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Glucosamine and Chondroitin Sulphate Content of Shark Cartilage (*Prionace glauca*) and its Potential as Anti-Aging Supplements

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Abstract: The purpose of this study was to determine the content of glucosamine and chondroitin sulphate of shark cartilage and its use as an anti-aging supplement. It was carried out at the Laboratory of Fish Processing, HangTuah University (HTU), for preparation and isolation of bioactive compounds ; Laboratory of Biochemistry, Faculty of Medicine, HTU for clinical trials, and Laboratory of Pharmacy for capsulation. Results showed that glucosamine and chondroitin isolated from the shark cartilage were 28.36% and 6.06%, respectively, of the weight of shark bone powder which is 18.39% of fresh cartilage.
Keywords : shark cartilage, glucosamine, chondroitin.

Introduction

Sharks are a cartilaginous fish recently largely caught for fins to be served as healthy menu, so that their flesh and bone are often wasted in the ocean. Their unique history and life in the ocean has encouraged numerous studies to obtain bioactive compounds that could be beneficial for human. Shark cartilage is especially composed of complex protein and carbohydrate bound with certain tissues without nerves and blood support [1]. Based on clinical studies, shark cartilage was able to control the growth and the spread of tumor cells, help reducing bone pain, avoiding rheumatic diseases, strengthening and maintaining bone function, relieving soreness and gout, maintaining body health and vitality and avoiding crooked spinal abnormalities.[2]

On the other hand, the ability to synthesize glucosamine in human body will decrease as people get older. This change is affected by the decline in the ability of proteoglycans to produce glucosamine that will lead to osteoarthritis disease [3].

Previous findings [4] revealed that bone protein and calcium levels were 30.74 % and 10,673.59 ppm, respectively. Total amino acid of the spinal column is 23.66 % protein, with limited amino acids, leucine and phenylalanine, and it is a good source of threonine. Identification of functional group indicated that glucosamine and chondroitin isolated from shark cartilage were glucosamine sulphate and c-typed-chondroitin sulphate, respectively. Glucosamine sulphate was osteoarthritis medicine[5], while chondroitin sulphate was a good supplement for the health of bones and joints[6]. However, the identification studies are still needed on its anti- inflammatory effects and clinical trials before later used as a basic supplement material for anti-aging.

In general, treatment is an attempt to nullify the symptoms and cure the disease, including disease prevention as well. A fundamental principle of treatment includes also the rule that clinical benefit of a drug given should be higher than the possible risk from its consumption. Objective judgment on the benefit and the

safety of the drug needs knowledge of clinical test methodology, a scientifically methodological tool used to assess the clinical benefit of the drug. The method can also be certain theraphic intervention considering the influencing factors, while adverse effects on individual or population are not desired [7].

Materials and Method

Materials and Equipment

Materials used in this study were both the back part and side part of shark cartilage, pro-analytical standard chemicals, glacial acetic acid, 4.5N ammonia, aquadest, and ammonium.

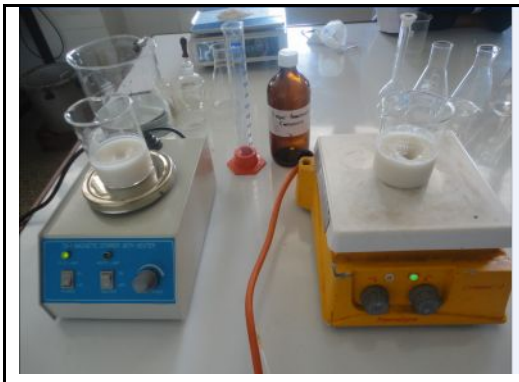
The equipment used in glucosamine and chondroitin isolation were knives, cutting board, dryer, blender, thermometer, a baking pan, screen no. 80 of tyler standard sieve series (0.177 mm), plastic clips, cuvet, magnetic stirer, centrifuge, freeze dryer, pen markers, pH-meter and incubators.

Method

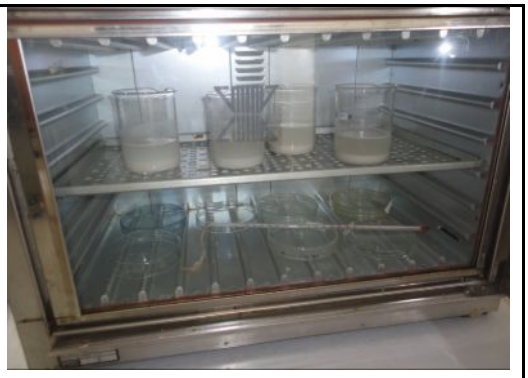
This study used a descriptive method through glucosamine and chondroitin content examination of the shark cartilage. The cartilage samples were collected from PT "Angin Timur" in the frozen condition directly stored in the freezer up to preparation. Flesh debris attached to the bone was removed to avoid disturbances in drying process. The bones were then chopped into very small pieces and dried in a drying machine at 50^{o}C±2^{o}C for 24 hours. The dry shark cartilage was then ground using blender machine and sieved through screen no. 80 to gain a homogenous particle size of flour. It was then put in the plastic bags and kept in the refrigerator until extraction was done.

