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Characterization of β-glucan obtained from *Candida albicans* of caprine mastitis

Saddam Hussein Mahmoud* and Shaimaa Nabhan Yassein

Faculty of Veterinary Medicine, University of Baghdad, Baghdad, Iraq

Abstract

Background: Beta-glucan (β-glucan) is a polysaccharide containing β-glycosidic bonds that is an important structure part of different yeast cells.

Aim: The purpose of the study is to characterize β-glucan obtained from *Candida albicans (C. albicans)* isolated from caprine mastitis.

Methods: The β-glucan was extracted by using utilizing an Alkaline-acidic extraction technique. The dry weight of extracted β-glucan was 7.47/150 g with 4.98%.

Results: The findings demonstrated that the extracted β-glucan had similarity in the primary peak 5.78 of liquid samples using the method of high-performance liquid chromatography when compared to the standard form of β-glucan. However, scanning electron microscopy studies revealed that the standard of β-glucan was distinct in morphology but similar to β-glucan isolated from *C. albicans* in terms of particle sizes in the range of 1.60–2.65 m and the lack of cell wall traces. The findings of an investigation using energy-dispersive X-ray fluorescence spectroscopy (EDS/EDX) of extracted and standard β-glucan, showed the principal elements discovered were carbon (C), oxygen (O), and nitrogen (N). Aluminum (Al), silicon (Si), nickel (Ni), and gold (Au) were also present, but in less amounts.

Conclusion: The extracted β-glucan displayed a high degree of similarity and purity to the standard β-glucan, according to the findings of Fourier transform infrared spectroscopy (FT-IR) research.

Keywords: Subclinical mastitis, Mycotic mastitis, HPLC, Electron microscopy, Iraq.

Introduction

Mycotic mastitis is less common than other agents; it has grown significantly in the last ten years and is now recognized as a major cause of mycotic mastitis in ruminants (Mohammed and Yassein, 2020). More than 26 species of fungi have been linked to mycotic mastitis, which typically develops after bacterial mastitis has been treated with antibiotics (Al-Muhna, 2014). Yeasts are regarded as an essential part of the microflora of dairy products and are typically present in high concentrations in milk due to their high protein, sugar, fat, and organic acid content. Yeasts have the potential to seriously harm public health by causing metabolic degradation in milk (Spanamberg *et al.,* 2009). Moreover, fungi impact milk quality and shelf life (Hasan and Yassein, 2018). *Candida albicans (C. albicans)* is the most infective species which is responsible for more than half of the cases of candidiasis in various countries (Caggiano *et al.,* 2017; Singh *et al.,* 2020). *Candida albicans* only contains β-glucan and does not contain α-glucans; also, the cell walls of *C. albicans* contain both β-1,3 and β-1,6-glucans, but no intrachain combined with β-1,3/1,6 linkage (Ruiz-Herrera *et al.,* 2006).

Beta-glucan (β-glucan) is a component of cell walls in algae, bacteria, fungi, yeast, and plants (Pengkumsri *et al.,* 2017). It belongs to a class of polysaccharides called β-glycosidic polysaccharides that are composed of glucose polymer units (Vetvicka *et al.,* 2021), and high molecular weight found in the cell wall of many types of yeast. Yeast β-glucan is composed of a mixture of β-1, 3 and β-1, 6 glucan (Many and Vizhi, 2014). The normal yeast cells contain 30 to 45% β-1,3 glycan and 5%–10% β-1,6 glycan (Soares and Soares, 2012); while the cell wall is primarily composed of β-1,3/1,6-D-glycan (50%–60%) and mannoproteins (40%) (Klis *et al.,* 2006). Glucans are classified as (alpha-glucan, β-glucan, and alpha-β-glucan) based on the position of glycosidic linkages (1, 3, 1, 4, and 1, 6 glucans) according to the anomeric conformation of glucose (Synytsya and Novak, 2013) and soluble or particulate (Bashir and Choi, 2017). Yeast cell wall β-glucan consists of 1→3 β-linked glucopyranosyl residues, with a small number of 1→6 β-linked branches (Upadhyayv *et al.,* 2017). Several potential properties of β-glucans have been reported in the literature, such as being

***Corresponding Author:** Saddam Hussein Mahmoud. Faculty of Veterinary Medicine, University of Baghdad, Baghdad, Iraq.
Email: *saddam.hussein1101f@covm.uobaghdad.edu.iq*

antioxidant, anti-inflammatory, anti-cholesterol, anti-aging, a blood glucose regulator, and an antitumor agent (Chamidah *et al.,* 2017; Amer *et al.,* 2021). Moreover, β-glucan can act as an immunomodulatory molecule, which acts via the modification of the host immune response by the activation of innate immune cells including macrophages, neutrophils, and granulocytes (Muta, 2006).

The yeast cell wall contains three forms of glucan, which differ in terms of molecular connection and branched (Saluk-Juszczak *et al.,* 2010). The inner part contains 30 to 35% insoluble β-glucan, the intermediate layer contains 20% to 22% soluble β-glucan, and the outside part contains around 30% glucoproteins (Akramiene *et al.,* 2007). B-glucans are polysaccharides that display a variety of biological characteristics as a result of their diverse chemical make-up as the same as all dietary fibers (Vlassopoulou *et al.,* 2021). This study aims to extract β-glucan from *C. albicans* and detect the characterization by using scanning electron microscopy (SEM), high-performance liquid chromatography (HPLC), Fourier transform infrared spectroscopy (FT-IR), and energy dispersive X-ray fluorescence spectroscopy (EDS/EDX).

Materials and Methods

Source of C. albicans isolate

The isolate of *C. albicans* was isolated from subclinical mastitis in goats which was purified and examined macroscopically and microscopically, then exposed to the confirmatory diagnosis by implied specific tests such as germ tube production test, urease test, chlamydospore formation, and cultivation on CHROM agar according to other studies (Quinn *et al*., 2004; Washinton *et al*., 2006; Deorukhkar and Roushani, 2018).

Extracting of β–glucan from *C. albicans* cell walls according to other studies (Mahdi and Mohsin, 2015; Bacha *et al.,* 2017; Pengkumsri *et al*., 2017).

