Research article

Evaluation of Some Nutritional Values and Antioxidant Activities of Dried Tea Leaves in Seasonally

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Abstract Tea Leaf is one of the traditional food in Myanmar. It is eaten as well as drunk with two main forms which are wet tea leaf (Myanmar name, Lahpet so) and dried tea leaf (Myanmar name, Lahpet chauk). Tea leaves can be picked starting from April until October. At Namhsan area, Northern Shan State in Myanmar, tea leaves are generally divided into six kinds depending on the plucked season. There are "Shwephi-Oo", "Shwephi-Hnaung", "Khakan-Oo", "Khakan-Hnaung", "Kha-Naing", and "Kha-Hawt" or "Hnin Tet". In this study, some nutritional value and antioxidant activities of six kinds of dried tea leaves in Namhsan region were evaluated by the aiming of which kind of dried tea leaves have the best quality for consumers. The analyses included examination of the dried tea leaves were their nutritional value (carbohydrate, protein, and amino acids), assessment of selected minerals, caffeine, catechin mixture, dietary fiber and total ash. Nutritional contents of tea leaves were examined by preliminary phytochemical screening method and the selected mineral contents (Ca, Zn, Fe, Mn) were analyzed by atomic absorption spectrophotometry (AAS). Total ash contents were determined by ignition method. Antioxidant activities of dried tea leaves were evaluated by the DPPH free radical scavenging assay. Caffeine and catechin mixture (flavonoid) were extracted by using chemical reagents such as dichloromethane and chloroform. From the observed results, dried tea leaves in Namhsan region were rich in mineral contents (2.33-5.48 mg/kg of Ca, 1.40-1.94 mg/kg of Zn, 1.06-1.77 mg/kg of Fe and 8.06-21.06 mg/kg of Mn), 0.525-3.413% protein, 8-11.5% crude fiber, 4.20-7.39% total ash, 0.075-0.200% caffeine, 0.4-2.0% catechin mixture and high antioxidant activities (2.3-5.9 μg/L, IC_{50} value of DPPH scavenging assay) were found. Based on the studied nutritional values and antioxidant activity results, Shwephi-Oo dried tea leave was confirmed that the best quality.

Keywords dried tea leave, nutritional value, antioxidant activity, phytochemical screening, free radical scavenging, AAS

INTRODUCTION

Tea is grown in different states of Myanmar, mainly in Shan state. The best quality and major cultivation are in Namhsan region, Northern Shan State. The total tea plantation area is around 70,000 hectares with an annual production of 78 million Kg green leaves. Namhsan area is a mountainous

region with deep slope that forms a natural drainage system, vital for tea plantation (website 1). There are generally six kinds of tea seasonal names in use for the tea leaf. These are "Shwephi-Oo" if the tea leaf is picked 3rd week of March to 2nd week of April,"Shwephi-Hnaung" if it is picked 3rd week of April to 2nd week of the May, "Khakan-Oo" if it is picked 3th week of May to 2nd week of July, "Khakan-Hnaung" if it is picked mid-July to 2nd week of August, "Kha-Naing" if it is picked 3rd week of August to end-September, and,"Kha-Hawt" or "Hnin-Tet" if it is picked 1st October to end-November. The period of these seasons may be differed in locally.

Tea leaves contain thousands of the chemical compounds. When tea leaves are processed, the chemical compounds within them break down, form complexes with one another and form new compounds. The most important compounds in fresh tea leaves are polyphenols, amino acids, enzymes, pigments, carbohydrates, methylxanthines, minerals and many volatile flavor and aroma compounds. These components are responsible for producing teas with desirable appearance, aroma, and taste (Tony, 2019). All types of tea leaves (green tea, black tea, white tea, oolong tea, and pu-erh tea) contain unique antioxidants called flavonoids. The most potent of these, known as Epigallocatechin gallate (EGCG), may help against free radicals that can contribute to cancer, heart disease, and clogged arteries. All these teas also have caffeine and theanine, which affect the brain and seem to heighten mental alertness.

