THE 5th INTERNATIONAL CONFERENCE ON HEALTH POLYTECHNICS OF SURABAYA (ICOHPS)

2nd International Conference of Medical Laboratory Technology (ICoMLT)

Characteristics of Chitosan in Gonggong Snail Shells in 2022

Romalina1, Dewi Puspa Rianda2, Hevi Horiza3, Iwan Iskandar3

1Prodi DIII Nursing Poltekkes Ministry of Health Tanjungpinang

2Prodi DIII Nursing Poltekkes Ministry of Health Tanjungpinang

3Prodi DIII Sanitation Poltekkes Ministry of Health Tanjungpinang

3Prodi DIII Sanitation Poltekkes Ministry of Health Tanjungpinang

Corresponding author: ***romalina@poltekkes-tanjungpinang.ac.id***

ABSTRACT

Chitosan biopolymer is effectively depolymerized to release N-acetyl-β-D-glucosamine to initiate fibroblast proliferation during the wound healing process. This research was the first step to explore natural wound topical ingredients of the Riau Islands which were appropriate for use in the wound healing process. The objective of this research was to extract and describe the characteristics of chitosan produced from gonggong snail shells. The extraction of chitosan in the shell of the gonggong snail begun with cleaning it from dirt, drying it and drying it under the sun. Then the shell of the gonggong snail was crushed to form flakes and then the process of demineralization, deproteinization and deacetylization was conducted. Then the chitosan obtained was characterized to determine the quality of the chitosan produced. The characterization included tests for water content, ash content, solubility in 2% acetic acid and ionization degree. This research was carried out in the Chemical Laboratory of Padang State University in May 2022. The stages of this research were preparation of gonggong snail shell samples, manufacture of chitosan and then determine the characteristics of chitosan. After doing the research, the results of the characteristics of chitosan included water content of 0.71% in 100 grams of sample, 95.90% ash content in 100 grams of sample, soluble solubility and ionization degree of 79.8821% in 100 grams of sample.

Keywords: *Shell, Gonggong snail, Characteristics, Chitosan*

BACKGROUND

The Bahamas, Fiji, Philippines, Indonesia and Papua New Guinea were the first five countries to receive approval from the United Nations as archipelagic states.

Indonesia is the largest archipelagic country in the world consisting of 17,504 islands with a population of 270,203,917 in 2020. The Riau Islands is one of the youngest provinces in the Republic of Indonesia. Geographically, the Riau Islands province is bordered by neighboring countries, such as Singapore, Malaysia, and Vietnam. Riau Islands Province consists of 9,982.88 km2 of land and 415,231.79 km2 of sea (96%) is water with 2,408 islands (Pemerintah Provinsi Kepulauan Riau, 2021).

The Riau Islands has enormous potential in making marine and fisheries a driver of economic growth. Riau Islands Province has great resource potential in coastal and marine areas. The fisheries sector is the main source of livelihood for the people of the Riau Islands Province. Most of the fishery production in the province is marine capture fisheries with production in 2013 of 140,597 tons. Utilization of the sea and its various resources can not only be relied upon in encouraging the economic growth of a region. However, through the right strategy, the marine and fisheries sector can be used as a solution for a sustainable source of income for the community, to reduce poverty and income inequality between regions. The determination of the Fisheries Management Area of the Republic of Indonesia (WPP-RI) needs to be carried out to ensure optimal and sustainable utilization of fish resources. WPP-RI is a fisheries management area for fishing, fish cultivation, conservation, research, and fishery development which includes inland waters, archipelagic waters, territorial seas, additional zones, and the Indonesian Exclusive Economic Zone (ZEEI) (BPS KEPRI, 2016).

