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Phytochemical analysis of Myanmar Green Tea: implications to antioxidant properties and health benefits

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Many people around the world drink green tea for its reputed health benefits, which are believed to be attributed to the presence of polyphenols. Polyphenols contained in tea are classified as catechins, which are chemicals with potent antioxidant properties and thus, act as scavengers of free radicals. This antioxidant property of green tea is dependent on the gentle steaming method which prevents oxidation and thus preserving the polyphenols in its original form. Myanmar Green Tea (Nara Organic Green Tea; Kachin Special Group) and Plain Tea (Htoo Super Plain Tea), commercially available in the market, were subjected to qualitative and quantitative analysis of its constituents including alkaloids such as caffeine, catechin containing polyphenols, and tannins. The results showed that Myanmar Green Tea has a higher percentage of polyphenols than Plain Tea, thus supporting the preservation of antioxidant properties and its health benefits. Presence of alkaloids including caffeine and related compounds is responsible for the stimulant effect of both plain tea and green tea. The study supported the importance of the processing methods in making tea if the beneficial effects are to be preserved.

INTRODUCTION

Millions of people around the world continue to use herbal remedies because they believe in them [1]. Among these remedies are various forms of tea, scientifically known as *Camellia sinensis*, of which the leaves are widely consumed, both as traditional food and medicine. Green tea, which is highly reputed throughout the world for its health benefits, is believed to be attributed to the presence of antioxidant properties [2]. In Myanmar, there are 3 varieties of tea; plain tea, black tea and fermented tea (La-phet). Most people in Myanmar believed green tea and plain tea to be the same. However, in reality, the processing methods of these two forms are different. In Myanmar, of the well known brands, the Nara Organic Green Tea is processed by modernized stream distillation method while the "Htoo" Super Plain Tea is processed by the traditional method of drying, either by sunlight and/or by oven heating. Thus, it becomes interesting to know which of the methods used in Myanmar, is the best to preserve the antioxidant properties and the reputed health benefits, through research into the phytochemical constituents and tests for antioxidant activity in these two brands.

Currently, antioxidants are believed to help protect the body from free radical damage. Long-term use of vitamins and nutrients to prevent aging and diseases has been known and recent evidence has pointed out that such substances found in natural sources like fruits, leaves and vegetables rich in plant flavonoids are excellent antioxidants [eg. ascorbic acid from Zee-Phyu-Thee, retinoids and carotenoid from carrots, allicin in garlic and polyphenols (catechins) in tea leaves]. Thus, research to preserve the antioxidant properties of natural food and

food products, in preventing aging, diseases of sedentary living such as atherosclerosis, hypertension, cancer, diabetes and obesity, and even in smoking and environmental pollution has become an interesting topic of the 21st century [3-13].

Objectives

- To determine the phytochemical constituents in the tea leaves subjected to different processing methods during their preparation.
- To compare the free radical scavenging activity of Nara Green Tea and Htoo Super Plain Tea.

MATERIALS AND METHODS

Nara Green Tea consists of young tea leaves prepared by steam distillation process and Htoo Super Plain Tea consists of mature tea leaves prepared by heating and drying. Both samples were purchased locally from the market. Qualitative comparison of phytochemical constituents in Nara Green Tea and Htoo Super Plain Tea was done by the method of Harborne, 1984 [14] and quantitative measurement of the active compound of alkaloid and polyphenols was done by Unani Formulation, 1987 [15].

Total alkaloid

Ten grams of sample from each brand were accurately weighed, and extracted with 0.5N H_2SO_4 before being filtered. The filtrate was mixed with 30 ml chloroform and shaken for 30 minutes. The lower chloroform layer was collected using a separating funnel and the remaining upper acid layer made alkaline by adding an excess of dilute NH_4OH and shaken for 20 minutes. After standing for 10 min, the chloroform layer was separated and later removed using a Rotatory Evaporator. The remaining dried residue was dehumidified in a vaccum desiccator until a constant weight was obtained and the final weight was taken as total alkaloid. This separated alkaloid compound, which should represent the caffeine content, was checked by standard color test and Thin Layer Chromatography (TLC), and further quantitated by UV-1601 spectrophotometer.

