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# Research Article
# Description of Isolated LAB Producing β-glucan from Egyptian Sources and Evaluation of its Therapeutic Effect

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## Abstract

**Background:** Lactic Acid Bacteria (LAB) have great therapeutic and nutritional benefits. Among these are: Improved nutritional value of food, control of intestinal infections, improved digestion of lactose, immune modulator effect, control of some types of cancer and control of serum glucose level. **Objective:** The aim of this study was carried out to evaluate the therapeutic effect of β-glucan extracted from LAB was isolated from different food sources in Egypt. **Materials and Methods:** The isolation was performed from local Egyptian boza, cider, cheese and yoghurt. Only seven species from 27 was identified as LAB. Only one showed to produce β-glucan and identified by VITEC® 2 as *Pediococcus parvulus*. The optimization of isolate growth was examined in different temperature, pH and media. **Results:** The study revealed that the optimum temperature was 37°C, pH was 6 and the most selective medium for growth was *Pediococcus* selective medium (PSM). Beta-glucan production was assessed by FT-IR analysis, HPLC and HNMR spectroscopy. The therapeutic effect of *Pediococcus parvulus* β-glucan as antioxidant against DPPH was more significant, cholesterol lowering effect of β-glucan gave valuable results compared to simvastatin drug, the antibacterial effect and control of cancer *in vitro* against Ehrlich Ascites Carcinoma (EAC) were also gave valuable results. **Conclusion:** The extracted β-glucan from Egyptian *Pediococcus parvulus* had different therapeutic effect *in vitro* including, antioxidative properties, antimicrobial, control of cancer and cholesterol lowering effect.

Key words: *Pediococcus parvulus*, lactic acid bacteria, biological activities, β-glucan production, PSM

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Beta-glucan has been reported to be associated with many health-promoting effect, (1, 3) β-glucans are found in bacteria and eukaryotic organisms. These polysaccharides include the linear glucans and 6-substituted (1, 3) β-glucans that have branch-on-branch or cyclic structures. The intake of oat β-glucan at daily doses of at least 3 g may reduce plasma total and Low Density Lipoprotein (LDL) cholesterol levels by 5-10% in normocholesterolemic or hypercholesterolemic subjects[1]. Beta-glucan also improves the glycemic index of meals and beneficially influences glucose metabolism in patients with type 2 diabetes or metabolic syndrome, as well as in healthy subjects. Furthermore, a blood-pressure-lowering effect of β-glucan in hypertensive subjects seems fairly well substantiated[2]. The β-glucans that exist as non-digestible polysaccharides derived from different food sources have demonstrated not only health promoting effects, but also the potential as a novel source of prebiotics[3]. Consumption of oat bread for 4 weeks, compared with wheat bread, increases serum NO level and brachial artery diameters but has no effects on FMD. These findings reveal the importance of fiber consumption and suggest more strategies to improve dietary fibers especially in hypercholesterolemic patients. Further studies are warranted in this regard[4] people fed β-glucan-enriched pasta for 2 months showed increased populations of beneficial bacteria in their intestinal tracts and reduced populations of non-beneficial bacteria. They also showed reduced LDL (bad) cholesterol[5]. Concerning prokaryotes, several bacteria including *Agrobacterium* and *Rhyzobium* species can produce these polymers one such product, β-glucan has been approved as a food additive by the Food and Drug Administration (FDA) and essentially is a linear (1, 3) β-glucan which may have few inter or intra chain (1, 6) linkages[6]. The β-glucan production is rarely found in LAB. It has only been reported to be synthesized and secreted by a small number of strains isolated from alcoholic beverages, namely: *Pediococcus parvulus* IOEB8801 and *Oenococcus oeni* IOEB0205 from wine and *P. parvulus* 2.6R, CUPV1, CUPV22, *L. diolivorans* G77 and *O. oeni* I4 from cider[7]. The potential health effects of β-glucan can decrease the level of saturated fats in the blood and may reduce the risk of heart disease and control of serum glucose level[8]. Some studies have suggested that cereal-derived β-glucan may also have immune modulator properties; β-glucans are further used in reduction of serum glucose level[9]. The β-glucan contributes to glycemic control, several factors were found to influence such an interaction, including dose, food form and molecular weight. Dose of β-glucan is important in the regulation of its effects. Beta-glucan induced a significant reduction in insulinemia in comparison to the control pasta without any apparent effect on glycemia[10]. Similarly, in healthy subjects the ingestion of 50 g rye bread, containing 5.4 g of glucan reduce postprandial insulinemic responses without parallel reduction in glucose responses as compared with the control bread[11]. The β-glucans were found to be strongly effective in modulating plasma lipid parameters. The ingested dose of oat β-glucan appears as a limiting factor, the US Food and Drug Administration and Health Canada have accepted 3 g as an effective daily intake of oat β-glucan to reduce serum LDL cholesterol[12].

The effects of soluble dietary fibers, including β-glucan on arterial blood pressure have been the least studied among the components of the metabolic syndrome, in one meta analysis, increased dietary fiber consumption provided a safe and acceptable means to reduce blood pressure in patients with hypertension[13].

