International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 14, Special Issue 3, 2022

Original Article

FORMULATION AND ACTIVITY TEST OF SUNFLOWER OIL (*HELIANTHUS ANNUUS* L.) LIQUID SOAP AS ANTI ACNE

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Received: 15 Dec 2021, Revised and Accepted: 21 Mar 2022

ABSTRACT

Objective: This study aims to obtain a liquid soap containing sunflower oil which has a good physical characteristics and stability, also has potential as an antibacterial towards acne bacteria.

Methods: Sunflower oil is formulated into liquid facial wash with various concentrations 10% (F1); 15% (F2); and 20% (F3). Evaluation of preparations includes organoleptic test, pH value, specific gravity, viscosity, foaming ability, foam stability, freeze and thaw stability, and antibacterial activity against *Propionibacterium acnes* which causes acne by using the disc diffusion method. Data obtained was analyzed using Kruskal Wallis statistical test, One Way ANOVA, and descriptive test.

Results: Based on the results of the evaluation of the preparations, it was found that the organoleptic examination of F1, F2, and F3 liquid soap preparations had a thick liquid form, milky white in color, with the smell of rose. Liquid facial wash preparations of F1, F2, and F3 meet the evaluation requirements of pH, specific gravity, viscosity, ability to form foam, stable during storage in various degrees. Based on the analysis of the One Way ANOVA statistical test, there was a significant difference ($p<0.05$) in the diameter of the inhibition zone between each group of formulas.

Conclusion: It was concluded that the liquid facial wash preparations of sunflower oil F1, F2, and F3 were physically stable during storage, and based on the antibacterial activity test it was known that F3 had the greatest bacterial inhibition with a strong inhibition category (32.19±0.36 mm)

Keywords: Liquid facial wash, Antibacterial, *Propionibacterium acne*, Sunflower oil

DOI: https://dx.doi.org/10.22159/ijap.2022.v14s3.11 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Country with a tropical climate and is located on the equator which is exposed to sunlight every day, which can stimulate the oil gland tissue on the skin. Dust, dirt, and oil are easily stuck on human skin and can clog pores and trigger acne formation. Acne commonly occurs during puberty, but it can occur regardless of gender or race. Although it is not dangerous, it can leave skin scars [1-3]. The cause is unknown, but many factors, including stress, hereditary factors, hormones, drugs, and bacteria, in particular *Propionibacterium acnes*, *Staphylococcus albus*, and *Malassezia furfur*, play a role in the etiology. *Propionibacterium acnes* participates in the pathogenesis of acne by producing lipase, which breaks down free fatty acids from skin lipids. These fatty acids can cause tissue inflammation and contribute to the forming of acne [2, 4].

One of the plants that empirically and based on scientific data has anti-acne properties is the sunflower (*Helianthus annuus* L.). Sunflower seeds contain terpenoids and linoleic acid, which can help in preventing and eliminating acne. Damage the bacterial cell membrane. Linoleic acid works as an anti-inflammatory. This content can be obtained in the form of oil by cold pressing (cold press) on sunflower seeds [5, 6]. The content of linoleic acid in sunflower seed oil has the potential to have antibacterial activity and the results showed the effectiveness of anti-acne against *Propionibacterium acnes* at concentrations of 10%, 15%, and 20% with inhibitory diameters of 9.92 mm, 10.65 mm, and 13.65 mm respectively [7, 8]. The sunflower seed oil contains terpenoids and linoleic acid, so it can inhibit *Propionibacterium acnes* and has been formulated into cream preparations with a minimum inhibitory concentration (MIC) of sunflower seed oil of 1.5% with an inhibitory diameter of 15.28 mm [7, 9].

The purpose of this study was to determine whether sunflower seed oil (*Helianthus annum* L.) can be used as an anti-acne in liquid facial soap preparations and has good stability during storage. Soap is a cleansing cosmetic that is used to clean the skin, dirt, fat, and sweat and makes the skin fresher [9, 10]. Currently, liquid soap is more in demand by the public compared to solid soap, because its use is more practical, more efficient, not contaminated with bacteria, and leasen contact to hand during usage. Facial soap is more often used as an alternative to anti-acne because it is widely known to the public and is more practical and economical to use [11, 12].

