Determination of Total Flavonoid Content of Commonly Consumed Commercial Tea

Arnt Win[*], Aye Mon Thida Nyo[**], Kyae Mon Lwin[*]

[*] Department of Chemistry, Kyaukse University, The Republic of the Union of Myanmar
[**] Department of Chemistry, University of Mandalay, The Republic of the Union of Myanmar

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Abstract **-** In this research work, the leaf of *Camellia sinesis* (L.) Kuntze, Myanmar name Let-phet, was selected to qualify and quantify the flavonoids present in it. The commercial teas were purchased from Ywa Ngan Township, Southern Shan State, Myanmar. Firstly, to get the ready-to-drink product, the infusion of commonly consumed commercial tea was prepared by the hot distilled water. Moreover, the infusion prepared from commercial tea was checked for qualitative test of flavonoids. In addition, total flavonoid content in 1 cup (200 mL) of this ready-to-drink product was evaluated by the Aluminium Chloride ($AlCl_3$) method using PD 303-UV spectrophotometer, at 415 nm. The total flavonoid content of this selected sample was expressed as mg quercetin equivalent (QE) per 200 mL of ready-to-drink product prepared from 2 g of commercial tea.

Index Terms - *Camellia sinesis* (L.) Kuntze; flavonoids; Commercial tea; Aluminium Chloride method; UV spectrophotometer.

I. INTRODUCTION

Tea is one of the oldest non-alcoholic beverages in the World. The origin of the tea is considered to be around the Myanmar (Burma) regions. From there it has been spread to China, Indonesia and India. Taxonomically it is known as *Camellia sinensis* (L.) Kuntze and belongs to the family Theaceae [1]. It grows best in tropical and subtropical areas with adequate rainfall, good drainage and slightly acidic soil [2]. There are two varieties of tea. *C. sinensis* var. *sinensis* (China tea) is grown extensively in China, Japan and Taiwan, while *C. sinensis* var. *assamica* (Assam tea) predominates in South and Southeast Asia, including Malaysia [3].

It is no surprise to find various types of tea being offered in stores and restaurants in almost every parts of the world. Tea is the second most consumed beverage in the world, after water, with an estimate of 20 billion cups enjoyed every day. Three main types of tea are green tea, black tea and oolong tea. These teas are characterized by the degree of fermentation where fermentation is the process of oxidizing green tea to produce black tea [4-8]. Tea is manufactured in three basic forms. Green tea is prepared in such a way as to preclude the oxidation of green leaf polyphenols. During black tea production, oxidation is promoted so that most of these substances are oxidized. Oolong tea is a partially oxidized product. Fresh tea leaf is unusually rich in the flavanol group of polyphenols known as catechins, which may constitute up to 30% of the dry leaf weight [9]. Drinking tea not only quenches thirst but also brings health benefits. While black tea may decrease blood pressure; green tea may regulate body temperature, blood sugar and enhance digestion; and oolong tea is excellent for its anti-diabetic, anti-obese and anti-inflammatory properties [10].

Tea is one of the most widely consumed beverages in the world. *C. sinensis* has been used for tea beverage since 3000 B.C. consisting of the leaf and bud of the plant *Camellia sinensis* [11]. Flavonoid and caffeine are the most abundant type of phenolic compound and alkaloid found in tea respectively [7,12]. Phenolic compounds are considered the most important constituent of tea since it is the largest component and act as bioactive ingredient that enhances the therapeutic action of tea [7,10].

The reasons for the worldwide popularity of tea were unique aroma and characteristic flavor, but recently, its popularity has increased due to its potential health benefits against cardiovascular diseases and cancer as well as pharmaceutical activities such as antihypertensive, antiateriosclerotic, hipocholesterolemic and hypolipidemic properties mostly from activities of antioxidant flavonoids present in tea. Monomeric flavonoids (flavan 3-ols or tea catechins) present in *C. sinensis* leaf are transformed to polymeric theaflavin and thearubigin by oxidation occurring during tea fermentation. The distinctive color, decreased bitterness and astringency and characteristic flavor are derived from the fermentation process giving fermented teas a marked distinction from non-fermented green tea. Even though teas are available in many different fermentation levels from green to black, the difference in phytochemicals and volatile compounds in tea with different degrees of fermentation has not been fully investigated yet within the same tea leaf [13].

This present research work is aimed at determination of total flavonoid content from commonly consumed commercial tea sample available in Myanmar market. It is essential to have knowledge of the actual levels of bioactive ingredients in the commercial tea consumed in various parts of the Myanmar in relation with human health. In the present research the commercial tea was analyzed for determining the total flavonoid content.

