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Inhibition Efficacy of Thai Pomelo Peel Extract Against α-amylase and α-glucosidase in Vitro

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Abstract

High blood sugar, known as hyperglycemia, occurs when there is an excessive amount of glucose in the bloodstream due to issues with regulating blood sugar levels. Insufficient insulin production can lead to the development of type 2 diabetes. One approach to managing Type 2 diabetes involves reducing glucose levels after meals. This can be done by inhibiting the enzymes responsible for breaking down carbohydrates in the digestive system-specifically α glucosidase and α amylase. Acarbose is an oral medication that acts as a potent enzyme inhibitor, slowing down the breakdown of carbohydrates and delaying glucose absorption. However, using this medication may come with significant side effects, including potential liver complications. Researchers have explored the enzyme inhibiting properties of pomelo peel extract, which is rich in flavonoids. Pomelo, a citrus fruit widely cultivated globally but particularly in Thailand and Asia, boasts varieties like Thong Dee and Kao Nam Pheung known for their high flavonoid content. In a study examining these cultivars effects on digestive enzymes, both Kao Nam Pheung and Thong Dee demonstrated inhibitory abilities in vitro. However, when compared to the traditional drug acarbose, which is used as an enzyme inhibitor, both extracts showed lower effectiveness. The IC_{50} values of Kao Nam Pheung and Thong Dee were higher than that of acarbose for both α-amylase inactivation (IC_{50} = 21.87 and 21.63 and 7.71

mg/mL) and α-glucosidase inactivation (IC_{50} = 2,222.84, 1924.89 and -2,144.80 µg/mL) respectively.

Keywords: Pomelo Peel, Carbohydrate Enzyme Inhibitor, α-amylase, α-glucosidase

Introduction

This study examines the potential of Thai pomelo peels as a natural method for managing type 2 diabetes, a condition associated with issues in carbohydrate absorption and elevated blood sugar levels (Sansenya & Payaka, 2022). While medications like Acarbose are effective in controlling blood sugar by inhibiting digestive enzymes, they may also come with side effects (Huang et al., 2021; Alssema et al., 2021). In this study, researchers explore alternative natural options. Pomelo peels, which are rich in flavonoids (plant compounds), show promise in inhibiting digestive enzymes similar to Acarbose (Wang et al., 2024; Singh et al., 2020; Ha et al., 2022). The focus of the research is on two Thai pomelo varieties, Thong Dee and Kao Nam Pheung, known for their high flavonoid content (Kongsri & Nartvaranant, 2019).

The significance of this research is twofold. Success could lead to natural diabetes treatments with fewer side effects. Furthermore, utilizing pomelo peels could help reduce agricultural waste generated by the citrus industry (Yin et al., 2023). This approach offers potential benefits for both enhanced health outcomes and a more sustainable food system.

1. Scope and Significance of the Study

Studying the use of Thai pomelo peel extract for managing type 2 diabetes naturally. Researchers will extract beneficial compounds from two types of Thai pomelos to evaluate their ability to block essential enzymes. The findings could pave the way for a new nutritional supplement with less adverse effects compared to existing drugs. This investigation seeks to establish a foundation for upcoming studies, ultimately enhancing the well-being of individuals living with diabetes.

2. Diabetes Mellitus: Overview of Diabetes

Diabetes arises from problems with insulin, impacting blood sugar levels. In 2014, around 8.5% of adults were diagnosed with diabetes, resulting in 1.5 million fatalities by 2019. Making adjustments to one’s lifestyle such as dietary modifications, physical activity and steering clear of harmful habits can help lower the chances of

developing the condition. Various forms of diabetes call for distinct treatment approaches, which may involve medication to regulate blood sugar levels. In severe instances, insulin therapy might be deemed necessary.

