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Study on the Bioactive Compounds of Shark (*Prionace glauca*) Cartilage and its Inflammatory Activity

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Abstract: The objective of the study was to identify the bioactive compounds of shark (Prionace glauca) cartilage and observe its antiinflammatory activity. This study was descriptive and used laboratory experiment separated into 3 phases: preparation and extraction, bioactive compound identification using Fourier Transform Infra Red (FTIR) spectroscopy, and in vivo antiinflammatory activity test using wistar rats. Results showed that IR-spectra of chondroitin and glucosamine isolated from the shark cartilage had very similar absorption peak to the standard glucosamine sulphate and chondroitin sulphate. The IR-spectra of standard chondroitin possessed strong absorption peak at the wavelength of 1627.87 cm^{-1} and 1413.72 cm^{-1} indicating that the presence of carboxyl groups with amine and sulphate. The mean percent of inflammatory inhibition was 13.40%, 4.02%, 4.15%, 3.88 % and 2.01 %, respectively, for indomethacine, shark cartilage powder,chondroitin extract, glucosamine extract and the combination of chondroitin-glucosamine extracts. The extract of chondroitin suphate had higher inflammatory activity than that of other treatments, but not significantly different from that of shark cartilage powder.
Keywords: Shark cartilage, anti-inflammatory, in vivo.

Introduction

Indonesia is a maritime country of rich biopharmaceutical products. Sharks are one of the fishes possessing high number of species and believed to have compounds potential to anti-cancer. It has been used in either conventional form or in modern pack. The use of shark cartilage as medicine in Indonesia is uncommon yet. The fish is fished for their fins as delicious soup dish, while the flesh and cartilage are often wasted. Information on bioactive compound purificatiom and anti-angiogenic activity will increase the economic value of sharks[1].

Shark cartilage is composed of collagen, amino acid, mineral, and glycosaminoglycan. It contains more than 40% of pure collagen proteins plus essential amino acids and non essensial amino acids. Collalagen and amino acids are required for tissue elasticity, fissured skin, ligament, and tendon healing, and growth and rejuvenation of skin and connective tissues[2]. Previous study[3] revealed that shark cartilage held 30.74% proteins and 10,673.59 ppm of calcium. Total content of amino acid was 23.66% of protein, but its amino acid composition was higher than PAP (Provisional Amino Acid Pattern). Glucosamine and chondroitin are amino glucose as major components of shark cartilage. Both compounds act to stimulate the formation of glycosaminoglycan, proteoglycan, and hyaluronic acid. Clinical studies have revealed that glucosamine helps reducing arthritis symptomes. In short period, glucosamine has been tested and demonstrated effective symptoms osteoarthritis patients. Glucosamine could also take protective role against modification disease in osteoarthritis case by providing anti-cathabolic effect and anti-inflammatory activity on chondrocytes[4-6]. This

study was aimed at identifying the bioactive compounds of shark cartilage using FTIR (Fourier Transform Infra-Red) spectroscopy and knowing the antiinflammatory activity of the compounds.

Materials and Method

Materials

Shark cartilage samples were obtained in frozen condition from frozen fish processing company of "CV Angin Timur". These were taken to Fisheries Product Processing Laboratory of Hang Tuah University, Surabaya, using cool boxes. The frozen samples were melted in flowing water and cleansed from flesh remnants attaching to the cartilage and non-cartilaginous tissues. The cartilage was then stored at -20^{o}C up to extraction. All chemicals used were pro-analytical quality, such as ammonium carbonate, glacial acetic acid, indomethacin, carrageenan, ether, and Na-CMC. For antiinflammatory activity test, wistar-strained rats were employed.

Equipment

This study used knife, thermometer, dryer, pan, blender, a 80-mesh screen, plastic clip, cuvet, magnetic stirrer, centrifugator, freeze dryer, IR-spectrophotometer, marker, pH pen, rat cage, rat drinking water supply, measuring tube, incubator, stirrer, sonde, and plethysmometer.

