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Original Article

ANTI-BACTERIAL ACTIVITY OF CHITOSAN NANOPARTICLES GEL FROM CRAB SHELL WASTE (PORTUNUS PELAGICUS) AGAINST STAPHYLOCOCCUS AUREUS AND ESCHERICHIA COLI BACTERIA

YUSAN L. Y.[1*], SUBAGIO H.[2]

[1]Program Study of Pharmacy, Faculty of Pharmacy, Hang Tuah University, Surabaya, Indonesia. [2]Department of Fisheries, Faculty of Engineering and Marine Science, Hang Tuah University, Surabaya, Indonesia
*Corresponding author: Yusan L. Y.; *Email: liza.yusan@hangtuah.ac.id

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ABSTRACT

Objective: This study aims to develop a chitosan nanoparticle gel from crab shell waste (*Portunus pelagicus*) by testing the anti-bacterial activity against *Staphylococcus aureus* and *Escherichia coli*.

Methods: Chitosan nanoparticles gel formulation was made using spontaneous method. The gel formulation used 1% concentration of chitosan nanoparticles. Optimization of gel base and formula, including organoleptic test, homogeneity, pH determination, adhesion, spreadability and viscosity test were evaluated. Anti-bacterial activity was determined against *Staphylococcus aureus* and *Escherichia coli* bacteria.

Results: The results showed that the formulation produced a stable gel with semisolid characteristics without any coarse grains, clear white and homogeneous. The pH of the formula was 5.94-6.46 and included the type of Oil in Water (O/W) gel. The formula showed adhesion of 1.05-1.97 seconds, spreadability of 5.33-5.00 cm and viscosity ranging from 7444-7792 cp. Chitosan nanoparticles gel showed the highest anti-bacterial activity with the zone of inhibition on *Staphylococcus aureus* of 15.37±1.34 mm and *Escherichia coli* of 18.33±0.25 mm with 250 mg chitosan nanoparticles gel.

Conclusion: *Portunus pelagicus* shell waste chitosan nanoparticles produced a stable gel and showed moderate anti-bacterial activity with the highest inhibition zone at 250 mg.

Keywords: Chitosan nanoparticles gel, *Portunus pelagicus*, Anti-bacterial activity, *Staphylococcus aureus*, *Escherichia coli*

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INTRODUCTION

Chitosan, the second most common natural polysaccharide obtained from *crustacean* shells, is a partially deacetylated derivative of chitin and a mixture of β-(1-4) linked d-glucosamine and N-acetyl-d-glucosamine [1, 2]. Chitosan, a naturally occurring biopolymer, is an excellent candidate for designing wound dressing materials due to its antimicrobial and hemostatic properties [1, 3]. In most studies, it has been shown that chitosan inhibits microbial growth [1, 2]. Besides, its non-toxic, biodegradable, biocompatible, and anti-bacterial and anti-inflammatory properties allow chitosan to be used in various contexts, one of which is in pharmaceutical preparations [1, 4, 5].

Chitosan Nanoparticles (CS-NPs), a chitosan derivative possessing outstanding physicochemical properties, are considered as delivery systems due to their various advantages, such as their ability to pass through the smallest capillaries due to their small volume and pass through gaps in cells and tissues [6-10]. They can also reduce toxic side effects and improve drug usability. Nanoparticles prepared from various materials and composites have shown anti-bacterial power against various types of bacteria [11, 12]. CS-NPs have been prepared through various techniques, including ionotropic gelation, microemulsions, solvent diffusion emulsification, polyelectrolyte complexes, and inversion micelle methods [8]. CS-NPs have shown improved biological activities, including antimicrobial [6, 13], anticancer [14], anti-inflammatory and antioxidant [15, 16], and antinociceptive and immunomodulatory activities [16, 17].

