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ORIGINAL PAPER

Biological and prebiotic activities of polysaccharides from *Taraxacum officinale* F. H. Wigg., *Cichorium intybus* L., and *Gundelia tournefortii* L

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Abstract

Probiotics are rapidly multiply by using prebiotic compounds in benefit microorganisms such as bacteria and fungi and/or some plant-derived compounds like oligosaccharides. In this study, the effects of polysaccharides isolated from three medicinal plants belonging to the Asteraceae family including *Taraxacum officinale* F. H. Wigg., *Cichorium intybus* L., and *Gundelia tournefortii* L. on the growth of *Lactobacillus rhamnosus* (NIMBB006), and their antibacterial activity against four bacteria (*Escherichia coli, Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi*, and *Bacillus subtilis*) and antioxidant capacity were investigated. The main polysaccharide components were identified using liquid chromatography–mass spectrometry (LC–MS/MS). The highest amounts of extracted polysaccharide were detected in two herbs i.e. *C. intybus* and *G. tournefortii* with high antioxidant activity. The maximum values of the antibacterial properties were related to polysaccharide isolated from *T. officinale* against four studied bacteria and polysaccharide isolated from each three herb against *S. typhi*. Totally, the extracted polysaccharides from *T. officinale* (2%) was significantly more effective in increasing the growth of *L. rhamnosus* (NIMBB006) as a native probiotic in comparison to two commercial prebiotics i.e. inulin and dextrose. In conclusion, the major constituents such as cyanidin-3-O-β-glucoside, N-acetylcysteine, and glutamic acid in *T. officinale* may play a major role in biological properties.

Keywords Bioactivity · Dandelion · Chicory · *Lactobacillus rhamnosus* NIMBB006 · Polysaccharides · Probiotic · Tumble thistles

Introduction

Today, the applied of the medicinal plants and their vital role in advancing national and global goals to achieve the health and vitality of drug self-sufficiency communities and food security, conservation of genetic resources, and active presence in global markets are very significant [1]. Plant-derived polysaccharides possess a variety of biological and prebiotic activities. In particular, most of the plant-derived polysaccharides have drawn attention from investigators in the food and biomedical fields due to their effectiveness as antioxidant, antitumor, anti-cancer, anticoagulant, antiplatelet, antithrombotic [2–5], immunoregulation [3, 6], antihyperglycemic [3], antiviral against COVID-19 [6], antidiabetic [3], antibacterial and antimicrobial [3], and hypocholesterolemic activities [7], and gut microbiota modulation effect [8], and several other nutritional benefits [9, 10]. Therefore, the wide applications of plant-derived polysaccharides in various industries have stimulated many researchers to discover and evaluate new plant polysaccharides. Results of investigations [10, 11] have found that the polysaccharides isolated from the plants have least side effects. In addition, these polysaccharides as prebiotic compounds when reach the human intestine have a major impact on gut microbial ecology and brain function [12]. Probiotics are rapidly multiply by using prebiotic compounds in benefit microorganisms such as bacteria and fungi and/or some plant-derived compounds like oligosaccharides. The probiotics can change the intestinal flora and eliminate pathogens by lowering intestinal pH and producing antimicrobial

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compounds, which effective in the prevention and treatment of gastrointestinal diseases [13].

Chicory (*Cichorium intybus* L.) belonging to the Asteraceae family is an important medicinal plant in Iran. Inulin, sesquiterpene lactones, coumarins, vitamins, and polyphenols as the main components in different parts of the plant have a lot of nutritional and medicinal value [14]. Chicory can delaying the onset of diabetes and management of osteoarthritis and cardiovascular disease [15]. Hepatoprotective, anti-inflammatory, antioxidant, immunological, cardiovascular, antidiabetic, and anticancer properties are the main of biological activities of *C. intybus* [16].

Dandelion (*Taraxacum officinale* F. H. Wigg.) belonging to the family Asteraceae [1] is used to produce commercially available dietary supplements and pharmacological preparations, which are mainly recommended as anti-inflammatory, antioxidant, anti-diuretic, anti-cancer, laxative, and hepatoprotective properties [17, 18]. In a previous study, the inhibition of SARS-CoV-2 using the extract from the *T. officinale* leaves has reported [19].

Thistle-like (*Gundelia tournefortii* L.) belonging to the family Asteraceae family as an ancient herbaceous perennial plant grows in the Mediterranean regions [1, 20]. *G. tournefortii* as an important herb in traditional medicine of Iran used to purify blood and liver [20]. The aerial parts of the herb especially young stem and leaves are used as flavoring in yogurt or vegetable [1]. The parts of *G. tournefortii* due having some main components such as minerals, vitamins, fatty acids, sterol, tocopherols and other bioactive phenolic compounds are highly appreciated for their nutritional value and health promoting properties including anti-cancer, anti-proliferative, apoptotic effects [21], antidiabetic activity [22], etc.

To our knowledge, no documented studies have been reported to recognize the prebiotic, antibacterial, and antioxidant activities of polysaccharides isolated from chicory, thistle-like, and dandelion. Generally, the main aims of this investigation were *i*) to detect polysaccharides of extracted from *T. officinale, C. intybus*, and *G. tournefortii* using LC-MS, *ii*) to evaluate the effects of polysaccharides isolated from three medicinal plants on the growth of *Lactobacillus rhamnosus* (NIMBB006), *iii*) to determine the antibacterial activity of polysaccharides against four bacteria, and *iv*) to evaluate the antioxidant capacity of polysaccharides using DPPH assay.

