Prithvi Academic Journal
[A Peer-Reviewed, Open Access Multidisciplinary Journal]
Indexed in NepJOL with JPPS Star Ranking
ISSN 2631-200X (Print); ISSN 2631-2352 (Online)
URL: https://ejournals.pncampus.edu.np/ejournals/paj/

Centre for Research & Innovation
Prithvi Narayan Campus
Tribhuvan University
Pokhara, Nepal
http://pncampus.edu.np/

RESEARCH ARTICLE

Phytochemical Screening, Evaluation of Antioxidant and Antidiabetic Activities of Green Tea Available in Nepal

Surya Kant Kalauni, PhD[1], Jeetendra Karki[2], Menaka Sharma[3], Lekha Nath Khanal, PhD[4]

[1] Central Department of Chemistry, Tribhuvan University, Kirtipur, Nepal
[2]District Treasury Controller Office, Dhanusha, Nepal
[3]Independent Researcher Based in Kathmandu, Nepal
[4]Department of Chemistry, Prithvi Narayan Campus, TU, Pokhara, Nepal

Article History: *Submitted 31 January 2024; Reviewed 27 March 2024; Accepted 01 May 2024*
Corresponding Author: *Lekha Nath Khanal*, ***Email***: *lnkhanal@pncampus.edu.np*
DOI: *https://doi.org/10.3126/paj.v7i1.65744*

Copyright 2024 © The author(s). The publisher may reuse all published articles with prior permission of the concerned authors. This work is licensed under a Creative Commons Attribution 4.0 International (CC BY 4.0) License.

ScanToAccess eCopy

ABSTRACT

Green tea has gained popularity among consumers in recent years due to its several health benefits. It exhibits antioxidant properties, antidiabetic effects, and helpful to reduce cholesterol, stress, and anxiety. This study aims to identify the phytochemicals and assess the antioxidant and antidiabetic capacity of methanol extracts of green tea common in Nepal. The total phenolic and flavonoid contents were evaluated by Folin-Ciocalteu and aluminum chloride colorimetric methods. The antioxidant capacity was assessed using the 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging technique, while the starch-iodine method was employed to measure the α-amylase inhibition capability. Phytochemical screening showed the abundance of alkaloids, flavonoids, polyphenols, and carbohydrates in the tea. The total phenolic and flavonoid contents were 54.20 ± 1.41mg GAE/g and 91.0.50 mg QE/g respectively. The tea extract was found to show significant antioxidant and α-amylase inhibition capacities with half-maximal concentration (IC_{50}) values of 53.79 ± 3.93 and 66.47 ± 1.94 μg/mL respectively. The outcomes of this study ratify the advantages of green tea in the management of various health issues associated to free radicals and hyperglycemia.

KEYWORDS: α –Amylase, antioxidant, DPPH, green tea, starch-iodine method

INTRODUCTION

The practice of drinking tea is considered stimulating and health promoting from ancient times. Tea, a worldwide consumed and refreshing brew, is made by steeping hot water in extracts from the leaves, leaf nodes, and internodes of the tea plant, *Camellia sinensis* L. of the family Theaceae (KC et al., 2020). It is obvious that all teas originate from the leaves of the young tender plant. The variations in the manufacturing process, particularly the degree of fermentation that involves complex alterations in chemical

compositions, different brands of teas are designed. Green tea is manufactured with no fermentation, oolong tea is semi-fermented, and fully fermented and post-fermented teas are known as black tea and puerh tea, respectively (Kong et al., 2014).

A diverse range of teas are practiced by a broad spectrum of consumers worldwide. Several studies have revealed the order of antioxidant capacity of different teas as green tea>oolong tea >black tea (Nkubana & He, 2008). Biological activities of teas are influenced by different factors like brewing, temperature, addition of lemon, milk, ginger, honey, sugar, etc. However, evidence-based studies have offered contradictory results, highlighting the necessity for additional comprehensive research in this area (Bartoszek et al., 2018). Numerous bioactive compounds including alkaloids, carbohydrates, amino acids, proteins, etc. are abundant in tea. The stimulating as well as therapeutic properties of teas are due to the presence of a wide spectrum of biologically active compounds particularly polyphenols and catechins. Caffeine, theophylline, and theobromine are the well-known alkaloids, and (-) epicatechin gallate (ECG), (-)-epicatechin (EC), (-) epigallocatechin (EGC), and (-)-epigallocatechin gallate are the major catechins abundant in tea plants (Namita et al., 2012).

