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Physical and Chemical Characteristic, Irritation Index and Anti-inflammatory Activity from Ointment of *Syzygium aromaticum* Oil by Adding an Enhancer

(Sifat Fisika dan Kimia, Indeks Iritasi dan Aktivitas Anti-inflamasi dari Salep Minyak Atsiri *Syzygium aromaticum* dengan Penambahan *Enhancer*)

MUHAMMAD ALFIAN[1*], NINING SUGIHARTINI[2], SAPTO YULIANI[3]

[1]Departement of Pharmaceutical Technology, Faculty of Halal Industry, Universitas of Nahdlatul Ulama Yogyakarta, Yogyakarta, Special District of Yogyakarta, 55162, Indonesia
[2]Departement of Pharmaceutical Technology, Faculty of Pharmacy, Universitas of Ahmad Dahlan, Yogyakarta, Special District of Yogyakarta, 55162, Indonesia
[3]Departement of Pharmacology, Faculty of Pharmacy, Universitas of Ahmad Dahlan, Yogyakarta, Special District of Yogyakarta, 55162, Indonesia

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Abstract: This research aimed to evaluate how enhancers affect the properties of *Syzygium aromaticum* oil in both water-soluble and hydrocarbon bases. Physical and chemical characteristic tests, irritation index evaluations, and anti-inflammatory activity assessments were carried out to analyze the *Syzygium aromaticum* oil ointment. The pH tests were conducted to measure the chemical characteristics and viscosity, adhesivity, spreadability tests were conducted to measure the physical characteristics of the ointment. Rabbit-based tests were used to determine the irritation index, mice induced by crotton oil were used in anti-inflammatory activity assessments. The pH tests revealed no significant differences between both of ointments. The ointment in the water-soluble base had higher viscosity and adhesivity but lower spreadability than the ointment in the hydrocarbon base. The epidermis thickness of water-soluble and hydrocarbon-based ointments was 3.81±0.40 µm and 4.22±0.15 µm, while the number of cells with COX-2 expression was 18.24±6.51 and 21.01±5.96. The addition of enhancers did not cause any irritation in either ointment. The study concluded that the formula of *Syzygium aromaticum* oil in a water-soluble ointment with the addition of propylene glycol and oleic acid (70:30) as enhancers provided better physical and chemical characteristics, a lower irritation index, and higher anti-inflammatory activity compared to the hydrocarbon.

Keywords: Enhancer, ointment, *Syzygium aromaticum* oil.

Abstrak : Berdasarkan penelitian sebelumnya, salep minyak cengkeh basis larut air memiliki sifat fisik dan indeks iritasi yang optimal dengan konsentrasi 2,5% dan 5% pada basis hidrokarbon. Pengembangan dilakukan dengan penambahan asam oleat dan propilen glikol sebagai *enhancer* dengan komposisi 7:3 dalam basis larut air dan 0:100 dalam basis hidrokarbon. Tujuan penelitian ini untuk mengetahui pengaruh penambahan *enhancer* terhadap sifat fisik, kimia, indeks iritasi dan aktivitas anti-inflamasi salep dalam basis larut air dan hidrokarbon. Salep dievaluasi sifat fisik (viskositas, daya lekat dan daya sebar), kimia (pH), indeks iritasi menggunakan kelinci dan aktivitas anti-inflamasi menggunakan mencit dengan menghitung tebal epidermis dan ekspresi COX-2. Hasil menunjukkan tidak terdapat perbedaan nilai pH yang signifikan antara kedua salep (P>0,05). Salep dalam basis larut air memiliki viskositas dan daya rekat yang lebih tinggi, daya sebar lebih rendah daripada hidrokarbon. Penambahan *enhancer* pada kedua salep tidak menimbulkan iritasi, tebal epidermis kelompok salep larut air dan hidrokarbon rerata 3,81±0,40 µm dan 4,22±0,15 µm, sedangkan jumlah sel yang mengekspresikan COX-2 rerata 18,24±6,51 dan 21,01±5,96. Dapat disimpulkan formulasi minyak cengkeh dalam basis larut air dengan penambahan *enhancer* propilen glikol dan asam oleat (70:30) menghasilkan sifat fisik, kimia, indeks iritasi dan aktivitas anti-inflamasi yang lebih baik dibandingkan dengan salep hidrokarbon.

