CHAPTER VI
DISCUSSION

6.1 Antioxidant Activities

Medicinal plants are the richest bioactive compounds that function as nutraceuticals and chemical entities for synthetic drugs. The extracted contents of bioactive compounds in plants can be differentiated under different extraction processes. The parameters influencing the extracted quality and quantity are extraction procedures, solvent systems and plant materials. Even though many studies have been focused on the antioxidant property of tea extracts, little information regarding antioxidant activity of herbal tea and its comparison with conventional teas was available. Thus, the aim of this part of the project was to optimize and investigated the recovery yield of extracted substances at the highest quality and quantity regarding their antioxidative property using conventional teas and Thai herbal teas from different plant parts including fruits, leaves, flowers, stems and roots.

6.1.1 Optimized extraction conditions

Extraction process is widely employed as a separation procedure to obtain bioactive compounds from plant materials. In fact, different plants may require particular extraction conditions to achieve maximum recovery yield of bioactive compounds. However, several factors such as detecting procedure, type of extraction solvent and particle size of medicinal plants could influence the rate of extraction and quality of extracted bioactive compounds.

Antioxidant detecting procedure is significant factor that impacts reliability of the verified antioxidant capacity. Based on their functions, antioxidants are classified as primary (or chain breaking) and secondary (or synergists) compounds. Primary antioxidants are able to inhibit chain reactions by acting as hydrogen donors or free radical acceptors. On the other hand, the action mechanism of the secondary antioxidants is based on chelating property of prooxidant and free metals. Various

methods have been employed to evaluate the antioxidant capacity *in vitro*. The assays can be classified into two categories based on the reaction mechanism, including HAT and SET reactions. However, due to the complex nature of biological systems and the multiple reaction characteristic, no individual assay for measuring antioxidant capacity will accurately reflect all radical sources and antioxidants [32]. Thus, in this study, four different methods including ORAC assay as HAT mechanism as well as FRAP, DPPH radical scavenging and Folin–Ciocalteu assays as SET mechanisms were employed to investigate antioxidant capacity. These methods measure the ability of primary antioxidants.

The simple and rapid DPPH radical scavenging assay was first attempted to screen antioxidant activity of tea extracts. This method measures the loss of DPPH free radicals after reacting with antioxidants. However, DPPH radicals are artificially generated, thus antioxidants may not reflect their real property with lipid radicals [108]. ORAC and FRAP assays were then performed to further confirm the results. ORAC assay uses a peroxyl radical, which is the most common radical in food and biological systems, to conduct the reaction under physiological condition. Thus, it is believed that this method resembles biological relevant. Besides, this assay can be adapted to detect both hydrophilic and hydrophobic antioxidants. On the other hand, FRAP assay is simple, quick, less labor and does not required specialized equipment. However, it cannot detect any compounds that possess radical quenching property (by hydrogen atom transfer), particularly thiols such as glutathione and protein [32].

Additionally, phenolic compounds are excellent natural antioxidants, and the most commonly detecting method for determining total phenolic contents is Folin–Ciocalteu assay. This method is simple, sensitive and precise; however, it is not applicable for lipophilic and non–phenolic substances, which can interfere with the assay. Thus, this method can lead to overestimation of total phenolic compounds [32].

Other factors that could influence the rate of extraction and quality of extracted bioactive compounds are type of solvent and solvent concentration. Selected extraction solvents play critical roles for optimizing particular bioactive compounds. Various solvents are used in the extraction procedures such as water, alcohol (ethanol and methanol), acetone, chloroform and ether. Nevertheless, water is the solvent in a fundamental condition for making tea infusion, while ethanol is the commonly organic

solvent to extract antioxidant compounds from various plants. Water is universal solvent that can be used to dissolve water soluble substances such as anthocyanins, starches, tannins, saponins, terpenoids, polypeptides and lectins. Ethanol, on the other hand, is a versatile solvent that can be used to dissolve both polar and non–polar substances such as tannins, polyphenols, polyacetylenes, flavonols, terpeniods, sterols and alkaloids. Nevertheless, both solvents are commonly employed due to the safety of finished products that can be used internally by consumers [109].

Additionally, antioxidant of plant extracts depends on aqueous ethanol concentrations used in the extraction process. The effects of different extraction solvents of all conventional teas and Thai herbal teas suggested that antioxidant activities were optimized under the range of 30–70% (v/v) aqueous ethanol. According to these results, the extraction solvent of 50% (v/v) aqueous ethanol was chosen to compare antioxidant activity between herbal teas and conventional teas. It was previously found that various extraction conditions could provide a variety of measurable concentrations of antioxidants from teas [4–6]. Recent studies had shown that 40% (v/v) aqueous ethanol was the most effective solvent system for extraction of catechins in white and green teas, comparing to tea extracts with 0, 10 and 75% (v/v) aqueous ethanol [110]. Besides, antioxidant activity of black tea extracted with 50% (v/v) aqueous ethanol was higher than those under 80% (v/v) aqueous ethanol extraction [111]. This solvent system (50% (v/v) aqueous ethanol) was also employed to extract high quantity of antioxidant compounds from green tea and black tea [112]. Thus, it could be suggested that different polarities in each solvent contribute to the efficiency of antioxidant extraction. It was found that polarity index values of 0, 25, 50, 75 and 100% (v/v) aqueous ethanol are 9.0, 8.1, 7.1, 6.2 and 5.2, respectively [113]. Thus, the extracts with difference ethanol concentrations likely contain particular bioactive compounds with difference polarity. As results, the aqueous mixture of ethanol is a better solvent than absolute ethanol or pure water for extracting both antioxidants and phenolics from teas.