The contemporary literature provides a plenty of scientific evidence to support the health benefits of tea. Consumption of teas, particularly green and black teas, has been revealed beneficial to cardiovascular and metabolic disorders (Liu et al., 2024). The polyphenolic compounds in the teas are reported to exhibit antiaging, antidiabetic and several therapeutic benefits. The evidence of cancer-preventing effects of green tea has been established from the data of cell culture and other study reports on animal and human subjects (Khan & Mukhtar, 2013). The scientific community is increasingly attentive on exploring the effectiveness of tea and its extracts in managing diabetes, and cardiovascular complaints. A study has reported that tea can potentially lower blood sugar levels and safeguard pancreatic β-cells in diabetic mouse models, suggesting a potential risk reduction for type 2 diabetes (Fu et al., 2017). There are several meta-analysis, cohort, and case-control studies of green teas on the risk of type 2 diabetics. They have reported the precautionary and defensive properties of teas against diabetics on human subjects from the Unites States, China, Japan, and Vietnam (Meng et al., 2019).

Nepal is a landlocked country with numerous hilly districts, including Jhapa, Ilam, Panchthar, Dhankuta, and Bhojpur, where tea is a major cash crop. Jhapa, in particular, is well-known in the global market for producing high-quality tea having subtle and pleasant flavor. This loftier quality is supposed to be concomitant with the distinctive climate, elevation, soil, and processing expertise of the region. However, there is presently inadequate researches regarding the chemical profiling of leaves and tea types from various clones of the plant in the country (Dhakal, 2023). This study aims at the evaluation of antioxidant and antidiabetic potential by one of commonly used green teas in Nepal. The methanol extract of the tea is evaluated for antioxidant activity by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging, and α-amylase inhibitory activity by the iodine-starch method. The study highlights the biological usefulness of the Sencha green tea of Nepal in terms of antidiabetic and antioxidant competency.

MATERIALS AND METHODS

Preparation of Sample

Exactly 100 g of dry sample of green tea (Sencha tea) was bought from the local market of Kathmandu and ground into fine powder by using mortar and pestle. The powder was dipped into 300 mL of 80% methanol and was intermittently shaken for the

extraction. The process was repeated for three lots of solvents. The mixture was filtered and the filtrate was concentrated by using a rotatory evaporator to get semi-solid residue and stored in a refrigerator at 4ºC. The extraction yield was calculated by using the formula:

$$\%\,Yield = \frac{Mass\ of\ semisolid\ extract\ (g)}{Mass\ of\ dry\ plant\ material\ (g)}\ X\ 100$$

Phytochemical Screening

The phytochemical examination of methanolic extract of green tea was carried out following the procedure of (Iqbal et al., 2015; Shaikh & Patil, 2020; Tiwari et al., 2020). The test was performed for the detection of alkaloids, flavonoids, carbohydrates, reducing sugars, tannins, polyphenols, saponins, cardiac glycosides, steroids, and terpenoids.

Test for Alkaloids

The presence of alkaloids was detected by the Mayer's test and Dragendroff's test.

Mayer's Test. 1 mL of the extract was diluted with HCl and drops of Mayer's reagent were added. The formation of a white precipitate showed the presence of alkaloids.

Dragendroff's Test. Drops of Dragendroff's reagent were added to 2 mL of the extract solution. The formation of reddish-brown precipitate confirmed the presence of alkaloids.

Test for Saponins

About 1 g of the extract was boiled with 5 mL of distilled water in test a tube and filtered. The formation of foam on shaking the filtrate suggests the presence of saponins.

Test for Carbohydrates

The presence of carbohydrate was observed by Molisch Test. 2 drops of α-naphthol were added to 2 mL of extract solution, shaken well, and 1 mL of Con.H_2SO_4 was poured from the side of the test tube. The formation of a violet ring takes place.

Test for Reducing Sugars

The test for the presence of reducing sugars in methanol extract of the green tea was carried out by the following test methods.

Benedict's Test. 0.5 mL Benedict's reagent was boiled with 1 mL of the extract. The appearance of light orange or red precipitate by the reducing sugars.

Fehling's Test. 1 mL of Fehling's solution (Fehling A + Fehling B) was boiled with 1 mL of the extract. The formation of red precipitate shows the presence of reducing sugars in the extract.

Test for Flavonoids

The presence of flavonoids in the extract was detected by alkali test, lead acetate test, and Shinoda's test.

Alkali Test. A few drops of conc. NaOH were added to 1 mL of the extract. An intense yellow color that dissolves with HCl shows the presence of flavonoids.

Lead Acetate Test. A few drops of 10% lead acetate solution were added to 1 mL of the plant extract. A yellow precipitate indicates the presence of flavonoids.

Shinoda's Test. A small piece of Mg ribbon was put in 5 mL alcoholic solution of the extract and shaken with 1 mL of HCl. A pink to crimson color indicates flavonoids.

Test for Polyphenols

The presence of polyphenolic compounds in the methanol green tea extract was identified by ferric chloride test and lead acetate test.

