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ANTIFUNGAL AND WOUND HEALING ACTIVITIES OF CHITOSAN NANOPARTICLES FROM GREEN MUSSEL SHELL (*Perna viridis*) AND JERNANG (*Daemonorops draco*) ETHANOL EXTRACT DRESSING PATCH

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ABSTRACT

The development of chitosan nanoparticles and jernang (*Daemonorops draco*) as wound medicine materials have received a lot of attention from researchers. In this research the chitosan nanoparticles and jernang ethanol extract were combined. The antifungal of the materials determined based on inhibition test of *Candida albicans* and *Malassezia furfur*. The wound healing activities determined based on the in vivo wound healing test on Wistar rat (*Rattus novergicus).* The materials applicated on wound dressing patch. Chitosan was synthesized from green mussel shell waste (*Perna viridis* L). The result showed that the yield of chitosan was 64.67% and degree of deacetylation (DD) was 69.53%. Meanwhile, chitosan nanoparticles can be prepared based on ball mill process. The result showed that average of the particles size was 437.6 nm (nanoparticles size is 1-1000 nm). Jernang was obtained by maceration process using ethanol organic solvents technical. The yield was 22.41% and showed positively of alkaloid, saponin, flavonoid and triterpenoid test. F3 has highest activities on antifungal and wound healing test results.

Keywords: antifungal, dressing patch, green mussel shell, jernang, wound healing

1. INTRODUCTION

Skin constitutes the largest multi-layer organ of human body, which includes the epidermis and dermis, and it also acts as a barrier for body protection [13]. Skin has high potential to be injured. Nowadays, study of wound healing dressing technology still become one of interesting topic. The dermal patch technology has proven to be fastest, easiest, safest, and most economical way to help wound to heal [12]. Epithelial wounds healing is a tightly regulated physiological process. Wound healing or wound repair is an intricate process in which the skin or organ or tissue repairs itself after injury. It comprises of a continuous sequence of inflammation and repair in which epithelial endothelial inflammatory cells, platelets and fibroblasts briefly come together outside their normal domains, interact to restore a semblance of their usual discipline and having done to resume their normal function [3,11]. This study aims to determine wound healing activities of dressing patch which contained nanoparticles chitosan and jernang extract. Chitosan in nanoparticles size has larger surface than microparticles or other bigger size. So that, interaction of the material and environment can be more reactive [2,5].

Chitosan is a natural polysaccharide comprising of copolymers of glucosamine and N-acetylglucosamine and can obtained by deacetylation of chitin. Preparation of chitosan carry out in three stages, demineralization, deproteination, and deacetylation from chitin to be chitosan [5,7]. Chitin, poly (b-(1-4)-N-acetyl-D-glucosamine), is a natural polysaccharide of major importance. Among the novel families of biological macromolecules, whose relevance is becoming increasingly evident are chitin and its main derivative, chitosan. Chitin is the major component in outer cover of insects, the shell of shrimps, crabs, cartilage of the squid, and mussel [1,4].

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Green mussel shell (*Perna viridis*) is kind of mussel that is consumed by a lot of people. The waste of green mussel shells is still not used optimally so that there are still waste in the environment. Waste of green mussel shells can be used as a source of chitosan through the deacetylation process, so the economic value of green mussel shells can be improved [16].

Chitosan is a natural polycationic that is multi-functional material with good biocompatibility, no immunogenicity, and no skin irritation. In 2001, it was approved by Food and Drug Administration of Uniter States (FDA) as a GRAS (Generally Recognized as Safe) substance. Currently, a number of FDA-approved chitosan-based hemostatic products including Celox® (MedTrade Products Ltd., Cheshire, UK) is commercially available. Chitosan is a natural cationic alkaline polysaccharide; the positive charge of $-NH^{3+}$ on chitosan chain electrostatically interacts with the anions on the surface of RBC, leading to intensive aggregation of RBC around the wound site to form blood clots which quickly stop bleeding. Therefore, the degree of protonation of amino groups on chitosan chains plays an important role in the adsorption of red blood cells. Studies have shown that the ability of chitosan to initiate coagulation was related to the percent of deacetylation and was more dependent on the number of protonated amine groups. However, it still is a challenge to enhance their hemostatic potential [5,20].

