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COMPOSITION AND ANTIOXIDANT ACTIVITY OF THE POLYSACCHARIDES FROM GINGER (*Zingiber officinale* L.)

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ABSTRACT

Extracted from ginger rhizome (*Zingiber officinale* L.) with boiling water crude polysaccharides. polysaccharides extracted was separated into four fractions through chromatographic analysis on DEAE-cellulose and Sephadex G-200 column, GP1, GP2, GP3 and GP4 were separated. It has been identified at the molecular weight of polysaccharides (GP1, GP2, GP3 and GP4) separated by The gel permeation chromatography (GPC) analysis were approximately 136, 27.7, 11.8 and 11.4 kDa, respectively. Using a GC-MS analysis was to identify the monosaccharides analysis, the results showed the presence of monosaccharides following (mannose, glucose and galactose) larger amounts of monosaccharides (rhamnose, xylose and fructose), I found that the amount is less compared to other monosaccharides. An evaluation of antioxidant activity that GP4 compound was good potential for scavenging activity of DPPH radical, ABTS radical and higher scavenging activity of hydroxyl radicals compared to other studied polysaccharide, should be explored as antioxidants in the new studies.

Keywords: Ginger, fractions polysaccharide, monosaccharides, antioxidant activity

INTRODUCTION

Ginger (*Zingiber officinale* L) for more than 2,000 years and is used as one of the types of spices (Bartley and Jacobs, 2000). Ginger root contains many polyphenol compounds such as 6-gingerol and its derivatives, and have a high an antioxidant activity (Chen, *et al*. 1986 and Herrmann, 1994). In previous studies for each of the (Ahmed, *et al*. 2000 and Ahmed and Sharma, 1997), appear that that long term dietary feeding of ginger had hypoglycemic and hypolipidemic effects and the antioxidant effect compared to the effect of ascorbic acid. Sharma, *et al*. (1996) studied feeding the rabbits on the ginger extract Hypolipidemic and anti atherosclerotic effects also demonstrated in cholesterol. Tyrosinase inhibitory activity and the superoxide scavenging of ginger is well known (Masuda, *et al*. 2004 and Khanom, *et al*. 2003). The study of antioxidant properties and chemical composition of ginger root through (Shirin Adel and Jamuna 2010), in which he explained that ginger is a good source most of the antioxidant components exhibit higher activities. The antioxidant activities of fractions polysaccharides were evaluated based on DPPH, the ability to scavenge superoxide, hydroxyl radicals, to binding to Fe(II) ions and reducing power (Zhang *et al*. 2011 and Hasan *et al*. 2012).

The aim of this study the separation and purification of polysaccharides from ginger roots and components of monosaccharides and evaluate their antioxidant activities in *vitro*.

MATERIALS AND METHODS

Materials and chemicals

Ginger (*Z. officinale* L) was collected from the local market Zagazig city (Egypt). The fresh roots were washed, air dried, cutting into small pieces then grind to fine powder and kept in glass jars at room temperature for use. Diethylaminoethyl cellulose (DEAE-cellulose). Dextrans of different molecular weights and sephadex G-200 were purchased from Sigma. Chemicals used in this study were of analytical grade and purchased from El-Gomhoria Co.

Extraction of polysaccharides

The powder of ginger was extract polysaccharides passed through several stages began the process of adding petroleum ether and ethanol 80%, extracted with re-distilled water at 100°C for 2h, 3 times and then the aqueous extract were compiled filtrated and concentrated under vacumm. It was removed from the protein in the crude polysaccharides using the detector Sevag regent (chloroform/butanol 4:1, V/v) described in the method (Navarini *et al*., 1999). After removing the protein from being the solution of polysaccharide fraction was dialyzed against deionized water and then precipitated polysaccharide using 80% ethanol. The precipitate was collected by centrifugation (1200 rpm, 20 min, 4°C), washed successively with ethyl acetate and acetone then dissolved in water and lyophilized to yield the crude polysaccharide (CP). The extract yield of crude polysaccharide was 6.47%.