Ferric Chloride Test. 2/3 drops of 10% $FeCl_3$ solution were added to the aqueous extract and shaken. The formation of blue or dark green color or precipitate shows the presence of phenolic compounds in the extract.

Lead Acetate Test. 2 mL of lead acetate solution was added to the aqueous solution of the extract. The formation of white precipitate takes place.

Test for Tannins

About 0.5 g of the extract was boiled with 3 mL of distilled water and filtered. The appearance of brownish green to black color on addition of few drops of $FeCl_3$ solution to the filtrate indicates the presence of tannins.

Test for Terpenoids

Salkowski's test was carried out to detect the presence of terpene compounds in the extract. 1 mL of the extract was shaken with 2 mL chloroform and 2 mL of Conc. H_2SO_4 and heated the test tube in water bath. A reddish-brown color indicates the terpenoids in the extract.

Test for Cardiac Glycosides

Keller-Kellani test method was performed to detect the presence of cardiac glycosides in the extract. About 1 mL of glacial acetic acid, and 1mL of 5 % $FeCl_3$ were added into 3 mL of the extract solution and conc. H_2SO_4 were introduced along the side of the test tube. The appearance of reddish brown or violet ring at the junction indicates cardiac glycosides.

Test for Steroids

Liberman Burchard test was performed to identify the presence of steroids in the green tea extract. About 2 mL of the extract was taken in a test tube and 1mL of acetic anhydride and 2 mL of conc. H_2SO_4 were added and mixed well. The formation of dark blue/green color indicated the presence of phytosterols in the extract.

Quantification of Total Phenolic and Flavonoid Contents

The total phenolic content (TPC) in the green tea extract was estimated by Folin-Ciocalteu method (Khanal et al., 2022a; Pawar & Dasgupta, 2018). Briefly, gallic acid solutions of 10, 20, 40, 60, 80, and 100 μg/mL were prepared from a stock solution (1 mg/mL) by dilution. Aliquots of 20 μL of the gallic acid solutions and the tea extract (5 mg/mL) were mixed with 100 μL of FCR solution (1:10 diluted with distilled water) and 80 μL of 1N Na_2CO_3 solution. The mixture was put in the dark at lab temperature for 30 minutes and the absorbance was measured at 765 nm by a microplate reader (Bio Tek Multimode reader, UK). Distilled water with FCR and Na_2CO_3 solutions in the same volumes were used as blank and the experiments were performed in triplicates. The TPC was calculated from the standard curve and expressed as mg GAE/g.

The total flavonoid content (TFC) in the green tea extract was calculated by using the aluminum chloride colorimetric method (Khanal, 2023; Makhubu et al., 2019). A standard curve was constructed by taking absorbance of quercetin at the concentrations 10-100 μL in methanol. For the green tea extract 20 μL of extract (5 mg/mL) was mixed with 110 μL of distilled water, 5 μL of 1M potassium acetate, 5 μL of 10% $AlCl_3$, and 60 μL of ethyl alcohol in the wells of microplate in triplicate. The mixture was slightly shaken and incubated in the dark for 30 minutes and the absorbance was measured at 415 nm against blank that contained all the components except the sample. The TFC in the extract was calculated from the calibration curve by regression analysis and presented as mg QE/g of the dry extract.

Antioxidant Activity

The antioxidant activity of methanol extract of green tea was evaluated by DPPH free radical scavenging method (Khanal et al., 2022b; Xiao et al., 2020). Briefly, the sample solutions of the extract and ascorbic acid of 250, 125, 62.5, 31.25, 15.6, and 7.8 μg/mL were prepared by serial dilution in 50% dimethyl sulphoxide (DMSO). Aliquots of 100 μL of the extracts and ascorbic acid were mixed with 100 μL DPPH solution in triplicates. The blank was prepared by putting the DPPH with the solvent. The mixtures were well-shake and incubated for about 30 minutes in the dark and the absorbance were recorded at 517 nm. From the data obtained the DPPH radical scavenging capacities of the extracts and standard were calculated. By using the following formula:

$$\%\, Scavenging\ capacity = \frac{Ab - As}{Ab} X100$$

A_b = Absorbance of blank, A_s = Absorbance of sample

From the plot of percentage scavenging capacity against concentrations, the concentration inhibiting 50% of the radical was calculated by regression analysis.

