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Antioxidant, anti-cholinesterase,anti-*α*-glucosidase and prebiotic properties of beta-glucan extracted from Algerian barley

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Abstract

Beta-glucan, such as barley-derived beta-glucan (BBG), are homopolysaccharides that have attracted attention by their nutritional and therapeutic properties. The aim of this study was to evaluate the antioxidant power of BBG extracted from local Algerian variety of barley (SAIDA 183), and its acetylcholinesterase, alpha glucosidase inhibitory activity as well as its prebiotic potential by fermentation with lactic acid bacteria isolated from camel's milk, namely lactococcuslactisssplactis (Lc.l.l) and leuconostocmesenteroidesspmesenteroides (Ln.m.m). The results revealed that BBG exhibited low activity against DPPH and ferric-reducing power (IC_{50} 4018.61 ± 656.69 and $A_{0.5}$ at 359.88 ±63.64 μg/mL respectively), in contrast to other antioxidant tests (ABTS, Beta-carotene and CUPRAC) where BBG demonstrated a moderate activity (IC_{50} 529.91 ±26.37, IC_{50} 161.013±13.322, $A_{0.5}$ 529.79 ± 48.65 μg/mL). The scavenging ability of hydroxyl radical and superoxide radical by BBG with an IC_{50} at 2268.38±101.57 μg/mL and IC_{50} 345.26± 62.32 μg/mL, respectively, while enzymatic inhibition by BBG exhibited for AChE at IC_{50} 859.164 ±64.46 μg/mL , BChE at IC_{50} at 725.470 ±30.95 , *α*-Amylase inhibitory activity at IC_{50} 2986.785 ± 37.046 . The bacterial growth of the two strains used in this study is favorably affected by the use of BBG as the only carbon source, in comparison with glucose as a control. In light of these findings, it can be concluded that BBG have shown moderate antioxidant and enzyme inhibitory activities and can be used as a prebiotic by acting synergistically with probiotics in functional food matrices.

Keywords: β Glucan; antioxidant; anti-cholinesterase; anti-*α*-glucosidase and prebiotic

Introduction

The polysaccharides non-cellulosic β-d-glucans occur as a principal component of the cell walls of bacteria, fungi, algae and cereals such as barley and oats (2.5 -11.3% and 2.2 - 7.8%, respectively). The primary structure of beta glucan consisting of β -d-glucopyranose units connected by (1 → 4) and (1 → 3) glycosidic bonds in case of cereals and (1 → 6) glycosidic bonds in case of fungal sources [1] . The physicochemical properties of β-glucans (BBG) differ depending on characteristicsof their primary structure, including linkage type, degree of branching, molecular weight, and conformation [2] . Various benefits of BBG in health have been approved. Including stimulate the immune system, modulating humoral and cellular immunity, growth inhibition of tumor cells and thereby have beneficial effect in fighting bacterial, viral, fungal and parasitic infections[1]. BBG have been demonstrated to reduce blood lipid levels, including cholesterol and triglyceride levels and control the postprandial glycaemia, it also has potential against different types of reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide and hydroxyl radicals who are closely involved in various human diseases, such as cerebral ischemia, diabetes, Alzheimer, inflammation, atherosclerosis and cancer, as well as aging processes [3] . Many reports show that BBG exhibit scavenging these free radicals in preventing oxidative damage in living organisms [4] . It was reported that the BBG possess neu-

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roprotective effect and improve spatial memory deficits in rat model [5] .

Antioxidant activity of these macromolecules is related to species, variety, environmental factors and several structural parameters (solubility, viscosity, molecular weight, composition, type of glycosidic linkages and degree of branching by side-chain functional groups [6] . Recent studies have shown that derivatives of BBG by carboxylmethylation, sulphation or phosphorylation as well as physical modifications such as γ-irradiation [7] have an effect on the antioxidant and biological properties of these macromolecules.

Several studies have demonstrated the beneficial effect of probiotics, prebiotics or the combination of both (synbiotic effect) in humans as well as in animals [8] . Long-chain carbohydrates such as BBG are not assimilated by the human digestive system, and will subsequently be fermented by the intestinal flora, generating fermentation metabolites such as Short Cain fatty acids (SCFA), which have a positive effect on human and animal health. [9] .

However, the antioxidant activity, anti- cholinesterase activity and prebiotic potential of Algerian barley β-glucan (SAIDA 183 cultivar) have not been extensively investigated. The objective of the present study was to investigate the in-vitro antioxidant effect of beta-glucan isolated from SAIDA barley cultivar, by testing its ability to scavenging different free radicals such as ABTS and DPPH and reactive oxygen species (ROS), such as hydroxyl radical and superoxide radical implicated in many pathological processes. Furthermore, inhibition of cholinesterase enzyme is implicated in various pharmacotherapeutical applications; its inhibition by BBG is also investigated in this work. Finally, the ability of two strains of lactic acid bacteria (2 strains) to grow in a medium containing BBG as a sole source of carbon is studied, in order to confirm the prebiotic potential of BBG.

