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RESEARCH ARTICLE

Extraction and Characterization of Pectin from Orange Peel

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Abstract

Fruit and vegetable processing waste contributes to around 30% of the total weight of the fruits and vegetables. These wastes can be converted into valuable goods for potential benefits. Therefore, this study was focused on extracting pectin from agricultural waste (orange peels) using two methods: alcohol precipitation and enzymatic extraction. The extracted pectin was compared in terms of various properties. Alcohol precipitation resulted in higher yield percentages (16.99 ± 0.8 % wet; 6.46 ± 1.01 % dry respectively) compared to the enzymatic method (16.84 ± 0.42 % wet; 3.66 ± 0.34 % dry, respectively). FT-IR and XRD spectra indicated the presence of highly methylated semi-crystalline pectin in both methods with potent antioxidant property (40.57%). This research work provides comparative extraction method of pectin from agricultural waste i.e. orange peels which has diverse applications in the food, pharmaceutical industries and biomedical applications.

Keywords: Pectin; Alcohol precipitation; Enzymatic extraction; Esterification.

Introduction

Pectin is a high molecular weight polysaccharide contributes structural integrity to the cell wall as well as cell adhesion in plants [1]. It is water-soluble, chelator-soluble, or protopectin. Adetunji *et al.*, [2] explained that D-galacturonic acid (GalA), which is a modified form of D-galactose, serves as the primary building block for the complex pectin molecule [2]. The pectin chain, α-D-galacturonans, consists largely of D-galacturonic acid linked by α- (1→4) linkages [3] (Figure 1). The carboxyl groups of pectin are partially esterified with methanol and the hydroxyl groups leading to differences in the degree of methyl esterification (DE or DM) are partially acetylated with acetic acid [4].

Pectin is widely used in food systems as emulsifying, stabilizing and thickening agent. Besides of its technological applications, this polysaccharide has numerous health benefits, which lead to an increase in global demand for it [5].

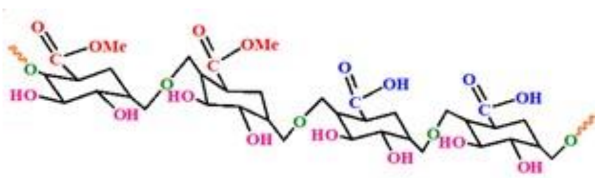

Figure 1: *Pectin a polymer of a- galacturonic acid with a variable number of methyl ester groups*

The application possibilities of pectin are very wide and numerous, ranging from the major categories of food applications, and the industrial and pharmaceutical sectors. The usages of pectin are mainly divided into three categories: food sector, health and pharmacy, and food packaging [6]. It is well known that peel of citrus fruits contains maximum amount of pectin than any other fruits or vegetable therefore orange peel was used in this study.

Hence, the aim of this study was to compare the amount of pectin that can be extracted from orange peel, which is thrown as waste material, by alcohol precipitation and enzymatic method then investigate their characteristics.

Materials and Methods

Materials

Orange peel powder, 99 % ethanol (Changshu Hongsheng Fine Chemical Co. Ltd), cellulase enzyme (Fizmerk India Chemicals), HCl (Merck Life Science Pvt. Ltd.), cheesecloth, sodium azide (Thermo Fisher Scientific, India), citric acid (HiMedia Laboratories Pvt. Ltd.), buffer solution (pH 4) (HiMedia Laboratories), distilled water (Marech Pvt. Ltd.),

Material Preparation

400 g orange peels were collected from local market (Gorkhali fruits and juice center), Sorakhutte, Kathmandu, Nepal. The peels were cut into smaller pieces of 5 cm length and then washed with large amount of water to remove the glycosides, the bitter taste of peels. The pieces were then air dried, ground with the help of Nima NM-8300 Mini portable electric mixer grinder (2 blade) and sieved through sieve (600 µm) to obtain 385 g dry, powdered orange peels [7]-[9]. The peel powder was then divided into two parts (A and B) to carry out alcohol precipitation method and enzymatic precipitation method **(Fig. 2a, b)**.

