FORMULATION AND EVALUATION OF ANTI-INFLAMMATORY EMULGEL OF *Brucea javanica* (L.) Merr SEED EXTRACTS

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Abstract

Anti-inflammatory drugs are needed to overcome excessive inflammatory reactions that can interfere with activities. The existence of side effects in the use of synthetic anti-inflammatory drugs causes the search for natural drugs with high therapeutic effects and low side effects to continue. Seeds of Makassar (*Brucea javanica* (L.) Merr) plant are known to have anti-inflammatory activity. Topical anti-inflammatory administration is currently being developed because it can minimize side effects compared to oral. This study aimed to formulate 96% ethanol extract of Makasar fruit seeds in the form of an emulgel and to determine the physical properties and anti-inflammatory activity of the emulgel in vitro. Makasar fruit seeds were extracted by sonication method using 96% ethanol solvent (3 x 35 minutes). The extract was then formulated into an emulgel preparation with an extract concentration of 1%. Emulgel was then tested for its physical properties and anti-inflammatory activity in vitro using protein denaturation inhibition method. The results showed that the physical properties of emulgel base (F0), extract emulgel (F1), and Na-diclofenac emulgel (F2) met the requirements for homogeneity, pH, adhesion, spreadability, and viscosity tests. F0, F1, and F2 had inhibition percentages of 3.74±1.58%,; 23.07±0.72%; and 33.49±0.29, respectively. According to one-way ANOVA statistical test, the three tested groups had significant differences.

***Keywords**: anti-inflammatory, emulgel, Makassar seed, Brucea javanica (L.) Merr*

Introduction

Inflammation is body's natural response to injury, irritation, or infection characterized by redness, heat, swelling, pain, and loss of tissue function (Abbas and Litchman, 2009). However, if the inflammatory reaction is excessive and can interfere our activity, anti-inflammatory drugs are needed to control inflammation below the harmful level. Based on Riskesdas (2013) data, 20.516 (19.8%) households store anti-inflammatory drugs throughout Indonesia and West Nusa Tenggara Province ranks seventh in anti-inflammatory drug storage (Soleha et al., 2018). Anti-inflammatory drugs on the market are synthetic drugs that have side effects on the skin such as itching and rashes on all skin types (Katzung, 2018). So we need anti-inflammatory drugs from natural ingredients that have a high therapeutic effect with low side effects. One of natural ingredients as an anti-inflammatory is Makassar plant.

Makassar (*Brucea javanica* (L.) Merr) is a plant that grows wild in Indonesia. The fruit part of the Makassar plant is often used as a traditional medicine to prevent dysentery, diarrhea, and malaria (Dalimartha, 2006). According to previous studies, ethyl acetate fraction of Makasar plant seeds in vitro and in vivo can inhibit the production of inflammatory mediators and induce the production of anti-

inflammatory cytokines, namely IL-10 (Yang *et al.*, 2018). Makasar fruit seed oil can inhibit NF-kB signal transduction in DSS-induced rats so that it can have anti-inflammatory activity (Huang *et al.*, 2017). Research by Amini et al. (2020) reported that a cream containing ethanolic extract of Makasar fruit seeds could provide protection against skin inflammation in mice due to UV rays.

The provision of topical anti-inflammatory drugs is currently being developed because it can minimize the side effects that occur compared to oral treatment. This is because topical preparations have a rate of drug reaching the systemic circulation 5-17 times lower than orally (Francio et al., 2017; Kienzler et al, 2010), so that topical preparations are not expected to be absorbed systemically. In this research, a topical anti-inflammatory preparation of Makasar fruit seed extract in the form of an emulgel will be developed. Emulgel is an emulsion mixed with a gelling agent. Emulgel preparations are generally more stable than other topical dosage forms, such as ointments that go rancid easily due to their high oil content, creams that break easily, and hygroscopic powder forms (Raj and Balakrishman, 2016). In addition, emulgel preparations can have high acceptability because they are easy to wash, spread easily on the skin, moisturize, and do not leave stains (Mahajan and Basarkar, 2019).

The anti-inflammatory emulgel will then be tested for its effectiveness in vitro using the protein denaturation inhibition method. So the purpose of this study was to determine the physical properties and anti-inflammatory activity of the obtained emulgel.

Research Methods

Tools and materials

Sonicator (Elmasonic), Spectrophotometer UV / Vis Analytic Jena Specord 200 plus, Viscometer (Brookfield Ametek LV DV2T).