The more processed the tea leaves, usually the less polyphenol content. Polyphenols include flavonoids. When the tea leaves are oxidized or fermented, so they have lower concentrations of polyphenols than fresh green tea leaves; but their antioxidizing power is still high (Julie, 2009). Taste and aroma, as well as price and brand are the main factors impacting consumers' preferences with regard to tea of their choice; on the other hand consumers less frequently pay attention to the chemical composition and nutritional value of tea (Maria, 2017). Therefore, in this study, some nutritional values and antioxidant activity of dried tea leaves were examined by dividing the six kinds based on their plucked periods.

OBJECTIVE

This study assessed the nutritional values and antioxidant activities of dried tea leaves in seasonally and confirmed the best quality dried tea leave based on the studied results.

MATERIALS AND METHODS

Sample Collection

Tea leaves were plucked in one of the tea farms from Zayangyi village in Namhsan region, Nothern Shan State, Myanmar. "Shwephi-Oo" kind of tea leaves were plucked in 2nd week of April 2018, "Shwephi-Hnaung" was plucked in 4th week of April 2018, "Khakan-Oo" was plucked in 4th week of May 2018, "Khakan-Hnaung" was plucked in 3rd week of July 2018, "Kha-Naing" was plucked in 3rd week of August 2018 and, "Kha-Hawt" was plucked in 1st week of October 2018. After plucking, all kinds of tea leaves were cleaned and dried at room temperature for two weeks. The dried tea leaves were stored in the air tight plastic bags before the analyses were performed.

Sample Preparation

Aqueous extract of all kinds of dried tea leaves were prepared for the drinking purpose of dried tea leave infusion with boiling water.

Preliminary Phytochemical Analysis of Dried Tea Leaves

Preliminary phytochemical investigations of aqueous extract of dried tea leaves were carried out to know what chemical constituents were involved in the dried tea leaves according to the standard procedure of qualitative test for preliminary phytochemical screening. The procedure and results are presented in Table 1 and 2, respectively.

Table 1 Procedure for preliminary phytochemical screening

Constituents	Procedure	Observation
Alkaloid	Dragendroff's test, Few mL of sample filtrate + 1-2mL of Dragendroff's reagent	Reddish brown precipitate
Carbohydrate	Barfoed's test, 1 mL of sample filtrate + 1 mL of Barfoed's reagent (heated for2 min)	Red precipitate (monosaccharides)
Glycoside	Borntrager's test, 2 mL of sample filtrate hydrolysate + 3 mL chloroform (shaken well) + chloroform layer separate + 10 % ammonia solution	Pink colored solution
Protein and amino acids	Ninhydrin test, 2 mL of sample filtrate + 2 drops of Ninhydrin solution (10 mg Ninhydrin + 200 mL acetone)	Purple colored solution (Amino acids)
Flavonoid	Conc. H_2SO_4 test, plant extract + conc. H_2SO_4	Orange color
Phenolic compounds	Ferric chloride test, Extract aqueous solution + few drops of 5 % ferric chloride solution	Dark green/bluish black color
Tannins	Braymer's test, 1 mL of sample filtrate + 3 mL distilled water + 3 drops of 10 % ferric chloride sol:	Blue-green color

Determination of Some Nutritional and Mineral Contents of Dried Tea Leaves

Some nutritional (protein, total ash and crude fiber) contents of dried tea leaves were examined. The crude protein contents of the samples were determined by Macro-kjeldahl method (AOAC, 1990). Total ash was obtained by igniting tea sample in muffle furnace until it is free from carbon (Food Chemistry, 2012). The 5 g of each dried tea leave sample was taken in a tarred silica dish/crucible. It was heated at 100°C in an oven until moisture is expelled and incinerated at as low a temperature as possible. Then the dish was placed in furnace at 525 ± 20°C for about 1 hour and leave until white ash is obtained. The dish was transferred in a desiccator for cooling and weighted the dish. The results were expressed as % total ash.