isolation and purification

The freeze-dried sample (CP) was re-dissolved in deionized water and force through a filter (0.4µm), then applied to a column (300 x 25 mm) of DEAE-cellulose and eluted successively with deionized wate, 0.05, 0.1 , 0.2, 0.4 and 0.8 M NaCl solutions for 250 min, respectively, at a flow rate of 1.0 mL/min. fractions (10mL) were collected by a fraction collector. All of these fractions were analzed for the carbohydrate content by the phenol–sulfuric acid assay (Dubois, *et al*. 1956). four peaks were collected, and four fractions of which were further purified on a sephadex G-200 gel filtration column (700 x15 mm), and eluted with deionized water, at a flow rate of 0.2 mL/min. fractions (5ml) were collected and analyzed for the polysaccharides content.

Molecular size distribution of crude polysaccharides

The molecular weight of fractions were evaluated and determined by the gel permeation chromatography (GPC) with a Waters HPLC apparatus (Waters 515,Waters Co. Ltd., USA) equipped with an ultrahydrogel column (300 x 7.7 mm), a model 2420

refractive index detector (RID). The detailed operation conditions were mobile phase: 0.2M buffer phosphate (pH7); flow rate: 0.8ml/min; column temperature 30°C: room temperature 30°C; injection volume; 25µL; running time; 25 min. the column and detector compartment were maintained at 30 and 35°C, respectively The calibration curve for molecular weight determination was made using a series Dextran standards with different molecular weights (4,400, 9,900, 21,400, 43,500, 124,000, 196,000, 277,000 and 401,000 Da), following the method described by Alossp and Viachoglannis (1982). Empower software was used for the calculation of average molecular weights.

Analysis of monosaccharide composition

Monosaccharide composition of polysaccharides were determined using gas chromatography–mass spectrometry (GC–MS) (QP2010, Shimadzu, Japan). Ten milligrams of sample were hydrolyzed with 2 mL of 2 M trifluoroacetic acid (TFA) at 120°C for 6 h in a sealed glass tube according to the method of Erbing *et al*. (1995). The final solution was concentrated *in under vacumn* and the excess of acid was removed by repeated co-distillations with absolute ethanol.

Then the hydrolyzed products were prepared for acetylation. The acetylation was carried out with 10 mg of hydroxylamine hydrochloride and with 0.5 mL of pyridine by getting heated in a water bath for 30 min at 90°C. After incubation, the mixture was cooled at room temperature, and then 0.5 mL of acetic anhydride was added and mixed thoroughly by vortexing. The tube was sealed and incubated in a water bath for another 30 min at 90°C. After cooling, approximately 1µL of clear supernatant was loaded onto a Rtx-5SilMS column (30 m x 0.32 mm x 0.25µm) of the GC–MS. Alditol acetates of authentic standards (glucose, mannose, rhamnose, galactose, xylose and arabinose) with myo-inositol (2 mg) as the internal standard were prepared and subjected to GC–MS analysis separately in the same way. The operation was performed in the following conditions: N_2: 1.0 mL/min; injection temperature: 240°C; detector temperature:240°C; column temperature programmed: 160°C for 2 min, then increased to 240°C at 5°C/min and finally holding for 5 min at 240°C.

Infrared spectral analysis

The IR spectra of the polysaccharide fractions were determined using a Fourier transform IR spectrophotometer (FTIR) (PerkinElmer, USA). The purified polysaccharide fractions were ground with KBr powder and then pressed into pellets for FTIR measurement in the frequency range of 4000–500 cm^{-1}(Kumar *et al*. 2004).