Antidiabetic activity

The α–amylase inhibition assay was performed by taking acarbose as positive control with slight modification (Kusano et al., 2011; Sudha et al., 2011). The undigested starch due to enzyme inhibition was detected at 630 nm (blue, starch-iodine complex) by using a spectrophotometer. Substrate was prepared by dissolving 500 mg starch in 25 mL of (0.4 M) NaOH by heating at100°C for 5 minutes. After cooling, pH was adjusted to 7.0 with 2N HCl and the final volume made up to 100 mL with distilled water. Aliquots of 40 μL of substrate solution were pre-incubated at 37°C for 3 minutes with 20 μL of acarbose or plant extract at varying concentrations (10, 20, 40, 80,160, and 640 μg/mL), followed by 20 μL of 3 U/mL α-amylase (20 mM phosphate buffer with 6.7 mM NaCl, pH 6.9), and incubated at 37°C for 15 minutes. Termination of the reaction was carried out by adding 80 μL of HCl (0.1 M). Finally, 100 μL of iodine reagent (2.5 mM) was added, and the absorbance was read at 630 nm using a 96-well plate reader.

The percentage of inhibition was calculated using the formula:

$$\%\, Inhibition = \{1 - \frac{(Abs2 - Abs1)}{(Abs4 - Abs3)} X\ 100\}$$

Abs1: Absorbance of the incubated mixture containing plant sample, starch and amylase.
Abs2: Absorbance of incubated mixture of sample and starch.
Abs3: Absorbance of the incubated mixture of starch and amylase.
Abs4: Absorbance of incubated solution containing starch only.

The concentration of the standard and the extract that inhibited 50% of the enzyme activity was calculated by the regression analysis.

RESULTS AND DISCUSSION

Phytochemical Screening, Estimation of TPC and TFC

Here, we used the cold maceration method for the extraction using 80 % methanol as the extracting solvent. The extraction was done for three successive steps with the solvent and the extraction yield was calculated. The yield of the extraction was 20% in this study. The quantity of the extract depends on several factors like extracting solvent, time, temperature, methods, etc. state of maturity of the plant material, etc. (Bakar et al., 2020).

Table 1

Results of Phytochemical Screening of Methanol Extract of Green Tea

S. No	Phytochemicals	Test	Results
1	Alkaloids	Dragendroff's test, Mayer's test	+++
2	Flavonoids	Alkali test, Shinoda test and lead acetate test	+++
3	Saponins	Froth test	-
4	Carbohydrates	Molisch test	+++
5	Reducing sugar	Benedict's test, Fehling's test	++
6	Polyphenols	Lead acetate test, $FeCl_3$ test	+++
7	Tannins	$FeCl_3$ test	+
8	Terpenoids	Salkowski's Test	-
9	Cardiac glycosides	Keller-Kellani test	++
10	Phytosterols	Liberman Burchard test	+

Note: (+++) = abundant, (++), (+) = trace presence, and (-) = absence

Here, we used the green tea that is prepared without fermentation of the green twig of the plant and the results of phytochemical screening are presented in Table 1. The results showed the abundance of alkaloids, flavonoids, polyphenols, and carbohydrates in prominent levels. On the other hand, reducing sugar, tannins, cardiac glycosides, and phytosterols were found in trace amounts. The extract did not give the positive test for the presence of saponins and terpenoids.

Several food supplements, chemical compounds, and pharmaceutical components are abundant in tropical medicinal plants. Similar to our results, the presence of phenols, flavonoids, and tannins were reported in alcoholic extract of black and green tea (Samadi & Fard, 2020). A wide spectrum of secondary metabolites including alkaloids, flavonoids, terpenoids, polyphenols, steroids, terpenoids, etc. abundant in the plants used in folk medicine exhibit significant antioxidant, antimicrobial, anticancer, antidiabetic, anti-inflammatory, etc. activities have been used for the development of novel drugs (Shazhni et al., 2018). Phenolic compounds have significant role in the management of oxidative stress and de novo metabolic disorders like cancer, diabetics, Alzheimer's disease, neurodegenerative diseases, etc. The estimation of these compounds was performed by one of the most common FCR method taking gallic acid as standard. The calibration curve of gallic acid was constructed with equation of straight line ($y = 0.015x + 0.0025$, $R^2 = 0.9929$). Table 2 shows that the methanol extract of the green tea has the TPC of 54.20 ± 1.41 mg GAE/g. Similarly, the equation of the quercetin calibration curve ($y = 0.018x + 0.0406$, $R^2 = 0.983$) was used to calculate the TFC in the green tea extract. The total flavonoid content was found to be 91.83 ± 0.50 mg QE/g. Our result is in agreement with the results of Indarti et al. (2019) where the green teas shows the significant amounts of phenolic and flavonoid compounds.