Various mechanisms underlying the antihypertensive effects of soluble dietary fibers have been hypothesized. Insulin resistance is a major underlying mechanism contributing to the development of hypertension[14].

## MATERIALS AND METHODS

**Isolation of lactic acid bacteria:** Different local samples (boza, cheese, cider and yoghurt) were purchased from different area in Egypt. Each sample was aseptically weighed and homogenized by adding 225 mL of physiological saline solution. Further decimal dilutions were prepared from this homogenized mixture. It was spread-plated onto *Lactobacillus agar* acc. to DE MAN, ROGOSA and SHARPE (MRS) agar (Oxoid)[15]. De Man Rogosa Sharpe supplemented with 0.05% L-cysteine (Sigma) (MRS-C) was used to isolate Lactic Acid Bacteria (LAB).

The plates were placed in an anaerobic flask (Oxoid) in the presence of a gas generating kit (Anaerobic system BR0038B, Oxoid) and incubated at 30°C for 2 days. The colonies of bacteria having different appearances were randomly subcultured from MRS agar plated and plating on MRS agar to obtain purified isolates. All isolates were examined for Gram reaction, catalase and oxidase test and isolates were stored to further analysis.

**Staining with aniline blue:** Cell suspensions were prepared from slant agar cultures after incubation for 2 days by dilution with an 225 mL sterilized water and streaked on a solid

*Pediococcus* selective medium containing the dye. The medium was composed of 1% glucose, 0.005% dye, 2% agar and was adjusted to pH 7.2. When testing organisms produce acid, $CaCO_3$ (0.3%) was added to neutralize the medium during incubation, because a pH range of 5-7 is required for adequate staining with aniline blue. Staining of colonies with dye was observed by naked eye[16].

**Factors affecting growth:** Growth at different temperatures (5, 10, 15, 20, 25, 30, 35, 40 and 45°C), different pH values (2, 4, 6, 8 and 10) and carbohydrate fermentation tests were carried out by using the API 50 CHL kit according to the manufacturer's instruction (Biomerieux, France).

**Long term preservation of isolates:** Isolates showing homofermentative, Gram positive and catalase negative characteristics were preserved in MRS broth medium contained 20% (v/v) glycerol as frozen stocks at -80°C. Glycerol stock samples were prepared by mixing 0.5 mL of overnight cultures and 40% glycerol.

**Identification cultures by selective media:** The culture was examined microscopically by staining and morphological characteristics were noted. Cells were Gram stained by the method of Bergey's manual of determinative bacteriology[17]. The growth of isolate in different selective medium, MRS agar[10], nutrient agar[18] and *Pediococcus* selective medium (PSM)[19] was performed. Isolate suspension of 100 µL was spread on the plate of each medium and incubated under anaerobic conditions[20] at 37°C for 72 h. The colonies were isolated and examined in terms of morphology and Gram staining. The viable count of isolate in all media was also done and compared using colony count method.

**Identification of isolated bacteria:** The bacterial colony isolated by selective media (PSM) was identify by VITEC® 2 version 7.1 (A National Service of Egyptian Armed Forces Projects).

### Optimization of lactic acid bacteria growth

**Growth at different temperatures:** Fifty microliters of overnight cultures were transferred into MRS Brumocresol agar[21] optimum temperature assessed when the color of medium converted from purple to yellow. By using pour plate method incubated for 24 h at (15, 20, 25, 30, 35, 40 and 45°C). The optimum temperatures was detected by plate count method.

**pH optemization:** The pH of each supernatant obtained by centrifugation after incubation was determined by using pH meter (Corning pH/ion analyser 350). The optimum pH was detected by plate count method[22].

**Extraction and purification of EPS:** For extraction and purification of EPS, bacterial cells were removed from fermented media by centrifugation (16,000×g, 4°C for 30 min). The EPS present in the supernatant was precipitated by addition of two volumes of cold acetone and incubation overnight at 4°C[23].

After centrifugation at 14,000×g for 10 min at 4°C, the precipitate was washed 3 times with 70% acetone and sediment by centrifugation.

### Detection of extracted β-glucan

**FT-IR spectroscopy analysis:** The potassium bromide (KBr) pressed disc technique (approximately 1 mg of sample mixed with 200 mg of KBr) was used for preparing the samples. The FT-IR spectra were recorded on a Nicolet Nexus FT-IR spectrometer in the wavenumber range of 750-4000 $cm^{-1}$ at a resolution of 4 $cm^{-1}$. Twenty scans for each specimen were made[24].

**High Performance Liquid Chromatography (HPLC):** The samples and standard of β-glucan were analyzed by HPLC separation with column Luna 5 µ C18 (250×4.6) mm internal diameter (id). The mobile phase was acetonitrile (ACN) 100% with a flow rate[25] of 0.5 mL $min^{-1}$.

