

EVALUATE THE POTENTIAL OF MODIFIED AND UNMODIFIED PECTINS DERIVED FROM THE BIOWASTE AS AN ANTI- CANCEROUS COMPOUND

Submitted in partial fulfilment of the requirements for the award of Bachelor of
Technology degree in Biotechnology.

By

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SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
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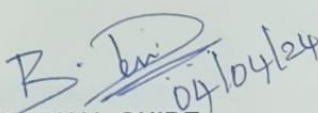
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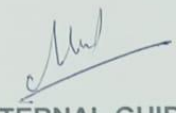
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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **ARUL HANCY EVITAA.K.I (40230005)**, **JEBETHA.SB (40230015)** who carried out Project entitled "**EVALUATE THE POTENTIAL OF MODIFIED AND UNMODIFIED PECTINS DERIVED FROM THE BIOWASTE AS AN ANTI-CANCEROUS COMPOUND**" under our supervision from September 2023 to April 2024.


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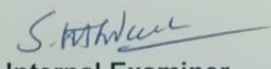

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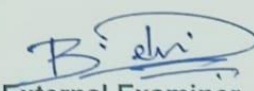

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We, **ARUL HANCY EVITAA.K.I (40230005), JEBETHA.SB (40230015)** hereby declare that the Project Report entitled “**EVALUATE THE POTENTIAL OF MODIFIED AND UNMODIFIED PECTINS DERIVED FROM THE BIOWASTE AS AN ANTI-CANCEROUS COMPOUND**” done by me under the guidance of **Dr. Devi** and **Dr. Michael Rahul Soosai**, at the Department of Chemical Engineering, Sathyabama Institute of Science and Technology, Chennai, is submitted in partial fulfilment of the requirements for the award of Bachelor of Technology degree in Biotechnology.

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ABSTRACT

Cancer is a group of diseases that cause cells in the body to change and spread out of control. Most types of cancer cells eventually form a lump or mass called a tumor, and are named after the part of the body where the tumor originates. Biowastes generated from the food processing industries cause environmental issues due to nitrogen, phosphorous, macronutrients, and water contents. These nutrients make the wastes more susceptible to fermentation, thus causing pollution. However, these biowastes contain a high level of marketable bioproducts extracted for value-added products like pectin. The current study deals with the pectin extraction from pineapple peel (PP) waste employing the ultrasound-assisted extraction (UAE) technique. Further, response surface methodology (RSM) was employed to determine the optimum conditions for maximum pectin extraction using independent variables like ultrasonication time (15–30 min), liquid to solid (LS) ratio (10–20 mL/g), temperature (50–80 °C) and pH (1–2). A maximum pectin yield (16.24%) was attained at 15.20 mL/g of LS ratio, 21.88 min of ultrasonication, 70.83 °C and pH 1.0. The extracted pectin was purified using anion exchange chromatography (DEAE cellulose), and the purity index was 89.5–90%. The purified pectin fractions were analyzed through column chromatography and characterized by SEM, FT-IR, TGA, XRD. The polysaccharide content was quantified using the phenol-sulfuric assay. Other functional properties like emulsification, oil and water holding capacity were also measured. In addition, based on antinutritional and antioxidant properties, the extracted PP pectin was confirmed to be a toxic-free compound. A detailed structural and physio-chemical properties study confirmed the pectin from PP was of good quality and could be utilized as a value-added product in the pharmaceutical industry.

Keywords: *Chromatography, SEM, Ultrasonification, FT-IR, TGA, XRD.*

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LIST OF ABBREVIATIONS

DNA	Deoxyribonucleic acid
GC-MS	Gas chromatography-Mass spectrometry
SEM	Scanning electron microscopy
FT-IR	Fourier transform infrared
UAE	Ultrasonic-Assisted Extraction
TGA	Thermogravimetric Analysis
XRD	X-Ray powder Diffraction
RSM	Response surface methodological
GalA	Galacturonic Acid
CC	Column Chromatography
KMNO ₄	Potassium permanganate
LS	Liquid to solid ratio
MS	Mean square
NaBH ₄	Sodium Borohydride
NaCl	Sodium chloride
NaN ₃	Sodium azide
NaOH	Sodium hydroxide
NH ₄ OH	Ammonium hydroxide
NH ₄ SCN	Ammonium thiocyanate
OD	Optical density
OHC	Oil holding capacity
OVAT	One variable at a time polygala polygalacturonic acid
PP	Pineapple Peel
PRESS	Predicted error p-value Probability of obtaining test results

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CHAPTER 1

INTRODUCTION

Pectin, a Heteropolysaccharide commercially derived from the cell wall of higher plants, is mainly used in food as a gelling agent in jams and jellies and a stabilizer in fruit juice and milk drinks. It has also received significant interest as a source of dietary fibre. Furthermore, pectin is proven to have diverse biological activities including lipid and cholesterol level-lowering effects, serum glucose and insulin content lowering effects, gastric emptying delay, and anti-cancer activities. Pectin has been shown to induce apoptosis in human colonic adenocarcinoma cells and to have anti-metastatic properties. Dietary pectin can bind metal ions, particularly lead ions, thus reducing their retention in the body and diminishing their toxic effects. On the other hand, pectin enhances intestinal solubility and absorption of ferric iron. Pectin with a low degree of esterification or having a large volume of linear oligogalacturonide segments shows significant mucoadhesion capacity in the gastrointestinal tract. In this way, pectin forms a physical barrier protecting epithelium against opportunistic microbial invasion during stress. Pectin is derived from plants and can be utilized as a bioplastic material for a range of applications. It is a carbohydrate polymer derived mostly from natural sources that serve as a structural component of plants' cell walls. Pectin is a biocompatible polysaccharide with biological activity that can take on many shapes depending on the source or extraction method. It is a poly α 1–4-galacturonic acid containing carboxylic acid residues that have been methylated to various degrees. Since it has low cost, biodegradability, water solubility, and non-toxicity, pectin can be utilized for a variety of reasons. Pectin and structurally modified pectin 10 are the most promising pharmaceutical and medicinal applications as well as used for drug delivery systems (DDS). Increasing interest in pectin is due to its easy availability in nature and increasing availability in the pharmaceutical industry. Due to their exceptional physical, chemical, and biological capabilities, biotechnologists and microbiologists have developed various types of biopolymers for specific applications in the biomedical and pharmaceutical industries.

Over the last few years, there has been a growing awareness of environmental preservation and resource sustainability. As a result, natural resource management is regaining prominence in material technology as a viable alternative to non-renewable reserves. Fruit processing industries generate many highly perishable biowastes that are set down in the environment, adding significantly to the accumulation of solid wastes and leading to pollution. Thus, a greater concern is needed for processing these wastes by the relevant industries and that the disposal of wastes is also properly monitored by pollution monitoring authorities (Minjares-Fuentes et al., 2014).

Pineapple (*Ananas comosus*) is amongst the most prominently traded tropical fruits globally after avocado, mango, and bananas. Moreover, it is considered as one of the most popular fruit juices after orange and apple.

Pineapple industries produce major wastes from 50% of total fruit mass, with peel accounting for 29–40%, the core for 9–10%, the stem for 2–5%, and the crown for 2–4%. However, this pineapple peel (PP) wastes contain a high level of marketable bioproducts, which could be utilized for value-added products, including pectin, dietary fiber, and enzymes (Ketnawa et al., 2012). Therefore, bioproduct recovery from PP wastes can considerably develop the economics of processing units and reduce environmental problems. Pectin is a plant cell wall polysaccharide consisting of a complex mixture of D-galacturonic acid monomers, some of which are esterified or acetylated with methyl groups, linked up primarily within α -(1 $\rightarrow$ 4)-

List of symbols and abbreviations

β_0 Intercept coefficient β_{ij} Quadratic model of the linear model β_j Coefficient of the linear model β_{jj} Second-order terms of the linear model η Pectin solution viscosity η_r Relative viscosity η_s Solvent viscosity ^{13}C NMR Carbon-13 (C13) nuclear magnetic resonance 1D NMR One-dimensional nuclear magnetic resonance ^1H NMR Proton nuclear magnetic resonance 2D NMR Two-dimensional nuclear magnetic resonance ANOVA Analysis of variance AOAC Association of official analytical chemists AUA Anhydrouronic acid c Concentration of pectin $\text{C}_{12}\text{H}_{22}\text{O}_{11}$ Lactose $\text{C}_{12}\text{H}_{22}\text{O}_{11}$ Maltose $\text{C}_{12}\text{H}_{22}\text{O}_{11}$ Sucrose $\text{C}_4\text{H}_9\text{OH}$ Butanol $\text{C}_5\text{H}_{10}\text{O}_5$ Arabinose $\text{C}_5\text{H}_{10}\text{O}_5$ Ribose $\text{C}_5\text{H}_{10}\text{O}_5$ Xylose $\text{C}_5\text{H}_5\text{N}$ Pyridine $\text{C}_6\text{H}_{12}\text{O}_6$

Fructose C₆H₁₂O₆ Glucose C₆H₁₂O₆ Galactose C₆H₁₂O₆ Mannose C₆H₈O₆
 Ascorbic acid C₇H₈ Toluene Ca²⁺ Calcium ion CaSO₄ Calcium sulfate
 CCD Central composite design CCP Commercial citrus pectin CF₃COOH
 Trifluoroacetic acid CH₂Cl₂ Dichloromethane CH₃COOH Acetic acid CHCl₃
 Chloroform COSY NMR Correlation spectroscopy nuclear magnetic resonance cts
 Counts d1 Pectin solution density d2 Density of the solvent DD Double distilled DE
 Degree of esterification DEAE Diethylaminoethyl cellulose df Degree of freedom DM
 Degree of methoxylation DPPH 2,2-diphenyl-1-picrylhydrazyl DW Dry weight EC
 Emulsifying capacity ES Emulsifying stability FeCl₂ Ferric chloride FT-IR Fourier
 transform of infrared radiation Gal A Galacturonic acids GC-MS Gas
 chromatography-mass spectrometry H₂O Water HCl Hydrochloric acid HgCl₂
 Mercuric chloride k Independent process parameter variables KMnO₄ Potassium
 permanganate LS Liquid to solid ratio MS Mean square MW Molecular weight
 NaBH₄ Sodium borohydride NaCl Sodium chloride NaN₃ Sodium azide NaOH
 Sodium hydroxide NH₄OH Ammonium hydroxide NH₄SCN Ammonium thiocyanate
 OD Optical density OHC Oil holding capacity OVAT One variable at a time polyGalA
 Polygalacturonic acid PP Pineapple peel PRESS Predicted error p-value Probability
 of obtaining test results R² Coefficient of determination R_f Retention factor RSM
 Response surface methodology R_t Retention time SE Solvent extraction SEM
 Scanning electron microscope SS Sum of square ST Sonication time T Temperature
 t₁ Pectin solution flow time in the viscometer t₂ Solvent flow time in the viscometer
 TGA Thermogravimetric analysis CC Column chromatograph, UAE Ultrasound-
 assisted extraction V_f Emulsion volume V_i Total volume of the solution V_t Centrifuged
 emulsion volume after incubation w Weight of sample taken (%AUA calculation)
 WHC Water holding capacity W_o Weight of dried extracted PP pectin W_t Weight of
 treated PP biomass X_i, X_j Process variables XRD X ray diffraction y Volume of NaOH
 from methoxyl content Y Response of pectin yield z Volume of NaOH from equivalent
 weight C.S. Shivamathi et al. Food Hydrocolloids 123 (2022) 107141 3 glycosidic
 bond backbone present in the middle lamella of the cell wall region of terrestrial
 plants (Ochoa-Villarreal et al., 2012).

Pectin is most commonly found in fruits like citrus fruits, apples, pears, plums, guavas, etc. However, pectin usage in recent years has drastically increased in food industries as a substitute for commercial food additives (Thakur et al., 1997). Pectin is a multifunctional food ingredient utilized to produce jams, jelly preparation, and edible films in the pharmaceutical industry. Around 80–90% of 7000 tonnes of commercial pectin worldwide are used to produce jelly and related products (Madhav & Pushpalatha, 2006). The global pectin market demand has led to about 45, 000 tons yearly, with a 4–5% increase per year (Methacanon et al., 2014). Pectins are categorized according to their degree of methoxylation (DM), low methoxy pectin when the DM is less than 50%. Such pectins are used as stabilizers, emulsifying agents, texturizers, and colorants in the food industries due to methyl esterified galacturonic acid, which possesses a food protecting capacity (Lara-Espinoza et al., 2018; Sims,ek, 2004). Further, high methoxy pectins (DM greater than 50%) are used in the pharmaceutical industries due to their reported potential properties like anti-cancer, cholesterol-lowering properties, and control of blood glucose uptake level (Kammerer et al., 2014; Majee et al., 2018). Citrus peel or apple pomace are the two exclusively used sources of commercial pectin. Although pectin exists widely in various plant tissues, the number of sources utilized for industrial pectin production is limited (Boukroufa et al., 2015).

Pectin in the cancer treatment The incidence of cancer is increasing owing to metastases and tumor cell medication tolerance, even though a wide range of scientific investigations have been stepped up to tackle this disease. Pectin has been shown in studies to play a role in the prevention of metastasis, which is especially true of pectin which has been broken down into smaller fragments with a lower molecular weight that the body can absorb. Pectin that has been altered by the use of chemicals, heat, radiation, and enzymes has stronger anticancer properties than unaltered pectin. The proliferation and migrating of colon carcinoma cells have been shown to be inhibited by functionally engineered pectin that contains neutral sugar sequences with a low degree of branching and is rich in galactose. The prostate is a part of the male reproductive system, which includes the penis, prostate, seminal vesicles, and testicles.

