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Comparison of Methods for Glucosamine Production from *Achatina fulica* Shells Waste

(Perbedaan Penggunaan Metode untuk Produksi Glukosamin dari Limbah Cangkang Bekicot *Achatina fulica*)

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ABSTRACT

Osteoarthritis is a degenerative joint disease. This disease occurs when the joint feels painful due to mild inflammation that arises due to activity with the bone of end friction joints. Glucosamine has been proven to stimulate the production of cartilage and inhibit the enzyme that destroys cartilage. Glucosamine which was obtained from the hydrolysis of chitin occurs in a variety of animals such as the shell of crustacea, insects, arthropods, and the shell of molluscs (snail shells). There is a large snail population in Indonesia. Some restaurants and people make use by product snails as food sources. However, this process leaves snail shell as a by product. The high yield of glucosamine produced from snail shells use methods such as reflux+water bath and reflux+magnetic stirrer was 1.47 %. Glucosamine hydrochloride was obtained through autoclave at 121 °C and pressure 1 atm for 70 minutes. The presence of chitin, chitosan, glucosamine was confirmed using FTIR spectroscopy. A joint pain ointment was prepared using glucosamine hydrochloride from snail shells concentration at 1 % w/w. Results of this research will be very useful for the reduction of agricultural pests while simultaneously generating a new product that can lift the economic value of snail shells.

***Keywords**: Chitin, Chitosan, Glucosamine, Osteoarthritis, Snail Shells*

ABSTRAK

Osteoartritis adalah penyakit sendi degeneratif. Osteoartritis disebabkan oleh gesekan ujung tulang penyusun sendi. Glukosamin terbukti dapat menstimulasi produksi tulang rawan dan menghambat enzim yang menghancurkan tulang rawan. Glukosamin disintesis dari hidrolisis kitin yang terdapat pada cangkang crustaceae, insekta, arthropoda, mollusca (bekicot). Populasi bekicot tinggi di Indonesia. Beberapa restoran dan orang menggunakan bekicot sebagai makanan. Cangkang beki-

cot menjadi limbah karena tidak dimanfaatkan. Produksi glukosamin terbesar terdapat pada metode dengan penggunaan refluk+waterbath dan refluks+magnetic stirrer sebesar 1.47 %. Glukosamin hidroklorida didapatkan dari penggunaan autoclave pada tekanan 1 atm dengan suhu 121 °C selama 70 menit. 3 menghasilkan rendemen glukosamin tertinggi sebesar 2.40 %. Kitin, kitosan, dan glukosamin dikonfirmasi menggunakan FTIR. Salep nyeri sendi dibuat mengunakan glukosamin hidroklorida dari cangkang bekicot dengan konsentrasi 1 %. Hasil penelitian ini akan bermanfaat untuk mengurangi limbah cangkang bekicot dengan menghasilkan produk baru yang memanfaatkan limbah cangkang bekicot sehingga mampu meningkatkan nilai ekonomi cangkang bekicot.

***Kata kunci**: Cangkang bekicot, Glukosamin, Kitin, Kitosan, Osteoartritis*

1. INTRODUCTION

Osteoarthritis is a degenerative joint disease. The joints become painful due to mild inflammation that arises due to tampering with the bone end friction joints. Osteoarthritis is marked by the depletion of the joint cartilage, which is progressively accompanied by the formation of new bone in trabecula subcondral and osteophytes on the cartilage of the joints (Gartner *et al*. 2011). Osteoarthritis generally strikes the older people. This can be due to menopause in a phase which occurs by a release of a mineral in the bone (Martin & Craig 2004). Glucosamine is composed of glucose and the amino acid of glutamine (Cahyono 2015). Glucosamine (2-amino-2-deoxi-β-D-glucopyranose) is a substance found in the matrix of joint cartilage and joint fluid. Glucosamine is a major precursor for the biosynthesis of various macromolecules such as hyaluronic acid, glycosaminoglycan, proteoglycans, glycoproteins, and glycolipids. Glucosamine is present in almost all of the soft tissues in the human body, but the highest concentrations are to be found in cartilage (Miller and Clegg 2011). Glucosamine (2-amino-2-deoxi-β-D-glucopyranose) is an amino acid monosaccharide of a disaccharide units glicosaminoglican which is proteoglycans, as well as being the basic substance of cartilage (Nurjannah *et al*. 2016). Glucosamine is proven to stimulate the production of cartilage and inhibit enzymes which destroy cartilage (Isbagio 2009). Glucosamine compounds can be synthesized from hydrolysis of chitosan compounds that are a derivative of chitin. Chitin is the main organic material found in the group of crustaceans, insects, fungi, molluscs, and arthropods (Victor *et al*. 2016; Kusumaningsih *et al*. 2004).

