ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 13, Issue 4, 2020

Online - 2455-3891
Print - 0974-2441

Research Article

SEASONAL VARIATIONS IN ANTIOXIDANT CAPACITIES AND PHENOLIC CONTENTS OF TEA LEAF EXTRACTS

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Received: 18 January 2019, Revised and Accepted: 15 February 2020

ABSTRACT

Objectives: The objective of the study was to estimate the seasonal variations in the antioxidant capacities, total polyphenol content (TPC), total flavonoid content (TFC), and tannin content (TC) of tea leaf extracts from two different plantation sites.

Methods: Samples were collected from two tea gardens in Tuli and Ungma situated at N 26°39'19.3 E 094°39'22.7 and N 26°17'30.6 E 094°28'29.2, respectively, under the Mokokchung district of Nagaland, India. TPC, TFC, and TC from sample extracts were determined using Folin–Ciocalteu reagent, aluminum chloride colorimetric, and Folin–Ciocalteu assay. Apart from these, antioxidant capacities were analyzed using ferric reducing ability of plasma (FRAP) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay.

Results: The concentrations of total polyphenol, flavonoid, and tannin varied from 552.029±8.079 to 305.647±1.744 mg gallic acid equivalent/g, 238.770±0.508–148.457±1.653 mg catechin equivalent/g, and 26.453±0.485–20.173±0.173 mg tannic acid equivalent/g, respectively. FRAP and DPPH assay displayed value ranging from 2.564±0.023 to 1.074±0.023 mmol Fe(II) equivalent/g and 3.612±0.053–2.076±0.028 mmol Trolox equivalent/g. Significant seasonal variations in concentrations of these compounds were observed and a positive correlation between antioxidant capacities and phenolics of tea leaf extracts was established.

Conclusion: Tea (*Camellia sinensis* (L.) O. Kuntze) has been regarded as a plant of immense medicinal and therapeutic value since time immemorial. The tea leaf extracts analyzed in this study gave high TPC, TFC, and TC, as well as high antioxidant activity in terms of DPPH and FRAP value. Studying such properties in tea leaves contributes more to our understandings of health benefit potentials in tea leaves and the quality of tea leaves on the basis of seasons and sites where they are planted.

Keywords: Seasonal variations, Antioxidant capacities, Tea leaves, Mokokchung district of Nagaland, Phenolics.

 DOI: http://dx.doi.org/10.22159/ajpcr.2020.v13i4.36866

INTRODUCTION

Tea (*C. sinensis* (L.) O. Kuntze) is one of the most popular beverages and the economic crop of the world with remarkable biological activities. Some of the dependent factors for successful tea cultivation include temperature, rainfall, humidity, and solar radiation [1]. There are different types of processed tea leaves consumed by the world among which the most commonly available ones include green tea, black tea, and oolong tea [2,3]. Black tea is the most commonly produced one representing 76–78% of the total tea produced and consumed in the world [4]. Aroma, test, and various positive physiological functions make tea desirable for consumption [5]. Important factors that determine the taste, flavor, and health benefits of a specific type of tea are the variations in leaves composition [6]. Tea has been consumed by people since ancient times. In olden times, tea was consumed for improving blood circulation, body resistance, and to eliminate toxins [7]. Studies reported wide health benefits of tea consumption including a reduction in cardiovascular mortality [8] and protective effect against many different types of cancer [9]. *In vitro* studies reported the preventive effect of tea against cellular DNA damage caused by arsenic-mediated oxidative stress [10]. These benefits of tea have been attributed mainly to its phytochemicals such as polyphenols which are important constituents of the human diet widely distributed in vegetable foods an [11]. The phenolics are known to possess diverse biological properties including antibacterial, antitumor, and antimutagenic properties [12]. Tea has been reported to possess a sufficient amount of polyphenols including flavonoids and catechins and their derivatives. Tea polyphenols have a phenolic hydroxyl group attached to the flavan-3-ol structure due to which they have strong antioxidant properties and can scavenge free radicals [13,14]. Flavan-3-ols, commonly known as catechins, are the major polyphenolic compounds in tea and it includes (−)-epicatechin (EC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), (−)-epigallocatechin gallate (EGCG), (−)-gallocatechin (GC), and (−)-gallocatechin gallate (GCG) [15]. Apart from many health benefiting properties, tea catechins are also responsible for the bitter taste and dark color of black tea due to oxidation and condensation to theaflavin, theaflavin-3-gallate, theaflavin-3′-gallate, and theaflavin-3-3′-digallate and thearubigins during fermentation [16].

