RESEARCH ARTICLE

Study on physicochemical properties, structural morphology, and prebiotic potential of extracted pectin from a novel source: Bael pulp (*Aegle marmelos*) residue

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The production of a large volume of agricultural residue during the processing of fruits has resulted in environmental problems. Therefore, the production of bioactive compounds from agricultural residue will provide a direction to overcome these issues. These residues are a potential source of many bioactive compounds, such as pectin. The present study deals with the extraction and characterization of pectin from the Bael pulp residue produced during the processing of Bael fruit. Pectin was extracted by using hot acid extraction methods and evaluated for its physicochemical properties by using Fourier transform infrared spectroscopy (FTIR), scanning electron microscope (SEM), X-ray diffractometer (XRD), and the values of moisture content, ash content, equivalent weight, methoxyl content, total anhydrouronic acid content, and degree of esterification. The results were compared with those of commercial pectin. Bael pulp residue yielded 24.02 ± 0.63% of pectin by using HCl and 22.1 ± 0.7% by using H_2SO_4. The physicochemical characterization of extracted pectin, such as equivalent weight (852.32 ± 8.43), methoxyl content (8.28 ± 0.35%), total anhydrouronic acid (67.69 ± 0.35%), and degree of esterification (69.39 ± 0.57%), was within the acceptable range and comparable with the commercial pectin. The values of moisture content (4.62 ± 1.14%) and ash content (5.22 ± 0.66%) indicated that the extracted pectin was in pure form. SEM and XRD patterns observed the rough and amorphous nature of extracted pectin. The antioxidant activity of pectin and total phenolic content were also observed. The IC_{50} value was found to be 15.74 µg/mL, showing an adequate amount of antioxidant activity in isolated pectin with a total phenolic content of 58.5 mg Gallic acid equivalent (GAE)/g. Additionally, the prebiotic potential of extracted pectin was confirmed by the viability assay of two probiotics (*Bacillus clausii* and *Saccharomyces boulardii*). Based on these results, Bael pulp residue could be a good alternative source of high-value pectin that could be used to make health products and other biological applications.

Keywords: antioxidant activity; Bael pulp residue; FTIR; pectin; prebiotic; SEM; XRD.

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Introduction

During the processing of fruits, a large number of solid wastes are produced, which are a potential source of bioactive compounds and are generally discarded by food processing industries and lead to many environmental problems. The maximum food waste is produced by beverage industries [1]. The proper utilization of these solid wastes can not only expand the number of sources for numerous valuable nutraceutical and bioactive substances but also aid in the eradication of

environmental problems.

Bael also known as *Aegle marmelos* is one of the traditional, underutilized, medicinal, and nutritive fruits, majorly cultivated in the southeast region of Asia [2]. The edible portion of Bael fruit is processed for the production of various products like jam, squash, refreshing beverages, toffee, and syrup [3]. Bael fruit juice is a traditional drink with lots of nutritive value and rejuvenates the body with its cooling effect. The processing of Bael fruit juice generates a large amount of non-edible Bael pulp waste/residue, and unsystematic dumping and burning of these residues may create many environmental problems. The Bael pulp leftover includes several beneficial biomaterial and nutraceutical chemicals, as well as a high concentration of antioxidants and dietary fibers [4], and it has significant potential to be utilized as a valuable resource by extracting these crucial compounds from the residues. It was reported that Bael pulp contains approx. 8.8 g of pectin per 100 g. Pectin is one of the essential natural biomaterials present in Bael pulp residue. Pectin is a valuable bioactive polysaccharide used as gelling agent and stabilizer in the food and pharmaceutical industry [5]. The limited sources of commercial pectin make it difficult to achieve the high demand for pectin which is increasing from time to time. It is estimated that the consumption of pectin may increase up to 48,735 metric tons by the year 2026 [6]. So, proper utilization of Bael pulp waste may become one of the potential sources of pectin while also reducing environmental concerns. The structure and characteristics of pectin are based on the source and method used for the extraction of pectin [7]. The variation in properties increases the application of pectin to be used in food, cosmetic, pharmaceutical, and nutraceutical industries, and also used as a natural edible coating in packing perishable foods [8].

Hot acid extraction is the most convenient and widely used technique for the industrial production of pectin [9]. The international pectin producer association also describes that pectin is produced by using mineral acids as a processing aid in water [10]. Apparently, no data is available on the physicochemical characterization of pectin together with its structural, functional group analysis, and prebiotic potential extracted from Bael pulp residue. In order to give information on the distinctive properties of pectin that can be used as a functional bioactive ingredient in the nutraceutical, medicinal, and non-medicinal industries, the current study intended to extract the most valuable and demanding biomaterial pectin from the novel source Bael pulp residue. It also planned to evaluate the antioxidant activity, total phenol content, and structural morphology by using Fourier-transform infrared spectroscopy (FTIR), scanning electron microscope (SEM), and X-ray diffractometer (XRD), as well as prebiotic potential.

