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Transdermal Delivery Enhancement of Gel Containing Niosomes Loaded with *Volvariella Volvacea* Extract

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Abstract: Our previous study demonstrated the potent *in vitro* collagen biosynthesis stimulating and antioxidant activities of *Volvariella volvacea* extract. This study was performed the loading of *V. volvacea* extract (VV) in niosomes (NV). The physicochemical characterization of the VV loaded niosomes and gel containing niosome of the extract (GNV) was studied. Rat skin transdermal absorption by Franz diffusion cells at 6 hours of NV, GNV was compared with *V. Volvacea* extract solution (SV). GNV and NV showed higher chemical stability of total phenolic contents than SV. NV exhibited negative zeta potential values with the mean size of 254±20.32 nm. Both GNV and NV retarded the cumulative amounts and fluxes of the total phenolic in the extract in the first hour of skin permeation, while enhanced the skin permeation at the 6^{th} hour in the experiment. GNV gave the highest percentages of the total phenolic content through rat skin to the receiving solution followed by NV and SV, respectively. This study has demonstrated the potential of gel containing niosomes of the *V. volvacea* extract appeared to be the suitable system for topical anti-aging application of *V. volvacea* extract because of the enhancement of chemical stability and rat skin transdermal absorption of *V. volvacea* extract.

Keywords: Niosomes, stability, transdermal absorption, *Volvariella volvacea*.

INTRODUCTION

Our previous study has demonstrated the potent *in vitro* collagen biosynthesis stimulating and antioxidant activities of the sonicated aqueous extract of *Volvariella volvacea* [1] which has known as a paddy straw mushroom or straw mushroom or "Hed Fang" in Thai. It is specie of edible mushroom cultivated throughout the East and Southeast Asia and used extensively in Thai cuisines. *V. volvacea* (Family: Phuteaceae) is a popular variety among people because of its distinct flavor, pleasant tastes, higher protein content and shorter cropping duration compared to other cultivated mushrooms. Presently, this mushroom is available in most of the supermarkets. It originally grows in rice straw stack in tropical zone that has a high temperature and a rainy climate especially in Chiang Mai, Thailand. Many studies have found that some species of mushrooms are having therapeutic properties such as antioxidant, antimicrobial, anticancer, cholesterol lowering and immuno stimulatory effects [2]. Mushrooms are widely consumed and have been valued as an edible and medical resource. They accumulate a variety of secondary metabolites, including phenolic compounds, polyketides, terpenes and steroids [3]. Mushrooms are traditional in Thailand and also commonly used as food ingredients. Since the potent *in vitro* collagen biosynthesis stimulating and antioxidant activities of the sonicated aqueous extract of *V. volvacea* (VV), it was loaded in niosomes to enhance its stability, then developed as a cosmetic product and investigated their transdermal delivery [1]. Niosomes are widely used to deliver drugs, cosmetics, and plant extracts for pharmaceutical and cosmeceutical purposes [4], especially for the continuous release and targeted delivery [5]. Nonionic surfactants e.g. Span, Tween 61 can be used to form bilayer vesicles because of not only low cost, but also high chemical stability [6]. The bilayer spheroidal structures of these vesicles are usually composed of non-ionic surfactants mixed with cholesterol. These vesicles are advantageous for drug and cosmetic delivery because of their modification of pharmacokinetics, bioavailability and environment protection of the entrapped substances [7, 8]. Transdermal delivery is a convenient method of active ingredient administration that provide the controlled delivery of substance through skin with minimal discomfort [9]. This method offers many benefits over delivery via oral [10, 11], intravenous, or injection routes [12, 13] which often result in adverse side effects or fail to deliver the active ingredient to the skin or insufficient concentrations to treat a variety of systemic diseases [14]. However, niosomes were introduced to overcome those problems because niosomes can delivery active ingredient to the target such as anti-cancer drug and anti-infective agent [15].

Thus, the present study has investigated the physicochemical properties and transdermal absorption of the sonicated aqueous extract of *V. volvacea* (VV) loaded niosomes (NV) and gel containing niosomes of VV (GNV) in comparing to VV solution (SV).

MATERIALS AND METHODS

Materials

Tween61 (polyoxyethylene sorbitan monostearate), gallic acid, Carbopol® 980, Folin–Ciocalteu reagent and sodium

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carbonate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Cholesterol from Wako Pure Chemical Industrial Ltd. (Osaka, Japan) was used. All other chemicals and reagents were analytical grade.