Materials and methods

Chemicals

Gallic acid, quercetin, ascorbic acid, inulin, dextrose, ethanol, and methanol (HPLC grade) were purchased from Merck Co. (Darmstadt, Germany) and the Folin–Ciocalteu reagent and the 1,1-diphenyl2-picryl-hydrazil (DPPH) were purchased from Sigma-Aldrich Co. (Steineheim, Germany).

Plants materials

The fresh parts of *Taraxacum officinale*, *Cichorium intybus*, *Gundelia tournefortii* were collected from the natural habitats of Shahrekord (altitude 2050–2150 m, longitude 50° E, latitude 32° N), southwestern Iran during spring 2019. Identification of the plants was consequently confirmed with the help of the authentic specimens deposited at the Herbarium of Research Center for Medicinal Plants, Shahrekord Branch (IAUSHK), Iran. Voucher numbers of *T. officinale*, *C. intybus* and *G. tournefortii* are IAUSHK-174, IAUSHK-112, and IAUSHK-154, respectively. After transportation of whole parts of the plants to the Research Center for Medicinal Plants of I.A.U., the plants parts were washed with water, cleaned with filter papers to remove all traces of dust and insects. The plants were dried in shad 25 °C for six days. At the end, the herbs were stored in desiccator until to use for extraction and fractionation of polysaccharides [23].

Extraction and fractionation of polysaccharides

T. officinale, *C intybus*, *G. tournefortii*, and mixed of these herbs were grinded and extracted with distilled water and then homogenized. The homogenate was autoclaved for 2 h, followed by cooling to room temperature before centrifugation at 3,600 g for 20 min. The filtered supernatant was then treated with 95% ethanol (1:4 *v/v*) for about 15 h at 4 °C. Crude polysaccharides were obtained by centrifugation (6000 × g for 30 min) by centrifuge Z 206 A German and then were dissolved in distilled water. The supernatant was collected and precipitated with 95% ethanol (1:4 *v/v*) for 15 h at 4 °C. The precipitate containing the polysaccharides was pelleted by centrifugation at 10,000 g for 20 min and then resuspended in distilled water. The supernatant was filtered through a membrane filter and freeze dried (Alpha 1–2 LDplus, Germany) to obtain a crude polysaccharide extract [23].

Bacterial strains and growth conditions

The strain of *Lactobacillus rhamnosus* NIMBB006 isolated from breast milk with registration code MT995144 was prepared on NCBI / BLAST site from Shams Bavaran Salamate Noor Company. The strain was activated in MRS liquid culture medium at 37 °C for 16 h before use.

DPPH radical scavenging assay

Briefly, 50 µL of each polysaccharide at concentration of 1 mg/mL was filled in 1.5-mL micro-tubes. Then, 100 µL of

DPPH solution (0.15 mM in 80% methanol) was added to each micro-tube. The blend was shaken strenuously and left to stand for 30 min in the dark room [24, 25]. Ascorbic acid (1 mg/mL) was taken as positive control. Absorbance of the resulting solution was measured in triplicate at 517 nm by Cytation 3 (Bio Tek Instruments, Winooski, VT) after centrifugation by centrifuge (Z 206 A, Germany) at 6000 g for 10 min and inhibition activity was calculated. The scavenging ability was defined as:

$$\text{Inhibition activity (\%)} = [1 - (A_{\text{sample}} / A_{\text{control}})] \times 100.$$

The antiradical activity was explicated as IC_{50} (µg/mL).

Antibacterial test

Clinical isolates of two Positive-Gram bacteria i.e. *Staphylococcus aureus* ATCC 29,213 and *Bacillus subtilis* NCTC 5398 and three Negative-Gram bacteria including *Escherichia coli* ATCC 25,312 and *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella typhi* PTCC 1609 were obtained from Collection Center of Industrial Microorganisms of Iran (IROST). The density of bacteria culture required for the test was adjusted to 0.5 McFarland standards (1.0×10^7 CFU/mL) and measured using a spectrophotometer (Eppendorf, AG, Germany). The bacteria were cultured in an agar medium using a sampler from a 200 µL liquid medium and placed on a pre-prepared solid medium. The liquid was spread over the surface of the medium to cover the entire surface of the medium; this was done for all plates in the same way. Then, using sterile pins a antibiogram was inserted in each plate. The extracts (1 mg/L of 50 µL) were added to the dedicated disks and then kept in an incubator at 37 °C for 48 h [26]. After the prescribed period, the diameter of the halo formed around each disk in each plate was measured by the caliper [26, 27] and compared to positive control or antibiotic such as Ciprofloxacine and Azithromycin (5 µg).

LC-MS/MS analysis

Identification of the polysaccharide constituents of the medicinal plants extracts was performed using liquid chromatography–mass spectrometry (LC–MS) according to [28]. Also, LC analysis was accomplished on an Agilent 1200 (Agilent Technologies, Waldbronn, Germany) equipped with an auto sampler and coupled to an Agilent 6410 triple quadrupole tandem mass spectrometer. Separation processes were performed using a Waters XBridge Shield RP-C_{18} (2.1 mm × 100 mm; 1.7 µm) column. Solvents were methanol and deionized water. Solvents were delivered at a total flow rate of 0.35 mL/min. The solvent was run by isocratic elution. The MS spectra were acquired in the positive ion mode. MS analysis was conducted using an Agilent 6410 triple quadrupole tandem mass spectrometer ESI + 4000 V with the full scan (m/z 100–1200); at source temperature of 140 °C. A 5 µl volume of the extracts were injected onto the analytical column for analysis. Afterward, the obtained data were statistically analyzed by MestreNova software Workstation.

