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Comparative evaluation of antioxidant, hypoglycemic and hypolipidemic potentials of Black tea from three major tea growing zones of India

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ABSTRACT

Tea is a very popular commercial crop and India is the world's largest consumer of tea in the world and the second largest producer of tea. Black tea is mostly preferred in Indian context and its multifaceted health benefits are being largely explored. This research article made a comparative study of antioxidant, hypoglycemic and hypolipidemic effect of Assam, Darjeeling and Nilgiri varieties of black tea. Research results have shown that Assam variety of tea has the highest antioxidant, hypoglycemic and hypolipidemic potentials followed by Nilgiri and Darjeeling variety.

INTRODUCTION

Tea is a very popular commercial crop, a worldwide popular beverage and being a source of several pharmacologically active molecules has currently attracted research limelight owing to its multifaceted pharmacologic actions. India is the world's largest consumer of tea in the world and the second largest producer of tea. As per historical records, the prevalence of tea drinking in India is since 750 BC [1]. Since 1947, India has approximately 563,980 hectares of land under tea cultivation and the largest tea cultivating states include Assam (304,400 hectares), West Bengal (140,440 hectares), Tamil Nadu (69,620 hectares) and Kerala (35,010 hectares). The versatile health aspects of tea are already being extensively studied [2, 3]. This research article focuses to study the antioxidant, hypoglycemic and hypolipidemic potentials of three different varieties of black tea (Darjeeling tea, Assam tea and Nilgiri tea) grown in three different states of India.

MATERIALS AND METHODS

Research material

Black tea of Darjeeling variety (DBT); Black tea of Assam variety (ABT); Black tea of Nilgiri variety (NBT)

Preparation of three different varieties of Black tea infusion

2 g of black tea of each variety was separately added to 100 mL of boiling water in three different containers and steeped for 15 min (ISO TC 34/SC 8; http://www.rsc.org). Extracts were filtered by Whatmann No.1 filter paper for removal of residues. Filtrates were centrifuged (Remi, R-8C Lab Centrifuge) at 5000 rpm for 20 min and the supernatants were collected, concentrated (rota evaporation) and dried in hot air oven (50 ^{o}C). Later extracts were scrapped and stored at –20 ^{o}C for future estimations [4].

Aqueous black tea extracts of three different varieties were coded as DBT for Darjeeling variety tea, ABT for Assam variety of tea and NBT for Nilgiri variety of tea.

In vitro antioxidant effect of three different varieties of Black tea infusion

DPPH radical scavenging

The antioxidant activity of three different tea samples (DBT, ABT and NBT) was determined on the basis of the scavenging effect on the stable DPPH free radical activity as per literature methods [5-7]. Briefly, 0.2 mM DPPH solution was prepared by dissolving 0.08 g of DPPH in methanol in a 100 mL standard flask and volume made up to mark with methanol. Next 1.5 mL of 0.2 mM DPPH solution and 1.5 mL of sample solutions in different concentrations were mixed. In another series 1.5 mL of different concentrations of sample solutions were mixed with 1.5 mL of methanol. All solutions were kept for 30 min at room temperature and allowed to react. Absorbance was read at 517 nm (5-7). Calculations were done basing on the equation:

$$\%\ \text{of scavenging activity} = \frac{(\text{Ab} + \text{As}) - \text{Am}}{\text{Ab}}$$

Where, Ab= absorbance of 1.5 mL DPPH+1.5mL methanol;

Am= absorbance of 1.5 mL DPPH+1.5 mL sample solution; As = absorbance of 1.5 mL sample solution +1.5 mL methanol. Plotting was done of percent inhibition *versus* concentration, and the concentration of sample required for 50% inhibition is regarded as IC_{50} value for each of the test samples.

ABTS assay

The assay was carried out according to literature with slight modifications [8-10]. The main principle is based on the ability of tea samples (DBT, ABT and NBT) to scavenge 2,2'-azino-bis (ethylbenzthiazoline-6-sulphonic acid or $ABTS^{+}$) radical cation. The radical cation was prepared by mixing 7 mM ABTS stock solution with 2.45 mM potassium per-sulfate (1/1, v/v), leaving the mixture for 4-16 h for completion of reaction and getting a stable absorbance. The $ABTS^{\cdot+}$ solution was diluted with ethanol to get an absorbance of 0.700±0.05 at 734 nm for measurements. The photometric assay was conducted on 0.9 mL of $ABTS^{\cdot+}$ solution and 0.1 mL of tested samples (100 and 200 μg/mL), mixed for 45 sec and measurements taken immediately at 734 nm after 15min. The anti-oxidative activities of the tested samples were calculated by determining the decrease in absorbance at different concentrations by using the equation:

$E = [(A_c - A_t)/A_c] \times 100$ where A_t and A_c are the respective absorbance of tested samples and $ABTS^{\cdot+}$ expressed as μM.