Materials and methods

Sample collection

Bael pulp residue was collected from the local Bael juice stall in Delhi, India. Collected pulp residue was washed thoroughly and strained for a few hours in open to eliminate excess water and then was placed in a hot air oven maintained at 60°C for 22-24 hours for complete removal of moisture followed by milling into fine powder by using a mixer grinder. The powdered sample was kept in a small air-tight container and stored at 3 to 5°C for further use.

Extraction of pectin

Pectin from Bael pulp residue was isolated by using hot acid extraction method described by Maskey, *et al*. [11] with a slight alteration. Two different acids, 1 M HCl and 1 M H_2SO_4, were used to identify the effect of particular acid on the final extract. Initially, water was brought to a boil and maintained at 90°C in the heating mantle flask. The powdered sample was added in a 1:6 ratio in hot water followed by particular acid and brought the pH of the solution to 1.5. The solution was continuously agitated for 60 min and maintained at 90°C on the heating mantle.

The solution was cooled down instantly by using an ice water bath and brought to 50°C followed by filtration using three layered muslin cloth. For maximum precipitation of pectin, 1:2 volume of 96% ethanol was mixed with the filtrate and kept overnight. The precipitate was obtained by filtration process using cheesecloth and washed with 70% ethanol followed by absolute alcohol to eliminate impurities. The purified pectin was kept in a hot air oven at 50°C till the complete removal of moisture before being ground into a fine pectin powder. The fine pectin powder was packed in small airtight pouches and stored at 4-6°C for experimental use. The following equation was used to calculate the Yield % of isolated pectin:

$$\text{Pectin yield \%} = \frac{\text{Weight of dried pectin powder (g)}}{\text{Weight of Sample (g)}} \text{ X } 100\%$$

Physicochemical characterization of pectin powder

(1) Equivalent weight (EW):

The EW of isolated pectin was determined by the method of Ranganna [12]. In a nutshell, 5.0 g of pectin was kept in a beaker and moistened with 5 mL of ethanol. 1.0 g of NaCl was mixed with a moist solution followed by 100 mL of distilled water. Further, six drops of phenol red indicator were added to the mixture and titrated against 0.1 N NaOH until the color of the mixture turns pink and remained for 30 sec. The EW was calculated by using the below-given formula:

$$\text{EW} = \frac{\text{Weight of the sample (g)} \times 1000}{\text{Volume of alkali (mL)} \times \text{Normality of alkali}}$$

(2) Methoxyl content (MeO):

The MeO of pectin was determined by the method of Ranganna [12] with some modifications. 25mL of 0.1 N NaOH was mixed with the neutral solution obtained from equivalent weight (EW) determination. The mixture was stirred and kept undisturbed for 30 min. After 30 min, 25 mL of 0.1 N HCl was poured slowly into the mixture with continuous stirring and titrated against 0.1 N NaOH to a similar endpoint marked in EW determination. The MeO of the sample was determined by using the following formula:

$$\text{MeO (\%)} = \frac{\text{Volume of alkali (mL)} \times \text{Normality of alkali} \times 31}{\text{Weight of the sample (g)} \times 1000} \times 100\%$$

(3) Total anhydrouronic acid (total AUA):

The titter of EW and MeO was used to calculate the total AUA content of isolated pectin by using below given equation [13].

$$\text{AUA \%} = \frac{(176 \times 0.1z \times 100\%) + (176 \times 0.1y \times 100\%)}{W \times 1000}$$

where 176 is the molecular unit of AUA (1 unit) in gram. z is the titer of EW in mL. y is the titer of MeO in mL. W is the weight of the Bael pulp residue pectin.

(4) Degree of esterification (DE):

The values of methoxyl (MeO) and total AUA were used to determine the DE of isolated pectin by using the below given equation [13].

$$\text{DE\%} = \frac{176 \times \text{MeO\%}}{31 \times \text{AUA \%}} \times 100\%$$

Moisture content and ash content:

The moisture and ash contents of Bael pulp residue pectin were determined by using the Association of Official Analytical Chemists (AOAC) method [14].

(1) Moisture content:

Approximately 1 g of pectin sample was placed on a dried clean pre-weighted dish. The sample was kept in a hot air oven at 105°C for overnight and weighed after cooling in a desiccator. The moisture content was calculated by the following equation:

$$\text{Moisture content (\%)} = \frac{\text{weight of dried sample}}{\text{weight of pectin}} \times 100\%$$

(2) Ash content:

Approximately 2 g of pectin sample was placed into a tared crucible and kept in a muffle furnace for 4-5 hours at 550°C and weighed after cooling in a desiccator. The ash content was calculated by using the following formula:

$$\text{Ash content (\%)} = \frac{\text{weight of ash}}{\text{weight of pectin}} \times 100\%$$

Antioxidant activity

The method given by Hendel, *et al*. [15] was used to determine the antioxidant activity of isolated pectin with slight modification. Briefly, 2 mL of 0.1 mM 2,2-Diphenyl-1-picrylhydrazyl (DPPH) solution was added to 2 mL of sample solution of different concentrations and incubated at room temperature for 30 min. The methanol was used as a control and the absorbance was taken at 517 nm by using Shimadzu UV 1800 UV-Visible spectrophotometer (Shimadzu, Kyoto, Japan). The percentage of inhibition (I %) was calculated by using the following formula.

$$\text{I \%} = \frac{A_0 - A_1}{A_0} \times 100\%$$

where A_1 is the absorbance of the sample and A_0 is the absorbance of a control. The linear regression curve was used to calculate the IC_{50} level of the samples.

