The comparison between total phenolic content and antioxidant activity of gyokuro, sencha and matcha from some tea store in Chiang Rai and Bangkok

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Abstract

The objective of this study is to compare the total phenolic content and antioxidant activity between gyokuro, sencha and matcha from Chiang Rai and Bangkok. This research is very helpful for customers to choose the best green tea based on scientific studies. The result, sencha samples from Bangkok noted that sencha contains the most total phenolic content (179.07 ± 1.15 mgGAE/1g extract, gyokuro (91.64 ± 0.15 mgGAE/1g extract) and matcha (78.51 ± 0.45 mgGAE/1g extract), and sencha is shown to have the highest antioxidant activity (IC_{50} equal to 0.0077 ± 0.0001 mg/ml), gyokuro (IC_{50} 0.0140 ± 0.0006 mg/ml) and matcha (IC_{50} 0.0191 ± 0.0005 mg/ml). Samples from Chiang Rai noted that sencha contains the most total phenolic content (105.56 ± 0.31 mgGAE/1g extract), matcha (101.08 ± 0.12 mgGAE/1g extract) and gyokuro (72.91 ± 0.07 mgGAE/1g extract). Sencha has the highest antioxidant activity (50% inhibitory concentration (IC_{50}) equal to 0.0084 ± 0.0001 mg/ml), matcha (IC_{50} is equal to 0.00168 ± 0.0005 mg/ml) and gyokuro (IC_{50} is equal to 0.0232 ± 0.0004 mg/ml. Based on the amount of total phenolic content and antioxidant activity, it can be concluded that sencha is the best type of green tea for consumption. Most green tea samples contain higher antioxidant activity than the BHT standard, especially the sencha sample from Bangkok has higher antioxidant activity than the BHT standard by around 2-folds. Many factors are involved in the amount of total phenolic content and antioxidant activity such as sunlight, storage duration, pH value, oxygen, temperature, ingredients in products etc.

Keywords : Camellia sinensis, Green tea, Total phenolic content, Antioxidant activity, DPPH

1. Introduction

Green tea is one of the most popular tea consumed as beverage, and has been known to have many benefits to the human body. Green tea is different from other teas, because its leaves are not fermented like black tea and oolong tea. Instead, green tea is prepared by having its leaves pass through a few steps, starting from the tea leaves being harvested from the *Camellia sinensis* plant, then through the quickly heated process by pan firing or steaming, and finally dried to prevent too much oxidation from occurring that would turn the green leaves brown and alter their fresh-picked flavor. There are many factors that can be reasons why there are many kinds of green tea because it depends on variety of *Camellia sinensis* used, growing conditions, horticultural procedure, manufacturing time of harvest and leaf grades (Zhang *et al*., 2018). There are many brands of beverage with different kinds of green tea products, such as gyokuro, sencha, matcha, etc. All of these types of tea leaves are also called green tea, but each contains different levels of antioxidant, as well as differing levels of antioxidant activity. Thus, from this research we want to determine which kinds of green tea contains the most antioxidant and can be the most effective one for human health benefits.

Green tea is a kind of beverage that many people are interested in because there are a lot of benefits as it contains healthy bioactive compounds. Catechin is available in flavonoid group about 60-70%. Most of catechin includes (-)-epigallocatechin-3-gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG) and (-)-epicatechin (EC) (Khan& Mukhtar, 2018). Green tea is contained with polyphenol antioxidants, including a catechin called Epigallocatechin gallate (EGCG). EGCG is one of the most powerful antioxidant in green tea. *Camellia sinensis* has a lot of beneficial properties such as anticancer, anti-inflammation, antioxidant effects and etc. Anti-oxidation effects of green tea are mainly involved in inhibition of free radicals and lipid peroxidation. The study showed that drinking green tea four glasses per day within 4 months can lead to reduce the urinary levels of 8-hydroxydeoxyguanosine (Musial, Kuban-Jankowska and Gorska-Ponikowska, 2020).