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Kata kunci : *Enhancer*, minyak atsiri *Syzygium aromaticum*, salep.

*Corresponding author
e-mail: muhammadalfian@unu-jogja.ac.id

INTRODUCTION

RESEARCH has demonstrated that the physical properties of *Syzygium aromaticum* oil as a topical treatment can be influenced by the specific formula used. Increasing the concentration of essential oils on a water-soluble base has been found to have minimal impact on adhesion. However, noteworthy adhesion differences were observed between concentrations of 5% and 15% when applied on a hydrocarbon base[1], Additional studies have further highlighted variations in the physical traits and irritation tests of *S. aromaticum* oil, depending on whether it was incorporated into a water in oil or oil in water cream preparation[2].

During the course of development, additional compounds, including propylene glycol and oleic acid, were incorporated to enhance the formulation. Their purpose was to increase the permeability of eugenol, the active ingredient in *S. aromaticum* oil, so that it could penetrate the skin more effectively. These enhancers have demonstrated the ability to improve the transportation of active ingredients into the skin's layers[3]. Through experimentation with an emulgel and an absorbent basis, it was discovered that the properties of an enhancer can be enhanced by optimising the mixture composition between propylene glycol and oleic acid. The findings indicated that an enhancer made of 100% propylene glycol offered the most optimal physical properties. These results demonstrate the importance of selecting the appropriate enhancer composition to improve the overall physical properties of a product[4].

The objective of this research was to explore the impact of hydrocarbon and water-soluble bases, as well as enhancer composition, on the physical characteristics, irritation index test, and anti-inflammatory activity of *S. aromaticum* oil. Previous studies suggest that the ideal concentration of clove flower essential oil in a water-soluble base was 5% with an enhancer composition of 70% oleic acid and 30% propylene glycol[1,5]. According to the study conducted, it has been discovered that the optimal concentration of *S. aromaticum* oil for hydrocarbon consideration was 2.5%. Moreover, it has been observed that 100% propylene glycol can serve as a potent enhancer[6]. These research findings offer significant contributions to the creation of *S. aromaticum* oil for topical drug delivery.

MATERIALS AND METHODS

MATERIALS. The *S. aromaticum* oil was obtained from the Center of Essential Oils Studies (CEOS) of Indonesian Islamic University, Yogyakarta, Indonesia. The materials were used in the preparation of a water-soluble base ointment and hydrocarbon base ointment with pharmaceutical grade: PEG 400 (Hallstar, USA), PEG 4000 (PT Darnait Esa Artha, Indonesia), Propylene glycol (PT Brataco,Indonesia) oleic acid (Vigoun, USA), liquid paraffin (Vigoun, USA), Vaseline (Dow Chemical, Indonesia) and distilled water (PT Brataco,Indonesia). The irritation test used rabbit (strain albino, male rabbits from New Zealand, 3 months of age, weight 2.5 - 3 kg. The procedure was evaluated and approved by the Ethics Committee of Ahmad Dahlan at 2015 University number 011504040.

Equipments. Equipment descriptions were as follows: Water bath (Memmerth, Buechenbach, Germany), analytical balance (Ohaus, New J, USA), viscometer Rheosys spindle Cone & Plate 2.0/30mm (Rheosys, New Jersey, USA), pH meter (Lutron PH-208, Taipei, Taiwan), and lightening microcscope (Olympus, Tokyo, Japan).