Total phenolic content (TPC)

The TPC of Bael pulp residue pectin was obtained by using the method of McDonald, *et al*. [16]. Briefly, 0.1 mL of 0.5 M Folin-Ciocalteu reagent was added to 0.5 mL of extract solution and incubated for 15 min. Further, 2.5 mL of saturated sodium carbonate was mixed with the solution and incubated at room temperature for 30 min. The absorbance was recorded at 760 nm by using Shimadzu UV 1800 UV-visible spectrophotometer (Shimadzu, Kyoto, Japan). TPC was expressed in terms of Gallic acid equivalent mg/g of the sample on a dry basis.

Fourier Transform Infrared spectroscopy (FTIR) analysis

The functional group of Bael Pulp residue pectin was determined by using a Bruker Alpha FT-IR spectrophotometer (Bruker, Germany). The FTIR spectra were observed by using attenuated total reflectance (ATR) mode in the frequency range between 4,000−600/cm.

Scanning Electron Microscopy (SEM)

SEM imaging was analyzed by using a high-resolution field emission scanning electron microscope (HR- FESEM) (Sigma VP, Zeiss, Germany). An accelerating voltage of 5 kV was used to capture an image of the nanostructure of Bael pulp residue pectin.

X-ray diffractometric analysis (XRD)

The X-ray diffraction (XRD) patterns were acquired by an X-ray diffractometer (Rigaku Smart Lab Guidance, Rigaku, Japan). X-Ray Diffractometer was used to perform a diffraction analysis of extracted pectin and record the intensity and diffracted angle of x-ray radiation. The scanning level was 2°/min at a 2θ diffraction angle which ranged from 5 to 90.

Prebiotic potential of pectin

The prebiotic effect of Bael pulp residue pectin was determined by evaluating the viability of two probiotics (*Bacillus clausii* and *Saccharomyces boulardii*) at different concentrations of pectin. Briefly, the *Bacillus clausii* and *Saccharomyces boulardii* were isolated from Enterogermina capsule (Sanofi S.p.A, Origgio, Varese, Italy) and Econorm capsule (Biocodex, Beauvais, France), respectively. The cultures were prepared by the method described by Sarwar, *et al*. [17]. The cells were harvested from the culture by centrifugation at 10,000 rpm at 4°C for 5 min. The supernatant was discarded, and the cell pellet was suspended in 1 mL of autoclave water for further use. Nutrient agar (sodium chloride, peptone, beef extract, agar, and distilled water) and potato dextrose agar (potato, dextrose, agar, and distilled water) were prepared with different concentrations of Bael pulp residue pectin (0.1-0.5%) to examine the growth of *B. cluasii* and *S.*

boulardii, respectively, and compared with control (without pectin).

Statistical analysis

Statistical significance of the variables viz., equivalent weight, methoxyl content, total anhydrouronic acid, and degree of esterification were evaluated through one-way analysis of variance (ANOVA) at $P < 0.05$ level by using *IBM® SPSS®* Version 25 (IBM, Armonk, New York, USA). The data was expressed as the mean ± standard deviation (SD) of the triplicate.

Results and discussion

Extraction of pectin

Heating the plant sample in hot acidified water is the most frequent method for isolating pectin from plant sources. Hydrochloric acid and sulphuric acid were applied for the pectin extraction from Bael pulp residue. The extraction process resulted in light brown, odorless, fine powder of pectin from Bael pulp residue. The hot acid extraction process yielded 24.02 ± 0.63% of pectin by using 1 M HCl and 22.1 ± 0.70% of pectin by using 1 M H_2SO_4, respectively, by maintaining a constant temperature of 90°C for 60 min. The yield was slightly higher in HCl as compared to H_2SO_4. Higher ionic strength acids have a better ability to precipitate pectin because of their increased affinity for cations like Ca^{2+} which stabilizes the pectin molecule. There is no data exist on the amount of pectin isolated from Bael pulp residue. The pectin isolated from mango peel, banana peel, and apple pomace yielded 8.8%, 2.8%, and 12.5% of pectin, respectively, by using the conventional HCl method [18], which were all lower than that of pectin yielded from Bael pulp residue. The maximum yield was reported in citrus peel at 25.5% [18]. Other research found that dragon fruit peel yielded 6.27% of pectin [19], and watermelon rind yielded 12% of pectin [20]. Pectin could also be extracted from many other organics as well as inorganic acids. However, HCl is the most widely used reagent because it is cost-effective as compared to other organic acids [21].