3. Flavonoids - Structure and Potential for Enzyme Inhibition

Flavonoids are natural substances present in plants such as honey, grapes, legumes and fruits, featuring a distinct three ringed shape (Tang et al., 2024). There exist 14 varieties of flavonoids, like flavones and flavanols, each possessing unique traits (Shen et al., 2022). These components have the ability to hinder enzymes like α amylase and α glucosidase responsible for starch breakdown by attaching to them. It is essential to comprehend the molecular makeup of flavonoids when exploring their potential health advantages through enzyme inhibition; specific structural attributes are necessary for effective enzyme blockage (Lim et al., 2019).

4. Pomelo

Pomelo, the largest citrus fruit in the Rutaceae family (Yin et al., 2023), is well loved worldwide, especially in Asia where it comes in three mains; Chinese, Thai and Indonesian (Wang et al., 2024). Thailand stands out as a major pomelo producer, known for its diverse range of cultivars that boast a variety of colors varieties (Kongsri & Nartvaranant, 2019; Makkumrai et al., 2021). Packed with carotenoids, flavonoids and fiber, pomelo is celebrated for its antioxidant properties attributed to the polyphenols present in both its flesh and peel. The different types of Thai pomelos offer varying levels of polyphenols and antioxidants, hinting at potential health advantages. Moreover, certain Thai pomelo varieties like Kao Nam Pheung and Thong Dee have been shown to have enzyme inhibiting effects related to carbohydrate digestion - a promising aspect for managing blood sugar levels. These distinct characteristics of the cultivars are shaped by factors such as soil composition and climate influences (Sornsanit et al., 2019).

5. Enzyme Inhibition

This passage delves into the concept of enzyme inhibition and its potential application in controlling hyperglycemia, a condition characterized by elevated blood sugar levels. Enzyme inhibitors are molecules that attach to enzymes, impeding their ability to carry out specific functions in different biological processes (Lim et al., 2019). They play a vital role in the development of medications and are commonly utilized in

treating various human ailments (Huang et al., 2021). To explore the potential of enzyme inhibition in managing blood sugar, this delves into the use of enzyme inhibition as a method to control blood sugar levels and sheds light on a potential avenue of research involving Thai pomelo varieties. When examining enzyme Inhibition and blood sugar regulation, the inhibition of specific enzymes, namely α amylase and α glucosidase, shows promise as an effective approach to managing hyperglycemia, a condition characterized by elevated blood sugar levels.α Amylase; This enzyme plays a role in breaking down complex carbohydrates such as starch into simpler sugars like maltose and dextrins in both the mouth and small intestine (Tran et al., 2023). For α-glucosidase, it further breaks down these disaccharides and any remaining starch molecules into glucose, the primary sugar that our bodies absorb (Kittiwisut et al., 2021).

Colorimetric assays are well established techniques used to assess how substances inhibit the activity of α-amylase and α-glucosidase. These assays employ specific substrates that are broken down by the target enzymes during the process (Ha et al., 2022; Mahnash et al., 2022). The level of enzyme activity is indirectly determined by tracking the absorbance of a product formed during the enzymatic reaction. The α-amylase Assay indicates a decrease in absorbance when α-amylase is inhibited by the test extract, as shown by the reduced levels of p nitrophenol (Ha et al., 2022; Mahnash et al., 2022). Similarly, the α-glucosidase Assay demonstrates a decrease in absorbance, suggesting inhibition of α-glucosidase by the test compound (Kittiwisut et al., 2021).

Although not explicitly discussing the application of these assays to Thai pomelo cultivars, the passage hints at an area ripe for future exploration. With a growing interest in natural health products and the known impact of enzyme inhibition on blood sugar regulation, it is important to investigate whether these cultivars have potential for inhibiting α amylase and α glucosidase. By conducting these tests, we can determine if these cultivars exhibit such inhibitory properties, offering new possibilities for developing innovative functional foods or nutraceuticals.

6. The Connection Between Thai Pomelo Varieties

Although there has been an analysis that does not specifically discuss the ability of Kao Nam Pheung and Thong Dee pomelo extracts to inhibit α-amylase and α-glucosidase, the information provided hints at a promising direction for future

exploration. With the growing interest in natural health products and the known importance of enzyme inhibition in managing blood sugar levels, it is worthwhile to investigate the potential of these Thai pomelo varieties for inhibiting α-amylase and α-glucosidase. Conducting additional studies using the mentioned enzyme inhibition tests could reveal whether these cultivars possess such inhibitory properties and their effectiveness. If successful, these results may open doors for creating innovative functional foods or nutraceuticals.

