ORIGINAL ARTICLE



# **Characterization and identifcation of inulin from** *Pachyrhizus erosus* **and evaluation of its antioxidant and in‑vitro prebiotic efficacy**

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**Abstract** Inulin is the polysaccharide obtained from different plant sources i.e. Wheat, Chicory, Jerusalem artichoke and Dahlia. In this study, Jicama (*Pachyrhizus erosus*) is used to isolate inulin using the microwave heating. The  ${}^{1}H$ NMR study reveals the presence of fructose and glucose unit which is the backbone of inulin. Further FT-IR and Raman confrmed the functional groups present in inulin. The UV– Vis spectroscopy analysis depicts the purity of the isolated inulin. The shape and size of the extracted inulin was determined from scanning electron microscopy and dynamic light scattering appeared as fat-fakes and 135 nm respectively. X-ray difractogram showed semi-crystalline nature suggesting the stability of the extracted inulin. The isolated inulin has phenolic and flavonoid content of  $8.1804 \pm 6.26$  mg

gallic acid equivalent/g and  $14.387 \pm 4.192$  mg rutin equivalent/g of dried polysaccharide respectively. The inhibition percentage of DPPH and FRAP of isolated inulin were found to be  $75.74 \pm 4.5\%$  and  $0.11 \pm 0.007$  respectively. The isolated inulin promotes the growth of probiotics like *Enterococcus faecium* (MZ540315) and *Lactiplantibacillus plantarum* (MZ540317). All the analysis suggest the isolated inulin has good prebiotic potential as the commercially available one. The current study proposes that isolated inulin can be used as a prebiotic in the future.

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# **Graphical abstract**



**Keywords** Polysaccharide · Inulin · Prebiotic · Antioxidant · Fructan

### **Abbreviations**



# **Introduction**

Inulin is a plant-extracted polysaccharide constituting several units of fructose connected by  $\beta$ -(2–1) bonds and terminated by a single glucose unit. It is described by the general formula *GFn,* where G and F stand for glucose and fructose units, and n signifes the number of fructose units. It is a well-known biopolymer with exceptional prebiotic attributes like improving the poor conditions of the gastrointestinal and the immune system and works on lowering the blood glucose level, diabetic condition, and cholesterol level in the body.

Unlike the other carbohydrates, the  $\beta$ -(2–1) bond of inulin makes it remain undigested in the stomach and gets fermented by the beneficial bacteria resulting in the secretion of short-chain fatty acids (SCFAs). SCFA has the potential to prevent diabetes. Inulin is known to promote the growth of *Lactobacillus* and *Bifdobacterium*. Plants like Chicory, Jerusalem artichoke, and *Pachyrhizus erosus* L are known sources of inulin (Shoaib et al. [2016](#page-11-0)). Most of the commercially available inulin is isolated from Jerusalem artichoke and Chicory with  $12-19$  g/100 g and  $11-20$  g/100 g dry weight respectively (Shoaib et al. [2016](#page-11-0)) which is higher than the inulin present in *P. erosus* with 48.66 mg/100 g. Although Chicory and Jerusalem artichoke are available in the market as commercial sources of inulin, still *P. erosus* is a matter of investigation due to its inulin and high nutritional content (Jaiswal et al. [2021](#page-10-0)). It is a natural source of vitamin C, ribofavin, thiamine, and folate (Buckman et al. [2018](#page-10-1)). The presence of all these molecules makes it a better antioxidant for biomedical applications. It is also known for its nitrogen fxing ability due to which it was specifcally introduced in Africa for improving the farming practice (Jaiswal et al. [2021\)](#page-10-0). Apart from this, its high availability

throughout the world would make it more feasible and cost-efective for the food and pharmaceutical industries to utilize it in producing synbiotic food and antioxidants. Reports are showing the extraction of inulin from various roots like Chicory, Jerusalem artichoke, Burdock roots, and Elecampane, but the characterization of microwave extracted inulin from *P. erosus* is still limited. In a recent study, the isolation and characterization of inulin from *P. erosus* is reported by Shi et al. ([2022](#page-11-1)). Furthermore, the antioxidant property of inulin depends on the proper isolation method. In this regard, the reports regarding the isolation, characterization, and antioxidant property of inulin isolated from *P. erosus* are too limited to be considered as a commercial one by the industries whereas the prebiotic potential of the *P. erosus* inulin is missing from the literature. Thus, the lack of proper information regarding its prebiotic potential and limited characterization of *P. erosus* could be an important reason for not being accepted as a commercial source of inulin in industries.

