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CHARACTERIZATION OF B-GLUCAN EXTRACTED FROM *SACCHAROMYCES CEREVISEAE* AND *CANDIDA ALBICANS*

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ABSTRACT

β-glucan is a polysaccharide with β-glycosidic linkages and it's a major structural component of various yeast cells. The goal of this research was to focus on the characterization of β-glucan extracted from both types of yeasts (*Saccharomyces cereviseae and Candida albicans*).The extraction of β-glucan depend on acid-base hydrolysis method and the yield of β-glucan extracted from *Saccharomyces cereviseae* was 63.35gm (28.15%) while the yield of β-glucan extracted from *Candida albicans* was 9.35gm (4.15%) from 225gm of both yeasts also the results explained that High performance liquid chromatography (HPLC) analysis of extracted β-glucan had similarity to the standard of β-glucan in major peak 3.22 of liquid samples extracted from two types of yeasts. On the other hand, Scanning electron microscope (SEM) revealed that standard of β-glucan was similar to both β-glucan extracted from *Saccharomyces cereviseae* and *Candida albicans* in size of particles in range (1.11-2.60µm) and no presence of cell wall trace but different in morphology.

Keyword : β-glucan, *Saccharomyces cereviseae, Candida albicans,* HPLC, SEM.

Introduction

β-glucan is polysaccharides. Complex and high molecular weight found in the cell wall of many types of yeast. Yeast β-glucan composed from mixture of β-1, 3 and β-1, 6 glucan (Many and Vizhi, 2014).

Yeast β-glucan has been made up 55-65%w/w of cell wall (Mongkontanawat *et al.,* 2018).The nature of β-glucan isolated from yeast are insoluble (Lesage and Bussey, 2006) and don't soluble in water because of chitin (Sobieralski *et al*., 2012) in addition many study suggested that insoluble (1, 3/1, 6) β-glucan have biological activity than of soluble (1, 3/1, 4) (Rahar *et al*., 2016). There are three types of glucan present in yeast cell wall which differ with type of linkage and branching of molecules (Saluk-Juszczak and Krolewska, 2010). The inner layer of yeast cell wall there is between 30-35% of insoluble β-glucan, in the middle layer between 20-22% of soluble β-glucan and the outer layer approximately 30% of glucoproteins (Akramiene *et al*., 2007). The percentage of β-glucan in the cell wall of *Saccharomyces cereviseae* in 1,3-β-D-glucan 50-55% but in 1,6-β-D-glucan 5-10% (Kwiatkowski *et al*., 2009) while in Candida albicans in β-1,3-glucan 40% but β-1,6-glucan 20% (Tronchin *et al*., 2008) as well as in (blastospore)58-60% but in (mycelium) 54-56% (Ruiz-herrera *et al*., 2006). The cell wall polysaccharides can be separated from each other by alkaline extraction, which solubilizes the α-mannoprotein fraction and leaves β-glucan particles in suspension. The β-glucan particulate can be separated from the soluble α-mannoprotein fraction by centrifugation and spray-dried to yield light, yellow colored, fine powder, free of any smell or taste, containing ~65% β-glucan (Kwiatkowski and Kwiatkowski, 2012). The extraction of β-glucan may be occur by chemical methods by NaOH, HCl, acetic acid, citric acid (Lee *et al*., 2001; Hunter *et al.,* 2002; Pelizon *et al.,* 2005) this method need high temperature (boiling) , physical methods like (sonication, high pressure) and enzymatic (lytic enzymes) lysis of yeast cells (Many and Vizhi, 2014). The aims of this study were extraction of β-glucan from both yeasts (*Saccharomyces cereviseae and Candida albicans*) and study the characterization of β-glucan through Scanning electron microscope (SEM).

Material and Methods

Strain, media and standard

Saccharomyces cerevisiae (Dried bakery yeast) was collected from different local markets in Baghdad. The yeast was activated by (0.1)g of sample in (10)ml of distilled water mixed and incubated at 30ºC for 30 min. after that cultured by streaking on YEPD agar plates and incubated at 30 ºC for 48h. according to (Ibrahim, 2014).

