EX VIVO PERMEATION STUDY OF NANOSTRUCTURED DOSAGE FORM CONTAINING MANGO SEED KERNEL EXTRACT USING FRANZ CELL

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ABSTRACT

Background: *mango seeds (Mangiferin indica L.) have the ability to inhibit P. acnes, S. aureus, and E. coli bacteria and inhibit inflammation, potentially for transdermal therapeutic dosage forms. The nano-emulsion dosage forms were prepared based on the SNEDDS system with nano-oil droplets containing mango seed extract. These dosage forms contributed to carrying the active ingredient deeply into the impact site, bringing the highest efficiency, but this needs to be proven. Thus, it is necessary to study the process to evaluate the permeability of these formulas to prove their availability improvement.* ***Objectives****: To assess the transdermal permeability and active substance release of nanostructured dosage forms by a validated procedure.* ***Methods:*** *an ex vivo experiment was designed by adding a sample to diffuse through Franz cells. In addition, to ensure the quality and effectiveness of preparations containing mango seeds, developing and validating a process for quantifying the total polyphenols in the preparation are extremely necessary. Total polyphenols were quantified by color complexing with Folin-Ciocalteu reagents, with maximum absorption at 765 nm.* ***Results:*** *the procedure had been validated according to the International Conference on Harmonization (ICH) on the criteria of specificity, system compatibility with RSD = 1.02%, linearity built on the concentration range of 10–50 µg/ml with* R^2 *= 0.998, accuracy, precision with %recovery in the range of 97.73% to 102.56%. The results showed that more than 300 mg/g of polyphenol was released after 6 hours from the tested nanostructured dosage form, about 4.3 times as many as the total amount of polyphenols in the comparative cream.* ***Conclusions****: the quantification of polyphenols diffused through Franz cells helps evaluate the quality of the preparation. The procedure had been validated according to the International Conference on Harmonization (ICH) and could be applied to evaluate nanostructured dosage forms containing mango seed kernel.*

Keywords: *mango seed kernel extract, Franz cell, polyphenols, nanostructured dosage forms.*

I. INTRODUCTION

Mango's scientific name is *Mangifera indica* L (*M. indica*) in the family Anacardiaceae. Some studies in the world indicate that mango seeds have antioxidant effects applied to protect food [1]; in addition, there are anti-inflammatory, antibacterial, and anti-fungal effects applied to the digestive system, skin disease treatment, etc. [2], [3], [4], [5], [6]. According to a study by Ha Cao Thien et al. (2022), mango seed kernel extract (MSKE) has significantly better antibacterial and anti-inflammatory activity than mango seed coat extract [7]. On the other hand, there has been no further research on biological activity or the application of these activities to make preparations in Vietnam. In addition, we noticed that nowadays, a common trend among consumers is using cosmetics made from natural medicinal herbs because of

their proximity, safety, and high efficiency. Therefore, MSKE's effects can be applied and developed into therapeutic dosage forms through the skin.

The self-nanoemulsifying drug delivery system (SNEDDS) is a form of pre-emulsion, usually formulated in anhydrous forms to form tablets to improve the bioavailability of the drug when taken orally. SNEDDS is a homogeneous mixture of oil and surfactant or co-surfactant. When entering the stomach, it will slowly come into contact with the aqueous phase of gastric digestive juice. Under the contractile action of the stomach, it will form a nanometer-sized oil and droplet-sized emulsion [8]. For cosmetic products used on the skin, SNEDDS is prepared to enhance the permeability of therapeutic dosage forms through the skin.

To evaluate the efficacy of these dosage forms, it is essential to develop an *ex vivo* Franz static permeation cell experiment to assess transdermal permeability and release of the active ingredient in the formulation containing MSKE.

This study aims to develop and validate the process of quantifying the total polyphenols loaded in the preparation by UV-Vis spectroscopy. The process is validated with the following criteria: specificity, system compatibility test, linearity, precision, and accuracy. This validated procedure is then applied to quantify the total amount of polyphenols in creams diffusing through Franz cells.

II. MATERIALS AND METHODS

2.1. Materials, chemicals, and instruments

Materials, chemicals, and reagents: gallic acid (98%) was supplied by Sigma Aldrich, sodium hydroxide (NaOH), potassium monobasic phosphate (KH_2PO_4), and sodium carbonate (Na_2CO_3) were mixed for day use, and Folin-Ciocalteu reagent was purchased from Nanjing Duly Biotech. Nanostructured dosage forms of MSKE were prepared using the SNEDDS technique.

