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The Chitosan-Sodium Alginate Submicro Particles Loading Herbal of Ethanolic Extract of Leaves *Senna alata*. L for Curing Of Bacterial Infection on Skin

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Abstract

This research was performed to detect the activity of the optimum formula of chitosan and sodium alginate submicro particles loading of the ethanol extract of leaves *ketepeng cina* (*Senna alata* L.) to *P. acne* growth. Submicro manufactured particles was used three variations of the formula from three of volume used of calcium chloride (CaCl₂): 20, 40, and 100 mL based on ionic gelation method. The results obtained of %EE in formulas 1, 2 and 3 were 78.56%, 81.71%, and 77.48%. Formula 2 with a value of % EE of 81.71% was used as an optimum formula which indicates that the particles are well protected by chitosan and sodium alginate polymers so as to prevent particle damage during the homogenization process. The results of the diameter measurements showed that the optimal formula enters in the submicro particle range with the value obtained is 525.9455 nm. The submicro formula of chitosan alginate particles loading the *ketepeng cina* leaf ethanol extract was barely homogeneous which based on the results obtained by the PSA was 0.433 of PDI. The zeta potential value was +3.5 mV. The results of X-ray diffraction analysis or X-Ray Diffraction produce a pattern shaped amorph with the resulting peak does not have a wide distance and pattern. In vivo testing using *ketepeng cina* leaf extract as submicro was decreased the *P. acne* lesion faster than using only *ketepeng cina* leaf extract because the submicro particle preparation has a small particle that is below to 600 nm making it easier to penetrate the skin pores to reach the target. The results of the analysis of the curing of many lesions on the skin of mice have a significance value of homogeneity. The one-way ANOVA test of < 0.05 was a significant effect of the test group on the decreasing in the number of lesions on the skin of the mouse.

Keywords

ketepeng cina, submicroparticles, chitosan, sodium-alginate, *P.acne*.

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1. INTRODUCTION

Indonesia is in a tropical climate with high humidity and facilitates the growth of fungal and bacterial pathogens that cause infectious diseases, especially skin infections. Research conducted to determine the development of skin infections shows dermatitis by bacteria ranks first followed by dermatomycosis. The extrapolated incidence rate is the same as the big cities in Indonesia. This case might increase with different disease variations in the interior (Karimkhani et al., 2017). Bacteria are the main causes of infections such as *Propionibacterium acnes* (*P. acne*) and fungi involved in exacerbating infections. In the pathology of human skin diseases, fungi that can aggravate the disease are caused by dermatophyte fungi from the family arthrodermataceae with more than 40 species, one of which is the genus *Trichophyton* (Jenifer et al., 2015).

One of the traditional medicines as an anti-infection is *ketepeng cina*.

Based on other traditional uses of the *ketepeng cina* plant (as seen in Figure 1), it is used as an antiparasitic, ring-worm, scabies, phlegm, malaria, constipation, inflammatory skin inflammation, antiviral, syphilis, herpes, influenza, and bronchitis. The results showed this plant has the potential to stimulate an immune response. *Ketepeng cina* leaf extract (*Senna alata* L.) contains alkaloids, anthraquinones, flavonoids, saponins, tannins, terpenes and steroids. In vitro research shows that ethanol extract of *ketepeng cina* leaves has anti-fungal activity. The test was conducted on skin bacteria and fungi that cause dermatophytosis, namely *Trichophyton*. Antifungal activity of *Trichophyton mentagrophytes* (Somchit et al., 2003).

Flavonoids that work in a bacterio-static and fungistatic way by inhibiting enzymes in cellular activity in pathogenic cells (Sheikah et al., 2015). Increased antifungal activity of ethanol extract of *ketepeng cina* leaves can be done by formulating extracts



Figure 1. The leaves of ketepeng cina (*Senna alata L.*)

in the form of submicro particles. Drugs or extracts formulated with particle submicro technology in pharmaceutical preparations will help drug penetration into the skin faster because the particle size of the preparations is smaller, so that it will facilitate targeted drug delivery and have a maximum therapeutic effect. The manufacture of submicro particle preparations in topical drug delivery systems requires polymers because it is useful as a carrier that functions to bring the active drug into the cell more quickly and efficiently. Polymers are also capable of coating these active substances and can be degraded and accepted by the body as well as low systemic toxicity (Andreas et al., 2015).

