BUKTI KORESPONDENSI

ARTIKEL JURNAL INTERNASIONAL BEREPUTASI

Judul Artikel	Antiinflammatory Activity of *Syzigium aromaticum* Essential Oil in Emulgel
Jurnal	Jordan Journal of Pharmaceutical Science Volume 13 No 2 Tahun 2020
Penulis	Nining Sugihartini, Muhammad Fariez Kurniawan, Tedjo Yuwono

No.	Perihal	Tanggal
1.	Bukti konfirmasi submit artikel	**8 Desember 2018**
2.	Bukti konfirmasi review dan pertanyaan reviewer	**16 Februari 2019**
3.	Bukti konfirmasi submit revisi pertama	**19 Maret 2019**
4.	Bukti konfirmasi artikel accepted	**20 Mei 2020**

Tahap Submit

Bukti konfirmasi submit artikel di jurnal pada tanggal 8 Desember 2018 tersaji pada lampiran 1.

Tahap Revisi

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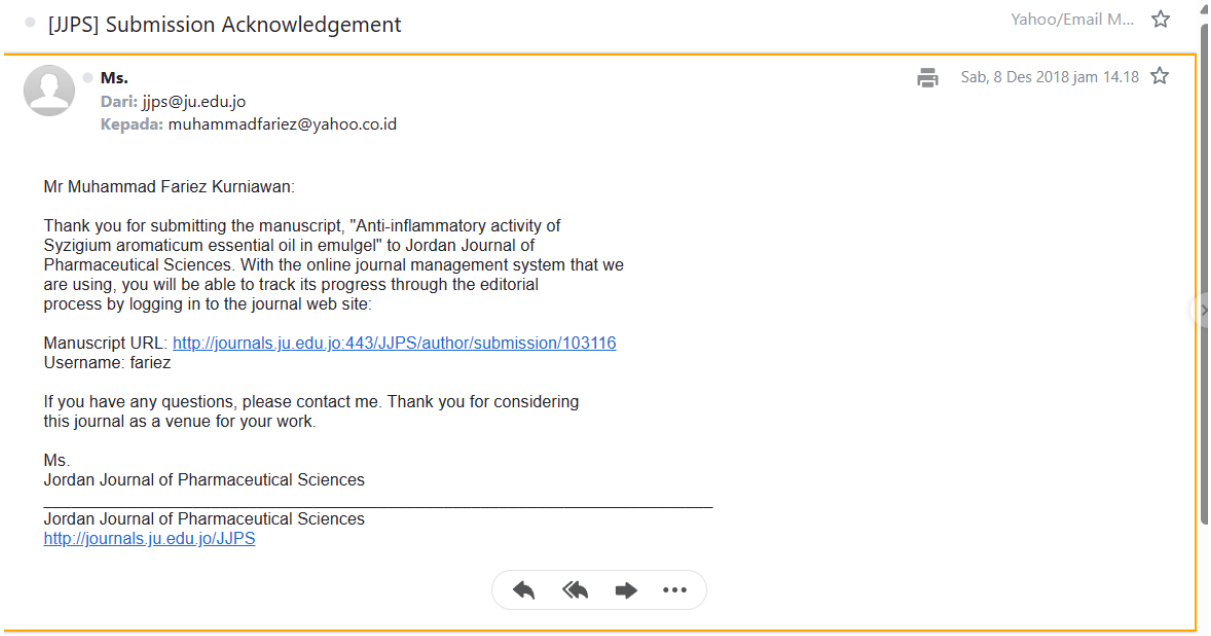

[JJPS] Submission Acknowledgement

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Ms.
Dari: jjps@ju.edu.jo
Kepada: muhammadfariez@yahoo.co.id

Sab, 8 Des 2018 jam 14.18

Mr Muhammad Fariez Kurniawan:

Thank you for submitting the manuscript, "Anti-inflammatory activity of Syzigium aromaticum essential oil in emulgel" to Jordan Journal of Pharmaceutical Sciences. With the online journal management system that we are using, you will be able to track its progress through the editorial process by logging in to the journal web site:

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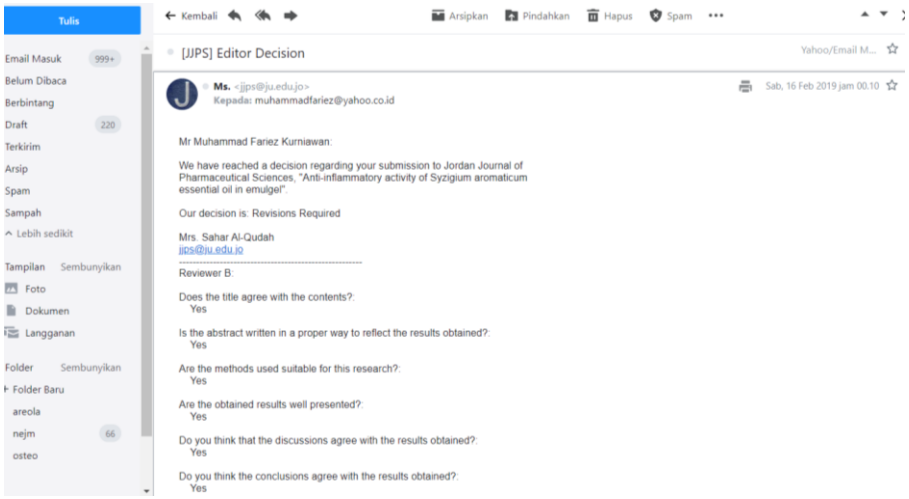

