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Optimization of Emulgel Formulation of The Defatted Ethyl Acetate Extract of *Mychorriza arbuscule* Induced Ginger Rhizomes (*Zingiber officinale* Rosc.)

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Abstract

The research aim was to develop an emulgel formulation of the defatted ethyl acetate extract of *Mychorriza arbuscule* fungi induced ginger rhizomes (MIG) as a topical anti-inflammation dosage form by using optimization technique. A complete randomized design with two factors and two levels was used. The concentration of gelling base (hydroxyl propyl methyl cellulose, HPMC, at levels 0.75 and 2%) and the concentration of the extract (at levels 8 and 16%) were used as independent variables while the viscosity and the percentage of edema inhibition of the dosage form were taken as the response variables. Four (2^2) formulations were prepared: F1, Fa, Fb, and Fab. As a marker, 6-gingerol was used which concentration was determined by TLC-densitometry with silica gel GF_{254} as a stationary phase and *n*-hexane : ethyl acetate (13:7) as a mobile phase. Data were analyzed using multi regression analysis, the optimum formula was obtained from the intercept of the superimposed contour plot. The TLC densitometry system showed that the marker and the extract had a retention factor of 0.3. The 6-gingerol concentration of F1 was 0.31%; Fa 0.38%; Fb 0.82%; Fab 0.81%; and the defatted extract 3.67%. The viscosity of each formula was 10 (F1), 200 (Fa). 5 (Fb) and 150 (Fab) dPa.s, respectively. The percentage of edema inhibition of the dosage form was 31.6% (F1), 31.3% (Fa), 45.5% (Fb) and 43.7% (Fab), respectively. The optimum formula of the emulgel is at HPMC concentration of 2% and the extract 12% which has a viscosity of 127.129 dPa.s and edema inhibition 37.8%.

Keywords: ginger extract, *Mychorriza arbuscule* fungi, optimization technique, emulgel, oedema inhibition

1. Introduction

Ginger has been used traditionally in Indonesia to treat swollen skin, nausea, vomiting and as a stimulant [1]. The literature review shows that ginger extract has several pharmacological activities such as antiemetic, antimicrobial, anti-inflammation, analgesic, antioxidant, anticarcinogenic, and immunomodulatory [2-5]. The major compounds in the extract are 6-gingerol, 6-shogaol, paradol, methyl gingerol, gingerdiol, dehidrogingerdion, and gingerdion [2]. Gingerol and shogaol are known to exhibit anti-inflammatory activity by suppression the biosynthesis of inflammatory mediators [5, 6]. A previous study shows that MIG has higher shogaol and gingerol contents; the major compound of its defatted ethyl acetate extract is 6-gingerol [7, 8].
The bioactive components of ginger are very potential to be developed as an anti-inflammatory agent [5].
A pharmacokinetic study shows low bioavailability of 6-gingerol (1.79%) and 6-shogaol (0.32%) in human after the ingestion of ginger extract [9]. Poor bioavailability of phenolic compounds from plant extract is associated with low concentration of the free phenolic forms in the blood due to either low absorption or

extensive metabolism in the intestinal epithelium and the liver [9, 10]. A transdermal dosage form of ginger extract may overcome this problem by providing sufficient quantities of the free gingerol and shogaol in the inflamed area. The objective of this study is to develop an emulgel formulation of the defatted ethyl acetate extract of MIG by using optimization technique. As a control, an emulgel containing diclofenac sodium (Voltaren emulgel Novartis) marketed in Indonesia is used.

2. Materials and Methods

2.1 Materials

Fresh MIG was supplied by PT Andalas Agrotama Padang (Indonesia); 6-gingerol was purchased from Sigma Aldrich®; *n*-hexane, ethyl acetate, sulphuric acid, and acetic acid were purchased from Merck®; HPMC K100M was from Wuhan Senwayer Century Chemical® (China); methanol, p-anisaldehyde, sodium hydroxide, potassium dihydrogen phosphate, propylene glycol, liquid paraffin, tween 80, span 80, methyl paraben, propyl paraben, and carrageenan were purchased from Bratachem® (Indonesia). All reagents and solvents were of pharmaceutical grade.

2.2 Experimental animals

Male albino rats (*Rattus norvegicus*) weighing 140-160 g were used as experimental animals. The animals were kept under observation before the experiment for acclimatization. The chosen animals were housed in plastic good aerated cages at the normal atmospheric temperature (24 ± 2°C) and normal 12 hours light/dark cycle. They were given access to water and standard diet of known composition. All animal procedures were undertaken with the approval of Institutional Animal Ethics Committee of the Faculty of Medicine, Andalas University.