Total phenolics

Ten grams of sample from each brand were extracted with water and filtered. The filtrate was shaken with petroleum ether (PE) for 30 minutes. The lower PE layer was discarded and the upper aqueous layer was precipitated by saturated solution of lead acetate. The precipitate was digested for 5 minutes in a water bath and filtered. The dried residue was suspended in alcohol, in slightly warmed water bath (38ºC), after which the clear alcoholic solution obtained was further concentrated using a Rotatory Evaporator. The dried residue was dehumidified in a vacuum desiccator until constant weight was obtained. The obtained polyphenolic compounds were separated by TLC and the catechin (epigallocatechin gallate) was checked by color test and UV-1601 spectrophotometer.

Measurement of DPPH radical scavenging activity by spectrophotometric method [16, 17]

DPPH (1,1-Diphenyl-2-picryl-hydrazyl) stock solution, 60 µM (DPPH 2.36 mg in 100 ml of 95% ethanol), was freshly prepared and stored in brown colored volumetric flask at +5ºC (no longer than 24 hrs) before use. Serial dilutions of the watery extract of test samples in concentrations of 0.625, 1.25, 2.5, 5 and 10 µg/ml were prepared in a 50% ethanolic solution. The test sample was also prepared by mixing 2 ml of 60 µM DPPH solution and 2 ml of watery extract (test solution) vigorously by a vortex mixer. Blank solution was prepared by mixing equal volume of test sample solution and 50% ethanolic solution. All solutions were allowed to stand at room temperature for 30 minutes after which measurement of absorbance was done at 517 nm using a UV-1601 Shimadzu Spectrophotometer. Absorbance measurements were done in triplicate and calculated to obtain the % inhibition using the following formula:

% inhibition = DPPH alone - (sample-blank) /DPPH alone x 100
% inhibition = Percent inhibition of test sample
DPPH alone = Absorbance of control solution
Sample = Absorbance of test sample solution
Blank = Absorbance of blank solution

RESULTS

The findings indicated that the contents of alkaloid compound, polyphenol, glycoside and steroids were higher in Nara Green Tea than in Htoo Plain Tea. Detail results are shown in Table 1.

Table 1. Comparison of phytochemical constituents of Nara Green Tea and Htoo Super Plain Tea

Phyto-test	Reagent	Observed (color)	Results	
			Nara	Htoo
Alkaloid	Dragendroff's reaget	Orange ppt	+	+
Glycoside	NaOH solution	Yellow	++	+
Flavonoid	conc: HCl/Mg	Red	+	+
Polyphenol	10% $FeCl_2$ sols:	Blue	+	+
Steroid	Acetic anhydride and conc: H_2SO_4	Green blue	++	+
Terpene	Acetic anhydride and conc: H_2SO_4	Pale yellow	-	-
Tannin	1% $FeCl_2$ soln:	Yellowish brown	+	+
Reducing sugar	Fehling soln:	Brick red	+	++
CN glycoside	Picric paper	Brown	-	-

(+) detected (-) not detected

Total alkaloid content was higher in Nara Green Tea than Htoo Plain Tea (1.24% and 1.12%, respectively). Caffeine content was slightly higher in Nara Green Tea (4.8%) than in Htoo Plain Tea (3.2%) and the presence of caffeine was confirmed by TLC and UV spectrophotometer, showing a definite spectrum with a peak at 272.5 nm, detected in 0.1N HCl solution [14] (Fig.1).

The content of total phenolic compounds was higher in Nara Green Tea than in Htoo Plain Tea (3.6% and 2.6%, respectively). Catechin compound was detected in total phenolic by UV spectrophotometer, showing a definite spectrum with a peak at 281.0 nm in methanol solvent (Fig. 1).

