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# ANTI-ACNE POTENCY OF WATER-SOLUBLE CHITOSAN PEEL-OFF GEL MASK AGAINST PROPIONIBACTERIUM ACNES

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## ABSTRACT
Acne is a skin disease that one of the causes is bacteria. Acne can be treated with chemical antibiotics, but this medication could cause resistance, therefore it is required natural ingredients that can be used as an antibacterial against the *Propionibacterium acnes* bacteria. One of the natural ingredients that can be used is chitosan, but chitosan has a weakness that is only soluble in organic acid solvents so that its utilization is limited therefore it needs to be modified into water-soluble chitosan and to facilitate its application then the preparation of the *peel-off* mask. The purpose of this study is to know the effect of the addition of water-soluble chitosan with different concentrations of 150, 170 and 190 mg/ml and *peel-off* gel mask capability inhibits acne bacteria. The used method is an experimental method which includes antibacterial, time dries, pH, viscosities, organoleptics and irritation. The results showed that the best concentration *peel-off* gel mask in 190mg/ml, it was able to inhibit *Propionibacterium acnes* and were relatively strong. The test of drying time, pH and viscosities still meet the standard of the specified mask while organoleptic test of the color and smell best concentration is 150 mg/ml because it is considered similar to the control sample. *Peel-off* mask the soluble-chitosan is classified as safe because it does not cause erythema and edema after being tested on mouse skin.

## KEY WORDS
Peel-off gel mask, water-soluble chitosan, antibacterial, irritation.

Acne is a skin problem that can reduce a person's confidence. There are several factors that trigger the formation of acne including increased sebum production, sloughing of keratinocytes, bacterial growth and inflammation (Chomnawang *et al*., 2005). Propionibacterium acnes is a bacterium that triggers the formation of acne because it can produce metabolites that can react with sebum, thereby increasing inflammation (Laianto, 2014). There are several ways that can be done to treat acne, one of which is by topical treatment (Wasitaatmadja, 1997). This treatment is usually done by giving antibiotics such as doxycycline, tetracycline and clindamycin (Oprica, 2004). In addition to antibiotics from chemicals, there are other alternatives that can be used, namely antibiotics from natural ingredients. One of the natural ingredients that can be used as an antibiotic is chitosan.

Chitosan is a derivative of chitin through deacetylation process (Silvia *et al*., 2014). Chitosan is one of the natural ingredients that have potential as an antimicrobial because it contains lysosim enzymes and aminopolysacharida groups that can inhibit bacterial growth (Mulyaningsih *et al*., 2015). However, chitosan has a weakness that is difficult to dissolve in water and only soluble in most organic acid solvents such as formic acid, acetic acid, tartaric

acid, and citric acid (Irianto and Ijah, 2011), so it is not optimal in its use, especially as an anti- acne its application on the face that has a pH requirement of 4.5 - 8.0 (SNI, 1996). Therefore, it is necessary to modify the shape and structure of chitosan to increase its solubility. One way that can be done is by depolymerization using H2O2 (Tanasale *et al*., 2016). This is reinforced by research (Widiyanti, 2018), which explains that the use of 3% H2O2 by heating at 40°C for 4 hours can produce a solubility of 90%.

The application of water-soluble chitosan as an anti-acne drug can be in the form of a mask, one of which is a peel-off gel face mask. Water soluble chitosan peel off gel mask is a product whose application is carried out directly on the skin surface, so in addition to the resulting benefits, it is necessary to pay attention to the safety factor of the peel off gel mask product. A health or cosmetic product according to Wasitaatmadja (2010) must meet pre-clinical requirements before the product is used commercially in humans, therefore in this study, erythema and edema were tested using male wistar rats (*Rattus norvegicus*) before use in humans so that the level of security can be known so that it becomes a safe product.

## METHODS OF RESEARCH

The tools used in this study were Bunsen, petri dish, test tube rack, spatula, glassware, suction ball, ose needle, washing bottle, triangle, autoclave, incubator, measuring flask, digital scale, analytical balance, hot plate, pH meter, oven, water bath shaker, mortar and pestle.

The materials used in this study were chitosan obtained from the results of Widiyanti's research (2018), male wistar rats (Rattus norvegicus), Propionibacterium acnes bacteria, MHA (Mueller Hinton Agar), NA (Sodium Agar), NB (Sodium Broth), HPMC (Hydroxypropyl Methylcellulose), calico, tissue, aluminum foil, spirit, alcohol, plastic wrap, label, gauze, distilled water, 2% acetic acid, 3% H2O2, 10% NaOH, PVA (Polyvinyl Alcohol), glycerin, methylparaben, propylparaben.

The method used in this research is an experimental method which is carried out in two stages of research, namely the first stage of research and the second stage of research.