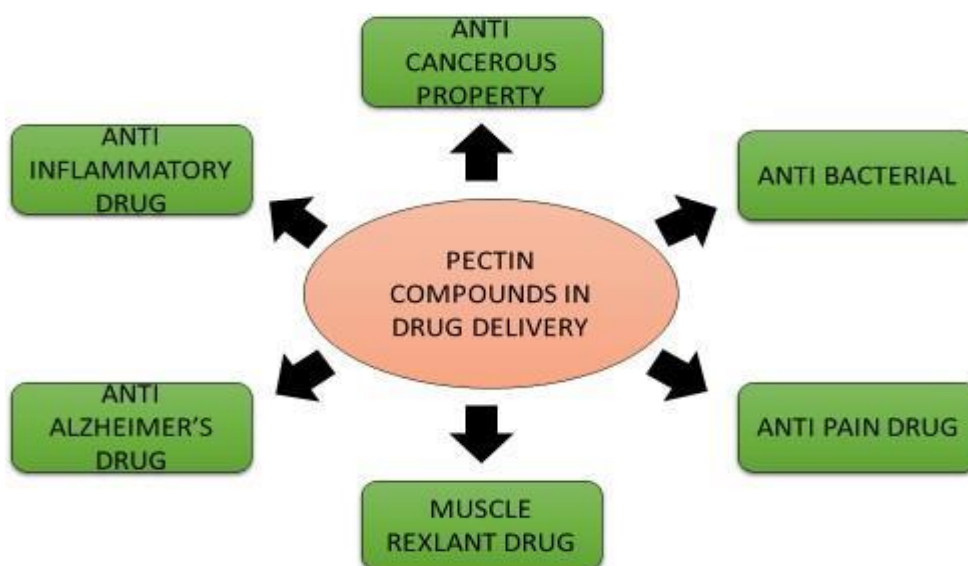


Fig 1.1 *pectin compounds in drug delivery*

1.1 TYPES OF PECTIN AND APPLICATIONS

Pectins are a diverse group of polysaccharides found in various plant tissues, and they can be classified into different types based on their chemical properties, structural features, and functional characteristics. Each type of pectin has specific applications across various industries. Here are some common types of pectin and their applications:

1.1.1 High Methoxyl Pectin (HMP)

Gel- formation properties: HMP forms gels in the presence of high sugar and low pH, commonly used in jams, jellies, and fruit spreads.

Stabilizer and thickener: It provides viscosity and stability in dairy products like yogurts and desserts.

Pharmaceutical applications: HMP is used in controlled-release drug delivery systems and oral suspensions.

1.1.2 Low Methoxyl Pectin (LMP)

Gelling agents for low-sugar applications: LMP forms gels under mild conditions without high sugar content, suitable for sugar-reduced or sugar-free jams and jellies.

Stabilizer in acidic dairy products: LMP enhances texture and stability in acidic dairy products like cream cheese and sour cream.

Health supplements: LMP is used in dietary supplements and pharmaceutical formulations due to its gelation properties.

1.1.3 Amidated Pectin

Thermal Stability: Amidated pectin exhibits improved stability at high temperatures, making it suitable for bakery fillings, fruit preparations, and pasteurized products.

Emulsifier and stabilizer: It enhances emulsion stability in salad dressings, sauces, and mayonnaise.

Pharmaceutical applications: Amidated pectin is used in tablet coatings and as a binder in pharmaceutical formulations.

1.1.4 Modified Pectins:

Cross-linked pectin: Enhances gel strength and stability, used in confectionery products, gummy candies, and fruit snacks.

Enzymatically modified pectin: Improves solubility and dispersibility, suitable for instant drink mixes, powdered supplements, and pharmaceutical suspensions.

Chemically modified pectin: Alters properties like gelling ability, viscosity, and stability for specific applications in food, pharmaceuticals, and cosmetics.

1.1.5 Low-Ester Pectin

Formulation stability: Low-ester pectin provides stability in acidic conditions, used in fruit fillings, pie fillings, and acidic sauces.

Reduced sugar applications: Enables gel formation with lower sugar content, beneficial for diabetic-friendly products and reduced-calories formulations.

Functional beverages: Low-ester pectin is used in juice concentrates, sports drinks, and functional beverages for viscosity control and mouthfeel enhancement.

1.1.6 Native Pectin

Clean label applications: Native pectin extracted using gentle methods retains its natural properties and is suitable for clean label products with minimal processing.

Organic and natural formulations: Native pectin is preferred in organic and natural food products, baby foods, and health-conscious formulations.

These different types of pectin offer a wide range of functionalities and applications, making them versatile ingredients in the food, pharmaceutical, cosmetic, and biotechnological industries. The choice of pectin type depends on the desired texture, stability, processing conditions, and end-product requirements.

1.2. ANTI NUTRITIONAL PROPERTIES OF PECTIN

Pectin, primarily known for its beneficial properties such as dietary fiber and potential health benefits, also possesses certain anti-nutritional properties. These properties are not inherently harmful but can interfere with the absorption of certain nutrients or cause digestive discomfort in some individuals. Here are the main anti-nutritional properties associated with pectin:

1.2.1 *Binding to Minerals*

Pectin can bind to minerals such as calcium, magnesium, zinc, and iron, forming complexes that are less available for absorption in the digestive tract. This can lead to reduced bioavailability of these essential minerals, especially in individuals with low mineral intake or those relying heavily on pectin-rich foods.

1.2.2 *Inhibition of Digestive Enzymes*

Pectin may inhibit the activity of digestive enzymes such as amylase, lipase, and protease to some extent. This inhibition can affect the breakdown and absorption of carbohydrates, fats, and proteins in the digestive system, potentially leading to reduced nutrient absorption and utilization.

1.2.3 *Fermentation and Gas Production*

As a soluble fiber, pectin is fermentable by gut bacteria in the large intestine. While fermentation of pectin produces short-chain fatty acids beneficial for gut health, it can also lead to increased gas production (flatulence) and bloating in some individuals, especially those not accustomed to high-fiber diets.

1.2.4 *Impact on Blood Sugar Levels*

Pectin's gel-forming properties and ability to slow down digestion can influence blood sugar levels. While this can be beneficial for individuals with diabetes or insulin resistance by preventing rapid spikes in blood glucose, it may also interfere with nutrient absorption and affect overall glycemic control in sensitive individuals.

1.2.5 *Potential Allergenicity*

Although rare, some individuals may experience allergic reactions to pectin or pectin-containing foods. Allergic responses can range from mild symptoms like itching and hives to more severe reactions such as anaphylaxis.

People with known allergies to fruits or other plant-derived substances should exercise caution when consuming pectin-rich foods.

1.2.6 Intestinal Bulk and Transit Time

Pectin's ability to increase intestinal bulk and promote regular bowel movements can be beneficial for digestive health. However, excessive intake of pectin or sudden increases in fiber consumption without adequate fluid intake may lead to gastrointestinal discomfort, constipation, or diarrhea in susceptible individuals.

It's essential to note that the anti-nutritional properties of pectin are generally mild and primarily affect certain population groups or individuals with specific dietary sensitivities. For most people, moderate consumption of pectin-rich foods as part of a balanced diet is safe and offers various health benefits, including improved digestive function, cholesterol management, and satiety. As with any dietary component, moderation and individual tolerance should be considered.

1.3 PECTINS COMPOUNDS

The prostate is a part of the male reproductive system, which includes the penis, prostate, seminal vesicles, and testicles. The prostate is located just below the bladder and in front of the rectum. It is about the size of a walnut and surrounds the urethra (the tube that empties urine from the bladder). It produces fluid that makes up a part of semen. The men are at risk for prostate cancer. Out of every 100 American men, about 13 will get prostate cancer during their lifetime, and about 2 to 3 men will die from prostate cancer. The most common risk factor is age. The older a man is, the greater the chance of getting prostate cancer. Some men are at increased risk for prostate cancer. You are at increased risk for getting or dying from prostate cancer if you are African-American or have a family history of prostate cancer.

There are three compounds found after the extraction of pineapple peal waste. They are listed below.

1. RIBOSE
2. GALATURONIC ACID
3. ARABINOSE

1.4 PROSTATE CANCER RECEPTOR

1. Human androgen receptor
2. Human prostate specific antigen
3. Human prostate derived ets factor

CHAPTER 2

LITERATURE SURVEY

2.1 Pectin-based nanoparticles for targeted drug delivery:

Drugs can be loaded into a polysaccharide matrix or be bound to the external surface, improving their aqueous solubility and stability (e.g., proteins). They are used to enhance slow enzymatic degradation, targeting mucosal recognition, receptor binding, adhesion, and transport systems. Polysaccharides have presented advantages and benefits when they are used as biomaterials for the manufacture of nanoparticles. Compared to nanoparticles synthesized from other materials, such as metals or synthetic polymers, polysaccharide nanoparticles do not present toxicity problems due to their biocompatibility and biodegradability, thus providing safety, better storage, and physiological stability.

Nanotechnology-based drug delivery systems aim to target different routes of administration. When designing polysaccharide-based nanoparticles for use in biomedicine and other industries, it is important to consider the route of administration, retention time, and therapy. These factors may be dependent on the polysaccharide used, its size, and its structure. One possible disadvantage when using polysaccharides for manufacturing nanoparticles is their high molecular weights. In addition, in many cases, the low solubility of some polysaccharides limits their potential in chemical modification. Due to the polysaccharide properties, the size of polysaccharide-based nanoparticles has been considered between 10 and 1000 nm. These nanoparticles can carry drugs, proteins, peptides, or DNA material to target a specific organ or cell. Depending on the technique, the drug or other active compound can adhere, dissolve, encapsulate, or entrap into the nanoparticle matrix. The significant interest in this technology is due to the fact that the nanoparticles can target specific organs or Diana cells, and reduce side effects. Nanoparticles have a larger surface area, and their smaller size allows them to enter smaller capillaries and cells.

Nanoparticle size and surface charge are highly important because many secondary properties, such as surface area, toxicity, degradation, targeting, and absorption mechanism, are related.

Polysaccharide-based nanoparticles have shown significant potential in biological and pharmaceutical applications. Natural polysaccharides used to fabricate nanoparticles include chitosan, alginate, pectin, guar gum, dextran, hyaluronic acid, and arabinoxylan.

2.2 Pectin-based hydrogels for sustained drug release:

Over the last 50 years, hydrogels based on biodegradable natural polymers have been widely used in drug delivery systems. Hydrogels have expanded in the drug delivery field as their three-dimensional structures have exclusive properties such as being hydrophilic in nature, biocompatibility, biodegradability, moist environments in surrounding tissues, and low cost. Thus, in this section, various forms of Pec-based hydrogels in drug delivery applications are showcased.

In another work, hydrogels were fabricated by graft polymerization and magnetic nanoparticles (MNPs) comprised in Pec-based hydrogels through the in-situ method for the controlled release of the diclofenac sodium (DS) drug. Due to the presence of MNPs, there was a substantial enhancement in the mechanical properties, swelling capacity, drug loading efficiency, and drug release performances. Due to the porous network and high surface of the MNPs, the MNP-based pectin hydrogels displayed 68.84% of drug loading efficiency, however, without MNPs, the Pec-based hydrogels showed only 44.84% of loading efficiency.

It was identified that around 95% of the DS drug was released from the MNP based Pec hydrogels, suggesting that swelling controlled the diffusion mode of the drug release profiles.

Other research investigations have been reported to improve the therapeutic effects and lessen the toxic effects of the systematic drug administration of vancomycin hydrochloride (Vanco HCl), a hydrogel scaffold of silk fibroin/oxidized pectin (SF/OP) designed based on the Schiff-base reaction. To obtain a sustained release profile of the drug, electrospun fibers of poly(L-lactide) (PLLA) were incorporated into the hydrogel and unveiled high 97% of drug loading efficiency, followed by a 61% decrease in drug release content.

For the first 24 h, the drug release profile from hydrogel was 39.97% and in the hydrogel/fiber system, a 13.83% decrease in the drug release content was observed, followed by promising sustained release up to 192 h. In addition, the designed hydrogel drug delivery systems proved to be non-toxic against human adipose derived mesenchymal stem cells (hAD-MSCs).

2.3 Pectin as a carrier for bioactive compounds:

Bioactive compounds (essential oils, vitamin, minerals, polyphenols, carotenoids and other compounds) are those substances obtained from fruits, vegetables, oils, legumes, nuts and other plant sources have good effects on health through cellular activities as well as physiological activities.

Another important nanocarrier is solid lipid nanoparticles (SLNs) which are like nano emulsions however their dispersed phase is solidified in the oil component. This helps in reducing leakage of encapsulated bioactive component.

The utilization of nature-derived polymers has been well-described in the literature for food and biomedical applications. Among different biopolymer-based carriers, there is an increasing interest in designing protein-based delivery devices, as GRAS (generally regarded as safe) materials, by virtue of their extraordinary attributes, e.g., high nutritional value, abundant renewable sources, biodegradability/biocompatibility, non-antigenicity, and outstanding binding capacity of diverse bioactive molecules. They demonstrate unique functional features, i.e., emulsification, gelation, foaming, and water-binding capacity, in addition to their easy preparation and scale-up during manufacture.

Of particular interest for bioactive delivery via protein-based delivery systems are whey protein-based vehicles (WPVs) due to their diverse functionalities and ability to offer a plethora of architectural options capable of a specific application. Through encapsulation, WPVs (i.e., micro/nanoparticles, hydrogels, nanogels, emulsions, Pickering emulsions, conjugates, complexes, nanotubes, etc.) can enhance the bioavailability of bioactive components, protect them from harsh environmental conditions (like light, O₂, temperature, enzymes, etc.), and thus lead to controlled/sustained release.