Nanomaterials have been prepared and tested in various ways, such as polymeric, lipidic, inorganic and inorganic-organic hybrid nanoparticles, nanocrystalline microemulsions, polymers, dendrimers, nanogels, nanofibers, and nano silver dressings [18-21]. These nanocarriers can be designed with various surface and volume chemistries, sizes, geometries, and architectures to optimize drug release, targeting, and blood circulation time. Positively charged surfaces, for example, often facilitate uptake of nanoparticles by cells [22, 23]. Polyethylene Glycol (PEG) on nanocarriers, for example, causes blood opsonins to repel each other and significantly increases the duration of the nanomaterial in circulation. Nanomaterial size impacts biological distribution and cell uptake [24-26].

According to a study, at all tested levels, *Staphylococcus aureus (S. aureus)* exhibited more growth suppression than *Escherichia coli (E. coli)*. For *S. aureus, E. coli*, and *Streptococcus mutans* (*S. mutans)*, the Minimal Bactericidal Concentration (MBC) was found at 25, 50, and 12.5% of the Bexident Post (BP) gel topical formula gel containing chitosan, Chlorhexidine, panthenol, and allantoin, respectively. The Minimal Inhibitory Concentration (MIC) for the same strain was found to be 12.5, 25, and 3.125% [27]. To treat infections caused by *S. aureus* and *E. coli,* such as gangrenous wounds, a compound from natural sources is needed, as *S. aureus* can develop into *Meticillin-Resistant Staphylococcus Aureus* (MRSA), which is more resistant to some antibiotics (*beta-lactams*) and more difficult to treat [28].

Over the past few decades, researchers have investigated the use of biocompatible polymers from natural or synthetic sources for tissue engineering applications due to their multifunctionality and flexibility. Wound dressings made from biocompatible polymers have shown promising results in overcoming problems in tissue regeneration, making them a good choice for the treatment of different types of wounds under various conditions. To overcome wound healing problems, choosing the right wound care product is very important [29]. In this study, chitosan nanoparticles from crab shell waste (*Portunus pelagicus*) that have been further characterized were developed into a topical gel formula to be applied as a pharmaceutical preparation in gangrenous wounds, where the loss of electrolytes from the wound and bacterial invasion can sometimes be life-threatening.

MATERIALS AND METHODS

Raw material

Fishermen in Pangkah Kulon Village, Ujungpangkah Subdistrict, Gresik Regency, East Java, Indonesia, stockpile a lot of crab

(*Portunus pelagicus*) shell waste, whose meat was purchased by crab meat export companies. A clean water stream was used to clean the grass, dried in an oven at 80 °C, and then pulverized with a 60 mesh sieve. The chemicals and solvents used were of analytical grade. Hang Tuah University's Joint Basic Laboratory, Universitas Hang Tuah Microbiology Laboratory, Universitas Airlangga Institute of Life Sciences, Engineering, and Techniques (LIHTR), Institut Teknologi Sepuluh Nopember (ITS) Chemistry Laboratory, and ITS Energy and Environment Laboratory provided the research tools.

Preparation of nanochitosan gel

Crab shell waste CS-NPs were made in gel form. The gel base was made by first heating distilled water at 80 °C for about 2 min; after warming, add carbomer little by little with stirring using a magnetic stirrer at a speed of 200 rpm. Then add methylparaben and stir until dissolved. After dissolving, add PEG, then add glycerin. Then enter the crab shell CS-NPs into the gel base, stirring gently until dissolved and mixed for about 5-8 min until a homogeneous preparation is obtained.

