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# 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

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method is used to determine the influence of composition 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. Themale 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 ofgelling agent Carbopol 940 with 30 mL hot distilled water for24 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 Emulgelcomprising 50% of propylene glycol and 50% of oleic acid

FIII: Formulation of Emulgelcontaining 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 wasthe healthy control which was no treatment (KS), the positive control which was treated with induction of inflammation and then was smeared withvoltaren® emulgel which has been shown to be efficacious as an anti-inflammatory (KP) and the negative control which was treatedwith induction of inflammation (KN). The treatment group was the group

which was induced inflammatory and then was smeared withemulgel 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 ofthe 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

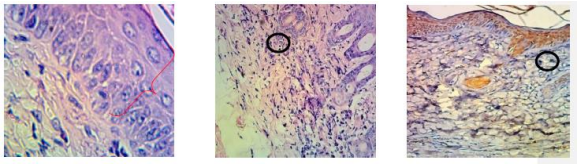

**Figure 1:Representative staining with hematoxylin and eosin (HE)(A) Epidermal thickness (B) inflammatory cells and (C) cells with expressing of COX-2 with immunohistochemicalstaining in 400x magnification.**

**Table 2.Epidermal thickness, the amount of inflammatory cell and the amount of cellwith 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).

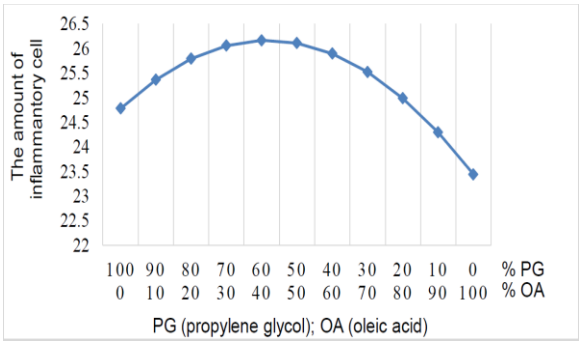


**Figure 2: The relation between propyleneglycol and oleicacidcompositionas an enhancer in emulgel to some many inflammatorycells**

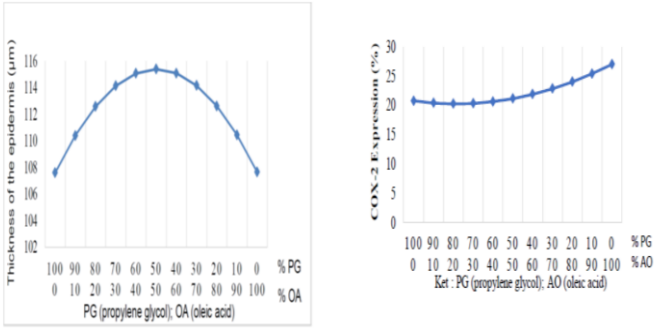


**Figure 3: The relation between propyleneglycol and oleicacidcomposition in emulgel as an enhancer on epidermal thickness.**

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.

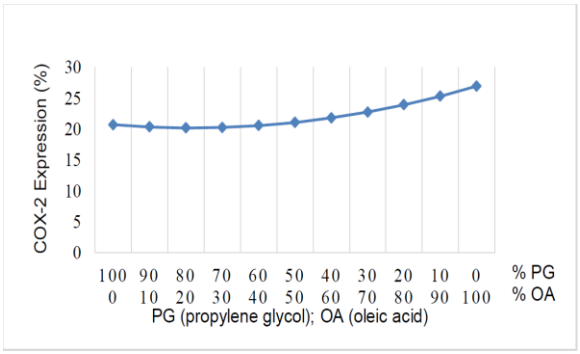


**Figure 4: The relation between propyleneglycol and oleicacidcomposition in emulgel as an enhancer to% COX-2 Expression**

The result of the statistical test between healthy control and all of the formula emulgelwas 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 emulgeljust 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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## REFERENCES

(1) Kamatou, G.P. Vermaak, I. and Viljoen, A.M. 2012. Eugenol - From the remote Maluku Islands to the international market place: a review of a remarkable and versatile molecule. *Molecules* 17(6):6953–6981.

(2) Daniel, A.N. Sartoretto, S.M. Schmidt, G. Assef, M.C. Ciomar. A. Amaldo, B. Cuman, R.K. 2009. Artigo anti-inflammatory and antinociceptive activities of eugenol essential oil in experimental animal models. *Brazilian Journal of Pharmacognosy* 19:212–217.

(3) Kaur, L.P. and Guleri, T.K. 2013. Topical gel: a recent approach for novel drug delivery. *Asian Journal of Biomedical & Pharmaceutical Science* 3(17):1–5.

(4) Fox, L. Gerber, M. Plessis, J. and Hamman, J. 2011. Transdermal drug delivery enhancement by compounds of natural origin *Molecules*16:10507–10540.

