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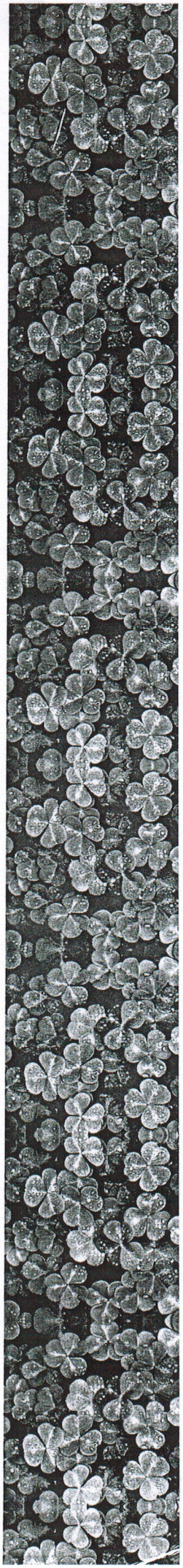

Penerbit

PERHIMPUNAN PENELITI BAHAN OBAT ALAMI (PERHIPBA)

The Indonesian Association of Natural Drug Researchers

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DAFTAR ISI

OPTIMATION COMPOSITION OF OLEIC ACID, PROPYLENE GLYCOL AND VOLATILE OIL OF Curcuma xanthorrhiza AS ENHANCER OF TRANSPORT OF EPIGALLOCATHECIN GALLAT IN GREEN TEA EXTRACT WITH SIMPLEX LATTICE DESIGN METHODE

{Optimasi komposisi asam oleat, propilen glikol dan minyak atsiri temulawak *(Curcuma xanthorrhiza)* sebagai enhancer pada transport epigalokatekin galat dalam ekstrak teh hijau dengan metode Simplex Lattice Design}

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Abstrak

The hijau mengandung epigalokatekin galat (EGCG) yang dapat dipergunakan sebagai pencegah kanker kulit. Efektivitasnya perlu ditingkatkan dengan menambahkan senyawa enhancer agar kemampuan penetrasinya di kulit meningkat. Pada penelitian ini ingin diketahui komposisi optimum campuran enhancer antara propilen glikol (PG), asam oleat (OA) dan minyak atsiri temulawak (*Curcuma xanthorrhiza*) (VO) dengan metode *Simplex Lattice Design*.

Penelitian ini menggunakan 7 komposisi enhancer berdasarkan metode *Simplex Lattice Design*. Komposisi dari OA, PG dan VO adalah 100-0-0, 0-100-0, 0-0-100, 50-50-0, 0-50-50, 50-0-50, 33-33-33. Uji transport dilakukan dengan alat uji difusi tegak dengan menggunakan kulit mencit sebagai membran. Transport EGCG dari larutan donor (Larutan 20 mg% ekstrak teh hijau dalam larutan dapar asetat pH 4) ke larutan aseptor (larutan PBS 0,1 M pH 6,2) diukur dengan menggunakan metode KCKT selama 26 jam. Data dianalisis dengan piranti lunak *WinSAAM* untuk mendapatkan parameter jumlah obat yang siap ditransport (P (2); lag time (DT (4)) dan koefisien difusi dari kompartemen 3 ke 2 (L (2.3)). Komposisi optimum enhancer ditetapkan dengan menggunakan piranti lunak *Design Expert*.

Hasil penelitian menunjukkan bahwa komposisi campuran 30,20% OA; PG 35,85% dan 33,95% VO adalah komposisi optimum untuk membantu EGCG menembus lapisan kulit. Komposisi ini memberikan nilai *desirability* yang paling tinggi berdasarkan piranti lunak *Design Expert* dengan nilai prediksi untuk $P(2) = 2475{,}56\ \mu g$; $DT(4) = 15{,}98$ jam dan $L(2,3) = 75{,}53$.

Kata kunci: asam oleat, propilen glikol, minyak atsiri temulawak *Curcuma xanthorrhiza*, EGCG, senyawa enhancer.

Abstract

Green tea contains epigallocatechin gallat (EGCG) which can use as chemoprevention of skin cancer. The ability of its penetration into the skin need to be increased by using enhancer to increase it's effectivity. In this study, the best composition of propylene glycol (PG), oleic acid (OA) and volatile oil of *Curcuma xanthorrhiza* (VO) as enhancer will be evaluated using *Simplex Lattice Design* method.

