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ISOLATION AND PURIFICATION OF HYALURONIC ACID LIKE COMPONENTS FROM *IPOMOEA BATATAS (SWEET POTATO)*

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ABSTRACT

Hyaluronic acid is a substance that is present in the human body. It is found in the most elevated fixations in liquids in the eyes and joints. Individuals take hyaluronic acid for different joint issue problems, including osteoarthritis. Hyaluronic acid is utilized in certain eye surgeries like, corneal transplantation, and repair of a disengaged retina and other eye wounds. Hyaluronic acid is additionally utilized as a lip filler in plastic surgery. A few people apply hyaluronic acid to the skin for mending wounds, cosmetics, skin ulcers, and as a lotion. Hyaluronic acid has been advanced as a "wellspring of youth." In the present investigation, *Ipomoea batatas* (sweet potato) was chosen for the study. The samples were extracted with methanol and chloroform. Goat brain and Visceral fluid as standard was chosen for the study. Estimation was carried out to check the presence of total carbohydrates, proteins and reducing sugars. Qualitative and quantitative studies were carried out which indicated the presence of hyaluronic acid like components in sweet potato and brain. Further ion exchange chromatography was carried using DEAE-Sepharose. The elution obtained from ion exchange showed a peak at 230nm which indicates the presence of hyaluronic acid like components. The peak eluents were further purified by gel permeation chromatography using Sephadex G-100. The result indicates the presence of hyaluronic acid like components. The results of the present work suggested that hyaluronic acid like components could be present in Sweet potato.

KEYWORDS: Hyaluronic acid, Sweet potato, chromatography, Visceral fluid.

INTRODUCTION

Hyaluronic acid (HA) is a high molecular weight biopolysacharide, discovered in 1934, by Karl Meyer and his assistant, John Palmer in the vitreous of bovine eyes. Hyaluronic acid is a naturally occurring biopolymer, which has important biological functions in bacteria and higher animals including humans. It is found in most connective tissues and is particularly concentrated in synovial fluid, the vitreous fluid of the eye, umbilical cords and chicken combs. It is naturally synthesized by a class of integral membrane proteins called hyaluronan synthases, and degraded by a family of enzymes called hyaluronidases (J. Necas et al., 2008). As with the joints, doctors also inject hyaluronic acid into the skin to eliminate wrinkles, due to the important part HA plays in collagen health. Additionally, HA is used in skin care creams and lotions since it helps to moisturize the skin and combat the dryness and loss of elasticity that occurs in aging skin that has been depleted of youthful HA stores. Topical hyaluronic acid has been shown to accelerate wound healing - in part by protecting tissue from oxygen free-radical damage in a number of studies. Scientists have noted its beneficial effects both immediately after the injury occurs and in long-term wounds as well. HA treatment has been reported to cause a 70 percent reduction in the surface area of wounds. Studies also have documented HA's effectiveness in leg ulcers and pressure wounds.

Hyaluronic acid is an important tool in the armamentarium of supplements used for two of the most common concerns of aging - osteoarthritis and aging skin. New research is revealing HA's effectiveness for a variety of other concerns as well, including rosacea, vaginal dryness, oral health and precancerous lesions of the cervix. Recommending this supplement to patients with joint pain and aging skin may reduce pain and significantly improve their quality of life. (Chris D. Meletis, N.D.) The present study aims at isolating and purifying hyaluronic acid like components from plant source such as sweet potato.

MATERIALS AND METHODS

Chemicals

Acetone, chloroform, methanol, sodium acetate, cysteine, disodium ethylenediaminetetraaceticacid, sodium chloride, absolute ethanol, dinitrosalicyclic acid, glucose, maltose, anthrone, sodium carbonate, sodium hydroxide, sodium potassium tartarate, copper sulphate purchased from Himedia. Folin-ciocalteu reagent, bovine

serum albumin, hydrochloric acid purchased from Fisher Scientific. DEAE-Sepharose matrix, CM-Sepharose matrix, Sephadex G-100 purchased from Sigma.

Equipment
Weighing balance (Auy 220 Shimadzu and Elb 300), Homogenizer, Rocker, Vacuum pump, REMI R-8C centrifuge, Elico LI-120 pHmeter, Systronics UV-VIS Spectrophotometer 117.

Plant source
Ipomoea batatas (sweet potato) was purchased from near by market in H.B.R Layout, Bangalore, Karnataka. Fresh tubers of these samples were selected randomly and used for the study.