Phenolics are the most abundant antioxidants in plants as well as in beverages such as tea, coffee and wine. These compounds are generally soluble in organic solvent with less polarity than water. Numerous of natural phenolics are currently reported to be biosynthesized in plant sources. Dietary phenolics are

classified into three main groups: phenolic acids, flavonoids and tannin. These compounds, especially flavonoids and their derivatives, are commonly phenolics founds in most plants as well as tea leaves. As well, they are capable of solubilizing in water and can be easily extracted with polar solvents such as aqueous ethanol. However, this is not the case for tannins, which bind to proteins upon cell disruption during the extraction. Thus, it may be presumed that flavonoids (especially catechins), phenolic acid and tannin (such as tannic acid) are the antioxidant compounds, which obtained from tea extracts under both water and aqueous ethanol solvent.

6.1.2 Antioxidant properties of aqueous ethanol extracted teas

Under the optimized solvent system (50% (v/v) aqueous ethanol), the antioxidant activities of all conventional teas indicated that green tea exhibited the highest antioxidant activity, followed by white tea, oolong tea, pu–erh tea and black tea, respectively. These results were corresponded to the previous studies, in which green tea was found to possess stronger antioxidant properties than oolong tea and black teas, as being detected by DPPH, FRAP, ORAC, ABTS assays [10, 114, 115]. Besides, it was found that the main chemical constituents of green tea are catechins, a monomeric form of polyphenols with an effective antioxidant property [33]. The potent antioxidant activities of catechins in green tea are due to their three adjacent hydroxyl (–OH) groups on the β–ring as in EGCG and EGC. These compounds are more effective as scavengers toward free radicals than ECG and EC, which contain two adjacent hydroxyl groups. Besides, the contents of EGCG and EGC in green tea is greatly higher than those in oolong and black teas [116], which contains thearubigins and theaflavins as major antioxidants [20]. Pu–erh tea that passes through fully fermented process is found to contain lower concentrations of tea polyphenols and tea catechins than other types of tea [17]. Thus, it could be suggested that the process of tea fermentation (oxidation) could cause a decrease in antioxidant activities.

As for herbal teas, the results suggested that stevia, Indian gooseberry and cat's whisker herbal teas exhibited the highest antioxidant activity. These herbal teas even provided comparable antioxidant activities to those of conventional teas. Ethanolic leaf extract of stevia possess a potential as a significant source of natural antioxidant agent [117]. Major phenolic compounds in stevia are steviol glycosides

(e.g., stevioside and rebaudioside A) (Figure 6.1A, B) [118]. These compounds may attribute the strong antioxidant properties in stevia tea due to their hydroxyl (OH) groups. Likewise, Indian gooseberry is rich in phenolic compounds that acted effectively as the antioxidant agents. It has been reported that gallic acid and tannic acid are the major phenolic acids of Indian gooseberry as being extracted with 70% (v/v) aqueous ethanol [119]. Additionally, Indian gooseberry contains high levels of ascorbic acid (445 mg/100 g of dry fruit), which is widely known for its antioxidant activity [120, 121]. In addition, cat's whisker is also claimed to possess antioxidant activity. It was found that rosmarinic acid, a caffeic acid derivative, is a major component responsible for the antioxidant activity of cat's whisker extract [122]. This compound has ability to eliminate hydrogen peroxide (H_2O_2) and sequester free radicals through the hydroxyl (OH) groups in its structure (Figure 6.1C) [123].

The comparison of antioxidant capacity and total phenolic contents in conventional teas and Thai herbal teas suggested that the level of phenolic compounds in conventional teas were generally higher than those of Thai herbal teas. Since phenolic compounds are phytochemicals that play a major role in the protection of oxidation reaction, antioxidant activity of many plants has been attributed to the presence of phenolic compounds. Antioxidant activities of conventional teas were consistent with the total phenolic contents, in which green tea exhibited the highest quantity of phenolic compounds, followed by white tea, oolong tea, black tea and pu–erh tea, respectively. As well, Indian gooseberry exhibited the highest quantity of phenolic compounds among fifteen Thai herbal teas, followed by stevia, beal fruit and cat's whisker. Thus, it can be concluded that tea samples possess relatively high antioxidant activity due to contribution of phenolic compounds. Additionally, ethanolic extract of stevia, Indian gooseberry and cat's whisker herbal teas can be used as an accessible source of natural antioxidants with consequent health benefits. These results are supportive information for further development in healthy food supplement like commercial EGCG from green tea extract.