7. Research Hypotheses

1) Hypothesis 1. Thai pomelo peel extract has the efficacy to inhibit α-amylase activity.

This hypothesis suggests that extracts obtained from the peel of Thai pomelo varieties might possess the ability to hinder the function of the enzyme α-amylase. It is pointed out that flavonoids - natural components present in pomelo peels (among other parts) - can inhibit α-amylase by attaching to it (Lim et al., 2019).

2) Hypothesis 2. Thai pomelo peel extract has the efficacy to inhibit α-glucosidase activity.

This hypothesis proposes that extracts from Thai pomelo peels might also be effective in inhibiting the enzyme α-glucosidase. Similar to α-amylase, this highlights the inhibitory effect of flavonoids on α-glucosidase (Lim et al., 2019).

Research Methodology

A research project delved into the use of Thai pomelo peel extract to control blood sugar levels by blocking enzymes. The study concentrated on how effective the extract was at inhibiting certain enzymes, testing different concentrations to gauge its strength. Two types of pomelo, Kao Nam Pheung and Thong Dee, were studied to see if there were variations in their inhibitory effects. The goal of the research was to determine whether the type of pomelo influenced the levels of enzyme inhibitors in the peels, taking into account factors such as flavonoid content.

The study involved a positive control using Acarbose, an α amylase inhibitor, for reference. Pomelo peel extract's ability to inhibit enzymes was tested by comparing it with Acarbose. The research aimed to see how effectively the extract could block α amylase and α-glucosidase enzymes, which are crucial for the experiment's results.

Various concentrations of the extract and a control were employed to analyze enzyme activity and inhibition levels. This research offers valuable findings on the effectiveness of the extract.

This study used a structured experiment to analyze the effects of pomelo peel extract and variety on enzyme inhibition. Results could lead to the development of functional foods or nutraceuticals utilizing Thai pomelo.

1. Research Sample

Two types of pomelo including Thong Dee (TD) and Kao Nam Pheung (KNP) were chosen from Nakhon Pathom province in Thailand, both harvested at the age of 6.5 to 8 months.

2. Research Tools

Research tools include analytical balance, vortex mixer, micropipette, microplate reader, UV-Vis spectrophotometer, pH-meter, rotary evaporator, while various chemicals consist of Distilled Water, 95% Ethanol (C_2H_5OH), α-amylase enzyme, α-glucosidase enzyme, Acarbose, *p*-nitrophenyl-α-D-glucopyranoside (PNP-G), Quercetin, Sodium phosphate buffer (pH6.8) conc. 50 mM, Sodium carbonate (Na_2CO_3) conc. 1.0 mM, Hydrochloric acid (HCL), Tris (Hydroxymethyl) aminomethane, Calcium chloride ($CaCl_2$), Dimethyl Sulfoxide (DMSO), Aluminum chloride ($AlCl_3$) and Soluble Starch.