The β-glucan has been extracted via a combination of bases and acids ($NaOH/CH_3COOH$ extraction). *Candida albicans* had been grown on sabouraud dextrose agar at 37ºC for 3–5 days. After the colony had been collected, 1.5 l of 1 M sodium hydroxide (NaOH) was added to 225 g of harvested yeast, then the mixture was heated in a magnetic stirrer at 80ºC for 2 hours. Following that, cell pellets were obtained by cold centrifuging (6,000 × g for 25 minutes) at 4ºC within three folds from water that was distilled, cells were suspended and centrifugation was repeated again. Cell pellets had been dissolved by adding 1.5 l of 1M acetic acid (CH_3COOH). It was then stirred for 2 hours at 80ºC using a magnetic stirrer, centrifuged at 6,000 × g for 25 minutes, and used to collect the pellet at 4ºC. The supernatant was discarded after being washed three times with water and centrifuged (6000 ×g for 25 minutes) at 4ºC. The pellets had been mixed in 600 cc from 100% ethanol using a magnetic stirring device for 1 hour. To precipitate β-glucan, ethanol was added (Divya *et al.,* 2020), and the solution was centrifuged (6,000 × g for 25 minutes) at 4ºC. The pellets were dried in an oven at 60ºC for 24 hours. The typical β-glucan (β-1, 3-glucan) of *Euglena gracilis* has been utilized through this investigation, which was provided by Sigma.

β-Glucan Characterization

SEM

Standard β-glucan and β-glucan isolated from *C. albicans* were analyzed for morphology (shape and size) using a scanning electron microscope (SEM) (ThermoScientific™ Axia™ChemiSEM) at various magnifications and accelerated voltages of 10 KV. The specimens were then covered by a 2-nm-thick gold layer using a magnetron sputtering system (Bacha *et al.,* 2017).

EDS/EDX analysis

The unique live quantitative energy-dispersive X-ray spectroscopy (EDS) connecting (live structural data: works EDS through scanning several signals at the same time, identifying the shape and fundamental cosmetics for an object in actual period). The β-glucans were analyzed through the EDS/EDX technique (Bacha *et al.,* 2017).

FT-IR of β-glucan

The chemical structure of *C. albicans* β-glucan was investigated using Fourier transformed-infrared (IR) spectrometry (Shimadzu IRPrestige-21, Japanese) in the Ministry of Science and Technology. The spectrum of FTIR (an enhanced IR spectrometer) has been used to identify the functional compounds in the glucan molecule in comparison to the standards. It was accomplished using the FTIR technique with a wavelength range of 400–4,000 cm^{-1} with an accuracy of 8 cm^{-1}. This procedure required combining a comparable amount of β-glucan specimen and conventional glucan using potassium bromide (KBr), and analyzing this combination with an FTIR analyzer (Salim *et al.,* 2017; Rasheed and Haydar, 2023).

High-performance liquid chromatography (HPLC) technique of β-glucan

HPLC separation was used to examine the samples and standard of β-glucan using column Lichrosphere C18 (50 × 4.6 mm), with particle size 3 mm. Mobile phase: deionized water detection: a refractive index detector and RF Shimadzu detector. The flow rate was 1.2 ml/minutes, the temperature at 30°C and the pH was 3.5. The samples were prepared using 10 mg had been dispersed into 250 ml to form a 40 µg/ml conventional, and then 20 µl had to be injected through an HPLC cartridge for measurement. The purification was performed utilizing liquid chromatographic Shimadzu 10AV-LC fitted by the bipolar supply pump models LC-10A Shimadzu, and the peak results were examined with a UV-Vis 10 A-SPD spectrophotometer (Salim *et al.,* 2017; Mohamed and Al-Shamary, 2022; Rasheed and Haydar, 2023).

The detection happened in UV light with a wavelength of 305 nm. As described by other studies (Salim *et al.,* 2017; Mohamed and Al-Shamary, 2022), the sample of β-glucan isolating *C. albicans* and conventional β-glucan have been examined by HPLC separating at the Ministry of Science and Technology, and the concentration of samples was estimated using the equation:

$$\text{Concentrationof Samples}(\mu g / ml)(ppb) = \frac{\text{Sample area} \times \text{Standardconcentration}}{\text{Standards region}} \times \text{Diluting factor.}$$

Statistical analysis

The *t*-test in the GraphPad Prism Software was applied to estimate significant differences at $p < 0.05$ (Gharban *et al*., 2023).

Ethical approval

This study gets a license from the Scientific Committee of the Department of Internal and Preventive Veterinary Medicine, College of Veterinary Medicine, University of Baghdad (Baghdad, Iraq).

Results

Extraction of β-glucan from C. albicans

During the current investigation, alkaline-acidic extraction procedures were used to extract the β-glucan. The dry weight for β-glucan isolated from *C. albicans* using this approach was 7.47/150 gm (4.98%). The physical characteristics of β-glucan isolated out of *C. albicans* are characterized by the powder's yellow color (Fig. 1).

SEM image of β-glucan obtained from C. albicans

The findings for SEM evaluations, including the conventional forms of β-glucan and β-glucan derived from *C. albicans*, were examined at seven magnifications (5, 10, 20, 30, 50, 100, and 300 m). In general, the standard β-glucan has particles that are the same size as β-glucan isolated from *C. albicans* but differ in morphology. Standard β-glucan has homogenous, irregularly shaped particles that aggregate to produce irregular masses with spherical to rounded particles. The recovered *C. albicans* β-glucan particles resembled platelet-like aggregators, and depicts bigger, homogenous clusters of particles with a size range of 1.60–2.65 µm (Figs. 2 and 3).

EDS/EDX

The EDS-EDX was one of the prospective technologies for fast element detection. It is considered alternative equipment for detecting elements in bio-materials. The results of the EDS/EDX investigation of standard β-glucan revealed that the primary element discovered were carbon, oxygen, and nitrogen with less amount of Aluminum (Al), (Table 1, Fig. 4). While the findings of this screening obtained β-glucan showed the same primary element that found in standard β-glucan with lesser quantification in aluminum, silicon (Si), nickel (Ni), and gold (AU) (Table 2, Fig. 5).