The production of glucosamine from shrimp shells is a comman procedure. But, production of glucosamine from the shell of the snail still novel. Snails are quite abundant in nature, especially in tropical countries like Indonesia. A tropical climate stimulates a high growth of the snail. Snails can thrive in environmental conditions in which the availability of nutrients is limited. As a result, uncontrolled growth of the snail and they often become pests agricultural in Indonesia. On the other hand, there are the areas that utilize snail meat as a food sources, namely in the area Posoklaten, Kediri, Indonesia. This region utilizes snail meat as a satay dish. However, this process leaves the shells as a by product. Based on the high growth of snails and the high of utilization the snail

meat without utilizing. The shell results in waste by product. This makes the economic value of snail shells low. Results of this research will be very useful for the reduction of agricultural pests while simultaneously generating a new product that can lift the economic value of snail shells.

2. METHODOLOGY

Extraction of Chitin (Modification of Dewi *et al.* 2016)

Snail shell flour which has been sifted, is deproteinised with 1:10 (m/v) between the snail shell flour and NaOH 3.5 % with a temperature of ± 65 ℃ for 2 hours. Then, the product is demineralised using 1:15 (m/v) between the dry flour results in deproteination and HCl 1N reflux at ± 40 ℃ for 30 minutes to remove the minerals in the flour. Furthermore, the process of depigmentation is by 1:10 (m/v) dried flour and NaOCl 0.315 % reflux at ± 40 ℃ for 1 hour to eliminate the colour. The flour obtained is a chitin powder which can be used to manufacture chitosan. The presence of chitin is confirmed using FTIR spectroscopy.

Production of Chitosan (Modification of Dewi *et al.* 2016)

Deacetylation of chitin was by 1:20 (m/v) on the circuit of reflux while stirring using magnetic stirrer at ± 110 ℃ for 1 hour. Solids obtained are filtered, then neutralized and dried in the oven. The powder obtained is a chitosan powder used to manufacture glucosamine. The presence of chitosan is confirmed using FTIR spectroscopy.

Synthesis of Glucosamine Hydrochloride (Ernawati. 2012)

Mixing the chitosan powder with HCl 8 % in a rasio of 9:1 (m/v) and placed in an autoclave at 1 atm for 70 minutes. Samples that have been autoclaved are washed with isopropyl alcohol until they reach a pH of 5.0 and then dried. The powder obtained is glucosamine hydrochloride.

Characterization of Glucosamine

The yield of glucosamine obtained is measured. Characterization includes determination of the yield glucosamine and confirmation using FTIR spectroscopy.

The Manufacture of Ointment for Joint Pain

Glucosamine that has been produced and has been characterized is mixed with white vaseline at 1 % (w/w). Firstly, a powder of glucosamine and ethanol 96 % was heating until it blended. Then, white vaseline was added until blended. The mixture was cooled and placed in an ointment bottle.

Statistical Analysis

Characterization of chitin, chitosan, glucosamine was carried out using FTIR spectroscopy with an IR spectral field transmittance range of 4000-400 cm^{-1}. FTIR spectrum of chitin shells of snails showed a tape of loan on the wave number 3464 cm^{-1} indicating the vibration of the cluster -OH and the cluster N-H vibrations which overlap. An absorption band at a range of wavenumber 2947-2870 cm^{-1} indicated the presence of vibration span C-H on $-CH_2-$ aliphatic functional group. Absorption bands at wavenumber 1666 cm^{-1} indicated the presence of the functional groups amide C=O.