3. Research Procedures

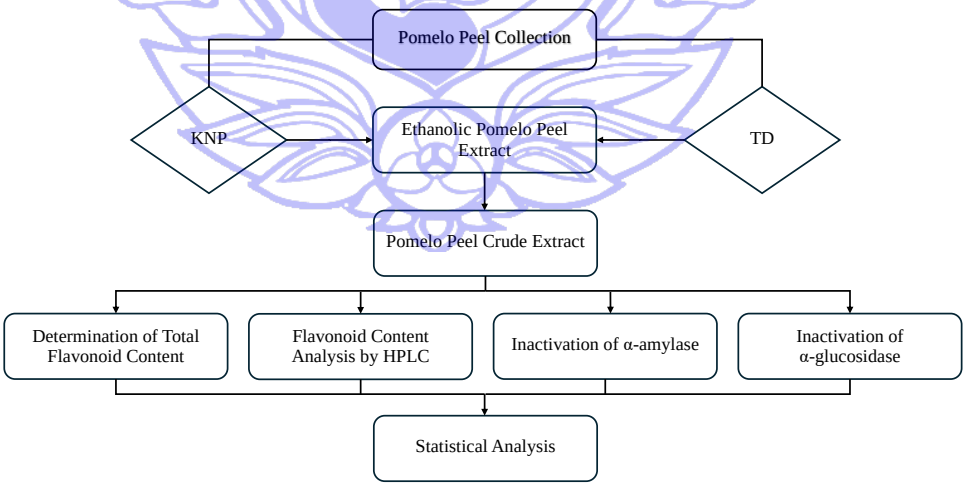

Figure 1 Laboratory framework

Getting the extract from pomelo peel (PPE) for studying enzyme inhibition is quite a detailed process. Each step can be broken down as follows.

1) Obtaining the Raw Material - To start off, one kilogram of pomelo peel was gathered from each selected type. Thong Dee (TD) and Kao Nam Pheung (KNP).

2) Cutting and Drying - The peels collected were cut into small pieces measuring 0.5 cm by 0.5 cm for convenience. These pieces then went through a freeze-drying process at 20°C for 26 hours to remove moisture while keeping the important components intact.

3) Preparing Powder - Once dried successfully, the peels were ground into fine powder uniformly to ensure consistent surface area for better extraction in the next step.

4) Ethanol Extraction - The finely ground powder was mixed with an ethanol solution in a 1:2 weight to volume ratio (w/v). Ethanol acted as a solvent, effectively extracting desired compounds from the pomelo peel. This mixture sat for two hours to allow proper interaction between the solvent and powder.

5) Filtering and Concentration - After extraction, the mixture underwent thorough filtration using filter paper.

The liquid extract, which contained the desired compounds, was separated from the solid residue through a filtration process. This filtration was carried out five times to ensure optimal extraction efficiency. The filtrate, which may contain enzyme inhibitors, was then concentrated using rotary evaporation. During this process, the volume of the filtrate was reduced to around 350 mL at a temperature of 60°C over a period of 45 minutes. Rotary evaporation served to concentrate the extract while minimizing any thermal damage to the desired compounds. Regarding storage, the final product known as concentrated pomelo peel extract (PPE) was stored in a dark and cool environment at 4°C. This storage condition aided in preserving the bioactivity of the extract for subsequent analysis. By meticulously following these steps, the study acquired a concentrated pomelo peel extract suitable for evaluation in enzyme inhibition tests. The extract was subjected to tests against α-amylase and α-glucosidase enzymes to assess its potential in managing blood sugar levels.

This approach allows for both a qualitative analysis (identifying quercetin) and a quantitative analysis (measuring the quantity of quercetin). As for Enzyme

Deactivation, the section outlined the procedures for evaluating the ability of samples (PPE) to hinder two enzymes; alpha amylase and alpha glucosidase.

1) Alpha Amylase Inactivation;

a. An adapted version of a previously published method was employed.

b. Soluble starch was prepared and its pH was adjusted accordingly.

c. Sample solutions were prepared at varying concentrations.

d. A mixture of starch solution and sample solution was pre incubated.

e. Alpha amylase solution was introduced, followed by another incubation period.

f. The reaction was halted and iodine solution was introduced.

g. The absorbance of the mixture at 650 nm was gauged using a microplate reader.

h. A higher absorbance indicates reduced enzyme activity (greater inhibition by the sample).

i. A standard curve was developed to determine the concentration of sample required to inhibit 50% of enzyme activity (IC50).

j. All measurements were conducted in triplicate.

2) Alpha Glucosidase Inactivation;

Similar to the alpha amylase assay, an altered version of a previously published method was adhered to. Samples were dissolved in a buffer solution at different concentrations. The enzyme solution and a substrate called *p*-nitrophenyl-a-D-glucopyranoside were combined with the sample solution in a microplate. After letting it sat for a while, the reaction was halted by adding sodium carbonate solution. The amount of light absorbed by the mixture was checked at 405 nm using a microplate reader. Just like with the alpha amylase test, a higher absorbance means less enzyme activity. To figure out the IC_{50} for inhibiting alpha glucosidase, a standard graph was made. Each measurement was taken three times to ensure accuracy.