In the first stage of research, namely testing the inhibition of water-soluble chitosan against Propionibacterium acnes bacteria with concentrations of 130, 140 and 150 mg/ml. The best concentration of the first phase of research is used for the second phase of research. The experimental design used was a simple RAL (Completely Randomized Design) followed by the BNT test.

Making water soluble chitosan using the Widiyanti method (2018) is to weigh 10 grams of chitosan, then add 200 ml of 2% acetic acid and stir until homogeneous. 3% H2O2 was added as much as 26 ml then homogenized and heated using a water bath shaker at 40 °C for 4 hours then added with 10% NaOH until the pH was neutral and continued with filtering using a calico cloth. The separated chitosan was then dried in an oven using a temperature of 50 °C for 24 hours after 24 hours the water-soluble chitosan crystals were crushed using a mortar and pestle to a fine powder.

In testing the inhibition of the pitting method against acne bacteria according to Rusli et al., (2016) with modifications, the first thing to do is to take 20 L of Propionibacterium acnes bacteria culture. The bacteria were then inoculated into a petri dish containing MHA (Mueller Hinton Agar) media and flattened with a triangle, allowed to stand for 5 minutes and bore holes were made. The wells that have been made are filled with water-soluble chitosan concentration (130 mg/ml, 140 mg/ml, 150 mg/ml) and the negative control is distilled water and the positive control is tetracycline antibiotics; incubation at 37°C for 18-24 hours. The zone of inhibition was observed and measured using a caliper.

The best results from the first stage of research were continued in the second stage of research, namely water soluble chitosan after being applied to a peel off gel mask with a concentration of 150, 170, 190 mg/ml, negative control in the form of a mask preparation without the addition of water soluble chitosan and positive control in the form of commercial products. Analyzed inhibition, speed of drying time, pH, viscosity, organoleptic and irritation test using male wistar rats (Rattus norvegicus). The inhibition test procedure for the pitting

method was carried out the same as the first stage of the study.

The process of making water soluble chitosan peel off gel masks based on the Budiman et al. (2017), method with modifications, first PVA (Polyvinyl Alcohol) and HPMC (Hydroxy Propyl Methyl Cellulose) were developed using distilled water at 90°C in different containers. The next container contains methylparaben and propyleparaben which have been dissolved in glycerin. The water soluble chitosan sample was dissolved in sufficient aquadest. All the ingredients from the various containers were mixed into one container and stirred until homogeneous.

The method used for testing the irritation of the water-soluble chitosan peel off gel mask preparation is the Draize method with modifications (Trisnayanti et al., 2015). First, the acclimatization process was carried out on the test rats for 3 days. The rats were then shaved on the back with an area of 2 x 2 cm2 then smeared with a gel preparation (concentrations 150, 170 and 190 mg/ml) of 0.25 grams each. The back of the rat was covered with gauze and plaster then waited for 24 hours after 24 hours the gauze and plaster were removed then observed for erythema and edema, the next stage the gauze and plaster was closed again and the same observations were made at 48 and 72 hours.

## RESULTS AND DISCUSSION

In this study, the water-soluble chitosan used was the best result from Widiyanti's research (2018) which used 3% H2O2 treatment with a heating temperature of 40°C and within 4 hours. In this study, the characteristics of water soluble chitosan were obtained, namely yield of 3.5%, water content of 10.6%, solubility of 90%, and the degree of deacetylation of 94.21%. Characteristics are important because they can indicate the quality of the resulting product. In Widayanti's research (2018), the water-soluble chitosan produced has fairly good characteristics because it has a solubility of 90%. Increasing the solubility of chitosan is important because it can facilitate the application of chitosan in medicine and food (Du *et al*., 2009), in addition to the degree of solubility of the resulting deacetylation (DD) is also quite high, namely 94.21%. The degree of deacetylation (DD) is an important characteristic because it can affect the quality of water-soluble chitosan (Bahri *et al*., 2015). The higher the degree of deacetylation in chitosan, the higher its antimicrobial activity (Rabea *et al*., 2003).

In this study the method used is the well method. This method was used to determine the inhibition of water-soluble chitosan against the acne-causing bacteria *Staphilococcus epidermidis*. Analysis of ANOVA data showed significantly different results (Fcount > F 5%) so that further BNT tests were carried out to show different notations at each concentration as shown in Table 1.