Instruments: instruments used in the present study consist of UV/Vis V-730 spectrophotometer (Kern-Germany) instruments, an analytical balance (Kern AES – Germany) with an accuracy of 0.0001 g, a magnetic stirrer, a laboratory water bath (Membert – Germany), and a Franz cell (Vietnam).

2.2 Methods

2.2.1. Method validation

Franz static permeation cells had a receptor compartment volume of 13.45 mL and an area for permeation of 2 cm^2. The solution in the receptor compartment was a phosphate buffer at pH 7.4. Permeation membranes were taken from the young white porcin ears' epidermal skin. After purchased, they were cleaned with distilled water and stored immediately in a NaCl 0.9% solution at 4–8 ^oC. Hairs, subcutaneous fat tissues, and blood vessels were removed using scissors to obtain a full-thickness skin of about 500 µm, an area of 2x2 [10], [11]. Receptor compartment solution: phosphate buffer pH 7.4 was maintained at 37 ± 0.5 °C. The receptor compartment solution and permeation membranes temperature was maintained at 37 ± 0.5 °C. Agitation was maintained at 400 rpm. Before applying the preparation to the permeation membrane surface in the donor compartment, the membranes were stabilized at this condition for about 30 minutes.

Determination of the maximum wavelength of total polyphenols: the gallic acid standard solution was diluted employing phosphate buffer pH 7.4 to gain a gallic acid solution

concentration of 30 µg/mL. Then, the solution was reacted with a Folin-Ciocalteu reagent. The absorbance of the solution was scanned in the wavelength range of 450–900 nm using a UV/Vis V-730 spectrophotometer with phosphate buffer pH 7.4 and Folin-Ciocalteu reagent as a blank.

Determination of total polyphenol concentration: the recovered solution was reacted with Folin-Ciocalteu reagents to quantify the total polyphenol concentration. The reaction samples were photometrically measured at the maximum absorption wavelength (expected at 765 nm). Total polyphenol content is calculated according to gallic acid. For the test sample: accurately aspirate 1,0 ml of the recovered solution into the test tube, add 5.0 ml of Folin-Ciocalteu reagent, and shake well for 2 minutes. Left at room temperature for 10 minutes. Add 4,0 ml of sodium carbonate (Na_2CO_3) at 7.5%. Shake well for 2 minutes, cover tightly, and leave at room temperature for 60 minutes.

Validation of the method: appraisal according to the International Conference on Harmonization (ICH) guidelines [9] includes the following indicators:

Specificity: scanning the spectrums of gallic acid standard solution, the cream, and the placebo diffused through the Franz static permeation cells in 6 hours. All were reacted with a Folin-Ciocalteu reagent.

System Suitability Test: preparation of a gallic acid standard solution with a 10 µg/ml concentration from the original standard solution. Accurately aspirated 1.0 ml of this solution, reacted with the Folin-Ciocalteu reagent, and measured absorption at 765 nm (n = 6).

Linearity: on a series of standard solutions with a concentration range of 10–50 µg/ml, we constructed a regression equation representing the linear dependence between concentration and absorption. Requirements: $R^2 \geq 0.99$.

Precision: prepare samples on the intraday (n = 6) and interday (n = 3) days. Intraday precision was evaluated by measuring the total amount of polyphenols in six samples on the same day. Interday precision was evaluated by continuously measuring the total amount of polyphenols in three days. All test samples were reacted with a Folin-Ciocalteu reagent and measured at 765 nm. Calculation of results: From the sample's absorption, calculate the concentration of total polyphenols in the sample. From the concentration of total polyphenols in the sample, we measured the total amount of polyphenols in the preparation that diffused through the Franz static permeation cells in 6 hours by the formula: $Q_n = V \times C_n$. Where: V (ml): volume of the receptor compartment; C_n (g/ml): concentration of sample received at t_n; Q_n (µg): the total amount of polyphenols in the preparation that diffuses through the Franz static permeation cells.

Accuracy: 1.0 mL of reference solution of 16, 20, and 24 µg/ml was added to the test tubes containing 1.0 mL of test sample taken from the receptor comparison solution in 6 hours (n = 9). All samples were reacted with the Folin-Ciocalteu reagent, and absorption was measured at 765 nm. The accuracy was determined based on a percentage of recovery (%Recovery) parameter.