1.1 Botanical Description

Family Name	-	Theaceae
Botanical Name	-	*Camellia sinesis* (L.) Kuntze
Myanmar Name	-	Let-phet
English Name	-	Tea
Genus	-	Camellia

Figure 1: The plant, flower and leaves of *Camellia sinesis* (L.) Kuntze

II. MATERIALS AND METHODS

2.1 Sample Collection

The commonly consumed commercial teas, *Camellia sinesis* (L.) Kuntze were purchased from Ywa Ngan Township, Southern Shan State, Myanmar.

2.2 Extraction Procedure

Distilled water (200 mL) was firstly heated 97ºC, to which the bag containing 2 g of selected sample was immersed for 10 minutes. During the first 30 seconds, the bag was submersed 10 times. After 5 minutes, the procedure was repeated, and at the end of the 10 minutes, the tea bag was carefully removed and the tea was immediately conditioned in a beaker. This beaker was covered to avoid water evaporation and after cooling, it was filtered and then centrifuged with 3200 rpm for 30 minutes. The volume of infusion was adjusted again to 200 mL. 200 mL of ready-to-drink product was obtained.

2.3 Qualitative Test for Flavonoids

Ferric Chloride Test:

A few drops of neutral ferric chloride solution were added to 1 mL of extract solution. Formation of blackish red color indicates the presence of flavonoids.

Shinoda's Test:

To 1 mL of extract solution, a small piece of magnesium ribbon or magnesium foil was added and a few drops of concentrated HCl were added. Change in pink red colour shows the presence of flavonoids.

Lead- acetate Test:

To 1 mL of extract solution, a few drops of aqueous basic lead acetate solution were added. Formation of precipitate indicates the presence of flavonoids.

2.4 Quantitative Determination of Total Flavonoid Content

2.4.1 Principle

The basic principle of Aluminium chloride colorimetric method is that aluminium chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition it also forms acid labile complexes with the ortho-dihydroxyl groups in the A- or B- ring of flavonoids. Quercetin is reported to be suitable for building the calibration curve. Therefore standard quercetin solutions of various concentrations were used to build up the calibration curve [14-17].

2.4.2 Preparation and Determination of Standard Quercetin

10 mg of the standard quercetin was taken in a test tube. 100 mL of MeOH was added to the standard compound. The stock solution was obtained. It was diluted with MeOH in various ratios to obtained four ranges of concentration, such as 25 µg/mL, 50µg/mL, 75 µg/mL, and 100 µg/mL respectively. Then, 4.0 mL of solution was prepared for each concentration. 0.5 mL of each standard quercetin solution was taken in test tube and 1.5 mL methanol, 0.1 mL of 10% aluminium chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL distilled water were added separately to each tubes.

These tubes were left at room temperature for 30 min after which the absorbance of the reaction mixture was measured at 415 nm with UV/ Visible spectrophotometer. The calibration curve was plotted by using the resulted absorbance data of standard quercetin solutions at concentrations 25 µg/ mL to 100 µg/ mL in methanol. The calibration curve of standard quercetin is shown in Figure 2 [14-17].

2.4.3 Determination of Total Flavonoid Content of Commonly Consumed Commercial Tea

The total flavonoid content of commonly consumed commercial tea was measured by aluminium chloride ($AlCl_3$) according to the spectrophotometric method using quercetin as a standard. Firstly, 0.5 mL of of ready-to-drink product was taken in test tube and 1.5 mL methanol, 0.1 mL of 10% aluminium chloride, 0.1 mL of 1M potassium acetate and 2.8 mL distilled water were added into tube.

This tube was left at room temperature for 30 min after which the absorbance of the reaction mixture was measured at 415 nm with UV/ Visible spectrophotometer. The assay was performed in triplicate. The total flavonoid content of ready-to-drink tea leaf product was expressed as mg quercetin equivalent (QE) /200 mL (1 cup) of ready-to-drink product prepared from 2 g of commercial tea [14-17].

III. RESULTS AND DISCUSSION

Evaluation of Total Flavonoid Content in Commonly Consumed Commercial Tea

Special Test for Flavonoid

The infusion obtained from selected commercial tea with hot distilled water was examined by using the special qualitative tests of flavonoid. The resulted data are tabulated in Table 1.

Table 1: The results of qualitative test for flavonoid

No	Experiment	Observation	Inference
1.	Ferric Chloride Test:	Blackish red colour was appeared	Flavonoid may be present
2.	Shinoda's Test:	Colour turns to pink red colour	Flavonoid is present
3.	Lead Acetate Test:	Precipitate was produced	Flavonoid is present

From these results, it was observed that the infusion of the selected sample consists of flavonoid compounds.