Yeast Pepton Dextrose Agar was prepared from (pepton 20g, glucose 20g, yeast extract 10g and agar 20g). This media was used to culture of *Saccharomyces cerevisiae* at 30 ºC for 48h.

Candida albicans were obtained from Microbiology Dept., Veterinary Collage/ Baghdad Univerisity. Sabouraud Dextrose Agar (Himedia-India) was used for culturing of *Candida albicans* at 37 □C for 48h.

Standard β- glucan (β- 1, 3-glucan) from *Euglena gracilis* was used throughout this study and supplied from Sigma.

Extraction of β –glucan from *Saccharomyces cereviseae* and *Candida albicans* cell wall by using alkaline-acid treatment according to (Pengkumsri *et al*., 2017) with some modification

β-glucan extraction from S*accharomyces cereviseae* cell wall

Mixing of (225) g of dried yeast with (1.5) L of 1M NaOH and the mixture was incubated at 80 ºC in magnetic stirrer heating for 2hr. then the cell pellet was collected by cold centrifuge at (6000xg) for 25 min.at 4 ºC and suspended in 3 fold of distilled water. The process was repeated and the supernatant was discarded and the pellet was taken to dissolve in (1.5) L of 1M acetic acid (CH_3COOH), after that the mixture was incubated at 80 ºC in magnetic stirrer for 2hr.then the pellet was collected by centrifugation at (6000xg) for 25 min.at 4 ºC and washed with water 3 times and centrifuged at (6000xg) for 25 min.at 4ºC and the supernatant was discarded, after that the pellet was mixed with (600) ml of absolute ethanol with magnetic stirrer for 1hr.and the suspension was centrifuged at (6000xg) for 25 min. at 4 ºC then pellet was dried by hot oven at 60 ºC for 24hr.

β -glucan extraction from *Candida albicans* cell wall

Candida albicans was cultured on SDA at 37 ºC for 3-5 days then the culture was harvested and 225g was taken and mixed with 1.5L of 1M NaOH. after that, the same procedure had been mentioned for β -glucan extraction from *Saccharomyces cereviseae* cell wall was performed.

Analysis of β –glucan by High Performance Liquid Chromatography (HPLC) technique

The standard of β–glucan 0.1mg was dissolved in (2.5) ml of deionized water, and the final concentration 40µg/ml, and then filtrated by Millipore 0.22µm.

The samples of (β –glucan extracted from *Saccharomyces cereviseae* and β -glucan extracted from *Candida albicans*) (0.5) mg were dissolved in (12.5) ml of deionized water, and the final concentration 40µg/ml, and then filtrated by Millipore 0.22µm.

The samples (β –glucan extracted from *Saccharomyces cereviseae and* β -glucan extracted from *Candida albicans*) and standard of β –glucan were analyzed by HPLC separation according to (Sabah, 2017) with column phenomenex C18 (50mm x 2.0mm), 3µm particle size. The mobile phase was deionized water with Flow rate 1ml/min.at 30 ºC. The injection volume for sample and standard solution was 20µL.This separation was occurred on liquid chromatography Shimadzu 10AV-LC equipped with binary delivery pump model LC-10A Shimadzu; the eluted peaks were monitored by UV-Vis 10 A-SPD spectrophotometer and the Concentration of samples were calculated according to equation:

$$\frac{\text{Concentration of samples}}{(\mu g/ml)} = \frac{\text{area of sample} \times \text{Conc. of std.} \times \text{Dilution factor}}{\text{Area of standard}}$$

Morphological characterization of β –glucan extracted from *Saccharomyces cereviseae* and *Candida albicans*

The morphology (shape and size) of standard β –glucan and β –glucan extracted from *Saccharomyces cerevisiae* and *Candida albicans* were visualized by a Scanning Electron Microscope (SEM) (TESCAN-VegaIII) at an accelerated voltage of 10KV at different magnification.