Physicochemical characterization of pectin

The chemical properties of Bael pulp residue pectin were evaluated based on different parameters shown in table 1. The chemical characteristics of pectin can vary depending on the source, solvent, and method used for extraction [22]. The EW of pectin indicates the gelling properties of pectin. High EW implies more gel-forming abilities [23]. The EW of Bael pulp residue pectin was found to be 852.32 ± 8.43 which was slightly lower than that of obtaining from commercial pectin (931.74 ± 10.08) and lies between the range 368 to 1,632 reported by Azad, *et al*. [24] in lemon pomace pectin and higher than the EW of pectin extracted from cocoa husk (510.68 to 645.19) [25]. Pectin with high methoxyl content indicates high spreading capacity and sugar-binding potential [26]. The MeO of pectin usually ranges between 0.2-12% based on the source used and the isolation techniques [27]. The MeO of isolated pectin was found to be 8.28 ± 0.35% which was slightly higher than that of commercial pectin. The methoxyl content of extracted pectin was higher than that of dragon fruit peel pectin which ranged from 2.98-4.34% [28] and mature lemon pomace pectin (4.24%) [24], and almost consistent with the methoxyl content of banana-papaya peel pectin (8.37%) [29] and mango peel pectin (8.89%) [30]. The food chemical codex [31] suggested that the total AUA (anhydrouronic acid) content of pectin should not be less than 65%**.** The total anhydrouronic acid (AUA) of isolated pectin was found to be 67.69 ± 0.40% which indicated the purity of extracted pectin and relatively comparable to AUA content of pectin isolated from different varieties of pomegranate peel ranged from 47.71 to 68.51% [32] and citrus peel ranged from 51.04 to 67. 89% [33], and also similar to the AUA content of banana-papaya mixed peel pectin (69.97%) [29]. The AUA content of commercial pectin was found as 64.29 ± 0.53%. The degree of esterification (DE) of pectin can vary depending upon fruit, ripeness of fruit, and extraction technique [34]. DE describes the gel-forming ability of pectin and its application in the food industry [35] as DE > 50% defined as high MeO pectin and DE < 50%

Table 1. Physicochemical characteristics of Bael pulp residue pectin and commercial pectin.

Parameters	Bael Pulp Residue Pectin	Commercial Pectin
Equivalent weight (EW)	852.32 ± 8.43[b]	931.74 ± 10.08[a]
Methoxyl content (MeO %)	8.28 ± 0.35[a]	7.99 ± 0.62[b]
Total anhydrouronic acid (AUA %)	67.69 ± 0.40[a]	64.29 ± 0.53[b]
Degree of esterification (DE %)	69.39 ± 0.57[b]	70.61 ± 0.11[a]

Note: values were expressed as means of triplicate measurements. Means with different alphabets (a: higher; b: lower) in the same row were significantly different at $P < 0.05$.

defined as low MeO pectin [36]. The isolated pectin with the DE value of 69.39 ± 0.57% could be used as high MeO pectin which was comparable to the DE of commercial pectin (70.61 ± 0.11%). The DE of pectin extracted from varied types of banana peels was between 63.15-72.23% [37] and banana-papaya peel pectin was 67.91% [29]. A significant difference was observed ($P < 0.05$) among all the parameters between Bael pulp residue pectin and commercial pectin. The statistical means of EW and DE of Bael pulp residue pectin were significantly lower than that of commercial pectin, while the statistical means of MeO % and AUA % of Bael pulp residue pectin were significantly higher than that of commercial pectin. Similarly, Khamsucharit, *et al*. also observed a significant difference ($P < 0.05$) among all the parameters between varied types of banana peels, citrus peel, and apple pomace pectin [37].

Purity of pectin

The moisture content of any product describes the shelf life of that product. Low moisture content implies to longer shelf life by decreasing the microbial activity, which may degrade the pectin quality [38]. High water content increases microbial growth, which may stimulate the production of pectinase enzyme that can decrease the value of pectin [39]. For longer shelf life and carefree storage, the moisture content of pectin should not be more than 12% [40]. The moisture content of extracted pectin was found to be 4.62 ± 1.14%, which indicated that extracted pectin had a longer shelf life and low water content as compared to many other reported values such as mixed banana-papaya peel pectin (7.2%) [29], papaya peel pectin (7.3%) [34], banana peel pectin (8.94%), mango peel pectin (8.82%) [30], different citrus peel pectin (6.4-10%) [41], white dragon fruit peel pectin (5.02-7.3%) [42], and similar to Vietnamese mangosteen rind pectin (4.64%) [43]. The ash content indicates the presence of inorganic contaminants in the product. According to IPPA [10], the acceptable range of ash content is > 10% to define its purity level. The ash content in the present study was 5.22 ± 0.66%, which was under the acceptable range of purity. Many researchers reported more or less ash contents as compared to the present study, which might be because of different sources of pectin and extraction techniques. However, the present value was almost consistent with the ash content of Indonesian mangosteen rind pectin (3.91 ± 0.17%) [44], cocoa peel pectin (1 to 5%) [38], orange peel pectin (2.45 - 6.66%) [45], and papaya peel pectin (4.8%) [34]. Some other researchers reported 1.2-3.2% of ash content in soya hull pectin [46] and 3.3% in lemon peel pectin [47]. Hence the moisture and ash contents of Bael pulp residue pectin found in the present study were considered pure and good-quality pectin.