Sevent enhancer composition based on the concept of *Simplex Lattice Design* method were used. They consist of OA, PG and VO: 100-0-0, 0-100-0, 0-0-100, 50-50-0, 0-50-50, 50-0-50, 33-33-33 respectively. Transport test was performed in vitro in vertical type diffusion cells using mice skin as the diffusion membrane. Transport of EGCG from donor solution (20 mg% green tea extract in acetate buffer solution of pH 4) to acceptor solution (0.1 M PBS pH 6.2) was determined by HPLC for 26 hours. Data were analyzed using software WinSAAM to obtain the amount of drug that was ready to be transported (P (2); lag time (DT (4)) and diffusion coefficient of the compartment 3 to compartement 2 (L (2.3)). Design Expert Software was used to determine the optimum composition of the enhancers.

The result showed that the enhancer mixture of 30.21% OA; PG 35.85% and 33.94% VO was the optimal composition to facilite the penetration of EGCG from green tea extract into the skin layers. This composition gave the highest of desirability in Design Expert Software. The prediction number of P(2) was 2475.56 μg; DT(4) was 15.98 hours and L(2,3) was 75.53.

Key words: oleic acid, propylene glycol, volatile oil of *Curcuma xanthorrhiza*, EGCG, enhancer.

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INTRODUCTION

Green tea contains EGCG that is widely used as skin cancer prevention. In order to increase its activity, it is need to increase its ability to penetrate the skin. The primary barrier to across the skin is stratum corneum (SC). A common approach to increase the capability of drug to penetrate the SC is the used of enhancer such Propylene glycol (PG).

PG could increase transdermal delivery of Propanolol HCl (1). This is due to its ability to solve the keratin in-

stratum corneum (2) and to increase the solubility of drug and the drug interaction with protein stratum corneum (3).

In relation to PG, Oleic Acid (OA) can affect the lipid of layer (4) and than increase permeability of skin. Recently, volatile oils (VO) was also used as enhancer due to their high capability in penetration of skin and low irritancy potential (5). Their terpene constituents have been investigated as potential penetration enhancers (6). VO contains terpen which can increase transport of drug across membrane of skin.

Based on these considerations the aim of the present study was to find out the optimum composition of OA, PG and VO as skin transport enhancer of EGCG using Simplex Lattice Design method.

METHOD

Materials

This study used EGCG p.a (E Merck),Vertical type diffusion cell (obtained from ITB Bandung), HPLC (Shimadzu), thermoline, propylene glycol, oleic acid (pharmaceutical grade from Brataco), aqua destilata, phospat bufer saline (Na2HPO4 p.a, KH2PO4 p.a, KCl p.a, NaCl p.a), Buffer asetat (Na asetat, ammonium asetat, asam asetat glasial), skin of male mice (Balb C), Curcuma xanthorrhiza (obtained from Yogyakarta)

Preparation of the Enhancer and Donor Solution

Mixture of OA, PG and VO was used as enhancer solution. This research used 7 enhancer composition based on the concept of Simplex Lattice Design as shown in table I. The donor solution was 20 mg% green tea extract in acetate buffer solution of pH 4.

Preparation of Diffusion Membrane

The two months old of mice were sacrificed by excess aether inhalation and back skin hair was shaved using an electric razor. The back skin was surgically removed and adhering subcutaneous fat was carefully cleaned. The full thickness of skin was soaked in the 0.1 M of PBS (Phosphate Buffer Saline) solution for approximately 30 minutes and saturated with the enhancer solution for 3 hours using the vertical difusion cell with the aceptor compartment containing 0.1 M PBS solution at pH 6.2. Finally the skin can use as membrane in the in vitro diffusion test.

Table I. Composition of enhancer based on simplex lattice design method

Formulation	Oleic Acid (%)	Propylene glycol (%)	Volatile oil of *Curcuma xanthorrhiza*, L (%)
I	100	0	0
II	0	100	0
III	0	0	100
IV	50	50	0
V	0	50	50
VI	50	0	50
VII	33	33	33