Results and Discussion

Extraction of β –glucan from *Sacchromyces cereviseae* and *candida albicans*

In this study, β-glucan was extracted by using a method depended on Alkaline-acidic extraction steps. By this method, the dry weight of β–glucan obtained from *Sacchromyces cereviseae* was 63.35gm (28.15%) (63.35/225gm), which higher than the yield of β-glucan extracted from *Candida albicans* was 9.35gm (4.15%) (9.35/225gm) as illustrated in table (1). This result refered to different in texture in these yeasts which used for extraction of β-glucan. Alkaline acidic method that used in this study is characterized by its ability to extract β –glucan with higher amount (Many and Vizhi, 2014) comparing with other methods for extraction of glucan like (Al-maliki, 2012) who extracted β –glucan from *Saccharomyces cervices* and the yield of β –glucan about (67gm, 13.4%) from 500gm, and Ibrahim (2014) who suggested that the yield of β –glucan about (17.6gm, 8.8%) from 200gm, Also, Al-Jumaiee *et al*. (2019) who indicated that the yield of β-glucan from the same yeast 5.95% from 4gm. β –glucan may be extracted from *Saccharomyces cervices* with different degree of purity depending on the method used and biological activity (Anthony *et al*., 2005). On the other hand, very little reportseas carried out about the extraction of β-glucan from *Candida albicans* (Lowman *et al*., 2003) and the yield of β-glucan extracted from *Candida albicans* in this study was lower when compare with the study by (Miura *et al*., 2002) who revealed the percentage of soluble β- glucan from the same yeast was 25.9% in (yeast form) but 7.5% in (mycelial form) from dried yeast cells. Many procedures used for extraction of glucan from yeast were depend on alkaline-acid with differences in time, type and concentration of the chemicals components, the advantage of using alkaline-acid extraction method was the treatment with base, acid and organic solvent which lead to dissolve or remove most proteins, mannan, nucleic acid and others (James *et al.,* 1991).

Ahmad *et al*. (2010) suggested that many factors may be effected on the extraction and properties of β –glucan like temperature and PH, also the extraction condition may be affected the physical, chemical and functional properties of β –glucan.

The other results revealed that the morphological features of β-glucan extracted from *Sacchromyces cereviseae* characterized by bright white color of powder as in figure (1-A), this result was concerning with (AL-Zubaidy, 2013) whereas the powder of β-glucan obtained from *Candida albicans* represented by yellow crystal particles as shown in figure (1-B).which agreement with (Ruiz-Herrera *et al.,* 2006).

Table 1 : Percentage of β-glucan extracted from both yeasts

Method	Types of yeast	Weight of yeast	Weigt of β-glucan extracted (gm)	Percentage of extraction (%)
Alkaline-acidic (NaOH-CH_3COOH)	*Sacchromyces cereviseae*	225	63.35	28.15
Alkaline-acidic (NaOH-CH_3COOH)	*candida albicans*	225	9.35	4.15

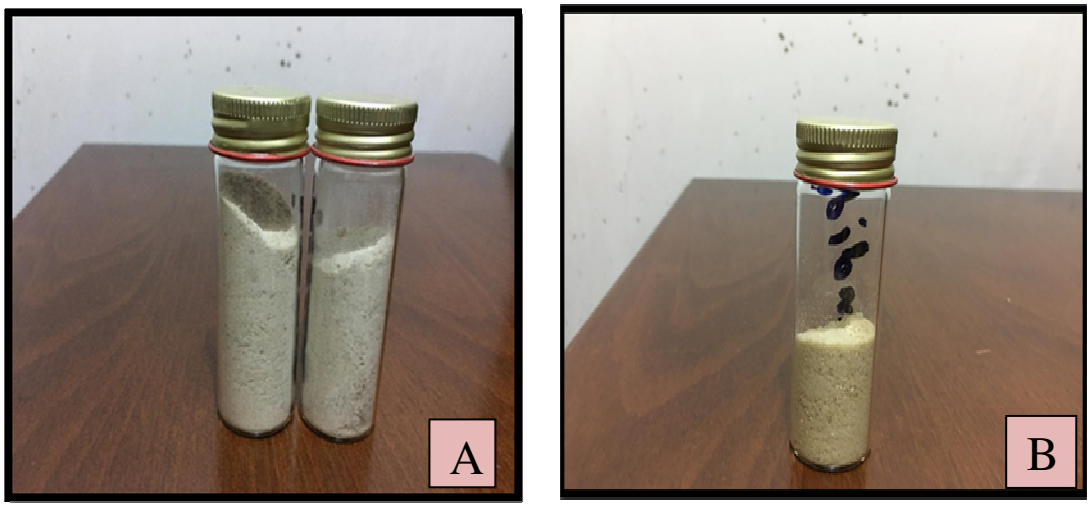

Fig. 1 : Shows powder of β-glucan extracted from A- *Saccharomyces cerviseae*, B- *Candida albicas*