Chemical composition of tea varies with factors including climate, season, variety, horticultural practices, and the age of the leaf [17] which may bring variations in concentrations of leaves chemical compounds even between the same varieties cultivated under different agricultural practices and among different seasons. Seasonal variation in tea leaves total phenolic, antioxidant activity, plant nutritional elements, and fatty acids was reported by others [18]. Ahmed *et al*. [19] reviewed that out of 18 studies, 14 studies amounting to 78% demonstrated a decrease in phenolic compounds or their bioactivity concentrations with a seasonal shift from the spring and or first tea harvest to other seasons.

Nagaland state of India surrounded by Myanmar, Assam, Manipur, and Arunachal Pradesh on its East, West, North, and South belongs to one of the biodiversity hotspots of the world. Despite its neighboring state, Assam being one of the oldest and largest tea producers in the country, tea plantation practice in Nagaland is only a few years old. However, since the past few years, there has been an increase in tea plantation in some districts such as Mokokchung. A study has been conducted on a seasonal basis in the concentration of important tea leaves constituents total phenol, total flavonoid, tannin content (TC), and also on the antioxidant capacities from extracts of air-dried tea leaves grown in

Nagaland. This study will provide valuable information on the quality of tea grown in different plantation sites of Nagaland and will aid in the potential effects of climatic and plantation sites in leaf compounds.

METHODS

Chemicals and reagents

Folin–Ciocalteu reagent, sodium carbonate (Na_2CO_3), sodium nitrite ($NaNO_2$), sodium phosphate, methanol, and hydrochloric acid (HCl) were purchased from Sisco Research Laboratories Pvt. Ltd., India. Gallic acid monohydrate was purchased from Hi-Media, India, and catechin LR hydrate, ascorbic acid, aluminum chloride ($AlCl_3$), sodium hydroxide (NaOH), sodium potassium tartrate tetrahydrate, ammonium molybdate, 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), iron (III) chloride 6-hydrate, iron (II) sulfate 7-hydrate, tannic acid, sodium acetate, 1,1-diphenyl-2-picrylhydrazyl (DPPH), and (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich, India.

Tea leaves sampling

The two tea gardens situated Tuli and Ungma at N 26°39'19.3 E 094°39'22.7 and N 26°17'30.6 E 094°28'29.2 in Mokokchung district, India, were chosen for the present study. Both the tea gardens are 10 years old and growing the same variety of tea plant. Young shoots were collected in all four seasons. Tea leaves were washed with distilled water and allowed to dry in shade at room temperature. Air-dried leaf samples were cut into pieces and ground to a fine powder in a mill and stored in an airtight sterile container for analysis. Leaves collected during different seasons from Tuli and Ungma during spring, summer, autumn, and winter were designated as Tspr, Tsum, Taut, and Twin and Uspr, Usum, Uaut, and Uwin, respectively.

Extraction of air-dried tea leaves

A 5.0ml of 70% methanol/water extraction mixture, preheated to 70°C for ½ h, was poured to an extraction tube containing 0.2000±0.001g air-dried tea sample. Tubes were heated in water bath for 10min where mixing on the vortex mixer at the beginning, after 5min, and at the end of 10min was carried out. Tubes were centrifuged at 1095× *g* for 10min after bringing them to room temperature. The supernatant was transferred into a 10ml test tube. The residues were extracted again and the extracts were combined. The final volume was made up to 10 ml with cold methanol/water extraction mixture and this sample solution was used to determine various secondary metabolites.

Determination of total flavonoids

Total flavonoid content (TFC) in the standards and the extracts was measured using aluminum chloride colorimetric assay [20]. To 1 ml of extracts, 4 ml of distilled water was added followed by 0.3 ml 5% $NaNO_2$. The solutions were allowed to stand for 5 min after which 0.3 ml of 10 % $AlCl_3$ was added followed by 1 min incubation in room temperature and addition of 2 ml 1 M NaOH. Distilled water was added to make the total volume up to 10 ml. Blank was prepared using distilled water. The standard solution was prepared using catechin in different concentrations following the same procedure. The absorbance was measured at 510 nm against blank in ultraviolet (UV)–visible spectrophotometer. TFC of tea leaves was then expressed as mg catechin equivalent (CE)/g fresh weight of tea leaves. All samples were analyzed in triplicates.