Fig. 1. β-glucan obtained from *C. albicans.*

FT-IR of β-glucan

The characterization of Cladophora and Spirulina feed stock by FTIR suggests that the presence of functional groups of diverse elements such as the Hydroxyl unit (–OH) and amides (N–H stretch) have been accountable for adsorption on the surface activities. The current study's FT-IR examination confirmed that the extracted β-glucan had a high degree of similarity and purity when compared to the standard (Figs. 6 and 7). FT-IR findings of standard and extracted β-glucan included the presence of proteins and other associated components. The FT-IR analyses of standard and extracted β-glucan of the fungus cells revealed 5 broad bands. The -OH bending has been seen between 3,000 and 4,000 cm^{-1}, corresponding to a peaking of 3,377.35 cm^{-1} for derived β-glucan and 3,425.58 cm^{-1} for standard β-glucan. The 2nd band at 2,922.16 cm^{-1} for extracted and 2,924.09 cm^{-1} for standard indicated CH and CH_2 stretching.

The 3rd band at 1,651.07 cm^{-1} for derived β-glucan and the band at 1,730.15 cm^{-1} for standard β-glucan belongs to NH teams suggesting the existence of protein. 4th band at 1,076.28 cm^{-1} for derived β-glucan and the wide band at 1,078.21 cm^{-1} for standard β-glucan, is caused by COC bonds, which are bonds of glycosidic *via* cycle architecture, the peak at 10,746 cm^{-1} of the obtained β-glucans confirms the existence for starches. The 5th band of Carbohydrates comprised architecture may be indicated by bands within 1,043.49 cm^{-1} for extracted β-glucan and band at 1,035.77 cm^{-1} for standard β-glucan representing CO bond.

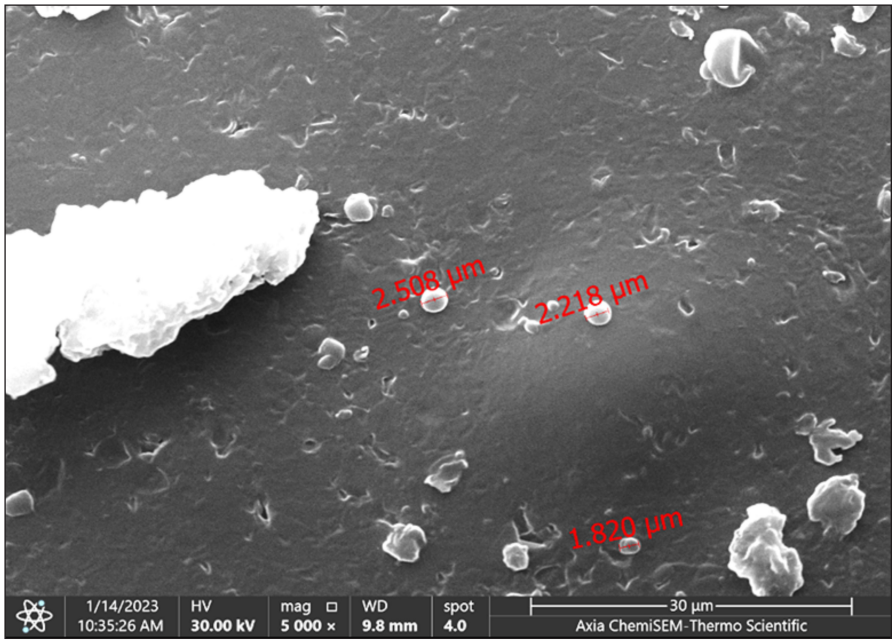


Fig. 2. SEM of conventional β-glucan in magnification power: 30 µm (1.60–2.65 µm).

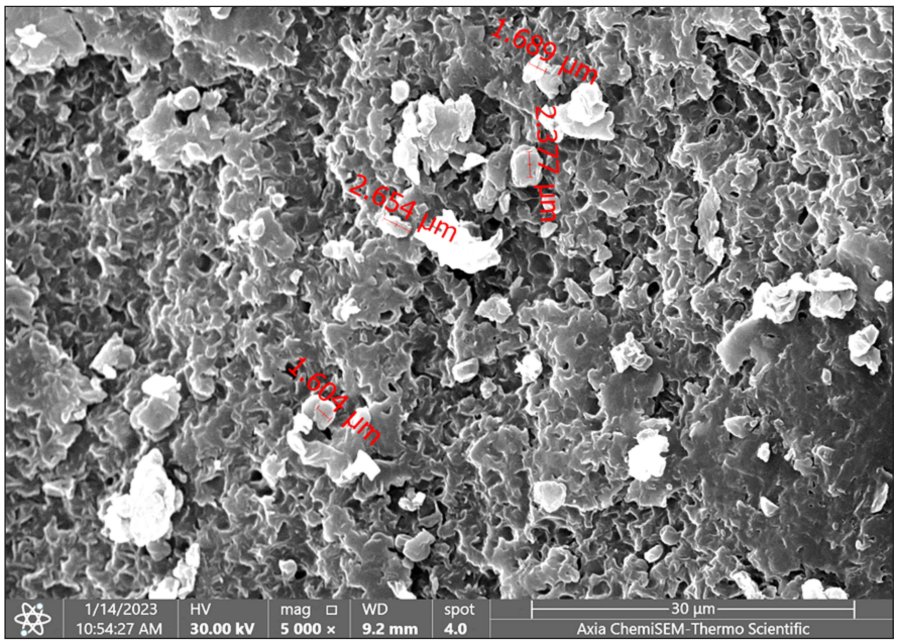


Fig. 3. SEM of β-glucan obtained from *C. albicans* at magnification power of 30 µm (1.60–2.65 µm).

High-performance liquid chromatography analysis of β-glucans

This method was employed during this investigation to determine the purity and quality of β-glucans that were extracted from *C. albicans* and also to support the standard β-glucan's structural similarity. In the present work, the extracted β-glucan's HPLC analysis result showed its structural resemblance to the β-glucans standard. HPLC test showed that the major peak of a liquid sample of β-glucan extracted from *C. albicans* was 5.78, which represented the purity of the extracted β-glucan. This peak had a similar duration of retention as the conventional of β-glucans (Figs. 8 and 9), and a concentration of β-glucans (conventional and specimen

Table 1. Analysis of EDS/EDX for standard β-glucan with total acquisition time: 28 seconds.