Methods

Preparation of the Sonicated Aqueous Extract of V. volvacea (VV)

V. volvacea were collected from Chiang Mai Province in Thailand, during January to June in 2013. The specimen was authenticated by a botanist at Faculty of Pharmacy, Chiang Mai University, Thailand and deposited at Faculty of Pharmacy, Chiang Mai University in Thailand. Then, the mushrooms were washed, cut into pieces, dried at 40 ± 2°C in a hot air oven, ground to powder and kept in an airtight plastic bag at 4 ± 2°C until use. For the extraction process, 100 g of the dried mushroom powder were sonicated in 1000 ml of distilled water for 2 h (with the pulse on for 5.0 sec and off for 2.0 sec) at room temperature (25 ± 2°C) by the Probe sonicator (Vibra Cell™, Sonics & Materials Inc., U.S.A.). The mixtures were filtered through Whatman No. 1 filter paper and the mushroom residues were re-extracted twice under the same conditions. The filtrates were pooled and concentrated under vacuum by a rotary evaporator (R-124 Buchi, Switzerland) with the bath temperature of 45 ± 1°C and the pressure of 70 mbar, and lyophilized. The dried extracts were stored at 4 ± 2°C prior to use. The sonicated aqueous extract of *V. volvacea* (VV) extract was obtained and the percentage yields were calculated on a dry weight basis.

Preparation of Loaded Niosomes

For VV loaded niosomes (NV), the amounts of 20 mM of Tween61 mixed with cholesterol (at 1:1 molar ratio) were dissolved with chloroform in a round bottom flask. The solvent was removed by a rotary evaporator (Eyela, Tokyo Rikakikai Co. Ltd., Tokyo, Japan) to get a thin film and the residual solvent was dried overnight in a vacuum desiccator. An amount of 20 ml of distilled water together with the VV at various concentrations (0.5, 1.0, 2.0 and 3.0 % w/v) were added to the film and mixed at 50 ± 1°C for 15 minutes by a rotary evaporator. The dispersion put in the ice bath was sonicated for 2 minutes by an ultrasonic generator (US-300T, Nissei, Tokyo, Japan).

Physicochemical Characteristics of the Loaded Niosomes

The VV loaded niosomes (NV) were stored in transparent vials covered with aluminum cap for 3 months at various temperatures (4±2, 25±2 and 45±2°C) and investigate at initial and after stored for 1,2 and 3 months. In the evaluating process, the samples were withdrawn and assayed for physical characteristics (appearance, particle size and zeta potential) and the total phenolic contents. Moreover, the NV was evaluated their stability by the accelerated heat cool cycle for 6 cycles.

Physical Characteristics of Niosomes

The Maximum Loading of the VV

The concentrations of the VV entrapped in niosomes were increased from 0.5 to 3.0 % w/v. The maximum loading of the VV in niosomes was determined from the maximum concentration of the VV which gave no precipitation.

Appearances

The NV was investigated for the sedimentation, separation layer and color optically at initial and 1, 2, 3 months.

Particle Sizes

The particle sizes of various concentrations of VV in NV were measured by dynamic light scattering (DLS) by the Zetasizer 300HSA (Malvern Instruments, Malvern, UK) based on photon correlation spectroscopy. The niosomal dispersions were diluted to 15 times with distilled water. The diameters of loaded niosomes were carried out for 100 s at room temperature (25±2°C). The time-dependent correlation function on the scattered light intensity was measured at a scattering angle of 90° and wavelength at 535 nm.

Zeta Potential

The zeta potential values of VV loaded niosomes were determined using the Zetasizer 300HSA (Malvern Instruments, Malvern, UK). The analysis time was kept for 60 s. The average zeta potential was determined. The time-dependent correlation function on the scattered light intensity was measured at a scattering angle of 90°. Samples were diluted 15 times with freshly filtrated Millipore water for the particle size and zeta potential measurement.

Preparation of Gel Containing the Niosomes Loaded with VV (GNV)

The 2.0 % w/v of NV was incorporated into the gel base containing Carbopol® 980. Briefly, 0.3% (w/w) Carbopol® 980 was dispersed in the niosomal dispersion with gentle stirring, resulting in the gel containing niosomes loaded with 2.0 % (w/v) of NV (gel of VV niosomes, GNV).

