The 2nd International Conference on Herbal and Traditional Medicine 2017

"Value-Added of Herbs and Phytotherapy : Challenges for the 21st Century"

Proceedings

HTM

25-27 January, 2017
Asia Hotel, Bangkok, Thailand

ISBN: 978-616-223-985-4

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First Published : January 20, 2017 300 copies

ISBN : 978-616-223-985-4

Tel. 6643 202 378, Fax. 6643 202 137
Email: htm2017@kku.ac.th Facebook: https://www.facebook.com/htmthailand2017/

Published by: The Organizing and Scientific Committee of HTM 2017
The 2nd International Conference on Herbal and Traditional Medicine 2017,
Khon Kaen University, Khon Kaen 40002, Thailand

Khon Kaen University Printing House,
123 Mittraphap road, Nai Muang, Muang, Khon Kaen, 40002 Thailand
Tel 6643 347 100 Email: kkuprinting@hotmail.com

Editorial Board

CONTENTS

Speakers Abstracts

Abstracts of Oral Presentation

Scientists Presentation

CATEGORIES 1 : The quality of crude drugs and herbal medicines

CATEGORIES 2 : Biotechnology for the production of secondary metabolites

CATEGORIES 3 : Discovery of natural products from new sources

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Abstracts of Oral Presentation

Students Presentation

CATEGORIES 1 : The quality of crude drugs and herbal medicines

THURSDAY JANUARY 26, 2017

Rajthevee Grand Ball Room 1, Floor 3

Scientists

Time	Code-ID	Name - Surname
13.00-13.15	T4-O-RD-011	Dr. Naphatsorn Kumar
13.15-13.30	T5-O-RD-012	Dr. Alok Nahata
13.30-13.45	T5-O-RD-013	Assist. Prof. Dr. Bahtiar Anton
13.45-14.00	T5-O-RD-014	Dr. Tri Widyawati Tri
14.00-14.15	T5-O-RD-015	Miss Siti Syarifah Siti

Students

Time	Code-ID	Name - Surname
14.15-14.30	T5-O-ST-019	Mr. Apichakan Sampannang
14.30-14.45	T5-O-ST-020	Miss Pimsuda Kulpradit
14.45-15.00	T5-O-ST-021	Lisdiana Lisdiana

Rajthevee Grand Ball Room 2, Floor 3

Students

Time	Code-ID	Name - Surname
13.00-13.15	T4-O-ST-011	Mrs. Susana Elya Sudradjat
13.15-13.30	T4-O-ST-012	Mrs. Gayatri Rizkiana
13.30-13.45	T4-O-ST-013	Miss Titiporn Thongyen
13.45-14.00	T5-O-ST-014	Miss Dhaneshree Bestinee Naidoo
14.00-14.15	T5-O-ST-015	Mrs. Erna Harfiani
14.15-14.30	T5-O-ST-016	Miss Dana Joanne Von Trono
14.30-14.45	T5-O-ST-017	Mr. Jaturon burawat
14.45-15.00	T5-O-ST-018	Miss Supatcharee Arun

HTM 2017

Categories 4 :

New herbal drug formulation

(Oral Presentation)

Student Presentation

T4-O-ST-011

Formulation and Penetration Study of Etosom Gel Myristicin of Nutmeg Oil (*Myristica fragrans H.*)

Susana Elya Sudradjat [1], Abdul Mun'im [2], Kris Herawan Timotius [3] , Effionora Anwar [4]