Element	Atomic%	Atomic% Error	Weight%	Weight% error
C	46.3	0.4	39.6	0.4
N	7.1	1.0	7.1	1.0
O	46.5	0.6	53.0	0.7
Al	0.2	0.0	0.3	0.0
p value	0.0362*	0.0453 *	0.0356 *	0.0448

Significance * ($p < 0.05$).

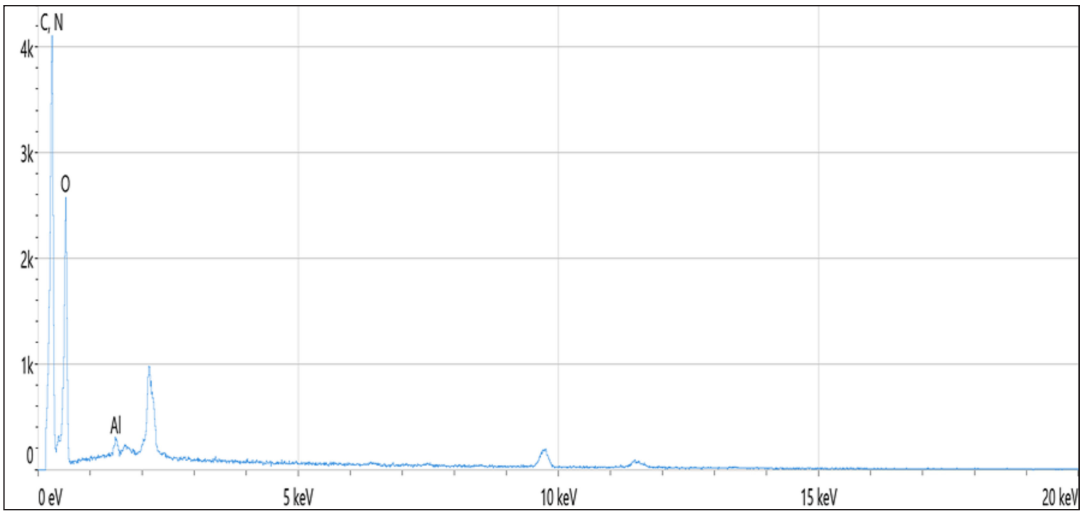


Fig. 4. Analysis of EDS/EDX for standard β-glucan with maps resolution: 768 × 512.

Table 2. Analysis of EDS/EDX for extracted β-glucan with total acquisition time: 28 seconds.

Element	Atomic%	Atomic% error	Weight%	Weight% error
C	48.1	0.4	38.3	0.3
N	6.5	0.9	6.0	0.8
O	44.5	0.6	47.3	0.6
Al	0.2	0.0	0.4	0.0
Si	0.1	0.0	0.2	0.0
Ni	0.0	0.0	0.2	0.0
Au	0.6	0.0	7.7	0.4
p value	0.0088 **	0.0497 *	0.012 *	0.0483

Significance * ($p < 0.05$), ** ($p < 0.01$).

of β-glucans obtained) discovered using HPLC (Table 3).

Discussion

The current study's findings are consistent with those of Jameel and Yassein (2021) who extracted β-glucan utilizing a technique based on Alkaline-acidic extraction stages with (4.15%); also, the morphological characteristics of the *C. albicans* derived β-glucan powder recorded as yellow crystal particles. In addition, Al-Jumaiee *et al.* (2019) reported a yield of 5.95% from 4 g of the same yeast. According to Ruiz-Herrera *et al.* (2006) powder from β-glucan produced by *C. albicans* is characterized by yellow crystal particles.

