

Extraction, Identification, and Gel Formulation of Mangiferin from Mango (*Mangifera indica* L.) Leaves Extract

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Abstract: Mango (*Mangifera indica* L.) leaves contain flavonoid which has anti-inflammatory and antioxidants effects that are beneficial on healing diabetic ulcers. The extract made in gel formulation because it was easy to dry, forming the washable film layer that provides a cool sensation on the skin. Gel components influence the stability of formula. To ensure gel quality, safety, and benefits, physical stability test was needed to fulfill the specifications and stability during storage. This study aimed to extract and identify the mangiferin as an active compound in mango leaves and to formulate *Mangifera indica* leaves extract gel as a wound healer. Extraction of *Mangifera indica* leaves used soxhlet method with ethanol 70% and determined using TLC-densitometry method. The optimum formula of the gel was determined by variations of CMC-Na concentration as gel base, and the compliance of the gel characteristics. The analysis of characteristics included spreadability test, homogeneity test, adhesivity test, and pH test. The result of extraction was determined by TLC-Densitometry as 330,52 mg/gram of viscous extract. The formula with 5% CMC-Na gel base complied with the required characteristics and was the optimum formula, which stability analysis did not show any changes in pH, colour, consistency, adhesivity and spreadability during storage.

1 INTRODUCTION

Diabetes Militus (DM) is a disease characterized by the occurrence of hyperglycemia and carbohydrate, fat, protein metabolism disorders, as a result of disorder or insulin deficiency by β Langerhans cells of the pancreas gland, or caused by the lack of responsiveness of body cells to insulin. One of the complications of DM occurring is diabetic ulcers or diabetic lesions, which are skin lesions caused by high blood glucose levels resulting in vascular resuscitation and further vascular neuropathy (Fatimah, 2015, Sarwono, 2009). Based on the data of the Indonesian Ministry of Health (2014), the prevalence of diabetic ulcer wounds in Indonesia reaches 54%. This disease is often found in developing countries; Indonesia was ranked seventh with a number of 10 million diabetic patients in 2015 (IDF, 2015).

Using antioxidants as a treatment on diabetic wounds is the most effective approach related to wound healing of diabetes. One of the types of plants that is potential as a wound healer of diabetes is mango (*Mangifera indica*). Mango leaves contain active compound of mangiferin that acts as

antioxidant and capable of lowering blood sugar levels in diabetes therapy. Moreover, extracts of mangiferin has a potential for the healing of wounds in diabetes (Fithriyani *et al.*, 2014, Khandare, 2016). Mangiferin total from ethanol extracts of *Mangifera indica* is 102 mg/gram of mangiferin compounds. This plant grows a lot in the community and only the fruit are commonly consumed, not yet optimally utilized in increasing the value of use (Fithriyani *et al.*, 2014).

Utilization in the community is seen as not optimal yet because it has not been processed into useful drugs; therefore, it needs formulations to form products, i.e. preparations in the form of a gel. The gel is a semi-solid material consisting of a suspension made of inorganic particles that are small or large organic molecules including penetration by a liquid. The gel preparation is chosen because it is easy to dry out, forming a layer of film that is easily washable and provides a cool sensation on the skin (Ansel, 2008, Panjaitan *et al.*, 2012).

Formulation of gel in this study used CMC-Na as the gel agent. CMC-Na is a polymer derivate cellulose that quickly expands when supplied with hot water and neutral, clear crystal and has a strong

Table 1: Formula of mango leaves extract (MLE) gel.

