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Development of an anti-acne cream based on natural oils: Investigation of the effect of ingredients on rheology, texture properties, and physical stability

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Abstract

Acne vulgaris is a very common skin problem. *Cutibacterium acnes* (formerly *Propionibacterium acnes*), a common skin organism, is most notably recognized for its role in acne vulgaris. Standard oral and topical treatments have significant side effects including skin irritation, indigestion, and also, cause resistance to antibiotics. In current study, o/w creams based on ostrich and sesame fixed oils containing *Mentha piperita*, *Origanum vulgare* and *Lavandula officinallis* essential oils as active ingredients were developed. The results showed that using Cetomacrogol 1000/Cetostearyl alcohol (CSA) as emulsifier/co-emulsifier could produce stable o/w creams containing 10-30 % w/w of fixed oils with various consistencies. Long-term (room temperature/24 months), accelerated (40 °C/ three months), thermocycling (-10 °C for 24h/40 °C for 24 hr) and centrifuge test (3000 rpm, 30 min at 50 °C) studies all showed the very high physical stability of the formulations. The rheological studies showed the shear-thinning thixotropic behavior of creams and Ostwald equation as the best model describing behavior of the creams. Texture analysis showed the significant effects of ingredients on creams hardness and adhesiveness having a reasonable correlation to spreadability test results. The results showed that aqueous and oil soluble thickening agents had the highest effect on rheological and texture parameters. Finally, the optimum cream was standardized using gas chromatography equipped to mass detector (GC-MS).

Keywords: Acne, Essential Oil, Natural Oil, o/w cream, Rheology, Texture Analysis.

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1. Introduction

World Health Organization believes that acne is one of the most commonly treated skin conditions and should be recognized as a chronic disease with psychological consequences (1). Acne is the eighth most common disease in the world and affects 9.4% of the world's population (2). Acne and scarring acne can have serious psychological consequences, including anxiety, depression, and social withdrawal. Physical scars, persistent hyperpigmentation, or both are complications of acne that usually cost a lot to treat. They are also often difficult to treat. The effects and scars of acne can remain on the patient's skin for years. The scar may even remain in people who have self-limiting acne in adulthood (3). An increase in the prevalence of adult acne has been reported since early 1979. Today, clinical studies based on objective findings and questionnaire studies show that about 40% of adults may have acne. It usually starts in about 20% of cases in adulthood and about 80% in puberty. Adult acne is more common in wom-

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en than men. The main site of lesions in women is the face and in men the back of the body (4). Topical products have the advantage of being applied directly to the area, thus reducing systemic absorption, increasing exposure to pilobase units, and increasing effectiveness. However, the main side effect of topical acne products is skin irritation. Topical products are available in various formulations including creams, gels, lotions, solutions and detergents (5). One of the most common treatments for acne is topical antibiotics which are often used for mild to moderate inflammatory acne. Their activity is against the microorganism *P. acnes*, so they act on the surface of the skin to reduce the stimuli and cause to reduce inflammation of the lesions. Antibiotic resistance is one of the most important public health concerns in almost every part of the world. Bacterial resistance is due to selective pressure on bacteria which can be the result of appropriate and inappropriate use of antibiotics. Antibiotic resistance can be due to the use of antibiotics against *P. acnes* or to other pathogenic organisms (6). In recent years, continuous use of these antibiotics has led to increased resistance to *P. acnes* strains. There are more effective and safer treatment options for acne. Numerous complementary and alternative therapies (CAMs) have been mentioned or promoted for use as acne treatments that are generally considered safe. Herbal remedies have more benefits due to the combination of a wide range of active ingredients. Some researchers believe that herbs may reduce antibiotic resistance if used as an alternative or in combination with antibiotics. However it still needs to be confirmed by clinical studies (5). Traditional medicine has used essential oils and extracts of medicinal plants to treat acne for thousands of years (7).