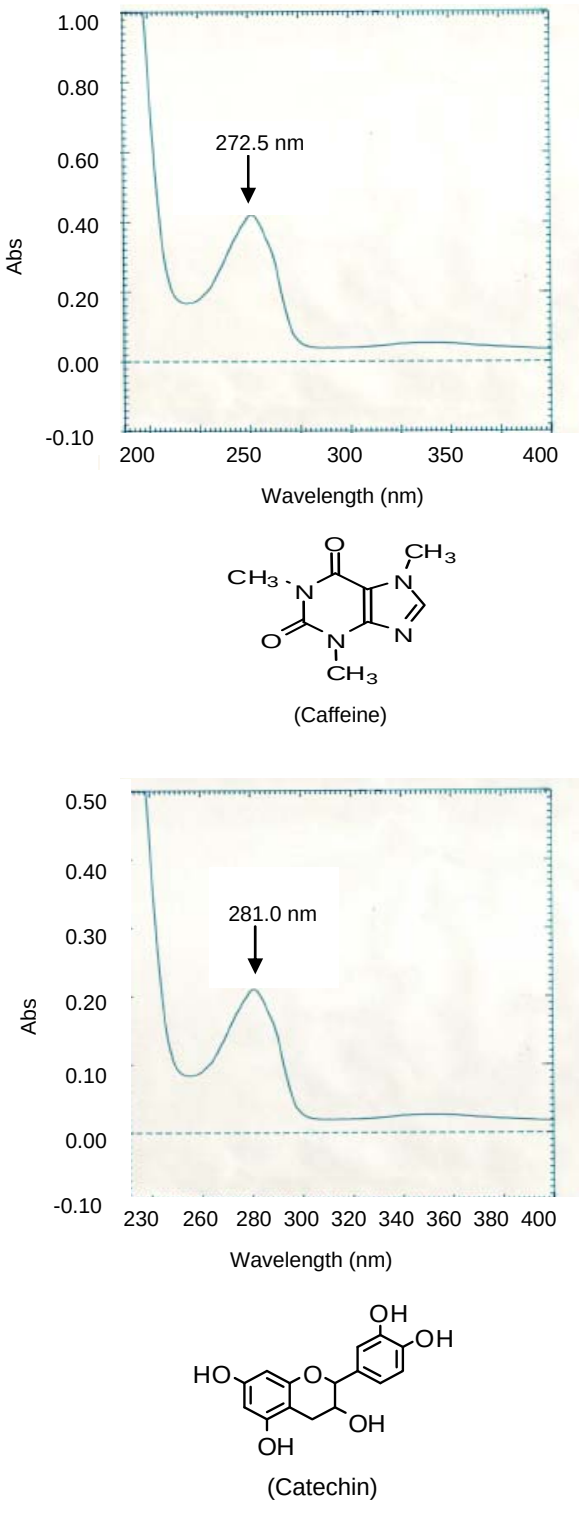


Fig. 1. UV-spectrum of caffeine and catechin compound in Nara Green Tea

Determination of radical scavenging activity of DPPH method is based on the change in absorbance of watery extracts solution in

various concentrations. Determination of absorbance values was carried out at wavelength 517 nm using spectrophotometer. Decrease in absorbance indicates increase in radical scavenging activity.

Free radical scavenging activity of watery extracts, usually expressed in term of % inhibition, is shown in Table 2.

Table 2 . Inhibition % of oxidation of watery extracts from tea leaves

No.	Extracts conc: (µg/ml)	% inhibition				
		0.625 µg/ml	1.25 µg/ml	2.5 µg/ml	5.0 µg/ml	10 µg/ml
1	Watery extract of Htoo plain tea		59.8	62.5	75.5	94.o
2	Watery extract of Nara plain tea		58.5	80.4	82.6	85.9
3	Ascorbic acid	69	83.2	94.6	98.9	99.5

From these results, it was also observed that increase in concentration showed to increase % inhibition, i.e, increase free radical scavenging activity.

DISCUSSION

Oxygen, the molecule of life for all aerobic creatures, undergoes chemical reactions with carbohydrate molecules, in the blood and the living cells, with the resultant generation of energy molecules such as ATP (adenosine triphosphate). During such process, undesirable side products known as free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) are also generated regularly, both during normal organ function or excessive oxidative stress. The reactive species superoxide ($O_2^{\cdot}$), hydroxyl-radical ($^{\cdot}OH$), nitrogen oxide ($NO^{\cdot}$) and hypochlorous acid (HOCL) are all products of normal human metabolic pathway, which can exert harmful effects under certain conditions [18,19].

