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Antibacterial Action, Antioxidant Activity and Anticoagulant Effect of Pectin Extracted from Peels of Algerian Citrus Sinensis †

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Abstract: In this study, we characterised the pectin extracted from peels of Algerian Citrus sinensis and evaluated its antibacterial action, antioxidant activity and anticoagulant effect. Pectin was extracted under acidic conditions using hydrochloric acid for PCT-1 and citric acid for PCT-2 and determining their physicochemical properties using Fourier-Transform Infrared spectroscopy (FTIR), X-ray powder diffraction (PXRD), differential scanning calorimetry (DSC), yield, the degree of methylation, water content and ash content. In addition, the FTIR results showed desired banding characteristics, and their thermal properties evaluated using DSC showed that the thermal degradation was around 240 °C. XRD results show that PCT-1 and PCT-2 are amorphous and have similar characteristics to commercial pectin. On the other hand, the antibacterial action showed that PCT-1 and PCT-2 have no effect on *Pseudomonas aeruginosa* and *E. coli* bacteria, unlike *Staphylococcus epidermidis*, where it showed considerable antibacterial action. The antioxidant activity of PCT-1 and PCT-2 was observed using the 2,2 diphenyl-1-picrylhydrazyl (DPPH) method; the absorbance values recorded for PCT-1 and PCT-2 confirmed their antioxidant potential explained by the presence of several free hydroxyl groups in the PCT-1 and PCT-2 structure. On the other hand, our findings indicate that PCT-1 and PCT-2 do not have a marked anticoagulant effect but have acceptable potential and can be used as anticoagulants for the treatment of thrombotic diseases with fewer side effects compared to the widely used heparin. These results suggest that pectin from the peels of Algerian Citrus sinensis has potential properties as a biomaterial for several biomedical applications.

Keywords: pectin; extraction; antibacterial action; antioxidant activity; anticoagulant effect

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1. Introduction

Improving the quality of life is currently one of the most hotly debated human concerns, where environmental protection and sustainable development have become major challenges for mankind. It is with this in mind that research focuses on progress and innovation in biotechnological processes, with the aim of making everyday life easier for everyone by developing high-performance materials that respond to challenges and constraints due mainly to the depletion of natural resources and pollution. To solve this problem, research is focusing on materials of natural origin, known for their excellent biodegradability and biocompatibility properties. However, the food processing and agri-food industries that use citrus fruits generate considerable quantities of by-products in the form of pulp, seeds and peels, which account for 50% of raw fruit [1]. Recycling the bio-waste generated by industrial processes, which produces large quantities, before recovering it, has become a priority and a challenge. Citrus comprises around 16 species in the Rutaceae family, widely

grown in subtropical regions [2]. The peels are bio-waste that can be recycled and used as a fertiliser. The bark is a bio-waste that can be recovered and used as a potentially exploitable resource for the production of pectin, a co-product obtained mainly through extraction [3]. Pectin is a complex branched polysaccharide present in the primary cell wall of plants, obtained by extraction from plants such as apple and orange peels and is considered to be the most complex macromolecule in nature, composed of 17 different monosaccharides containing more than 20 different linkages, which are enriched with repeated methyl ester galacturonic acid units [4]. Generally, it is widely used in the food industry as a gelling agent, thickener, emulsifier and stabiliser; it is considered for soluble fibres with a high water retention capacity, heavy metal adsorption properties and other medical applications [5]. On the other hand, pectin inhibits the development of several parasites and infections and is known as a good antibacterial and antifungal agent, anti-tumour, antiviral and healing [6]. The main objective of this work is (i) the recovery of C. sinensis bio-waste from orange peels through extraction processes using the acid hydrolysis of pectin using citric acid and hydrochloric acid, (ii) the evaluation of the physicochemical, structural and thermal properties, (iii) and the study of the antibacterial, antioxidant and anticoagulant activity of pectin.

2. Materials and Methods

The raw material, sweet orange fruit (C. sinensis), was obtained from farms located in the Sid Matmar region, Relizane province, North-West Algeria (35°43′59.999″ N 0°33′0″ E).