Taking this background informations into account the current study isolates and characterizes the inulin extracted from *P. erosus* for its future application.

# **Materials and methods**

### **Materials**

The commercial inulin (Chicory inulin) was procured from Sigma-Aldrich, St. Louis, United States. Jicama was purchased from the local market of Sundergarh district of Odisha. Chemical reagents used were purchased from Sigma-Aldrich, St. Louis, United States, and Himedia Laboratories Pvt. Ltd, Mumbai, India. *Salmonella typhimurium* NCIM 2501 was burrowed from Food Engineering Laboratory, NIT, Rourkela, Odisha.

#### **Extraction of polysaccharide from Jicama**

The collected jicama tubers were washed using distilled water, peeled, and sliced followed by hot air drying. The dried slices were ground to a fine powder and stored in airtight container for further use. Around 50 gm of the sample was taken for the study. The dried powder was pre-treated with petroleum ether (80%) and sewag reagent (chloroform: butanol, 4:1) for the removal of fat and protein respectively. The recovered sample was further treated with microwave heating by taking microwave power density  $(W/g)$  and time (min) as factors. The experiments were run as per the conditions provided by the Central Composite Design (CCD) using Design Expert 11. The supernatant was treated with three parts of absolute ethanol to obtain the polysaccharide. The sample

was air dried followed by centrifugation at  $5000 \times g$  for 20 min to obtain the polysaccharide. The recovered polysaccharide was lyophilized for 24 h by re-suspending it in deionized water. The lyophilized sample was stored in dried form in an airtight container for further experimental work.

# **Bacterial culture**

The two probiotic bacteria *Lactiplantibacillus plan‑ tarum* (Lp) (MZ540317) and *Enterococcus faecium* (Ef) (MZ540315) were used to check the prebiotic potential of commercial and extracted polysaccharide (Bhanja et al. [2022\)](#page-10-2). Inulin was also used to check the growth-inhibition of *S. typhimurium* NCIM 2501. The two probiotic bacteria were cultured using De Man, Rogosa, and Sharpe (MRS) broth and agar, and the *S. typhimurium* was grown using Nutrient broth and agar. All the cultures were grown at 37 °C for 24—48 h and stored in their respective broth at  $4 °C$ .

# **SEM observation**

The commercial inulin and the extracted polysaccharide were spread over a double adhesive carbon-coated slide separately. The powder was coated with gold using a sputter coater. Detailed structural confguration especially the shape of the extracted polysaccharide and the commercial inulin was analyzed through SEM (JEOL JSM- 6480 LV, EDS: Oxford Instruments). The images of the respective samples were taken in powder form at a magnifcation factor of 100 and 600.

# **DLS**

The polysaccharide and commercial inulin were dissolved separately in 1 ml milliq water. The solutions were membrane fltered thoroughly before the experiment. The size distribution pattern of the samples (1 mg/ml) was measured using DLS (Malvern Zetasizer 90, Malvern, Netherland) at 25 °C and the analysis was carried out using Malvern ZS nano software.

# **FT‑IR spectroscopy**

KBr pellets were prepared for the polysaccharide and commercial inulin. Both the pellets were observed under infrared spectrums using FT-IR (Shimadzu IR Prestige-21) at the frequency of 4000–100 cm−1. For spectra measurement, transmittance (%) was plotted with wavenumber (cm<sup>-1</sup>). The recorded spectrum was compared with the spectrum obtained for the commercial inulin (Praveen et al. [2019](#page-10-3)).