High Performance Liquid Chromatography (HPLC) analysis of β-glucan

In the current study, the result of HPLC Analysis of the extracted β-glucan confirmed the structural similarity with the standard of β-glucan. The analysis revealed are major peak 3.22 of liquid samples of β-glucan extracted from *Saccharomyces cerviseae and Candida albicans* as shown in figure (2) which representing the purity of the extracted β-glucan from these yeasts. Such peak showed the same retention time of the standard of β-glucan in figure (3) and the sequences of the eluted material of β-glucan (standard, sample of β-glucan extracted from *Saccharomyces cerviseae* and sample of β-glucan extracted from *Candida albicans*) was shown as in table (2).The major peak of β–glucan extracted from *Saccharomyces cervices* about 3.22 which agreement with the work of (Ibrahim, 2014) that showed the major peak of β –glucan extracted from *Saccharomyces cervices* in HPLC analysis about 3.78.in addition HPLC was considered the efficient method for detecting of the β –glucan.

Table 2 : The results of sequences of eluted material of β-glucan detected by HPLC

Sequences	Subject	Retention time	Area	Concentration
1	Standard of β-glucan	3.227	189277	40mg/ml
2	sample of β-glucan extracted from *Saccharomyces cerviseae*	3.22	264137	40mg/ml
3	sample of β-glucan extracted from *Candida albicans*	3.22	155007	40mg/ml

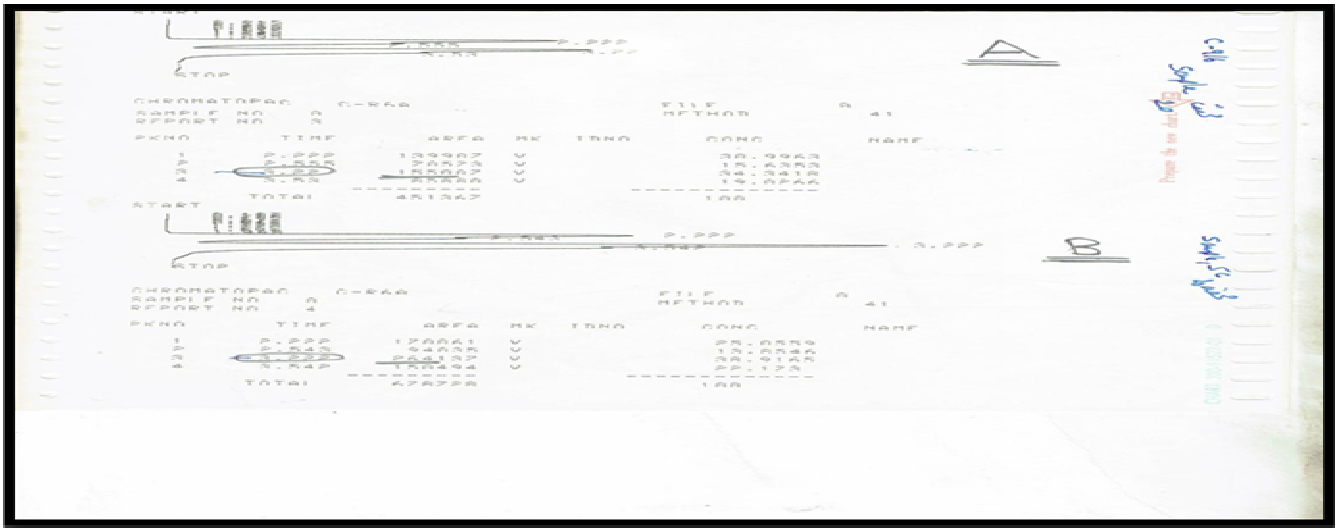

Fig. 3 : HPLC analysis of β-glucan extracted from A- *Candida albicans* and B- *Saccharomyces cerviseae.*

