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Original Article

OPTIMIZATION OF DIMETHYL SULFOXIDE AS AN ENHANCER ON EX VIVO PENETRATION OF *SESEWANUA* (*CLERODENDRUM FRAGRANS* WILD) LEAF EXTRACTS EMULGEL

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ABSTRACT

Objective: The present study aims to investigate the effect of using DMSO as an enhancer on the ex vivo penetration of an emulgel from *sesewanua* leaf extracts.

Methods: The *sesewanua* emulgel was prepared into four formulas, SWE1-SWE4, with different DMSO concentrations: 0%, 3%, 5%, 7%, and compared with QCE, quercetin without DMSO. Further, the penetration test of the *sesewanua* leaf ethanol extract emulgel was performed by determining the rate and cumulative penetration of quercetin within the skin of the mice by employing the Franz diffusion cell approach.

Results: The SWE4 emulgel containing 7% of DMSO was the best formula that enhanced the penetration of the emulgel, with a cumulative penetration at 331.423 µg/cm^2, the penetration rate at 2.762 µg/cm^2/minute, and better dispersibility of 4.6 cm and the results of the one-way ANOVA suggested a significant influence ($p<0.05$). DMSO with a concentration of 7% of the *sesewanua* emulgel was proven to increase 2,4-fold the rate and cumulative amount penetration. DMSO can be considered as a penetration enhancer of natural compounds for anti-inflammatory treatment.

Conclusion: The use of natural ingredients as topical anti-inflammatory continues to be developed to avoid the first-pass effects. The used of DMSO in topical emulgel preparation become a simple way to enhance the penetrant of active inggredient.

Keywords: Quercetin, Antiinflammation, DMSO, Franz cell diffusion, Flavonoid

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INTRODUCTION

Recent advances in science and technology have significantly altered the way we discover, treat, and prevent specific diseases in all aspects of human life [1]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most commonly prescribed medications in the world, but it has a number of side effects, primarily on the gastrointestinal tract, such as ulcers, bleeding, perforation, and obstruction [2]. It is a great need to develop secure and valid drugs for long-term use from natural sources for the establishment of new treatments for clinical use [3].

Sesewanua (*Clerodendrum fragrans* Wild) been used for the material in *bakera* spa (a traditional spa of people in North Sulawesi) and as an antipyretic [4–8]. It contains 13.47% of quercetin, a subclass of flavonoid compound, which has anti-inflammatory activities [9] and has moderate anti-cancer potential [10]. The above finding has underpinned the urge to formulate ethanol extracts of *sesewanua* leaves in the form of topical emulgel.

Emulgel is defined as a multi-drug delivery system comprising emulsion and gel. Several properties of emulgel are thixotropic, greaseless, easily spreadable, moisturizing, long shelf-life, transparent, considerably stuck longer, and easy to wash [11]. Emulgel has been successful in delivering active ingredients to their target, such as clove oil [12], diclofenac sodium [13], methotrexate [14], itraconazole [15], Mimosa pudica polyphenols [16], benzyl benzoate [17], chlorphenesin [18], Mentha essential oil [19], acyclovir [20], tramadol [21], etc.

The outermost layer of skin (stratum corneum) consists of corneocytes enclosed by the lipid bilayers. This is the main barrier to delivering the drug to the skin. When a drug is poorly water-soluble, it can be limited to the superficial stratum corneum (SC) following topical administration [14, 22–25]. For this reason, a specific enhancer is essential in formulating *sesewanua* ethanol extract emulgel.

Penetration enhancers are substances that aid in the absorption of penetrants through the skin by temporarily thinning the skin's impermeability. Penetrant enhancer has no therapeutic effects, but it helps drug transportation to the skin [26]. Dimethyl Sulfoxide (DMSO) is the most common enhancer that has been proven effective in enhancing drug penetration that capable of changing the conformation of stratum corneum keratin from α–helical conformation to β–sheet conformation and increasing drug fluxes through its interaction with lipid on the stratum corneum and change the protein structure, thereby resulting in changes in the fluxes' partition coefficient [27, 28].