MATERIALS AND METHODS

Instruments

The tools used in this research are analytical balance (Fujitsu, Japan), pH meter (Hanna Instrument, Singapore), hotplate (Thermo Scientific, Singapore), magnetic stirrer (Tianjin, China), refrigerator (GEA, Germany), vortex (VWR International, Singapore), Erlenmeyer flask (Pyrex, USA), test tube (Pyrex, USA), funnel (Pyrex, USA), measuring cylinder (Pyrex, USA), Bunsen burner (Usbeck, Germany), tweezers (Misumi, Japan), caliper (Tricle brand, China), beaker glass (Pyrex, USA), pipette glass dropper (Pyrex, USA), petri dish, incubator (Labotect, India), autoclave (Labtech Daihan, Korea), oven (Memmert, Germany), UV-Vis spectrophotometer (Shimadzu, Jepang), micropipette (Mettler Toledo, Greifensee, Switzerland), Ose needle (Local manufacturer, Bandung, Indonesia), viscometer (Brookfield, Middleboro, Massachusetts), pycnometer (Ochoos, USA).

Materials

The ingredients used in this study are sunflower oil (Lansida Group, Indonesia), stearic acid (*industry grade* 99.7%, Bratachem), sodium lauryl sulfate (*industry grade* 97%, Bratachem), sodium chloride *food grade* 98% (CV. Eteris Nusantara), glycerin *cosmetic grade 99.5%* (Brataco), adeps lanae *cosmetic grade 99.9%* (Brataco), triethanolamine *industry grade* 97% (Bratachem), sodium metabisulfite *cosmetic grade* (Bratachem), aqua dest (CV Rudang Jaya), Nutrient Agar media (CV Rudang Jaya), sodium chloride 0.9%

(Merck, CV Rudang Jaya), clindamycin paper disc (Oxoid) and *Propionibacterium acnes* ATCC 11827.

Sunflower oil antibacterial activity test

The tools used in the antibacterial activity were sterilized in the oven at 150 °C for 2 h and some in the autoclave. The ose needles and tweezers are sterilized by burning with a Bunsen flame [13]. Then a total of 5 grams of nutrient agar (NA) media were weighed and then dissolved in 250 ml of distilled water, heated to boiling while stirring until completely dissolved. The mixture was sterilized in an autoclave at 121 °C, for 15 min, then stored in an aseptic cabinet. Colonies of *Propionibacterium acnes* were taken using a sterile needle and then implanted on media so that it was slanted and incubated at 37 °C for 24 h. *Propionibacterium acnes* bacteria were then suspended in physiological sodium chloride solution, then the transmittance was measured using UV-Vis spectrophotometry with a range of 25% at a wavelength of 580 nm [14-16].

For making inoculum media, a total of 0.3 ml of the tested bacterial colonies were put into a petri dish, then 15 ml of nutrient agar medium was added, then homogenized and allowed to solidify at a temperature of 15°-30 °C [15, 17].

The paper disc was dripped with 10 µl of sunflower seed oil that had been prepared at a concentration of 5%, 10%, 15%, 20%, and 25% using micropipettes. The negative was a preparation without sunflower oil and the positive control used was clindamycin. Each paper disc was placed on the media in a petri dish containing a bacterial suspension using sterile tweezers. The Petri dishes were then incubated for 24 h at 37 °C in the incubator. After incubation, the width of the inhibition zone from the bright zone formed around the paper disc was observed and measured using a caliper [14, 16].

Preparation of liquid facial wash

All ingredients are weighed according to the formula listed in the table 1.

Table 1: Formulation of liquid facial wash from various concentrations of sunflower oil

Ingredients	F0	F1	F2	F3
Sunflower oil	-	10%	15%	20%
Stearic acid	2.5	2.5	2.5	2.5
Sodium Lauryl Sulfate	28	28	28	28
Sodium chloride	1.67	1.67	1.67	1.67
Glycerin	0.7	0.7	0.7	0.7
Adeps lanae	0.5	0.5	0.5	0.5
Triethanolamine	0.15	0.15	0.15	0.15
Sodium metabisulfite	0.1	0.1	0.1	0.1
Oleum Rosae	0.1	0.1	0.1	0.1
Aquadest ad	100	100	100	100

The aqueous phase was dissolved first, where sodium lauryl sulfate (SLS) is dissolved in warm water at a temperature of 40-70 °C, then sodium metabisulfite is added until it dissolves, and sodium chloride is added homogenously. The oil phase consisting of stearic acid, glycerin, adeps lanae, and TEA was heated at a temperature of 60-70 °C in a beaker until it melted while stirring with a stirrer at 100 rpm. Making soap base by mixing the water phase and the oil phase at a temperature of 60-70 °C for 30 min at a speed of 300 rpm. The process was then continued by adding sunflower oil into the liquid soap. In the end, oleum rosae is added as a fragrance [18, 19].