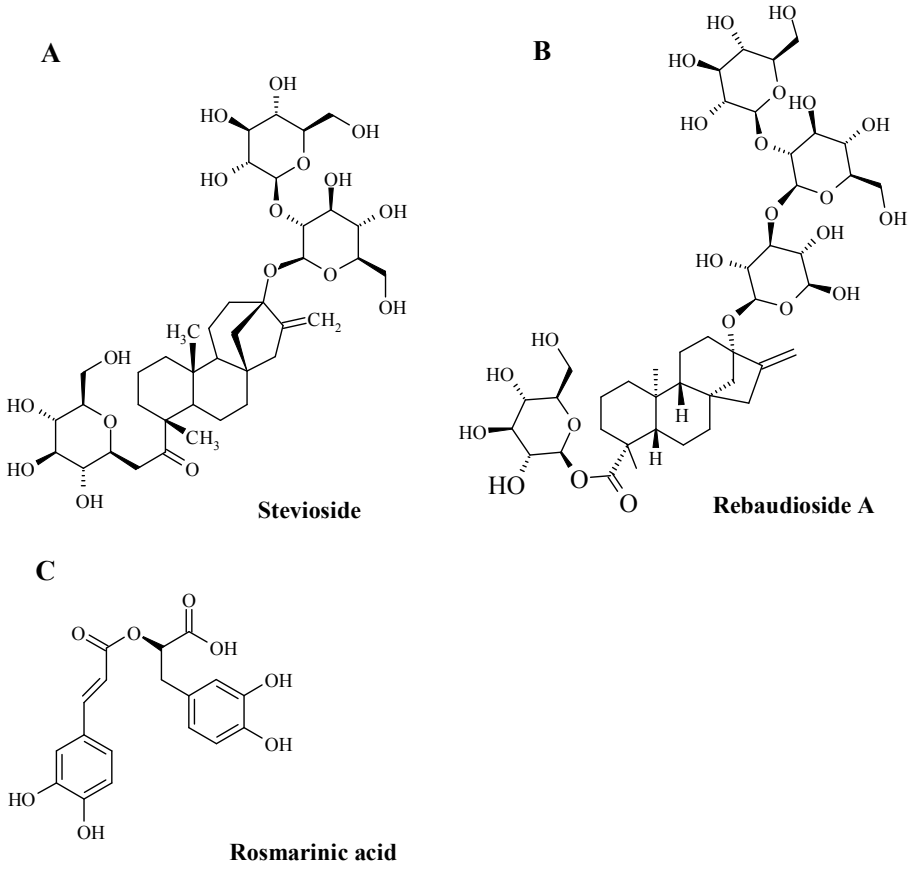

Figure 6.1 The chemical structures of (A) stevioside, (B) rebaudioside A and (C) rosmarinic acid. Stevioside and rebaudioside A are main component of steviol glycosides that extracted from the leave of stevia. Rosmarinic acid is the compound that found in the leaves of cat's whisker extracts.

6.1.3 Antioxidant properties of tea infusions

The antioxidant properties of tea infusions were investigated in order to determine the type of tea regarding its antioxidative effect toward tea consumers. It was found that the antioxidant activity of tea extracted with 50% (v/v) aqueous ethanol was significantly higher than those of tea infusions, suggesting that a binary solvent system (aqueous ethanol) is more suitable than a mono–solvent system (water) in the extraction of phenolic compounds from teas. The different polarities of solvents seemed to be a significant contribution toward the quantity of extracted antioxidants from teas. However, the yield of phenolic compounds in water extraction was increased by increasing of extraction temperature and time. [124]. Thus, it was

possibly suggested that extraction temperature and time could affect the quantity of extracted antioxidants.

The antioxidant activities of all conventional tea infusions were consistent to the results of tea extracted with 50% (v/v) aqueous ethanol with green tea being the richest source of antioxidants followed by white tea, oolong tea~pu–eh tea and black tea, respectively. These results suggested that tea fermentation could influence the quantity of extracted antioxidants in both water and aqueous ethanol solvent systems. Likewise, the results of herbal tea infusion indicated that only antioxidant activities of Indian gooseberry, stevia and cat's whisker herbal teas were comparable to those of conventional teas, whereas bitter cucumber herbal tea exhibited the lowest antioxidant activity. These results were corresponded to the previous investigation on the comparison of conventional teas and some herbal teas [10, 125]. It was found that the antioxidant activity of stevia herbal tea was higher than those of oolong tea and black tea [10, 125]. Besides, stevia herbal tea was found to exhibit higher antioxidant activity than those of cat's whisker, roselle, safflower, mulberry lemon grass, bitter cucumber, jewel vine and ginger herbal teas [10, 11, 125]. Likewise, Indian gooseberry herbal tea was reported to exhibit strong antioxidant activity, which was even higher than that of stevia herbal tea [11].