Abstract

Introduction: Myristicin is a sedative drug to be, that can reduce sleep disorders and improve patient health. Transdermal drug delivery is a non-invasive technique and not influenced by absorbtion in the gastro intestinal tract. The aims of this study is to formulate myristicin etosom and measured the penetration ability of myristicin through Spraque Dawley rat abdomen skin as membrane diffusion membrane. Myristicin had been isolated from nutmeg oil by destilation and its content was determined. Myristicin was used as an active ingredient in myristicin etosom gel and myristicin gel. **Methods**: Myristicin is formulated into etosom in various concentrations of phosphatidyl choline and etanol. Myristicin etosom is prepared with different formulas and characterized by its vesicle size, entrapment effiency, and polydispersity index. Physical stability of the products were investigated, including the influence of the temperatures, organoleptic test, pH, and particle size. The penetration of myristicin from each dosage form was evaluated by means of Franz diffusion cell using rat abdomen skin as a membrane. **Results** : The result shows that etosom sizes varied from 118.9±4.80 to 778.5±54.28 nm depending on the concentration of soya phosphatidyl choline (SPC) and ethanol. The formulation exhibited entrapment efficiencies from 28±0.44 to 94±1.75%. Flux of penetration of myristicin etosom gel had higher penetration (84.05±1.71 µg cm^{-2} $hour^{-1}$) than the penetration of non etosom gel (60.21±2.46 µg cm^{-2} $hour^{-1}$). The sum of cumulative penetration of myristicin etosom gel and myristicin gel were 2642.6±44.92 µg cm^{-2} and 1893.1±19.32 µg cm^{-2} respectively. The percentage of the myristicin penetration from etosom gel was 52.85±2.84% and myristicin from gel was 37.86 ±1.74%. **Conclusion : The results show that both myristicin etosom gel and myristicin gel are physically stable and penetration ability of myristicin etosom gel is higher than myristicin gel.**

Keywords: Etosom, Gel, Skin penetration, Cycling test, Flux

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Susana Elya Sudradjat [1], Abdul Mun'im[2], Kris Herawan Timotius [3] , Effionora Anwar [4]

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Methods: Myristicin is formulated into etosom in various concentrations of phosphatidyl choline and etanol. Myristicin etosom is prepared with different formulas and characterized by its vesicle size, entrapment effiency, and polydispersity index. Physical stability of the products were investigated, including the influence of the temperatures, organoleptic test, pH, and particle size. The penetration of myristicin from each dosage form was evaluated by means of Franz diffusion cell using rat abdomenskin as a membrane.

Results : The result shows that etosom sizes varied from 118.9±4.80 to 778.5±54.28 nm depending on the concentration of soya phosphatidyl choline (SPC) and ethanol. The formulation exhibited entrapment efficiencies from 28±0.44 to 94±1.75%. Flux of penetration of myristicin etosom gel had higher penetration (84.05±1.71 μg cm-2 hour-1) than the penetration of non etosom gel (60.21±2.46 μg cm-2 hour-1). The sum of cumulative penetration of myristicin etosom gel and myristicin gel were 2642.6±44.92 μg cm-2 and 1893.1±19.32 μg cm-2 respectively. The percentage of the myristicin penetration from etosom gel was 52.85±2.84% and myristicin from gel was 37.86 ±1.74%.

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INTRODUCTION

Myristicin (syn. 4-methoxy-6-(2-propenyl)1,3-benzodioxole) is known for its sedative effect and as a major component of the essential oil either from seed or mace of nutmeg (*Myristica fragrans* Houtt).[1-3] (Figure 1). Myristicin is a derivate of phenylpropanoid compounds, in the form of a clear liquid, not soluble in water but soluble in organic solvents.[4]

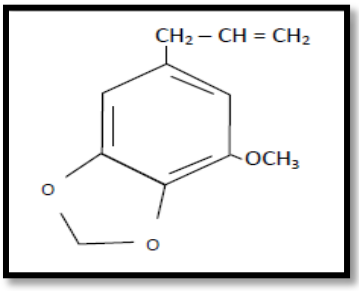

Figure 1 Chemical structure of Myristicin

The bioavailability of myristicin is low when administered orally. It will metabolized into 1,2-dihyroxymyristicin[5] to enhance the bioavailability of myristicin, transdermal drug delivery with a patch is the best choice. It is a non-invasive method, and it doesn't depend on the absorption mechanism in the digestive tract. The development of the transdermal method, including the application of lipid-based vesicles, will solve the permeability barrier of the stratum corneum.[6] The lipid-based vesicles might merge with skin lipids due to their similar biocompatibility characters. Ethosome is better than liposome since its lipid vesicles may penetrate and enter deeper into the skin or stratum corneum. The synergetic combination of phospholipid and ethanol is important for the efficacy of drug delivery through the skin layer. Therefore, ethosome is considered better than transfersome and liposome.[7]