Material and Methods

β-Glucan extraction

For this study, whole barley flour from the SAIDA 183 cultivar was used, provided by ITGC (Technical Institute of Great Cultures), Constantine, Algeria. The whole barley was milled to flour by a high-speed electric mill (Model MF 10.1, IKA), equipped with 0.50-mm screen. The β-Glucan was extracted from whole barley flour by a hot water extraction method according to [10] , 50 g of flour was suspended in 500 ml of water, adjusted to pH 7 with sodium carbonate (20%, w/v) followed by vigorous stirring for 30 minutes at 55°C. The mixture was centrifuged (refrigerated centrifuge 3-30 KS, Sigma-Aldrich, Germany) for 15 min at 15,000 x g at 4°C to remove the solids. To precipitate the proteins, the pH of the supernatant was adjusted to 4.5 with 2M HCl and then centrifuged again (20 min at 21,000 × g, 4°C). β-glucan was precipitated by adding an equal volume of absolute ethanol (99.9%) to the supernatant slowly with stirring. After 12 hours at 4°C, the precipitate was recovered by centrifugation (10 min at 3300 × g, 4°C). The pellet was resuspended in ethanol, and then air-dried in an oven (Memmert)at 25°C with forced air circulation for 90 min. The β-glucan extract was stored at 4°C.

Determination of β-glucan content

The gum was analyzed for β-glucan content using megazyme assay kit for mixed linkage β-glucan analysis (Megazyme International Ireland Ltd., Wicklow, Ireland) by hydrolysing with lichenase (1000 U/ml) and b-glucosidase (40 U/ml). β-glucan concentration is expressed as % (weight) of the total sample.

Determination of total phenolic concentration

The phenolic concentration of β-glucans extracted from barley was determined by the Folin–Ciocalteu's phenol reagent [11] . First, 250 μL of β-glucans or gallic acid standard were added to 1.25 mL of freshly made10-fold diluted Folin–Ciocalteu reagent (0.2N). After 05 min at room temperature, 1 mL of 7% (w/v) Na_2CO_3 solution was added and mixed. After incubation for 60 min, the absorbance was monitored at 750 nm versus a blank. Eight concentrations (10-80 μg/mL) of Gallic acid solutions in methanol were prepared and used as standard. The phenolic concentration was expressed as μg gallic acid equivalent (GAE)/mg of sample. The sample was analyzed in triplicate.

Determination of total flavonoid concentration

The amount of flavonoid was determined according to the colorimetric method described by O¨ ztu¨rk et al [12] . The β-glucan's flavonoid content was based on the complexation with Al^{3+}. 500

μL of β-glucans solution in methanol was added to test tubes containing 1.9 mL methanol, 50μL of potassium acetate (1M) and 50μL aluminum nitrate (10%). After incubation at room temperature for 40 min, the absorbance was determined at 415 nm. Quercetin was used as a standard at varying concentration (0-200 μg). The flavonoid content was expressed as μg quercetin equivalent/mg of sample. The sample was analyzed in triplicate.

DPPH free radical scavenging activity

The radical scavenging activity of β-glucans was determined using DPPH assay according to Shah et al with minor modifications [13] . The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517nm.The

reaction requires preparing a mixture of 40 μL of β-glucans at various concentrations (0.0625–4 mg/mL) with 160 μL DPPH solution (0.02% prepared in DMSO).For each concentration, a blank is prepared by mixing40 μL of samples with 160 DMSO. A control containing 40 μL DMSO and 160 μL DPPH is also prepared. Alpha-tocophe´rol (4mg/mL) was used as standard. Af- ter incubation for 30 min in the dark, the decrease in the absorp- tion of the DPPH solution was measured at 517nm using a 96- well microplate reader (Enspire Multimode plate reader, Perkin Elmer). Scavenging activity was expressed as IC_{50} (Concentra- tion in μg/mL of β-glucans that reduces the absorbance of DPPH by 50%). The anti-radical activity is determined using the for- mula:

$$DP\ P\ H\ radical\ scavenging\ activity(\%) = \frac{[A\ control\ \underline{A}\ sample]}{[A\ control]}\ x\ 100$$