In this research, 3 kinds of popular green tea were chosen based on customer recommendation and preference, namely the gyokuro, sencha and matcha. There are different from its manufacturing and production processes. Gyokuro: the plants are shaded from direct sunlight for approximately 3 weeks before the spring harvest. Once harvested, the leaves are rolled and dried naturally (Gyokuro dewy-colored Japanese green tea, 2019) then sencha: after the sencha leaves are picked, they are steamed immediately and taken away for preparation. Sencha is then dried after the steaming process and, when dried enough, rolled into a variety of shapes until it is completely dried and shriveled (Sencha, 2021). Matcha's manufacturing process differs from gyokuro in that the leaves are not rolled at all. After steaming gyokuro leaves, they are thoroughly dried, then ground into a super-fine powder, and that powder is known as matcha (Matcha, 2021).

In this research green tea samples are taken and from 2 places. First place, the green tea sample were purchased from a tea store in Amphoe Mueang, Chiang Rai province (material green tea were grown in Chiang Rai province). The second place, the green tea samples were purchased from the tea store in Bangkok which are imported from Japan.

2. Objectives

2.1 To compare the amount of total phenolic content of gyokuro, sencha and matcha green tea by using Folin-Ciocalteu

2.2 To compare antioxidant activity of gyokuro, sencha and matcha green tea using DPPH radical scavenging (DPPH method).

3. Materials and methods

This research has based on Rusak G, Sola I & Vujcic Bok V (2021)'s protocol because it is involved in studying matcha and sencha green tea extracts with regards to their phenolics pattern and antioxidant and antidiabetic activity during in vitro digestion, and also it is the latest review. And to find the crude extraction of each type of green tea is based on Hui Ru tan (2019) and L.K. Takao (2014)'s protocol (Hui Ru Tan *et al*., 2018; Takao, Imatomi and Gualtieri, 2015)

3.1 Chemicals and materials

All chemicals and reagents are from (OHAUS Adventurer, USA), (PYREX, Germany), (MARTIN CHRIST, Germany). Enzymes are from SIGMA-ALDRICH, Switzerland), (ACROS ORGANICS, China) and (KEMAUS, Australia). Samples of gyokuro, sencha and matcha are purchased from the same tea stores in Chiang Rai and Bangkok.

3.2 Preparation of green tea extract

Each sample of gyokuro, sencha and matcha is prepared by mixing 10 grams of each sample mix 100 mL of deionized water at 80^{o}C, stirred for 5 minutes then filtered by Whatman No.1 filter paper and vacuum pump unit. Filtered green tea extract is kept in refrigerator at 0^{o}C. Then filtered green tea extract is submitted to freeze-dry for 3-days and wait until all the substance of each extracted green tea is completely dry. Next, the separation process between liquid and each extracted green tea sample has done by rotary evaporator, and wait until liquid from each extracted green tea sample has completely evaporated. Then the crude extraction of each green tea sample is collected.

3.3 Methods for total phenolic content by using Folin-Ciocalteu

1) Prepare Folin-Ciocalteu reagent at a concentration of 10% in 100 mL of distilled water.

2) Prepare Sodium carbonate (Na_2CO_3) at a concentration of 2.5% (w/v) in 100 ml of distilled water

3) Prepare Gallic acid at a concentration of 3 mg/mL in distilled water.

4) Prepare sample at a concentration of 10 mg/mL in distilled water, and diluted at concentration 0.3125 and 0.0781 mg/mL. Dilute the gallic acid and sample by distilled water to be concentrated at 0.3, 0.15, 0.075, 0.0375, 0.0188, 0.0094 and 0.0047 mg/mL then pipette the substance from each concentration in the amount of 20 µL and mix with 100 µL of Folin-Ciocalteu reagent and shake, then keep in the darkness for 5 minutes. Next, add 80 µL of Sodium carbonate anhydrous (Na_2CO_3), shake and keep in the darkness for 20 minutes. Finally, measure the light absorbance by UV- visible spectrophotometer at 760 nm. Each sample is dome triplicate and use distilled water as reference solution.

3.4 Methods for antioxidant activity by using DPPH method

The antioxidant activity of green tea samples is measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH). The DPPH is prepared at concentration of 0.004 g/ 1 mL in EtOH 100 ml. Prepare standard BHT in EtOH (50mg/1mL) and dilution at different concentrations (1, 0.5, 0.1, 0.05 and 0.025 mg/mL). Prepare tea extract in EtOH at different concentrations (1, 0.5, 0.1, 0.05 and 0.0025 mg/mL).

