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Halal Source of Medication: Glycosaminoglycan Derived Medicinal Plant

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Abstract

Background Orthosiphon stamineus, is a herbal plant traditionally used in various types of illnesses for example to treat kidney problems, diabetes, rheumatoid, nephritis, muscle pain and also wound healing. These properties majorly attributed by phenolic compounds according to previous researchers. Throughout this study, another novel compound that is glycosaminoglycans (GAGs) were discovered from this magical plant. The best part of this finding is that, the source is halal which until now none studies upon medicinal plant were carried for GAGs activity. Additionally, the particular GAGs are facing limitation for their source leaving mostly come from non-halal source. Objective: This study is endeavouring to extract GAGs from O. stamineus plant using an optimized extraction method. Second objective is to characterize the targeted compound chemical structures. Results: Both FTIR and NMR analysis showed presence of GAGs structure. Total sulfated GAGs were found to be 106.62 mg/g surpassing several other sources of GAGs. In extraction method, this study able to increase its yield up to 47.93% proving the promising yield of this plant as new source. Conclusion: It can be concluded that O. stamineus could be the next GAGs contributor, preparing for a recent halal medication.

Keywords— medicinal plant, halal glycosaminoglycan (GAG), extraction, characterization

INTRODUCTION

GAG is a novel compound that receives a primary attention these days for its multifunctional uses [1]. GAG is used in various fields including pharmaceutical, healthcare, cosmetics and textile [2]; the most popular role of it is a lubricant for osteoarthritis disease [3]. GAG is a strong component of sugar built up from four active classes attached to protein core. The classes include chondroitin sulfate, heparin, keratan sulfate and hyaluronic acid which are present dominantly in all animal cell structures and extracellular matrix functioning as regulator for proteins [4]. Each GAG structures are built up with amino sugar, either N-acetylglucosamine or N-acetylgalactosamine together with uronic acid can be divided into two types, iduronic and glucuronic acid [5]. Researchers have been studying about GAG deriving from organism and microorganism in conjunction of investigating the best source of GAG with high quantity and quality. One of the examples is from *Escherichia coli* capsular polysaccharide [2] where [6] studied on Salmon for the expression pattern on sulphated GAG for development of vertebral column. However, GAG is facing a limited source to be utilized for a worldwide scale not to mention from halal origin making it as a primary concern for this present study despite many researches were made. Considering the incident, this study is introducing a new source from medicinal plant, *O. stamineus.* To the best of our knowledge, none of previous studies have discussed on GAG compound isolated from medicinal plant yet.

O. stamineus or Cat Whiskers is a plant rich with flavonoids compound [7]. It is a herb type of plant, 0.3 – 1 m heights with four-angled stems, has white/dull lilac flowers and widely distributed from East Asian countries including Malaysia and also Africa [8]. It is easily found along roadsides and forest edges which also can be propagated by stems cutting [9]. From recent finding [10], *O. stamineus* is proven to have an important class of constituent that is polyphenol which can be divided into eupatorin, sinensitin, caffeic acid and rosmarinic acid. Another study by [11] suggested *O. stamineus* action by triggering diuretic and uricosuric actions when treated in rats. The statement also has been reported in [12] discussion on crucial active component containing terpenoids and polyphenols.

Most of the studies investigated on secondary metabolites of this plant due to its substantial abilities, and somehow its sugar composition was left out. Accordingly, the aim of this study is to isolate GAG compound from *O. stamineus* in an attempt of reviving this plant as a new source of GAG by specifying its structural presence through characterization process.

MATERIALS AND METHODS

A. Materials

The *O. stamineus* sample was obtained from Delima Jelita Herbs situated in Alor Setar, Kedah. Blyscan kit came along with standards of heparin and chondroitin sulfate was purchased at Biocolor (UK). All other chemicals including consumables and non-consumables were purchased from Chemolab and Bumi Telus (Malaysia).

