https://doi.org/10.48048/tis.2024.7243

Utilization of Chitosan from Fresh Water Lobster (*Cherax quadricarinatus*) Shells in Anti-Acne Gel Preparations

Ridwanto Ridwanto*, Vici Delvio Rosa, Zulmai Rani and Ziza Putri Aisyia Fauzi

Department of Pharmacy, Faculty of Pharmacy, Universitas Muslim Nusantara Al Washliyah, Sumatera Utara 20147, Indonesia

(*Corresponding author's e-mail: rid.fillah66@gmail.com)

Received: 24 June 2023, Revised: 13 July 2023, Accepted: 25 August 2023, Published: 15 December 2023

Abstract

Cutibacterium acnes is one of the bacteria that cause acne on the face. Freshwater crayfish (*Cherax quadricarinatus*) shells have been abandoned and are not used by the community; thus, their shells are processed into economically valuable compounds such as chitosan. Chitosan has antibacterial characteristics that can inhibit acne bacterial development. The purpose of this study is to see if chitosan derived from crayfish shells may be stable in the form of an anti-acne gel and has antibacterial action against *Cutibacterium acnes*. Anti-acne gel was made from crayfish shells at concentrations of 2, 4 and 6 %, then gel formulations were evaluated and antibacterial efficacy against *Cutibacterium acnes* germs was tested. Chitosan produced by freshwater lobster shells was 68.75 %. The results of the test of freshwater crayfish shells against *Cutibacterium acnes* revealed that the average diameter of the inhibition was 6.34 mm at a concentration of 2 %, 6.57 mm at a concentration of 4 % and 6.90 mm at a concentration of 6 %; thus, a concentration of 6 % has the best inhibition. According to the organoleptic test, there was no change in shape or color. According to the homogeneity test, there are no lumps or coarse grains. According to the spreadability test, all preparations were within a 56 cm range. The adhesion test shows a satisfactory adhesion time of more than 1 s. The pH test revealed that all preparations were within the normal skin pH range, indicating that the recipe satisfied the specifications. Anti-acne gel prepared from the shell of a freshwater crayfish displays antibacterial action against *Cutibacterium acnes* as a cause of acne, with an inhibitory diameter of 6.11 mm at 2 % concentration, 6.8 mm at 4 % concentration and 7.11 mm at 6 % concentration. The diameter of the inhibitory zone in the positive control was 11.9 mm.

Keywords: *Cherax quadricarinatus*, Chitosan, Anti-acne gel, *Cutibacterium acnes*

Introduction

Acne (acne vulgaris) is an inflammatory skin illness that affects the pilosebaceous glands (oil glands). Acne is frequently caused by excessive oil production in the sebaceous glands, resulting in clogged skin pores [1]. Acne develops as a result of polysebaceous obstruction and inflammation caused by the bacteria *Cutibacterium acnes*, *Staphylococcus epidermidis* and *Staphylococcus aureus*. Because oily skin clogs pores, these anaerobic bacteria grow quickly and produce acne. *Cutibacterium acnes* causes inflammation by damaging the stratum corneum and stratum germinativum by secreting chemicals that can destroy the pore walls [2], as well as the formation of pus on the epidermal layer, which is known as pimples [3]. *Cutibacterium acnes* contributes to acne pathogenesis by generating lipases, which break down free fatty acids from skin lipids. When exposed to the immune system, these fatty acids can cause tissue inflammation and promote acne. Getting Rid of Acne Teens treat acne with the chemicals benzoyl peroxide and retinoic acid, as well as medicines like clindamycin or doxycycline. If antibiotics are used inappropriately, there will be side effects, one of which is the emergence of resistance, where resistance is the ability of bacteria to survive against antibiotics; in other words, antibiotics will no longer be able to kill bacteria, necessitating the development of the latest generation of antibiotics. As a result, it is critical to find natural remedies with few side effects that can alleviate acne problems caused by bacterial proliferation [4].

