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Extraction and Isolation of Pectin Punica granatum from for its Antimicrobial Activity

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Abstract:

Pectin is a naturally occurring polysaccharide which was obtained by various sources of fruits and has garnered a lot of attention in the last few years. The necessity to utilize natural pectin has increased due to its biodegradability, safety and eco- friendliness nature that are being valued by researchers and consumers. Pectin is a derivative of polygalacturonase acid which is methylated and taken off produced mainly in fruit peels, such as citrus, apple, and pomegranate in mildly sour conditions.

Pectin possesses several pharmaceutical applications such as the use as a solidifying substance, a binding substance and a diluent in pills. In addition to its functional properties, "it possesses great biological activities of antimicrobial, antibacterial, and antifungal properties. These properties have over the years made pectin to be widely utilized in the food industry as a functional ingredient

Keywords: Punica Granatum, acid extraction method, pectin, Anti-microbial activity.

Introduction: Pectin is a complex polysaccharide and may be obtained not only out of Punica granatum (pomegranate), but also many other fruit substances. It is of great importance in the organization of the plant cell walls and is considered a good functional ingredient to be utilized in food and pharmaceutical industry. Due to its gelling, thickening, as well as stabilizing properties, pectin can be extensively applied in various industrial processes.

Fruit processing waste is one of the most valuable sources of pectin. Good sources of major proportions of pectin include mango peel (3050%), banana peel (around 20%), pomegranate peel (4050%), citrus peel (1.215%), orange peel (1030%), and watermelon rind (328%). In addition to its technological uses, pectin has been asserted to possess several biological functions which include; anti-inflammatory, antimicrobial, antioxidant, antidiabetic and anti-hypertensive. These characteristics make it suitable in management of various health conditions such as skin infections such as acne vulgaris and Staphylococcal infections.

Pectin is widely used as a thickening, emulsifying and a texturizing agent in food industry. It is also applied as a stabilizer, filling agent, and fat substitute in most of processed foods. In jam and jelly making, pectin is one of the commonest products used where it is used as a gel-forming material, and as a result, it has been used to impart the desired texture and consistency to the product. Other foodstuffs that use pectin include in the preparation of beverages, syrups, and saues among others.

Different procedures are applicable in extracting pectin in plants. These methods can be divided into conventional and contemporary methods. The traditional methods entail either acid or alkaline extraction

methodologies which are usually used in the industrial procedures. Recently, it has been invented that some new alternatives of extraction that are encompassed are Enzyme-Assisted Extraction (EAE), Microwave-Assisted Extraction (MAE), Ultrasound-Assisted Extraction (UAE), and Subcritical Water Extraction (SWE). These new methods have the benefits of needing less energy to extract, less time taken to extract, more production and sustainability. Among these practices, the hot acid extraction is one of the most common practices of commercial practices because pectin of good quality is highly produced. The pomegranate peel is a natural source and therefore, it can be considered as a source of pectin since the pectin content of the fruit is between 40 and 50 percent and therefore, could be utilized to extract pectin and in researching its biological action particularly antimicrobial activity.

Chemical constituents of punica Granatum peel:

The Punica granatum peel is also enriched with diverse bioactive substances, which make it biologically active. Flavonoids and tannins occur in large amounts and are commonly found in large proportions in wild varieties than in cultivated fruits. Besides these phenolic compounds there are also complex polysaccharides that occur in the peel. Other research have indicated the potential existence of alkaloids like pelletierine, but this is yet to be concluded. To illustrate, the Dragendorff test can be positive, and the Mayer test can be negative, so it should be confirmed.

The main chemical constituents isolated from pomegranate skin/pericarp/peel are:

Hydroxycinnamic acids:caffeic acid, chlorogenic acid, p-coumaric acid

Hydroxybenzoic acids: Gallic acid, Ellagic acid.

Cyclitol carboxylic acids: Qunic acid .

Flavon-3-ols/Flavonoids and their glycosides: Catechin,

Epicatechin, Epigallocatechin-3-gallate, Quercetin,

Kaempferol, Luteolin, Rutin, Kaempferol-3-O-glycoside,

Kaempferol-3-O-rhamnoglycoside, Naringin.

Anthocyanins: Cyanidin, Pelarginidin, Delphinidin.

