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A comparative study of pectins from peel and pulp of mangrove apple fruit: the functional properties and bioactivities

Ranisiska RANISISKA [1], Serti PARE [1], Embun Sekar LANGIT [2], Reni Tri CAHYANI [1*]

1 Department of Fisheries Product Technology, Faculty of Fisheries and Marine Sciences, Borneo Tarakan University, Tarakan, Indonesia.
2 Department of Agribusiness, Faculty of Agriculture, Borneo Tarakan University, Tarakan, Indonesia.
* Corresponding Author. E-mail: renitri_c@borneo.ac.id (R.C.); Tel. +62-813-4545 40 58.

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ABSTRACT: This study investigated pectin's functional properties and bioactivities derived from the peel and the pulp of the mangrove apple fruit, further elucidating its prospective bioactivity against metabolic syndrome through in vitro assessments. The study exhibited a discernible disparity in functional properties and bioactivity between the two pectins (sig < 0.05). The peel and pulp-derived pectins were classified under the high methoxyl pectin category and enriched with flavonoid and phenolic compounds, further showcasing anti-diabetic potential. Such findings underscore the potential of these pectins as therapeutic agents in managing metabolic syndrome.

KEYWORDS: *a*-glucosidase; Antidiabetic; Flavonoid; Methoxyl content; Phenolic content.

1. INTRODUCTION

In this research, the mangrove apple -endemic to tidal regions of mangrove forests, has drawn attention due to its medicinal potential. Its fruit boasts a rich composition of bioactive compounds, encompassing 67.67 mg of phenolic compounds (GAE/g), 37.06 mg of flavonoids (RE/g), and 5.41 mg of carotenoids (BC/100g). Moreover, its impressive antioxidant capacities are evidenced by its DPPH activity at 98.32%, FRAP at 67.72%, ABTS at 91.24 mg/g, and an inhibition of acetylcholinesterase enzyme activity at 47.18% when observed at a concentration of 250 µg/ml [1]. Beyond its pharmacological merits, the mangrove apple fruit, characterized by its nutritional value, sour flavor, and distinct aroma, has been deemed fit for consumption, finding its way into a myriad of culinary applications [2]. In most cases, its pulp undergoes processing, with its peel, rich in polysaccharides like pectin, often overlooked.

Pectin, a complex polysaccharide originating from plant cell walls, serves pivotal roles in the realms of food, medicine, and biomedicine, attributable to its multifaceted functionalities: gelling, thickening, and emulsifying, to name a few. Its inherent bioactive compounds further bolster its reputation, offering anti-inflammatory, immunomodulatory, and antidiabetic advantages [3]. Embracing its status as a dietary fiber, pectin's therapeutic potential for metabolic ailments such as diabetes mellitus is noteworthy. It demonstrates capabilities to modulate blood glucose, ameliorate hyperlipidemia, and enhance insulin responsiveness, particularly by steering protein expression within the Pl3K/Akt signaling pathway [4]. Indeed, its broad-spectrum bioactivity, including wound healing, antibacterial, cardioprotective, and antidiabetic properties, makes pectin an indispensable component of natural therapeutic arsenals [5].

Pectin's structural and biochemical attributes exhibit distinctive variations contingent upon the origin of the primary material, facilitating its adaptability across a myriad of applications [6]. Recognizing the pervasive and multifaceted utility of pectin in sectors spanning food, beverage, and pharmaceuticals, there emerges a pressing demand for alternative sources for its production. Conventionally, commercial pectin extraction predominantly relies on the peels of oranges and apples. The presence of pectin in the pulp of the mangrove apple has been identified, with a notable yield of 7.30% [7]. Yet, scholarly literature remains silent on the potential pectin content within the peel of this fruit. Furthermore, the bioactive properties of pectins derived from both the peel and pulp of the mangrove apple remain uncharted. Augmenting our

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understanding of the functional attributes and bioactivity of pectin from various mangrove fruits, with a spotlight on the mangrove apple species, is pivotal. Such advancements would bolster the sustainable harnessing of mangrove resources, ensuring they attain their full economic and functional potential. In light of these gaps, the present study endeavors to juxtapose the functional properties and bioactivity potential of pectin extracted from the peel and pulp of the mangrove apple fruit.

2. RESULTS AND DISCUSSION

2.1. Functional properties of pectins

2.1.1. Yield

The yield plays a pivotal role in the realm of pectin production, serving as an indicator of the efficiency of the production process. In this study, the yield ranged from 7.23 to 21.05 wt%. Upon analysis, the fruit peel pectin demonstrated a superior yield, surpassing the yield of fruit pulp pectin (Table 1). Such findings resonate with the observations on pectin derived from persimmon peel, which exhibited a higher yield, ranging from 7.2 to 9.1 wt%, when juxtaposed with the pectin procured from the pulp, which was in the range of 4.7 to 6.2 wt% [8]. It is noteworthy that the pectin yield from the mangrove apple fruit pulp in the present investigation aligns closely with the previous findings, with a reported yield of 7.66 wt% [9].

A statistical analysis employing the Independent Sample T-test revealed a discernible disparity between the pectin yields from the peel and the pulp of the mangrove apple fruit (sig < 0.05). A plausible rationale behind this difference might stem from the variation in the volume of the acid solution introduced to the sample suspension to achieve an equal pH. As the sample suspension from the fruit's pulp had a lower initial pH than its peel, it required less acid solution during the pH adjustment process, thus predisposing the final volume of the extraction media. An inferior pectin yield might be attributed to insufficient extraction media required to solubilize pectin compounds. The solid-to-liquid ratio in the pectin extraction will have an impact on dissolution capacity, which ultimately determines the yield [10].

2.1.2. Ash content

Ash content serves as a pivotal quality marker for pectin, given its direct correlation with pectin's purity. In this study, the ash content ranged from 0.87 to 1.45 wt%. A comparative assessment revealed that fruit peel pectin registered the most elevated ash content, subsequently trailed by fruit pulp pectin (Table 1). This analytical observation aligns with the previous findings, which documented an ash content of 1.45 wt% in mandarin orange peel pectin [11].