Some natural ingredients that can suppress the growth of acne-causing bacteria include seaweed, chitosan and so on [5,6]. Chitosan is a polysaccharide formed by the deacetylation of chitin, which is often derived from crustacean animal skin debris, such as freshwater crayfish shells [7]. Furthermore,

chitosan can be produced from reed shells [8]. Lobster sellers typically only sell the meat, while the lobster shells are discarded and left to rot. If allowed unchecked, this will result in environmental degradation and harm to the environment's beauty. Gel is a popular type of anti-acne preparation on the market since it is easier to apply, spreads fast on the skin and has clear colors. This makes gels more appealing to many people than conventional anti-acne treatments. Based on the description above, researchers are interested in utilizing chitosan from crayfish shells as the main ingredient for making anti-acne gel against *Cutibacterium acnes*.

Materials and methods

In this study, the materials used were freshwater lobster shells taken from Central Aceh District, acetic acid, NaOH pro analysis (p.a)., HCl p.a., $AgNO_3$, Phenolphthalein (PP) indicator, Aquadest, Na CMC, propylene glycol and methylparaben. The tools used in this study were: Pyrex glassware, stir bar, dropper pipette, test tube, mesh 50 sieves, oven, furnace, water distillation apparatus, blender (Miyako), pH meter, analytical balance, aluminum foil, petri dish, hot plate, mortar, tamper, parchment paper and desiccator.

Research procedure

Sample processing

Live crayfish weighing up to 10 kg were removed from their shells and meat before being washed and dried in an oven. To make a fine powder, the shell is mashed and sieved.

Deproteination process

The shell powder obtained from demineralization was mixed with a 3.5 % NaOH solution in a solvent-to-sample ratio of 1:10 (w/v). To obtain a solid in the form of supersenicate, the mixture was heated at 40 - 50 °C for 4 h while stirring at a speed of 50 rpm and then centrifuged for 15 min at a speed of 2,000 rpm. The final filtrate was examined with the PP indicator; if there is no brick-red color change, the remaining OH-ions have vanished. Furthermore, the solid is filtered and chilled before being washed with distilled water to extract chitin. The produced solid was dried in an oven at 80 °C for 24 h [9].

Demineralization process

The shell powder, which had been crushed to a mesh size of 50, was added to a 1.5 N HCl solution in a 1:15 (w/v) ratio. To create a supersenicate form, the mixture was heated at 40 - 50 °C for 4 h while stirring at 50 rpm and then centrifuged for 15 min at 2,000 rpm. To eliminate any leftover HCl, the substance is rinsed with distilled water. The final filtrate was tested with $AgNO_3$ solution; if no white precipitate formed, the remaining Cl-ions have vanished. The solids were then dried for 24 h in an oven at 80 °C [8].

Depigmentation process

As a result of the demineralization process, each was placed in a different beaker, then 0.315 % NaOCl solution was added with a ratio of 1:10 (w/v) between the sample and the solvent, then heated at 40 °C for 1 h, then the residue obtained was washed with distilled water until the pH was neutral, then dried in an oven with 80 °C milk, then cooled in a desiccator, and the results were weighed [10].

Deacetylation process

Following the demineralization and deproteination operations, the products were deacetylated by adding 60 % NaOH in a 1:20 (w/v) ratio. The mixture was agitated and heated at 40 - 50 °C for 4 h with a stirring speed of 50 rpm, then centrifuged for 15 min at a speed of 2,000 rpm to achieve a supersenicate solid. The collected solids were neutralized with distilled water until the pH reached neutral. The solid was then dried for 24 h in an oven at 80 °C [11].

Chitosan characterization

Water content

The weight of 0.5 g of chitosan sample was known after it was placed in a different porcelain cup. For 2 h, samples were cooked in an oven at 100 - 105 °C. It was then chilled in a desiccator for 30 min and weighed until it reached a steady weight [12].

Ash level

The sample is weighed up to 0.5 g and then placed in a different crucible, the weight of which is known. The sample is then ashed in a furnace at 600 °C till white ash is recovered. The cup is then chilled in a desiccator and weighed [13].

Chitosan solubility

Chitosan solubility is a parameter that can be used to reference chitosan quality standards. The higher the solubility of chitosan, the higher its quality. Chitosan was dissolved in acetic acid at a concentration of 2 % in a 1:100 (g/mL) ratio [14].