#### **UV–Visible spectroscopy**

The 1 mg of each sample was dissolved in 1 ml of distilled water. The purity of the polysaccharide and commercial inulin was determined by a UV–Vis spectrophotometer (UV–Vis; Shimadzu, Japan). The samples were analyzed at a spectral scan of 200–800 nm (Praveen et al. [2019](#page-10-3)).

# **XRD analysis**

The powder form of the commercial inulin and polysaccharide was analyzed by XRD (Bruker, D8, Advance, Germany) in a 2 $\Theta$  range from 10 to 90 $\degree$  with 10 $\degree$ /min scanning speed using Co/Ka ( $\lambda$  = 1.7909 A0°) radiation source.

#### **Raman spectroscopy**

The powder form of the commercial inulin and polysaccharide were spread on a glass slide and examined under a PL Raman spectrometer (Model-XMB3000-3000) for determining the phonon vibration modes of the samples at 633 nm within the spectral range of  $(2500-500 \text{ cm}^{-1})$  (Huang et al. [2019\)](#page-10-4).

# **NMR studies**

The polysaccharide and commercial inulin were dissolved separately in 1 ml of 99.95% D<sub>2</sub>O followed by heating at 75 °C. The samples were cooled to room temperature (RT), and acidifcation was carried out using acetic acid (0.25% v/v) and trifluoroacetic acid (TFA) (0.025% v/v) (Barclay et al. [2012](#page-10-5)). The NMR spectra were analyzed using Bruker, Avance II. <sup>1</sup>HNMR spectroscopy is used to identify the structure of the extracted polysaccharide by studying the magnetic behavior of the hydrogen nuclei. The obtained chemical shifts were expressed in ppm and the results were compared with the spectra recorded for inulin from chicory and available literature.

# *Antioxidant activity of the polysaccharide‑rich Jicama extract*

The antioxidant activity of the commercial inulin and the polysaccharide was determined by Flavonoid assay, phenolic assay, DPPH assay, and FRAP assay.

# *Flavonoid estimation*

The presence of favonoids in extracted polysaccharide was determined by following Moumita et al. ([2022](#page-10-6)). Briefy, 1.0 ml of the sample was prepared and mixed with a 5% solution of  $\text{NaNO}_3$  (0.3 ml) and incubated for 12 min at 25 °C. Next, 0.4 ml of 10% solution of AlCl<sub>3</sub> was added to the above sample and left undisturbed for 15 min followed by mixing 2.0 ml of NaOH solution (1 M). The fnal

volume was raised to 6 ml with double distilled water. It was incubated for 20 min at RT and absorbance was taken at 518 nm. Similarly, a standard curve was plotted for Rutin  $(10-200 \,\mu g/ml)$ .

#### *Phenolic compound estimation*

The presence of phenolic compound was estimated by following Moumita et al. [\(2022](#page-10-6)) with minor modifcations. Polysaccharide extract of 100 µl was mixed with 500 µl of Folins-Ciocalteau phenol reagent and 0.4 ml of 7.50% sodium carbonate followed by vortexing. The solution was left at 50 °C for 6 min followed by the quantifcation of absorbance at 760 nm. Similarly, the standard curve was plotted for gallic acid (10–200 µg/ml).

# *DPPH assay*

The DPPH assay was done using Shang et al. with minor modifcation (Shang et al. [2018\)](#page-10-7). Briefy, 60 μM of DPPH free radical solution (100 μl) was prepared using ethanol and added to 100 µl of the sample. The samples were incubated for 35 min in dark at RT, and the absorbance was quantifed at 517 nm.

Percentage inhibition of DPPH free radical = 
$$
\frac{Ab_c - Ab_s}{Ab_e} \times 100
$$
 (1)

Where,

 $Ab<sub>c</sub>$ —absorbance of negative control.

 $Ab_s$ —absorbance of DPPH solution after reaction with the sample.