Statistical scrutiny, utilizing the Independent Sample T-test, underscored a discernible discrepancy between the ash content of pectin derived from the peel and the pulp of the mangrove apple fruit (sig < 0.05). This variation is conceivably attributable to the fruit peel's augmented concentration of inorganic constituents relative to the pulp. The fruit peel, being the outermost protective layer, is inherently predisposed to accumulate inorganic pollutants, thereby amplifying its ash content [12]. Echoing this perspective, another posit articulated that the ash content serves as an indicator of the extent of inorganic contaminants present in pectin. Such contaminants can potentially hinder the gelation process [13]. Notably, the pectin evaluated in this study conforms to the standards set forth by the International Pectin Producers Association, which stipulates an upper limit of 10% for ash content [14].

2.1.3. Water content

The water content of pectin stands as a crucial indicator of its quality, significantly influencing its shelf life. In this study, the water content ranged from 16.55 to 20.25 wt%. Upon evaluation, the pectin derived from fruit peels exhibited the most pronounced water content, while that from the fruit pulp (Table 1). In a parallel vein, the substantial water content in bael fruit pectin, which was 16.66 wt%, has been identified [15].

A statistical assessment conducted via the Independent Sample T-test discerned a notable variance in the water content between pectin sourced from the peel and that from the pulp of the mangrove apple fruit, (sig < 0.05). This discrepancy is arguably attributable to the differential volumes of acid solutions used during extraction. Given that the fruit's pulp is intrinsically more acidic than its peel, the requisite volume of acid solution to attain an equal pH would be comparatively reduced. Consequently, the amplified volume of acid solution incorporated into the peel's sample suspension facilitates greater water retention within the sample. It was reinforced by the fact that polysaccharides, endowed with hydrophilic hydroxyl groups, manifest a robust propensity to bond with water [16].

Regrettably, the pectin assessed in this investigation failed to align with the benchmarks stipulated by the International Pectin Producers Association, which mandates a water content not exceeding 12% [14]. This elevated water content can plausibly be ascribed to the suboptimal drying techniques employed. Corroborating this, a present recorded a water content of 15.03% in orange peel pectin, hinting at the pivotal role of the drying process's efficiency in determining pectin's final water content [17].

2.1.4. Equivalent weight

In this study, the equivalent weight ranged from 311.23-443.58 mg. The equivalent weight of pectin from fruit peel was observed to be the higher than the pulp, as tabulated in Table 1. These findings surpass the previous data reported, where the equivalent weight of pectin derived from pear skin oscillated between 119.73 mg and 155.00 mg. Such disparities in equivalent weights can be indicative of variations in pectin's gelation capabilities [18].

A statistical analysis utilizing the Independent Sample T-test elucidated a significant difference in the equivalent weights between pectin derived from the peel and the pulp of mangrove apple fruit, with a significance level below 0.05. However, the pectin values from this study did not align with the stipulated standards set by the International Pectin Producers Association, which prescribes an equivalent weight ranging from 600-800 mg [14].

The diminished equivalent weights observed in both specimens can be attributed to an overly acidic pH coupled with an extended extraction duration. A report posited that the equivalent weight corresponds to the quantity of non-esterified galacturonic acid groups. A surge in acid concentration can precipitate a decline in the equivalent weight. Furthermore, an overly prolonged extraction process might result in decreased equivalent weight, stemming from the esterification of pectin [19]. Myriad factors can modulate the equivalent weight of pectin. Elements such as pH, inherent acidity, and the quantum of free acid inherent in pectin are instrumental, and these tend to diverge based on the provenance of the raw materials [20].

Table 1. Functional properties of pectins from peel and pulp of mangrove apple fruit

Functional properties	Peel	Pulp
Yield	21.05[a]	7.23[b]
Ash	1.50[a]	0.87[b]
Water	20.25[a]	16.55[b]
Equivalent weight	443.58[a]	311.23[b]
Methoxyl	29.66[a]	13.54[b]
Galacturonic acid	167.67[a]	170.65[b]
Degree of esterification	80.86[a]	57.51[b]

Different superscript letters following the numbers indicate significant differences ($\alpha<0.05$)

2.1.5. Methoxyl content

In this study, the methoxyl content ranged from 13.54-29.66%. The methoxyl content in pectin sourced from fruit peel was notably higher that the fruit pulp, as delineated in Table 1. These values notably exceed the previous findings, which discerned that the methoxyl content in pectin derived from pumpkin skin ranged from 6.20% to 7.23% [21]. An elevated methoxyl content is emblematic of enhanced thickening capabilities [17].

A statistical evaluation utilizing the Independent Sample T-test revealed a significant disparity in the methoxyl content between the pectin sourced from the peel and that from the pulp of the mangrove apple fruit, achieving a significance level below 0.05. The pectin methoxyl content in this investigation conforms to the benchmarks set by the International Pectin Producers Association, which mandates a methoxyl content in excess of 7.12% for high methoxyl pectin [14]. This is corroborated by previous research, highlighting that pectin extracted from mangrove apple fruit boasted a substantial methoxyl content, quantified at 10.64% [7].

High methoxyl pectin uniquely gels under conditions of pH less than 3.5, accompanied by sugar concentrations exceeding 55%. In contrast, its low methoxyl counterpart achieves gelation within a pH spectrum of 2-6, and interestingly, its gelling capacity remains unaffected by the presence or absence of sugar [22]. In addition, high methoxyl pectin has etched its relevance in the food processing domain, particularly within the confines of jam and jelly manufacturing, owing to its multifaceted functionality as a gelling, emulsifying, stabilizing, and thickening agent [23].

2.1.6. Galacturonic acid

In this study, the galacturonic acid ranged from 167.67-170.64%. The galacturonic acid content was notably lower in the peel pectin, while the pulp manifested a slightly greater, as tabulated in Table 1. This data resonates with the previous findings, which discerned that the galacturonic acid concentration in mangrove apple fruit pectin oscillated between 111.14% and 311.07%. The provenance of the raw materials plays a pivotal role in determining the variability of galacturonic acid content within pectin. Elevated glucuronate levels are often a consequence of the intensified hydrolysis of protopectin into pectin. This transition is modulated by an array of determinants encompassing pH, temperature, and the duration of extraction [19].