More so, WPVs typically degrade *in vivo* through biological processes, and naturally occurring hydrolysates are deemed as generally safe.

Many recent review articles offer good insight into the development of various protein-based delivery systems; nevertheless, rather little attention has been given to the latest and most commonly utilized encapsulation platforms based on WPVs.

This review underlines the recent progress of WPVs, in which the vehicles matrix is utilized to promote targeting, bioavailability, (bio) stability, and release of the bioactive molecules. Nevertheless, owing to the wide spectrum of research papers that have been published and the restricted space available for this review, it is almost impossible to offer a comprehensive overview of the field. Instead, we tried to highlight the bioactive-comprising systems that make use of whey proteins as delivery vectors. After an overview of different barriers to oral bioactive delivery, we illustrated the progress made on WPVs in order to tackle them and discuss strengths and weaknesses of these delivery systems and the road ahead.

2.4 Pectins as biomaterials in tissue engineering

Biomaterials have been widely used in tissue engineering applications at an increasing rate in recent years. The increased clinical demand for safe scaffolds, as well as the diversity and availability of biomaterials, has sparked rapid interest in fabricating diverse scaffolds to make significant progress in tissue engineering. Hydroxyapatite has drawn substantial attention in recent years owing to its excellent physical, chemical, and biological properties and facile adaptable surface functionalization with other innumerable essential materials. This focused review spotlights a brief introduction on Hydroxyapatite, scope, a historical outline, basic structural features, various synthetic strategies, and their scientific applications concentrating on functionalized hydroxyapatite in the diverse area of tissue engineering fields such as bone, skin, periodontal, bone tissue fixation, cartilage, blood vessel, liver, tendon, and corneal are emphasized. Besides clinical translation aspects, the future challenges and prospects of hydroxyapatite based biomaterials involved in tissue engineering are also discussed. Furthermore, it is expected that researchers may find this review expedient in gaining an overall understanding of the latest advancement of hydroxyapatite based biomaterials.

CHAPTER 3

AIM AND OBJECTIVE

AIM

The process of optimization and characterization of pectin derived from underexploited pineapple peel biowaste as a value-added product.

OBJECTIVE

To optimize and characterize the pectin from pineapple peel as a value-added product.

Functional properties like Emulsification, oil, water holding capacity were also measured.

To extract the solution by Ultrasonification technique.

In-silico system of pharmacology approach.

CHAPTER 4

MATERIALS AND METHODS

4.1 RAW MATERIALS AND CHEMICALS

Pineapple (harvested in august 2023) peel was obtained from a fruit juice shop in Kanchipuram, Tamil Nadu, India. First, the ripened matured PP was manually removed, chopped into small pieces and sun-dried. Further, the peels were desiccated at 50 °C in a hot air drier until constant dry peel weight was obtained. Finally, the samples were powdered and sieved using an orbital sieve shaker (TMC associates, Chennai). To avoid bacterial and fungal contamination, the samples were packed in airtight containers. All the analytical grade chemicals hydrochloric acid (HCl), ammonium hydroxide (NH₄OH), absolute alcohol (99%), sodium hydroxide (NaOH), potassium permanganate (KMnO₄), phenolphthalein, methyl orange, mercuric chloride (HgCl₂), sodium chloride (NaCl), silica powder, trifluoroacetic acid (CF₃COOH), ammonium thiocyanate (NH₄SCN), sodium azide (NaN₃), butanol (C₄H₉OH), chloroform (CHCl₃), acetic acid (CH₃COOH), xylose (C₅H₁₀O₅), lactose (C₁₂H₂₂O₁₁), fructose (C₆H₁₂O₆), ribose (C₅H₁₀O₅), mannose (C₆H₁₂O₆), sucrose (C₁₂H₂₂O₁₁), arabinose (C₅H₁₀O₅), maltose (C₁₂H₂₂O₁₁), glucose (C₆H₁₂O₆), galactose (C₆H₁₂O₆), Mayer reagent, calcium sulfate (CaSO₄), DPPH (2,2-diphenyl-1-picrylhydrazyl), ascorbic acid (C₆H₈O₆), ferric chloride (FeCl₃) were purchased from HiMedia Laboratories Pvt Ltd, Mumbai, India. Other requisite chemicals, namely DEAE cellulose, sodium borohydride (NaBH₄), pyridine (C₅H₅N), toluene (C₇H₈), and dichloromethane (CH₂Cl₂).

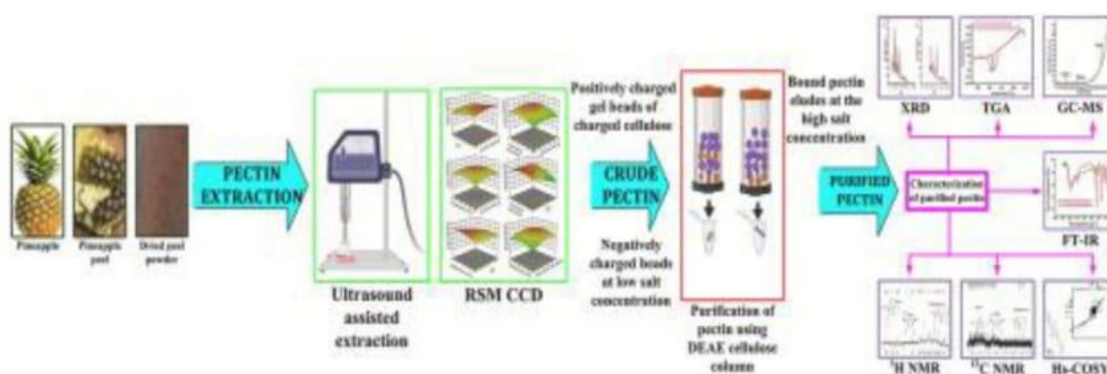


Fig 4.1: (a) Dried pineapple peel; (b) Powdered pineapple peel.

4.2 ULTRASONIC- ASSISTED EXTRACTION OF PECTIN

The powdered PP (1 g DW) was dissolved in 20 mL DD H₂O and 1N HCl was used to vary the solution pH (1–3). Pectin was extracted using an ultrasonicator (Sonics Vibra Cell VCX 130, USA) with a 2 cm tip probe C.S. Shivamathi et al. Food Hydrocolloids 123 (2022) 107141 4 (Wang, Ma, et al., 2016). The process was operated at a pulse of 10 s on and 10 s off-cycle, at 20 kHz of the ultrasonic frequency with 75% amplitude. The solution was cooled to 25°C and centrifuged (REMI C-24 plus, India) for 15 min at 8000 rpm. The obtained supernatant was mixed with twice the amount of absolute ethanol and incubated overnight at ambient temperature (Bagherian et al., 2011). The colloidal mixture formed was filtered using Whatman No.1 filter paper, then the retentate was separated and washed twice with 96% ethanol. The content was then dried for 2 h at 50°C.

Graphical Abstract



4.3 PURIFICATION PROFILE

Pectin of both the selected mulberry varieties was mixed with water and boiled to make a solution separately. 20 ml of N/10 bromine water was added to 50 ml of the cooled pectin solution. Oxidation was allowed to proceed for varying periods at different temperatures. Excess bromine water was removed by shaking 2–3 times and added ether until the aqueous layer was colorless. Pectin samples were then precipitated by 95% alcohol and after 2 h were filtered through a muslin cloth. Filtered pectins were finally passed through various grades of alcohol and dried in an oven at 100°C for 20 h.

4.4 STRUCTURAL AND THERMAL CHARACTERIZATION

For the characterization study, the morphological and functional properties of purified pectin were compared with CCP. SEM analysis was used for the morphological characterization at 20 kV, 200 μ m magnifications (Zeiss, Germany) (Klaver et al., 2015). The functional groups were identified using FT-IR (SHIMADZU IR, Japan) in the 4000 to 500 cm^{-1} wavelength range. XPERT-PRO instrument (Malvern Panalytical, UK) was used to record the XRD profile in the range of 2θ (100–800 cm^{-1}). An STA 6000 system (Perkin Elmer, USA) was used for TGA, obtained over a temperature range of 40–300°C (heating ramp rate of 10°C/min) (Vaziri et al., 2018). The pectin was solubilized in D₂O and the chemical shifts were expressed as δ (ppm) in relation to the resonance of an internal standard. (Grassino et al., 2016; Westerlund et al., 1991).

4.5 ANTINUTRITIONAL PROPERTIES

Antinutritional properties are biological components that can reduce the nutrient value of a bioactive compound. Therefore, antinutritional properties like oxalate, phytic acid, saponin, and tannin content need to be studied for evaluating the application of pectin in the food or pharma industry.

4.6 FUNCTIONAL PROPERTIES

4.6.1. *Water holding capacity (WHC) and oil holding capacity (OHC)*

Briefly, 1 g (DW) of purified PP pectin was added to 10 mL of DD H₂O or refined vegetable oil. The solution was vigorously mixed for 1 min and then centrifuged at 3000 rpm for 30 min (Bayar et al., 2018).

Finally, the pellet was collected and weighed. The amount of water or oil held by 1 g of PP pectin determined its WHC or OHC, respectively.

4.6.2. Emulsifying property

In 5 mL of corn oil, 2 and 4% of purified PP pectin dissolved in DD H₂O were suspended together. Then, the suspension was mixed vigorously for 3 min and centrifuged for 3 min at 5000 rpm, and using the below equation the emulsifying capacity was calculated (Kazemi et al., 2019a):

$$EC (\%) = V_f / V_i \times 100$$

To assess the emulsion stability, the solution was incubated at 50°C for 60 min and then centrifuged for 15 min at 5000 rpm.

$$ES (\%) = V_t / V_f \times 100$$

The total volume of the solution was denoted as the V_i ; emulsion volume was denoted as the V_f ; centrifuged emulsion volume after incubation was denoted as V_t .

4.7. PECTIN DERIVED FROM PINEAPPLE PEEL IN THE REGULATION OF INSILICO SYSTEM OF PHARMACOLOGY APPROACH.

The biological application of pectin nanocomposite is also explained in this review, along with a recent publication. As a result, the goal of this review was in-depth analysis to summarize biological application of pectin and its modified nanocomposites. Due to their exceptional physicochemical and biological characteristics, pectin and its nanocomposites are remarkable materials for medicinal applications. In addition to enhancing the immune system, controlling blood cholesterol, and other things, they have been shown to have anticancer, antidiabetic, antioxidant, anti-inflammatory, immunomodulatory, and antibacterial properties. Because of their biocompatibility and properties that allow for regulated release, they have also received a lot of interest as drug carriers in targeted drug delivery systems. They have been used to administer medications to treat cancer, inflammation, pain, Alzheimer's, bacteria, and relax muscles.

This review found that pectin and its derivatives have better drug delivery efficiency and are viable candidates for a wide range of medicinal applications. It has been advised to conduct further research on the subject of toxicity in order to produce commercial formulations that can serve as both therapeutic agents and drug carriers.

The prostate cancer proteins or ligands are linked with the pectin compounds and it was checked for the target binding. This shows that the 8 pectin compounds have some anti-cancerous activity. With the help of some bioinformatic tools like MALSOFT, SWISS ADME, PRO TOX-II, SWISSTARGET PREDICTION, PYROX and molecular docking tools the binding activity of the pectin compound and the prostate cancer ligands were found out.

4.7.1 Retrieval of the bioactive compound

The bioactive compound of pectin like RIBOSE, GALATURONIC ACID and ARABINOSE were retrieved from the PubChem database (pubchem.ncbi.nlm.nih.gov). The following steps were followed for the retrieval of the active compound:

The PubChem database was searched using the keyword 'RIBOSE, GALATURONIC ACID and ARABINOSE'.

The search results were filtered to find the compound record of pectin compounds. The chemical structure of pectin compounds was retrieved from the database in the desired format.

The molecular weight, chemical formula, and other relevant information of the active compounds were also extracted from the database.

4.7.2 In-Silico Drug-Likeness and molecular property prediction:

The 3 pectin compounds were then subjected to In-silico drug-likeness prediction and molecular property prediction. This was carried out using the MolSoft server, which provides various tools for predicting the molecular properties of compounds. The following molecular properties are predicted:

- To assess the ability of 3 pectin compounds to penetrate the bloodbrain barrier, the MolSoft server is utilized to obtain the BBB Score.
- The drug-likeness model score is determined using MolSoft software, based on the Rule of Five, to predict the suitability of BetaSitosterol for oral medication.
- The MolSoft server is used to calculate the lipophilicity and aqueous solubility of Beta-Sitosterol by determining the LogP and LogS, respectively.
- The MolPSA/TPSA is computed by the MolSoft server to predict drug delivery characteristics. A PSA of less than 90 Å² is typically necessary for molecules to cross

the blood-brain barrier. Additionally, the molecular polar surface area and volume are inversely proportional to the percentage of absorption (%ABS).

- The MolSoft server determines the molecular weight, hydrogen bond 15 donors, and hydrogen bond acceptors of 3 pectin compounds. The utilization of In-Silico methods to predict the drug-likeness and molecular properties of 3 pectin compounds can yield valuable insights into its physicochemical characteristics that may be applicable to its potential use as a drug against human reproductive receptors.

4.7.3 In- Silico prediction of ADME properties:

The Swiss ADME server (www.swissadme.ch) was used to evaluate the unique ADME 16 properties of 3 pectin compounds. The results were presented in various formats, such as tables, graphs, and an Excel spreadsheet. The SMILES notation was used to describe the multiple input molecules, with each molecule entered on a separate line. The bioavailability radar produced by the Swiss ADME software offers a rapid assessment of the drug-likeness of the molecules being studied, taking into consideration six key physicochemical characteristics, namely, lipophilicity, size, polarity, insolubility, saturation, and flexibility.