Alcohol Precipitation Method

Pectin was extracted from dried orange dried peel powder by following the protocol mentioned in Ref. [7] **(Fig. 2a).** 30 g of dried peel powder was taken in a different beaker (1000 mL) containing 500 mL of distilled water and HCl was added to maintain pH 3 in the beaker. The mixture was then boiled using Bunsen burner for 1 hour, filtered through cheesecloth then filtrate was precipitated with 200 mL of 99 % ethanol, stirred, left for 30 minutes to allow the pectin to float on surface. After that, the floating pectin flocculent was separated from ethanol and water by filtering through cheesecloth. Then after the weight of extracted pectin was taken and pectin was dried in oven (Tanco) at 60 ℃ for 5 hr. Afterwards each dried pectin was powdered by using pestle and mortar which was later weighed to obtain dry pectin powder. The procedure was repeated thrice to obtain three different amounts of pectin.

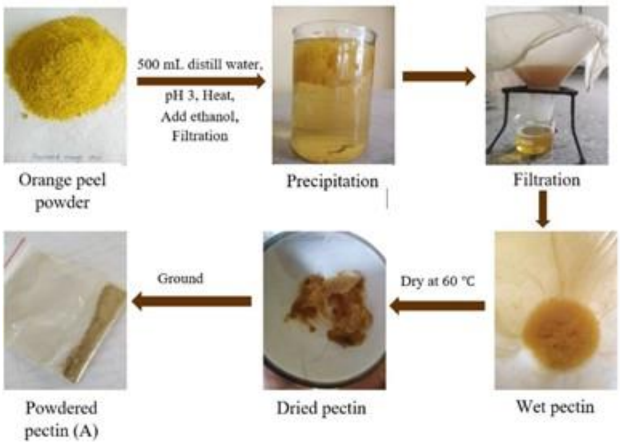

Figure 2a: *Flow chart of pectin extraction by alcohol precipitation method.*

Enzymatic Precipitation Method

Pectin was extracted from dried orange dried peel powder by following the protocol mentioned in Ref. [10] **(Fig. 2b)**. For this, 30 g of powdered orange peels was taken in a beaker (1000 mL) which was digested in magnetic stirrer (Remi 2 ML) under stirring (500 rpm) with the aid of buffer solution (pH 4) with 0.01 % (w/w) sodium azide and 0.05 g cellulase enzyme for 15 h at 30 ℃. The pH was adjusted to 5.2 with citric acid in each beaker with the help of Universal pH indicator paper [pH 1.0-14.0]. Then the insoluble obtained after enzymatic digestion was filtered through cheesecloth and precipitated by adding 200 mL of ethanol (99 %). Thereafter, obtained pectin was separated by filtering through cheesecloth and wet weight of pectin was measured [11]. After that, pectin was dried in oven (Tanco) at 60 ℃ for 5 hr. Then dry pectin was powdered using pestle and mortar which was weighed to obtain dry powdered pectin [10], [12]. The procedure was repeated thrice, resulting in the acquisition of three different amounts of pectin.

***Figure 2b**: Flow chart of pectin extraction by enzymatic method.*

Characterization

Qualitative Analysis

The color of extracted pectin obtained by both methods were observed. Both samples were visually observed and noted down. The solubility of both samples (pectin) was observed in cold and hot water. For this, 0.01 g of pectin was taken in two different conical flask containing 10 mL of 99 % ethanol and 50 mL of distilled water. It was then shaken vigorously to find out solubility in cold water. Afterwards, both the flasks were heated at 80 ℃ in magnetic stirrer separately and hence solubility in hot water was observed.

Quantitative Analysis of Pectin

Percentage yield, equivalent weight, methoxyl group content (MeO), Total anhydrouronic acid content (AUA), degree of esterification, moisture content, ash content and pH were determined following the method mentioned in Ref. [7], [13], [14].