Liquid soap characteristic examination

Organoleptic test

Appearance test by looking directly at the color, shape, and smell of the liquid soap that is formed. According to SNI, the ideal standard for liquid soap is to have a liquid form and a distinctive odor and color [13].

pH test

Before testing, the pH meter was calibrated using a solution of pH 7 (neutral) and pH 4 (acidic). One ml of the liquid soap was diluted with distilled water ad 10 ml. The pH meter electrode is dipped into the solution, leave it until the pH value is stable, the pH indicated by the pH meter was then recorded [20].

Specific gravity test

Determination of specific gravity using a pycnometer. This is done by weighing the empty pycnometer (W0), weighing the pycnometer filled with aqua distillate (W1), and weighing the pycnometer filled with sample (W2). Specific gravity is calculated by the formula in the equation below [21, 22].

Eq.1: Specific gravity = W2-W0/W1-W0

Viscosity test

Viscosity was measured with a Brookfield viscometer. An amount of 300 ml of the tested sample was placed in a material holding container, the height of the container was adjusted so that the spindle could move. Set spindle no.2 with a speed of 100 rpm, placed on the hanger and set. The viscometer is turned on for±10 seconds so that the viscosity value is obtained on the viscometer screen [23].

Foam height and foam stability test

The sample was weighed as much as 1 g, put into a test tube, then added up to 10 ml of distilled water, shaken by inverting the test tube, and immediately measuring the height of the foam produced. Then, the tube was allowed to stand for 5 min, then the height of the foam produced was measured again after 5 min [23, 24].

$$\text{Eq.2: Foam stability} = \frac{\text{foam height after test}}{\text{foam height before test}} \times 100\%$$

Stability test

The stability test using the Freeze and Thaw method aims to see if there is phase separation in the preparation during the storage process. Stability tests were carried out by placing 4 ml of the preparation into 8 vials and tightly closed. A total of 4 vials were used as controls, stored at 25 °C. The remaining 4 vials will be used for the Freeze and Thaw cycle by storing the vials at 4 °C for 24 h, then moved to 40 °C storage for 24 h. After 1 cycle was accomplished, the sample was being observed for its organoleptic changes. The test was done and repeated for 6 cycles [25, 26].

Data analysis

The data obtained were analyzed descriptively and presented in the form of data, tables, or figures. The preference test was statistically analyzed using Kruskal Wallis and the bacterial inhibition zone of facial wash was statistically analyzed using one-way ANOVA.

RESULTS AND DISCUSSION

Sunflower oil antibacterial activity test

Sunflower oil antibacterial activity test to determine the effective concentration to be used in the formulation of liquid soap preparations. The results obtained show that at a concentration of 5%, 10%, 15%, 20% and 25% of sunflower oil itself only have a moderate inhibitory power. Less than 5 mm inhibition zone shows a

weak bacterial inhibition activity, moderate category showed by a diameter ranging of 6-10 mm and the strong category was in 10-20 mm inhibition zone [4, 14]. The results of the measurement of the diameter of the inhibition zone can be seen in table 2.

Table 2: Bacterial inhibition activity

Sample	Average diameter of inhibition(mm)±SD (mm)
Control (-)	0.00±0.00
Control (+)	16.2±0.5
SO 5 %	6.24±0.08
SO 10 %	6.44±0.1
SO 15 %	6.27±0.1
SO 20 %	6.37±0.1
SO 25 %	6.06±0.05

*Data was given in mean±SD, n= 3 experiments, SO: Sunflower oil

Each concentration of oil provides inhibitory activity with a moderate category. This is because the use of too high an oil concentration makes it difficult for the oil to diffuse into the agar medium. The negative control used was chloroform. The positive control used an antibiotic clindamycin 2 g/disk which is a broad-spectrum antibiotic and is often used in the treatment of acne. The concentration with the greatest inhibitory power was 10% which was used as MIC so the concentration of sunflower seed oil (*Helianthus annuus* L.) used in the liquid facial soap formulation was 10%, 15%, and 20%. [14].