Table 1: The composition of CS-NPs gel

Function	Ingredient*	F0(%)	F1(%)
Active substance	Nanochitosan	-	1
Gelling agent	Carbomer	1	1
Preservative	Methylparaben	0.2	0.2
Co-surfactant	Propylene glycol	2	2
Emollients	Glyserin	2	2
Solvent	Distilled water	Ad 100	Ad 100

*Registered simple patent with registration number S00202409225 dated September 11, 2024

Characterization of nanochitosan gel

Physical evaluation

Physical characterization was performed using organoleptic test on texture, color, clarity, and odor of the gel by visual observation [30]. Furthermore, homogeneity test was conducted to assess the quality of CS-NPs gel.

pH determination

The pH determination was tested using a pH meter (LAQUA Instrument, US) [30]. The electrode was washed with distilled water before and after measurement. Next, the pH was measured using a standard calibration procedure and the electrode was dipped into the of CS-NPs gel preparation in a beaker glass replicated three times. The electrodes were rinsed after each measurement to avoid contamination from other samples.

Determination of adhesion

The adhesion test is carried out to see the nature of adhesion resistance. 0.5 g gel preparation was placed on a glass object, which was then covered with another glass object [31]. Then, pressed with a load of 50 g for 5 min. The glass object is hooked between the stative and given a load and then the time of adhesion of the preparation is measured, which is replicated three times.

Determination of spreadability

The spreadability test was carried out by taking 0.5 g of cells and placing a watch glass, then another watch glass was placed on it and allowed to stand for 1 minute. Then, 150 g of load was added and allowed to stand for 1 minute and measured the constant diameter, which was replicated three times.

Viscosity measurement

Viscosity of CS-NPs gel was carried out to determine the consistency of the gel, using a brookfield viscometer with spindle number 61 at 50 rpm, and the test was replicated three times [30].

Anti-bacterial activity against *S. aureus* and *E. coli*

Anti-bacterial activity was tested using the pitting diffusion method with several concentrations. Petri dishes were sterilized using an oven at 170 °C, while the media were sterilized using an autoclave at 121 °C for 15 min. Bacterial suspension isolates were subcultured into the media and standardized with 0.5 McFarland. 0.1 ml of each suspension was pipetted and then placed in a petri dish, followed by incubation at 37 °C for 1 x 24 h [32]. Subsequently, measurements were made in triplicate for each sample.

Data analysis

Data were presented in the form of mean value and standard deviation (SD). Data analysis in this study used statistical analysis with the IBM SPSS Statistics 25 application. The data obtained (inhibition of anti-bacterial activity test) was analyzed using a 3-factorial ANOVA test with α 5%.

RESULTS AND DISCUSSION

Characterization of nanochitosan gel

The selection of each additive used to make the crab shell waste CS-NPs gel preparation is very important for the safety and effectiveness of the nanoemulgel results. Important components in nanoemulgels are oils, surfactants, and cosurfactants. Oil is one of the important components as it also acts as a penetration enhancer [33]. In this study, crab shell waste (*Portunus pelagicus*) was made in the form of nanoemulgel because it was developed for topical drug preparations on gangrenous wounds, which generally occur infections so that they can overcome skin inflammation and help overcome infections caused by bacteria.

Based on the results of the physical evaluation repeated 3 times in table 2, it is known that both the gel base preparation and the 1% CS-NPs gel preparation of crab shell waste (*Portunus pelagicus*) developed for the treatment of diabetic ulcers have good characteristics because they meet the requirements in terms of homogeneity, pH, viscosity, adhesion, spreadability and dry time. Details of the characterization of CS-NPs gel results are presented in table 2.

Table 2: Physical evaluation results of crab shell CS-NPs gel preparation

Parameters	Gel base	CS-NPS 1% GEL	Requirements
Organoleptic	Shape: gel without any coarse granules Color: clear white Odor: odorless	Shape: gel without any coarse granules Color: clear white Odor: odorless	Without any coarse grains
Homogeneity	Homogeneous	Homogeneous	Homogeneous
pH	5.94±0.03*	6.46±0.02*	4.5-6.5
Stickiness	1.05±0.05*	1.97±0.15*	<10 sec
Spreadability	5.33±0.15*	5.00±0.00*	5-7 cm
Viscosity	7444±0.00*	7792±0.66*	6000-50000 cp