The crude fiber contents in the dried tea leave samples were examined by the method given in "The chemical analysis of Foods" (Joslyn, 1970).

The selected mineral contents (Ca, Zn, Fe, and Mn) were analyzed by atomic absorption spectrophotometer. 1 g of each dried tea sample was dissolved in 10 mL of 1 M nitric acid and boiled to complete the dissolution and filtrated. The precipitate was washed with 1 M nitric acid and filtered. The obtained filtrate was transferred to 25 mL volumetric flask and fill up to the level with double distilled water. Then the digested solution was analyzed with AAS spectrophotometer (SHIMADZU AA-7000). The results are described in Table 2.

Determination of Caffeine, Catechin Mixture and Antioxidant Activities of Dried Tea Leaves

Caffeine and catechin mixture (flavonoid) were extracted by using chemical reagents such as dichloromethane or chloroform and ethyl acetate. 10 g of ground tea leaves were extracted with 300 mL of pure water at 80 °C for 40 min. Leaves were removed by filtration using a filter paper (Whatmann, No. 1). Aqueous tea infusion was initially partitioned with chloroform then second

partition was carried out with ethyl acetate (Row and Jin, 2006). Caffeine and catechin mixture were calculated by the equation 1 and 2 respectively (Ezgi, 2015).

% Caffeine (w/w) = (*mass of caffeine extract / mass of tea sample*) x 100 (1)

% Catechin (w/w) = (*mass of catechin extract / mass of tea sample*) x 100 (2)

Antioxidant activities of dried tea leaves were evaluated by the DPPH free radical scavenging assay according to Lee *et al*, 2004. The results are described in Table 3. All experiments were performed in the laboratory of Department of Chemistry, Taunggyi University, Southern Shan State, Myanmar.

RESULTS AND DISCUSSION

Fig. 1 shows the collected six kinds of air dried tea leaves from one of the tea farm in Zayangyi Village, Namhsan Region, Nothern Shan State, Myanmar.

Fig. 1 The six kinds of dried tea leaves

Table 2 Preliminary phytochemical screening of six kinds of dried tea leaves

Sample (H_2O extract)	Alkaloid	Carbohydrate	Glycoside	Protein and amino acids	Flavonoid	Phenolic compounds	Tannins
Shwephi-Oo	+	-	+	+	+	+	+
Shwephi-Hnaung	+	-	+	+	+	+	+
Khakan-Oo	+	-	–	+	+	+	+
Khakan-Hnaung	+	-	–	+	+	+	+
Kha-Naing	+	-	–	+	+	+	+
Kha-hawt	+	-	–	+	+	+	+

**(+) = Present, (-) = absent*

The qualitative tests of preliminary phytochemical screening for aqueous extract of six kinds of dried tea leaves showed the results which described in Table 2. According to these results, alkaloid, protein and amino acids, flavonoid, phenolic compounds and tannins were observed in all kinds of dried tea leaves. Glycoside was found in the two kinds, Shwephi-Oo and Shwephi-Hnaung. Carbohydrate was found to be absent in all kinds of dried tea leaves. By viewing these results, it is recommended that all kinds of dried tea leaves infusion with boiling water could significantly contribute to the health management and our daily need of secondary metabolites.

