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Climatic variation of antioxidative properties, phenolic and mineralnutraceuticals in tea (*Camellia sinensis* (L.) Kuntze) grown in North Bengal, district Darjeeling

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Abstract
Climatic variation of anti-oxidative properties, Phenolics and mineral nutraceuticals in fresh tea shoots, consisting of one apical bud and two adjoining leaves sampled from TV1, TV20, TV26, TV29, TV30 (Tocklai Vegetative) clone and Tingamara, Dangri Manipuri and Sundaram B/5/63(Seed jat) grown in Terai region in Darjeeling district, North Bengal was investigated during three harvest season (March, June and December) in 2007, 2008 and 2009. The total Phenolics of all clones were lower in cool months of December in three years (average 36.02-93.29 mg GAE/g dry weight basis). Thereafter, the levels of total Phenolics increased throughout the warmer months from March to September. Antioxidant activity determined by Antioxidant activity determined by DPPH based free-radicals scavenging assay showed similar trends which increased from 1st harvest (March) to 3rd harvest (December). All clones showed nearly 100% antioxidant activity at 2nd and 3rd harvest season which higher than standard synthetic antioxidant BHA (Butylated hydroxyl anisole). However, seasonal variation of minerals (N, P, K, Ca, Mg, Na, Fe, Cu, Mn, and Zn) showed different results according to clones used. These results seem to suggest that the harvest time is crucial to determining the antioxidant potential of fresh tea shoots.

Keywords: *Camellia sinensis,* Fresh shoots, climatic variation, antioxidants, Phenolics-minerals.

1. Introduction

Tea-elixir is drunk in almost every country around the world and has reached a ceremonial status as a social and medicinal beverage. Tea (*Camellia sinensis* L.Cruntz) is one of the world's oldest panaceas. The tea plant was first discovered by the ancient Chinese, who then spent many centuries perfecting the art of tea cultivation to manufacturing, which resulted in a variety of types available today.

Willson KC *et al*.1992 [18] reported that the tea plant is an evergreen of the *Camellia* family that is native to Northern India and China. There are two main varieties of the tea plant. The broad leaf variety, known as *Camellia sinensis var assamica*, grows best in the moist, tropical climates found Northeast India and the Szechuan and Yunnan provinces of China (.The small leaf variety, known as *Camellia sinensis var sinensis*, thrives in the cool, high mountain regions of central China and Japan. India and China are the two major tea producers and exporters and followed by Sri Lanka, Kenya and Turkey, respectively.

The production of tea in India mainly started in the early 1758 AC of the British India along the Brahmaputra valley of Assam and Terai, Doors and Hills of Darjeeling. Many of the tea plantations are centered on the Darjeeling and Jalpaiguri District of West Bengal from the Nepal border to Jorhat and Kachar of Assam, reaching the altitude of around 1000 m to 3000m from msl.

Helliwell*et al*. 1999[6] stated since 3000 B.C., traditional Chinese medicine has recommended green tea for headaches, body aches and pains, digestion, enhancement of immune system, detoxification, as an energizer and to prolong life. The health benefits of tea are confirmed and therapeutic value of tea for the prevention and treatment of many diseases has become more and more commonly known (Misra *et al*., 2008). Tea is also contains minerals and trace elements such as K, Mn, Cr, Ni and Zn which are essential to human health. Fernandez *et al*. 2002[4] showed that regular consumption of tea may contribute to the daily dietary requirements of several elements and tea could be an important source of manganese and the large amount of potassium in comparison with sodium that could be beneficial for hypertensive patients.