In each concentration, the test will include 1 tube of reference solution and 3 tubes of control solution, 1 tube of blank sample and 3 tubes of sample solution then add DPPH into each tube at different concentrations, shake and keep in the dark for 30 minutes and wait for the reaction to be completed. After that, light absorbance is measured by UV-visible spectrophotometer at 517 nm. All the tests were carried out in triplicate. Then Calculate % inhibition at 50% by comparison with BHT standard solution, then calculate IC_{50}. IC_{50} stands for 50% inhibitory concentration which means concentration value of tested sample that can inhibit 50% of reaction.

% inhibition = [(Abs Control – (Abs Sample – Abs Blank sample))/Abs Control] X 011

3.5 Statistical analysis

All results are evaluated using Program SPSS (MFU) version 21.0. Results gathered from the comparison of the amount of total phenolic content and antioxidant activity in 3 types of green tea are done by one-way ANOVA. Let the confidence interval be 95% ($P \leq 0.05$).

4. Results

Green tea extraction Each the material green tea 10 g were mixed with distilled water 100 mL and stirred at 80^{o}C for 5 minutes. After the process of freeze-drying is done, the crude extract of green tea from Chiang Rai has shown the yield of gyokuro, sencha, matcha extracts are 19.34, 20.16, and 30.75 % yield, respectively. While, the crude extract from Bangkok has shown gyokuro, sencha, matcha extracts are 15.59, 17.33, and 28.98 % yield, respectively (Table 1). Matcha has more yield in terms of crude amount per extraction than other kinds of green tea due to its finely ground powdered appearance. Because matcha is a finely ground powdered appearance, it is easier to be processed and thus yields more crude extract than other types of green tea.

Table 1 Results of green tea extraction

Green tea samples	Amount of initial substance (grams)	Final amount of crude extraction (grams)	%yield
Gyokuro in Chiang Rai	10	1.9343	19.3430
Sencha in Chiang Rai	10	2.0159	20.1590
Matcha in Chiang Rai	10	3.0745	30.7450
Gyokuro in Bangkok	10	1.5591	15.5910
Sencha in Bangkok	10	1.7331	17.3310
Matcha in Bangkok	10	2.8980	28.9800

Total phenolic content

Total phenolic content (TPC) of green tea extract from Chiang Rai; the result shows that sencha contains the highest level of total phenolic content, having a mean of 105.5575, matcha and gyokuro, having the means of 101.0803 and 72.9142. Additionally, the result generated from the One Way Analysis of Variance also confirms that the difference in the type of tea significantly lead to the difference in the level of total phenolic content at 95 percent confidence level, having the P-value of <0.001 which is lower than 0.05. Green tea extract from Bangkok, sencha contains the highest level of total phenolic content, having a mean of 179.0741, gyokuro and matcha, having the mean of 91.6373 and 78.5108. The result generated from One Way Analysis of Variance also suggests that the difference in the types of tea significantly lead to the difference in the level total phenolic content at 95 percent confident level as the P-value is equal to <0.001 (Table 2). The result indicated that sencha show the highest level of total phenolic content both from Chiang Rai and Bangkok, and the TPC of sencha from Bangkok show the highest level , and more than gyokuro and matcha almost two folds.

Table 2 The difference in the level of total phenolic content (mg GAE/1 g extract) of green tea, each three types from Chiang Rai and Bangkok

Independent Variable		$\bar{x}$	S.D.	df	F	P
Green tea Types (Chiang Rai)	Gyokuro	72.9142	.07050	8	24655.821	<0.001
	Sencha	105.5575	.30730			
	Matcha	101.0803	.12211			
Green tea Types (Bangkok)	Gyokuro	91.6373	.15365	8	17261.767	<0.001
	Sencha	179.0741	1.15411			
	Matcha	78.5108	.45003			

Note: df is the degree of freedom, which refers to the maximum number of logically independent values, which are values that have the freedom to vary, in the data sample. F-value is calculated as variation between sample means / variation within the samples. The higher F- value means higher the variation between sample means relative to the variation within the samples. The higher the F-value means, the lower corresponding P-value. A p-value is a statistical measurement used to validate a hypothesis against observed data. A p-value of 0.05 or lower is generally considered statistically significant.