Jernang (*Daemonorops draco*) or known as dragon's blood is one of high value non-wood forest products originated from Indonesian forest. Dragon's blood which is red colored resin secreted from rattan's fruits has been utilized traditionally [14]. Jernang is used an ingredient in making medicines, efficacious gum can stop bleeding (static blood dispel, reduce pain (relieve pain), injury trauma due to fractures (traumatic injuries causing fracture) bruising medication, sprains stop bleeding due to bruising and stop bleeding protects the festering wounds surface to rot, growing the flesh tissue, and relieving the pain in chronic wounds (chronic non-healing sores) [16,18]. According to phytochemical test, jernang contained a group of metabolic compounds secondary to flavonoids, terpenoids, and antibacterial activity tests giving clear zones (+) to E. coli, Salmonella and S. Aureus bacteria. from the results of previous studies carried out the making of liquid wound medicine from resin with its resin formulation [19]. Jernang is soluble in alcohol, ether, fat oil, and oil volatile, partly soluble in chloroform, ethyl acetate, petroleum spirits and carbon disulfide and insoluble in water. Jernang can be extracted by maceration process using ethanol technical to more economical, effective and safer way [19]. Effectiveness of nanoparticles chitosan and jernang as wound healing will increase if the materials have antifungal activities. So that, antifungal of the materials is kind of important test in wound healing study.

2. METHODS

The research samples were green mussel shell (*Perna viridis*) waste from Takisung beach, Tanah Laut, South Kalimantan and Jernang (*Daemonorops draco*) from Bireun, Peulimang, Nangroe Aceh Darussalam (NAD). The test animal was white rat (*Rattus novergicus*). The test microbials were using *Candida albicans* and *Malassezia furfur*. The materials used in this study were NaOH (Merck®, Germany), HCl (37% purity Merck®, Germany), CH_3COOH (99% purity Merck®, Germany), aqua distillated, ethanol (96% purity Merck, Germany), wound dressing patch without active material (Plesterin®) and chitosan (Gauze®). The instrument used were Fourier Transform Infrared (FTIR), Scanning Electron Microscope (SEM), Particle Size Analyzer (PSA), drying sheed, jaw crusher, ball mill and hot plate.

2.1 Nanoparticles Chitosan Isolation

Preparation green mussel shell was including cleaning, drying, and milling to 50 mesh. Then chitosan was isolated by deproteination, demineralization and deacetylation of the powder sample. The deproteination sample was soaked in NaOH 3% 3:1 (mL NaOH/g sample) and stirred for 1 hour then heated to 80 ^oC for 30 minutes. Then demineralization sample was soaked in HCl 1.25% 3:1 (mL HCl/g sample) then heated to 75 ^oC for 1 hour. The deacetylation sample was soaked in NaOH 60% 20:1 (mL NaOH/g sample). Sample was neutralized and dried after each process. The resulting chitosan prepared for characterization by FTIR. The chitosan sample was ball milled to be

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nanoparticles size and prepared for characterization by PSA and SEM. Nanoparticles chitosan (nano chitosan) prepared to be solution using acetic acid 2%.

2.2 Jernang Extraction

Jernang ethanol extract (JEE) was prepared by maceration process using ethanol solvent 2:1 (jernang powder: ethanol). Maceration process was 3 x 24 hours. Then sample filtered until the solvent from the results was clear. The maceration solvent results were evaporated until the thick ethanol is extracted. The extracted was characterization by FTIR. JEE prepared to be solution using ethanol.

2.3 Prepared of Dressing Patch

Dressing patch was prepared 5 treatment groups formulation. Each formulation group was dripped into the patch of Plesterin® equally then dried.

Table 1. Formulation groups

Group	Formulation
F1	Nano Chitosan 1%: JEE 5% (1:1)
F2	Nano Chitosan 2.5%: JEE 5% (1:1)
F3	Nano Chitosan 5%: JEE 5% (1:1)
Control (-)	Acetic acid 2%: Ethanol (1:1)
Control (+)	*Celox solution 5%*

2.4 Antifungal Activities Test

The antifungal activities of 5 group formulation were carried out by disc diffusion method using disc paper. The method was doing by SDA media 20 mL applicated to each petri dish until set. Then added the 0.1 inoculum *Candida albicans* and *Malassezia furfur*. Disc paper was soaked in each formulation group. Media surface was swab using cotton bud then disc paper placed. Petri dish incubated in 37 ^{o}C for 24 hours and inhibition area was measured.

2.5 Wound Healing Activities Test

Wound healing test was doing to white rat. Rat must be health and has weight between 100-200 gram. Back of rat was shaved then clean by ethanol 70%. Back of rat was injured, length of the wound was ±1 cm. After that the formulation and controls dressing patch applicated to the wound. The formulation and control dressing patch were applicated to the wound and measured each day until day 8th.

