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Evaluation of *alpha* amylase inhibition activity, antioxidant capacities and caffeine content of commercially available ready to drink green tea sample in collected from Nepal

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The present study aim to evaluate the chemical constituents and biological activities of the commercially available green tea sample. High performance liquid chromatography equipped with UV detector was used to quantify the caffeine content in the tea extract. The caffeine content in the green tea sample range from 7.18 to 22.90 mg/g DW. Whereas, total phenolic, total flavonoids content varied from 9.19 to 11.41 mg GAE/g DW, 7.03 to 9.25 mg/g DW, respectively. Further, analyzed green tea extract revealed the range of *in vitro* antioxidant activities measured by DPPH with IC_{50} values from 53.46 to 64.54 µg/ml. Furthermore, evaluated tea extract were found to be potent *alpha* amylase inhibitors.

Keywords: Black tea, green tea, HPLC, caffeine, polyphenols, flavonoids.

Introduction

Tea is one of the most widely consumed beverage in the world[1] and is popular in Asian countries[2]. Hundreds of different brands of tea are now produced and distributed worldwide. Mostly tea is produced in Southeast Asia and Central Africa. Depending on the level of oxidation, teas are categorized into three types: green tea (unfermented), oolong tea (partially fermented) and black tea (fermented)[3]. Among them, green tea is one of the most popular and widely consumed dietary supplements in the world[4]. Numerous studies have recorded the beneficial effects of tea, which include antioxidant[5], anti-carcinoma[6], anti-inflammation[7], and antimicrobial properties[8]. Furthermore, tea are regarded as chemopreventive agent for toxic chemicals and carcinogens[9].

The chemical composition of green tea varies with genetic strain, climatic conditions, soil properties, plucking season, position of the leaf, processing and storage[10–12]. Mostly green tea contains the different secondary metabolites such as polyphenols, alkaloids (caffeine, theophylline and theobromine) as well as amino acids and several micro and macronutrients.

Among several secondary metabolites present in green tea, phenolic compounds are most interesting and bioactive molecules[13]. The major phenolic compounds in tea are flavan-3-ols (i.e. catechins) which include: (+)-catechin (C), (–)-epicatechin (EC), (–)-epigallocatechin (EGC), (–)-epigallocatechin gallate (EGCG), (–)-gallocatechins (GC), (–)-epicatechin gallate (ECG), and (–)-gallocatechin gallate (GCG)[14]. It has been considered that 12–15% catechin content considered as a good quality of tea. Catechin, are the major metabolites contributing to the flavor and astringent characteristics of tea. The composition of catechin varies with the process of fermentation, leaf type also with the harvesting season as well.

Furthermore, global diabetes cases are increasing each year and are one of the major health problems in the developing countries as well. The basic characteristics of diabetes is hyperglycemia i.e. uncontrolled high glucose level. The animal model study in diabetic mice revealed that green tea extract increases the insulin sensitivity and lower the blood glucose level[15]. It is evident that tea polyphenols involve in lowering the absorption of glucose in body by inhibiting the carbohydrate hydrolyzing enzymes such as pancreatic *alpha* amylase. Such a inhibition of *alpha* amylase by green tea extract delay the carbohydrate digestion, resulting in the reduction of glucose adsorption rate as well. Hence, the present study aims to quantify the major chemical constituent's and their biochemical properties as well as to

investigate the *alpha* amylase inhibition tendencies of the commercially available green tea.

Experimental

Collection of green tea sample and extraction:

The commercially available ready to drink green tea and black tea were purchased from the market. Each tea samples (20 g) was extracted successively using 200 ml of hot water. Filtration of individual extracts was done through Whatman No. 1 filter paper and Vacuum Evaporator (Hanil P201502902-1) used to evaporate the liquid solvents from the extract to acquire dehydrated extracts. Subsequently next to drying, crude extracts were weighed. Stock vials were used to store and kept in refrigerator (0–4°C) for further use.