ABTS radical scavenging assay

Radicals scavenging activity of the polysaccharides against radical cation ($ABTS^+$) were measured using the methods of Re *et al*. (1999) with some modifications. $ABTS^+$ was produced by reacting 7 mmol/L of $ABTS^+$ solution with 2.45 mmol/L of potassium persulphate, and the mixture would be kept in the dark at room temperature for 16 h. In the moment of use, the $ABTS^+$ solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. Each sample (0.2 mL) with various concentrations (0.01–2.0 mg/mL) were added to 2 mL of $ABTS^+$ solution and mixed vigorously. After reaction at room temperature for 6 min, the absorbance at 734 nm was measured. The $ABTS^+$ scavenging effect was calculated by the following equation:

$$\textbf{Scavenging effect (\%)} = (1 - A_{sample}/A_{control}) \times 100$$

Where $A_{control}$ is the absorbance of control without sample, A_{sample} is the test sample without $ABTS^+$.

Hydroxyl radical scavenging assay

The hydroxyl radicals scavenging activity of the purified polysaccharides were measured according to the method of Wang *et al*. (2008). Different concentrations (0.01–2.0 mg/mL) samples were incubated with 1.0 mL of ortho-phenanthroline (7.5 mmol L^{-1}), 5.0 mL of phosphate buffer (0.2 M, pH 6.6), 1.0 mL of ferrous sulfate (7.5 mmol L^{-1}) and 1.0 mL of H_2O_2(0.1 %) were mixed and diluted to 25 mL with distilled water. After incubation at room temperature for 30 min, the absorbance was measured at 510 nm.The hydroxyl radical scavenging effect was calculated by the following equation:

$$\textbf{Scavenging effect (\%)} = (1 - A_{sample}/A_{control}) \times 100$$

Where $A_{control}$ is the absorbance of control without sample, A_{sample} is the test sample without H_2O_2.

DPPH radical scavenging assay

The DPPH radicals scavenging activity of the purified polysaccharides were measured according to the method of (Braca et al., 2001; Shimada et al., 1992) with some modifications. Vitamin C was used as reference material. Three milliliters of sample (0.01–2.0 mg/mL) was added to 1 ml of 0.1 mmol/L methanol solution of DPPH. The absorbance at 517 nm was measured after the solution was kept at room temperature for 30 min. The DPPH radical scavenging effect was calculated by the following equation:

$$\textbf{Scavenging effect (\%)} = (1 - A_{sample}/A_{control}) \times 100$$

Where $A_{control}$ is the absorbance of control without sample, A_{sample} is the test sample without DPPH.

Statistical analysis

The data for various biochemical parameters were analyzed by one way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) using statistics software package (SPSS for Windows, V. 13.0, Chicago, USA). P values <0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

Isolated polysaccharides and purification

The raw polysaccharide fractions of ginger roots by using the method of purification ((Navarini *et al*., 1999 and Glicksman, 1969) using ion exchange chromatography and that succession isolated with water and sodium chloride, at different concentrations (0.05, 0.1, 0.2, 0.4, 0.8 M and is described in the method (Woolfe *et al*., 1977) were obtained on four polysaccharide fractions, first names appearing GP1, GP2, GP3 and GP4 were obtained from the GP1 only water while the other polysaccharide fractions (GP2,

GP3 and GP4) by sodium chloride concentrations (0.05, 0.1, 0.2 M, respectively. As shown in Fig. (1). Also by using sephadex G-200 column were separated and purified polysaccharide fractions obtained from Ginger has been collecting 4 ml of each fraction was lyophilized as he appeared four peaks are purified GP1, GP2,GP3 and GP4, respectively, as shown in Fig. 2