Antioxidant activities of these tea infusions were related to the presence of phenolic compounds. These results were consistent to the result of tea extracted with aqueous ethanol. Thus, it can be concluded that the phenolic compounds were the major antioxidant compositions under studied conditions. This result was consistent with the previous study, which reported the positive correlation between total phenolic content and antioxidant activity of tea extract [10–12, 125]. Total phenolic contents of conventional teas were also generally higher than those of Thai herbal tea with the exception of Indian gooseberry, stevia and cat's whisker herbal teas. Interestingly, these herbal teas contained even higher phenolic compounds than those of conventional teas. Thus, stevia, Indian gooseberry and cat's whisker herbal teas were classified into a group of high phenolic contents with high antioxidant capacities. These herbal teas can be used as a potential food beverage with antioxidants properties.

As in dietetic perspective, an average daily intake of polyphenols with the diet is reported to be approximately 1 g, which should be sufficient to destroy free radicals in human body [126]. The main polyphenol dietary sources are fruit, vegetables and beverages. Following the recommendation to consume five servings of fruits and vegetables daily, it would result in a total polyphenols intake of >500 mg [127]. However, it also depends on food choice, which could be much higher. Daily consumption of a cup of green tea could easily increase polyphenols intake by 50–100 mg [128]. In this study, tea infusions from stevia, Indian gooseberry and cat's whisker herbal teas showed higher phenolics than those of conventional teas. Thus, daily consumption of these herbal tea infusions could increase the quantity of polyphenols to the level of targeted intake in addition to fruit and vegetable consumption. In addition, for individual who suffers from the side effect of caffeine, some herbal teas may be a safer beverage than those of conventional teas (approx. 11–28 mg/g of dry matter) [129].

6.2 Anti–glycation Activity

Glycation is an oxidative stress related glycosylation process. It is a non–enzymatic reaction between amino groups of protein or lipid molecules with reducing agents such as sugar, resulting in the formation of AGEs. AGEs has been shown to cause oxidative stress in various cell types and also implicate in the progression of diabetes complication and many age–related diseases. Thus, medicinal plants or natural products with combined antioxidant and anti–glycation properties are highly desired, because they act more effective toward treatment of various disorders. Interestingly, some teas possess not only antioxidant capacity but also anti–glycation properties.

Based on previous studies, it had been implied that the efficiency of anti–AGEs formation was depended on the capacities of primary antioxidants and quantity of phenolics [10, 130–132]. Because of this relationship, it was hypothesized that the results of glycation would follow the same trend as antioxidant capacities and total phenolic content. Thus, only tea infusions were screened for potential anti–glycation activity, because it was in a form of daily life consumption. The anti–glycation activity

was evaluated according to the ability to fight against the formation of fluorescent AGEs in the BSA/glucose and BSA/MG systems. Since BSA/glucose systems provided higher detection of anti–glycation capacity than BSA/MG system in all cases, it was suggesting that glucose is an effective reducing agent that can induce AGEs formation with a higher rate than MG.

Based on our experimental results, green tea and white tea exhibited the most potent anti–glycation capacities among five conventional teas. This result was corresponded to the previous studies, which indicated that green tea exhibited higher inhibitory activity against the formation of AGEs than those of oolong tea and black tea [10]. However, the anti–glycation activity of white tea and pu–erh tea has been rarely reported. Based on our experimental results, the anti–glycation activity of conventional teas was depended on the phenolic contents and their antioxidant activity, in which white tea and green tea showed the highest quantity. Thus, it could be suggested that fermentation processes of tea could influence the quantity of phenolics with antioxidant properties, and thus function against glycation formation.

The anti–glycation capacities of conventional teas were generally higher than those of herbal teas in exception of stevia, Indian gooseberry and cat's whisker. These herbal tea infusions possessed inhibitory activities that were even higher than those of green tea and white tea. According to previous studies, infusion from stevia was found to exhibit stronger anti–glycation ability than those of black tea and oolong tea; however, its activity did not exceed that of green tea [10]. However, our experimental results suggested that the level of phenolic compounds of stevia infusion was higher than those of conventional teas, which supports the highest inhibitory activity of stevia. Thus, our experimental results were consistent to the previous studies, in which it was demonstrated that the anti–glycation activity is strongly correlates to the phenolic contents [2, 131, 132]. Thus, plants with high phenolics may possess the potential function against glycation. However, several tea infusions such as bael fruit, bitter cucumber, white mulberry, Asiatic pennywort, pandanus, jiaogulan, lemon grass, jewel vine and ginger occupied low anti–glycation activity, which may be due to low phenolic compounds as being observed in the Folin–Ciocalteu experiment.

It was found that phenolic compounds, particularly flavonoids, are responsible for the anti–glycation activity of herbal infusions [2, 10]. Likewise, tannin, an abundant phenolic compound found only in conventional tea, has been reported as the main compound that is responsible for the anti–glycation activity in green tea [43]. These compounds may retard the process of AGEs formation by preventing further oxidation of Amadori products and metal–catalyzed glucose oxidation. Thus, AGEs are believed to participate in the pathogenesis of diabetes and diabetes complication. Base on the antioxidant and anti–glycation capacities, it may be suggested that the consumption of herbal infusions such as stevia, Indian gooseberry and cat's whisker may serve as alternative health beverages for prevention of oxidative stress as well as diabetes and diabetic complications.