Analysis of Fourier Transform Infrared (FTIR)

An FTIR spectrophotometer with wave numbers ranging from 4,000 to 400 cm^{-1} was used to determine the chitosan functional groups produced. The baseline approach was developed and can be used to determine the degree of deacetylation based on FTIR data [15].

$$\% DD = 1 - (A1651/A3421 \times 1/1.33)$$

Gel preparation formulation

The formulation of crayfish shell chitosan gel preparations was carried out by comparing the 4 formulations. Each formula had a different chitosan concentration. Chitosan formulations are shown in **Table 1**.

Tabel 1 Acne gel preparation formulation.

No	Material	Formulas			
		F0	**F1**	**F2**	**F3**
1	Chitosan (g)	-	2	4	6
2	Glacial Acetic Acid (g)	-	100	100	100
3	Na-CMC (g)	2.5	2.5	2.5	2.5
4	Glycerin (g)	8	8	8	8
5	Methyl Paraben (g)	0.12	0.12	0.12	0.12
6	Propylene Ethylene Glycol (g)	4	4	4	4
7	Distilled water ad (mL)	100	100	100	100

The preparation of instruments and materials is the first step in the process of creating acne gel formulations. The base gel was then created by weighing 2 chitosan, 4 chitosan and 6 g and dissolving them in 100 mL of 2 % glacial acetic acid in a 250 mL beaker glass. The mortar was then filled with 40 mL of boiling distilled water, which was uniformly sprinkled with 2.5 g of CMC Na, before being closed and stored in a dark place for 30 min. After 30 min, the Na-CMC was aggressively crushed till it became homogenous and translucent. Glycerin and PEG were then added, and the mixture was homogenous. After that, methylparaben was added, which had been dissolved in distilled water and crushed until homogenous.

Evaluation of acne gel preparations

Physical and biological evaluations are included in formula evaluation. Examining the stability of the preparation, evaluating the pH, testing the adherence and spreadability, examining the viscosity and testing for skin irritation are all part of the physical examination. Using the agar diffusion method, the biological evaluation involved assessing the antibacterial activity of an anti-acne gel formulation against *Propionibacterium acne*.

Acne gel physical stability test

The physical stability of the acne gel is tested using organoleptic tests, which include the texture, color and aroma of the preparations after being left for 24 h [16].

pH test

A pH meter is used to test the pH, which begins with calibration with a pH 4 buffer solution. The pH value is read on the pH meter scale and shown as a number on the digital pH meter [17].

Stickiness test

Gel weighing up to 0.25 g is placed on a glass object whose area has been determined. Then, for 5 min, another glass object is placed on top of the gel and loaded with 1 kg. The test glass object was then inserted, the load weighing 80 g was released, and the time until the 2 glass objects were released was recorded [18].

Spreadability test

The 0.5 g of gel were weighed and placed in the middle of a round glass. The round glass cover was weighed first, then placed on top of the gel mass and left for 1 min. The length of the diameter of the gel that spread was measured, then 50 g of additional weight were added, it was allowed to stand for 1 min, and the diameter of the gel that spread was recorded. The addition of the load is continued until a constant diameter is obtained [19].

Homogeneity test

The gel sample is homogeneously tested by smearing it on a piece of glass or another suitable transparent material. The preparation must be homogenous in composition and free of coarse granules [7].

Viscosity power test

The gel preparation's viscosity was tested using a Brookfield viscometer with spindle No. 6 in accordance with SNI 0364412000.

Irritation test on volunteers

The patch test technique was used to examine the irritation of the gel preparation by putting the formula preparation to the back of the volunteer's hand. This test was performed on 20 volunteers using a positive control, that is, no active ingredient mixture, and then evaluating the symptoms that occurred. If irritation develops, a skin reaction will occur after the preparation is applied to the skin. Irritation that occurs immediately after attachment is referred to as primary irritation, but irritation that occurs many hours later is referred to as secondary irritation [20].

Microbiological testing of preparations

The diameter of the growth inhibition of *Cutibacterium acnes* bacteria was measured in a microbiological test of chitosan anti-acne gel from crawfish shells using the agar diffusion method. The test technique is as follows: The test medium wells are dripped with 50 L of the gel preparation using a micropipette, then incubated at 35 °C for 24 h, and the diameter of the inhibition area (clear zone) is measured using a caliper [21].