Table 2
Results of TPC TFC, Antioxidant and Antidiabetic Activities of Green Tea

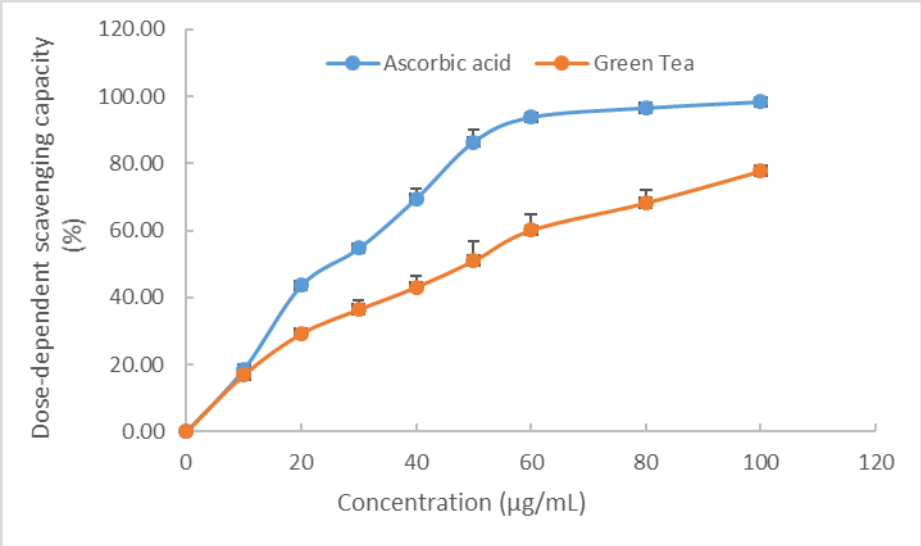
Sample	TPC (mg GAE/g)	TFC (mg QE/g)	DPPH test (IC_{50}) (µg/mL)	α-amylase test (µg/mL)
Green tea extract	54.20 ± 1.41	91.83 ± 0.50	53.79 ± 3.93	66.47 ± 1.94
Acarbose	-	-	-	39.12 ± 0.27
Ascorbic acid	-	-	31.14 ± 0.82	-

Note: All the values are mean ± SD (n=3)

Antioxidant Activity

The DPPH method is one of the popular methods of evaluating antioxidant capacity of the plant extracts by the scholars. It is fast, easy and gives a clear results on the basis of hydrogen atom or electron donating capacity of the antioxidant compounds in the plant (Kaewseejan et al., 2015). The dose-dependent variation of percentage scavenging capacity of the extracts with corresponding concentrations were compared to that of ascorbic acid. Figure 1 shows the positive relation of concentration with scavenging capacity of the green tea and ascorbic acid. The results of the study in Table 2 shows the half maximal concentration (IC_{50}) of green tea as 53.79 ± 3.93 μg/mL that is lower than that of ascorbic acid (31.14 ± 0.82 μg/mL). The antioxidant capacity of ethanol extract and its fractions of *Camellia sinensis* leaves was evaluated by the DPPH method.

Figure 1
Plot of DPPH radical scavenging capacity at different concentrations



The ethyl acetate fraction had the highest activity with IC_{50} values of 3.92 μg/mL followed by aqueous fraction 7.4 μg/mL and the lowest activity was shown by the extract 9.01 μg/mL (Indarti et al., 2019). The DPPH inhibiting capacity of black and green tea from Iran was compared with t-butylhydroquinone (TBHQ) by Samadi and Fard (2020) and reported the higher scavenging capacity of ethanol (89.53%) and water extracts (81.05%) than that of black tea.

The higher scavenging capacity is explained due to the higher proportions of tannins in alcoholic and aqueous extracts of the green tea. Similarly, various types of

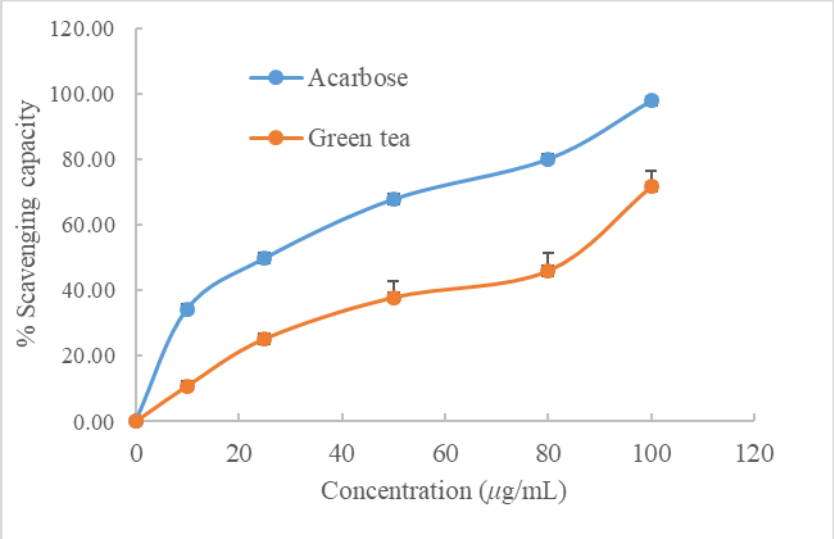
teas, including green tea, black tea, rosemary tea, lemongrass tea, and others from South Korea, were assessed to determine their total phenolic content, total flavonoid content, as well as antioxidant and antibacterial activities. Green tea exhibited the highest antioxidant activity among all the samples evaluated in the study. The antioxidant capabilities of the teas demonstrated a positive correlation with total phenolic content (TPC) and total flavonoid content (TFC), highlighting the potential impact of phenolic and flavonoid compounds on antioxidant capacity (Oh et al., 2013). The Traditional Matcha green tea of Japanese origin was found to exhibit higher antioxidant potential than the Daily Matcha and the activity was corroborated to the presence of flavonoids and polyphenols. The brewing process that involves the chemical changes of the bioactive compounds in the tea also affects the antioxidant potential.