Effect of polysaccharides on growth and acidifying activity of probiotics

Probiotic bacterial strain (*Lactobacillus rhamnosus* MT995144) was obtained from Microbiology Culture Collection, Shams Bavarane Salamate Nour Co., Iran, which was stored at − 80 °C in the MRS medium supplemented with 20% (*v/v*) glycerol. The strain was refreshed in MRS broth at 37 °C for 24 h before use. Carbohydrate-free Man-Rogosa-Sharp (MRS) medium supplemented with 0.05% (*m/v*) l-cysteine was used as basal culture medium to study acidifying activity of the polysaccharides. Inulin and dextrose was used as positive control, while the basal medium was used as blank control. At first, the polysaccharides of *T. officinale*, *C. intybus*, and *G. tournefortii*, combination of three plants, inulin, and dextrose were filter-sterilized and added to the sterilized basal medium to give a final concentration 5% (*w/v*). The medium supplemented with different of the polysaccharides was implanted in to test tubes (10 mL in each), inoculated with 1×10^6 CFU/mL of the probiotic, and incubated at 37 °C. The bacterial numbers were counted after incubation for 0, 6, 24, and 48 h. The pH of the medium was measured using a pH meter at the same time. The bacterial enumeration was performed as following procedures. One milliliter of the culture was transferred to sterile test tubes and serially diluted with sterile 1% (*w/v*) peptone solution. Subsequently, 100 µL of each dilution was spread on the plates of MRS agar, followed by an aerobic incubation at 37 °C for 48 h. Bacteria were enumerated and expressed as log CFU/mL. The increase of bacterial numbers between 0, 6, 24, and 48 h was calculated according to the formula below:

$$\text{Increase of bacterial number} = \log B - \log A.$$

where A was the bacterial number at 0 h (CFU/mL) and B was the bacterial number after incubation for 6, 24, and 48 h (CFU/mL) [29].

Statistical analysis

All assays were done in triplicates and results are expressed as the mean ± standard deviation (SD). The data were statistically analyzed in one-way ANOVA using the program SPSS (21.0). Means were compared with Tukey's test at $p \leq 0.05$ level.

Results and discussion

The yield of extracted polysaccharide

Results of this study indicate that there were significant differences in the yields of extracted polysaccharide among the studied species (Fig. 1.). The highest yield (1.41 mg or 14.1% *w/w*) was obtained from the *C. intybus* extract, which can be due to the presence of enzymes in the cell wall of plants and bacteria, the ability to break down its sugars [30]. In fact, chicory plant cell wall enzymes perform better in sugar metabolism than other plants. Results of previous investigation indicated that the yields of the water-extracted polysaccharides, chelate-extracted polysaccharides, and acid-extracted polysaccharides from the chicory (*C. intybus*) root pulp were 14.3% [31]. In addition, the results of previous investigation on characterization and biological activities of polysaccharides from dandelion (*Taraxacum offificinale*) leaves indicted that the yield of extracted polysaccharide from *T. officinale* was 14.05% *w/w* [32]. No information on polysaccharide yield of *Gundelia tournefortii* is available. Results of present study shows the effects of the physicochemical properties and structure of polysaccharides, from cell walls in raw materials, that have an impact on their biological activities, including molecular weight, monosaccharide composition, water solubility, chain structure, and uronic acid content. In addition, the structure of certain natural polysaccharides limits their biological activity. Chemical modification and degradation of these structures can enhance the pharmacological properties of natural polysaccharides to a certain extent. At the same time, the processing method affects the structure and yield of polysaccharides on the cell wall and in the cell [33]. Moreover, efficient degradation of the cell walls of mushrooms can be achieved by enzymes, producing more polysaccharides from the inside cells, whose extraction rates depend on the type and amounts of enzymes, temperature, pH, the ratio of liquid to solid material, reaction time, etc. [34].

Identification of polysaccharides compounds by LC-MS

Results of LC-MS analysis are shown in Figs. 2, 3, 4 and 5, found that the composition of purified polysaccharide from *T. officinale*, *C. intybus*, and *G. tournefortii* and poly herbal were uronic acid, monosaccharides, and glycosidic linkage. In general, high-molecular mass bound polysaccharieds in the extracts of these herbs and their mixed contained Cyanidin-3-O-β-glucoside, Glutamic acid and N-acetylcysteine, N-Acetylglucosamine, N-Acetylgalactosamine, glucuronic acid, and uronic acid (Figs. 2, 3, 4 and 5). Results of a previous study showed that the main polysaccharides isolated from the *T. officinale* leaves were galacturonic acid, D-glucose, D-galactose, L-arabinose, L-rhamnose, D-xylose, and D-mannose [35]. In an investigation found that the N-acetylcysteine treatment had a similar restorative effect on the APAP-induced pathological changes [36]. Results of previous investigation found that cyanidin-3-O-β-glucoside isolated from the buds of *Rosa damascena* in comparison with the other isolated compounds like flavonoids may be effective to improve the cardiovascular system [37, 38]. The polysaccharides isolated from *T. officinale,*