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Kepada: muhammadfariez@yahoo.co.id

Sab, 16 Feb 2019 jam 00.10

Mr Muhammad Fariez Kurniawan:

We have reached a decision regarding your submission to Jordan Journal of Pharmaceutical Sciences, "Anti-inflammatory activity of Syzigium aromaticum essential oil in emulgel".

Our decision is: Revisions Required

Mrs. Sahar Al-Qudah
jjps@ju.edu.jo

Reviewer B:

Does the title agree with the contents?:
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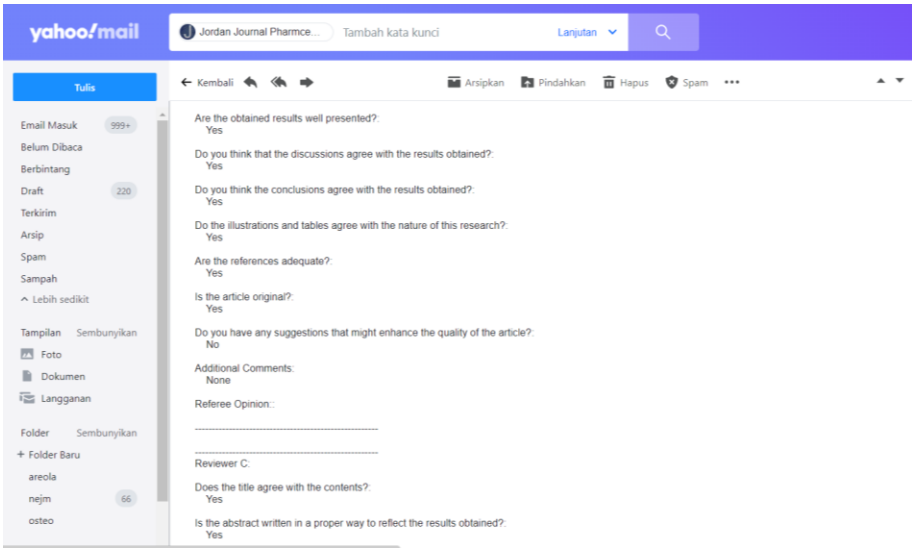
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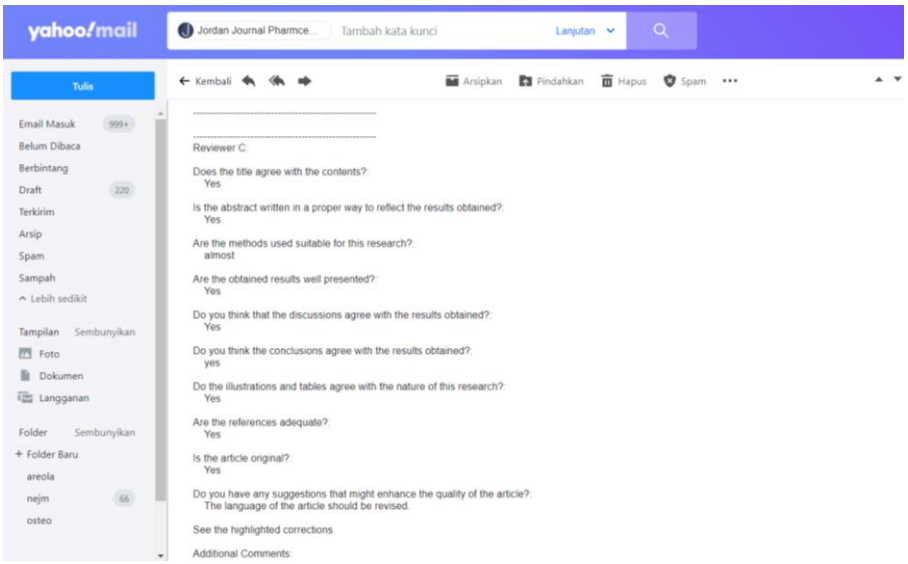
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Reviewer C:
Does the title agree with the contents?:
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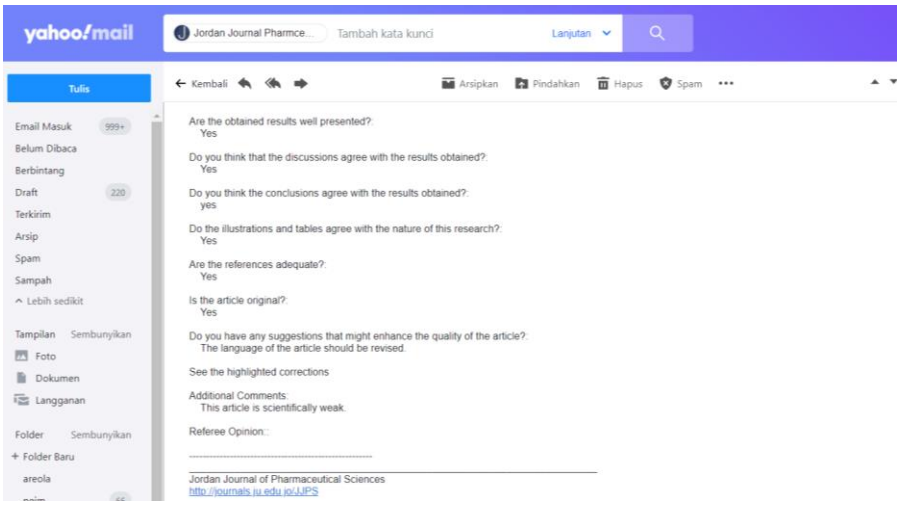
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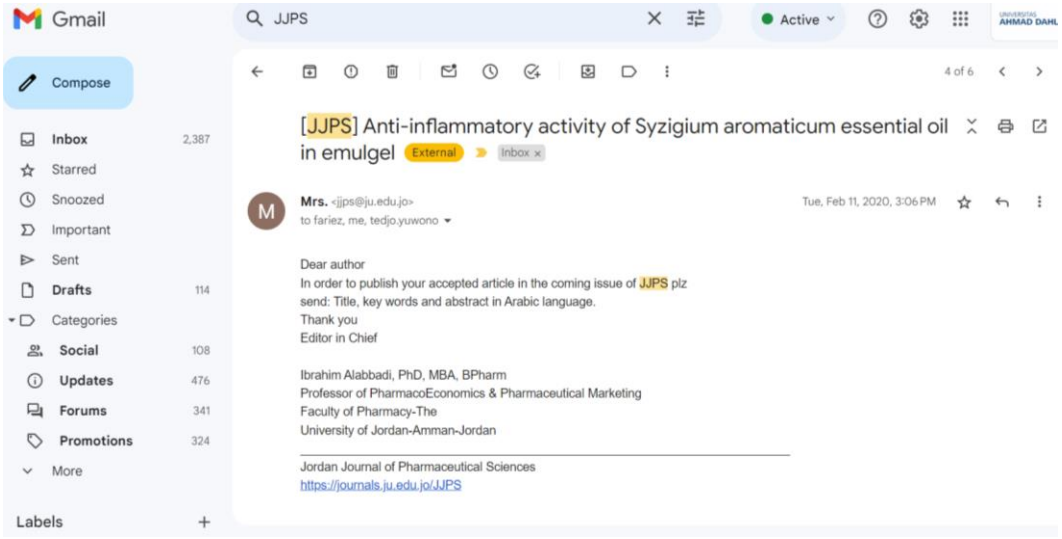
Lampiran 3. Permintaan dan bukti penambahan judul dan abstrak bahasa arab