The glucosamine isolation was done through the following procedures [8]: 10 g of the shark cartilage flour (pass through screen no. 80) were dissolved into 100 ml of buffer solution-0.1M ammonium carbonate-pH 8 for 24 hours at room temperature through continuous agitation using a magnetic stirrer. Ammonium carbonate buffer solution was made as follows: 10.8 g of ammonium carbonate were weighed, added with 500 ml of 4.5N of NH_4OH, and stirred up to homogeneous.

Chondroitin isolation used the procedure as follows [9]: 10 g of the shark cartilage flour (pass through screen no. 80) were dissolved in 100 ml of acetic acid solution at pH 4.5 for 7 hours at a temperature of 37^{o}C. Moreover, both glucosamine and chondroitin isolates were then centrifuged at 10,000 rpm for 3 minutes. The supernatant was collected and kept in the refrigerator up to frezee-dried. The process of glucosamine and chondroitin isolation can be seen in Fig. 1.



Isolation of Glucosamine



Isolation of Chondroitin

Figure 1. Isolation of Glucosamine dan Chondrotin

The supernatant obtained from shark cartilage extraction was then dried in a freeze dryer at a temperature of – 80^{o}C, pressure of 20 p.a. and 50 ml of 96 % alcohol for 36 hours. The product obtained from freeze dryer was white-colored coarse-grain for glucosamine and pink-colored clod for chondroitin. The

extraction of glucosamine and chondroitin was done gradually because of limited capacity of the incubator and magnetic stirrer.

Results and Discussion

Shark cartilage flour

The shark cartilage and its flour yield are presented in Fig. 2 and Table 1. The cartilage is white-yellowish, and mean yield produced is 18.39%. Previous finding found that glucosamine isolated from shark cartilage had nearly similar functional group to the commercially supplement and based on the functional group, the glucosamine belonged to glucosamine sulphate. [10]

Pieces of Shark Cartilage After Washing

Pieces of Shark Cartilage After Cutting

Fig 2. Fresh Shark Cartilage

Table 1. Yield of cartilage flour

Process	Weight of whole cartilage (g)	Weight of cartilage (g) flesh free	Weight of dried cartilage (g)	Weight of cartilage flour (g)	Yield (%)
Process 1	3000	2572	771.6	601.85	20.06
Process 2	1000	842.6	244.4	185.71	18.57
Process 3	2500	2000	509.6	386.4	15.46
Process 4	2500	2200	616.6	486.64	19.46
Average					18.39

Cartilage flour analysis showed that only about 18.39% of the dried flour could be produced from the shark cartilage, indicating sufficiently high water content of the cartilage. In the previous study, it was shown[4] that shark cartilage proximate found that shark cartilage dominantly contained water and protein (Table 2).

Table 2. Mean proximate content of shark cartilage

Sample	Water Content (%)	Ash (%)	Protein (%)	Fat (%)	Carbohydrate (by difference)
TS	70.00±3.46	5.21±0.08	3.92±0.60	1.77±0.08	19.10±3.17
PS	74.00±2.31	6.43±0.15	3.64±0.37	1.64±0.04	14.29±2.39

Note : TS : side part of fresh cartilage
PS : backbone part of fresh cartilage

The proximate content of side cartilage and the backbone part was firstly analyzed to distinguish the flour of each cartilage source. Both cartilage flour sources were dominated by carbohydrate approaching to 70%. Water content was below 6 % obtained through mechanical dryer. The proximatee content of the

cartilage flour is presented in Table 3 [4]. Side part of cartilage is a slightly flat cartilage usually taken from the dorsal part, while the backbone part is the shark vertebra.

Table 3. Mean Proximate Content of Shark Cartilage Flour

Sample Code	Water Content (%)	Ash (%)	Protein (%)	Fat (%)	Carbohydrate (by difference)
TT	5.20±0.06	19.52±0.34	3.59±0.18	1.96±0.62	69.73±0.25
TP	5.40±0.20	21.80±0.26	3.44±0.05	2.02±0.71	67.34±0.89

Note : TS : side part of cartilage flour
PS : backbone part of cartilage flour

Glucosamine and Chondroitin Yield

Glucosamine is one of amino glucose compounds extensively found in the cartilage and possesses very important role in health and joint malleability. Its function in the body is to produce synovial fluid working as lubricant of the cartilage that the cartilage moves well[11]. Synovial fluid defficience will cause joint disturbances, such as stuffy joint, and could cause *Osteoarthritis* (OA) as well. Glucosamine sulphate (2-Amino-2-deoxy-D-glucose sulphate) is salt of glucosamine with chemical structure of $C_6H_{13}NO_5(H_2SO_4)$ that is a natural compound in human body consisting of glucose and glumatime amino acid. Glucosamine is also main element of GAG (glycosaminoglycan) in the cartilage and synovial fluid.

