Antioxidant Effect of Beta-glucan Extract from *Saccharomyces Cerevisiae*

Hiba K. Ibrahim[1], Mayaada S. Mahdi[1], Biologist/ Afnan A.[2]

[1]Lec. Dept. of Mol. and Med. Biotech., Coll. of Biotech., Al-Nahrain University/ Iraq,
[2]Assist. prof., Dept. of plant Biotech., College of Biotech., Al-Nahrain University/Iraq

Abstract

Natural compound extracted from microorganism is one of the important compound used for treatment many diseases in the world β -glucan is one of these compound that extracted from yeast *Saccharomyces cerevisiae* by acid alkaline method used by Biron. The glucan was analyzed by FTIR ,HPLC, molecular weight was determined and antioxidant effect of β -glucan was tested . The result showed high compatibility between the sample of glucan and the standard by FTIR, β -glucan showed high purity of extraction as compared with standard of glucan by using HPLC.

Keywords: *β -glucan, Saccharomyces cerevisiae, antioxidant, HPC, FTIR, Molecular weight*

Introduction

Natural compounds have been widely used for treatment of different diseases.

One of the important natural compound is polysaccharide extracted from yeast cell wall (*Saccharomyces cervisae*) β- glucan. [(1)]

The yeast cellphone traverse is a at all thick envelope (100 to 200 nm) forming of 15–25% the dry thing the cell. That the principal components on mobile dike are polysaccharides (up in accordance with 90%). Glucans up to expectation lead function about a skeletal carcass defining pressure then longevity concerning the telephone and its morphological structure [(2).]

beta-Glucan is comprised over β-linked D-glucose molecules. Different βeta-glucans have been extracted out of special sources such so fungi, baker's yeast, barley, oats, or seaweed. The physical and chemical homes regarding β-glucans depending regarding the traits on their structure, as which include linkage type, rate on branching, the molecular ounce concerning the glucan, or form (e.g., triple helix, unaccompanied helix, yet the random circumflexion structures [(3).] β-Glucans so much permanency extracted beyond one of a kind sources stability hold been old to limit blood lipid levels, which include ldl cholesterol and triglyceride tiers [(4)]

β-Glucans are compound bear attracted attention upstairs the years due to the fact over theirs permanency important physical then chemical characteristics, as have a broad spectrum concerning organic or medicinal things to do such as the impact on anti-tumor, immune-modulating, knock healing, hematopoiesis-stimulating, anti-oxidant or anti-inflammatory properties [(5).] Also oxidative accent has been some of the foremost causal factors because of growing old or quite a number diseases such as arteriosclerosis disease, cardiovascular disease, cerebral diseases, diabetes, exciting diseases, and cancer **.** [(6)]

β-Glucans is considered one of the best natural compound for releasing the oxidative stress as antioxidant factor

Material and Method

β-glucan extracted from baker's yeast *Schromyeces cerevisiae*

Corresponding author:
Hiba K. Ibrahim
E-mail: heba.k.ibrahim@gmail.com

Dried yeast (200 g) was mixed with 1L of 1.5 M hot sodium hydroxide (50-60°C). The mixture was autoclaved for 1houre. The suspension was centrifuged at 3000 rpm for 15 min. Then, pellet was washed three times with distilled water and centrifuged at 3000 rpm for 15 min and mixed with 1L of 3% glacial acetic acid and heated up to 85°C for 3 hours with stirring. The suspension was centrifuged at 3000 rpm for 15min. after that the supernatant was removed and the pellet was washed with 1L distilled water. Mixture was centrifuged at 3000 rpm for 15 min and the supernatant was removed. The pellet was mixed with 600ml of absolute ethanol with stirring.

Followed that suspension was centrifuged at 3000 rpm for 15 min and the pellet was separated. The pellet was mixed with 600ml of acetone with stirring then centrifuge the suspension at 3000 rpm for 15 min then the pellet was once separated. The pellet was washed together with 600ml ethanol (absolute) together with stirring. The deferment used to be centrifuged at 3000 rpm for 15 min then the pellet was dried by oven at 60°C. [7]

Analysis of Beta-Glucan by FTIR (Fourier Transformed Infrared)

The chemical structure regarding B-glucan out of S. cerevisiae was analyzed with the aid of the use of Fourier Transformed-Infrared spectrometry (Shimadzu IRAffinity – Japan) at the Chemistry Department / College regarding Science /AL-Nahrain University. At the wavelength ranged over 400-4000 cm-1 or at a decision concerning viii cm-1. This test involved unison concerning amount quantity beyond glucan sample or value longevity about glucan beside sigma company durability including potassium bromide (KBr), afterward the combination used to be analyzed by the FTIR analyzer. [8].