Jordan Journal of Pharmaceutical Sciences, Volume 13, No. 2, 2020

Anti-inflammatory Activity of *Syzygium Aromaticum* Essential Oil in Emulgel

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ABSTRACT

The essential oil of clove has acted as an anti-inflammatory. This study aims to detect the influence of the various component of oleic acid and propylene glycol as an enhancer to the anti-inflammatory activity of essential oil of clove in emulgel. The composition of oleic acid -(AO) and propylene glycol -(PG) in emulgel was based on Simplex Lattice Design method are FI (100% 0% AO-PG), FII (50% AO-50% PG), FIII (0% AO- 100% PG). Emulgel was evaluated for anti-inflammatory activity by using male mice strain BALb/C which was induced inflammatory with croton oil. The results of the study showed the increasing concentration of propylene glycol caused the decreasing of the value of COX-2 ($p>0.05$) and the thickness of epidermis ($p <0.05$). On the other hand, the increasing concentration of propylene glycol caused an increase in the number of inflammatory cells ($P>0.05$). The optimum composition of enhancer in emulgel of essential oil of clove was 100% of propylene glycol.

Keywords: Anti-inflammatory, Emulgel, Enhancer, Oleic acid, Propylene glycol.

INTRODUCTION

The main component of clove is eugenol that has acted as an anti-inflammatory. The mechanism as an anti-inflammatory is inhibiting the activity of enzyme cyclooxygenase-2 and lipoxygenase-15 enzyme (1). Other studies have shown that eugenol at doses of 200 and 400 mg/kg can reduce pleural exudates without altering some many leucocytes in the blood. This indicates the anti-inflammatory effect of eugenol (2). These potentials need to be developed in the appropriate dosage forms for the benefit of the wider community. Currently, the emulgel is preferable because it is more stable, the drug release can be controlled and more comfortable application than cream and ointment (3). On the other hand, one of the challenges of dosage forms that are applied on the skin is the penetration ability of the active substance in penetrating the skin layer especially the stratum corneum (4). One attempt to enhance the ability of active ingredients to penetrate the skin layer is by the addition of enhancers (5). The chemicals that can be used as enhancers are oleic acid and propylene glycol (6).