Liquid soap characteristic examination

Organoleptic test

After 8 w of storage, it was found that F0 (0%) had a clear viscous liquid consistency with a characteristic odor of oleum rosae, at F1 (10%) it was in the form of a thick liquid with a characteristic odor. oleum rosae, and white in color, at F2 (15%) in the form of a thick milky white liquid, with a characteristic odor, oleum rosae at F3 (20%) in the form of a thick, milky white liquid with a characteristic odor of oleum rosae. The higher the concentration of sunflower seed oil, the more concentrated the color produced in the liquid facial soap preparation. The overall odor of the liquid facial soap preparation is the characteristic odor of oleum rosae and the overall form of the liquid facial soap is in the form of a thick liquid.

pH test

From the pH identification, it was found that during 8 w of storage, the pH range of base = 7.04-7.29; F1 = 7.21-7.27; F2 = 7.18-7.41 and F3 = 7.19-7.42. These results met the pH requirements of SNI (1996) soap, which range 6-8. After observing for 8 w, it was seen that an increase in the pH of the preparations in the four formulas was influenced by temperature. As the temperature increases, the bonds holding the protons in preparations are broken and causing the pH to increase.

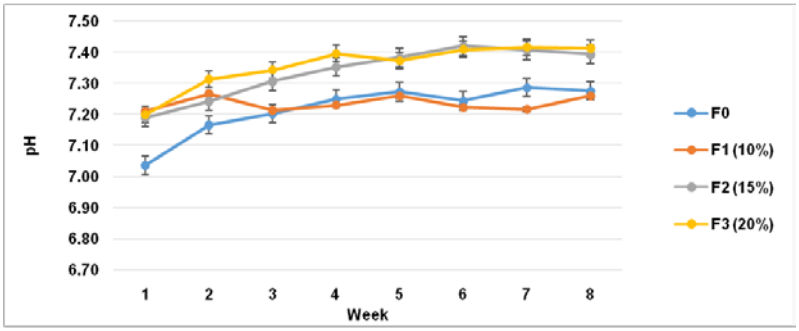

Fig. 1: pH evaluation of liquid facial soap during storage

Specific gravity test

Examination of the specific gravity of a substance is the ratio of the weight of the substance in the air to the weight of water with the same volume and temperature [13]. Determination of specific gravity using a pycnometer which has the advantage of being easy to work with. The results of eight weeks of storage showed specific gravity F0= 1.0388-1.0348 g/ml; F1 = 1.0378-1.0315 g/ml, F2 = 1.0361-1.0301 g/ml and F3 = 1.0296-1.0209 g/ml. This specific gravity is related to the viscosity, i.e. the greater the viscosity of a substance, the greater the specific gravity. Based on the above results, the specific gravity of liquid soap in the first formula to the third formula has met the criteria] which ranges from 1.01-1.10 [13, 14].

Viscosity test

Viscosity is one of the parameters that can affect the level of acceptance of a product by consumers [12, 24]. According to SNI (Indonesian national standard) 06-4085-1996, the viscosity requirements of liquid soap are in the range of 500-20000 cPs, so it can be concluded that the viscosity of sunflower seed oil soap F0, F1, and F2 meets quality requirements, while F3 does not meet required [13, 14].

Table 3: Viscosity result

Formulation	Observation	
	Week-1 (cPs)	Week-8 (cPs)
0	523.8	516.0
1	517.4	511.4
2	509.1	506.3
3	503.4	497.6

Foam height and foam stability test

The foam height test was carried out to see the amount of foam produced by liquid facial soap. Sodium lauryl sulfate (SLS) as a surfactant plays an important role in producing facial soap foam, so the higher SLS concentration will cause more foam to form [13].

Based on [13] the foam height requirement of liquid soap is 13-220 mm. Based on the results obtained, the four formulations met the SNI requirements and the criteria for good foam stability, which if within five minutes, the foam should be able to hold between 60%-70% [13, 14].

Table 4: Foam stability result

Formulation	Foam height (cm)				Foam stability	
	Week-1		Week-8		Week-1	Week-8
	0 min	5 min	0 min	5 min		
F0	8	6.5	7.4	5.5	81.2%	79.7%
F1	6.0	5.5	5.8	5,2	91.6%	89.6%
F2	5.5	5.3	5.3	5.0	96.3%	94.3%
F3	5.5	5.1	5.0	4.5	92.7%	90%

Fig. 2: Foam stability test

Stability test

From the stability observation, it was found that F0, F1, and F2 were stable without any phase separation, while F3 phase started to separate on the 4th cycle. The content of glycerin is hygroscopic which is suspected to absorb moisture from the air, which causes the water content in the preparation to become increase. By this condition, preparation tends to become unstable [19, 23].

Fig. 3: Stability test results after 6 cycles

Activity test preparation of liquid soap sunflower seed oil

The results of the antibacterial activity test the first week before the storage of liquid soap preparations from sunflower seed oil (*Helianthus annuus* L.) against *Propionibacterium acnes* showed the inhibitory diameter at F1 = 26.23+0.30 mm; F2 = 27.41+0.53 mm; F3 = 32.19+0.36 mm; in negative control = 12.16 mm and in positive control is 18.05 mm. Preparations F1, F2, and F3 were included in the category of strong inhibitory power, and the positive control was included in the category of moderate bacterial inhibition, which was 18.05 mm.