In this research, *Sesewanua* has been used historically as an anti-inflammatory was made in a topical dosage form. Emulgel preparations have been selected using DMSO as a penetrant enhancer. The objective is to investigate the effect of various DMSO concentrations on the ex vivo *sesewanua* extract emulgel and the physical stability of the emulgel formula with the best penetration qualities in terms of cumulative penetration and penetration rate.

MATERIALS AND METHODS

Materials

The materials encompassed *sesewanua* leaf simplicia collected from Tilango District, Gorontalo Regency in January 2020, SWR/J mice aged 6 to 8 w weighing at 20 to 30 g, DMSO (CV. Chem-Mix P. A Grade), Veet® (fur remover), ethanol 96%, quercetin (CV. Chem-Mix P. A Grade), Carboxy Methyl Cellulosa (CMC) Sodium (CV. Chem-Mix P. A Grade), Nipagin (CV. Chem-Mix P. A Grade), Nipasol (CV. Chem-Mix P. A Grade), Poly Ethylene Glicol (PEG) 400 (CV. Chem-Mix P. A Grade), metabisulfite sodium (CV. Chem-Mix P. A Grade), tween 80 (CV. Chem-Mix P. A Grade), span 80 (CV. Chem-Mix P. A Grade), and aquadest (Dept. Pharmacy Chemistry Laboratory Polkesgo, Pharmaceutical Grade).

Ethical approval

All research procedures have received ethical approval from the Health Polytechnic Ethics Commission of the Ministry of Health of Gorontalo with No. LB.01.01/KEPK/52/2020

Preparation of *sesewanua* extract emulgel

Table 1: Emulgel formulation

Materials	SWE1	SWE2	SWE3	SWE4	QCE
Oil phase emulsion					
Sesewanua extract	5%	5%	5%	5%	-
Quercetin	-	-	-	-	5%
Olive Oil	10%	10%	10%	10%	10%
Poly Ethylene Glycol 400	25%	25%	25%	25%	25%
Cetyl Alkohol	2%	2%	2%	2%	2%
Span 80	2.028%	2.028%	2.028%	2.028%	2.028%
Nipasol	0.02%	0.02%	0.02%	0.02%	0.02%
Water phase emulsion					
DMSO	0%	3%	5%	7%	0%
Tween 80	0.972%	0.972%	0.972%	0.972%	0.972%
Aquadest	14.34%	14.34%	14.34%	14.34%	14.34%
Metabisulfit Sodium	0.1%	0.1%	0.1%	0.1%	0.1%
Gels					
CMC Sodium	1%	1%	1%	1%	1%
Nipagin	0.18%	0.18%	0.18%	0.18%	0.18%
Poly Ethylene Glycol 400	25%	25%	25%	25%	25%
Aquadest	14.34%	14.34%	14.34%	14.34%	14.34%

SWE1: *Sesewanua* Emulgel with 0% DMSO, SWE2: *Sesewanua* Emulgel with 3% DMSO, SWE3: *Sesewanua* Emulgel with 5% DMSO, SWE4: *Sesewanua* Emulgel with 7% DMSO, QCE: Quercetin Emulgel

The concentration of DMSO made refers to previous research [29–31]. The materials for the emulgel formula were grouped into three parts, namely oil-phase emulsion, water-phase emulsion, and gel base. All of them were firstly mixed according to the respective part. Cetyl alcohol melted and mixing with olive oil, PEG 400, span 80, nipasol, and extract at 60 °C. Meanwhile, the water phase (tween 80, Metabisulfite sodium, water, and DMSO) mixed was done by mixing the materials in a beaker using a stirrer. In this phase, DMSO was added according to the concentrations that had been determined. Following the process was the preparation of gel components by heating the water, adding and mixing CMC Sodium until well-hydrated. Moreover, the nipagin and PEG 400 were added to form a gel base. After the formation of the gel base, all three components from the oil phase, water phase, and gel were mixed using a stirrer set at the speed of 2000 RPM, resulting in four emulgels with the DMSO concentration SWE1-SWE4 and emulgels with quercetin QCE.

Analyzing the penetration of *sesewanua* extract emulgel

Quercetin standard curve

The absorption of quercetin solutions at the concentration of 20 ppm, 40 ppm, 60 ppm, 80 ppm, 120 ppm, 140 ppm, 160 ppm, and 180 ppm was measured at the wavelength (λ) of 415 nm using a UV-Vis spectrophotometer. After that, we the drawing of the calibration curve and its regression equation.