Test Animals

Male wistar rats, *Rattus novergicus*, of each 100 – 120 g BW were obtained from CV. Surabaya Mouse Service. They were kept in cages at room temperature. Drinking water and feed were given *ad-libitium*. They were then separated into 6 groups each of which was 4 rats. Two groups were employed as positive and negative controls. The former was orally induced with indomethacin of 10 mg/kg BW, and the latter with that of 1 ml of 2% Na-CMC. Other 4 groups were treatment groups that were daily administered with cartilage powder, extracted glucosamine, extracted chondroitin, and the combination of glucosamine-chondroitin extract each of which with the dose of 100 mg/kg BW. The test animals were previously acclimated for 2 weeks to adapt to the laboratory conditions. All research procedures have followed the guidance and the regulation of the ethical clearance committee of Hang Tuah University.

Bioactive compound extraction

Frozen shark cartilage samples were melted at 4^{o}C, then chopped (about 1 mm^3 size), dried, milled, and sieved through a 80-mesh sieve to obtain fine powder. The cartilage powder was then dissolved in 0.1 M ammonium carbonate pH 8 (10 gr of cartilage powder per 100 ml of ammonium carbonate), agitated with magnetic stirrer for 24 hours at room temperature, cengtrifuged, and the supernatant was dried in a freeze dryer to gain glucosamine compound[7]. Chondroitin extraction was done through incubation of 10 g of shark cartilage powder in 100 ml of pH 4.5 water at 37^{o}C for 7 hours, pH is controlled through acetic acid addition. This condition is an optimal treatment for chondroitin extraction of cow's nose cartilage[8]. After incubation and then centrigugation, the supernatant was dried in a freeze dryer for 12 – 18 hours to obtain chondroitin compound.

Bioactive Compound Identification with FTIR Spectrocopy

FTIR spectroscopy is an established and continuously growing analytica tehnique that enables to do simple, reliable, and fast analyses of various types of samples[9]. IR spectrocopy is highly useful for qualitative analysis (identification) of organic compounds since each organic compound produces unique spectrum with suitable structural peak for different features. Each functional group also absorbs the infra-red light at the unique frequency. For instance, a carbonyl group, C=O, always absorbs the IR light at the wavelength of 1670 cm^{-1} to 780 cm^{-1}, that causes the carbonyl bond stretchable[10]. A chemical bond can vary with its energy level by giving specific frequency. This has become the principle of infra-red spectroscopiy measurement, in which if the sample is exposed to infra-red light the functional group of the sample will absorb the IR radiation and vibrate through stretching, bending, forming or a combination of vibration. The absorption/vibration can directly be correlated with chemical (biochemical) types, and the spectra of IR absorption can be illustrated as characteristics of chemicals or biochemicals[11].

In Vivo Anti-inflammatory Test

In vivo anti-inflammatory test followed the method of Winter *et al*. (1962)[12]. This study used 24 inviduals of male wistar rats divided into 6 groups each of which held 4 individuals. Before the experiment started, carrageenan had been prepared by dissolving 0.5% carrageenan in aquadest (w/v). Each animal was subcutaneously injected with 0.1 ml of 0.5% carrageenan in the rear right foot after the rat foot had been measured as initial data (v_0). The intumescence was measured after 1, 2, 3, 4, and 5 hours of carrageenan injection. The intumescence volume of the rat foot was measured using a plethysmometer, and the percent of the intumescence from inflammation was calculated as follows:

$$\% \text{ Volume of edema } = \frac{Vt - Vo}{Vo} x100\% \text{ , and then}$$

percent inhibition of edema was calculated as follows:

$$\% \text{ Edema inhibition} = \frac{R - S}{R} x100\%$$

where

Vt : Volume of rat feet at t
Vo: Volume of rat feet at t_o
R : Mean inflammation of negative control group
S : Mean inflammation of treatment groups

Results and Discussion

Bioactive Compounds of Shark Cartilage

Infra-red spectral absorption was read at the wavelength of 400-4,000cm^{-1}. Glucosamine isolated from the shark cartilage possesses nearly similar functional group to standard glucosamine, glucosamine sulphate. The IR spetra of shark cartilage powder and glucosame powder of shark cartilage are presented in Fig. 1 and 2.