Recent study reported that a single dose of green tea catechin extract (500 mg/day) is equivalent to drinking six cups of green tea per day, which improved the plasma antioxidant capacity of healthy adults [133]. As well, it may support the prevention of AGEs formation in the body. Therefore, consumption of six cups (or less) of stevia, Indian gooseberry and cat's whisker herbal infusion may be help to prevent oxidative stress as well AGEs formation as those of green tea.

6.3 Lipase Inhibitory Activity

Obesity is the result of an energy imbalance, which focuses on dietary fat intake that is over–emphasized at the expense of total energy intake. Pancreatic lipase is a key enzyme for fat digestion and absorption. Thus, the reduced utilization of ingested fat though the inhibition of lipase results in caloric deficit and possibly yields a positive effect on weight control. The effects of tea on obesity have continuingly received increasing attention in attempt to develop healthy beverage and investigate possible anti–obesity agents from natural sources. The mechanisms of actions may be related to particular pathways such as the modulations of energy balance, appetite suppression and nutrients metabolism. Nevertheless, the lipase inhibitory activities of conventional teas and Thai herbal teas are currently not well established and, thus, leading to incomplete information. Besides, little investigation on the comparison of lipase inhibitory activities of conventional and herbal tea extracts under the same

extraction conditions is available. Therefore, in this study, all tea samples that extracted with aqueous ethanol and tea infusions were screened and compared their potential lipase inhibitory activity in order to provide supportive evidence of the fundamental knowledge to promote the usage of these conventional and herbal teas as the potential choices of healthy beverages for overweight and obesity patients.

6.3.1 Lipase inhibitory properties of aqueous ethanol extracted teas

Amongst five conventional teas, white tea showed the highest inhibitory activity, followed by green tea~oolong tea, pu–erh tea and black tea, respectively. These results were consistent to prior study, which suggested that white tea possessed stronger lipase inhibitory activity than those of green tea and black tea [7]. It is highly possible that the different phenolic composition between types of tea during the fermentation processes could affect the lipase inhibitory activity. Phenolic compounds in tea have been suggested to possess anti–obesity thought the influence of lipid digestion. Besides, these compounds may interfere or inhibit the emulsification, hydrolysis and micellization of lipids, resulting in reduced overall uptake [134]. Tea catechins, especially EGCG in green tea (Figure 6.2A), have also been confirmed to acts as lipase inhibitors [4, 135]. Catechins in green tea extract directly inhibit gastric and pancreatic lipases as well as stimulation of thermogenesis *in vitro* [5]. It was also found that after 3 months of 375 mg catechin consumption, body weight and waist circumference were decreased by 4.6% and 4.48%, respectively [5]. These results suggested that green tea extract could be the potentially natural source for obesity treatment *via* its inhibitory activity toward lipases as well as stimulation of thermogenesis.

Additionally, strictinin (Figure 6.2B), a hydrolyzed tannin found in oolong tea, could effectively inhibit lipase even at low quantity (IC_{50} = 0.472 μM) [136]. A previous study also suggested that strictinin in green tea and white tea is a significant bioactive compound that functions against lipase enzyme [7]. This study also suggested that teas with high quantity of strictinin could increase inhibition of lipase and act synergistically with catechins to increase lipid–lowering effect.

Furthermore, recent study has reported that pu–erh tea may prevent obesity by functioning against the activity of pancreatic lipase through gallic acid (Figure 6.2C) [137]. However, the inhibitory activity of pu–erh tea is unlikely contributed from the acts of gallic acid alone. Synergistic activity with other components such as catechins and polymerized flavonoids was also suggested. These compound complexes might decrease absorption of fat and/or increase the stability of gallic acid [137]. Nevertheless, the results suggested that consumption of pu–erh tea may provide beneficial to consumers regarding their preventive function against obesity similarly to the consumption of green tea, white tea and oolong tea.

On the other hand, low lipase inhibitory activity in black tea might be furnished from low concentration of tea catechin contents. The major active component of lipase inhibitor in black tea is theaflavins [138]. It has been suggested that a benzotropolone nucleus in theaflavins (Figure 6.2D) was important to the activity [136]. Although black tea showed only little lipase inhibitory activity [7], it could suppress intestinal lipid absorption and body weight gain in mice fed with a high fat diet [138].