Recovery test

The absorption of the level of quercetin, each at 120 PPM, was identified at the wavelength of 415 nm using a UV-Vis spectrophotometry. The content of the quercetin was measured using the equation of the quercetin standard curve.

Preparing the mice skin

Mice were purchased from Rizky Agency in Gorontalo Province, Indonesia. Mice were housed in cages, in controlled temperature (20-22 °C) and room under a 12 h day/night cycle. The animals were used in the study after acclimatizing for a week to allow free access to water and food. The mice were euthanatized before carefully removing their fur. The abdomen skin was cut to the size of the diameter 0.03 cm and membrane area 0.002826 cm2 and then soaked in phosphate buffer pH 7.4 for 30 min.

Diffusion test

The penetration test was performed utilizing the Franz diffusion cell [32–35]. The receptor compartment was filled with phosphate buffer 7.4 pH, with a temperature kept at 37±1 °C, and stirred at a constant speed. Further, the mice's skin, with the stratum corneum part facing upward, was placed between the donor and receptor compartment. The 1g sample was then applied to the skin. After every 0, 30, 60, 90, and 120 min, 3 ml of sample was removed from the receptor compartment using a syringe and then replaced with the 7.4 pH phosphate buffer in the same volume. The absorbance of each sample was measured on the wavelength of 415 nm using a UV-Vis spectrophotometer.

Preparation evaluation

Homogeneity test

SWE1-SWE4 and QCE were weighed as much as 0.1 grams and smeared evenly on the slide, no coarse grains should be seen.

Spreadability test

SWE1-SWE4 and QCE were weighed as much as 0.5 grams, placed on a transparent glass lined with graph paper covered with transparent glass, and given a load of 150 g. The diameter of the distribution was measured 4 times.

Stability test

SWE1-SWE4 and QCE were placed in a glass container, placed alternately at 4 °C and 40 °C for 24 h for 7 cycles. Emulgel stability was observed.

Data analysis

The correlation between each *sesewanua* extract emulgel and the cumulative penetration was analyzed using the one-way ANOVA with SPSS 16.

RESULTS

Recovery test

The accuracy test using a percentage recovery method was aimed at determining the accuracy of the method, i.e. whether or not the method, in determining the level, was capable of depicting the actual level of the substance of the quercetin within the emulgel. Provided in table 2 are the values of the percentage of recovery method.

Table 2: Quarcetine standard absorbance data

Concentration (µg/ml)	Absorbance (A)	Deviation standard	Equation of line
60	0.240	0.0006	
80	0.346	0.0012	r = 0,9878
100	0.415	0.0010	a = 0,0196
120	0,470	0.0010	b = 0,0043
140	0.565	0.0006	y = 0,0043x-0,0196
160	0.657	0.0010	
180	0.786	0.0015	

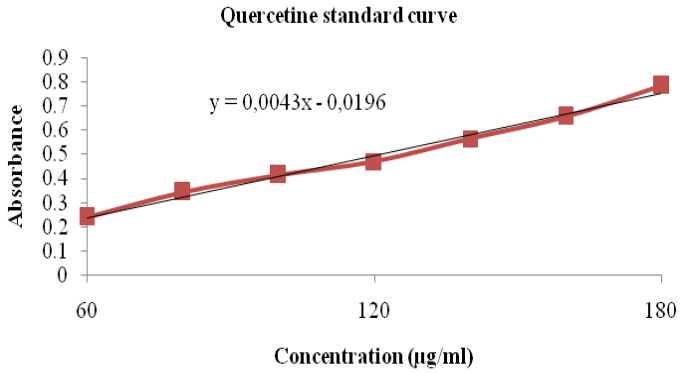

Fig. 1: Graph of quercetin's standard curve, data represents mean±SD (n=3)