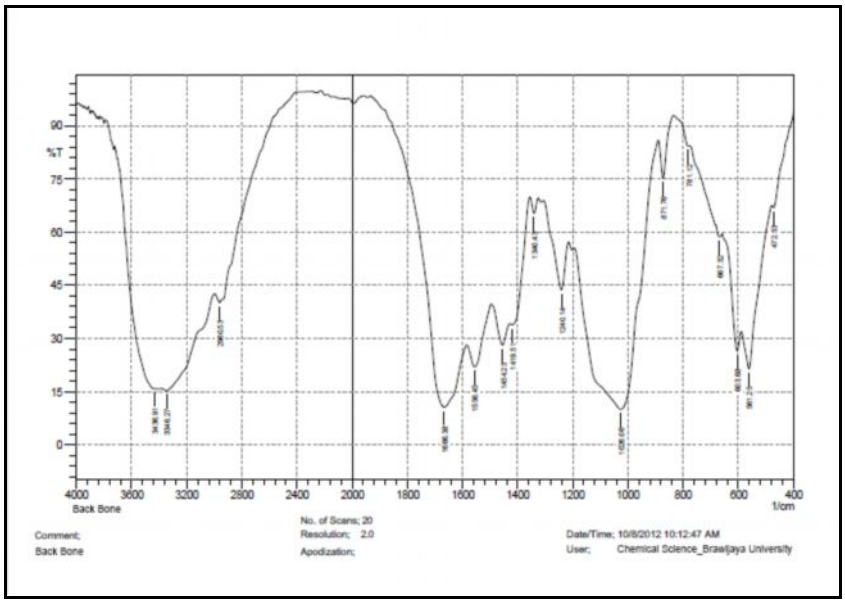

Figure 1. FT-IR Spectra of Shark Cartilage Powder

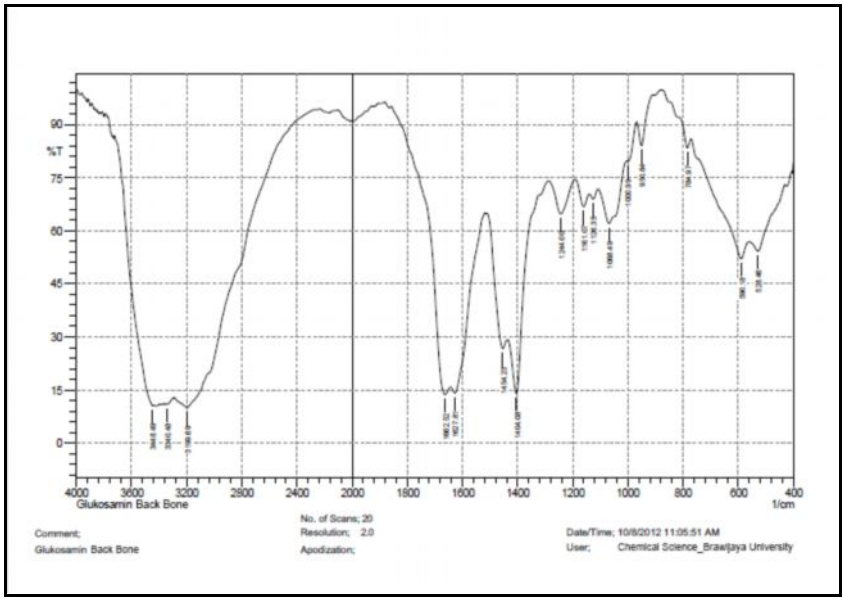

Figure 2. FT-IR Spectra of Glucosamine Isolated From Shark Cartilage

Fig. 2 demonstrates that the IR spectra of glucosamine isolated from shark cartilage is indicated with wide band above ther wavelength of 3000 cm^{-1} and strong absorption at the wavelength of about 1,650 cm^{-1} and 1,050 cm^{-1}. Hydroxide functional group is dominat group and possesses wide and strong IR absorption characteristics at the wavelength of 3,423.41 cm^{-1} dan 3,448.49 cm^{-1}. The asymetrical NH group has the IR absorption characteristics at the wavelength of 1,662.62 cm^{-1} and 1,627.81 cm^{-1}, while the symetrical NH group occurs at the wavelength of 1,454.23 cm^{-1} and 1,404.00 cm^{-1}. The stretching and deformation of –C-O-H bond vibration occurring at the wavelength of 1,627.81cm^{-1} and 1,454.23cm^{-1} are corresponding to the presence of carboxylate association with amine and sulphate. It indicates that glucosamine isolated from the shark cartilage is classified as glucosamine sulphate. Exogenous glucosamine generally consisted of glucosamine hydroxide and glucosamine sulphate. Ini Japan, glucosamine sulphate is an osteoarthritis drug, not nutritional supplement[13]. The infrared spectrum of standard chondroitin sulphate chondroitin isolated from the cartilage can be seen in Fig. 3 and 4.