The gonggong snail is one of the soft animals (Mollusca) belonging to the gastropod class from the Strombus canarium species (Zaidi et al. 2009 in Ricky 2016). The results showed that 3 types of gastropods were found; Strombus turturella, Natica Gualtieriana, and Lambis on the coast of Bintan Regency, Riau Islands. The highest abundance value of gonggong snails was in the Lepah River) with a total of 0.5 ind/m2, in Sungai Kecil with a total of 0.04 ind/m2 and while in Sekera gonggong snails were not found. The habitat of gonggong snail was found on the sand substrate at all stations. The pattern of distribution showed that gonggong snails live in groups. Community activities on the coast of Bintan Regency that affect the abundance of gonggong snails include fishing to meet the needs of local consumption and tourists, sand mining in the sea and on the beach. Utilization of gonggong snails in culinary as a source of protein for the people of Riau Islands. This is in line with the research which showed that the protein profile of the thick-shelled and shelled gonggong meat was 100 individuals, respectively, taken from Madong Village, Bintan Island, Riau Islands Province using histone protein primers of H2A and H2B in the 75 bp target gene. Antimicrobial activity in thick shell boiled Bintan gonggong meat extract containing histone protein as a favorite food in Bintan has the ability to inhibit S. aureus and E. coli bacteria, so it is strongly suspected that boiled gonggong of Bintan can be used as a candidate for functional food of boiled gonggong typical of Bintan Islands. Riau (Rosady, Astuty and Prihadi, 2016 and Viruly et al, 2019).

Based on the research conducted by Ricky (2016) that the catch of fishermen on the gonggong snails was directly sold to “seafood” restaurants which were used as culinary tourism by local or foreign tourists who came to the Riau Islands. The gonggong snail used in culinary tourism is only its meat while the shell itself becomes waste. The gonggong snail shells containing chitin and chitosan which are widely used in everyday life, for example as adsorbents for heavy metal waste and dyes, preservatives, antifungal agents, cosmetics, pharmaceuticals, flocculants, anti-cancer, and anti-bacterial (Stephen. 1995; Lee, et all., 1999: Liu, et all., 2006 in Pratiwi, 2014).

The waste from the gonggong snail shells is used to produce chitosan (Kusumawati 2009 in Pratiwi, 2014; Suhardi, 2012). Likewise with research by Horiza et al, 2018 that found chitosan content in gonggong snail shells. The next research explained that the main biochemical activities of chitin and chitosan-based materials in the wound healing process are through activation of polymorphonuclear cells, activation of fibroblasts, production of cytokines, migration of giant cells, and stimulation of type IV collagen synthesis. Chitosan biopolymer is effectively depolymerized to release N-acetyl-β-D-glucosamine to initiate fibroblast proliferation during the wound healing process (Singh.R, Shitiz.K and Singh.A, 2017 in Kurniawaty, 2019).

Wounds are loss or damage of body tissue caused by many things such as trauma, temperature differences, exposure to chemicals, contact with electricity and bites. A healthy body has a natural ability to protect and restore itself. Increased blood flow to the damaged area, clearing of cells and foreign matter and early cellular development are part of the healing process. The healing process can occur normally or with assistance. For example, protecting the wound area from dirt by maintaining good hygiene helps to promote tissue healing (Black, 2014).

Optimal wound care leads to a good wound healing process in a short time, thereby reducing wound care costs and increasing productivity. General wound care consists of wound bed preparation and wound closure. Wound bed preparation is carried out through debridement, bacterial control, and management of wound exudate. Wound closure is carried out when the wound has been well prepared and can be done per secundam, perprimam, skin graft, flap, and using stem cells. Wound assessment, determination of action, and selection of dressings in wound care with any diagnosis is carried out based on the condition and problem of the wound. Wound conditions can be identified by the color and surface of the wound. The color of the wound can be adjusted according to the type of wound, namely acute wounds, necrotic wounds (black), slough wounds (yellow necrotic), granulation wounds, infectious wounds (yellow green), and epithelialized wounds. The wound surface can be in the form of wet wounds, dry wounds, and moist wounds. Wound problems can include bacterial infections, necrotic tissue, and exudates. Bacterial infections can be controlled with antibiotics, antibacterial materials and debridement. Necrotic tissue can be treated with debridement. Exudates can be overcome by giving abortive products (Wintoko and Yadika, 2020).

The principle of wound care is tissue management, inflammation and infection control, moist balance environment, epithelial advancement or edge (TIME) with the right modern dressing. There are several factors that influence the choice of modern dressing, including: type of wound, wound description, wound characteristics, and bacterial profile. Nursing care for patients with wounds includes: assessment (holistic and wound assessment) of wounds, formulating nursing diagnoses: damage to skin/tissue integrity, nursing planning (TIME) and implementation (wound care) and evaluation (Poerwantoro, 2013).