Results and discussion

Chitin isolation and chitosan synthesis

The study's findings revealed that the percentage yield of chitin was 12.99 %. The chitin isolation stage in this work began with demineralization and continued with deproteination for 4 h at a temperature of 40 - 50 °C. Apart from the heating temperature and stirring time, this had an effect on the % yield of chitin produced. To obtain chitosan, the next step is deacetylation. At this step, the chitin was soaked in 60 % p.a. NaOH in a 1:20 (w/v) ratio, then stirred with a stirrer for 4 h at a temperature of 40 - 50 °C. The acetyl group in chitin is removed by deacetylation in a strong hot base by breaking the connection between the carbon in the acetyl group and the nitrogen in the amine group. Chitosan is formed from chitin via a hydrolysis reaction of an amide by a base. Chitin serves as the amide, and NaOH serves as the base. After an addition reaction in which the -OH group entered the $NHCOCH_3$ group, the CH_3COO group was removed to create an amine, namely chitosan [9]. The yield of chitosan achieved in this investigation was 63.72 %. The FTIR analysis of crayfish shell chitosan are in **Figure 1**.

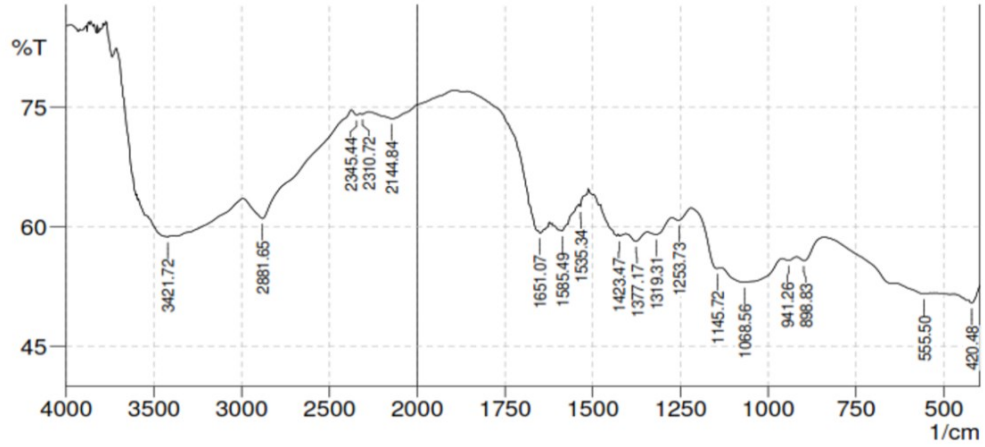


Figure 1 FTIR analysis results of crayfish shell chitosan.

Based on the spectra above, the wave number in chitosan is 3,421 cm^{-1} in lobster shell chitosan as a result of the stretching vibration of the -NH group. The appearance of an absorption band at wave number 1,068 cm^{-1} suggests that the C-O group is stretching. The stretching vibration of the C-H groups of the alkanes appears to be responsible for the absorption at wave number 2,881 cm^{-1}. A wave number of 1,377 cm^{-1} indicates the existence of a $-CH_3$ group linked to an amide ($-NHCOCH_3$). The stretching band of an amide-NH group can be seen in the absorption band at wave number 1,651 cm^{-1}. The bending vibrations of NH amides are known in the region of 1,570 - 1,515 cm^{-1}. Because the $-CH_3$ group's broad absorption in the amide overlaps with the bending vibration of the amide NH group, it is not apparent in this spectra. The sharp absorption at wave number 898 cm^{-1} indicates that the concentration of silica minerals in chitin is still low [22].

Chitosan characterization

The chitosan obtained was analyzed in order to assess its quality. The chitosan characterization are shown in **Tabel 2**. An organoleptic inspection (texture, color and odor), a water content test, an ash content test and solubility in 2 % acetic acid were all performed. The study's characterization of chitosan results were compared to the quality of SNI No. 7949, Year 2013.

Table 2 Chitosan characterization.

Parameter organoleptic examination	SNI (No. 7949, Year 2013)	Vaname shrimp skin chitosan
Texture, color	Powder, light brown to white	Powder, yellowish white
Water content (%)	≤ 12	0.189
Ash level (%)	≤ 5	2.8
The solubility of chitosan in glacial acetic acid is 2 %.	Late	Late

Based on the table above, the chitosan obtained has met the standard values so that it can be used for various applications.