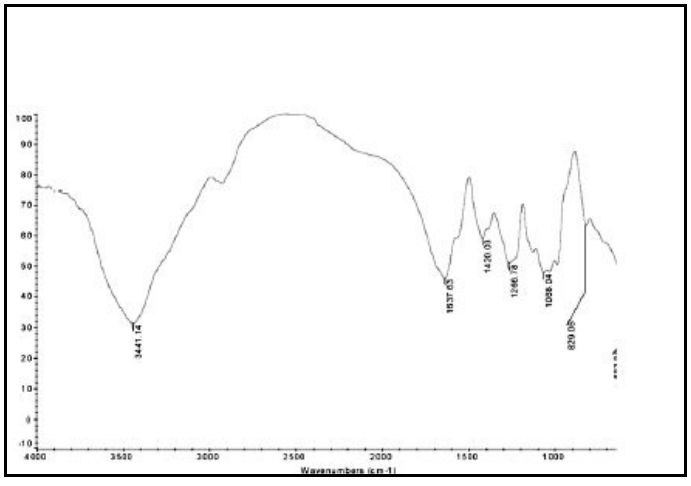

Figure 3. FT-IR Spectra of Standard Chondroitin Sulphate (Manjusha, 2011)

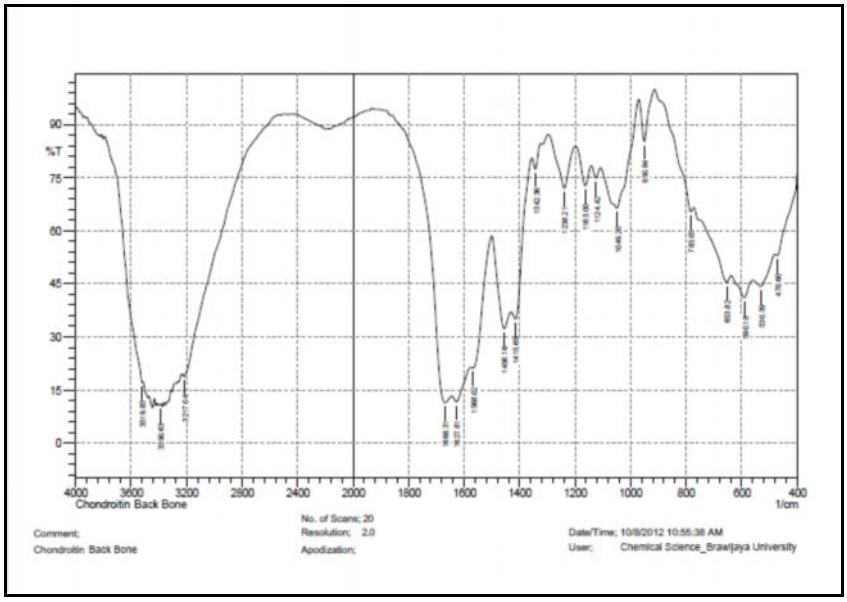

Figure 4. Chondroitin Isolated From Shark Cartilage

The IR-spectrum of chondroitin isolated from the shark cartilage (Fig. 4) is compared with the standard IR-spectrum of standard chondroitin sulphate (Fig. 3). In the case of standard chondroitin sulphate C, stretching and deformation of –C-O-H- bond vibration at the wavelength of 1,637.63 cm^{-1} and 1,420.03cm^{-1} is corresponding to the carboxylate association with amine and sulphate, while the peak intensity of chondroitin isolated from shark cartilage occurs at the wavelength of 1,661.31cm^{-1} and 1,415.05 cm^{-1} (Fig. 4). The wide band above the wavelength of 3,000cm^{-1} determines the stretch of hydroxyl vibration of polysacharride and water involving hydrogen bond. Sulphate bond of standard chondroitin sulphate (Fig. 3) started from the wavelength of 1,266.78 cm^{-1} to 829.06 cm^{-1}. The same peak intensity was found at the wavelength of 1,233.21cm^{-1} to 783,05cm^{-1} in chondroitin isolated from shark cartilage indicating sulphate group. Chondroitin sulphate is particularly useful for taking care of cartilage structure and function, ostearthritis joint pain, and anti-inflammatory activity[14].