Animal source
Fresh Goat brain was collected from Dhodi slaughter house, Frazer town, Bnagalore, Karnataka. Visceral fluid was collected from common fish's eye from Thanisandra, Bangalore, Karnataka. The brain and the visceral fluid was used as a standard.

Extraction of the sample
Extraction was carried out according to the protocol from Claudia Severo da Rosa, 2012. The samples were washed thoroughly and weighed separately. Brain (100g) and sweet potato (50g) was taken. The samples were crushed and immersed in volume of acetone to get 50% extract. These samples were kept in rocker for 1 hour. Chloroform: Methanol (2:1) ratio was prepared for the three samples of 100ml. The samples were kept in the chloroform: methanol solution and incubated for 24hours at 25°C. After the incubation period, Digestion buffer (100Mm sodium acetate pH 5.0, 5.0mM cysteine and 5.0mM disodium EDTA) was prepared in ratio approximately 2ml buffer to 100mg of tissue. The samples were hydrated in the digestion buffer for 44 hours at 5°C. After hydration the mixture was centrifuged at 3200rpm for 30minutes. Supernatant was discarded and the pellet was washed with 3ml of 2.0M NaCl and absolute ethanol. Absolute ethanol was added in 2:1 ratio and incubated for 24hours at -16°C. After the incubation period, the sample was centrifuged again at 3200rpm for 30minutes. Supernatant was discarded and the pellet was washed with 80% ethanol. The solution was centrifuged again (3200rpm/30min), the supertanant was discarded and the pellet was dried for 24 hours at 25°C. The final solid was re-suspended in 5ml of distilled water and further assays were carried out.

Estimation of total carbohydrates by anthrone method
Total sugars were estimated by Anthrone method in the samples. (Yemm EW and Willis AJ. 1954). The readings were noted and a standard curve plotted to determine the total carbohydrate content in the samples. The results were expressed asmg/ml of total carbohydrate.

Estimation of reducing sugars by DNS method
Reducing sugars were estimated by DNS method in the samples (Nelson. 1944). The readings was noted and a standard curve plotted to determine the reducing sugars content in the samples. The results were expressed as micromoles/ml of reducing sugars.

Estimation of proteins by Lowry's method
The protein content in the samples was estimated by Lowry's method (Oliver H Lowry et al. 1951). The readings were noted and a standard curve plotted to determine the protein content in the samples. The results were expressed as micrograms/ml.

Purification
Extraction was followed by Partial purification such as Ion exchange chromatography and Gel permeation chromatography

DEAE- Ion Exchange Chromatography
The sample (1ml) was loaded onto the column and the column washed with two bed volumes of start buffer. The bound proteins were eluted with stepwise increase in the ionic strength (0 M to 1.5 M NaCl). Fractions of 5ml were collected. The absorbance was taken for the eluents in UV spectrophotometer (200-270nm).

CM-Sepharose Ion Exchange chromatography
The sample (1ml) was loaded onto the column and the column washed with two bed volumes of start buffer. The samples were eluted with stepwise increase in the ionic strength (0 M to 1.5 M NaCl). Fractions of 5ml were collected. The absorbance was taken for the eluents in UV spectrophotometer (200-270nm). The absorbance was compared between the two matrices.

Sephadex G-100 Gel-filtration chromatography
Sephadex G – 100gel was equilibrated with 10mM phosphate buffer, pH 7 and packed into a column under gravity (125mm X 15mm). The column was equilibrated with two bed volumes of 10mM phosphate buffer, pH 7 at a flow rate of 12ml/hr. The DEAE –Sepharose fraction, Hyaluronic acid containing fractions were subjected to gel permeation chromatography separately using Sephadex G – 100. The samples were eluted with start buffer and fractions of 2ml were collected. Absorbance was taken at 230nm in UV Spectrophotometer. A single peak of glycosaminoglycan was obtained.

RESULTS AND DISCUSSION
The results were obtained and the data is represented in the form of graphs which help in the comparison and understanding of the presence of hyaluronic acid. Presence of carbohydrates indicates that hyaluronic acid is present which was further purified by ion exchange and gel permeation chromatography.

Estimation of total carbohydrates by anthrone method

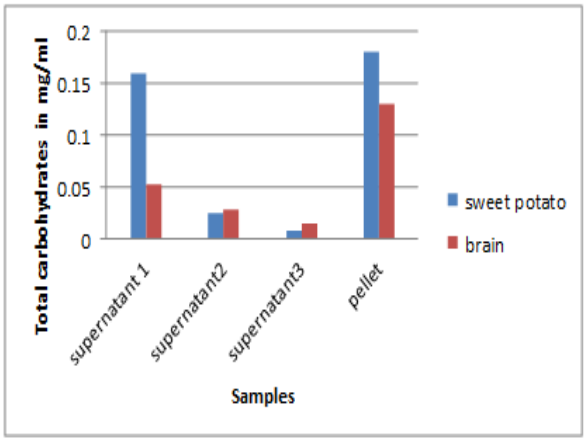


Figure 1: Bar Graph showing a comparison between total carbohydrate content in the two samples.