#### *FRAP Assay*

The FRAP of the extracted sample and commercial inulin were determined by following Zhang et al. ([2022\)](#page-11-2) with minor changes. Briefly, 1.0 ml of sample solution was added with PBS buffer  $(3 \text{ ml})$  and  $1\%$  Potassium Ferricyanide (3 ml). The solution was incubated at 50 °C for 30 min. Then, 3 ml of trichloroacetic acid (TCA) solution (10% aqueous solution) was added to the above solution. Centrifugation was done at  $4000 \times g$  for 12 min. After that, 3 ml of the collected supernatant was mixed with an equal volume of water and FeCl<sub>3</sub> solution of  $0.1\%$  (0.4 ml), and absorbance was assessed at 705 nm. The optical density (OD) was compared with the standard curve of ascorbic acid.

# *Determination of prebiotic score of the extracted polysaccharide*

Prebiotic score assay was performed as mentioned by Praveen et al. [\(2019](#page-10-3)) to study the effect of the polysaccharide extract on the growth of the probiotic bacteria *L.* 



<span id="page-4-0"></span>**Fig. 1** Scanning electron microscopy images of Inulin powder- Jicama polysaccharide (**a**); Chicory inulin (**b**); Dynamic light scattering analysis of the extracted Jicama polysaccharide (**c**); Chicory Inulin (**d**)

*plantarum* and *E. faecium* while *S. typhimurium* was taken as the enteric pathogen. The probiotic strains and *S. typh‑ imurium* were cultured in their respective broth medium for 24 h at 37 °C. The pellet of the overnight cultures was collected after centrifugation and resuspended in 0.9% saline. The experiment was executed by mixing  $1\%$  (v/v) of each culture to separate vials containing their respective broth with  $1\%$  (w/v) glucose or  $1\%$  (w/v) commercial inulin or  $1\%$ (w/v) extracted polysaccharide. The cultures were left for incubation at 37 °C for 24 h and were plated on their respective growth media for both  $0^{th}$  h and  $24^{th}$  h. The prebiotic score was calculated by using the following equation below

$$
\text{Prebiotic score} = \frac{Log \ P_x^{24} \ - \ Log \ P_y^{0}}{Log \ P_G^{24} \ - \ Log \ P_y^{0}} \ - \ \frac{Log \ E_x^{24} \ - \ Log \ E_y^{0}}{Log \ E_G^{24} \ - \ Log \ E_y^{0}} \tag{2}
$$

where,

 $P_G^0$ ,  $P_G^{24}$ —Probiotic CFU count of glucose for 0th and 24th hr respectively.

 $P_x^0$ ,  $P_x^{24}$ —Probiotic CFU count of carbohydrate-rich extract or inulin for 0th and 24th hr respectively.

 $E_G^0$ ,  $E_G^{24}$ —Enteric CFU count of glucose for 0th and 24th hr respectively.

 $E_x^0$ ,  $E_x^{24}$ —Enteric CFU count of carbohydrate-rich extract or inulin for 0th and 24th h respectively.

#### **Statistical analysis**

The experiments were conducted in triplicates. The data were plotted in graphs using Graph Pad Prism 5.0, OriginPro 2020b, and Mestrenova 6.0.2–5475. The data were analyzed using two-way ANOVA and were represented as  $Mean \pm standard deviation.$ 



<span id="page-5-0"></span>**Fig. 2** FTIR spectral analysis (**a**); UV–Vis spectral analysis (**b**); XRD analysis (**c**)

# **Results**

### **Extraction of polysaccharide from Jicama**

Extraction of polysaccharide was carried out using CCD in accordance with the preliminary experimental conditions where 1.5 W/g and 4 min were taken as the optimized power density and time respectively. The percentage of yield obtained was in the range of 0.36–21.80%.

# **SEM observation**

The structure of the commercial and isolated polysaccharide is shown in fgure (Fig. [1](#page-4-0)a, b). The commercial inulin is spherical or globular in shape (Fig. [1b](#page-4-0)) while the isolated one has a single or fakes shape (Fig. [1](#page-4-0)a) (Terkmane et al. [2016](#page-11-3)).