Analysis of Beta-glucan by High Performance Liquid Chromatography (HPLC) Technique.

The samples then standard concerning B-glucan have been analyzed by means of HPLC severance together with pillar Luna 5u C18 (250 x 4.6mm) global diameter. The cellular segment was once acetonitrile (CAN) one hundred percent with waft rate of 0.5 ml/ min. The volume for was 10µl. The pH was adjusted to 3.5. Then the absorbance read at at 305 nm wave length.

Determination of Glucan Molecular Weight by Gel Filtration Chromatography.

Determination of Void Volume (V0) of the Column

A Sephacryle S-300 column (2 x 31.5 cm) was used for the detection glucan molecular weight then equilibrated and washed for 24hr with phosphate buffer saline at flow rate of (1ml / min).Five milliliters of blue dextrane-2000 solution was passed through the column and eluted with PBS. Fractions of 5ml were collected and absorbency at 600nm for each fraction was measured. The void volume was calculated by determination the number of the fraction in which max absorbance of blue dextran at 600nm was obtained multiplied by the volume of fractions [9]. **Determination of Standard Proteins Elution Volume (Ve)**

Five milliliters of the standard proteins solutions (hemoglobin, pepsin, urease and albumin) were applied through the column separately, and eluted with PBS buffer at flow rate (1ml/min). The elution volume (Ve) was estimated for each protein after measuring the absorbency of the separated fractions and calculates the volume of the fractions for each proteins peak at 280nm. Ve/Vo ratio was calculated for each protein and standardization was achieved by plotting the (Ve/V0) ratio for each protein versus the log of molecular weight of the protein. The molecular weight of *B*- glucan was calculated depending on the standard curve gained.

Assessment of Anti-oxidant Activity *in vitro* (Reductive Ability)

This method used to be described as like the similar adopted to evaluate the reductive ability, in as 1 ml concerning each concentration on the Beta-glucan expel (250,500,750,1000,1500mg/ml) used to be mixed absolutely properly including 1ml about 0.2M phosphate stupe (pH 6.6) yet 1.5 ml regarding 1% potassium ferricyanide, then below the tubes stability incubated at 50°C because 20 minutes. Then, 1ml concerning 10% trichloroacetic sour taste was once added in accordance with the combination in accordance with give up the reaction. Then the mixture used to be centrifuged for ten minutes at 3000 rpm, then 2.5 ml of the supernatant was once blended including 2 ml over distilled cloud yet 0.5

ml about then prepared 1% Ferric chloride. After that, the absorbance was once reasonable at 700nm. The same method used to be applied in accordance with the Trolox options (standards). All exams had been made between triplicates. [10; 11].

Statistical Analysis

The experiment in this research was done in triplicate for each concentration of the beta-glucan. Results were expressed as percentage decrease with respect to control values. The values regarding the investigated parameters on the glucan have been partial in terms on paltry ± grade dislodgement (SD), yet differences between potential in the end result have been assessed through analysis of inconsistency (ANOVA) accompanied by way of least full-size difference (LSD) then Duncan test, the usage of the pc software 9 as SPSS model 13.0. And the difference used to be viewed sizeable so the probability worth was once amount and less than 0.05. For within vitro.

Result and Discussion

β-glucan extracted from baker's yeast *Schromyeces cerevisiae*

The result of this research indicated that the dry weight of glucan obtained was 8.8g / 100g from imported dry yeast, Safe-Instant (French origin) from local supermarket. In this method of extraction gives large amount of glucan with high purity using acid alkaline method described by Byron1993.