Chemical Stability of the Total Phenolic Acid in Niosomes Loaded with VV (NV) and Gel Containing the Niosomes Loaded with VV (GNV)

The remaining total phenolic acid contents in the loaded niosomes (NV) and gel containing the niosomes loaded with VV (GNV) were determined at 0, 1, 2 and 3 months in comparing to the VV solution (SV) which dissolved in distilled water. Total phenolic contents (TPC) in the form of gallic acid in the GNV, NV and SV were determined using the Folin–Ciocalteu reagent [16]. Briefly, 500 ul of GNV, NV or SV was extracted by 500 ul of 98% ethanol, then mixed with 1 N Folin–Ciocalteu reagent and 20% w/v of

sodium carbonate (Na_2CO_3) at ambient temperature (25±2ºC). After incubation for 30 minutes, the absorbance of blue color developed in each assay mixture was recorded at 760 nm by a Microplate reader (Model 550, BIORAD, U.S.A.). The TPC in GNV, NV or SV were expressed in mg of gallic acid equivalents (GAE) per gram of extract.

Transdermal Absorption by Vertical Franz Diffusion Cells

Preparation of the Rat Skin

The male Sprague-Dawley rats (10–12 weeks, 150-200 mg) were obtained from National Laboratory Animal Centre, Mahidol University, Nakhon Pathom in Thailand. The hair on the abdominal skin was shaved off and left overnight. The rats were sacrificed and the abdominal skin was separated. The subcutaneous fat was then trimmed off by a scalpel. The skin was freshly used. The investigational protocol for all procedures has adhered to the "Principles of Laboratory Animal Care."

Sample Preparation

The samples used for the transdermal study were gel containing the niosomes loaded with VV (GNV), VV loaded niosomes (NV) and VV solution (SV). All formulations contained 2.0 % w/v of VV.

Transdermal Absorption Experiment

The rat skin was mounted on the receiving compartment solution of the Franz diffusion cells with the stratum corneum (SC) side facing upwards to the donor compartment. One milliliter of each sample was placed in the donor compartment and covered with paraffin film. The available diffusion area of the rat skin was 2.46 cm^2. The receiver chamber was filled with 14 ml of phosphate buffer saline (pH 7.4), controlled at 37±2 ^oC and constantly stirred at 100 rpm with a small magnetic bar throughout the experiment. The diffusion cells were withdrawn at 1, 3 and 6 hours. Total phenolic contents (TPC) in the whole skin and the receiving compartment solution were extracted and determined using the Folin–Ciocalteu reagent [16]. All experiments were done in triplicate.

Extraction and Determination of TPC in the Treated Samples

After the experiment, the rat skin was removed from the diffusion cells and swung twice in 100 ml of DI water. The rinsed water was discarded. The skin was cut into small pieces and pooled in a vial containing 5 ml of distilled water. The vial was vortexed for 5 min and centrifuged at 18,000 rpm, 4 ^oC for 10 minutes. The supernatant was collected and dehydrated using a freeze dryer (CHRIST, Martin Christ, Germany). Then, the freezed-dried powder was reconstituted with 1 ml of distilled water and determined for total phenolic contents (TPC) in the form of gallic acid in each sample using the Folin–Ciocalteu reagent.

Data Calculation and Statistical Analysis

The fluxes at the steady state (mg/cm^2/h), of the samples were calculated using the linear part of the correlation between the cumulative amounts of total phenolic compound (mg/cm^2 that permeated the rat skin by unit area and time with the linearity and LLOQ of the cumulative amounts of total phenolic were 0.991 and 0.010 mcg/ml, respectively**.** Data were expressed as the mean of the three experiments ± the standard deviation (SD) and were analyzed using ANOVA with LSD test. Statistical analysis differences yielding p <0.05 were considered significant.

RESULTS AND DISCUSSION

Physical Characteristic of Niosomes Loaded with VV Extract (NV)

Since the sonicated aqueous extracts of *V. volvacea* not only showed the highest total phenolic and polysaccharide contents but also gave the highest DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging, lipid peroxidation inhibition and collagen biosynthesis stimulating activities in our previous study [1], VV extract was selected to develop as a topical anti-aging cosmetic product in this study. The nanotechnology, niosomes, was introduced not only to enhance the stability of loaded substance but also modify its pharmacokinetics and bioavailability [8]. Moreover, the niosomes can protect VV extract from the environment, light, heat, and others chemicals that leads to the oxidation process.