Antiiflammatory activity test

Carrageenan is a suitable model for acute inflammatory study of rats[15,16]. Foot intumescence model from carrageenan induction is very sensitive to evaluating orally antiinflammatory agent, particularly natural products, and highly reliable for acute antiinflammatory studies[17]. Subcutaneous injection of carrageen in the rat foot causes edema formation that is a biphasic event[18,19]. Phase 1 (0 – 2.5 hours after carrageenan injection) is mainly caused by inflammatory mediator release, such as histamine, serotonin, from surrounding damaged tissues. Phase 2 (3 – 6 hours after carrageenan injection) is supported by releasing prostaglandin produced by the macrophage and mediated by bradykinin and leukotrienes[17]. *In vivo* anti-inflammatory activity was observed once an hour for 5 hours of post-carrageenan induction. The effect of the bioactive compounds isolated from shark cartilage on the rat foot induced with carrageenan is given in Fig. 5 and the anti-inflammatory activity in Table 1 .

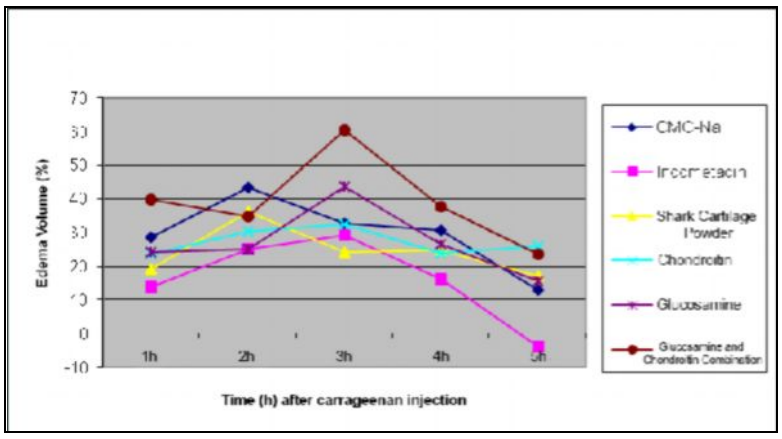

Figure 5. The effect of shark cartilage bioactive compounds on the carrageenan-induced rat foot.

Fig. 5 shows that maximum edema volume occurs in the third hour. It is in line with the previous study that edema grows fast in 3 hours of post-carrageenan induction and stays at the maximum volume for about 5 hours after induction[20]. The administration of shark cartilage powder, glucosamine isolate, chondroitin isolate, and glucosamine and chondroitin isolate combination gives similar effect to that of indomethacin application, edema reduction at the third hour and reaching the initial volume at the fifth hour.

In pharmacokinetics, the ability of compound to reduce edema of the test animal foot from carrageenan injetion is expressed as inflammatory ability[21]. This is useful to determine whether two similar dose formulations can release the same dose in the body. The value of antiinflammatory ability is obtained by comparing the area below the edema curve volume of the treatment animal with the area below the negative control curve. The area under the curve (AUC) is the area below the curve of drug concentration in the plasm plotted against time. The AUC formula is as follows:

$$AUC_{0\text{-}5} = \frac{V_0 + V_1}{2}(t_1 - t_0) + \frac{V_2 + V_1}{2}(t_2 - t_1) + ... + \frac{V_5 + V_4}{2}(t_5 + t_4)$$

and the anti inflammatory ability was calculated as follows:

$$\text{Anti inflammatory ability} = \frac{(AUCx - AUCp)}{AUCx} x100\%$$

where

AUC : Area under curve
AUC_x : Area under edema percentage curve against time in average negative group
AUC_p : Area under the percent edema curve against time in average treatment group
V_0 : Initial volume (before induction)
$V_{1\text{-}5}$: Edema volume of $t_1 - t_5$
0-5 : Measurement time of edema volume ($t_0 - t_5$)
$t_{1\text{-}5}$: Measurement time

Table 1. Data of AUC and Anti inflammatory Ability

Treatment Group	AUC	Anti inflammatory (%) Ability
Negative Control: Na-CMC 2%	0.373	
Positive Control: Indomethacin	0.323	13.40
Test I: Cartilage Powder	0.358	4.02
Test II: Chondroitin	0.3755	4.15
Test III: Glucosamine	0.3585	3.88
Test IV: Combination glcosamine and chondroitin	0.3655	2.01