The submicro form of chitosan particles has many advantages, namely non-toxic, stable during use (Agnihotri et al., 2004). Chitosan also acts as a tight junction opening (TJ) between cells so that it can be an enhancer that functions to bring the active drug substance into the cell. This biopolymer also has the advantage of being able to maintain the release of the active substance which is encapsulated in a long period of time (Rodriguez et al., 2002).

Chitosan is positively charged with negatively charged polyanion in sodium alginate by a joining process to prevent damage to the active ingredients encapsulated in submicro particles. Calcium chloride is most often used and is non-toxic and is widely used as a drug delivery. Calcium chloride (CaCl_2) readily coats sodium alginate because Ca^{2+} ions are bound to function as a cross linker.



2. EXPERIMENTAL SECTION

2.1 Materials

The materials which were used in this study were *P. acne* ATCC 25746, ketepeng cina Leaf Extract (*Senna alata L.*), Citric Acid (Sigma aldrich), Calcium Chloride (Merck), Ethanol (Merc), Chitosan Polymers (Sigma Aldrich), Sodium Alginate (Sigma Aldrich), Aquadest (Otsuka), and Aqua Pro Injection (Otsuka).

2.2 Methods

2.2.1 Sampling and extract preparation

Samples of ketepeng cina (*Senna alata L.*) were obtained from Muara Enim, South Sumatra. Fresh ketepeng cina leaves are as much as 15 kg (picked directly from the leaves) were washed c

and then chopped roughly. Furthermore, ketepeng cina leaves are dried in the sun then covered with black cloth until they dry. Simplicia were then put into a dark bottle and macerated using 70% ethanol. After that the solvent was inserted until the surface of the sample completely shaken and stored in a dark place while occasionally stirring. Remaceration process was carried out two times. After 5 days, the macerat was then filtered using Whatman paper so that the filtrate was obtained. All of the filtrate obtained was collected and concentrated using a rotary evaporator at a temperature of 65°C with a speed of 40 rpm to obtain a thick ethanol extract.

2.2.2 Screening of flavonoid compounds

Flavonoid screening was done using ethanol extract of ketepeng cina leaves as much as 1 mL each inserted into 2 test plates. Plate 1 was added with 2 drops of 10% NaOH, plate 2 added 2 drops of HCl 2N and 50 mg of Mg powder. The positive reaction of flavonoids was shown by the change of yellow-orange-red color on plate 1 and yellow or red on both plates.

2.2.3 Formulation

Based on literally which was studied known that extract of ketepeng cina extract as much 300 mg and 30 mg chitosan Sule and Okonko (2010) need CaCl_2 of 0,0018 mM dan natrium alginat 3.2 mg (Moradhaseli et al., 2012). Furthermore, the differences of formula (F1, F2, F3) were based on the volume of crosslinker CaCl_2 as seen in Tabel 1.

Table 1. Formula of submicro partikel loading of ketepeng cina extract

Compounds	ammount		
	F1	F2	F3
Extract	300 mg	300 mg	300 mg
Chitosan	30 mg	30 mg	30 mg
Na Alginate	3.2 mg	3.2 mg	3.2 mg
CaCl_2 0.018 mM	20 μL	40 μL	100 μL

2.2.4 Manufacturing of submicro particles

Ketepeng cina leaf extract was added to 30 mL of chitosan solution Moradhaseli et al. (2012) into the vial using a magnetic stirrer with a speed of 750 rpm for each formula as mass 1 (procedures for submicro-particles preparation). Sodium alginate solution was placed on a magnetic stirrer with a speed of 750 rpm taken 30 mL as mass 2. Mixing mass 1 with mass 2 was conducted by using drop-by-drop method which was facilitated by micro pipette on a magnetic stirrer. The mixing process takes place at a speed of 750 rpm for 4 hours. Calcium chloride solution was added with various concentrations of 20, 40 and 100 μL for each formula.