Percentage Yield

Percentage yield in wet content in each of the samples was calculated from the wet weight of extracted pectin and weight of peel powder taken by using the formula-1.

$$\% \text{ yield} = \frac{\text{weight of pectin extracted}}{\text{weight of peel powder taken}} \times 100\ \% \ldots\ldots\ldots \ (1)$$

The wet percentage yield was calculated thrice for each extraction by applying above formula to obtain mean weight.

Equivalent Weight

0.25 g pectin was weighed and moistened with 5 mL ethanol. Then 1 g NaCl, 100 mL distilled water followed by a few drops of phenol indicator (Fisher scientific) was added. Finally, the mixture was titrated with 0.1 M NaOH to obtain the end point. Then equivalent weight was measured by the formula-3 [7].

$$\text{Equivalent weight} = \left(\frac{\text{weight of pectin}}{\text{volume of alkali}} \times \text{Molarity}\right) \times 100 \ldots\ldots(3)$$

Above process was repeated thrice for both method of extraction to calculate mean equivalent weight.

Methoxyl Content (MeO)

25 mL of 0.25 N NaOH was added to the neutral solution obtained from equivalent weight measurement which was then allowed to stand for 30 minutes at room temperature. 25 mL of 0.25 N HCl was added to it and titrated with 0.1 N NaOH. After reaching end point, volume of 0.1 N NaOH was noted from burette. Then methoxy content was determined by the formula-4 [14].

$$\text{MeO}\ (\%) = \frac{\text{mL of alkali} \times \text{normality of alkali} \times 3.1}{\text{weight of sample} \times 1000} \times 100\% \quad (4)$$

The above procedure was repeated three times for both extraction methods.

Total Anhydrouronic Acid Content (AUA)

Total anhydrouronic acid content (AUA) of pectin was obtained by applying formula-5 [14].

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

100% (5)

Where, 176 = molecular weight of AUA, 0.1 = Normality of NaOH taken y = volume of NaOH obtained from equivalent weight determination, z = volume of NaOH obtained from methoxyl content determination

Determination of Degree of Esterification (DE)

The degree of esterification of pectin was measured on the basis methoxyl and Anhydrouronic acid content and calculated by following formula-6 [14].

$$DE = \frac{176 \times MeO\ (\%)}{31 \times AUA\ (\%)} \times 100\ \% \text{ .... } (6)$$

Moisture Content

In a dried, empty petri dish 0.3 g of the pectin sample was transferred into a hot air oven (Tanco) and was placed for 1 h. After 1 hour, the Petri dish was removed; cooled in a desiccator and final weight was noted to get the moisture content in the sample by using the formula-7 [14]:

$$\text{Moisture content } = \frac{\text{weight of residue}}{\text{weight of sample}} \times 100\ \% \text{..... } (7)$$

Ash Content

Ash content was determined by heating the samples taken for moisture content at 555 ℃ for 2 h in the muffle furnace (Accuma × India) following AOAC method. Then the weight of ash was taken separately after which, the ash content was calculated using following formula-8 [13], [15].

$$\text{Ash content} = \frac{\text{weight of ash}}{\text{weight of sample}} \times 100\ \% \text{ .... } (8)$$

Potential of Hydrogen (pH)

The pH was studied by preparing a buffer at pH 7.0 and the glass electrode was standardized with standard buffer solution with the electrode. Then the electrode was rinsed with distilled water and inserted into the prepared pectin solution to determine pH of the solution [13].