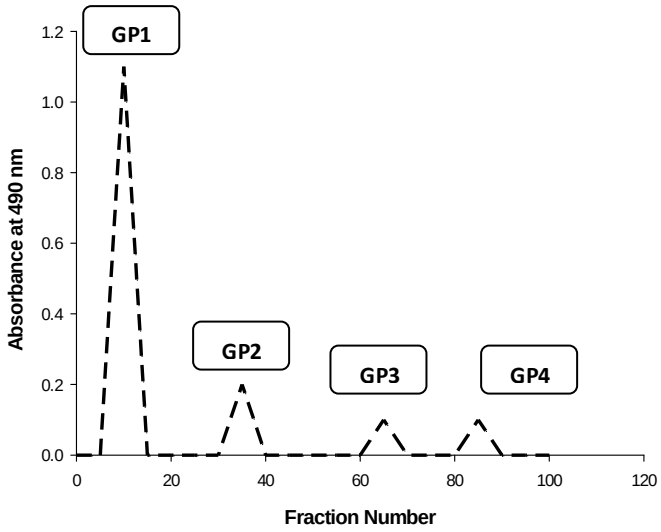

Fig.1 Ion exchange column chromatogram of eluted crude polysaccharide (CP) on DEAE-cellulose (300 x25 mm).

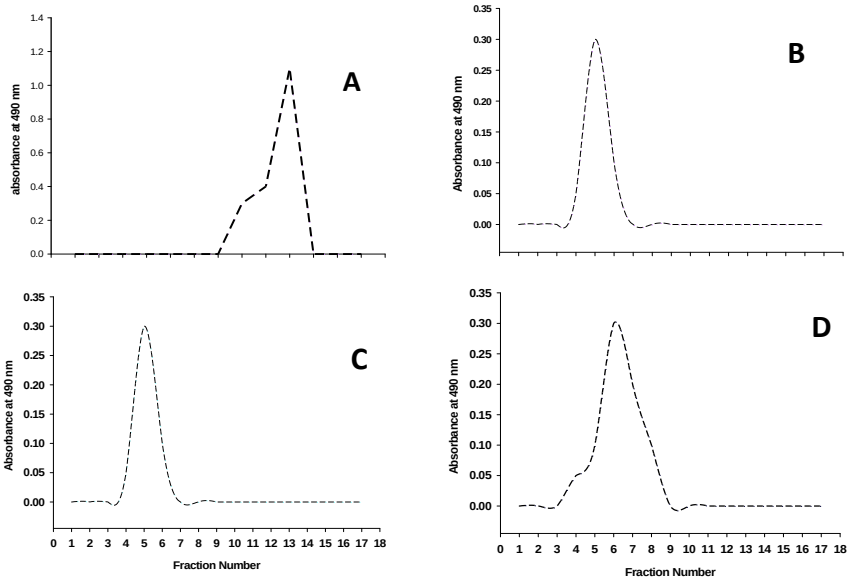

Fig.2 SephadexG-200 chromatography of GP1(A), GP2(B), GP3(C) and GP4(D)

Molecular weight and of chemical composition polysaccharide fractions

It was estimated molecular weight of polysaccharide fractions of separated and purified from ginger roots according to the method (Dreher, et al. 1979). the chromatograph analysis showed the molecular weights of the polysaccharide fractions were separated (GP1, GP2, GP3 and GP4 were approximately 136, 27.7, 13.2 and 12.8 kDa, respectively. GCMS analysis Table 1, show that monosaccharides composition of the four polysaccharides fractions consists of the same sugars. From the same table, it could be observed that GP1 and GP2 fractions had the highest content of mannose and glucose. Average values of 13.97 and 16.29% were dectected for mannose in GP1 and GP2 respectively. While glucose gave 43.65 and 51.26% in the same two fractions, respectively. On the other hand fraction GP3 and GP4 contained the highest amount of galactose content. Average values of 65.0 and 47.1% were found for this sugar in these two fractions, respectively.

