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

TEMSURENLA JAMIR*, AJUNGLA T
Department of Botany, Nagaland University, Lumami, Nagaland, India. Email: arentem123@gmail.com
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 90°43'92.2 and N 26°17'30.6 E 90°42'28.9, 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 3.61±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.

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 [23]. Black tea is the most commonly produced one representing 76–78% of the total tea produced and consumed in the world [4]. Aroma, taste, 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 [12]. Even today, tea is consumed for these and the additional benefit of lowering the risk of many diseases [13].

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. The changes in total phenol, total flavonoid, tannin content, and 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 (Na₂CO₃), sodium nitrite (NaNO₂), 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 (AlCl₃), 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 5.0 ml of 70% methanol/water extraction mixture, preheated to 70°C for ½ h, was poured to an extraction tube containing 0.2000±0.001 g air-dried tea sample. Tubes were heated in a water bath for 10 min where mixing on the vortex mixer at the beginning, after 5 min, and at the end of 10 min was carried out. Tubes were centrifuged at 1095×g for 10 min after bringing them to room temperature. The supernatant was transferred into a 10 ml 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% NaNO₂. The solutions were allowed to stand for 5 min after which 0.3 ml of 10% AlCl₃ 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% Na₂CO₃ 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 as 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 Na₂CO₃ 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 FeSO₄·7H₂O 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>Tsum>Uspr>Usum>Uaut>Twin>Uwin with the highest and the lowest TFC up to 26.45±0.485 mg TAE/g and 20.17±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 3.61±0.053 mmol TE/g was seen in Tspr while the lowest value with 2.07±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 3.61±0.053 mmol TE/g and 3.15±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   | 20.409±0.443   | 0.768**        | 22.485±0.438   |
| Tspr   | 23.74±0.368    | 0.671          | 24.707±0.375   |
| Tsum   | 18.60±0.320    | 1.047**        | 21.64±0.335    |
| Taut   | 16.02±0.350    | 2.070±0.020    | 18.10±0.365    |
| Twin   | 15.78±0.165    | 2.806±0.015    | 18.61±0.375    |
| Ungma  | 5.02±0.340     | 0.865**        | 5.02±0.351     |
| Uspr   | 3.18±0.309     | 1.80±0.015     | 4.00±0.319     |
| Usum   | 1.64±0.309     | 2.236±0.015    | 3.87±0.319     |
| Uaut   | 1.48±0.315     | 2.181±0.020    | 3.67±0.325     |
| Uwin   | 1.48±0.315     | 2.181±0.020    | 3.67±0.325     |

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   | 2.56±0.023   | 3.61±0.053    |
| Tspr   | 2.24±0.011   | 3.14±0.050    |
| Tsum   | 1.92±0.025   | 2.90±0.015    |
| Taut   | 1.09±0.013   | 2.18±0.026    |
| Twin   | 3.33±0.036   | 3.56±0.040    |
| Usum   | 2.07±0.020   | 2.90±0.016    |
| Uaut   | 2.37±0.045   | 2.48±0.038    |
| Uwin   | 1.07±0.023   | 2.07±0.029    |

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         | FRAP         | Ungma         |
|                        | 0.953**      | 0.947**      | 0.956**       |
|                        | 0.887**      | 0.853**      | 0.865**       |
|                        | 0.899**      | 0.944**      | 0.972**       |
|                        | 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

SD: Standard deviation. Different ** in the same line indicates significant differences by Tukey’s test (p<0.05)

showed significant differences by Tukey’s test (p<0.05)

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 TPC, TFC, and TC in tea leaf extracts is indicated in Table 3. TF antioxidant capacity by DPPH and FR 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 (R²=0.953) in tea leaf extracts of Tuli and with TC (R²=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 (R²=0.947) and with TC (R²=0.793) in tea leaf extracts of Ungma.

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 polyphenols are powerful chain breaking antioxidants that can directly contribute to the work of others [31] and 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 physicochemical 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 flavonol [39]. Lasano et al. [40] indicated a great association of antioxidant activity with TPC and TFC in unfermented Strobilanthes crispata 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 leaves extracted in this study gave high TP, THC, 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.