ABTS free radical scavenging activity

The decolorization test of 2,2'-azinobis- (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) free radical cations was carried out using an improved method of O¨ ztu¨rk et al with slight modifications [12]. A stock solution of $ABTS^+$ was produced by the oxidation of ABTS 7.0 mM in water and 2.5 mM potassium persulfate for 16 h in the dark at room temperature, after that, the solution $ABTS^+$ was diluted with absolute ethanol to an absorbance of 0.8-0.9 at 734 nm before being used in the test. Then, 160 μLof $ABTS^+$ solution was added to 40 μL of β-glucans in DMSO at different concentrations. After 10 min, the absorbance was monitored at 734 nm by using a 96-well microplate reader. DMSO was used as a control, while BHA was used as standard. Results were expressed as IC_{50}.The capability to scavenge the $ABTS^+$ radical was calculated using the following equation:

$$ABTS\ radical-scavenging\ activity\ (\%) = \frac{[A\ control\ \underline{A}\ sample]}{[A\ control]}\ x\ 100$$

Antioxidant activity with the β-carotene bleaching method

The antioxidant activity of β-glucans was evaluated with the beta-carotene bleaching protocol according to the method of Ö ztu¨rket al ,with slight modifications [12]. 0.5 mg of β-carotene was solubilized in 1 mL of chloroform, then 25 µL of linoleic acid and 200 mg of Tween 40 were added. After, the chloroform in the mixture was evaporated; 50 mL of oxygenated-ultra pure water was added with vigorous stirring. To 40 μL of the sample at different concentrations was added 160 μL of β-carotene/linoleic acid mixture, followed by initial absorbance measured at 470 nm using a 96-well microplate reader. After incubation of the plate for 2 h at 50 °C., the absorbance was measured again. DMSO was used as a control. BHT was used as standard. Using the following equation, the anti-oxidantactivity of β-glucans was calculated in terms of percent inhibition:

Antioxidant activity % = [1 – (As $_{t=0}$ – As $_{t=120}$/Ac$_{t=0}$ – Ac$_{t=120}$)] x 100

Where As$_{t=0}$: absorbance of the sample at t = 0, As $_{t=120}$: absorbance of the sample at t = 2h, Ac $_{t=0}$: absorbance of control at t = 0, Ac $_{t=120}$: Absorbance of control at t = 2h.

Cupric reducing antioxidant capacity (CUPRAC)

For measuring of reducing antioxidant capacity of β-glucans, the cupric ions (Cu2+) method was used according to Özyürek el al with slight modification [14] . The solutions of 10 mM $CuCl_2.2H_2O$ and 1 M NH_4CH_3COO were prepared in bidistilled water, while the Neocuproine solution at 7.5 mM concentration was prepared in ethanol 96%. 40μL of β-glucans solution at different concentrations was added to the reaction mixture consisting of 60μL Cu(II), 50μL Nc and 50μL NH_4CH_3COO solutions. Then, the reaction mixture was let to stand for 30 minutes to achieve equilibrium. Absorbance was measured of the resulting cuprous-neocuproine complex at 450 nm using a 96-well microplate reader against a reagent blank.The extract concentration providing 0.5 of absorbance (EC50) was calculated from the graph of absorbance at 450 nm against β-glucans concentration. BHA and BHT were used as standard.

Ferric- reducing antioxidant power (FRAP)

The ability of to reduce Fe^{3+} was estimated by the method described by Shah et al [7], which is based on the capacity of molecules to convert the oxidation form of iron Fe^{3+} to its reduced state Fe^{2+}.10 μL of β-glucans solution at varying concentrations was mixed with 40μL of 100 mM potassium phosphate buffer (pH 6.6) and 50μL of 1% w/v potassium ferricyanide. The mixture was incubated at 50°C for 20min, the reaction was terminated by adding 50μL of trichloroacetic acid (10% w/v), 40μL of water and 10μL of 0.1% w/v FeCl3 were then added. The absorbance was then measured at 700nm using a 96-well microplate reader. 1mg/mL of EDTA was used as standard. The intensity of the absorbance is proportional to the reducing power of the reaction mixture. The extract concentration providing 0.5 of absorbance (EC_{50}) was calculated from the graph of absorbance at 700 nm against β-glucans concentration.

Scavenging of superoxide radical by alkaline DMSO method

The superoxide radical scavenging ability was measured in terms of inhibition of generation of $O2^-$ using alkaline DMSO according to Sanchez-Moreno with modifications [15] . Method adapted to 96-well microplates. The reaction mixture consisted of 40 μL of β-glucans at varying concentrations (0-4mg/mL in