Ellagitannins: Punicallin, Punicalagin, Corilagin, Casuarinin,

Gallagyldilacton, Pedunculagin, Tellimagrandin, Granatin A,

Granatin B.

Alkaloids: Pelleteriene

Punica granatum peel has a broad spectrum of bioactive compounds which make it have biological properties. Some of these constituents include flavonoids and tannins which occur in high concentrations and in most cases, occur in higher amounts in wild varieties than in cultivated varieties. Besides such phenolic compounds, the peel also has complex polysaccharides which are also significant in terms of its functional attributes. The peel may also contain some alkaloids like pelletierine as suggested by some studies, but the evidence does not conclusively prove this. An example is a chemical test like the Dragendorff test where a positive reaction may

appear, and Mayer test may yield a negative reaction showing that the test needs to be further confirmed through further analysis.

Table 1: Pectin yield from various fruit and agricultural by-products

No.	Source	Pectin yield (%)	References
1.	Apple pomace	4.60–20.92	[36]
2.	Citrus: orange peel	10.90–24.80	[37]
3.	Grapefruit peel	21.60–28.00	[32]
4.	Lemon peel	20.96–30.60	[31]
5.	Lime peel	9.00–33.60	[29]
6.	Sugar beet pulp	4.10–24.96	[38]
7.	Banana peel	2.40–21.70	[39]
8.	Carrot peel	8.90–9.10	[40]
9.	Mango peel	9.20–31.80	[41]
10.	Papaya peel	11.00–50.00	[42]
11.	Pomelo peel	6.00–24.00	[43]
12.	Watermelon peel	3.00–28.00	[44]
13.	Durian rinds	2.00–10.00	[45]
14.	Blueberry wine pomace	3.00	[46]
15.	Passion fruit peel	10.00–30.30	[11]
16.	Jackfruit peel	8.94–14.50	[47]
17.	Rapeseed cake	6.85	[48]
18.	Cocoa husk	3.38–12.60	[49]
19.	Dragon fruit peel	2.00–27.00	[11]
20.	Creeping fig seeds	5.00–6.00	[50]
21.	Mangosteen rind	12.00	[51]
22.	Tangerine peels	19.90	[52]

Methodology:

Pressurized carbon dioxide and deionized water method

Application: Pressurized Carbon dioxide extraction is applied as a method in coal mining.

One has devised a new method of extraction where pressurized carbon dioxide is applied to acidify water and generate carbonate ions (CO 3 2 -) that naturally chelates calcium ions (Ca 2 +) (Tsuru et al., 2021). The authors of Tsuru et al. (2021) argue that this approach yielded less pectin compared to the use of standard hydrochloric acid (HCl) and chelating agents, including sodium hexametaphosphate, to extract pectin in orange peel. Nonetheless, the pectin that was retrieved after using pressurized carbon dioxide extraction exhibited a greater level of esterification (DE).

In particular, this procedure resulted in about 4 percent pectin that had over 90 percent DE as opposed to HCl extraction that gave about 10 percent pectin with less than 80 percent DE. Besides, the pectin derived by the carbon dioxide process had a higher molecular weight as compared to the pectin derived by the hydrochloric acid process under the same conditions. Even though this method is not yet fully developed, it has a good potential of being a viable alternative and greener method of extraction.

Subcritical water extraction:

Subcritical water extraction is used when pressurized water is used in temperatures that are more than 100 C. In such circumstances, the water becomes less polar and that enhances its capability of infiltrating the tissues of plants and dissolving the pectin in the waste products of plants. Nonetheless, an overly hot temperature can cause the loss of pectin, browning process, and dissolution of other impurities.

As such, every raw material has its optimum extraction conditions. For example:

Citrus peel: best temperature in the range of 120 o C.

Apple pomace: best temperature is approximately 150 o C.

Pomelo peel: best in 120 o C and 3 Mpa pressure.

Pectin recovery is also affected by other parameters like extraction time and liquid to solid ratio. As an example, the pectin extraction was the highest at about 9.15 minutes with the 17 mL/g liquid to solid ratio.

The pectin extracted using this method tends to have lower molecular weight and a reduced level of esterification than pectin extracted using conventional methods. Nevertheless, by using subcritical water extraction in combination with the use of ultrasound treatment namely 29.1 of pectin of sugar beet pulp can be attained which can be significantly improved.