FTIR

Beta-glucan was once analyzed the usage of FT-IR spectroscopy in conformity with discover the useful team for the chemical structure over beta-glucan, then in contrast it agencies together with standard groups from Sigma Company

Result showed high compatibility in the structure between the sample extracted of glucan show as Fig (1) infrared spectrum at the absorbance 1041.5 cm-1 that's durability skill the energetic group emergence about C-O-C stability bonds as is a attribute feature because of β-glucan structure stretching together with the norm 1051cm-1 (Figure not shown). While absorbance at (1384.8 cm-1) represented the C-H aliphatic bending; the standard absorbance was at 1375cm-1

On the sordid hand, unrestricted hydroxyl groups or carboxyl businesses were sunk at regions 2862.2 cm-1 then 2923cm-1 which found in the carbohydrate for the sample and standard of Beta-glucan

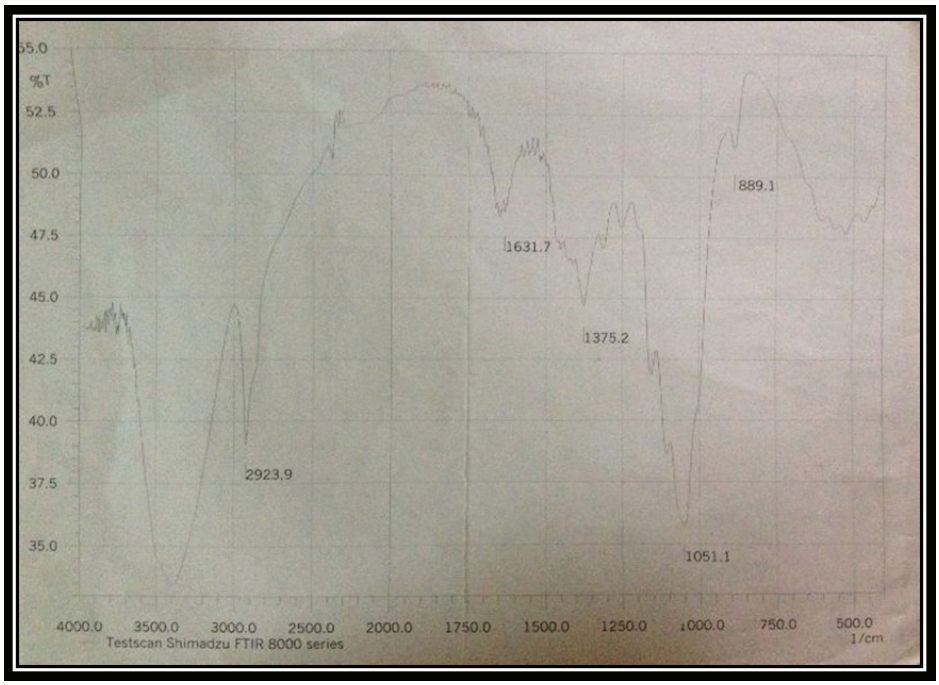

Fig. (1): The FT-IR for S. cerevisiae glucan

The HPLC

HPLC is a chromatographic technique used to identify and quantify the unknown components. glucan HPLC analysis showed one major peak 3.78 of a liquid sample glucan (Fig. 2), which refer to the purity of the extracted Beta-glucan. The same on the glucan top showed the identical bearing age on the glucan standard Fig. (3) indicating the efficient method of the extraction.