Determination of total polyphenols

Total polyphenol content (TPC) in the standards and the extracts was measured by the Folin–Ciocalteu reagent assay [21]. To 1 ml of extracts, 9 ml distilled water was added followed by 1 ml of Folin–Ciocalteu reagent. The mixture was shaken and after 5 min, 10 ml of 7% Na_2CO_3 solution was added. Distilled water was added to make the total volume up to 25 ml. Blank was prepared using distilled water. The standard solution was prepared using gallic acid in different concentrations following the same procedure. Standard solution, blank, and sample solution were incubated at room temperature for 90 min. The absorbance was measured at 750 nm with an UV–visible spectrophotometer. The total phenolic content of tea leaves was then expressed mg gallic acid equivalent (GAE)/g weight of tea leaves. All samples were analyzed in triplicates.

Determination of total tannins

TC in the standards and the extracts was measured by Folin–Ciocalteu reagent assay [22] with slight modification. To 1 ml of extracts, 1 ml of Folin–Ciocalteu reagent was added followed by 4 ml of Na_2CO_3 solution and 4 ml of distilled water. The standard solution was prepared using tannic acid in different concentrations following the same procedure. Standard solution, blank, and sample solution were incubated at room temperature 30 min at room temperature. The absorbance was measured at 725 nm using UV–visible spectrophotometer. The TC was expressed as mg tannic acid equivalent (TAE)/g weight of tea leaves.

Ferric reducing ability of plasma (FRAP) assay

FRAP assay of the leaves extracts was carried out following Benzie and Strain [23] with slight modification as given by Wong *et al*. [24]. FRAP solution was prepared by mixing 300 mmol/l, pH 3.6 sodium acetate buffer, 10 mmol/l TPTZ solution in 40 mmol/l HCl, and 20 mmol/l iron (III) chloride solution 10:1:1 volume ratio. To 0.1 ml of extracts, 3 ml of the freshly prepared and 37°C warmed FRAP solution was added. The mixture was allowed to react for 4 min and absorbance was measured at 593 nm with UV–visible spectrophotometer. The standard solution was prepared using $FeSO_4 \cdot 7H_2O$ solution and the antioxidant activity was then expressed as mmol Fe(II) equivalent (FE)/g weight of tea leaves. All samples were analyzed in triplicates.

DPPH assay

DPPH radical scavenging activity of the leaf extracts was carried out following Brand-Williams *et al*. [25] as given by Lee *et al*. [26]. To 0.1 ml of extracts, 3.9 ml of 0.12 mM DPPH solution was added. The standard solution was also prepared using different concentrations of Trolox. Decrease in absorbance was determined at 515 nm after 30 min. Results were expressed as mM Trolox equivalent (TE)/g weight of tea leaves.

Statistical analysis

Results from the experiments carried out in triplicates were expressed as mean±standard deviation. Using the SPSS for Windows, version 18.0 (SPSS, Chicago, IL), one-way ANOVA was used for calculating significance differences for multiple comparisons by Tukey's *post hoc* test. Significant difference was based on $p<0.05$. Pearson's correlation analysis was conducted to assess a correlation between variables.

RESULTS

TPC, TFC, and TC

The TPCs of the tea leaves were in the order Tspr>Tsum>Taut>Uspr>Usum>Uaut>Twin>Uwin with the highest and the lowest TPC up to 552.029±8.079 mg GAE/g and 305.647±1.744 mg GAE/g, respectively (Table 1). The TFCs of the tea leaves were in the order Tspr>Uspr>Tsum>Usum>Uaut>Taut>Twin>Uwin with the highest and the lowest TFC up to 238.770±0.508 mg CE/g and 148.457±1.653 mg CE/g, respectively (Table 1). Tannin concentration in tea leaf extracts was in the order Uspr>Tsum>Tspr>Usum>Uaut>Taut>Twin>Uwin with the highest and the lowest TC up to 26.453±0.485 mg TAE/g and 20.173±0.173 mg TAE/g, respectively (Table 1). Between the sites, tea leaf extracts from Tuli gave higher concentrations of the compounds studied except for TC where Uspr showed the highest value (Table 1).