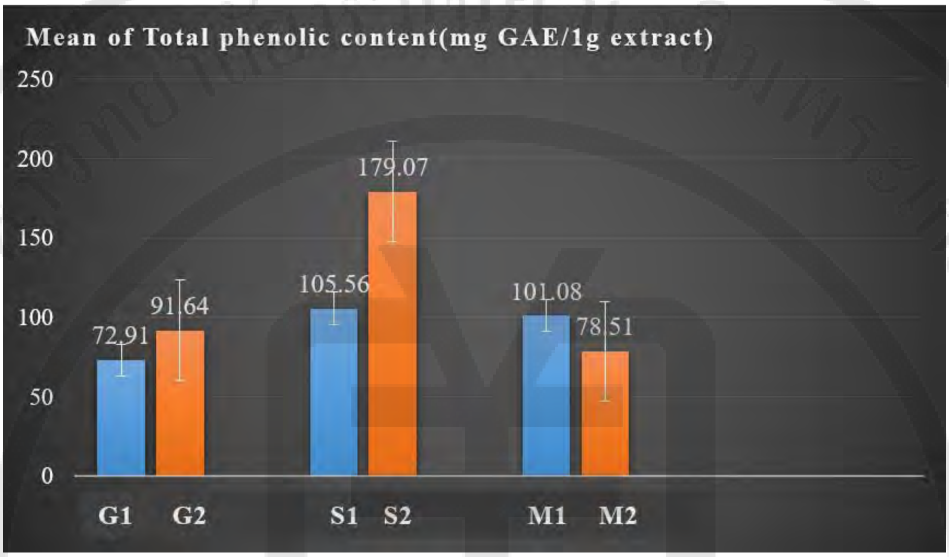

Figure 1 The total phenolic content of three types of green tea from Chiang Rai and Bangkok (Mean ± SD, n=3); G1, S1 and M1 refers to gyokuro, sencha and matcha from the same tea store in Chiang Rai. G2, S2 and M2 refers to gyokuro, sencha and matcha from the same tea store in Bangkok.

Antioxidant activity

All green tea extract from Chiang Rai and Bangkok are subjected to test antioxidant activity by using DPPH method. The green tea extract from Chiang Rai showed the antioxidant activity in IC_{50} range 0.0083-0.0233 mg/mL. Sencha extract showed the highest antioxidant activity in IC_{50} at 0.0083 mg/mL, matcha and gyokuro, having the means of 0.0167 and 0.0233 mg/mL. Moreover, the result generated by One Way Analysis of Variance also shows that the difference in the types of green tea significantly leads to the difference in the level of IC_{50} at 95 percent confidence level as P-Value is equal to <0.001 which is lower than 0.05. The antioxidant activity of green tea extract from Bangkok, the sencha extract showed the highest antioxidant activity in IC_{50} at 0.0080 mg/mL, gyokuro and matcha, having the means of 0.0140 and 0.0190 mg/mL. The result generated from One Way Analysis of Variance also confirms that the difference in the types of green tea significantly leads to the difference in the level of IC_{50} at 95 percent confidence level as the P-Value is equal to< 0.001 which is lower than 0.05 (Table 3).

Table 3 The difference in the level of IC_{50} (mg/mL) of three types of green tea from Chiang Rai and Bangkok

Independent Variable		$\bar{x}$	**S.D.**	**df**	**F**	**P**
Green tea Types (Chiang Rai)	Gyokuro	0.0233	.00058	8	508.333	<0.001
	Sencha	0.0083	.00058			
	Matcha	0.0167	.00058			
Green tea Types (Bangkok)	Gyokuro	0.0140	.00100	8	136.500	<0.001
	Sencha	0.0080	.00000			
	Matcha	0.0190	.00100			
BHT (Standard)		0.0172	0.0005			

Note: df is the degree of freedom, which refers to the maximum number of logically independent values, which are values that have the freedom to vary, in the data sample. The F-value is calculated as variation between sample means / variation within the samples. The higher F- value means higher the variation between sample means relative to the variation within the samples. The higher the F-value means, the lower corresponding P-value. The p-value is a statistical measurement used to validate a hypothesis against observed data. A P-value of 0.05 or lower is generally considered statistically significant.