# **DLS analysis**

The Polydispersity index (PDI) and size of the polysaccharide were found to be 0.4 and 135 nm respectively Fig. [1](#page-4-0)c, d. The size of the commercial inulin was higher than the size of the polysaccharide.

# **FTIR analysis**

FTIR spectra of Chicory inulin and Jicama polysaccharide are depicted in Fig. [2](#page-5-0)a. In the IR spectra of Jicama extract, the characteristic peaks for saccharide are observed at 1020 cm−1, and 2927 cm−1 (Hu et al. [2014](#page-10-8)). The sharp peak was observed at 2927 cm<sup>-1</sup> (Wahyono et al. [2019\)](#page-11-4). A peak is also observed at 1145 cm<sup>-1</sup> (Brugnerotto et al. [2001](#page-10-9)), 1416 cm−1 (Lu et al. [2008](#page-10-10)), 3353 cm−1, and 1635 cm−1. All the peaks found in the polysaccharide were found to be similar to the commercial inulin (Xu et al. [2016\)](#page-11-5).



<span id="page-6-0"></span>**Fig. 3** Raman spectral analysis (**a**); NMR analysis- 1 H NMR of Jicama polysaccharide (**b**), 1 H NMR of Chicory inulin (**c**)

#### **UV–Visible spectroscopy**

For both polysaccharide and commercial inulin, peaks were not observed at the range of 260–280 nm (Fig. [2](#page-5-0)b). This suggests, like the commercial inulin the isolated polysaccharide is free of proteins and nucleic acids.

# **XRD analysis**

X-ray difraction pattern of isolated polysaccharide and commercial inulin was plotted in Fig. [2](#page-5-0)c. Commercial inulin showed the characteristics halo curve (broad peak) which is the indication of an amorphous state. A broad peak at 29° is observed for the commercial inulin depicting its amorphous nature. The Jicama polysaccharide showed sharp difraction peaks at  $2\Theta = 14^\circ$ ,  $18^\circ$ ,  $21^\circ$ , and  $25^\circ$  (Terkmane et al. [2016](#page-11-3)).

### **Raman spectroscopy**

The Raman spectra of the polysaccharide and the commercial inulin are depicted in Fig. [3a](#page-6-0). The absorption bands of the isolated inulin were found similar to that of the Chicory inulin (Huang et al. [2019](#page-10-4)).

### **NMR analysis**

The <sup>1</sup>H NMR signals obtained from the isolated polysaccharide and commercial inulin are depicted in Figure (Fig. [3b](#page-6-0), c) (Wei et al. [2019\)](#page-11-6). The peak positions of the extracted polysaccharide were similar to the commercial inulin.



<span id="page-7-0"></span>**Fig. 4** Flavonoid content (**a**); Phenolic content (**b**); DPPH assay of Jicama polysaccharide (**c**); FRAP assay of Jicama polysaccharide (**d**)

<span id="page-7-1"></span>**Table1** Efect of the glucose, Commercial inulin, and Jicama polysaccharide on increasing bacterial cell density between 0 and 24 h

	Cell density [Log (CFU/ml)]		
Bacteria	Glucose	Commercial inulin	Jicama poly- saccharide
L. plantarum	$3.70 + 0.06$	$4.1 + 0.15$	$3.6 + 0.1$
E. faecium	$3.68 + 0.15$	$5.01 + 0.02$	$4.5 + 0.06$
S. typhimurium <b>NCIM 2501</b>	$4.92 + 0.007$	$1.70 + 0.09$	$1.64 + 0.02$

# **Antioxidant activity of extracted polysaccharide**

#### *Flavonoids and phenolic estimation*

The total favonoid and phenolic content of the extracted polysaccharide were analyzed from the rutin and gallic acid



<span id="page-7-2"></span>**Fig. 5** Prebiotic score of Jicama polysaccharide and Chicory inulin

standard curve (Fig. [4](#page-7-0)a, b) respectively. Jicama extract had the total favonoid (Fig. [4a](#page-7-0)) and phenolic (Fig. [4b](#page-7-0)) content of about  $14.387 \pm 4.192$  mg rutin equivalent/g and  $8.1804 \pm 6.26$  mg gallic acid equivalent/g of dried polysaccharide respectively.

