INHIBITION EFFICACY OF THAI POMELO PEEL EXTRACT AGAINST α-AMYLASE AND α-GLUCOSIDASE IN VITRO

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MASTER OF SCIENCE
IN
ANTI-AGING AND REGENERATIVE SCIENCE

SCHOOL OF ANTI-AGING AND REGENERATIVE MEDICINE
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Chawit Songkrasin

Independent Study Title	Inhibition Efficacy of Thai Pomelo Peel Extract Against α-Amylase and α-Glucosidase in Vitro
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ABSTRACT

Hyperglycemia is a disorder characterized by an excessive concentration of glucose circulation in the bloodstream, and it can be brought on by impairment to the blood sugar regulation system. An inadequate amount of insulin leads to type 2 diabetes. One kind of treatment for Type 2 diabetes is lowering postprandial glucose levels. This can be achieved by blocking the digestive tracts carbohydrate hydrolytic enzymes, which will delay the absorption of glucose: α-glucosidase and α-amylase Acarbose is a medication taken orally. The medication works as a potent enzyme inhibitor to delay the pace at which carbohydrates break down, so slowing down the absorption of glucose. Regretfully, using the medication can have certain major side effects that are typically experienced concurrently, such as liver disease. Pomelo peel extract has been studied on such an enzyme inhibitor property as riches in flavonoid content. Pomelo (*Citrus maxima*) is one of the citrus fruits that are the most abundant fruit grown throughout the world especially in Thailand and Asia, Thong Dee and Kao Nam Pheung are the famous predominant commercial cultivars in Thailand and are the top cultivar that contain higher number of flavonoids among others Both are the selected cultivars on this. the result in this study showed both Kao Nam Pheung and Thong Dee showed the ability to inhibit digestive enzymes in vitro. However, in comparison to the conventional drug acarbose that used as enzymes inhibitor, both

extracts showed lower efficacy as the IC_{50} of Kao Nam Pheung and Thong Dee are greater, compared to IC_{50} of acarbose on 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-Hydrolyzing Enzyme Inhibitor, α-Amylase, α-Glucosidase

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CHAPTER 1

INTRODUCTION

1.1 Background and Rationale

Serious health issue including diabetes mellitus can be brought on by abnormalities in carbohydrate absorption. Impairment of blood sugar control system can cause hyperglycemia condition, which is defined as an excessive concentration of glucose circulation in bloodstream, is the cause of diabetes that is one of the most severe and chronic disorder. Type 2 diabetes results from insulin insufficiency. This type accounts for roughly 90% of all diabetes cases globally. Reducing postprandial glucose level is one of the therapy strategies for treating Type 2 diabetes. This can be accomplished by delaying the absorption of glucose by inhibiting the digestive tract carbohydrate hydrolytic enzymes: α-amylase and α-glucosidase (Sansenya & Payaka, 2022).

Acarbose is an orally administrated drug, approved by Food and Drug Administration (FDA), and become as essential therapeutic tactic for regulating postprandial glycemia (Huang et al., 2021). The drug functions as a powerful enzyme inhibitor to slow down the absorption of glucose by delaying the rate of carbohydrate breaking down. Unfortunately, some serious adverse effects on using the drug, are usually accompanied simultaneous such as liver disorder (Alssema et al., 2021). It is vital to use naturally occurring substitutes for manufactured inhibitors to prevent or lessen the negative effect (Sornkhwan et al., 2022). Reviews on abilities of phenolic compounds like flavonoids that found in plants, act as acarbose-like functions, that have been published during past decades.

Recently, Pomelo peel extract has been studied on such an enzyme inhibitor property (Wang et al., 2024). Pomelo (Citrus maxima) is one of the citrus fruits that are the most abundant fruit grown throughout the world especially in Thailand and Asia,

containing valuable beneficial bioactive phytochemical (Singh et al., 2020). Scientists found a very significant positive correlation between phenolic content in the peel against the digestive enzymes' inhibitor compared to their pulp (Ha et al., 2022). Thong Dee and Kao Nampheung are the famous predominant commercial cultivars in Thailand and are the top cultivar that contain higher number of flavonoids among others (Kongsri & Nartvaranant, 2019a). Both are the selected cultivars on this study. Although their pulps are popular by its taste, their peel may be concerned as an agricultural waste, which may affect the environmental problem (Yin et al., 2023)

This research result may aid in the development of diabetes-fighting medication by functional natural food in Thailand by these representative Thai pomelos, as well as help reduce the excessive amount of agro-industrial waste from pomelo peel

1.2 Research Objectives

1.2.1 To study efficacy of Thai pomelo peel extract on α- amylase inhibition

1.2.2 To study efficacy of Thai pomelo peel extract on α- glucosidase inhibition

1.3 Hypothesis

1.3.1 Thai pomelo peel extract have the efficacy on α- amylase inhibition

1.3.2 Thai pomelo peel extract have the efficacy on α- glucosidase inhibition

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1.4.1 Two Thai cultivar pomelo selected as sample to be extracted

1.4.2 Functional inhibition test on α- amylase and α- glucosidase enzymes in laboratory (in vitro)

1.5 Significant of The Study

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1.5.2 To be the database for continually research

1.5.3 To improve well-being health

1.6 Variable

1.6.1 Independent variable: pomelo peel extract, pomelo cultivar, the concentration of the extract and acarbose

1.6.2 Dependent variable: the ability of α-amylase inhibition, the ability of α-glucosidase inhibition

1.7 Conceptual Framework

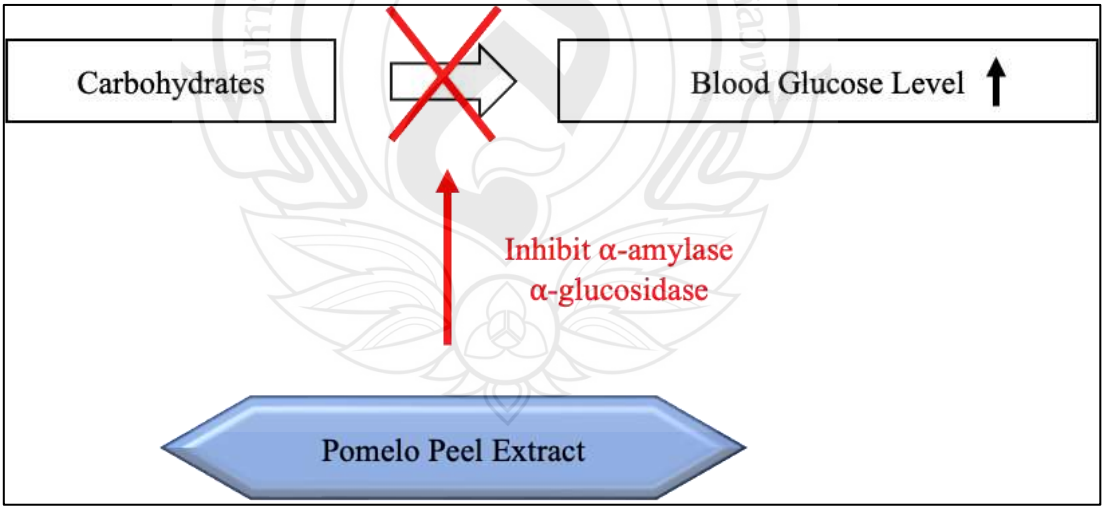


Figure 1.1 Conceptual Framework

In patients with diabetes, there is increasingly in postprandial blood glucose. Pomelo peel extract could reduce hyperglycemia due to delay carbohydrate digestion and absorption by inhibit α-amylase and α-glucosidase enzymes.

1.8 Definition of Specific Terms

1.8.1 Pomelo peel means a thick rind, both green (flavedo) to white (albedo) in color, surrounding the fruit.

1.8.2 Pomelo peel extract means the pomelo peel that dried by freeze dryer and extracted by 95% ethanol.

1.8.3 The ability of α-amylase inhibition means the ability of pomelo peel extract to delay the function of α-amylase enzyme (positive %inhibition).

1.8.4 The ability of α-glucosidase inhibition means the ability of pomelo peel extract to delay the function of α-glucosidase enzyme (positive %inhibition).

1.8.5 The efficacy of pomelo peel extract on α-amylase and α-glucosidase inhibition means the percent of α-amylase and α-glucosidase inhibition from pomelo peel extract is greater than or equal to acarbose standard solution at the same concentration or IC50 is less than or equal to acarbose.

1.8.6 IC50 means the half maximal inhibitory concentration that shows the quantitative measure to indicate how much of pomelo peel extract is needed to inhibit the enzyme.

CHAPTER 2

LITERATURE REVIEW

2.1 Diabetes Mellitus

2.1.1 Overview of Diabetes

Diabetes, characterized as a chronic condition, emerges when the pancreas fails to produce sufficient insulin or when the body develops resistance to the insulin it generates. Insulin, acting as a pivotal hormone, plays a vital role in maintaining blood sugar levels within the body. Hyperglycemia, commonly referred to as high blood sugar or high blood glucose, stands as a frequent complication stemming from untreated diabetes, ultimately leading to significant damage across various physiological systems, particularly affecting neurons and blood vessels. Statistics from 2014 revealed that 8.5% of individuals aged 18 and older were afflicted with diabetes. By 2019, the gravity of the condition became starkly evident, as diabetes emerged as the primary reason of 1.5 million deaths, with nearly half of all diabetes- associated fatalities taking place prior to the age of 70 (World Health Organization [WHO], 2020).