The In Vitro Difusion test of EGCG

The in vitro permeation studies was performed in vertical diffusion cells. The previously hydrated-membrane was mounted between donor and acceptor compartment. Three milliliters of donor solution (20 mg % green tea extract) was placed on the skin surface of the donor compartment. The acceptor compartment was filled with 0.1 M PBS solution at pH 6.2. During the experiments the solution in the acceptor phase was maintained at 37°C and stirred at 6 scale. Three milliliters solution were collected from the acceptor side at the designated time intervals (0.5, 1, 2, 3, 4, 5, 6, 24, 25 and 26 h) and 3.0 mL of 0.1 M PBS at pH 6.2 was added into the acceptor side immediately after each sample collection. EGCG concentration in the collected samples was determined by HPLC using C18 column with an UV detector at 280 nm. Elution was carried out at room temperature using mobile phase consisting of acetonitrile, Aquabides, methanol and phosphate acid 0,1% (3:7:1:14 v/v/v/v) at a flow rate of 1 mL min–1.

Data Analysis

The amount of EGCG transported to medium aceptor was represented as the amount of cumulative EGCG for 26 hours and analysed using WinSAAM software to obtain the following parameters : drug that is ready to transport (P (2)); lag time (DT (4)) and diffusion coefficient (L (2.3)). Analysis was continued using Design Expert Software to determine the optimum composition of the enhancers.

RESULTS AND DISCUSSION

The addition of enhancers could increase the amount of EGCG which was ready to transport across the membrane (P(2)), the diffusion coefficient (L(2,3)) and the decrease of the lag time (DT(4). Based on three parameters Design Expert software calculated parameter of the desirability (figure 1). The highest of the desirability is found in the middle of the contour plot. It's means that the optimum composition to enhance transport EGCG across membrane is in the mixture of OA, PG and VO.

Calculation in the software of Design Expert showed that the optimum composition (OC) was 30.20% of OA; 35.85% of PG and 33.95% of VO. It was indicated that using the combination of enhancers was better than single enhancer on facilitating the EGCG transport. There was possibility of synergistic effect of the enhancers as shown at the all of contour plot. This result similar to the other studies which indicated that the combination of OA and iso propyl alcohol (50%:50%) as enhancer could give the highest cumulative percent release of Lisinopril dyhidrate (7). Other combination enhancer was reported, such as 20% PG with 5% terpene and 30% ethanol which could significantly increase the percutaneous absorption of midazolam in comparison to the control (8); conjugation of OA with PG could also increase the transport of Lidocaine (9), and terpene in co-solvent systems (propylene glycol/water) also found to increase transport of 5-fluorouracil (10).

The increase of drug that was ready to transport might be caused by the contribution of all the component in the OC on the increase of EGCG partition coefficient. It was indicated that PG its self has the ability to increase the partition coefficient in the skin and OA can improve the diffusion parameter as well as the partition coefficient (11). In addition terpen also able to increase both skin partitioning and disruption of stratum corneum lipid-

bilayer (12).

The increase of the diffusion coefficient might be caused by the ability of the enhancer combination on the changed of the lipid organization in the stratum corneum (11). OA, as the most popular enhancer (13), can disrupt the stratum corneum lipid lamellar structure and increase the epidermal permeability with perturbation of stratum corneum lipid bilayers and lacuna formation (14). PG can facilitate the OA activity by solvating α-keratin in corneocytes (15). In addition, champhor, a major terpene compound of this VO, might also be used as an enhancer, such it was demonstrated that other terpene (1,8-cineole, menthone, (+)-limonene and nerolidol) was able to disrupt stratum corneum lipids (10, 11).

Increasing of the diffusion coefficient enhanced the speed of transport across the stratum corneum (11). This condition caused the time period that was required of EGCG to start absorption (lag time) shorter.

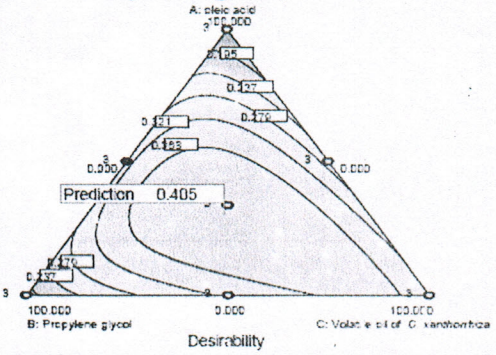

Figure 1. Contour Plot of *desirability* in transport of EGCG with variation enhancer combination. The optimum combination of enhancer found on the 0.405. The proportion of combination was 30.20% of Oleic acid; 35.85% of Propylene glycol and 33.95% of Volatile oil of *C. xanthorrhiza.*

Based on the software of design expert, it could predicted the number of P(2), L(2,3) and DT(4) on the OC enhancer were 2475.5 ug, 75.51 and 15.97 hours respectively. This result was significantly different compared to the untreated membrane with the P(2), L(2,3) and DT(4) are 16549.44 ug, 31.39 and 22.85 hours respectively. This indicated that the OC has the ability to increase the EGCG transport significantly ($p<0.05$).