The Maximum Loading of VV in Niosomes

VV extract was loaded in neutral niosomes (20 mM of Tween61 mixed with cholesterol at 1:1 molar ratio) which was the best formulation from our previous study [17]. When the loading concentration of VV was more than 2.0 % (w/v) of VV, the sedimentation of VV extract was observed at the bottom of bottle. Thus, the maximum loading of VV in niosomes was 2.0 % (w/v). VV extract may localize at the hydrophilic core of niosomes and slowly release to the environment. Niosomes loaded with VV extract at 2.0 % w/v (NV) was a light brown opalescent suspension

Particle Sizes and Zeta Potential of Niosomes Loaded with VV Extract (NV)

Size and zeta potential of the loaded niosomes were presented in Fig. (**1**). The 2.0 % NV gave the average size of 254±20.32 nm, PDI of 0.322 and zeta potential of -50.07±3.11 mV which was outside the range of ± 30 mV range demonstrating the physical stability of the dispersion [18]. After stored at 4 and 25°C for 3 months, the loaded niosomes showed the range of size of about 230-280 nm, while the niosomes at 45°C showed more fluctuate size of about 250-300 nm. This might be due to the heat from the storage temperature changed the gel structure of niosomal membrane from the closely packed gel to the loosely packed liquid crystalline structure resulting in the variation of