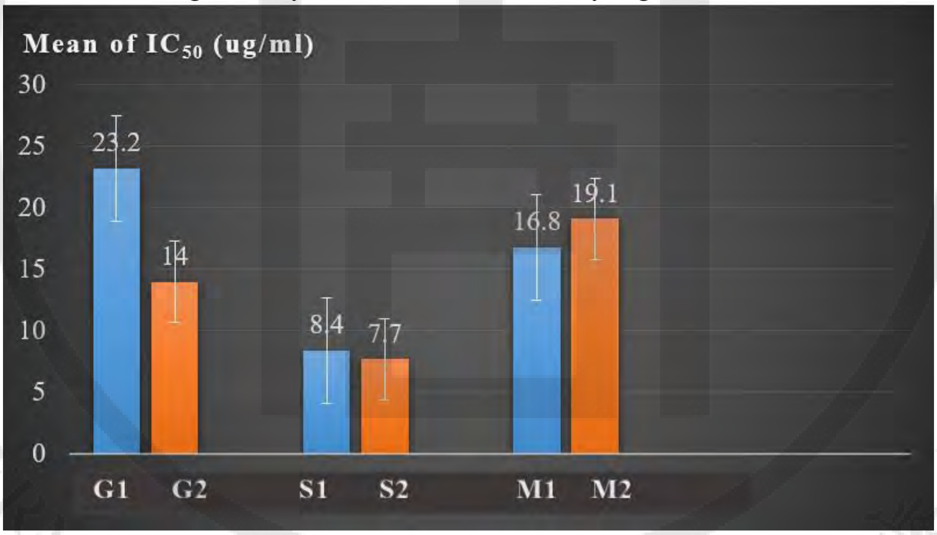

Figure 2 The antioxidant activity of three types of green tea from Chiang Rai and Bangkok (Mean ± SD, n=3); G1, S1 and M1 refers to gyokuro, sencha and matcha from the same tea store in Chiang Rai. G2, S2 and M2 refers to gyokuro, sencha and matcha from the same tea store in Bangkok.

5. Discussion

Matcha has more yield in terms of crude amount per extraction than other kinds of green tea due to its finely ground powdered appearance. Because matcha is a finely ground powdered appearance, it is easier to be processed and thus yields more crude extract than other types of green tea, as shown in the result of this experiment.

We concluded that sencha contains the most total phenolic content and has the highest antioxidant activity. Due to the manufacturing process, sencha is grown in sunlight directly and is different from gyokuro and matcha, which are grown under shade. The cultivation process of sencha is involved in photosynthesis reaction because it is grown in sunlight directly, different from gyokuro and matcha. Photosynthesis can produce secondary metabolites, in this thesis secondary metabolites are focused on phenolic and flavonoid which are founded in green tea. Secondary metabolites are divided in many classes and many biosynthetic pathways. In this thesis focus only on pathways of phenolic compounds which are important substances involved in antioxidant activity of green tea.

Phenols are found in green tea and occur in the pathways, starting from photosynthesis that is involved in the glycolysis reaction, which converts glucose into pyruvate. Then pyruvate oxidation converts pyruvate into acetyl -CoA, next acetyl-CoA is converted into malonyl-CoA by the enzyme acetyl-CoA carboxylase. Malonyl-CoA builds polyketide synthesis, finally lead to the production of phenols and flavonoid (Michael Wink, 2010; Maike Petersen, Joachim Hans and Ulrich Matern, 2010), this reaction is the reason sencha has the highest antioxidant activity and the most total phenolic content. In conclusion, sencha is cultivated via exposure to sunlight directly; sunlight help stimulate the process of photosynthesis, which produces secondary metabolites, which are phenols and flavonoid that can be found in green tea. Meanwhile, gyokuro and matcha are grown under shade, in which photosynthesis does not take place. That is the reason gyokuro and matcha have lower antioxidant activity and contain less total phenolic content (Figure 3).