The results of the antibacterial activity test in the last week after storage of liquid soap preparations of sunflower seed oil (*Helianthus annuus* L.) against *Propionibacterium acnes* showed the inhibitory diameter at F1 = 22.83+0.39 mm; F2 = 23.40+0.38 mm; F3 = 23.85+0.95 mm; in the negative control 11.67 mm and in the positive control was 18.26 mm. Preparations F1, F2, and F3 were included in the category of strong inhibitory power, and the positive control was included in the category of moderate inhibition of bacteria, which was 18.26 mm.

The inhibition of the negative control formed was caused by the presence of SLS which is bacteriostatic for gram-positive bacteria and glycerin as an antimicrobial agent [14, 27, 28, 29] The value of the diameter of the inhibition zone of the positive control had moderate antibacterial activity, this was because the clindamycin disc used was influenced by poor disc storage factors and the disc had been opened for a long time. Clindamycin discs also had a low concentration of 2 g/disk, while the preparations on discs had concentrations of 1000 g, 1500 g, and 2000 g per disk, thus making the inhibitory power of the positive control smaller than the preparations.

Table 5: Inhibition towards propionibacterium *acnes* of sunflower seed oil liquid soap

Observation for week-1	Control (+) (mm)	Inhibition zone diameter (mm)				Control ±SD (mm)
		I	II	III	Mean±SD (mm)	
F1	18.05	26.49	26.3	25.9	26.23±0.30	12.16±0.32
F2		27.98	26.94	27.31	27.41±0.53	
F3		32.69	32.01	31.87	32.19±0.36	
Observation for Week-8	Control (+) (mm)	Inhibition zone diameter (mm)				Control ±SD (mm)
		I	II	III	Mean±SD (mm)	
F1	18.26	23.39	22.58	22.53	22.83±0.39	11.67±0.36
F2		23.83	23.07	23.31	23.40±0.38	
F3		22.78	24.62	24.15	23.85±0.95	

The data was presented in mean±SD, n=3

Based on the results obtained in the activity test of the preparation, there was an increase in the diameter of the inhibition zone from before formulation compared to after formulation. This is caused by a mixture of oil with surfactants and water that forms an emulsion [22]. Which states that mixing oil with surfactants and water provides greater antibacterial activity than the oil itself. This is because the amount of surfactant concentration used is 30% and the oil dispersion in the emulsion makes the oil homogeneity better to provide increased antibacterial activity.

The results of the one-way ANOVA statistical analysis showed p-value <0.05, which means that there was a significant difference in inhibition diameter in each formula both before and after storage.

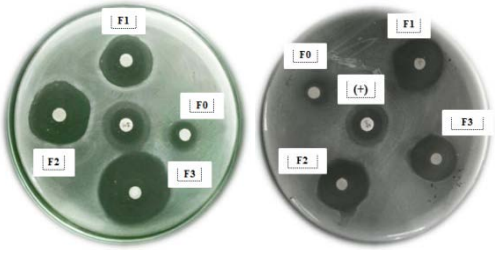

Fig. 4: Inhibition activity of liquid soap before storage (left) and after storage (right)

CONCLUSION

Based on the research, it can be concluded that sunflower seed oil can be used as an anti-acne in liquid soap which is stable during storage. Liquid soap containing 10% and 15% sunflower seed oil met the requirements of its characteristic examination (pH, specific gravity, viscosity, foam height, foam stability, and stable without any phase separation during the accelerated stability test). Based on the One Way ANOVA statistical test, there was a significant difference ($p<0.05$) between the formulas on the diameter of the bacterial inhibition zone, which indicates that sunflower seed oil liquid soap was effective as an anti-acne in the very strong inhibition category (>20 mm).

ACKNOWLEDGMENT

This research was conducted at the Pharmaceutical Laboratory, Laboratory of Natural Material Pharmacy, Research and Biopharmaceutical Laboratory, and Sekolah Tinggi Ilmu Farmasi Riau.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

BI designed the study, supervised, analyzed, and interpreted the data, as well as partook in writing the original draft of the article. GN designed the study analyzed and interpreted data, as well as partook in writing the original draft of the article. RSP designed the study, conducted their search, interpreted data, and partook in writing the original draft of the article. CKL, MDCS, and L individually revised the final article. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript

CONFLICT OF INTERESTS

The authors have no conflict of interest to declare.

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