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Total Flavonoid Content in Commonly Consumed Commercial Tea

The calibration curve was plotted against by using the resulting data of standard quercetin solution as shown in Table 2 and Figure 2.

Table 2: The results of absorbance values of standard quercetin solutions

No	Test Sample	Concentration (µg/mL)	Absorbance
1	Standard 1	25	0.165
2	Standard 2	50	0.308
3	Standard 3	75	0.495
4	Standard 4	100	0.636

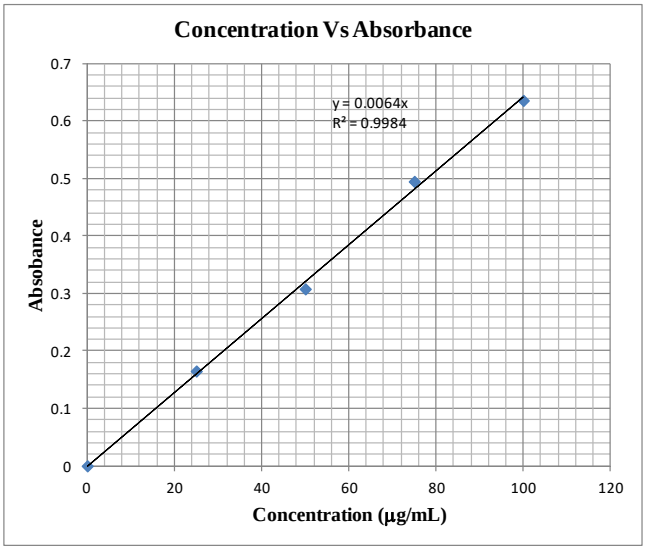

Figure 2: Concentration absorbance calibration curve for standard quercetin

In addition, the total flavonoid content of the infusion prepared from selected commercial tea was carried by aluminium chloride spectrophotometric method using the quercetin as a standard. The absorbance of prepared sample solution (0.5 mL or 500 µL) was measured by PD 303-UV spectrophotometer at 415 nm with respect to the blank solution. The results are described in Table 3.

Table 3: The results of absorbance values and concentrations of extract solutions of commonly consumed commercial tea

No	Name of Sample	Flavonoid (mg/200mL)	Flavonoid (mg/200 mL) Mean ± Standard Deviation
1.	Commonly consumed commercial tea	19.7	19.6 ± 0.17
		19.4	
		19.7	

From this result, the amount of total flavonoid content of analyzed sample was obtained by using the standard graph. The total flavonoid content present in the infusion prepared from selected commercial tea was found as 19.6 ± 0.17 mg quercetin equivalent (QE) per 200 mL (1 cup) of ready-to-drink product prepared from 2 g of commercial tea. Therefore, this result suggests that 1 cup (200 mL) of ready–to-drink product or infusion prepared from 2 g of commercial tea contains the significant amount of the total flavonoid content.

Flavonoid compounds that are secondary metabolites are antioxidant. The capacity of flavonoids to act as antioxidants depends on their molecular structure. The position of hydroxy groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities. Quercetin, the most abundant dietary flavanol, is a potent antioxidant because it has all the right structural features for free radical scavenging activity.

IV. CONCLUSION

The total flavonoid content of the infusion obtained from the commercial tea could be evaluated by UV spectrophotometer using the Aluminium chloride method at 415 nm. It was observed that the total flavonoid content of the ready-to-drink product is 19.6 ± 0.17 mg quercetin equivalent (QE) per 200 mL (1 cup). The resulted data of the current study showed that the selected sample, the ready-to-drink product prepared from commercial tea, had the considerable amount of total flavonoid compounds. The results of present research are important since the beneficial health effects of dry tea leaf are mostly connected with antioxidants, mainly catechins. It is important to inform the consumer that the total natural antioxidant flavonoid content in 1 cup (200 mL) of infusion prepared from commercial tea had 19.6 ± 0.17 mg quercetin equivalent (QE) which is significant amount.

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First Author – Dr Arnt Win, PhD, Associate Professor, Department of Chemistry, Kyaukse University, [Myanmar], PH-+95-92021984.
e-mail: arntthein@gmail.com

Second Author – Dr Aye Mon Thida Nyo, PhD, Associate Professor, Department of Chemistry, University of Mandalay, [Myanmar],PH-+95-9977256425.
e-mail: arntmonnyo@gmail.com

Third Author – Dr Kyae Mon Lwin, PhD, Professor and Head, Department of Chemistry, Kyaukse University, [Myanmar], PH-+95-9798808027.

Correspondence Author – Dr Arnt Win, PhD, Associate Professor, Department of Chemistry, Kyaukse University, [Myanmar], PH-+95-92021984.

e-mail: arntthein@gmail.com