Antidiabetic Activity

Intestinal α-glucosidases and pancreatic α-amylases are the two principal enzymes involved in the digestion of long chain carbohydrates into monomeric glucose that is easily absorbed in the blood stream. The inhibition of their activities interrupt the digestion of carbohydrates and increase the concentration of blood sugar leading to diabetes. Any substance or enzymes that can inhibit the activity of carbohydrates could be the potential candidate of therapeutic solutions against type 2 diabetes mellitus (Dedvisitsakul & Watla-iad, 2022). Here, we have evaluated the α-amylase inhibition capacity of the green tea extracts by iodine-starch method taking acarbose as standard. The dose-dependent variation of the extracts was compared with that of acarbose is shown in Figure 2. Both samples exhibited a direct relationship with concentration, and the curve of the green tea extract lies below the standard curve, suggesting a lower capability to hinder the enzyme's activity. The concentration corresponding to 50% inhibition of the enzyme (IC_{50}) shown Table 2 for green tea (66.47 ± 1.94 μg/mL) indicates a noteworthy ability to inhibit α-amylase activity, albeit to a lesser level compared to acarbose (39.12 ± 0.27 μg/mL). Our study reports are in agreement with several studies reporting the antidiabetic potential of green tea.

Figure 2

Dose-dependent scavenging capacity of α -amylase inhibition capacity



In another *in vivo* study on diabetic rats, the aqueous extract of a green tea (Puerh tea) significantly increased the glucose uptake by HepG2 cells and inhibited the

activity of porcine pancreatic amylase (PPA), sucrose and maltase (Du et al., 2012). The oral gavage feeding of alcoholic extract of green teas of Iranian origin on streptzotocin-induced diabetic rats at the dose of 200 mg/kg significantly reduced the serum glucose and hepatic malondialdehyde (MDA) concentration and total antioxidant capacity (TAC) (Haidari et al., 2013). The antioxidant and antidiabetic activities of two green teas namely Matcha and Sencha were evaluated together with their reverse phase high performance liquid chromatography (RP-HPLC) profiling of the polyphenols. They reported the higher antidiabetic potential of the Matcha tea on α-glucosidase inhibition assay (Rusak et al., 2021).

CONCLUSIONS

Phytochemical screening showed the presence of alkaloids, flavonoids, polyphenols, and carbohydrates in prominent levels. The extract found to exhibit significant antioxidant capability with an IC_{50} value of 53.79 ± 3.93 μg/mL in the DPPH method, and α-amylase inhibition capacity with an IC_{50} value of 66.47 ± 1.94 μg/mL. The findings of this research emphasize the potential benefits of incorporating green tea into one's diet for relieving oxidative stress and hyperglycemia. Moreover, it underscores the need for more extensive investigations using different solvents, methodologies, and *in vivo* studies. Such comprehensive research could shed light on the utilization of green tea in developing herbal therapeutics to address health complications associated with free radicals.

AUTHOR CONTRIBUTIONS

LNK: Concept design, data analysis, review and finalization of the manuscript. SKK: concept; development, data curation, finalization of the manuscript. JK and MS: lab work, data analysis, preparation of first draft.

ACKNOWLEDGEMENTS

Authors are thankful to the Central Department of Chemistry for providing lab facility and chemicals.