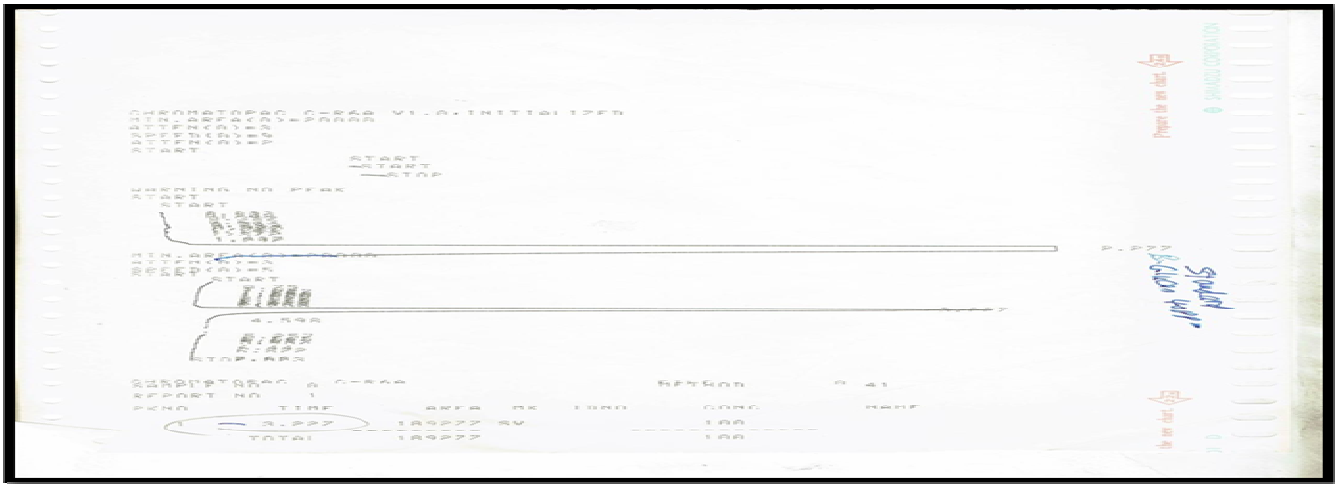

Fig. 4 : HPLC analysis of standard β-glucan

Scanning Electron Microscope (SEM) of β-glucan extracted from *Saccharomyces cerviseae* and β-glucan extracted from *Candida albicans*

The results of Scanning Electron Microscope (SEM) of three samples including standard of β-glucan, β-glucan extracted from *Saccharomyces cerviseae* and β-glucan extracted from *Candida albicans* in (10 µm) magnification were investigated. In general the standard of β-glucan is similar to both extracted samples (β-glucan extracted from *Saccharomyces cerviseae* and β-glucan extracted from *Candida albicans*) in size of particles with no presence of cell wall trace, but different in the morphology, however the shape of β-glucan extracted from *Saccharomyces cerviseae* is nearly similar to the standard of β-glucan.

The particles of standard β-glucan are uniform, irregular aggregation forming irregular masses with spherical to rounded particles. In addition, the particle size of standard β-glucan about (1.11-2.60) µm as shown in figure (4-A).

While the particles of β-glucan extracted from *Saccharomyces cerviseae* are irregular (geometric shape) with sharp edges and aggregated particles forming large mass. In addition, the particle size of this sample about (1.56-2.36) µm as shown in figure (4-B). These results were agreement with (Bacha *et al*., 2017; Upadhyay *et al*., 2017). Very rare researches about Scanning Electron Microscope for β –glucan extracted from *Candida albicans* and the result of this study indicated that the particles of β-glucan extracted from *Candida albicans* appeared as aggregators of booklet-like platelets with the size of particles from (1.2-1.84) µm as shown in figure (4-C).

