ISBN 978-979-95107-7-8

The International Conference on Pharmacy and Advanced Pharmaceutical Sciences

Faculty of Pharmacy UGM

PROCEEDING

The International Conference on Pharmacy and Advanced Pharmaceutical Sciences

Faculty of Pharmacy UGM
Yogyakarta Indonesia
October 2009

UNIVERSITI SAINS MALAYSIA

NAIST

PROCEEDING

The International Conference on Pharmacy and Advanced Pharmaceutical Sciences Yogyakarta, Indonesia, 2009

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Published by:
Faculty of Pharmacy Universitas Gadjah Mada
Sekip Utara, Yogyakarta, 55281,
Indonesia

ISBN : 978-979-95107-7-8

First Edition, 2010

Printed in Yogyakarta, Indonesia

Preface from the Editor

The proceeding was produced based on papers and posters presented at the international Conference on Pharmacy and Advanced Pharmaceutical Sciences, held in Yogyakarta, Indonesia, 5 – 6 October 2009.

The proceeding clearly reflects broad interest; from there are participants coming from all around the world. Many contributions on Pharmaceutical Sciences there are quite a substantial number of papers on Pharmacist role in general. The papers presented file into a broad spectrum in Pharmaceutical sciences including Pharmacology, Toxicology, Analytical Chemistry and Drug Design, Drugs Synthesis, Formulation of Drugs, Pharmacy Social, Pharmacoepidemy, Traditional Medicine Natural Product Chemistry and Phytochemistry, etc.

In addition there are substantial numbers of paper deal with professional aspect of Pharmacist in general health care.

In this an opportunity, I would like to express my appreciation to the editorial team of the proceeding who have been working hard to review manuscripts, and making the first edition of this proceeding be possible.

I would like also to thanks to all invited speakers and presenters who participated in the International Conference on Pharmacy and Advanced Pharmaceutical Sciences and your contribution to this proceeding.

Finally, I hope this proceeding will give contribution to the advanced scientific research in the field of pharmaceutical sciences

Yogyakarta, July 2010

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Welcome Message
From the committee

Welcome to Yogyakarta

On behalf of the Scientific and Organizing Committees, it is a great pleasure for me to welcome all participants to Yogyakarta, to the International Conference on Pharmacy and Advanced Pharmaceutical Science 2009.
The international conference is organized by the faculty of Pharmacy UGM to celebrate its 63th anniversary and the Lustrum XII of Gadjah Mada University, as a collaboration work between the Faculty of Pharmacy UGM with the Nara Institute of Science and Technology (Japan) and the Universiti Sains Malaysia (Malaysia). In this conference 15 lectures within the field of Pharmaceutical Care and Advanced Pharmaceutical Science will be given by invited speakers. Besides, 55 posters and 75 paper will be presented in the parallels presentation sessions. Herewith, we express our gratitude to all speakers and presenter, who would like to share their advance knowledge in this scientific event.

The Organizing Committee gratefully acknowledges the Nara Institute of Science and Technology and the Universiti Sains Malyasia, for the nice collaboration in bringing forth this conference. A special acknowledgment is addressed to the Rector of Gadjah Mada University and the sponsors, for all supports that make this symposium possible. Furthermore, personally, I want to express my deep appreciation to the members of the Organizing Committee, for the good teamwork and their great effort given in the preparation for this symposium.

Finally, I wish all participants a scientifically rewarding and an enjoyable meeting in Yogyakarta.

Chairman

Dr. Hilda Ismail, M.Si., Apt.

Remark of the Dean Faculty

Assalamu'alaikum wr. wb.
Distinguished ladies & gentlemen.

First of all, on be half of the Faculty of Pharmacy Universitas Gadjah Mada, I would like come to all of you in Yogyakarta, thank you very much for your attention to come and to attend the international Symposium on Pharmacy and Advanced Pharmaceutical Sciences. I hope we are all in health condition.

Ladies and gentlemen,
The symposium is organized by the Faculty of Pharmacy UGM in collaboration with the Faculty of Pharmaceutical Sciences Universiti Sains Malaysia and the Nara Institute of Science and Technology Japan, and held as part to celebrate the 63th anniversary of the Faculty of Pharmacy UGM.
In the symposium , I hope we can communicate our recently information concerning social / clinical pharmacy and pharmaceutical sciences. I hope the symposium will be very fruitfull, very useful for all of us .
I addres special thanks to the plenary speakers both from domestic and aboard, the oral and poster presenters, as well as to those who come just to know the development of clinical or social pharmacy and pharmaceutical science. Your willingness to come , to communicate and to share your experiences is highly appreciated.