3. RESULTS AND DISCUSSION

3.1 Nanoparticles Chitosan Isolation

Table 1 present the results of green mussel shell chitosan characterization according to national standard, SNI 749:2013. The table showed that chitosan has high content of ash. That means the acid in demineralization process was not effective. Beside that green mussel shell a lot of minerals contained such as $CaCO_3$. The mineral content ($CaCO_3$) of shell was found 51,62% (Abdulkarim 2013). The Deacetylation degree of the chitosan is lower than standard. It could be decreasing the effectiveness of the activities. The result showed higher than reported by Abdukarim *et al* (2013), 15,14%).

Table 2. Characterization of chitosan

Parameter	Study Results	SNI 7949:2013
Colour	White	Light Brown white
Texture	Powder	Powder
Odor	Smell of fish	No smell
Deacetylation degree (%)	69,53	Min. 75
Solubility in Acetic Acid 2%	Soluble	Soluble
pH	7	7-8
Water contained (%)	2,46	Max. 12
Ash (%)	55,12	Max. 5
Yield (%)	64,67	-

Table 3. Characterization of nanoparticles chitosan

Parameter	Study Results	Nano Chitosan Standard [1,9]
Colour	White	White
Particle Form	Powder, irregular crystal	Layered amorf
Particle size (nm)	437.6	1-1000 nm

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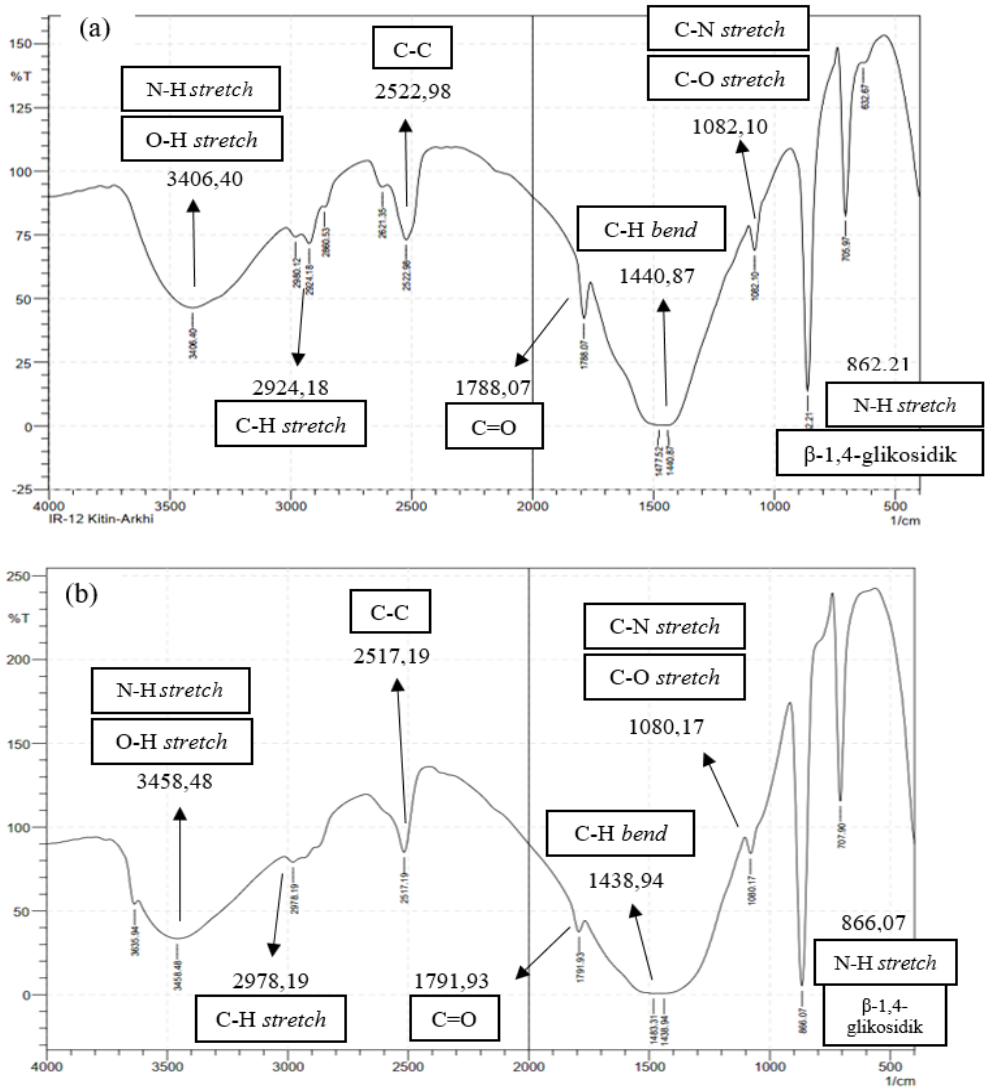