3. Statistical Analysis

All experiments were repeated thrice to make sure they were precise. SPSS software analyzes the data and compares results from different groups (samples vs. positive control). The statistical method used one-way ANOVA, 95% confidence level as the data followed a normal distribution.

Results

Exploring the Potential of Thai Pomelo Peels for Digestive Enzyme Inhibition

The research presents exciting possibilities for using Thai pomelo peels as natural inhibitors of digestive enzymes. However, further exploration is needed before considering them as a viable treatment option due to several important factors. Firstly, the study was carried out in a controlled laboratory setting (in vitro). Although the extracts show promise in inhibiting enzymes in this environment, their behavior within a living organism (in vivo) remains uncertain. Factors including digestion, absorption and metabolism can significantly impact the effectiveness of the extracts in the body. Secondly, the research indicates that the inhibitory effect of the extracts is weaker compared to acarbose, a commonly used medication for diabetes management. Additional research is required to determine the ideal dosage of these extracts to achieve an inhibitory effect equivalent to or better than existing medications. Thirdly, safety and potential side effects of the extracts were not evaluated in the study. It is essential to conduct clinical trials to identify any adverse reactions or interactions with other medications before deeming these extracts safe for human consumption. Despite these limitations, the study underscores the promising potential of Thai pomelo peels. In order to maintain reliable outcomes in upcoming studies and possible clinical trials, it is crucial to establish a standardized approach for gathering and safeguarding the peels. This involves regulating variables such as the time of harvest, drying techniques and storage conditions, all of which can influence the effectiveness of the extracts in the end product.

Moreover, should additional research validate the efficacy of the extracts, securing a dependable source of raw materials will be vital. Creating a sustainable method for collecting and preserving pomelo peels will guarantee a steady provision for future research efforts and potential commercial ventures. By addressing these constraints through further exploration, Thai pomelo peels may emerge as a valuable natural asset for managing diabetes and other conditions associated with blood sugar regulation.

Discussion and Suggestion

From the conducted experiment, two Thai pomelo cultivars peel were selected as samples - Kao Nam Pheung and Thong Dee - to study the efficacy on inhibitory effect of pancreatic enzymes (α-amylase and α-glucosidase). The enzymes involved in the level of postprandial blood glucose.

The yield of Kao Nam Pheung and Thong Dee peel extract were 4.63% and 4.70% respectively. The main phytochemical compound in this study is flavonoid. Flavonoid was found in many studies show the beneficial action of digestive enzyme inhibitor (Wang et al., 2024). The total flavonoid content of Kao Nam Pheung peel extract was 5.80 ± 0.96 (mg quercetin/g of crude extract) and Thong Dee peel extract was 6.78 ± 0.64 (mg quercetin/g of crude extract). Both cultivars gave the same level of the yield as they were grown at the same area. They were fed with the same protocol, got the same soil and environment (Makkumrai et al., 2021).

As there are many specific types of flavonoid, quercetin is the standard selection on quantitation of flavonoid study. The HPLC analysis was utilized to measure the quantitation of quercetin of samples in this study. The quantitation of quercetin in Kao Nam Pheung peel extract was 0.244 (mg/g) and Thong Dee peel extract was 0.807 (mg/g). The higher number showed in Thong Dee as pinkish fruit. The important aspect found from few studies. The pinkish pulp pomelo contained higher predominant flavonoids and bioactive compound than the yellowish one (Yin et al., 2023).

According to the study, it was discovered that the result of inactivation of α-amylase and α-glucosidase by calculating the percentage (%) inhibitory compared to acarbose as a positive control. Firstly, Acarbose, Kao Nam Pheung and Thong Dee can inhibit α-amylase activity as shown in Table 1 and Table 2.