Table 1. sugars composition (mol%) of fraction polysaccharides from ginger (*Zingiber officinale* L.)

fractions	mol (%)					
	Arabinose	Xylose	Rhamnose	Mannose	Glucose	Galactose
GP1	3.43	1.89	2.00	13.97	43.65	34.85
GP2	1.59	0.79	2.00	16.29	51.26	28.14
GP3	7.48	2.13	4.26	7.55	13.58	65.00
GP4	4.70	1.50	11.89	11.64	22.47	47.10

FT-IR spectras of polysaccharide fractions

The work of the IR four polysaccharide fractions separated from ginger roots and that has been purified according to the method (Kumar *et al*. 2004) to identify effective group function type of structure polysaccharide fractions and the type of cycle pyranose or furanose constituent sugar unilateral monosaccharides, as well as the linking α or β through region IR absorption in the region has been shown by the illustration in fig.3. The absorption in the region of polysaccharide fractions were found to be similar. The band between 3600 and 3200 cm^{-1} GP1: 3421 cm^{-1}, GP2: 3421 cm^{-1}, GP3: 3429 cm^{-1}, GP4: 3413 cm^{-1}), represented the stretching of the hydroxyl groups. The small band at around 2923 cm^{-1}(GP1 :2926 cm^{-1}, GP2: 2928 cm^{-1}, GP3: 2928 cm^{-1}, GP4: 2929 cm^{-1}) was attributed to the C–H stretching and bending vibrations. The bound at 1629 cm^{-1} (GP1), 1638 cm^{-1} (GP2), 1645 cm^{-1} (GP3) and 1635 cm^{-1} (GP4) were due to the bound water (Yuhong and Fengshan, 2007 and Park, 1971). Showed all separated polysaccharide fractions absorbed in the region 1210 - 1090 and this region shows where ring vibrations overlapped with stretching vibrations of C-OH side group as well as the O-C group glycosidic bands vibration. The absorptions at 1030, 1081 and 1157 cm^{-1}GP1, at 1026, 1082 and 1159 cm^{-1}GP2, at 1024, 1081 and 1158 cm^{-1} GP3, at 1031, 1078 and 1152 cm^{-1} (GP4) indicated a pyranose form of monosaccharides (Zhao, *et al*., 2005). Absorptions at 874 cm^{-1} (GP1), 856 cm^{-1}GP2 and 858 cm^{-1}GP3 were typical for α-configuration in pyranose form (Barker *et al*., 1954). A characteristic peak at around 897 cm^{-1} was found in GP4, indicating the β-configuration of the monosaccharides (Coimbra et al., 2002). On the basis of the results, it could be conclud that the polysaccharide fractions GP1, GP2 and GP3 were consisted of α-configuration in pyranose form monosaccharides while, GP4 was consists of β-configuration in pyranose form monosaccharides.

Scavenging effect on ABTS radical

the method $ABTS^+$ in assessing total antioxidant activity overall whether individual compounds or mixtures of compounds in various plants in accordance with the method (Huang *et al*., 2008 and Katalinic *et al*., 2006), and the measured absorption at a wavelength of 734 nanometers, and this way also $ABTS^+$ in measuring the antioxidant activity in extracts of organic as well as water extracts and thus can be used to measure Isolated from ginger roots polysaccharide fractions activity as described in the method (Han *et al*. 2008 and Wu *et al*. 2006). In the search activity was an estimate of the polysaccharide fractions separated using the $ABTS^+$ were obtained results shown in Figure 4, which indicate that sugar GP4 best antioxidant activity compared to sugars other even gave results close to that of vitamin C as when you use a concentration of 2 mg was GP4 approximately 79.2 while Activity vitamin C at the same concentration of 98% while the other polysaccharide fractions GP1, GP2 and GP3 results were much lower than the GP4, which gave GP2 better than GP3 and was the least GP1 compared to when the same polysaccharide fraction concentration GP4 was better than other polysaccharide fractions sign by $p < 0.05$ it is so clear that the GP4 is the best antioxidant activity using the $ABTS^+$. It also noted increased emphasis increasingly antioxidant for all separated polysaccharide fractions. These result indicate that GP4 had the strongest scavenging power for $ABTS^+$ radical and should been explored as possible antioxidants.