2.1. Extraction of Pectin

The bark was washed, cleaned, cut, dried at 50 °C, crushed and sieved using a hand sieve to obtain a fine powder.

2.1.1. Extraction with Citric Acid

The amount of 40 g of bio-waste was added to citric acid (1/25 *w*/*v*, 0.1 N, pH = 2), stirred until homogenised, and the samples were acidified at 70 °C in a water bath for 40 min. The mixture was stored for 24 h at room temperature. The precipitated pectin was recovered via centrifugation (6000 rpm, 10 min). The samples were filtered, and 95% ethanol (1:2 *v*/*v*) was added to allow the pectin to precipitate. Samples were stored (24 h, 25 °C) to allow pectin flotation, which was separated via filtration and washed twice with ethanol (70%). The final product (PCT-1) was dried in an oven (65 °C).

2.1.2. Extraction with Hydrochloric Acid

In total, 40 g of pre-treated bio-waste was added to hydrochloric acid (1/20 *w*/*v*; 0.1 N) and boiled in a reflux system (90 °C, 45 min). After 6 min, the reaction medium was placed on ice to stop the hydrolysis reaction. The filtrate thus obtained was then recovered via filtration and precipitated in ethanol. The filtrate was washed with 60, 80 and 98% EtOH, then centrifuged (10,000 rpm, 20 min). PCT-2 was then dried and before being ground [7].

3. Results and Discussion

3.1. Yield of Pectin

PCT-1 and PCT-2 are perfectly soluble in cold or hot water; this may be explained by the increased crystalline structure of the pectin entities remaining on the macromolecule, which itself depends on the origin of the natural material. However, pectin was insoluble in most organic solvents, in agreement with the literature [8]. The yields of PCT-1 (6.3%) and PCT-2 (4.7%) were mainly affected by the choice of the acid used, with a slight increase in citric acid compared to hydrochloric acid. Researchers have explained that the yield is influenced by the extraction time, and this decrease observed for PCT-1, where the acid treatment lasted overnight, was explained by the cleaving action of the acid on the glycoside and ester bonds of the pectin, which led to a decrease in the yield. This is in

contrast to other extraction processes, such as microwave extraction, which showed higher yields [9].

3.2. Degree of Methylesterification (DE)

The number of esterified carboxy groups was calculated from the volume of a 0.1 N sodium hydroxide solution used for the final titration; the degree of methylesterification (DE) is defined as the ratio of esterified galacturonic acid to the galacturonic acid groups present [10]. The results showed that DE is affected by the extraction time, pH and yield. PCT-2 with DM (57%, known as highly methylated pectin HM) obtained at pH 3.3 at a shorter extraction time (45 min) was favourable for the preparation of sugar-rich products [11]. By contrast, PCT-1 had a DM (40%, considered as low methylated pectin LM). The reduction in carboxylate functions is responsible for the depletion of the repulsive forces of the polysaccharides, which favours pectin gelation, giving more precipitated pectin at a lower pH, in agreement with the work of Yapo et al. [12], who confirmed that the yield increased with increasing acid strength for pectins obtained by extraction from apple pomace and sugar beet pulp.

3.3. FTIR Characterisation

FTIR spectra showed a broad peak (3700–3000) cm^{-1} corresponding to the stretching of (OH) due to hydrogen bonds in galacturonic acid. The peaks (2954–2941) cm^{-1} of PCT-1 and PCT-2, respectively, are attributed to CH bond stretching, and those of (1730–1620) cm^{-1} correspond to esterified and free carboxyls, respectively (Figure 1). In addition, the bands at (1100–1020) cm^{-1} are attributed to COC-stretching vibrations, thus confirming the presence of pyranoses in the structure of pectins. On the other hand, the asymmetric stretching around (1643–1626) cm^{-1} is attributed to carbohydrate functions, and the spectra in the region (1300–800) cm^{-1} correspond to the main carbohydrate chemical groups in polysaccharides, the bands (between 1100 and 990) cm^{-1} was attributed to galacturonic acid and finally the peaks (920–820) cm^{-1} referred to the absorption of D-glucopyranosyl and α-D-mannopyranose, respectively, with results in agreement with other works [13].