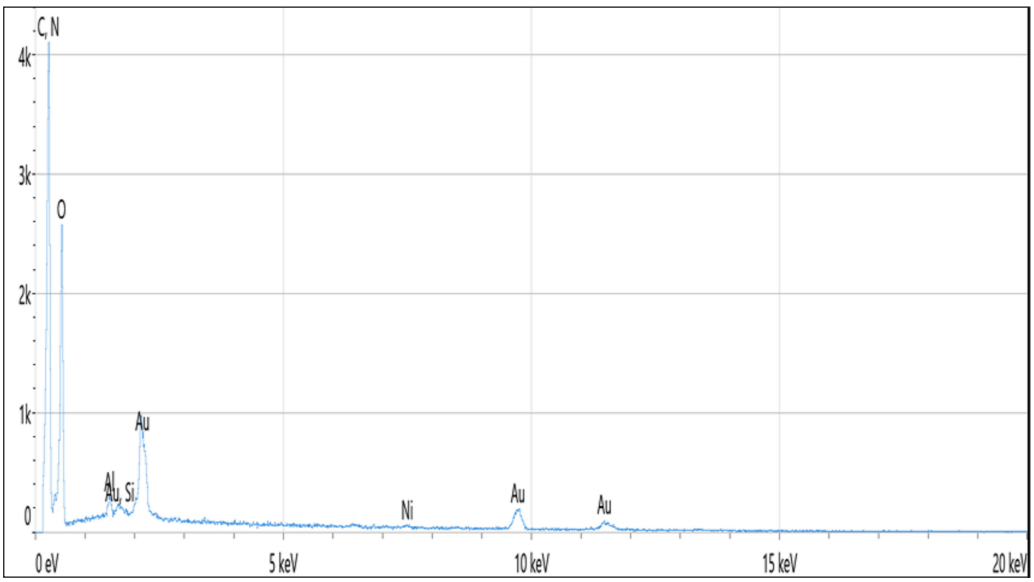
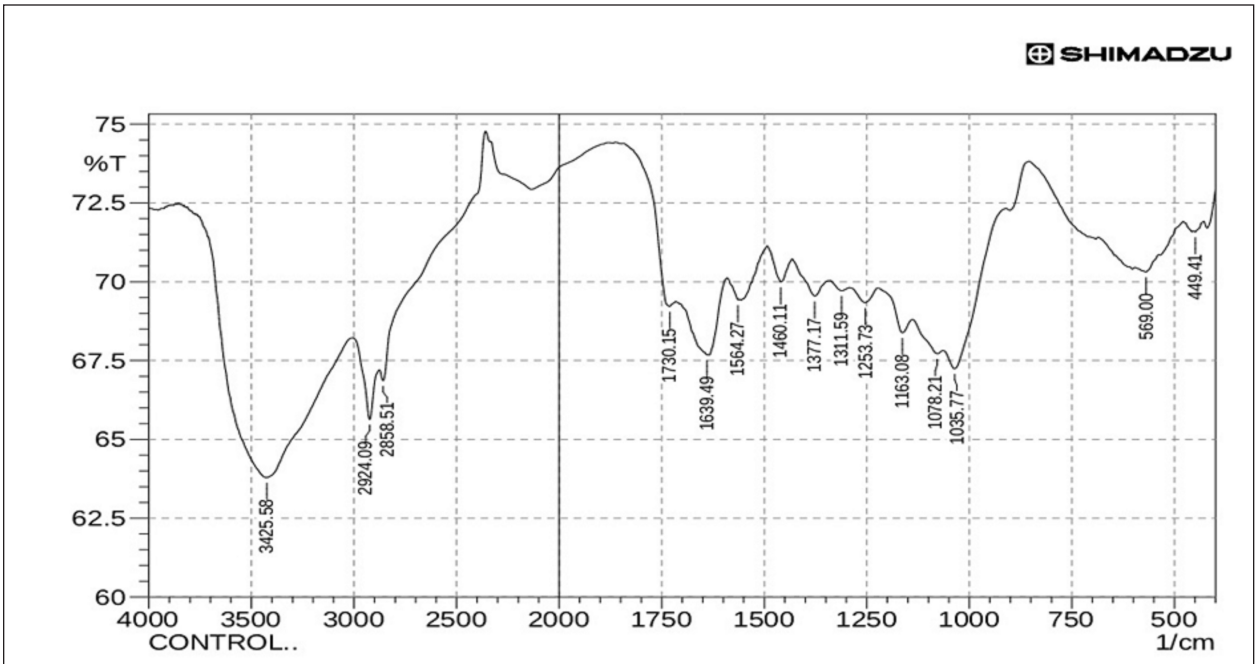
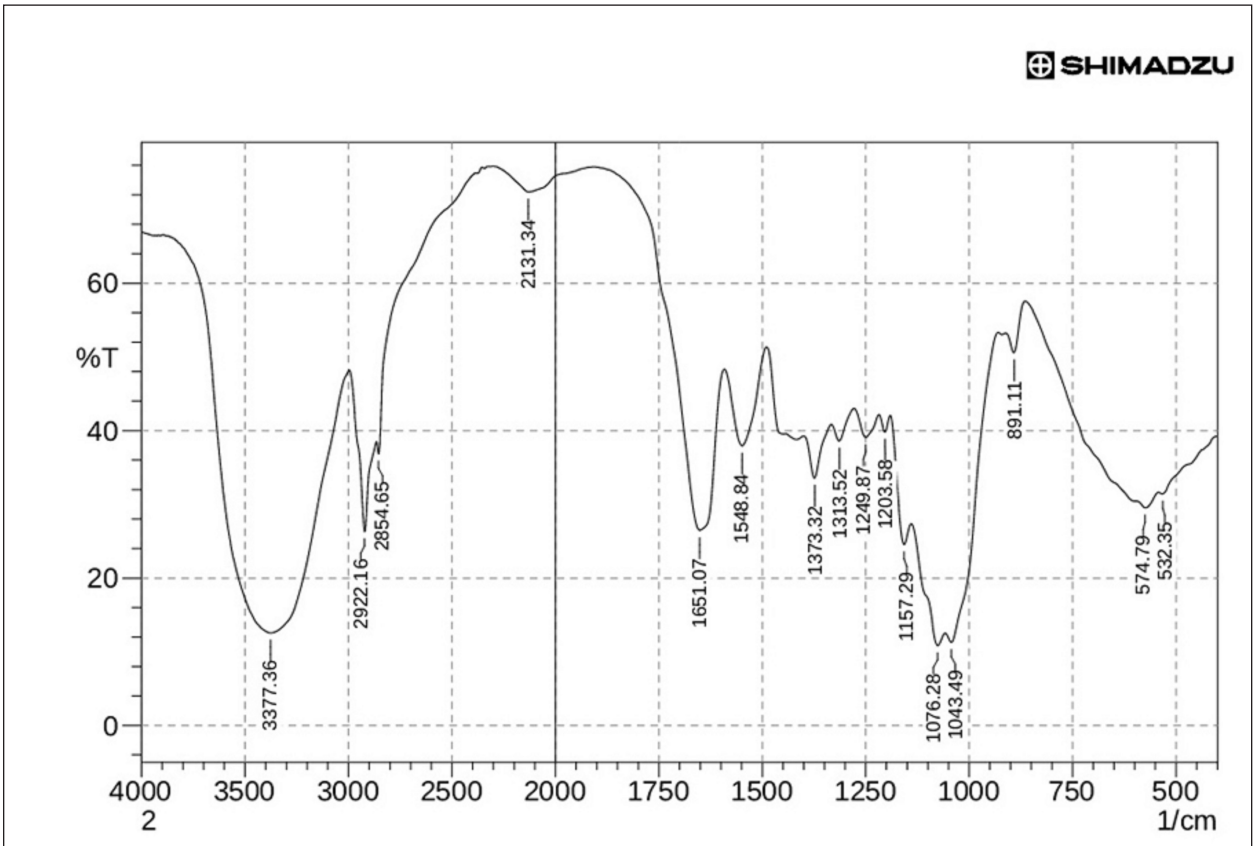


Fig. 5. Analysis of EDS/EDX analysis for extracted β-glucan with maps resolution of 768 × 512.



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	449.41	71.582	0.077	453.27	428.2	3.619	0.007
2	569	70.309	0.266	586.36	538.14	7.32	0.041
3	1035.77	67.246	1.419	1064.71	910.4	24.369	0.503
4	1078.21	67.719	0.282	1138	1064.71	12.215	0.083
5	1163.08	68.384	0.715	1222.87	1138	13.622	0.107
6	1253.73	69.336	0.471	1292.31	1222.87	10.941	0.103
7	1311.59	69.714	0.192	1338.6	1292.31	7.223	0.03
8	1377.17	69.545	0.772	1433.11	1338.6	14.633	0.215
9	1460.11	69.994	0.916	1492.9	1433.11	9.09	0.171
10	1564.27	69.425	0.109	1591.27	1560.41	4.825	0.004
11	1639.49	67.678	0.033	1645.28	1637.56	1.307	0.001
12	1730.15	69.213	0.693	1843.95	1716.65	17.967	-0.318
13	2858.51	66.865	0.595	2875.86	2358.94	77.84	0.598
14	2924.09	65.632	1.946	3007.02	2875.86	22.802	0.592
15	3425.58	63.794	0.107	3711.04	3421.72	51.839	2.464

Fig. 6. Analysis for standard β-glucans using FTIR.