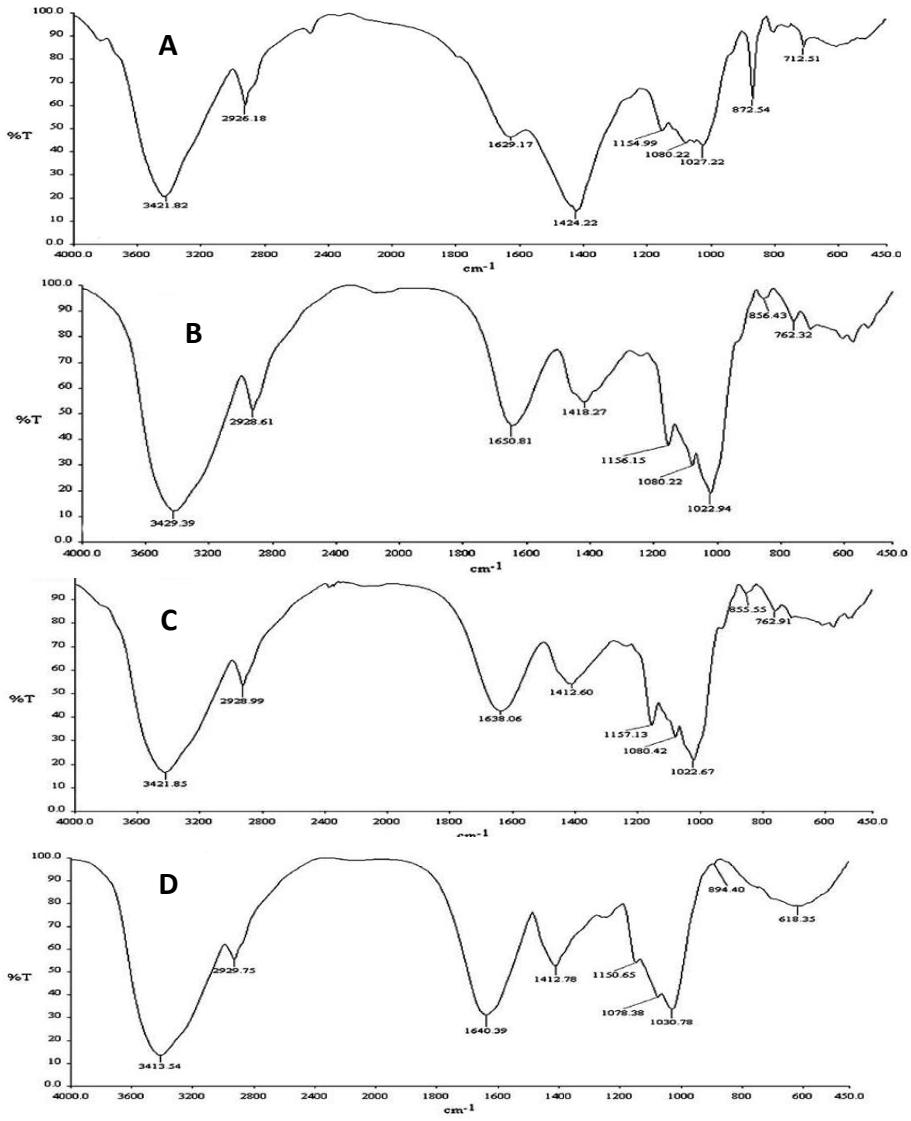

Fig.3 FTIR spectra of the polysaccharide fractions (GP1(A), GP2(B),GP3(C) and GP4(D)