This research was the first step to explore natural wound topical ingredients from the Riau Islands which were appropriate for use in the wound healing process. This can support independent nursing practice carried out by researcher as an effort to bring services closer to the community.

RESEARCH METHOD

Extraction of chitosan in the shells of gonggong snails obtained from seafood waste must first be cleaned from dirt, then dried or dried under the sun. After the dried shells was obtained, the gonggong shells were crushed or mashed to form flakes, and then a demineralization, deproteination, and deacetylation process was conducted which aimed to remove minerals, proteins and acetyl groups in the gonggong shells to produce chitosan (Susilowati, 2014). The stages of this research were preparation of gonggong snail shell samples, isolation of chitin from gonggong snail shells and deacetylation (Horiza et all, 2018).

This demineralization process aimed to remove inorganic salts or mineral content contained in the shell of the gonggong snail. Removing minerals (demineralization) in the gonggong snail shell by means of gonggong snail shell + with 1.5 M HCL in a ratio of 1:5 (w/v) stirred for 4 hours at a temperature of 65°C. After neutralization and oven at 80°C for 24 hours.

Then a deproteinization process was carried out which aimed to separate or release protein bonds from chitin. Followed by deproteinization by means of demineralized snail shells + NaOH 3.5% ratio 1:10 (w/v) stirred for 4 hours at a temperature of 65 - 70°C. After neutralization and in the oven at 80°C for 24 hours.

Then the depigmentation process through the shell of the gonggong snail as a result of the deproteinization process + NaOCL 0.315% ratio 1:10 (w/v) was stirred for 1 hour at 40°C. After neutralization and in the oven at 80°C for 24 hours.

The next stage was the conversion of chitin to chitosan called the deacetylation process. The deacetylation process is the process of removing the acetyl group (-COCH3) from chitin by using an alkaline solution to turn it into an amine group (-NH2). The shells of gonggong snails resulting from the depigmentation process + 50% NaOH at a ratio of 1:20 (w/v) were stirred for 4 hours at 120°C. After neutralization and oven at 80°C for 24 hours.

The chitosan obtained was characterized to determine the quality of the chitosan produced. The characterization carried out included tests for water content, ash content, solubility in 2% acetic acid and the degree of ionization. Moisture content and ash content are parameters used as quality standards for chitosan. The water content of chitosan is influenced by the relative humidity of the air around its storage area, since chitosan is easy to absorb moisture from the surrounding air. Chitosan polymer groups (amine, N-acetyl and hydroxyl groups) will hydrogen bond with H2O from the air (Dompeipen et al, 2016 in Rochmawati, Nabila, Ainurrohmah 2018). Testing the water content using the gravimetric method with the following equation:

The ash content indicated the success rate of demineralization, so the low ash content indicated the purity of a chitosan. Determination of ash content aimed to determine the mineral contained in blood clams. In addition, the ash content can also be used to measure the solubility of chitosan in the solvent. If the ash content is high, then the minerals contained are still high and if the ash content is low, the minerals contained in the sample are small.

The solubility of chitosan was obtained by taking a sample of gonggong snail shells, weighed 1 gram and then dissolved in 100 ml of 2% acetic acid. Meanwhile, the acetylation degree in 100 grams of gonggong snail shell samples was carried out using the FTIR method (Mardiana, 2021). The calculation of the deacetylation degree (DD) from the infrared spectra of chitosan was carried out by comparing the absorbance at the wave number for the amide NH group (A1655) with the absorbance at the wave number for the primary amine group (A3450), with an absorbance value of 1.33 in the complete deacetylation process. The equations used are:

$$\%DD = [100 - (\frac{A\ 1655}{A\ 3450} \text{ x } \frac{100\ A\ 1655}{1{,}33\ A\ 3450}\ x\ \frac{100}{1{,}33})]$$

A : Log $(\frac{P\ o\ P\ o}{p\ \ p})$

Po : % transmittance on flat line

P : % transmittance at minimum peak

A1655 : amide group absorption band

A3450 : absorption band of hydroxyl group (OH)

RESEARCH ETHICS (Komite Etik Penelitian dan Pengembangan Kesehatan Nasional Kementerian Kesehatan RI, 2021).