DMSO), 130 μL of alkaline DMSO (1 mL DMSO containing, 5 mM NaOH in 0.1 mL water) and 30 μL NBT (1 mg/mL in DMSO). The mixture was incubated at 25 C for 5 min, and absorbance was measured at 560 nm using a 96-well microplate reader. Ascorbic acid was used as positive control. The scavenging activity is determined using the equation:

$$Scavenging\ activity = \frac{[A\ sample - A\ control]}{[A\ sample]} x\ 100$$

Hydroxyl radical scavenging activity

Scavenging activity of hydroxyl radical of the extract was measured according to the method of Rajamanikandan et al [16] .200 μL of the final reaction solution consisted of aliquots (80 μL) of various concentrations of β-glucans, 24μL$FeSO_4$ (8mmol/L), 20 μL of H_2O_2 (20mmol/L) and 80 μL sodium salicylate (3mmol/L). The reaction mixture was incubated for 1 h at 37 C. L-Ascorbic acid was used as the standard. The color development was measured at 560 nm using a 96-well microplate reader against a blank. The scavenging activity is determined using the formula:

$$Scavenging\ activity = \frac{[A\ control - A\ sample]}{[A\ control]} x\ 100$$

Determination of anticholinesterase activity

The enzymatic assay for Acetylcholinesterase (AChE) and butyrylcholinesterase activity was performed utilizing a slightly modifying spectrophotometric method by Ellman et al [17] .In this study,AChE from electric eel and BChE from horse serum were used, while acetylthiocholine iodide (ACHI) and butyrylthiocholine chloride BCHC) were employed as substrates of the reaction. Briefly, 110 μL of 0.1 M sodium phosphate buffer (pH 8.0), 20μL of AChE (or BuChE) solution and 50 μL of β-glucans solution at different concentrations (1000 μg-62.5 μg) were added in a 96-well microplate. After an incubation period of 15mn at 25˚C, the reaction was then initiated by the addition of 10μL of DTNB and 10 μL of substrate (ACHI or BCHC). The amount of DMSO in the mixture does not exceed 1.20%. The formation of the yellow 5-thio-2-nitrobenzoate anion as a result of the reaction was monitored spectrophotometrically at 412 nm using a 96-well microplate reader against a blank. Galanthamine was used as positive control. The results were given as IC50 value (μg/mL) corresponding to the concentration shows 50% inhibition. Percent AChE enzyme inhibition was calculated using following formula:

$$\%\ Inhibition = \frac{[A\ control - A\ sample]}{[A\ control]} x\ 100$$

In-vitro Yeast α-Glucosidase Inhibition Assay

Yeast α-glucosidase enzyme inhibition assays using p-Nitrophenyl-α-d-glucopyranoside (p-PNP) as substrate were carried out on 96-well microplates in accordance with the method described by Zhang et al ,with minor modifications [18] . Briefly, the reaction mixture contains:α-glucosidase (15 μL, 1.0 unit/mL in 100 mM phosphate buffer, pH 6.8) mixed with different concentrations of BBG (7mg/mL – 0.219 mg/mL) and 40 μl of phosphate buffer in a 96-well plate. After incubation at 37 ˚C for 10 min, 45 μL of p-NPG solution (2mM in 0.1 mM phosphate buffer, pH 6.8) was added to start the reaction. Acarbose was used as standard (7 mg/mL – 0.219 mg/mL). Enzymatic activity was monitored by measuring the p-nitrophenol released from PNP-glycoside at 405 nm every 45 sec for 3 min. The control samples were prepared without BBG. Results were expressed as a concentration of BBG resulting in 50% inhibition of enzyme activity (IC_{50}). The inhibition percentage was calculated according to the following formula:

$$\%\ Inhibition = \frac{[A\ control - A\ sample]}{[A\ control]} x\ 100$$

In-vitro prebiotic potential of BBG

The growth simulation of probiotic bacteria, namely lactococcuslactisssplactis and LeuconostocMesenteroidesSspMesenteroides, in presence of BBG or glucose as sole source of carbon were determined according to modified method of Das et al [19] .The overnight grown probiotic bacterial culture (˜1.5 x 10^8 CFU/mL) was inoculated separately to the 10 ml MRS broth, containing 1% (w/v) of glucose (as positive control) or 1% (w/v) BBG and incubated at 37 ˚C under anaerobic conditions for 24 h. The growth of the bacterial culture was classified as CFU/ml, by counting colonies, which appeared on spreading 100 μL of culture after 24 h of incubation on MRS agar plate, and the plates were incubated at 37 ˚C for 18 h. The prebiotic activity assay was expressed as total counts of probiotic bacteria (log 10 CFU/mL).

Statistical analysis

All experiments were performed in triplicate (n = 3) and data are presented as mean ± SD. The concentration giving 50% inhibition (IC50) was calculated with microsoft excel 2013 and kaleida graph version. The dose–response curve was obtained by plotting the percentage inhibition versus concentration. Statistical significance was determined by one-way analysis of variance (ANOVA) using XLSTAT. Significant differences between means were determined by Turkey method and differences at the level $P<0.05$were considered to be significant.