Figure 1. FTIR spectra of chitin (a) and chitosan (b)

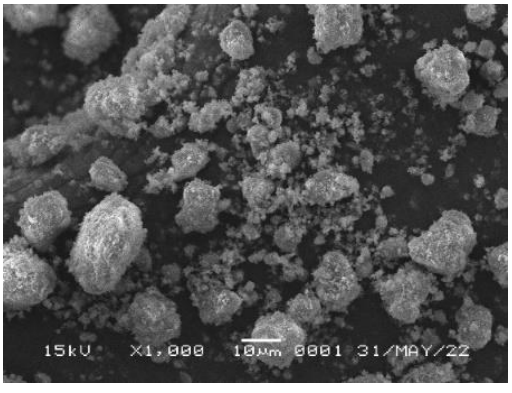

Figure 2. Morphology of nanoparticles chitosan of SEM scanning 1000x

Figure 1 showed the FTIR spectra of chitin and chitosan respectively. The FTIR spectra gave characteristic bands of -NH at 3406.40 cm^{-1} in chitin spectra and at 3458,48 cm^{-1} in chitosan spectra [10]. There was peak that characteristic of carbonyl group at 1788,07 cm^{-1} in chitin spectra and at 1791,93 cm^{-1} in chitosan spectra. Intensity of carbonyl peak of chitosan was decreased that chitin. It means that deacetylation process was changed acetamide ($-NHCOCH_3$) become amine ($-NH_2$) [1].

3.2 Jernang Extraction

Characteristic of extract in this study present in Table 4. Results showed that JEE has high quality according to national standard SNI 1671-2010. Ash content in this study was lower than 4% which is standard of super quality of jernang resin. The colour of JEE was as good as highest quality standard.

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Table 4. Characteristic of jernang ethanol extract

Variable		Results
Yield (%)		22,41
Ash (%)		0,42
Colour		Dark red
Phytochemical	Alkaloid	+
	Saponin	+
	Flavonoid	+
	Tanin	-
	Steroid	-
	Triterpenoid	+

3.3 Antifungal Activities Test Results

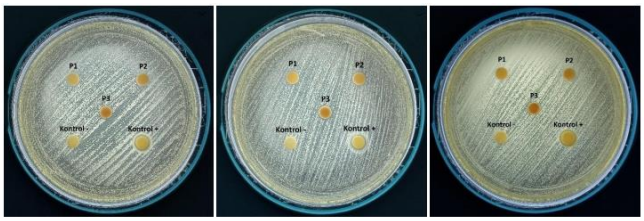

Figure 3. Antifungal test results for *Candida albican*

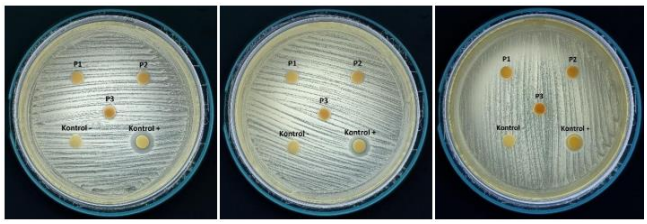

Figure 4. Antifungal test results for *Malassezia furfur*

Table 5. Antifungal test results of formulation

Sample	*Candida albicans*		*Malassezia furfur*	
	Inhibition Diameter Average (mm)	Results	Inhibition Diameter Average (mm)	Results
F1	6.23	Active	6.08	Active
F2	6.57	Active	6.17	Active
F3	6.58	Active	6.25	Active
C (+)	7.60	Active	10.05	Active
C (-)	6.00	Inactive	6.00	Inactive

Table 4 showed the antifungal test results of 5 group formulation solution. The results showed that F2 and F3 has highest antifungal activities of *Candida albicans* and F3 has highest antifungal activities of *Malassezia furfur*. F3 contained nano chitosan 5% and JEE 5%. The increases of nano chitosan concentration increased the antifungal activities. But F3 antifungal activities was still lower than positive control.