As explained in detail in international ethical guidelines and various research rules in various jurisdictions, the criteria/standards of ethical adequacy. Before the research was carried out in the laboratory, the researcher proposed ethical clearance at the Polytechnic of the Ministry of Health of Malang with the number Reg.No.:300/KEPK-POLKESMA/2022. It is declared ethically appropriate according to 7 WHO standards, namely: social values, scientific values, equal distribution of burdens and benefits, risks, inducements/exploitation, confidentiality and privacy, and approval after an explanation referring to the 2016 CIOMS Guidelines.

1. Social Value and/or Clinical Value

   Research is ethically acceptable if the research has an impact not only on the individuals participating, but also on the community where the research was conducted and/or to whom the research results will be applied. Research is ethically justifiable because it attempts to produce information relevant to significant health needs for low-resource communities. Parameters of social value are the existence of novelty phenomena and efforts to disseminate results. Social value is actually difficult to quantify quantitatively. However, qualitatively there are generally 4 important factors, namely: the quality of the information (knowledge) produced must be meaningful, its relevance is meaningful to the health problems of the local community, its contribution to the creation or evaluation of interventions, policies, or implementations that promote individual or community health, and information to understand interventions, contributions to health promotion, alternative ways of dealing with problems, etc.

2. Scientific Value (Scientific Design)

Research can be accepted ethically if it is based on a valid scientific method. The scientific value parameter refers to the ability of the research to produce i.e. valid and reliable information, according to the objectives stated in the protocol, the basis for further research, and relevant data for clinical decision making, health, social policy, or resource allocation.

3. Equal distribution of burdens and significances

Research is ethically acceptable if the risks have been minimized (by preventing potential harm and minimizing possible negative impacts) and the significances of research outweigh the risks. In addition, it was ensured that benefits and burdens were distributed evenly, no group status/level was subject to greater risk/burden. Subjects were included for scientific considerations, not recruited based on socioeconomic status, on the basis of authority, or ease of manipulation or selection. Research ethics ensures that no group or individual bare more burdens than they should when participating in research.

4. Potential Risks and Significances

In considering the limits of the acceptable level of risk, and the balance of risk against benefit, it is necessary to consider referring to the previous basic moral and ethical theories and statements of the research code of ethics. Minimal risk, generally no requirement for subject-specific protective measures required for all studies involving subjects.

5. Inducements, Financial Benefits, and Replacement Costs

In research, it was necessary to avoid suspicion of the existence of "exploitation and the importance of moral aspects. Statements related to aspects of benefits and harm), vulnerability, and consent are important. Researchers need foresight and sensitivity to seek to determine how exploitation relates to other ethical concepts. Recruitment of subjects with socially and economically disadvantaged conditions is more profitable for researchers and sponsors. It is ethically acceptable and permissible to reimburse an individual for any costs associated with participating in research, including transportation costs, childcare, lost income while participating in research and reimbursement of time spent participating in research.

6. Protection of Privacy and Confidentiality

Violation of the privacy and confidentiality of research subjects, such as, is not respecting the subject of embarrassing things and invisible losses, such as social stigma, rejection by family or society, or loss of opportunities, for example in work or getting a place to live.

7. Consent After Explanation (PSP) or Informed Consent (IC)

Consent after explanation (PSP)/Informed Consent (IC) is consent given by a competent individual. The individual has received the required information, has understood it, and made a decision without being subjected to coercion, undue influence or persuasion, or intimidation.

RESULTS AND DISCUSSION

Chitin is the main component of the exoskeleton of invertebrates, crustaceans and insects where this component functions as a supporting and protective component. Chitosan is a biopolymer that is unique in that in an acidic solution, chitosan has the characteristics of a cation and is positively charged, while in an alkaline solution, chitosan will precipitate. Chitin and chitosan are linear polymers which are polycationic. The gonggong snail is a type of sea snail (Strombus canarium L.1758), is one of the soft animals (Mollusca), many live on the coast of Bintan Island and its surroundings, such as Dompak Island, Lobam Island, Mantang Island (Pratiwi, 2014; Zaidi et al. 2009 in Ricky et al, 2016).