Lipophilicity	The partition coefficient between n-octanol and water, log Po/w, is a crucial property for drug discovery. SwissADME provides access to five predictive models, including atomistic, topological, and physics-based methods, to estimate log Po/w. Consensus log Po/w is calculated as the mean of the predictions from these methods.
Water solubility	Solubility greatly impacts drug development, as it affects handling, absorption, and formulation.

	SwissADME offers three predictive models to estimate water solubility, including two topological methods (ESOL and Ali et al.) and a fragmental method (SILICOS-IT). All predictions are in the form of log S, with additional qualitative solubility classes and solubility units provided.
Pharmacokinetic parameters	SwissADME is a web tool that predicts the pharmacokinetic properties of small molecules. It provides predictions for skin permeability, passive human gastrointestinal absorption, blood-brain barrier permeation, substrate or inhibitor of P-gp, and inhibitor of major CYP isoenzymes.
Drug likeness	Drug-likeness is a qualitative assessment of a molecule's likelihood to become an oral drug. SwissADME provides access to rule based filters, including the Lipinski, Ghose, Veber, Egan, and Muegge methods, to filter chemical libraries for drug-like molecules.

Medical chemistry properties	SwissADME offers pattern recognition methods to identify potentially problematic fragments in a molecule, including PAINS and Structural Alert filters. It also provides a rule-based method for lead likeness and discusses the importance of considering synthetic accessibility in the selection of virtual molecules.
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CHAPTER 5

RESULTS AND DISCUSSION

5.1 EXTRACTION

The powdered PP (1 g DW) was dissolved in 20 mL DD H₂O and 1N HCl was used to vary the solution pH (1–3). Pectin was extracted using an ultrasonicator (Sonics Vibra Cell VCX 130, USA) with a 2 cm tip probe C.S. Shivamathi et al. Food Hydrocolloids 123 (2022) 107141 4 (Wang, Ma, et al., 2016). The process was operated at a pulse of 10 s on and 10 s off-cycle, at 20 kHz of the ultrasonic frequency with 75% amplitude. The solution was cooled to 25°C and centrifuged (REMI C-24 plus, India) for 15 min at 8000 rpm. The obtained supernatant was mixed with twice the amount of absolute ethanol and incubated overnight at ambient temperature (Bagherian et al., 2011). The colloidal mixture formed was filtered using Whatman No.1 filter paper, then the retentate was separated and washed twice with 96% ethanol. The content was then dried for 2 h at 50°C.

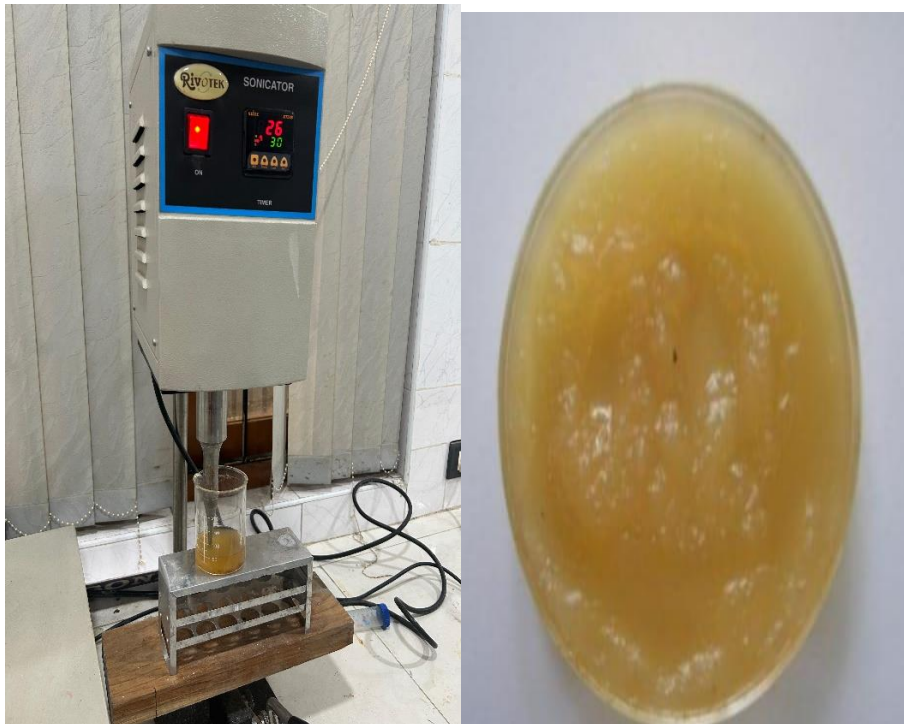


Fig.5.1: (a) Ultrasonication;(b) Pectin Extraction

5.2 PURIFICATION AND CHARACTERIZATION OF PP PECTIN

The purification of extracted PP pectin was performed using anionexchange chromatography (DEAE cellulose) eluted with stepwise elution with increasing NaCl gradient. The column purified fractions were used to quantify the pectin present in the PP extract using phenolsulfuric assay (Keijbets & Pilnik, 1974). Since DEAE cellulose covered a relatively narrow range, thus leading to a higher resolution, only one major peak could be distinguished, indicating that the pectin was quite homogeneous in molecular weight and charge density (Bonnin et al., 2002; Schols et al., 1995). The purity index of PP pectin was 89.5–90%, thus suggesting only one type of polymer. Similar studies have reported that the distribution of ester groups throughout the galacturonan chain impacts pectin fractionation on DEAE-cellulose, confirming that a higher concentration of buffers is needed for the desorption of the less methylated pectin (Mukhiddinov et al., 2000).

The purified pectin was then characterized by column chromatography compared with standard sugar monomers. The derivatized pectin was analyzed by GCMS (Fig.5.3) to confirm sugars and pectin levels. The obtained results revealed the presence of xylose (0.5–1.0%), ribose (0.7–2.0%) and pectin (1.5–4.0%).



Fig.5.2: Purified Pectin

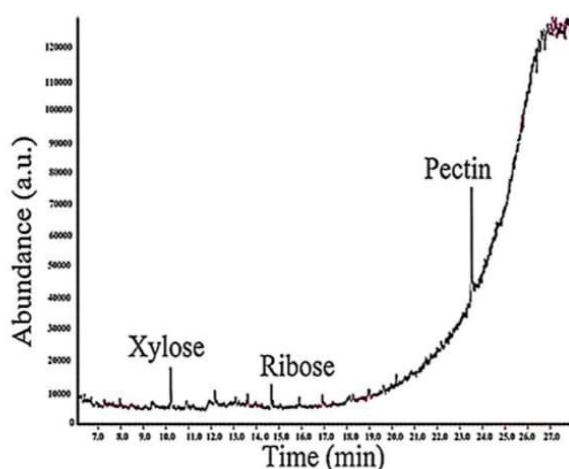


Fig 5.3: GC-MS analysis of purified pp pectin after derivatization process.

To study the functional groups of PP pectin, fourier-transform infrared (FT-IR) spectra were analyzed and compared with CCP. The FT-IR spectrum of PP pectin showed resemblances in their transmission patterns to that of CCP. In Fig. 5.4, the wavenumber range of $940\text{--}1150\text{ cm}^{-1}$ is the fingerprint spectrum of carbohydrates. The prominent peaks related to the attributes of pectin polysaccharides appeared at 1099 , 1024 , and 962 cm^{-1} , which were ascribed to C–C stretching, C–H stretching, and C–O bending, respectively were similar to the report on pectin from the lime peel (Rodsamran & Sothornvit, 2019). Galacturonic group, carboxylic group, methyl, and ester group represent the wavenumber region of $1720\text{--}1760\text{ cm}^{-1}$. Particularly, the peak at 1730 cm^{-1} denotes the existence of galacturonic acid (Rodsamran & Sothornvit, 2019). Moreover, the region between 1800 and 1500 cm^{-1} represents the degree of methylation, which was comparatively less in PP pectin than CCP (Abid et al., 2017). In the region of $2970\text{--}2930\text{ cm}^{-1}$, the CCP showed more intense C–H absorption compared to the PP pectin. This can be described by a higher C–H bond concentration after amidation by long-chain aliphatic amines. The peaks ranged between 3000 and 3500 cm^{-1} , referred to as the inter and intra-molecular hydrogen binding of O–H groups associated with the galacturonic acid units (Kazemi et al., 2019b). The peaks at 1632 and 1748 cm^{-1} (fingerprint region of pectin) represent the free carboxylic and esterified groups of polymers containing galacturonic acid, respectively (Karbuz & Tugrul, 2021).

Therefore, the FT-IR spectra for the PP pectin sample substantiate the presence of pectin (polygalacturonic acid).

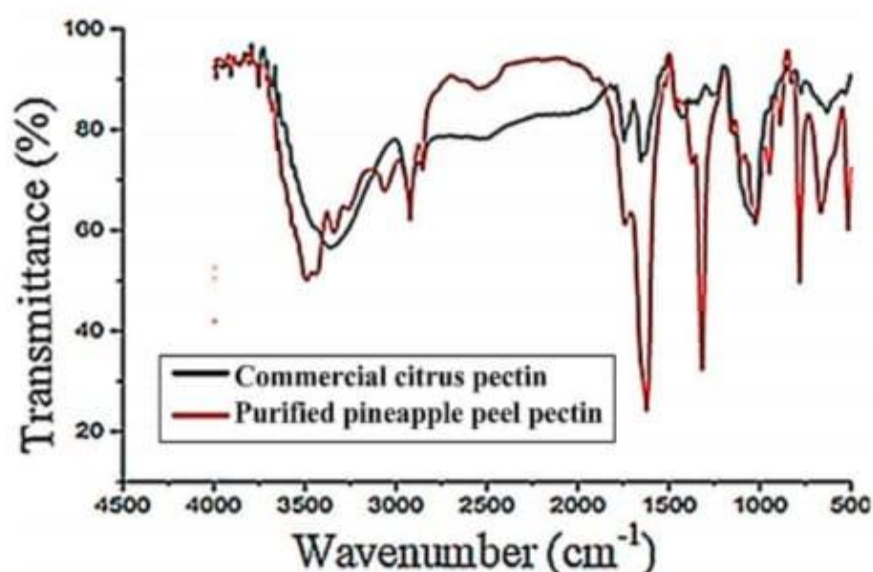


Fig 5.4: FT-IR analysis of commercial pectin and pectin from pp.

Pectin crystallinity differs based on the raw material and extraction procedures employed. Therefore, X-ray diffraction (XRD) analysis was employed to study PP pectin structure (amorphous or crystalline) compared to CCP. The XRD patterns of pectin between 5° and 65° (2 θ) (Fig. 5.5) indicated that both CCP and PP pectin structures were relatively crystalline, rather than amorphous, because of the appearance of sharp and narrow peak range signals. Thus, the obtained results suggest the crystalline nature of PP pectin. Similar results were also reported for *Tamarindus indica* L. pulp, eggplant peel, and pistachio green hull pectin (Kazemi et al., 2019b & 2019c; Sharma et al., 2015.).

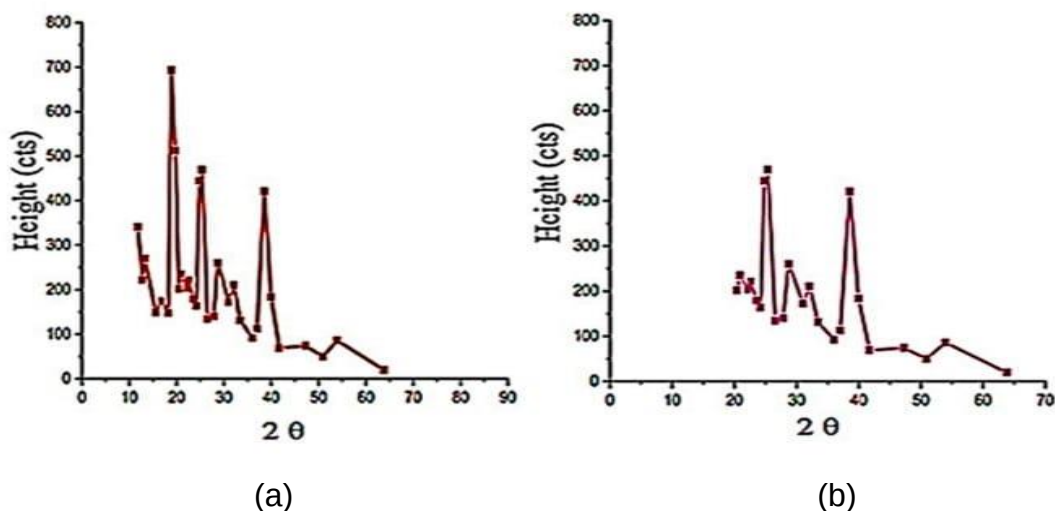


Fig 5.5 XRD diffraction analysis (a) Commercial pectin (b) PP pectin

The potential for structural modification or evolution, during the pectin recovery process, of PP pectin was checked by scanning electron microscopy (SEM). In this study, SEM was used to examine the structural changes in treated (after sonication) PP materials. Because of the rapid rise in inside pressure and temperature, as well as the ultrasound energy penetration into the solvent and sample, the cell wall in the treated samples was ruptured and destroyed during ultrasound treatment (Fig.5.6), whereas no signs of such disintegration was noticed in the untreated sample (Sharma & Ahuja, 2011). As a result, chemical substances from the interior plant materials were released into the solvent system, improving the pectin recovery.