Structural Characterization

X- Ray Diffraction (XRD)

Crystallinity degree of extracted pectin (Pectin A and Pectin E) was determined by using X-ray diffraction technique at Nepal Academy of Science and Technology Nepal (NAST), Khumaltar, Lalitpur. The obtained data was used to identify crystallinity and average crystallite size. Average crystallite size was calculated from the diffractogram by using Scherrer equation-9 [16].

$$D = K\lambda / \beta cos\theta \text{.............................. } (9)$$

Where, D = average crystallite size

K = Scherrer constant

λ = x-ray wavelength

β = line broadening at FWHM in radians

θ = Bragg's angle in degrees

Fourier Transform Infrared Spectroscopy (FT- IR)

The FT-IR spectra of extracted pectin and raw orange peel sample, were obtained in transmittance mode using SHIMADZU spectrophotometer (IR Prestige-21) at Nepal Academy of Science and Technology (NAST), Khumaltar, Lalitpur. The FT-IR spectrum of the sample was obtained at the wavelength in the range of 4000–500 cm^{-1} at a resolution of 4 cm^{-1}.

Biological activity

Antibacterial Properties

Antibacterial properties were analyzed in Himalaya Research Institute of Biotechnology, Koteshwor, Kathmandu, Nepal where each extracted pectin was analyzed against *Bacillus subtilis* ATCC 6051 (Gram positive) and *Escherichia coli* ATCC 8739 (Gram negative) strains bacteria utilizing Agar well diffusion method [17], [18]. The pectin sample was applied to small sterile discs known as "antibiotic discs" or "test discs." Next, sterile agar plates were prepared and evenly spread with the bacterial culture. The pectin-impregnated test discs were then carefully positioned on the agar surface and left to incubate for 24 hours. After the incubation period, the presence of circular zones of inhibition around the test discs was examined and recorded

Antioxidant Activity

The antioxidant activity was analyzed in the Department of Biotechnology, National Institute of Science and Technology (NIST), Lainchour, Kathmandu, Nepal using DPPH radical scavenging assay. In this test, quercetin (20 µg/mL) was used as positive control and 100 µL DPPH with 100 µL 50% DMSO was used as negative control. The absorbance was recorded using UV-spectrophotometer at 517 nm. The antioxidant property of pectin was calculated by using equation-10 [4].

$$\% \, inhibition = \frac{abs\ of\ control - abs\ of\ sample}{abs\ of\ control} \times 100\%..(10)$$

Results and Discussion

The color of extracted pectin by both procedures was found brown (Table 1). However, surface contamination, ambient conditions, the types of fruits used, and accidental contamination may have all affected to the color difference; this could be result of insufficient ethanol used for precipitation and purification while conducting the procedure [13].

Solubility in Cold and Hot water

Pectin extracted using both methods were partially soluble in cold water but entirely soluble in hot water which aligned along with the literature [7] (**Table 1**).

Percentage Yield in Wet Content:

The mean wet percentage yield of pectin obtained from the alcohol precipitation (16.99 ± 0.8%) and from enzymatic method (16.84 ± 0.42%) was found almost similar (**Table 1**). Khamsucharit and coworkers reported that the yields of pectin extracted from different sources

Table 1: Results of qualitative analysis and quantitative analysis of extracted pectin

S. N.	Parameters/Sample	Pectin A (alcoholic method)	Pectin E (enzymatic method)
1	Color	Brown	Brown
2	Solubility	Slightly soluble in cold water Soluble in hot water	Slightly soluble in cold water Soluble in hot water
3	Yield (%)	16.99 ± 0.8 (wet content) 6.46 ± 1.01 (Dry content)	16.84 ± 0.42 (wet content) 3.66 ± 0.34 (Dry content)
4	Equivalent weight (mg/mol)	179.62 ±7.26	175.42 ± 7.26
5	Methoxy content (%)	5.44±0.15	5.46 ± 0.04
6	Anhydrouronic acid (%)	40.68 ± 0.97	42.77 ± 2.17
7	Degree of esterification (%)	75.89 ±0.85	72.603 ± 3.15
8	Moisture Content (%)	81.33 ±0.88	83.1 ± 13.4
9	Ash Content (%)	6.77±0.38	4.55 ± 1.34
10	pH	4.4	4.63