2.3 Methods

2.3.1 Preparation of the defatted ethyl acetate extract of MIG

The MIG was washed, dried, weighed and finely cut. The rhizomes were defatted with n-hexane and then extracted with ethyl acetate for 5 days at room temperature; the extraction was done in triplicates. The collected ethyl acetate extract was concentrated and dried *in vacuo* with a *rotary evaporator* (Buchi®) [8].

2.3.2 Experimental design

Four emulgel formulations were prepared according to a 2^2 factorial design employing the factors and levels described in Tables 1, while the formula composition is shown in Table 2.

Table 1. Factors and Levels for the 2^2 Factorial Design

No	Factor	Level	
		Low	High
1	HPMC K100M	0.75%	2.5 %
2	The defatted ethyl acetate extract of MIG	8%	16%

Table 2. Composition of the defatted ethyl acetate extract of MIG Emulgel Formulations

Formula	F1 (%)	Fa (%)	Fb (%)	Fab (%)
HPMC K100M	0.75	2.5	0.75	2.5
The defatted ethyl acetate extract of MIG	8	8	16	16
Propylene glycol	10	10	10	10
Liquid paraffin	5	5	5	5
Tween 80	2	2	2	2
Span 80	1	1	1	1
Methylparaben	0.1	0.1	0.1	0.1
Propylparaben	0.1	0.1	0.1	0.1
Purified water to	100	100	100	100

2.3.3 Preparation of the defatted ethyl acetate extract of MIG emulgel formulations

The gelling base was prepared by dispersing HPMC K100M in heated purified water (80^{o}C) then left cooled. The oil phase of the emulsion was prepared by dissolving span 80 in liquid paraffin while the aqueous phase by dissolving Tween 80 in purified water; methyl and propyl parabens were dissolved in propylene glycol which then mixed with the aqueous phase. Both oil and aqueous phases were separately heated to 70^{o}C. The oily phase was added to the aqueous phase with gentle stirring to room temperature to obtain an emulsion. The emulsion was then mixed with the gelling base with gentle stirring to obtain an emulgel. The emulgel base was added to the MIG extract and stirred vigorously to ensure homogeneity.

2.3.4 Examinations of the emulgels

Formed emulgels were examined for physical characteristics, including colour, homogeneity, consistency, and phase separation. The pH values of 1% aqueous solutions of the emulgels were measured by a pH meter (Voltcraft®, Germany). The viscosity was determined at 25^{o}C by using a cone and plate viscometer (Rion VT-04F). Physical stability was observed during the storage at 25^{o}C for 3 months.

2.3.5 Quantitative analysis of 6-gingerol in emulgels

The concentration of 6-gingerol in the extract and emulgel formulations was determined by TLC-densitometry method (Camag®) by using TLC plates 60 F_{254} as the stationary phase and n-hexane : ethyl acetate (13:7) as the mobile phase. The spot was sprayed with p-anisaldehyde, heated for 10 minutes and scanned with TLC scanner at wavelength 525 nm (Camag®). The methods were validated over the concentration of 62.5, 125, 250 and 500µg/mL.

2.3.6 The assay of anti-inflammatory activity

The anti-inflammatory effect was examined by using the method of carrageenan-induced paw oedema in rats as described by Winter et al, 1962 [11]. The rats were divided into 6 groups, each consisted of 3 rats. Group I, II, III, and IV were the test groups given emulgel F1, Fa, Fb, and Fab, respectively. Group V was a control group received only the vehicle (gel base) and group VI was a reference group, received Voltaren® emulgel. Inflammation was induced by injecting 0.2 mL of 0.5% carrageenan suspension to the plantar surface of the right hind paw of each rat. The test groups of rats were treated by applying ± 0.2 g of the emulgels topically to the right hind foot of the rats after carrageenan injection. The volume of the inflamed paw was determined using a mercury plethysmograph (U. Basile, Italy) right after carrageenan injection (initial volume) and 6 hours after application of the emulgels (volume at time t). The percentage of oedema and that of oedema inhibition in treated rats versus control were calculated using Equations 1 and 2.

$$\% \text{ edema} = \frac{Vt - V0}{V0} \times 100\% \qquad \text{(Equation 1)}$$

$$\% \text{ edema inhibition} = \frac{a-b}{a} \times 100\% \quad \text{(Equation 2)}$$

Where Vt = the paw size at time t, Vo = the initial paw volume, a = the average paw volume of control group and b = the average paw volume of treated groups.