REFERENCES

Bakar, F. I. A., Bakar, M. F. A., Abdullah, N., Endrini, S., & Fatmawati, S. (2020). Optimization of extraction conditions of phytochemical compounds and anti-gout activity of *Euphorbia hirta* L. (Ara Tanah) using response surface methodology and liquid chromatography-mass spectrometry (LC-MS) analysis. *Evidence-Based Complementary and Alternative Medicine, 2020,* 1–13. https://doi.org/10.1155/2020/4501261

Bartoszek, M., Polak, J., & Chorążewski, M. (2018). Comparison of antioxidant capacities of different types of tea using the spectroscopy methods and semi-empirical mathematical model. *European Food Research and Technology*, *244*(4), 595–601. https://doi.org/10.1007/s00217-017-2986-z

Dedvisitsakul, P., & Watla-iad, K. (2022). Antioxidant activity and antidiabetic activities of Northern Thai indigenous edible plant extracts and their phytochemical constituents. *Heliyon*, *8*(9), 1–13. https://doi.org/10.1016/j.heliyon.2022.e10740

Dhakal, S. (2023). Economic analysis of tea farming in Jhapa District, Nepal. *Food & Agribusiness Management*, *4*(2), 71–76. https://doi.org/10.26480/fabm.02.2023.71.76

Du, W., Peng, S., Liu, Z., Shi, L., Tan, L., & Zou, X. (2012). Hypoglycemic effect of the water extract of Pu-erh tea. *Journal of Agricultural and Food Chemistry*, *60*(40),

10126–10132. https://doi.org/10.1021/jf302426w

Fu, Q. Y., Li, Q. S., Lin, X. M., Qiao, R. Y., Yang, R., Li, X. M., Dong, Z. B., Xiang, L. P., Zheng, X. Q., Lu, J. L., Yuan, C. B., Ye, J. H., & Liang, Y. R. (2017). Antidiabetic effects of tea. *Molecules*, *22*(5), 1–19. https://doi.org/10.3390/molecules22050849

Haidari, F., Omidian, K., Rafei, H., Zarei, M., & Shahi, M. M. (2013). Green tea (*Camellia sinensis*) supplementation to diabetic rats improves serum and hepatic oxidative stress markers. *Iranian Journal of Pharmaceutical Research*, *12*(1), 109–114.

Indarti, K., Apriani, E. F., Wibowo, A. E., & Simanjuntak, P. (2019). Antioxidant activity of ethanolic extract and various fractions from green tea (*Camellia sinensis* L.) leaves. *Pharmacognosy Journal*, *11*(4), 771–776. https://doi.org/10.5530/pj.2019.11.122

Iqbal, E., Salim, K. A., & Lim, L. B. L. (2015). Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of *Goniothalamus velutinus* (Airy Shaw) from Brunei Darussalam. *Journal of King Saud University - Science*, *27*(3), 224–232. https://doi.org/10.1016/j.jksus.2015.02.003

Kaewseejan, N., Sutthikhum, V., & Siriamornpun, S. (2015). Potential of *Gynura procumbens* leaves as source of flavonoid-enriched fractions with enhanced antioxidant capacity. *Journal of Functional Foods*, *12*, 120–128. https://doi.org/10.1016/j.jff.2014.11.001

KC, Y., Parajuli, A., Khatri, B. B., & Shiwakoti, L. D. (2020). Phytochemicals and quality of green and black teas from different clones of tea plant. *Journal of Food Quality*, *2020*, 1–13. https://doi.org/https://doi.org/10.1155/2020/8874271

Khan, N., & Mukhtar, H. (2013). Tea and health: Studies in humans. *Current Pharmaceutical Design*, *19*(34), 6141–6147. https://doi.org/10.2174/1381612811319340008

Khanal, L. N. (2023). Evaluation of antioxidant capacity of methanol extracts of leaf and stem bark of *Tinospora cordifolia*. *Prithvi Journal of Research and Innovation*, *5*, 102–113. https://doi.org/https://doi.org/10,3126/pjri.v5i1.60696 and

Khanal, L. N., Sharama, K., Pokharel, Y. R., & Kalauni, S. K. (2022a). Characterization of essential oil, estimation of phenolic and flavonoid content and biological activities of *Ephedra pachyclada* BOISS. *Journal of Institute of Science and Technology*, *27*(1), 27–35. https://doi.org/https://doi.org/10.3126/jist.v27i1.40590

Khanal, L. N., Sharma, K. R., Pokharel, Y. R., & Kalauni, S. K. (2022b). Phytochemical analysis and in vitro antioxidant and antibacterial activity of different solvent extracts of *Beilschmiedia roxburghiana* Nees stem barks. *Scientific World Journal*, *2022*, 1–7. https://doi.org/10.1155/2022/6717012

Kong, D., Wu, J., Sun, S., Wang, Y., & Xu, P. (2014). A Comparative study on antioxidant activity and Inhibitory potential against key enzymes related to type 2 diabetes of four typical teas. *Journal of Food and Nutrition Research*, *2*(9), 652–658. https://doi.org/10.12691/jfnr-2-9-20

Kusano, R., Ogawa, S., Matsuo, Y., Tanaka, T., Yazaki, Y., & Kouno, I. (2011). α-Amylase and lipase inhibitory activity and structural characterization of Acacia bark proanthocyanidins. *Journal of Natural Products*, *74*(2), 119–128. https://doi.org/10.1021/np100372t