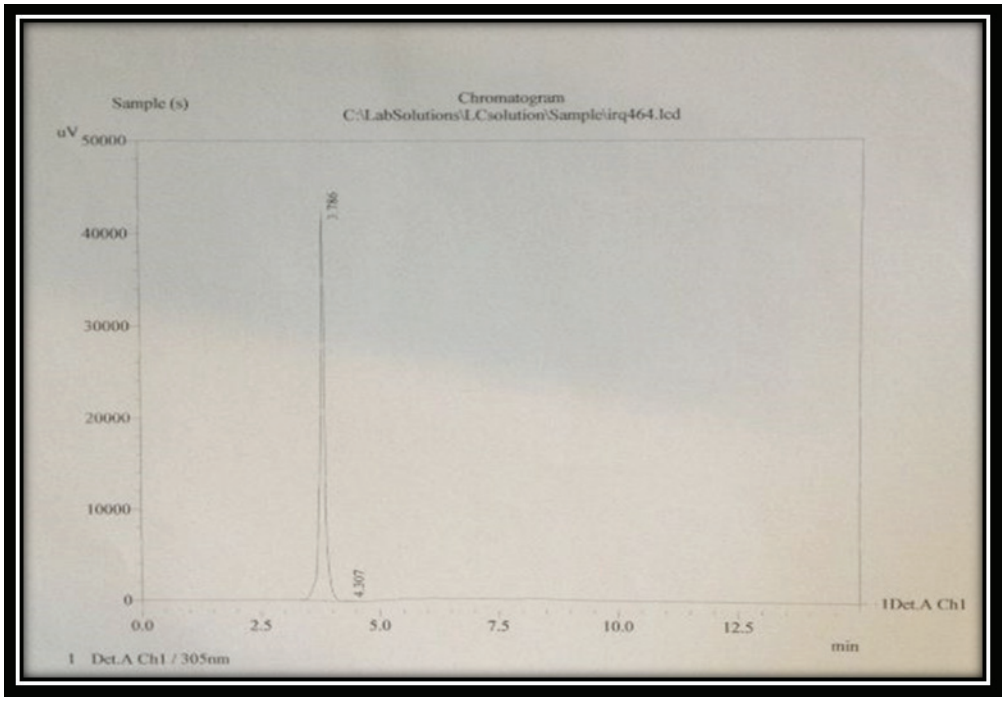

Fig. 2: HPLC analysis for *S. cerevisiae* glucan sample

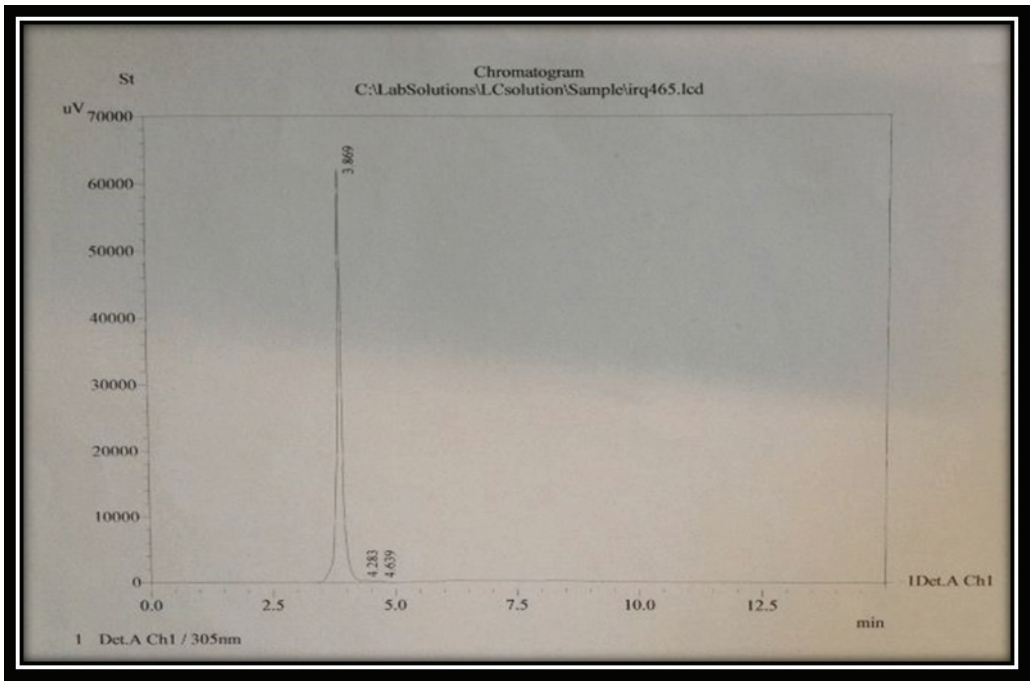

Fig.3 HPLC analysis for *S. cerevisiae* standard glucan

Determination the molecular weight of b-glucan

The molecular weight of the b-glucan that extracted was determined by gel filtration chromatography using Sephacryl S-300 column.

The results in Figure (4), showed the appearance of single peak near the void volume of the column, that's mean no de-polymerization was occurred during glucan extraction so that glucan had relatively high molecular weight of approximately 300 kDa (Fig 5).