**High Nuclear Magnetic Resonance (NMR) detection of β-glucan:** Proton nuclear magnetic resonance (proton NMR, hydrogen-1 NMR or 1H NMR) is the application of nuclear magnetic resonance in NMR spectroscopy with respect to hydrogen-1 nuclei within the molecules of a substance, in order to determine the structure of its molecules. In samples where natural hydrogen (H) is used, practically all the hydrogen consists of the isotope 1H (hydrogen-1; i.e. having a proton for a nucleus). A full 1H atom is called protium. Simple NMR spectra are recorded in solution and solvent protons must not be allowed to interfere. Deuterated (deuterium = 2H, often symbolized as D) solvents especially for use in NMR are preferred, e.g., deuterated water ($D_2O$), deuterated acetone $(CD_3)_2CO$, deuterated methanol ($CD_3OD$), deuterated dimethyl sulfoxide $(CD_3)_2SO$ and deuterated chloroform ($CDCl_3$). However, a solvent without hydrogen, such as carbon tetrachloride, $CCl_4$ or carbon disulphide, $CS_2$ may also be used[26].

**Evaluation of biological actevities of β-glucan**

**Antioxidant activity by *in vitro* technique with 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay:** The DPPH (0.1 mM) was prepared by dissolving 1.9 mg from DPPH in 100 mL of absolute methanol, then keeping in the dark place for 1 h and 1.0 mL of this solution was added to 3.0 mL of extract solution in methanol at different concentrations. Thirty minutes later, the absorbance was measured at 517 nm. A blank was prepared by adding extract. Concentrations (1-18 μg $mL^{-1}$) were used as standard, lower absorbance of the reaction mixture indicates higher free radicals scavenging activity[27]. The scavenging ability was defined as follows:

$$\frac{1-\text{A517 (sample)}}{\text{A517 (blank)}}\times 100\%$$

**A blank was prepared without adding extract:** Concentrations (1-18 μg $mL^{-1}$) were used as standard, lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

**Cholesterol lowering effect of β-glucan:** Cholesterol binding assay: (a) Different concentrations (100, 200, 300, 400 and 500 μg mL) of β-glucan were prepared from stock of 10 mg of lyophilized cells per milliliter suspended in 1 mL of cholesterol-ethanol solution (100 μg of cholesterol dissolved in 1 mL of 60% ethanol), vortexed and incubated at 37°C for 1 h in a shaking water bath. The mixture was then centrifuged at 1118×g for 10 min and unbound cholesterol in the supernatant was determined by enzymatic analysis and the tests were carried out in triplicate. The same previous different concentrations from simvastatin standard (38956) which was purchased from sigma Aldrich were used. An enzymatic colorimetric kit was used for the determination cholesterol was obtained from Biodiagnostic Company Dokki, Giza, Egypt. The absorbance of the sample and standard against blank was measured at 517 nm[28]. The percentage of cholesterol lowering effect was determined by the equation:

$$\text{Cholesterol reduction (\%)} = \text{A517 standard-}\frac{\text{A517 sample}}{\text{A517 standard}}\times 100$$

***In vitro* assessment of antitumor activity of β-glucan:** The initial inoculums of Ehrlich Ascites Carcinoma (EAC) were purchased from the National Cancer Institute, Cairo University, Egypt. The EAC cells were propagated in NODCAR laboratories by weekly intraperitoneal injection of 0.2 of 1:5 $mL^{-1}$ saline solution of freshly drawn ascetic fluid ($0.2\times10^6$ EAC cells) from a donor mouse bearing 6-8 days old ascitic tumor, into three mice to ensure that the ascetic fluid will still propagate and can then be drawn from at least one life mouse. Transplantation was carried out using sterile disposable syringes under aseptic conditions. The tumor growth was as rapid as to killing the mice within 18-20 days due to the accumulation of ascetic fluid and rarely the tumor showed distal metastasis or spontaneous regression. After making appropriates dilution, the non-viability was tested for trypan blue exclusion method[29] as follows:

$$\frac{\text{No. of non-viable cells}}{\text{Total cells}}\times 100$$

**Antibacterial activity of β-glucan:** Antibacterial activity of β-glucan against tested *E. coli* bacteria in comparison with cefalaxin hydrate antibiotic was studied by agar well diffusion method according to Perez *et al.*[30] using 200 μL from 1 mg $mL^{-1}$ concentration of β-glucan for each well. After 24 h of incubation at 37°C, all plates were observed for zone of growth inhibition and the diameter of zones was measured in millimetres. All tests were performed in triplicate and the antibacterial activity was expressed as the mean of inhibition diameters (mm) produced.

**Media, strain and antimicrobial agents:** Nutrient agar medium (NA), antimicrobial cephalexin hydrate 22238 reference standards was purchased from sigma Aldrich Company. Reference *E. coli* 25922 strain was purchased from (ATTC) American type collective center was used.

**Statistical analysis:** Data obtained was subjected to analysis of variance and the means were compared using the Least Significant Differences (LSD) test at the 0.05 levels, as recommended by Snedecor and Cochran[31].