Component	F1	F2	F3
Mango leaf extract (MLE)	4%	4%	4%
CMC-Na	5%	5%	5%
Gliserin	5%	5%	5%
Propilenglikol	2,5 %	2,5 %	2,5 %
Nipagin	0,25%	0,25%	0,25%
Aquadest ad	50 grams	50 grams	50 grams

bond between molecules (Aponno *et al.*, 2014). In this study, the variation of gel base CMC-Na was analyzed to find the optimum formula. It is specified based on gel characterized, i.e. spreading test, adhesion test, homogeneity, consistency, and pH. CMC-Na has advantages over Carbopol; pH of CMC Na is higher than carbopol, the spreading power of CMC-Na is greater than carbopol gel, and also the extraction into CMC-Na does not affect the spreadability, while the gel of carbopol decreased power of scatterplot (Maulina and Suhigartini, 2015).

2 MATERIALS AND METHOD

2.1 Materials

Mango leaves (*Mangifera indica*), 70% Ethanol, ethyl acetate, glacial acetic acid, formic acid, methanol, CMC Na, Tragakan, propilenglycol, Carbopol, glycerin, Methyl paraben, and Aquadest.

2.2 Methods

2.2.1 Preparation of Ethanolic Extract of Mango Leaves

Mango leaves from the area of Sleman, Yogyakarta

were picked and dried under the blazing sun and previously washed beforehand; they were covered with black cloth in the process to avoid direct contact with sun rays. To obtain even drying, leaves were then moved into oven for 2-3 hours at a temperature of 500-600 °C, before being ground to make powder leaves and sieved with sieve mesh no. 40.

About 625 grams of leaves powder was transferred into a soxhlet tool and then added with 1500 mL ethanol as solvent. Extraction was performed for 48 hours (Sachin *et al.*, 2014). The extract obtained was collected and concentrated on evaporator to evaporate in a waterbath until viscous extract was obtained.

$$Yield = \frac{\text{Weight of viscous extract}}{\text{Weight of powder}} \times 100\% \quad (1)$$

2.2.2 Preparations and Determination of the Optimum Formula of Mango Leaf Extract Gel

Mango leaves extract (MLE) gel was formed from MLE and excipients; the composition of the gel formulated by a trial-error method in the preformulation step. According to Adnan (2016), MLE gel with CMC-Na as gel base has the composition as in Table 1.

These formulas compared and evaluated to choose the optimum one. The evaluation includes organoleptic test and homogeneity, consistency, pH, adhesive test and spreading test. The results obtained are indicated in Table 2.

2.2.3 Organoleptic Test and Homogeneity

The organoleptic test was performed by directly observing the colour and smell. Homogeneity test carried out by applying the gel on a piece of glass (Maulina and Sugihartini, 2015).

Table 2: Physical evaluation of MLE gel.

Evaluation	Concentration		
	F1	F2	F3
Organoleptic Colour	Very thick brownish green	Thick brownish green	brownish green
Smell	Very strong	Strong	Less
Homogeneity	Homogenous	Homogenous	Homogenous
pH	5	5	5
Spreadibility	8,117 cm	6,4 cm	4,13 cm
Adhesivity	3,4 second	5,4 second	56,7 second
Consistency	Stable	Stable	Stable

2.2.4 pH Testing

pH test was done using pH paper universal that was dipped into the diluted samples. Colour change on the pH paper was compared with the universal standard (Maulina and Sugihartini, 2015).

2.2.5 Spreadability Test

About 0.5 grams of gel was placed on the glass and another round glass was placed on top of it, and left for 1 minute. After that, 150 grams of weight was added and left for 1 minute. The diameter was then measured (Astuti *et al.*, 2010).

2.2.6 Adhesivity Test

About 0.25 grams of sample was placed between two glass objects; given a load of 1 kg for 5 seconds, which was then lifted and changed with 80 gram of weight. The time of the release of the gel determined as adhesivity of gel (Miranti, 2009).

2.2.7 Consistency Test

Consistency test was done using centrifugation test. Gel samples were centrifuged at 3000 rpm for 5 minutes, and then observed of physical changes (Djajadisastra *et al.*, 2009).