Values are means (n = 3) ± standard deviations

were significantly different and varied from 10.91 to 24.08% and it also depends on the fruit maturation, decreases with increase in fruit maturation [19]. The highest yield of orange pectin was 16.01% at 1.27 pH and lowest yield was 11.01 % at 2.22 pH [20]. Twinomuhwez mentioned that the pectin yield increases as the pH decreases. Since at lower pH values, there is higher H^+ ions, which leads to the more hydrolysis of protopectin. Furthermore, lowering the pH value may cause the release of pectin from the raw peel due to the breaking of pectin-hemicellulose bonding [20]. These results shows that the orange peels are good sources of pectin which can be extracted using alcohol precipitation and enzymatic method.

Percentage Yield in Dry Content

While considering the mean dry percentage yield, the alcohol precipitation method resulted in a higher value of 6.46 ± 1.01% compared to the enzymatic method's mean dry percentage yield i.e. 3.66 ± 0.34% (**Table 1**).

Equivalent Weight

The mean equivalent weight obtained by alcohol precipitation method and enzymatic method were 179.62 ± 7.26 g and 175.42 ± 7.26 mg/mL respectively. However, Bagde *et al.,* [7] reported the equivalent weight of lemon and Orange 200 and 166.67 mg/mL respectively.

Methoxyl Content (MeO)

Mean methoxyl content for the alcohol precipitation method and enzymatic method were 5.44 ± 0.15 % and 5.46 ± 0.04% respectively (**Table 1**). The methoxyl content of extracted pectin varies from 0.2 to 12% depending on the source and the way of extraction [7]. Hence, the percentage methoxyl content obtained by both methods falls within the given range. Because the experimental values obtained were less than 7%, the pectin had a low ester characterization, showing that the pectin is of good quality.

Moisture Content

The enzymatic method had slightly higher mean moisture content (83.1 ± 13.4% *vs.* 81.33 ± 0.88%) than alcohol precipitation method (Table 1). The moisture content of orange peel's pectin reported in Ref. [7] and [14] 80% and 70% respectively.

Anhydrouronic Acid Content (AUA):

The enzymatic method resulted in a slightly higher mean anhydrouronic acid (AUA) content compared to the alcohol precipitation method (42.77 ± 2.17% and 40.68 ± 0.97%) (Table 1). anhydrouronic acid (AUA) was ranged from 38.84 to 41.30%, based on extraction temperature and extractant pH [21].

Degree of Esterification (DE)

The alcohol precipitation method gave higher mean degree of esterification (75.89 ± 0.85% *vs.* 72.603 ± 3.15%) than that obtained from enzymatic method (**Table 1**). The level of degree of esterification reported in this study corresponded within the range (73.26 to 76.59%) [21], [22]. The degree of methylation was found greater than 50% indicates that the orange peel pectin has high-methoxyl content and rapid set pectin. Therefore, orange peel pectin can be used in the manufacture of jam and jelly because degree of methylation affects the gelling ability of pectin [23].

Ash Content

The mean ash content was higher in alcohol precipitation method (6.77 ± 0.38 % *vs.* 4.55 ± 1.34%) (**Table 1**). Similarly, Kar & Arslan, reported 6.07% ash content [23].

Potential of Hydrogen (pH):

The pH for alcohol precipitation method and enzymatic precipitation method was 4.4 and 4.63 respectively (**Table** 1). pH values of orange peel and lemon peel pectin were reported 4.5 and 3.9 respectively [7].

Structural Characterization:

X- Ray Diffraction:

XRD (X-ray diffraction) diffractograms of the orange peel pectins (Pectin A and Pectin E) were presented in **Fig. 3**. The peaks obtained from XRD analysis by alcohol precipitation method were at 12°, 16°, 18°, 27° and 29° while by enzymatic method was 16°, 26°, 29°, and 30°. The diffractograms indicated peaks and amorphous regions, suggesting that both types of pectin possess a semi-crystalline structure. It was observed that both pectins had similar peaks to the peaks reported in the literature and showed amorphous nature [5], [24]-[26]. Using the Scherrer equation, the average crystallite size achieved by the alcohol precipitation method was 13.05 nm, whereas the enzymatic approach yielded a size of 16.45 nm which is shown in Table 2 and 3.