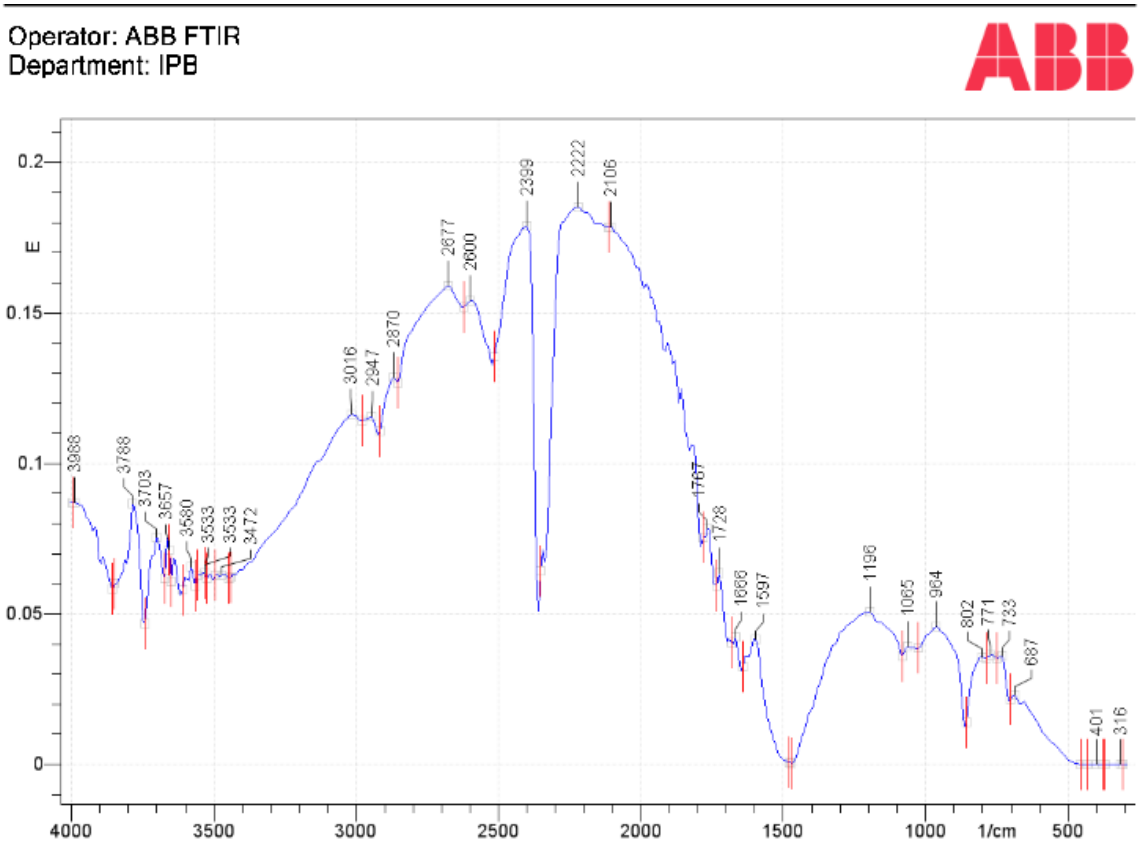

Figure 1 The IR spectra and chitin in the spectral field range 4000-400 cm^{-1}

The C-O bond span is identified in the wave number 1234 cm^{-1} and 1065 cm^{-1}, span of C-O is can come from C-O-C or C-O-H groups. Absorption bands at wavenumbers 1497 cm^{-1} indicated the presence of a $-CH_3$ cluster (Kurniasih and Kartika 2011). Confirmation of the structure of functional groups by using FTIR spectroscopy transmittance in spectral field 4000-400 cm^{-1} indicated that the sample of chitin has a functional group -OH, N-H, $-CH_2-$, C=O, CO-, -CH3. This characterization of the results prove that the resulting powder is composed of chitin.

Chitin powder is used in the of manufacture of chitosan. Chitin powder has been obtained previously mixed with NaOH 60 % (v/v). The end result of this stage is chitosan powder. Chitosan powders are characterized using FTIR. Use of FTIR are conducted to ensure functional groups on chitosan powder have been obtained. The result of the characterization of chitosan using FTIR spectroscopy transmittance in spectral field 4000-400 cm^{-1} is shown in the Figure 2.

FTIR spectrum of chitosan shells of snails showed a tape of loan at wavenumber 3472 cm^{-1} indicating the vibration of the cluster -OH and the cluster N-H vibrations which overlap. An absorption band at a range of wavenumbers 2947-2870 cm^{-1} indicate the presence of a vibration span C-H on $-CH_2-$ for an aliphatic grouping. Absorption bands at wavenumbers 1666 cm^{-1} indicating the presence of functional groups amide C=O. The C-O bond span is identified in the wavenumber 1196 cm^{-1} and 1065 cm^{-1}, the span C-O is can come from