According to lipase inhibitory activity of herbal teas, ginger exhibited the highest lipase inhibitory activity among fifteen herbal teas, followed by cat's whisker, chrysanthemum, jewel vine, Asiatic pennywort and stevia, respectively. These herbal teas were found to exhibit a potential lipase inhibitory activity that may be affected by the presence of their polyphenolic compounds, especially catechin, epicatechin and quercetin [13]. Among these herbs extract, only the ethanolic extract of chrysanthemum has been previously reported to exhibit lipase inhibitory activity (IC_{50} >100 μg/mL) [139]. However, animal based model studies had previously demonstrated that Thai herbs are potential dietary sources of bioactive components with medicinal properties such as anti–obesity (as hypolipidemic agent). Ethanolic extract of ginger had showed the hypolipidemic effect in cholesterol–fed rabbits [140]. Besides, it significantly reduced total serum cholesterol and triglycerides as well as increased high density lipoprotein–cholesterol levels in diabetic rats [141]. Daily administration of cat's whisker and Asiatic pennywort aqueous ethanol extracts had also been reported to significantly decrease triglyceride level in blood of rats [142, 143]. Consumption of stevia extract was found to significantly reduce the levels of

cholesterol, triglyceride, low density lipoprotein–cholesterol in human with hypercholesterolemia [144].

In addition to these *in vivo* experiments, it can be suggested that all conventional teas and some herbal teas such as ginger, cat's whisker, chrysanthemum, jewel vine, Asiatic pennywort and stevia may provide health benefit toward prevention of obesity and hyperlipidemia. The mechanisms of actions may be related to certain pathways such as thermogenesis and inhibition of lipase enzyme. Thus, these teas may be useful for further development into dietary supplement and medication with potential usefulness in preventing obesity and hyperlipidemia.

Based on our experimental results, conventional teas with the IC_{50} of 0.06–0.36 mg/mL were the most effective toward lipase inhibitory activity. Nevertheless, these IC_{50} values were 500–3000 folds higher than that of orlistat (0.24 μM or 0.12 μg/mL) as being investigated under similar experimental conditions. Orlistat is a weight–controlled drug for management of obesity. It can act as a gastrointestinal lipase inhibitor that competes with dietary fats for the active sites on the lipase enzyme. Orlistat is capable of preventing the absorption up to 30% of dietary fat at a therapeutic oral dose of 120 mg, three times a day [64]. However, several studies have reported about undesirable side effects of this drug such as flatus with discharge, oily stool, abdominal pain and malabsorption of fat soluble vitamin [65]. Thus, the development of natural products for obesity treatment with low side effect is significant matter. This study investigated tea extracts that may, however, contain a mixture of many different chemicals. Thus, the isolation of pure compounds was needed to investigate for the further study. It is possible that, pure compounds of tea extracts could effectively inhibit lipase even at low quantity, which may be useful for further development medication that acts as lipase inhibitor with fewer side effects than orlistat.

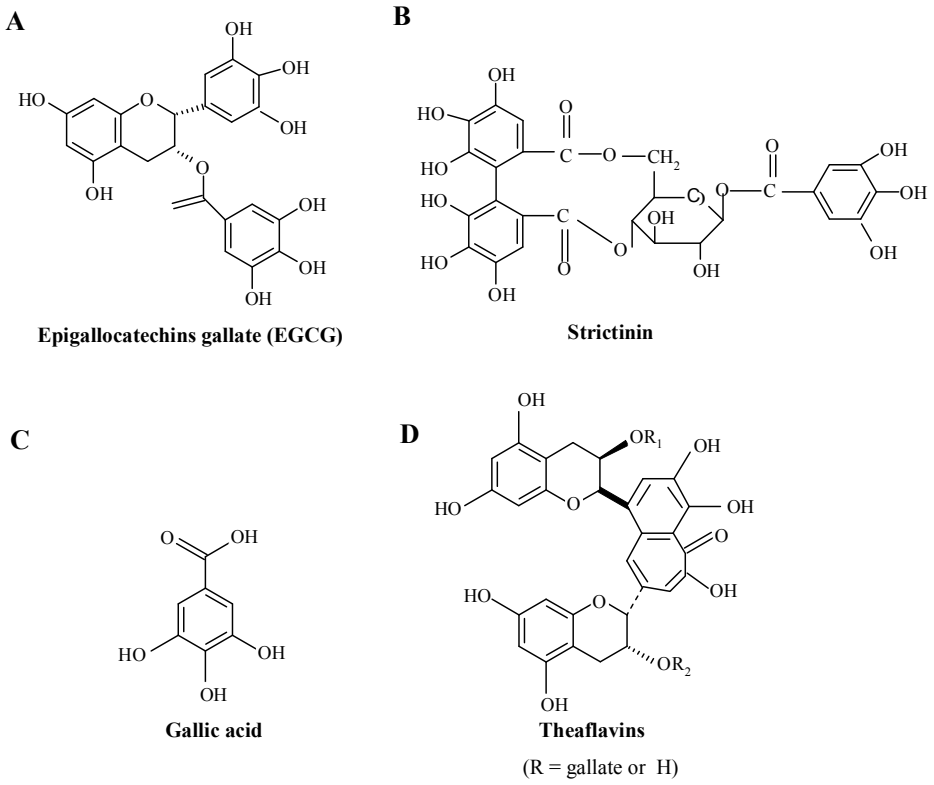

Figure 6.2 The chemical structures of (A) EGCG, (B) strictinin, (C) gallic acid and (D) theaflavins. These compounds are found in tea leaves (*C. sinensis*).