Glucosamine is obtained from skin or shell extraction of crustacean, such as skrimp, lobster, and crab, which through deproteinization and decalciumization, becomes chitin then hydrolized to be glucosamine. It could be found in nearly all body tissues including cartilage. Shark whose bones are cartilageous contain mucopolysacharides, including chondroitin and glucosamine.[12,13]

Chondroitin Sulphate (CS) is an unbranched long chained-heteropolysacharide called glycosaminoglycans (GAGs). GAGs is a heteropolysacharide possessing negative edge binding protein called mucopolysacharide. CS is major component of the extracellular matrix which plays a role in maintaining the structural integrity of the tissue. Cartilage is an important structural component for defense against pressure [14]. CS is anionic polysacharide consisting of disacharide unit structure, N-Acetylgalactosamine 4- or 6-sulphate and D-glucoronic acid. In the cartilage tissue, the polysacharide is covalently bound to protein to make proteoglycan. CS has extensive application in pharmaceutical, cosmetic, food industries. For instance, CS has been known possessing chondroprotective and anti-arthergenic effects on test animals. [9]

Glucosamine and chondroitin sulphate products are used for symptomatic therapy of knee and hip osteoarthritis with some potential effect of structural modification[15,6]. The product of glucosamine and chondroitin isolated from blue shark in this research is presented in Table 4 and Table 5, respectively.

Table 4. Yield of glucosamine isolated

Process	Cartilage flour (g)	Supernatant (ml)	Powder (g)	Yield (%)
1	40	227	10.59	26.48
2	40	178	10.96	27.40
3	40	185	11.05	27.63
4	40	224	12.40	31.00
5	40	218	10.52	26.30
6	40	230	12.56	31.40
7	40	225	11.33	28.33
	Mean yield			28.36

Table 5. Yield of chondroitin Isolated

Process	Cartilage flour (g)	Supernatant (ml)	Powder (g)	Yield (%)
1	240	1380	18.85	7.85
2	240	1260	11.32	4.72
3	240	1363	16.59	6.91
4	240	1356	12.54	5.23
5	240	1440	15.02	6.26
6	240	1320	13.71	5.71
7	240	1409	15.51	6.46
8	240	1356	12.82	5.34
	Mean Yield			6.06

Previous study[4] also found that the Fourier Transform Infra-Red (FTIR) spectrum of chondroitin isolated from shark cartilage had a broad and strong tape characteristic at the wavelength of 3000 cm-^{1} and strong absorption at the wave number of 1,668.31 cm^{-1}, 1,627.81 cm^{-1} , 1,456.16 cm^{-1}, and 1,415.65 cm^{-1} in back part of cartilage, while the FTIR spectrum of side part shows that chondroitin had a sharp and strong tape at the wavelength of 1,672.17 cm^{-1}, 1,627.81 cm^{-1}, 1,454.23 cm^{-1} , and 1,413.72 cm^{-1}, respectively.

The FTIR spectrum of chondroitin sulphate C of commercial products marketted in Indonesia under VD trademark had a peak of strong absorption at the wave number of 1,637.63 cm^{-1} and 1,420.03 cm^{-1} indicating the presence of carboxyl groups, amine and sulphate. Strong peak recorded at 1,627.81 cm^{-1} and 1,415.65 cm^{-1} in chondroitin isolated from the shark backbone is similar to the peak of 1,627, 81 cm^{-1} and 1,413.72 cm^{-1} of side part cartilage that indicates the existence of carboxyl group with amine and sulphate. Both compounds are similar to the commercial VD product marked on the lebels that chondroitin used originates from frozen bovine trachea, while glucosamine is generated from shrimp shell.

In first isolation of 40 g of shark cartilage powder extracted in 400 ml of ammonium carbonate buffer solution, 227 ml of supernatant was obtained, and after freeze dried, 10.59 g (26.48 %) of white glucosamine powder was obtained, while chondroitin isolation using 240 g of shark cartilage powder extracted in 2.4 liter of acetic acid solution - pH 4.5 obtained 1,380 ml of supernatant, after freeze drying, gained 18.85 g (yield 7,85 %) of chondroitin powder was obtained. This process is still needed to be performed up to 200 g of each glucosamine and chondroitin obtained. Isolated glucosamine cannot be continued to *in vivo* test because the ammonia smell was still strong, indicating that ammonia residue is still inside and not safe for human.

Conclusion

Glucosamine and chondroitin successfully isolated from shark cartilage were around 28.36 % and 6.06 %, respectively, of the shark cartilage flour weight, with mean weight of 18.39% of the fresh cartilage.

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