Results & Discussion

Extraction and recovery of β-glucan

Yield of gum product represent the weight of gum obtained from 100 g of whole barley flour. In this experiment, yield of gum

was 4.99 ± 0.21 %. This pelletdid not represent the whole quantity of β-glucan isolated (In addition to β-glucan, this gum also contained someimpurities such asfat, protein, starch and minerals). Todetermine the efficiency of extraction method, β-glucan recovery (wt. of β-glucan in gum product/wt. of β-glucan in 50g flour) should be calculated. The percentage of recovery was identified and it was found to be of 79.29± 2.48%. The extraction of the BBG was carried out at a pH of 7 and a temperature of 55 °C. Several studies have shown that the yield of the extraction is strongly linked to the extraction conditions (extraction solvent, T° and pH) [10] found that there was an increase with T° in the percentage of β -glucan recovered at pH 7.0 and 8.0 and decreased at higher pH (9-10). The extraction procedures used by [20] are characterized by the addition of a refluxing step with ethanol at 80%. Highest recovery (83.1%) was obtained in hot water extraction method followed by 81.4% and 80.4% in enzymatic and acidic extraction process. Least recovery (78.1%) was obtained in alkaline extraction process.

Determination of total phenolics and flavonoid contents

Phenolic compounds may contribute directly to antioxidative action. They are also very important plant constituents because their hydroxyl groups confer scavenging ability. The total phenolics content of BBG was determined by Folin–Ciocalteu assay and expressed in terms of microgram of gallic acid equivalent GAE/mg. Using the standard curve ofgallic acid (R^2 =0,7152). The total phenolic content of BBG was 76.47 ± 2.12 μg GAE/mg. Thondre et al revealed that the extraction of phenolic compounds in barley β-glucan with 70% methanol gave better yield than 70% ethanol [21] .The flavonoid content determined by the aluminum nitrate method for BBG was reported in μg equivalent quercetin /mg(R^2 = 0.9867). The results show that BBG have flavonoid content (47.06 ±2.12 μg QE/mg) (Table 1). Based on these data, it can be deduced that the flavonoids represent 61.54% of the total phenols in the methanol extract. The study achieved by Keddari et al revealed that the amounts of the flavonoids in soluble dietary fiber and insoluble dietary fiber extracts from durum wheat bran were 0.52 ± 0.012 and 0.46 ± 0.119 mg EQ/g, respectively [22] .The content of polyphenol and flavonoids in cereals is largely affected by species, variety, environmental factors and extraction methods.

Antioxidant activity

Evaluation of the antioxidant power of the various extracts is carried out by the DPPH test, which is considered to be a relatively stable free radical. The discoloration of DPPH is directly proportional to the ability of bioactive molecules to reduce it. The DPPH scavenging activity of BBG at different concentrations and alpha-tocopherol as standard are represented in Table 2.From these results, it can be seen that the BBG showed 50% inhibition IC_{50} at a concentration of 4018.16 ±656.59 μg/mL. The levels of inhibition of DPPH recorded in the presence of the various concentrations of BBG are lower than observed with standard BHA, BHT and α-Tocopherol (6.14±0.41, 12.99±0.41&13.02±5.17 respectively). Shah et al reported that the β-glucan from oat and barley at a concentration of 20 mg/mL produced 20.96 and 15.22 % scavenging effect which increased up to 52.23 and 47.31 % at 100 mg/mL respectively but were less in comparison to that of the standard α-tocopherol (69.28%), at the same concentration [13].

According to Kozarski et al , carbohydrates have a low antioxidant power, due in large part to the presence of hydrogen in their structure. In the case of carbohydrate polymers, where the monosaccharides are linked together by glycosidic linkages, this activity increases (multiplier effect), but it is still relatively low [23] . Wang et al reported that the scavenging effect is much more effective while the hydroxyl groups in polysaccharide are substituted by carboxymethyl or sulfate groups [6]. It illustrates that the present of sulfate groups and carboxymethylgroups acting on β-glucan can enhance the antioxidant capability comparing with any compounds with single sulfate or carboxymethyl groups.

The ABTS assay is based on the generation of a blue/green $ABTS^+$ that can be reduced by antioxidants and the decolorization reflects the capacity of an antioxidant to donate electrons or hydrogen atoms to deactivate the radical species. The results shown in Table 2 indicate that BBG demonstrate high ABTS+ scavenging effects. The IC_{50} value of the BBG was found to be 529.1 ± 26.37 when compared with standard BHA and BHT (IC 50 1.81±0.10&1.29±0.30 respectively). Khan et al reported an increase in the ABTS scavenging activity with the increase in the concentration of irradiated β-glucan and the degree of irradiation [24] .