Mandal *et al*. 2010[11] revealed that there has been increasing interest in finding plants with high antioxidant capacities since they can progression of many chronic diseases. Serafini *et al*. 1996[16] analyzed young tea shoots contain more than 35% of their dry weight in polyphenols. Non fermented green tea contains predominantly flavanols, flavandiols and phenolic acids like gallic acid, coumaric acid or caffeic acid, with those in green tea being higher than those in black tea. Misra *et al*. 2008[12] reported that Phenolic compounds that are present in young tea shoots (also referred to as fresh green leaves, fresh tea shoots, or flushes) are known to be one of the main factors in determining the quality of the resulting tea drink. There were studies related to chemical composition of tea shoots and its constituent catechins are best known for their antioxidant properties, which has led to their evaluation in a number of diseases associated with reactive oxygen species (ROS), such as cancer, cardiovascular and neurodegenerative diseases. Several epidemiological studied by Yang *et al*. 2002[20] as well as studies in animal models have shown that green tea can afford protection against various cancers such as those of the skin, breast, prostate and lung. However the variation of chemical content present in tea shoots in different harvest time in particular in North Bengal has not been studied so far. Therefore the aim of the study to investigate the seasonal variation of total Phenolics, antioxidant activity and minerals in young tea shoots of eight tea clones/jats in Terai region of North Bengal.

2. Materials and Methods

Chemicals

All chemicals used were analytical grade and were bought from Sigma (USA).

Collection and preparation of tea samples

Fresh tea shoots (*C. sinensis*), comprises of one apical bud and two adjoining leaves, around 250 g, were hand plucked from commercial clones, TV1, TV20, TV26, TV29, TV30, Tingamara, Dangarimonipuri and Sundaram(B/5/63).The clones are initially identified by Baganbabu (Head of the workers) and Assistant Manager of the TE and finally by the Tea experts. The clones are found together in the same plantation in Paharghumia TE located at Naxalbari area in Terai of North Bengal. The clones were same age. The samples were collected from these commercially produced clones at three harvest season (30th March, 30th June and 5th December) in 2007, 2008 and 2009 respectively. There were no statistically differences between years in terms of chemical profiles among shoots therefore the results of three years were pooled. The collected fresh shoots from 16 plants per clones were cleaned and cut into small pieces before being dried in a hot air-blowing oven at 45 °C for 14 hrs. Semi dried samples were ground to a fine paste with a Pestle and mortar and kept at room temperature prior to extraction. The semi dried shoots were used for the analysis of antioxidant activity, total Phenolics and minerals.

DPPH based free radical scavenging activity

The free radical scavenging activities of each fraction were assayed using a stable DPPH, following standard method of Blois MS. 1958[2]. The reaction mixture contained 2 ml of 0.1mM DPPH and 0.1 ml of each methanol fraction. Simultaneously, a control was prepared without sample fraction. The reaction mixture was allowed to incubate for 5 min at room temperature in the dark and scavenging activity of each fraction were quantified by decolourization at 515 nm. Percentage of free radical scavenging activity was expressed as % inhibition from the given formula:

$$\text{Percent inhibition} = \frac{\text{Abs. of control} - \text{Abs. of sample}}{\text{Abs. of control}} \times 100$$

Antioxidant capacities of the samples were compared with those of BHA and the blank.

Determination of total Phenolics in tea shoots

Total Phenolics in the methanol extracts were determined spectrometrically using Folin-Ciocalteu reagent as described by Slinkerd *et al*. 1977[17]. Gallic acid was used as a standard and results were expressed as mg Gallic acid equivalents per g dry weight basis.

Determination of mineral elements

The tissues sampled were oven-dried at 68°C for 48 h and ground. The Kjeldahl methodand a Rapid Kjeldahl Distillation Unit were used to determine total N. Macro (P, K, Ca and Mg), and micro elements (Na, Fe, Mn, Zn and Cu) were determined after wet digestion of dried and ground sub-samples in a H_2SO_4-Se-salisilic acid mixture as suggested by Bremner. 1996[3]. In the diluted digests, P was measured spectrophotometrically by the indophenol blue method after its reaction with ascorbic acid by UV/VIS spectrophotometer. K, Ca, Na analyze by flame photometer and Mg, Fe, Mn, and Zn, analysis were determined by atomic absorption spectrometry (ECIL made AAS).