2.3.7 Satistical analysis

The effect of formulations expressed as (the average oedema ± standard of deviation) were analysed by one-way ANOVA ($\alpha = 0.05$) to determine the significant differences between means followed by Duncan Multiple Range Test at 5% significance level. The viscosity of emulgels and oedema inhibition were analysed using multi regression analysis by *Design Expert* and an optimum formula was selected from the superimposed contour plot of the viscosity and oedema inhibition responses.

3. Resuts and Discussion

A previous study reports that the defatted plant extract yields a higher total phenolic content [12]. In this study, a pre-extraction procedure with hexane was conducted to remove fatty oil, oleoresins, volatile oils and other lipophilic contents in MIG which might cause irritation for topical application and to obtain a higher gingerol and shogaol contents in the extract [5]. The higher phenolic content of the defatted extract might be due to an increase in the polarity after the removal of fatty substances [12].

The MIG extract concentration and the viscosity of dosage forms are two main factors influencing the anti-inflammatory activity of the emulgel formulation. Their levels were determined previously by measuring oedema inhibition of the extract at concentrations range of 2 – 16 % and determining the viscosity of the emulgel base at HPMC concentration of 0.75 – 2.5%. The low and high levels of the extract were 8 and 16% which obtained oedema inhibition of 32.90 and 49.65% respectively, the latter was not significantly different than that of the reference ($p>0.05$). The HPMC levels of 0.75 (low) and 2.5% (high) gave a consistency ranged from 20 – 250 dPa.s which were in the range of the viscosity of gel dosage forms [13].

The emulgels obtained were pale brown to brown opaque semi-solids with a specific ginger odour. The physicochemical characteristics of the emulgels are summarized in Table 3. There was a slight difference in the colour depending on the concentration of MIG extract in the emulgels. The pH value of the emulgel base was 6.5, and that of the MIG formulations were 3.8 (F1), 2.6 (Fa), 3.7 (Fb) and 2.6 (Fab), respectively. These values were much lower than the skin pH which may increase the risk of irritation upon application to the skin. Skin patch test on human volunteers showed that F1 and Fa (8% MIG extract) did not cause irritation but Fb and Fab (16% MIG extract) did. Therefore, the formula containing the highest level of extract might not be an optimum formulation. A gel base containing buffer component might be required to maintain the emulgels pH in the range suitable for a topical use.

Table 3: Physicochemical properties of the ethyl acetate extract of MIG emulgels

Parameters	**Formulations**				
	F1	**Fa**	**Fb**	**Fab**	**Emulgel base**
pH value	3.8	2.6	3.7	2.6	6.5
6-gingerol content (%)	0.31	0.38	0.82	0.81	-
Physical stability (25^{o}C, 3 mos.)	Stable	Stable	Stable	Stable	Stable
Viscosity (dPaS)	10	200	5	150	20-250

Data analysis of viscosity response (Y_1) to the concentration of HPMC (X_1) and the MIG extract (X_2) by using factorial design 2^2 obtains the following equation (equation 3) [14]:

$$Y_1 = 91.25 + 83.75\ X_1 - 13.75\ X_2 - 11.25\ X_1X_2. \quad \text{(Equation 3)}$$

This indicates that HPMC has a positive influence on the viscosity by +83.75. The MIG extract as an active ingredient had a negative effect on the viscosity by -13.75. An interaction between the two factors shows a

negative influence on the viscosity by -11.25. The contour plot showing the viscosity response of the gelling agent and the MIG extract levels is given in Figure 1.