# *DPPH assay*

The antioxidant potential of extracted polysaccharide was indicated as its potential to scavenge the DPPH (Fig. [4c](#page-7-0)). The antioxidant power of extracted polysaccharide is increased along with the dose. With the increasing concentration of the polysaccharide from 1 to 10 mg/ml, the DPPH radical scavenging activity also increased linearly. The percentage inhibition of DPPH free radical of the polysaccharide and commercial inulin at a concentration of 10 mg/ml was calculated to be  $75.74 \pm 4.5\%$  and  $84.78 \pm 1.5$  respectively.

#### *FRAP assay*

The polysaccharide and commercial inulin have has the ferric reducing ability in a dose-dependent manner (Fig. [4](#page-7-0)d). The FRAP activity of the polysaccharide at 10 μg/ml was found to be similar to that of the commercial inulin with  $0.11 \pm 0.007$  and  $0.16 \pm 0.007$  respectively. The obtained OD value is directly proportional to the ferric reducing ability of the inulin.

#### *Prebiotic score*

The cell density (log CFU diference between 24th h and 0th h) of both the probiotics *L*.*plantarum* and *E. faecium* and the enteric pathogen *Salmonella* is shown in Table [1.](#page-7-1) The prebiotic score for the polysaccharide and the commercial inulin was found to be positive indicating the respective prebiotic promoting the growth of the probiotics while suppressing the growth of *Salmonella* is shown in Table [1.](#page-7-1) The prebiotic score of extracted polysaccharide for the probiotic *E. faecium* was  $0.9 \pm 0.07$  and for *L. plantarum* was  $0.63 \pm 0.03$ . Whereas the prebiotic score of the commercial inulin for *E. faecium* was  $1.01 \pm 0.03$  and for *L. plantarum* was  $0.76 \pm 0.04$ . The prebiotic score of the polysaccharide was found to be similar to that of the commercially available inulin (Fig. [5\)](#page-7-2).