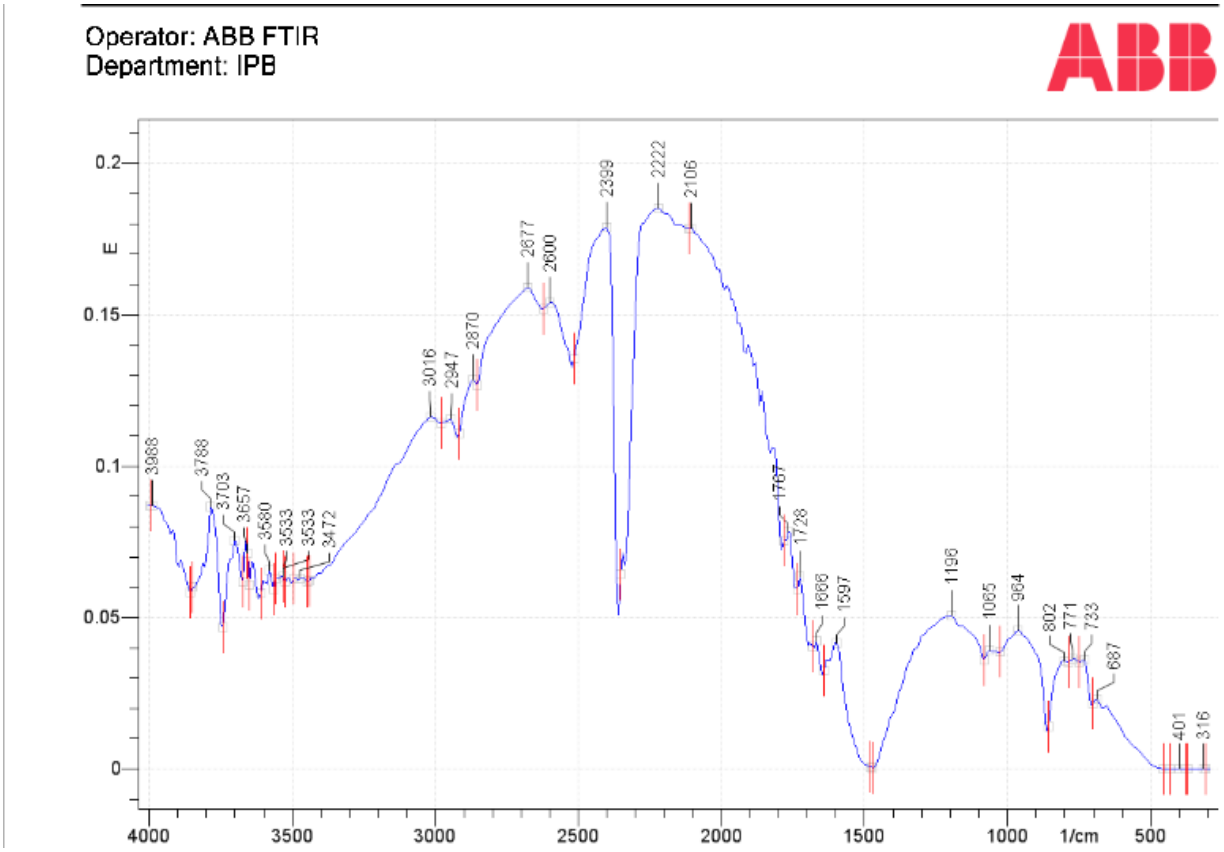

Figure 2 The IR spectra of chitosan in a spectral field range of 4000-400 cm^{-1}

C-O-C or C-O-H. There was no wavenumber uptake for the cluster -CH_3. This indicates the occurrence of deacetylation processes which has led to the loss of the methyl group (-CH_3) (Kurniasih and Kartika 2011). Characterization of chitosan using FTIR is known to have functional groups of -OH, N-H, -CH_2-, C=O, CO-. This characterization of the results prove that the resulting powder is chitosan.

The chitosan powders which have been obtained at this stage of purification of glucosamine is continued. The chitosan powders have been obtained were mixed with HCl 8 % and put it on autoclave at a pressure of 1 atm for 70 minutes. The function of the pressure in the autoclave is not to break the acetyl group but, rather just cut the polymer of chitosan into smaller units so the Cl^- ion from HCl more easily bind to the cluster of amine. Chitosan forms NH_3Cl. The presence of hydroxyl groups O-H and NH_3Cl on carbon units create glucosamine hydrochloride which is soluble in water (Ernawati 2012). The end result of this stage is glucosamine powder. Glucosamine powder was characterized using FTIR spectroscopy transmittance in spectral field range 4000-400 cm^{-1}. Use of FTIR spectroscopy transmittance to ensure functional groups on the glucosamine powder is obtained. The results of the characterization of glucosamine is shown in Figure 3.