Antioxidant activity (DPPH Assay)

The antioxidant activity of extracted pectin was determined by using the DPPH method. In this method, the antioxidant compound scavenged the free radical of DPPH due to which the color of the sample changed from purple to yellow [48]. The antioxidant activity of standard ascorbic acid and Bael pulp residue pectin were determined in different concentrations and illustrated in Table 2. The results showed that inhibition percentage

Table 2. Antioxidant activity of ascorbic acid and Bael pulp residue pectin.

Concentration (µg/mL)	Ascorbic Acid		Bael pulp residue pectin	
	% Inhibition	IC_{50}	% Inhibition	IC_{50}
12.5	51.95 ± 0.94	4.96 ± 1.28	48.17 ± 0.12	15.32 ± 0.40
25.0	54.63 ± 0.73		54.43 ± 0.18	
50.0	63.33 ± 0.30		61.38 ± 0.07	
100.0	81.42 ± 0.93		70.60 ± 0.24	
150.0	90.24 ± 1.21		90.93 ± 0.25	

of extracted pectin was found to increase with the increase in concentration and maximum at 150 µg/mL with 90.93 ± 0.25% scavenging activity. The IC_{50} value of Bael pulp residue pectin was calculated by the linear regression method and found to be 15.32 ± 0.40 µg/mL, which implied that Bael pulp residue pectin had an adequate amount of antioxidant activity. The lower the IC_{50} indicated higher scavenging activity. However, sour orange peel pectin also has approximately 90% of inhibition at 25 mg/mL concentration [49], whereas fig pectin has 79.8% of scavenging activity at 20 mg/mL [50].

Total phenolic content (TPC)

The TPC of Bael pulp residue was estimated by the Folin-ciocalteu method. The phenolic compound of any extract holds redox properties and is responsible for scavenging activity [51]. The result was derived from a calibration curve of Gallic acid having R^2= 0.985. The TPC of isolated pectin was 56.83 ± 2.88 mg Gallic acid equivalent per gram (mg GAE/g) of pectin powder. The literature reported the variation in TPC of the pectin from a different source such as persimmon waste streams pectin had 53.3 ± 2.27 to 111.7 ± 9.4 mg GAE/g [52] of TPC, which was almost similar to extracted pectin. However, *Tamarindus indica L*. pectin contained 79.66 ± 4.71 mg GAE/g [53] of total phenolic content.

FTIR spectral analysis of extracted pectin

The functional group identification of Bael pulp reside pectin and commercial pectin was accomplished by using Fourier transform infrared spectroscopy (Figure 1). All polysaccharides showed visible peaks approximately at 3,400, 2,939, and between 990-1,200/cm, representing O-H stretching, C-H stretching of CH, CH_2 and CH_3, saccharides, respectively [54]. The extracted pectin showed the major peak at 3,281/cm, which corresponded to the O-H group. Similarly, one major peak at 3,309/cm has been observed in pomegranate peel pectin due to inter and intramolecular hydrogen bonds caused by O-H stretching [29]. Another major peak of extracted pectin was observed at 2,923/cm corresponding to C-H stretching and bending vibration of CH_2 groups. Similarly, peaks at 2,919, 2,920, and 2,929/cm have been identified in *Terminalia* puree pectin, *Terminalia* powder pectin, and apple pectin, respectively [55], and peak at 2,925/cm has been found in mixed banana-papaya peel pectin [26].

The extracted pectin demonstrated a strong band centered at about 1,738/cm assigned to stretching of the C=O ester carbonyl group, which was similar to the commercial pectin that also showed a band at 1,729.28/cm. The second band of extracted pectin was observed at around 1,630/cm corresponds to stretching mode of COO^- carboxylate ion [56]. *Terminalia* puree and powder pectin showed two major peaks at 1,735 and 1,619/cm, and apple pectin demonstrated at 1,732 and 1,608/cm [55]. The mixed banana-papaya peel pectin also showed a peak centered at 1,740/cm [26], and a pomegranate peel pectin peak centered at 1,731 and 1,619/cm [29]. The quince pectin spectrum showed a less intense band at 1,384/cm corresponding to symmetrical stretching vibrations due to the COO^- group of polygalacturonic acid [57], which is similar to the extracted pectin with a less intense band approximately at 1,368/cm.