Table 1 % Inhibitory of α-amylase

Concentration (mg/ml)	%inhibitory of α-amylase			F-test	df	p-value
	Acarbose (n=3)	Kao Nam Pheung (n=3)	Thong Dee (n=3)			
	mean±SD	mean±SD	mean±SD			
15.38	76.76 ± 1.97[a]	35.57 ± 1.86[b]	32.40 ± 1.31[b]	606.639	2,6	<0.001
12.31	75.70 ± 0.74[a]	31.07 ± 1.00[b]	31.49 ± 1.13[b]	2089.317	2,6	<0.001

Table 1 (continued)

Concentration (mg/ml)	%inhibitory of α-amylase					
	Acarbose (n=3) mean±SD	Kao Nam Pheung (n=3) mean±SD	Thong Dee (n=3) mean±SD	F-test	df	p-value
9.23	57.20 ± 2.07[a]	19.37 ± 1.15c	24.32 ± 1.47[b]	684490.358	2,6	<0.001
6.15	44.28 ± 3.59[a]	7.55 ± 0.81[b]	10.93 ± 1.68[b]	226.577	2,6	<0.001
3.08	19.72 ± 0.85[a]	6.36 ± 0.08[b]	5.49 ± 0.37[b]	658.633	2,6	<0.001
2.77	15.82 ± 0.33[a]	5.96 ± 0.38[b]	5.03 ± 0.15[b]	1053.303	2,6	<0.001
2.31	9.15 ± 1.30[a]	4.38 ± 0.15[b]	4.75 ± 0.09[b]	36.651	2,6	<0.001
1.85	4.12 ± 1.18	3.37 ± 0.32	3.68 ± 0.07	0.862	2,6	0.469
1.54	4.16 ± 2.18	3.38 ± 0.73	3.16 ± 0.20	0.468	2,6	0.648
1.23	4.19 ± 2.32	2.75 ± 0.17	2.05 ± 0.18	1.967	2,6	0.220
0.77	3.76 ± 1.67	2.32 ± 0.09	1.81 ± 0.15	3.257	2,6	0.110
0.31	3.22 ± 4.31	1.57 ± 0.14	0.85 ± 0.12	0.715	2,6	0.526
0.15	0.75 ± 3.14	0.59 ± 0.13	0.40 ± 0.09	0.478	2,6	0.642

Both Kao Nam Pheung and Thong Dee had lower %inhibitory than acarbose in every concentration. The statistical analysis showed the difference between groups at final concentration 2.31, 2.77, 3.08, 6.15, 9.23, 12.31 and 15.38 mg/ml (*p-value < 0.05*). At the highest final concentration, Acarbose, Kao Nam Pheung and Thong Dee had the highest % inhibitory (76.76 ± 1.86, 35.57 ± 1.86 and 32.40 ±1.31 mg/ml, respectively). The efficacy of extract on inhibitory action by comparing each IC_{50} as showed acarbose had the highest efficacy on inhibitory effect (IC_{50} = 7.71 mg/ml) which is greater than Kao Nam Pheung and Thong Dee (IC_{50} = 21.87 and 21.63 mg/ml, respectively).

Table 2 % Inhibitory of α-Glucosidase

Concentration (µg/ml)	%inhibitory of α-glucosidase					
	Acarbose (n=3) mean±SD	Kao Nam Pheung (n=3) mean±SD	Thong Dee (n=3) mean±SD	F-test	df	p-value
5000	99.90 ± 0.09	99.44 ± 0.59	99.66 ± 0.12	1.286	2,6	0.343
4500	99.75 ± 0.14	99.11 ± 0.43	99.24 ± 0.33	3.261	2,6	0.110
3750	99.59 ± 0.16[a]	82.06 ± 5.63[b]	93.55 ± 0.46[a]	22.451	2,6	0.002
3000	99.47 ± 0.23[a]	76.60 ± 3.08[c]	83.45 ± 1.27[b]	110.918	2,6	<0.001
2500	99.34 ± 0.34[a]	64.47 ± 2.90[c]	74.98 ± 2.01[b]	228.653	2,6	<0.001
2000	88.03 ± 0.52[a]	45.50 ± 8.69[c]	61.17 ± 0.61[b]	54.646	2,6	<0.001

