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Research Paper

Formulation Of Ionic-Gelation Submicron Particles Loading Extract Papaya Leaves (*Carica Papaya L.*) With Lactic Acid Isolates

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Abstract

A study regarding ionic-gelation submicron particle of papaya leaves (*Carica papaya L.*) extract with lactic acid of weeds potential for antidiarrhea has been conducted. Preparation of papaya leaves ethanolic extract and lactic acid isolate into particles was done by ionic gelation method. This study aimed to determine: the major compound of extract, the total quercertine of extract, the percent value of encapsulation efficiency of the optimum formula which was varied by $(CaOH)_2$ of the three formulas, and physical properties of particles. Formula 1 was using $(CaOH)_2$ of 12.5 gram; formula 2 $(CaOH)_2$ of 17,5 gram; formula 3 $(CaOH)_2$ of 22.5 gram. The results showed formula 1 as the optimum formula that has the highest %EE. The average %EE values of F1; F2; F3 respectively were 80,82%; 80,41%; 80,31%. The results of particle characterization using the PSA in the optimum formula produced particle size values with an average of 253.6 nm, PDI of 0.218, and zeta potential +8 mV respectively.

Keywords

Ionic-gelation, submicron-particles, Carica papaya L., lactic acid isolates

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1. INTRODUCTION

Swamp forages has been a major wild weeds in swamp area (Li et al., 2015). Swamp forages can be utilized in the process of production which results in the formation of organic acid as a metabolite by lactic acid bacteria. These bacteria convert carbohydrates into lactic acid. Isolate of lactic acid is a probiotic which has the functions as a growth promoter for normal flora in the human intestine (Moorthy et al., 2015).

Forages of swamp are also found in Indralaya (south sumatra province) Indonesia. Kumpai weed (*Hymenache acutigluma*) is one of the. Lactic acid isolate can be used in antidiarrheal therapy because it has the function of feeding the normal flora in the intestine to compete with the Escherichia coli as the pathogenic bacteria that causes diarrhea.

Alternative natural ingredients that can be combined with lactic acid isolates are papaya leaves (*Carica papaya L.*) which have antibacterial activity to inhibit the growth of pathogenic bacteria (Baskaran et al., 2012). Diarrhea is defecation with liquid or semi-liquid stool and more than usual fecal water content (more than 200g or 200mL / 24h). Cotrimoxazole is the most important antibiotic in treating diarrheal diseases. The over-use of antibiotics can lead to resistance (Bow et al., 1998) such as the resistance pattern that shown of *Escherichia coli* (as much as 95%) to cotrimoxazole, so it is relevant reason to use alternatives from natural ingredients without resistances such as leaf extract of Carica papaya (Vale et al., 2015). The treatment of specific diarrhea caused by bacterial infection is expected to provide the maximum therapeutic effect(Shen et al., 2018) to pathogenic bacteria by using the new dosage form (Jyoti et al., 2019) in the form of particles (Chandrasekaran et al., 2016) which encapsulated the extract properly.

This study aims to elevate the potential of papaya leaves combined with lactic acid isolates as antidiarrheal herbal medicines formulated in the form of particles for development of new dosage form for oral administration (Banala et al., 2015). The formulation of antidiarheal herbal medicine can reduce the use of synthetic drugs for the treatment of diarrhea. The *in-vivo* test was performed on Wistar strain male rats which induced by *Escherichia coli*.

2. EXPERIMENTAL SECTION

2.1 Materials

The materials used in this study were: extract of papaya leaves (*Carica Papaya L.*) and lactic acid isolate from department of pharmacy Sriwijaya University, ethanol 96% (Merck®), quercetin (Sigma-Aldrich®), *Escherichia coli*, chitosan (Sigma-Aldrich®), sodium alginate (Sigma-Aldrich®), citric acid (Sigma-Aldrich®),

Figure 1. Papaya (*Carica papaya L*) leaves

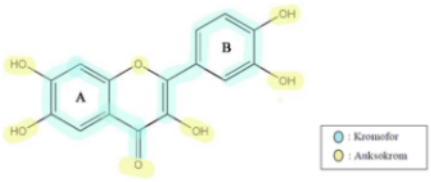

Figure 2. Structure of quercertine and the position of chromophore and auxocrome side

calcium chloride (Sigma-Aldrich®), NaCMC (Sigma-Aldrich®), sodium hydroxide (Merck®) and aquadest.

2.2 Methods

2.2.1 Extraction of Papaya Leaves

Papaya leaves were obtained at Inderalaya, South Sumatra which were taken from dark green leaves, then washed with running water. The drying process was protected from direct sunlight exposure for 14 days. Leaves were pollinated using a blender. The powder was sifted so that the obtained simplicia powder was appropriate to be extracted. Preparation of extracts was done by maceration method.

3 Kg of papaya leaves powder were dissolved in 96% ethanol with solvent replacement every 48 hours for 5 days. After that, the residue was filtered. Then the residue was macerated again with ethanol 96% for 2 days and filtered as the mixing filtrate. Finally the filtrates were mixed and then evaporated with a rotary evaporator at 60°C to obtain a thick extract.