Table 3: Percentage value of recovery

Teoritic value (µg/ml)	Replication		A	µg/ml	% Recovery	Mean of % recovery
120	1	1	0.490	118.51	98.7583	99.5990±0.995
		2	0.493	119.21	99.3411	
		3	0.500	120.84	100.6976	
	2	1	0.494	119.44	99.5348	99.2118±0.296
		2	0.492	118.97	99.1472	
		3	0.491	118.74	98.9534	
	3	1	0.490	118.51	98.7583	98.9529±0.512
		2	0.489	118.27	98.5658	
		3	0.494	119.44	99.5348	
	4	1	0.487	117.81	98.1782	98.7204±0.524
		2	0.496	119.91	99.2248	
		3	0.490	118.51	98.7583	
	5	1	0.490	118.51	98.7583	99.0175±0.623
		2	0.495	119.67	99.7286	
		3	0.489	118.27	98.5658	

n=3

Diffusion test

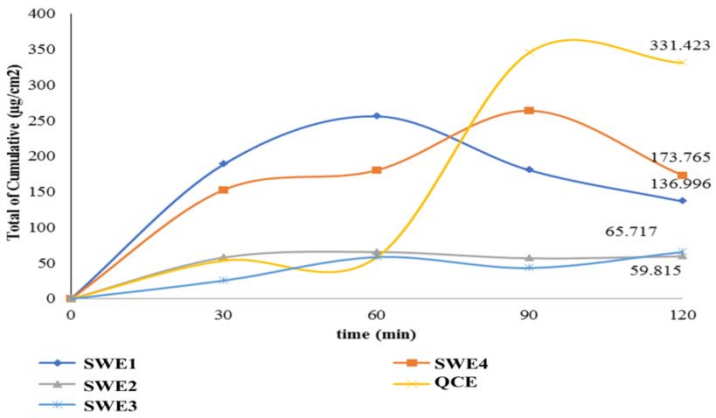

Fig. 2: Cumulative penetration amount of emulgel preparation *Sesewanua* extract optimization DMSO; Data represents mean, (n=3)

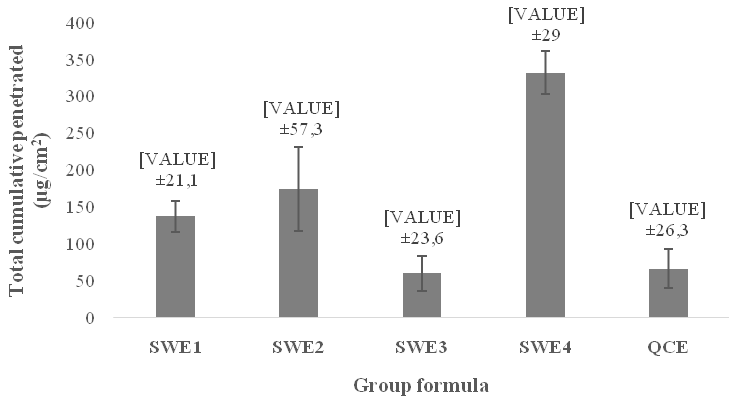

Fig. 3: Total cumulative amount of penetrated *sesewanua* extract emulgel; Data represents mean±SD, (n=3)

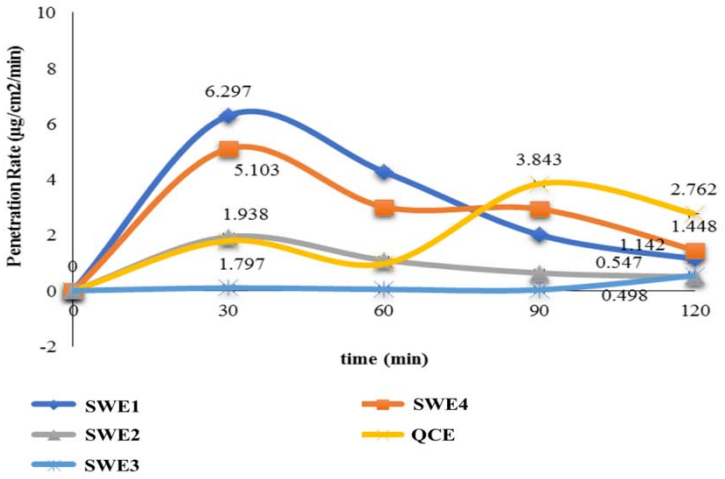

Fig. 4: Flux penetration rate of emulgel, Data represents mean, (n=3)