A meticulous analysis using the Independent Sample T-test illuminated a significant discrepancy in the galacturonic acid content between the pectin derived from the peel and that from the pulp of the mangrove apple fruit, registering a significance value of less than 0.05. The pectin's galacturonic acid concentration in this study met the threshold delineated by the International Pectin Producers Association, which mandates a minimum of 35% [14]. The integrity and purity of pectin are intrinsically linked to its galacturonic acid content, a factor of paramount significance when considering its applications in the formulation of jams and jellies. For optimum performance, the galacturonic acid concentration in pectin is advocated to be no less than 65% [24].

2.1.7. Degree of esterification

In this study, the degree of esterification ranged from 57.51-80.86%. The degree of esterification was higher in pectin sourced from fruit peel than the pulp, as evidenced in Table 1. These findings are in congruence with previous research, which reported a degree of esterification of 67.32% in pectin derived from mangrove apple fruit [7]. The heterogeneity in the degree of esterification of pectin can be attributed to myriad factors, including the habitat of the source, the botanical classification, and the specifics of the extraction protocol [25].

A rigorous analysis utilizing the Independent Sample T-test revealed a statistically significant disparity in the degree of esterification between the pectin derived from the peel and pulp of the mangrove apple fruit, with a significance value below 0.05. The pronounced esterification levels observed in both samples can likely be ascribed to an overly protracted extraction process. Fundamentally, the degree of esterification offers insights into the abundance of methyl ester groupings within the pectin molecular structure. Pectin boasting an esterification degree below 50% falls under the category of low methoxyl pectin, whereas pectin with a degree surpassing 50% is typified as high methoxyl pectin. Intriguingly, the maturation phase of the fruit plays a pivotal role in influencing the degree of esterification, with a more mature fruit generally manifesting a diminishing degree of esterification [6]. It is heartening to note that the pectin evaluated in this study comfortably meets the stringent benchmarks of the International Pectin Producers Association, requiring a degree of esterification exceeding 50% for high ester pectin [14].

2.1.8. Functional group

The Fourier-transform infrared (FTIR) spectra derived from the peel and pulp of mangrove apple fruit pectin are delineated in Figure 1. The absorption peaks evident in both pectin samples underscore the stretching modes of O-H bonds inherent in the galacturonic acid polymer, manifesting prominently at a wavenumber of 3425 cm^{-1}. This observation was corroborated by a report, which posited that vibrations within the 3600-3400 cm^{-1} range are indicative of the hydroxyl groups (-OH) in both sampled and commercial pectin [26]. Simultaneously, the pectins exhibited stretching vibrations of $-CH_2$ at wavenumbers 2934 cm^{-1} and 2922 cm^{-1}. The finding was congruent with the previous report which stated that wavenumber spanning 3000-2900 cm^{-1} in pectin signal the C-H groups, elicited by the symmetric stretching vibrations of CH_2 [27].

Further spectral analysis revealed the presence of free carbonyl ester groups (C=O) and COO at wavenumbers 1745 cm^{-1} and 1632 cm^{-1}, respectively. A pronounced intensity within the absorption band proximate to 1739 cm^{-1} corresponds to the asymmetric tension of the methyl ester carbonyl. Conversely, the absorption band circling 1637 cm^{-1} mirrors alterations in the methoxylation degree, as deciphered from symmetric tension vibrations in the carboxylate ion [8]. These observations resonate with the previous findings, which identified absorption bands ranging between 1760-1745 cm^{-1} and 1640-1620 cm^{-1} in both sampled and commercial pectin [28].

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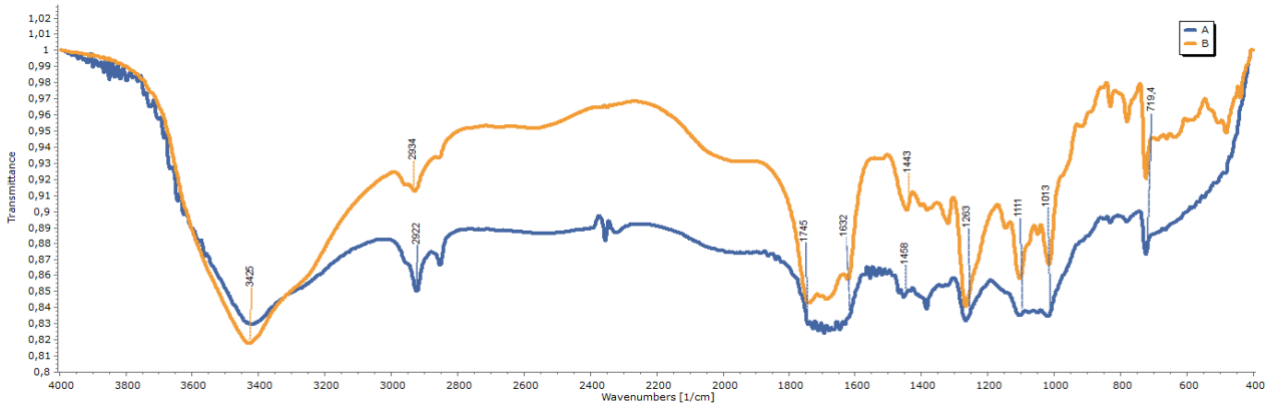

Figure 1. FTIR spectrums of pectins from peel and pulp of mangrove apple fruit: A- fruit peel pectin; B- fruit pulp pectin

Peaks discerned at 1458 cm^{-1} and 1443 cm^{-1} in the pectin from the mangrove apple fruit's peel and pulp signify the CH_3 methyl group. A previous present pinpointed -CH_3 stretching vibrations within the 1443 cm^{-1} absorption band in commercial pectin [29]. The absorption bands appearing at 1263 cm^{-1}, 1111 cm^{-1}, and 1012 cm^{-1} potentially signify the presence of -CH-O-CH- linkages in both pectin samples. The stretching vibrations of C-O ester bonds are manifest within the spectral range of 1300-1000 cm^{-1} [30]. Lastly, vibrations within the absorption band region proximate to 719 cm^{-1} likely represent characteristic chemical groupings in pectin. Absorption bands of medium intensity falling below 920 cm^{-1} are emblematic of the galacturonic acid molecule's structure, engendered by the vibrations of the C-O-C bond [31].

