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COMPARATIVE STUDY BETWEEN POLYSACCHARIDES ISOLATED FROM OKRA AND PURSLANE

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ABSTRACT: Such investigation has as its goal to study polysaccharides from okra and purslane. The chemical composition of okra and Purslane is different. Okra contains 87.49% carbohydrates, 72.55% total soluble polysaccharides, 21% extraction percentage, against 79.51% carbohydrates, 63.0% total soluble polysaccharides, 17.5% extraction percentage for purslane. The infrared analysis reveald absorption in the region of 1725.18, 1732.38 and 1723.3 cm⁻¹ for okra and purslane indicated the presence of CH₂ group. The maximal absorption occurs at around 1626.54 cm⁻¹ of Purslane reflects the polysaccharide property and relates to the stretching vibration of C=O of the N-acetyl group or protonated carboxylic acid. The peaks at 1598.44 and 1408.51 cm⁻¹ of okra, 1588.32 and 1417.09 cm⁻¹ of purslane were characteristic to the symmetric stretching of the COO group, indicating that plants are acidic polysaccharides. The peak located at 1318.56 and 1244.31 cm⁻¹ of okra, 1376.02 and 1322.88 cm⁻¹ of purslane correlated with the ester sulphate groups' stretching vibration (S=O). The ingestion at 1144.54 and 1036.39 cm⁻¹ of okra, 1024.08 cm⁻¹ of purslane in the range of 1200 – 1000 cm⁻¹ which are anomeric regions, were attributed to the existence of C-O bonds in polysaccharides was shown by the compounds C-O-C and C-O-H that the monosaccharides in okra and purslane have α-pyranose ring. The presence of characteristic absorption at 893.03, 821.35 and 780.94 cm⁻¹ of okra, 826.94, 778.58 and 719.28 cm⁻¹ of purslane during anomeric area $950 - 700 \text{ cm}^{-1}$ of plants were assigned to the coexistence of both α and β glycosidic connections. An estimated of the molecular weight of the polysaccharides of okra was 5.29 \times 10^4 g/mol and purslane 1.25×10^4 g/mol. HPLC analysis of okra polysaccharides was indicated the monosaccharides of using a molar ratio manuronic acid, glucose, mannose, fucose, and arabinose at 0.52, 1.22, 0.96, 0.35, 1.0, respectively. Purslane polysaccharides contained manuronic acid, glucose, galactose and arabinose with molar ratio 0.36, 1.51, 1.0, 1.6, respectively. The ¹H NMR spectra of okra and purslane polysaccharides clearly differs from each other in the pattern of resonances for their glycosyl anomeric and ring protons. The chromatogram of ¹H NMR spectrum exhibited four peaks corresponding to the analysis of linkage and component reported in previous values. Among them, signals at 5.19-5.15 ppm were assigned to α -Arabinose residues, 4.87–4.91 ppm signals might represented the residues of α -Glucose pyranose.

Key words: Polysaccharides – Okra – Purslane – Monosaccharides – Molecular weight– HPLC

INTRODUCTION

Polysaccharides are polymeric carbohydrate molecules composed of long chains monosaccharide units bound together by glycosidic bonds. Natural occurring polysaccharides exhibit distinct structural molecular weight, features in terms of monosaccharide composition, glycosidic linkage patterns, configuration (α or/and β), charging properties, degree of branching. Polysaccharides are playing an important role in living things and could be used as a therapeutic candidate due to their impressive pharmacological properties (Kim et al., 2018). Polysaccharides have recently investigated as having potential applications in a variety of industries, including cosmetics, food, paper, medicine, petrochemicals. The glycemic index usually refers to the glycemic response of available carbohydrates from food relative to the equal

amount of actual carbohydrates. The speed and amount of starch digested in the lumen of the intestine play an important role in the regulation of glucose and insulin homeostasis (Jonsson *et al.*, 2020).