Pectin Extraction with the help of microwave:

Microwave assisted extraction (MAE) involves the use of microwave energy to heat plant materials using dielectric heating. In the process, the polar molecules, especially water molecules, will rapidly rotate due to the microwave radiation and the process will result in the internal heating of the tissues in the plants.

The internal heating interferes with the cell wall structure and helps the pectin to leak into the extraction medium. Consequently, the microwave-assisted extraction can be used to extract high quality pectin at a better extraction rate and in a shorter time than the traditional procedures.

Pectin was extracted with the help of an enzyme:

Enzyme assisted extraction is viewed as an environmentally friendly and efficient process of acquiring pectin. In this method, special enzymes are used which break down the components of the plant cell walls and liberate pectin more efficiently.

Enzymes typically used in this technique are polygalacturonase, hemicellulase, cellulase, protease, amylase, xylanase, 0 -galactosidase, endopolygalacturonase and pectinesterase. They are enzymes that degrade structural polysaccharides that are in the plant cell wall and this enables pectin to be released more readily.

Due to this, enzymatic extraction frequently results in increased pectin yield, enhanced quality and less adverse effect on the environment compared to chemical extraction technologies, and consequently makes it an appealing alternative to chemical extraction.

Acid Extraction of Pectin:

The most popular technique of isolating pectin is acid extraction, which efficiently changes insoluble protopectin to soluble pectin, resulting in an increase in extraction. This is normally done with both organic and mineral acids.

Organic acids are found in various forms, such as acetic acid, citric acid, lactic acid, malic acid and tartaric acid and commonly used mineral acids are hydrochloric acid, nitric acid, oxalic acid, phosphoric acid and sulfuric acid.

The release of galacturonic acid is increased by increasing the acidity of the extraction medium (low PH) which increases the quality of pectin. Normally extraction is carried out at pH of 1-3 and temperatures of 60 o C to 85 o C. The condition of high level of hydrogen ions encourages hydrolysis of protopectin which makes soluble pectin to be liberated (7).

Biological Activities:

Antimicrobial Effect

Pomegranate peel extract has been found to have great antimicrobial activity against numerous microorganisms. Research on the antimicrobial efficacy has indicated that the greatest antibacterial effect can be witnessed in Staphylococcus aureus. Methanolic extracts of pomegranate peel have demonstrated notably important antibacterial effects among other solvents.

Pomegranate has a wide range of antimicrobial action which prevents the growth of Gram-positive bacteria, Gram-negative bacteria, fungi and moulds. It is considered that the antimicrobial activity of pomegranate peel is explained primarily by its pectin and other bioactive compounds, such as phenolic substances.

Other researchers have also reported that the pomegranate peel extract has high antimicrobial activity than other plant parts. The peel has high levels of flavonoids and tannins which are highly correlated with this activity. Pomegranate peel extract has thus picked up as a natural antimicrobial agent against a wide range of microbial and fungus pathogen (4).

Anti-Inflammatory Effects:

Inflammation is an innate immunity process of the body, which is provoking infection, injury, or exposure to adverse stimuli. It is usually divided into two phases, which can be acute inflammation and chronic inflammation. Acute inflammation is a defense mechanism that ensures that the harmful agents are removed and that tissue healing is initiated. Nevertheless, sustained inflammation may cause chronic inflammatory diseases which will cause tissue damage.

Chronic inflammation has been linked with the occurrence of a number of diseases such as chronic obstructive pulmonary disease (COPD). Besides, inflammation is a development of other non-communicable diseases, including obesity, diabetes, insulin resistance, atherosclerosis, and neurological disorders (5).

Conclusion:

Pectin that is obtained as the extract of Punica granatum (pomegranate) peel is one of the valuable natural polysaccharides which has pharmaceutical and biological significance. Several extraction techniques are employed to extract pectin with the desired yield and quality such as; acid extraction, microwave-assisted extraction, enzyme-assisted extraction and subcritical water extraction". The most popular of the techniques is acid extraction due to its simplicity, efficiency and excellent recovery rate.

The existence of bioactive compounds like flavonoids, tannins, and phenolic acids in the pomegranate peel are the reasons that lead to its high antimicrobial effect against different microorganisms. As a result, pectin extracted on pomegranate peel has potential uses in pharmaceutically and food industries. In addition, the application of fruit waste products like pomegranate peel in the extraction of pectin helps in the practice of sustainability, and in advancing greener methods in the industrial and research process.

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