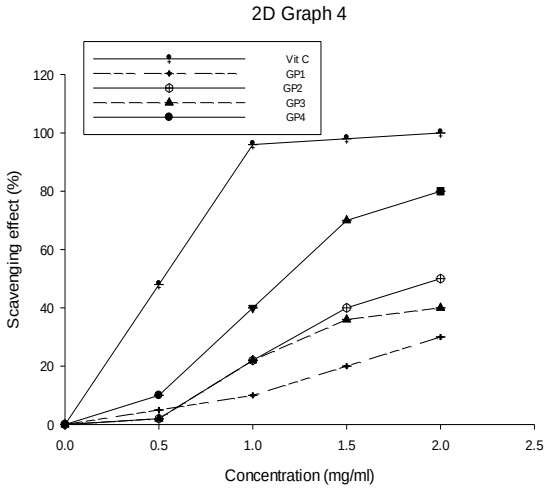

Fig. 4 The sc*a*venging activities of different concentration polysaccharide fractions on ABTS+ radical.

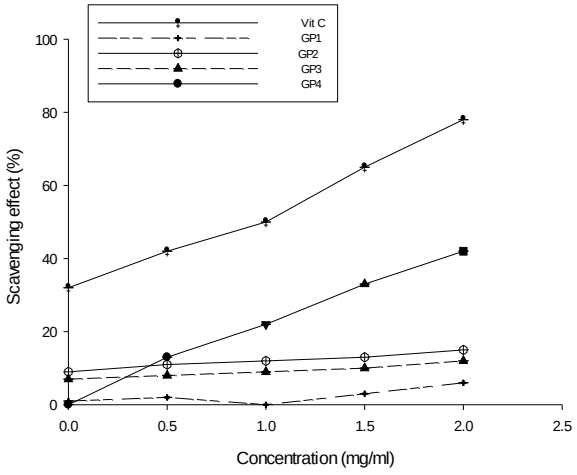

Fig. 5. The sc*a*venging activities of different concentration polysaccharide fractions on hydroxyl radical.

Hydroxyl radical scavenging activity of polysaccharide fractions

Hydroxyl radical way derivative depends on the hydroxyl group derivative is responsible for the oxidative damage of vital molecules as described (Ke *et al*., 2009).

As in the way the hydroxyl radical was compared to polysaccharide fractions (GP1, GP2, GP3 and GP4) with each at different concentrations of them (0.1 - 2.0 mg mg/mL) as well as vitamin C and the results were obtained and shown in Fig. 5 indicate that polysaccharide fraction GP4 is the best among other polysaccharide fractions (GP1, GP2 and GP3) in activity antioxidant ($P< 0.05$), but was significantly lower than vitamin C at the same concentration range of 0.1–2.0 mg/mL (Hasan *et al*., 2012 and Shirin Adel and Jamuna 2010). Shows of fig.5 that the polysaccharide fraction GP4 gives at a concentration of 2 mg/mL antioxidant activity about of 42% while the polysaccharide fractions (GP1, GP2 and GP3) did not increase the degree of antioxidant activity of 12% at the same concentration using this method Hydroxyl. It was also noted that the increased antioxidant activity with increased concentrations of the polysaccharide fractions

DPPH scavenging activity of polysaccharide fractions

The results obtained showed decreased activity sugars GP1 observant activity does not appear from the use of different concentrations until the concentration of 2%. In Fig. 6 is the estimation antioxidant activity separated polysaccharide fractions of ginger root in a assay DPPH and compared it with vitamin C showed

The results obtained showed decreased activity sugars GP1 observant activity does not appear from the use of different concentrations until the concentration of 2%. Showed the polysaccharide fraction GP4 antioxidant 39% when using the ability of the concentration of 1%. Note from the results that polysaccharide fractions differed in activity antioxidant attributed these differences in antioxidant activity to the structural composition of these polysaccharide fractions in the average molecular weights of the polysaccharide fractions were different through GPC analysis. The molecular weights of GP1 was the maximal among the four samples, approximately 136 kDa,. Although the other polysaccharide fractions own small molecular weight, (GP2, GP3 and GP4) they exhibited stronger antioxidant activity than GP4 fraction (Zhang *et al*. 2011). In the Infrared spectra analysis, we found GP4 was composed of *β*-configuration in pyranose form monosaccharides, on the antipode, GP1, GP2 and GP3 were composed of *α*-dominating configuration in pyranose form monosaccharides. The antioxidant effects test, showed that GP4 with β-configuration in pyranose form sugars exhibited stronger biological activity (Hasan *et al*. 2012). From your GCSM been identified to the monosaccharide compositions, consisting of numerous sugars that the results showed that glucose and galactose highest in the monosaccharides that are of ginger were different rate within each type of sugars and also found a clear difference in structure between the GP4 and other polysaccharide fractions, proportion rhamnose 11.89% in the GP4 also galactose 47.10%