Antioxidant capacities by DPPH and FRAP assay

The highest antioxidant capacity with 3.612±0.053 mmol TE/g was seen in Tspr while the lowest value with 2.076±0.028 mmol TE/g was seen in Uwin (Table 2). For both the tea leaf extracts, the season with the highest capacity to neutralize DPPH radicals with 3.612±0.053 mmol TE/g and 3.152±0.040 mmol TE/g was observed to be spring. The results obtained from FRAP assay showed that tea leaf extracts had a significant reducing power (Table 2). FRAP value indicates the antioxidant capacity of the sample extracts because of their ability to reduce ferric ions to ferrous ions. Similar to DPPH assay, this assay also

Table 1: Total polyphenol, total flavonoid, and tannin content (mg/g) in tea leaf extracts of Tuli and Ungma. Data represent the mean±SD

Sample	TPC (mg/g)	TFC (mg/g)	TC (mg/g)
Tuli			
Tspr	553.521±6.816[a]	238.770±0.508[ab]	24.507±0.285[a]
Tsum	540.029±9.383[b]	186.703±1.008[ab]	25.086±0.040[b]
Taut	496.662±6.147[ab]	160.531±1.087[a]	22.485±0.438[ab]
Twin	386.159±9.703[ab]	157.890±1.915[b]	20.409±0.443[ab]
Ungma			
Uspr	502.834±4.147[a]	234.616±1.858[a]	26.453±0.485[ab]
Usum	412.280±2.332[a]	173.613±2.201[a]	24.379±0.351[a]
Uaut	390.164±4.308[a]	164.188±3.046[a]	23.429±0.391[b]
Uwin	305.241±0.323[a]	148.457±1.653[a]	20.173±0.173[ab]

SD: Standard deviation. Different [abc] in the same line indicates significant differences by Tukey's test ($p<0.05$)

Table 2: Antioxidant capacities in tea leaf extracts of Tuli and Ungma (mmol/g). Data represent the mean±SD

Sample	FRAP (mmol/g)	DPPH (mmol/g)
Tuli		
Tspr	2.564±0.023[a]	3.612±0.053 [a]
Tsum	2.245±0.011[b]	3.146±0.050 [a]
Taut	1.692±0.245[ab]	2.806±0.015 [a]
Twin	1.092±0.013[ab]	2.181±0.026 [a]
Ungma		
Uspr	2.336±0.036[a]	3.152±0.040[a]
Usum	2.070±0.020[b]	2.904±0.016[a]
Uaut	2.374±0.465[c]	2.484±0.038[a]
Uwin	1.074±0.023[abc]	2.076±0.028[a]

SD: Standard deviation. Different [abc] in the same line indicate significant differences by Tukey's test ($p<0.05$)

showed spring season with 2.336±0.036 mmol FE/g as the highest reducing capacity. However, in the case of Ungma, it was autumn season with 2.374±0.465 mmol FE/g that gave the highest value.

Correlation analysis

Strong positive correlations were observed between the antioxidant and total phenolic content of tea leaves in our study. Pearson's correlation coefficient analysis between FRAP and DPPH assay with TFC, TPC, and TC in tea leaf extracts is indicated in Table 3. TE antioxidant capacity by DPPH and FE antioxidant capacity by FRAP was observed to have highly significant correlations with TPC, TFC, and TC of tea leaf extracts at $p<0.01$ (Table 3). However, between the tea gardens, the highest correlations of the DPPH assay were established with TPC (R^2=0.953) in tea leaf extracts of Tuli and with TC (R^2=0.972) and in tea leaf extracts Ungma. Similarly, the FRAP assay in tea leaf extracts of Tuli also established the highest correlations with TPC (R^2=0.947) and with TC (R^2=0.793) in tea leaf extracts of Ungma.

Table 3: Correlation analysis between the antioxidant capacities and total phenolic, total flavonoid, and tannin content in tea leaf extracts

Antioxidant capacities	Correlations		
	TPC	TFC	TC
Tuli			
DPPH	0.953**	0.887**	0.899**
FRAP	0.947**	0.853**	0.944**
Ungma			
DPPH	0.956**	0.865**	0.972**
FRAP	0.768**	0.575	0.793**

**Correlation is significant at the 0.01 level. TPC: Total polyphenol content, TFC: Total flavonoid content, TC: Tannin content, FRAP: Ferric reducing ability of plasma