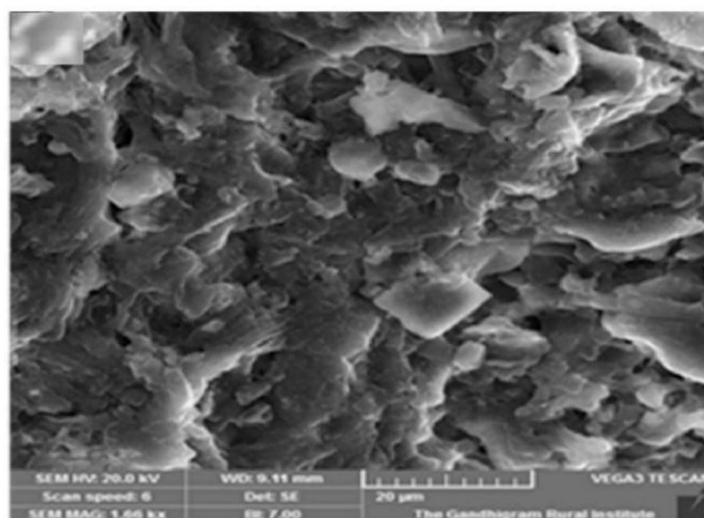


Fig: 5.6. PP powder after ultrasound-assisted extraction process

TGA was used to examine the thermal characteristics and designate changes throughout the thermal degradation of PP pectin. The endothermic and exothermic thermal effects of PP pectin and CCP were conducted by TGA (Vaziri et al., 2018). The results indicated that endothermic peak formed due to the heat of fusion that arises due to water holding capacity by the presence of the hydrophilic group (Fig. 5.7). The endothermic peak observed in PP pectin at 420 °C and for CCP at 480 °C are due to galacturonic acid that decreases the transition temperature, which lowers the degree of methylation. The peak at 980 °C, the exothermic peaks of the PP pectin, and 970 °C for CCP indicated the degradation properties of pectin. Compared to the CCP, the PP pectin showed a pyrolytic breakdown which is the primary source of deterioration in this temperature range. Pectin used in food products is exposed to elevated temperature; thus, pectin with high-temperature resistance might be further preferred in the food or pharma industries (Combo et al., 2013). Moreover, in the current work, the thermal analysis confirmed that PP pectin had better thermal stability similar to CCP, showing that PP pectin could withstand thermal food processing, similar to Wang, Ma, et al. (2016) report on pectin from grapefruit peel.

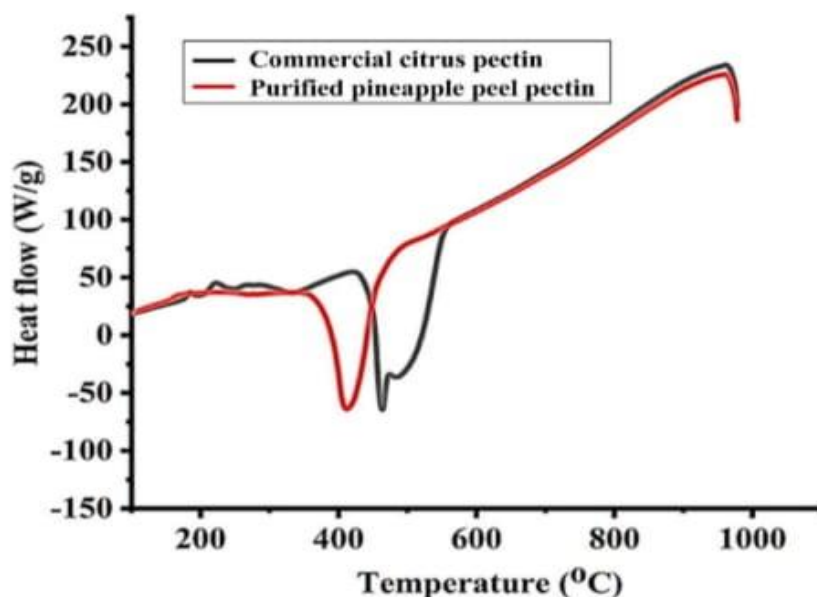


Fig:5.7: TGA of commercial pectin and pectin from pp

5.3 ANTI-NUTRITIONAL PROPERTIES OF PP PECTIN

The study of antinutritional property in purified PP confirmed that the PP pectin lacked antinutritional factors such as alkaloids, saponins, oxalate, phytic acid and tannin. The absence of antinutritional factors indicates the high nutritional value of PP pectin (Zaidel et al., 2017).

5.3.1 Alkaloid content

Alkaloids are secondary plant metabolites that protect plants from predation by various microorganisms (Torralbo et al., 2012). Moreover, due to alkaloids' bitter taste and potential toxicity, insects and herbivores are usually repulsed (Fereidoon, 2014; Gemedé & Ratta, 2014). On the other hand, alkaloids are considered antinutrients due to their physiological effects on humans, including disrupting the gastrointestinal tract cell membrane and nervous system with inappropriate electrochemical transmission (Fernando & Pathmeswaran, 2012). Based on Dragendorff and Mayer's experiments, alkaloids were not found in the PP pectin, indicating good quality pectin.

5.3.2 Saponins content

Saponins are treated as antinutritional factors due to their toxicity, hemolytic activity to fish, growth impairment, bitterness, astringency and throat irritation. Moreover, saponins decrease the bioavailability of nutrient uptake and reduce enzyme activity like trypsin and chymotrypsin, affecting protein digestibility (Gemedé & Ratta, 2014). In addition, because of amphipathic glycosides presence in saponins, they develop foam when vortexed in an aqueous solution. In this study, the commercial citrus pectin and PP pectin did not produce foams, thus indicating the absence of saponins.

5.3.3. Oxalate content

Oxalate is a salt form of oxalic acid and is an antinutritional factor. In addition, oxalic acid binds with minerals, thus preventing uptake in the gastrointestinal tract, causes severe irritation to the gut lining, and majorly results in kidney disease (Gemedé & Ratta, 2014; Onibon et al., 2007). In this study, based on the titrimetric method, both CCP and the PP pectin displayed a pink color formation, which shows the absence of oxalate content in PP pectin (Lucas, 1975).

5.3.4. Phytic acid content

Phytate is a salt form of phytic acid that is considered an antinutritional content majorly because of its property to bind vital dietary divalent and trivalent minerals ions, proteins, and starch, subsequently reducing their bioavailability and mineral deficiency (Gemede & Ratta, 2014). In this study, based on titrimetric method both PP pectin and CCP resulted in a brownish yellow color formation that confirms the absence of phytic acid.

5.3.5 Tannin content

Tannin is astringent, bitter plant polyphenolic compound that can react with proteins and several other organic molecules resulting in decreased protein digestibility. In addition, tannins inhibit amylase, trypsin, chymotrypsin, lipase activities and interfere with the absorption of vitamin B12 (Gemede & Ratta, 2014; Mojab et al., 2010). The CCP and extracted PP pectin showed brown color formation in the titrimetric method, confirming the absence of tannic acid content. Thus, the antinutritional results, associated with the absence of alkaloid, aflatoxin, saponins, oxalate, phytic acid, and tannin, improve the safety and nutritional quality of the PP pectin.

5.4 FUNCTIONAL PROPERTIES OF PP PECTIN

5.4.1. Water holding capacity (WHC) and Oil holding capacity (OHC)

The quantity of water or oil fixed per gram of sample is known as the water holding capacity (WHC) and oil holding capacity (OHC). In the present study, OHC was estimated to be 0.84 g/g. However, it was lower than CCP (0.94 g/g). Previous reports on OHC showed 1.46 g/g obtained from eggplant calyx pectin and 1.32 g/g from orange peel (Hosseini et al., 2019; Kazemi, 2019). It was inferred that the oil retention power was correlated with the hydrophilic nature and overall charge density (Bayar et al., 2018). The WHC of the optimized purified PP pectin shows 2.45 g/g higher than CCP (3.02 g/g), whereas a previous report on WHC of eggplant calyx pectin showed 4.62 g/g and orange peel with 3.10 g/g (Bayar et al., 2018; Hosseini et al., 2019; Kazemi, 2019). It was inferred that many factors can influence the WHC such as charge dependency, pectin structure and porosity.

5.4.2. Emulsifying property

The emulsion activity of the purified PP pectin showed 41.42% which was lower than the CCP (46.5%). However, these results were comparable to previous reports with emulsion activity of 53.42% from orange peel pectin, 44.97%–47.71% from potato pulp pectin, 37.03% apple pomace pectin and 45.87% apricot pulp pectin (Baississe et al., 2010; Hosseini et al., 2019; Yang et al., 2018). Generally, the emulsion activity is more stable at very low temperatures, however, the emulsion solution that was stored at 4 °C showed good emulsion activity with 90.21%, which is indicating the purified PP pectin emulsion has good stability (Bayar et al., 2018).

5.5 INSILICO SYSTEM OF PHARMACOLOGY APPROACH

5.5.1 In-silico prediction of target identification

We utilized the Swiss Target Prediction tool to determine the possible targets of 3 pectin compounds, which predicts the biological targets of small molecules by comparing their structure to known ligands. The results are displayed in a table with a confidence score that denotes the probability of the predicted target being correct.

5.6 MOLECULAR DOCKING ANALYSIS

The study aims to use PyRx software for molecular docking analysis of 3 pectin compounds with human reproductive receptors. Protein structures of androgen receptor, estrogen receptor alpha, luteinizing hormone receptor, follicle-stimulating hormone receptor, and progesterone receptor are obtained from PDB and prepared using Molegro molecular viewer software. Docking analysis will provide insights into binding mode and interactions between Beta-Sitosterol and target receptors. The binding affinity will be evaluated by calculating the binding energy of the best binding pose obtained from the docking analysis. In summary, the proposed research aims to investigate the drug potential of 3 pectin compounds for human reproductive receptor- related diseases using a systems pharmacology approach. The results obtained from this study could have important implications for the development of new therapeutic options and natural product drug discovery methods.

5.7 PECTIN COMPOUND DETAILS

Table.5.1 General Characteristics of Phytoconstituents of pectin compounds

PUBCHEM ID	PHYTOCHEMICAL NAME	SMILES	MOLECULAR FORMULA	MOLECULAR WEIGHT
101119315	Ribose	<chem>OC1COC(C(C1O)O)O</chem>	C ₅ H ₁₀ O ₅	150.13 g/mol
439215	Galaturonic acid	<chem>OC1OC(C(=O)O)C(C(C1O)O)O</chem>	C ₆ H ₁₀ O ₇	194.14 g/mol
439195	Arabinose	<chem>OC1COC(C(C1O)O)O</chem>	C ₅ H ₁₀ O ₅	150.13 g/mol

Table.5.2 MalSoft prediction of pectin compounds

PUBCHEM ID	PHYTOCHEMICAL NAME	SMILES	MOLECULAR FORMULA	MOLECULAR WEIGHT	NO. OF HBA	NO. OF HBD
101119315	Ribose	<chem>C1C(C(C(C(O1)O)O)O)O</chem>	C ₅ H ₁₀ O ₅	150.05	5	4
439215	Galaturonic acid	<chem>C1(C(C(OC(C1O)O)C(=O)O)O)O</chem>	C ₆ H ₁₀ O ₇	194.04	7	5
439195	Arabinose	<chem>C1C(C(C(C(O1)O)O)O)O</chem>	C ₅ H ₁₀ O ₅	150.05	5	4

MolLogP	MolLogS	MolPSA	MolVol	NO.OF.STEREO CENTERS	BBB SCORE	DRUG- LIKENESS SCORE
-2.93	0.49	73.40 A2	117.58 A3	4	1.96- 6(HIGH)	-1.37
-2.97	-0.21	100.94 A2	147.09 A3	5	1.77- 6(HIGH)	-0.3
-2.93	0.49	73.40 A2	117.58 A3	4	1.96- 6(HIGH)	-1.37

Table.5.3 Lipophilicity of phytoconstituents of pectin compounds

PHYTOCHEMICAL NAME	llogp	XLOGP3	WLOGP	MLOGP	SILICOS- IT	CONSENSUS LOG P e/w
Ribose	-0.39	-3.02	-2.58	-2.32	-1.7	-2
Galaturonic acid	-0.19	-2.34	-3.13	-2.89	-2.72	-2.25
Arabinose	-0.39	-3.02	-2.58	-2.32	-1.7	-2

Table.5.4 Water solubility of phytoconstituents of Pectin compounds

PHYTOCHEMICAL NAME	ESOL				Ali				SILICOS-IT			
	LOG S	SOLUBILITY		CLASS	LOG S	SOLUBILITY		CLASS	LOG S	SOLUBILITY		CLASS
		mg/mL	mol/L			mg/mL	mol/L			mg/mL	mol/L	
Ribose	1.13	2.03e+03 mg/mL	1.35e+01 mol/L	Highly soluble	1.69	7.34e+03 mg/mL	4.89e+01 mol/L	Highly soluble	2.23	2.56e+04 mg/mL	1.71e+02 mol/L	Soluble
Galaturonic acid	0.5	6.09e+02 mg/mL	3.14e+00 mol/L	Highly soluble	0.2	3.08e+02 mg/mL	1.59e+00 mol/L	Highly soluble	3.07	2.26e+05 mg/mL	1.16e+03 mol/L	Soluble
Arabinose	1.13	2.03e+03 mg/mL	1.35e+01 mol/L	Highly soluble	1.69	7.34e+03 mg/mL	4.89e+01 mol/L	Highly soluble	2.23	2.56e+04 mg/mL	1.71e+02 mol/L	Soluble