2.2.5 Encapsulation Efficiency (%EE)

Purification of submicro particles of chitosan sodium alginate ethanol extract carrier ketepeng cina leaves was carried out by

means of 30 ml of solution put into Vivaspin® 300 kDa and then centrifuged for 15 minutes to obtain 2 phases. The unabsorbed phase is separated and added with 30 ml of Aqua Pro Injection (API) into the reabsorbed and re-centrifuged phase, repeated 3 times to obtain unabsorbed particles.

The determination of %EE was conducted by making a calibration curve. The standard curve solution was made from 100 µg / mL quercetin parent solution by piping 0.1; 0.5; 1.0; 2.0; and 2.5 mL, dissolved in 10 mL methanol p.a. (the standard solution level becomes 1; 5; 10; 15; 20; and 25 µg / mL). Preparation of a 10% AlCl₃ solution was carried out with 2.5 g of AlCl₃ dissolved with aquadest up to 25 mL. 1.5 mL of each solution concentration was reacted with 0.1 mL of 10% AlCl₃, 0.1 mL of sodium acetate 1 M, then diluted with distilled water to the mark. The solution is shaken and allowed to stand for 30 minutes.

2.2.6 Determination of crystallinity

The crystallinity was measured using XRD. The optimum formula was first measured at a temperature of -8°C with a 24 hour period then at room temperature for 24 hours, for 6 days alternately continued on the XRD with the sample compacted and leveled surface on an aluminum holder to then be measured scanning mode which is done is continuous scan on axis 2θ.

2.2.7 Determination of particles properties

Particles properties including diameter, distribution, and zeta potential of the particle using the Particle Size Analyzer (PSA) through the Dynamic Light Scattering (DLS) method. Purified submicro-particles suspension of chitosan sodium alginate particles carrying of *ketepeng cina* leaf extract was taken as much as 50 µL, diluted 100 times (5 mL) using distilled water, then it was taken 50 µL and poured into PSA cuvette.

2.2.8 Inhibition of bacterial infection on skin

Sources of infection (*P. acne*) colonies for in vivo testing were carried out by random sampling. Samples taken were *P. acne* cultures which had been in sloping agar for 2 days, *P. acne* colonies were taken to be diluted with 0.9% NaCl until the turbidity was equivalent to standardization of 0.5 McFarland (1.5×10^8 CFU) (Santos et al., 2006). Submicro particles chitosan sodium alginate particle carrying of *ketepeng cina* leaf extract which has been made and then applied to the infection area. Observations were made on days 5, 10, and 15 with an assessment based on a decrease in the diameter of the infection site or the loss of red spots found on the skin of mice with infection. The test treatment group can be seen in Table 2.

3. RESULTS AND DISCUSSION

3.1 Screening of flavonoid compound

Phytochemical screening for the identification of positive flavonoid compounds using Mg and HCl is characterized by a yellow discoloration. Positive results using NaOH reagents are marked with a yellow-orange-red discoloration. The addition of Mg and HCl metal reagents in the test sample experienced a significant

Table 2. Group of Animal Test

No	Group	Treatment
1	Control normal	Water
2	Control negative	Bacterial suspension
3	Control positive	Bacterial susp + antibiotics
4	Extract	Bacterial suspension + Extract
5	NPs	Bacterial suspension + NPs

change because in the flavonoid sample reduced the benzopyron nucleus to form a flavilium salt which was marked with a yellow color change. The addition of NaOH reactor in the test sample changed color to yellow-orange-red 2. Calculation of total flavonoid levels in *ketepeng cina* was 27.91 mg per 1 g sample from the regression equation on the standard (quercetin) calibration curve. The total amount of flavonoid was 27.91 mg/L.

3.2 The efficiency of capsulation (% EE)

The particles were purified with a membrane size of 300 kDa with the assumption that the free extract would come out of the membrane, while particles larger than the extract would be retained in the membrane. Hence the level of extract that escapes would be able to indicate how much the efficiency of capsulation (% EE). The %EE results obtained in formulas 1, 2, and 3 were 78.566%, 81.718%, and 77.448% respectively. Formula 2 has the largest % EE value as an optimum formula.

3.3 Determination of crystallinity

The results obtained in XRD were particles from crystalline polymers but after becoming submicro particles, the shape of the particles turns into amorphous. The shape of the particle is said to be amorphous when diffraction patterns occur. Amorphous does not have separate peaks at a certain distance and the resulting pattern is wide, whereas the crystal shape has separate peaks at a certain distance and the resulting pattern is not wide as seen in Figure 2. This research is in accordance with the expected that the particles must be amorphous. The reason the particle shape must be amorphous is that it dissolves more easily and is absorbed quickly.