Diabetes exacts a heavy toll, adding 460,000 deaths from kidney disease and contributing to 20% of cardiovascular fatalities due to elevated blood glucose levels.

From 2000 to 2019, there was a 3% increase in the age-standardized death rate attributed to diabetes. Especially in lower-middle-income countries, diabetes-related mortality surged by 13% during this period. Despite these concerning trends, there's a glimmer of progress on a global scale. The likelihood of premature death between the ages of 30 and 70 from major non-communicable diseases, including cardiovascular diseases, cancer, chronic respiratory disorders, and diabetes, decreased by 22% over the same timeframe.

Impaired glucose tolerance (IGT) and impaired fasting glycaemia (IFG) represent transitional stages between normal blood sugar levels and diabetes. While

persons with IGT or IFG face an elevated risk of developing type 2 diabetes, it's significant to note that this outcome is not inevitable.

Diabetes can have severe health consequences, affecting various parts of the body over time. Diabetic adults face a significantly higher risk, ranging from 2 to 3 times, of experiencing heart attacks and strokes compared to non-diabetic individuals. Neuropathy, or nerve damage, particularly in the feet, can lead to complications such as foot ulcers, infections, and, in severe cases, amputation, especially when coupled with reduced blood flow. Diabetic retinopathy, a leading cause of blindness, stems from prolonged damage to the delicate blood vessels in the retina, affecting nearly 1 million people worldwide. Additionally, diabetes stands as a major contributor to renal failure. Moreover, persons with diabetes are at increased risk of poor outcomes from various infectious diseases, including COVID-19.

Prevention

Lifestyle measures are key in preventing the onset of type 2 diabetes. To mitigate the risk of type 2 diabetes and its complications, individuals should focus on:

2.1.1.1 Maintain a healthy body weight through proper diet and regular exercise.

2.1.1.2 Participate in physical activities, striving for a minimum of 30 minutes of moderate-intensity exercise on a regular basis. Additional activity may be necessary for weight management.

2.1.1.3 Adopt a nutritious diet, emphasizing the avoidance of excessive sugar and saturated fats.

2.1.1.4 Refrain from tobacco use, as smoking is linked to an increased risk of diabetes and cardiovascular disease.

2.1.2 Etiologic Classification of Diabetes Mellitus (American Diabetes Association [ADT], 2011)

2.1.2.1 Type1 diabetes

Type 1 diabetes is distinguished by insufficient insulin production, requiring daily insulin therapy. In 2017, there were 9 million persons with type 1 diabetes, the primary of them resides in countries with high incomes. It is an etiology and methods of prevention are unknown.

Common symptoms of type 1 diabetes comprise increased urine production (polyuria), excessive thirst (polydipsia), persistent hunger, weight loss, alterations in eyesight, and weariness, which may manifest abruptly.

2.1.2.2 Type2 diabetes

Type 2 diabetes stems from insulin resistance, affecting over 95% of individuals with diabetes. This condition is primarily linked to excess body fat and physical inactivity.

Symptoms resembling those of type 1 diabetes may be present; however, they are often less severe. Consequently, the condition may remain undetected for several years after onset, by which time complications may have already arisen.

Formerly restricted to adults, this form of diabetes is now progressively affecting younger individuals at an escalating rate.

2.1.2.3 Gestational diabetes

Gestational diabetes is characterized by hyperglycemia with blood glucose levels higher than normal but lower than those diagnostics of diabetes. It occurs during pregnancy and poses an elevated risk of adverse outcomes during pregnancy and delivery for affected women. Additionally, both mothers and their children face a heightened risk of developing type 2 diabetes later in life.

The diagnosis of gestational diabetes is typically made using prenatal screening instead of solely depending on symptom reporting.

2.1.2.4 Other Specific types

Other types of diabetes can stem from a variety of conditions, such as genetic defects in beta-cell function or insulin action, disorders of the exocrine pancreas, endocrine disorders, drug or chemical-induced factors, infections, rare forms of immune-mediated diabetes, and various genetic syndromes.

2.1.3 Pharmacological Treatment of Hyperglycemia (American Diabetes Association [ADT], 2013)

The pharmaceutical therapy of hyperglycemia is predicted on the two most important metabolic abnormalities in type2 diabetes: insulin resistance and decreased insulin secretion. Consequently, each hypoglycemic medication addresses one of these anomalies, and combination therapy is frequently required to treat both. Sulphonylureas

and glinides promote insulin secretion directly, but thiazolidinediones and metformin enhance insulin sensitivity. α-glucosidase inhibitors reduce carbohydrate absorption, hence lowering the demand for insulin secretion after meal. If patients are really symptomatic or have a very high blood glucose level, it is doubtful that dietary and lifestyle modifications would achieve goal values. In this scenario, immediate pharmaceutical treatment should be initiated.

2.1.3.1 α-glucosidase inhibitors.

glucosidase inhibitors, such as acarbose, miglitol, and voglibose, lower postprandial blood glucose and, to a lesser extent, fasting glucose, thereby enhancing overall glycemic management. They have a weight-neutral or weight-reducing effect and can be used in conjunction with diet or in combination with sulphonylureas, metformin, and insulin as first-line therapy. These medications may decrease HbA1C by roughly 1%. To reduce the likelihood of gastrointestinal side effects, a low initial dose is recommended, followed by a gradual increase.

2.1.3.2 Metformin

In some countries, metformin is indicated as first-line therapy for non-obesity people. Metformin is recommended as first-line therapy for obese and overweight patients. Metformin can lower HbA1c as effectively as sulfonylureas and insulin without causing significant weight gain, as proven by the UK Prospective Diabetes Study (UKPDS). Importantly, it is the only hypoglycemic drug demonstrated to prevent cardiovascular disease and mortality. Metformin does not cause hypoglycemia or weight gain, but it is usually associated with bothersome gastrointestinal side effects, which are frequently dose-dependent and can manifest after several years of treatment. Due to the danger of lactic acidosis, Metformin should not be administered to patients with compromised renal function, hepatic illness, or septic shock, or during major surgery. If the serum creatinine concentration is greater than 150 mol/L (1.7 mg/100 mL), metformin should not be administered.

2.1.3.3 Sulphonylureas

Sulphonylureas enhance beta cell insulin secretion and reduce HbA1C by 1–2%. The UKPDS confirmed that these medications minimize the progression of microvascular problems by 25% . Intensive blood glucose management with sulphonylureas or insulin versus standard therapy and the risk of complications in type2

diabetes patients. However, they typically result in weight gain and can cause hypoglycemia, particularly in the elderly and those with renal or hepatic illness. Consequently, sulphonylureas are typically utilized as second- or third-line treatments. Gliquidone is the preferred sulphonylurea for renal insufficiency.

2.1.3.4 Thiazolidinediones

Thiazolidinediones like rosiglitazone and pioglitazone increase cellular responsiveness to insulin, but they do not increase insulin synthesis. They lower HbA1C by 1–2% without hypoglycemia. Weight gain, mostly from subcutaneous fat, is a typical side effect. Fluid retention can cause cardiac failure in pre-existing heart disease patients. Active liver disease or transaminase levels exceeding 2.5 times the upper limit of normal should prevent thiazolidinedione use. Rosiglitazone and pioglitazone have not been linked to abnormal liver function tests, although they should be monitored. Thiazolidinedione treatment may increase weight and fluid retention. Rosiglitazone and pioglitazone have showed significant changes to surrogate indicators for heart disease, suggesting a long-term benefit. Outcome studies are presently investigating this and its potential to prevent diabetes.

2.1.3.5 Insulin

Insulin is most frequently administered when oral medications alone can no longer maintain sufficient glycemic control. As type2 diabetes is a progressive condition characterized by gradual loss of beta cell function, insulin is frequently required to achieve adequate glycemic control and should be explored for all patients on maximal oral therapy with a HbA1c > 6.5%. Inadequate glycemic control, despite maximal oral therapy, can frequently be improved by increasing adherence to lifestyle goals. Nevertheless, early initiation of insulin therapy warrants serious consideration if unintentional weight loss is observed at any point during the course of diabetes, including the time of diagnosis.

Insulin should typically be taken with oral medicines since they minimize weight gain, reduce hypoglycemia associated with insulin therapy, and there is evidence that metformin is essential for cardiovascular protection.