Table 2: Calculation of average crystallite by alcohol precipitation method

K	λ (A⁰)	2θ (degree)	2θ (radian)	FWHM	Size (nm)	average size (nm)
0.9	0.15	12.405	0.216	0.250	31.037	
0.9	0.15	16.499	0.288	1.548	5.048	
0.9	0.15	18.572	0.324	1.387	5.647	13.052
0.9	0.15	26.973	0.470	0.658	12.076	
0.9	0.15	29.624	0.517	0.698	11.453	

Table 3: Calculation of average crystallite size by enzymatic method

K	λ (A⁰)	2θ (degree)	2θ (radian)	FWHM	Size (nm)	average size (nm)
0.9	0.15	16.216	0.283	6.914	1.129	
0.9	0.15	26.549	0.463	0.303	26.223	16.45
0.9	0.15	29.498	0.514	0.449	17.805	
0.9	0.15	30.299	0.528	0.388	20.644	

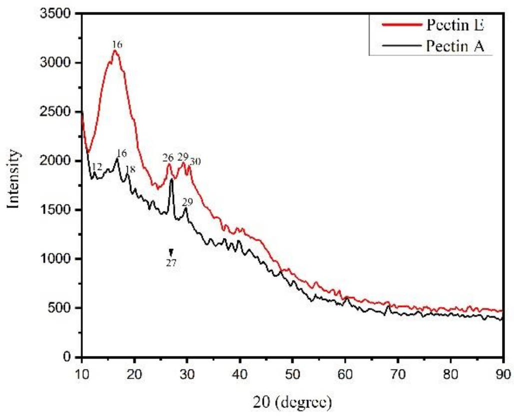

Figure 3: *XRD spectra of extracted pectin extracted (Pectin A & Pectin E)*

Fourier Transform Infrared Spectroscopy:

Figure 4 exhibits the infrared spectra of raw orange peel powder (raw OP), pectin A and pectin E. The spectra reveal distinct chemical shifts at 3390 cm^{-1}, 2932 cm^{-1} and 1068 cm^{-1} by alcohol precipitation method while 3435 cm^{-1}, 2929 cm^{-1}, and 1073 cm^{-1} by enzymatic method representing inter and intra-molecular hydrogen bonds of O-H, C-H of CH_3 and CH_2, and C-O of glycoside compounds, respectively [5], [24], [27]. The signals detected at 1740 cm^{-1} and 1759 cm^{-1} by enzymatic and alcohol precipitation method respectively are related to the C=O stretching vibration of methyl esterified carboxyl groups. Additionally, the absorption peaks from 1626 cm^{-1} by enzymatic method and at 1645 cm^{-1} by alcohol precipitation method are associated with the C=O stretching vibration of free carboxyl groups in galacturonic acid (GalA) units [5].

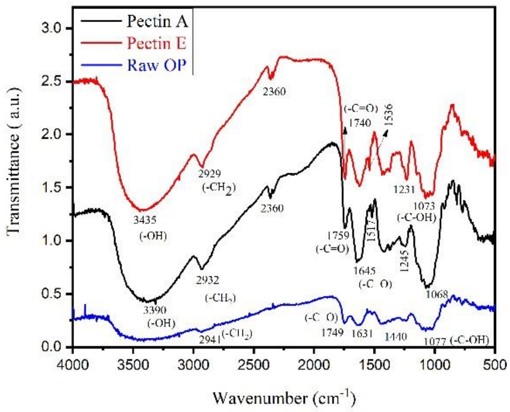

Figure 4: *FTIR spectra of orange peel: Orange peel powder (Raw OP), Pectin (A) and Pectin (E)*

Biological Activity

Antibacterial Property

Both pectin (Pectin A and Pectin E) didn't show any inhibitory zone which resulted that pectin was inactive towards both *Bacillus subtilis* ATCC 6051 (Gram positive) and *Escherichia coli* ATCC 8739 (Gram negative) strains bacteria [17], [18] (**Fig. 5**).