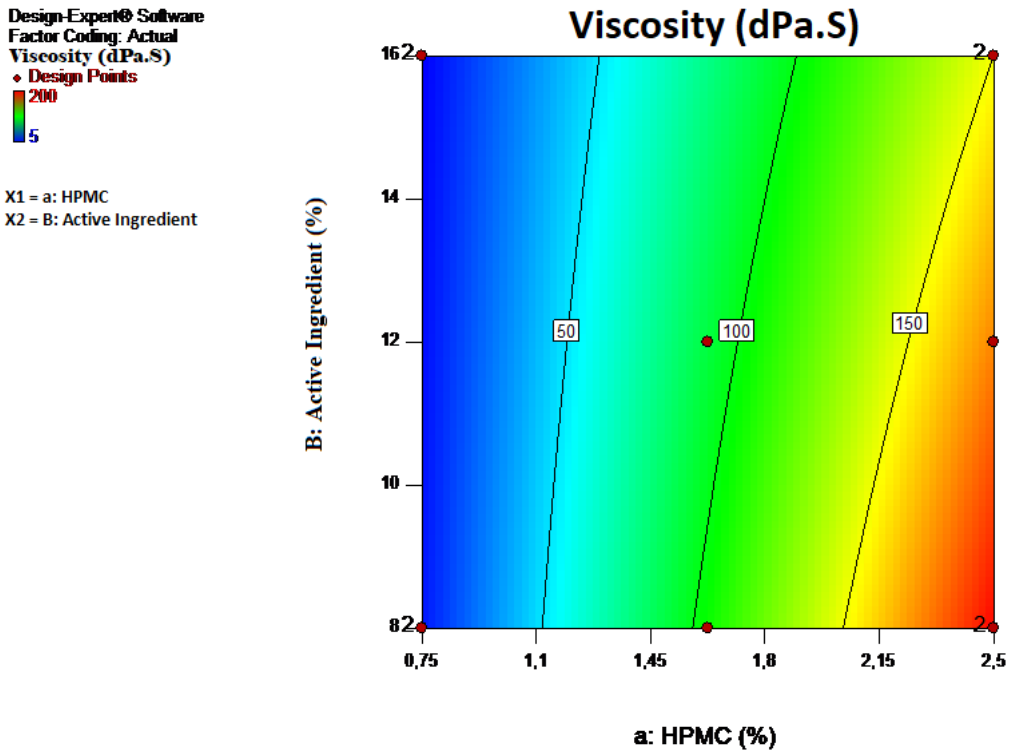

Figure 1: Contour plot for the viscosity response of the MIG extract emulgels as a function of the active ingredient and gelling base levels

Quantitative analysis of 6-gingerol was carried out at the maximum wavelength of 525 nm and results in a spot with the retention factor of 0.3. Validation of the method shows a linear regression between concentration of 6-gingerol versus absorbance (y = 1509.65 + 23.124 x; r = 0.995). The 6-gingerol content of the MIG extract is 3.67% and that of the emulgels are in Table 3.

The effect of the prepared emulgels on the reduction of rat's paw oedema is presented in Table 4. The equation for the inflammatory inhibition response (Y_2) based on factorial design 2^2 [14] is as follow:

$$Y_2 = 38.025 - 0.525\ X_1 + 6.5\ X_2 - 0.375\ X_1X_2 \quad \text{(equation 4)}$$

Equation 4 shows a negative influence of HPMC on the inflammatory inhibition for -0.525 which means that an increase in the gelling base concentration reduces the anti-inflammatory effect of the emulgels. This might be due to a decrease in drug liberation as the viscosity increased [15]. On the other hand, the active ingredient has a positive effect on the anti-inflammatory activity of the emulgels by +6.5. An interaction between the HPMC effect and the anti-inflammatory effect is negative (-0.375). Figure 2 shows the relationship between the HPMC level and the concentration of the MIG extract on the anti-inflammatory activity of the emulgels.

Table 4: Effect of MIG emulgels on carrageenan-induced paw oedema in rats

Groups	Treatment	n	Average edema ± SD (%)	Edema inhibition (%)
Control	Control	3	103.33 ± 5.77^{d}	0.0
Test	F1	3	70.67 ± 3.52^{c}	31.6
	Fa	3	70.90 ± 1.56^{c}	31.3
	Fb	3	56.33 ± 3.18^{a}	45.5
	Fab	3	58.17 ± 3.18^{ab}	43.7
Reference	Voltaren®	3	53.00 ± 2.60^{a}	48.7

The effect of the prepared emulgels on the reduction of rat's paw oedema is presented in Table 4. Analysis of the average oedema using one-way ANOVA shows a significant difference between formula. Furthermore, analysis by Duncan Multiple Range test suggests that the average oedema of Fb group was not significantly different than that of the reference group. The average oedema of the Fab group shows no significant difference than that of Fb, but it is different significantly than that of the reference group. These results indicate that emulgel formulations of the defatted ethyl acetate extract of MIG decrease inflammatory significantly ($P<0.05$). Fb shows a good percentage of oedema inhibition, but it might not be the best formulation. The edema inhibition of emulgels Fb and Fab (containing ± 32 mg of 6-gingerol) is comparable with that of the ethanol extract of ginger at dose 50 mg/kg BW intraperitoneally which is 43% after 1-hour administration [11].