2.2.8 Data Analysis

The data obtained was processed in statistics using SPSS 24 software. Normality test (Shapiro-Wilk) and homogeneity test (Levene) were performed on the data. To see the relationship between the treatment groups, the variations were analyzed in one way (ANOVA) if the data was distributed normally and homogenously. If the data was not normal, Gaussian and then Kruskal-Wallis analysis were done.

3 RESULTS AND DISCUSSION

Mashed dried mango leaves formed the smooth brownish-green colour with a distinctive odour. Ten pounds of dry mango leaves produced 0.8 kilograms of powder. The water content in the dried mango leaves was $8\% \pm 0.5$, which met the standard IE ($<10\%$). If water content $>10\%$, it can cause the onset of enzymatic processes and cause damage due to microbes, which can change the chemical content of it.

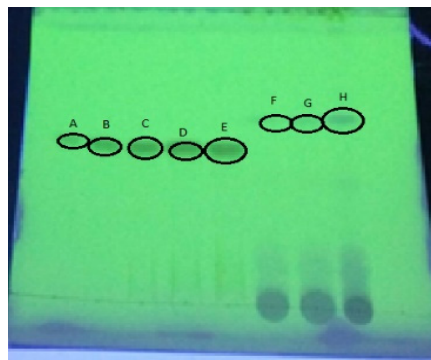


Figure 1: TLC plate of MLE in UV 245.

Mango leaf extract obtained by the soxhlet method, using ethanol 70% as the solvent, was left for 24 hours and the ethanol 70% was vaporized on the waterbath to obtain extracts. The extract obtained had a bitter taste and a distinctive smell. Yield and extract obtained was 12.5%.

The identification of the xanthan group carried out was qualitative and quantitative. Qualitative analysis was done by FeCl_3 5% and HCl 1M. The colour that was changing to brownish blackish colour proved that the extract contained compound of the xanthan (mangiferin), positive. Quantitative analysis was done with the Densitometry method to obtain TLC mangiferin levels using the stationary phase of GF 254 and mobile Phase Chloroform: Methanol: comparison with Formiat Acid 90:10:3. Test results are presented in Figure 1.

TLC densitometry analysis aimed to determine levels of mangiferin and quite economical since it used relatively little motion phase and relatively short time and the measurement of levels of samples simultaneously. From the analysis, the obtained concentration of mangiferin was 330.52 mg/gram of extract.

In the formulation gel, MLE was the active ingredient. CMC-Na served as gel base, while propilenglycol and Glycerin as humectant that increased the stability of formula. CMC-Na was used as the gel base in the formula because it has good stability in acid and alkaline condition (pH 2-10). Propilenglikol in gel formula was used as a humectant to maintain the stability while keeping the moisture content in the material properties of the gel. This material can be stable at pH 3-6. The most influential factors in the physical quality of the gel preparations are the base and humectant. The base gel will form a structure which is an important factor in the gel formulation. Humectant serves to keep the

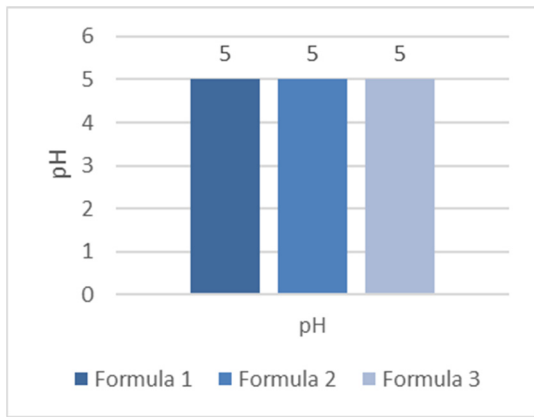


Figure 2: pH diagram with variations of the base formula of CMC Na 2,5%% (FI), 5% (FII), dan 7,5% (FIII).

gel formulation by absorbing moisture and reduce evaporation of water from the formulation. Methyl paraben was used as a preservative because gel has a high moisture content that can lead to the occurrence of microbial contamination.