Okra (Abelmoschus esculentus) is one of the most widely known and utilized species of the family *Malvaceae* and an economically important vegetable crop grown in tropical and sub-tropical parts of the world. Okra has a high fibre content that slows down the pace at which the body absorbs sugar from the intestines, hence stabilising blood sugar levels. Because of its fibre and other nutrients, okra is beneficial for lowering blood sugar levels and dealing with diabetes (Andras et al., 2015). The fiber likewise helps support blood sugar levels level simply by slowing down sugar assimilation through the intestines. The frequent usage of okra might help avoid kidney disease. Within the research, those who consumed okra every day decreased clinical indications of kidney damage a lot more than the ones that simply consumed a diabetic diet. This ties along with diabetes, as almost 50% of kidney disease cases are generated by diabetes (Aladele et al., 2018). A genus of succulent plants known as purslane (Portulaca oleracea L.) is found throughout the tropics. In India, four species are found in the wild, and two exotics have assimilated. Purslane, sometimes referred to as P. oleracea, is a herbaceous weed. According to the traditional approach, it may treat piles, ulcers, leprosy, dysentery, and diarrhoea as well as decrease inflammation and tiny tumours. The plant is said to have qualities that are diuretic, antiscorbutic, anti-vulnerary, anti-aperient, and refrigerant (Chugh et al., 2019). In diabetic mellitus mouse models, the effects of crude polysaccharide from purslane (CPP) on body weight, blood sugar, total cholesterol, highlipoprotein cholesterol (HDL-c), triglyceride, and serum insulin levels were examined. Fasting blood glucose (FBG), total cholesterol, and triglyceride values all drop during CPP therapy. CPP dramatically boosted the mice's body weight, blood insulin level, and HDL-c content (Rahimi et al., 2019). In the present study, polysaccharides from okra and purslane were extraction and isolation chemical

analysis.

MATERIALS AND METHODS

1- Plant collection and identification

Fresh samples of okra (Abelmoschus esculants L) and purslane (Portulaca oleracea L.) were collected from plants grown in Minya Al-Qamh lands in Sharkia Governorate, Egypt, in summer (July 2020). The botanical department of Menoufia University's faculty of agriculture is where the plants were identified. Samples of plants were cleaned, allowed to air dry for 24 hours, and then dried at 50 °C. The dried materials were ground into a fine powder and stored for examination in the refrigerator.

2: Chemical analysis

2-1: Polysaccharides extraction

Dry sample (50 g) was weighed 450 ml of 95% ethanol was added and the extract was then let to cool after being immersed in a water bath at 80 °C for four hours, then filtered and the filtrate was disposed of. The filtration process was repeated 5 times, and each time the filtrate was removed. At 40 °C, the precipitate was dried. The precipitate was combined with 500 ml of distilled water, which was then heated to 100°C for 4 hours while being stirred. The mixture was then allowed to cool and filter the solution. This process was repeated 4 times to complete the extraction process. The filtrate was taken, then 95% ethanol was added at a ratio of 1:4 volumes, then it was placed in the freezer for 5 days to complete the sedimentation process, then a centrifuge was made in order to get the sediment. The precipitate was taken and placed in a Petri dish at room temperature. Then we added 10 ml of acetone, and 1 ml of diethyl ether. The precipitate was left to dry to get polysaccharides from okra, aloe Vera and purslane (Qu et al., 2013).

2-2: Determination of total carbohydrate

A known weight (0.2 g) of dried sample was completely hydrolyzed for 6 hours with HCl (1 N) on boiling water bath under reflux condenser. The solution was then filtered and the filtrate was clarified by the leading and deleadin method using lead acetate solution (137 g/L) and excess of lead salts was precipitated using 1/3N

disodium hydrogen phosphate solution. The extract was measured into a measuring flask (50 ml). The combind filtrate was completed to the mark with distilled water the sugars were determined according to the method of Dubois et al., (1956) as follow; An aliquot of 1 ml of the sugars solution was quantitatively transferred into a test tube and treated with 1 ml 0.5% aqueous phenol solution followed by 5 ml of concentrated sulphuric acid added by a fast delivery pipette. The blank experiment was carried out using 1 ml of distilled water instead of the sugar solution. The absorbance of yellow orange color was measured in spectrophotometer at wavelength 490 nm. A standard curve was prepared using known concentration of glucose. The established curve was used to convert the colorimeter absorbance into milligrams of glucose.