The results of research conducted by Sari et al. (2015) showed that emulgel of clove essential oil concentration 10-15% had an excellent profile of physical properties and did not cause irritation in the test animals (7). Based on the result, this research will be formulated at 10% essential oil of clove in emulgel with the addition of a mixture of oleic acid and propylene glycol as an enhancer. The combination of propylene glycol and oleic acid is capable of producing a synergistic effect to increase the absorption of some drugs (8). Oleic acid has been shown to increase the anti-inflammatory drug Lumiracoxib (9). Similarly, propylene glycol was widely used in topical products due to its excellent skin penetration ability (10). The simplex lattice design method is used to determine the influence of composition

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variation of oleic acid and propylene glycol as enhancers to the anti-inflammatory activity of essential oil of clove in emulgel based on the parameters of epidermal thickness, the number of inflammatory cells and the percentage of cells with COX-2 expression.

1. MATERIAL AND METHODS

1.1 Material

This research used essential oil of clove (MABC) that was obtained from the Center for Essential Oils Studies (CEOS) Universitas Islam Indonesia, Sleman, Yogyakarta. The ingredients of emulgel with the pharmaceutical degree were carbopol 940, oleic acid, propylene glycol, triethanolamine (TEA), sorbitol, paraffin liquid, span 80, tween 80, Methylparaben, Propylparaben, and distilled water. The male mice strain BALB/c (weight 25-30 g) used in the anti-inflammatory test. The croton oil (Sigma) was used to induce inflammation on the back skin of mice. The equipment in this study is glassware (Pyrex) water bath (Memmerth), the analytical scale (Ohaus), the microscope (Olympus).

1.2 The Formulation of Essential Oils of Clove in Emulgel

The emulgel formula referred to the results of the previous study as presented in Table 1 (7). Emulgel preparation had started with the soaking of gelling agent Carbopol 940 with 30 mL hot distilled water for 24 hours. After that, the water phase and oil phase was melted on the water bath at 60°C. Both of the two periods were mixed. The essential oil of clove was added after the mixture getting cold. Finally, the combination of the emulsion was added into the Carbopol 940 solution to form a homogeneous mixture.

Table 1. Formulation of Essential Oil of Clove in Emulgel

Materials (g)	FI	FII	FIII
Essential Oil of Clove	10	10	10
Carbopol 940	4	4	4
Propylene glycol	10	5	-
Oleic acid	-	5	10
TEA	8	8	8
Sorbitol	2	2	2
Paraffin liquid	1.25	1.25	1.25
Span 80	2.5	2.5	2.5
Tween 80	17.5	17.5	17.5
Methylparaben	0.18	0.18	0.18
Propylparaben	0.02	0.02	0.02
Distilled water to	100	100	100

FI: Formulation of Emulgel containing 100% of propylene glycol

FII: Formulation of Emulgel comprising 50% of propylene glycol and 50% of oleic acid

FIII: Formulation of Emulgel containing 100% of oleic acid

1.3 The Anti-inflammatory Test

In this test, the mice were divided into two groups consisting of the control group (3 groups) and treatment (3 groups). The control group was the healthy control which was no treatment (KS), the positive control which was treated with induction of inflammation and then was smeared with voltaren® emulgel which has been shown to be efficacious as an anti-inflammatory (KP) and the negative control which was treated with induction of inflammation (KN). The treatment group was the group which was induced inflammatory and then was smeared with emulgel with 100% of propylene glycol (FI), 50% of propylene glycol and 50% of oleic acid (FII) and 100% of oleic acid (FIII). Each group consisted of 6 mice. The procedure of inflammatory induction was initially with shaving the back of the mice in the area of 2x2 cm. After 24 hours, the back of the mice was dropped with 0.1 ml of 4% solution of croton oil. After 30 minutes, the back of the mice was smeared with 100 mg of F1, FII, FIII and positive control (voltaren®). The treatment was done for three days. After that, the mice were sacrificed, and the back of the skin was taken to make of histopathological preparation. The subsequent preparations were stained with HE and COX2.

Based on the results of the painting could be the measured thickness of the epidermis, the number of inflammatory cells and the percentage of cell with COX-2 expression (11). This research received approval from the UAD Committee of Ethics NO. 011508062 in 2015.