B. Extraction

This study was conducted by combining hot water extraction and treating sample with ultrasonic disintegrator with some modifications according to method in [13]. Extractions were performed by having plants sample weighed and mixed with 1 L distilled water which then treated 30 minutes with ultrasonic machine. Extracts were then brought to be extracted in hot water at temperature ranging from 60 to 70 °C. This process was allowed for 30 minutes and samples proceeded to centrifugation at 12,000 rpm. Double extraction was done by separating debris and supernatant in order to obtain higher yield of extract. Isolation of GAG took place by precipitation with 80% ethanol overnight.

C. Total sulfated GAG determination

Total sulfated GAG was carried out following Blyscan kit manual.

D. FTIR (Fourier Transform Infrared Spectroscopy)

Crude samples were analysed using Fourier transform infrared spectrophotometer (FTIR). A fraction was taken and grind with Potassium Bromide (KBr) powder. In order to obtain a transparent result for viewing, the fraction must be pressed into pellets before undergo measurement within frequency range of 4000-400 cm-1.

E. NMR (Nuclear Magnetic Resonance)

^{1}H (NMR) characterization was performed at Central Laboratory in UMP.

RESULTS AND DISCUSSION

A. Optimization of GAG extraction

GAGs as matter of fact are complex structure that could not be easily analyzed due to its binding to protein core, thus extraction method plays an important role to break out the bond between them. There are many ways to elicit GAG out of one particular organism, somehow these extraction method not able to provide high amount of targeted compound for medicinal plant. Extraction methods should not be easily chosen without optimizing as most of medicinal plants are herbs type thus preservation on its herbal activities are prioritized. Additionally, plant also consists of cell wall as their rigid protective layer which in this case needs to be broken for the sake of obtaining GAGs within. Water extraction or decoction is an established technique for pharmaceutically extracting medicinal plants by boiling the sample in a specified volume of water applying 1:14 or 1:16 as starting ratio [14]. The boiling water will be able to drag out GAG from inner side of plants only after its cell wall is broken; this is when ultrasonic disintegrator came into the picture.

For this study, extraction and sonication was carried out two times. Supernatant from both extractions were pooled and precipitated with 80% of ethanol. Precipitation using ethanol is compulsory to obtain low molecular weight compound as in GAG. 80% concentration was chosen according to [15] that also tested on ethanol concentration efficacy in producing high yield of crude. Precipitate obtained was however proportional to the concentration of ethanol use [16]. They have tested ethanol concentration starting from 10 to 90% (v/v) and found out 70-80% was optimum concentration to extract out polysaccharide. They also highlight on individual characterization of targeted compound firstly must be studied before starting precipitation as molecular size and structure affected polysaccharide precipitation in ethanol. This new extraction method successfully gave out a remarkable outcome after calculated. New trial increased yield by giving at most 47.93% of yield when compared to previous method that gave 15.74% out of 100 g powder sample (Table 1). *O. stamineus* exhibited appreciable amount of GAG by 5.334 µg in an aliquot of 50 µl while 676.35 mg (10.67%) from total yield (6.34 g). Thus, 106.62 mg/g of GAG was found per gram freeze-dried matter according to Blyscan assay standard curve (Fig 1).

Table 1

	Extraction yield	
	Weight (g)	Percentage (%)
First extraction	1.58	15.74
Optimized extraction	6.34	47.93

Table 1 shows extraction yield from optimized method of 100 g raw sample. Powder was obtained after undergo freeze-drying for 3 days having optimized extraction produced three times higher yield than previous method. While crude was obtained right after separation process before freeze drying, the crude was in gel-like state, reckoned as yield of crude over initial weight of raw sample times with hundred percent.

GAG content in *O. stamineus* found to be higher than chicken egg as studied by [1]. In the study conducted, GAG in chicken egg was detected in all components of egg and separately measured according to each GAG classes. The plant surprisingly surpass the amount of GAG consist in mollusk [17] of 23.90 %. From a study by [18], bovine cartilaginous tissues exhibited almost 80% of GAG yield from estimate 10 g of wet sample. According to the result, it can be speculated that *O. stamineus* able to give a comparative amount of GAG to bovine. Confirmation on its structure was then made using software analysis.