Total carbohydrate (%) = Total carbohydrate/ weight of sample \times 100

2-3: Determination of total soluble polysaccharides

Total soluble polysaccharides expressed as glucose were determined calorimetrically according to the method of Dubois *et al.*, (1956) as previously described.

Total soluble polysaccharides (%) = Total polysaccharides/ total carbohydrates × 100

2-4: Extraction percentage

We used 10 g of the dry sample and the polysaccharides were extracted by the same way as before. The sample was dried to get remove moisture at a temperature of 40 0 C and the extraction rate was calculated (Yao *et al.*, 2005). The extraction (%) = weight of extract / weight of sample \times 100

2-5: Infrared Fourier Transform analysis (FTIR)

The FTIR-UNIT Bruker Vector 22 Spectrophotometer was used to analyse the FTIR spectra of polysaccharide from okra, aloe vera, and purslane in order to determine the functional groups (Brock-Neely, 1957).

2-6: Molecular weight of polysaccharides

Samples (100 mg) were dissolved in 2 ml of solvent and filtrated by 0.45 µm filter then the samples were injected in gel permeation chromatography (GPC) device (Jun *et al.*, 2009). Molecular weight and RI signal readings at each elution volume were used to compute the number average molecular weight (Mn) and weight average molecular weight (Mw), respectively, in accordance with the definitions of Mn and Mw. The Mw/Mn ratio was used to construct the polydispersity index (PI) (You *et al.*, 2013).

2-7: Determination of monosaccharides composition

2-7-A: Hydrolysis of polysaccharides from plants

A complete acid hydrolysis was performed by adding 88% formic acid to known weight of polysaccharides for five hours at 100 0 C in a closed tube. The formic acid was then dried by evaporation under vacuum at 40 0 C, and rinsed multiple times in distilled water to get rid of the extra formic acid. The hydrolyzates were concentrated under vacuum and stored frozen in a sealed vial until analysis (Chaplin and Kennedy, 1994).

2-7-B: Determination of monosaccharides by HPLC

The monosaccharides were analyzed by HPLC (Agilate Pack, series 1200), equipped with Aminex carbohydrate HP-87C column (300 mm × 7.8 mm). Deionized water was used as the mobile phase at flow rate 1 ml/min. Chromatography peaks were identified by comparing the retention timed with the respective retention times of known standard reference material. Retention time and peak area were used to calculation of sugar concentration by the data analysis of agilat Packard (Randall *et al.*, 1989).

2-8: spectra of NMR

A JEOL JNM-ECX 400 spectrometer (JEOL, Japan) was used to record the ¹H and ¹³C NMR spectrum of the hydrolyzed EPS (400 and 100 MHz respectively) in D2O/H2O solution (60 mg of substance in 0.7 ml D2O and 0.005 ml acetonitrile) with and without 70% HCLO4. The signals (δ H 2.06 ppm, δC 119.68 and 1.47 ppm)

of acetonitrile were used as a chemical shift reference. The spectra were processed using the software packages ACD/NMR Processor Academic Edition, ver. 12.01 and Delta 4.3.6. The NMR spectra of the native polysaccharide were recorded using a Bruker AV 600 NMR instrument (600 MHz) at 40 0C with HDO signal suppression. To prepare the NMR sample, 20 mg of the polysaccharide were lyophilized and dissolved in 99.9% D2O. The NMR spectra were processed by Mestre Labs Mestre Nova software and referenced to internal DSP at δ H 0.00, δ C – 1.59 (Podgorskii *et al.*, 2011).