Determination of total phenol content:

Total phenolic content estimation was carried out using Folin Ciocalteu's methods following the standard protocol[16,17]. Aliquots of 1 ml sample (10, 20, 40, 60, 80, 100 μg/ml) was placed into separate test tubes and followed by the addition of 0.5 ml of Folin Ciocalteu's reagent, 4.5 ml of distilled water and mixed well. Further, after 5 min, 4 ml of 7% sodium carbonate was added and blue color mixture was shaken well and incubated at 40°C in water bath and absorbance were recorded at 760 nm. The experiments were performed in triplicates. The blank was made using reagent blank with solvent. Standard gallic acid was used for the quantification of total phenolic content. The results were expressed as mg gallic acid equivalent/g dry weight of sample (mg GAE/g DW).

Determination of total flavonoid content:

Total flavonoids content was determined using aluminum chloride colorimetric assay following the standard protocol[18,19]. Quercetin was used as a standard for calculation to TFC. The 1 ml of sample aliquots solution (100, 200, 400, 600, 800, 1000 μg/ml) was added into separate test tubes, followed by the addition of 0.3 ml of 5% sodium nitrite solution, 4 ml of distilled water. After, 5 min 0.3 ml of 10% aluminum chloride was added and 2 ml of 1 *M* sodium hydroxide was added into the resultant solution and final volume is adjusted to 10 mL with distilled water. In this way prepared solution were mixed well until the yellowish color was developed. The absorbance was then measured at 510 nm spectrophotometer using UV-Visible instrument. Distilled water was used to perform blank and quercetin was used as a standard. The experiment was carried out in triplicates. The total flavonoids content was expressed as mg of quercetin equivalents/g of dry mass (mg QUE/g DW).

Free radical scavenging activity determination:

Free radical scavenging activity were measured using DPPH (2,2-diphenyl 1-picryl hydrazyl) as following the standard protocol[20,21]. Different concentrations of extracts (0.3 mL) were mixed with freshly prepared methanol solution of DPPH radicals (0.004% (w/v), 2.7 mL). In this way prepared solution was vigorous shaken and stored in dark for about 60 min (until stable absorption values were obtained). The range of reduction of the DPPH radical was measured by determining the absorption at 517 nm. For reference standard ascorbic acid was used and DPPH solution was used as the control.

Alpha amylase activity:

The *alpha* amylase inhibition assay was carried out using standard protocol[22,23]. The reaction mixture were prepared by mixing 0.2 *M* potassium phosphate buffer solution (pH 6.9), 1 % (w/v) starch solution in phosphate buffer saline (pH 7) and different concentrations of extract (200–1000 μg/ml) were added followed by the enzymatic digestion with 100 μL amylase (13 U/ml) solution. In this way prepared reaction mixture were incubated for 10 min at 37°C to proceed the hydrolysis reaction. Starch solution 2 ml was added and incubated at 37°C for another 10 min and 310 μL of the reaction mixture of each sample was poured to another test tube followed by addition of stop solution (HCl 0.1 *N*, 2 ml). Further iodine solution (5 mM I_2 and 5 mM KI, 500 μL) was added to the reaction mixtures and absorbance was recorded at 620 nm. As a control same reaction without extract were used. The results were expressed as mean±SD of triplicate experiments.

HPLC analysis:

The crude extracts were analyzed using a Shimadzu Prominence-i LC2030 equipped with an UV detector at 254 nm and C18 column (dimension 4.6×150 mm). The mobile phase were filtered using membrane filtration technique (PALL 66548 0.45 μm). The flow rate was 1 mL min^{-1} and the injection volume was 5 μL. For the determination of the caffeine isocratic condition were used with 1:1 ratio of methanol and water system.

Statistical analysis:

The experiment was designed in a completely randomized design (CRD) with three replicates and data so generated for different attributes was analyzed using Origin. The significance was determined via one-way ANOVA using SPSS.