Estimation of total carbohydrates of the samples was carried out by anthrone method. The above figure 1 illustrates that there is highest yield of the total carbohydrates in the pellets obtained of both the sample compared to the supernatants. 1mL aliquot of each sample were used for the assay all of which showed high yield in the pellet. The total carbohydrate concentration in the pellet was found to be 0.18mg in sweet potato and 0.13mg in brain. The lowest yield of total carbohydrates was found in supernatants 3, 2 and 1.

Estimation of reducing sugar by DNS method

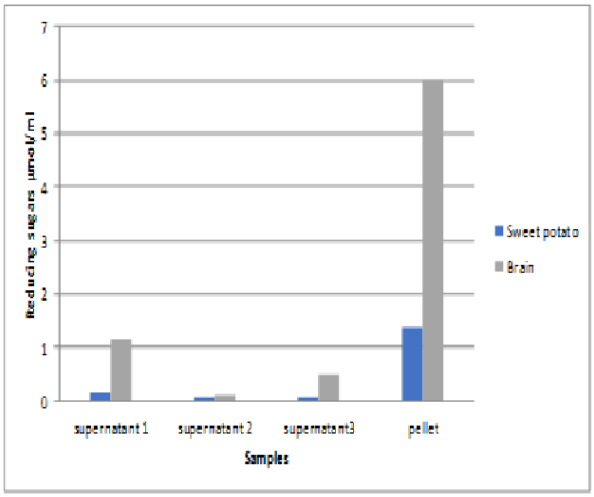


Figure 2: Bar graph showing comparison of the two samples in reducing sugars.

Estimation of reducing sugars of the samples was done by dinitro salicyclic method. The above figure 2 illustrates that there is a significant high yield in reducing sugars in the pellets of both the samples as compared to the supernatants. 1ml aliquot of each sample was used for the assay. The reducing sugar concentration in the pellet of each extract was found to be 1.388µmol/ml in sweet potato and 6µmol/ml in brain. The lowest yield of reducing sugar content was found in supernatants. These results showed that the pellets had highest levels of reducing sugar after extraction indicating that can be subjected to further purification.

Estimation of proteins by Lowry's method

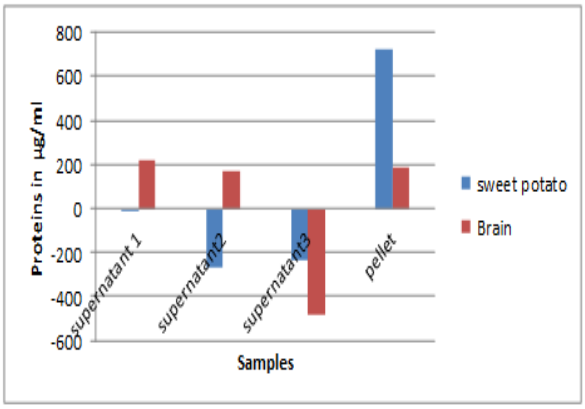


Figure 3: Bar graph showing comparison of protein content in two samples.

Protein content of the samples was estimated using Lowry method. The above figure 3 illustrates that there is very low concentration of protein content in each sample after extraction. This indicated that proteins were effectively degraded during the extraction enabling the samples to be further purified for HA like components. 1mL aliquot of each sample were used for assay and the concentrations were depicted in micrograms permL. All the samples yielded low concentrations of protein.

Partial purification of HA like components
Ion exchange chromatography (DEAE- sepharose)