RESULTS AND DISCUSSION

1: Determination of total carbohydrate and total soluble_polysaccharides of okra and purslane

Data presented in Table (1) show that total carbohydrate in okra was 87.49%, which are similar to previously reported observations by according to Agarwal et al. (2001) and Saini et al. (2017) stated that okra contain high value of carbohydrate 86.8%. However, the total amount of carbohydrates in purslane was 79.51%. This result is in line with that of Teixeira and Carvalho, (2008), who found that total carbohydrate of purslane 81.32%. While the high level of total soluble polysaccharides was found in Okra which reached to 72.55 % and low value in pursalne registered 63.0 %. These results were confirmed by Chunmei and Shu, (2014). They studied and estimated purslane in more methods which total soluble polysaccharides reached 61.4%. However, Dhruve et al. (2015) and Alam et al. (2018) discovered that okra had 73.9% total soluble polysaccharides.

2: Extraction percentage of okra and purslane polysaccharides

It can be noted from Table (1) that the highest extraction percentage was for okra where it was 21%, while the lowest extraction percentage was for purslane reaching 17.5%. These results were agreement with Zhao *et al.*, (2015) they conducted a study on the purslane plant and the extraction percentage was 19.8%, while the extraction percentage of okra was 20.3% which is a result consistent with Chen *et al.* (2016) and Nevenka *et al.* (2018).

3: Fourier Transform Infra-Red analysis (FT-IR)

Functional groups of okra and purslane were identified using FT-IR spectroscopy as shown in Figure (1 a, b). A broad stretching intense characteristic peak at 3238.26, 3275.99 and 3269.07 cm⁻¹ for okra and purslane for OH stretching vibration range 3600 - 3200 cm⁻¹ of the polysaccharide and the weak C-H₂ stretching band at 2922.14 cm⁻¹ for okra, 2955.53, 2916.36 and 2848.4 cm⁻¹ respectively for purslane. The peak in the region of 1725.18, 1732.38 and 1723.3 cm⁻¹ for okra and purslane showed the presence of CH2 group. The Purslane absorption peak at about 1626.54 cm⁻¹ demonstrates the typical IR absorption of polysaccharides and corresponds to the stretching vibration of C=O of the N-acetyl group or protonated carboxylic acid. The peaks at 1598.44 and 1408.51 cm⁻¹ of okra, 1588.32 and 1417.09 cm⁻¹ of purslane were characteristic to the symmetric stretching of the COO- group, indicating that plants are acidic polysaccharides. The peak for located at 1318.56 and 1244.31 cm⁻¹ of okra and 1376.02 and 1322.88 cm⁻¹ of purslane was in accordance with the ester sulphate groups' stretching vibration (S=O). the ingestion at 1144.54 and 1036.39 cm⁻¹ of okra and 1024.08 cm⁻¹ of purslane in the range of 1200 - 1000 cm⁻¹ which are anomeric regions, were attributed to C-O-C and C-O-H This implied the existence of C-O bonds in polysaccharides that the monosaccharides in okra and purslane have α-pyranose ring. The presence of characteristic absorption at 893.03, 821.35 and 780.94 cm⁻¹ of okra and 826.94, 778.58 and 719.28 cm⁻¹ of purslane during anomeric area 950 - 700 cm⁻¹ of plants were assigned to the coexistence of both α and β-type glycosidic linkages. an additional observable absorption peak at 631 - 580 cm⁻¹ indicates C-H flexural vibration that is out of plane in the sample. These results are similar with Eberendu et al., (2019) who found that FT-IR spectra of purified okra fractions are showed characteristic absorbance of polysaccharides. The intensity of bands in the region 3600-3200 cm⁻¹ was due to the hydroxyl stretching vibration of the polysaccharide, which was expected to be broad. The absorption bands at 2988.4 cm⁻¹ in the range of 3200–2800 cm⁻¹ were due to the C-H stretching vibration (Dore et al., 2013). Each polysaccharide showed high