*Mean±SD (n=3)

The physical appearance of nanoemulsion and nanoemulgel preparations is very important. The physical appearance of nanoemulgels (organoleptic) is related to the acceptance of the preparation by patients. The physical appearance of nanoemulgel preparations is as follows clear white in color, has a texture that is almost the same as a regular gel without any coarse grains, odorless, and acceptable. O/W is the type of nanoemulgel used because it is formed when surfactants are dissolved in the water phase [33]. O/W nanoemulgels have a tendency to solubilize both hydrophobic and hydrophilic active components in their structure, which enhances the delivery of active components or drugs [34, 35].

The pH of the preparation is one of the physical properties that affect efficacy because it involves the process of drug release in the workplace, human skin is known to have a normal pH between 4.5 and 6.5 [36], the pH of the gel results of CS-NPs of crab shell waste (*Portunus pelagicus*) is still below 6.5. When an acute wound occurs, the pH of the skin changes to become more alkaline, which is around 7.4. After that, naturally, the body will respond by restoring the acidity of the skin to heal the wound. During this process, the environment around the wound will turn acidic. This acidic environment will inhibit the growth of bacteria so that infection does not occur. This is one of the activities in wound healing [37]. In order for a wound healing preparation to help this process, its pH should not be more than 6.5 [36]. Carbopol as a gelling agent, naturally has an acidic pH, so it needs to be neutralized to achieve the targeted pH in the formulation [38].

Other important aspects that affect the quality of topical preparations are spreadability and viscosity. Both of these factors affect the retention time of the preparation and its ease of application on the skin surface, which will ultimately have an impact on therapeutic effectiveness [39]. Smaller spreadability means higher viscosity, which will make the preparation more difficult to apply to the skin. Too high spreadability, which means too low viscosity, is also undesirable as it will shorten the retention time on the skin surface. Therefore, ideal values of spreadability and viscosity are required. The resulting spreadability of the CS-NPs gel was 5.0±0.00 cm, which meets the requirements of 5.0-7.0 cm, while the viscosity was 7792±0.66 cp. A lower concentration of Carbopol will increase the ability of the gel to spread [38]. When the concentration of gelling agent is lower, the viscosity will decrease and the spreadability will increase. The viscosity shift test was conducted to evaluate the physical stability of the gel in a storage environment. The smaller the percentage of viscosity shift, the better the physical stability of the gel [40].

Wound healing is a complex process, and throughout the process, it is important to keep the wound area sterile, free from contamination and bacteria that can cause infection, and hydrated [41]. Recent developments in wound management have found that a moist environment will accelerate the wound healing process, especially in the process of reepithelialization [42]. Carbopol, when dispersed into water and alkalized, will form a rigid three-dimensional network that has the ability to absorb water and retain the absorbed water content in its network [43]. PEG is a humectant that has an important role in topical preparations by maintaining skin moisture and increasing water absorption from the epidermis to deeper parts of the skin (dermis) [44]. Because water greatly facilitates microbial growth, to prevent this from happening in hydrogel formulations that contain more than 80% water, methylparaben is added as a preservative in the formula. Thus, the wound will not become contaminated or infected, and infection will not inhibit the wound-healing process [40].

Based on the results of the physical evaluation in table 2, it is known that the gel base preparation for diabetic ulcers added with 1% CS-NPs of crab shell waste has good characteristics because it meets the requirements in terms of homogeneity, pH, viscosity, adhesion, and spreadability. Anti-bacterial activity test was also conducted on the crab shell CS-NPs gel preparation, with the results shown in table 3. The test was carried out by agar well diffusion method using positive control Amoxcillin 500 mg capsule and for negative control using gel base (F0) used for CS-NPs gel which was carried out 3 times replication in each concentration of crab shell CS-NPs.