Special thanks also I address to my colleague the Dean of Faculty of Pharmacy USM who has been coordinating USM students to attend this symposium. The hope is not to set up networking between the pharmacy students of USM and UGM.

Therefore, during almost whole day discussing scientific matter related to human health and welfare, I hope we can make a wonderful opportunity to make a scientific closer relationship while we enjoy the cultural performances of Yogyakarta presented by our pharmacy student.

Finally, I hope that this meeting will give benefits to all of us, and we may see each other again in a similar event in the near future.

I look forward to thank you all for attending this event.

Wassalamu'alaikum wa rahmatullahi wa barakatuh,
Dean of Faculty of Pharmacy UGM

Prof. Dr. Marchaban, DESS., Apt.

Speech of the Senior Vice Rector
For Education, Research and Community Services,
Gadjah Mada University

Assalamu'alaikum wa rahmatulLahi wa barakatuh,

On behalf of the Rector, I would like to welcome all of you to our campus Gadjah Mada University and to our home town Yogyakarta. It is a great honor for me and Gadjah Mada University to host the Two-day International Conference on Pharmacy and Pharmaceutical Sciences that is conducted by the Faculty of Pharmacy, Gadjah Mada University. The increasing problems and new cases of some diseases in the world, both the infectious and the degenerative diseases, have demanded the development of medical and pharmaceutical sciences and technologies for supporting the developments of early detection methods of the diseases, the accurate diagnoses, as well as the appropriate and effective medications or therapy. Pharmaceutical Science and Technology have been developing very fast within recent years. The development trend shows using much more biotechnological approach in both diagnose establishment and medication administrations. For examples the usage of some serums, enzymes, hormones, vaccines, etc., and their recombinant products. The science and technology for finding prevention method against infectious diseases or degenerative diseases now have been developing so amazing, for example the usage of growth hormones, vaccines, and stem cells for it.

Gadjah Mada University has been committed to become World Class University; therefore international networking in education, research and publication is much needed. I really support to this international conference on Pharmaceutical Science and Technology which can keep us in touch with the state of the art of pharmaceutical science. I do believe that by conducting this kind of international meeting, we can get and exchange new information and best practices on pharmaceutical science and technology, and it is very important to inspire our young researchers and enhance our research networking internationally. In this occasion, I would like to express my great gratitude to all the guest speakers and speakers, who have contributed their advanced presentations in this international conference. I also would like to extend my gratitude to the Organizing Committee from the Faculty of Pharmacy, Gadjah Mada University, who has already successfully arranged this international conference. I would also thank to all institutions or companies who have sponsored and supported this conference.

Finally, have a fruitful conference and enjoy Yogyakarta. Thank you
Wassalamu'alaikum wa rahmatulLahi wa barakatuh,

Senior Vice Rector for Education, Research and Community Service
Gadjah Mada University

Prof. Dr. Retno Sunarminingsih, M.Sc., Apt.

CONTENTS

The Effect Of β-Cyclodextrin And Oxybenzone-Octyl Dimethyl Paba (3:7% W/W) Addition On The Penetration Of Kojic Acid In Vanishing Cream (Based on Activity Inhibition of Tyrosinase)

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Abstract

This research was aimed to investigat the effects of the addition of β-cyclodextrin (BCD) and a combined of oxybenzone and octyl dimethyl PABA (3:7% w/w) on the penetration of kojic acid based on inhibion activity of tyrosinase. The presence of BCD was expected to make the preparation effective, while the addition of oxybenzone and octyl dimethyl PABA was intended to overcome photosensitive properties of kojic acid. Effectiveness determination of vanishing cream preparation was conducted by testing activity inhibition of tyrosinase that was expressed as percentage inhibition. Tyrosinase was an enzyme that plays an important role in the process of melanin formation in human skin. Inhibition activity of tyrosinase by kojic acid was determined in vitro by observing absorption values of dopachrome (an intermediate product of melanin formation) using spectrophotometer. Based on results of effectiveness tests of kojic-acid vanishing cream preparation by means of measurement of activity inhibition of tyrosinase, it was found that addition of sunscreen (oxybenzone and octyl dimethyl PABA without BCD) had no significant effect on the activity inhibition of tyrosinase. While, addition of BCD or combined of BCD-sunscreen significantly reduced inhibition of tyrosinase activity in vitro.