absorbance in the region 1200–950 cm⁻¹, which was within the so-called fingerprint region. Hence, in the fingerprint region, the strongest absorption band at 1038.3 cm⁻¹ indicates that the substance is polysaccharide and suggests that the monosaccharide in it has a pyranose ring (Yuan and Macquarrie, 2015 and Abdelhedi *et al.*, 2016). The peaks at 1554.1, 1463.1, and 1403.2

cm⁻¹ were characteristic of the carboxyl groups or carboxylate. This result is consistent with its uronic acid content approximately 14% quantified through the carbazole–sulfuric acid method using d-glucuronic acid as standard. Moreover, the characteristic absorptions at 843.1 cm⁻¹ indicate that α -configurations exist in the polysaccharides (Lai *et al.*, 2010).

Table (1): Total carbohydrate, total soluble polysaccharides and extraction percentage of okra and purslane

Test	Okra	Purslane
Total carbohydrate (%)	87.49	79.51
Total soluble polysaccharide (%)	72.55	63.0
Extraction percentage (%)	21.0	17.5

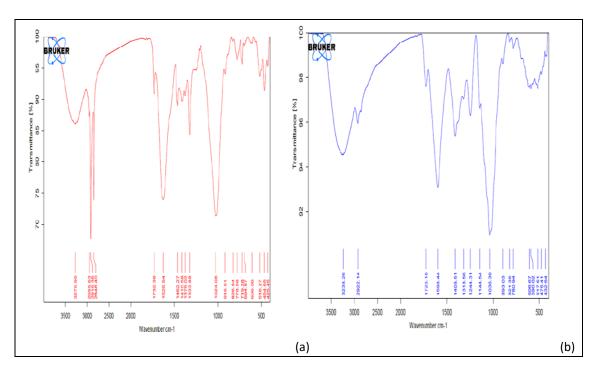


Fig. (1): FT-IR spectra polysaccharide from okra (a) and purslane (b).

4: Molecular weight of polysaccharides from okra, aloe vera and purslane

The number average molar mass also named average molecular weight of the number (Mn), the weight average molecular weight (Mw), also known as the mass average molar mass, and the molar mass distribution or polydispersity index (PI= Mw/Mn) were determined using Gel Permeation Chromatography (GPC). Results are displayed in Table (2) and Figure (2 and 3) explain that the mass average molecular weight (Mw) was 5.29×10^4 g/mol, the number average molecular weight (Mn) was 4.85×10^4 g/mol with polydispersity Index (PI) 1.09 for okra polysaccharides, the mass average molecular weight (Mw) was 1.25×10^4 g/mol, the number average molecular weight (Mn)was 1.23×10⁴g/mol with polydispersity Index (PI) 1.02 for purslane polysaccharides. These results are nearly with that of Uraku et al., (2011) they The Mwdistribution polysaccharides obtained from okra was 5.11× 10⁴ and *PI* was 1.03, while Hwang *et al.*, (2018) they conducted a study on the purslane that polydispersity Index 1.08 by Samuel et al., (2019).

5: Monosaccharides of polysaccharides from okra and purslane by HPLC fraction

Polysaccharides from okra, aloe vera and purslane were hydrolysed and the outcomes of the High Performance Liquid Chromatography (HPLC) analysis are provided in Table (3). From the following table we find the okra polysaccharide consisted high molar ratio of glucose, arabinose, mannose, mannouronic acid and fucose in amount of 1.22, 1.0, 0.96, 0.52 and 0.35, respectively. The results are in line with those of Zhengzheng et al., (2019) they found the monosaccharride composition of okra contain mannose, glucose, mannouronic acid, galactose, and arabinose with molar ratio 1.0, 1.76, 0.38, 0.27 and 1.13, respectively. As for purslane polysaccharide contains high value of arabinose, glucose, galactose and mannouronic acid with molar ratio 1.6, 1.51, 1.0 and 0.36, respectively. Results similar to those found by Tarkergari et al., (2013) and Youguo et al., (2019) they found portulaça oleracea are contained monosaccharides such as mannouronic acid, glucose and arabinose which recorded 0.55, 1.39 and 1.81 molar ratio respectively.