## RESULTS AND DISCUSSION

Table 1 shows that the bacteria existing in boza were two types of isolates; yoghurt produced one type and two types of both cider and cheese. These isolates were identified according to Bergey's manual of determinative bacteriology[17] and compared with strains identified by Hoda *et al.*[32]. These bacteria were *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus acidophilus* and *Lactobacillus fermentum*. One type of isolates had further identification by culture on selective media (PSM), staining with aniline blue and confirmed by VITEC® 2 version 7.1. According to chemicals and morphological characteristics for tested microbial isolate[17]. The isolate was identified as (*Pediococcus parvulus*).

Table 1: Bacterial isolates with different sources

| Sources | Identification |
|---|---|
| **Boza (B)** | |
| B1 | *Pediococcus parvulus* |
| B2 | *Lactobacillus casei* |
| B3 | Non identified |
| B4 | Non identified |
| B5 | Non identified |
| **Yoghurt (Y)** | |
| Y1 | Non identified |
| Y2 | Non identified |
| Y3 | *Lactobacillus casei* |
| Y4 | Non identified |
| Y5 | Non identified |
| Y6 | Non identified |
| **Cider (C)** | |
| C1 | Non identified |
| C2 | *Lactobacillus plantarum* |
| C3 | *Lactobacillus acidophilus* |
| C4 | Non identified |
| C5 | Non identified |
| **Cheese (Ch)** | |
| Ch1 | *Lactobacillus fermentum* |
| Ch2 | *Lactobacillus plantarum* |
| Ch3 | Non identified |
| Ch4 | Non identified |

Table 2: Effects of pH on the growth of *Pediococcus parvulus*

| pH | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|
| Colony unit | -ve | $10^1$ | $10^2$ | $10^3$ | $10^2$ | -ve | -ve |

Table 3: Effects of temperature on the growth of *Pediococcus parvulus*

| Temperature (°C) | 5 | 10 | 15 | 20 | 25 | 30 | 35 | 40 | 45 | 50 |
|---|---|---|---|---|---|---|---|---|---|---|
| Colony unite | -ve | $10^1$ | $10^2$ | $10^3$ | $10^4$ | $10^4$ | $10^5$ | $10^3$ | $10^2$ | -ve |

**Aniline blue:** Figure 1 clear that *Pediococcus parvulus* produce β-glucan by appearing blue color with aniline blue media[28].

**Effects of pH on the growth of *Pediococcus parvulus*:** Table 2 shows the possibility of the isolate to grow at different pH values. The growth observed at pH values between of 4.0 and 7.0 with optimum pH 6 indicated that the isolated bacteria preferred to grow in neutral environment[33].

**Effects of temperature on the growth of *Pediococcus parvulus*:** The effect of temperature on the growth of the isolate showed that the isolate produced maximum number of cells when cultivated at 37°C. The bacterial growth in PSM broth disappeared at temperature of more than 45°C. It was also observed that the growth of the isolate decreased at temperature below 35°C. The results showed that optimum temperature for maximum growth of *Pediococcus* was 35°C in PSM (Table 3). The bacterial growth in PSM medium declined at temperature more than 45°C. Also was observed that the growth decreased at temperature below 35°C[34]. Reported that lactic acid bacteria could grow even at 45°C with an optimum growth within 35-40°C and that is no growth at 10°C.

Fig. 1: Beta-glucan production by *Pediococcus parvulus*

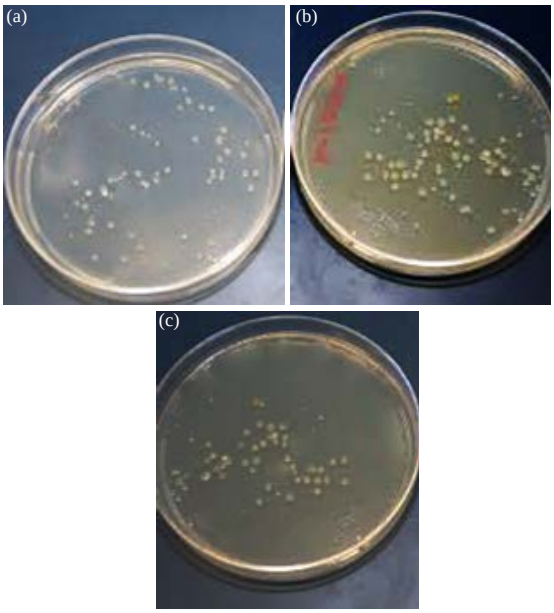


Fig. 2(a-c): *Pediococcus parvulus* colonies shape grow on agar medium (a) MRS medium, (b) PSM medium and (c) Nutrient agar

**Effect of different media on the *Pediococcus parvulus* growth:** The morphology and characteristics of the tested isolate in different selective growth media are presented in Fig. 2. The properties of the colonies were similar in all media except the color of the colony which was yellowish in nutrient agar. While was white in the other media. There were significant differences in the viable count between PSM agar

and other media (nutrient agar and MRS medium). The growth of isolate in PSM and other media appeared with number of colonies to be different in three tested media and the more selective medium for isolate is PSM (Fig. 3). Similar isolates facultative can grow in both aerobic and anaerobic conditions[35].