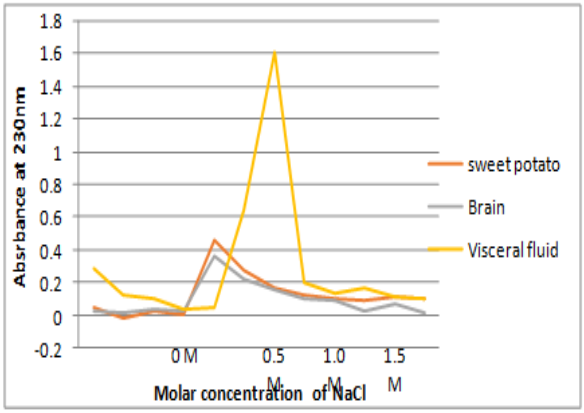
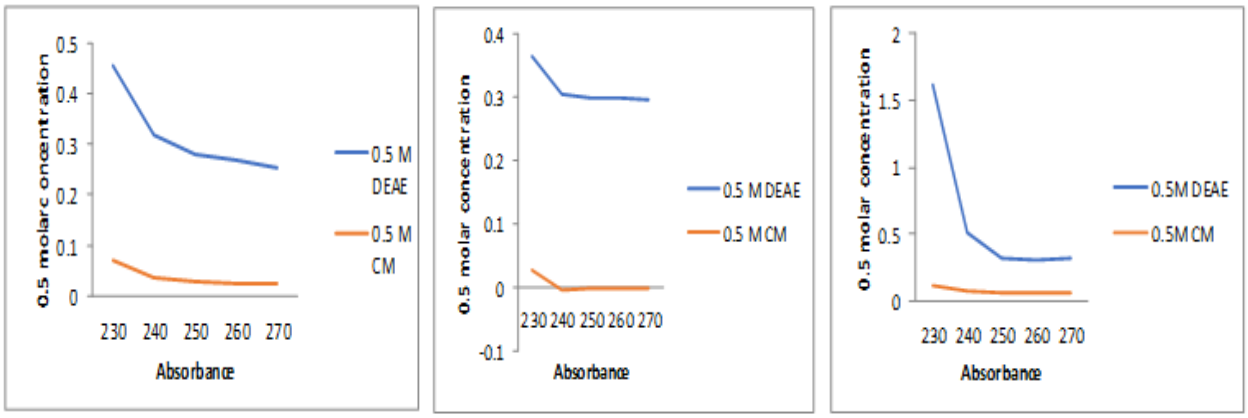


Figure 4: Line graph showing comparison of absorbance between different elutions of three samples at 230nm. Highest peak was obtained at 0.5M elution in all the three samples.

Partial purification was carried out using ion exchange chromatography. The figure 4 depicts that elutions obtained at 0.5M concentration gave highest absorbance at 230nm, the column flow rate was maintained at 25ml/hr. The number of fractions collected where 14 and each fractions were analysed for HA like components at 200 -270nm in which 0.5M NaCl elution fraction showed maximum absorbance at 230nm.