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	532.35	31.407	0.743	542	405.05	62.065	0.222
2	574.79	29.522	1.432	597.93	543.93	28.012	0.582
3	891.11	50.586	4.558	912.33	864.11	13.118	0.735
4	1043.49	11.288	5.613	1056.99	931.62	75.315	5.075
5	1076.28	10.856	4.904	1138	1058.92	62.643	4.395
6	1157.29	24.539	7.919	1190.08	1139.93	26.5	2.794
7	1203.58	39.788	2.31	1219.01	1192.01	10.466	0.323
8	1249.87	39.081	3.519	1278.81	1219.01	23.381	1.213
9	1313.52	38.606	3.013	1332.81	1278.81	21.181	0.786
10	1373.32	33.576	6.307	1398.39	1334.74	27.276	1.99
11	1548.84	37.938	11.637	1591.27	1489.05	37.168	6.224
12	1651.07	26.474	26.867	1909.53	1593.2	86.483	22.015
13	2131.34	72.377	3.452	2301.08	1909.53	50.893	3.828
14	2854.65	36.838	2.404	2864.29	2385.95	94.923	-34.169
15	2922.16	26.322	16.347	2995.45	2866.22	57.072	9.679
16	3377.36	12.558	43.667	3842.2	2997.38	470.187	260.057

Fig. 7. Analysis for extracted β-glucans using FTIR.

On the other hand, Hassan *et al.* (2014) mentioned that the yeasts can produce up to 12% of their weight in β-glucan when they are extracted using an alkaline acid. In this work, the amount of β-glucan produced by *C. albicans* had been lesser than that of Miura *et al.* (2003) discovered that the amount of soluble β-glucan in the identical fungus is 25.9% in (yeast forms) but 7.5% in (mycelial forms) in dry yeast cells.

Ibrahim (2014) discovered that the dry weight of the resulting glucan was 8.8/100 g dry yeast. AL-Rubaee (2008) claimed the ability to obtain glucan 6.25/100 g dry-weight baker's yeast. The current study agrees with Limberger-Bayer *et al.* (2014) who observed that morphological characteristics for both of the samples, derived β-glucans and conventional β-glucans, were identical, exhibiting a porosity and sponge look and not any obvious signs of the creation of cell walls.

The results of a study by Jameel and Yassein (2021) performed by SEM on the particles of conventional β-glucan demonstrated the existence of homogeneous, irregular aggregating generating irregular mass with cylindrical to round particles The findings of very few

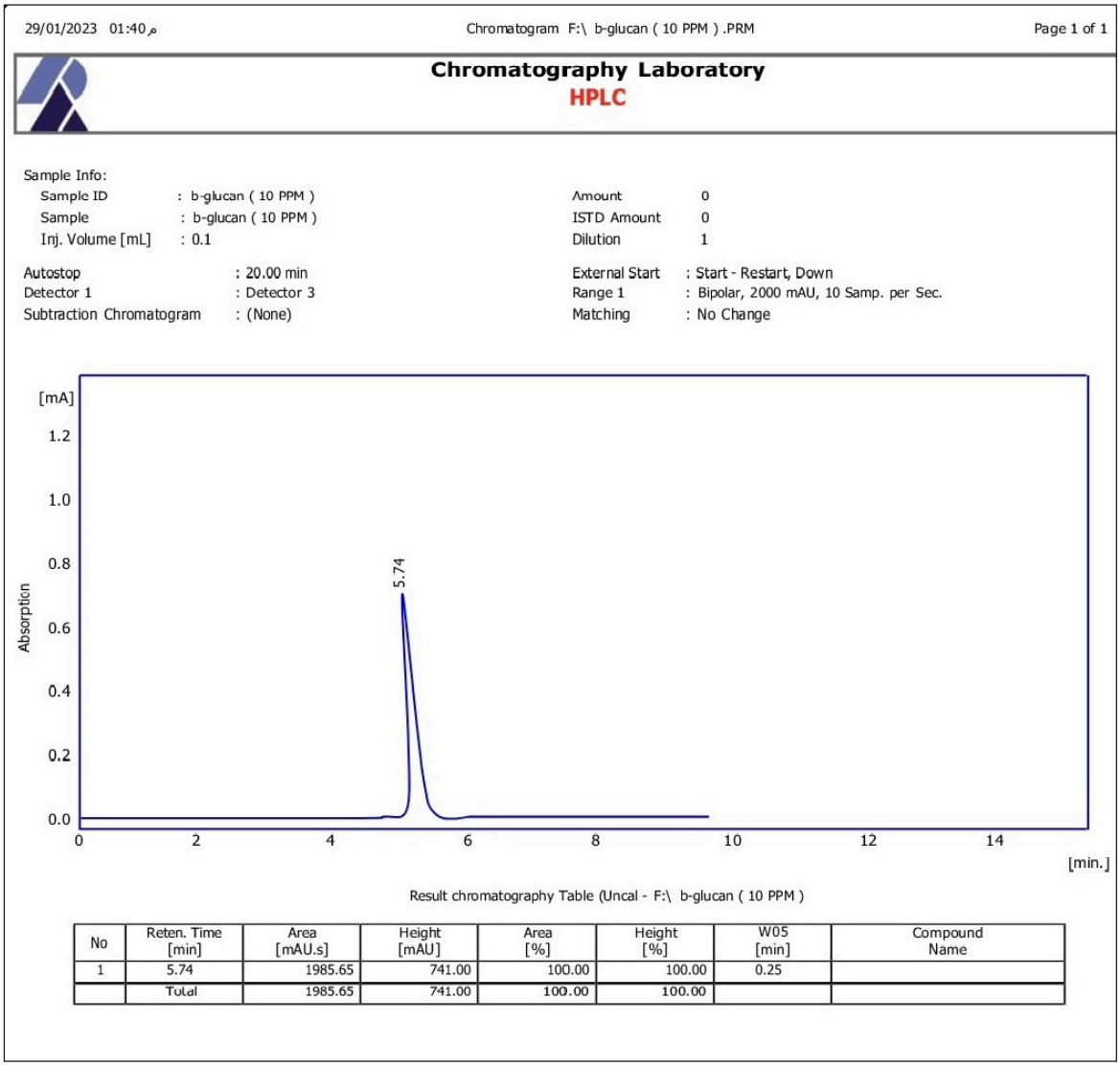


Fig. 8. HPLC analysis of standard β-glucans.

studies on SEM for β-glucan obtained from *C. albicans* showed that the particles of β-glucan obtained from *C. albicans* having an aggregation of booklet-like platelet having particle sizes ranging from 1.2 to 1.84 µm.