3.4 Wound Healing Test

Wound healing process was observed using white rat. Excision wound on rat back is secondary wound that showed the change of epidermis tissue clearly. Observation of wound healing was doing for 8 days. Table 5 presented the wound healing process figure.

Figure 5 showed the percentage of wound healing which measured by calculated differences of length the day observation and day 0. The increased percentage of wound healing showed the fast of wound healing. The results showed that F3 was fastest of healing than control and other formulated dressing patch in day 2 was 32,14%. That's mean in 2 days, F3 dressing patch was more effective to heal the wound than the other sample. F3 dressing patch contained nano chitosan 5% and JEE 5%. Wound healing activities of nano chitosan and JEE is potential to become wound healing materials.

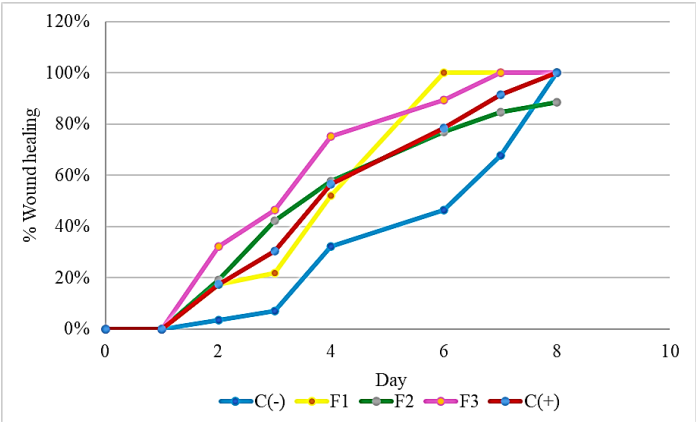

Figure 5. Graphic of wound healing process

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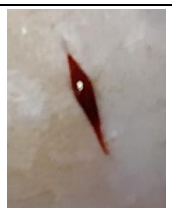
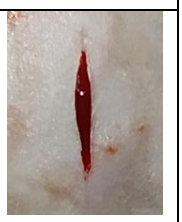
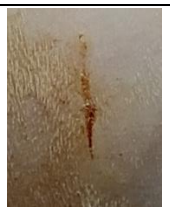
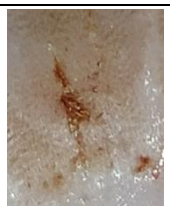
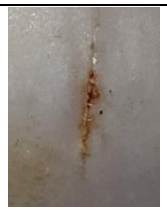
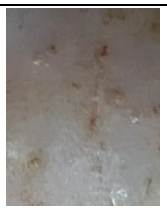
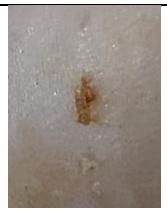
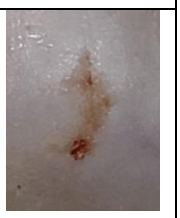
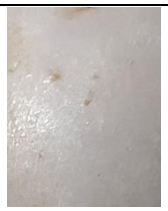
Table 6. Wound healing process

Day to-	Group				
	C(-)	F1	F2	F3	C(+)
0					
1					
2					
4					
8					

4. CONCLUSION

Nano chitosan 5% and JEE 5% has highest activities of antifungal (*Candida albicans* and *Malassezia furfur*) and has highest activities of wound healing. The materials were potentially as wound healing materials.

REFERENCES

[1] Abdulkarim A., Isa M. T., Abdulsalam S., Muhammad A. J., and Ameh A. O., 2013, Extraction and Characterisation of Chitin and Chitosan from Mussel Shell, *Civil and Environment Research*, 3(2): 108-116.

[2] Arsyi, Nita Zul, Eko N., Darari N. A., dan Eni Budiyati, 2018, Karakterisasi Nano Kitosan dar Cangkang Kerang Hijau dengan Metode Gelasi Ionik, *Jurnal Teknologi Bahan Alam*, 2(2):106-111

[3] Azzahrah, Nur Fatimah, Abdul Wahid J., dan Yuko Mulyono A., 2019, Efektifitas *Patch* Sederhana dari Ekstrak Daun Kayu Jawa (*Lannea coromandelica* (Haoutt.) Merr.) Terhadap Penyembuhan Luka Sayat pada

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e-ISSN: 2776-4508; p-ISSN: 2776-1711
Publisher : Universitas Pakuan

Tikus (*Rattus novergicus*), *As-Syifaa Jurnal Farmasi*, 11(02):169-180.