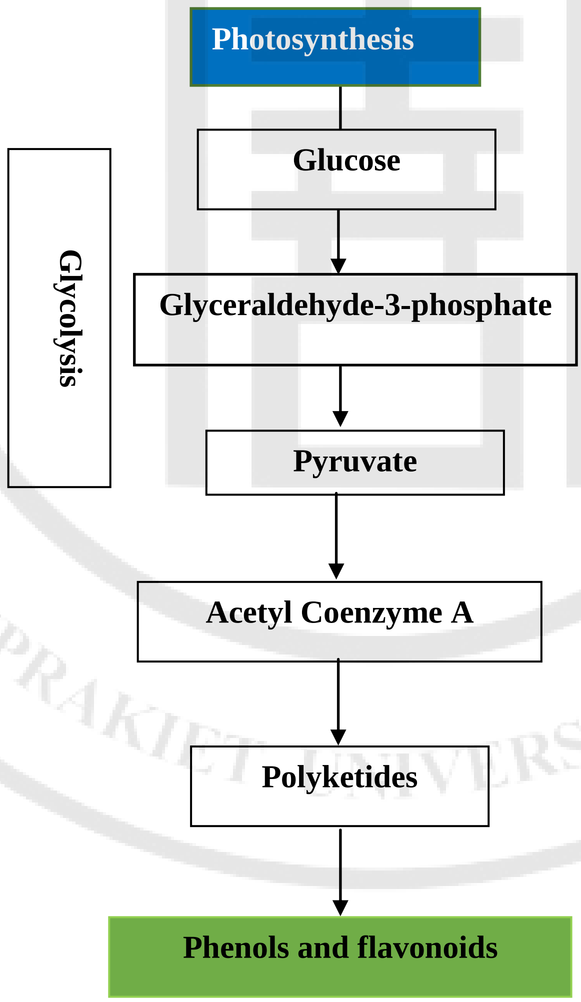

Figure 3 The main pathways leading to secondary metabolites

Flavonoid and catechins being the main substances that can be found in green tea. They are produced via shikimate pathway, which starts from photosynthesis. The shikimate pathway starts from eryhthrose-4-phosphate and phosphoenolpyruvate that are converted to chorismate which is the precursor of many aromatic secondary metabolites. The shikimate pathways are closely related to many aromatic amino acids such as L-tryptophan, L-phenylalanine and L-tyrosine (Michael Wink, 2010; Maike Petersen, Joachim Hans and Ulrich Matern, 2010). From this shikimate pathway is another reason sencha has the highest antioxidant activity and contain the most total phenolic content (Figure 4).