SEM investigation revealed the ridge-like character of the β-glucan, as well as the shiny exterior and undulated borders. The oval to elliptical form of the granular particles indicates that the natural architecture of β-glucan particles has been preserved, as mentioned by Raikos *et al.* (2018). In yeast cells, the FTIR spectrum was utilized to analyze three major sections, which correspond to polysaccharides (925–1,190 cm^{-1}), proteins (1,500–1,700 cm^{-1}), and lipids (2,800–3,000 cm^{-1}), (Liu *et al.,* 2015; Pengkumsri *et al.,* 2017; Al Dujaily and Mahmood, 2021).

IR spectroscopy can monitor the molecule oscillations from covalent bonding; the IR range 4,000–500 cm^{-1} contains data about basic oscillations as mentioned by Limberger-Bayer *et al.* (2014). The FTIR technique has been utilized to identify functional groups within the structure of the chemical of β-glucan and compare it to standard groups (Rumberger *et al.,* 2005; Ibrahim *et al.,* 2006; Hassan *et al.,* 2014; Salim *et al.,* 2017). The samples FT-IR spectroscopy was utilized to examine differences in primary ingredients (carbohydrates, fat, and protein) and overall composition (Rieder *et al.,* 2017; Salim *et al.,* 2017; Ibrahim *et al.,* 2020; Abd and Mohammed-Ridha, 2021).

Limberger-Bayer *et al.* (2014) analyzed commercial and purified-isolated β-glucan. Because yeast's outside cell walls are predominantly made of carbohydrates, cells of yeast displayed five bands, one of which was 3,409.873 cm^{-1}. The polysaccharides include a lot of OH groups. In the case of O–H teams, the –OH

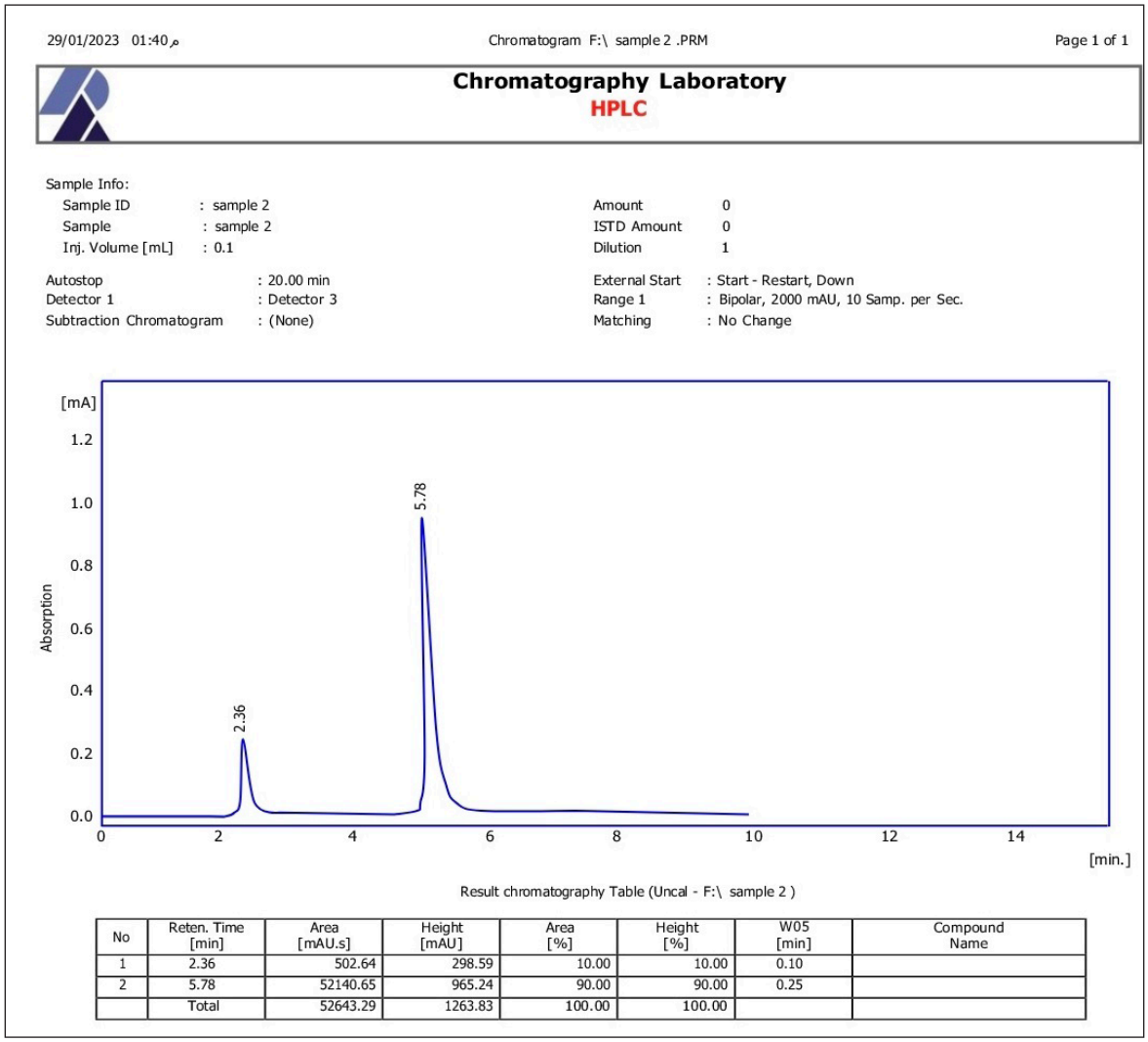


Fig. 9. HPLC analysis of extracted β-glucans.