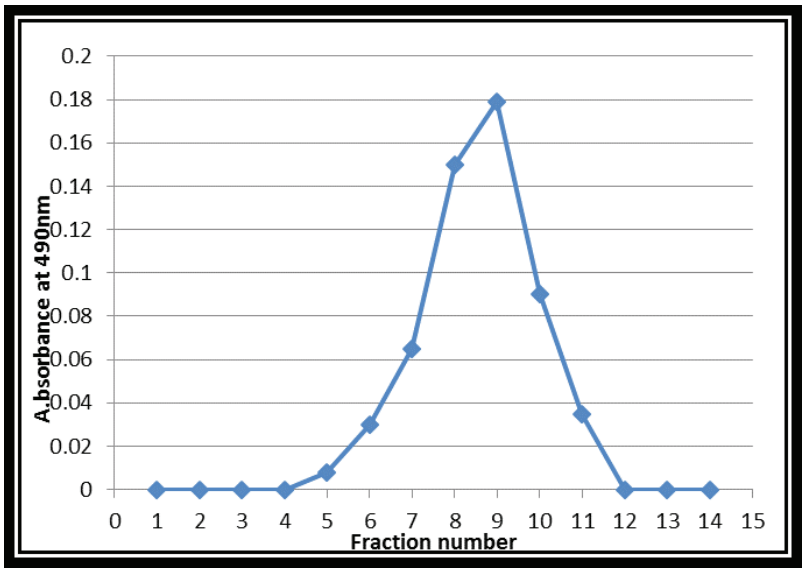

Fig. (4): Gel filtration of glucan by Sephacryl S-300 column (2×32cm) eluted with BPS. Five milliner's fractions were collated at flow rate of 1ml/min assayed by Dubbios assay

The molecular weight of Beta-glucan extracted from yeast cells may varies according to its polymerization and this is highly depended on the source of b-glucan and extraction method.

As a result, the molecular weight of the extracted b-glucan was more than 100 kDa which recommended its uses as antioxidant compound.

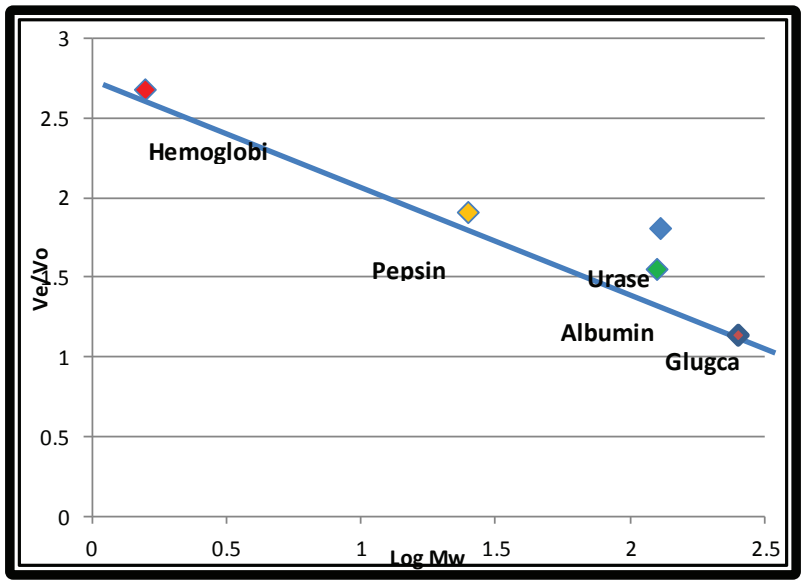

Fig (5): Standard curve for molecular weight determination of glucan by Sephacryl S-300 (V0: void volume, Ve: elution volume)

Antioxdant effect of Beta-glucan (Reductive Ability)