3.4 Particles properties

The results of the particle size analysis conducted on submicro particle suspension samples obtained were 525.9455 nm. The PDI value produced in the measurement of particle distribution or PDI and zeta potential with PSA were 0.433 and +3.5 mV. Particles were quick to agglomeration because of their distribution which was higher than 0.3 as PDI. The zeta potential was also not stayed in range > +25 or < -25 mV.

3.5 Observation on inhibition of bacterial infection on skin

Lesions appear on day of fifth and continue to grow into infections day after day. Data from Table 3 shows that on the 15th day the number of lesions could be overcome and what was left by the submicro preparations of *ketepeng cina* leaf particles.

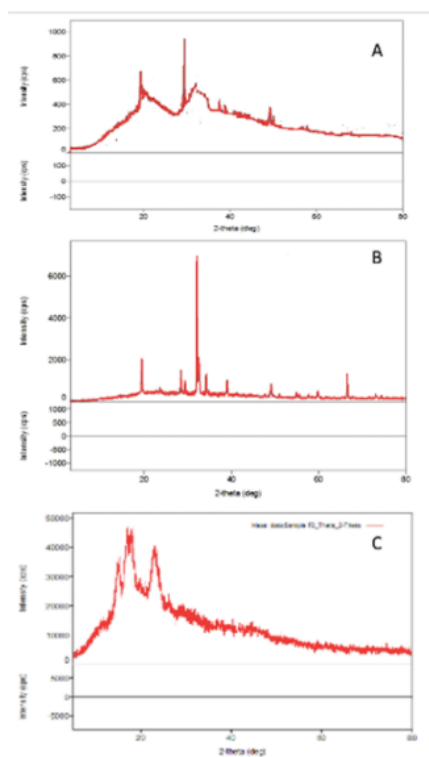


Figure 2. XRD pattern of chitosan (A), sodium alginate (B), and submicroparticles (C)

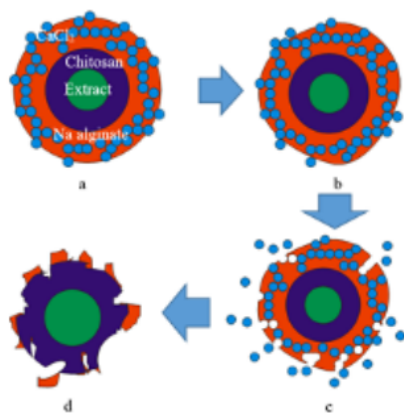


Figure 3. Illustration of release the bioactive compound from chitosan-Na-alginate particles

The number of lesions in general was decreased but the normal group certainly did not show a decrease. The number of lesions remaining on the 15th day after submicroparticles application and release their active compound (as illustrated in Figure 3) was 2.3 number of lesions which was less than while in treatment with extract (only used *ketepeng cina* leaf extract). The results were quite significant although not as good as the decrease in the number of lesions given by positive control.

Table 3. Results of in vivo test for inhibition the number of lesions by *P. acne*

Days	Control Normal	Control Negatif	Control Positif	Extract	NPs
D-1	11	12	11	11	11
D-5	10	10	9	8	9.6
D-10	9	7.6	8	6.3	5.3
D-15	8	6	5.6	3.6	2.3

4. CONCLUSIONS

Based on the results which has been described, the summary of this research were *ketepeng cina* contain flavonoids. Formula 2 with a value of % EE of 81.71% was used as an optimum formula. The diameter measurements showed that the optimal formula enters in the submicro particle range with the value obtained is 525.9455 nm. The submicroparticles distribution was obtained by the PSA was 0.433 of PDI. The zeta potential value was +3.5 mV. The results of X-ray diffraction analysis or X-Ray Diffraction produce a pattern shaped amorf. The results of the analysis of the curing of many lesions on the skin of mice have a significance value of homogeneity. The one-way ANOVA test of < 0.05 was a significant effect of the test group of NPs on the decreasing in the number of lesions compare to the extract.

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