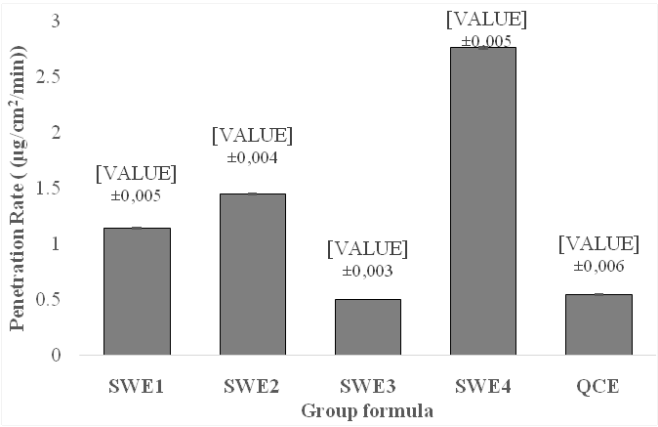

Fig. 5: Penetration rate of *sesewanua* extract emulgel, Data represents mean±SD, (n=3)

Table 4: One way ANOVA statistical test cumulative penetration amount and penetration rate emulgel preparation 120 min DMSO optimization

Criteria	Formula				
	SWE1	SWE2	SWE3	SWE4	QCE
Cumulative Amount Penetrated (µg/cm2)	136,99±21,1-*	173,76±57,3-**	59,81±23,6+	331,42±29-***	65,71±26,3-
Penetration Rate (µg/cm2/min)	1,142±0,005-*	1,448±0,004-**	0,498±0,003+	2,76±0,005-***	0,547±0,006-

Description: -***Very Significantly Different Increase Against QCE,-**Different Moderate Significant Increase Against QCE,-*Significantly Different Increase Against QCE,+No Significant Difference in Its Decrease Against QCE, Data represents mean±SD (n=3)

Evaluation of emulgel

Homogeneity and stability

Table 5: Homogeneity and stability/freezthaw test of *sesewanua* emulgel

Test	SWE1	SWE2	SWE3	SWE4	QCE
Homogeneity	Homogenous	Homogenous	Homogenous	Homogenous	Homogenous
Stability/Freezthaw	No phase separation occurs	No phase separation occurs	No phase separation occurs	No phase separation occurs	No phase separation occurs

n=3

Dispersibility test

Table 6 below shows the dispersibility of *sesewanua* extract emulgel.

Table 6: Dispersibility of *sesewanua* extract emulgel

Formula	Dispersibility (cm)
SWE1	4.025±0.05
SWE2	3.700±0.18
SWE3	3.625±0.05
SWE4	4.600±0.00
QCE	4.15±0.01

Data represents mean±SD, (n=3)

DISCUSSION

DMSO is one of the sulfoxide chemical penetrant enhancers. DMSO was chosen because it is cheap, easy to obtain, and has proven successful in increasing the penetration of the active ingredient [27, 28]. Chemical penetration enhancers are done in one of three ways: by chemical interaction with intracellular proteins, by improving the distribution of drugs in the stratum corneum, or by destroying highly ordered lipids. stratum corneum [36]. There were variations of the DMSO, 3%, 5%, and 7%, because DMSO showed a better action at that concentration [29–31], listed in table 1.

Based on quercetin standard absorbance data (table 2), the recovery test (table 3) and graph of quercetin's standard curve (fig. 1) result of five replications of the test reported that the percentage recovery met the prerequisite of 120 ppm formulated and recovered quercetin using the raw curve drawn for at least 99%. Such a result is consistent with the study by [37] suggesting that the compound level examined using a percentage recovery at>1% has the range of percentage recovery from 97 to 103%. The notion signifies that the method is accurate in determining quercetin's content in the penetration of *sesewanua* extract emulgel.