Bovine Serum Albumin (Sigma-Aldrich), Makasar fruit, Carbopol 940 (Brataco), Ethanol 96% technical (Brataco), Menthol (Merck), Methyl paraben (Merck), Diclofenac sodium pa (Smart-Lab), Solid NaCl (salt) kitchen), liquid paraffin (Merck), Propyl paraben (Merck), Propylene glycol (DOW USP Grade), Span 80 (Merck), TEA (Merck), Tween 80 (Merck), and Tris Base (Merck).

Sample collection and processing

Samples of ripe Makasar fruit with purple-black color were collected in the Sesaot area, West Lombok Regency, West Nusa Tenggara. Sample identification was carried out at the Advanced Biology Laboratory, Faculty of Mathematics and Natural Sciences, University of Mataram with letter number 08/UN18.7/LBL/2021. The sample is then separated from the fruit skin and dried. Makasar fruit seeds are then ground into simplicia powder.

Extraction

Makassar fruit seed powder as much as 100 g was extracted by sonication method (3x35 minutes; 25℃) using 96% ethanol with a ratio of Makassar fruit seed powder: 96% ethanol was 2:5. Subsequently, a filtration process was carried out to obtain the extract and then concentrated with a rotary evaporator at a temperature of 40℃ (Dearny, 2016).

Preparation of anti-inflammatory emulgel

Emulgel preparation refers to the formula used by Burki et al., (2020) with the modifications listed in Table 1. Emulgel preparation is carried out in 2 stages. The first stage is the manufacture of an oil-in-water emulsion and a gel base. The second step is mixing the emulsion and gel base. The oil phase of the emulsion was prepared by mixing Span 80, propylene glycol, methyl paraben, propyl paraben, and menthol which had been dissolved in liquid paraffin at 70℃. The aqueous phase was made by mixing distilled water and tween 80 at 70℃. Then

the oil phase is slowly mixed into the water phase. The mixture is stirred until it forms an emulsion consistency. Furthermore, the gel base was made by dispersing Carbopol 940 into hot distilled water and stirring until homogeneous. After that, the emulsion was added slowly to the gel base while stirring until the mixture was homogeneous. Then *Brucea javanica* extract was added to the emulgel base and homogenized. The last step, added TEA until the pH is in the range of 6–6.5 (Mahajan and Basarkar, 2019; Sekar and Ismail, 2018).

Table 1. Formulation of anti-inflammatory emulgel

Materials	Composition (%)			Function
	F0	F1	F2	
Brucea javanica seed extract	0	1	0	Active agent
Na dichlofenac	0	0	1	Active agent
Carbopol 940	1,5	1,5	1,5	Gelling agent
Paraffin liquid	4	4	4	Thickener
Tween 80	2,5	2,5	2,5	Emulgator
Span 80	1	1	1	Emulgator
Propilen glikol	5	5	5	Co-solvent
Metil paraben	0,18	0,18	0,18	Preservative
Propil paraben	0,2	0,2	0,2	Preservative
Menthol	0,1	0,1	0,1	Penetration booster
Aquadest	Ad 100	Ad 100	Ad 100	Solvent
TEA	20 tetes	20 tetes	20 tetes	pH adjusting

Physical properties testing of anti-inflammatory emulgel

1) Homogeneity Test

The emulgel that has been made is applied to the glass of the object evenly and thinly. The preparation conditions are stated to be homogeneous i.e. there should be no coarse grains (Riski et al., 2016).

2) pH test

The pH indicator was put into 1 gram emulgel which had been dissolved with 10 mL aquadest. Furthermore, the pH indicator is compared with the standard contained in the container. The pH requirements for topical preparations are pH 4.5-6.5 (Trenggono and Fatma, 2007.

3) Spreadability Test

A total of 0.5 grams of emulgel is placed between 2 slides and then given a load of 100 grams. The diameter of the preparation was measured after 1 min (Azkiya et al., 2017). Good dispersion is indicated by a diameter of 3-5 cm (Garg et al., 2002).

4) Adhesion Test

A total of 0.5 grams of emulgel is placed between 2 slides. Put a weight of 1 kg for 5 minutes. Furthermore, the two slides were separated by pulling the slide above using a load of 80 grams and then recording the time until the two slides separated. The requirement for good adhesion time is not less than 4 seconds and the longer the time the emulgel preparations are separated, the better the emulgel produced (Hastuty et al., 2018).