Results and discussion

Tea extracts have been reported to have diverse phytonutrients. Major phytonutrients are phenolic and flavonoid. These results revealed that the green tea sample have the potential health benefits. Furthermore, different varieties of commercially available green tea were available in the market. The quality of the green tea depends on the harvesting time, fermentation condition as well as the quality of tea leaves. In this present study as a measure of the quality of the leaves, its total phenolic content, flavonoids content, antioxidant capacities, *alpha* amylase inhibitory activities as well as the amounts of caffeine content in the green tea sample collected from the markets were evaluated. The results were compared with the black tea sample. Quantitative determination of total flavonoids and phenolic content was determined as described in material and methods. The TPC and TFC of six tea sample were summarized in Table 1. Results revealed that five selected green tea sample revealed the range of TPC and TFC 9.02 to 11.41 mg GAE/g DW and TFC 7.03 to 9.17 mg QUE/g DW, respectively. Whereas, black tea content 9.65±0.53 mg GAE/g DW and 9.25±0.63 mg QUE/g DW, respectively. We observed the slight variation in the TPC and TFC content in black and green tea. Green tea sample have relatively higher amounts of phenolic and flavonoids content. Such as high abundance of phenolic and flavonoids in green tea sample signify the potential health benefits.

Furthermore, antioxidant tendencies of the commercial green tea extract were analyzed using DPPH radical scavenging activity as described in material and methods. The percentage inhibition of radical was found to increase with the increase in concentration of extract. The IC_{50} value (concentration required for 50% inhibition) of five green tea sample range from 46.94 to 63.29 μg/ml. Whereas, black tea sample has IC_{50} value of 64.54 μg/ml (Table 1). Our results revealed that green tea is relatively higher antioxidant activities than black tea (Fig. 1).

Furthermore, chemical components and its potential bioactivities depend on the harvesting time and the tea leaves as well as the storage condition.

The *alpha* amylase inhibition activity was performed as

Table 1. Total polyphenol content (TPC), total flavonoid content (TFC), antioxidant activity and *alpha* amylase inhibition assays of green tea extract in different solvent, the results were expressed in terms of gallic acid/g DW and quercetin/g of sample for three independent measurements (triplicate, n = 3, mean ± SD)

Green tea	TPC (mg GAE/g DW)	TFC (mg QUE/g DW)	DPPH antioxidant IC_{50} (μg/ml)	α-Amylase inhibition activity IC_{50} (μg/ml)
Sample 1 (A)	10.81±0.55	7.66±0.64	63.29 ±0.31	1355.51±3.42
Sample 2 (B)	11.41±0.17	9.17±0.54	63.39±0.26	707.07±2.14
Sample 3 (C)	9.29±0.14	6.06±0.23	57.89±0.16	695.40±2.37
Sample 4 (D)	9.02±0.25	8.85±0.36	46.94±0.04	394.31±2.40
Sample 5 (E)	9.19±0.43	7.03±0.24	53.46±0.06	549.53±3.53
Black tea (F)	9.65±0.53	9.25±0.63	64.54±0.25	1541.16±7.16

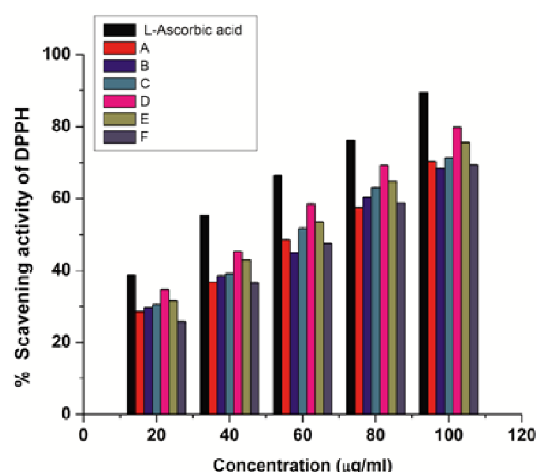

Fig. 1. DPPH radical scavenging activity of green tea extract. Where, A-E = green tea sample and F = black tea.

described in material and methods. Of the six tea crude water extract analyzed, Sencha tea was most effective in inhibiting the *alpha* amylase (Fig. 2). The green tea extract revealed the range of inhibitory activities against *alpha* amylase enzyme. The inhibitory activities of the green tea sample were concentration dependence. The higher the concentration higher the inhibitory activities. In our experiments at concentration of 1 mg/ ml of the tea extract, 77.71% of inhibition