Preparation evaluation

Organoleptic test of acne gel preparations

Anti-acne gel derived from lobster shell chitosan evaluated. The gel preparations' color, fragrance and form did not change significantly from week 0 to week 3. The odor created by these preparations is due to the inclusion of perfume as an aroma, whereas the larger dosage affects the turbidity in the gel preparation.

Homogeneity testing

The gel preparation does not form coarse grains, so it can be concluded that the anti-acne gel preparation is said to be physically homogeneous and the gel ingredients used in the formulation are completely dissolved and mixed.

pH testing of acne gel preparations

The pH test determines how much pH the gel produces. Gel preparations that are morally safe for use on the skin, namely those that fall within the pH range of 4.5 - 6.5, are safe and do not irritate the skin. The pH measurement from week 0 to week 3 are shown in **Tabel 3**.

Table 3 pH measurement from week 0 to week 3.

Formulas	pH average (Mean ± SD)	
	Week 0	Week 3
Blank	7 ± 0.57	6.8 ± 0.05
2 %	6.6 ± 0.57	6.6 ± 0.11
4 %	5.3 ± 0.57	6.5 ± 0.15
6 %	6.5 ± 0.57	6.6 ± 0.05

Based on the data above, it shows that the preparation meets the pH requirements that are the same as the skin pH, namely 4 - 8.

Stickiness test

The goal of assessing the gel's adhesion is to determine its capacity to adhere to the skin. A gel that is too tightly adhered to the skin will clog the pores. Furthermore, if the gel is too weak to adhere to the skin, it will not have any therapeutic impact. An excellent gel has a time of adhesion of more than 1 s. Stickness gel of chitosan are shown in **Tabel 4**.

Table 4 Stickiness test results.

Formulas	Stickiness (per s)
2 %	1.53
4 %	2.37
6 %	2.06

The table above shows that gel preparations have very good adhesion because the adhesion lasts longer than 1 s.

Spreadability testing

The gel dispersion test determines the gel's ability to spread when applied to the skin. The stronger the gel's spreading capacity, the easier it is to use. Spreadability gel of chitosan are shown in **Tabel 5**.

Table 5 Spreadability test.

Formulas	Spreadability (cm)
Blank	5.5
2 %	5.7
4 %	6
6 %	6

Testing the spreadability of anti-acne gel preparations showed that the preparations had good spreadability, which was in the range of 5 - 7.

Viscosity testing

The purpose of viscosity testing is to determine the thickness of the gel. A good gel has a consistency that is neither too thick nor too runny. Viscosity gel of chitosan are shown in **Tabel 6**. If the gel formula is excessively thick, the active component may not be released. A good gel viscosity range is 2,000 - 4,000 centipoise (cP), which is equivalent to 20 - 40 density Pascal second (dPas) [23].

Table 6 Viscosity testing.

Formulas	Average viscosity (Mean ± SD)	
	Week 0	Week 3
2 %	4,480 ± 0.57	3,450 ± 1.52
4 %	4,420 ± 1.00	3,323 ± 1.52
6 %	4,150 ± 1.00	3,360 ± 0.57
Blank	3,390 ± 0.57	3,145 ± 1.00

Based on the data above, it shows that the anti-acne gel meets the requirements of a good gel, which are in the range of 2,000 - 4,000 centipoise (cP).

Volunteer ırritation test

The irritation test of the anti-acne gel from chitosan lobster shells was witnessed by the selected volunteers based on the results of the irritation test. The subjects in this study did not experience any irritation as a result of the anti-acne gel. At the time of testing, the most annoying symptoms observed were skin redness, itching and roughness. Based on the table, it is possible to conclude that this preparation is suitable and safe for usage.