Kusumawati, 2009 in Pratiwi (2014) states that the benefits of chitosan include agriculture, chitosan offers a natural alternative in the use of harmful chemicals to the environment and humans. Chitosan creates defense mechanisms in plants (such as vaccines for humans), stimulates growth and stimulates certain enzymes (phytoalexin chitinase, pectinnase, glucanase and lignin synthesis): This new organic controller offers an approach as a biocontrol tool; (2) in the field of water treatment, chitosan can be used as a raw material for the manufacture of ultrafiltration membranes; (3) in the food sector, chitosan has been widely used in food compositions in Japan, Europe and the United States, as a fat trap which is a breakthrough in the field of diet; and (5) in medicine, chitosan is used for bacteriostatic, immunology, anti-tumor, cicatrizant, homeostatic and anti-coagulant, ointment for wounds, malignancy, orthopedics and surgical suture closure. Chitosan can be used to accelerate wound healing and bone damage.

According to Pratiwi (2014) that the safety of using chitosan products has received approval from BPOM No. HK.00.05.52.6581 for use in food products. In America, chitosan has been approved as a Generally Recognized As Safe (GRAS) product by the FDA. Besides being safe, the chitosan produced by PT. Araminta Sidhakarya has also received a halal certificate from LPPOM-MUI No. 00170043490307 (as preservative) and 00170043510307 (as coating).

The manufacture of chitosan by means of gonggong snail shells obtained from seafood waste must first be cleaned of dirt, then dried or dried in the sun. After the shells were dry, the gonggong snail shells were crushed or mashed to form flakes, and then a demineralization, proteination, and deacetylation process was carried out which aimed to remove minerals, proteins and acetyl groups in the gonggong shells to produce chitosan (Susilowati, 2014).

Mineral Removal (Demineralization)

This demineralization process aimed to remove inorganic salts or mineral content present in the gonggong snail shells. Mineral Removal (demineralization) in the gonggong snail shell by means of gonggong snail shell + with 1.5 M HCL in a ratio of 1:5 (w/v) stirred for 4 hours at a temperature of 65°C. After neutralization and it was put into the oven at 80°C for 24 hours.

Figure.1 Demineralization

Protein Removal (Deproteination)

This process aimed to separate or release protein bonds from chitin. Protein removal (Deproteinization) by means of demineralized snail shells + NaOH 3.5% ratio 1:10 (w/v) stirred for 4 hours at a temperature of 65 - 70°C. After neutralization and it was put into oven at 80°C for 24 hours.

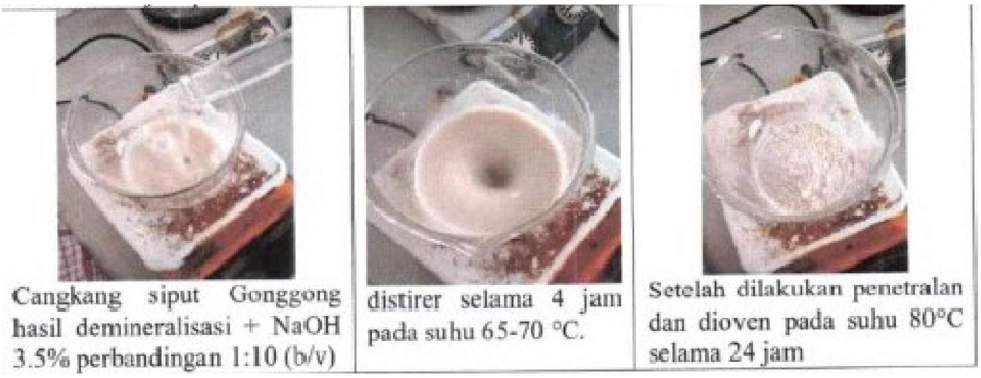

Figure.2 Deproteinization

Depigmentation

The gonggong snail shells resulting from the deproteinization process + NaOCL 0.315% ratio 1:10 (w/v) were stirred for 1 hour at 40°C. After neutralization and it was put into the oven at 80°C for 24 hours.

Figure.3 Depigmentation

Acetyl group Removal (Deacetylization)

The conversion of chitin to chitosan is called the deacetylation process. The deacetylation process is the process of removing the acetyl group (-COCH3) from chitin using an alkaline solution to turn it into an amine group (-NH2). The gonggong snail shells resulting from the depigmentation process + 50% NaOH at a ratio of 1:20 (w/v) were stirred for 4 hours at 120°C. After neutralization and it was put into oven at 80°C for 24 hours.