Table 1 demonstrates that the highest anti-inflammatory ability occurs in rat treated with indomethacin (positive control). Indomethacin is non-steroid indol derivative anti-inflammatory drug which is chemically 1-(p-chlorobenzoil)-5-methoxy-2-methyl-indola-3-acetic acid. Indomethacin works more effectively than aspirin or other non steroid antiinflammatory drugs, is the strongest prostaglandin synthetic inhibtor. It is well absorbed after oral administration and partly related with plasm protein (about 90%). Metabolisms occur in hepar in unchangable form, and this drug is excreted into the bile and urine[22]. Shark cartilage[23] contains hialuronic acid, a compound responsible for the viscosity of synovial fluid and synthesized by synovial membrane cells. The liquid part of synovial fluid is believed originating from transudate. The synovial fluid acts also as source of nutrition for joint cartilage. It was also found[24] that as glycosaminoglycan, hialuronic acid worked as wound healing as well.

Glucosamine is a basic component of glycoproteins. It is known to be able to reduce the cartilage proteolytic process and reform the damaged glucosaminoglycan. *In vitro* study demonstrated that glucosamine had antiinflammatory effect through inhibition of inflammation-nediating cell production. This effect was demonstrated in experimental rats with arthritis[25]. Chondroitin sulphate is a derivative of cartilage components, either from shark, cow (*bovine cartilage*) or chicken. Previous studies in France and Italy in 1998 found that oral or injection administration could help increasing the joint movements and reducing the joint pain[26].

Conclusion

The value of anti-inflammatory ability shows the presence of anti inflammatory effect of shark cartilage powder, chondroitin isolate, glucosamine isolate, and the combination of glucosamine–chondroitin. The highest percentage of the anti-inflammatory ability occurs in chondroitin test group and nearly similar to that of the cartilage powder. Chondroitin isolate production is more difficult and needs higher cost, so that the use of the cartilage powder is more effective and efficient.

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References

1. Sajuthi, D. 2001. Purification of Antiangiogenesis Compound From Indonesian Shark Cartilage (Elasmobranchii) and In Vitro assay With Endothelial Cell Culture. *Bulletin of Chemistry*. 1 : 81-85.
2. Zhion. 2013. Shark Cartilage Benefits, Shark cartilage Side Effects. http://www.Zhion.com/shark cartilage.html. accessed on November 24, 2014.
3. Agustin, T.I., Febriani, M., Yatmasari, E. 2012. Proximate composition and amino acid profile of shark cartilage (*Prionace glauca*). National Proceeding MPHPI Seminar. University of Brawijaya. ISBN : 978-602-9286-19-9 (B4) 1 - 8.
4. DeAngelo, N.A., and Gordin, V., (2004), "Treatment of patients with arthritis-related pain", *J. Am. Osteopath. Assoc.*, Vol.104, pp.2-5, at:http:// www.jaoa.org/cgi/content/full/104/11_suppl/2S, accessed on July 5, 2010
5. Scotto, A.D., Corsi, A., Grillo, M.G., Cicione, C., Calamia, V., Panzini, G., Sansone, A., Giordano, C., Politi, L., and Scandurra, R. 2008. "Effects of intra-articular administration of glucosamine and a peptidyl-glucosamine derivative in a rabbit model of experimental osteoarthritis: a pilot study", *Rheumatol Int.*, Vol.28(5), pp.437-443, PMID:17924112.
6. Huskisson, E.C., 2008, "Glucosamine and chondroitin for osteoarthritis", *J Int Med Res.*, Vol.36(6), pp.1161-1179, PMID:19094424.
7. Fontenele, J.B., Viana, G.S., Xavier, J.F., de Alencar, J.W., (1996), "Anti-inflammatory and analgesic activity of a water-soluble fraction from shark cartilage", *Braz J Med Biol Res.*, Vol.29(5), pp.643-646,
8. Nakano, T., N, Ikawa and L. Ozimek. 2000. An economical method to extract chondroitin sulfate-peptide from bovine nasal cartilage. *Can. Agric. Engin*. 42:205-208.

