*k}±34.1	6.76^{fk}±0.31	3935^{f*k}±34.5	6.62^{fk}±0.26	3875^{f*k}±43.1	6.19^{fk}±0.17	3720^{f*k}±37.9

Measurement data were done in triplicate, data shows mean ± standard deviation $^{a}S_1$ vs. S_2; $^{b}S_1$ vs. S_3; $^{c}S_1$ vs. S_4; $^{d}S_1$ vs. S_5; $^{e}S_1$ vs. S_6; $^{f}S_1$ vs. S_7; $^{g}S_2$ vs. S_3, $^{h}S_4$ vs. S_5, $^{k}S_6$ vs. S_7 Mean bearing at least one common superscript with * differ significantly (P<0.05) The sample codes corresponding to the formulations containing: S_1- basic formulation without preservative, S_2- basic formulation loaded with 0.2% methylparaben, S_3-basic formulation loaded with 0.3% methylparaben, S_4- basic formulation loaded with 1% TTO, S_5-basic formulation loaded with 2% TTO, S_6- basic formulation loaded with 1% LEO, S_7- basic formulation loaded with 2% LEO.

determined periodically, after every week (Tables 3 and 4). The average of three triplicates and standard deviation were calculated.

According to Table 3, the organoleptic properties of samples S_1, S_2, S_3, S_4, and S_6 are stable in different temperatures of storage for four weeks; there were no changes in color, odor, and liquefaction. Samples loaded in 2% EOs (S_5 and S_7) presented liquefaction after the second week of storage at 40°C; sample S_7 presented changes in color as well. As a result, we can conclude that formulations with higher concentrations of EOs are unstable when stored at high temperature.

The pH testing of samples was performed in triplicate, which was 6.56 on average at all temperatures during storage. By using statistical analysis at a 5% level of significance, it was evident that the change in the pH of different samples was insignificant at different intervals of time at different temperatures. Viscosities were also found to decrease in both the base and formulations when stored at various temperatures, particularly at 40°C.

As presented in Table 4, the decrease of viscosity was significant, for the samples loaded with 2% essential oil, stored at 40°C (Figure 4). Once the viscosity is decreased the liquefaction begins to be presented.

According to Table 4, we can notice that: after the first week of storage and onwards, the variation in viscosity is significative for the samples loaded in 2% EOs (stored at 25°C and 40°C) compared to the basic formulation, or to the sample loaded in 1% EOs. Yeo. *et al.*, 2018 worked on stability determination of the various cosmetic formulations containing glycolic acid; the

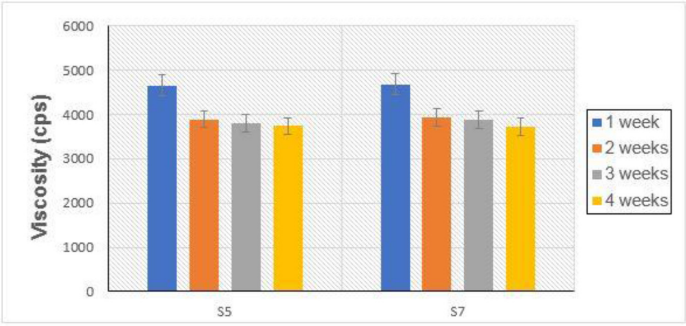

Figure 4: Viscosity value variation of samples S_5 and S_7. (S_5, formulation with tea tree essential oil 2%; S_7, formulation with lemongrass essential oil 2%)

obtained results showed that the gel and cream formulations have proper stability in the centrifugal test, pH stability, viscosity, and the observation of odor and color. On the other hand, the ointment did not have stability during the storage at 40°C.[40] Stability study of a cosmetic emulsion loaded with Tamarindus seeds extract conducted by Muhammad. *et al.*, 2019 showed good stability of the formulation at 8°C, 25°C, 40°C, and 40°C with 75% RH (relative humidity) over the 12-week study period.[41]

In our investigation, we tried to use EOs at various concentrations as sometimes a larger concentration is needed in order to obtain the desired antimicrobial activity. However, a high concentration of EOs causes a number of problems such as inappropriate viscosity of the formulation, phase separation, or unfavorable odor. As was noted in earlier studies, EOs and synthetic preservatives can be

combined to produce better antimicrobic results using the lowest amount of preservative ingredients.[42]

Contamination tests

Cosmetics must be produced, stored, transported, and delivered safely. The presence of both water and organic ingredients in cosmetic emulsion favors the growth of micro-organisms. In many cases, this has an impact on the structure of the adding agents, affecting the stability of the finished product. As a result, it's critical to evaluate a product's microbiological stability to assure its safety. The selection of the preservative and its concentration are the most important factors in cosmetic formulation.[42,43]

Recently, essential oils that have antibacterial properties and have been recommended as natural preservatives have received a lot of interest. Although TTO and LEO have excellent antimicrobial action, fewer studies have examined their self-preserving properties in cosmetic compositions.[15,44] For this reason, we decided to use them as natural preservatives in our cosmetic formulations.