The physical properties test of the gel are required to guarantee the quality of gel formulation. Test results from the third formula shown in the Table 2. pH of topical formulation requirement is 5-7 because the normal pH skin IE 4.5 – 6.5 (Martin,1983). Test results are presented in Figure 2.

The gel in this research has pH of 5, which is appropriate for skin pH. If the pH is not a match for the skin, it will irritate the skin and reduce comfort when applied to the skin.

Spreading power test done to learn the ability of the gel to spread on the skin. The terms of the power of spread in the topical formulation are 5-7 cm

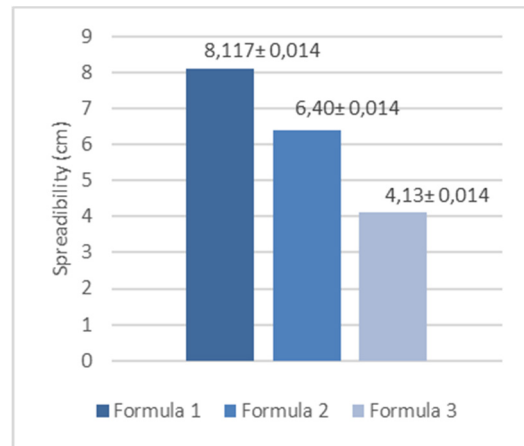


Figure 3: Spreadability diagram with variations of the base formula of CMC Na 2,5 %(FI), 5% (FII), dan 7,5% (FIII).

(Ulaen 2012, *et al.*). Increasing spreadability of topical formulation will simplify in the application. Figure 3 presented the test results.

In this research, the spreading power that can qualify in specified. The higher concentration of gel base will cause the smaller area of spreading. It is because in the high concentration of base gel, it contains the lower of water. It makes increasingly viscous gel consistency so that the spreading power decline. Statistic analysis for the third formula, there are F1, FII and FIII, normal distributed data and homogeneity of data that show a different result. It is indicated by one way ANOVA statistics with value $p = 0,200$ or $> 5\%$ of the value that is assigned. So that the third formula meets the requirements.

To see the ability of the gel in attaching to the skin, adhesivity test was performed. Power requirement for the material to latch onto the skin is no shorter than 4 seconds (Ulaen, *et al.*, 2012). Figure 4 presented the test results.

The formulas that met the requirements were formula II and III. It is because the lower consistency made shorter time in contact with skin. In addition, the increase of concentration caused a thick consistency that also was increasingly adhesive. Statistic analysis of normal distributed and homogeneity by ANOVA retrieved result of the sig (5%; $p < p = 0.00$) and the data is not homogeneous (5%; $p < p = 0.04$), so then the Kruskal Wallis test produced a significant value of ($p 5\%; < p = 0,026$). It means the difference of concentration affected adhesivity of the gel. Consistency test showed that segregation in gel formulation did not occur. It indicated that the gel formula were stable in storage.

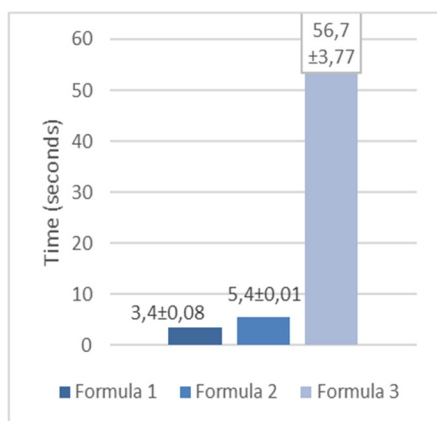


Figure 4: Adhesivity diagram with variations of the base formula of CMC Na 2,5 %(FI), 5% (FII), 7,5% (FIII).

4 CONCLUSION

The results showed the MLE gel formulation were green-brown with a distinctive smell, homogeneous, pH 5, spreading 4.13-8.117 cm, latching for 3.4-56.7 seconds, and stable in storage. The most optimal formula is gel with a concentration of CMC Na 5%.

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