The diffusion test parameters in the present work consisted of the cumulative compound penetration (Q) and fluxes (J). The amount of cumulative penetration for SWE1, SWE2, SWE3, and SWE4 are displayed in fig. 2 and 3. According to the curve, we can see the concentration of penetrant enhancer DMSO in the *sesewanua* extract emulgel formula, compared to SWE1, contributes to an increase in the quercetin of the emulgel passed through the membrane. Jatav *et al.*, [38] show that such an increase in DMSO can lead to a rise in the cumulative penetration amount of active, hydrophilic substances. DMSO is capable of speeding up drug penetration, either hydrophilic or lipophilic, by its mechanisms of extracting the fat layer through the transcellular pathway. The process results in the replacement of the bonding of water through the intracellular pathway. Consequently, the quercetin possessing polar properties can pass through the skin layer, precisely the thick stratum corneum, through two different pathways [39, 40].

A diffusion test result showing a contrasting result is seen in SWE3, where SWE3 experiencing a decline in its cumulative penetration amount is due to the consistency of the *sesewanua* extract. In formula SWE3, the extract had a different thickness than those used in other formulas, i.e., SWE1, SWE2, and SWE4. Anita Sukmawati and Suprapto [41] opine that increased viscosities may result in a drop in transdermal drugs' total cumulative chemical penetration. This condition is the cause of the drop in the rate and cumulative penetration in SWE3 after 120 min. In the present study, the most significant increase in the cumulative of *sesewanua* extract emulgel penetration is seen in SWE4 with DMSO 7%. Simply put, the higher the DMSO concentration is, the greater the cumulative amount of emulgel penetrated. DMSO has successfully acted as a penetrant enhancer with a concentration of 7%. It was proven to increase 2,4-fold the rate and cumulative amount penetration of the ethanolic extract of *Sesewanua* leaves in the emulgel preparation. This is similar to the research of Damayanti in that DMSO increased the theophylline transport from 17,9 µg to 139,1 µg [31]. But this finding is not similar to Jantharaprapap, that found DMSO did not improve the permeability of meloxicam emulgel [30] and Bhutkar found that found Aloe vera gives a better permeability than DMSO in lidocaine emulgel [29].

Other results indicated that the cumulative amount of sesewanua emulgel penetration in SWE1 without DMSO was greater than QCE, with the pure quercetin material functioning as the active substance material (and later serving as the target in determining the cumulative penetration). This situation occurs because the quercetin contained in the extract had been mixed with other compounds with oil properties, thus causing a longer quercetin contact in the extract; this is in line with the physical and chemical properties of skin that consists of several fat layers [27]. Such a notion corresponds to the results seen in Ramadon's study [42] that compare the cumulative of polar and non-polar carriers penetration. As a result, the non-polar carriers have greater cumulative penetration than the positive polar.

Emulsion-based systems containing *sesewanua* extract do not adhere well to the skin due to their liquid state and have poor, short-term effects. Therefore, to solve the problem and improve retention, an emulsion was formulated into an emulsion gel formulation, same as curcumin [43]. The results of the one-way ANOVA revealed that the cumulative amount in minute 120 of the emulgel formula SWE1, SWE2, and SWE4 increased significantly compared to QCE. In other words, the emulgel and the other emulgel with enhancer DMSO can further improve the total cumulative penetration of the *sesewanua* emulgel extract. Different results were shown in the result of the SWE3 test, where it had positive values, contrasting with QCE with its negative values (table 4). Such a result implies that the decline in the cumulative amount in SWE3 is insignificant statistically.

A rise in the cumulative amount penetrated is proportional to the flux value (penetration rate), thereby increasing the flux value of the emulgel with DMSO and allowing the emulgel to pass through the skin layer. The increase in the flux is displayed in fig. 4 and 5.

In the evaluation of emulgel, the dispersibility indicates that all emulgels have met the requirement of good dispersibility, as displayed in table 6. Such a result resonates with the one found by [44], claiming that emulgel with good dispersibility is the one with dispersibility from 5 to 7 cm. Referring to the data, we can see that SWE4 emulgel with DMSO at 7% has the widest dispersion, thus increasing the cumulative total of the penetration of *sesewanua* extract to the skin.

CONCLUSION

The use of natural ingredients as topical anti-inflammatory continues to be developed to avoid the effects of the fist pass. The

use of DMSO in topical emulgel preparation has become a simple way to enhance the penetrant of natural active ingredients.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS

The authors declared no conflict of interest in this study.

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