Ethosome vesicle has been applied form the delivery of several drugs, such as erythromycin, lamivudine (antivirus), insulin, minoxidil (hair grower), diclofenac (anti-arthritis), trihexyphenidyl (antiparkinson), miconazole (anti fungi), salbutamol sulfate, propranolol, and testosterone. It is also applicable to herbal medicine for several skin diseases, such as scabies, anti-inflammation, etc.[8] Presently benzodiazepines are widely used as sedative drugs, but they have a long half time that can cause cumulative effects due to multiple doses.[9] In this study, we studied the delivery of myristicin that carried by ethosome, with the hope that ethosomal myristicin can be applied as a control-released sedative drug. Myristicin-loaded ethosomes were incorporated

into a gel. Then, penetration in vitro tests was carried out. This study is the first on myristicin trans delivery with the help of ethosome. The previous myristicin studies were only done through the oral, and inhalation routes.[1,10] This study is aimed to find the best formulation of the myristicin loaded ethosome by comparing various combinations of soy phosphatidylcholine and ethanol, to evaluate the stability, and the skin penetration ability.

MATERIAL AND METHODS:

Chemicals and reagents: Myristicin used in this study was obtained from Balittro (Balai Penelitian Tanaman Rempah dan Obat, Research Center for Plant Spices and Medicine, Bogor, Indonesia). The myristicin was isolated from nutmeg oil by the fractional vacuum distillation. The myristicin content is 83,45%.[11]

Lipoid S-100 (96,5% soy L-α-Phosphatidylcholine) was a grant from Lipoid GmBH (Germany). Ethanol 96 %, dichloromethane, and methanol (HPLC-grade) were bought from Merck (Germany). Carbopol 940® was obtained from Sinobio (China). All other solvents and reagents were an analytical grade, bought from Sigma (America).

Instrumentation:

HPLC was conducted using Schambeck (Germany) equipped with a reversed-phase column (5 μm, ACE® C18, 250×4.6 mm) and UV/Vis detector (278 nm), and run at room temperature. Methanol was used as the mobile phase.

Formulation of myristicin loaded ethosome

The thin-film hydration method was used to form myristicin loaded ethosomes. Nine different ethosomal formula were designed (Table 1). Firstly, phospholipid and myristicin were dissolved in dichloromethane-ethanol (2:1). The solvent was then evaporated by rotary evaporator at 37^0C at 50 rpm with increasing the rotary's speed every 30 minutes up to 150 rpm. Afterward, the obtained thin layer was blasted with nitrogen gas and then kept in a refrigerator for one night. After being kept overnight in the refrigerator, the thin layer was hydrated with aquabidest-ethanol-propylene glycol solvent at 37^0C at 50 rpm and increased the rate every 30 minutes up to 150 rpm. To get the smaller size of the ethosome vesicle, the sonication of ethosome suspension is 5 minutes. The resulted suspension was kept in the vial and kept in the refrigerator until it is needed to be used.[7]

Table 1: Formulae of ethosomal myristicin

	Concentration (%)								
Composition	E1	E2	E3	E4	E5	E6	E7	E8	E9
Myristicin	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
PC	2	2	2	3	3	3	4	4	4
Ethanol	20	30	40	20	30	40	20	30	40
PG	5	5	5	5	5	5	5	5	5
Aqua	ad 100	ad 100	ad 100	ad 100	ad 100	ad 100	ad 100	ad 100	ad 100

PC=phosphatidylcholine; PG= propylene glycol

Particle size analysis of ethosomes

Dynamic laser scattering (Zetasizer Nano ZS, Malvern Instruments, UK) was used to measure the size of the particle and the zeta potential. Ethosome suspension was diluted with an appropriate solvent. All measurement were in triplicate.[7]