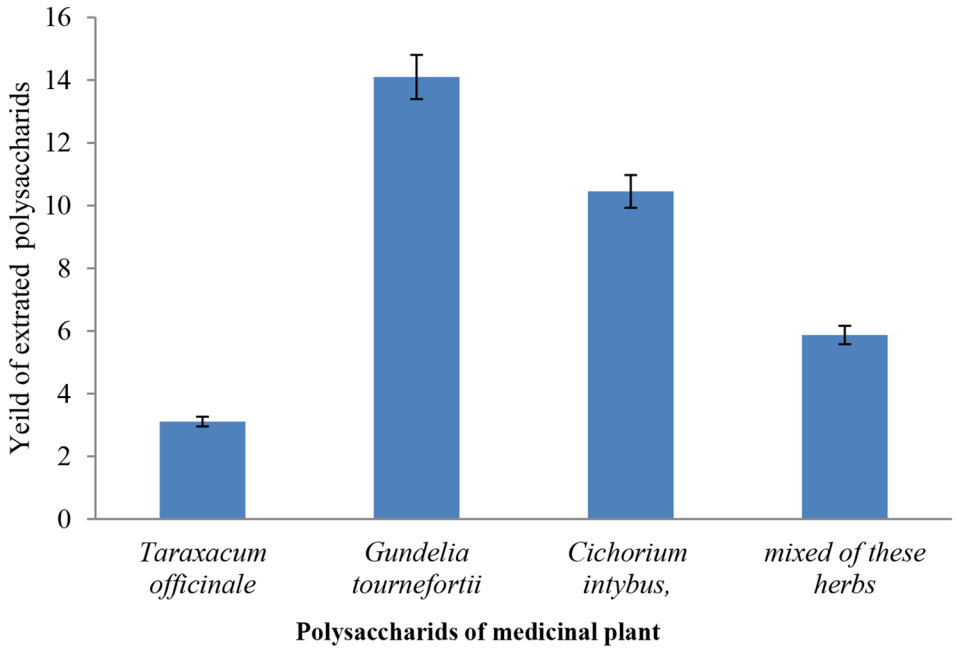

Fig. 1 The yields of extracted polysaccharide from the medicinal plants All analyses were the mean of triplicate measurements ± standard deviation † Means with different letter in a column are statistically significant at 5% level probability