Table 3. Concentration of β-glucans (conventional and samples of β-glucans obtained from *C. albicans* detected by HPLC.

Subject	Retention time	Area	Concentration (µg/ml) (ppb)
Standard of β-glucan	5.77	16,994.43	
Extracted β-glucan from *C. albicans*	5.78	52,643.29	131.3 µg/ml
p value	0.0735	0.0209 *	

Significance * (P<0.05)

stretches may be seen at 4,000–3,000 cm^{-1}, which indicates the peak 3,434 cm^{-1} for derived β-glucan and 3,424 cm^{-1} for conventional β-glucan. The derived spectral fingerprint is 2,928 cm^{-1}, but the commercial fingerprint is 2,919 cm^{-1}. Moreover, Mikkelsen *et al.* (2010) observed that the significant absorbance in 1,651 cm^{-1} for obtained β-glucans and 1,631 cm^{-1} for conventional β-glucans was caused by the stretched on the proteins' CN and NH categories, confirming the existence of amide linkages and protein in the sample. As a result, the peak in 1,074 cm^{-1} for the derived sample and 1,025 cm^{-1} for the conventional produced suggests the existence of glycosidic linkages and cycle monosaccharide architecture. The spectra revealed

peak absorbance in 987 cm^{-1} for the obtained β-glucans and 899 cm^{-1} for the conventional β-glucans, indicating the presence of β-glycosidic anomeric linkages. Bacha *et al.* (2017) discovered precise spectrum data relating to conventional and extracted β-glucan from yeast cells. Conventional (3,421.678 cm^{-1}) and extracted β-glucan (3,440.964 cm^{-1}) have demonstrated minimal proteins. Furthermore, the highest strength of the –OH team in isolated β-glucan is larger and wider than in conventional β-glucan. The stretching vibration of $–CH_2$ groups in isolated β-glucan has a broader range than the stretching vibration of $–CH_2$ groups in conventional β-glucan. In addition, the proteins (–NH) band in the extracted β-glucan (1,641.109 cm^{-1}; 1,558.14 cm^{-1}) have been displaced to less wave lengths compared to those in conventional β-glucan. It implies the separated specimens contain less protein, confirming the pure nature of the extracted β-glucan. Further crucially, the isolated β-glucan's spectrum absorption at 1,079.780 cm^{-1}, 1,045.339 cm^{-1}, and 923.879 cm^{-1} supports the existence of glycosidic linkages, suggesting that the β-glucan has not been disrupted.

The results reveal that the existence of C–O–C links in the spectrum of IR light around absorption (1,028.0 cm^{-1}) have typical characteristics of the β-glucan architecture stretched to the conventional (1,055.0 cm^{-1}), (Hozova *et al.,* 2007). The absorbing capacity (1,371.3 cm^{-1}) indicates the existence of C–H aromatic bends, whereas reference absorption (1,315.4 cm^{-1}) (Karreman *et al.,* 2006). The current work is comparable to Ibrahim *et al.* (2006) who discovered similar indicating that the FT-IR spectrum of the obtained β-glucans had the look of conventional β-glucans having an elevated level of clarity. Free hydroxyl groups and carboxyl groups, on the other hand, were taken in locations (2,927.7 cm^{-1}) and (2,922.0 cm^{-1}) identified in carbohydrates.

Boutros *et al.* (2022) discovered several characteristic beta glucan bands at wave numbers 890 (beta-linked polymer), 1,076 (C–O stretch), 1,372 (CHOH stretch), 2919 (C–H stretch), and 3,390 (OH stretch) in the samples. Hassan *et al.* (2014) showed that the IR spectrum at the absorbance of 1,041.5 cm^{-1} indicates the existence of C–O–C bonds which is a hallmark property of β-glucans architecture stretched to the conventional 1,051 cm^{-1}.

Another study performed by Salim *et al.* (2017) found this method was applied to assess the purity and quality of *Pleurotus eryngii* β-glucans as well as to demonstrate its similarity in structure to the conventional β-glucans. The HPLC test showed the existence of the extracted glucan as a prominent peak (2.087) of a liquid glucan. Such a peak displayed the same glucan standard retention time (2.193). Also, the HPLC examination of a liquid sample of β-glucan exhibited a single significant peak at 3.78, confirming the pure nature of the obtained β-glucans. The fact that this peak shared an identical persistence period as the β-glucans conventional indicates that the extraction process was successful as mentioned by Hassan *et al.* (2014).

The same peak (at 3.78) was recorded by Ibrahim (2014) when analyzing the β-glucans obtained from *Saccharomyces cerevisiae* by HPLC. According to Jameel and Yassein (2021), the extracted β-glucan's HPLC analysis result showed its structural resemblance to the β-glucan standard. The β-glucans obtained from *S. cerevisiae* and *C. albicans* displayed a large peak at 3.22, which represents the pureness of the β-glucans isolated from both fungi. This signal had a similar duration of retention as the conventional for β-glucan, according to the HPLC analysis. The most effective approach for identifying β-glucan was thought to be HPLC. On the other hand, Al-Aubydi and Abed (2011) demonstrated that all elements of β-glucans obtained from mushrooms were separated using the HPLC, which provided an effective approach for identifying the β-glucan.

Conclusion

The results of the current study indicated that it had been beneficial in extracting β-glucans from *C. albicans* and demonstrated this type of extraction, which depends on an acid-base technique, may produce large amounts of extracts. In comparison to the conventional, SEM, EDS/EDX, FT-IR spectroscopy, and HPLC analysis verified the obtained β-glucans from *C. albicans* were β-glucans. However, the current investigation, suggests that this kind of β-glucan may be utilized in studies in science.

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Authors' contribution

SHM: Isolation of *C. albicans*, obtaining and characterization of β-glucan, and carrying out the HPLC. SNY: Performing of SEM, quantitative EDS and FT-IR, and statistical analysis of data. The final copy of the manuscript was read and approved carefully by both authors.

Conflict of interest

All authors have no conflict of interest to disclose.

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Data availability

All data supporting the findings of this study are available within the manuscript.

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