2. RESULTS AND DISCUSSION

The results of the anti-inflammatory test are presented in table 2. Furthermore, the data were analyzed statistically to know the significant difference between groups. The results of the statistical analyses showed that between the healthy control group (KS) and negative control group (KN) were substantial differences in the data of epidermal thickness, the amount of inflammatory cell and the percentage of the cell with COX-2 expression. It showed that croton oil could induce inflammation. The previous study was used it as inductor of inflammation (12). The mechanism of croton oil was by activating the phospholipase-A2 enzyme that converts phospholipids to arachidonic acid (13). Using croton oil on the topical application may cause irritation and inflammation, so it was used to induce inflammation (14). In this study, the epidermal thickness was used as one of the parameters to evaluate the anti-inflammatory activity of emulgel. Since it was highly correlated with a reduction in levels of the inflammatory markers (15). The data of epidermal thickness, the amount of inflammatory cell and the percentage of cells with COX-2 expression are shown in Table II. The microscopic picture of skin tissue with hematoxylin-eosin (HE) and cells with expressing COX-2 with immunohistochemical staining with 400x magnification was shown in Figure.1

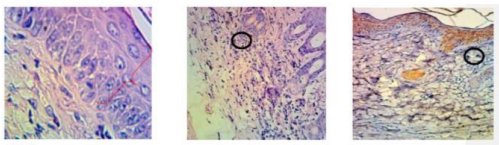

Figure 1: Representative staining with hematoxylin and eosin (HE) (A) Epidermal thickness (B) inflammatory cells and (C) cells with expressing of COX-2 with immunohistochemical staining in 400x magnification.

Table 2. Epidermal thickness, the amount of inflammatory cell and the amount of cell with COX-2 expression in various treatment groups

Parameter	KS	K+	K-	FI	FII	FIII
Thickness of the epidermis (µm)	81.9 ±26.88	107.2 ±8.42	228.0 ±12.95	156.69 ±26.76	181.60 ±21.44	193.69 ±21.21
The amount of inflammatory cells	13.17 ±2.31	59.67 ±2.50	70.83 ±3.66	24.77 ±3.71	26.11 ±4.87	23.44 ±5.32
The % of cell with COX-2 expression	18.16 ±4.95	31.23 ±2.41	43.63 ±3.57	20.74 ±7.49	21.11 ±5.33	26.98 ±6.51

KS = Healthy control
K+ = Positive control
K- = Negative control
FI = Formula I
FII = Formula II
FIII = Formula III

The statistical test was also performed between the data of the treatment groups (FI, FII, FIII) and the data from emulgel without enhancer group. The results of tests showed the thickness of the epidermis, the number of inflammatory cells and the percentage of cells with COX-2 expression of emulgel with the addition of enhancers were lower than the emulgel without enhancers. A significant decrease was found in the amount of inflammatory cell data. Based on the calculation of Simplex Lattice Design it was known that the smallest amount of inflammatory cells and epidermal thickness were obtained in the composition of 100% oleic acid as presented in Figures 2 and 3. Oleic acid is one of the widely used enhancers (16) could alter the structure of the fatty layer on the stratum corneum (17) and therefore might increase the permeability of the epidermal layer as well as by the formation of lacuna (18).

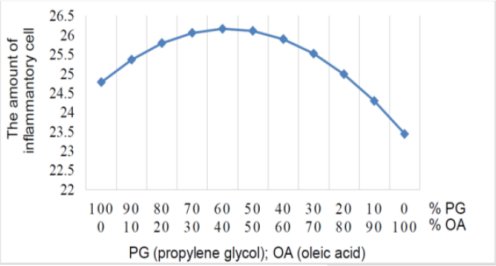

Figure 2: The relation between propylene glycol and oleic acid composition as an enhancer in emulgel to some many inflammatory cells

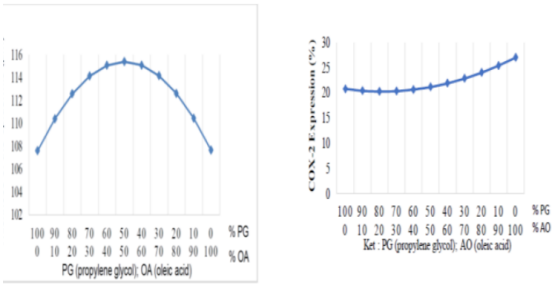

Figure 3: The relation between propylene glycol and oleic acid composition in emulgel as an enhancer on epidermal thickness.

Different results were shown in the percentage of cells with COX-2 expression. Based on figure 4 it was known that the rate of cells with the smallest COX-2 expression was obtained in a 100% propylene glycol enhancer composition. Propylene glycol was an enhancer that widely was used in topical preparations. Its mechanism was interacting with the fat portion of the stratum corneum. Also, it can decrease the skin's defense function and increase the solubility of drugs in the stratum corneum, so there was an increase in the flux of drugs passing through the skin (19,20,21). The increase of flux causes eugenol in clove essential oil could enter the skin and caused the decreasing of the percentage of cells with COX-2 expression. The previous study showed that the increasing composition of proplene glycol raised the anti-inflammatory activity of essential oil of clove in absorption base (22) and in lotion (23).