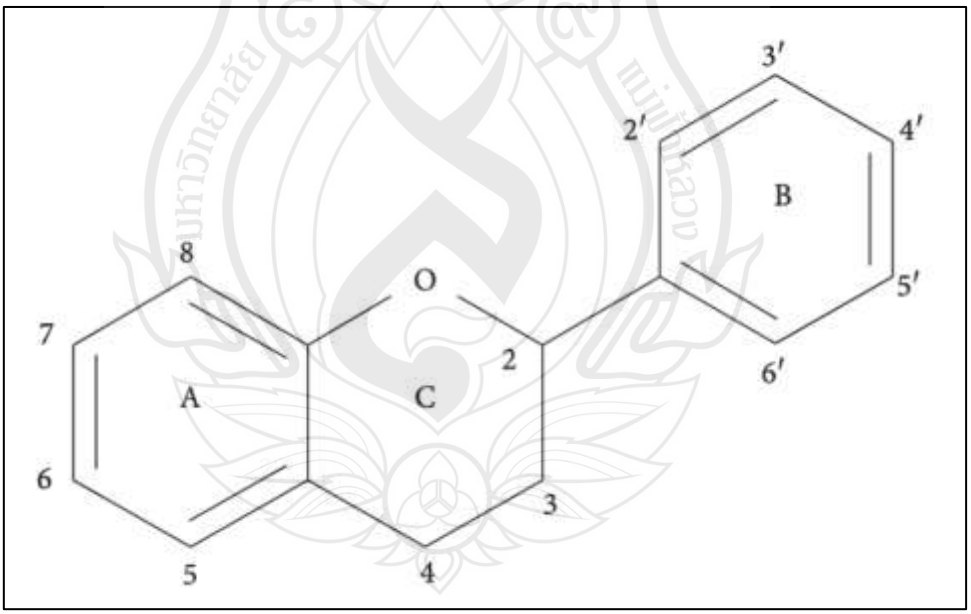
2.2 Flavonoid

Structure of Flavonoids

Flavonoids are compounds with relatively low molecular weight, distinguished by a common skeleton (C6-C3-C6). This structure comprises two phenyl rings (A and B) connected by a pyran (heterocyclic) C ring. In rings A and C, carbon atoms are numbered between 2 and 8, while in ring B, they are numbered between 2′ and 6′ (Tang et al., 2024).

This fundamental structure permits a number of ring replacement schemes. Critical to the functioning of flavonoids are:

1. The presence of a catechol or O-dihydroxy structure in the B ring
2. A double bond at positions 2 and 3 in ring C
3. Hydroxyl groups at positions 3 and 5 in rings C and A, respectively



Source Lim et al. (2022)

Figure 2.1 Basic Structure of Flavonoid

Flavonoids originate from a biosynthetic pathway within plant tissues. This process entails the merging of three molecules of malonyl-CoA and one molecule of p-coumaroyl-CoA with an additional molecule of p-coumaroyl-CoA, resulting in the formation of an intermediate called chalcone. Chalcones, aided by specific enzymes, serve as precursors for synthesizing a diverse range of flavonoids. The biosynthetic pathway illustrates the series of enzyme-mediated processes leading to the synthesis of various types of flavonoids.

Based on the molecule's chemical composition and the arrangement of substituent groups in rings A, B, and C, flavonoids are primarily categorized into 14 distinct (Shen et al., 2022). The most prevalent varieties are listed below:

1. Flavones: These flavonoids exhibit a typical chemical composition of 2-phenyl-1-benzopyran-4-one and are predominantly present in food items like honey and grapes.

2. Flavanones: Hydroxylated flavanones exist either in their free form or as glycosides in various plant parts such as flowers, leaves, and fruits. They are widely distributed, particularly in higher plants.

3. Isoflavanones: Typically found in legumes, isoflavanones feature a base skeleton of 3-phenylchromen-4-one, formed as a result of the biosynthetic process in which a benzene ring shifts from position 2 to position 3 within the central ring.

4. Flavonols: Characterized by a backbone structure of 3-hydroxyflavone, flavonols are prevalent in a diverse range of fruits and vegetables.

5. Aurones: Aurones undergo a conversion of the pyranic ring of chalcones into a furan ring.

6. Anthocyanidins: Found abundantly in wild fruits, particularly those with a purple hue, anthocyanidins have a structured based on the foundation of 2-phenylbenzopyrylium

7. Anthocyanins: These flavonoids closely resemble anthocyanidins but feature a glycosylated segment attached to the oxygen substituent of carbon 3 , frequently found in red fruits.

The majority of flavonols and flavones are commonly found as O-glycosides, predominantly linked to carbon 3 of ring C and less frequently to carbon 7 of ring A. The aglycone refers to the portion of a flavonoid molecule devoid of sugars, while

glycosylated flavonoids contain sugar segments within their structure. Understanding the molecular composition of flavonoids is crucial for various purposes, including recognizing that the antioxidant potential of flavonoids relies on their hydroxyphenolic groups and determining suitable solvents for extracting flavonoids from plant material. Nonpolar flavonoids, such as flavonols, flavones, flavanones, and, isoflavones have an affinity for solvents like ethyl acetate, diethyl ether, dichloromethane, and chloroform. Conversely, polar flavonoids demonstrate an affinity for aqueous and pure alcohols.

2.2.1 Flavonoid's Structure Requirement for The Selective Inhibition of Enzymes (Lim et al., 2022)

Flavonoids sourced from plants possess the ability to hinder starch-digesting enzymes through non-covalent binding to the active site residues of these enzymes. Their three-ring structure and chemical characteristics make flavonoids well-suited for investigating the inhibitory specificity of α-amylase and α-glucosidase. Lim et al. (2019) outlined the general structural prerequisites of three flavonoids—quercetin, luteolin, and eriodictyol—as natural inhibitors. They found that the presence of a double bond between C2 and C3 of the C-ring in quercetin and luteolin was critical for inhibiting pig pancreatic α-amylase. Additionally, the hydroxyl group (OH) at C3 of the C-ring in quercetin and eriodictyol was associated with the inhibition of α-glucosidases from the rat gut.

The study, marking a novel contribution, elucidated the distinct structural prerequisites for flavonoids to inhibit human pancreatic α-amylase in contrast to two intestinal α-glucosidases (Tran et al., 2021). It revealed that flavonoids possessing specific chemical configurations can selectively impede these two categories of starch-digesting enzymes. Particularly noteworthy, for α-amylase inhibition, the flavonoid structure necessitates a double bond between C2 and C3, coupled with hydroxyl (OH) groups at positions A5, B3, and B4.

To block α-glucosidases, the structure must have OH groups at positions B3, B4, and C3.

Example of Flavonoids that have ability of inhibition on both enzymes are Quercetin and Luteolin

2.3 Pomelo

Pomelo (*Citrus maxima*), a member of the Rutaceae family, is the largest citrus fruit, which is one of the leading fruit crops worldwide (Yin et al., 2023). According to the 2019-2020 world citrus production report, grapefruit and pomelos have a strong rate of production. They are frequently paired because of their similar appearances.

A considerable area of Asia has a climate that is suitable for pomelo growth. In Asia, pomelo cultivars are typically classified into three main categories: Chinese varieties, thriving in subtropical regions: Thai varieties, renowned as the top tropical varieties; and Indonesian cultivars, which exhibit considerable variability and occasionally yield fruit of mediocre quality (Wang et al., 2024). The pomelo is one of the ten most economically successful fruit crops in Thailand. The bulk of Thailand has tropical wet and dry climates or savanna. Pommelos may be grown wherever: Chainat, Chaiya- phum, Prachinburi, Nakhon Si Thammaratand, Nakhon Nayok, Nakhon Pathom, Chiang Rai, Samut Songkhram, are Pichit, Kanchanaburi the main producing provinces (Makkumrai et al., 2021). Among the many cultivars growing in Thailand, they can be split into two categories based on their color: white or pink one. The white group also includes Pattavee, Kao Phuang, Kao Hom, Kao Tanggwa, Kao Nampheung, Kao Paen, and Kao Yai. Tong Dee and Tha Knoi are represented by the pink (Kongsri & Nartvaranant, 2019a).

Health-promoting substances like carotenoids, flavonoids, linonoids, and fiber have been found to be abundant in pomelo. The active anti-tumor, anti-fungal, anti-inflammatory, and blood clot inhibitory capabilities of citrus fruit extracts have also been discovered (Xiao et al., 2021). The citrus fruit's health advantages have primarily been linked to its antioxidant activity; moreover, it has been suggested that the fruit's total polyphenol content acts as its primary antioxidant (Hou et al., 2019). The membrane of the segment, the juice sacs, and the segment all have effects that are related to edible tissue sections. In comparison to the juice sacs and segments, they discovered the segment membrane had a higher concentration of antioxidants (Tsai & Wong, 2019). Not only flavonoids present in the edible portion of citrus fruit, but they have also been identified in the non-edible portions, primarily the pomelo peel. The

citrus fruit peel exhibited good total radical antioxidant potential in the citrus fruit peel (ferric reducing antioxidant power, FRAP) (Zhang et al., 2020). Because of the possibility for synergistic effects of the bioactive chemicals contained in food, fruit, and vegetables, the study of the antioxidant capacity of these items has drawn considerable attention. There have been reports of flavonoids in pomelo.