CONCLUSION

The result showed that the enhancer mixture of 30.21% Oleic Acid; Propylene Glycol 35.85% and 33.94% Volatile Oil of Curcuma xanthorrhiza was the optimal composition to help EGCG in green tea extract to penetrate the mice skin layers.

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REFERENCES

1. Amnuaikit C, Ikeuchi I, Ogawara K, Higaki K, Kimura T. 2005. Skin permeation of propranolol from polymeric film containing terpene enhancers for transdermal use. Int. J. Pharm. 289(1,2) :167-178.
2. Trommer H, Neubert R.H. 2006. Overcoming the stratum corneum: the modulation of skin penetration. A review. Skin Pharmacol. Phys.19 (2) 106-21.
3. Moolgaard B. 1993. Synergistic effect in percutaneous enhancement in Walters K.A, Hadgraff J. (Eds). Pharmaceutical Skin Penetration Enhancement. 229–239. Marcel Dekker. New York.
4. Jiang J, Wang R.K. 2005. How different molarities of oleic acid as enhancer exert its effect on optical clearing of skin tissue in vitro. J. X-Ray Sci. Technol.13:149-159.
5. Jiang S.J, Hwang S.M, Choi E.H, Elias, Ahn S.K, Lee S.H. 2000. Structural and functional effect of oleic Acid and iontophoresis on hairless mouse stratum corneum. J. Invest. Dermatol. 114:64–70.
6. Britto M.B, Barin G.B, Araujo A.A.S, de Sousa D.P, Cavalcanti S.C.H, Lira A.A.M, Nunes R.S. 2009. The action modes of lippia sidoides (cham) essential oil as penetration enhancers on snake skin. J. Therm. Anal. Calorim. 97(1): 323-327.
7. Das M.K, Bhattacharya A, Ghosal S.J. 2006. Effect of different terpene-containing essential oils on percutaneous absorption of trazodone hydrochloride through mouse epidermis. Drug Delivery.13:425-431.
8. Banweer J, Pandey S, Pathak A.K. 2010. Formulation, optimization and evaluation of matrix type transdermal system of Lisinopril dihydrate using permeation enhancers. Drug Invention Today. 2(2):134-137.
9. Ota Y, Hamada A, Nakano M, Saito H. 2003. Evaluation of percutaneous absorption of midazolam
10. by terpenes. Drug Metab. Pharmacokinet. 18(4):261-266.
Ben-Shabat S, Baruch N, Sintov A.C. 2007. Conjugates of unsaturated fatty acids with propylene
11. glycol as potentially less-irritant skin penetration enhancers. Drug Dev. Ind. Pharm. 33(11):1169-1175.
Yamane M.A, Williams A.C, Barry B.W. 1995. Terpene penetration enhancers in propylene
12. glycol/water co-solvent systems: effectiveness and mechanism of action. J. Pharm. Pharmacol. 47(12A):978-989.
Songkro S.2009. An overview of skin penetration
13. enhancers:penetration enhancing activity, skin irritation potential and mechanism of action. Songklanakarin J. Sci. Technol. 31(3):299-321.
Fang J.Y, Tsai T.H, Lin Y.Y, Wong W.W, Wang M.N,
14. Huang J.F. 2007. Transdermal delivery of tea cathecins and theophylline enhanced by terpenes:a mechanistic study. Biol. Pharm. Bull. 30(2):343-349.
15. Kogan A, Garti N. 2006. Microemulsions as transdermal drug delivery vehicles. Adv. Colloid Interface Sci.369(85):123-126.
Jiang S.J, Zhou X.J. 2003. Examination of the
16. mechanism of oleic acid-induced percutaneous penetration enhancement: an ultrastructural study. Biol. Pharm. Bull. 26(1):66-8.
Ammar H.O, Salama H.A, Ghorab M, El Nahhas S.A, Elmotasem H. 2006. A transdermal delivery system for Glipizide. Curr. Drug Delivery. 3: 333-341.