Statistical Analysis

The experiment was a completely randomized design with three replications. Data were subjected to analysis (version 11.0 SPSS. Inc.) of variance (ANOVA) and the Duncan's multiple range test (DMRT) was used for making comparison prescribed by Gomez *et al*. 1984[5], $P < 0.01$ was regarded as significant.

3. Results and Discussion

The total phenolic content of young fresh tea shoots belongs to eight clones is given in table 1. The statistically important differences on total phenolics were obtained in different harvest times in tea clones ($p<0.01$). Total phenolic content were continuously increased from 1st harvest to 3rd harvest in all clones, which found to be 90.57-253.11, 48.75-12.75, 81.48-225.11, 36.02-114.20, 197.84-86.02, 65.11-249.71, 93.29-294.20, 93.29-294.20 mgGAE/g DW in TV1, TV20, TV26, TV29, TV30, Tingamara, Dangri Manipuri and Sundaram clone respectively (table 1). Misra *et al*. 2008[12] previously reported that fresh tea shoots are extremely rich in phenolic compounds which can constitute up to 300 mg/g of dry material. It is also reported that green tea shoots is very rich for total phenolics and total phenolic content of green tea shoots 4-5 times higher than phenolic rich Ashwagandha (*Withnia somnifera*) plant.

The great difference of tea shoots in terms of total phenolics at different harvest time is supposed to the effect of change of ecological parameters. North Bengal takes less sunlight in December month (3rd harvest) than the other harvest times. This may affect the biosynthesis of total phenolics. On the other hand, in this month in North Bengal region, the differences between day and night temperatures are also higher and the rainfall was irregular. In this stress conditions, tea clones produce more phenolics. It has been shown that the biosynthesis of Phenolic compounds in tea shoots can be effectively induced by stronger sunlight and length of daytime. That is why in shaded tea flushes the concentrations of polyphenols are much lower. Same findings also observed by Mahanta *et al*. 1992[10]. On the basis of this information, the differences in total phenolic levels between fresh tea shoots harvested in different months in North Bengal may not be just a temperature effect but also a day length and sunlight effect. However, further studies are required to elucidate the induction of the biosynthesis of total phenolics by day length and sunlight exposure correlating to the UV index. Previously, under field conditions, the phenolic composition of tea shoots varies considerably with seasonal, genetic, and agronomic factors and mechanisms that induce seasonal variations on total phenolic content in tea shoots may include one or all three of the following environmental conditions i.e day length, sunlight, and/or temperature, which vary markedly across seasons. Achuthan *et al*. 2003[1] said that the highest total phenolic levels in tea shoots are important for public by reducing the risk of atherosclerosis and coronary heart disease, which can be caused by oxidation of low-density lipo proteins. The present total phenolic content results would indicate that there is potential to produce higher quality black tea during the December months in North Bengal. These results are in agreement with the findings of Yao *et al*. 2005[19] which found more phenolics occurred in relatively warmer months in the shoots. On the other hand, the differences were observed on total phenolic content in shoots among tea clones (table 1). Obanda *et al*. 1997[13] showed the level of phenolics in green tea shoots varied among clones. Antioxidant activity of fresh tea shoots in seven clones is given in table 1. There were statistically differences among harvest times in all tea clones except TV1 ($p<0.05$). Antioxidant activity was increased from 1st harvest to 3rd harvest times in all tea clones (table 1). Free radical scavenging activity was found to be between 96.72-99.49, 77.47-98.22, 89.25-98.94, 86.87-99.00, 86.47-96.55, 89.72-99.28, 90.55-99.97 and 90.55-99.97% in TV1, TV20, TV26, TV29, TV30, Tingamara, Dangri Manipuri and Sundaram clone/jat, respectively (table 1). Antioxidant activity (% Free radical scavenging) of BHA (200 mg/l) was 91.18%. All extracts sampled from 3rd harvest