Further statistical tests were used to evaluate the effect of the compositions of enhancer to the anti-inflammatory activity of emulgel. The difference in the enhancer composition did not make a significant difference in the thickness of the epidermis, the number of inflammatory cells and the percentage of cells with COX-2 expression. This means the use of oleic acid or propylene glycol either individually or mixed would have the same effect. This was reinforced by the result of the statistical test between the group that contains various enhancer compositions with positive controls as well as healthy controls. All formulas showed significant differences in the number of inflammatory cells and the percentage of cells with COX-2 expression (except in FIII). While on the data of epidermal thickness there was no significant difference. This means that the emulgel formula with the addition of enhancers has the same capability as the products on the market to reduce the thickness of the epidermis.

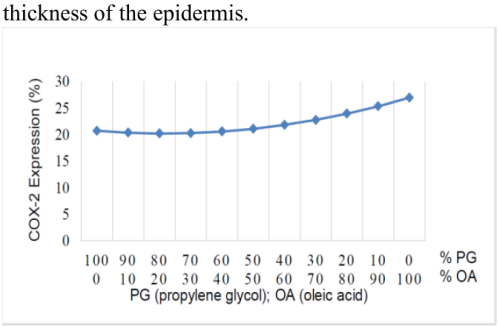

Figure 4: The relation between propylene glycol

and oleic acid composition in emulgel as an enhancer to% COX-2 Expression

The result of the statistical test between healthy control and all of the formula emulgel was significant differences, especially in the number of inflammatory cells. The thickness of the epidermis, the number of inflammatory cells and the percentage of cells with COX-2 expression in healthy controls were still smaller than the formula of emulgel. It showed that the emulgel administration has not been able to restore the condition to normal. This was probably due to the application of emulgel just only for three days, so it was not enough to restore the skin to its original condition.

CONCLUSION

The addition of enhancers could increase the anti-inflammatory activity of essential oil of clove. The variation of enhancer composition does not affect the anti-inflammatory activity of emulgel based on epidermal thickness, the number of inflammatory cells and the percentage of the cell with COX-2 expression.

ACKNOWLEDGEMENT

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النشاط المضاد للالتهابات من زيت القرنفل الضروري في هلام