The FTIR spectrum glucosamine shells of snails showed a tape of loan at wavenumbers 3472 cm^{-1} indicating the vibration of the cluster -OH and cluster N-H vibrations which overlap. Absorption bands at a range of wavenumbers 2986-2870 cm^{-1} indicating the presence of

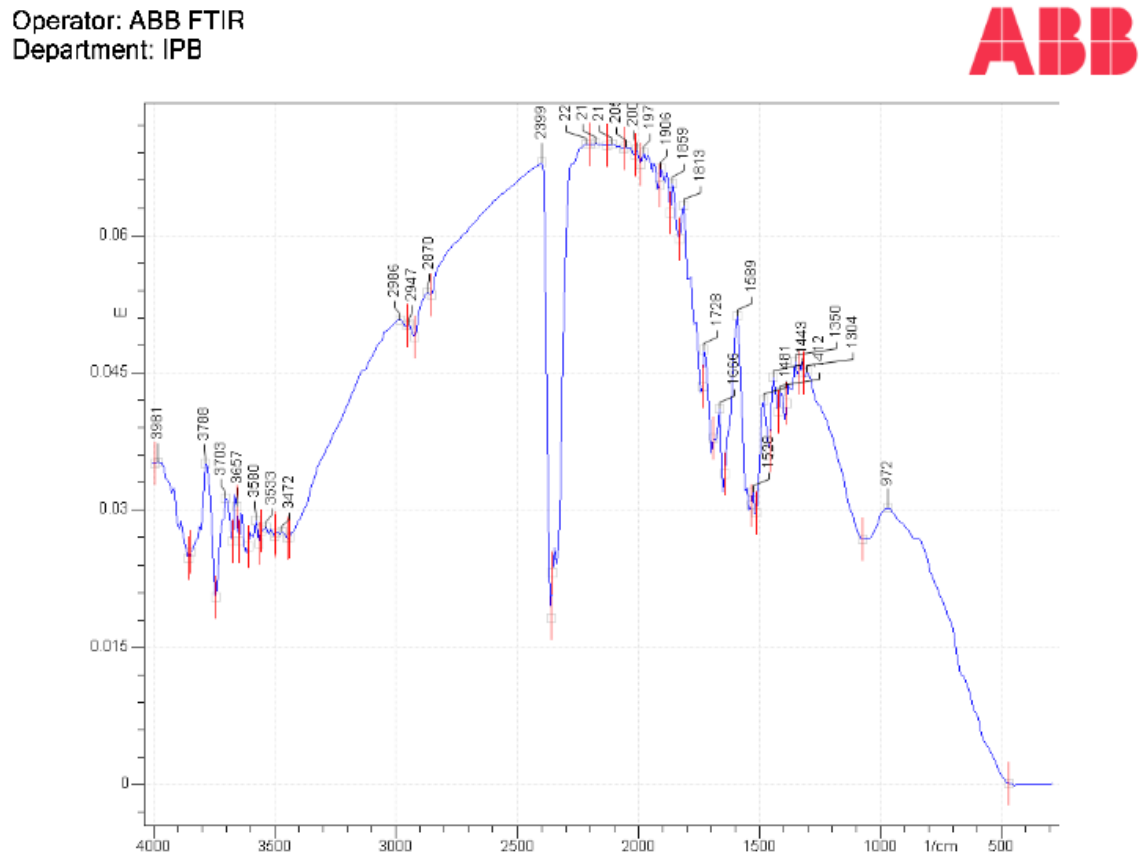

Figure 3 The IR spectra of glucosamine on spectral field range 4000-400 cm^{-1}

vibration span C-H on -CH_2- on the aliphatic group. Wavenumbers at 1666 cm^{-1} indicate the presence of functional groups of an amide C=O. The stretch of C-O-C is not identified because there is no absorption bands at wavenumbers 1170.79 - 898.83 cm^{-1} that indicating the presence of functional groups of C-O-C (Dewi *et al*. 2016). In addition, there is no cluster to absorption bands -CH_3. Characterization of glucosamine using FTIR spectroscopy transmittance is known to have functional groups -OH, N-H, -CH_2-, C=O. This characterization result proves that the resulting powder is glucosamine.