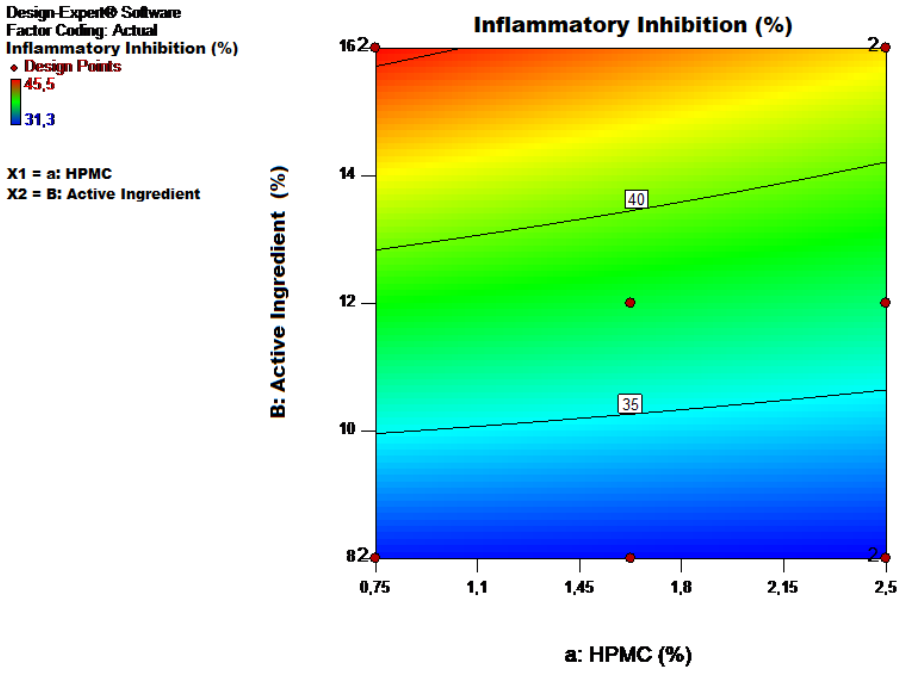

Figure 2: Contour plot for the anti-inflammatory inhibition of the MIG extract emulgels as a function of the active ingredient and gelling base levels

An optimum formulation of the MIG extract emulgel is predicted from the superimposed contour plot of the viscosity and inflammatory inhibition responses. Figure 3 indicates the optimum HPMC level of 2% and the MIG extract concentration of 12% results in an emulgel with a viscosity of 127.129 dPaS and an inflammatory

inhibition of 37.8%. A preparation of the optimum formula and determination of the amount of 6-gingerol released are yet to be studied.

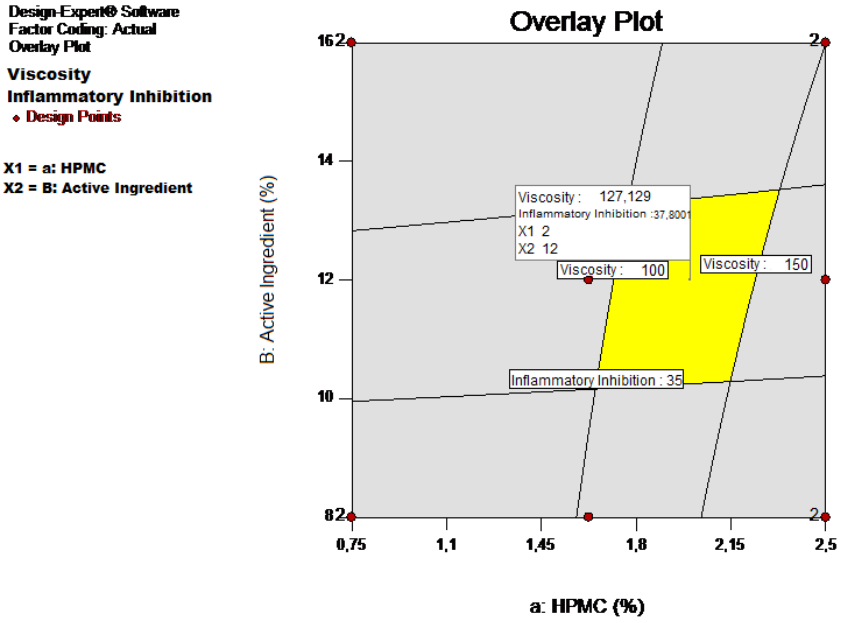

Figure 3: A superimposed contour plot of the optimum emulgel formulation of the MIG extract

4. Conclusion

The optimum emulgel formulation of the MIG fraction consists of the HPMC level 2% and the MIG fraction level 12% which results in a viscosity of 127.129 dPaS and the inflammatory inhibition of 37.8%.

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