Total antioxidant capacity (FRAP assay)

Total antioxidant activity was determined by the FRAP assay as per the standard operating procedure laid down by literature with slight modifications [8-10]. The procedure is based on the reduction of ferric to ferrous form in the presence of antioxidants in the black tea infusion. The stock solutions included 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl and 20 mM $FeCl_3$; and 300 mM acetate buffer (pH 3.6). A 30 mM acetate buffer was prepared by dissolving 3.1g of sodium acetate in 16 mL glacial acetic acid and volume adjusted with distilled water to 1 L; 40 mM HCl was prepared by diluting 1.46 mL concentrated HCl (11 M) with distilled water upto 1 L; 10 mM TPTZ solution was prepared by dissolving 0.031 g TPTZ in 10 mL of 40 mM HCl. The working solutions were freshly prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ and 2.5 mL of $FeCl_3$. The temperature of the solution was raised to 37 $^{\circ}$C prior to use. 200 μL of tea samples were allowed to react with FRAP solution (2900–3000 μL) for 30 min in the dark. Absorbance of the colored product

formed (ferrous tripyridyl triazine complex) was recorded at 595 nm. Results were expressed in μM equivalent to $FeSO_4$ by extrapolation from the calibration curve.

In vitro hypoglycemic studies of three different varieties of Black tea infusion

Alpha amylase inhibitory assay

The study was carried out following standard literature methodologies using starch azure in phosphate buffer containing calcium chloride as the substrate [11, 12]. Dilutions of DBT, ABT and NBT were prepared using 1mL of 0.1% dimethyl sulfoxide (DMSO). Next, 0.1 mL of porcine pancreatic amylase in tris-HCL buffer (2 units/ mL) was added to the tube containing dilutions and substrate at 37°C. The absorbance was measured at 595 nm. Acarbose (A_{car}) in the concentration range of 1.25, 2.5, 5, and 10 µg/mL in distilled water was used to create the calibration curve. The assay was performed in triplicate and the inhibitory potentials are expressed in terms of IC_{50} values. Absorbance was calculated using the formula:

$$\alpha\,\text{amylase activity} = \frac{[(Ac+)-(Ac-)-(As-Ab)]}{[(Ac+)-(Ac-)]} \times$$

Alpha glucosidase inhibitory assay

The assay procedure was developed as per literature [11, 12]. Yeast α-glucosidase enzyme solution was prepared by dissolving at a concentration of 0.1U/mL in 100 mM phosphate buffer, pH 7.0, containing bovine serum albumin and sodium azide which was used as enzyme source. This enzyme solution was added to test samples in increasing concentrations (1, 1.5, 2, 2.5, 3, 3.5 µL mL^{-1}). The reaction was initiated by adding 0.20 mL of para-nitro phenyl-α-D-gluco-pyranoside solution (pNPG); 2mM pNPG in 50 mM sodium phosphate buffer pH 6.9) which acted as the substrate. The reaction was terminated by adding 1 mL 0.1 M Na_2HPO_4. The α-glucosidase inhibitory activity was determined at 405nm by measuring the quantity of *p*-nitro phenol released from pNPG. The assay was performed in triplicate and the inhibitory potentials are expressed in terms of IC_{50} value. Acarbose (A_{car}) was dissolved in distilled water to prepare a series of dilutions (1.25, 2.5,5,10 mg/ml) and was used as the positive control. The percent inhibition was calculated according to the formula:

$$\%\,\text{inhibition} = \frac{\text{Abs400 control} - \text{Abs400 extract}}{\text{Abs400 control}}$$

In vitro hypolipidemic studies of three different varieties of Black tea infusion

Pancreatic lipase inhibitory assay

The hypolipidemic properties of DBT, ABT and NBT were studied by *in vitro* lipase inhibitory activity by evaluating the hydrolysis of pNP laurate and recorded spectrophotometrically at 400 nm as per the literature methodologies [13-15]. Porcine pancreatic lipase dissolved in distilled water (10 mg/mL), centrifuged at 8000 rpm for 10 min and the supernatant was used for experimentation. 100 mM Tris HCl (pH 8.2) as the assay buffer and pNP laurate was used as substrate (0.08% w/v of pNP laurate dissolved in 5mM sodium acetate buffer, pH 5.0, containing 1% Triton X-100, heated to boiling and then cooled to room temperature). The assay was carried out in a total volume of 1000 µL, containing 300 µL Tris-HCl buffer, 450 µL substrate solution, 100 µL of extracts in different concentrations and 150 µL of lipase. The blank consisted of 400 µL assay buffer, 450 µL substrate solution and 150 µL of lipase. After mixing with test extracts, lipase and substrate, the mixtures were incubated at 37ºC for 30 min, heated in boiling water bath for 10 min to stop the reaction. After centrifugation of the mixture for 10 min at 8000 rpm, the readings were recorded against blank at 400 nm.