The antioxidant potential of a plant extract can be evaluated by determining the ability to inhibit β-carotene oxidation. Ta- ble 2 shows the total antioxidant activity of the extracts of BBG compared with BHT and BHA as standards. Total antioxidant activity increased with increasing amount of BBG in the reaction. All the concentrations tested of BBG showed lower antioxidant activity than BHTand BHA. The BBG showed 50% inhibition at a concentration of 161,01±13,32 μg/mL, whereas standard BHA and BHT with IC50 value of 1.05±0.03 and 0.91±0.01 respectively. These results are similar to those observed by Nandi et al with an inhibition of 68 % at 400 μg/mL and IC50 at concentration of 180μg/mL [25]. The β-carotene-linoleic acid method is important to understand the type of H-donor antioxidant and the level of inhibition of lipid peroxida-

tion [26]. This method is also important to understand the antioxidants responsible for singlet oxygen scavenging causing radicals in lipids.

The Antioxidant Copper Reduction Capacity Test (CUPRAC) is a method of measuring the antioxidant potential used for a wide variety of polyphenols, including acids Phenolics, hydroxycinnamic acids, flavonoids, carotenoids, anthocyanins, as well as thiols and synthetic antioxidants, vitamins C and E[14]. The cupric reducing antioxidant capacity of the BBG is shown in Table 2,with the increase in concentration, there is an increase in the absorbance of the samples thus, increased percentage reduction. The reducing antioxidant capacity of BBG was much weaker than those of BHA and BHT. The BBG concentration corresponds to the absorbance 0.5 was 529.79 ± 48.65 μg/mL, whereas absorbance 0.5 of BHA and BHT was 5,35±0.71 & 8.97±3.94 respectively. Haghshenas et al reported that β-glucan addition in shrimp nugget formulation exhibited increasing significantly ($p < 0.05$) antioxidant activity [27]. TEACCUPRAC value of free phenolic extract of the sample with β-glucan and control sample were 3.12 and 1.02 mmoltrolox/g sample respectively.

The antioxidant activity of the BBG was evaluated using

the FRAP method. The results are shown in Table 2. The reducing power capacity increased with the amount of BBG in the mixture.The BBG concentration which gives the absorbance was359.88 ±63.64 μg/mL. These results were weakly compared to the standards used in this study(ascorbic acid, alpha-tocopherol and tannic acid), which showed concentrations corresponding to 0.5 absorbance equal to 6.77±1.15, 34.93 and 5.39±0.91 μg/mL respectively. Several previous studies have proven the reducing power of β-glucan from different sources, Nandi et al reported that the *B*-glucans from from edible mushroom Russulaalbonigra, at concentration of 500 μg/ml showed reducing power of 0.5 [25]. This result suggests that BBG has power to provide electron to reactive free radicals, converting them into more stable non-reactive molecules

Superoxide anion ($O2^{l}$) is one of the six major reactive oxygen species (ROS) causing oxidative damage to cellular components as it is the precursor of several reactive species[3, 26]. Therefore, superoxide radical scavenging assay is very important to reveal potential antioxidant property. In this assay the concentration of superoxide concentration is proportional to the color intensity at 560 nm. Table 2 shows the superoxide radical scavenging activity of different concentrations of BBG. The BBG showed a dose-response relationship. The IC 50 value of BBG was found to be a 345.26± 62.32 μg/mL; the IC 50 value of alpha tocopherol and tannic acid are 31.52±2.22 & 7.59±1.16 respectively. In a study published by Wang et al. carried out on three derivatives of β-glucan. The results show that the scavenging of the radical superoxide increased with the concentration increasing and can reached 69.68 % for thecarboxylmethyl (1- 3)-β-d-glucan sulfatederivative at a concentration of 1600 μg/mL [6].

The hydroxyl assay shows the capacities of BBG to inhibit hydroxyl radical. In our protocol, Hydroxyl radicals were generated by Fenton reaction (Fe^{2+}–ascorbate–H2O2 system). The BBG exhibited obvious scavenging activity on hydroxyl radical in aconcentration-dependent manner. The IC50 value of the BBG was determined 2268,38±101.57μg/mL.The β-glucan extracted from edible mushroom Entolomalividoalbum studied by Maity et al exhibited an IC_{50} for the Hydroxyl radical scavenging ability at 400 μg/mL [28] . Kofuji et al reported that pectin from apple or citrus, chitosan, and xanthangum which are used as food additives showed hydroxyl radical scavenging activity inferior to that of β-glucan extracted from barley [29].