Nutrients can be divided into two categories: macronutrients, and micronutrients. Macronutrients are those nutrients that the body needs in large amounts. These provide the body with energy (calories). There are seven main classes of nutrients that the body needs. These are carbohydrates, proteins, fats, vitamins, minerals, fibre and water. It is important that everyone consumes these seven nutrients on a daily basis to help them build their bodies and maintain their health. Micronutrients are those nutrients that the body needs in smaller amounts. There are 7 essential plant nutrient elements defined as micronutrients [boron (B), zinc (Zn), manganese (Mn), iron (Fe), copper (Cu), molybdenum (Mo), chlorine (Cl)]. They constitute in total less than 1% of the dry weight of most plants (website 2). Table 3 shows the observed results of some nutritional and mineral contents of six kinds of dried tea leaves. From these results, it is found that the nutritional values are more or less differ from one another according to their plucked seasons. The protein (3.413%), crude fiber (11.5%) and zinc (1.9409 mg/kg) contents are the richest in "Kha-Naing" kind. The richest iron content (1.7661 mg/kg) is found in "Khakan-Oo" kind. The other parameters, total ash (7.39%), calcium (5.4764 mg/kg) and manganese (21.0559 mg/kg) are observed as higher significance in "Shwephi-Oo" kind. Despite the little difference in contents, on the basis of these observation, all kinds of tea leaves appeared that the presence of significance sources of nutritional and minerals for daily necessary human diet.

Table 3 Some nutritional and mineral contents of six kinds of dried tea leaves

Sample	Amount of parameters						
	Protein (%)	Crude fiber (%)	Total ash (%)	Ca (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	Fe (mg/kg)
Shwephi-Oo	0.525	8	7.39	5.4764	1.5873	21.0559	1.3953
Shwephi-Hnaung	0.525	8	6.60	5.4413	1.5079	15.1911	1.3514
Khakan-Oo	2.625	10.5	5.79	3.0048	1.8865	10.8227	1.7661
Khakan-Hnaung	1.138	10	5.39	4.4226	1.4070	8.0566	1.0664
Kha-Naing	3.413	11.5	6.60	3.3061	1.9409	11.6994	1.7569
Kha-hawt	2.144	8	4.20	2.3382	1.7858	11.1704	1.5577

Table 4 shows the amount of caffeine, catechin mixture and antioxidant activities of six kinds of dried tea leaves. The caffeine and catechin mixture contents were significantly higher in "Shwephi-Oo" kind. Numerous studies have indicated that catechins and other polyphenols in tea exhibit powerful antioxidant activities. They function as antioxidants in vitro by scavenging nitrogen species and reactive oxygen generated due to a variety of oxidative stress and by sequestering metal ions. The stable organic free radical, DPPH, has been considered as a useful reagent for determining free radical scavenging capacity of antioxidant materials (Tao, et al, 2019). The observed antioxidant results were compared with that of ascorbic acid as standard materials. The IC_{50} values (the effective concentration of 50% inhibition) were showed that all kinds of tea leaves have significance antioxidant activities. Among them, "Shwephi-Oo" has highest caffeine, catechin and antioxidant activity. It is considerable that "Shwephi-Oo" is the foremost plucked season kind at the beginning of summer. It was free from rain and the leaves were fresh and best strength. Therefore, it is confirmed that the quality of tea leaves depend upon the plucked season as well as other geographical conditions.

Table 4 The amounts of caffeine, catechin mixture and antioxidant activities of six kinds of dried tea leaves

Sample	Amount of parameters			
	Caffeine (%)	Catechin mixture (%)	Antioxidant activity (DPPH scavenging assay, IC_{50} value), (μg/L)	ascorbic acid (Standard)
Shwephi-Oo	0.200	2.0	2.3	1.2
Shwephi-Hnaung	0.075	1.3	2.7	
Khakan-Oo	0.100	0.4	4.8	
Khakan-Hnaung	0.100	0.6	4.1	
Kha-Naing	0.125	0.8	4.7	
Kha-hawt	0.075	0.5	5.9	

CONCLUSION

From the experimental results, six kinds of dried tea leaves in Namhsan region have valuable nutritional values and high antioxidant activity. Among them, the selected mineral contents, total ash, catechin mixture, caffeine content and antioxidant activity were found higher rich in "Shwephi-Oo" than another five kinds of dried tea leave apart from protein, crude fiber, Zn and Fe contents. Therefore, "Shwephi-Oo" kind of dried tea leave is generally regarded as the best quality for consumers.

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