Table 1: Seasonal variation of antioxidant activity and total phenolic content of tea shoots

Tea clone	Harvested time	Free radical scavenging activity (%)	Total phenolic content mgGAE/g DW
TV1	March	99.49a	253.11a
	June	92.86b	114.93b
	December	96.721ns	90.57c
TV20	March	98.22a	123.75a
	June	92.60b	109.48b
	December	77.47c	48.75c
TV26	March	98.94a	225.11a
	June	96.66ab	84.02b
	December	89.25b	81.48b
TV29	March	99.00a	114.20a
	June	96.77ab	84.02b
	December	86.87b	36.02c
TV30	March	96.55a	86.02b
	June	97.46a	92.20b
	December	86.47b	197.84a
Tingamara	March	99.28a	249.71c
	June	94.19ab	123.11b
	December	89.72b	65.11c
Dangri Manipuri	March	99.97a	294.20a
	June	97.35ab	96.75b
	December	90.55b	93.29b
Sundaram B/5/63	March	99.97a	294.20a
	June	97.35ab	96.75b
	December	90.55b	93.29b

*Values in the same column with different lower-case letters in same clone are significantly different at $P<0.01$. ns: non significant

in all clones had higher antioxidant activity than BHA (table 1). Similar to 3rd harvest, all clones also showed higher antioxidant activity at 2nd harvest time than BHA, except TV20 clone. However, in 1st harvest time, only TV1 clone had higher antioxidant activity than BHA (table 1). The other clones had lower antioxidant activity than BHA at 1st harvest time. In previous studies conducted on tea by Karori *et al*. 2007[8], the antioxidant activity of different tea products in different solvent was found between 56-83%. Halliwell *et al*. 1999[6] revealed that commonly consumed products such as tea, coffee and cocoa have possessing significant amount antioxidant activity. The results for antioxidant activity clearly outline that tea shoots could be one of the richest sources among plants in terms of antioxidant activity. The great difference of tea shoots for antioxidant activity at different harvest time is supposed to the effect of change of ecological parameters. The composition of tea shoots varies with climate, season, variety, and age of the shoot. There were strong relationships between antioxidant activity and total phenolics in all harvest dates in all tea clones ($R=0.998$, $p<0.05$). Juliani *et al*. 2002[7] are also reported strong relationships between antioxidant activity and total phenolics in tea shoots which support our findings. The mineral contents of tea shoots at different harvest times in seven tea clones are shown in table 2. Differences among the different harvest times were observed based on the mineral compositions in all tea clones (table 2). The amount of N in tea leaves was the highest at 3rd harvest time in TV26, TV29, TV30 and Tingamara clones between 2.27-2.87%, respectively. However, in TV1 and Dangri manipuri clones the amount of N was the highest in 2nd harvest (2.63%). In TV20 clone the amount of N was the highest at 1st harvest time (2.96%), respectively (table 2). The amount of P in tea leaves were between 0.151- 0.280% and it was the highest at 3rd harvest time in TV29, Tingamara, Dangri manipurit and Sundaram clones/jats (table2). Similar to N, the TV20