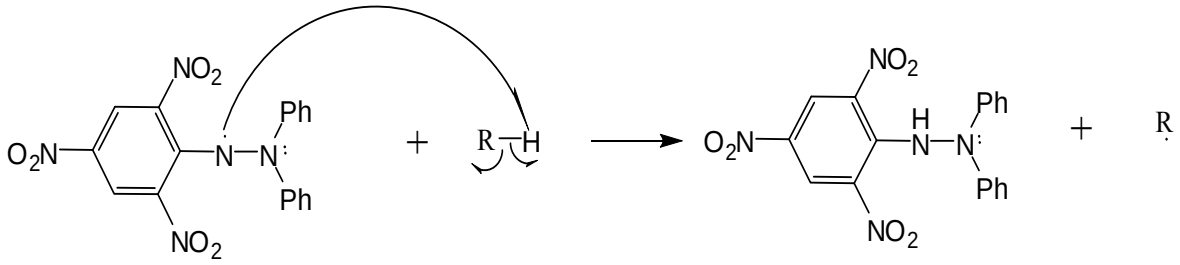
Over and above, environmental pollution, radiation, cigarette smoke and herbicides can form harmful free radicals that damage protein, DNA and other essential molecules. These gradual accumulations of proteins and DNA within the cells are now known to cause aging as well as diseases such as cancer and cardiovascular diseases [18].

Antioxidants are substances that scavenge free radicals damaging compounds in the body that alter cell membranes, tamper DNA (genetic material) and even cause cell death. Free radicals occur naturally in the body when subjected to oxidative stress, but environmental toxins including ultraviolet light, radiation, cigarette smoke, and air pollution can also increase the number of damaging particles. Free radicals are believed to contribute to the aging process as well as the development of cancer and cardiovascular diseases. It has been reported that polyphenols inhibit the growth of cancer cells, and may even kill them without harming the normal tissues [11, 18, 19].

Green tea is prepared from unfermented tea leaves and contains phenolic acid, polyphenols whose antioxidant effects has been known to be greater than Vitamin C. It has been reported that consumption of three cups of green tea per day (3 g soluble components, or 240 to 320 gm polyphenols) or 300 to 400 mg per day of standardized green tea extract (extracts should contain 80% total polyphenols and 55% epigallocatechin) is recommended for healthy living [19, 20].

Polyphenols in green tea are classified as catechins and involve 6 main compounds; catachin, gallocatechin, epicatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate (EGCG). This EGCG is considered to be the most active component in green tea, and thus, serves as a standard parameter determining the antioxidant property of a compound, and its presence is seen in green tea in the present study.

Commonly used assays for measurements of radical scavenger activity are as follows: Conjugated Diene Assay, Lipid Peroxide (PD) Assay, Thiobarbituric Acid Reactive Substances (TBARS), Carotene Linoleic



Acid System, 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) assay, and Cyclic Volumentry (CV). The present study utilized the measurement of radical scavenging activity of DPPH, a stable free radical, using the spectrophotometric method [17].

The principle of DPPH method is that, in the presence of a stable free radical (DPPH), an antioxidant donates a hydrogen atom to quench the stable free radical. Due to the ethanolic component, DPPH solution is violet and at 517 nm, which changes upon neutrilization by free radicals from violet to pale yellow. Spectroscopic measuring of absorbance at 517 nm allows the determination of DPPH proportion being neutralized by the test substance, the decolouration of the initial color being proportional to the anti-radicalizing (antioxidant) power of test substances by Thin Layer Chromatographic method. This method can be applied either when the antioxidant in its pure form or in a mixture.

From these results, it was found that the absorbance decreases with increase in concentration (i.e., increase in concentration increase the radical scavenging activity).

The findings in the present study indicated that the polyphenols present in the tea leaves may differ depending on the processing methods used in preparing them. The difference can be due to the some polyphenols being destroyed (oxidized) to some extent during the process of drying in sunlight and/or heating in the oven. Thus, in spite of the use of same tea leaves, the polyphenol content was lower in the Htoo Plain Tea when compared to Nara Green Tea which is made from young tea leaves which are processed by gentle steaming.

This finding supported the importance of steam processing of green tea which prevents the EGCG compound from being oxidized.

Conclusion

The present study showed that the antioxidant activity is higher in Nara Organic Green Tea than Htoo Super Plain Tea. However, the difference in the antioxidant activity of same tea leaf used is due to different processing methods of the two brands. The antioxidant property of polyphenol from Nara Organic Green Tea is dependent on the gentle steaming method which preserves polyphenol in its original form. Therefore, destruction of the active compound is the result of difference in the health benefits in taking tea [20, 21].

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