2.2.2. Determination of the release of a nanostructured dosage form containing mango seed kernel extract by the ex vivo Franz static permeation cells experiment

The release of the MSKE-loaded nanostructured dosage form was determined by determining the total amount of polyphenols that diffused through the Franz static permeation cells in the prepared formulation.

The test nano-emulsion cream composition was prepared with the formula: oil phase including vitamin E, lanolin, stearyl alcohol, and cetyl alcohol; surfactant including tween,

span, and polyethylene glycol 400 (PEG 400) as co-surfactant; water phase including glyceryl, MSKE, and distilled water. The ratio of nano-emulsion phases is 15:50:35. The water phase was slowly administered with continuous stirring to adjust the nano-emulsion volume. The MSKE concentration administered was 5% w/w. To compare the ability to diffuse through the skin and release the active ingredient of nano-emulsion cream containing MSKE simultaneously, we designed the experiment under the same conditions as nano-emulsion cream. A comparative cream (normal cream) was prepared with the same formula but a different preparation method for nano-emulsion cream. The method involved heating the oil and water phases and then mixing the two phases vigorously and homogeneously. 30mg of cream was placed on the membrane surface and continuously contacted the surface for 6 hours in the donor compartment. After 6 hours, 2.0 mL of solution was removed from the receptor compartment to recover and analyze.

2.2.3. Statistical Analysis

All experiments were conducted in triplicate, and the data were reported in terms of mean ± SD (standard deviation). A student's t-test (independent sample t-test) was utilized for statistical purposes to determine the total amount of polyphenol diffused through the *ex vivo* Franz static permeation cells between two comparisons**,** with a p-value of <0.05 for significant comparisons.

III. RESULTS

3.1. Method validation

The maximum wavelength of gallic acid standard solution in phosphate buffer pH 7.4 obtained from the absorbance scanning in the range of 450-900 nm is 765 nm (Figure 1c). Therefore, the maximum wavelength at 765 nm was determined as a quantification wavelength for further total polyphenols assays in this study.

Specificity: results of the spectrogram (Figure 1) indicated that at the absorption peak (765nm) of the cream sample, there was no absorption in the placebo sample. Thus, the process achieves specificity.

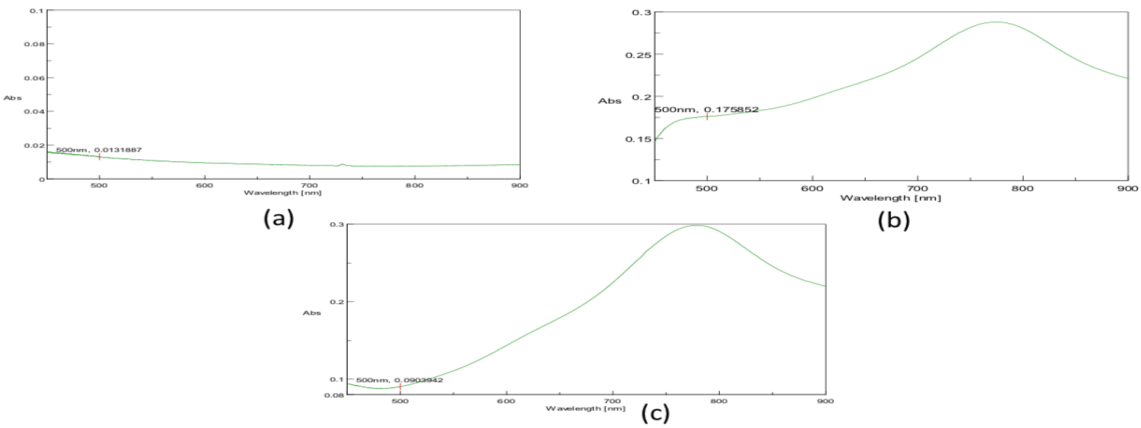

Figure 1. UV spectrum of samples(a) Placebo in 6 hours reacted with Folin-Ciocalteu reagents; (b) Cream in 6 hours reacted with Folin-Ciocalteu reagents; (c) Gallic acid standard solution reacted with Folin-Ciocalteu reagents.