9. Manjusha, K.P. 2011. Isolation and Characterization of Glycosaminoglycans and a Study of Its Bioactive Potential in Two Commercially Important Species of Cephalopods, *Loligo duvauceli* and *Septa pharaonis*. Ph.D. Thesis. Cohin Univ. Sci. Tech. India.
10. Silverstein, R.M and Webster F.X. 1996. Identification of Organic Compound, 6th Edition. State University of New York : John Wiley & Sons Ltd. accessed October 20, 2013
11. Ellis, D.I. and Goodacre, R. 2006. Metabolic Fingerprinting in Disease Diagnosis : Biomedical Applications of Infrared and Raman Spectroscopy. Jurnal of Royal Society of Chemistry. 131 : 875–885
12. Mutee, A.F., S.M. Salhimi, M.F.Yam, C.P. Lim, G.Z. Abdullah. 2010. In vivo Anti-Inflammatory and in Vitro antioxidant Activities of *Peperomia pellucida*. International Journal of Pharmacology 6 (5) : 686-690.
13. Osaki,T., Kazuo A., Seiji K., Yoshimori T., Takeshi T., Tomohiro I., Yoshiharu O. and S. Minami. 2012. Metabolomic Analyses of Blood Plasma After Oral Administration of D-Glucosamine Hydrochloride to Dogs. Journal of Marine Drug. Vol 10., 1873-1882. ISSN 1660-3397. Open acces. www.mgpi.com/journal/marinedrugs. Diakses 30 2014
14. Somasheker P.L., Tripathy A.S., Chandrashekar J., Sathis K.P., Palakashi G.O., 2010. Spectrophotometric Determination of Chondroitin Sulfate in Bulk Drug and Pharmaceutical Formulation.Der Pharmacia Letter. 2 (6) : 283 – 288. Available online at www.scholarsresearchlibrary.com. accessed on October 1st, 2012.
15. Sawadago, W.R., Rainatou B., Marius L. and N. Some. 2006. Anti-inflammatory, Analgesic and Antipyretic activities of Dicliptera verticillata. International Journal of Pharmacology 2 (4) : 435 – 438. Accessed on October 24, 2013.
16. Tandon, V.R. and R.K. Gupta, 2006. Anti-inflammatory Activity and Mechanism of Vitex negundo Linn. International Journal Phamacol. 2 : 303 – 308. accessed on October 24, 2013
17. Antonio, M.A. and A.R.M.S. Brito, 1998. Oral Anti-inflammatory and Antiulcerogenic Activities of a Hydroalcoholic Extract and Partitioned Fractions of Turnera ulmifolia (Turneraceae). Journal Ethnopharmacol. 61 : 215 – 228. Accessed on October 20, 2013
18. Di Rosa, M., 1972. Biological Properties of Carrageenan. J. Pharmacy Pharmacol. 24 : 89-102.
19. Vinegar, R., W. Schreiber and R. Hugo., 1996. Biphasic Development of Carrageenan Edema in Rats. J. Pharmacol. Exp. Ther., 166 : 96 - 103
20. Morris, Christoper J. 2003. Carrageenan-Induced Paw Edema in the Rat and Mouse. In P. G. Winyard and D. A Willoughby (Ed.). *Methode in Molecular Biology,* Vol. 225: Inflammation Protocols Ipp.115-121). Totowa, NJ: Humana Press Inc.
21. Martin, K. and M. Guidoux. 2010. Area Under Plasma Drug Concentration-Time Curve. Biology faculty, De Lausanne University. *www.sepia.unil.ch/pharmacology.* Accessed on September 24, 2013.
22. Katzung, B.G., 2002, Basic and Clinical Pharmacology. Third Ed., 693-694, Kedokteran EGC Book Publ, Jakarta
23. Ramdani, Wahyu Caesar. 2011. Muskuloskletal. *http:// angsukri. blogspot.com/ 2011/07/muskuloskletal.html*. Accessed on October 21, 2013.
24. Marks, D.B., Allan D. M., C.M. Smith. 2012. Biochemistry of Basic medical: A clinical approach. EGC Publisher, Jakarta,
25. Kolanski, S.L., 2001. *Complementary and Alternative Therapies for Rheumatic Disease*. Hosital Practice. Volume 36, Issue 4, 2001.
26. Thomson, G. 2005. Cartilage Supplements. The Gale Encyclopedia of Alternative Medicine. Second Edition. Volume 1. Jacquelinel. Longe, Projected Editor. Printed in the United States of America.

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