2.2.2 Identification of Flavonoid Compounds

The 1 g thick papaya leaf extract (*Carica papaya L.*) is included in the test tube. 5 mL of ethanol is added, then heated for 5 minutes. 0.2 mg of Mg powder and 2 drops of concentrated HCl are added to the tube. Another method can be done by adding 2 drops of 10% NaOH. If it produces yellow, orange and red it indicates flavonoid. This work to fit the previous research regarding identification of papaya extract by Gogna et al. (2015).

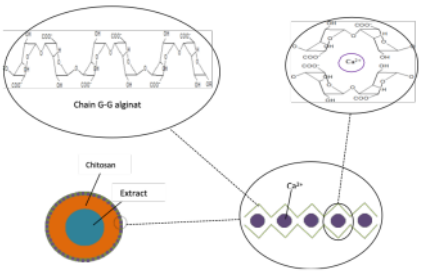

Figure 3. Illustration of combining ionic polymer to form submicron particle

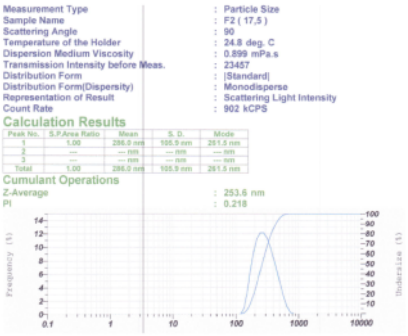

Figure 4. The size and the distribution of submicron particles

2.2.3 Determination of moisture content

Determination of water content was carried out using the gravimetric method. 1 g of extract is weighed carefully in a container that has been anchored. The extract was dried at 105°C for 30 minutes and weighed. Drying is continued until constant weight.

2.2.4 Formula

The papaya ethanol extract nanoparticle formula with lactic acid isolates was shown in Table 1. Variation of the formula in this study was F1 isolates of lactic acid was added $Ca(OH)_2$ as much as 12.5 grams, F2 isolates of lactic acid + $Ca(OH)_2$ as much as 17.5 grams and F3 of 22.5 grams of lactic acid was added $Ca(OH)_2$ isolates were used as much as 10 mg, chitosan as much as 35 mg, sodium alginate 50 mg and calcium chloride 0.018M. The difference in this formula is the concentration of $Ca(OH)_2$ which is mixed into lactic acid isolates to obtain the optimum formula.

2.2.5 Preparation of Gelation-Ionic Submicron particles

Citric acid of 50 mg was dissolved in 10 mL aquadest and then 3 ml of citric acid solution was pippeted and mixed into 1000 mg extract of papaya leaves as mass 1. The remaining 7 mL citric acid solution was mixed with 35 mg chitosan, sodium alginate of 50 mg was dissolved into 10 mL hot water, then solution was stirred until homogeneous. Chitosan was dissolved in 7 mL citric acid solution then it was mixed into 10 mL sodium alginate solution for each formula as mass 2. Mass 1 and mass 2 were

Table 1. The Formula of Ionic-Gelation Submicron Particles

Materials	Amount of materials in formula		
	F1	F2	F3
Extract Papaya Leaves (mg)	1000	1000	1000
Isolat lactic acid 8,24×107CFu/mL (mg)	10	10	10
$Ca(OH)_2$ (g)	12.5	17.5	22.5
Citric acid (mg/mL)	50	50	50
Chitosan (mg)	35	35	35
Alginate sodium (mg)	50	50	50
$CaCl_2$ 0,018M (mg)	9	9	9

mixed drop by drop on the stiring condition using a magnetic stirrer. The mixing process was allowed to run at a speed of 750 rpm for 1 hour. Pre-particles were formed and then stored at chill temperature. A solution of 9 mg of calcium chloride in 10 mL of aquadest was mixed for the three formulas. Lactic acid+$Ca(OH)_2$ isolate of 10 mg for each formula.

2.2.6 Determination of Total Quercertine and %Encapsulation

This process was started for preparation a calibration curve with a concentration of 2,4,6,8,10 ppm of quercertine at the wavelength that has been obtained. Preparations was used to calculate percent efficiency of encapsulation by separating preparations using centrifuges. Separate supernatants from each formula were added 0.1 mL of 10% sodium acetate and 0.1 mL of $AlCl_3$ 10% were measured at the maximum wavelength at 436 nm using a UV-Vis spectrophotometer to obtain the absorbance value. The absorbance results are made in the form of a calibration curve in the regression equation (y = a + bx) with a value of R ≥ 0.990.

2.2.7 Characterization of Submicron Particles

The potential diameter, polydispersity index (PDI), and zeta were measured using a particles size analysis (PSA) with the dynamic light scattering (DLS) method. The particles suspension was taken as much as 50 μL and diluted 100 times to 5 mL using distilled water, then 50 μL was taken and put into the cuvette PSA, then the monochromatic light was fired by the instrument, the light was refracted at 90° and 173° and the detector would capture the results refraction of light to produce potential zeta data, while the results of scattering light at an angle of 90° will be captured by the detector so that it had diameter data and particle distribution (Fithri et al., 2017).

