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High Performance Liquid Chromatography (HPLC) for Detection of Glucosamine and Chondroitin Sulfate Compounds

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Abstract

Glucosamine and chondroitin sulfate are compounds found in shark cartilage (*Carcharhinus sorrah*). The two compounds have many health benefits, that is wound healing and helping the process of angiogenesis. This study aims to determine the content of glucosamine and chondroitin sulfate compounds in shark cartilage (SC) extract. The method used was *High Performance Liquid Chromatography* (HPLC) with potassium phosphate buffer solution at pH 3. The results of this research were SC extract contained glucosamine and chondroitin sulfate compounds with a retention time of 1.914 minutes.

Keywords: HPLC; shark; glucosamine; chondroitin sulfate.

INTRODUCTION

Shark cartilage (SC) has several benefits, including controlling the growth and spread of tumor cells, cancer, helping to reduce bone pain, avoiding rheumatic diseases, strengthening and maintaining bone function, relieving pain and gout, maintaining body health and vitality, and avoid curvature of the spine (Sulityowati *et al*., 2015; Dean and Summers, 2006). According Martel-Pelletier (2015) SC can also treat osteoporosis and osteoarthritis because it contains chondroitin sulfate. The extraction and purification of chondroitin sulfate was first carried out in 1960 (Miller and Clegg, 2011).

Research conducted by Sulityowati *et al* (2015) showed that shark cartilage powder contained 28.36% glucosamine and 6.06% chondroitin. Chondroitin sulfate powder is white to cream in color with a pH value between 5.5 to 7.5. This compound is easily soluble in water and is hygroscopic. Chondroitin sulfate becomes unstable when exposed to direct light and at high temperatures (Marzuki *et al*., 2014). According Garnjanagoonchorn *et al*. (2007) in Xie *et al* (2014) states that dry SC contains glycosaminoglican 10-40% and collagen type II 25-55%. Glycosaminoglycan (GAG) is components of the extracellular matrix of connective tissue. Chondroitin sulfate is a type of GAG whose components consist of N-acetyl-galactosamine, sulfuric acid and glucuronic acid. Chondroitin sulfate can be used in joint ailment therapy, anti-inflammatory, arthritis, atherosclerosis and cancer (Siagian, 2014; Widyaningsih *et al*, 2016), and immunostimulant (Bargahi and Rabbani-Chadegani, 2008). According to Kerri *et al*. (2003) and Huskisson (2008) the chemical structure of chondroitin sulfate and glucosamine were shown in Figures 1 and 2.

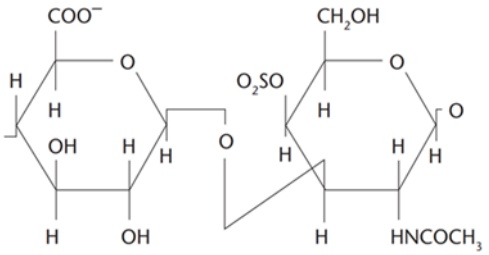

Figure 1. The chemical structure of chondroitin sulfate compound.

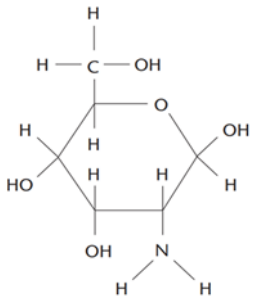

Figure 2. The chemical structure of glucosamine compound.

Chondroitin sulfate is a polysaccharide anion consisting of the disaccharide unit Naacetylgalactosamine 4- or 6-sulfuric acid and D-

glucuronic acid repeated in cartilage tissue. These polysaccharides covalently bind to proteins to form proteoglycans. Chondroitin sulfate has a wide range of applications in the pharmaceutical, cosmetic and food industries (Nakano *et al*., 2000). According to Siagian (2014), chondroitin is found in hyaline cartilage and can be distinguished structurally by the position of the sulfate ion in the monosaccharide bonds.