### Identifaction of β-glucan extracted from *Pediococcus parvulus*

**FT-IR analysis of β-glucan:** The FT-IR analysis in order to examine the structure of the EPS produced by *Pediococcus parvulus* was carried out, FT-IR analysis show as indicated in Table 4 that a carbohydrate structure characterized by a broad absorption band at the 3,485 $cm^{-1}$ region and it assigned the hydroxyl stretching vibration of the polysaccharide, indicating a strong O-H band. The two bands at 2,820 and 3,000 $cm^{-1}$ indicating a C-H stretching band assigned to the C-H asymmetric stretch and C-H symmetric stretch of $CH_2$ and $CH_3$ groups, respectively. The absorption at 1,370 $cm^{-1}$ also indicates CH band variation. In addition to the characteristic of carbon hydrogen absorptions, the attributions are also related to vibrations of the O=C-O structure (1,170 and 1,120 $cm^{-1}$) and strong C-O bending bands (at 1,250 and 0,970 $cm^{-1}$) by glycoside bond vibration of the EPS (attributed to asymmetrical and symmetrical stretching vibrations of the carboxylic group) (Fig. 4 and Table 4).

Table 4: Fourier transforms infrared spectroscopy (FT-IR) of β-glucan

| Range ($cm^{-1}$) | Assignment |
|---|---|
| 3000-3730 | O-H hydrogen-bond bridges |
| 2820-3000 | CH and $CH_2$ stretching vibration |
| 1395-1430 | C-O H bending vibration |
| 1360-1470 | $CH_2$ bending vibration |
| 1170-1120 | C-O-C asymmetric vibration |
| 970-1250 | C-O stretching vibration |

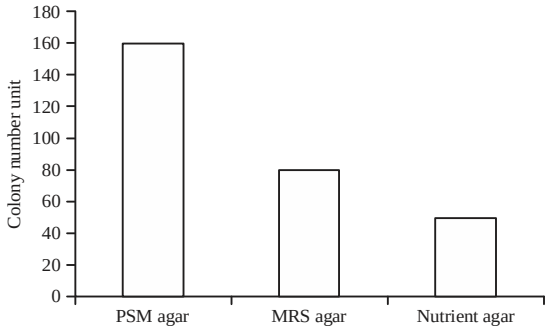


Fig. 3: *Pediococcus parvulus* growth in different media measured by number of colonies

**HPLC analysis of (1→3)(1→6)-β-D-glucan:** Figure 5b HPLC shows exopolysaccharide content extracted from *Pediococcus parvulus* containing β-glucan at retention time 9.044 with area (121.5104) and concentration 0.463 mg $L^{-1}$.

Figure 6 shows that, $^1H$ NMR ($CDCl_3$): d 7.17 (s, 1H), 7.05 (s, 1H), 6.92 (s, 1H), 5.39-5.5.13 (m, 4H), 4.92-4.26 (m, 10H), 4.13-3.41 (m, 13H), 3.28-3.04 (m, 6H), 2.54-1.95 (m, 7H), 1.88 (s, 1H), 1.21 (s, 3H), 1.54-1.01 (m, 3H), 8.83 (d, 2H, J = 8.0 Hz) according to FT-IR, HPLC and H NMR, this compound is β-glucan[32]. Some physicochemical and rheological properties of the exopolysaccharide (EPS) produced by *Pediococcus parvulus* 2.6 were examined. Structural characterization by NMR ((1) H and 2D-COSY) showed that the same EPS, a 2-substituted (1, 3)-β-D-glucan was synthesized irrespective of sugar source used for growth (glucose, fructose or maltose[23]. The molecular masses of these β-glucans were always very high (>10 (6) Da) and influenced by the culture medium or sugar source. The steady shear rheological experiments showed that all concentrations of the β-glucan aqueous solutions exhibited a pseudoplastic behavior at high shear rates. Viscoelastic behavior of β-glucan solutions was determined by dynamic oscillatory analysis. A critical concentration of 0.35% associated with the appearance of entanglements was calculated. The β-glucan adopts an ordered hydrogen bond dependent helical

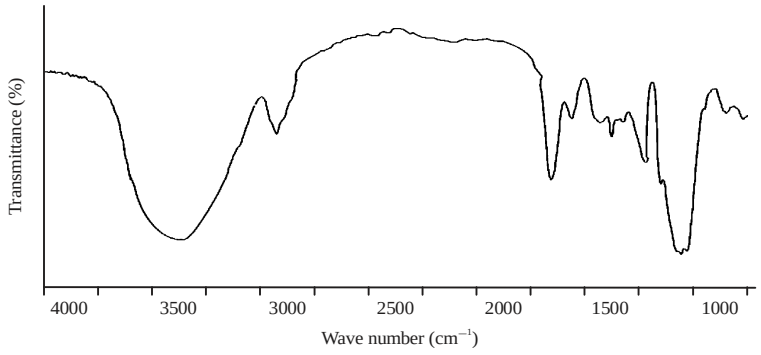


Fig. 4: Fourier transforms infrared spectroscopy (FT-IR) of β-glucan produced by *Pediococcus parvulus*