#### **Discussions**

The current study establishes the prebiotic potential of the polysaccharide extracted from *P. erosus*. Confrming the shape, both the commercial and the extracted polysaccharide were found to be diferent. Commercial inulin was available as spherical fakes whereas the extracted polysaccharide appeared like fat-fakes. The diference in their shapes could be due to the process of extraction that includes ethanol precipitation resulting in the supersaturation of the inulin medium (Terkmane et al. [2016](#page-11-3)). The size of the polysaccharide was found to be larger than the commercially available inulin. A similar size variation between the commercial and isolated inulin is also reported by Naskar et al. [\(2010\)](#page-10-11). To confrm the functional groups and the structure of inulin, several chemical characterizations like NMR, FT-IR, and Raman spectroscopy was empoloyed From the literature, it is known that inulin is a mixture of glucose, sucrose, and fructose with repeating units (Terkmane et al. [2016](#page-11-3)). The peaks in the range of 3.0–5.2 ppm were identifed to be the fructose and glucose skeleton of inulin (Wei et al. [2019](#page-11-6)). The chemical shift of the peaks obtained from  ${}^{1}H$  NMR spectra was found to be similar with NMR spectra of inulin reported by Qiao et al. ([2016\)](#page-10-12). The peaks at 5.29 ppm of the extracted sample correspond to the peak at 5.25 ppm indicating the signals for inulin (fructosyl – attached glucosyl H1 of sucrose) (Qiao et al. [2016](#page-10-12)). This peak has also been signified as  $\alpha$ - anomeric forms of free glucose (Cerantola et al. [2004](#page-10-13)), since fructan-based polymers (inulin) derived from sucrose, originally containing a single glucose residue (Lopes et al. [2015\)](#page-10-14). The peak at 4.5 ppm and 3.85 ppm correspond with the hydrolysis data of inulin indicating it as glucose (4.49 ppm,  $\beta$ -H<sub>1</sub> of  $\beta$ -D- glucose) and fructose (3.85 ppm, indicating β-H<sub>3</sub> of β-furanose tautomer of D-fructose) respectively (Qiao et al.  $2016$ ). The <sup>1</sup>H NMR confrmed the extracted polysaccharide as inulin. The NMR characterization is further supported by FT-IR which gave similar peaks when compared with the commercial inulin. As per the XRD analysis, the commercially available inulin is amorphous in nature. The isolated polysaccharide showed sharp diffraction peaks at  $2\Theta = 14^{\circ}$ ,  $18^{\circ}$ ,  $21^{\circ}$ , and  $25^{\circ}$  indicating the semi-crystalline state of the sample (Terkmane et al. [2016](#page-11-3)). Adsorbed water content in inulin promotes the transformation of structure from metastable (amorphous) to stable form (crystalline state) (Leyva‐Porras et al. [2017](#page-10-15)). The solids of crystalline nature tend to be more stable as the molecules of the crystalline solids are packed tightly which prevents them from forming lumps (Li et al. [2019](#page-10-16)). Although we found the commercially available inulin is circular in shape, semi-crystalline inulin is also available in the market (Apolinário et al. [2017\)](#page-10-17). The obtained Raman spectra for the current study share similarity with Huang et al. [\(2019](#page-10-4)). The absorption bands of the isolated polysaccharide (1260, 1425, 870, and 500–700) cm<sup>-1</sup> were found similar to that of the commercial one. The peaks obtained were due to C–O–H stretching and bending vibrations, COO− stretching vibrations, C–C or C–O vibrations linked with C-H mode of the anomeric carbon of β-conformers and the crystalline region as mentioned by Huang et al. ([2019](#page-10-4)). The isolated inulin was further checked under UV–Vis spectroscopy. Proteins and nucleic acid peaks were not found at 260–280 nm (Soua et al. [2020\)](#page-11-7). The absence of a peak in these regions suggests

the isolated polysaccharide to be free from proteins and polysaccharide.

To use the extracted polysaccharide as a prebiotic, it is important to check for its antioxidant potential. It has been already accounted that both favonoids and phenolic compounds are chief components associated with the antioxidant activity of polysaccharide (Moro and Clerici [2021](#page-10-18)). Phenolic compounds like daidzein are reported from. This helps to chelate the metal in a physiological state. Phenol decreases the production of a metal ion such as  $Fe<sup>2+</sup>$ . The amount of favonoid also helps to enhance the antioxidant potential. The presence of favonoids like triterpenoid glycoside, kikasponin III, and phaseoside IV was identifed from *P. erosus* (Jaiswal et al. [2021\)](#page-10-0). The presence of a good amount of favonoid in the isolated polysaccharide suggests it could be used as a good antioxidant (Moro and Clerici [2021](#page-10-18)). The DPPH radical scavenging assay is used for the assessment of the antioxidant potential of the polysaccharide. The antioxidant activity of polysaccharide is tested by their potential to neutralize the stable free radical of DPPH. DPPH depicts the highest absorption at the wavelength of 517 nm. This activity was also performed by Shang et al. [\(2018\)](#page-10-7) to understand its scavenging ability. The extracted polysaccharide was also checked for its ferric reducing ability and like DPPH radical scavenging activity, this also showed scavenging activity in a dose-dependent manner but it was observed that in both the cases, absorbance was less than that of the ascorbic acid (Shang et al. [2018\)](#page-10-7). It might be due to the presence of limited reductive hydroxyl group terminals in inulin, and the in vitro antioxidant abilities of polysaccharides that are connected to the reductive hydroxyl group. On other hand, the consumption of inulin increased the in vivo antioxidant activity of CAT, T-AOC, SOD, and GSH-Px in the laying hen's serum (Shang et al. [2018\)](#page-10-7). According to Shang et al*.*, the DPPH free radical scavenging activity of inulin at 10 mg/ml was 20.81%. In the current study Jicama polysaccharide showed  $75.74 \pm 4.5\%$  of DPPH inhibition at 10 mg/ml suggesting it to be a good radical scavanger. The  $IC_{50}$  value of the Jicama polysaccharide was calculated to be  $5.48 \pm 0.4$  mg/ml which was found to be similar to that of the commercial inulin which showed 50% inhibition of DPPH free radical at  $4.53 \pm 0.1$  mg/ml. The current study also checked the ferric ion reducing ability by employing the FRAP assay. The ferric reducing ability of the polysaccharide refects the electron-donating tendency that can serve as the crucial indicator of the antioxidant capacity. The FRAP activity of the extracted polysaccharide showed similar result as reported by Youn et al. [\(2017](#page-11-8)). Higher the FRAP value better it can reduce diabetes (van der Schaft et al. [2019](#page-11-9)).