Table 1 – Table of Inhibitory Zones of Water Soluble Chitosan Against *P.acnes* Bacteria

| Concentration (mg/ml) | Inhibition Zone Diameter (mm) | Barrier Zone Category |
|---|---|---|
| | *P.acnes* | *P.acnes* |
| Control - | 0±0a | No |
| 130 | 16,42±0,35b | Strong |
| 140 | 19,37±0,26c | Strong |
| 150 | 21,17±0,22d | Very strong |
| Control + | 28,23±0,31e | Very strong |

In the test of water-soluble chitosan inhibition against *Propionibacterium acnes* bacteria, the concentrations were 130, 140 and 150 mg/ml. The greatest inhibitory power of water-soluble chitosan against these bacteria was found at a concentration of 150 mg/ml which was 21.92 mm in *Propionibacterium acnes* bacteria as shown in Table 1. The resulting inhibition zone is also large. According to Pelczar and Chan (2010), the more active substances added, the higher the antibacterial activity produced.

The results of data analysis showed that the antibacterial peel off chitosan gel mask was water soluble against *P.acnes* bacteria ($P<0.05$).

Table 2 – Inhibitory Zone of Water Soluble Chitosan Peel Off Gel Mask against Acne Bacteria P.acnes

| Sample | Inhibition Zone Diameter (mm) | Barrier Zone Category |
|---|---|---|
| | *P.acnes* | *P.acnes* |
| M0 | 0±0a | No |
| M1 | 11,98±0,65b | medium |
| M2 | 13,38±0,37c | medium |
| M3 | 14,32±0,32d | Strong |
| RA | 17,00±0,35e | Strong |

*Note: RA = control + (Commercial products); M0 = Peel off formula gel mask 0 (0 mg/ml); M1 = Peel off formula gel mask 1 (150 mg/ml); M2 = Peel off formula gel mask 2 (170 mg/ml); M3 = Peel off formula gel mask 3 (190 mg/ml).*

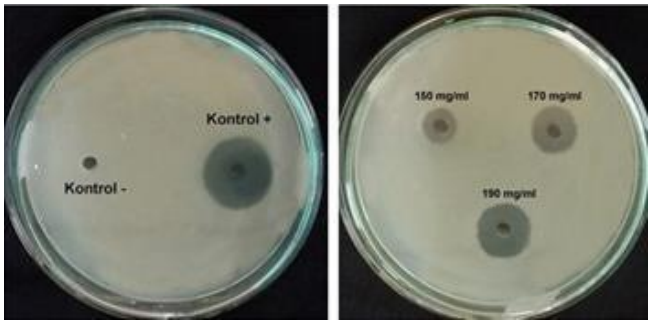


Figure 1 – Inhibition zone *P.acnes*

The results of the inhibition zones of water soluble chitosan peel off gel masks against acne bacteria *Propionibacterium acnes* with different concentrations of water soluble chitosan 150, 170 and 190 mg/ml resulted in different inhibition zones. In the bacteria the largest inhibitory zone was found in M3 with a concentration of 190mg/ml. The diameter of the largest inhibition zone in *Propionibacterium acnes* was 14.82 mm.

In Figure 1 it can be seen that the water soluble chitosan peel off gel mask produced the diameter of the inhibition zone found in the bacterium *Staphilococcus epidermidis*. This is presumably because the acne bacteria *Propionibacterium acnes* is classified as gram-positive bacteria. Gram-positive bacteria in general have a simpler cell wall structure, namely 90% of the cell wall consists of a peptidoglycan layer while the other layer is teichoic acid (Fardiaz, 1989).

The inhibition produced by water-soluble chitosan before being applied and after being applied into the form of a mask showed a decrease in the diameter of the inhibition zone, this was presumably due to the addition of other ingredients such as HPMC (Hydroxy Propyl Methyl Cellulose), PVA (Polyvinyl alcohol), glycerin, methylparaben, propyleparaben, and aquadest which allows it to prevent water-soluble chitosan as an active substance from entering into bacterial cells, resulting in a decrease in inhibition. This is reinforced by the statement of Riski and Fitriyanti (2015), that if the active substance is added to other ingredients, the inhibitory ability will decrease.

Table 3 – Water Soluble Chitosan Peel Off Gel Mask Test Results

| Sample | Drying time (min) | pH | Viscosity (cP) |
|---|---|---|---|
| M0 | 25,5±0,54a | 6,16±0,54 | 14322a |
| M1 | 27,3±0,21b | 6,38±0,23 | 16490c |
| M2 | 28,6±0,60d | 6,49±0,35 | 18188d |
| M3 | 29,3±0,18e | 6,62±0,29 | 20486e |
| RA | 28,0±0,32 | 6,47±0,38 | 15254b |

The drying time test was carried out to determine how long the gel preparation can dry and form a film layer. In Table 3, it can be seen that the peel off gel mask preparations that do not contain water-soluble chitosan (M0) have a faster drying speed compared to the three peel off gel mask preparations containing water-soluble chitosan (M1, M2, M3). This is

presumably because the addition of water soluble chitosan prolongs the evaporation time of water in the peel off gel mask preparation. The same results were also seen in the positive control (commercial anti-acne gel peel off mask) which had a longer drying time than the negative control (M0). Based on the opinion of Izzati (2014), the number of active substances added to the peel off gel mask preparation can prevent the water in the material from evaporating so that it slows down the drying time. From these results, it can be said that the drying time of the water soluble chitosan peel off gel mask preparation still meets the good dry time of the peel off gel. According to Vieira (2009), the drying time for a good peel off gel mask is 15-30 minutes.