Table (2): Molecular weight polysaccharides of okra and purslane

Sample	Mw (g/mol)	Mn (g/mol)	PI
Okra polysaccharide	5.29×10^{4}	4.85×10^4	1.09
Purslane polysaccharide	1.25×10^4	1.23×10^{4}	1.02

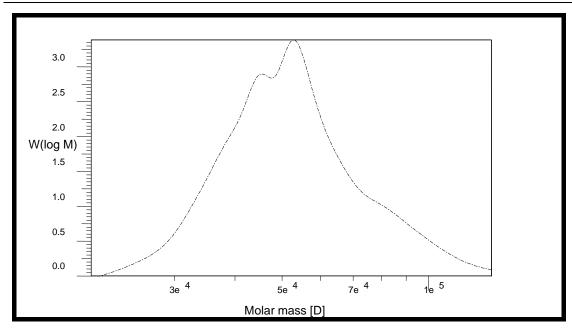


Fig. (2): Molecular weight of okra polysaccharides.

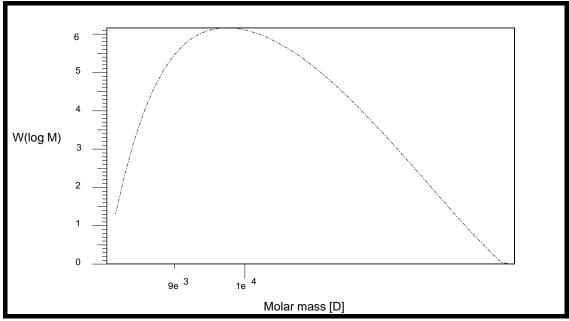


Fig. (3): Molecular weight of purslane polysaccharides.

Table (3): Monosaccharides molar ratio of okra and purslane by HPLC fraction

Monosugars	Okra	Purslane
Mannouronic acid	0.52	0.36
Glucose	1.22	1.51
Mannose	0.96	0.00
Fucose	0.35	0.00
Arabinose	1.00	1.60
Galactose	0.00	1.00

6: NMR Spectra of polysaccharides

The ¹HNMR spectra comparing PS from different plant okra and purslane are shown in Figures (4 and 5). Each spectrum clearly differs from the other in the pattern of resonances for their glycosyl anomeric and ring protons. These results show, as is indicated by the composition differences, that the structure of the PS1 from okra is different from these PS3. chromatogram of ¹H spectrum exhibited four peaks corresponding to the analysis of linkage and component reported in previous values. Among them, signals at 5.19–5.15 ppm were assigned to α-Arabinose residues, 4.87–4.91 ppm signals might represented the residues of α-Glucose pyranose. The 1.15-1.25 ppm signal indicated the -CH₃, which was corresponding to the typical characteristic absorption at 2939.52 cm⁻¹ in FTIR spectrum. Moreover, all of these glycosyl residues are -anomers, according to the chemical shifts and J_{1,2} coupling constants of their anomeric protons, while the remaining glycosyl residue's anomeric configuration cannot be determined from the one-dimensional proton spectrum due to its small J_{1,2} coupling, indicating that this residue is in the manno configuration. The results are in line with those of Vlahov