5.8 SWISS ADME ANALYSIS

Table.5.5 Pharmacokinetic parameters of the Pectin compound

PHYTOCHEMICAL NAME	GI ABSORPTION	BBB PERMEANT	P-gp SUBSTRATE	CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4	LOG Kp (cm/s)
Ribose	Low	No	No	No	No	No	No	No	-9.36 cm/s
Galaturonic acid	Low	No	No	No	No	No	No	No	-9.15 cm/s
Arabinose	Low	No	No	No	No	No	No	No	-9.36 cm/s

Table.5.6 Drug Likeness of the Phytoconstituents of Pectin compounds

PHYTOCHEMICAL NAME	LIPINSKI	GHOSE	VEBER	EGAN	MUEGGLE	BIOAVAILABILITY SCORE
Ribose	Yes	No	Yes	Yes	No	0.55
Galaturonic acid	Yes	No	Yes	Yes	No	0.56
Arabinose	Yes	No	Yes	Yes	No	0.55

Table.5.7 Medical chemistry properties of Pectin compounds

PHYTOCHEMICAL NAME	PAINS	BRENK	LEADLIKENESS	SYNTHETIC ACCESSIBILITY
Ribose	0 alert	0 alert	No	3.8
Galaturonic acid	0 alert	0 alert	No	3.94
Arabinose	0 alert	0 alert	No	3.8

5.9 PROTOX-II PREDICTION ANALYSIS

Table.5.8 Pro Tox-II toxicity prediction of pectin compounds

Phytochemical name	SMILES	PUBCHEM ID	Hepatotoxicity	Carcinogenicity
Ribose	<chem>OC1COC(C(C1O)O)O</chem>	101119315	YES	NO
Galaturonic acid	<chem>OC1OC(C(=O)O)C(C(C1O)O)O</chem>	439215	YES	NO
Arabinose	<chem>OC1COC(C(C1O)O)O</chem>	439195	YES	NO

Immunotoxicity =UPPER(	Mutagenicity	Cytotoxicity	Aryl hydrocarbon Receptor (AhR)	Androgen Receptor (AR)
YES	NO	NO	NO	NO
YES	NO	NO	NO	NO
YES	NO	NO	NO	NO

Androgen Receptor Ligand Binding Domain (AR-LBD)	Aromatase	Estrogen Receptor Alpha (ER)	Estrogen Receptor Ligand Binding Domain (ER-LBD)	Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)
NO	YES	YES	YES	NO
NO	YES	YES	NO	NO
NO	YES	YES	YES	NO

Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	Heat shock factor response element (HSE)	Mitochondrial Membrane Potential (MMP)	Phosphoprotein (Tumor Suppressor) p53	ATPase family AAA domain containing protein 5 (ATAD5)
NO	NO	NO	NO	NO
NO	NO	NO	NO	NO
NO	NO	NO	NO	NO

5.10 SWISSTARGET PREDICTION ANALYSIS

Table.5.9 Swiss-Target analysis to find the targets of the pectin compounds

NAME	Target	Common name	Uniprot ID	CHEMBL ID	Target Class	Probability*
Ribose	Beta-glucose	GBA	P04062	CHEMBL2179	Enzyme	0.064951
	Heat shock protein HSP 90-alpha	HSP90A A1	P07900	CHEMBL3880	Other cytosolic protein	0.033971
	Vascular endothelial growth factor A	VEGFA	P15692	CHEMBL1783	Secreted protein	0.033971
	Gamma-secretase	PSEN2 PSENEN NCSTN APH1A PSEN1 APH1B		CHEMBL2094 135	Protease	0.033971
	Acidic fibroblast growth factor	FGF1	P05230	CHEMBL2120	Secreted protein	0.033971
	Basic fibroblast growth factor	FGF2	P09038	CHEMBL3107	Secreted protein	0.033971
	Heparanas	HPSE	Q9Y25 1	CHEMBL3921	Enzyme	0.033971
	Cyclin-dependent kinase 1	CDK1	P06493	CHEMBL308	Kinase	0.023833
	Aldosereductase	AKR1B1	P15121	CHEMBL1900	Enzyme	0.023833

Galatur o nic acid	Vitamin D receptor	VDR	P114 73	CHEMBL1 977	Nuclear receptor	0.08057
	Gamma- secretase	PSEN2 PSENEN NCSTN APH1A PSEN1 APH1B		CHEMBL2 094 135	Protease	0.07178 7
	Serotonin 2b (5-HT2b) receptor	HTR2B	P415 95	CHEMBL1 833	Family A G protein- coupled receptor	0.07178 7
	Alpha-2a adrenergic receptor	ADRA2 A	P089 13	CHEMBL1 867	Family A G protein- coupled receptor	0.07178 7
	Adrenergic receptor alpha- 2	ADRA2 C	P188 25	CHEMBL1 916	Family A G protein- coupled receptor	0.07178 7
	Alpha-2b adrenergic receptor	ADRA2 B	P180 89	CHEMBL1 942	Family A G protein- coupled receptor	0.07178 7
	Dopamine D1 receptor	DRD1	P217 28	CHEMBL2 056	Family A G protein- coupled receptor	0.07178 7
	Dopamine D2 receptor	DRD2	P144 16	CHEMBL2 17	Family A G protein- coupled receptor	0.07178 7
	Alpha-1d adrenergic receptor	ADRA1 D	P251 00	CHEMBL2 23	Family A G protein- coupled receptor	0.07178 7
	Serotonin 2a (5-HT2a) receptor	HTR2A	P282 23	CHEMBL2 24	Family A G protein- coupled receptor	0.07178 7

	Serotonin 2c (5-HT2c) receptor	HTR2C	P28335	CHEMBL225	Family A G protein-coupled receptor	0.071787
	Dopamine D3 receptor	DRD3	P35462	CHEMBL234	Family A G protein-coupled receptor	0.071787
	Cytochrome P450 2D6	CYP2D6	P10635	CHEMBL289	Cytochrome P450	0.071787
	Serotonin 6 (5-HT6) receptor	HTR6	P50406	CHEMBL3371	Family A G Protein coupled receptor	0.071787
	Alpha-1a adrenergic receptor (by homology)	ADRA1 A	P35348	CHEMBL229	Family A G Protein coupled receptor	0.071787
	Serotonin 1b (5-HT1b) receptor (by homology)	HTR1B	P28222	CHEMBL1898	Family A G Protein coupled receptor	0.071787
	Nuclear receptor ROR-gamma	RORC	P51449	CHEMBL1741186	Nuclear receptor	0.071787
	Protein phosphatase 2C alpha	PPM1A	P35813	CHEMBL2437	Phosphatase	0.071787
	Glycine receptor subunit alpha-1	GLRA1	P23415	CHEMBL5845	Ligand-gated ion channel	0.071787
	Glycine receptor subunit alpha-2	GLRA2	P23416	CHEMBL5871	Ligand-gated ion channel	0.071787
	Kappa Opioid receptor	OPRK1	P41145	CHEMBL237	Family A G Protein coupled receptor	0.071787

5.11 MOLECULAR DOCKING ANALYSIS

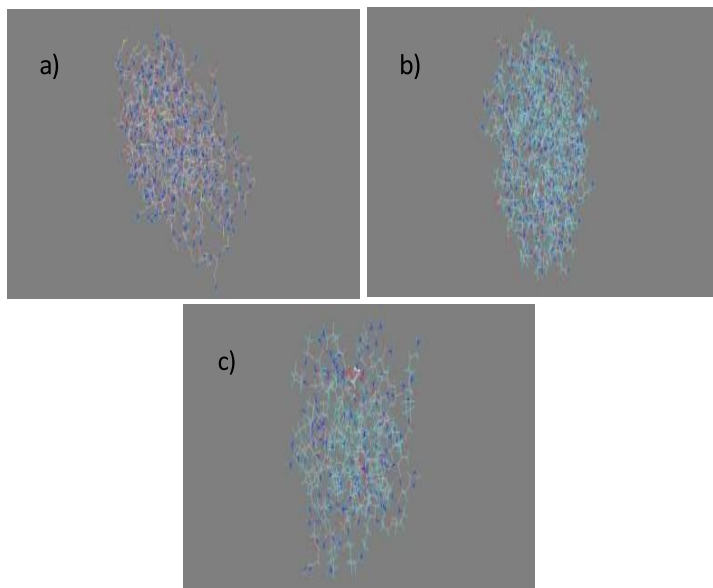


Figure 5.8. a) Binding of ribose with Human androgen receptor b) Binding of ribose with Human prostate specific antigen c) Binding of ribose with Human c) a) b) 27 prostate derived Ets factor.

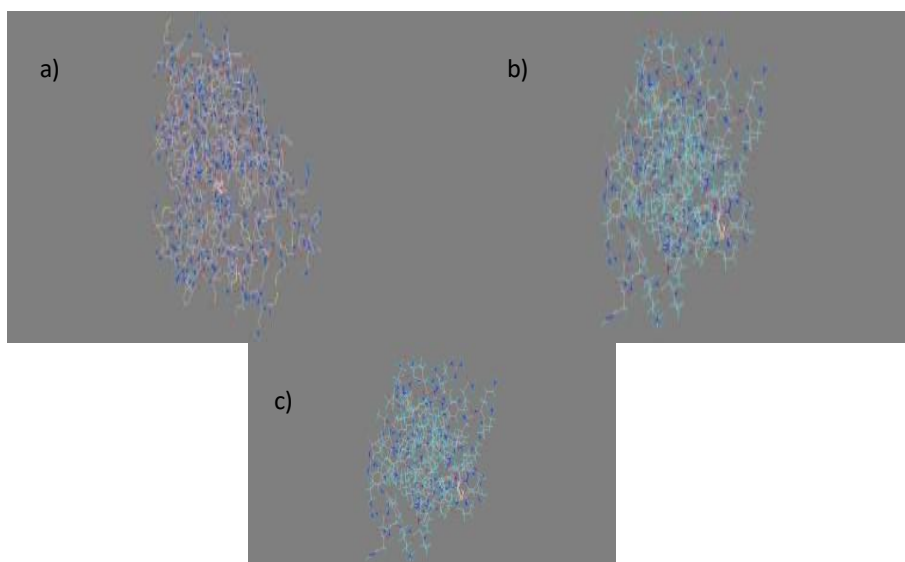


Figure 5.9. a) Binding of galaturonic acid with Human androgen receptor b) Binding of galaturonic acid with Human prostate specific antigen c) Binding of galaturonic acid with Human prostate derived Ets factor.

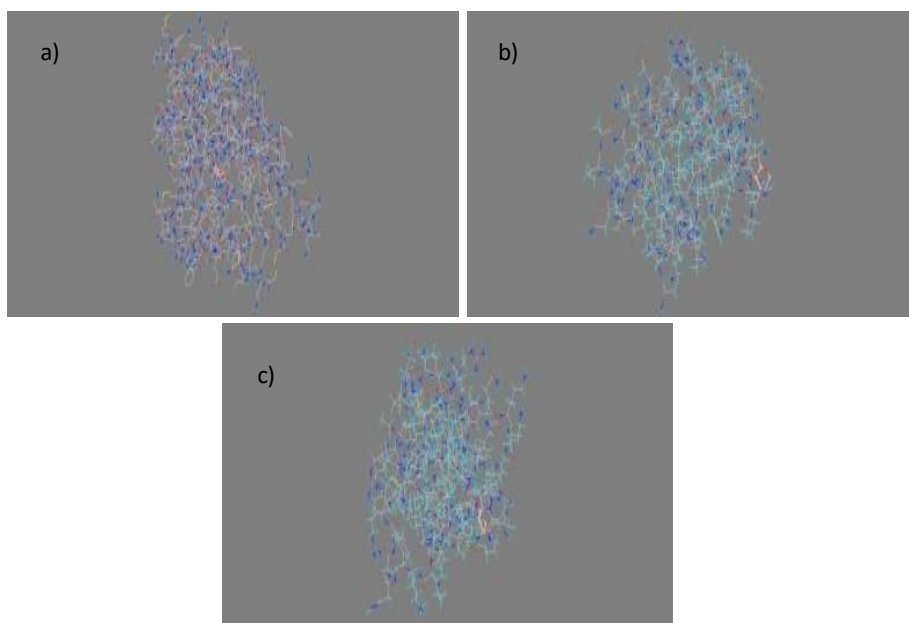


Figure 5.10. a) Binding of arabinose with Human androgen receptor b) Binding of arabinose with Human prostate specific antigen c) Binding of arabinose with Human prostate derived Ets factor.