In 2014, Pichaiyongvongdee and team (Pichaiyongwongdee et al., 2014) studied the overall polyphenolic content and antioxidant characteristics in four distinct tissue types (seeds, segment membranes, albedo and flavedo) of 7 Thai pomelo cultivars (Thong Dee, Tha Khoi, Kao Nampheung, Kao Yai, Kao Tangkwa, Pattavee and Kao Paen). The findings revealed that the highest total polyphenol content was observed in the seeds of all cultivars (ranging from 3,108.78 to 4,957.97 μg/g), followed by the albedo (ranging from 1,176.58 to 3,384.81 μg/g), flavedo (ranging from 1,096.27 to 2,163.63 μg/g), and segment membranes (ranging from 825.68 to 2,266.66 μg/g) in descending order. The antioxidant qualities (DPPH) were greatest in the seeds, while the albedo, flavedo, and segment membranes ranked in decreasing order. The flavedo and seeds from the cultivar Thong Dee (TD) had the highest total polyphenol content and antioxidant capabilities (1, 1 dyphenyl- 1- picrylhydrazyl: DPPH and ferric mitigating antioxidant power; FRAP). The albedo and segment membranes of Tha Knoi (TK) showed the greatest total polyphenol content and antioxidant characteristics (DPPH and FRAP). The seeds of Kao Tanggwa (KTG) have the most antioxidant characteristics (DPPH) of any other species. For the peel including flavedo and albedo, Tha Knoi, Thong dee and Kao Nampheung are the top 3 which rich in total polyphenol content. The coefficient of determination (R2) for the linear association between the total polyphenol content and the DPPH is 0.702, while the R_2 for the FRAP is 0.659. Thus, pomelo's edible tissues may be a source of bioactive chemicals that are rich in antioxidant (Satari & Karimi, 2018).

Morphological characteristics of Kao Nam Pheung and Thong Dee

Kao Nam Pheung and Thong Dee were original grown in central part of Thailand especially Nakhon Pathom province. As their taste and unique quality, they were marked as geological indications or GI. (Kongsri & Nartvaranant, 2019a, 2019b). Kao Nam Pheung weighs roughly 1.8 kg and has a diameter of 17–19 cm. The peel white portion and the segment walls are easily separated from the pulp by their white

color. The pulp is fairly big, sticky, and has a honey-colored or brownish-yellow color. Among other cultivars, the flavor is exceptionally sweet, and the texture is crisp and juicy without being watery. Meanwhile, Thong Dee is the most popular commercial cultivar in Thai land sold in the domestic, Asian, and European markets. Thong Dee fruit is oblate and medium-sized, measuring 14-16 cm in diameter and weighing 0.9-1.1 kg. The rind is silky green, but the albedo and segment walls are pinkish. The fresh is pinkish, about 1 cm thick on the outside, and the rest is yellowish. The juice sacs are soft, extremely juicy, and watery, and they stick firmly to one other yet separate readily from the segment walls. It has a strong ascorbic acid content and a sweet-sour taste (Makkumrai et al., 2021).

Sometimes the same cultivar can present different taste and quality if they are grown in different area. They may be caused by several reasons such as

2.3.1 The Specificity of the Plant and Area

The nutrient in soils, environment or maintenance practice from gardener. Those result in certain flavor and morphological characteristic of the fruit in each area.

2.3.2 The Same Name but Different Species

In the past, gardeners generally used seeds for breeding. Pomelo is self-incompatibility which means they need to cross species breed to form the seeds. This results in variant by seeding. In the present, plant propagation uses by non-sexual production breeding like stem cutting, layering, cleft grafting and approach grafting, the new will be certainty the same as mother tree.

2.3.3 Other Events

Natural disaster like flooding, climate crisis or somatic mutant may affect the differentiation of fruits (Sornsanit et al., 2019).

2.4 Carbohydrate – Hydrolyzing Enzymes

2.4.1 α-amylase

The digestive enzyme α- amylase converts complex carbohydrates like starch into monosaccharides, glucose (Tran et al., 2023).

Dextrins and maltose are typically produced when the alpha bonds of large alpha – linked polysaccharides, such as starch and glycogen, are hydrolyzed. On the other hand, α-amylase is the most common type of amylase found in people and other mammals. Additionally, α-amylase is found in seed that have starch as a food reserve. Fungi also produce this enzyme into organic substance that is decomposing.

α- amylase is more prevalent in pancreatic juice than anywhere else, except for the mouth. Achrodextrin, erythodextrin, and amylodextrin are examples of soluble starches produced through the digestion of large insoluble starches by salivary amylase. The salivary α-amylase, however, further break down these tiny starches into maltose. Linear (1, 4) glycosidic linkages are the target of α-amylase activity (Xiao et al., 2022). Additionally, the stomach's gastric pH renders salivary α- amylase inactive. Pancreatic α-amylase randomly cleaves the (1,4) glycosidic linkages of amylose, releasing dextrin, maltose, or maltotriose.

2.4.2 α-glucosidase

α- glucosidase is an enzyme found in small intestine' s brush border. Furthermore, α- glucosidase acts on terminal and non – reducing glycosidic bonds. α-glucosidase, on the other hand, only produced one – glucose molecule. As a result, α-glucosidase converts starch and disaccharides to glucose. The enzyme will be found in the endocellular compartment of both germinated and non – germinated cereals in plants which may differ from human's and animal's origin. In addition, α-glucosidase regulates the composition of plant polysaccharides during the maturation stage.

2.5 Enzyme Inhibition

Enzyme inhibitors have numerous uses in the pharmaceutical, biochemical, and environmental industries, and have a significant impact on the healthcare and medical industry. The majority of medications in clinical usage are enzyme inhibitors, which are used to treat a number of significant human disorders. In the foreseeable future, specific enzyme inhibition will remain a key focus of pharmaceutical research (Huang et al., 2021). Enzymes are protein molecules that serve as biological catalysts. The specificity of enzymes ensures a high degree of coordination and a harmonic interplay between vital metabolic functions. Enzyme activity depends on various parameters, including enzyme concentration, substrate quantity, the electrochemical reaction of the medium (pH), and the presence of activators or inhibitors.

Enzyme inhibitors inhibit the catalytic action of enzymes by interfering with any step of the catalytic cycle. They are chemicals with a low molecular weight that, in minute quantities, can diminish or totally inhibit enzyme function. Human enzyme inhibitors like antithrombin and antitrypsin play a regulatory role in enzyme activity within the body, ensuring their proper function under physiological conditions. Some metabolic processes generate intermediary molecules that are natural enzyme inhibitors. The inhibition of products is a limited method of controlling or modulating the flux of substrates via the route. If enzymes are susceptible to product inhibition, the output of the pathway's end products will be reduced (Lim et al., 2019).

An inhibitor can change a single amino acid or several side chains essential for the catalytic activity of an enzyme. A ligand binds to an enzyme's essential side chain to prevent any alteration to the catalytic site (Xu et al., 2023). Conceptually, enzyme inhibitors can be categorized as either selective or nonspecific. Reversibly or irreversibly, inhibitors can diminish or fully stop the enzyme's catalytic activity. Usually, irreversible inhibitors alter the chemical structure of the enzyme. Depending on whether they bind to the enzyme, the enzyme-substrate complex, or both, reversible inhibitors can elicit various forms of inhibition. The majority of medicines that inhibit enzymes connect with their targets through simple, reversible binding processes.

Inhibitors that are reversible may be classified into three types; competitive, noncompetitive, or noncompetitive.

1. A competitive inhibitor competes with the substrate for access to the active site.

2. A noncompetitive inhibitor binds to both the enzyme in its free form and the enzyme-substrate complex with equal affinity.

3. A noncompetitive inhibitor generates an inactive enzyme- substrate-inhibitor complex by binding exclusively to the enzyme-substrate complex.

2.5.1 Anti-α-Amylase Assay

2.5.1.1 The 2-chloro-4-nitrophenol colorimetric method utilizing acarbose as a standard. Amylase is an enzyme present in saliva and the liver that aids in digestion. It plays a role in breaking down transformed starch into oligosaccharides and disaccharides, which are subsequently digested through enzymatic hydrolysis in the small intestine (Ha et al., 2022). In the experiment, a clear, colorless solution of 2-chloro-4-nitrophenyl-D-maltatrioside (CNPG3) was utilized. It functions as a substrate in the reaction in which α- amylase hydrolyzes CNPG3 to produce glucose and 2-chloro-4-nitrophenol (CNP), which is the clear, yellow solution. It absorbs light between 400 and 650 nm (Mahnashi et al., 2022).

2.5.1.2 The colorimetric microplate assay is the method that tests the enzymatic reaction with porcine pancreatic α- amylase, p- nitrophenyl- α- D-maltopentaoside (PNPG-5 and extract sample in 96-well plate microplates. The enzyme inactivation was measured across a wavelength range of 400 to 650 nm using a microplate reader in conjunction with the Gen5 data analysis program. And also, be able to use starch as the substrate.