Keyword : kojic acid, β-cyclodextrin, oxybenzone, octyl dimethyl PABA, tyrosinase, enzym, inhibition

Introduction

Kojic acid is a skin lightening agent which has very small molecular size, so easily absorbed up through the basal membrane. Because of its small molecule, kojic acid hardly absorbed through the lipid membrane of its target sites, the melanocytes (Manosroi et al, 2005) and can penetrate into the systemic (Nakayama et al., 2005). It is likely absorbed through voids between cells on the skin. Losses due to kojic acid to the systemic is not able to work effectively inhibits melanin formation took place in the basal membrane of skin that results are not optimal lightening effect. Addition of β-cyclodextrin reported can decrease penetration of kojic acid (Nakayama et al., 2005). BCD can be used in a number of 0,5-10 parts per 1 weight part of kojic acid, which is the optimal composition of 4 parts cyclodextrin every a part of kojic acid (Hatae et al, 1988).

Generally, skin lightening maybe caused the skin more photosensitive, so in cosmetics usage needed the sun-rays filter (Zulkarnain, 2003). To obtain maximum protection, frequently sunscreen used in combination of anti UV-A with anti UV-B, ei: oxybenzone and octyl dimethyl PABA. In this study used the composition (3:7) % w/w which is the optimum composition in accordance with the results of previous studies (Wati, 2005).

This research investigated the effects of the addition of β-cyclodextrin and a combined of oxybenzone and octyl dimethyl PABA (3:7% b/b) on the penetration of kojic acid based on activity inhibition of tyrosinase. Effectivity determination of vanishing cream preparation was conducted by testing activity inhibition of tyrosinase that was expressed as percentage inhibition. Tyrosinase was an enzyme that played an important role in the process of melanin formation of human skin. Activity inhibition of tyrosinase by kojic acid was determined in vitro by observing absorption values of dopachrome (an intermediate product of melanin formation) using spectrophotometer.

Methodology

Materials

The materials used in this study if no others have mentioned pharma-ceutical grade standards. Materials for the manufacture of test preparations including Kojic acid, Oxybenzone, Octyl dimethyl PABA, BCD (p.a), stearic acid, Cetyl alcohol, Span 80, Tween 80, and 70% sobitol solution. While material for inhibition of tyrosinase activity test is the Mushroom tyrosinase, L-Tyrosine, $NaH_2PO_4.2H_2O$ (p.a) and Na_2HPO4 (p.a).

Instrument

Double beam UV-VIS Spectrophoto-meter, Fourier Transform Infrared Spectrophotometer, Micropipet various volumes, Pumpkin measure, Research Hanson dissolution testing, thermo-meters, pH meter CG SCHOOT Glass 842 Mainz, Milipore Membrane Filters, Tue diffusion, Analytical scales, Vortex, light microscopy, Electrothermal Melting Point Apparatus.

Preparations :

Vanishing cream base

14% stearic acid, 2% cetyl alcohol, Methyl paraben 0.1%, Propyl paraben 0.05%, 0.5% Span 80, 4.5% Tween 80, 3% sorbitol solution 70%, Aqua ad 100%.

Table 1 Formulation

Component	Percentage (% b/b)			
	F_1 (control)	F_2	F_3	F_4
Kojic acid	1	1	1	1
β-cyclodextrin	–	4	–	4
Oxybenzone	–	–	3	3
Octil dimethyl PABA	–	–	7	7
Vanishing cream base(F_0)	Ad 100	Ad 100	Ad 100	Ad 100

Characteristics Determination of the cream preparation:

Determination of the physical quality of cream preparations include organoleptic (shape, colour, odour, texture), pH and spreading-ability.

Determination of the spreading-ability:

Determination of cream spreading-ability was performed uslng a pair of glass plate (20 X 20 cm). The cream preparation (1 gram) was put in the middle of the first glass plate that given the scale. Then put the second glass plate on the first glass plate and measured the diameter of cream spreading. After that put ballast on the second glass plate then measured the diameter spreading-ability of the cream. The weight of ballast that put on the second plate was increased until spreading-ability of the cream was constant.