REFERENCES

1. Wijeratne MA. Vulnerability of Sri Lanka tea production to global climate change. In: Water, Air, and Soil Pollution. Vol. 92. Berlin: Springer; 1996. p. 87-94.
2. Bizuayehu D, Atlabachew M, Ali MT. Determination of some selected secondary metabolites and their in vitro antioxidant activity in commercially available Ethiopian tea (Camellia sinensis). Springerplus 2016:5:412.
3. Chan EW, Soh EY, Tye PP, Law YP. Antioxidant and antibacterial properties of green, black, and herbal teas of Camellia sinensis. Pharmacognosy Res 2011;3:266-72.
4. McKay DL, Blumberg JB. The role of tea in human health: An update. J Am Coll Nutr 2002;21:1-3.
5. Hajimahmoodi M, Hanifeh M, Oveisi MR, Sadeghi N, Jannat B. Variation of total phenolic, antioxidant activity, and composition of fresh and processed tea leaves. J Food Sci Technol 2013;50:139-47.
6. Hara Y, Luo SJ, Wickremasinghe RL, Yamanishi T. Special issue on tea. Springerplus 2014;3:1-3.
7. Temsurenla Jamir was in charge of manuscript drafting. Temsurenla Jamir and Ajungla have equal contribution in concept development, sample collections, and data analysis.
8. Otherman A, Ismail A, Ghani NA, Adenan I. Antioxidant capacity and phenolic content of cocoa beans. Food Chem 2007;100:1523-30.
9. Fu L, Xu BT, Gan RY, Zhang Y, Xu XR, Xia EQ, et al. Total phenolic contents and antioxidant capacities of herbal tea and tea infusions. J Sci Food Agric 2011;5:282-94.
10. Tejapatri NA, Arsianti A, Qorina F, Fihrotuminisa Q. Photochemical analysis and antioxidant properties by DPPH radical scavenger activity of Ruellia brittoniana flower. Int J Appl Pharm 2019;11:24-8.
11. Chan EW, Lim YY, Chew YL. Antioxidant activity of Camellia sinensis leaves and tea from a lowland plantation in Malaysia. Food Chem 2007;102:1214-22.
12. Gosh D, Mondal S, Ramakrishna K. Photochemical profiling using LC-Q-TOF-MS analysis and in vitro antioxidant activity of a rare salt-secreting mangrove Aegialitis rotundifolia exb. Leaves extract. Int J Pharm Sci Pharm 2019;7:37-47.
13. Soares JR, Dinis TC, Cunha AP, Almeida LM. Antioxidant activities of some extracts of Thymus zygis. Free Rad Res 1997;26:469-78.
14. Rajasekar T, Shamya MA, Joseph J. Screening of phytochemical, antioxidant activity and anti-bacterial activity of marine seaweeds. Int J Pharm Sci 2019;1:61-6.
15. Higdon JV, Frei B. Tea catechins and polyphenols: Health effects, metabolism, and antioxidant functions. Crit Rev Food Sci Nutr 2003;43:89-143.
16. El Sheikh R, Amin SA, Gouda AA, Abdalla AH. Determination of phenolic components and antioxidant activity of some Egyptian tea samples. Int J Pharm Sci Nutr 2013;4:152-61.
17. Ouwor BO, Obanda M, Nyirenda HE, Mandala WL. Influence of region of production on clonal black tea chemical characteristics. Food Chem 2008;108:263-71.
18. Erncisi S, Othan E, Ozdemir O, Sengul M, Gungor N. Seasonal variation of total phenolic, antioxidant activity, plant nutritional elements, and fatty acids in tea leaves (Camellia sinensis var. Sinensis clone Derepazari 7) grown in Turkey. Pharm Biol 2008;46:683-7.
19. Ahmed S, Griffin TS, Kramer D, Schaffner MK, Sharma D, Hazel M, et al. Environmental factors variably impact tea secondary metabolites in the context of climate change. Front Plant Sci 2019;10:939.
20. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem 1999;65:555-9.
21. Singleton VL, Rossi J. Colorimetry of total phenolics with phospomolybdic acid. Am J Enol Vitic 1965;16:144-58.
22. Siddiqua A, Premakumari KB, Sultana R, Vithya V, Savitha S. Antioxidant activity and estimation of total phenolic content of Muntingia calabura by colorimetry. Int J Chem Tech Res 2010;2:205-8.