5) Viscosity Test

Emulgel is placed on a Brookfield viscometer until the spindle is immersed to the limit mark. The spindle used is spindle 63 with a speed of 0.3; 0.6; and 0.9 rpm for 5 seconds at room temperature (Ratnapuri et al., 2019).

In vitro anti-inflammatory activity assay

The anti-inflammatory activity assay was determined by the protein denaturation inhibition method (Farida et al., 2018; Sekar and Ismail, 2018):

1) Preparation of Test Solution

a. Preparation of emulgel solution F0, F1, and F2 10,000 ppm

A total of 100 mg of emulgel was weighed and 0.2% BSA solution was added until the volume became 10 mL then vortexed.

b. Preparation of emulgel test solution F0, F1, and F2

A total of 1 mL of 10,000 ppm emulgel solution was pipetted into a 10 mL volumetric flask and 0.2% BSA solution was added to the mark and then vortexed. 5 mL of the solution was pipetted as the emulgel test solution.

2) Control preparation

a. Negative control preparation

A total of 500 L of distilled water was added with 0.2% BSA solution to a volume of 5 mL.

3) In vitro anti-inflammatory activity test

Each solution (test solution and negative control) was incubated for 30 minutes at ±25℃ then heated for 5 minutes at ±72℃ with a water bath, then cooled for 25 minutes at room temperature. Then the absorbance was measured with a UV-Visible spectrophotometer at a wavelength of 660 nm. Calculations of the percentage of protein denaturation inhibition were showed in equation 1.

$$\%\ inhibition = \frac{A\ negative\ control - A\ tested\ solution}{A\ negative\ control} \times 100\% \quad (1)$$

If the resulting % inhibition > 20% is considered to have anti-inflammatory activity.

Data analysis

Anti-inflammatory activity result was analyzed using One Way ANOVA test of SPSS Software.

Results and Discussion

Ethanol extract of Makassar fruit seed

The crude extract obtained is yellow with characteristic aroma of Makassar fruit seeds in the form of a semisolid paste. The percentage of yield obtained in this study using sonication method was 10.04%. These results showed an increase compared to previous studies that used the same solvent but different extraction methods, namely soxhletation and maceration with percentages of 3.62% and 7.4%, respectively (Amini et al., 2020; Risnadewi et al., 2019). The results obtained are in accordance with previous studies which said that extraction with sonication method increased the yield percentage by 6% to 35% with polyphenol compounds as targets (Garcia et al., 2010).

Physical properties of anti-inflammatory emulgel

The resulting emulgel is in the form of a semisolid. The color of Emulgel F1 which contains Makasar fruit seed extract is pale yellow in contrast to the other two white formulas which can be seen in Figure 1. The aroma of the three formulas is menthol-scented because there is the addition of menthol as a penetration enhancer and fragrance. The homogeneity test showed that all emulgel preparations were homogeneous, characterized by the absence of coarse particles when the preparation was smeared on a slide.

The pH of the emulsion preparation obtained is in the range of 4.5-6.5 so that it can be said to be in accordance with the pH of the skin. The pH value of a preparation plays an important role because the pH of the preparation is too alkaline can cause dry and rough skin while if the pH of the preparation is too acidic can cause skin irritation (Trenggono and Fatma, 2007).

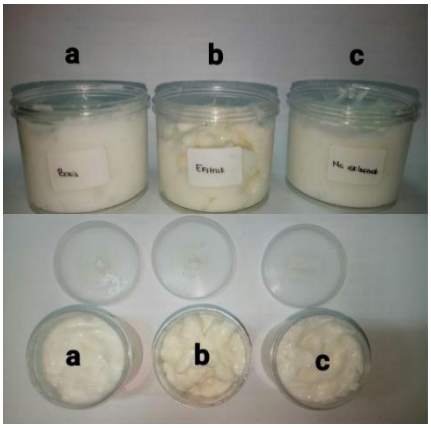

Figure 1. Results of emulgel preparation, (a) Emulgel F0, (b) Emulgel F1, (c) emulgel F2

The results of spreadability test of the emulgel prepared were in accordance with the requirements of a good topical preparation because it was in the 3-5 cm range. Preparations that have a spread between 3-5 cm are semi-rigid (semi-stiff) preparations (Hastuty et al., 2018).

The results of the adhesion test of the emulgel preparation were obtained for more than 4 seconds so that it met the requirements of a good topical preparation (Garg et al., 2002). The longer the preparation is able to stick to the skin, the more active substances can diffuse into the skin, and the maximum effect is obtained (Hastuty et al., 2018).