Table 3: Anti-bacterial activity test results of 1% CS-NPs gel of crab shell waste against *S. aureus* and *E. coli*

CS-NPs gel concentration1% (mg)	Zone of inhibition (mm)							
	S. aureus				*E. coli*			
	Replication			Mean±SD	Replication			Mean±SD
	1	2	3		1	2	3	
100	9.40	9.60	9.60	9.53±0.12	9.60	9.10	10.20	9.63±0.55
150	11.40	10.15	11.60	11.05±0.79	12.20	11.10	13.20	12.17±1.05
200	14.80	11.50	13.30	13.20±1.65	16.60	16.35	15.20	16.05±0.75
250	16.90	14.80	14.40	15.37±1.34	18.30	18.10	18.60	18.33±0.25
Control (+)*	18.30	19.70	16.90	18.30±1.40	33.70	32.20	36.10	34.00±1.97
Control (-)**	0.00	0.00	0.00	0.00±0.00	0.00	0.00	0.00	0.00±0.00

Data presented as mean±SD in which each treatment was repeated three times (n=3), *Positive control: Amoxcillin caps 500 mg, **Negative control: gel base (F0)

The results of the anti-bacterial activity test in table 3, show that the crab shell waste CS-NPs gel (*Portunus pelagicus*) can inhibit *S. aureus* and *E. coli* bacteria. An empty zone around the disk indicates the absence of bacterial growth. The wider the zone formed, the better the anti-bacterial effect. Zone of inhibition<12 mm is categorized as weak, 12-20 mm indicates moderate activity, and>20 mm is classified as strong category as anti-bacterial [45]. Therefore, the crab shell waste CS-NPs gel showed moderate anti-bacterial activity against *S. aureus* and *E. coli* bacteria.

In this study, the F0 sample was used as a negative control which was tested for anti-bacterial activity with the composition of the gel base without any content of CS-NPs from crab shell waste (*Portunus pelagicus*). The results showed that F0 did not inhibit the anti-bacterial activity of *S. aureus* and *E. coli*. This finding indicates that the crab shell waste (*Portunus pelagicus*) CS-NPs gel has anti-bacterial activity, and the inhibition of *S. aureus* and *E. coli* bacteria is not influenced by the gel base. Based on the inhibition zone data, then conducted a 3-factorial ANOVA test obtained the results of a significance value of p = 0.001, indicating that the results were significant, namely $p<0.05$. Then each data on the concentration and sample of the crab shell waste CS-NPs gel (*Portunus pelagicus*) shows a significant difference.

Several studies have developed CS-NPs gel preparations mostly derived from plants such as *Pandanus amaryllifolius R.* extract [33], *Leucaena Leucocephala* (Lam.) *De Wit*leaves extract. [40], or Chitosan/Chlorhexidine Gel Commercial [28]. In addition, there are studies on CS-NPs that can heal wounds on the skin [46], and CS-NPs that shows potential as a promising anti-bacterial agent [47]. Developments in the use of nanotechnology in the effective and individualized management of diabetes in the future will result in better treatment, fewer complications and a better standard of living for diabetic patients [48].

CONCLUSION

This study found that CS-NPs gel derived from crab (*Portunus pelagicus*) shell waste had moderate anti-bacterial activity against *S.*

aureus and *E. coli* bacteria. The zone of inhibition of *S. aureus* was largest at 15.37±1.34 mm and *E. coli* at 18.33±0.25 mm with 250 mg CS-NPs gel. The more CS-NPs gel content in the tested crab shell waste, the greater the bacterial inhibition zone. This CS-NPs gel is expected to be used in gangrene patients as it allows faster wound recovery. Therefore, further research will use these findings to determine how CS-NPs gel from crab shell waste functions as an anti-bacterial and anti-inflammatory *in vivo* studies.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally, where the first author Yusan LY conducts research from start to finish, while the second author Subagio H contributes in obtaining crab shell waste and helps isolate crab shell waste chitosan.

CONFLICT OF INTERESTS

Declared none

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