2.2. Bioactivities of pectins

2.2.1. Total flavonoid content

In this study, the total flavonoid content (TFC) ranged from 17.46-34.37 µg EQ/g. TFC was observed to be the most abundant in fruit peel pectin. In contrast, the fruit's pulp exhibited a considerably lower TFC value, as delineated in Table 2. This disparity suggests that the fruit's peel possesses a richer flavonoid compounds reservoir than its pulp. In a corroborative study, citrus fruit peels harbor a heightened concentration of flavonoid compounds relative to the pulp, which is instrumental in endowing them with robust antioxidant properties [32].

An Independent Sample T-test discerned a statistically significant variance in the TFC of pectin derived from the peel and the pulp of the mangrove apple fruit, with a significance level below 0.05. Intriguingly, while pectin and flavonoids are distinct molecular entities, the flavonoids' presence within pectin can be attributed to their interplay during the fruit's processing phase. Flavonoids are endogenously present within plant cellular structures and do not typically associate with pectin compounds under natural conditions. However, upon fruit processing, these flavonoid compounds are extricated from their cellular confines, enabling them to associate with other molecular entities, including pectin. This association is facilitated through a myriad of bonds such as noncovalent, hydrogen, hydrophobic, and ionic interactions [33].

Elucidating this phenomenon further, a report revealed that pectin, when extracted utilizing the acoustic cavitation technique, contains flavonoids and phenolic acids. These compounds predominantly adhere to the pectin's surface, conferring upon it heightened biological activity [34]. It's pertinent to note that the flavonoid content within pectin can be modulated by the presence of intermediary compounds, which foster the bond between flavonoids and pectin. To exemplify, pectin samples infused with iron showcased an enhanced affinity for binding to quercetin compared to their non-iron counterparts [35].

Table 2. Bioactivities of pectins from peel and pulp of mangrove apple fruit

Parameters	Peel	Pulp
Total flavonoid content (µg EQ/g)	34.37^{a}	17.46^{b}
Total phenolic content (µg GAE/g)	694.45^{a}	583.33^{b}
Antidiabetic activity (%)	28.83^{a}	80.38^{b}

Different superscript letters following the numbers indicate significant differences ($\alpha<0.05$)

2.2.2. Total phenolic content

In this study, the total phenolic content (TPC) ranged from 583.33-694.45 µg GAE/g. The TPC in fruit peel pectin was higher than the pulp (Table 2). These findings exceed a report, which underscored that pectin derived from fruit waste exhibited a phenolic range of 2.08-4.67 µg GAE/g, encompassing compounds such as gallic acid, benzoic acid, caffeic acid, and coumaric acid. The inherent antioxidant prowess of phenolic compounds, capable of mitigating free radical activity, underscores their significance. Even in diminutive quantities, the phenolic compounds encapsulated within pectin remain invaluable owing to their potent biological activity [28]. An Independent Sample T-test has further discerned a statistically significant discrepancy in the TPC of pectin obtained from both the peel and the pulp of the mangrove apple fruit, with a significance level less than 0.05.

The observed diminished TPC in both types of pectin may be attributed to the suboptimal pH levels during the extraction process. This hypothesis is substantiated by previous findings, which delineate that pectin extracted from apple pomace under pH 2.5 conditions demonstrated a peak TPC of 12.98 mg GAE/g. In stark contrast, a more acidic environment, pH 1.5, yielded a meager TPC of 2.16 mg GAE/g [36]. Furthermore, the pectin's molecular weight can significantly modulate its phenolic content. Pectin characterized by a high molecular weight boasts an enhanced capacity to retain phenolic antioxidants within its intricate matrix. A pronounced phenolic content in pectin can also manifest due to the simultaneous extraction of phenolic compounds during pectin production [37].

2.2.3. Antidiabetic activity

Both peel and pulp-derived pectins exhibited antidiabetic activity (AA) at a concentration of 10 mg/ml, ranged 28.83-80.38%, as delineated in Table 2. Specifically, the pectin from the fruit pulp manifested strong inhibition of α-glucosidase, whereas its counterpart from the peel demonstrated a comparatively moderate inhibition. This pronounced difference in inhibitory capacity in the pulp pectin can likely be attributed to the inherent variations in its chemical makeup and structural intricacies. An Independent Sample T-test elucidated a statistically significant variance in the AA of the pectins sourced from the peel versus the pulp of the mangrove apple fruit, as evidenced by a significance level of less than 0.05.

Pectin's constitution can be diverse, encompassing galacturonic acid and an ensemble of up to 16 distinct monosaccharides. The composition and structural arrangements of these elements hinge critically on their source, ambient conditions, and the methods employed in processing. Such disparities invariably cast a profound influence on the physiological and physicochemical attributes of pectin [38].

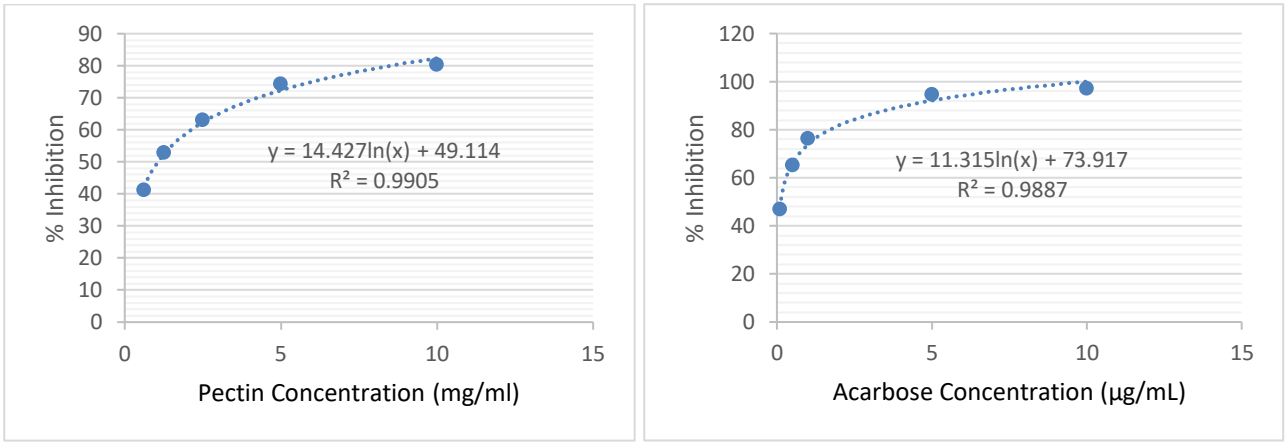

Figure 2. The correlation between the concentration of pectin from mangrove apple fruit pulp and the percentage of α-glucosidase inhibition and the correlation between the concentration of acarbose and the percentage of α-glucosidase inhibition