Myristicin entrapment efficiency

Ethosomal vesicles were separated from unentrapped myristicin by centrifugation at 15.000 rpm for 30 minutes at 40 ^{0}C. After centrifugation, the sediment was separated from the supernatant. The sediment was lysed by using methanol and then filtered at 0.45 µm. The solvent was diluted with methanol before analyzing it with HPLC. The experiment was done in triplicate at 25 ^{0}C, and the entrapment efficiency (EE) was calculated as followed.[12]

EE= (the entrapped myristicin)/(the total amount of myristicin) x 100%

Morphology of myristicin-ethosome

The morphology of myristicin ethosome was observed by the transmission electron microscopy (TEM FEI, type: Tecnai G2 Spirit 120 KV). The samples were placed on the grid and stained with 1% phosphotungstic acid.[13]

Gel Preparation

The gel was made from carbomer and triethanolamine neutralized. After that, propylene glycol was added to the gel base, then it was homogenized. The composition of the gel formula is in Table 2. The ethosomal gel myristicin was made by incorporated ethosomal myristicin into gel formula (EG), and myristicin was dispersed for non-ethosomal gel (NEG). Both gels were then homogenized for 15 min. The preparation of the ethosomal gel of myristicin and myristicin gel is in Table 2.

Table 2: The formulation of ethosomal gel and non-ethosomal gel

	Formula (%w/w)	
Composition	**EG**	**NEG**
Myr-ethosome	Equivalent to Myr 1mg/g	-
Myr	-	Equivalent to Myr 1mg/g
Carbopol 940®	1	1
Triethanolamine	0.5	0.5
Propylene glycol	7.5	7.5
Aquabidest ad	100	100

Myr: myristicin; EG: ethosomal gel; NEG: non-ethosomal gel.

Physicochemical Evaluation of Gel Dosage Forms

Physicochemical evaluation of gel, such as organoleptic test, homogeneity, pH, viscosity, and rheology properties, were conducted.

Gel Stability Test

According to Djayadisastra, these tests were conducted by recording the effect of storing the samples at different temperatures (4±2 ºC, 25±2 ºC, and 40±2 ^{0}C) for 12 weeks. The organoleptic (color, odor, and homogeneity), viscosity, pH, and observed the syneresis with interval two weeks of each observation. The cycling test was done for 6 cycles within 12 days.[14]

Myristicin ethosomal gel penetration with Franz diffusion cell

Penetration test was done by using the abdominal skin of female Spraque-Dawley rats, weighing 250-300 g. The test has been done in triplicate. The test was performed by using Franz diffusion cell, having 16 ml compartment volume, and its membrane area was 1.31 cm^2. The phosphate buffer (pH 7.4, 1% tween) was used to fill the compartment. The test was run under 37 ^{0}C and stirred at 300 rpm.[15]

The analysis was done by putting one gram of ethosomal gel myristicin or myristicin gel on the surface of the skin in the donor compartment. Then, took a half ml of the buffer from the recipient compartment after 30 minutes, 1, 2, 3, 4, 6, 8, and 12 hours. Afterward, gave the same amount of fresh buffer as a replacement, and then analyzed the samples by HPLC for their myristicin content.

Ethical clearance

The use of rats in this research has ethical approval from the Health Research Ethics Committee, Faculty Medicine, University of Indonesia, Cipto Mangunkusumo Hospital. The number of this authorization is 135/UN2.F1/ETIK/2017.

Statistical analysis

Data from the skin penetration experiment was performed using t-tests with significance level at $P<0.05$. The data were expressed as mean ±SD and checked by the Student's t-test.