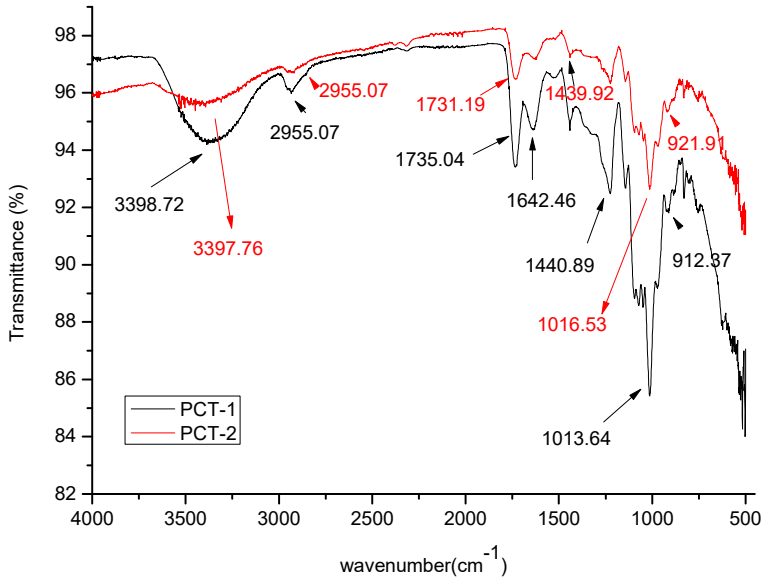

Figure 1. FTIR spectra of extracted Pictin.

3.4. XRD Characterisation

The crystalline nature of PCT-1 and PCT-2 was detected using XRD diffractometers and showed that PCT-1 and PCT-2 were amorphous and had similar characteristics to commercial pectin. Thermal analysis using DSC was carried out to assess the thermal behaviour of PCT-1 and PCT-2. The results showed that endothermic peaks (89 °C) for

PCT-1 and (77.7 °C) PCT-2 correspond to the mechanism of residual water retention explained by existing hydrogen bonds between the galacturonic acid units. Exothermic peaks were recorded (260 °C) for PCT-1 and (253 °C) PCT-2 and confirmed by previous work. In addition, PCT-1 had a higher degradation temperature than PCT-2, explained by the different operating conditions during the extraction process. Thermal analysis showed that PCT-1 had higher thermal stability than PCT-2, reflected by greater changes during heating [14].

3.5. Antibacterial Activity

The in vitro results of antibacterial activity were expressed by measuring the diameter formed by the inhibition zone around the colonies, symbolised by signs based on the sensitivity of the bacteria to the samples prepared. The antibacterial activity of the prepared biomaterials against the target strains *E. coli*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* was investigated. Several studies have demonstrated the antibacterial action of polysaccharides [15]. Our results show that PCT-1 and PCT-2 have no antibacterial effect on the following two strains of bacteria: *Pseudomonas aeruginosa* and *E. coli*. This is probably explained by a high level of resistance to the antibacterial action of the extracted pectins in contrast to the *Staphylococcus epidermidis* strain, where the antibacterial activity of PCT-1 and PCT-2 was shown with a maximum inhibition zone diameter of almost 14 mm and the absence of any effect on the growth of *E. col*. This can be explained by its mechanism of action on the cell wall, which can lead to a change in cell permeability that induces significant antibacterial activity. In addition, it was observed that the inhibition diameter decreased with the decreasing pectin concentration. On the other hand, this resistance is due to the disruption of membrane functions. The high resistance of Gram (-) bacteria is linked to the complexity of the cell envelope of these micro-organisms, which contains a double membrane, unlike the simple membrane structure of Gram (+) bacteria. PCT-1 and PCT-2 in solution against the target strains *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* and *E. coli* are illustrated in the Figure 2:

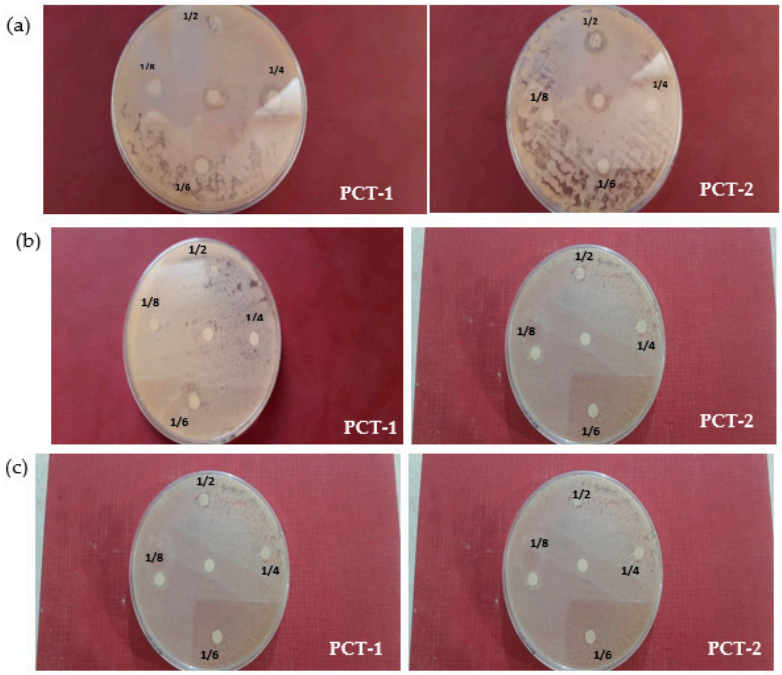

Figure 2. Antibacterial activity of PCT-1 and PCT-2 in solution against the germ control of the antibacterial activity, inoculated on an agar culture medium, incubated at 37 °C, using the disk diffusion method (**a**) *Staphylococcus epidermidis*, (**b**) *Pseudomonas aeruginosa* and (**c**) *Escherichia coli*.

3.6. Antioxidant Activity

The DPPH method is considered a simple, accurate and productive method for determining the antioxidant activity of plant extracts and pure compounds such as flavonoids. The scavenging of DPPH radicals by antioxidant polysaccharides is linked to their ability to donate hydrogen [16]. The antioxidant activity of pectin is explained by the presence of different free hydroxyl groups in the polysaccharide structure. The results obtained show that absorbance values decrease as the content used increases.

3.7. Anticoagulant Activity

Following coagulation tests, partial thromboplastin time (PTT) and prothrombin time (PT) were determined. PCT-1 showed a TQ (15.3 s) and TCK of PCT-1 (33.4 s). The anticoagulant activities of the prepared biomaterial fractions showed a slight difference from the anticoagulant activity of the normal control (no anticoagulant). However, Yoon et al. also showed a low anticoagulant activity of polysaccharides of plant origin compared with the compounds of animal origin [17].

4. Conclusions

The aim of this study was to develop biomaterials with antibacterial, antioxidant and anticoagulant properties obtained by extraction from a bio-waste of therapeutic interest, pectin. The extraction protocol was carried out successfully, with pectin extracted by hydrolysis and citric acid being more stable than that using HCl. Antibacterial analysis showed that the two pectins obtained exerted no antibacterial effect on the two strains, *Pseudomonas aeruginosa* and *E. coli*, unlike the *Staphylococcus epidermidis* strain, where considerable antimicrobial activity was clearly indicated. The results of the antioxidant activity were satisfactory, explained by the presence of various free hydroxyl groups in the structure of pectins. The samples prepared in this work have interesting potential and can be used as anticoagulants in the treatment of thrombotic diseases with fewer side effects than the widely used heparin. New perspectives can be envisaged by a broader study of the antibacterial activity of other pathogenic bacteria and an evaluation of antioxidant activities.

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