(5) Subedi, R. Oh, S. Chun, M. and Choi, H. 2010. Recent advances in transdermal drug delivery. *Archives of Pharmaceutical Research*33(3):339–351.

(6) Songkro, S. 2009. An overview of skin penetration enhancers: Penetration enhancing activity, skin irritation potential and mechanism of action. *Songklanakarin Journal of Science and Technology* 31(3):299–321.

(7) Sari, D.K. Sugihartini, N. Yuwono, T. 2015. Evaluasi uji iritasi dan uji sifat fisik sediaan emulgel minyak atsiri bunga cengkeh (*Syzygium aromaticum*). *Pharmaciana* 5(2):115-120

(8) Raut, S. Nemade, L. Desai, M. Bonde, S. Dongare, S. 2014. Pharmacy review & research drug delivery systems. *International Journal of Pharmaceutics* 4(1):33–40.

(9) Moreira, T.S.A. Pereira de Sousa, V. and Pierre, M.B.R. 2010. A novel transdermal delivery system for the anti-inflammatory lumiracoxib: influence of oleic acid on *in vitro* percutaneous absorption and *in vivo* potential cutaneous irritation. *American Assosiation of Pharmaceutical Scientist Pharmaceutical Science Technology*11(2):621–629.

(10) Lynde, C.B. Kraft, J.N. and Lynde, C.W.2006. Topical treatments for melasma and postinflammatory hyperpigmentation. *Skin Therapy Letter*11(9):1–6.

(11) Sugihartini, N. 2013. Optimasikompo sisi*enhancer* danemulgatorpadaformulasikrimfraksietilasestatekstrak tehhijau (*Camellia sinensis*, L) sebagaisediaantopikal anti inflamasi. *Disertasi*, Program Pascasarjana Universitas Gadjah Mada, Yogyakarta

(12) Shehadeh, M.B. Sosa, S. Suaifan, G.A.R.Y. Darwish, R.M. Giangaspero, A. Vassallo, A. et al. 2014. Topical anti-inflammatory potential of six salvia species grown in Jordan. *Jordan Journal of Pharmaceutical Sciences* 7(2):153-161.

(13) Shah, B. Seth, A. Maheshwari, K. 2011. A review on medicinal plants as a resource of anti-inflammatory agents. *Research Journal of Medicinal Plant* 5(2):1010-115

(14) Lan, M. Wan, P. Wang, Z.Y. Huang, X.L. 2012. Analisis GC-MS komponen Kimia DalamMinyak Biji *Croton tiglium*. *Zhong Yao Cai Journal* 35(7):1105-8.

(15) Khatri, S. Shemer, A. Rozenbilt, M. Dhingra, N. Czarnowicki, T. Finney, R. 2014. Cyclosporine in Patients with Atopic Dermatitis Modulates Activated Inflammatory Pathways and Reverses Epidermal Pathology. *Journal of Allergy and Clinical Immunology* 133(6):1626-1634.

(16) Kogan, A. and Garti, N. 2006. Microemulsions as transdermal drug delivery vehicles. *Advance in Colloidal and Interface Science*123-126, 369–385

(17) Jiang, S. Hwang, S. Choi, E. Ahn, S. and Lee, S. 2000. Structural and functional effect of oleic acid and iontophoresis on hairless mouse stratum corneum. *Journal of Investigative Dermatology*114:64–70.

(18) Jiang, S.J. & Zhou, X.J. 2003. Examination of the mechanism of oleic acid-induced percutaneous penetration enhancement: an ultrastructural study. *Biological Pharmaceutical Bulletin* 26(1):66–68.

(19) Lane, M.E. 2013. Skin penetration enhancers. *International Journal of Pharmaceutics* 447:12-21.

(20) Mohammed, D. Hirata, K. Hadgraft, J. Lane, M. 2014. Influence of skin penetration enhancers on skin barrier function and skin protease activity. *European Journal of Pharmaceutical Sciences* 51:118-122.

(21) Santos, P. Watkinson, A.C. Hadgraft, J. Lane, M.E. 2012. Influence of penetration enhancer on drug permeation from volatile formulations. *International Journal of Pharmaceutics* 260-268

(22) Sugihartini, N. Prabandari, R. Yuwono, T. 2019. The anti-inflammatory activity of essential oil of clove (*Syzygiumaromaticum*) in absorption base ointment with addition of oleic acid and propylene glycol as enhancer. *International Journal of Applied Pharmaceutics* 11(special issue 5):106-109

(23) Iriani, F.A. Sugihartini, N. Yuwono, T. 2017. The profile of anti-inflammatory activity of *Syzygiumaromaticum* volatile oil in lotion with variation composition of oleic acid and propylene glycol as enhancer. *Traditional Medicine Journal* 22(2):111-115.

# النشاط المضاد للالتهابات من زيت القرنفل الضروري في هلام

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## 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 (50 % OA – 50 % GP), 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-2 ($p< 0,05$) 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.

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