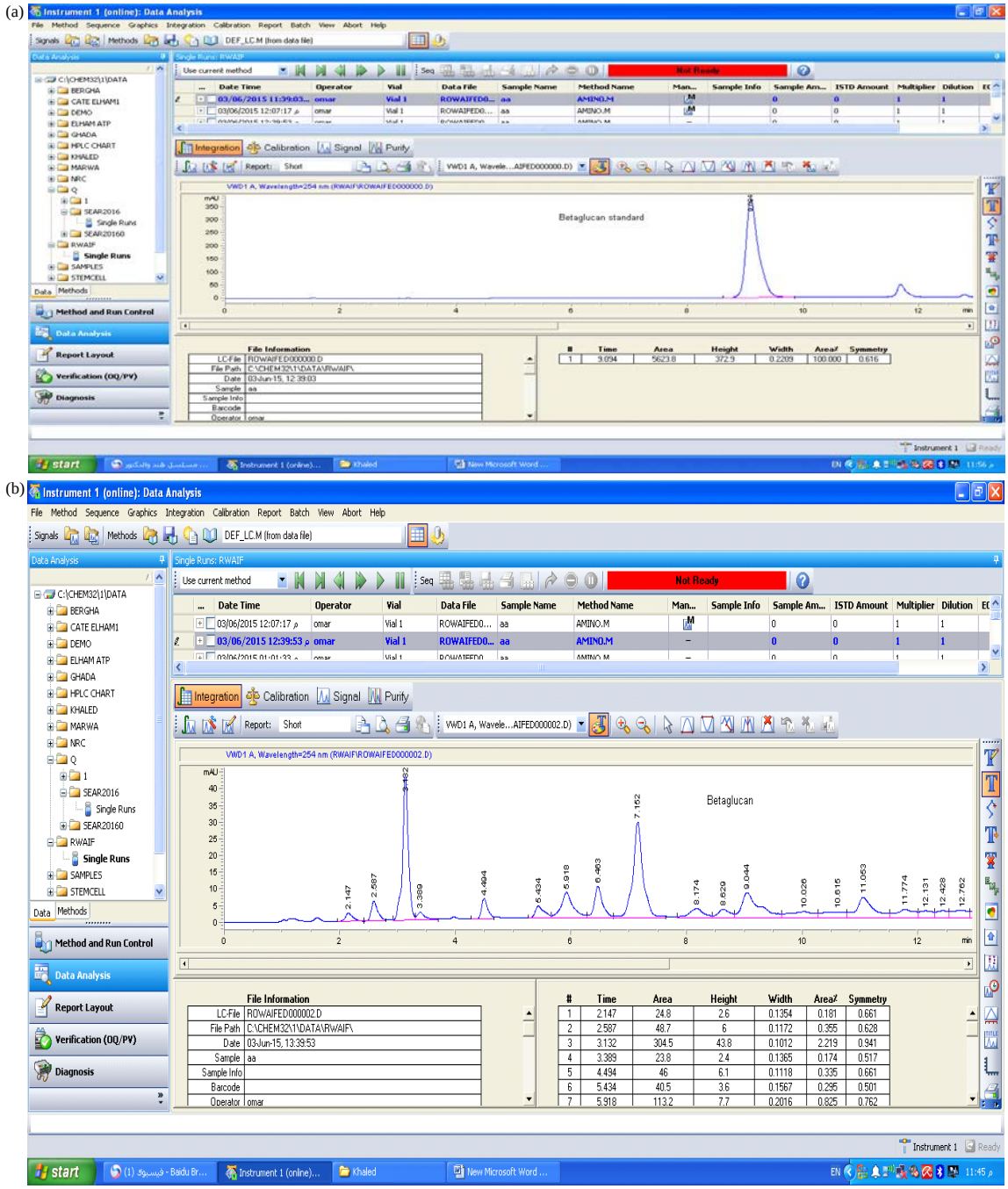


Fig. 5(a-b): (a) Standard β-glucan at retention time 9.024 with concentration 10 μg mL$^{-1}$ area (2811.6094) and (b) HPLC of exopolysaccharide extracted from *Pediococcus parvulus*

conformation in neutral and slightly alkaline aqueous solutions, which was partly denatured under more alkaline conditions[23].