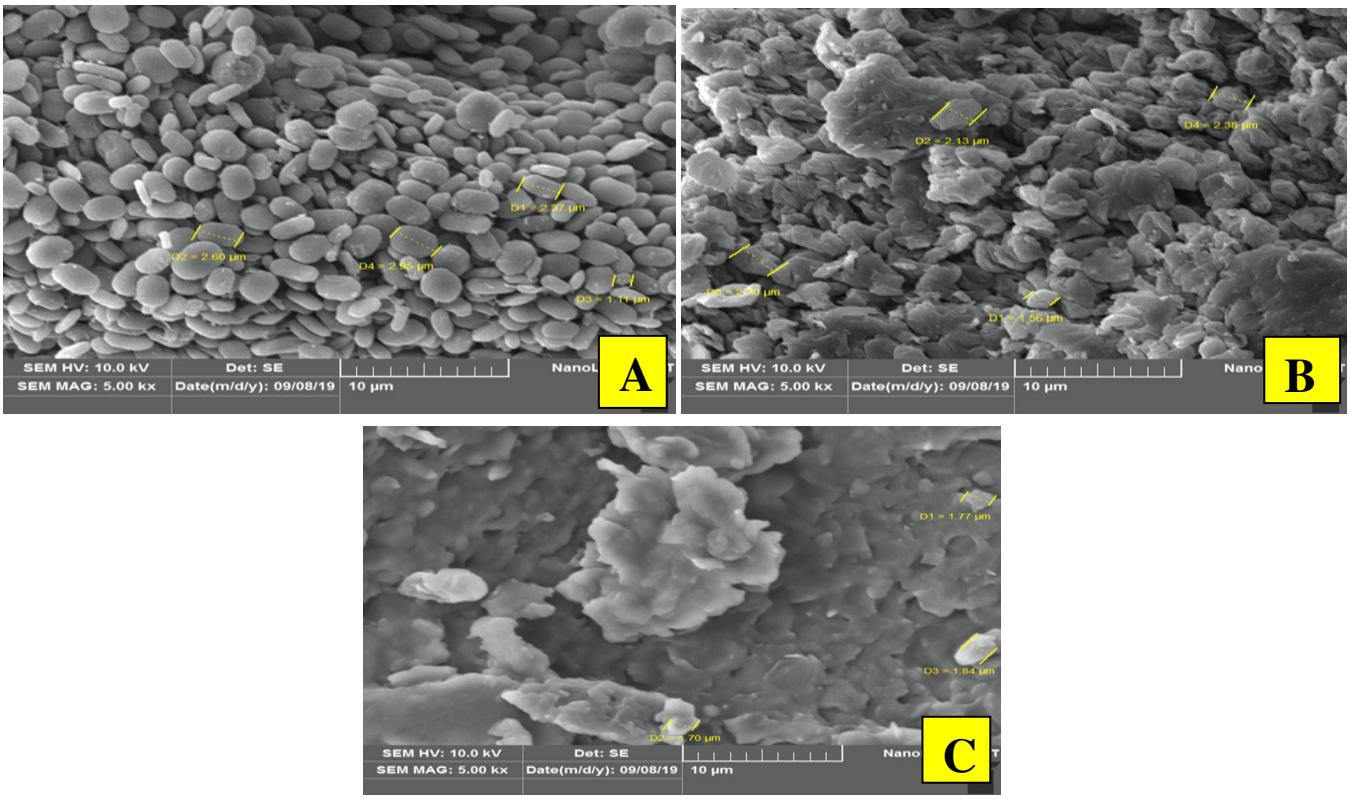

Fig. 5 : Scanning electron microscope (SEM) of A- Standard of β-glucan, B- β-glucan extracted from *Saccharomyces cerviseae* C- β-glucan extracted from *Candida albicans* in 10 µm magnification powers.

Conclusion

The present work suggested that successfully carried out the extraction of β- glucan from both yeasts (*Saccharomyces cereviseae and Candida albicans*) and showed that this extraction which dependent on acidic-base method can give high amount of extract, also the yield of β-glucan extracted from *Saccharomyces cereviseae* was more than the yield of β-glucan extracted from *Candida albicans* from 225gm of each yeast with a different color of extracted powder. In addition, the HPLC analysis and SEM confirmed that the extracted β-glucan from *Saccharomyces cereviseae and Candida albicans* were β-glucan when compared with the standard. On the other hand, this study is considering the first one for extraction and study the characterization of β–glucan from *Candida albicans* in Iraq. So, the current study recommends that this type of β-glucan can be used in the scientific researches.

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