METHODS. Preparation of an Ointment in Water Soluble and Hydrocarbon Base. To prepare both water-soluble and hydrocarbon base ointments, we used the fusion method[1]. The formula can be seen in Table 1. For the former, we heated and mixed PEG 400 with PEG 4000 until the mixture became homogeneous. Then we added a blend of enhancers, including propylene glycol and oleic acid, followed by *S. aromaticum* oil after cooling. For the latter, we combined vaseline album and paraffin liquid, heated and mixed them until they were homogeneous, and then added a mixture of enhancers containing propylene glycol and oleic acid. The addition of *S. aromaticum* oil took place after cooling.

The Evaluation of The Physical and Chemical Properties of The Ointment. The pH was evaluated when 0.5 g of ointment were diluted with 5 mL of distilled water. The pH level of the solution was measured by a pH meter.

The adhesivity test was evaluated by applying a precise amount of 0.25 g of ointment between two glass objects using adhesive tools. A weight of 1 kg was then carefully placed atop the glass objects for a duration of 5 minutes. Subsequently, the objects were subjected to a load testing tool, and an 80 g load was applied. The duration for the two glass objects to separate was recorded meticulously[7].

The spreadability test was measured with the utmost precision, and the circular glass substrate was prepared using established protocols. A measured quantity of 0.5 g of ointment was then meticulously applied to the surface of the substrate. The second circular glass substrate was cautiously positioned over the ointment, and the system was left undisturbed for one minute. The diameter of the ointment, as it

Table 1. Ointment formula.

Product	Ingredients (g)						
	SAO	PEG 4000	PEG 400	VA	PL	OA	PG
Base of water soluble ointment	-	42.5	42.5	-	-	-	-
Water soluble ointment without essential oil	-	42.5	42.5	-	-	3	7
Water soluble ointment without enhancer	5	42.5	42.5	-	-	-	-
Water soluble ointment	5	42.5	42.5	-	-	3	7
Base of hydrocarbon ointment	-	-	-	78.5	8.75	-	
Hydrocarbon ointment without essential oil	-	-	-	78.5	8.75	-	10
Hydrocarbon ointment without enhancer	2.5	-	-	78.5	8.75	-	
Hydrocarbon ointment	2.5	-	-	78.5	8.75	-	10

SAO = *Sygium aromaticum* oil; VA = vaseline album; PL = paraffin liquidium; OA = oleic acid; PG = propylene glycol.

spread outward, was methodically measured at regular intervals until it reached a stable state, and the final diameter was meticulously recorded and tabulated for further analysis[8]. The viscosity of ointment was measure by using a Rheosys Viscometer.

The Measurement of Irritation Index. In this experiment, a total of 12 rabbits were included and were divided into two groups for comparison. One group was given a hydrocarbon ointment, while the other group was given a water-soluble ointment. The experiment began by shaving the hair on the back of the rabbits, and then the backs were divided into six separate areas, each measuring 2x3 cm wide with a distance of 2 cm between them. These six areas were used for the application of the ointment base, ointment without enhancer, ointments without essential oils, enhancer, and untreated. The area receiving treatment was then covered with plaster. After an hour, the skin was observed for any red spots, and this evaluation continued for 24 hours, day 7, and day 14[9].

The Measurement of Anti-inflammatory Activity. The experiment conducted observed 11 sets of mice, each consisting of 6 mice aged between 2-3 months and weighing 20-30 g on average. The sets were then divided into 11 smaller sub-groups, which included normal control, negative control, positive control, water-soluble ointment, hydrocarbon ointment, water-soluble ointment without enhancer, hydrocarbon ointment without enhancer, water-soluble ointment without essential oil, hydrocarbon without essential oil, base water-soluble ointment, and base hydrocarbon ointment. The test was initiated by shaving the hair on the back of each mouse and then applying 0.1 mL of 4% croton oil between 2x2 cm areas on their backs. After 24 hours, the mice's backs were treated with each of the 11 sub-groups for 30 minutes. This treatment was repeated for three days. Upon completion of the treatment, the mice were euthanized, and their skin tissues were used to prepare hematoxylin and eosin staining. The thickness of the epidermis and COX-2 expression were then measured accordingly[2].