Table 1 Total Phenolic and Flavonoid content in BBG

Compound	Total phenolic content μg GAE/mg dried extract	Total Flavonoid Content μg QE/mg dried extract
β-Glucan	76.47±2.10	47.06±2.12

A IC50 and A0.50 values expressed are means ± SD of three parallel measurements ($p < 0.05$). B Reference compounds: BHA: butylated hydroxyanisole; BHT: butylated hydroxytoluene.

Determination of anticholinesterase activity

Several pathogenic pathways are implicated in the progression of Alzheimer's disease (AD) such as cholinergic deficit and oxidative stress. The brain of AD patients presents several signs of free radical attacks such lipid peroxidation, protein oxidation, damage to mitochondrial and nuclear DNA [30] . However, the antioxidants may reduce the Alzheimer's disease progression and minimize the neuronal degeneration by inhibition of the cholinesterase enzymes [31] . The BBG was further evaluated for its AChE and BChE inhibitory activities using the modified Ellman's colorimetric method in a 96-well microplate. Galantamine was used as the standard. In comparison with the standard, BBG showed a low activity compared to the standard. BBG showed IC_{50} 859.164 ±64.46 μg/ml compared to galantamine (6.27±1.15 μg/mL) (Table 3). This finding is in accordance with the previous reported by Haider et al [5]. In this study, β-glucan extracted from Saccharomyces cerevisiae exhibited dose dependent inhibition of AChE enzyme, at initial concentration of 20 mg/ml, β-glucan showed a percentage inhibition at 70%. The

Table 2 Antioxidant activity of BBG

Com-pound	DPPH assay (IC 50 μg/mL[a])	ABTS assay (TEAC value) (IC 50 μg/mL[a])	β-carotene bleaching assay (IC 50 μg/mL[a])	Cupric assay $A_{0.5}$ μg/mL[a]	FRAP assay $A_{0.5}$ μg/mL[a]	DMSO Alkaline assay (IC 50 μg/mL[a])	Hydroxy Scavenging activity IC50 μg/ml[a]
β-Glucan	4018.61 ±656.69	529.91 ±26.37	161.013 ±13.322	529.79 ± 48.65	359.88 ±63.64	345.26± 62.32	2268.38±101.57
BHA[b]	6.14±0.41	1.81±0.10	1.05±0.03	5,35±0.71	_	_	_
BHT[b]	12.99±0.41	1.29±0.30	0.91±0.01	8.97±3.94	_	_	_
Alpha Tocopherol[b]	13.02±5.17	_	_	_	34.93±2.38	31.52±2.22	_
Ascor-bicacid	_	_	_	_	6.77±1.15	7.59±1.16	_
Tanni-cacid	_	_	_	_	5.39±0.91	_	_

IC_{50} value of β-glucan was found to be 680 ± 80μg/mL. The BChE inhibitory activity of the BBG was increased with increasing concentration. Galantaine as positive control, showed the best percent inhibition at all concentrations and at different times. IC_{50} value of BBG was 725,470 ± 30.95μg/ml, while that of galantamine was 34.75±1.99μg/mL (Table 3). These results show that BBG could interact with both enzymes with different degrees of inhibition. It has been previously found that the BChE is an enzyme that can accept a wide range of substrates over ACHE, because of its low substrate specificity [32]. The active site of the AChE is composed of several phenylalanine residues which makes the catalytic pocket very narrow and therefore does not accept larger substrates, and would be preferable for the small substrates. In contrast, the BCHE which has a catalytic site rich in small amino acids residues (valine and leucine), making it able to hydrolyze larger substrates even in excess. This property of the two enzymes gives the BCHE the ability to cleave various esters, including those with larger acyl fragments, while AChE is an esterase with more specificity for ACH due to its structure but has a low ability to remove esters with larger acyl fragments. Consequently, in this experiment, the strong inhibition of BChE compared to that of AChE by BBG could be explained by the structural difference of their active site.

In-vitro Yeast α-Glucosidase Inhibition Assay

Alpha-glucosidase is a key enzyme involved in carbohydrate metabolism in the gut that represents one of the therapeutic approaches to fighting type 2 diabetes. In this study, we tested the ability of BBG to inhibit α-glucosidase activity in-vitro, and consequently a possible antidiabetic effect. The inhibitory effect of BBG against α-glucosidase is shown in Table 3. The BBG showed inhibition effect to α-glucosidase activity at dose-dependent manner. However, IC50 value of BBG was 2986.79 ± 37.05 μg/mL and that of acarbose was 115.22 ±10.91 μg /mL. These finding showed that BBG has a low inhibitory activity compared to acarbose. Dong et al,found that the activity of three intestinal disaccharidases (sucrase, lactase and maltase) is inhibited by oat β-(1,3)(1,4)-glucans in a dose-dependent manner in-vivo and in-vitro [33] . Brockman et al have demonstrated the effect of Barley beta glucan on postprandial blood glucose; this effect could be explained by its possible effect on the activity of intestinal α-glucosidase in addition to its effect on glucose absorption in the intestine [34].