In current study, we aimed to formulate an optimum and stable cream based on natural fixed oils and herbal essential oils with approved effects on acne including anti-inflamatory, antibacterial and wound healing properties. Ostrich and sesame fixed oils were selected as oil phase and *Menta piperita*, *Origanum vulgare* and *Lavandula officinallis* as active essential oils. Ostrich oil has been used topically for centuries to relieve pain in Egyptian, Roman and African cultures. Ostrich oil penetrates deep into the skin and its non-comedogenic properties provide skin moisture for hr without clogging the pores. This is due to its high levels of oleic acid and therefore can be used as a carrier in combination with various medicinal or cosmetic substances to transfer them under the skin barrier (8). The anti-inflammatory and antibacterial effects of its free fatty acids on sebum, especially linoleic and lauric acid, reduce inflammation and infection induced by *P. acnes*. Also, the herbal oils containing linoleic acid, especially sesame oil, may be used to reduce acne. Sesame has also been shown to have significant antibacterial activity against a variety of microorganisms (9). Lavender oil has remarkable antimicrobial and anti-inflammatory effects. Aqueous extract of *Lavandula officinallis* also shows anti-inflammatory effects attributed to the inhibitory activity of nitric oxide produced in macrophages (10, 11). Therefore, it is used as an additive to pharmaceuticals (salve and lotions for hard-to-heal wounds, eczema, and anti-rheumatic preparations), as well as cosmetics. The essence of the effect of lavender oil on skin microbiota depends on its quantitative and qualitative chemical composition. Essential oils have an affinity for lipid cell structures; therefore, they destroy the cell wall and membranes of bacteria, mainly Gram-positive ones (less often Gram-negative) and fungi, and, as a consequence, there is leakage and coagulation of the cytoplasm. In addition, lavender oil inhibits the synthesis of RNA, DNA, proteins, and polysaccharides, while, in fungi, it acts as anti-mycotics and inhibits the production of enzymes (12).

Origanum vulgare is a fragrant plant of the Lamiaceae family (13). In one study, it was shown that *Origanum vulgare* essential oil is effective in reducing the parameters involved in inflammation and cell stimulation during wound healing (13). Moreover, the oil has shown very good antibacterial effect on bacteria including *P. acne*. Screening for antimicrobial activity of seven oils showed oregano to exhibit the strongest antimicrobial activity with minimum inhibitory concentration (MIC) of 0.34 mg/mL and minimum bactericidal concentration (MBC) of 0.67 mg/mL against *P. acnes* (14). Also, the suppressive effect of ethanolic oregano extract was examined on live

P. acnes-induced *in vivo* and *in vitro* inflammation. Following ethanol extraction of oregano leaves, four compounds with strong antioxidant activity, including rosmarinic acid, quercetin, apigenin, and carvacrol, were identified by high-performance liquid chromatography.

Using the mouse ear edema model, it was demonstrated that ethanolic oregano extracts significantly suppressed *P. acnes*-induced skin inflammation, as measured by ear thickness (32%) and biopsy weight (37%). In a separate study, using the co-culture of *P. acnes* and human THP-1 monocytes, the extract reduced the production of interleukin (IL)-8, IL-1β and tumor necrosis factor (TNF)-α up to 40%, 37%, and 18%, respectively, as well as the expression of these three pro-inflammatory mediators at the transcriptional level. Furthermore, ethanolic oregano extracts inhibited the translocation of nuclear factor-kappa B (NF-κB) into the nucleus possibly by inactivating toll-like receptor-2 (TLR2) (15).

Mentha (also known as peppermint), a genus of plants in the taxonomic family Lamiaceae (mint family), is widely distributed throughout temperate regions of the world. Mentha contains various constituents that are classified as peppermint essential oil and non-essential components. peppermint essential oil, consisting mainly of menthol, menthone, neomenthol and iso-menthone, is a mixture of volatile metabolites with anti-inflammatory, antibacterial, antiviral, scolicidal, immunomodulatory, antitumor, neuroprotective, antifatigue and antioxidant activities (16).

The antibacterial potential of six extracts from leaf, stem and root of *Mentha piperita* against pathogenic bacteria such as *Bacillus subtilis*, *Streptococcus pneumonia*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris* and *Klebsiella pneumonia* with the organic (ethanol, methanol, ethyl acetate, chloroform, hexane and petroleum ether) extracts of the leaves are said to possess strong antibacterial activity against a range of pathogenic bacteria and its pharmacological effects have been reported by Punit Shahet al. (2004) (17). In one study, the organisms were isolated from skin infections like acne (whiteheads and black heads), A decoction of the chosen herbs was prepared at a concentration of 100 mg/mL and was tested against the isolated pathogens using agar well diffusion method. The highest inhibitory activity was observed with the methanol extracts. *Aloe vera* gel was extracted and a mixture of the herbs and the gel was prepared and tested against the isolated pathogens. A synergism in effect was observed indicating that it is a potential formulation to treat common skin ailments, especially acne that bothers most of the teenage population across the world (17). On the other hand, the cooling effect of *Mentha piperita* essential oil helps to reduce the irritation and inflammation caused by acne. Moreover, its antimicrobial properties can reduce the acne-causing bacteria and help treat cane (18).

Regarding the high potentials of mentioned fixed and essential oils, in current study, we aimed to develop a topical o/w cream incorporating the oils for acne treatment. The formulations were assessed in terms of organoleptic features, rheological behavior, texture analysis and physical stability.