Antioxidant Activity

In the conducted experiment, the absorbance of pectin was determined to be 0.564%, indicating a substantial presence of the compound in the sample (**Table 4**). The % inhibition (antioxidant activity) was calculated by using equation-10. Remarkably, this high absorbance value corresponded to an impressive antioxidant activity of 40.57 % which almost matches in literature reviewed of Gan *et* *al.*, (47.5%) [28]. Yang and coworkers [29] proposed that polysaccharides can reduce the very stable DPPH free radical (purple color) [30] to diphenyl picrylhydrazine (yellow color) due to the hydroxyl group of the monosaccharide unit. The hydroxyl group can furnish a proton to reduce the DPPH radical [29]. Thus, greater the hydroxyl group showed enhance antioxidant potential. This finding not only underscores the significant concentration of pectin in the sample but also highlights its potent antioxidant properties.

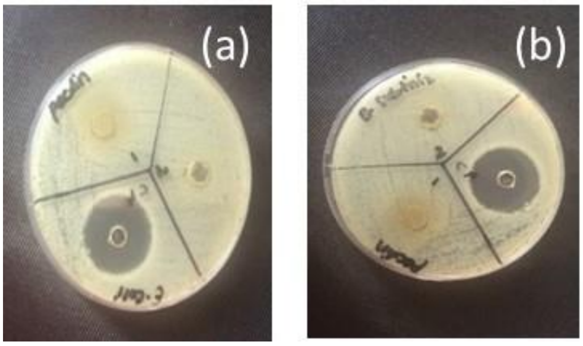

Figure 5: Antibacterial activity of pectin against *Escherichia coli* (A) and *Bacillus subtilis* bacteria (B)

Table 4: Absorbance of Quercetin, DMSO and Pectin by UV-spectrophotometer

S.N.	Standard	Remark	Absorbance	
			Initial	Final
1	20 g/mL quercetin	Positive control	0.042	0.042
2	50% DMSO	Negative control	0.733	0.338
3	Pectin	Sample (pectin)	0.359	0.564

Conclusions

The current study demonstrates that pectin can be extracted in varied amounts from discarded peels using various procedures, and that it can be used for a variety of purposes. Here, we compare the qualitative and quantitative parameters of two different pectins (Pectin A and Pectin E) and to see which one is more suitable for industrial applications. The presence of highly methylated pectin in both samples was confirmed by FTIR spectra and the calculated value of mean degree of esterification, while the semi-crystalline structure of pectin was elucidated by an XRD diffractogram in which alcoholic precipitation had smaller crystallite size. Although pectin does not have antibacterial properties, considerable antioxidant property has been displayed (40.47%).The research contributes to the understanding of utilizing orange peels as a potential source of pectin, which has various applications in the food and pharmaceutical industries. The high degree of methylated orange peel pectin can be used in the manufacture of jam and jelly and its antioxidant property is useful in pharmaceutical industries. Furthermore, research is suggested employing creative and diverse new methods with non-toxic environmentally friendly solvents for extraction of pectin.

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Author's Contribution Statement

Shanta Pokhrel: Conceptualization, Resources, Supervision, Funding acquisition, Writing-original draft preparation, Writing-review and editing, **Sunita Dahal:** Investigation, Formal analysis, Data curation, Writing-review and editing, **Samantha K.C.:** Investigation, Formal analysis, Data curation, Writing-review and editing

Conflict of Interest

The authors do not have any conflict of interest throughout this research work.

Data Availability Statement

The data supporting this study's findings are available from the corresponding authors upon reasonable request.

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