2.5.2 Anti-α-Glucosidase Assay

The α-glucosidase inhibitory activity was evaluated using the p-nitrophenol colorimetric technique with acarbose as the standard material. It is an enzyme found in the cell wall of the small intestine that hydrolyzes starches and carbohydrates into monosaccharides. This will be utilized in the experiment. As an enzyme reaction substrate, p-nitrophenyl-D-glucopyranoside (PNP-G) is a clear, colorless, thickened

solution. α-glucosidase interacts hydrolysis with PNP-G produces glucose and p-nitrophenol, a clear yellow solution with an absorbance at 405 nm. α-glucosidase can function effectively. However, if the experiment measures poor light absorption This means that the enzyme alpha glucosidase cannot function normally, i.e., the extracted material has rendered the enzyme inactive. from this sort of plant (Kittiwisut et al., 2021)

2.6 Related Study Review

2.6.1 Exploring the Inhibitory Capacity of Phenolic Compounds from Thai Colored Rice (*Oryza sativa* L.) Against α-Glucosidase and α-Amylase: Insights from *in vitro* and in *silico* Investigations

This study explored the efficacy of phenolic chemicals, including methyl vanillate, syringic acid, and vanillic acid, in inhibiting α-glucosidase and α-amylase. Additionally, it measured the phenolic component content of ten Thai colored rice cultivars: black rice (Khao Mali Nin, Khao Dam Nin, Khao Dam Snit, Khao Jao Dam Jean, Khao Gam Kliang, Khao Hom Nin, Khao Leum Pua, and Khao Gam 101), red rice (Khao Sin Lek), and purple rice (Khao Riceberry). The study also explored the correlation between the inhibitory effectiveness of colored rice extract and methyl vanillate, syringic acid, and vanillic acid (Sansenya & Payaka, 2022).

The results demonstrated that methyl vanillate, syringic acid, and vanillic acid were more effective at inhibiting α-glucosidase than α-amylase. Vanillic acid exhibited the strongest inhibitory activity against α-glucosidase and α-amylase, with IC50 values of 0.100 ± 0.01 and 0.130 ± 0.02 mmol/L, respectively. Four hydrogen bonds and three hydrogen bonds were identified between the amino acids in the binding sites of α-amylase and α-glucosidase, respectively, and vanillic acid. The inhibition mechanisms of these phenolic compounds on α-glucosidase were classified as a combination type of inhibition. The rice cultivars with the highest concentrations of methyl vanillate, syringic acid, and vanillic acid in their methanol extracts. The methanol extracts of all colored rice cultivars, including Khao Leum Pua, exhibited the most powerful suppression of α-glucosidase and α-amylase. The outcomes suggested that the phenolic

component concentration of all 3 kinds of colored rice with ten cultivars extracts was directly connected to their ability to inhibit α-glucosidase and α-amylase. This may conclude that the primary natural inhibitor against α-glucosidase and α-amylase was phenolic compounds.

2.6.2 Mechanistic Analysis of Porcine Pancreatic α-Amylase Inhibition by Flavonoids Derived from Dandelion

This work was conducted to quantify the inhibition and molecular mechanism of pancreatic α-amylase demonstrated by flavonoids from dandelion, with the goal of revealing their potential application in reducing postprandial hyperglycemia (Huang et al., 2021).

They investigated the dose-dependent inhibition of porcine pancreatic α-amylase by flavonoids extracted from dandelion, employing a combination of enzymatic kinetics, thermodynamic analysis, and measurement of fluorescence quenching. This research revealed that, under the current experimental conditions, dandelion flavonoids exhibit a greater inhibitory capability than acarbose. The IC_{50} values for dandelion flavonoids and acarbose were determined to be 0.0067 and 0.019 mg/mL. The Lineweaver-Burk analysis reveal that flavonoids derived from dandelion act as non-competitive inhibitors of pancreatic α-amylase, with K_m and K_i values of 10.51 mg/mL and 5.71×10^3 mg/mL, respectively. The reduction in Ksv with increasing temperature indicates that the fluorescence quenching of pancreatic α-amylase is likely a static phenomenon, attributed to the formation of the flavonoid-amylase complex in dandelion. The values of n at varying temperatures (298 K and 310 K, respectively) were observed to be nearly identical, indicating the existence of a singular class of binding sites for flavonoids from dandelion on pancreatic α-amylase. The negative ΔG (free energy change: $\Delta G = \Delta H - T\Delta S$) implies that dandelion flavonoids can spontaneously bind with pancreatic α-amylase. Additionally, ΔH (enthalpy change) and ΔS (entropy change) thermodynamic parameters were calculated to be 14.7 kJ/mol and 0.0829 kJ/(mol·K) respectively. This shows that hydrophobic interactions promote the interaction between dandelion flavonoids and pancreatic α-amylase, accompanied by a rise in entropy and a decrease in free energy. These findings indicate that flavonoids derived from dandelion are a promising inhibitor of pancreatic α-amylase.

This study offers a quantitative basis for utilizing dandelion flavonoids as a functional food ingredient in the development of healthier diets aimed at stabilizing or reducing blood glucose levels after meals.

2.6.3 *In Vitro* Evaluation of Antioxidant Capacity and Carbohydrate Digestive Enzyme Inhibition by *Citrus grandis* Peel Extract

This research studied on two local pomelo cultivars in Vietnam. They investigated the total polyphenolic content (TPN) on the pomelo peel extract (PPE) both flavedo and albedo parts from Thanh Kieu and Long Co Co, also tested the antioxidant activity (DPPH, FRAP and ABTS assay) and inhibitory properties against α-amylase and α- glucosidase (Ha et al., 2022).

In this investigation, Thanh Kieu flavedo PPE demonstrated the best antioxidant capacity at the IC_{50} values of 60.89 ± 0.31 μg/mL, 24.16 ± 0.06 μg/mL and 60.69 ± 0.21 μg/mL for DPPH, FRAP and ABTS assay, in conjunction with the highest TPC. PPE from two different kinds significantly inhibited α-amylase and α-glucosidase activity. Although having a lower TPC, the albedo PPE was discovered to have a stronger inhibitory effect on these enzymes with respect to IC_{50} values of 3.59 ± 0.02 mg/mL and 80.77 ± 0.34 μg/mL than the flavedo PPE. The results verified the efficacy of PPE derived from the flavedo and albedo of both cultivars (Long Co Co and Thanh Kieu) in treating hyperglycemia by inhibiting carbohydrate-digesting enzymes. To elucidate the inhibitory actions of PPE against these carbohydrate enzymes, further research is required to define the primary phytochemical ingredients (Mahnashi et al., 2022). This study indicated the potential anti-hyperglycemic impact of flavedo and albedo pomelo peel extract as a beneficial nutraceutical for the food and pharmaceutical industries.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Research Design

Laboratory Research at Medicine Plant Innovation Center of Mae Fah Luang University, Chiang Rai, Thailand

3.2 Research Sample

2 cultivars of pomelo selected (Thong Dee; TD and Kao Nam Pheung; KNP) at the same harvesting fruit age: 6.5-8 months, from Nakhon-Pathom province, Thailand.

3.3 Research Tools

The material that used in this study include the following:

3.3.1 Materials and Equipment

3.3.1.1 Analytical Balance

3.3.1.2 Vortex Mixer

3.3.1.3 Micropipette

3.3.1.4 Micropipette Tip

3.3.1.5 Microplate Reader

3.3.1.6 UV-Vis Spectrophotometer

3.3.1.7 pH-meter

3.3.1.8 Rotary evaporator

3.3.1.9 Freeze Dryer

3.3.1.10 Ultrasonic Machine

3.3.1.11 High-Performance Liquid Chromatography (HPLC)

3.3.1.12 Dropper

3.3.1.13 Stainless Test Tube Stand

3.3.1.14 Beaker

3.3.1.15 96-well plate

3.3.1.16 Cylinder

3.3.1.17 Volumetric Flask

3.3.1.18 Vial

3.3.1.19 Stirring Rod

3.3.1.20 Test Tube

3.3.1.21 Spatula

3.3.1.22 Mixer

3.3.1.23 Knife

3.3.2 Chemical

3.3.2.1 Distilled Water

3.3.2.2 Ethanol (C_2H_5OH)

3.3.2.3 α-amylase enzyme

3.3.2.4 α-glucosidase enzyme

3.3.2.5 Acarbose

3.3.2.6 *p*-nitrophenyl-α-D-glucopyranoside (PNP-G)