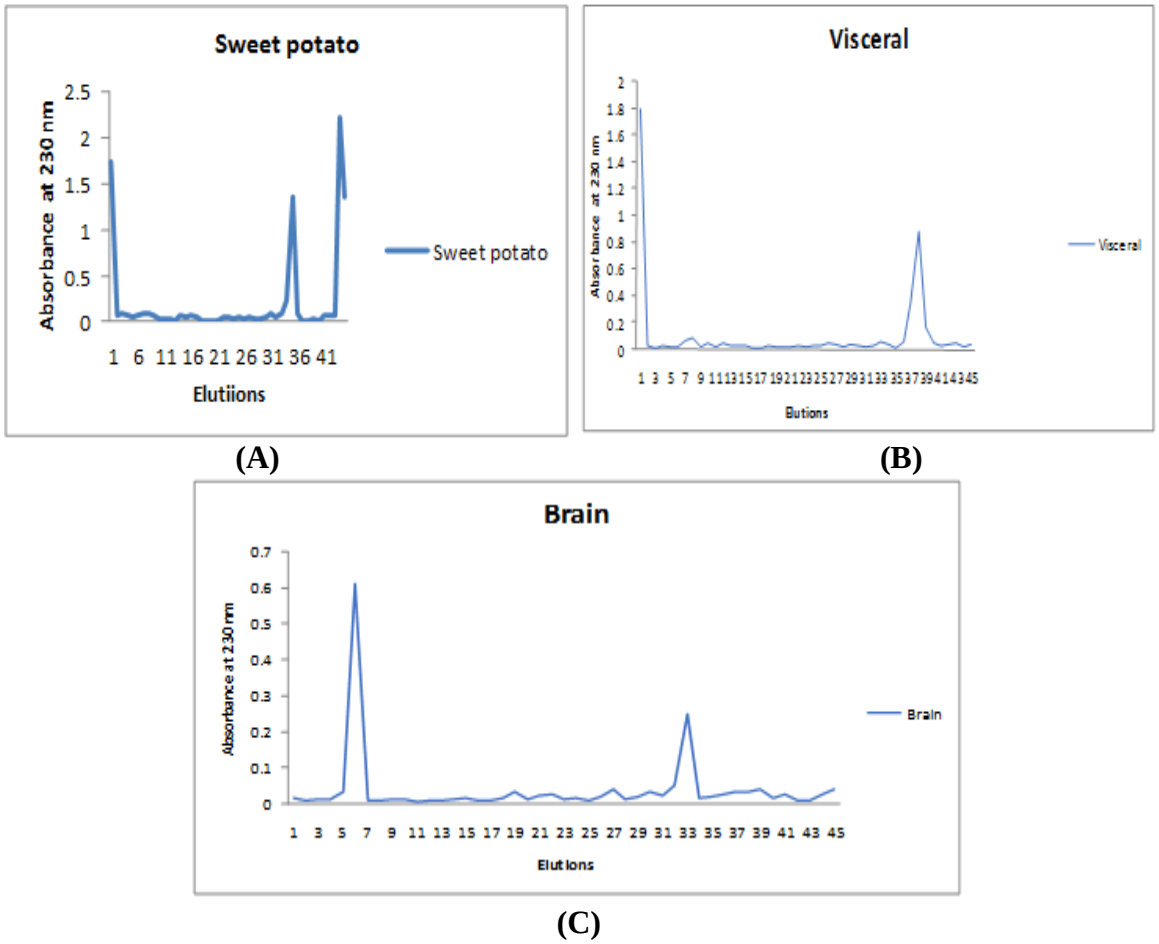


(A) Sweet potato **(B) Brain** **(C) Visceral fluid**

Figure 5: A comparison of absorption spectra at different wavelengths of ion exchange chromatography using anion exchanger (DEAE) and cation exchanger (CM) in A B and C

The above figures 5 depict the absorbance obtained after elutions in DEAE and CM matrix. It indicates that 0.5 M elutions of each sample showed highest peak at 230nm in DEAE matrix, showing that HA like components are present in the sample.

Gel permeation chromatography (Sephadex G-100 matrix)



(A) **(B)**

(C)

Figure 6: Peaks obtained at 230 nm for 1ml of 45 elutions in gel permeation chromatography in A, B and C

Gel permeation chromatography was performed with each sample eluted at 0.5M concentrations in Ion exchange chromatography. Sephadex G-100 matrix was used for separation where 100µL of each sample was loaded into the column. The flow rate was maintained at 12mL per hour and 45 elutions of 1mL was collected and then subjected to UV-vis spectra. The figure above shows peak obtained in each sample. The low molecular weight hyaluronic acids showed a peak at 230nm and showed that there was no modification of chemical

structure (Wu Yue, 2012). The peak indicating in this graph shows that there is presence of hyaluronic acid. When compared to the reference papers we get to know that the peaks obtained in the gel permeation chromatography is hyaluronic acid like components where peaks were obtained in different fractions. In sweet potato, there is a peak obtained at 21st eluent. If we compared to the standards (brain and visceral) the peaks obtained are almost similar. The peak indicates the presence of HA like components. In Gel permeation chromatography, the column used was 125mm X15mm. As G-100 is used, the fractions can have molecular weight between 10–90kDa. Hyaluronic acid is a high molecular weight molecule, therefore hyaluronic acid like components having higher molecular weight would have broken into smaller fragments which can have molecular weight between 10–90 kDa which is obtained in the graphs showing peaks, whose absorbance is read at 230nm. Based on this assumption the molecular weight of hyaluronic acid like components from Sweet Potato can be more than 100kDa, since it is eluted within the void volume. Also, existence of one more peak assures the presence of smaller molecule mass of HA of molecular weight 22kDa and 18kDa which would be fragments of the polymer.

CONCLUSION

Ipomoea *batatas* are tubers which were used as a source for the experiment. It is an outstanding source of nutrients and these are the best plant sources which possess hyaluronic acid like components. It is rich in starch, carbohydrates, vitamin-A, dietary fibre, manganese, copper, pantothenic acid, vitamin-B6 and niacin. It is low in saturated fat, protein and sodium, they also contain oleic acid and omega-3 fatty acids. Goat brain and visceral fluid were used as standard from animal sources. Also a rich source of hyaluronic acid. Significance of ion exchange chromatography indicates that hyaluronic acid being negative in charge binds to anion matrix giving a sharp peak at 230nm. The elution from ion exchange was further purified Sephadex G-100 through gel permeation chromatography. The results of gel permeation indicates that hyaluronic acid being a higher molecular mass component would have broken into smaller fragments having a molecular weight of 22kDa. Pure Hyaluronic acid is expensive as it is manufactured by recombinant technology. These methods can further be simplified or used to isolate hyaluronic acid which can be used in the industries.

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