Based on the results of the viscosity test listed in Table 2. it can be seen that the viscosity of the F1 and F2 emulgels containing the active substance was higher than that of the F0 emulgel base. The presence of the active substance in the preparation results in the absorption of the solvent in the preparation so that it can increase the viscosity value (Voigt, 1994). This is in line with the results of the dispersion and adhesion tests carried out. Increasing the viscosity of the preparation will increase the retention time at the site of application of the preparation, but will reduce the dispersibility of the preparation (Garg et al., 2002). This can be seen in Table 2, where F1 and F2 which contain the active substance have longer adhesion and decreased dispersion compared to F0.

Table 2. Physical properties of anti-inflammatory emulgel

Parameter	Formula		
	F0	**F1**	**F2**
Organoleptic	Form: Semisolid Color: White Scent: Menthol	Form: Semisolid Color: Dark yellow Scent: Menthol	Form: Semisolid Color: White Scent: Menthol
Homogeneity	Homogenous	Homogenous	Homogenous
pH	6.36	6.23	6.42
Spreadability (cm)	3.93	3.75	3.50
Adhesion (second)	7.5	10	8
Viskosity (cps)	0.3 rpm: 76,400 0.6 rpm: 64,400 0.9 rpm: 58,130	0.3 rpm: 77,200 0.6 rpm: 64,600 0.9 rpm: 59,470	0.3 rpm: 95,200 0.6 rpm: 84,600 0.9 rpm: 59,070

Anti-inflammatory activity of emulgel

Based on the results of the anti-inflammatory activity of emulgel preparations listed in Table 3. it can be seen that the percentage of inhibition of F0 which is the basis of emulgel is 3.74% which does not have anti-inflammatory activity because the percentage of inhibition produced is less than 20%. The percentage inhibition of the Makasar fruit seed extract emulgel (F1) and diclofenac sodium emulgel were 23.07% and 33.48%, respectively, so they had anti-inflammatory activity.

Although the F0 emulgel base preparation is considered to have no anti-inflammatory activity, the F0 emulgel preparation has a low percent inhibition of protein denaturation. The substance that is thought to play a role in producing the percent

inhibition in F0 is menthol. This is supported by research conducted by Yeggoni et.al (2017) that menthol can bind to albumin by creating hydrophobic interactions so that it can stabilize albumin and prevent protein denaturation.

Table 3. Emulgel anti-inflammatory test results

Emulgel	% Inhibition ± SD *
F0	$3,74 \pm 1,58^{a}$
F1	$23,07 \pm 0,72^{b}$
F2	$33,49 \pm 0,29^{c}$

Note: The difference in letters a, b, c shows a statistically significant difference

Ethanol extract of Makasar fruit seeds is known to contain secondary metabolites including phenolics, flavonoids, and terpenoids (Risnadewi, et al., 2019; Muliasari et al., 2019). Phenolics and flavonoids have aromatic rings and hydroxyl groups that can interact with amino acid residues in proteins by forming reversible non-covalent complexes such as hydrogen bonds and hydrophobic interactions. The interaction that occurs can stabilize the protein so that it can prevent protein denaturation when induced by heat (Zinellu et al., 2015). Terpenoids can interact with albumin protein to form hydrophobic interactions that can stabilize the tertiary structure of the protein so that it can inhibit protein denaturation (Basu et al., 2018; Berti et al., 2020).

The anti-inflammatory activity of emulgel preparations F0, F1, and F2 were statistically analyzed to see significant differences between the three preparations. The data obtained were normally distributed (sig > 0.05) and homogeneous (sig > 0.05) so that the data were tested parametrically with one-way ANOVA test and posthoc follow-up test. From the statistical test results, there were significant differences between the three test groups, so it can be said that the Makasar fruit seed extract emulgel had anti-inflammatory activity but its activity was lower than that of diclofenac sodium emulgel as a positive control.

Conclusion

Based on the research that has been done, it can be concluded that:

1. The physical properties of the F0, F1, and F2 emulgel preparations met the requirements for homogeneity, pH, adhesion, dispersibility, and viscosity tests.
2. Emulgel base (F0) has a percent inhibition value of 3.74±1.58% so it does not show anti-inflammatory activity. Meanwhile, the F1 and F2 formulations showed anti-inflammatory activity with the percentage inhibition values of 23.07±0.72% and 33.49±0.29%, respectively.

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