3.3.2.7 Quercetin

3.3.2.8 Sodium phosphate buffer (pH6.8) conc. 50 mM

3.3.2.9 Sodium carbonate (Na_2CO_3) conc. 1.0 mM

3.3.2.10 Hydrochloric acid (HCL)

3.3.2.11 Tris (Hydroxymethyl) aminomethane

3.3.2.12 Calcium chloride ($CaCl_2$)

3.3.2.13 Dimethyl Sulfoxide (DMSO)

3.3.2.14 Aluminum chloride ($AlCl_3$)

3.3.2.15 Soluble Starch

3.4 Research Procedures

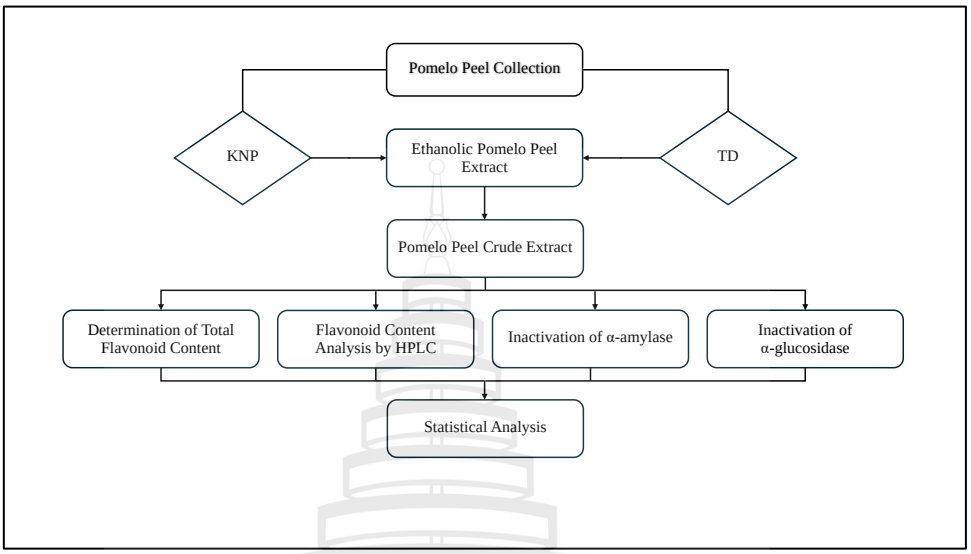


Figure 3.1 Laboratory Framework

Figure 3.2 Chopped Pomelo Peel

3.4.1 Preparation of Ethanolic Pomelo Peel Extract

3.4.1.1 Collected the pomelo peel from both cultivars (Thong Dee; TD and Kao Nampheung; KNP) each 1 kg.

3.4.1.2 Chopped the peels into size 0.5 cm × 0.5 cm

3.4.1.3 Dried those chopped peels by freeze dryer at -20 °C for 26 hours

3.4.1.4 Uniformly grounded to the fine powder

3.4.1.5 Mixed the powder with ethanol solution at the ratio of 1:2 (w/v)

3.4.1.6 Kept the mixture for 2 hours

3.4.1.7 Filtrated the mixture by filter paper with 5 times repetition

3.4.1.8 Concentrated the filtrate volume 350 mL at 60 °C for 45 min by using rotary evaporator

3.4.1.9 Stored the pomelo peel extract (PPE) in the dark room at 4 °C

%yield = [crude extract / dry peel] × 100

Figure 3.3 Pomelo Peel Crude Extract

3.4.2 Determination of Total Flavonoids Content and Flavonoid Content Analysis by High-Performance Liquid Chromatography (HPLC)

3.4.2.1 estimation of the total flavonoid was carried out using the aluminum chloride colorimetric method

3.4.2.2 prepared the quercitin solution by dissolving 5.0 mg quercitin in 1.0 mL methanol

3.4.2.3 mixed 0.5 mL diluted standard quercitin solutions with 0.5 mL of 2% aluminum chloride

3.4.2.4 mixed 0.5 mL PPE with 0.5 mL of 2% aluminum chloride

3.4.2.5 incubated both solutions for 60 min at room temperature

3.4.2.6 measured the absorbance of the reaction of the mixtures against blank at 420 nm wavelength with a Varian UV-Vis spectrophotometer (TECAN, infinite 200 PRO, Australia)

3.4.2.7 the concentration of the total flavonoid content in the test samples will be calculated from calibration plot and expressed as mg quercitin equivalent (QE)/g of dried material all the determinations are carried out in triplicate

3.4.2.8 analyzed the qualitative and quantitative flavonoid content (Quercetin) by High-Performance Liquid Chromatography; HPLC (Priscilla et al., 2011) by preparing sample at concentration 80 mg/mL in methanol (HPLC grade)

3.4.2.9 prepared quercitin reference standard at concentration 200, 100, 50, 25, 12.5, 6.25, 3.125 and 1.5625 μg/mL in methanol (HPLC grade)

3.4.2.10 filtered solution by syringe filter PTFE 0.2 μm (Agilent, USA) in a vial for analysis

Chromatographic conditions of HPLC

Instrument: Agilent 1290 Infinity II, a diode-array detector (DAD)

Column: Agilent Poroshell 120 EC-C18 (4.6 × 150 mm, 0.4 μm)

Temperature of column: 40 °C

Mobile phase: 50% methanol in 50% water (0.1% TFA) Run time: 15 min

Inject volume: 10 μL

Flow rate: 1 mL/min

Wavelength: 254 nm

3.4.3 Inactivation of α-Amylase

the inhibition of α-amylase activity protocol was performed by using a modified method of Ha and the team in 2022

3.4.3.1 dissolved 500 mg soluble starch in 25 mL of 0.4 M NaOH and heated for 5 min at 100 °C

3.4.3.2 cooled down in ice H2O

3.4.3.3 adjusted pH value of the solution to 7 with 2 M HCL and added H2O to adjust the volume to 100 mL

3.4.3.4 prepared the sample solutions by dissolving each of sample in acetate buffer (pH6.5) to make 2, 0.2 and 0.02 mg/mL solutions

3.4.3.5 mixed 40 μL of the mixture of substrate solution and 20 μL in the 96-well-plate

3.4.3.6 pre-incubated the mixture at 37 °C for 3 min

3.4.3.7 added 20 μL of α-amylase solution (50 μg/mL) on each well

3.4.3.8 incubated the mixture at 37 °C for 15 min

3.4.3.9 terminated the reaction by adding 80 μL of 0.1 M HCL

3.4.3.10 added 200 μL of 1 mM iodine solution

3.4.3.11 the absorption of the reaction mixture was observed at wavelength 650 nm by microplate reader (TECAN, infinite 200 PRO, Australia). Inhibitory activity (%) was calculated as follows:

$$\text{Inhibition (\%)} = \{1 - (\text{Abs } 2 - \text{Abs } 1) / (\text{Abs } 4 - \text{Abs } 3) \times 100\}$$

Where Abs 1 is the absorbance of incubated solution containing sample, starch, and amylase, Abs 2 is the absorbance of incubated solution containing sample and starch, Abs 3 is the absorbance of incubated solution containing starch and amylase, and Abs 4 is the absorbance of incubated solution containing starch.

3.4.3.12 built the standard curve of PPE concentration versus corresponding α-amylase inhibitory percentage and calculate the 50% inhibitory effect (IC_{50}) by using acarbose as positive control all the determinations were carried out in triplicate

3.4.4 Inactivation of α-Glucosidase

the inhibition of α-glucosidase activity protocol was performed by using a modified method of Kittiwisut et al. 2021.

3.4.4.1 each of PPE was individually dissolved in 0.1 M sodium phosphate buffer (pH6.8) to obtain the extract solution with final concentration in a range of 250-5000 μg/mL

3.4.4.2 added 50 μL of sample solution into 96-well-plate which each well was containing 50 μL of 1 mM p-nitro-phenyl-α-D-glucopyranoside

3.4.4.3 incubated the mixture at 37 °C for 5 min

3.4.4.4 added 50 μL of 0.05 U/mL of α-glucosidase enzyme in buffer

3.4.4.5 incubated the mixture at 37 °C for 30 min

3.4.4.6 added 50 μL of 0.3 M Na2CO3 to stop the reaction

3.4.4.7 the absorption of the reaction mixture was observed at wavelength 405 nm by microplate reader (TECAN, infinite 200 PRO, Australia).

3.4.4.8 built the standard curve of PPE concentration versus corresponding α-glucosidase inhibitory percentage and calculate the 50% inhibitory effect (IC50) by using acarbose as positive control

$$\%\text{inhibition} = \{[(A-B)-(C-D))/(A-B)]\} \times 100$$

Where A is the absorbance of blank reaction containing only 5% DMSO in phosphate buffer, B is the absorbance of control reaction containing 5% DMSO in phosphate buffer and α-glucosidase, C is the absorbance of PPE reaction containing PPE and α-glucosidase, D is the absorbance of control containing only PPE solution all the determinations were carried out in triplicate

3.5 Statistical Analysis

Each experimental data was in three replicates. SPSS software was utilized to conduct Kruskal-Wallis test and T-test/ Mann-Whitney U test apply to compare the mean values at the level of 95% confidence interval. ($p < 0.05$).

Compare the Difference between Each of the PPE and Positive Control Groups

The data found normally distributed, used independent t–test or One-way ANOVA for statistical analysis.

CHAPTER 4

RESULTS

The laboratory was performed at Medicine Plant Innovation Center of Mae Fah Luang University (MPIC)

4.1 Yield of Ethanolic Pomelo Peel Extract

Pomelo peels (Thong Dee and Kao Nam Pheung) were constituted by two parts including the flavedo (thin greenish peel) and the albedo (thick white peel). The pomelo peel extract (PPE) was collected from the flavedo and albedo of each variety by using ethanolic extraction.