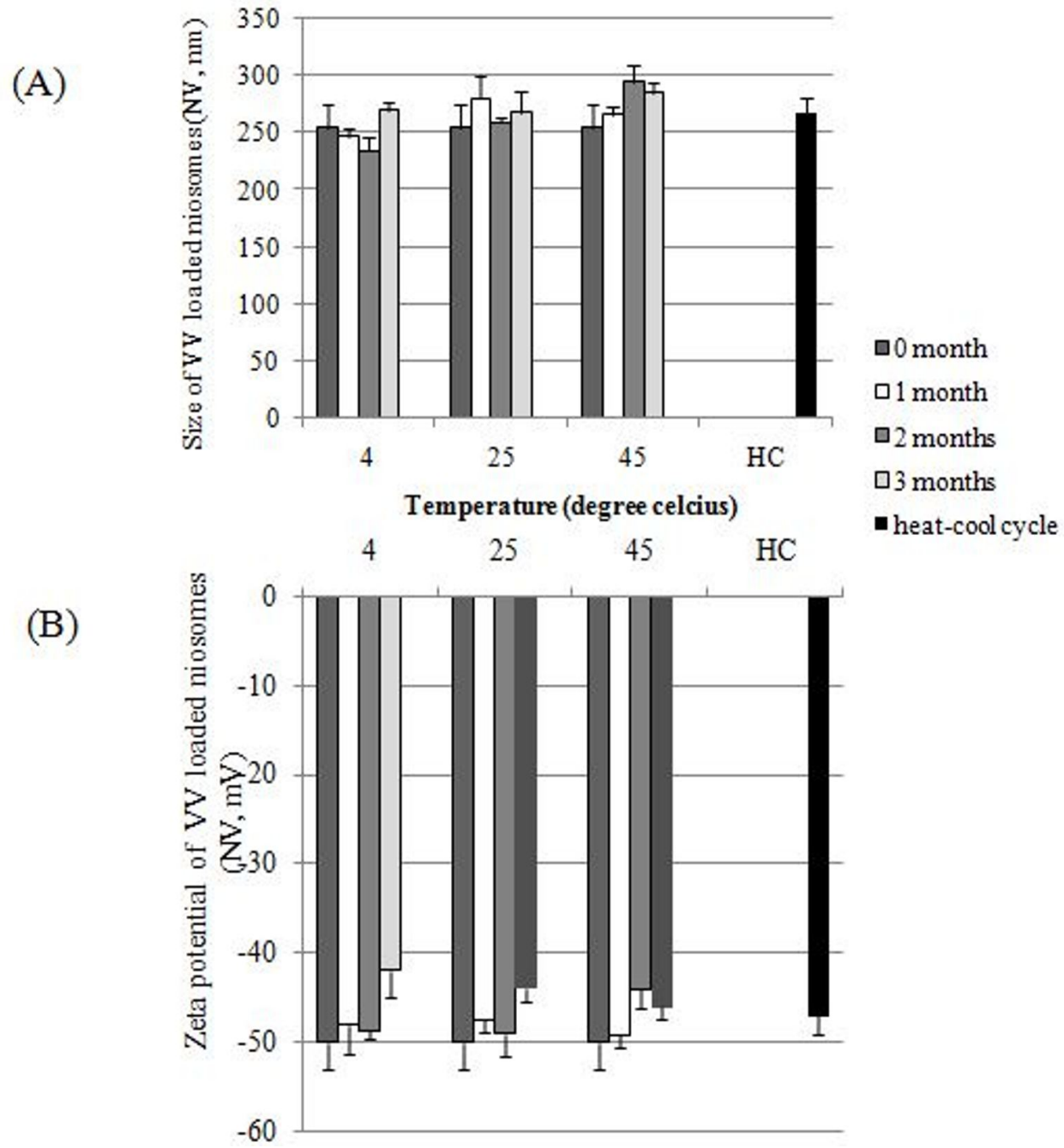

Fig. (1). The mean size (**A**) and zeta potential (**B**) value of niosomes loaded with VV extract (NV) at various storage temperature (4±2, 25±2 and 45±2°C) and heat cool cycle.

niosomal size [19]. However, the niosomal size after stored at three different temperatures for 3 months were in the acceptable range (230-300 nm). Moreover, the VV loaded in niosomes gave acceptable negatively zeta potential values of about -44 to -50 mV in all temperature conditions throughout 3 months.

Chemical Characteristic of Niosomes Loaded with VV Extract (NV)

The relative amount of total phenolic content in various formulations containing VV extract at 3 different storage temperatures (4±2, 25±2 and 45±2°C) for 3 months were shown in Fig. (**2**). Solution of VV (SV) showed the lowest amount of total phenolic compound throughout 3 months. After 3 months of storage, SV at 45±2°C gave the total phenolic content at 64.21±4.56 % of initial. This may be due to the influence from the high temperature and exposure time that affect the total phenolic content of VV extract in SV. When VV extract was loaded in niosomes to enhance the stability of VV, niosomes loaded with VV (NV) gave more remaining amount total phenolic compound of 1.26, 1.32 and 1.33 times than in SV at 4±2, 25±2 and 45±2°C in the 3rd month, respectively. Both NV and gel containing the