Determination of tyrosinase activity inhibition

Preparation of a solution of the reaction components.

The solution should be prepared for the implementation of this study was 0.1 M phosphate buffer pH 6.5, solution of tyrosinase (5370 units / mg solid in 0.1 M phosphate buffer pH 6.5 to 100.0 ml volume), a solution of 5.52 mM L-tyrosine, and 30% TCA.

Preparation of test sample solution.

The cream (around 3 grams) was put in the diffusion cell then covers with the Millipore membrane which was impregnated with isopropyl-myristate as modified lipid membrane. Then the preparation of cream in diffusion cell was put into the penetration chamber contain 500 ml of phosphate buffer pH 6.5 ± 0.05 at 37 ± 0.5ºC as diffusion medium, and then the paddle was stirred 100 rpm. The sample solution around 3 ml was collected at 6 hours after it penetrated.

Determination of the dopakrom maximum wavelength

L-tyrosine solution of 5.52 mM has taken a number of prepared and then added 0.5 ml 3 ml of buffer solution pH 6.5 ± 0.05. Then the mixture add with 1.0 ml of tyrosinase and oxygenated for 5 minutes. The mixture was incubated for 15 minutes at 26 ± 0.5 ºC. Then added 0.5 ml of 30% TCA. The solution is then inserted into cuvet and placed on the sample position in the spectro-photometer. Used as a blank solution 0.1 M phosphate buffer pH 6.5. Then do the reading of absorbance values from a wavelength of 400 nm to 500 nm, and the selected wavelength that gives the greatest absorption.

Inhibition Tyrosinase Activity Determination

L-tyrosine solution 5.52 mM added with 0.5 ml 3 ml of sample solution. Then the mixture added with 1.0 ml of tyrosinase and oxygenated for 5 minutes. The mixture was incubated for 15 minutes at 26 ± 0.5 ºC. Then add 0.5 ml of TCA 30% and observed the absorbance at dopacrom maximum wavelength. Inhibition tyrosinase activity is the percent inhibition values obtained from the later absorbance value calculated by the equation:

A = Absorbance at the maximum with the skin lightening
B = Absorbance at maximum λ without the skin lightening

Results and Discussions

Characteristics Test Results

Based on the results of organoleptic test, all formulas have the same smell and texture. Meanwhile, in terms of different colours. Formula that contains sunscreen cream white yellowish colours, while others are white.

Table 2. Organoleptic test results

Formula	Replication	Organoleptic		
		Color	Odor	Texture
F_1	1	White	-	Semisolid, smooth
	2			
	3			
F_2	1	White	-	Semisolid, smooth
	2			
	3			
F_3	1	White yellowish	-	Semisolid, smooth
	2			
	3			
F_4	1	White yellowish	-	Semisolid, smooth

Cream Type

The results of cream type test using water-soluble reagents (metylene blue) showed that the type of cream is O/W.

The pH Test Result

Based on the pH results in table 3, it was known that all formulas in the skin pH range (4 - 6.8).

Table 3. The pH test result

Formula	*pH	% CV
F_1	4,46 ± 0,01	0,34
F_2	4,24 ± 0,03	0,68
F_3	4,29 ± 0,03	0,70
F_4	4,04 ± 0,02	0,62

*) The average obtained from 3 replicates it, each determined 3 times at different places.

Spreading-ability

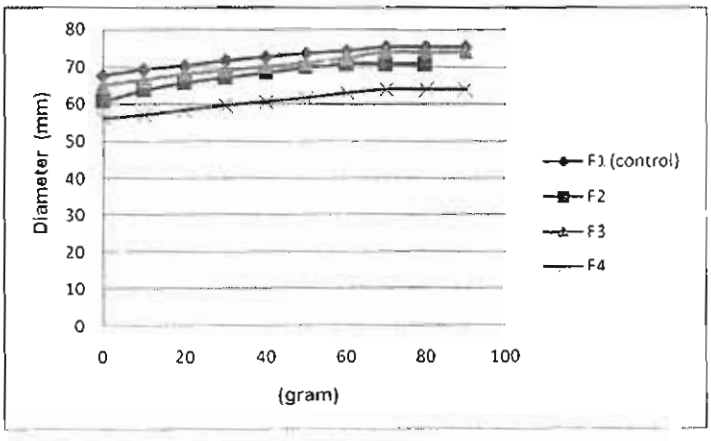

Figure 1. Profile of spreading-ability kojic acid cream on various formulas

Table 4. Sreading-ability result

	Replication	Dispersability (mm/g)	Average ± SD
F_1 (Control)	1	0,1179	0,1067 ± 0,01
	2	0,1083	
	3	0,0940	
F_2	1	0,1786	0,1595 ± 0,02
	2	0,1393	
	3	0,1607	
F_3	1	0,1083	0,1226 ± 0,01
	2	0,1369	
	3	0,1226	
F_4	1	0,1179	0,1155 ± 0,02
	2	0,0964	
	3	0,1321	