Figure 2 graphically represents the correlation between the concentration of pulp pectin and the corresponding percentage of α-glucosidase inhibition. Concurrently, it illustrates the relationship between acarbose concentration and its inhibitory impact on α-glucosidase. With escalating concentrations of pulp pectin, there was a commensurate surge in the inhibitory percentage, culminating in the curve equation y = 14.427 ln(x) – 50.545. This trend mirrored that observed in acarbose, which yielded the equation y = 11.315ln(x) + 73.917. Evaluating the antidiabetic potency of pulp pectin across a spectrum of concentrations

yielded an IC_{50} value of 1.06 mg/ml. This inhibitory potential aligns with previous report, which spotlighted the orange peel pectin's dual capabilities: glucose absorption and mixed-type non-competitive α-glucosidase inhibition. During their in vitro antidiabetic evaluation, the pectin registered an IC_{50} value spanning 1.18-2.52 mg/ml [39]. In stark contrast, the benchmark control acarbose showcased a more formidable IC_{50}, pegged at 0.12 µg/ml. These revelations underscore the somewhat diminished antidiabetic prowess of mangrove apple fruit pulp pectin vis-à-vis acarbose. Nonetheless, acarbose's effectiveness aligns with adverse side effects in human gastrointestinal systems, so discovering alternative α-glucosidase inhibitors is crucial [40]. It has been presented that pectins from mangrove apple fruit at a concentration of 10 mg/ml exhibited α-glucosidase inhibition activity from moderate to strong. Thus, using pectin as a food additive will provide additional benefits for human health, especially in managing metabolic syndrome.

3. CONCLUSION

The present study has effectively elucidated pectin's functional attributes and bioactivity derived from the peel and pulp of mangrove apple fruit. It was ascertained that the peel-derived pectin has a higher content than its pulp counterpart. Furthermore, salient differences in functional characteristics and bioactivity profiles were evident between the two pectin varieties. However, when assessed against IPPA benchmarks, the water content and the equivalent weight of the pectin fell short of the prescribed criteria. Despite these limitations, the study discerned that both pectin specimens belong to the high methoxyl pectin classification. Consequently, they hold promise as potential gelling agents, emulsifiers, stabilizers, and thickeners in various applications. Additionally, the presence of bioactive compounds such as flavonoids, phenols, and inherent antidiabetic activity suggests that they could be harnessed for therapeutic interventions targeting metabolic syndrome. To realize pectin of superior quality and augmented biological relevance, further investigative endeavors are warranted to refine the production process.

4. MATERIALS AND METHODS

4.1. Materials

In this study, mangrove apple fruits sourced from North Kalimantan, Indonesia, were utilized as raw materials. Upon collection, the fruits were meticulously washed to ensure cleanliness. Subsequently, they were dissected to segregate the peel (designated as A) and the pulp (designated to as B). To facilitate further analysis, the specimens were minimally sized, subjected to oven-drying at a temperature of 60°C, and subsequently pulverized using a blender. The resultant powdered material was then sieved through a 60-mesh sieve to achieve uniformity.

4.2. Methods

4.2.1. Pectin production

In a modified procedure, pectin was produced as follows: Initially, the powdered sample was combined with distilled water at a 1:40 weight-to-volume (w/v) ratio and agitated continuously for 30 minutes. To adjust the solution to a pH of 1.7, a 2N oxalic acid solution was introduced. The mixture was then subjected to a temperature of 70°C and maintained at this heat for two hours. Subsequent to this heating, the mixture underwent a filtration process using filter paper to obtain the filtrate. This filtrate was transferred to a beaker and heated to 95°C until its volume reduced to half of its original measure. Once cooled, ethanol (with a concentration of 96%) was added at a 1:3 (w/v) ratio. The resulting solution was allowed to precipitate for a full day. After this period, the formed pectin gel underwent a drying process in an oven at 60°C for six hours. Once dried, this pectin gel was pulverized and set aside, ready for subsequent analytical procedures [41].

4.2.2. Functional properties analysis

The yield of pectin was ascertained using the formula:

$$\text{(Eq. 1) \% Yield} = \frac{\text{Final weight}}{\text{Initial weight}} \text{x } 100\%$$

To determine the water content, the method was employed as follow: A sample, approximately weighing 1 gram, was placed in a pre-weighed porcelain cup. This was subjected to drying in an oven set at 105°C for three hours. Afterward, the sample was allowed to cool in a desiccator and then reweighed. This

drying and weighing process was iteratively conducted until a constant weight was achieved. The water content was then computed as [42]:

$$\text{(Eq. 2) \% Water} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \text{ x } 100\%$$

The ash content was determined using method as follow: About 1 gram of the sample was positioned in a pre-weighed porcelain cup, which was then transferred to a furnace. It was incinerated at 550°C until a light ash hue was observed. After cooling in a desiccator for an hour, it was reweighed. The ash content was then deduced using the relation [42]:

$$\text{(Eq. 3) \% Ash} = \frac{\text{Final weight}}{\text{Initial weight}} \text{ x } 100\%$$

The equivalent weight was discerned based on the method as follow: Roughly 0.1 gram of the sample was amalgamated with 1 ml of ethanol and 20 ml of distilled water, followed by an hour-long stirring. The mixture was then enhanced with 0.2 grams of NaCl and a few drops of PP indicator. Titration was subsequently performed with 0.1 N NaOH. The equivalent weight was calculated as [43]:

$$\text{(Eq. 4) Equivalent weight} = \frac{\text{sample weight (mg)}}{\text{Volume of NaOH (ml)x Normality of NaOH}}$$

In determining the methoxyl content, a method was adhered to as follow: To the titrated sample (from the equivalent weight determination), 10 ml of 0.25 N NaOH was added and stirred for an hour. Later, 10 ml of 0.25 N HCl was integrated and titrated using 0.1 N NaOH. The methoxyl content was derived using [44]:

$$\text{(Eq. 5) \% Methoxyl} = \frac{\text{Volume of NaOH x Normality of NaOH x 31}}{\text{sample weight (mg)}} \text{ x } 100\%$$

For the quantification of galacturonic acid, an approach was followed [44]:

$$\text{(Eq. 6) \% AGA} = \frac{176\,[100 - (\text{meq of NaOH at initial titration} + \text{meq of NaOH at final titration})]}{\text{sample weight (mg)}} \text{ x } 100\%$$

The degree of esterification, was ascertained using [20]:

$$\text{(Eq. 7) \% DE} = \frac{\text{Volume of NaoH at final titration}}{\text{Volume of NaOH at initial titration} + \text{Volume of NaOH at final titration}} \text{ x } 100\%$$

The functional groups were profiled using the Fourier Transform Infrared Spectroscopy (FTIR) instrument (Bruker Tensor 37, US), specifically in the spectral range of 4,000 to 400 cm^{-1}.