System suitability test: the measured absorption was 0.098 ± 0.001 (RSD = 1.02%). The result showed a relative standard deviation (RSD) of absorption of the standard solution after

reacting with the Folin-Ciocalteu reagent of 2%. Thus, this analysis method is compatible with the UV-Vis spectroscopy system.Figure 1. UV spectrum of samples(a) Placebo in 6 hours reacted with Folin-Ciocalteu reagents; (b) Cream in 6 hours reacted with Folin-Ciocalteu reagents; (c) Gallic acid standard solution reacted with Folin-Ciocalteu reagents

Linearity: accurately taking 1, 2, 3, 4, and 5 ml of the original standard solution into 10 ml flasks, filled to the line with phosphate buffer pH 7.4, These, solutions were shaken thoroughly to achieve equivalent concentrations of 10, 20, 30, 40, and 50 µg/ml gallic acid. Accurately aspirated 1.0 ml of the above solutions into test tubes and reacted with Folin-Ciocalteu reagents. Measuring the absorption of these solutions at 765 nm. The correlation between concentration and absorption of standard gallic acid is shown in Table 1 and Figure 2. Absorption into gallic acid concentration was linearly correlated with s correlation coefficient (r) R2=0.998, and the linear regression equation was y = 0.0102x – 0.0056.

Table 1. Absorption of reference solution at concentrations (n = 3)

No.	Concentration (µg/ml)	Absorption (Mean ± SD)
1	10	0.098 ± 0.001
2	20	0.196 ± 0.003
3	30	0.302 ± 0.001
4	40	0.392 ± 0.001
5	50	0.508 ± 0.002

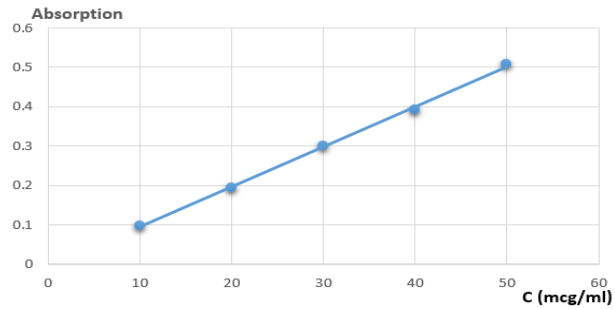

Figure 2. Plots of the calibration curve of the gallic acid standard

Precision: measuring the total amount of polyphenols that diffused through the membrane in 6 hours based on the intraday (n=6) and the interday (n=3). The results showed that the relative standard deviations (RSD) for both intraday and interday measurements were <2% (Table 2). Thus, the method is highly accurate and meets the analysis requirements.

Table 2. Precision results

Precision	The total amount of polyphenols that diffused through the membrane in 6 hours (µg)
Intraday (Mean ± SD)	300.54 ± 4.11* RSD= 1.37%
Interday (Mean ± SD)	295.62 ± 5.12* RSD= 1.73%

*Concentration was calculated from the linear regression equation.

Accuracy: based on the linear regression equation, calculate the recovery concentration and determine the percentage of recovery (%Recovery). The quantitative method exhibits high accuracy, with the recovery percentage ranging from 97.37% to 102.65%, averaging 100.12%, and showing an RSD of 1.57% (Table 3).

Table 3. The accuracy results (n = 9)

No.	Add concentration (µg/ml)	Recovery concentration (µg/ml)	%Recovery
1	16	15.58	97.37%
2	16	15.96	99.75%
3	16	16.14	100.86%
4	20	19.86	99.31%
5	20	20.16	100.78%
6	20	20.53	102.65%
7	24	23.75	98.98%
8	24	23.92	99.67%
9	24	24.41	101.72%
			Mean = 100.12%, RSD = 1.57%

3.2. Determination of the release of a nanostructured dosage form containing mango seed kernel extract by the *ex vivo* Franz static permeation cells experiment

Applying the validated procedure to quantify the total amount of polyphenols in creams containing MSKE diffused through the Franz static permeation cells experiment. The experimental results showed that the total amount of polyphenols in the nano-emulsion cream prepared by the self-nanoemulsifying drug delivery system that diffused through Franz static permeation cells reached 309.84 ± 2.67 µg. In contrast, the comparative cream (normal cream) reached 71.34 ± 0.95 µg (GAE) (Table 4). These results have contributed to evaluating the ability of drugs to release and penetrate through the skin in preparations containing MSKE. Moreover, they showed that the preparation based on the SNEDDS system containing MSKE helped to form a nano-emulsion's structure that has the ability of diffusion and active ingredient release more than normal cream, with a p-value of <0.05, this difference between the two preparations is statistically significant (α=5%).