Based on the results of one way ANOVA test, the addition of BCD (F_2) decreased its spreading-capacity, as well as addition of BCD on combination kojic-sunscreen (F_4)

Table 5. Sreading-capacity results

	Replication	Diameter at 60 g (mm)	Average ± SD
F_1 (Control)	1	75	74,33 ± 0,58
	2	74	
	3	74	
F_2	1	70	70,67 ± 0,58
	2	71	
	3	71	
F_3	1	73	74,00 ± 1,00
	2	74	
	3	75	
F_4	1	64	63,00 ± 1,00
	2	62	
	3	63	

Test Rresults Activity Inhibition of Tyrosinase

Table 6. Inhibition percent tyrosinase activity

Formula	% Inhibition			Average (%) ± SD	CV (%)
	Replikation				
	I	II	III		
F_1	61,73	62,10	63,07	62,3 ± 0,69	1,11
F_2	28,39	28,40	29,28	28,69 ± 0,51	1,78
F_3	60,50	60,65	62,26	61,14 ± 0,98	1,60
F_4	23,69	23,33	24,52	23,63 ± 0,61	2,56

Based on one way ANOVA test, known that the addition of BCD decreased inhibition percent compared to the control constraints. Nevertheless the addition of sunscreen alone does not affect the inhibition percent of tyrosinase activity.

Addition of BCD on combination kojic-sunscreen preparations showed the same phenomena such as decreased its inhibition percent of tyrosinase activity.

Decreasing of inhibition percent of tyrosinase activity after addition of BCD alone or BCD-sunscreen was might be caused by decreasing of kojic acid penetration. It was probably caused by some of the things that the release process of kojic acid from base and in the process of penetration through the membrane. Before reach basal membrane where the kojic acid site of action, kojic molecule should released from base further more penetrate through the skin.

From spreading-ability test was known that the addition of BCD alone and also BCD on combination with sunscreen preparations reduced spreading-capacity, it means increasing viscosity. Increasing viscosity will decrease the mobility of active ingredient molecules that caused barriers against the release of kojic acid.

After the release process, kojic acid has to penetrate through trans-epidermal route through diffusion mechanism and trans-appendageal. One of the factors affecting the diffusion of the active ingredient is a molecule characteristic, which is the size of molecules. In the research on the use of drug fendeline with BCD, showed that the diffusion through a semi-permeable membrane decreased which was caused by increasing its molecule size

(Duchene et al, 1986). In this case decreasing kojic acid penetration by BCD addition might be caused by increasing of molecules the size of kojic acid. There for it expected would inhibit its penetration to the systemic, and make it survive longer in basal membrane, which is synthesis of melanin site. While the addition of BCD on combination kojic-sunscreen (F_4) reducing inhibition tyrosinase enzyme activity significantly compared with the control, as well as formula with the addition of a single BCD (F_2). This is probably due to the interaction between these materials so that the amount of kojic acid penetrated decreased and tyrosinase activity in vitro also declined.

Conclusion

Based on research results can be concluded that:

1. Combination of sunscreen oxybenzone and octyl dimethyl PABA (3:7) % w/w in the preparation of the skin lightening 1% kojic acid in a vanishing cream base does not affect the penetration kojic acid based on inhibition of tyrosinase enzyme activity in *invitro*.
2. β-cyclodextrin decrease penetration kojic acid based on inhibition of tyrosinase enzyme activity in *invitro*.
3. The existence of β-cyclodextrin and combination sunscreen oxybenzone and octyl dimethyl PABA (3:7)% w / w decrease penetration kojic acid based on inhibition of tyrosinase enzyme activity in *invitro*.

Acknowledgement

This study was supported financially by Project Grant of Faculty of Pharmacy Airlangga University.

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