4.2.3. Bioactivities analysis

The quantification of total flavonoid content was executed employing the Colorimetric technique ($AlCl_3$). An aliquot of the sample (1 ml, 0.5 mg/ml) was combined with 1 ml of a 2% aluminum chloride solution and 8 ml of 5% acetic acid. Following vigorous agitation, the mixture underwent a 30-minute incubation. Absorbance readings were taken at 410 nm, using a calibration curve established with quercetin [45], as delineated in Figure 3.

The total phenolic content was assessed utilizing the Follin Ciocalteau approach. A sample volume of 0.2 ml (1 mg/ml) was amalgamated with 15.8 ml of distilled water and 1 ml of Follin Ciocalteu reagent. The blend was agitated and set aside for 8 minutes, post which 3 ml of 10% Na_2CO_3 was integrated. Following a 2-hour incubation, absorbance was measured at 765 nm. For reference and calibration, gallic acid was employed to establish a calibration curve [46], as delineated in Figure 3.

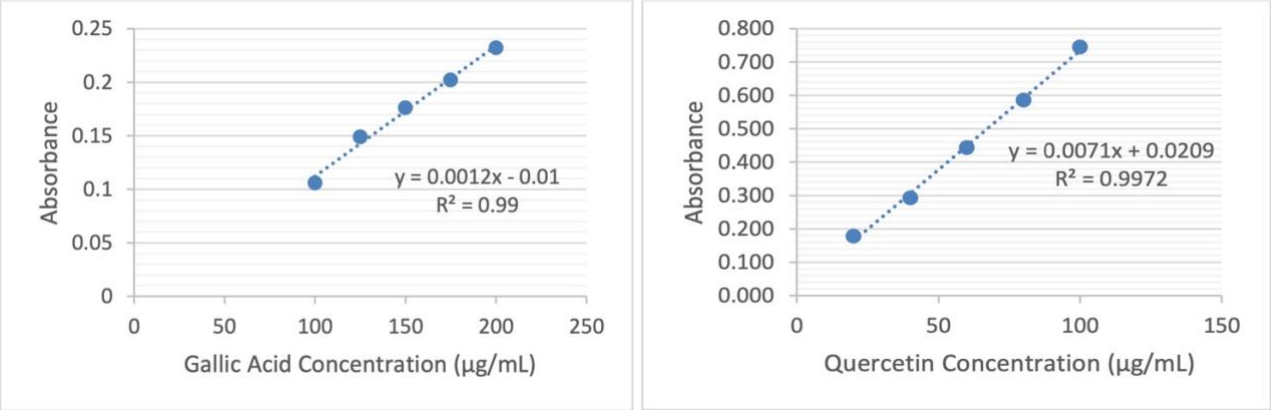

Figure 3. Calibration curve of quercetin standard and calibration curve of gallic acid standard

The antidiabetic potential was evaluated via the α-glucosidase inhibitory assay. Initially, 10 mg of the sample was solubilized in a blend of DMSO and a 0.1 M phosphate buffer at pH 6.9, resulting in various concentrations. Subsequently, 25 µl of a 0.04 U/ml α-glucosidase enzyme was dispersed in the aforementioned buffer. The assay mixture encompassed 50 µl of the 0.1 M phosphate buffer (pH 6.9), 25 µl of p-nitrophenyl-α-D-glucopyranoside solution (also in the 0.1 M phosphate buffer at pH 6.9), and 10 µl of the sample. After incubating this concoction at 37°C for 30 minutes, the reaction was quenched with 100 µl of a 0.2 M sodium carbonate solution. Absorbance was registered at 410 nm. The inhibitory concentration (IC_{50}) was deduced from a linear regression, yielding the equation y = a(x) + b [47].

4.2.4. Statistical analysis

An Independent T-test was employed, using SPSS version 25, to juxtapose the functional properties and bioactivities of the pectins. Functional groups were descriptively appraised. The functional attributes of the pectins were measured against quality benchmarks set by the International Pectin Producers Association (IPPA). The outcomes were depicted using graphs and tables.

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REFERENCES

[1] Abubakar FI, Abubakar MF, Hassan SHA, Sanusi SB, Kormin F, Sabran SF, Fuzi FZM. Comparison of phytochemicals, antioxidant and anti-cholinesterase activity of unripe and ripe fruit of sonneratia caseolaris. Food Res. 2020; 4(2): 507-514. http://dx.doi.org/10.26656/fr.2017.4(2).277.

[2] Dari DW, Junita D, Arsita Y, Meilina M, Meylani V. Chemical characteristics of juice of mangrove apple (sonneratia caseolaris) added with sugar. Int J Front Life Sci Res. 2022; 02(01): 018-028. http://dx.doi.org/10.53294/ijflsr.2022.2.1.0023.

[3] Roman-Benn A, Contador CA, Li M, Lam H, Ah-Hen K, Ulloa PE, Ravanal MC. Pectin: an overview of sources, extraction and applications in food products, biomedical, pharmaceutical and environmental issues. Food Chem Adv. 2023; 2: 1-13. http://dx.doi.org/10.1016/j.focha.2023.100192.

[4] Liu Y, Dong M, Yang Z, Pan S. Anti-diabetic effect of citrus pectin in diabetic rats and potential mechanism via PI3K/Akt signaling pathway. Int J Bio Macromol. 2016; 89: 484-488. http://dx.doi.org/10.1016/j.ijbiomac.2016.05.015.