RESULTS

Formulation of myristicin loaded ethosome

Myristicin was incorporated into nine formulations of ethosomes (see Table 1). From the nine different formulations, the particle sizes of four formulas were namely E1, E2, E4, and E5. and they were less than 200 nm. The rest were more than 200 nm. The highest entrapment efficiency was E4 that reached 94.1 %. (see Table 2)

Table 2: Particle size and myristicin entrapment efficiency

Parameter	Formula								
	E1	**E2**	**E3**	***E4***	**E5**	**E6**	**E7**	**E8**	**E9**
PS (nm)	**134.5±4.6**	**132.0±6.4**	**380.6±23.5**	***131.6±6.3***	**118.9±4.8**	**532.6±21.2**	**290.3±18.3**	**538.7±34.3**	**778.5±54.3**
EE (%)	**84.7±2.4**	**79.2±1.4**	**78.5±2.8**	**94.1±1.7**	**80.4 ±2.4**	**54.1±3.8**	**91.7±4.3**	**50.5±3.7**	**28.0±2.2**

PS= Vesicle Size; EE= Entrapment Efficiency

Therefore, formula E4 was considered as the best formula and used to formulate the myristicin loaded ethosomal gel. Analysis with Transmission Electron Microscopy (TEM) showed that ethosome (E4) had a spherical shape (Figure 2).

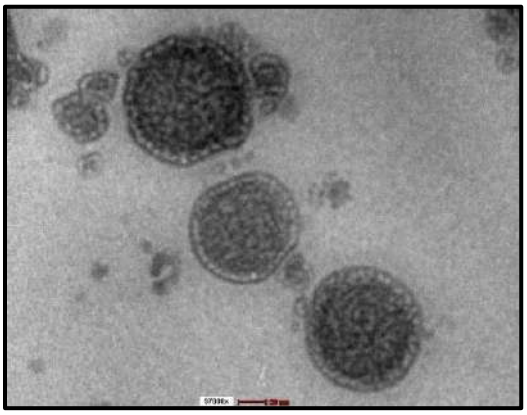

Fig. 2: Transmission electron microscope (TEM) image for myristicin loaded ethosome

The stability test showed that there were not many changes in odor or color for myristicin loaded ethosome gel (EG) that stored at various temperatures. However, non-ethosomal gel (NEG)

showed a slight color change when stored at a high temperature in eight weeks. The color change from white to pale yellow indicated the instability of myristicin in NEG. Myristicin contents were reduced when storing at 30 ^{0}C till 70 ^{0}C.[16] The pH of all gels decrease but still around 5.3 to 5.7. After 12 weeks of incubation, the viscosity of both gels decrease into 3.33 % for EG and 4.17 % for NEG (Figure 3).

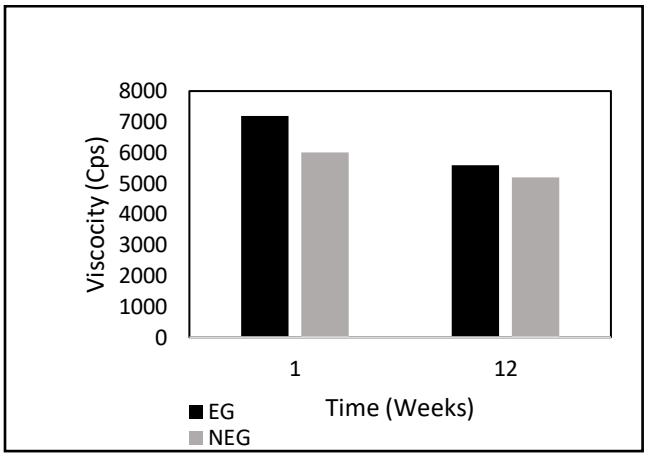

Fig. 3: Comparison of gel viscosity at week 0 and 12

The transdermal penetration test showed that EG and NEG were able to penetrate the myristicin through the rat skin in Franz Diffusion Cell. Flux of penetration of myristicin etosom gel had higher penetration (84.05±1.71 µg cm-2 hour-1) than the penetration of non etosom gel (60.21±2.46 µg cm-2 hour-1). (Figure 4).