Table 2: Seasonal variation of mineral content of tea shoots

Tea clone	Harvested time	N (%)	P (%)	K ppm	Ca ppm	Na ppm	Mg ppm	Zn ppm	Cu ppm	Fe ppm	Mn ppm
TV1	March	2.15b	0.21b	13900b	1202c	183b	506ab	448a	299a	636a	344a
	June	2.26a	0.298a	15700a	1604b	209ab	451b	405b	269ab	610b	341ab
	December	1.82c	0.168c	12300c	2205a	217a	631a	373c	252b	540b	302b
TV20	March	2.96a	0.276a	14520b	1603b	271a	633a	608a	412a	885a	479a
	June	2.85a	0.263a	13700c	1002c	183b	633a	583a	396ab	845b	460a
	December	1.64b	0.151b	19000c	2404a	253ab	578b	336b	227b	482c	272b
TV26	March	1.88b	0.177b	16300a	1006b	147b	631a	384b	260b	592b	310b
	June	1.94b	0.179b	13200b	803c	255a	594b	397b	270b	578b	325b
	December	2.87a	0.266a	15900a	2401a	253a	630a	590a	399a	857a	478a
TV29	March	2.66b	0.248b	14300b	1402b	145b	541b	465b	315b	679b	370b
	June	2.78ab	0.280a	14700ab	2003a	219a	558b	620a	421a	902a	504a
	December	2.87a	0.266ab	15300a	2206a	181ab	648a	570a	379ab	808ab	479ab
TV30	March	2.24a	0.209a	12300b	144b	217b	613ns	459a	311ns	669a	371a
	June	1.68b	0.154b	12300b	1206c	290a	540	341b	232	496b	276b
	December	2.27a	0.210a	19900a	2009a	181c	576	466a	315	676a	377a
Tingamara	March	2.27ns	0.211b	14600ns	1604c	127c	541ns	546ns	370ns	794ns	440ns
	June	2.45	0.259ab	14100	1908b	290b	570	580	401	808	472
	December	2.66	0.281a	14400	2401a	326a	612	621	420	900	508
Dangri Manipuri	March	1.82b	0.170b	15800b	2010c	185c	594ab	375c	252c	545c	311c
	June	2.63a	0.244ab	18200a	2203b	275a	486b	531b	366b	785b	440b
	December	2.60a	0.280a	13700c	2607a	217b	649a	630a	420a	901a	523a
Sundaram B/5/63	March	1.82b	0.168b	15700b	2011c	187c	595ab	371c	252c	549c	304c
	June	2.63a	0.246ab	18100a	2213b	280a	480b	540b	366b	781b	437b
	December	2.60a	0.282a	13800c	2601a	219b	649a	623a	420a	910a	509a

*Values in the same column with different lower-case letters in same clone are significantly different at $P<0.01$. ns: non significant

had highest P content at 1st harvest time as 0.276%. The K content of fresh tea shoots were between 12300- 19900 ppm among clones at different harvest times. Three out of eight clones had the highest K content at 3rd harvest time (TV20, TV29 and TV30). The other clones had higher K content at 2nd (TV1 and Dangri Manipuri clones) and 1st harvest (TV26 and Tingamara clones) (Table 2). All tea clones had the highest Ca content at 3th harvest time between 803-2607 ppm (table 2). Mg content was also highest at the 3rd harvest time in most of the clones, except TV20 and TV29 (table 2). Most of the tea clones had the highest Fe, Cu, Mn and Zn content at 3rd harvest time (table 2). The mineral composition of plants depended, not only on the species or varieties, but also on the growing conditions such as soil and geographical condition. In this study, while the existence of ten minerals was determined in all harvest times, K was predominant, followed by N, Ca, Mg and P, respectively (Table 2). It is previously reported by Owuor *et a*l. 1983[14] that N content of tea leaves were between 3.0-4.0% () which is higher than our results. Ozgumus *et al*. 1982[15] found that P content of tea leaves were between 0.31- 0.40% which close to our P results. In addition, our K results are in accordance with Kacar *et al.* 1979[9]. It is noteworthy that minerals are important not only for human nutrition, but for plant nutrition as well. Potassium, a mineral essential for controlling the salt balance in human tissue, can be detected. Zinc, a trace mineral that is especially important for normal functioning of the immune system, is present in good levels in tea shoots in seven tea clones. Calcium, a mineral which is essential to bone structure and function, is relatively high in seven tea clones.

4. Conclusion
It can be concluded that tea shoots are a valuable product, based on its rich and beneficial nutrient composition. It seems that the most important chemical components such as phenolics were highest at the first harvest time in Terai of North Bengal. However, certain growing conditions and cultural management techniques affecting the chemical components of tea shoots will be the subject of further research projects.

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