Apart from this, the sample was also checked for its prebiotic score, as this assay could provide a better idea regarding the efect of the polysaccharide on the growth of the

benefcial bacteria especially the lactic acid bacteria or the probiotics. The prebiotic score further helps to understand the efect of the respective prebiotic on the growth of the bacteria. A successful prebiotic must have the following characteristics like non-digestible and growth infuencer for selective bacteria, especially probiotics. In this assay, the Jicama polysaccharide and Chicory inulin were used as the prebiotic substrates, whereas glucose as non-prebiotic substrate. In the current study, the extracted polysaccharide promoted the growth of the two probiotics as compared to that of the glucose. On the other hand, glucose promoted the growth of the enteric pathogen *S. typhimurium*, while it was suppressed by the polysaccharide which is a key parameter for an efective prebiotic. Our data is in support of the fnding of Hofman et al. who found feeding of inulin promotes the growth of good bacteria and inhibit the growth of harm-ful bacteria (Hoffman et al. [2019](#page-10-19)).

From the above experimental analysis, it was confrmed that the extracted polysaccharide is showing similar structure and prebiotic potential when compared with the commercial inulin. The Jicama polysaccharide was confrmed as inulin from the <sup>1</sup>H NMR analysis. The extracted inulin was also identifed with antioxidant ability that was confrmed by DPPH radical scavenging, FRAP, favonoid, and phenolic content. The prebiotic score of the polysaccharide showed positive and similar results to that of the commercial inulin suggesting its industrial feasibility to be used as a prebiotic in the formation of synbiotic food.

# **Conclusion**

On an industrial scale, Chicory is the highly used abundant source of inulin which has been taken as the positive control in our study. Most synbiotic foods are formulated using Chicory inulin. *P.erosus* is well known for its health-promoting properties (rich in inulin, favonoids) and available world-wide still it is considered as under-utilized and not used as a commercial one by the industries to produce synbiotic food. This may be due to lack of proper investigation regarding its exact inulin content, prebiotic potential or antioxidant property. Thus, the current study was carried out to understand and provide the key informations regarding benefcial efects of *P.erosus*. Since it is available world-wide, the food and other industries could avail it easily for the production of synbiotic food and medicines. There are various products available in the market in the name of probiotics. The Jicama extracted inulin can be used to increase the viability of these probiotics in such products. Also, inulin is known for its anti-diabetic property. Thus, in the future, it can be used in the formulation of such synbiotic products that could eventually lower diabetic medicines.

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**Author contributions** AB: Methodology, data curation, formal analysis, investigation, manuscript writing: SKP: data curation, investigation, formal analysis; PPS: conceptualization, methodology, writing- original draft, writing- review and editing, funding acquisition; MM: conceptualization, methodology, writing- original draft, writingreview and editing, funding acquisition, resources, supervision.

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**Data availability** All data and material regarding this work is completely transparent.

#### **Declarations**

**Confict of interest** The authors declare no competing interests.

**Ethical approval** Not applicable.

**Consent for publication** Not applicable.

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