The 3 pectin compound interaction with the prostate cancer as the target receptor. The binding affinity obtained from the analysis is -3 kcal/mol, which suggests a strong binding interaction between them. The RMSD values obtained from the docking analysis indicate the degree of deviation between the predicted binding pose of 3 pectin compound and the crystal structure of the ligand in the protein. The RMSD/UB and RMSD/LB values suggest that the predicted binding pose of 3 pectin compound is in close agreement with the crystal structure of the ligand, with no significant deviation. The results suggest that has a high binding affinity for the androgen receptor protein and that the predicted binding mode is consistent with the crystal structure of the ligand in the protein. The results of the analysis revealed that 3 pectin compound has a good binding affinity with the progesterone receptor, with the best binding affinity of -8.6 kcal/mol. The root-mean-square deviation (RMSD) values were also calculated to evaluate the accuracy of the docking results, which showed

that the 3 pectin compound had an RMSD value of 0 Å for the best binding pose obtained, indicating that the ligand was well positioned in the binding pocket of the receptor. In addition, the RMSD/UB and RMSD/LB values were also calculated, which represent the upper bound and lower bound of the RMSD values, respectively. The results showed that the RMSD/UB values ranged from 9.223 to 59.944 Å, while the RMSD/LB values ranged from 3.46 to 53.704 Å for the different binding poses obtained. These values suggest that there is some level of variability in the positioning of 3 pectin compound within the binding pocket of the progesterone receptor. The 3 pectin compound binds with Luteinizing Hormone Receptor with high affinity. The Binding Affinity values for the different docked conformations range from -6.9 to -8.8, 30 which are indicative of strong binding. The RMSD/UB and RMSD/LB values indicate the root mean square deviation between the docked ligand and the unbound (UB) or bound (lb) ligand, respectively. The values for RMSD/UB and RMSD/LB range from 0 to 70.26 Å, with higher values indicating greater deviation from the bound conformation. It is worth noting that the conformation with the highest binding affinity does not necessarily have the lowest RMSD values. The 3 pectin compound was able to bind to the estrogen hormone receptor, PDB ID 3I1XB7, with varying degrees of binding affinity and RMSD values. The binding affinity values, which represent the strength of the interaction between the ligand and receptor, range from -6.9 to -5.6 kcal/mol. The lower the value, the stronger the binding. The RMSD values, which indicate the degree of deviation between the docked ligand and the crystallographic ligand in the receptor's binding site, range from 0 to 31.462 Å in the upper bound (ub) and from 0 to 28.938 Å in the lower bound (lb). A lower RMSD value indicates a better fit between the docked ligand and the receptor's binding site. The results suggest that 3 pectin compounds have the potential to bind to the estrogen hormone receptor with moderate to weak binding affinity and varying degrees of accuracy in its docking pose.

5.12 DISCUSSION

Pectins are a complex group of polysaccharides found in the cell walls of plants, particularly fruits and vegetables. They have shown potential in various biological activities, including anti-cancer properties. Pectins can interact with proteins involved in cancer pathways, such as cell cycle regulation and apoptosis, making them interesting candidates for anti-cancer therapy.

5.12.1.Preparation of Protein and Ligand Structures:

Obtain the 3D structure of the target protein involved in cancer pathways from a database like the Protein Data Bank (PDB).

Prepare the ligand structures of pectins in a suitable format (e.g., PDB, SDF, or MOL2). You may need to generate or obtain these structures based on the specific pectin molecules you are interested in.

5.12.2.Setting up PyRx:

Download and install PyRx on your computer. Ensure that all necessary dependencies are installed and configured.

Launch PyRx and set up a new project.

5.12.3 Loading Protein and Ligand:

Import the protein structure (PDB file) into PyRx.

Import the ligand structures (pectins) into PyRx.

5.12.4.Preparing the Protein:

Check and correct any structural issues in the protein if needed (e.g., missing atoms, incomplete residues).

Assign charges and atom types to the protein.

5.12.5.Preparing the Ligands:

Check and correct any structural issues in the ligands if needed.

Assign charges and atom types to the ligands.

5.12.6 .Docking Setup:

Define the docking parameters, such as search algorithm (e.g., AutoDock Vina), search space (grid box around the active site), and scoring function.

Set up any additional parameters specific to your study.

5.12.7.Performing Docking:

Run the docking simulation in PyRx. This process will generate multiple docking poses for each ligand within the active site of the protein.

5.12.8 Analysis

Analyze the docking results to identify potential binding modes and interactions between pectins and the protein.

Evaluate the binding affinity scores and select the most promising docking poses for further analysis.

5.12.9.Validation:

Validate the docking results through experimental studies such as in vitro assays or molecular dynamics simulations to confirm the predicted interactions and anti-cancer activity.

5.12.10.Interpretation and Reporting:

Interpret the results to understand how pectins interact with the target protein and their potential anti-cancer mechanisms.

Generate reports or visualizations to present your findings effectively.

CHAPTER 6

SUMMARY AND CONCLUSION

Ultrasound-assisted extraction techniques were used to extract pectin from PP waste. It was also found that an increase in sonication time above the optimum limit causes excessive heating, leading to structural changes and pectin disintegration. Above a pH of unity, the yield of pectin decreases, as above pH 1, structural degradation occurs via a hydrolyzing mechanism. Increases in the LS ratio above optimum level cause concentration differences between the external solvent and the inside plant cell, which results in the contents dissolving more effectively, leading to a rapid mass transfer rate and higher extraction yield.

As the temperature rises above optimum level, the solubility and diffusivity of solid from plant material may increase, resulting in a decline in yield. The structure of PP pectin was further confirmed by GC-MS, FT-IR, TGA, XRD, SEM. Monosaccharide sugar groups like rhamnose, galactose, ribose, polyGalA are present in PP pectin. The purified PP pectin has a good gelling property due to its ash and moisture content. Purified PP pectin is quite stable, has a good emulsion binding capacity and antioxidant activity. In addition, PP pectin has very good gelling and nontoxic properties. It is thus concluded that the PP waste might be considered a high potential feedstock for pectin production due to its high extraction yield compared to previous studies and as the better properties of the pectin produced than other fruit waste. Also, pectin purified from PP has high methoxy content and can be used in the pharmaceutical industry.

Systems pharmacology is an emerging field that combines computational modeling, data analysis, and experimental approaches to study the interactions between drugs and biological systems at a systems level. In the case of pectin compounds, system pharmacology has been used to elucidate the potential mechanisms by which it can modulate prostate function. The study suggests that pectin compounds have the potential to bind with several hormone receptors including Human androgen receptor, Human prostate specific

antigen and Human prostate derived Ets factor receptor with varying degrees of binding affinity and accuracy in docking pose.

The binding affinity values suggest that pectin compounds have a strong binding interaction with all of these receptors. However, it is important to note that these results are based on in silico predictions and further experimental validation is needed to confirm the actual binding affinity and mode of interaction between pectin compounds and these receptors. Nevertheless, these findings could inform further studies on the potential therapeutic applications of pectin compounds as a ligand for these hormone 32 receptors.

REFERENCES

- Abaza, R., Blake, J., & Fisher, E. (1968). Oxalate determination: Analytical problems encountered with certain plant species. *Journal of the Association of Official Analytical Chemists*, 51, 963–967.
- Abid, M., Cheikhrouhou, S., Renard, C. M., Bureau, S., Cuvelier, G., Attia, H., & Ayadi, M. A. (2017). Characterization of pectins extracted from pomegranate peel and their gelling properties. *Food Chemistry*, 215, 318–325.
- Abid, M., Renard, C. M., Watrelot, A. A., Fendri, I., Attia, H., & Ayadi, M. A. (2016). Yield and composition of pectin extracted from Tunisian pomegranate peel. *International Journal of Biological Macromolecules*, 93, 186–194.
- Allothman, M., Bhat, R., & Karim, A. A. (2009). Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. *Food Chemistry*, 115(3), 785–788.
- Altendorf, S. (2019). Major tropical fruits market review 2018. Rome, Italy: FAO, 1-0.
- Ameer, K., Shahbaz, H. M., & Kwon, J. H. (2017). Green extraction methods for polyphenols from plant matrices and their byproducts: A review. *Comprehensive Reviews in Food Science and Food Safety*, 16, 295–315.
- Azad, A. K., Ali, M. A., Akter, M. S., Rahman, M. J., & Ahmed, M. (2014). Isolation and characterization of pectin extracted from lemon pomace during ripening. *Journal of Food and Nutrition Sciences*, 2(2), 30–35.
- Bagherian, H., Ashtiani, F. Z., Fouladitajar, A., & Mohtashamy, M. (2011). Comparisons between conventional, microwave-and ultrasound-assisted methods for extraction of pectin from grapefruit. *Chemical Engineering and Processing: Process Intensification*, 50, 1237–1243.

Baississe, S., Ghannem, H., Fahloul, D., & Lekbir, A. (2010). Comparison of structure and emulsifying activity of pectin extracted from apple pomace and apricot pulp. *World Journal of Dairy & Food Sciences*, 5(1), 79–84.

Bayar, N., Friji, M., & Kammoun, R. (2018). Optimization of enzymatic extraction of pectin from *Opuntia ficus indica* cladodes after mucilage removal. *Food Chemistry*, 241, 127–134.

Begum, R., Aziz, M. G., Uddin, M. B., & Yusof, Y. A. (2014). Characterization of jackfruit (*Artocarpus heterophyllus*) waste pectin as influenced by various extraction conditions. *Agriculture and Agricultural Science Procedia*, 2, 244–251.

Bonnin, E., Dolo, E., Le Goff, A., & Thibault, J. F. (2002). Characterisation of pectin subunits released by an optimised combination of enzymes. *Carbohydrate Research*, 337(18), 1687–1696.

Boukroufa, M., Boutekedjiret, C., Petigny, L., Rakotomanomana, N., & Chemat, F. (2015). Bio-refinery of orange peels waste: A new concept based on integrated green and solvent free extraction processes using ultrasound and microwave techniques to obtain essential oil, polyphenols and pectin. *Ultrasonics Sonochemistry*, 24, 72–79.

Combo, A. M., Aguedo, M., Quiévy, N., Danthine, S., Goffin, D., Jacquet, N., Blecker, C., Devaux, J., & Paquot, M. (2013). Characterization of sugar beet pectic-derived oligosaccharides obtained by enzymatic hydrolysis. *International Journal of Biological Macromolecules*, 52, 148–156.

Ezzati, S., Ayaseh, A., Ghanbarzadeh, B., & Heshmati, M. K. (2020). Pectin from sunflower by-product: Optimization of ultrasound-assisted extraction, characterization, and functional analysis. *International Journal of Biological Macromolecules*, 165, 776–786.

FAO, Food, U. N. (2019). Agriculture organization corporate statistical database. Rome. FAO STAT F. (2018). Agriculture organization corporate statistical database. Agricultural Production—Crops. Retrieved from <http://www.fao.org/faostat/en/#data/QV>. (Accessed 14 April 2020).

Fereidoon, S. (2014). Beneficial health effects and drawbacks of antinutrients and phytochemicals in foods. *Applied Microbiology and Biotechnology*, 97, 45–55.

Fernández-Cruz, M. L., Mansilla, M. L., & Tadeo, J. L. (2010). Mycotoxins in fruits and their processed products: Analysis, occurrence and health implications. *Journal of Advanced Research*, 1(2), 113–122.

Fernando, R., Pinto, M. D., & Pathmeswaran, A. (2012). Goitrogenic food and prevalence of goitre in Sri Lanka. *International Journal of Internal Medicine*, 1(2), 17–20.

Fishman, M. L., Chau, H. K., Cooke, P. H., & Hotchkiss, A. T., Jr. (2008). Global structure of microwave-assisted flash-extracted sugar beet pectin. *Journal of Agricultural and Food Chemistry*, 56(4), 1471–1478.

Gemedo, H. F., & Ratta, N. (2014). Antinutritional factors in plant foods: Potential health benefits and adverse effects. *International Journal of Nutrition and Food Sciences*, 3(4), 284–289.

Grassino, A. N., Brnčić, M., Vikić-Topić, D., Roca, S., Dent, M., & Brnčić, S. R. (2016). Ultrasound assisted extraction and characterization of pectin from tomato waste. *Food Chemistry*, 198, 93–100.

Hosseini, S. S., Khodaiyan, F., Kazemi, M., & Najari, Z. (2019). Optimization and characterization of pectin extracted from sour orange peel by ultrasound assisted method. *International Journal of Biological Macromolecules*, 125, 621–629.

Hundie, K. B. (2020). Ultrasound-Assisted optimization of pectin extraction from orange peel using response surface methodology (RSM) and artificial neural network (ANN). *International Journal of Applied Science & Engineering*, 8(2), 69–87.

Ismail, A., Gonçalves, B. L., de Neeff, D. V., Ponzilacqua, B., Coppa, C. F., Hintzsche, H., Sajid, M., Cruz, A. G., Corassin, C. H., & Oliveira, C. A. (2018). Aflatoxin in foodstuffs: Occurrence and recent advances in decontamination. *Food Research International*, 113, 74–85.

Ismail, N. S. M., Ramli, N., Hani, N. M., & Meon, Z. (2012). Extraction and characterization of pectin from dragon fruit (*Hylocereus polyrhizus*) using various extraction conditions. *Sains Malaysiana*, 41, 41–45.

Kachapulula, P. W., Bandyopadhyay, R., & Cotty, P. J. (2019). Aflatoxin contamination of non-cultivated fruits in Zambia. *Frontiers in Microbiology*, 10, 1840.

Kammerer, D. R., Kammerer, J., Valet, R., & Carle, R. (2014). Recovery of polyphenols from the by-products of plant food processing and application as valuable food ingredients. *Food Research International*, 65, 2–12.

Kar, F., & Arslan, N. (1999a). Effect of temperature and concentration on viscosity of orange peel pectin solutions and intrinsic viscosity–molecular weight relationship. *Carbohydrate Polymers*, 40, 277–284.

Kar, F., & Arslan, N. (1999b). Characterization of orange peel pectin and effect of sugars, L-ascorbic acid, ammonium persulfate, salts on viscosity of orange peel pectin solutions. *Carbohydrate Polymers*, 40(4), 285–291.

Karbuzy, P., & Tugrul, N. (2021). Microwave and ultrasound assisted extraction of pectin from various fruits peel. *Journal of Food Science & Technology*, 58(2), 641–650.

Karim, R., Uddin, M. B., & Jubayer, M. (2014). Optimization of pectin isolation method from pineapple (*Ananas comosus* L.) Waste. *Carpathian Journal of Food Science & Technology*, 6.