RESULTS AND DISCUSSION

Physical and Chemical Properties Evaluation. The outcomes of the examinations conducted on the physical and chemical characteristics of the preparation are depicted in Table 2. According to the test results, the water-soluble ointment base exhibits higher viscosity in comparison to the hydrocarbon ointment base. This was attributed to the presence of PEG 4000 in the water-soluble base, which is solid and enhances the viscosity of the base [10]. Conversely, the hydrocarbon base contains liquid paraffin, which results in a softer texture[11]. Prior investigations have ascertained that the optimal concentration of *S. aromaticum* oil in a water-soluble base was 5%, with an enhancer composition of 70% oleic acid and 30% propylene glycol[5]. The study has also revealed that the ideal concentration of *S. aromaticum* oil for hydrocarbon consideration was 2.5%. Moreover, 100% propylene glycol serves as a potent enhancer[6]. The quantity of propylene glycol in the hydrocarbon base was higher compared to that in the water-soluble base. This humectant retains the moisture of the ointment, resulting in a lower consistency. Consistent with previous studies, the current investigation compares the formulations of *S. aromaticum* oil in two cream base types, namely, oil in water and water in oil. The cream of type W/O contains more propylene glycol than the cream of type O/W, thus resulting in a lower consistency than the cream of type O/W. Furthermore, it has been observed that oleic acid contributes to the viscosity of the ointment[2]. The water-soluble base contains oleic acid as an enhancer, while the hydrocarbon base does not. The viscosity tests showed that there was no significant difference between the viscosity of the water-soluble ointment base and the hydrocarbon ointment base. This implies that the difference in the material composition of the ointment formula does not affect the viscosity (P>0.05).

The viscosity of an ointment was a crucial factor that affected both its spreadability and adhesiveness. As viscosity increases, the ointment becomes more adhesive but less spreadable. Ointments with a water-soluble base typically exhibit higher viscosity than those with a hydrocarbon base, resulting in lower spreadability and higher adhesiveness. These findings are in line with previous investigations that have utilized *S. aromaticum* oil in absorption, lotion, oil in water cream, and water in oil cream[2]. Based on the data analysis, it can be concluded that both the hydrocarbon-based ointment and the water-soluble base ointment do not fulfill the requisite spreadability standards for semisolid dosage forms. The semisolid dosage forms are expected to possess a spreadability range of 5-7 cm2[12]. The outcome of the adhesivity examination indicates that the hydrocarbon-based ointment exhibits a minimal adhesion period of merely 0.09 minutes, which falls short of the prescribed 4-second duration for adhesivity ointments[13]. Therefore, there was a significant difference between hydrocarbon base ointment and water soluble base ointment in spreadability and adhesivity (P<0.05).

Irritation Test. The results of the irritation test are presented in Table 3. The tests revealed that the application of hydrocarbon ointments, water-soluble ointments, enhancers, and ointments without enhancers (comprised of the base and *S. aromaticum* oil) did not result in any irritation. The water-soluble base and ointments without essential oils (composed of the base and enhancer) demonstrated a lower rate of irritation.

Anti-inflammatory Test. One of the parameters to measure anti-inflammatory activity was the thickness of the epidermis. The epidermis has a thickness of 0.4 to 1.5 mm, which makes up the majority of keratin cells, which were divided into 5 layers/stratum[2] . Inflammation that occurs in the skin can be characterised by a thickening of the epidermal layer. Another parameter to analyse anti-inflammatory activity was the assessment of COX-2 expression, which aimed to see whether the preparation of water-soluble ointments and hydrocarbon ointments and *S. aromaticum* oil with the addition of enhancers can reduce, inflammation of the skin. Staining results with immunohistochemical techniques (IHC) using the COX-2 antibody appear brown in the cell cytoplasm, fibroblasts, macrophages, neutrophils, as well as the basal layer of the epithelium, with varying intensities indicating positive expression of COX-2 [2]. Results of the anti-inflammatory activity parameter by thickness of epidermis were presented in Figures 2 and 3, and the anti-inflammatory activity parameter by COX-2 expression was presented in Figure 1. There are significant differences between the normal control group and the negative control (P<0.05). This suggests that induction with crotton oil was capable of causing inflammation in the back skin of mice. Croton oil was used for the induction of inflammation because it contains phorbol esters and 12-0-tetradecanoylphorbol-13-acetate, which can cause inflammation, irritation, swelling, and increased NF-κB. Therefore, it can induce skin papillomas in mice and can induce hyperplasia and infiltration of leukocytes[14–17] , beside that, it can activate phospholipase A2 which can induce the release of arachidonic acid from cell membranes[18].