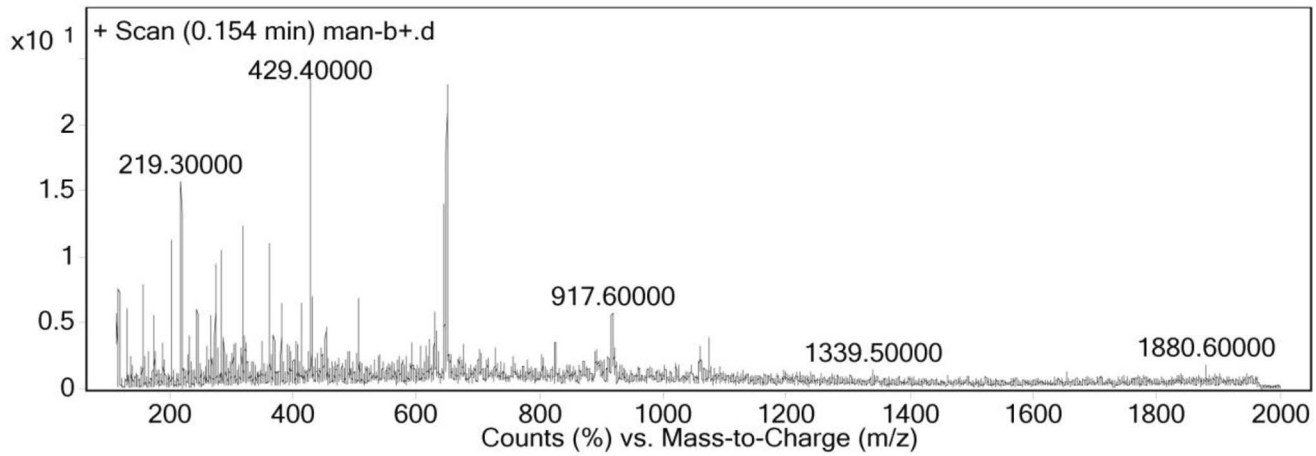

Fig. 2 Chemical structures of the compounds isolated from *T. officinale*

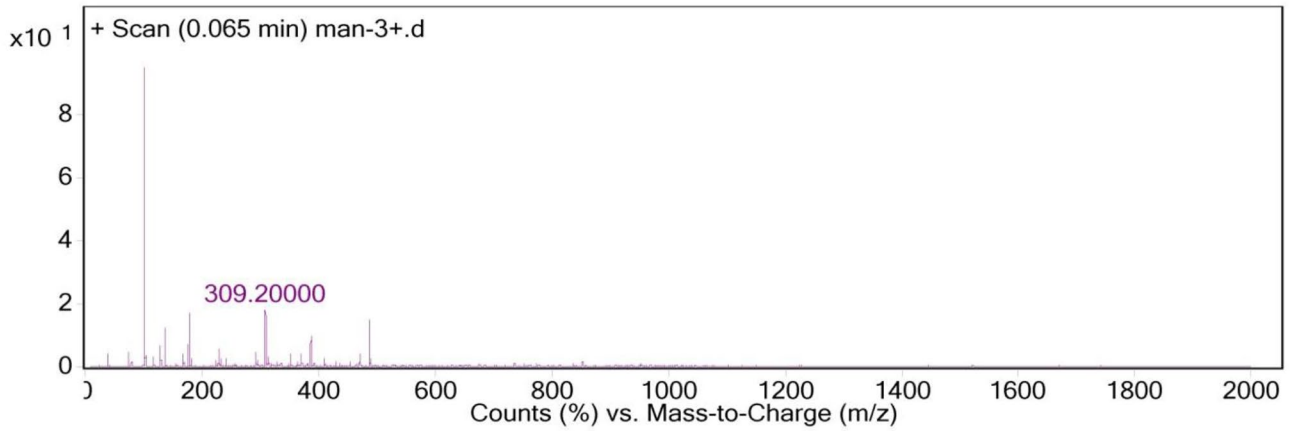

Fig. 3 Chemical structures of the compounds isolated from *C. intybus*

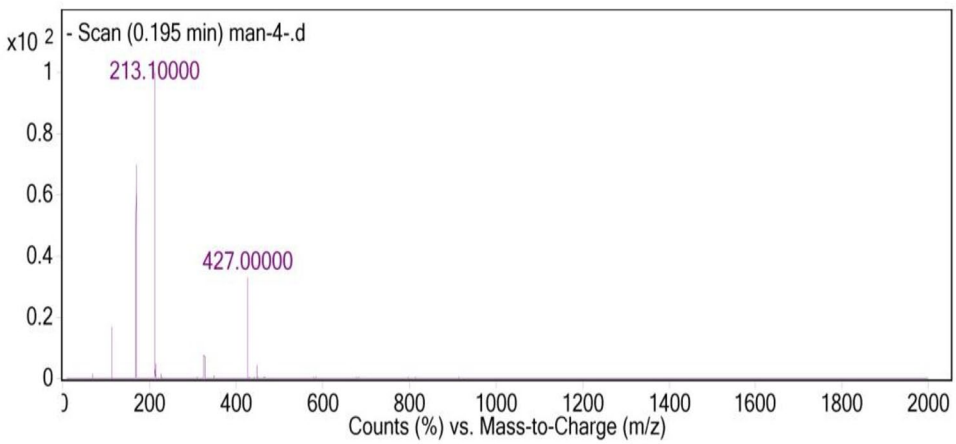

Fig. 4 Chemical structures of the compounds isolated from *G. tournefortii*

C. intybus, and *C. scolymus* divided into rigid fibrillars of chitin and glucans [39]. β-Glucans are considered a valuable functional ingredient of many food products due to their nutritional and health benefits as well as their use as thickening or stabilizing agents. Moreover, β-glucans can be used as fat replacers in low-fat yoghurts, ice creams, cheeses and butter-like products by enhancing the rheological, textural and sensory properties of food products [40]. Uronic

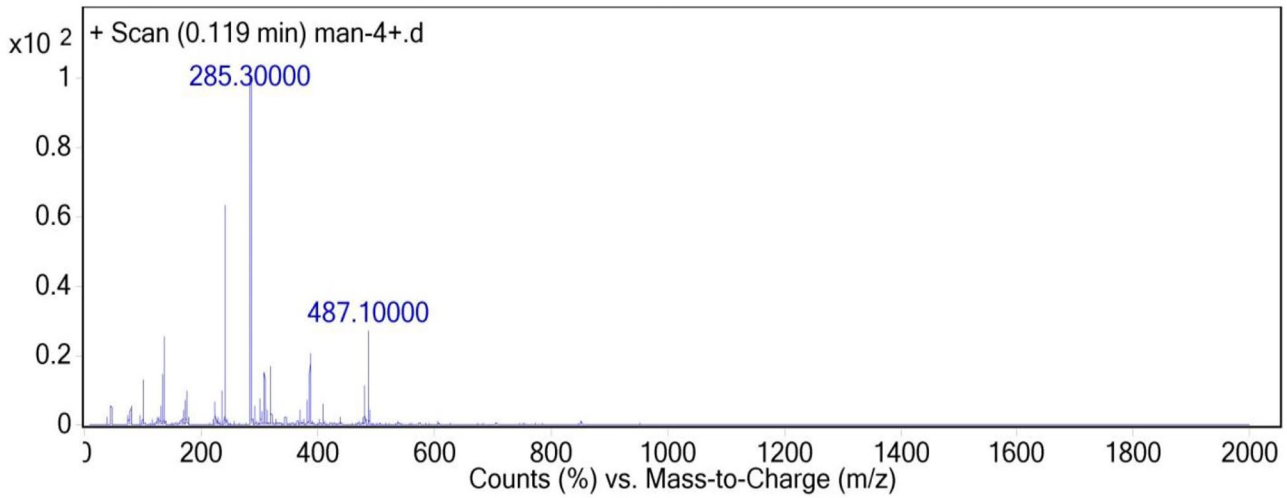

Fig. 5 Chemical structures of the compounds isolated from the mixed of these herbs

Table 1 Phytochemical and antioxidant properties of polysaccharides from *T. officinale*, *C. intybus*, *G. tournefortii*, and mixed of these herbs