[4] Dayanti, Efin Windi, Arimbi, Maya Nurwartanti Y., Hani Plumeriastuti, M. Thohawi Elziyad P. dan Prima Ayu W., 2021, Efektivitas Kitosan dari Limbah Kulit Udang Terhadap Angiogenesis dalam Penyembuhan Luka Eksisi pada Tikus Putih (*Rattus novergicus*) Jantan, *Media Kedokteran Hewan*, 32(2):60-69.

[5] Ghulaxe C. dan Verma R., 2015, A review on transdermal drug delivery system. *The Pharma Innovation Journal*, 4 (1): 37-43.

[6] Imtihani H.N., Wahyuono R.A. dan Permatasari S.N., 2020, *Biopolimer Kitosan dan Penggunaannya dalam Formulasi Obat,* Graniti: Gresik.

[7] Kurniawaty E. dan Putranta N.R., 2019, Potensi Biopolimer Kitosan dalam Pengobatan Luka. *Medula*, 9(3): 459-464.

[8] Mahlinda, Thalib A., Maurina L., Ridho K dan Supardan M., 2020, Ekstraksi Getah Jernang (Daemonorops draco) Sistem Basah dengan Dua Tahapan Proses: Perbedaan Rendemen dan Mutu. *Jurnal Riset Industri Hasil Hutan*, 12 (1): 29-30.

[9] Marlina D. T., 2011, Sintesis Kitosan Nanopartikel dengan Metode Sonokimia, Gelasi Ionotropik, dan Kompleks Polielektrolit, *Journal Prog.Kim.Si.*, 1(2): 130-137.

[10] Pavia, Donald L., Lampman, Gary M., Kriz, George S., dan Vyvyan, James R., 2009, *Introduction to Spectroscopy,* Brooks/Cole. Washington. 26-65.

[11] Nilani P., Pranavi A., Duraisamy B., Damoran P., Subhashini V., and Elango K., 2020, Assesment of wound healing potential of dermal patches. *International Journal of Pharmacy and Pharmacology*, 10(1): 1-6.

[12] Vivcharenko V, and Przekora A, 2021, Modification of Wound Dressing with Bioactive Agents to Achieve Improved Pro-Healing Properties, *Appl. Sci*, 11(4114): 1-16.

[13] Wahyuni, Wulan Tri, Sri Purwanti dan Irmanida Batubara. 2018. Antibacterial and Antibiofilm Activity of *Daemonorops draco* Resin. *Biosaintifika: Joernal of Biology & Biology Education.* 10(1):138-144.

[14] Waluyo, Totok K. dan Gunawan Pasaribu. 2015. Aktivitas antijamur, antibakteri dan penyembuhan luka ekstrak resin jernang. *Jurnal Penelitian Hasil Hutan*. 33 (4): 377-385.

[15] Waluyo T., dan Pasaribu G., 2013, Aktifitas Antioksidan dan Antikoagulasi Resin Jernang. *Jurnal Penelitian Hasil Hutan*, 21(4): 306-315.

[16] Wulandari W.T., Nurzaman, Pratita A.T.K. dan Idacahyati K., 2020, Pengaruh Variasi Konsetntrasi NaOH Terhadap Nilai Derajat Deasetilasi Kitosan dari Limbah Cangkang Kerang Hijau *(Perna viridis L*), *KOVALEN: Jurnal Riset Kimia*, 6(3): 171-176.

[17] Wang, Yu-Ye, Yu-Wei Dai, Jie Cao, Yu-Ting Chen, Chu-Xing Zhao dan Lin Jiang. 2020. Eight new flavonoids from the fruits of *Daemonorrops draco*. *Fitoterapia*. 143(2020) 104549. G RDX

[18] Yang Y., Liang Y., Chen J., Duan X, Guo B., 2021, Mussel-inspired adhesive antioxidant antibacterial heemostatic composite hydrogel wound dressing via photo-polymerazation for infected skin wound healing, *Bioactive Materials*, 8(2022): 341-354.

[19] Yusnelti dan Muhaimin, 2019, Utilization of Jernang Resin *(Daemonorops draco)* as the Basic Material for Making Liquid Wound Medicine, *Journal of Physics: Conference Series*, 1338 012011.

[20] Zhang Hu, Zhang D., Lu Si-Tong, Li Pu-Wang dan Li Si-Dong, 2018, Chitosan-Based Composite Materials for Prospective Hemostatic Applications, Marine Drugs, 16: 273.