## Evaluation of biological activities of β-glucan

**DPPH scavenging activity of β-glucan:** Figure 7 shows that the β-glucan extracted from *Pediococcus parvulus* isolate was reduced DPPH at 25 μg mL$^{-1}$ 38%, while at 100 μg mL$^{-1}$ the scavenging ability to DPPH stable free radical is 60%. The DPPH is a relatively stable organic radical, it has been widely used in the determination of antioxidant activities. The maximum antioxidant activity was 60% with 100 μg mL$^{-1}$. It is referred that the scavenging effect of different concentrations of β-glucan was more effective. Lin and

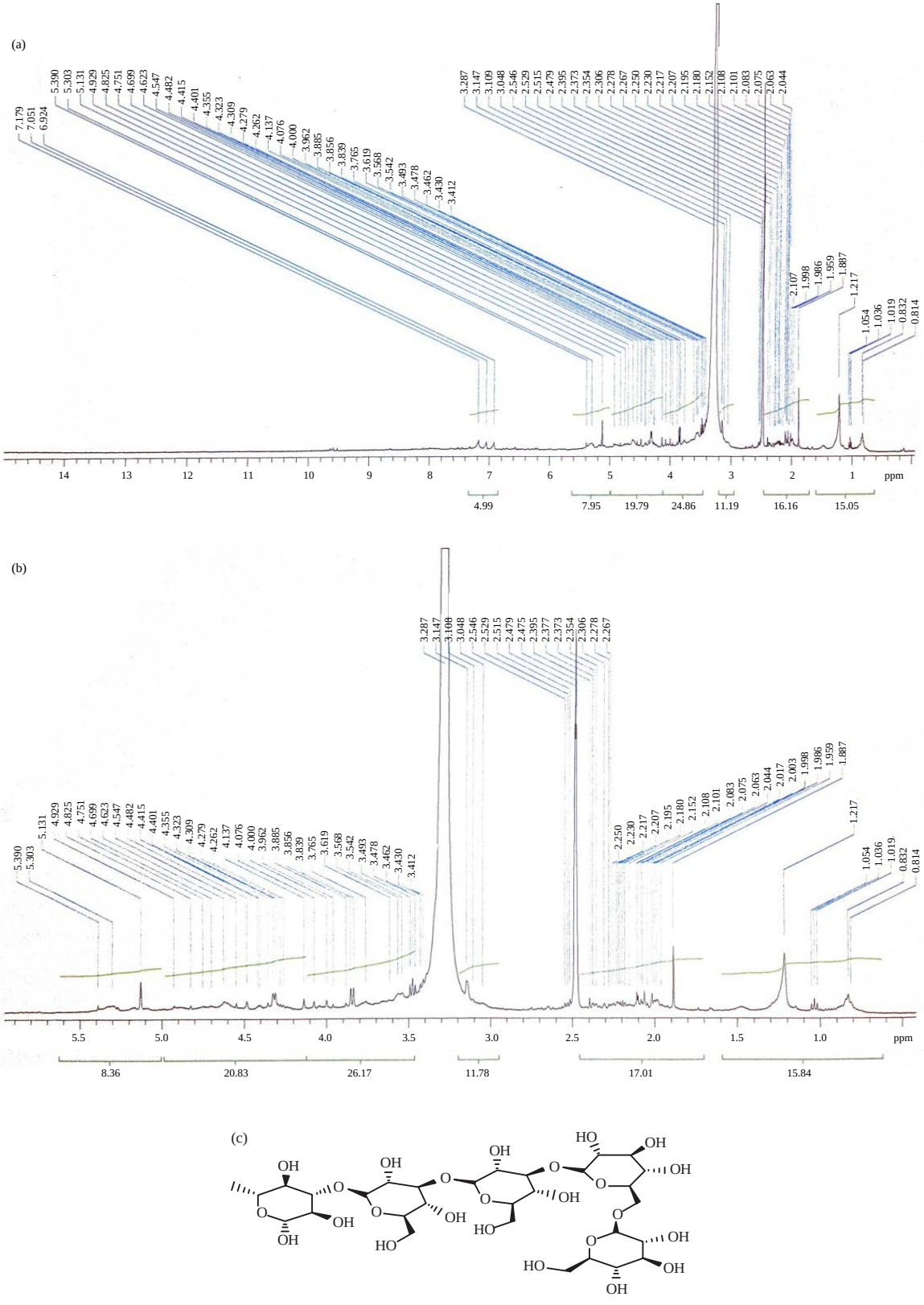


Fig. 6(a-c): (a and b) High nuclear magnetic resonance of β-glucan (H NMR) extracted from *Pediococcus parvulus* and (c) β (1-3)-(1-6)-D-glucane extracted from *Pediococcus parvulus*

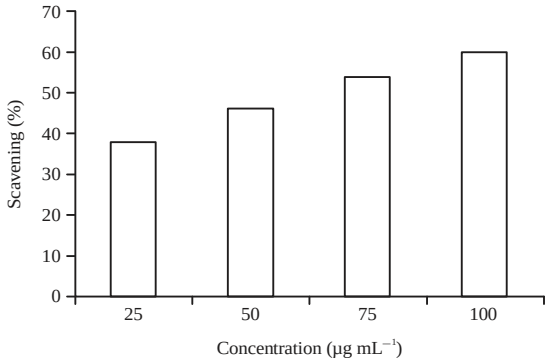


Fig. 7: DPPH scavenging potential of different concentrations of β-glucan extracted from *Pediococcus parvulus*