Figure.4 Deacetylization

Characteristics of Chitosan

From the research conducted in May 2022 at the Chemical Laboratory of FMIPA UNP, which measured the water content, ash content (Gravimetric method) and ionization degree (FTIR method) in the gonggong snail shell samples, it is preseanted in the following table:

Table 1 Water content in gonggong snail shells

No	Type of sample	Weight of original sample (initial weight (g))	Weight of sample after oven (final weight (g))	Loss of sample weight (initial weight-final weight)	Moisture content
1	Gonggong snail shell	1,0011 g	0,9940 g	0,0071 g	0,71%

Testing the water content using the gravimetric method obtained the following results:

$$Moisture\ content = \frac{loss\ of\ weight\ (g)}{weihgt\ of\ sample\ (g)} \text{ x } 100\%$$

$$= \frac{0{,}0071\ g}{1{,}0011(g)} \text{x } 100\%$$

$$=0{,}71\%$$

Table 2 Ash content in gonggong snail shells

No	Type of sample	Weight of original sample (initial weight (g)	Weight of ash after furnace (g)	Ash Content
1	Gonggong snail shell	1,0105 g	0,9691 g	95,90%

Testing the ash content using the gravimetric method obtained:

$$Ash\ content = \frac{Ash\ weight\ (g)}{weight\ of\ sample\ (g)}\ \text{x}\ 100\%$$

$$= \frac{1{,}0105\ g}{0{,}9691(g)}\ \text{x}\ 100\%$$

$$=95{,}90\%$$

Table 3 Sample analysis results of the gonggong snail shell

No	Parameter	Analysis result	Method
1	Moisture content	0,71% (in 100 grams of sample)	Gravimetri
2	Ash content	95,90% (in 100 grams of sample)	Gravimetric
3	Solubility	Soluble	Solubility
4	Ionization degree	79,8821% (in 100 grams of sample)	FTIR

The chitosan obtained was characterized to determine the quality of the chitosan produced. The characterization carried out included tests for water content, ash content, solubility in 2% acetic acid and the ionization degree. Moisture content and ash content are parameters used as quality standards for chitosan. The water content of chitosan is influenced by the relative humidity of the air around its storage area, since chitosan is easy to absorb moisture from the surrounding air. Based on the measurement results shown in table 3, it was found that the water content in 100 grams of gonggong snail shell samples through the gravimetric method was 0.71%. This is in accordance with the Protan Laboratory Standard 10% contained in research by Rohmawati, 2018 and Suprianto, 2012).

The ash content indicated the success rate of demineralization, so the low ash content indicated the purity of a chitosan. In addition, the ash content can also be used to measure the solubility of chitosan in the solvent. If the ash content is high, then the minerals contained are still high and if the ash content is low, the minerals contained in the sample are low. Based on the measurement results shown in table 3, it was obtained that the ash content in 100 grams of gonggong snail shell samples was 95.90. This is not in accordance with the Protan Laboratory Standard, which is 2% So it can be said that the mineral separation and washing process had not been effective (Rohmawati, 2018 and Suprianto, 2012)

Chitosan solubility is one of the main parameters of chitosan quality assessment standard. The higher the solubility of chitosan in 2% acetic acid, the quality of the chitosan produced was very good. The solubility of chitosan was observed by comparing the clarity of the chitosan solution with the clarity of the solvent. The solubility of chitosan was obtained by taking a sample of gonggong snail shells, weighed 1 gram and then dissolved in 100 ml of 2% acetic acid. The results in this research were chitosan can be dissolved in 2% acetic acid (Knoor, 2014 and Dompeipen et al, 2016 in Rohmawati, 2018 and Suprianto, 2012)

The deacetylation stage in chitosan extraction was carried out to change the acetyl group in chitin into an amine group. The deacetylation degree is a value that indicates the percentage of acetyl groups lost from chitosan and becomes a determinant of the quality of chitosan. The high deacetylation degree of chitosan causes the acetyl group contained in chitosan to be low, so the chitosan was positively charged and was able to bind strongly to polysaccharide anions, proteins and form neutral ions. The deacetylation degree of chitosan in this research was 79.8821%, this result did not meet the standard for pharmaceutical types in a research conducted by Trisnawati, Andesti, Saleh in 2013 where the degree of acetylation was > 95%.

