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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 have characterized the pectin extracted from peels of Algerian Citrus sinensis and evaluated its antibacterial action, its antioxidant activity and its anticoagulant effect. Pectin was extracted under acidic conditions using hydrochloric acid for PCT-1 and citric acid for PCT-2 and determined their physicochemical properties by Fourier Transformed Infrared spectroscopy (FTIR), X-ray powder diffraction (PXRD), differential scanning calorimetry (DSC), yield, degree of methylation, water content and ash content. In addition, the FTIR results showed desired banding characteristics, and their thermal properties evaluated by DSC showed that the thermal degradation was around 240 °C. XRD results showed that PCT-1 and PCT-2 were amorphous and have similar characteristics with 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 by 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 PCT-1 and PCT-2 structure. On the other hand, our findings indicate that PCT-1 and PCT-2 don't 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 peels of Algerian Citrus sinensis has potential properties as 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 a major challenge for mankind. It is with this in mind that research is focused 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 the 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, and 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 could be recycled and used as a fertiliser. Bark is a biowaste that could be recovered and used as a potentially exploitable resource for the production of pectin, a co-product obtained mainly by 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 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 gelling agents, thickeners, emulsifiers and stabilisers, and considered to be soluble fibres with a high water retention capacity, with heavy metal adsorption properties and other medical applications [5]. On the other hand, pectin inhibits the development of several parasites and infections, 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 biowaste from orange peels, by extraction processes using acid hydrolysis of pectin using citric acid and hydrochloric acid, (ii) the evaluation of the physico-chemical, structural and thermal properties, (iii) the study of the antibacterial, antioxidant and anticoagulant activity of pectin.

2. Materials and Methods

The raw material, sweet orange fruit (C. sinensis) from farms located in the Sid Matmar region, Rélizane 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 biowaste was added to citric acid (1/25 *w*/*v*, 0.1 N, pH = 2), stirred until homogenised, 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 by 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, separated by 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

40 g of pre-treated biowaste were 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 by filtration and precipitated in ethanol. The filtrate was washed with 60, 80 and 98% EtOH, then centrifuged (10,000 rpm, 20 min). PCT-2 is then dried and 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 acid used, with a slight increase for 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 lasts overnight, is explained by the cleaving action of the acid on the glycoside and ester bonds of the pectin, which leads to a decrease in yield. This is in contrast to other extraction processes, such as microwave extraction, which show higher yields [9].

3.2. Degree of Methylesterification (DE)

The number of esterified carboxy groups was calculated from the volume of 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 galacturonic acid groups present [10]. The results showed that DE is affected by 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), favourable for the preparation of sugar-rich products [11]. Whereas PCT-1 with 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 Characterization

FTIR spectra showed a broad peak (3700–3000) cm^{-1} corresponding to 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. 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} corresponds to the main carbohydrate chemical groups in polysaccharides, the bands between (1100–990) cm^{-1} were attributed to galacturonic acid and finally the peaks (920–820) cm^{-1} refer to the absorption of D-glucopyranosyl and α-D-mannopyranose, respectively, results in agreement with other works [13].

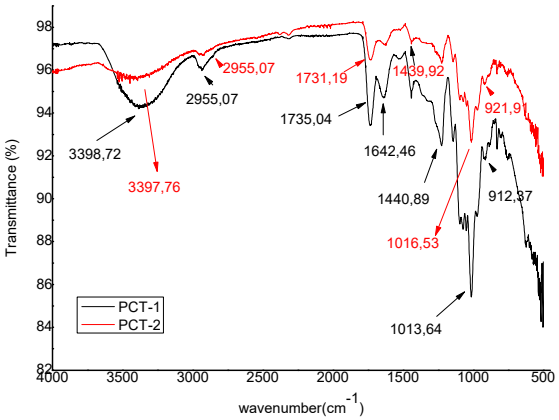

Figure 1. FTIR spectra of extracted Pictin.

3.4. XRD Characterization

The crystalline nature of PCT-1 and PCT-2 was detected by XRD diffractometers and showed that PCT-1 and PCT-2 were amorphous and have similar characteristics with commercial pectin. Thermal analysis by 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) for 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) for PCT-2, 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 a higher thermal stability than PCT-2, reflected by greater changes during heating [14].

3.5. Antibacterial Activity

The invitro results of antibacterial activity were expressed by measuring the diameter formed by 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 two strains of bacteria: *Pseudomonas aeruginosa* and *E. coli*, probably explained by a high level of resistance to the antibacterial action of the extracted pectins, in contrast to the *Staphylococcus epidermidis* strain where antibacterial activity of PCT-1, 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 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* is illustrated in the figures below:

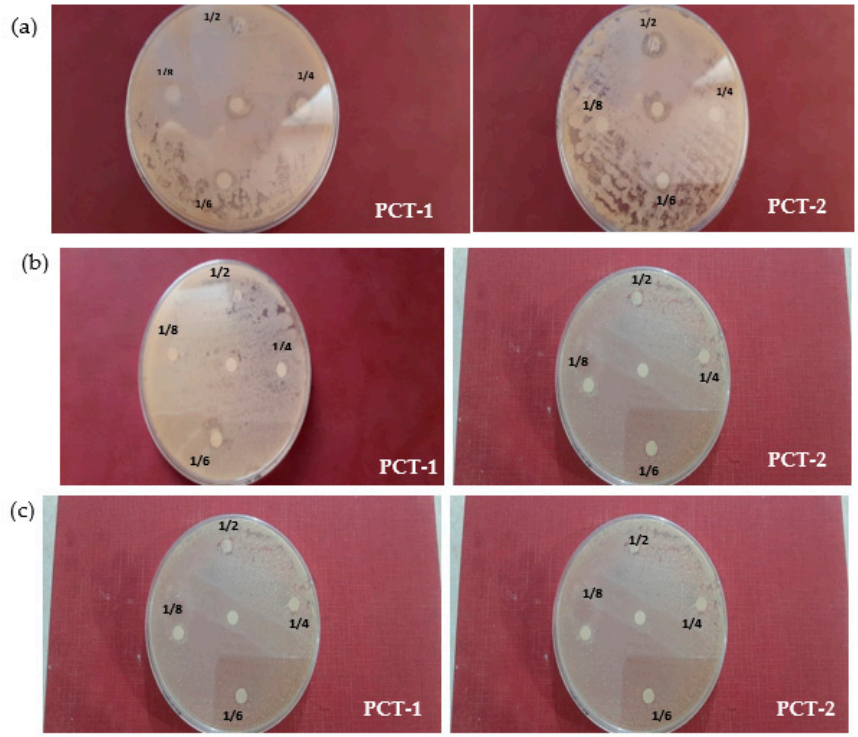

Figure 2. Antibacterial activity of PCT-1 and PCT-2 in solution against the germ control of the antibacterial activity, inoculated on agar culture medium, incubated at 37 °C, by Disk Diffusion Method (**a**) *Staphylococcus epidermidis*, (**b**) *Pseudomonas aeruginosa* (**c**) *Escherichia coli*.

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 was (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 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 biowaste of therapeutic interest, pectin. The extraction protocol was carried out successfully, with pectin extracted by hydrolysis using 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 on other pathogenic bacteria and to evaluate the antioxidant activities.

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