2. Materials and methods

2.1. Materials

Ostrich oil and Sesame oil were supplied by Talaye Sabz Sarvestan Co., Shiraz, Iran and Samar Co., Yazd, Iran, respectively. Lavender oil, Oregano oil and Mint oil were purchased from Johare Tame Co., Mashhad, Iran. Menthol as standard was purchased from Sigma-Aldrich Co., US. Stearic acid (SA),CSA, cetomacrogol 1000, and triethanolamine (TEA) were purchased from Merck Co., Germany. Carbapol (CP) 940 was provided by Lubrizol Inc., US. All other materials were atleast of analytical grade and provided by Merck Co., Germany.

2.2. Methods

2.2.1. Cream formulations

Fifteen cream formulations with different percentages were prepared according to Table 1. To prepare the creams, the deionized water was heated to 70 °C on Ben Marie (Behdad Co., Iran) and to correct the volume of evaporated water, the Beakers' water content was weighed before and after heating and the weight difference was corrected by adding deionized water. The purpose of

Table 1. The composition of cream formulations. The values are as % w/w.

Formula-tions	Stearic acid	Deionized Water	NaOH 1M	Carbopol 940	Cetomacrogol 1000	Cetostearyl alcohol	Ostrich Oil	Sesame Oil	Triethanol-amine	Glycerin
1	-	69	1.5	0.25	1.25	5	10	10	-	-
2	-	66.5	1.5	0.25	1.25	7.5	10	10	-	-
3	1	68	1.5	0.25	1.25	5	10	10	-	-
4	1	65.5	1.5	0.25	1.25	7.5	10	10	-	-
5	-	59	1.5	0.25	1.25	5	15	15	-	-
6	-	61.5	1.5	0.25	1.25	7.5	10	10	-	5
7	-	64	1.5	0.25	1.25	5	10	10	-	5
8	-	56.5	1.5	0.25	1.25	7.5	15	15	-	-
9	-	67.5	1.5	0.25	1.25	5	10	10	1.5	-
10	-	51.5	1.5	0.25	1.25	7.5	15	15	-	5
11	-	54	1.5	0.25	1.25	5	15	15	-	5
12	-	49	1.5	0.25	1.25	10	15	15	-	5
13	-	63.7	1.5	0.5	1.25	5	10	10	-	5
14	-	69	1.5	0.25	1.25	5	7.5	7.5	-	5
15		74	1.5	0.25	1.25	5	5	5	-	5

heating was to both help hydrate the CP940 and reduce the microbial load of the product. Then, the water-soluble components of the formulation including Cetomacrogol 1000, CP940, glycerin, methyl paraben (0.2%), and propyl paraben (0.03), were weighed and added to the water. The aqueous was mixed with an impeller (Heidolph SY1 H Co., Germany) at 1500 rpm for 20 minutes to completely hydrate the CP940. The fat-soluble components of the formulation (sesame oil, ostrich oil, CSA, and SA) were weighed and heated to 70 °C on Ben Marie to melt all the fatty components. The aqueous phase was heated on Ben Marie after hydration of CP940 to equalize its' temperature with the fatty phase. After reaching a temperature of 70 °C, the Beaker containing the fatty phase was placed under impeller at 2000 rpm and the aqueous phase was gradually added to it. After cream formation, sodium hydroxide (NaOH) 1M or TEA, was added to neutralize CP940 and form a gel. The essential oils (1 % w/w of each) were then added to the creams and mixed. The prepared creams were kept in sealed containers.

2.2.2. Organoleptic evaluations

To evaluate the appearance, texture and spreadibility of the creams qualitatively, the creams were placed on the skin and spread with a finger to evaluate their ability to smoothly disperse and assess their texture in terms of uniformity or the presence of tactile particles. Also, the color and odor of creams were assessed.

2.2.3. Long-term thermal stability

From each formulation, a sample was placed at refrigerator temperature (5±3 ºC) and a sample at room temperature (25±3 ºC) to evaluate physical stability. The samples were examined and compared at various time intervals during a 2-year period (19).

2.2.4. Accelerated thermal stability

All samples were stored in completely closed tubes for 3 months in incubator (Fan Azma Gostar Co., Iran) at 40±1 °C. The stability of the products was controlled in terms of phase separation and changes in product consistency during this period.

2.2.5. Thermal cycling study

All samples were placed in incubator at 40±1 °C. After 24 hr, the samples were removed from the incubator and transferred to the freezer (General Steel Co., Iran) at -10 °C for 24 hr. The changes were performed for 6 cycles and after each cycle, the appearance characteristics of the

products in terms of color, odor, presence of tactile particles, uniformity and non-separation of phases were investigated (19).