MATERIALS AND METHODS

Tools and Materials

The tools used in this research include: styrofoam ice box, measuring tape, sitting scale, digital scale, surgical instrument set, knife, tray, oven, aluminum bowl, blander (Miyako), sieve (size mesh 80), plastic bags, plastic clips, gloves, digital analytical scales, petri dishes, aluminum foil, surgical mats, rotary evaporator (RVO 400 SD Boeco Germany), beakers, vials glass, filtering funnels, hot plates, measuring cups, plastic pot bottles, HPLC tools (SHIMADZU), column C-18 (dimensions 4.6 x 250 mm, size of diameter pore 5µm), RID detector (*Refraksi Index Detector*), CPU, stainless steel spatula, tweezers, stirring rod. The material used in this research is shark (*Carcharhinus sorrah*) obtained from Depok Beach, Yogyakarta, ice cubes, methanol PA, acetonitrile, potassium dihydrogen phosphate powder (1.36 grams), distilled water, G-Nutri, Chondroitin Sulfate powder, and phosphoric acid (H_3PO_4).

Shark Cartilage Powder

Shark (*Carcharhinus sorrah*) was obtained commercially from the coast of Depok, Yogyakarta, then all flesh and tissue attached to the cartilage were removed. The cartilage was cut into ±1 cm in size and had been dried using an oven at 50^0 C for 24 hours. Furthermore, it was mashed using a blender, sieved with a sieve of 80 mesh. Shark cartilage powder was stored in a cool place prior to extraction (Sulityowati *et al*., 2015; Davis, C., 1994).

Shark Cartilage Extract

60 grams of shark cartilage powder was dissolved in 1200 ml of methanol as a solvent. The immersion time was 7 days. Every day the solution was stirred for 3 hours. Then after that filtered using filter paper. The filtrate obtained from the filtering was collected, while the residue was discarded. The filtrate was then processed using a rotary evaporator machine. The temperature used on the hot plate was 40^0C and the speed of the driving rotor was 2 turns. The result of the rotary evaporator was a thick, milky white liquid which was an extract of shark cartilage (Iffah *et al.*, 2018).

Detection of Glucosamine and Chondroitin Sulfate Compounds

Instrument setup HPLC (High Performance Liquid Chromatography)

The first step was to turn on the electric power, then turn on the stabilizer. After that turn on the pump on the tool. Next, turn on the water column by pressing the polar button. Then turn on the detector RID (*Refractive Index Detector*) [set the wavelength (γ)], then modular and finally turn on CPU (*Central Processing Unit*) on computer. If the chromatogram shows a flat base line then the instrument can be used.

Preparing the mobile phase

Mobile phase was using potassium phosphate buffer solution pH 3. The buffer solution was made by adding 1.36 grams of potassium dihydrogen phosphate powder into 800 ml of distilled water, then adding phosphoric acid (H_3PO_4). The ratio between the potassium phosphate buffer and the acetonitrile was 99.5:0.5. The flow rate is 1 mL/minute. The HPLC column used was C18 4.6×250 mm 5µm Merck. The temperature in the column used was 28^0C. The eluent will carry the components of the mixture to the RID (*Refractive Index Detectors*) detector. (Jahangir, *et al*., 2015; Nagarajan, *et al*., 2013).

RESULTS AND DISCUSSION

Based on the results of High Performance Liquid Chromatography (HPLC), it was found that the extract of shark cartilage (*Carcharhinus sorrah*) was proven to contain glucosamine and chondroitin sulfate compounds. Shark cartilage extract chromatogram based on HPLC method is shown in Figure 3.

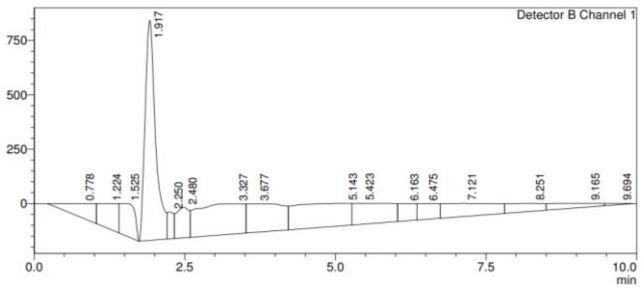

Figure 3. HPLC chromatogram on shark cartilage extract.