Based on Table 3, the results of the pH test on water-soluble chitosan peel off gel mask preparations showed that the preparations that did not contain water-soluble chitosan had a more acidic pH than those containing water- soluble chitosan. This is presumably because water-soluble chitosan has a pH close to neutral pH (pH 7) so that the more addition of water-soluble chitosan the higher the pH. In accordance with the Belangi statement (2018), that water-soluble chitosan in general has a pH that is not too low (acidic) or can be said to be close to neutral. The pH of the mask preparation ranges from 6.16 to 6.62, which means that the pH of the product is still within safe limits. Supported by the statement of Ahmad and Adhe (2013), which topical preparations should have a pH that is in accordance with the pH of the skin, which is between 4.5-8.0 because if it is too acidic it will cause irritation and if it is too alkaline it causes dry skin.

In the viscosity test, significantly different results were obtained (Fcount > F 5%) so that it was continued with the 5% BNT test which showed a different notation at each concentration as shown in Table 3. The highest average viscosity was found in the M3 treatment, which was 20486 cP while the lowest viscosity in the M1 treatment was 16490 cP. These results indicate that the higher the concentration of water-soluble chitosan added, the higher viscosity will increase. This is in accordance with the statement of Martin *et al*., (1993), if more concentrations of active ingredients are added, the viscosity value will increase. In this study, the viscosity value obtained was between 14322cP- 20486cP, which amount is still included in the permissible viscosity value in the peel off gel mask preparation. The viscosity of the gel preparation should be in the range of 3000 – 50,000 cP (SNI, 1996).

In the color organoleptic test, the highest result was obtained in the M1 sample, namely the concentration of water-soluble chitosan 150 mg/ml of 5.62 which means it is considered somewhat better than the control sample (R) while the lowest value is in the M3 sample, namely the concentration of water-soluble chitosan of 190 mg/ml with a value of 2.75 which means it is considered worse than the control sample (R). This is because the M1 sample has a slightly pale yellow color while the M3 sample has a more concentrated yellow color so that the panelists chose the M1 sample as a sample that is close to the control mask color as shown in Figure 2.

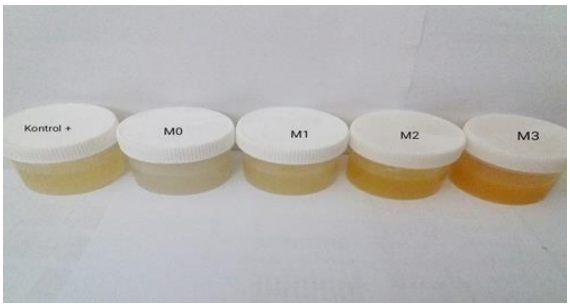


Figure 2 – Water Soluble Chitosan Peel Off Gel Mask

In the aroma test, the highest result is the M1 sample of 6.49 which means the sample is considered better than the control sample (R) while the lowest value is in the M3 sample which is 3.54 which means it is considered somewhat worse than the control sample (R). This is because the M1 sample has a less pungent aroma. While the samples of M2 and M3 masks have a slightly pungent and very pungent aroma. Maybe this is what makes the

panelists choose the M1 mask sample as the sample that is considered better than the control and the M3 mask sample as the sample that is slightly worse than the control.

The safety test on the water-soluble chitosan peel off gel mask was carried out by testing the irritation on the back skin of male wistar rats using the Draize test method. The irritation test was carried out with the aim of knowing the safety of a product, namely the presence or absence of side effects resulting from a water-soluble chitosan peel off gel mask.

The results of observations for 24, 48 and 72 hours after applying the water-soluble chitosan peel off gel mask product with formulations M0, M1, M2 and M3 obtained a Primary Irritation Index value of 0 which means that the rats did not experience irritation, either erythema or edema, so it can be said that the mask product safe. According to Natalia (2017), this says that if the primary irritation index value is small, it means that the product is less irritating.

## CONCLUSION

The best water-soluble chitosan peel off gel mask formulation was at a concentration of 190 mg/ml which produced an inhibition zone of 14.82 mm against Propionibacterium acnes bacteria. The results of the inhibition obtained from these bacteria are classified as strong category.

The water-soluble chitosan peel off gel mask formulation of all concentrations (150 mg/ml, 170 mg/ml and 190 mg/ml) showed no signs of irritation until 72 hours of application on the skin of male wistar rats.

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