Table 3 Enzyme inhibition activity by BBG

Compound	AChE (IC50 µg/mL[a])	BChE (IC50 µg/mL[a])	α-Amylase inhibitory activities (IC50 µg/mL[a])
B Glucan	859.164 ±64.46	725.470 ±30.95	2986.785 ± 37.046
Galantamine[b]	6.27±1.15	34.75±1.99	-
Acarbose	-	-	115.221±10.911

a IC50 values expressed are means ± SD of three parallel measurements ($p < 0.05$). b Reference compounds: Galantamine and Acarbose

In-vitro prebiotic potential of BBG

Prebiotics are mostly fibers that are non-digestible food ingredients and beneficially affect the host's health, by selectively stimulate the beneficial microbiota, and potentially suppress the harmful bacteria dominant [35]. There are several in-vitro tests used to screen and classify a food ingredient as a prebiotic, such as; 1) Resistant to the actions of acids in the stomach, bile salts and other hydrolyzing enzymes in the intestine, 2) Should not be absorbed in the upper gastrointestinal tract, 3) Be easily fermentable by the beneficial intestinal microflora and 4) stimulating the growth and/or activity of some genera of microorganisms in the colon that promote

gut health [35]. In-vitro anaerobic fermentation of BBG by two lactic acid bacteria, namely lactococcuslactisssplactis and Leuconostocmesenteroidessspmesenteroides are shown in table 4. The growth of these lactic acid bacteria in presence of BBG and glucose were comparable at 37˚C after 12 and 24 h incubation (p>0.05). The results showed that the β-glucan extract from barley stimulated the growth of lactococcuslactisssplactis and Leuconostocmesenteroidessspmesenteroides, by increasing its number from 08.14±0.024 to 10.45±0.009 and 7.99±0.021 to 10.21±0.021 log10 CFU/ml, respectively. It has been reported that the end products of fermentation, the SCFAs butyrate, acetate, and propionate, are considered to benefit the gut in many different ways. SCFAs production in the intestinal tract will lower the colonic pH, which limits growth of pathogens. These SCFA also supplied energy for colonic epithelium and modulation of cholesterol and lipid metabolism [9]. The data suggested that β-glucan extracts from barley had ability to be prebiotics for these lactic acid bacteria.

Table 4 In-vitro prebiotic potential of BBG

Bacteria	Glucose [a]		
	0 h	12 h	24 h
Lactococcuslactisssplactis	08.14±0.024	09.57±0.018	10.51±0.012
Leuconostocmesenteroidessspmesenteroides	07.99±0.021	09.12±0.032	10.18±0.019
	BBG [a]		
	0 h	12 h	24 h
Lactococcuslactisssplactis	08.14±0.024	09.26 ±0.036 [b]	10.45±0.009 [b]
Leuconostocmesenteroidessspmesenteroides	07.99±0.021	09.02±0.021 [b]	10.21±0.028 [b]

[a]Mean bacterial count ± standard error (n=3), [b] significantly different from the population at 0 h ($P \leq 0.05$).

Conclusions

Results indicated that the β-glucan extracted from Saida 183 barley variety has weak ability to scavenging free radical DPPH and weakly activity to ferric reducing power in contrast to that to ABTS. Moreover, the BBG showed a moderate activity toward a lipid peroxidation in the beta-carotene bleaching and cation bivalent chelation in CUPRAC method. These results indicated that wheat bran dietary fiber may replace synthetic antioxidant in food formulations and play a major role in human health. The present study provides the cognition enhancing potential of β-glucan by inhibiting ACHE activity. It can be considered as economic therapeutic option against cognitive disorders associated with decline in cholinergic neurotransmission. Additionally moderate alpha glucosidase inhibition activity has been reported this demonstrate as BBG as antidiabetic agent. In this work we reported the prebiotic action β-D-glucan of cereal origin on two probiotic LAB strains (Lactococuslactisssplactis and leuconostocmesenteroidessspmesenteroides). In particular, our results showed that all the investigated bacterial strains were able to use BBG as a substrate for their growth. Moreover, BBG could stimulate the growth of probiotic bacteria, namely. This study strongly proved that BBG can be used as a prebiotic ingredient in food industry to modulate intestinal microbiota into health beneficial direction.

Authors' contributions

Saad Mebrek performed the experiments and wrote the paper, Hanene Djeghim performed the experiments, Yamina Mehdi and Asma Meghezzi reviewed the data, Anwar Sirajudheen analyzed, reviewed and wrote the paper, Ali Awadh A. Nasser reviewed the paper, and Mohammed Benali conceived idea and designed the work.

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