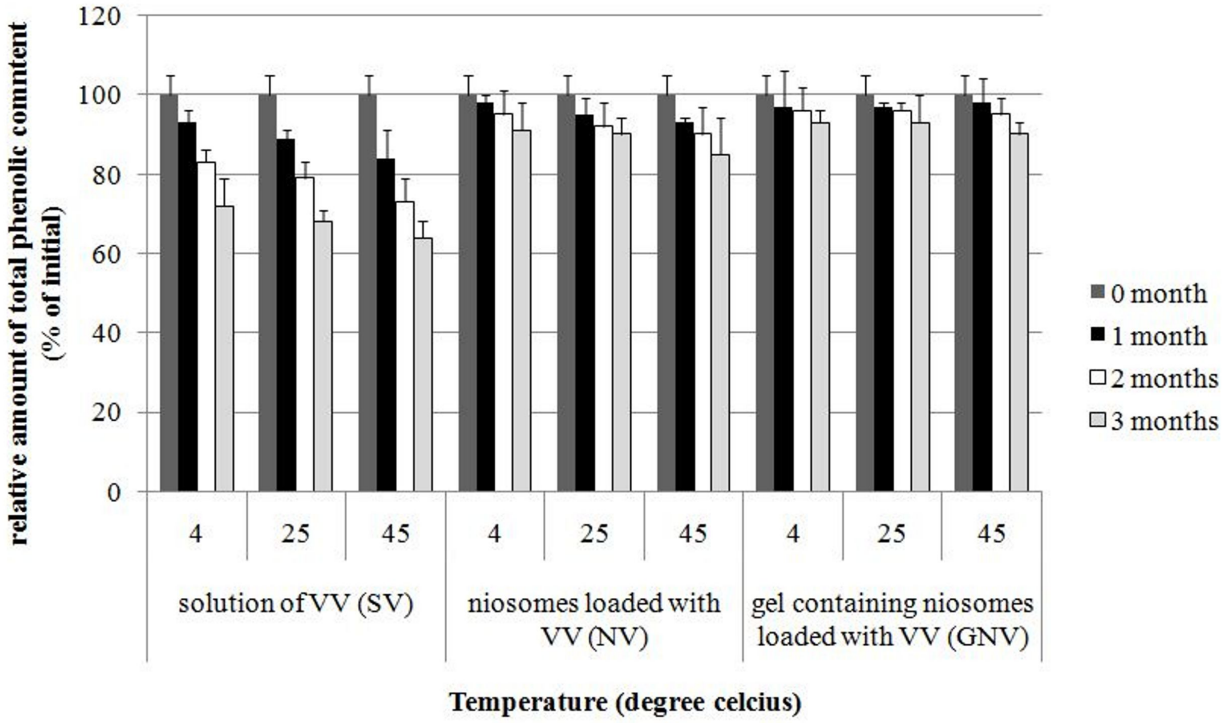

Fig. (2). Chemical stability of total phenolic content in various formulations containing VV extract at 3 different storage temperatures (4±2, 25±2 and 45±2°C).

niosomes loaded with VV (GNV) gave more than 80 % total phenolic content remaining through the stability period of 3 months at 3 different temperatures indicating the acceptable stability of total phenolic content after loading in niosomes. However, GNV showed higher content of total phenolic compound than those in NV of about 1.02, 1.03 and 1.05 times at 4±2, 25±2 and 45±2°C in the 3rd month, respectively, indicating the double environment protections from niosomes and also the gel structure that protects VV extract from the environment [20].

Transdermal Absorption of Various Formulations Containing VV Extract

The cumulative amounts of total phenolic compound in various systems in whole skin and the receiving compartment solution through transdermal absorption across the excised rat skin were shown in Fig. (**3**). The cumulative amounts of total phenolic compound in all systems gradually increased in both whole skin and receiving compartment solution throughout the period of 6 hours except the SV, which was stable after the 1st and 4th hour of the experiment. This may be due to the effect of the saturation of VV extract in the whole skin and then it was released to the receiving compartment solution, resulting in the gradually increasing of the cumulative amounts of total phenolic compound in the receiving compartment solution of SV system. The same mechanism, the VV extract saturation in whole skin and then release to the receiving compartment, was occurred in NV and GNV system. But the VV extract saturation did not cease the cumulative amounts of total phenolic compound in the whole skin, the cumulative amounts of total phenolic compound of NV and GNV were gradually decreased, which was agreed with the previous study [21]. This might be the advantage of niosomes in the transdermal delivery [22] that can load the substance inside them and penetrate through the skin via like dissolve like theory. Since the niosomes composition, cholesterol was found in SC [23]. The relative amount of total phenolic content from various systems containing VV extract (SV, NV and GNV) which delivered through vertical Franz diffusion cells comparing to the initial loading amount were shown in Fig. (**4**). The increasing rate of the relative amount of initial loading from NV and GNV were higher than SV. This may be from the slow release of niosomes that gradually released VV extract from the systems to the environment. But SV system showed the relative amount of total phenolic compound in the 1st hour in whole skin and the receiving compartment solution at about 4 and 1.75 % which were higher than those from NV system of about 1.74 and 2.75 times, respectively. This might be from the retard effect of niosomes system that cannot promptly release the substance to the environment as much as SV system. However, NV and GNV gave the retard effect at the 1st hour, but they could deliver higher total phenolic content than SV system at the 6th hour of about 1.45 and 1.38 times in the whole skin and 1.48 and 1.68 times in the receiving compartment solution, respectively. Moreover, NV system gave the highest amount of total phenolic compound in the whole skin at the 6th hour at 10.25 % of initial, while GNV gave the highest amount of total phenolic compound in