4.1.1 Yield of Kao Nam Pheung peel extract = 4.63%

4.1.2 Yield of Thong Dee peel extract = 4.70%

Table 4.1 Report of Yield of Ethanolic Pomelo Peel Extract

Sample	Fresh peel (g)	Dry peel (g)	Crude extract (g)	%Yield
Kao Nam Pheung	1,379	727.0	33.67	4.63
Thong Dee	2,380	432.1	20.30	4.70

4.2 Determination of Total Flavonoids Content and Flavonoid Content Analysis by High-Performance Liquid Chromatography (HPLC)

4.2.1 The Determination of Total Flavonoids

Estimation of total flavonoids in the plant extracts was carried out using the method of Tran et al. (2021). 0.5 mL of 2% $AlCl_3$ ethanol solution was added to 0.5

mL of sample. After one hour at room temperature, the absorbance was measured at 420 nm (TECAN, infinite 200 PRO, Australia). Extract samples were evaluated at a concentration of 10 mg/ml. Total flavonoid content was calculated as quercetin (mg/g) using the following equation based on

The calibration curve: y = 00176x + 0.0215, R^2 = 0.9996, where x is the absorbance and y is the quercetin equivalent (mg/g)

Table 4.2 Report of Determination of Total Flavonoids

Sample (n=3)	Total Flavonoids (mg quecetin/g of crude extract)
Kao Nam Pheung	5.80 ± 0.96
Thong Dee	6.78 ± 0.64

4.2.2 The Flavonoid Content Analysis by HPLC

The analysis to measure the quantitation of quercetin was performed by HPLC method. Prepared samples at concentration 80 mg/ml in methanol (HPLC grade). Then, prepared quercetin reference standard at concentration 200, 100, 50, 25, 12.5, 6.25, 3.125 and 1.5625 μg/ml in methanol, filtrated solution by syringe filter PTFE 0.2 μm (Agilent, USA) in vial for analysis

Table 4.3 Report of Quantitation of Quercetin by HPLC

Sample	mg quercetin/g in crude extract	% w/w
Kao Nam Pheung	0.244	0.024
Thong Dee	0.807	0.081

4.3 Inactivation of α-Amylase

The method to fine α-amylase inhibitory activity modified by Kusano. Acarbose was used as the positive control. % inhibitory activity was calculated by the absorbance at 650 nm which shown in Table 4.4 and Table 4.5

Table 4.4 %Inhibitory of α-Amylase

Concentration (mg/ml)	**%inhibitory of α-amylase**					
	Acarbose (n=3)	**Kao Nam Pheung (n=3)**	**Thong Dee (n=3)**	**F-test**	**df**	**p-value**
	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
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

From Table 4.4 shows %inhibitory of α-amylase of acarbose at all concentration is greater than %inhibitory of α-amylase of Kao Nam Pheung and Thong Dee. The statistical analysis shows the comparison between acarbose group, Kao Nam Pheung group and Thong Dee group at concentration 2.31, 2.77, 3.08, 6.15, 9.23, 12.31 and 15.38 mg/ml that have difference in the statistically significant 95% interval ($p<0.05$).

But there is no difference between groups at concentration 0.15, 0.31, 0.77, 1.23, 1.54 and 1.85 mg/ml.

IC_{50} Calculation

The IC_{50} was calculate by building the standard curve of pomelo peel extract concentration versus corresponding α-amylase inhibitory percentage.

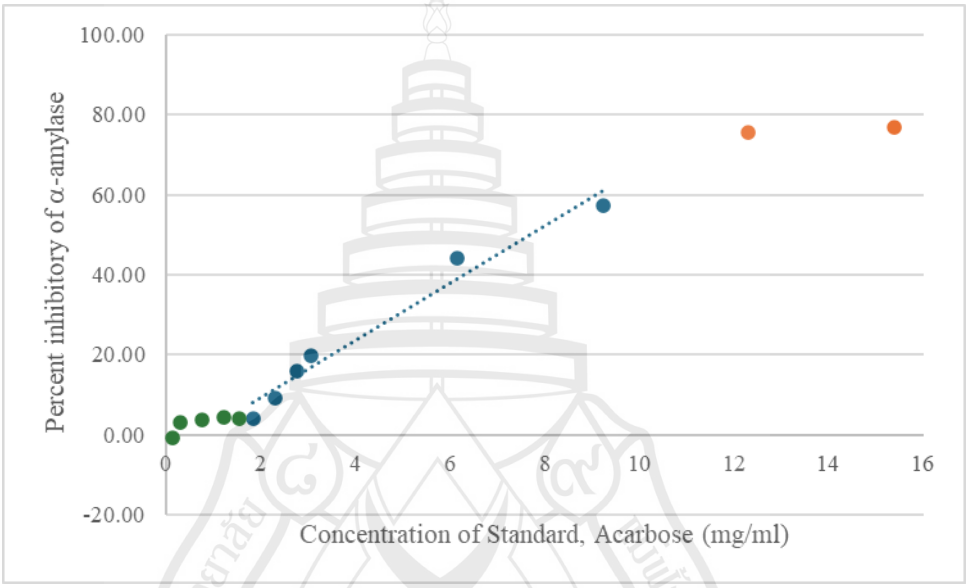


Figure 4.1 The Graph of Relation between the Percent Inhibitory of α-Amylase and the Concentration of Acarbose (mg/ml). $\hat{y} = (-5.3055) + 7.173x$, R Square = 0.967

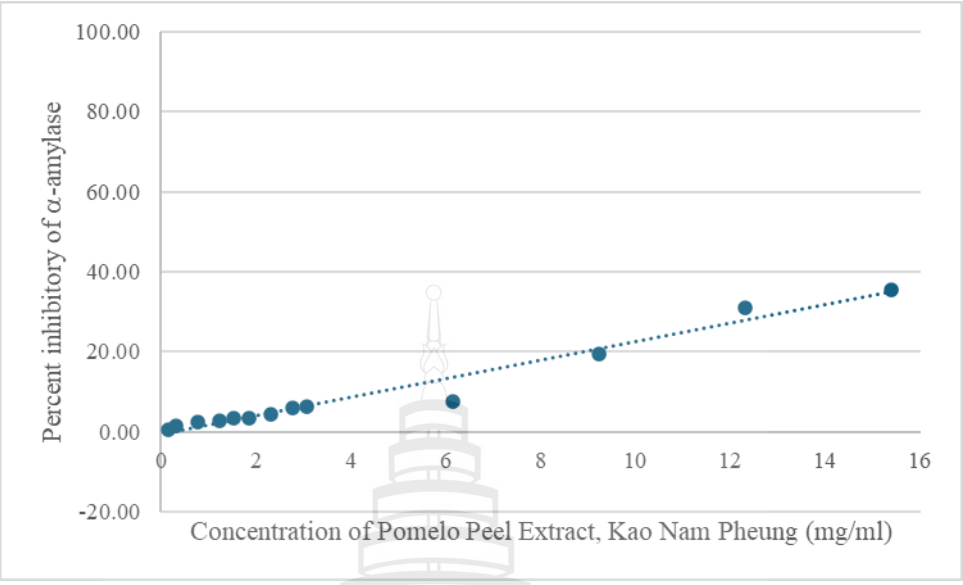


Figure 4.2 The Graph of Relation between the Percent Inhibitory of α-Amylase and the Concentration of Kao Nam Pheung (mg/ml). $\hat{y}$ = (-0.5929) + 2.3116x, R Square = 0.966

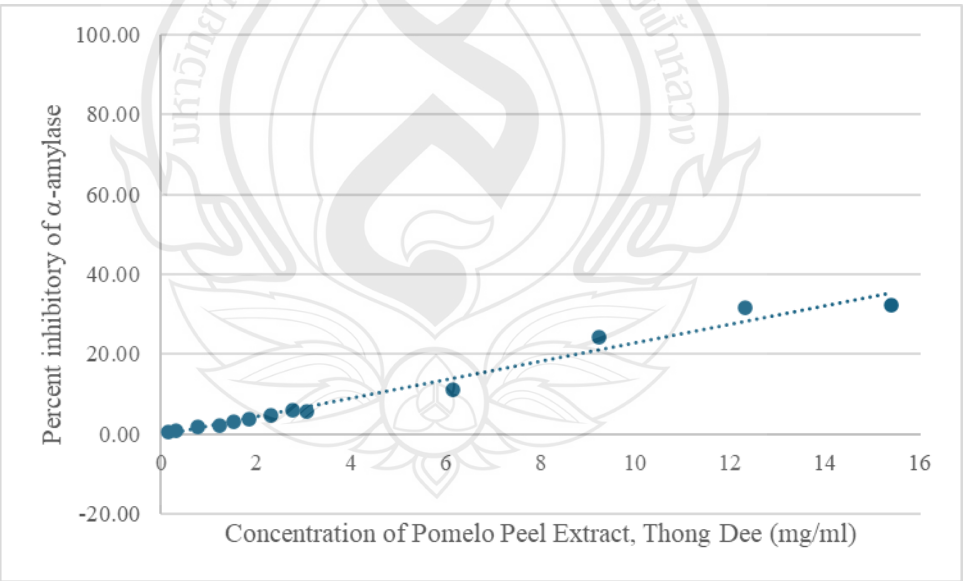


Figure 4.3 The Graph of Relation between the Percent Inhibitory of α-Amylase and the Concentration of Thong Dee (mg/ml). $\hat{y}$ = (-0.4478) + 2.3332x, R Square = 0.975

Table 4.5 IC_{50} of Experimental Extracts (α-Amylase)

Extract	IC_{50} (mg/ml)
Acarbose	7.71
Kao Nam Pheung	21.87
Thong Dee	21.63

4.4 Inactivation of α-Glucosidase

The inhibition of α-glucosidase activity protocol was performed using the modified method of Kittiwisut and the team. The absorbance of the reaction mixture was observed at wavelength 405 nm and calculated by using acarbose as the positive control which shown in Table 4.6.