B. FTIR analysis

FTIR was done to provide an idea on GAG composition existed in the samples. Two types of standard were referred in comparison with samples at range 400 – 4000cm-^{1}. Chondroitin sulfate and heparin standard showed stretching of -C-O-H- at 1638.49cm-^{1} and 1634.11cm-^{1} respectively which correspond to combination of carboxylate with amine and sulfate [19] while sample of *O. stamineus* exhibited a very close proximity to both standards at 1605.15cm-^{1}. *O. stamineus* showed presence of sulfate group starting from 1025.73cm-^{1} until 1099.76cm-^{1} (Fig 2). Results from FTIR suggested the existence of uronic acid in sample according to heparin standard band at 1634.11cm-^{1}. It was also due to the unsymmetrical stretch vibration of glucuronic acid for C=O group. Thus, the present study confirmed the identity of extraction yield from extract as sugar due to intense band ranging 3394.92cm-^{1} to 3630.14cm-^{1}.

C. NMR analysis

NMR was done in order to identify the content of the structures in crude sample as well as its chemical composition. Sample was prepared and clearly dissolved to prevent any undiluted residues that may interfere with NMR analysis. Based on Fig 3, some spectra show signals for hyaluronic acid ranging from 2.61 to 4.07 ppm which resembles protons on sugar ring [20]. Signal at 3.90 ppm exhibited protons for hyaluronic acid disaccharide unit [21]; [22] while 4.32 ppm for glucuronic acid group. The spectra also showed sign for heparin presence through 2.10 to 2.18 ppm signals [23]. From the results, it can be speculated that the sample is a GAG compound by a close similarity of signals exhibited during characterization using FTIR and NMR. The study revealed on crude of *O. stamineus* with tremendous amount of GAG that also can be utilized in high yield of extraction. FTIR and NMR give close proximity of spectra for GAG structure, uronic acid. These outcomes indicated *O. stamineus* potential as the next GAG source.

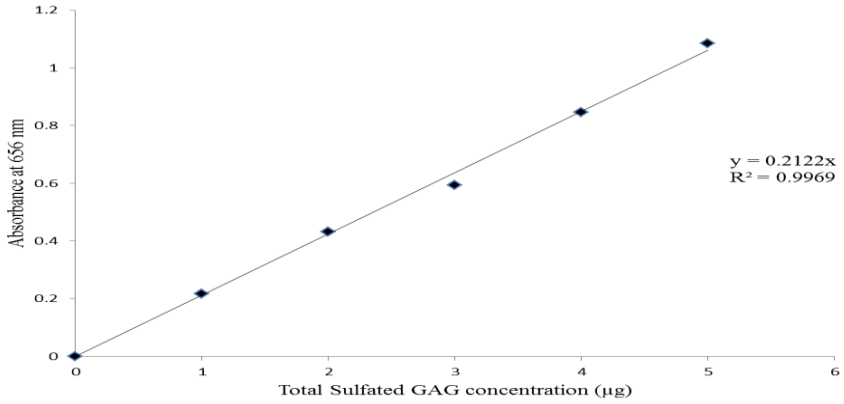

Figure 1, Graph shows calibration curve for Blyscan assay after seven trials taking from chondroitin sulfate standard. Concentration of sample starting from 1-5 µg was prepared and tested under Blyscan kit together with sample.