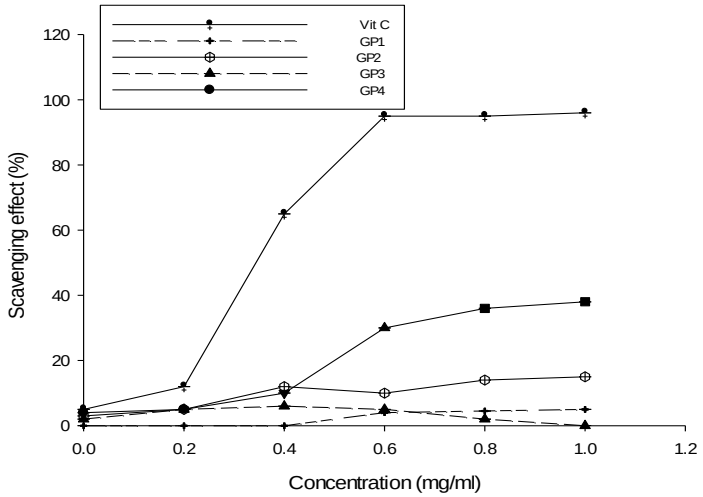

Fig.6 The sc*a*venging activities of different concentrations polysaccharide fractions on DPPH radical

CONCLUSIONS

In this wo*r*k were obt*a*ined on four major polysaccharide fractions (GP1,GP2,GP3 and GP4), purification been in different ways. Fraction GP1 higher molecular weight than other polysaccharide fractions, has been identified on the quality of monosaccharides components of these sugars found that glucose and rhamnose found that the fraction GP4 at a higher rate than the rest of the other fractions also found that GP4 the high antioxidant activity, through the methods that used to evaluate the antioxidant activity such as DPPH

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التركيب والنشاط المضاد للاكسدة للسكريات العديدة المستخلصه من الزنجبيل

حفناوى طه منصور حفناوى

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تم استخراج السكريات العديده القابله للذوبان في الماء من جذور نبات الزنجبيل (*Zingiber officinale* L) باستخدام الماء المغلي. تم تنقية هذه السكريات تباعا بواسطة االتحليل الكروماتوجرافي العمودي بمادتي DEAE السليلوز و مادة Sephadex-200. تم الحصول علي اربعة انواع من السكريات واطلق عليها GP1، GP2، GP3 و GP4 أظهر التحليل الكروماتوجرافي (GPC) أن متوسط الوزن الجزيئي (MW) من السكريات (GP1، GP2، GP3، GP4 و كانت حوالي ١٣٦ ، ٢٧.٧ ، ١١.٨ و ١١.٤ كيلو دالتون، علي التوالي. وكشف تحليل السكريات الأحادية عن وجود سكريات المانوز والجلوكوز والجلاكتوز وكميات صغيرة من الرامنوز والارابينوز بالاضافة الي سكر الزايلوز في السكريات الأربعة. وأشار تقييم النشاط المضادة للأكسدة ان المكون GP4 كان ذات إمكانات جيدة للنشاط الكاسح ABTS ، وارتفاع النشاط الكاسح للهيدروكسيل وDPPH مقارنة بالسكريات الأخرى، وينبغي استكشافها كمضادات للأكسدة في دراسات جديده .