3. RESULTS AND DISCUSSION

3.1 Simplicia Determination

Determination of samples was done at the Andalas University Herbarium (YOU), Biology Department, FMIPA Andalas University, Limau Manih Campus, Padang, West Sumatra. The results of the determination showed that the sample used in the study was pepaya as shown in Figure 1 with the namely Pepaya (*Carica papaya L.*) from the Caricaceae family.

3.2 Extraction of Papaya Leaves

The extraction process of papaya leaves was done by maceration using 96% ethanol. Maseration is done by inserting dry simplicia into a dark glass vessel to avoid interaction with light, then soaking while occasionally stirring, after 5 days of silence then filtered using a funnel coated with filter paper so that I get the filtrate I and residue. The residue was macerated with the solvent which was just left for 48 hours then filtered again using filter paper placed on top of the glass funnel and filtrate II was obtained. The first and second filtrates that were produced were then evaporated using a rotary evaporator until a thick extract was obtained. Thick extract is obtained as much as 85.56 g and has a yield value of 4.278%.

3.3 Identification of Flavonoid Compounds

The identification of compounds carried out on the ethanol extract of papaya leaves is a test of flavonoids and the structure was represented in Figure 2. The compound has an antibacterial activity that can kill pathogenic bacteria that cause diarrhea. The ethanol extract of papaya leaves added magnesium reagent showed positive results with the formation of brownish red color due to magnesium and HCl reacting with flavonoid compounds through the reduction of benzopiron nuclei in the structure of flavonoids with 10% NaOH reagent showed positive results with the formation of red brick colour, due to flavonoids including the phenolic compounds reacted with bases to produce a colour change.

3.4 Determination of Moisture Content

Determination of water content was carried out on ethanol extract of papaya leaves with the aim to determine the residual moisture content after the drying or thickening process. The high water content in the extract can also affect the quality of preparations that function as antibacterial so that the water content of the extract must be in accordance with the standards set by Materia Medika Indonesia which was < 10% which cannot be overgrown with microbes. The value of water content of the ethanol extract of papaya leaves obtained was 9.09%.

3.5 Determination of Total Quercertine and %EE

Determination of percent encapsulation efficiency begins with making a calibration curve that obtains a linear regression equa-

tion y = 0.079x - 0.094 with an R value of 0.999. Percentage of encapsulation efficiency (% EE) as shown in Table 2 was calculated by using a formula to determine the levels of ethanolic extract of papaya leaves in an encapsulated formula. The higher the% EE value, the more extracts encapsulated in the polymer were used. The value of the %EE were then analyzed using SPSS 22. The results of the analysis can be seen as the data were normally distributed or can be analyzed using the normality test. Based on the analysis of the normality test the %EE obtained a significance value of > 0.05 using the Shapiro-Wilk method which indicates that the value did not have a significant difference meaning the data is normally distributed. The One Way ANOVA method can be used for further step. The result of the analysis had a no significance value of ANOVA test of ($p > 0.05$). The value of %EE were in same level. Nevertheless, the highest value was shown by F1. The optimum formula was represented by F 1 which had the smallest concentration of $Ca(OH)_2$ compared to formula 2 and 3. High levels of Ca^{2+} ions in the preparation due to crystalline alkaline $Ca(OH)_2$ isolates in the preparation cause shrinkage in the size of the submicron particles the dimensions of the submicron particle preparation are reduced and only a few extracts can be encapsulated by alginate polymers.

Table 2. Results of %EE Calculation

Formula (F)	%EE±SD
1	80,82% ±0,0088
2	80,41% ±0,0342
3	80,31% ±0,0310

3.6 Evaluation and Characterization of Submicron Particles

Particles were formed by using chitosan, alginate, and ion calcium that was illustrated in Figure 3. In order to know the character of particles, the evaluation of physical properties was the main work which was to be conducted. Particle size distribution is important parameter (Banala et al., 2015) because it affects the ability of penetration, stability, and the potential for drug effects and safety. The smaller the PDI value, the better. This is because the smaller this shows that the particles are more uniform (Rawat et al., 2006). The results of the analysis of diameter measurements and particle distribution in the optimum formula (as shown in Figure 4) was 253.6 nm and the optimum formula shows results that enter the size range 200 - 500 nm.

4. CONCLUSIONS

Based on the research that has been done, conclusions can be drawn as follows: 1. The value of% EE produced in formulas 1, 2 and 3 is 80.82% ± 0.0088; 80.41% ± 0.0342; and 80.31% ± 0.0310 indicated that the F1 was an optimum formula. 2. The results of particle characterization using the PSA in the optimum formula produced particle size values with an average 253.6 nm, PDI 0.218, and zeta potential +8 mV respectively.

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