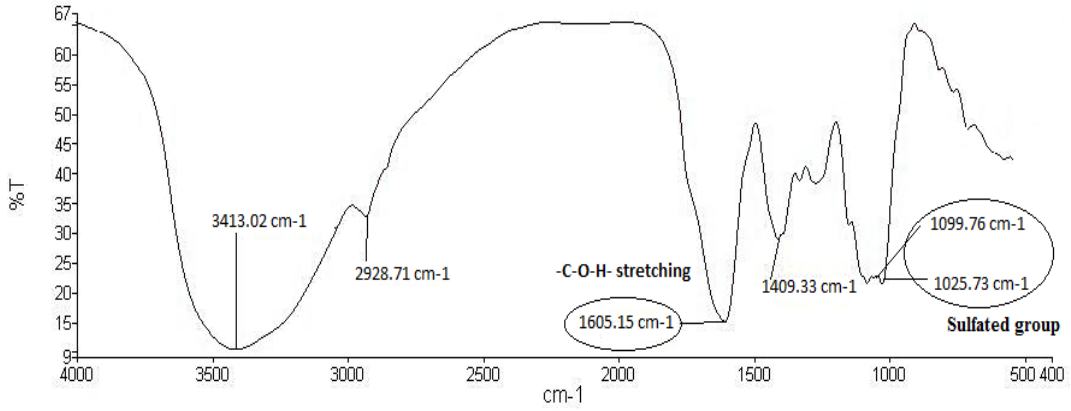

Figure 2 showing analysis result for FTIR using *O. stamineus* sample. A fraction was taken and grind with Potassium Bromide (KBr) powder. In order to obtain a transparent result for viewing, the fraction must be pressed into pellets before undergo measurement within frequency range of 4000-400 cm^{-1}.

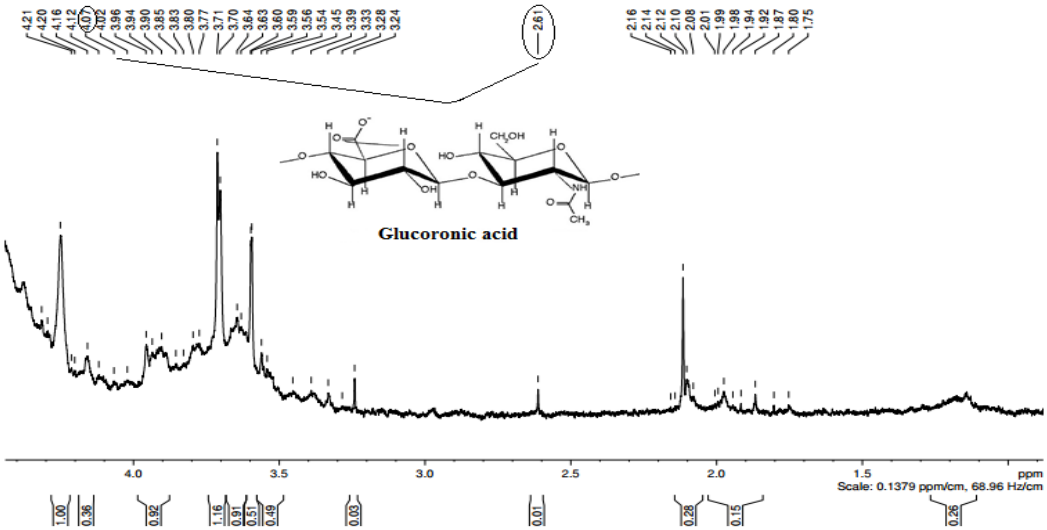

Figure 3 showing NMR peaks in zoom out view. 50 mg/ml of sample concentration was sent to Central Laboratory (UMP) for proton testing. Spectra show the sign of hyaluronic acid and heparin in range of 2.10 to 4.32 ppm.

CONCLUSION

In conclusion, the results obtained in the present study with medicinal plant confirm the presence of GAG compound in high amount as well as its satisfactory extraction yield. The GAG compound can be extracted at most 47.93% from 100 g of raw sample which is estimated can produced 63.40 g of GAG in 1 kg dry powder. This study also found competitively high yield of GAG in *O. stamineus* between other sources. Throughout the study, the most prevalent finding is also from extremely low

cost extraction method for larger production. *O. stamineus* is potentially can be the next source of halal GAGs.

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