Polysaccharides and synthesis antioxidant	DPPH radical scavenging activity (%)	IC_{50} (mg/mL)	Yield of extracted polysaccharide (mg/mL)	Yield of extracted polysaccharide (%)
Taraxacum officinale	41.56 ± 0.00 d†	812.00 ± 0.28 d	0.31 ± 0.00 d	3.11 ± 0.00 d
Gundelia tournefortii	63.50 ± 0.14 b	236.28 ± 0.22 b	1.045 ± 0.00 b	10.45 ± 0.00 b
Cichorium intybus,	59.50 ± 0.29 c	241.64 ± 0.37 c	1.41 ± 0.01 a	14.10 ± 0.01 a
Mixed	47.10 ± 0.63 d	901.66 ± 0.38 e	0.58 ± 0.01 c	5.87 ± 0.01 c
Ascorbic acid	97.36 ± 0.00 a	46.00 ± 0.00 a		

All analyses were the mean of triplicate measurements ± standard deviation (± SD)

† The averages in the columns with at least a common alphabet are not statistically significant (Tukey's test at $p \leq 0.05$ level)

acids consist of carboxylic acid; often these are formed by the oxidation of the primary hydroxyl group at the terminal carbon atom into a carboxylic acid [41]. As a part of the structure, these uronic acid groups have significant contribution in the elevation of the negative charge of a polysaccharide as well as in the effective scavenging action of free radicals through activation of hydrogen atoms [42]. A novel exopolysaccharide from *Lactobacillus planterum* LP6 aids protection against the lipid radical-induced damage of cell membrane in PC12 cells. Protection to the cell membrane is afforded by inhibiting lipid peroxidation and reduction of ROS invasion. Authors confirmed that higher uronic content has elevated negative charge of exopolysaccharide and granted the protective effect [43].

DPPH radical scavenging activity (1,1-diphenyl-2-picrilhydrazyl)

The DPPH is a stable free radical that is widely accepted as a tool for estimating the free radical scavenging activities. Antioxidant molecules can quench DPPH radicals (by providing a hydrogen atom) and convert them to a colorless product. According to results in Table 1; Fig. 6., there were significant difference ($P \leq 0.05$) between DPPH radical scavenging activity of the extracted polysaccharides from the studied species. Results indicated that the maximum values of DPPH radical scavenging activity were ascorbic acid (97.63 mg/mL) and the polysaccharide from *G. tournefortii* (60.50 mg/mL). In addition, for evaluation of antioxidant activity was expressed as IC_{50}. The lower IC_{50} value indicates a stronger ability of the extract to act as a DPPH scavenger. The lowest of IC_{50} values (the highest antioxidant capacity) were found to be 46.01 and 236.28 mg/mL for ascorbic acid and the polysaccharide from *G. tournefortii*, respectively (Table 1; Fig. 6.).

The mechanism of DPPH scavenging activity may be due to the hydrogen donation of polysaccharides from *T. officinale*, *G.tournefortii*, and *C. intybus*, which combines with radicals. Thus, it forms a stable radical to terminate the radical chain. It has been found that the antioxidant activities of polysaccharides are affected by various factors such as chemical contents, molecular mass, structure and extraction methods [44]. DPPH assay is easy, rapid and sensitive way to determine the antioxidant property of a

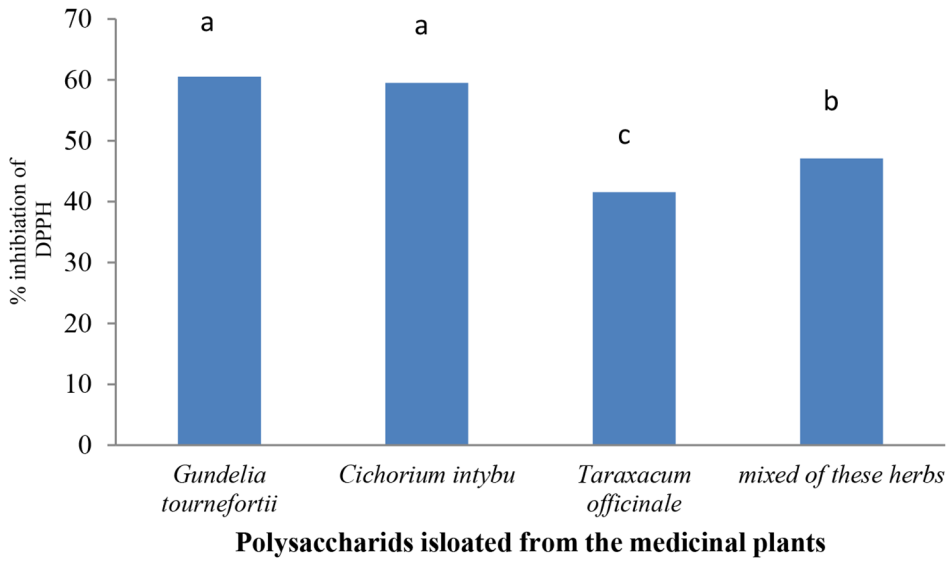

Fig. 6 DPPH radical scavenging activity of the polysaccharide from the medicinal plants
The averages in columns with at least a common alphabet are not statistically significant (Tukey's test at $p \leq 0.05$ level)