Table 2. Physical and chemical properties of *S. aromaticum* oil ointment.

Results of test	Water soluble ointment (SAO 5% ; OA 70:PG 30)	Hydrocarbon ointment (SAO 2,5%; PG 100)
pH	6.45	6.43
Viscosity (Pa.s)	3.52	3.05
Spreadability (cm^2)	2.21	4.32
Adhesivity (minute)	40.37	0.09

Table 3. Irritation test result.

Group	Irritation index	
	Water soluble ointment	Hydrocarbon ointment
Normal controls	0	0
Ointment base	0.07	0
Ointment with SAO and enhancer	0	0
Ointment without *Syzygium aromaticum* oil	0.07	0
Ointment without Enhancer	0	0
Enhancer	0	0

Treatment with an ointment of *S. aromaticum* oil in a water-soluble base and hydrocarbon base in mice resulted in the induction of inflammation and was able to reduce the thickness of the epidermis and the number of cells with COX-2 expression significantly. This suggests that *S. aromaticum* oil has anti-inflammatory activity. This data was supported by the significant differences between groups that were given the formula of ointment and groups that were given the formula of ointment without *S. aromaticum* oil. It was the same result when it was compared between groups that were given formulas of ointment and groups that were given bases of ointment. The activity as an anti-inflammatory was equal to the positive control group which was given anti-inflammatory dosage form that usually use in the medication.

The thickness of the epidermis in the group that was given *S. aromaticum* oil preparation in a water-soluble base is also significantly smaller than the group that was given clove essential oil in a hydrocarbon base. It was the same with the result of the number of cells with COX-2 expression. Water-soluble bases have hydrophilic characteristics, while hydrocarbon bases have lipophilic characteristics. The condition causes the *S. aromaticum* oil to be easier to release from the water solule base. Hydrocarbon bases were composed of material that was lipophilic, so *Syzygium aromaticum* oil tended to dissolve in it and was more difficult to release from base. This was similar to the previous study[6] . The activity of *S. aromaticum* oil aas an anti-inflammatory was more active in cream-type oil in water than in cream-type water in oil.

The inclusion of *S. aromaticum* oil as an enhancer in the ointment formula has been shown to enhance its anti-inflammatory properties. This was demonstrated by a noticeable decrease in the number of cells expressing COX-2 and in the thickness of the epidermis, particularly in the hydrocarbon-based ointment. Preparations without the enhancer had a thicker epidermis and a higher number of cells expressing COX-2 than those with the enhancer. The reduction in the thickness of the epidermis and the number of cells expressing COX-2 was more substantial in the water-soluble ointment base containing an enhancer composition that consisted of a mixture of propylene glycol and oleic acid.

Ointment-base hydrocarbons contain only one type of enhancer, propylene glycol. One of the enhancers that was widely used is oleic acid can change the structure of the fat layers of the stratum corneum and may increase the permeability of layers of the epidermis as well as the formation of lacuna. Propylene glycol acts as a co-solvent in oleic acid that would interfere with polar arrangement on the bilayer lipid layer so as to facilitate drug primarily hydrophilic penetration into the layers of the skin[19-21]. Besides that, diffusion coefficient of the drug in the skin can increase by adding an oleic acid[22].