23. Benzie IF, Szeto YT. Total antioxidant capacity of tea by the ferric reducing/antioxidant power assay. J Agric Food Chem 1999;47:633-6.
24. Wong CC, Li HB, Cheng KW, Chen F. A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. Food Chem 2006;98:101-7.
25. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT Food Sci Technol 1995;28:25-30.
26. Lee LS, Kim SH, Kim YB, Kim YC. Quantitative analysis of major constituents in green tea with different plucking periods and their antioxidant activity. Molecules 2014;19:973-86.
27. Scabberl A, Johnston JT, Saltmarsh M. Polyphenols: Antioxidants and beyond. Am J Clin Nutr 2005;81:2155-78.
28. Othman A, Ismail A, Ghani NA, Adenan I. Antioxidant capacity and phenolic content of cocoa beans. Food Chem 2007;100:1523-30.
29. Fu L, Xu BT, Gan RY, Zhang Y, Xu XR, Xia EQ, et al. Total phenolic contents and antioxidant capacities of herbal tea and tea infusions. J Sci Food Agric 2011;5:282-94.
30. Tejapatri NA, Arsianti A, Qorina F, Fihrotuminisa Q. Photochemical analysis and antioxidant properties by DPPH radical scavenger activity of Ruellia brittoniana flower. Int J Appl Pharm 2019;11:24-8.
31. Chan EW, Lim YY, Chew YL. Antioxidant activity of Camellia sinensis leaves and tea from a lowland plantation in Malaysia. Food Chem 2007;102:1214-22.
32. Gosh D, Mondal S, Ramakrishna K. Photochemical profiling using LC-Q-TOF-MS analysis and in vitro antioxidant activity of a rare salt-secreting mangrove Aegialitis rotundifolia exb. Leaves extract. Int J Pharm Sci Pharm 2019;7:37-47.
33. Soares JR, Dinis TC, Cunha AP, Almeida LM. Antioxidant activities of some extracts of Thymus zygis. Free Rad Res 1997;26:469-78.
34. Rajasekar T, Shamya MA, Joseph J. Screening of phytochemical, antioxidant activity and anti-bacterial activity of marine seaweeds. Int J Pharm Sci 2019;1:61-6.
35. Higdon JV, Frei B. Tea catechins and polyphenols: Health effects, metabolism, and antioxidant functions. Crit Rev Food Sci Nutr 2003;43:89-143.
36. El Sheikh R, Amin AS, Atwa MA, Gouda AA, Abdalla AH. Determination of phenolic components and antioxidant activity of some Egyptian tea samples. Int J Pharm Sci Nutr 2013;4:152-61.
37. Erncisi S, Othan E, Ozdemir O, Sengul M, Gungor N. Seasonal variation of total phenolic, antioxidant activity, plant nutritional elements, and fatty acids in tea leaves (Camellia sinensis var. Sinensis clone Derepazari 7) grown in Turkey. Pharm Biol 2008;46:683-7.
38. Ahmed S, Griffin TS, Kramer D, Schaffner MK, Sharma D, Hazel M, et al. Environmental factors variably impact tea secondary metabolites in the context of climate change. Front Plant Sci 2019;10:939.
39. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem 1999;65:555-9.
40. Singleton VL, Stewart D, Brennan R, Hackett CA, McDougall GJ. Over-seas analysis of quantitative trait loci affecting
phenolic content and antioxidant capacity in raspberry. J Agric Food Chem 2012;60:5360-6.

44. Jing LJ, Mohamed M, Rahmat A, Bakar MF. Phytochemicals, antioxidant properties and anticancer investigations of the different parts of several gingers species (Boesenbergia rotunda, Boesenbergia pulchella var attenuata and Boesenbergia armeniaca). J Med Plant Res 2010;4:27-32.

45. Ramos-Escudero F, Morales MT, Asuero AG. Characterization of bioactive compounds from monovarietal virgin olive oils: Relationship between phenolic compounds-antioxidant capacities. Int J Food Prop 2015;18:348-58.

46. Piluzza G, Bullitta S. Correlations between phenolic content and antioxidant properties in twenty-four plant species of traditional ethnoveterinary use in the Mediterranean area. Pharm Biol 2011;49:240-7.