specific compound or plant extract. DPPH method is based on the reduction of DPPH content in the presence of hydrogen donating antioxidant due to the formation of diphenyl-2-picrylhydrazine. Some studies have shown that this activity is due to the high content of uric acid. Some authors have suggested that they play an important role in the antioxidant properties of animals-derived polysaccharides [44]. Glutathione peroxidase and glutathione reductase have been reported to be two important antioxidant enzymes in the cell wall of bacteria and plants that protect cells from oxidative damage by removing reactive oxygen species (ROS) suggesting that the antioxidant capacity could be attributed to both enzymatic and non-enzymatic intracellular antioxidants [45]. Moreover, artichoke is high radical scavenging capacity categorized as an antioxidant rich vegetable, based on the comprehensive antioxidant food database [46]. Antioxidative activities of polysaccharides from *T. officinale* is low [47, 48]. The dandelion (*T. officinale*), in common with a considerable number of the Asteraceae family, contains in its roots a series of fl-D-fructofuranosidase. Neither of the hydrolases is inhibited by sucrose. The physiological role of these three hydrolytic enzymes is discussed [36]. Chicory extracts minimize the color of DPPH due to the power of hydrogen donating ability. The methanol extracts produced DPPH anion scavenging power (59 µg/mL) at 100 µg/mL concentration and 38 µg/ml for Ascorbic acid. The above value depicted DPPH anion scavenging power of extracts. Discoloration of violet DPPH to yellow clearly indicated the antioxidant effect of chicory extracts. The presence of reductant in a compound is responsible for their reducing ability [49]. Uronic acid, monosaccharides, and glycosidic linkage impacts antioxidant effects. In addition, polysaccharides conjugates may influence their radical scavenging ability [50]. Here, the low Mw; the triple helix stereo-configuration; the glycosidic bonds; and the high uronic acid, Glc, and Gal contents of Dandelion polysaccharide might be the main reasons for antioxidant activities of Dandelion polysaccharide [51]. Besides, the activities depend chiefly on the structural characteristics, including the amounts of carbohydrate, uronic acid, and sulfate and reducing end, Mws, viscosities, monosaccharide constituents, conjugates, positions and types of glycosidic linkages and branches, etc. For example, polysaccharides with more complex structures and lower Mws, involving more side-branches and residues, could alleviate the aging process to varying extents [52]. Likewise, biological activities of polysaccharides are drastically affected by their conformational features. More flexible and extended conformation, as well as good solubilities, confer polysaccharides to have more opportunities to bind to cell membranes and have stronger interactions with the correlated receptors, which lead to the higher antioxidant, anti-tumor, and immunomodulatory activities [53]. The peptide-protein conjugates of polysaccharides also provide the scavenging activity. The phenolic compounds like phenolic acids have also shown the scavenging ability.

Antibacterial effects

In this study, effects of different extracted polysaccharides from the herbs on antibacterial activity against four types of bacteria, including *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa*, and *S. typhi* were examined. The highest values of the antibacterial properties against *S. aureus*, *B. subtilis*, and *P. aeruginosa* were obtained from the extracted polysaccharide from *T. officinale* (Table 2). The maximum values of the antibacterial activity against *E. coli* and *S. typhi* were achieved from the extracted polysaccharide of *C. intybus*

Table 2 Antibacterial activity or zone of inhibition (mm) of the polysaccharides isolated from *T. officinale*, *C. intybus*, *G. tournefortii*, and mixed of these herbs against five bacteria

Polysaccharides and antibiotics	Zone of inhibition (mm)				
	Escherichia coli	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Salmonella typhi*	*Bacillus subtilis*
Taraxacum officinale	1.50 ± 0.00 c	2.50 ± 0.00 a	2.60 ± 0.58 ab	1.50 ± 0.00 c	3.00 ± 0.00 a
Gundelia tournefortii	1.67 ± 0.14 b	2.05 ± 0.29 b	2.25 ± 0.29 b	2.00 ± 0.00 a	1.60 ± 0.58 cd
Cichorium intybus	2.00 ± 0.00 a	1.60 ± 0.06 c	1.90 ± 0.58 c	1.80 ± 0.00 b	1.40 ± 0.58 d
Mixed of the herbs	1.50 ± 0.57 c	1.35 ± 0.09 d	1.70 ± 0.58 d	1.90 ± 0.58 ab	1.65 ± 0.29 cd
Azithromycin	1.50 ± 0.00 c	1.50 ± 0.00 cd	1.85 ± 0.87 c	1.50 ± 0.00 c	1.80 ± 0.00 c
Ciprofloxacin	1.50 ± 0.00 c	1.50 ± 0.00 cd	2.70 ± 0.00 a	1.50 ± 0.00 c	2.30 ± 0.00 b

All analyses were the mean of triplicate measurements ± standard deviation (± SD)

† The averages in the columns with at least a common alphabet are not statistically significant (Tukey's test at $p \leq 0.05$ level)

Table 3 Effect of polysaccharides from *T. officinale*, *C. intybus*, *G. tournefortii* on the growth of *L. rhamnosus* (NIMBB006)

Polysaccharides	Log cfu/mL				
	0 h	6 h	24 h	48 h	Increase of bacterial number = log B - log A (cfu/mL)
Taraxacum officinale,	8.54654	11.09760 a	14.48750 a	15.12000 a	6.57346 a
Gundelia.tournefortii	8.54654	11.01703 a	13.34914 b	14.27948 b	5.73294 b
Cichorium intybus	8.54654	10.40140 c	13.04643 b	13.95154 bc	5.405 bc
Mixed of these herbs	8.54654	10.77815 bc	13.15321 b	14.19191 b	5.64537 b
Inuline	8.54654	10.90309 b	13.12956 b	13.77815 c	5.23161 c
Dexterose	8.54654	10.00000 d	11.76996 c	11.00000 d	2.45346 d