6.3.2 Lipase inhibitory properties of tea infusions

The results suggested that the level of lipase inhibitory activity of tea extracted with 50% (v/v) aqueous ethanol was significantly higher than those of tea infusions. Thus, the compounds that act as lipase inhibitors in these teas may be more soluble in aqueous ethanol than in water. According the structure of possible anti–lipase agents, which are polyphenols, it could be confirmed that aqueous ethanol is more appropriate for the extraction of phenolic compounds from teas than water.

The lipase inhibitory activities of five conventional tea infusions were generally higher than those of Thai herbal teas. White tea showed the highest inhibitory activity, followed by green tea, oolong tea, pu–erh tea and black tea, respectively. These results followed a similar trend as teas extracted with 50% (v/v) aqueous ethanol and were also consistent to prior study [7]. Several previous studies reported that tea from *C. sinensis* can inhibit pancreatic lipase activity; however, IC_{50}

values of these teas were found to be varied [7, 138]. The rationale behind these variable results are unclear but could be related to differences in plant sources, enzymes origins, substrate types and extraction processes under particular studies.

Nevertheless, the results of herbal tea infusion indicated that among fifteen herbal teas, stevia and cat's whisker exhibited the highest lipase inhibitory activities, while ginger, which exhibited the highest anti–lipase activity as being detected under aqueous ethanol extraction, showed only little activity. It is highly possible that the major anti–lipase agents in ginger herbal tea may potentially solubilize in aqueous ethanol rather than in pure water. Previous study had demonstrated that several herbal tea infusions that exhibited lipase inhibitory activity including white mulberry > cat's whisker~Asiatic pennywort > stevia~safflower~jialgulan, respectively [13]. These results, however, are in contrast to our studies, in which the inhibitory activities of stevia > cat's whisker > Asiatic pennywort~jialgulan > safflower, respectively. Importantly, the inhibitory activities of these teas were generally less than the previous study, which may be due to the extraction process. The previous study was performed under a long incubation time for making tea infusion (2 hours in DI water at 90 °C) [13]. It could be explained in term of solubility of the bioactive compounds in some plants, which is time–dependent manner. However, the tea infusions in our experiments were prepared to imitate the process of daily tea making (5 minutes in DI water at 95 °C), thus the functions against lipase reaction were more applicable to daily lifestyle.

Therefore, a daily consumption of some herbal tea infusions such as stevia and cat's whisker herbal teas may be healthful for alternative prevention and treatment of obesity and hyperlipidemia. However, the clinical trials regarding daily consumption of these herbal teas are necessary to investigate for the supportive knowledge to promote the usage of these herbal teas as the healthy beverages for overweight and obesity patients.

6.4 ACE Inhibitory Activity

ACE is the key enzyme in a regulation of RAAS, the key mechanism in a body concerning a regulation of blood pressure, fluid and electrolyte balance. ACE cleaves AngI to produce AngII and also hydrolyzes and inactivates the vasodilator peptide, bradykinin. Thus, ACE inhibition is likely a target for antihypertensive agents and a development of natural products for alternative treatment of hypertension. In this study, all of tea samples extracted with aqueous ethanol and tea infusions were screened for potential ACE inhibitory activity.

ACE inhibitory activity was evaluated using Hip–His–Leu as a substrate. The activity was based on substrate hydrolysis and fluorescence measurement of *ortho*–phthaldialdehyde–His–Leu adduct. This method can be applied to directly quantify ACE inhibitory activity and IC_{50} values in biological samples that provide potential ACE inhibitors. However, this technique has a limitation of being laborious and costly. Thus, this study, ACE inhibitory activity was expressed as the percentage of inhibition under the same concentration of tea extracts.

6.4.1 ACE inhibitory properties of aqueous ethanol extracted teas

Many studies had investigated the properties of tea on the inhibition of ACE both *in vivo* and *in vitro*. It has been suggested that the *in vitro* ACE inhibitory activity of tea is probably due to their phenolic compounds. Since it was previously suggested that tea processing method defines different categories of tea as well as their chemical compositions [1], and in this study, five conventional teas extracted with aqueous ethanol showed the different quantity of total phenolic compounds, their functions against ACE reaction should be varied. However, according to the results of aqueous ethanol extract in this study, five conventional teas exhibited the equivalence effective ACE inhibitory activities. It was possible that the concentration of tea extracts (0.835 mg/mL) was too high under studied conditions or the detection time (20 min) were too long, thus causing the enzymatic reaction to almost reach its maximum velocity (V_{max}). At this stage of the reaction, the enzyme concentration is limited and could not convert any substrates into products. Thus, all inhibitory reactions would reach the same V_{max}, and the inhibitory activity could not be differentiated.