[5] Millan-Linares MC, Lapaz SM, Martin ME. Pectins and olive pectins: from biotechnology to human health. Biology. 2021; 10(9): 860. http://dx.doi.org/10.3390/biology10090860.

[6] Chandel V, Biswas D, Roy S, Vaidya D, Verma A, Gupta A. Current advancements in pectin: extraction, properties, and multifunctional applications. Foods. 2022; 11(17): 2683. http://dx.doi.org/10.3390/foods11172683.

[7] Patra PA, Basak UC. Physicochemical characterization of pectin extracted from six wild edible fruits in Odisha, India. Curr Res Nutr Food Sci. 2020; 8(2): 402-409. http://dx.doi.org/10.12944/crnfsj.8.2.05.
[8] Munoz-almagro N, Vendrell-Calatayud M, Mendez-Albinana P, Moreno R, Cano MP, Villamiel M. Extraction optimization and structural characterization of pectin from persimmon fruit (*Diospyros kaki* thunb. var. rojo brillante). Carbohyd Polym. 2021; 272: 118411. http://dx.doi.org/10.1016/j.carbpol.2021.118411.
[9] Jariyah, Murtiningsih, Widyawati A. Optimization of pectin extraction from pedada fruit (*Sonneratia caseolaris*). J Phys: Conf Ser. 2019; 1569: 032010. http://dx.doi.org/10.1088/1742-6596/1569/3/032010.
[10] Belkheiri A, Forouhar A, Ursu AV, Dubessay P, Pierre G, Delattre C, Djelveh G, Abdelkafi S, Hamdami N, Michaud P. Extraction, characterization, and applications of pectins from plant by-products. Appl Sci. 2021; 11(14): 6596. http://dx.doi.org/10.3390/app11146596.
[11] Mahmud MM, Belal M, Ahmed S, Hoque MM, Zzaman W. Microwave-assisted extraction and characterization of pectin from citrus fruit wastes for commercial application. Food Res. 2021; 5(5): 80-88. http://dx.doi.org/10.26656/fr.2017.5(5).592.
[12] Tyagi S, Gupta V, Jain P, Tripathi R. Comparative pharmacognostical and phytochemical account of the peel and pulp of the fruit of *C. pepo*. Lett Appl NanoBioScience. 2023; 12(4): 93. http://dx.doi.org/10.33263/lianbs124.093.
[13] Sanadarani MDJC, Prasadi VPN. Value added products from agriculture: extraction of pectin from pumpkin (*Cucurbita maxima*) waste. J Food Ind Microbiol. 2018; 4: 1. http://dx.doi.org/10.4172/2572-4134.1000125.
[14] IPPA, Pectin Commercial Production and Pectin In Organic Food Products, International Pectin Producers Association, Brussels, Belgium 2014.
[15] Maskey B, Dhakal D, Pradhananga M, Shrestha NK. Extraction and process optimization of beel fruit pectin. Food Sci Nutr. 2018; 6(7): 1927-1932. http://dx.doi.org/10.1002/fsn3.761.
[16] Prokhorov P, Luna-Barcenes G, Kumar-Krishnan S, Sanchez RAM, Reyes BEC, Vargas JH. Probing molecular interactions of polysaccharides in the presence of water. J Mol Struct. 2020; 1218: 128531. http://dx.doi.org/10.1016/j.molstruc.2020.128531.
[17] Susanti S, Legowo AM, Nurwantoro N, Silviana S, Arifan F. Comparing the chemical characteristics of pectin isolated from various indonesian fruit peels. Indones. J Chem. 2021; 21(4): 1057-1062. http://dx.doi.org/10.22146/ijc.59799.
[18] Lekhuleni I, Kgatla T, Mashau M, Jideani A. Physicochemical properties of south African prickly pear fruit and peel: extraction and characterisation of pectin from the peel. Open Agric. 2021; 6(1): 178-191. http://dx.doi.org/10.1515/opag-2021-0216.
[19] Jariyah, Kurniati E, Nurherdiana SD. Study of pectin extraction from pedada fruit and kepok banana peel. Adv Food Sci Sustain Agric Agroind Eng. 2022; 5(1): 40-48. http://dx.doi.org/10.21776/ub.afssaae.2022.005.01.4.
[20] Mamiru D, Gonfa G. Extraction and characterization of pectin from watermelon rind using acetic acid. Heliyon. 2023; 9(2): e13525. http://dx.doi.org/10.1016/j.heliyon.2023.e13525.
[21] Hamed AARH, Mustafa SE. Extraction and assessment of pectin from pumpkin peels. Biofarmasi J Nat Prod Biochem. 2018; 16(1): 1-7. http://dx.doi.org/10.13057/biofar/f160101.
[22] Gawkowska D, Cybulska J, Zdunek A. Structure-related gelling of pectins and linking with other natural compounds: A review. Polymers(Basel). 2018; 10(7): 762. http://dx.doi.org/10.3390/polym10070762.
[23] Robledo VR, Vazquez LIC. Pectin-extraction, purification, characterization and applications. In: Martin M. (Ed). Pectins-Extraction, Purification, Characterization and Applications. IntechOpen, London, 2019, pp. 1-19.
[24] Dasgupta S, Shah A. A comparative study of functional properties of pectin isolated from different fruit and vagetable source. Int J Pharm Sci Rev Res. 2020; 64(2): 141-145. http://dx.doi.org/10.47583/ijpsrr.2020.v64i02.023.
[25] Setyajati E, Prasetyo VK, Husin AS, Ratri MC, Junedi S, Sanjayadi S, Setiawati A. Comparative physicochemical properties of isolated pectin from various tropical fruit peel wastes. Trop J Nat Prod Res. 2023; 7(2): 2408-2413. http://dx.doi.org/10.26538/tjnpr/v7i2.17.
[26] Guzel M, Akpinar O. Valorisation of fruit by-products: production characterization of pectins from fruit peels. Food Bioprod Process. 2019; 115: 126-133. http://dx.doi.org/10.1016/j.fbp.2019.03.009.
[27] Aguilar-Veloz LM, Calderon-Santoyo M, Carvajal-Millan E, Martinez-Robinson K, Ragazzo-Sanchez JA. *Artocarpus heterophyllus* lam leaf extracts added to pectin-based edible coating for Alternaria sp control in tomato. LWT. 2022; 156: 113022. http://dx.doi.org/10.1016/j.lwt.2021.113022.
[28] Konrade D, Gaidukovs S, Vilaplana F, Sivan P. Pectin from fruit-and berry-juice production by-products: determination of physicochemical, antioxidant and reological properties. Foods. 2023; 12(8): 1615. http://dx.doi.org/10.3390/foods12081615.
[29] Wathoni N, Shan CY, Shan WY, Rostinawati T, Indradi RB, Pratiwi R, Muchtaridi M. Characterization and antioxidant activity of pectin from Indonesian mangosteen (*Garcinia mangostana* l.) rind. Heliyon. 2019; 5(8): e02299 . http://dx.doi.org/10.1016/j.heliyon.2019.e02299.
[30] Wathoni N, Yuan Shan C, Yi Shan W, Rostinawati T, Indradi RB, Pratiwi R, Muchtaridi M. Characterization and antioxidant activity of pectin from Indonesian mangosteen (*Garcinia mangostana* L.) rind. Heliyon. 2019;5(8):e02299. doi: 10.1016/j.heliyon.2019.e02299. Erratum in: Heliyon. 2020;6(1):e03074. https://doi.org/10.1016/j.heliyon.2019.e03074.
[31] Koziol A, Sroda-Pomianek K, Gorniak A, Wikiera A, Cyprych, Malik M. Structural determination of pectins by spectroscopy methods. Coatings. 2022; 12(4): 546. http://dx.doi.org/10.3390/coatings12040546.