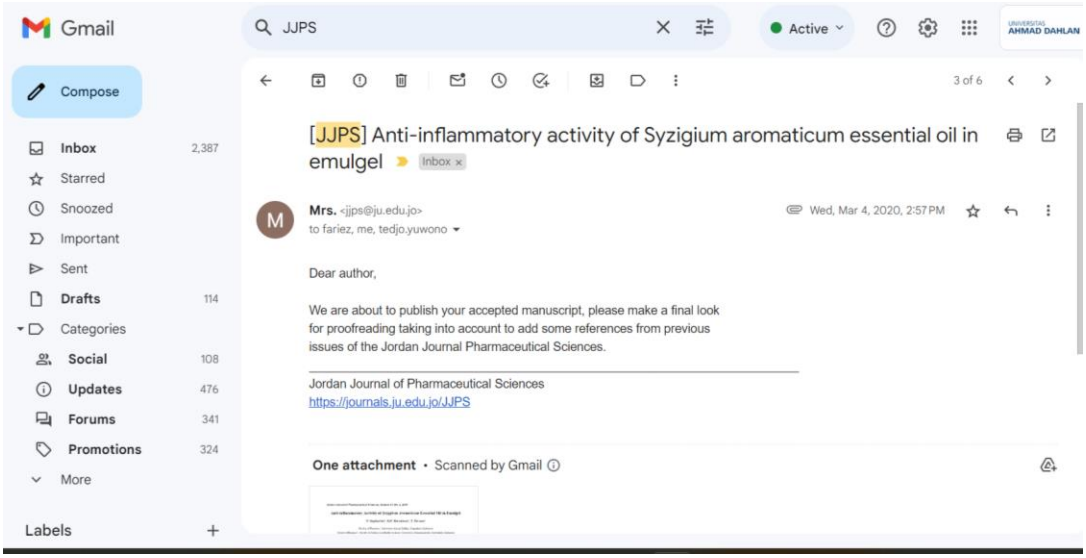
ملخص

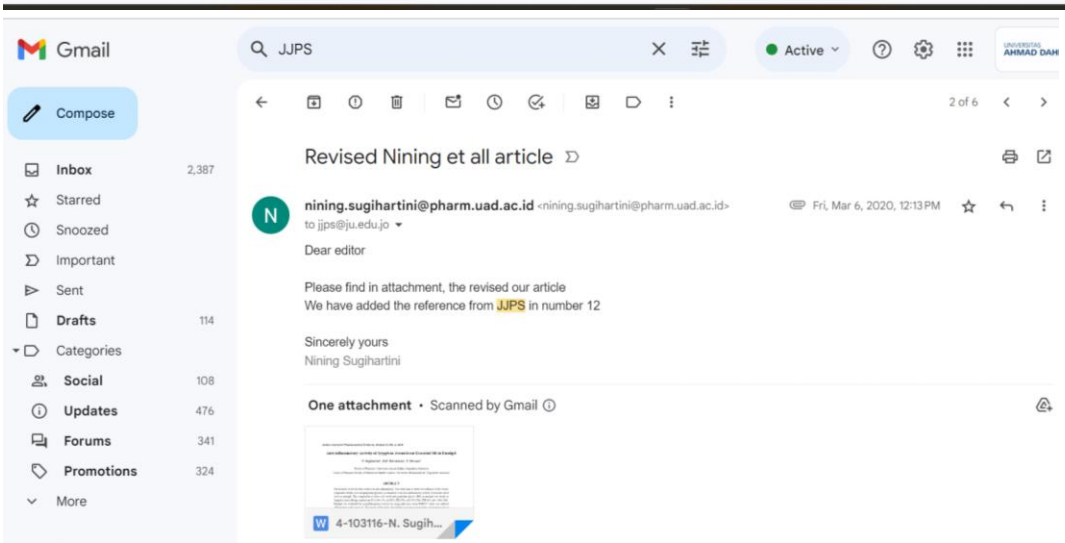
عمل زيت القرنفل الأساسي كمضاد للالتهابات. تهدف هذه الدراسة إلى معرفة تأثير المكونات المختلفة لحمض الأوليك وبروبيلين غليكول كمعزز للنشاط المضاد للالتهابات في الزيت العطري للقرنفل في هلام. استند تكوين حمض الأوليك

(OA) والبروبيلين غليكول (GP) في هلام على طريقة تصميم البسيط شعرية وهي IF (100 % OA – 0% GP), IIF (OA – 50 % GP % 50), IIIF (0% OA – 100 % GP). تم تقييم جل للنشاط المضاد للالتهابات باستخدام سلالة الفئران الذكور C/bLAB الذي تم إحداثه بالتهاب بزيت كروتون. أظهرت نتائج الدراسة أن زيادة تركيز البروبيلين غليكول تسبب في انخفاض قيمة XOC (p> 0,05)–2 وسمك البشرة (p < 0,05). من ناحية أخرى ، تسبب زيادة تركيز البروبيلين غليكول في زيادة عدد الخلايا الالتهابية (p> 0,05). كان التكوين الأمثل للمحسن في هلام الزيوت الأساسية من القرنفل 100 ٪ من البروبيلين غليكول.

الكلمات الدالة: المضادة للالتهابات، هلام، محسن، حمض الأوليك، البروبيلين غليكول.

Lampiran 4. Permintaan dan bukti penambahan pustaka





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(22) Sugihartini, N. Prabandari, R. Yuwono, T. 2019. The anti-inflammatory activity of essential oil of clove

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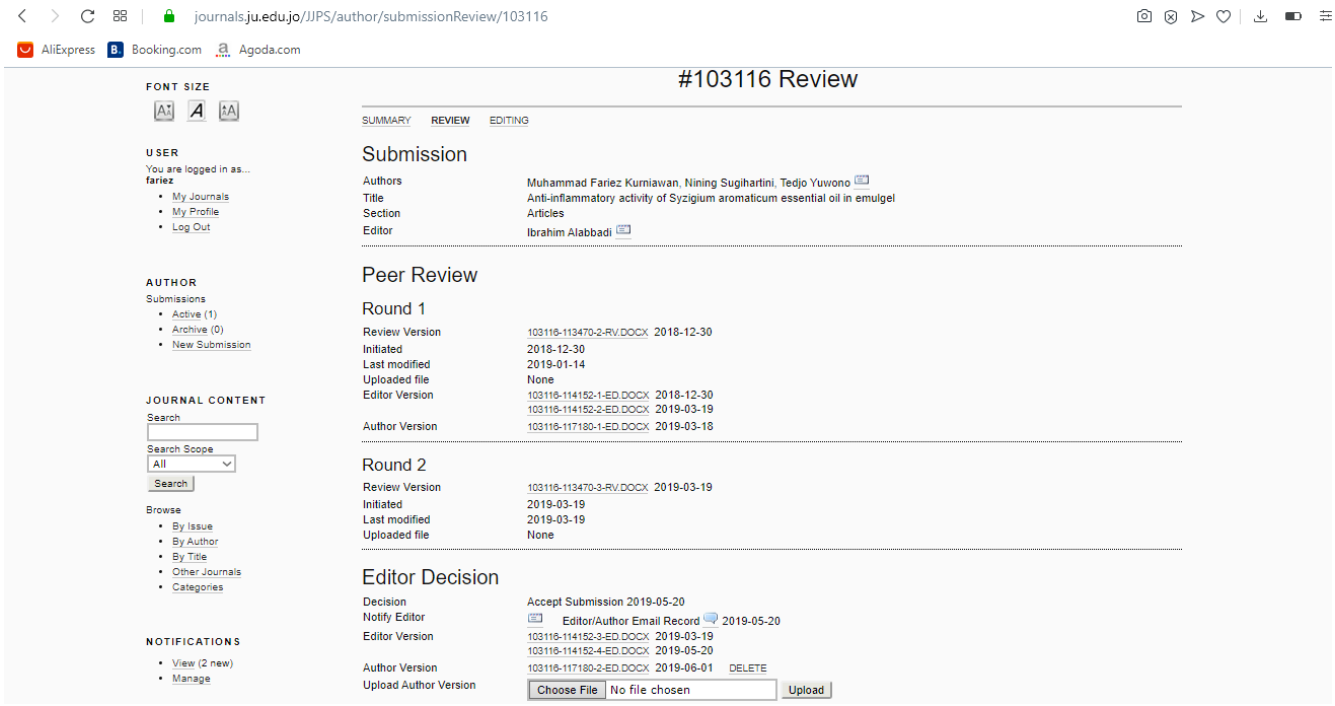