DISCUSSION

Health benefits of drinking tea are widely known and have been attributed mainly to the presence of different polyphenols. Polyphenols are the most abundant antioxidants in the diet with total dietary intake much higher than other classes of phytochemicals and known dietary antioxidants [27]. Considerable attention is being given to phenolics or polyphenols because of their physiological function such as antioxidant, antimutagenic, and antitumor activities [28]. It has been reported that the TPC assay is necessary for the quality control of manufactured tea products [29] because its composition in tea is critical for tea quality as well as its role as bioactive compounds. Results from the study clearly indicate that tea leaf extracts from both the gardens contained a reasonable amount of phenolic compounds. Significant decrease in TPC and TFC with the seasons from spring to winter was observed for both the sites in our study. It was also observed that the TPC and TFC were significantly higher in spring season as compared to other seasons, which give a deduction that plucking periods of tea leaves may be one of the factors responsible for the change in the amount of tea leaves composition. According to Turkmen *et al*. [30], seasonal variation, leaf handling and harvest methods, leaf maturity, and variety of tea clones are some factors that affect the polyphenol content of fresh tea leaves. Our result is comparable to the work of others [31] who also observed a declining trend in tea quality with the progress of season. It was also reported by the same authors that the teas which were plucked during the first flush in late April and early May had the highest quality. Variations in the concentration of each compound were observed between the samples despite being the plant of the same species and of the same age. These differences observed maybe the effect of variation in climatic conditions and soil physiochemical nature of the tea gardens. Apart from these, TCs were also determined where leaf extracts in spring season of Ungma were found to be the highest. Tannins are naturally occurring water-soluble plant polyphenols [32,33] that provide dark color and astringent taste [32]. In one site (Ungma), the TC was found to decrease progressively with the seasons; however, no significant relationship could be established between TC and season of the year in leaves extract of Tuli. Variations in leaf compositions among seasons and between the tea gardens are indicative of the effect of various factors, including geographic locations of tea plantations, soil properties, and management practices.

The present study also showed excellent antioxidant potential of tea leaf extracts through DPPH and FRAP assay. FRAP estimates the ability of compounds to act as an electron donor [34]. DPPH assay based on DPPH solution decolorization after the addition of a radical or an antioxidant species is a simple, rapid, sensitive, and reproducible method for the evaluation of the free radical scavenging effect of plant extracts [35]. DPPH at room temperature is a stable free radical and becomes a stable diamagnetic molecule by accepting an electron or by hydrogen transfer [36,37]. Tea phenolics are efficient in scavenging free radical, partly due to their ability to act as hydrogen or electron donors [38]. The concentration of DPPH and FRAP value in various seasons of the year differed greatly for both the sites. Correlation analysis of DPPH and FRAP assay with leaf tea polyphenols revealed high correlations, indicating its remarkable antioxidant potential. This finding is comparable with others who reported a similar trend of significant higher values of antioxidant activity along with total phenol and flavonoid [39]. Lasano *et al*. [40] indicated a great association of antioxidant activity with TPC and TFC in unfermented *Strobilanthes crispa* tea. It has also been shown that *in vitro* antioxidant powers vary according to teas and antioxidant capacity and the content of total phenolics in tea is strongly correlated [23] as phenolic compounds are powerful chain breaking antioxidants that can directly contribute to antioxidative action [41]. Other studies also showed a strong relationship between antioxidant activity and total polyphenols content in tea leaf extracts [42,43] as well as in extracts of other plants [44-46] authenticating the role of leaf constituents, especially polyphenols as potential health benefitting compounds.

CONCLUSION

The tea leaf extracts analyzed in this study gave high TPC, TFC, and TC, as well as high antioxidant activity in terms of DPPH and FRAP value. Variations were observed between leaf extracts of different seasons as well as differences in leaf extracts between sites. For both the sites, spring season yielded significantly higher value for all the compounds analyzed which is indicative of sampling sites and seasons of the year being one of the contributors for phenolic contents in tea leaves. The results also established a significantly positive correlation between polyphenols and antioxidant capacities in the studied tea leaf extracts confirming possible health benefits of tea leaves in Nagaland, India.

ACKNOWLEDGMENT

We are thankful to the University Grant Commission – Basic Scientific Research, Government of India, New Delhi, for providing fellowship for this work.

AUTHORS' CONTRIBUTIONS

Temsurenla Jamir was in charge of manuscript drafting. Temsurenla Jamir and T. Ajungla have equal contribution in concept development, sample collections, and data analysis.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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