Table 4.6 %Inhibitory of α-Glucosidase

Concentration (µg/ml)	%inhibitory of α-glucosidase			F-test	df	p-value
	Acarbose (n=3)	Kao Nam Pheung (n=3)	Thong Dee (n=3)			
	mean±SD	mean±SD	mean±SD			
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
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

From Table 4.6 shows %inhibitory of α-glucosidase of acarbose at all concentration is greater than %inhibitory of α-glucosidase of Kao Nam Pheung and Thong Dee. The statistical analysis shows the comparison between acarbose group, Kao Nam Pheung group and Thong Dee group at concentration 250, 500, 1250, 2000, 2500, 3000 and 3750 µg/ml that have difference in the statistically significant at level 95% ($p<0.05$). But there is no difference between groups at concentration 4500 and 5000 µg/ml.

IC_{50} calculation

The IC_{50} was calculate by building the standard curve of pomelo peel extract concentration versus corresponding α-glucosidase inhibitory percentage.

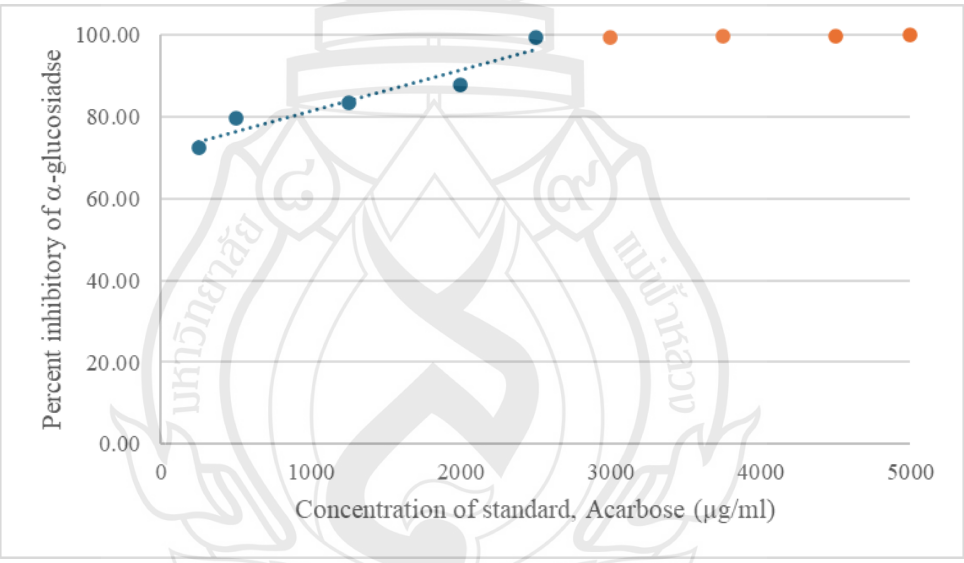


Figure 4.4 The Graph of Relation between the Percent Inhibitory of α-Glucosidase and the Concentration of Acarbose (µg/ml). $\hat{y} = (-71.448) + 0.01x$, R Square = 0.915

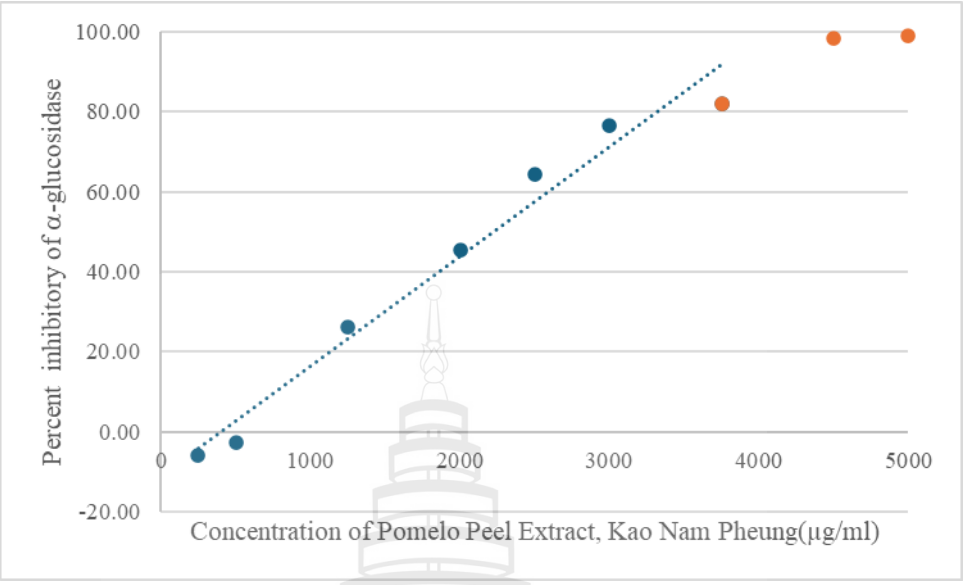


Figure 4.5 The Graph of Relation between the Percent Inhibitory of α-Glucosidase and the Concentration of Kao Nam Pheung (µg/ml). $\hat{y}$ = (-11.128) + 0.0275x, R Square = 0.973

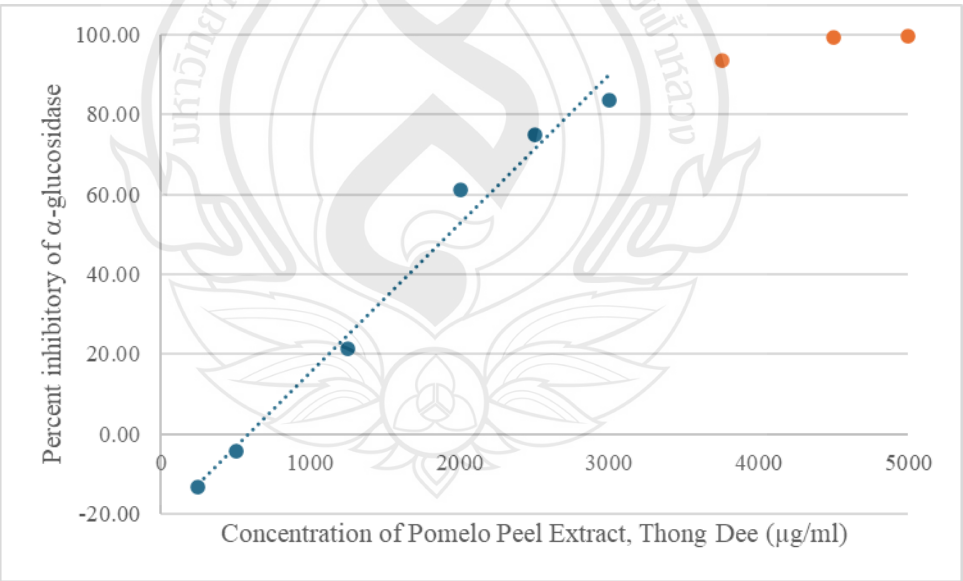


Figure 4.6 The Graph of Relation between the Percent Inhibitory of α-Glucosidase and the Concentration of Thong Dee (µg/ml). $\hat{y}$ = (-21.991) + 0.0374x, R Square = 0.984

Table 4.7 IC_{50} of Experimental Extracts (α-Glucosidase)

Extract	**IC_{50} (μg/ml)**
Acarbose	-2,144.80
Kao Nam Pheung	2,222.84
Thong Dee	1,924.89

CHAPTER 5

DISCUSSION

5.1 Discussion

From the conducted experiment, 2 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 involve in the level of postprandial blood glucose.

The yield of Kao Nam Pheung and Thong Dee peel extract are 4.63% and 4.70% respectively. The main phytochemical compound in this study is flavonoid. Flavonoid is 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 is 5.80 ± 0.96 (mg quercetin/g of crude extract) and Thong Dee peel extract is 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 fed with the same protocol, got the same soil and environment (Makkumrai et al., 2021).

As there are many specific kinds of flavonoid, quercetin is the standard selection on quantitation of flavonoid study (Munir et al., 2024). 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 is 0.244 (mg/g) and Thong Dee peel extract is 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 contains higher predominant flavonoids and bioactive compound than the yellowish one (Yin et al., 2023).

From the study showed the result of inactivation of α-amylase and α-glucosidase by calculating %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 4.4. 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 shown in Table 4.6, 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).

Secondly, Acarbose, Kao Nam Pheung and Thong Dee can also inhibit α-glucosidase activity as shown in Table 4.6. 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} as shown in Table 4.7, 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 showed the ability to inhibit digestive enzymes in vitro. However, in comparison to the conventional drug acarbose that used as enzymes inhibitor, both extracts showed lower efficacy. Among antidiabetic drugs, Acarbose is currently used because of its strong α-glucosidase inhibitory effect. However it proved by FDA, the adverse effect or contraindication is yet under concern (Alssema et al., 2021). Thai pomelo peel extract is one of the citric peel fruits that can be improved and need to be studied furthermore.

5.2 Suggestion

5.2.1 Suggestion in studying further in vivo or clinical trial phase

5.2.2 Suggestion in collection and preservation method of raw pomelo peels

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APPENDIX

APPENDIX

BUDGETS

1.1 Pomelo Peel	0 THB
1.2 Ethanolic Extraction	2400 THB
1.3 Total Flavonoid Content Assay	3000 THB
1.4 The HPLC Test	3600 THB
1.5 Enzyme Inactivation Test	6000 THB

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