Propylene glycol was a widely known ingredient used to enhance the efficacy of topical preparations. It acts by interacting with the fatty layer of the skin, which in turn weakens the defence function of the skin. This weakening effect leads to an increase in the solubility of drugs in the stratum corneum, the outermost layer of the skin. With the increased solubility, the flux of drugs passing through the skin also increases, thus making the drugs more effective in treating the targeted condition[5,23].

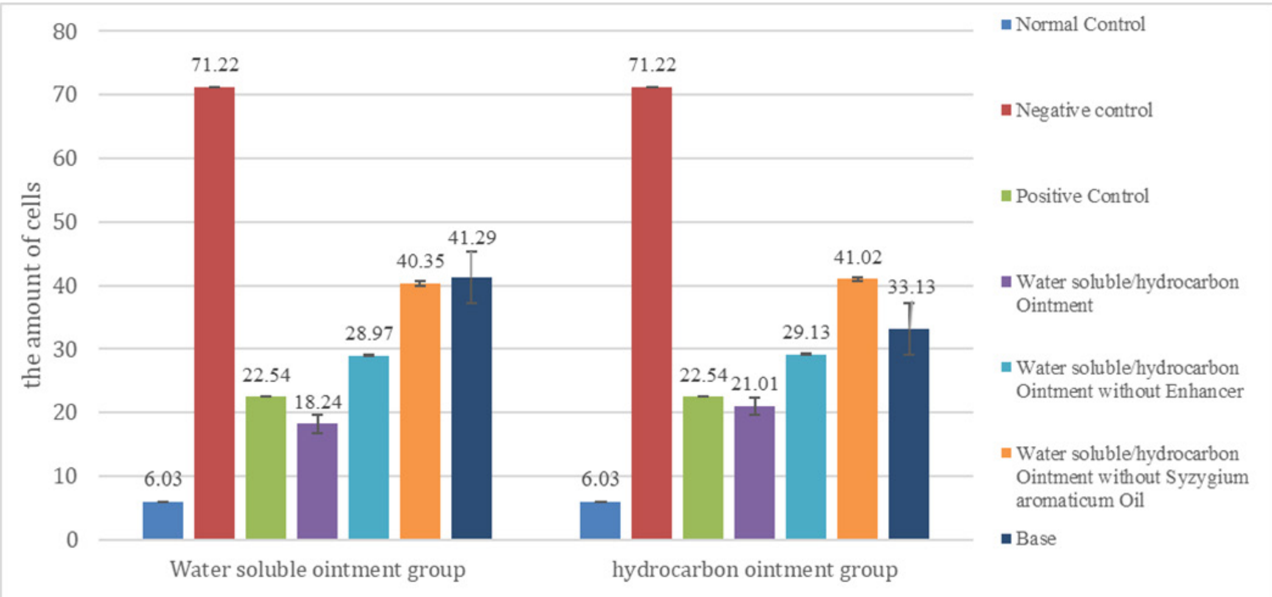

Figure 1. The results of anti-inflammatory test based on amount of COX-2 expression.