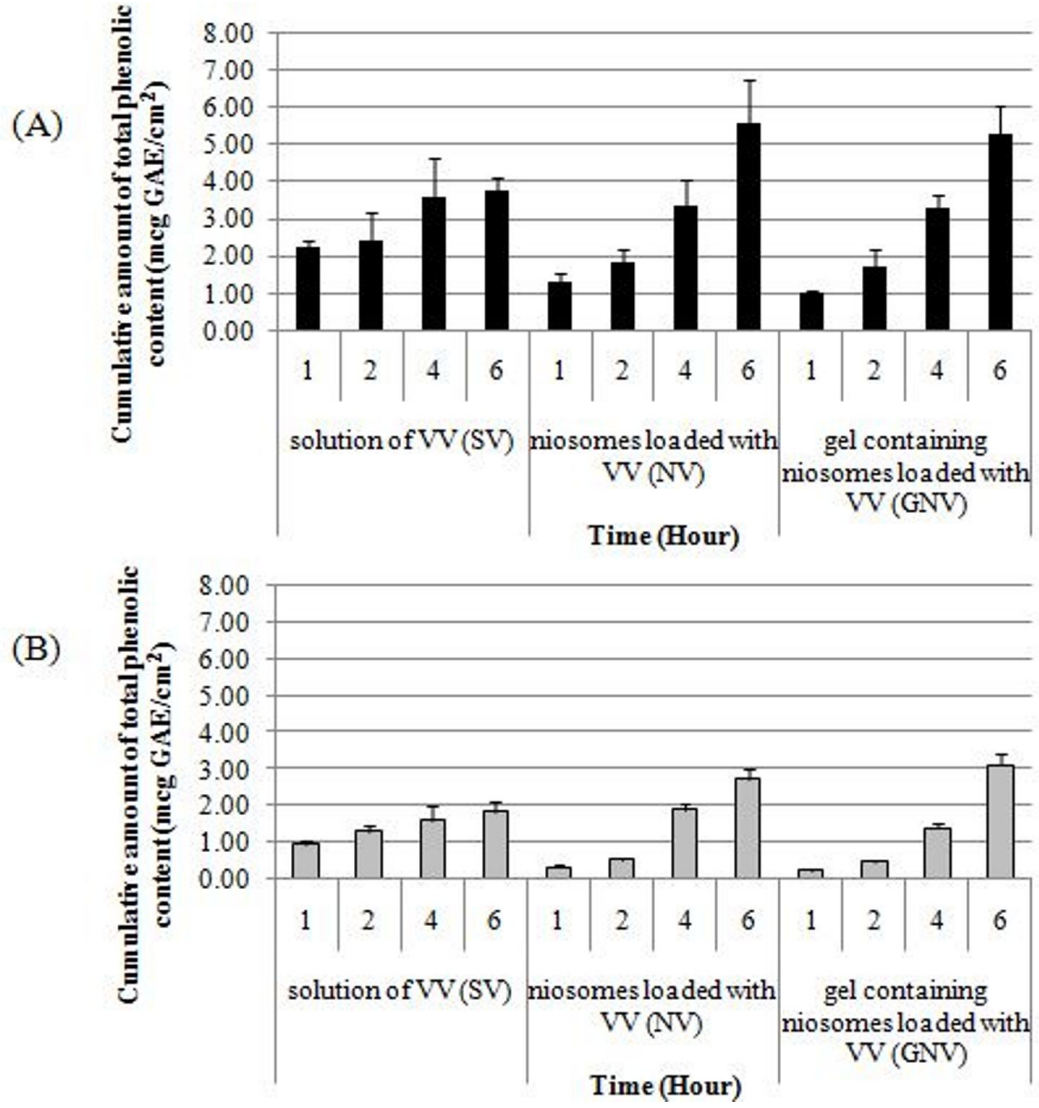

Fig. (3). The cumulative amounts (mcg GAE/cm^2) of total phenolic content from various systems containing VV extract in whole skin (A) and receiving compartment solution (B) through transdermal absorption across excised rat skin.

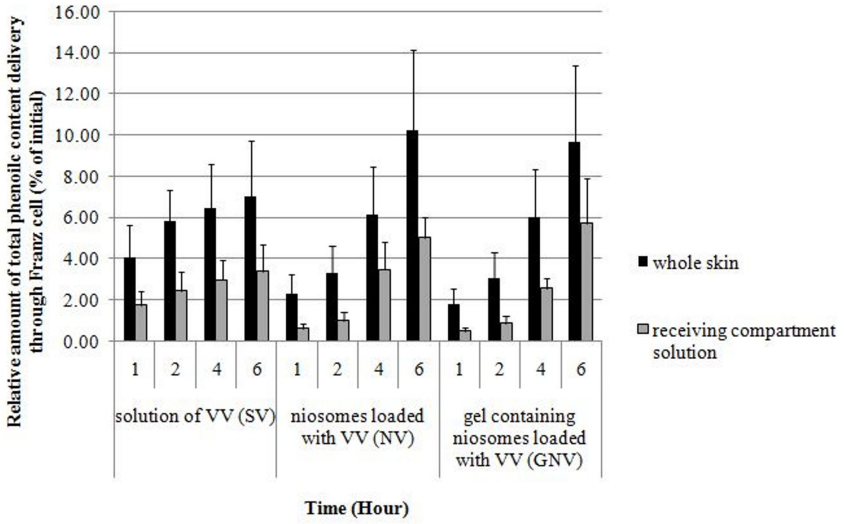

Fig. (4). Relative amount of total phenolic content from various systems containing VV extract (SV, NV and GNV) which delivered through vertical Franz diffusion cells comparing to the initial loading amount.