2.2.6. Centrifuge test

To test the stability of the creams, they were heated to 50 °C and centrifuged at 3000 rpm for 30 minutes. Then, the signs of instability were assessed as mentioned above (19).

2.2.7. Rheological study

Rheological properties of all formulations were investigated at room temperature. For this purpose, RC/S cone & plate rheometer (Brookfield Co., USA) equipped with cone spindle with a diameter of 25 mm (Spindle C50-1) was used. After placing the sample on the plate and lowering the cone, the formulation was given a 60-second time to regain its structure. The distance between the cone and the plate was considered to be 0.2 mm. Shear rate was applied from 0 to 400 sec^{-1} and viscosity was measured at 50 points. Rheometry was performed in triplicate for each sample. The rheograms were constructed by plotting shear stress and viscosity values against shear rate. The data were fitted to various models to find the best regression, and the consistency coefficient (K) and power index values (n) were calculated. Thixotropy and average viscosity were also calculated by the device software. It is worthy to note that for better comparison, a commercial cream (My® hand and face moisturizing cream) with acceptable consistency and spreadability on the skin was examined too.

2.2.8. Texture analysis

Texture analysis was performed for all samples using Texture Analyser1000 (Brookfield R/D Co., USA). In this study, the compression mode was considered and distance was selected as test target. The compression rig was placed on the surface of the creams and 5 mm was inserted into the samples, held for 10 seconds, and then returned. An initial force of 20 Milli Newton (MN) was considered for all the samples.

2.2.9. Spreadability test

To measure the extent of spreadability, we placed 0.5 g of the creams in the middle of two round glass plates, each weighing 49 g. After 3 minutes, the surface of the resulting circle was measured. Then, 200 g and 500 g weights were also placed on the top plate and the radius of the circles was measured 3 minutes after placing each weight. Finally, the area of creams spreading were calculated and reported.

2.2.10. Selection of final formulation

The most optimum formulation having acceptable texture, viscosity, adhesiveness, and physical stability was finally selected and evaluated for pH, microbial controls and chemical standardization.

2.2.11. pH determination

In order to evaluate the pH, 5 g of the selected formulation was weighed and transferred to beaker and 45 ml of deionized water was added to it. After complete mixing, the pH of the sample was measured using a Sartorius pH meter (IKA RW 20 Swiss) in triplicates. The pH of the cream was measured at the time of manufacture and during storage (19).

2.2.12. Preservative effectiveness test

Preservative efficacy test was performed according to the United States Pharmacopeia (USP 41 NF 36) method. After preparing the cream, microorganisms (*S. aureus* ATCC 6538, *E. coli* ATCC 8739, *P. aeruginosa* ATCC 9027, *C. albicans* ATCC 10231, and *A. brasiliensis* ATCC 16404) at a concentration of 1x105 CFU/g were added to the cream and then incubated at controlled room temperature (20-25 ºC) for 28 days. At the same time, a cream formulation containing all components except preservatives was prepared to evaluate the effect of the essential oils on bacterial growth.

2.2.13. Standardization by GC-MS

Chromatographic evaluations were performed using Agilent Technologies 7890A GC-MS. For this purpose, helium gas with a purity of 99.99 % was injected as a carrier gas. The temperature of the injector was 185 °C and the capillary column of HP-5MS with length of 30 m, internal

diameter of 0.25 mm and a stationary phase of 5 % phenylmethyl polysiloxane with a thickness of 0.25 µm was selected for analysis. The headspace setting was selected from settings. Syringe temperature, stirrer temperature and mixing time were 70 °C, 80 °C and 20 minutes, respectively. Menthol was used as a standard to standardize the final optimal formulation.

3. Results and discussions

3.1. Organoleptic evaluations

All formulations of the cream, except for F13, had acceptable ability to spread on the skin and did not cause a rough feeling when applied on the skin. Also, all creams were white in color and had a mint-like odor. However, after 2 years, some formulations showed signs of instability such as formation of fat granules, and separation of fat and water phases.

3.2. Long-term and accelerated thermal stability

All formulations with exception of F1 and F5 showed acceptable physical stability and no phase separation or coalescence was observed. Furthermore, the formulations texture remained homogeneous visually without a significant change in color and odor. These observations confirm that the Cetomacrogol 1000/CSA system could successfully emulsify high percentages of natural oils in water. Comparing F1 with stable formulations like F2, F3, F7, F9, and F13 shows that high concentrations of CSA (> 5 % w/w), the presence of SA (1 % w/w), glycerin (5% w/w), and using TEA as neutralizing agent in formulations could efficiently stabilize the emulsions because these formulations have the same percentages of oils and Cetomacrogol 1000 as emulsifier (Table 1). A similar comparison can be made between F5 with F8, F10, F11, and F12 (Table 1). These observations can be explained by the fact that the oil soluble (CSA and SA) and water soluble (CP940 and glycerin) thickening agents have the major contribution in stability of creams.