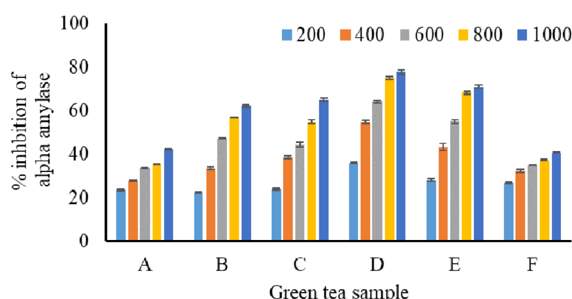

Fig. 2. Percentage *alpha* amylase inhibition activities of green tea extract. Where, A-E = green tea sample and F = black tea.

was recorded. The enzyme inhibitory capacities of the tea extract were expressed as IC_{50} (50% inhibition of *alpha* amylase) for all the tea extract. The lowest the IC_{50} values higher the inhibitory activities. Our result showed the range of inhibitory tendencies with IC_{50} values reange from 394.31±2.40 to 1355.51±3.42 µg/ml (Table 1). The smaller the IC_{50} value, the higher the inhibition percentage. Such a variation in the enzyme activities mostly depends on the quality of the tea leaves in different sample as well as its storage condition, climatic condition. Our results revealed that black tea has comparatively poor inhibitory activities against *alpha* amylase. The inhibitory activities of green tea is mostly because of the presence of diverse secondary metabolites such as catechin (C), epicatechin (EC), epicatechin gallate (ECG), gallocatechin (GC), epigallocatechin (EGC), and epigallocatechin gallate (EGCG) and several flavonoids like luteolin, genistein, daidzen. It is evident that tea catechin are mostly responsible for the enzyme inhibition. We believe that the amount of catechin in the different tea sample varies significantly, which is justifiable by our total phenolic and flavonoids results as well. Inhibition of *alpha* amylase should result in delayed digestion of carbohydrates and glucose absorption with reduction of postprandial hyperglycemic excursions.

HPLC method was used to quantify the caffeine content in tea crude extract. The compounds were identified based on the retention time and UV profile of the authentic compounds (Fig. 3). Quantification caffeine was performed

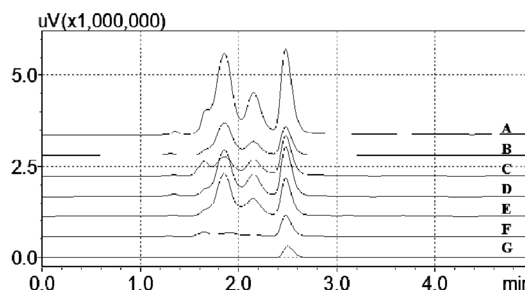

Fig. 3. HPLC chromatogram of green tea extract. Where, A-E = green tea, F = black tea and G = authentic caffeine.

through the external standard using calibration curve. The caffeine content of the green tea sample varies from 13.2 to 22.9 mg/g DW, whereas the black tea sample have 7.18 mg/g DW of caffeine (Fig. 4). Variation in the chemical components depends on the maturity of the leaves and harvesting time.

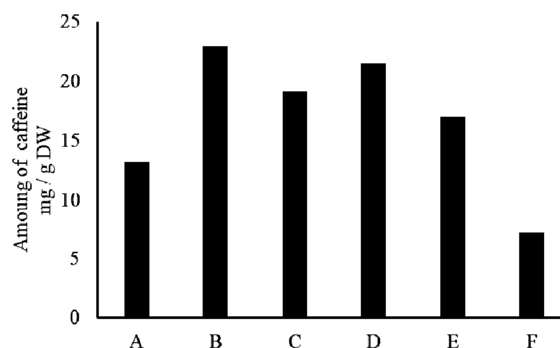

Fig. 4. Amount of caffeine in tea sample. Where, A-E = green tea, F = black tea.

Conclusion

There are different commercially available green tea sample available in market. However, its quality is often questionable. The quality of the green tea sample depends on the storage condition, quality of leaves used and processing techniques as well. The objective was to evaluate the biological significance of different green tea sample commercially available in the market. Our results revealed slight variation in TPC, TFC, antioxidant and *alpha* amylase inhibitory

activities. This might be due to quality of leaves used, harvesting time, processing technique as well as storage condition.