3. RESULTS

The glucosamine production process used a modification of the method of Dewi *et al*. 2016. This involved 3 stages. The first stage, the process of extraction of chitin through the process of deproteination, demineralisation, and pigmentation. The second stage, the process of production of chitosan through the process of deacetylation. The third stage, the synthesis of glucosamine by autoclaving at 1 atm for 70 minutes. The results obtained are shown in Table 1.

The resulting glucosamine powder is used as an ingredient mixed with white vaseline. The manufacture of ointments containing glucosamine is done by using the glucosamine have been obtained and the glucosamine from shrimp skins to distinguish colours, textures,

Table 1 The result of the production of glucosamine hydrochloride from snail shells

Methods	Chitin composition of Shell Waste	Chitosan composition of Chitin	Glucosamine composition of Chitosan	Total
Method 1 (reflux+water bath)	5.73 %	27.90 %	89.16 %	1.42 %
Method 2 (reflux+water bath and reflux+magnetic stirrer)	13.42 %	67.16 %	16.44 %	1.47 %

and smells of both the ointments. The ointment glucosamine from shells of snail and ointment glucosamine from shrimp skins at glucosamine 1 % (w/w). The ointment presented in Figure 4.

4. DISCUSSION

In Table 1 we show the two different methods used to produce glucosamine. Extraction of chitin and chitosan on production method 1 is done by using reflux+water bath. Extraction of chitin and chitosan on production method 2 is done by using reflux+water bath and reflux+magnetic stirrer. The process of demineralisation and depigmentation in method 2 was done by using reflux + water bath. Meanwhile, the process of deproteination and deacetylation on method 2 is done by using reflux + magnetic stirrer. Method 1 produces 5.73 % chitin from the snail shells, 27.90 % chitosan produced from chitin, 89.16 % glucosamine produced from chitosan. Method 2 produces 13.40 % chitin from the snail shells, 67.16 % chitosan produced from chitin, 16.44 % glucosamine produced from chitosan. Total yield shows that method 1 has glucosamine level of 1.42 %, method 2 has glucosamine yield of 1.47 %. Method 2 shows that has the highest total glucosamine yield. This shows that the use of magnetic stirrer can produce the highest yield amount of glucosamine. Use of a magnetic stirrer can accelerate the process of deproteination, demineralisation, depigmentation, and deacetylation.

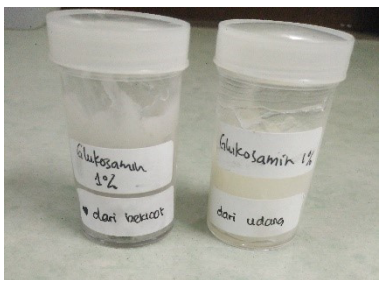

Figure 4 Ointment 1 % of glucosamine shells of snail and ointment 1 % of glucosamine skins of shrimp

The value of the yield of glucosamine is affected by factors such as the temperature, concentration of acidic, warm up, and pressure (Ernawati 2012). This occurs due to damage to the compound chitosan when hydrolyzed exceed the optimum time. Influence of pressure on yield of glucosamine hydrochloride is the higher pressures made the yield of glucosamine hydrochloride is getting smaller. The decline in yield is alleged to occur due to high pressure on the course followed by a rise in temperature, so the damage or degradation and can form a compound of furfural which ultimately lowers the yield glucosamine hydrochloride (Ernawati 2012).

Our ointments have a concentration 1 % (w/w) of glucosamine. Glucosamine 0.1 gram is mixed with 10 gram white vaseline. The production of ointments done by heating a mixture of glucosamine and ethanol 96 % blended well. Then, white vaseline was added

to the mixture until blended well. Furthermore, the mix of glucosamine and the white vaseline ointment produced has a white colour with a grey, smooth-textured, and was odourless. In contrast, the ointment of glucosamine skins of shrimp has a white colour with a yellowish, smooth-textured, and odourless.

Conclusion

Glucosamine is present snail shell waste. The greatest yield glucosamine production of shells used reflux+water bath and reflux+magnetic stirrer. Magnetic stirrer increased the reactions of production. An ointment of glucosamine was made by mixing 0.1 gram glucosamine and 10 gram of the white vaseline. The ointment of glucosamine from shells waste of snails has a white colour with grey, smooth-textured, and odourless.

5. ACKNOWLEDGEMENT

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