Table 2 (continued)

Concentration (µg/ml)	%inhibitory of α-glucosidase					
	Acarbose (n=3) mean±SD	Kao Nam Pheung (n=3) mean±SD	Thong Dee (n=3) mean±SD	F-test	df	p-value
1250	83.31 ± 0.95[a]	26.24 ± 2.49[b]	21.48 ± 4.43[b]	397.334	2,6	<0.001
500	79.48 ± 1.00[a]	-2.61 ± 3.79[b]	-4.33 ± 4.47[b]	583.785	2,6	<0.001
250	72.45 ± 0.76[a]	-5.97 ± 0.52[b]	-13.23 ± 3.78[c]	1344.544	2,6	<0.001

Secondly, Acarbose, Kao Nam Pheung and Thong Dee can also inhibit α-glucosidase activity as shown in Table 2. Both Kao Nam Pheung and Thong Dee had lower %inhibitory than acarbose in every concentration. Both Kao Nam Pheung and Thong Dee had lower %inhibitory than acarbose in every concentration. The statistical analysis showed the difference between groups at final concentration 250, 500, 1250, 2000, 2500, 3000 and 3750 µg/ml (*p-value* < *0.05*). At the highest final concentration, Acarbose, Kao Nam Pheung and Thong Dee had the highest % inhibitory (99.90 ± 0.09, 99.44 ± 0.59 and 99.66 ± 0.12 µg/ml, respectively). The efficacy of extract on inhibitory action by comparing each IC_{50} showed acarbose had the highest efficacy on inhibitory effect (IC_{50} = -2,144.80/µg/ml) which is greater than Kao Nam Pheung and Thong Dee (IC_{50} = 2,222.84 and 1924.89 µg/ml, respectively).

In conclusion, both Kao Nam Pheung and Thong Dee demonstrated the ability to inhibit digestive enzymes in lab tests. However, when compared to the traditional drug acarbose used as an enzyme inhibitor, both extracts showed less effectiveness. Acarbose is a commonly used antidiabetic medication due to its strong α glucosidase inhibitory properties. Despite being approved by the FDA, concerns regarding adverse effects or contraindications still exist (Alssema et al., 2021). The extract from Thai pomelo peel is among the citrus fruits that have potential for further research and enhancement.

This study indicates exciting potential in utilizing Thai pomelo peels as natural inhibitors of digestive enzymes. However, further investigation is warranted before considering it as a viable treatment option for the following reasons:

1. Laboratory vs. Real World; The study was carried out in a controlled lab environment (in vitro), showing that the extracts can inhibit enzymes. Yet, it is

uncertain if the same outcomes will manifest within a living organism (in vivo) due to factors like digestion, absorption and metabolism.

2. Dosage and Effectiveness; The research revealed that the extracts exhibit a weaker inhibitory effect compared to acarbose. More studies are necessary to determine the ideal dosage of these extracts to match or surpass the efficacy of current medications.

3. Safety Concerns; The study did not evaluate the safety profile or potential side effects of the extracts. Conducting clinical trials is essential to uncover any adverse reactions or interactions with other drugs.

Importance of Establishing a Collection and Preservation Protocol for Pomelo Peels;

Standardization; Variations in flavonoid content were noted between two pomelo cultivars (Kao Nam Pheung and Thong Dee). To ensure consistent findings in future investigations and possible clinical trials, adopting a standardized approach for collecting and preserving these peels is imperative. This approach can help manage variables such as when the crops are harvested, how they are dried and the conditions they are stored in, all of which can impact the strength of the extracts. Regarding sustainability; If additional studies validate the efficacy of these extracts, it will be essential to secure a dependable source of raw materials. Establishing a sustainable method for collecting and preserving pomelo peels ensures a steady provision for future research and potential commercial use. By delving into these aspects through further investigation, Thai pomelo peels could emerge as a valuable natural resource for treating diabetes and other health issues linked to regulating blood sugar levels.

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