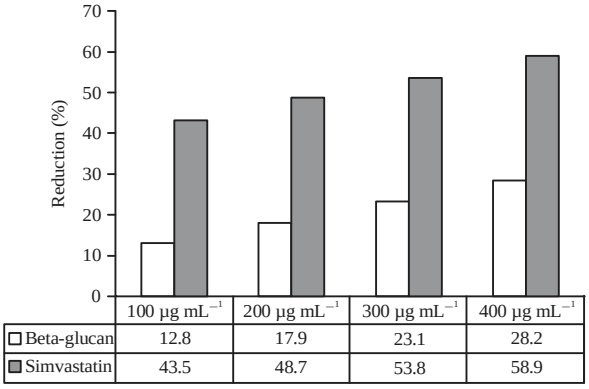


Fig. 8: Effect of β-glucan extracted from *Pediococcus parvulus* and Simvastatin in cholesterol lowering activity

Chang[36] mentioned that the radical scavenging ability of the lactic acid bacteria as intracellular extracts of *Bifidobacterium longum* and *Acidophilus* contribute good antioxidative effect on inhibiting linoleic acid peroxidation and scavenging the DPPH radical. *Lactobacillus acidophilus* isolated from infant faces of Egyptians infants showed having DPPH scavenging activities in case of cell lysate and intact cells[27].

**Effect of β-glucan extracted from *Pediococcus parvulus* and simvastatin in cholesterol lowering activity:** Figure 8 showed that the extracted β-glucan has cholesterol lowering activity at concentration 100 µg mL$^{-1}$ resulting 12.8 in comparison to simvastatin which showed 43.25% and the activity of β-glucan increased against cholesterol by using 400 µg mL$^{-1}$ in which the cholesterol lowering activities was 28.2%, while simvastatin at the same concentration showed 58.9%. The β-glucan possesses similar hypocholesterolemic property as other soluble dietary fibers[37]. However, the hypotriglyceridemic impacts of β-glucan have not been fully determined and warrant further investigation. Additionally, further studies need to be conducted in order to optimize β-glucan's hypolipidemic dose and to investigate the long-term effect of β-glucan supplementation on blood lipid chemistry. The eventual goal would be to combine β-glucan supplementation with other dietary means controlling blood lipids and to consequently prevent the need for cholesterol-lowering drugs in hyperlipidemic patients[37].

Table 5: Effect of different concentration of β-glucan on the viability of Ehrlich Ascites Carcinoma (EAC) cells

| Concentration (µg mL$^{-1}$) | 100 | 200 | 300 | 400 | 500 | 600 | 700 |
|---|---|---|---|---|---|---|---|
| Inhibition (%) | 5± | 15 | 25 | 50 | 55 | 75 | 85 |

Table 6: Antimicrobial activity of β-glucan

| Concentration (µg mL$^{-1}$) | Mean inhibition zone of cefalaxin (mm) | Mean inhibition zone of β-glucan (mm) |
|---|---|---|
| 200 | 33 | 24 |
| 100 | 25 | 20 |
| 50 | 21 | 16 |
| 25 | 17 | 13 |

Different concentrations of β-glucan (100, 200, 300, 400, 500, 600 and 700 µg mL$^{-1}$) were investigated. The effect on the viability of Ehrlich Ascites Carcinoma (EAC) cells was shown in Table 5. Results showed that different concentrations of β-glucan inhibited the proliferation of EAC cells *in vitro*. The concentration of β-glucan of 100 µg mL$^{-1}$ inhibited EAC by 5% while with 700 µg mL$^{-1}$ the inhibition percentage[38] was 85. The LAB and EPSs produced from *Lactobacillus* provided a beneficial physiological effects on human health, such as antitumor activity, immune modulating bioactivity and antimutagenicity[39]. The studied LA1 bacteria and their EPS may serve as an alternative for prolonged therapeutic option against cancer, without harmful side effects.

**Antimicrobial activity of β-glucan:** Table 6 shows the effect of β-glucan as antimicrobial activity of various tested concentrations against *E. coli* was divers depending on the antibiotic used and the concentration of β-glucan. In the case of cefalaxin antibiotic, the antibacterial activity of variuos concentrations was to inhibit *E. coli*. The β-glucan inhibit *E. coli* at concentration 200 µg mL$^{-1}$ with mean inhibition zone of 24 mm in comparison with cefalaxin 34 mm while at minimal concentration of 25 µg mL$^{-1}$ the inhibition zone was 13 and 17 for β-glucan and cefalaxin, respectively. From previous studies that the β-glucan extract obtained from *Chroococus turgidus* had substantial antibacterial and antifungal activities and that other extract could be used for further studies against these microorganisms[19].

## CONCLUSION

Beta-glucan extracted from *Pediococcus parvulus* was isolated from Egyptian boza and identified with VITEC® 2. β-glugan was identified by HPLC, FI-TR and $^{1}$HNMR. Beta-glugan had therapeutic effect against cholesterol, EAC cells, pathogenic bacteria and has scavenging ability to DPPH free radical.

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