The microbiological stability of formulations was assessed using a variety of bacteria as control, including *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa*, and *Candida albicans*. After the incubation period, 24 to 36 hrs., the petri dishes were removed and compared to the control to see if any microbial development had occurred. The efficacy of preservatives in cosmetic formulations is evaluated according to the European Pharmacopoeia guidelines;[45] as shown in Figure 5 no microbial growth was observed for all the formulations. The obtained results presented in Table 5 confirmed the microbial stability of our cosmetic formulations. TTO and LEO showed remarkable preservative capabilities and could therefore be considered alternative preservatives.

According to Herman *et al*., the antimicrobial activity, in cosmetic emulsion, of herbal extracts and essential oils (2.5%) (*Lavandula officinalis*, *Melaleuca alternifolia,* and *Cinnamonium zeylannicum*) was 3.5 times stronger than those of methylparaben (0.4%).[46] The study demonstrated the possibility of replacement of the synthetic preservatives with essential oil, however, in relatively high concentrations. A similar study demonstrated the efficacity of *Artemisia afra*, *Pteronia incana*, *Lavandula*

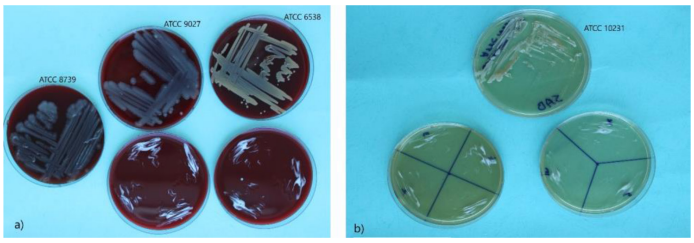

Figure 5: Microbiological stability of formulation: *bacterial (a) and* yeast *(b) stability*.

Table 5: Results of microbial quality studies.

	S_2	S_3	S_4	S_5	S_6	S_7
Pseudomonas aeruginosa ATCC 9027	N. P	N. P	N. P	N. P	N. P	N. P
Staphylococcus aureus ATCC 6538	N. P	N. P	N. P	N. P	N. P	N. P
Escherichia coli ATCC 8739	N. P	N. P	N. P	N. P	N. P	N. P
Candida albicans ATCC 10231	N. P	N. P	N. P	N. P	N. P	N. P

N.P- not presented, S_2- basic formulation loaded with 0.2% methylparaben, S_3-basic formulation loaded with 0.3% methylparaben, S_4- basic formulation loaded with 1% TTO, S_5-basic formulation loaded with 2% TTO, S_6- basic formulation loaded with 1% LEO, S_7- basic formulation loaded with 2% LEO.

officinalis, and *Rosmarinus officinalis* essential oils in reducing microbial contamination in cosmetic creams.[47] Efficacy of lemon, tea tree, and lavender essential oils alone as well as combined with synthetic preservatives in body milk (W/O) showed the possibility of the reduction of the level of methylparaben up to 8.5 times.[39] Synergistic effect of oils was studied by Maccioni *et al*., confirming the application of combined essential oils (*Laurus nobilis*, *Eucalyptus globulus,* and *Salvia officinalis*) giving the possibility to reduce significantly the amount of synthetic preservatives in cosmetic formulation.[48]

CONCLUSION

At the conclusion of our research, we found that cosmetic emulsions entrapped in 1% TTO and LEO as natural preservatives, showed promising stability, physico-chemical properties, and self-preserving effect under various situations. Although these values are within the normal range adding essential oils to the basic formulation resulted in a decrease in viscosity and an increase in pH. Due to the inherent self-preservative qualities of essential oils, the usage of synthetic preservatives in the cosmetic sector can be minimized or even completely eliminated. The formulations loaded with essential oils provide a novel alternative for the preservation of "paraben-free" cosmetic products.

Despite the substantial experimental work that was done in this study, these formulations still require more investigation. They should be examined for their ability to cause inflammation, their toxicity and, for their behavior "*in vivo*" using skin cell units.

In the near future, we will tend to include our natural products, especially native items from our regions, with all the unique characteristics and benefits that these spontaneous, or natural products possess.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

MPA: Methylparaben; **EO:** Essential Oil; **TTO:** Tea Tree Oil; **LEO:** Lemongrass Oil; **RPM:** Rotation per Minute; **CPS:** Centipoise; **CFU:** Colony-Forming Unit.

SUMMARY

The objectives of the present study were: to develop a cosmetic emulsion based on different concentrations of synthetic preservative and essential oils, applied as natural preservatives; to evaluate and subsequently compare their stability and antimicrobial activity. The prepared samples were evaluated related to organoleptic and physicochemical testing. The behavior of the samples related to light tests accelerated tests, and microbiological stability tests as well were performed for four weeks of storage. During the accelerated stability test, formulations loaded with 2% essential oils were not stable as noted a variation in the color, liquefaction, and a significant decrease in the viscosity. Cosmetic emulsions entrapped with 1% essential oils showed promising stability, physicochemical characteristics, and self-preserving effect. The formulations loaded with essential oils provide a novel alternative for the preservation of "paraben-free" cosmetic products.

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