Antibacterial activity test against Cutibacterium acnes

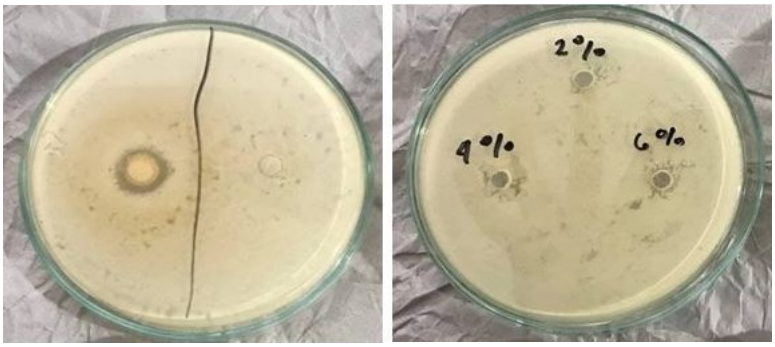
Antibacterial activity against *Cutibacterium acnes* can be seen in the **Table 7**.

Table 7 Antibacterial activity test against *cutibacterium acnes*.

Formulas	Obstacles zone (mm)			Average (mm) (Mean ± SD)
	Repetition 1	Repetition 2	Repetition 3	
2 %	6.11	6.8	6.13	6.34 ± 0.39
4 %	6.8	6.8	6.12	6.57 ± 0.39
6 %	6.8	6.8	7.11	6.90 ± 0.17
Positive control (+)	10.4			
Negative control (−)	0			

According to the table above, the diameter of the inhibitory zone on *Cutibacterium acnes* varies with concentration. The diameter of the inhibition zone was 6.34 mm at a concentration of 2 %, 6.57 mm at a concentration of 4 % and 6 mm at a concentration of 6 %, while the other positive controls obtained an inhibition of 10.4 mm and a negative control obtained resistance of 0 mm or did not provide a resistance response.

Inhibiting the growth of *Cutibacterium acnes*, the inhibition zone demonstrated antimicrobial sensitivity to chitosan from crayfish (*Cherax quadricarinatus*) shells. The smallest inhibition zone is at 2 % concentration, whereas the highest inhibition zone is at 6 % concentration. This study's inhibition category falls under the moderate inhibition category. This demonstrates that the higher the concentration, the stronger the suppression of bacterial growth. Inhibition zone of blank anda anti-acne gel of chitosan are shown in **Figure 2**.



(a) (b)

Figure 2 (a) inhibition zone of blank and (b) anti acne gel.

Conclusions

Chitosan derived from crayfish shells (*Cherax quadricarinatus*) can be manufactured into an anti-acne gel solution that is stable in storage and does not change color, shape or odor. Anti-acne gel derived from the shell of a freshwater crayfish (*Cherax quadricarinatus*) displays antibacterial efficacy against the acne-causing bacteria *Cutibacterium acnes*. Anti-acne gel prepared from the shell of a freshwater crayfish displays antibacterial action against *Cutibacterium acnes* as a cause of acne, with an inhibitory diameter of 6.11 mm at 2 % concentration, 6.8 mm at 4 % concentration and 7.11 mm at 6 % concentration. The inhibition zone diameter of Merck Acnes anti-acne gel was 11.9 mm.