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Do you think that the discussions agree with the results obtained?:
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Do you think the conclusions agree with the results obtained?:
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Mr Muhammad Fariez Kurniawan:

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Mrs. Sahar Al-Qudah
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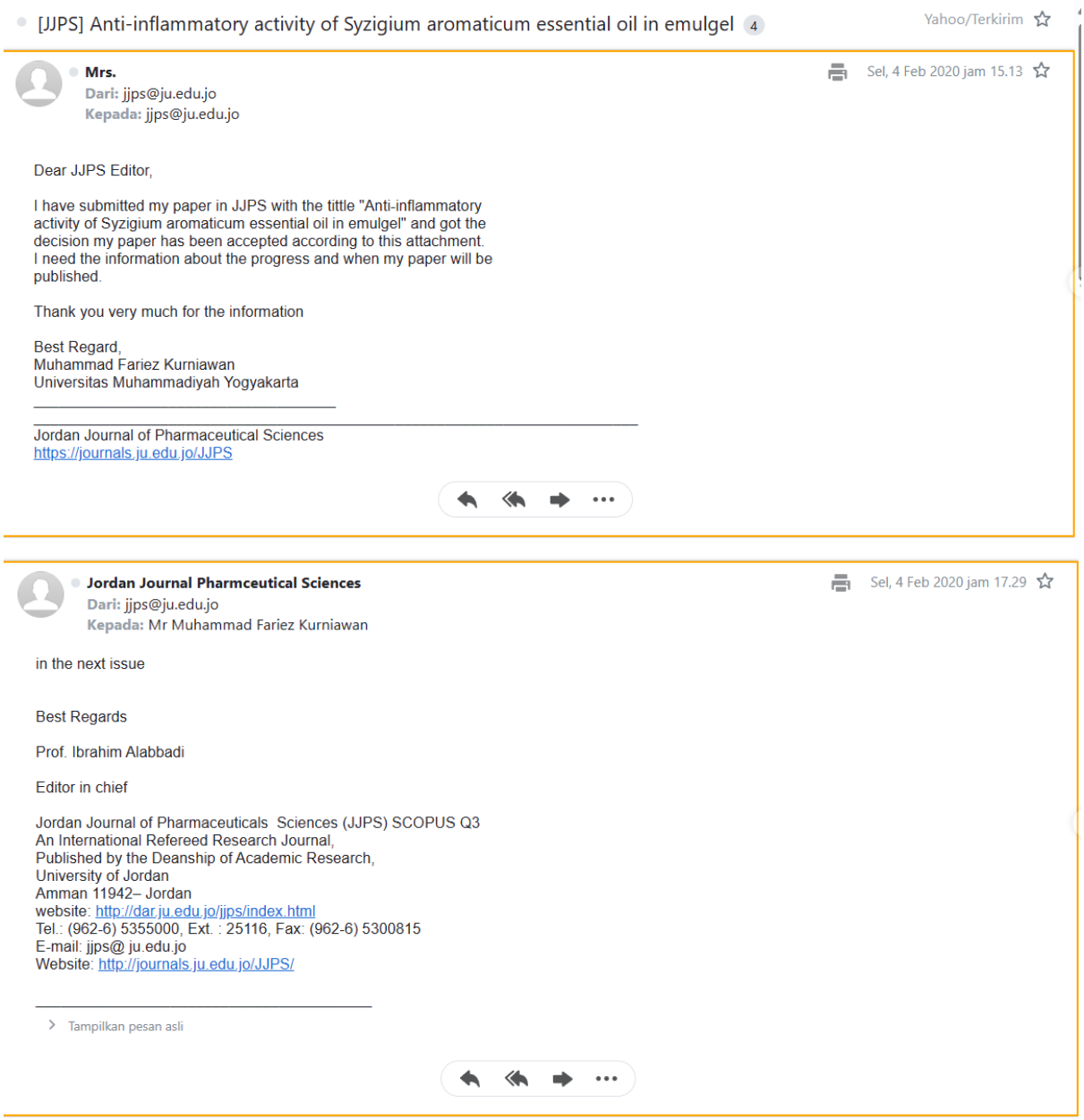

[JJPS] Anti-inflammatory activity of Syzigium aromaticum essential oil in emulgel 4

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Dari: jjps@ju.edu.jo
Kepada: jjps@ju.edu.jo

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Best Regard,
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Editor in chief

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