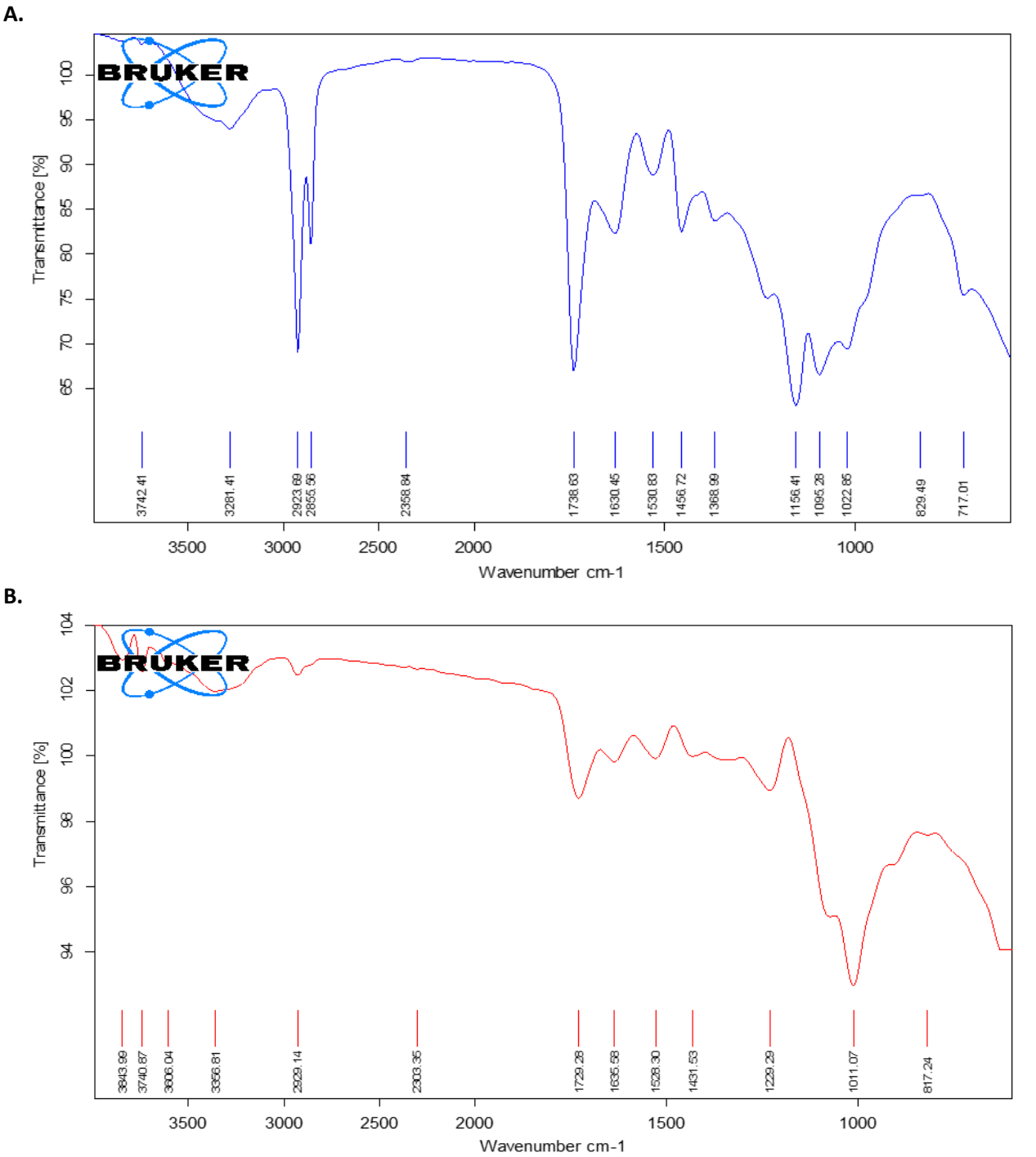

Figure 1. Fourier transform infrared spectroscopy (FTIR). **A.** Bael pulp residue pectin. **B.** commercial pectin.

The regions within 800-1,200/cm are assigned as 'fingerprint' regions and notify complex and unique functional groups of specific carbohydrates [58]. Thus, it is difficult to assign any particular vibration in this region [55, 59]. All peptic polysaccharide corresponds mainly by the bands at 1,104 and 1,000/cm, which shows the presence of galacturonic acid [57]. The extracted pectin showed the band approximately at 1,095 and 1,022/cm, while commercial pectin was at