References

[1] T Movita. Acne vulgaris. *Cont. Med. Educ.* 2013; **40**, 269-72.
[2] SH Anjelina. Antibacterial activity of ethanolic extract of kitolod (*Hippobromalongiflora*) leaf against *Staphylococcus aureus* and *Salmonella typhi*. *Asian J. Pharmaceut. Res. Dev.* 2020; **8**, 52-4.
[3] V Indrajati. *Herbal ahli atasi penyakit*. Penebar Swadaya Grup, East Jakarta, Indonesia, 2013.
[4] VE Kaban, N Nasri, HD Syahputra, MF Lubis and D Satria. Test the antibacterial activity of karenda leaf extract (*Carissa carandas* Linn.) against the bacteria propionibacterium acne and staphylococcus epidermidis. *J. Pharmaceut. Health Res.* 2023; **4**, 91-6.
[5] WA Setyati, AB Susanto, DBP Pamungkas, DB Makrima and JL Senduk. Isolation and identification of seaweed-associated bacteria and their antibacterial activity against skin disease agents. *Trends Sci.* 2023; **20**, 6517.
[6] SD Hardiningtyas, FA Putri and I Setyaningsih. Antibacterial activity of ethanolic Spirulina platensis extract-water soluble chitosan nanoparticles. *IOP Conf. Ser. Earth Environ. Sci.* 2022; **1033**, 012053.
[7] Z Rani, HM Nasution, VE Kaban, N Nasri and NB Karo. Antibacterial activity of freshwater lobster (*Cherax quadricarinatus*) shell chitosan gel preparation against *Escherichia coli* and *Staphylococcus aureus*. *J. Appl. Pharmaceut. Sci.* 2023; **13**, 146-53.
[8] NI Sepvina, R Ridwanto and Z Rani. Toxicity test of feather clam shell chitosan (anadara antiquata) using the brine shrimp lethality test (BSLT) method (*in Indonesian*). *Jurnal Ilmiah Ibnu Sina* 2022; **7**, 380-9.
[9] S Agustina and Y Kurniasih. Making chitosan from shrimp shells and its application as an adsorbent to reduce Cu metal levels. *In*: Proceedings of the Seminar Nasional FMIPA UNDIKSHA III Tahun, Buleleng, Indonesia. 2013.
[10] EJ Dompeipen, M Kaimudin and RP Dewa. Isolation of chitin and chitosan from shrimp shell waste. *Majalah Biam* 2016; **12**, 32-9.
[11] DE Rahayu and S Aulia. Reducing the color and TSS of liquid waste from Samarinda sarong weaving using chitosan from crab shell waste. *Jurnal Purifikasi* 2015; **15**, 1-11.
[12] AS Daulay and K Gurning. Isolation and characterization of chitosan from sea and freshwater waste, North Sumatera Province, Indonesia. *Rasayan J. Chem.* 2022; **15**, 780-5.
[13] A Fadli, D Drastinawati, O Alexander and F Huda. Effect of chitin/NaOH mass ratio and reaction time on the characteristics of chitosan synthesized from dried shrimp industrial waste. *Jurnal Sains Materi Indonesia* 2018; **18**, 61-7.

[14] MS Hossain and A Iqbal. Production and characterization of chitosan from shrimp waste. *J. Bangladesh Agr. Univ.* 2014; **12**, 153-60.
[15] TA Khan, KK Peh and HS Ch'ng. Mechanical, bioadhesive strength and biological evaluations of chitosan films for wound dressing. *J. Pharm. Pharmaceut. Sci.* 2000; **3**, 303-11.
[16] RR Robiatun, A Pangondian, R Paramitha, Z Rani and ED Gultom. Formulation and evaluation of hand sanitizer gel from clove flower extract (*Eugenia aromatica* L.). *Int. J. Sci. Tech. Manag.* 2022; **3**, 484-91.
[17] VE Kaban, N Nasri, HD Syahputra, R Fitri, Z Rani and MF Lubis. Gel preparation formulation from methanol extract of avocado seeds (*Persea americana* Mill.) as a wound healer in male rats (*Rattus norvegicus*). *Herbal Med. J.* 2022; **5**, 12-8.
[18] F Firlianty, R Rario, EB Naibaho and E Elita. Characteristics of HPMC gel from toman fish extract (*Channa micropeltes*). *Agrikan Jurnal Agribisnis Perikan* 2019; **12**, 8-12.
[19] A Ningtias and Z Rani. Formulation of cheek coloring preparations in solid form using buni fruit extract (*Antidesma bunius* (L.) Spreng). *Insologi Jurnal Sains Teknologi* 2022; **1**, 448-60.
[20] R Fitri, HD Syahputra, N Nasri, VE Kaban and Z Rani. Formulation of a biocellulose mask containing the essence of Aloe vera (L.) Burm. f combination with vitamin E as anti-aging. *ScienceRise Pharmaceut. Sci.* 2022; **6**, 36-42.
[21] I Indarto, W Narulita, BS Anggoro and A Novitasari. Antibacterial activity of binahong leaf extract against Propionibacterium acnes. *Biosfer Jurnal Tadris Biologi* 2019; **10**, 67-78.
[22] A Wafi, L Atmaja and YL Ni'mah. Analysis of tensile strength and elongation of gelatin - chitosan films. *Alchemy J. Chem.* 2020; **8**, 1-8.
[23] T Imanto, R Prasetiawan and ER Wikantyasning. Formulation and characterization of aloe vera powder nanoemulgel (Aloe Vera L.). *Pharmacon Jurnal Farmasi Indonesia* 2019; **16**, 28-37.