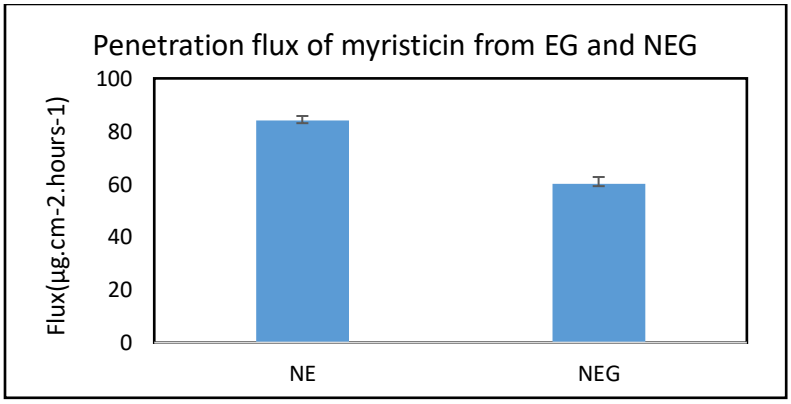

Fig. 4: Average penetration flux of myristicin from EG and NEG (average ±SD, n = 3

The sum of cumulative penetration of myristicin etosom gel and myristicin gel were 2642.6±44.92 μg cm-2 and 1893.1±19.32 μg cm-2 respectively. The percentage of the myristicin penetration from etosom gel was 52.85±2.84% and myristicin from gel was 37.86 ±1.74%.

DISCUSSION

The purpose of this study is to enhanced myristicin penetration and bioavailability, by formulating myristicin in ethosome.[7] Ethosomes are a good carrier for transdermal drug delivery, and the size is highly important. The size of these vesicles is reduced to 200 nm or 300 nm to be suitable for this route of administration.[17] The highest entrapment efficiency is 94,1% (E4). The combination of the nonpolar compound, 3% soy phosphatidylcholine and polar compound, 20% ethanol has key role to create an appropriate formation of the leaky vesicle membrane that decreasing the myristicin entrapment.[18]

The ethosomes can improve the stability of myristicin since they can shield myristicin from the temperature and oxidation exposure.[7,19] The declination in viscosity is related to the reduction in the pH of the gel. The rheological properties of the ethosomal gel indicate no changes after 12 weeks of storage. They tend to have pseudoplastic thixotropic rheology.

The low amount of penetration of NEG is due to myristicin hydrophilicity (Log P=3.53) that makes it difficult to penetrate the skin. This result is in accordance with the previous studies, where EG piroxicam and quercetin EG have higher penetration compare to NEG.[20,21] This myristicin entrapped in the ethosome will be in a phospholipid molecule that has the characteristics that resemble the lipid bilayer in the stratum corneum. Thus it can increase drug penetration through the skin because the phospholipids that absorb myristicin have the appropriate partition coefficient to be able to penetrate.[22] Ethosome formulated into gel dosage forms can control the drug delivery.[23] The appropriate formulation is important in order to have a significant drug release. The combination of hydrogel and ethosome increases drug release.[24] The use of gel as an ethosome carrier can increase the penetration of this myristicin. The penetration of myristicin in a NEG may cause by propylene glycol as a penetration enhancer. [25]

CONCLUSION

The best formulation of the myristicin loaded ethosome is depended on the combination of soy phosphatidylcholine and ethanol. It is stable, able to penetrate skin, and good bioavailability. It can be concluded that myristicin loaded ethosome can increase the penetration of myristicin.

CONFLICT OF INTEREST: The authors declare that there is no conflict of interest.

ABBREVIATION USED: EE: Entrapment efficiency; EG: Ethosomal Gel; Myr: myristicin; NEG: Non-Ethosomal Gel; OE: Oral Emulsion; PDI: Polydispersity index.

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Certificate of Participation

This is to certify that

Mrs. Susana Elya Sudradjat

Oral Presentation

Title: Formulation and Penetration Study of Etosom Gel Myristicin of Nutmeg Oil (Myristica fragrans H.)

The 2nd International Conference on Herbal and Traditional Medicine (HTM2017)
"Value-Added of Herbs and Phytotherapy: Challenges for The 21st Century"
January 25-27, 2017 Asia Hotel, Bangkok, Thailand

Associate Professor Dr. Paiboon Daosodsai
Dean, Faculty of Pharmaceutical Sciences, Khon Kaen University

Dr. Denpong Patanasethanont
Chairman, the 2nd HTM 2017 Organizing Committee