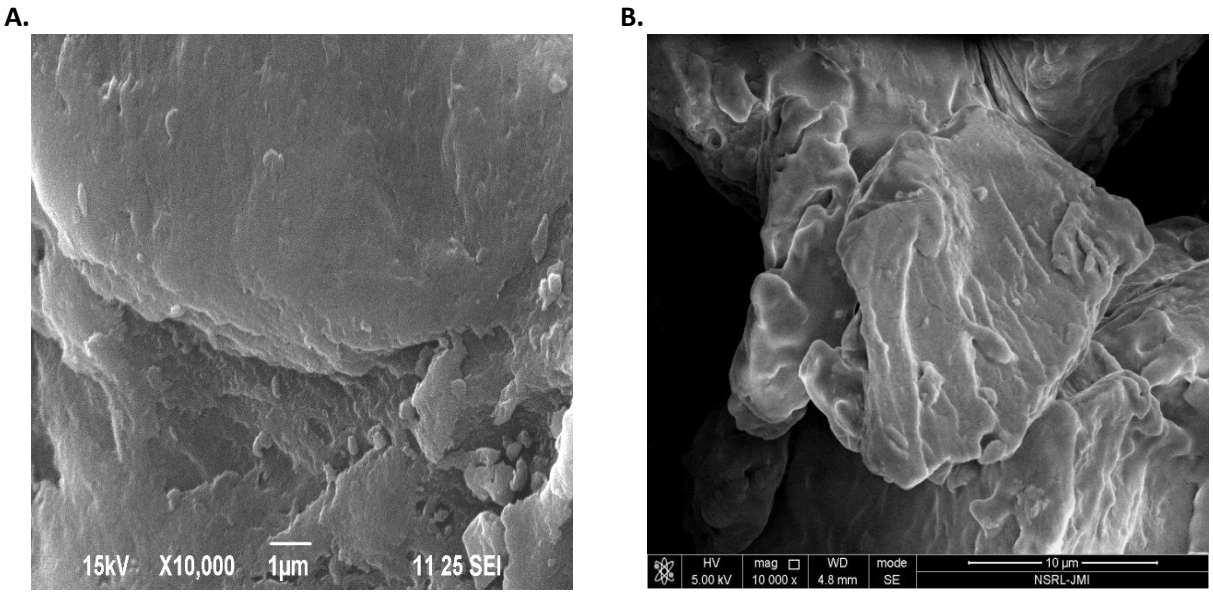

Figure 2. Scanning electron microscopy (SEM) images. **A.** Commercial pectin. **B.** Bael pulp residue pectin.

1,011/cm. The *Terminalia* puree and powder pectin also showed the major bands around 1,019/cm, while apple pectin showed the band at 1,011/cm [55]. The result suggested that the FTIR spectra of isolated pectin were similar to the commercial pectin and were consistent with the literature data. The fingerprint region suggested that extracted polysaccharide was pectin.

Scanning electron microscopy (SEM) analysis of extracted pectin

The SEM technique was used to analyze the nanostructure of Bael pulp residue pectin and compared with the commercial pectin (Figure 2). The surface of commercial pectin was smoother than that of Bael pulp residue pectin. The SEM image of extracted pectin demonstrated the roughness of the surface and relatively smaller particle size in contrast with commercial pectin. Commercial pectin may show different structural morphology due to the addition of sugar which modifies its structure [44]. This result was consistent with the micrograph of mango peel pectin [60], mangosteen rind pectin [44], and fig pectin [50]. The irregularities in particle size were also observed in jackfruit slimy sheath pectin [61].

X-ray diffractometric (XRD) analysis of extracted pectin

XRD diffractogram of the extracted pectin was presented in Figure 3. The XRD diffractograms technique is used for the structural analysis of biomolecules. The sharp peaks in XRD analysis indicate the crystalline nature of the product, whereas the broad background pattern indicates the amorphous nature of the product [62]. In the present study, the XRD pattern showed that the pectin extracted from the Bael pulp residue was more amorphous and less crystalline due to the broad background pattern, which was similar to the XRD of *Persea americana* peel pectin that also showed the pattern of the broad peaks due to the high amount of carboxylic group presented in galacturonic acid, which indicated the amorphous nature of pectin [63]. The amorphous structural nature has also been detected for pectin extracted from waste of golden variety of apple (*Spondias dulcis*) [64]. Non-crystalline XRD pattern has also been determined for pectin extracted from the common fig (*Ficus carica L*.) skin [50], mangosteen (*Garcinia mangostana L*.) rind [44], melon rind, pomegranate, and orange peel [65].