CONCLUSIONS AND RECOMMENDATIONS

After conducted the research, the results of the characteristics of chitosan include a water content of 0.71% in 100 grams of sample, 95.90% ash content in 100 grams of sample, its solubility and degree of ionization of 79.8821% in 100 grams of sample. It is necessary to conduct further research on the measurement of the acetylation degree which is recommended > 95% in the pharmaceutical field and the transformation of chitin into chitosan to be of high quality.

REFERENCE

Akmarina, C.A dan Sriwidodo. (2016) ‘ Artikel Review : Aplikasi Kitosan Dalam Bidang Farmasetik’, Farmaka, Suplemen Volume 14, Nomor 2, hal 318-330.

Black, J. M., & Hawks, J. H. (2014) *Keperawatan Medikal Bedah: Manajemen Klinis Untuk Hail Yang diharapkan.* Singapore: Elsevier

BPS Kepri.(2016),'Provinsi Kepulauan Riau dalam Angka 2016', diakses melalui https://kepri.bps.go.id/publication/2016/07/15/78c7670d6dcd5d34a0d6de1a/provinsi-kepulauan-riau-dalam-angka-2016.html

Horiza.H , Iskandar.I, Aldo.N. (2018) 'Degree of Acetylization Chitosan Gonggong Snail Shells', IOP Conference Series: Materials Science and Engineering 335

Kurniawaty.E dan Putranta.N.R. (2019) 'Potensi Biopolimer Kitosan Dalam Pengobatan Luka', Medula, Volume 9, Nomor 3, hal 459-464.

Komite Etik Penelitian dan Pengembangan Kesehatan Nasional Kementerian Kesehatan RI. (2021) *Pedoman Dan Standar Etik Penelitian Dan Pengembangan Kesehatan Nasional*, Lembaga Penerbit Badan Penelitian dan Pengembangan Kesehatan (LPB), Jakarta.

Noor, Juliansyah. (2014) *Metodologi Penelitian*. Jakarta: Kencana Prenada Media Group

Ricky et all. (2016) 'Kelimpahan dan Pemanfaatan Siput Gonggong (Strombus sp) di Kampung Madong Kelurahan Kampung Bugis Kota Tanjungpinang', Skripsi Jurusan Manajemen Sumberdaya Perairan, Fakultas Ilmu Kelautan dan Perikanan, Universitas Maritim Raja Ali Haji.

Pratiwi.R. (2014) 'Manfaat Kitin Dan Kitosan Bagi Kehidupan Manusia', Oseana, Volume XXXIX, Nomor 1, 35-43.

Poerwantoro, P. D. (2013). *Dasar-dasar perawatan luka modern dan pemilihan dressing untuk berbagai jenis luka.* Jakarta Timur: Pancar Gradia

Pemerintah Provinsi Kepulauan Riau (2021)'Profil Singkat Provinsi Kepulauan Riau'diakses melalui https://kepriprov.go.id/laman/tentang-kepri

Rochmawati et all. (2018) 'Karakterisasi kitosan yang diisolasi dari cangkang internal cumi-cumi', Sainteknol : Jurnal Sain dan teknologi, Vol 16, No 1, hal 105-111.

Viruly, et all. (2019) 'Protein Histon pada Siput Gonggong Bintan Strombus Sp. Sebagai Kandidat Pangan Fungsional', Jurnal Ilmu dan Teknologi Kelautan Tropis, Vol 11, No 1.

Rosady.V.P, Astuty.S, Prihadi.D.J. (2016) 'Kelimpahan dan kondisi habitat siput gonggong (strombus turturella) di pesisir kabupaten bintan, kepulauan riau' ,Jurnal Perikanan Kelautan Vol VII, No 2, hal 35 – 44.

Wintoko.R, Yadika.A.D.N. (2020) 'Manajemen Terkini Perawatan Luka' ,JK Unila, Volume 4, Nomor 2, hal 183-189.

Trisnawati.E,Andesti.D,Saleh.A. (2013) 'Pembuatan Kitosan dari Limbah Cangkang Kepiting Sebagai Bahan Pengawet Buah Duku dengan Variasi Lama Pengawetan',Jurnal Teknik Kimia, No 2, Vol 19, hal 17-26

Suprianto. (2012) ‘Karakterisasi Kitin dan Kitosan Udang Swallo (Metapenaeus monoceros)’,Akademia,Vol16, No 2