On the other hand, the acceleration study for 3 months at 40 °C showed complete stability for all formulations confirming that the creams can tolerate high temperature for long times. This finding is another proof of correct selection of creams components.

3.3. Thermal cycling stability

A very detrimental criterium showing physical stability of cream formulations is their resistance against thermal fluctuations. In this regard, most formulations could tolerate 6 cycles of thermal fluctuations showing no sign of instability including non-homogeneity and phase separation. Amongst them, F1 endured only 2 thermal cycles and coalescence was observed. Also, F5 became unstable in the sixth cycle. This result is in agreement with long-term and accelerated thermal stabilities confirming that the same mechanisms are involved in stability of creams under the experiments and signifies that the presence and the quantity of excipients are of crucial role in stabilizing formulations.

3.4. Centrifuge test

The stability of creams against extremely high stresses induced by centrifugation can guarantee stability of cream formulations during transportation and moreover, it can accelerate the instability processes within creams to predict the possibility of phase separation in long-term storages. Regarding this, F1, F3, F5, F6 and F8 could not maintain their stability against centrifuge stress and underwent phase separation. Although, no general rule could be deduced co-relating formulation parameters and test results, but these observations show that some formulations like F1 and F5 which could not pass thermal stability and thermal cycling tests, could not tolerate centrifuge test as well.

3.5. Rheological measurements

Table 2 demonstrates the rheological parameters of the formulations including the best describing mathematical model, consistency index (K), thixotropy, average viscosity (η), and flow behavior index (n). The results showed that most formulations (except F1, F2, F11 and F15) conform to Ostwald model. In other words, they show no initial resistance to flow ($\tau 0 = 0$) and begin to move when the force is applied. It should be noted that only the commercial cream conformed to Casson model (Table 2). In terms of consistency index,

Table 2. The rheological parameters of cream formulations measured by cone-plate rheometer.

Formulation	Model	Equation	K Ave ± SD	τ0 Ave ± SD	Thixotropy Ave ± SD	Viscosity (η) (mPa.s)	n
1	HB	Y=10.91+0.24X0.91	0.24±0.18	10.91±1.11	4005.87±227.80	0.23±0.01	0.91±0.14
2	HB	Y=34.18+7X0.5	7.00±2.53	34.18±9.60	4137.03±174.47	0.96±0.08	0.5±0.07
3	Ostwald	Y=54.79*X0.1	54.79±1.56	-	14186.83±1367.87	0.87±0.01	0.10±0.01
4	Ostwald	Y=65.08*X0.21	65.08±2.58	-	55017.36±6505.45	2.34±0.05	0.21±0.01
5	Ostwald	Y=22.59*X0.24	22.59±1.53	-	14734.48±1051.22	0.65±0.10	0.24±0.05
6	Ostwald	Y=65.45*X0.23	65.45±3.18	-	30821.88±189.06	1.72±0.09	0.23±0.01
7	Ostwald	Y=81.79*X0.21	81.79±3.17	-	23326.59±2947.67	2.03±0.14	0.21±0.01
8	Ostwald	Y=83.22*X0.15	83.22±9.87	-	26247.76±991.89	1.54±0.05	0.15±0.02
9	Ostwald	Y=24.18X0.27	24.18±3.17	-	12523.35±705.87	0.74±0.01	0.27±0.03
10	Ostwald	Y=23.72*X0.29	23.72±1.89	-	14798±917.92	0.80±0.01	0.29±0.02
11	HB	Y=24.63+0.19X0.88	0.19±0.16	24.63±4.64	5420.04±405.16	0.35±0.04	0.88±0.14
12	Ostwald	Y=97.41*X0.17	97.41±27.28	-	32332.96±3129.12	2.94±0.24	0.17±0.03
13	Ostwald	Y=244.52*X0.06	244.52±22.84	-	37798.24±4499.30	3.21±0.20	0.06±0.01
14	Ostwald	Y=74.33*X0.22	74.33±3.70	-	21968.44±692.27	1.88±0.06	0.22±0.01
15	HB	Y=35.44+6.64*X0.53	6.64±0.93	35.44±0.24	8036.08±616.9	1.18±0.02	0.53±0.02
My*	Casson	SQRT(Y)=SQRT(95.51) +SQRT(0.06*X)		95.51±2.50	11939.19±273.02	0.06±0.01	-

*A commercial, hand and face moisturizing cream.