Kazemi, M., Khodaiyan, F., & Hosseini, S. S. (2019a). Utilization of food processing wastes of eggplant as a high potential pectin source and characterization of extracted pectin. *Food Chemistry*, 294, 339–346.

Kazemi, M., Khodaiyan, F., & Hosseini, S. S. (2019b). Eggplant peel as a high potential source of high methylated pectin: Ultrasonic extraction optimization and characterization. *Lebensmittel-Wissenschaft & Technologie*, 105, 182–189.

Kazemi, M., Khodaiyan, F., Labbafi, M., Hosseini, S. S., & Hojjati, M. (2019c). Pistachio green hull pectin: Optimization of microwave-assisted extraction and evaluation of its physicochemical, structural and functional properties. *Food Chemistry*, 271, 663–672.

Keijbets, M. J., & Pilnik, W. (1974). Some problems in the analysis of pectin in potato tuber tissue. *Potato Research*, 17, 169–177. Ketnawa, S., Chaiwut, P., & Rawdkuen, S. (2012). Pineapple wastes: A potential source for bromelain extraction. *Food and Bioproducts Processing*, 90(3), 385–391.

Klaver, J., Desbois, G., Littke, R., & Urai, J. L. (2015). BIB-SEM characterization of pore space morphology and distribution in postmature to overmature samples from the Haynesville and Bossier Shales. *Marine and Petroleum Geology*, 59, 451–466.

Kumar, A., & Chauhan, G. S. (2010). Extraction and characterization of pectin from apple pomace and its evaluation as lipase (steapsin) inhibitor. *Carbohydrate Polymers*, 82 (2), 454–459.

Lara-Espinoza, C., Carvajal-Millán, E., Balandrón-Quintana, R., Lopez-Franco, Y., & Rascon-Chu, A. (2018). Pectin and pectin-based composite materials: Beyond food texture. *Molecules*, 23, 942.

Liu, L., Cao, J., Huang, J., Cai, Y., & Yao, J. (2010). Extraction of pectins with different degrees of esterification from mulberry branch bark. *Bioresource Technology*, 101, 3268–3273.

Lucas, G. (1975). Phytic acid and other phosphorous compounds of lima beans (*Phaseolus lunatus*). *Journal of Agricultural and Food Chemistry*, 23, 13–15.

Madhav, A., & Pushpalatha, P. B. (2006). Quality upgradation of jellies prepared using pectin extracted from fruit wastes. *Journal of Tropical Agriculture*, 40, 31–34.

Majee, S. B., Avlani, D., Ghosh, P., & Biswas, G. R. (2018). Therapeutic and pharmaceutical benefits of native and modified plant pectin. *Journal of Medicinal Plants Research*, 12(1), 1–6.

Methacanon, P., Krongsin, J., & Gamonpilas, C. (2014). Pomelo (*Citrus maxima*) pectin: Effects of extraction parameters and its properties. *Food Hydrocolloids*, 35, 383–391.

Minjares-Fuentes, R., Femenia, A., Garau, M. C., Meza-Velázquez, J. A., Simal, S., & Rossello, C. (2014). Ultrasound-assisted extraction of pectins from grape pomace using citric acid: A response surface methodology approach. *Carbohydrate Polymers*, 106, 179–189.

Mojab, F., Kamalinejad, M., Ghaderi, N., & Vahidipour, H. R. (2010). Phytochemical screening of some species of Iranian plants. *Iranian Journal of Pharmaceutical Research*, 77–82.

Moorthy, I. G., Maran, J. P., Ilakya, S., Anitha, S., Sabarima, S. P., & Priya, B. (2017). Ultrasound assisted extraction of pectin from waste *Artocarpus heterophyllus* fruit peel. *Ultrasonics Sonochemistry*, 34, 525–530.

Moorthy, I. G., Maran, J. P., Muneeswari, S., Naganyashree, S., & Shivamathi, C. (2015). Response surface optimization of ultrasound assisted extraction of pectin from pomegranate peel. *International Journal of Biological Macromolecules*, 72, 1323–1328.

Mukhiddinov, Z. K., Khalikov, D. K., Abdusamiev, F. T., & Avloev, C. C. (2000). Isolation and structural characterization of a pectin homo and ramnogalacturonan. *Talanta*, 53(1), 171–176.

Normah, O., & Hasnah, K. K. (2000). Pectin content of selected local fruit by-products. *Journal of Tropical Agriculture and Food Science*, 28, 195–202.

Nwabueze, T. U. (2010). Basic steps in adapting response surface methodology as mathematical modelling for bioprocess optimisation in the food systems. *International Journal of Food Science and Technology*, 45(9), 1768–1776.

Ochoa-Villarreal, M., Aispuro-Hernández, E., Vargas-Arispuro, I., & Martínez-Téllez, M.A. (2012). Plant cell wall polymers: Function, structure and biological activity of their derivatives. *Polymerization*, 4, 63–86.

Onibon, V. O., Abulude, F. O., & Lawal, L. O. (2007). Nutritional and antinutritional composition of some Nigerian fruits. *Journal of Food Technology*, 5(2), 120–122.

Pagan, J., & Ibarz, A. (1999). Extraction and rheological properties of pectin from fresh peach pomace. *Journal of Food Engineering*, 39, 193–201.

Pereira, P. H., Oliveira, T. I., Rosa, M. F., Cavalcante, F. L., Moates, G. K., Wellner, N., Waldron, K. W., & Azeredo, H. M. (2016). Pectin extraction from pomegranate peels with citric acid. *International Journal of Biological Macromolecules*, 88, 373–379.

Phatak, L., Chang, K., & Brown, G. (1988). Isolation and characterization of pectin in sugar-beet pulp. *Journal of Food Science*, 53, 830–833.

Picot-Allain, M. C., Ramasawmy, B., & Emmambux, M. N. (2020). Extraction, characterisation, and application of pectin from tropical and sub-tropical fruits: A review. *Food Reviews International*, 5, 1–31.

Ponmurugan, K., Al-Dhabi, N. A., Maran, J. P., Karthikeyan, K., Moothy, I. G., Sivarajasekar, N., & Manoj, J. J. (2017). Ultrasound assisted pectic polysaccharide extraction and its characterization from waste heads of *Helianthus annuus*. *Carbohydrate Polymers*, 173, 707–713.

Priyangini, F., Walde, S. G., & Chidambaram, R. (2018). Extraction optimization of pectin from cocoa pod husks (*Theobroma cacao* L.) with ascorbic acid using response surface methodology. *Carbohydrate Polymers*, 202, 497–503.

Putnik, P., Bursać Kovačević, D., Režek Jambrak, A., Barba, F. J., Cravotto, G., & Binello, A. (2017). Innovative “green” and novel strategies for the extraction of bioactive added value compounds from citrus wastes—a review. *Molecules*, 22, 680.

Raji, Z., Khodaiyan, F., Rezaei, K., Kiani, H., & Hosseini, S. S. (2017). Extraction optimization and physicochemical properties of pectin from melon peel. *International Journal of Biological Macromolecules*, 98, 709–716.

Ramli, N. (2011). Effect of ammonium oxalate and acetic acid at several extraction time and pH on some physicochemical properties of pectin from cocoa husks (*Theobroma cacao*). *African Journal of Food Science*, 5(15), 790–798.

Rodsamran, P., & Sothornvit, R. (2019). Preparation and characterization of pectin fraction from pineapple peel as a natural plasticizer and material for biopolymer film. *Food and Bioproducts Processing*, 118, 198–206.

Schols, H. A., Bakx, E. J., Schipper, D., & Voragen, A. G. (1995). A xylogalacturonan subunit present in the modified hairy regions of apple pectin. *Carbohydrate Research*, 279, 265–279. Sharma, R., & Ahuja, M. (2011). Thiolated pectin: Synthesis, characterization and evaluation as a mucoadhesive polymer. *Carbohydrate Polymers*, 85, 658–663.

Sharma, R., Kamboj, S., Khurana, R., Singh, G., & Rana, V. (2015). Physicochemical and functional performance of pectin extracted by QbD approach from *Tamarindus indica* L. pulp. *Carbohydrate Polymers*, 134, 364–374.

Shivamathi, C. S., Ganesh Moorthy, I., Vinoth Kumar, R., Soosai, M. R., Prakash Maran, J., Shyam Kumar, R., & Varalakshmi, P. (2019). Optimization of ultrasound assisted extraction of pectin from custard apple peel: Potential and new source. *Carbohydrate Polymers*, 225, 115240.

Sims, S. (2004). Production of commercially suitable pectin methylesterase and polyphenol oxidase from agro-industrial wastes. *Izmir Institute of Technology*. Suhaila, M., & Zahariah, H. (1995). Extraction and characterisation of pectin from various tropical agrowastes. *ASEAN Food Journal*, 10(2), 43–50.

Sun, Y., & Ren, T. Q. (2012). Research on experiment condition of pectin from pineapple peel. *Northern horticulture* (Vol. 21). Talekar, S., Patti, A. F., Vijayraghavan, R., & Arora, A. (2018). An integrated green biorefinery approach towards simultaneous recovery of pectin and polyphenols coupled with bioethanol production from waste pomegranate peels. *Bioresource Technology*, 266, 322–334.

Tang, W., Liu, D., Wang, J. Q., Huang, X. J., Yin, J. Y., Geng, F., & Nie, S. P. (2021). Isolation and structure characterization of a low methyl-esterified pectin from the tuber of *Dioscorea opposita* Thunb. *Food Chemistry*, 359, 129899.

Thakur, B. R., Singh, R. K., Handa, A. K., & Rao, M. (1997). Chemistry and uses of pectin—a review. *Critical Reviews in Food Science and Nutrition*, 37, 47–73.

Torralbo, D., Batista, K., Di-Medeiros, M., & Fernandes, K. (2012). Extraction and partial characterization of *Solanum lycocarpum* pectin. *Food Hydrocolloids*, 27, 378–383.

Ukiwe, L. N., & Alinnor, J. I. (2011). Extraction of pectin from Pineapple (*Ananas comosus*) peel using inorganic/organic acids and aluminum chloride. *Fresh Produce*, 5 (1), 80–83.

Vaziri, A. S., Alemzadeh, I., Vossoughi, M., & Khorasani, A. C. (2018). Comicroencapsulation of *Lactobacillus plantarum* and DHA fatty acid in alginate-pectin-gelatin biocomposites. *Carbohydrate Polymers*, 199, 266–275.

Virk, B., & Sogi, D. (2004). Extraction and characterization of pectin from apple (*Malus Pumila*. Cv Amri) peel waste. *International Journal of Food Properties*, 7, 693–703.

Wang, Y. (2020). Exploring the functionality of in situ produced dextran in high-protein or wholegrain enriched wheat bread. Wang, M., Huang, B., Fan, C., Zhao, K., Hu, H., Xu, X., Pan, S., & Liu, F. (2016). Characterization and functional properties of mango peel pectin extracted by ultrasound assisted citric acid. *International Journal of Biological Macromolecules*, 91, 794–803.

Wang, W., Ma, X., Jiang, P., Hu, L., Zhi, Z., Chen, J., Ding, T., Ye, X., & Liu, D. (2016). Characterization of pectin from grapefruit peel: A comparison of ultrasound-assisted and conventional heating extractions. *Food Hydrocolloids*, 61, 730–739.

Westerlund, E., Åman, P., Andersson, R., Andersson, R., & Rahman, S. (1991). Chemical characterization of water-soluble pectin in papaya fruit. *Carbohydrate Polymers*, 15, 67–78.

Xu, Y., Zhang, L., Bailina, Y., Ge, Z., Ding, T., Ye, X., & Liu, D. (2014). Effects of ultrasound and/or heating on the extraction of pectin from grapefruit peel. *Journal of Food Engineering*, 126, 72–81.

Yanbo, G., & Bing, J. (2020). Kinetic analysis and optimum design of extracting pectin from pineapple peel by ion exchange. IOP Publishing. IOP Conference Series: Earth and Environmental Science, 546(5).

Yang, S.-S., Cheng, K.-T., Lin, Y.-S., Liu, Y.-W., & Hou, W.-C. (2004). Pectin hydroxamic acids exhibit antioxidant activities in vitro. *Journal of Agricultural and Food Chemistry*, 52, 4270–4273.

Yang, J. S., Mu, T. H., & Ma, M. M. (2018). Extraction, structure, and emulsifying properties of pectin from potato pulp. *Food Chemistry*, 244, 197–205.

Yeoh, S., Shi, J., & Langrish, T. (2008). Comparisons between different techniques for water-based extraction of pectin from orange peels. *Desalination*, 218, 229–237.

Yuliarti, O., Goh, K. K., Matia-Merino, L., Mawson, J., & Brennan, C. (2015). Extraction and characterisation of pomace pectin from gold kiwifruit (*Actinidia chinensis*). *Food Chemistry*, 187, 290–296.

Yuris, A., & Siow, L. F. (2014). A comparative study of the antioxidant properties of three pineapple (*Ananas comosus* L.) varieties. *Journal of Food Studies*, 3(1), 40–56.

Zaidel, D. N. A., Rashid, J. M., Hamidon, N., Salleh, L. M., & Kassim, A. M. (2017). Extraction and characterisation of pectin from dragon fruit (*Hylocereus polyrhizus*) peels. *Chemical Engineering Transactions*, 56, 805–810.

Zakaria, N. A., Rahman, R. A., Zaidel, D. N., Dailin, D. J., & Jusoh, M. (2021). Microwave-assisted extraction of pectin from pineapple peel. *Malaysian Journal of Fundamental and Applied Sciences*, 17(1), 33–38.

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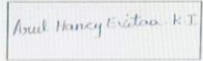
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