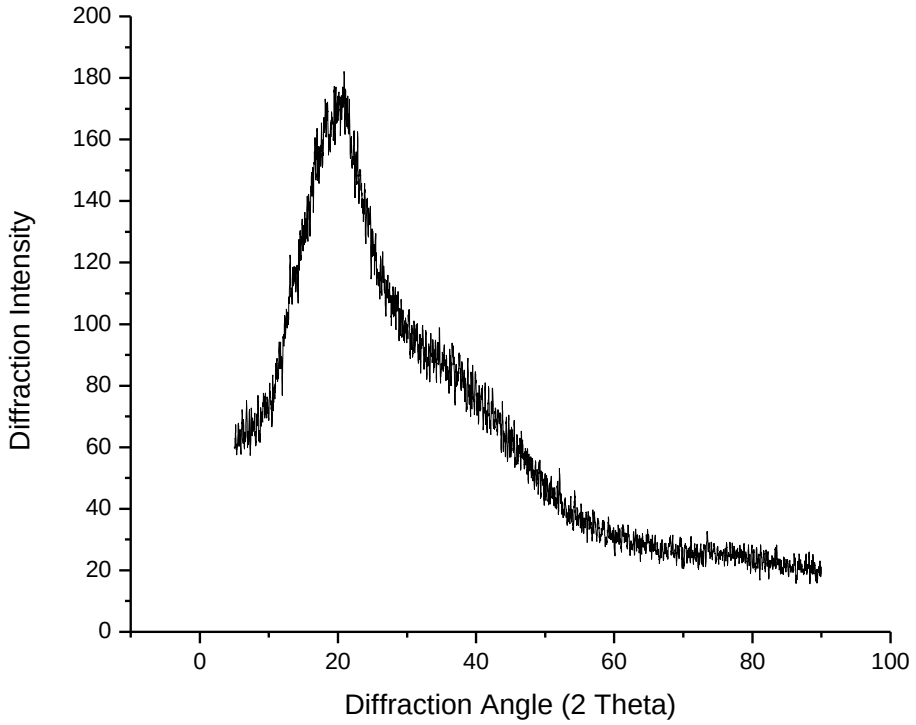

Figure 3. X-ray diffraction of Bael pulp residue pectin.

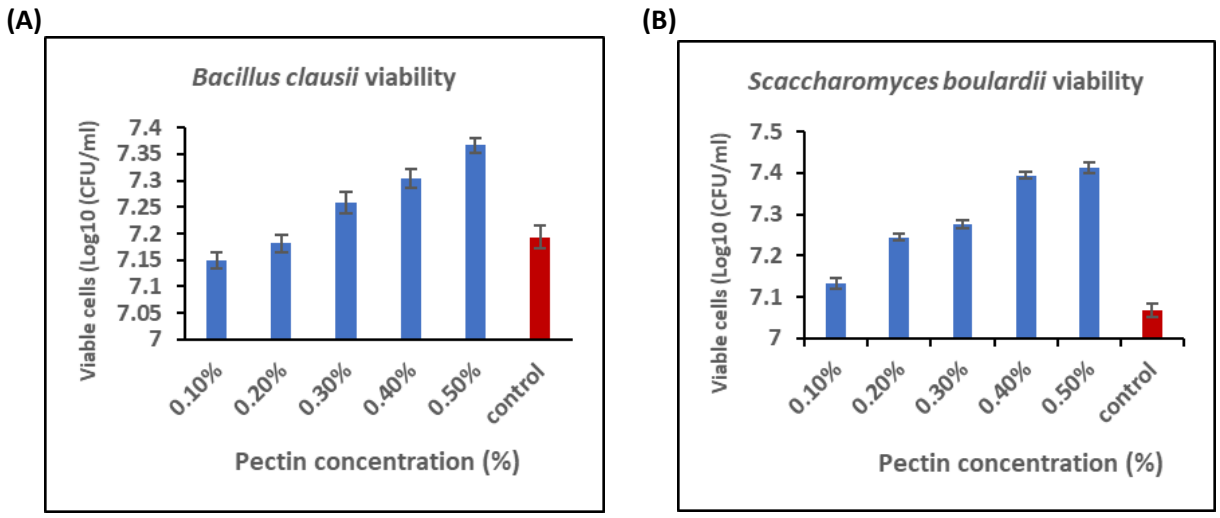

Figure 4. Viability of **(A)** *B. clausii* and **(B)** *S. boulardii* on different concentration of pectin and compared with control (without pectin).

Bael pulp residue pectin as prebiotic

The effect of different concentrations of Bael pulp residue pectin on viable cells was reported in Figure 4. The viabilities of *B. clausii* and *S. boulardii* were improved by increasing the concentration of pectin. There was a slight difference in the number of viable cells observed in *B. clausii* and *S. boulardii*. This implied that the Bael pulp residue pectin could stimulate the growth and multiplication of the probiotic cells and could be used as a potential prebiotic in functional foods.

Conclusion

Even though pectin is found in a wide variety of plant species, commercial pectin sources are scarce. As a result, new sources of pectin must be explored to obtain pectin of desired quality attributes. In the present study, the underutilized Bael pulp residue, a new source, was used for the extraction of highly valuable and natural polysaccharide pectin and various parameters were used to authenticate the quality characteristics of pectin. Our study supports the first-time utilization of Bael pulp residue for pectin extraction. The results showed that hot-acid extraction by using HCl at constant temperature (90°C), time (60 min), and pH (1.5) had more yield as compared to H_2SO_4. In these conditions, Bael pulp residue pectin was categorized as high methoxyl pectin. Also, total anhydrouronic acid confirmed the purity of pectin along with the moisture and ash contents of extracted pectin. The adequate antioxidant activity and total phenolic content were attributed to biomedical applications. Additionally, XRD and SEM analysis explained the amorphous nature and rough surface of pectin, respectively. The FTIR spectral pattern was also comparable with commercial pectin. The effect of pectin on the viability of *B. clausii* and *S. boulardii* validated the prebiotic potential of isolated pectin. The antioxidant activity of Bael pulp residue pectin as well as its physicochemical and structural studies suggested that it could be used as a functional, new high-value bioactive ingredient in the food, pharmaceutical, and cosmetic industries.

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