F13, F12, F8, and F7 showed the highest consistency, respectively. F13 is the only cream having 0.5 % w/w CP940 which produced the highest consistency compared to others with 0.25% w/w CP940 (Table 1). Also, F12 has 10% w/w of CSA as thickener, emollient and co-emulsifier which led to high consistency in formulation. A comparison among F11, F10, and F12 (containing 5, 7.5, and 10 % w/w CSA, respectively and K values of 0.19, 23.72, and 97.41, respectively) also confirms this conclusion (Table 2). A similar effect can be observed for SA with comparing F1 and F3 having no SA and 1 % w/w of SA, respectively. As CSA and SA are saturated fatty acids which are solid in room temperature, it is reasonable to increase the consistency of formulations. Other researchers have observed the same effect attributing it to stabilizing influence of CSA due to the formation of an extra crystalline CSA network (20).

In order to adjust the viscosity of an O/W cream by means of a co-emulsifier, it is necessary to use high concentrations of CSA which enables the formation of a pure crystalline co-emulsifying network. In another study, SA/TEA was found to influence on the process and formulation parameters including droplet size, consistency, hardness, compressibility, and adhesiveness (21). Microscopy and x-ray diffraction have shown that either pure cetyl or pure stearyl alcohol phase usually separates from water into a crystalline phase with an orthorhombic (β-form) or monoclinic crystal structure (γ-form). In the orthorhombic and monoclinic crystals, on the other hand, there is long-range bond orientational order of the alkyl tails, making these materials more solid-like. DSC and XRD studies have shown that stable emulsions are formed when the fatty alcohol is arranged in its hexagonally packed bilayer (22).

The structure and rheology of ternary systems formed by dispersing Cetomacrogol 1000 and CSA in water have been correlated with the properties of emulsions stabilized by a Cetomacrogol-CSA mixed emulsifier. Cetomacrogol 1000 can interact with CSA at low temperatures to form gel networks with properties similar to those formed during the standard method of preparation. The mixed emulsifier exhibits a self-bodying phenomenon, and the consistencies of the systems increase as the mixed emulsifier concentration rises. The networks responsible for the self-bodying phenomenon are not fully formed during the preparation of the ternary systems and emulsions.

They built-up slowly at 25 °C, and this buildup is responsible for the increase in consistency apparent in aged ternary systems and emulsions (23). Regarding the effect of oil content on viscosity, a comparison between F1 and F5 shows that higher percentage of oil phase (30 % versus 20 % w/w, respectively) causes higher consistency of formulations (0.24 and 22.59 for F1 and F5, respectively). Also, the formulations like F15 which had very low oil phase (10 % w/w), showed poor consistency (K = 6.64). Although, the oil phase consists the internal phase of creams and it is not expected to have a high impact on consistency, but considering the fact that the nature of natural oils is not completely hydrophobic and especially the short chain fatty acids can partially interact to aqueous phase, this observation can be reasonable. Similarly, some researchers showed that an increase in consistency index was resulted by increasing oil content and explained it by the increasing volumetric fraction of the emulsion dispersed phase (20).

In addition, our results showed that all creams had pseudoplastic and shear-thinning behavior. Among these, the highest n value was related to F1 and the lowest value was observed for F13 (0.91 and 0.06, respectively). As results show, the formulations complying with Herschel-Bulkley model have higher n values (0.5, 0.53, 0.88, and 0.91 related to F2, F15, F11, and F1, respectively) meaning a higher dependence to shear. Figure 1. shows the rheogram of F7 for example depicting a pseudoplastic shear-thinning pattern.

Regarding thixotropy, the highest values were related to F4, F13 and F12, respectively, and the lowest values were related to F1, F2 and F11, respectively (Table 2). A comparison among F2, F3, and F4 indicates that very high thixotropy of F4 is owing to having both high percentage of CSA and presence of SA in the formulation (Table 1). This observation could be explained with interaction of helices of these saturated fatty acids forming a three-dimensional network which takes longer times to recover after removing the shear. A remarkable point which is concluded with comparing F1 and F9 is that TEA has a much greater effect on increasing the consistency of the formulation compared to NaOH. It also gives the system a higher thixotropic property (Table 2). This effect can be due to the presence of TEA counter ion in the gel structure. However, it gave an unfavorable appearance to the creams and was avoided as neutralizer in the study.