Table 1. The cumulative amounts (mcg GAE/cm^2) and fluxes (mcg/cm^2/h) of total phenolic content from various systems containing VV extract in whole skin and receiving compartment solution through transdermal absorption across excised rat skin at 6 h by vertical Franz diffusion cells.

Samples	Cumulative Amounts (mcg GAE/cm^2)						Fluxes (mcg GAE/cm^2/h)					
	Whole Skin			Receiving Compartment Solution			Whole Skin			Receiving Compartment Solution		
solution of VV (SV)	3.714	±	0.412	1.846	±	0.265	0.619	±	0.069	0.308	±	0.044
niosomes loaded with VV (NV)	5.565	±	1.141*	2.735	±	0.274	0.928	±	0.190*	0.456	±	0.046
gel containing niosomes loaded with VV (GNV)	5.260	±	0.783	3.108	±	0.311*	0.877	±	0.131	0.518	±	0.052*

Notes: Each value represented mean ±S.D. of three experiments.
∗: significant difference ($p < 0.05$) in comparing to the SV system.

the receiving compartment receiving compartment solution at the 6th hour at 5.72 % of initial (Fig. **4**). The fluxes at the 6th hour (mcg/cm^2/h) of various systems containing VV extract (SV, NV and GNV) that permeated the rat skin were calculated using the linear part of the correlation between the cumulative amounts of total phenolic compound by unit area and time were shown in Table **1**. The fluxes of the whole skin at the 6th hour of NV system at 0.928±0.190 mcg/cm^2/h was significantly different from that of SV at 0.619±0.069 mcg/cm^2/h (p<0.05). This might conclude that niosomes are suitable for the penetration into the skin and remain in the skin which was not suitable for the delivery substance to the blood vessel, while gel system of GNV can deliver more substance to the receiving compartment solution with the significantly different flux of 0.518± 0.052 mcg/cm^2/h (p<0.05), that represented for the blood vessels that may from the occlusion effect of gel [24]. This study has suggested that the gel containing the niosomes loaded with VV (GNV) appeared to be the most suitable system to be used for topical anti-aging application with the highest transdermal penetration of total phenolic compound into the receiving compartment than niosomes system, the proper viscosity for convenient topical use and superior occlusion effect of the gel that will be beneficial for the saturation in the skin.

CONCLUSION

The sonicated aqueous extracts of *V. volvacea* were loaded in neutral niosomes (20 mM of Tween61 mixed with cholesterol at 1:1 molar ratio) to enhance the stability of loaded substance. The 2.0 % w/v of niosomes loaded with VV extract (NV) gave the average size of 254±20.32 nm and zeta potential of -50.07±3.11 mV demonstrating the good physical stability of the dispersion. Gel containing the niosomes loaded with VV (GNV) was prepared. . Both NV and GNV gave more than 80 % total phenolic content remaining through the stability period of 3 months at 3 different temperatures indicating the acceptable stability of total phenolic content after loading in niosomes. NV system gave the significantly highest amount of total phenolic compound in the whole skin at the 6th hour at the relative amount of 10.25 % of initial and the flux of 0.928±0.190 mcg/cm^2/h, while GNV gave the highest amount of total phenolic compound in the receiving compartment receiving compartment solution at the 6th hour at 5.72 % of initial and the flux of 0.619±0.069 mcg/cm^2/h showing that niosomes are suitable for the penetration into and remain in the skin, while gel system can deliver more substance to the receiving compartment solution. This study has suggested that the gel containing the niosomes loaded with VV appeared to be the most suitable system to be used for topical anti-aging application with the highest transdermal penetration of total phenolic compound into the receiving compartment, the proper viscosity for convenient topical use and superior occlusion effect that will be beneficial for the saturation in the skin.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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