All analyses were the mean of triplicate measurements ± standard deviation (± SD)

† The averages in columns with at least a common alphabet are not statistically significant (Tukey's test at $p \leq 0.05$ level)

and *G. tournefortii*, respectively (Table 2). The antibacterial activity of polysaccharides was evaluated against three Gram-negative and two Gram-positive bacteria. As shown in Table 2, the extracted polysaccharide from *T. officinale* had the highest antibacterial properties against Gram-positive (*S. aureus* and *B. subtilis*) and Gram-negative (*E. coli*, *P. aeruginosa*, and *S. typhi*) compared to antibiotic i.e. Azithromycin and Ciprofloxacin. Similarity, Shirzad, Hamedi [24] reported that among all the plant polysaccharides, the dandelion polysaccharides found to be more effective in suppressing the growth of the all microorganisms except *P. aeruginosa* which was found resistant against all the extracts of the plant at all concentrations. Opposite with our results, [54] reported that marshmallow root polysaccharide showed stronger inhibitory effect on Gram-positive bacteria. The antibacterial effect of polysaccharides can be exerted by enhancing the permeability of the cell membrane, inhibiting the adsorption of pathogenic to host cells, or blocking the transmembrane transport of nutrients or energy substances. Generally, polysaccharides extracted from the plants have been shown to have antibacterial activity, but the intensity and mechanisms underlying these effects can vary [55]. On the other hand, low molecular weight polysaccharides better attain antimicrobial inhibition action [50]. In addition, results of previous investigations indicated that polysaccharides can exert an antimicrobial effect by disrupting the integrity of membranes and effluxing the contents of soluble proteins [56].

Effect of polysaccharides on growth and acidifying activity of probiotics

Results of polysaccharides effect on the growth *L. rhamnosus* indicated that there were significant differences ($p < 0.0$) among the polysaccharide from the three herbs and their mixed and monosaccharides commercially available prebiotics such as inulin and dextrose (Tables 3 and 4). The maximum pH values followed the order of poly herbal > *T. officinale* > *G. tournefortii* > *C. intybus* > inulin > dextrose. The highest value of the prebiotic activity was obtained from the dandelion polysaccharides, however, the lowest of the prebiotic activity was achieved from positive control (dextrose). Results of a previous research [57] showed that the polysaccharide isolated from ginseng (*Panax ginseng*) had the prebiotic effect (*Lactobacillus casei*, *L. reuteri*, and *L. helveticus*). In addition, they reported that polysaccharides acidic and alkaline extracts polysaccharides and their sulfated polysaccharides derivatives from ginseng showed the best cytotoxic activity against human colon cancer cell, human liver cancer cell, and human breast cancer cell. The decrease in pH values reflected that some organic acids were produced, which the polysaccharides metabolized by the

Table 4 Effect of polysaccharides from *T. officinale*, *C. intybus*, *G. tournefortii* on acidifying activity of *L. rhamnosus* (NIMBB006)

Polysaccharides	pH				
	0 h	6 h	24 h	48 h	% Reduction of pH
Taraxacum officinale	6.50 ± 0.00	6.12 ± 0.00 a	5.28 ± 0.00 ab	5.14 ± 0.00 a	1.36 b
Gundelia.tournefortii	6.50 ± 0.00	5.60 ± 0.00 b	5.17 ± 0.01 b	5.13 ± 0.00 a	1.37 b
Cichorium intybus	6.50 ± 0.00	5.47 ± 0.00 b	5.18 ± 0.02 b	5.10 ± 0.00 a	1.40 b
Mixed of these herbs	6.50 ± 0.00	5.53 ± 0.00 b	5.41 ± 0.02 a	5.18 ± 0.00 a	1.20 c
Inuline	6.50 ± 0.00	5.00 ± 0.00 c	5.02 ± 0.00 b	5.07 ± 0.00 a	1.43 b
Dexterose	6.50 ± 0.00	5.75 ± 0.00 b	3.54 ± 0.00 c	4.44 ± 0.01 b	2.06 a

All analyses were the mean of triplicate measurements ± standard deviation (± SD)

† The averages in the rows with at least a common alphabet are not statistically significant (Tukey's test at $p \leq 0.05$ level)

tested bacteria and produced some short-chain fatty acids. In present study, the dandelion polysaccharides produced a stable pH in the culture medium after 48 h and had a good prebiotic activity [29].

Conclusions

The main polysaccharides extracted from three important medicinal plants including *T. officinale*, *C. intybus*, and *G. tournefortii* and their mixed were uronic acid, monosaccharides, and glycosidic linkage, which the highest amounts of extracted polysaccharide are related to *C. intybus* and *G. tournefortii*. The highest value the antioxidant capacity using DPPH assay was obtained from the polysaccharide from *G. tournefortii*. However, the polysaccharide from *T. officinale* had the maximum values of the antibacterial affects and prebiotic activity. Probably, the higher amounts of the polysaccharide compounds such as β-glucan, Cyanidin-3-O-β-glucoside, N-acetylcysteine in the extracted from the medicinal plants especially *T. officinale* is as an effective factor in biological properties. Overall, our findings suggest that polysaccharide fractions from edible *Taraxacum officinale, Cichorium intybus*, and *Gundelia tournefortii* might be useful in producing functional foods, nutraceuticals, and medicinal.

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