Overall, the formulations containing the medium quantities of thickening agents like F6, F7 and F14 showed moderate consistency, viscosity, and thixotropy which make a formulation optimum for topical applications.

3.6. Texture analysis

Texture analyzer is a quantitative tool to investigate the texture characteristics of semi-solid formulations. Table 3 summarizes the various parameters related to texture analysis of the formulations. F12 and F13 which had the highest consistencies (Table 2), also showed the highest hardness (63.67 and 175.33 mN, respectively)

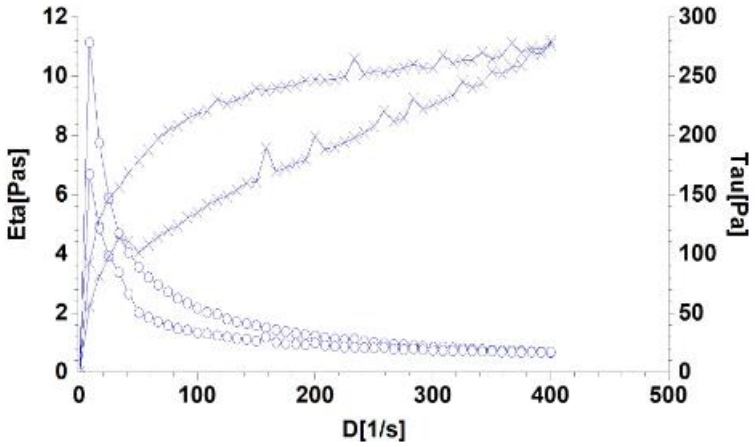

Figure 1. The rheogram of cream F7 showing shear stress (Tau)/viscosity (Eta) against shear rate (D).

Table 3. The texture parameters of the cream formulations measured by Texture Analyzer.

Formulation	Hardness Ave ± SD (mN)	Adhesiveness Ave ± SD (mJ)	Adhesiveness force Ave ± SD (mN)
1	27.00±1.41	0.14±0.04	26.50±6.36
2	36.67±1.53	0.12±0.02	22.67±2.52
3	26.33±0.94	0.15±0.02	17.67±1.53
4	63.33±3.79	0.18±0.01	44.33±2.31
5	51.00±3.61	0.09±0.01	19.00±1.73
6	35.67±2.08	0.13±0.01	25.00±0.00
7	36.00±1.00	0.14±0.03	28.67±4.62
8	39.00±1.73	0.14±0.02	28.33±3.06
9	24.67±0.58	0.11±0.02	16.33±1.53
10	28.00±2.83	0.18±0.01	20.00±0.00
11	25.33±1.15	0.14±0.03	15.67±0.58
12	63.67±2.08	0.18±0.02	49.67±5.51
13	175.33±21.83	0.22±0.08	75.67±21.59
14	39.67±5.69	0.16±0.03	27.67±3.79
15	30.33±1.53	0.11±0.02	22.67±2.89
My*	53.00±1.00	0.16±0.01	36.33±3.21

*A commercial, hand and face moisturizing cream.

and adhesiveness force (49.67 and 75.67 mN, respectively). This result indicates that the formulation components which have the large impact on viscosity and consistency including CP940, CSA and SA, have the highest influence on the hardness and adhesiveness as well. On the other hand, F11 with lowest consistency (Table 2) had the lowest hardness and adhesiveness force too (25.33 mN and 15.67 mN, respectively) confirming the above conclusion. The formulations with optimum consistency and viscosity (like F7 and F14) had the acceptable hardness and adhesiveness as well (Table 3). The adhesiveness is particularly of high significance regarding face creams as individuals don't usually tolerate sticky preparations for organoleptic reasons.

3.7. Spreadability test

According to the results (Table 4), F3 and F5 showed the highest spreadability, whereas F12 and F13 had the least spreadability. For F12 and F13, a reasonable relationship is observed between consistency/hardness and spreadability as higher consistency and hardness resulted in lower spreadability (compare Table 2 and Table 3 to Table 4). However, about F3 and F5 which showed the highest spreading, the hardness and consistencies were not as small accordingly. This means that this inverse relationship exists in the extreme limit of variables and in other areas, the effect of other parameters should also be considered.

3.8. Selection of final formulation

F7 was finally selected as the most optimum formulation having acceptable texture organoleptically, suitable spreadability, medium viscosity (2.03±0.14 mPa.s), low adhesiveness (28.67±4.62 mN), and high physical stability (2 years without phase separation). Therefore, it was evaluated for pH, microbial controls and chemical standardization as follow.

3.9. pH measurement

The measurement showed that the pH of formulation F7 was 6.0±0.1. The pH is relatively acidic which is in the accepted range for topical formulations (24).