(2013) and Adhvaryu et al. (2016) they shown that data obtained for okra polysaccharides containd both apexless α -(1, 2)-linked rhamnose (1.25 ppm) and branched α –(1, 2, 4)-linked rhamnose (1.33 ppm), the usual chemical changes discovered for H6 of these rhamnosyl moieties and 1 or 2 anomeric signals between 5.22 and 5.25 ppm had been attributed to rhamnosyl residues. Combining the anomeric signals at 5.01 and 4.96 ppm with the signal at 4.42 ppm, H-1 and H-4 of galacturonic acid were identified. Ebringerova et al. (2013), Gantner et al. (2015) and Oiu et al. (2017) they found the resonances present in the ¹H NMR spectra of β-Manp- $(1 \rightarrow or \leftarrow 4)$ -b-Manp- $(1 \rightarrow 4)$ Residues (4.84 ppm) or to H-1 of reducing terminal α -/ β -Man (5.27 ppm and 5.00 ppm) on the basis of similarity of chemical shifts with those of β -Man. Wu et al. (2009) and Ojha et al. (2010) who asserted that the proportion of intensities of β-Manp- $(1 \rightarrow \text{ or } \leftarrow 4)$ - β -Manp- $(1 \rightarrow \text{ to reducing})$ terminal Man H-1 resonances in samples suggesting that these fractions contained β-Manp- $(1\rightarrow 4)$ - β -Manp- $(1\rightarrow 4)$ -Man trisaccharide $(\beta$ -Man3) and β-Manp- $(1\rightarrow 4)$ -β-Manp- $(1\rightarrow 4)$ β-Manp-(1 \rightarrow 4)-Man tetrasaccharide (β-Man4).

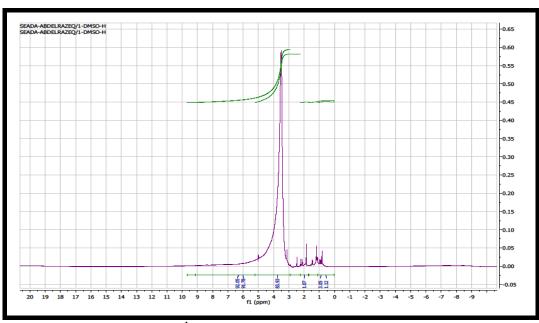


Fig. (4): ¹HNMR spectra of okra polysaccharides

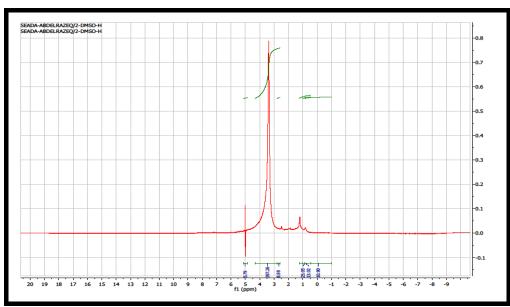


Fig. (5) ¹HNMR spectra of purslane polysaccharides

Conclusion

The chemical composition of okra and purslane elucidated that okra contains 87.49% 72.55% carbohydrates, total soluble polysaccharides, 21% extraction percentage, while Purslane contains 79.51% carbohydrates, 63.0% total soluble polysaccharides, 17.5% extraction percentage. While the infrared analysis proved in the spectral range that OH is present, CH₂, C=O, COO⁻, S=O, C-O-C, C-O-H and C-O groups, as well as alpha-pyranose rings, Both Purslane and okra have alpha and beta glycosidic linkages. The estimated molecular weight of the okra polysaccharides is 5.29 x 10 4 g/mol against 1.25 x 10 4 g/mol for purslane. HPLC analysis of okra polysaccharides reveald indicated the presence of manuronic acid, glucose, mannose, fucose and arabinose with molar ratio 0.52, 1.22, 0.96, 0.35, 1.0, respectively against manuronic acid, glucose, galactose and arabinose with molar ratio 0.36, 1.5, 1.0, 1.6 for purslane respectively.

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دراسة مقارنة بين السكريات العديدة المفصولة من الباميا والرجلة

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