The HPLC method is a suitable method to determine the presence of glucosamine and chondroitin sulfate compounds in shark cartilage extracts. Separation of analytes were using phosphate buffer solution and acetonitrile with a ratio of 99.5: 0.5. The buffer solution pH 3 is a mixture of phosphoric acid solution = 88 ml + 1000 ml aquades + 1.3 grams of potassium dihydrogen phosphate and the flow rate used was 1 ml/minute. In

Figure 1 it can be seen that the elution substance has formed a good symmetrical peak.

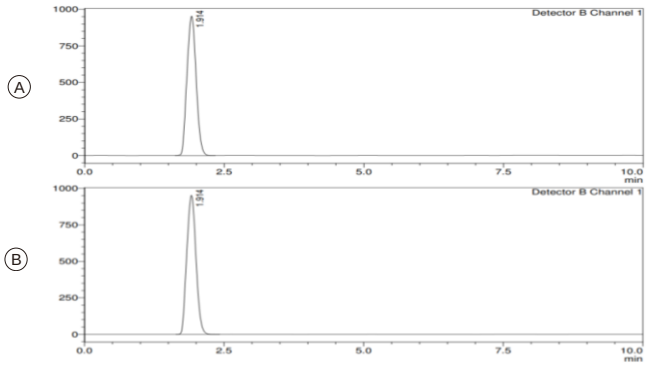

Figure 4. HPLC chromatogram results on Glucosamine (A) and Chondroitin sulfate (B) parameters.

From the HPLC output in the form of a chromatogram (Figure 3), it can be seen that the shark cartilage extract sample (*Carcharhinus sorrah*) has a retention time of 1.917. Meanwhile, the retention time for glucosamine and chondroitin sulfate parameters were 1.914 and 1.914 (Figure 4). This retention time proves that the shark cartilage extract contains glucosamine and chondroitin sulfate compounds.

According to research that has been done by Sulityowati et al. (2015) stated that in 40 grams of shark cartilage powder it contains 28.36% glucosamine and in 240 grams of shark cartilage powder there is 6.06% chondroitin. Glucosamine, 2-amino-2-deoxy-D-glucose (C6H14NO5) is a monosaccharide having a molecular weight of 197.2 Da (Agiba, 2017; Huskisson, 2008). This compound is the main component of glycosaminoglycans (GAGs) in cartilage and synovial fluid (Sulityowati et al., 2015). Glycosaminoglycans (GAGs) are heteropolysaccharides that have a negatively charged protein at the edge and function as a binder called mucopolysaccharide (Sulityowati et al., 2015). Glucosamine is found in almost all connective tissue, but the most abundant content is in cartilage (Dahmer and Schiller, 2008; Sulityowati *et al*., 2015).

Chondroitin sulfate (CS) is a heteropolysaccharide with long and unbranched chains called glycosaminoglycans with a molecular weight of 50-100 kDa, but after the extraction process the molecular weight becomes 10-40 kDa (Sulityowati et al., 2015; Henrotin et al., 2010). But according to Huskisson (2008) the molecular weight of chondroitin is 10,000-50,000 Da. CS compounds have the same properties as GC which are hydrophilic, can dissolve in water and produce a liquid that resembles sodium hyaluronate.

CONCLUSION

Based on observations that have been made using the High Performance Liquid Chromatography (HPLC) method, it can be concluded that the shark cartilage extract (*Carcharhinus sorrah*) contains glucosamine and chondroitin sulfate compounds with a retention time of 1.917 minutes.

***Conflict of Interests*:** Authors state that there is no conflict of interest in this research output.

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