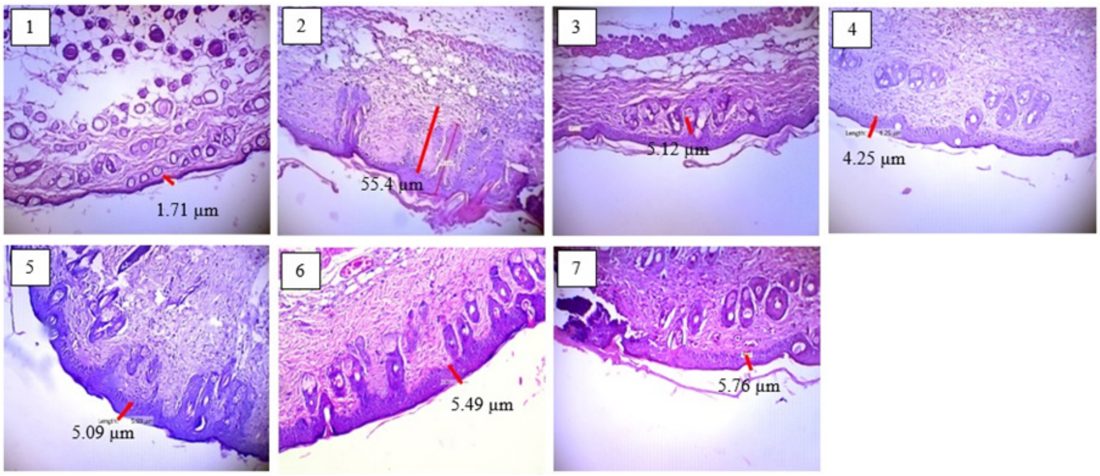

Figure 2. Microphotography thickness of the epidermis mice sign by red line, normal control (1), negative control (2), positive control (3), water soluble ointment (4), water soluble ointment without enhancer (5), water soluble ointment without *Syzygium aromaticum oil* (6) and base (7), zoom 100x.

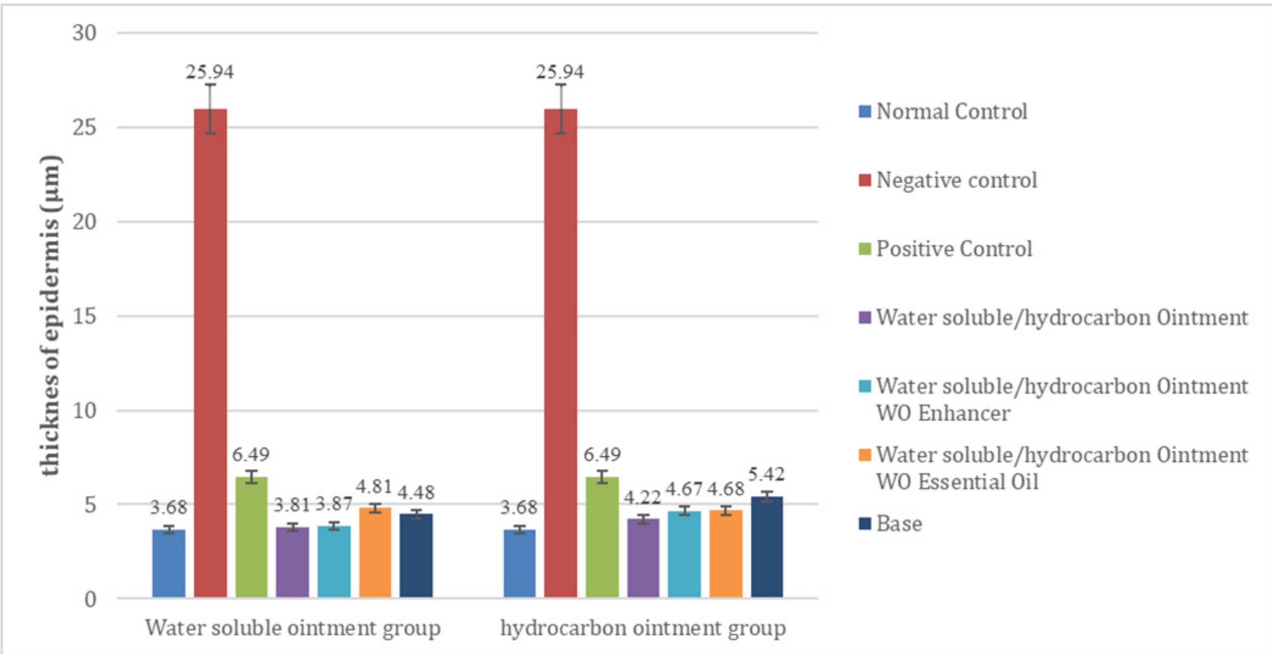

Figure 3. Results of anti-inflammatory test based on thicknes of epidermis (µm).

CONCLUSION

S. aromaticum oil in a water-soluble base ointment and addition by propylene glycol and oleic acid (70:30) as enhancers provides better physical and chemical characteristics, irritation index, and anti-inflammatory activity compared to hydrocarbon ointment.

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