[32] Chen Q, Wang D, Tan C, Hu Y, Sundararajan B, Zhou Z. Profiling of flavonoid and antioxidant activity of fruit tissues from 27 chinese local *Citrus* cultivars. Plants. 2020; 9(2):196. http://dx.doi.org/10.3390/plants9020196.
[33] Zhang Z, He Y, Zhang X. Flavonoid and pectins. In : Martin M. (Ed). Pectins - Extraction, Purification, Characterization And Applications. IntechOpen, London, 2019, pp. 1-17.
[34] Ciriminna R, Albanese L, Meneguzzo F, Pagliaro M. Integropectin: a new citrus pectin with uniquely high biological activity. Biol Life Sci Forum. 2021; 6(1): 76-77. http://dx.doi.org/10.3390/foods2021-11199.
[35] Chirug P, Nagar EE, Okun Z, Shpigelman. Effect of flavonoid structure and ph on iron-mediated pectin interaction. Food Hydrocoll. 2021; 116: 106654. http://dx.doi.org/10.1016/j.foodhyd.2021.106654.
[36] Gurev A, Cesko T, Cragancea V, Ghendov-Mosanu A, Pintea A, Sturza R. Ultrasound- and microwave-assisted extraction of pectin from apple pomace and its effect on the quality of fruit bars. Foods. 2023; 12(14): 2773. http://dx.doi.org/10.3390/foods12142773.
[37] Kumar M, Potkule J, Tomar M, Punia S, Singh S, Patil S, Singh S, Ilakiya T, Kaur C, Kennedy JF. Jackfruit seed slimy sheath, a novel source of pectin: studies on antioxidant activity, functional group, and structural morphology. Carbohydr Polym Technol Appl. 2021; 2: 100054. http://dx.doi.org/10.1016/j.carpta.2021.100054.
[38] Bai Y, Gilbert RG. Mechanistic understanding of the effects of pectin on in vivo starch digestion: A review. Nutrients. 2022; 14(23): 5107. http://dx.doi.org/10.3390/nu14235107.
[39] Liang T, Hu J, Song H, Xiong L, Li Y, Zhou Y, Mao L, Tian J, Yan H, Gong E, Fei J, Sun Y, Zhang H, Wang X. Comparative study on physicochemical characteristics, α-glucosidase inhibitory effect, and hypoglycemic activity of pectins from normal and huanglongbing-infected navel orange peels**.** J Food Biochem. 2022; 46(10): e14280. http://dx.doi.org/10.1111/jfbc.14280.
[40] Feng Y, Nan H, Zhou H, Xi P, Li B. Mechanism of inhibition of α-glucosidase activity by bavachalcone. Food Sci Technol. 2022; 42: 1-6. http://dx.doi.org/10.1590/fst.123421.
[41] Huang Y, Qi J, Liao J, Jiang W, Cao Y, Xiao J, Yang X. Oxalic extraction of high methoxyl pectin and its application as a stabiliser. Int J Food Sci Technol. 2021; 56(10): 5220-5229. http://dx.doi.org/10.1111/ijfs.15135.
[42] AOAC, Official Methods of Analysis, eighteenth ed., AOAC International, Gaithersburg, MD, USA 2005.
[43] Tugon TDA, Larasati RD, Adnan S, Sucimilawati E, Agustiani FS, Jaswir I. Characterization of banana peel pectin (musa acuminata colla) as a potential halal pharmaceutical excipient. Indones J Halal Res. 2023; 5(1): 41–52. http://dx.doi.org/10.15575/ijhar.v5i1.21285.
[44] Mendoza-Vargas L, Jimenez-Forero J, Raminez-Nino M. Evaluation of pectin extracted enzymatically from cocoa (theobroma cacao l) pod husks. Rev UDCA Actual Divulg Cient. 2017; 20(1): 131-138. http://dx.doi.org/10.31910/rudca.v20.n1.2017.70.
[45] Carolina F, Nastiti K, Rizali M. Thin layer chromatography of secondary metabolite profiles and determination of total flavonoid levels of the sawi langit extract that growing on peatlands. J Adv Med Pharm Sci. 2022; 1(1): 23-30. http://dx.doi.org/10.36079/lamintang.jamaps-0101.427.
[46] Santoso B, Wijaya A, Pangawikan SD. The Addition of crude gambir extract in the production of functional robusta coffee powder. Food Sci Technol. 2022; 42: e55721. http://dx.doi.org/10.1590/fst.55721.
[47] Syabana MA, Yuliana ND, Batubara I, Fardiaz D. Antidiabetic activity screening and nmr profile of vegetable and spices commonly consumed in indonesia. Food Sci Technol. 2021; 41(1): 254-264. http://dx.doi.org/10.1590/fst.14120.