As for the results of herbal teas, among fifteen Thai herbal teas, safflower, stevia and cat's whisker herbal tea showed the highest ACE inhibitory activities, which were similar to the quantity of ACE inhibitory activities from conventional teas. Laboratory studies had previously demonstrated that the extract of safflower, stevia and cat's whisker possessed potential hypotensive properties. Daily administration of safflower extract for five months showed the significantly reduced blood pressure as well as decreased plasma renin activity and AngII level of spontaneous hypertensive rats [145]. These results suggested that the decreased blood pressure is mediated by the renin–angiotensin system. In addition, many studies confirmed the antihypertensive properties of crude stevioside obtained from the leaves of stevia in hypertensive rats [146, 147]. It was also found that after three months and two years of stevioside consumption could significantly lower blood pressure in human [148]. Although the mechanism underlying the antihypertensive effect of stevioside remains unclear, it has been demonstrated that this effect appears to occur through a calcium antagonist mechanism similarly to that of verapamil, a calcium channel blocker [149]. Additionally, cat's whiskers leaves extract exhibited blood pressure–lowering effect in rats [150, 151] *via* the mechanism of vasodilating action, a decrease in cardiac output and diuretic action [150]. Interestingly, chrysanthemum herbal tea with secondly high anti–ACE activity has been frequently used in traditional Chinese medicine to treat hypertension; however, no scientific evidence is reported.

Thus, it can be suggested that the hypotensive effects of safflower, stevia, cat's whisker and chrysanthemum herbal teas may occur through the inhibition of ACE reaction in addition to other possible mechanisms as suggested previously. It is supposed that these plants may be useful for further development into dietary supplement and medication with potential preventing of hypertension.

6.4.2 ACE inhibitory properties of tea infusions

Among five conventional tea infusions, green tea and white tea exhibited the highest ACE inhibitoty activities, followed by oolong tea, black tea and pu–erh tea, respectively. These results were corresponded to the previously study, where it was found that the ACE inhibitory activity of teas was affected by tea processing method and associated with relative abundance of un–oxidized catechins, catechin polymers

and oxidized products [152]. Under the same study, it was also found that green tea exhibited ACE inhibitory activity through the mechanism of substrate–dependent pathway and predominantly direct enzyme inactivation. On the other hand, the mechanism of black tea was exclusively *via* enzyme inactivation [152].

The antihypertensive effects of tea infusions possess several potential biological mechanisms. EGCG in green tea inhibits human platelet aggregation *in vitro* [153] and also activates endothelial nitric oxide synthase as well as increases production of vasodilator nitric oxide *in vitro* [154]. In addition, flavanols in green tea and black tea can inhibit ACE activity in human endothelial cells and also increase nitric oxide concentration [8]. These mechanisms are involved in a decrease in blood pressure. As well, several animal and human studies also reported the relation between tea consumption and blood pressure. Black tea and green tea polyphenols attenuate blood pressure that increases through their antioxidant properties in stroke–prone spontaneously hypertensive rats [155]. Furthermore, the quantity of polyphenols used in this experiment was corresponded to those in ~1 L of tea, thus it may also provide protection against hypertension in humans. In addition, habitual consumption of green tea or oolong tea (120 mL/day or more) for 1 year significantly reduces the risk of developing hypertension in Chinese population [96]. Short term (two hours after consumption) and long term (daily consumption for 4 weeks) consumptions of black tea improved endothelium–dependent flow–mediated dilation of brachial artery in patients with coronary artery disease [156]. Additionally, oral intake of a single dose (400 mL) of green tea significantly inhibited ACE activity in healthy volunteers [97]. These studies suggested that conventional teas may have a potential to prevent cardiovascular disease, especially hypertension through inhibition of ACE activity in addition to improved vasodilator function of arteries.

According to the results of herbal teas, tea infusions from stevia, cat's whisker and Asiatic pennywort showed the highest inhibitory activity among fifteen Thai herbal teas, and the inhibitory activity was similar to those of conventional teas. These results were consistent to the results of aqueous ethanol extracted teas. On the other hand, infusions from safflower and chrysanthemum showed only little ACE inhibitory activity. Nevertheless, little information regarding ACE inhibitory activities of Thai herbal tea infusion is available, and the comparison of the inhibitory activities

among different herbal tea infusions under the same extraction conditions was not reported.

Interestingly, Asiatic pennywort possessed the potent ACE inhibitory activity in addition to safflower, stevia, cat's whisker and chrysanthemum herbal teas (from the results of aqueous ethanol extract). This finding is consistent with previous studies that have demonstrated that Asiatic pennywort extracts could inhibit ACE activity *in vitro* [100]. Previous study has been reported the relation of Asiatic pennywort and blood pressure, in which oral administration of Asiatic pennywort juice could decrease systolic blood pressure and heart rate as well as improve local blood flow in hypertensive rats [157]. These results suggested that the plant may prove useful in treatment of cardiovascular disorders, especially hypertension, and a possible mechanism is through inhibition of ACE.

Therefore, a daily consumption of some herbal tea infusions such as stevia, cat's whisker and Asiatic pennywort herbal teas may promote potential health benefits by reducing high blood pressure *via* the inhibition of ACE.