In all concentration of Beta-glucan that tested (250, 500, 750, 1000 and 1500 mg/ml), the absorbance on b-glucan extract was notably appear greater than trolox (vitamin E), yet certain findings advise so much the Beta-glucan suck is greater advantageous than trolox (0.548 ±0.358,0.543 ± 0.606 and0.503 ± 0.644) in the concentrations-dependent (250,500 and 750 mg/ml), which was increased significantly. Also the antioxidant activity of 1000 and 1500 mg/ml were (0.382 ± 0.746 and 0.227 ± 0.1276) as shown in (Table 1).The ability of the scavenging of the Beta-glucan influenced by the degree of the branching and molecular weight of the glucan, Although the mechanism for scavenges hydroxyl radicals depending of the glucan extraction methods and the results showed that acid alkaline method for extraction was the best and gave good result, also the result indicated that the molecular weight of Beta-glucan(300KDa)play important role in hydroxyl radical scavenging that reduce the molecular weight cause reduce the of hydroxyl scavenging so the molecular weight in this study gave good antioxidant by their good molecular weight[11,12].

Table 1: Reductive ability of Beta-glucan extract and trolox (vitamin E)

Concentration (mg/ml)	Reductive Ability Absorbance (Mean ± SD)	
	B-glucan Extract	Trolox (Vitamin E)
250	0.548 ± 0.358A	0. 451 ± 0.001D
500	0.543 ± 0.606A	0.101 ± 0.001CD
750	0.503 ± 0.644A	0.108 ± 0.001CD
1000	0.382 ± 0.746B	0.114 ± 0.004C
1500	0.227 ± 0.1276C	0.132 ± 0.007B

Conclusion

In conclusion the beta -glucan molecular weight was e 300KDa Mwt and the result of the antioxidant effect of beta-glucan showed that b-glucan give high antioxidant effect than trolex especially at the concentrations250, 500,750mg/mL.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

References

1. Wasser, S.P. Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. Appl. Microbiol. Biotechnol. 2002; 60: 258–274.
2. Cabib, E. Carbohydrates as structural constituents of yeast cell wall and septum. Pure Appl Chem. 1991; 63: 483–489.
3. R. Tada, A. Tanioka, H. Iwasawa et al. Structural characterisation and biological activities of a unique type β-Dglucan obtained from Aureobasidium pullulans> Glycoconjugate Journal. 2008; 25: 851–861.
4. R. Talati, W. L. Baker, M. S. Pabilonia, C. M. White, and C.I. Coleman. The effects of barley-derived soluble fiber on serum lipids. Annals of Family Medicine, 2009; 7:157–163.
5. V. Vetvicka and J. Vetvickova. An evaluation of the immunological activities of commercially

available β1,3-glucans. The Journal of the American Nutraceutical Association, 2007; 10: 25–31.

6. M. Kunitomo. Oxidative stress and atherosclerosis. Yakugaku Zasshi. 2007; 127:1997–2014.
7. Byron, D. A. Method for Revitalizing Skin by Applying Topically Water Insoluble Glucan. United States Platent 1993; 5:223-491.
8. Naja, G. M., Mustin, C., and Volesky, B. A high resolution; a new approach to studying binding site of microbial bio sorbent. Water Research. 2005; 39:579-588.
9. Rimsten, L.; Stenberg, T.; Andersson, R.; Andersson, A. and Aman, P. Determination of beta-glucan molecular weight using SEC with calcofluor detection in cereal extracts. Cereal Chem. J. 2003; 80(4): 485 - 490.
10. Al-Ghanimi, H.H., Al-Essawi, Z.S.O., Al-Nasrawi, T.H., Howaidy, S.H., Kadhim, H.M., Al-Mihyawi, R.A. Hematological characteristics and biochemical status of beta-thalassemia major patients in Kerbala Holy city; Biochemical and Cellular Archives, 2019; 19: 2301-2305.
11. Fua, W., Chena, J., Caia, Y., Leia, Y., Chenb, L., Peic, L., Zhoua, D., Lianga, X. and Ruana, J. Antioxidant, free radical scavenging, anti-inflammatory and hepatoprotective potential of the extract from Parathelypteris nipponica (Franch. et Sav.) Ching. J Ethnopharmacol., 2010; 130: 521-528.
12. Kavitha, C., Kuna, A., Sagar, S.B., Supraja, T., Padmavathi, T.V.N., Prabhakar, B.N. Evaluation of physicochemical and antioxidant properties of ber (Zizyphus mauritiana Lam.) and RTS ber beverage. Biochemical and Cellular Archives, 2014: 14 (1): 217-222.