Liu, B., Gu, S., Zhang, J., Zhou, H., Su, J., Wang, S., Sun, Q., Zhou, Z., Zhou, J., & Dong, C. (2024). Green tea consumption and incidence of cardiovascular disease in type 2 diabetic patients with overweight/obesity: A community-based cohort study. *Archives of Public Health*, *82*(1), 1–12. https://doi.org/10.1186/s13690-024-01242-3

Makhubu, F. N., Ashafa, A. O. T., Fouché, G., & Balogun, F. O. (2019). Phytochemical screening, free radical mitigation and antidiabetic potentials of *Pentanisia prunelloides* (Klotzsch ex Eckl. & Zeyh.) Walp. root extracts. *Journal of Food and Nutrition Research*, *7*(5), 391–401. https://doi.org/10.12691/jfnr-7-5-9

Meng, J. M., Cao, S. Y., Wei, X. L., Gan, R. Y., Wang, Y. F., Cai, S. X., Xu, X. Y., Zhang, P. Z., & Li, H. Bin. (2019). Effects and mechanisms of tea for the prevention and management of diabetes mellitus and diabetic complications: An updated review. *Antioxidants*, *8*(6), 1–25. https://doi.org/10.3390/antiox8060170

Namita, P., Mukesh, R., & Vijay, K. J. (2012). *Camellia sinensis* (green tea): A review. *Global Journal of Pharmacology*, *6*(2), 52–59.

Nkubana, A., & He, Q. (2008). A comparative study of antioxidant activity between black tea from Rwandan highlands with green and oolong teas from China. *International Journal of Food Safety, Nutrition and Public Health*, *1*(2), 159–166. https://doi.org/10.1504/IJFSNPH.2008.023016

Oh, J., Jo, H., Cho, A. R., Kim, S. J., & Han, J. (2013). Antioxidant and antimicrobial activities of various leafy herbal teas. *Food Control*, *31*(2), 403–409. https://doi.org/10.1016/j.foodcont.2012.10.021

Pawar, S. S., & Dasgupta, D. (2018). Quantification of phenolic content from stem-bark and root of *Hugonia mystax* Linn . Using RP-HPLC. *Journal of King Saud University - Science*, *30*(3), 293–300. https://doi.org/10.1016/j.jksus.2016.09.002

Rusak, G., Sola, I., & Bok, V. V. (2021). Matcha and Sencha green tea extracts with regard to their phenolics pattern and antioxidant and antidiabetic activity during in vitro digestion. *Journal of Food Science and Technology*, *58*(9), 3568–3578. https://doi.org/10.1007/s13197-021-05086-5

Samadi, S., & Fard, F. R. (2020). Phytochemical properties, antioxidant activity and mineral content (Fe, Zn and Cu) in Iranian produced black tea, green tea and roselle calyces. *Biocatalysis and Agricultural Biotechnology*, *23*, 1–6. https://doi.org/10.1016/j.bcab.2019.101472

Shaikh, J. R., & Patil, M. (2020). Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*, *8*(2), 603–608. https://doi.org/10.22271/chemi.2020.v8.i2i.8834

Shazhni, J. R. A., Renu, A., & Vijayaraghavan, P. (2018). Insights of antidiabetic, anti-inflammatory and hepatoprotective properties of antimicrobial secondary metabolites of corm extract from *Caladium hortulanum*. *Saudi Journal of Biological Sciences*, *25*(8), 1755–1761. https://doi.org/10.1016/j.sjbs.2018.03.013

Sudha, P., Zinjarde, S. S., Bhargava, S. Y., & Kumar, A. R. (2011). Potent α-amylase inhibitory activity of Indian Ayurvedic medicinal plants. *BMC Complementary and Alternative Medicine*, *11*(1), 5. https://doi.org/10.1186/1472-6882-11-5

Tiwari, S., Nepal, S., Sigdel, S., Bhattarai, S., Rokaya, R. K., Pandey, J., Khadka, R. B., Aryal, P., & Bhandari, R. (2020). Phytochemical screening , antibacterial - guided fractionation , and thin - layer chromatographic pattern of the extract obtained from *Diploknema butyracea*. *Pharmacognosy Research*, *12*(4), 437–443. https://doi.org/10.4103/pr.pr

Xiao, F., Xu, T., Lu, B., & Liu, R. (2020). Guidelines for antioxidant assays for food components. *Food Frontiers*, *1*(1), 60–69. https://doi.org/10.1002/fft2.10

To cite this article [APA style, 7th edition]:
Kalauni, S.K., Karki, J., Sharma, M., & Khanal, L.N. (2024). Phytochemical screening, evaluation of antioxidant and antidiabetic activities of green tea available in Nepal. *Prithvi Academic Journal, 7,* 9-19. *https://doi.org/10.3126/paj.v7i1.65744*