References

1. Y. F. Wang, J. Wang, J. Wu, P. Xu, Y. Q. Wang, J. J. Gao and D. Hochstetter, *J. Zhejiang Univ. Sci. B*, 2014, **15**, 173. doi: 10.1631/jzus.B1300186.
2. H. C. Lee, A. M. Jenner, C. S. Low and Y. K. Lee, *Res. Microbiol.*, 2006, **157**, 876. doi:10.1016/j.resmic.2006.07.004.
3. T. Yamamoto, L. R. Juneja, D. C. Chu and M. Kim, "Chemistry and applications of green tea", CRC Press, Boca Raton, 1997, pp. 109-121.
4. S. P. J. N. Senanayake, *Journal of Functional Foods,* 2013, **5**, 1529.
5. J. A. Vinson and Y. A. Dabbagh, *FEBS Lett.*, 1998, **433**, 44. doi: 10.1016/s0014-5793(98)00880-1.
6. Y. Miyata, T. Matsuo, K. Araki, Y. Nakamura, Y. Sagara, K. Ohba, and H. Sakai, *Medicines (Basel)*, 2018, **5**, 87. doi: 10.3390/medicines5030087.
7. P. Chatterjee, S. Chandra, P. Dey and S. Bhattacharya, *J. Adv. Pharm. Technol.* Res., 2012, **3**, 136. doi: 10.4103/2231-4040.97298.
8. W. C. Reygaert, *Front Microbiol.*, 2014, **5**, 434. doi: 10.3389/fmicb.2014.00434.
9. U. L. Lee and S. W. Choi, *ISRN Oncology*, 2011, **7**. http://dx.doi.org/10.5402/2011/403707.
10. L. S. Lee, S. H. Kim, Y. B. Kim and Y. C. Kim, *Molecules*, 2014, **19**, 9173. doi: 10.3390/molecules19079173.
11. S. Saklar, E. Ertas, S. Ibrahim *et al.*, *J. Food Sci. Technol.*, 2015, **52**, 6639. doi: 10.1007/s13197-015-1746-y.
12. S. Ahmed, S. T. Griffin, D. Kraner, M. K, Schaffner, D. Sharma, M. Hazel *et al.*, *Front Plant Sci.*, 2019, **10**, 939. doi: 10.3389/fpls.2019.00939.
13. J. Jin, H. Ying, M. Huang and Q. Du, *Pak. J. Pharm. Sci.*, 2015, 2267.
14. C. W. Reygaert, *BioMed. Research International*, 2018, 9. https://doi.org/10.1155/2018/9105261.
15. J. Yu, P. Song, R. Perry, C. Penfold and R. Ashley, *Diabetes Metab. J.*, 2017, **41**, 251. doi: 10.4093/dmj.2017.41.4.251.
16. B. P. Pandey, R. Thapa and A. Upreti, *Asian Pac. J. Trop. Med.*, 2017, **10**, 952.
17. S. A. Baba and S. A. Malik, *JTUSCI*, 2015, **9**, 449. https://doi.org/10.1016/j.jtusci.2014.11.001.
18. R. Ranjit, R. Shrestha, S. Paudel, J. Maharjan *et al.*, *Trop. J. Nat. Prod. Res.*, 2019, **3**, 265.
19. S. Chandra, S. Khan, B. Avula, H. Lata, M. Hye Yang *et al.*, *Evid. Based Complement Alternat Med.*, 2014, 9. http://dx.doi.org/10.1155/2014/253875.
20. T. Tong, Y. J. Liu, J. Kang, C. M. Zhang and S. G. Kang, *Molecules*, 2019, **24**, 2917. doi: 10.3390/molecules24162917.
21. R. Thapa, A. Upreti and B. P. Pandey, *J. Int. Ethnopharma.*, 2018, **7**, 66.
22. Q. Cheng, S. Cai, D. Ni, R. Wang, F. Zhou *et al.*, *J. Food Sci. Technol.*, 2015, **52**, 928. doi: 10.1007/s13197-013-1059-y.
23. A. Upreti, B. Byanju, M. Fuyal, A. Chhetri, P. Pandey, R. Ranjitkar and J. J. Bhatta, *eJTCM*, 2018, **9**, 312. https://doi.org/10.1016/j.jtcme.2018.07.001.