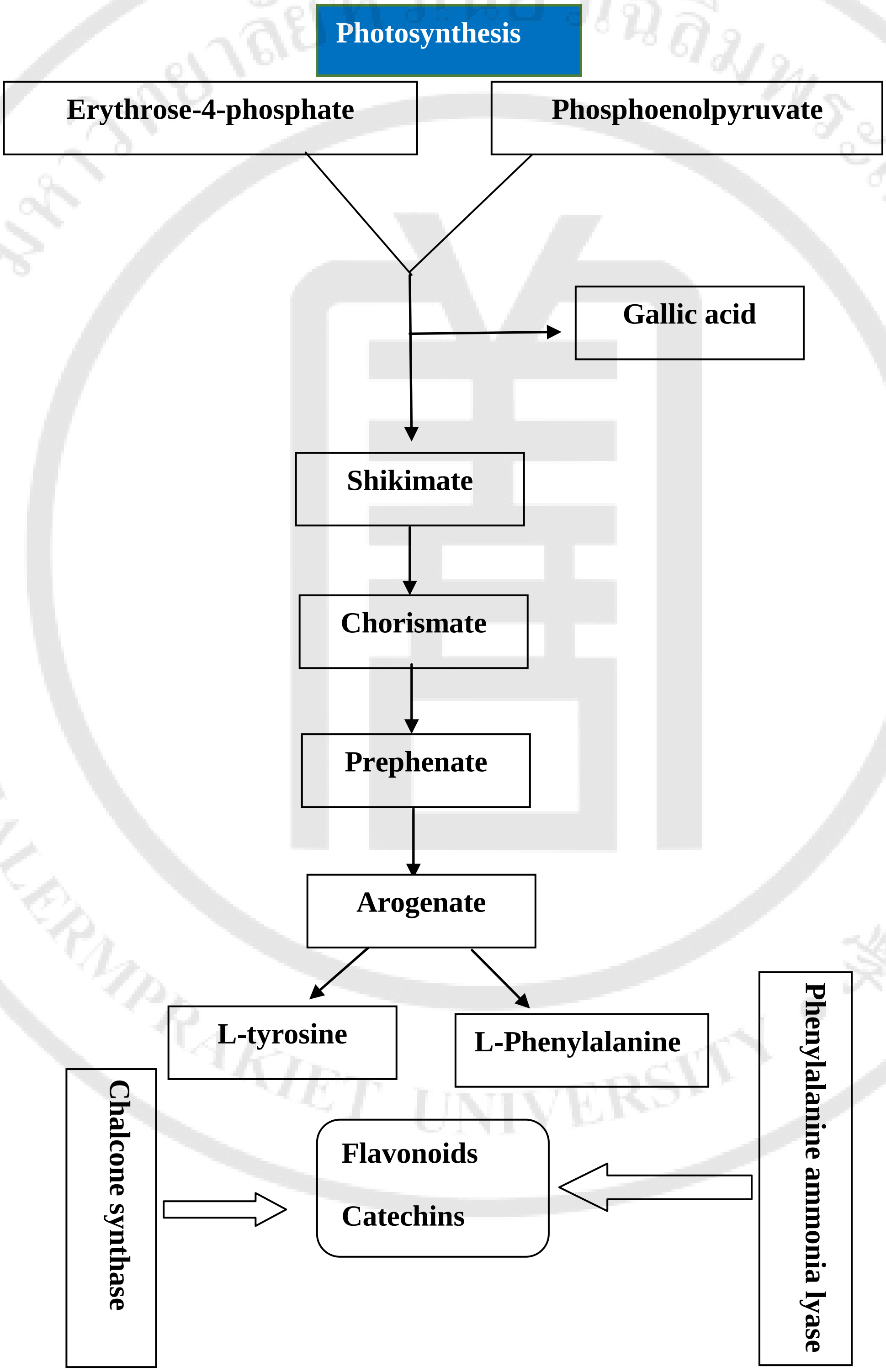

Figure 4 Several pathways of secondary metabolites

The main reason gyokuro and matcha has less amount of total phenolic content and less antioxidant activity than sencha is because both gyokuro and matcha do not go through photosynthesis reaction, because both are grown under shade. Another reason gyokuro and matcha from Chiang Rai and Bangkok or the same kind of green tea but from different places has different results is due to other factors, mainly temperature, pH, oxygen availability and presence of metal ions (Sang *et.al*, 2015), meaning catechin is highly stable in acidic pH and higher temperature can lead to unstable of tea polyphenol (Liang *et.al*, 2017). Leaf grades, plantation elevations and geographical locations are involved, and can lead to higher or lower total antioxidant activity and more or less amount of total phenolic content, meaning fresher green tea leaves and low elevation of green tea growth have higher antioxidant activity and contain more amount of total phenolic content than old green tea leaves and high elevation of green tea growth (Musial, Kuban-Jankowska and Gorska-Ponikowska, 2020). Sencha sample from the tea store in Bangkok (Originated from Japan) contain higher amount of total phenolic content and has higher antioxidant activity than sencha sample from Chiang Rai (grown in Amphoe Mueang, Chiang Rai) due to many factors, such as sencha leaves from Bangkok are fresher, grown at lower elevation, smaller size of tea leaves than sencha leaves from Chiang Rai. Total phenolic content and antioxidant activity depends on all of these factors (Musial, Kuban-Jankowska and Gorska-Ponikowska, 2020).

6. Conclusion

"Sencha" is the best green tea to drink because sencha contain the highest amount of total phenolic content mainly catechin, and highest antioxidant activity. This research uses deionized water as the solvent, because deionized water can be consumed, and thus served as the perfect solvent in terms of projecting the beneficial effects of green tea on the human body. With many different types of green tea in the market today, this research will most definitely be helpful for customers to make s good wise decision on which kind of green tea would benefit them most health-wise.

The gyokuro and matcha products in daily are mainly used in beverages but matcha is more commonly found in bakery ingredients. Although the result of total phenolic content and antioxidant activity from both Chiang Rai and Bangkok differ only slightly in results, gyokuro is more expensive than matcha.

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