3.10. Preservative efficacy testing

F7 as the selected formulation was evalu-

Table 4. The spreadability of creams under different weights presented as surface area (cm2).

Formulation	Plate (49 g)	Weight 200 g	Weight 500 g
1	11.34	31.13	38.97
2	17.34	33.16	57.34
3	30.43	47.15	65.7
4	16.97	38.5	55.89
5	20.41	48.98	70.65
6	20.42	47.69	54.08
7	20.35	34.96	50.11
8	16.25	35.75	50.23
9	19.63	41.55	53.43
10	19.63	40.69	50.82
11	20.02	35.23	49.56
12	4.66	12.54	17.34
13	5.42	13.5	20.77
14	13.52	32.15	42.39
15	16.97	40.62	58.06
My*	8.55	18.84	33.13

*A commercial, hand and face moisturizing cream.

ated for microbial testing. The results showed that the preservatives used were effective because no colonies had grown after 48 hr. Also, in the antimicrobial effectiveness test, the inhibitory effect of the cream formulation on the growth of bacteria was confirmed and no growth was observed after 14 days.

3.11. Formulation standardization

Menthol as the main component of Mint oil left the GC column in approximately 6.9 minutes (Figure S1). The results of GC-MS quantification test on formulation 7 showed that the product contained 0.45 % (w/w) menthol. As mentioned previously, the active components of essential oils in the cream have various therapeutic effects for treatment of acne including anti-microbial effect. Menthol has approved antibacterial and anti-fungal effects which make it a very effective agent for treatment of acne. Silva *et al.* (2019) proved that topical formulations based on menthol and saturated fatty acids could be suitable for wound healing. They showed antibacterial activity against *Staphylococcus epidermidis* and *Staphylococcus aureus*, including methicillin-resistant strains, and did not elicit any relevant cytotoxicity (25).

Furthermore, menthol has a remarkable anesthetic and cooling effect which efficiently ameliorate the pain of acne. It is noteworthy that the result shows that menthol quantity in the cream (about 0.5 % w/w) is absolutely safe. In low concentrations (0.1–1%) as a part of topical preparations, menthol exerts antipruritic and anti-inflammatory properties and is used for the treatment of pruritus and urticaria. In higher concentrations (1.25–16%), it can cause skin-sensitizing and irritant properties (26).

In addition, the GC-MS analyses showed the existence of the active constituents of the other essential oils used in the product including terpenoids thymol, carvacrol, carvone, linalool, and linalyl acetate which have potent anti-microbial, anti-inflammatory, wound-healing and anti-scaring effects. Many essential oils rich in 1,8-cineole, terpinen-4-ol, carvone, carvacrol, and thymol are excellent antibacterial, antiviral, or antifungal agents (25). A collection of these active agents makes the cream a perfect product for treatment of acne vulgaris.

4. Conclusion

Acne vulgaris is the most common skin disease. As acne is a multicausal disease, its treatment is a big challenge requiring to prescribe many

chemical drugs concomitantly which usually cause severe adverse effects. Hence, todays the physicians tend to use alternate natural products for acne therapy which mainly include herbal medicines. In current study, we aimed to formulate a o/w cream based on natural fixed oils including ostrich and sesame oils. These oils have a long history to treat acne owing to having nourishing fatty acids, anti-inflammatory and anti-oxidant agents. Moreover, the short to medium length of alkyl chains (12 to 20 carbons) compared to very long-chain of petroleum hydrocarbons (80 to 120 carbons) makes them suitable non-comedogenic alternate oils to mineral oils. On the other hand, a composition of *Mentha piperita*, *Origanum vulgare* and *Lavandula officinalis* essential oils could provide all the necessary functions needed for an anti-acne product including anti-microbial, anti-inflammatory, wound healing, anesthetic and analgesic activities. Our results showed that cetomacrogol 1000/CSA was an efficient emulsifying system enabling to formulate organoleptically acceptable and physically stable creams. The creams showed suitable shear-thinning behavior with a wide range of consistencies and adhesiveness. The observations indicated that the creams rheology and texture properties are mainly influenced by thickening agents used in both aqueous and oil phases including CSA, SA and CP. Moreover, the results showed that the creams with optimum consistency could tolerate all stability tests including long-term, accelerated, thermal cycling and centrifuging tests confirming the possibility of formulating a commercial product. A GC-MS analysis showed that the optimum cream had about 0.5 % w/w menthol and other constituents with approved effects on acne. Overall, the results of study showed that a natural anti-acne cream could be developed based on natural fixed and essential oils with excellent organoleptic properties and sufficient physical stability.

Conflict of Interest

None declared.

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