Table 4. The results of the release of the MSKE-loaded nanostructured preparation

No.	The total amount of polyphenols that diffused through Franz static permeation cells (µg)	
	Nano cream in 6 hours	Normal cream in 6 hours
1	306.93	70.55
2	310.43	71.07
3	312.17	72.39
Mean ± SD	309.84 ± 2.67	71.34 ± 0.95

IV. DISCUSSION

4.1. Method validation

Many studies around the world conclude that polyphenols contained in mango seeds have many uses, such as antioxidant [1], antibacterial, anti-inflammatory, and anti-fungal [2], [6]. Ha Cao Thien et al. have indicated that the antibacterial and anti-inflammatory abilities of the mango seed kernel are stronger than those of the mango seed coat [7]. To apply this effect of mango seed kernel extract in the transdermal therapeutic system, we added MSKE to the formulation of nano-emulsion preparations to bring this active ingredient to the target, achieving the highest efficiency. The Franz static permeation cells

experiment helped to evaluate the active ingredient diffusion and release of the preparation. The quantification of polyphenols diffused through Franz cells helps evaluate the preparation's quality. Quantitative procedures can be performed using methods such as HPLC, Raman spectroscopy, and UV-Vis spectroscopy. In this study, we used the UV-Vis spectroscopy method to quantify the active ingredient because of its simplicity, ease of implementation, and popularity. Appraisal results indicated that the method had high accuracy (100.12%, RSD <2%). Because of these above factors, this method is going to be used for developing quality assessment factors for products used for transdermal therapy.

4.2. Determination of the release of a nanostructured dosage form containing mango seed kernel extract by the *ex vivo* Franz static permeation cells experiment

The Franz static permeation cells experiment evaluated the nano-emulsion cream's ability to diffuse through the skin and release the active ingredient. The results showed that the total amount of polyphenols that diffused through Franz static permeation cells of the nano-emulsion cream containing MSKE was about 4.3 times as many as the total amount of polyphenols in normal cream. With a p-value of <0.05, this difference between the two preparations is statistically significant ($\alpha = 5\%$).

According to the study of Harwansh et al. (2011) [12], it evaluated the comparison of the Franz static permeation cells between nano-emulsions that had been optimized with the normal gel formula. The results showed a significant increase in the osmotic parameters of the nanoemulsion. In addition, the study concluded that the difference in permeability of the active ingredient between the two dosage forms was due to the oil droplet size of the nano-emulsion being much smaller than that of conventional gels.

According to another study by Akram A et al. (2019) [13], they designed an experiment to investigate the ingredients in many different formulations and optimize the formula by diffusion through Franz static permeation cells. The results indicated that the droplet size of the emulsion significantly influences the permeation rate. The small oil droplet size helped the oil adhere to the biological skin layer and better controlled the active ingredient's ability to transport into the cell membrane. In addition, it suggested that the oil phase composition and the surfactant were two factors that contributed to the microemulsion structure. The solubility and stability of the active ingredient are greatly improved when incorporated into the microemulsion system.

Our research results agree with previous studies' opinion that emulsion droplet size affects the permeability and release of active substances. Therefore, we designed a comparative experiment between two formulations with the same formula but different preparation methods. From the results obtained, we consider that, in addition to the influence of the composition and concentration of the oil phase and the surfactant, the preparation method is also one factor affecting the oil droplet size of the emulsion. Thus, it affects the ability to diffuse and release drugs through the skin.

According to the findings, the self-nanoemulsifying drug delivery system (SNEDDS) is a promising drug delivery method for creating formulations for therapeutic use when applied topically. Its ability to form a nanometer oil droplet-sized emulsion not only improves diffusion and contributes to the deep penetration of the active ingredient into the skin layers but stabilizes the active ingredient and the emulsion.

V. CONCLUSION

The quantification of polyphenols diffused through Franz cells helps evaluate the preparation's quality. The procedure has been validated according to the International Conference on Harmonization (ICH) guidelines and could be applied to evaluate nanostructured dosage forms containing MSKE.

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