RESULTS AND DISCUSSIONS

The comparative antioxidant potentials of three different varieties of black tea (DBT, ABT, and NBT) have been provided in Table 1 and Figure 1-2. As evident from experimental results, Assam variety of tea (ABT) exhibited highest antioxidant potencies followed by Nilgiri (NBT) and Darjeeling variety (DBT).

Table 1. DPPH radical scavenging activity of three different varieties of Black tea

Variety	50 μg/mL	100 μg/mL	200 μg/mL	300 μg/mL
DBT	14.14±1.36	30.36±1.95	63.27±1.51	64.81±1.24
ABT	17.04±2.32	34.87±1.34	66.52±3.19	69.45±1.21
NBT	15.94±2.61	32.27±2.24	64.89±2.56	66.59±2.34

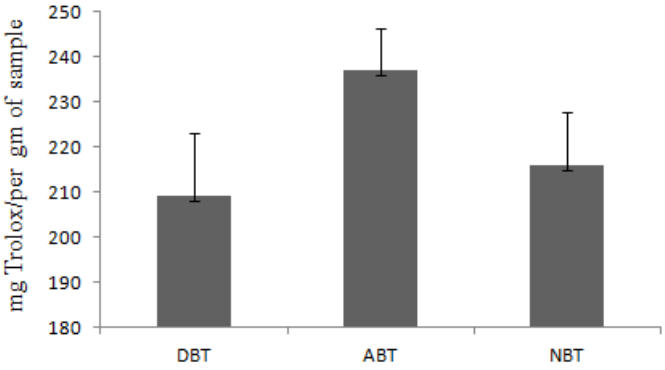


Figure 1. Comparative histogram of ABTS assay for three different varieties of black tea

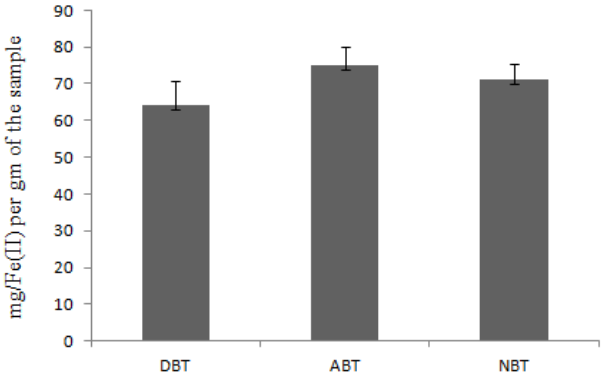


Figure 2. Comparative histogram of FRAP assay for three different varieties of black tea

The comparative hypoglycemic effect of three different varieties of black tea studied by alpha amylase and alpha glucosidase inhibitory effect have been presented in Figure 3-4 and the hypolipidemic effect in Figure 5. Considering the hypoglycemic and hypolipidemic effect of three different varieties of black tea produced in three different zones of India presented in terms of IC_{50} values, Assam variety tea exhibited highest hypoglycemic and hypolipidemic potentials (Figure 3-5) followed by Nilgiri and Darjeeling variety.

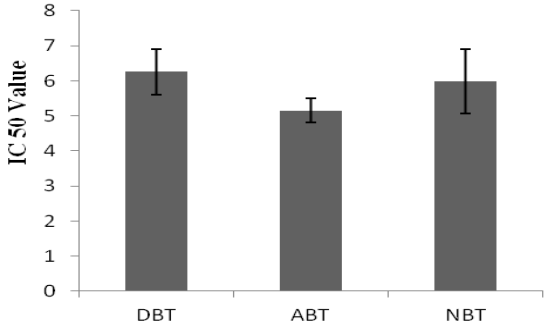


Figure 3. Comparative histogram of hypoglycemic effect of three different varieties of black tea studied by pancreatic alpha amylase inhibitory assay

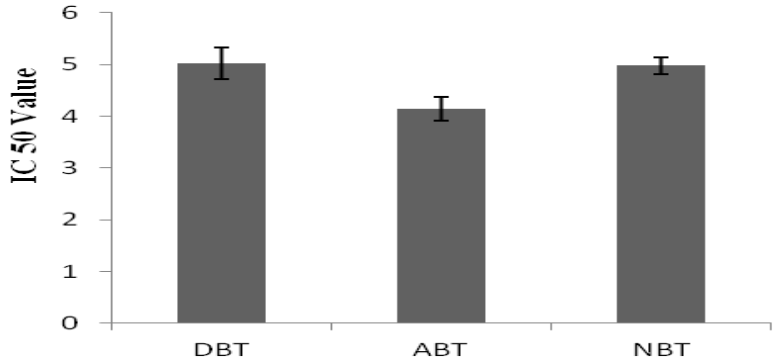


Figure 4. Comparative histogram of hypoglycemic effect of three different varieties of black tea studied by pancreatic alpha glucosidase inhibitory assay

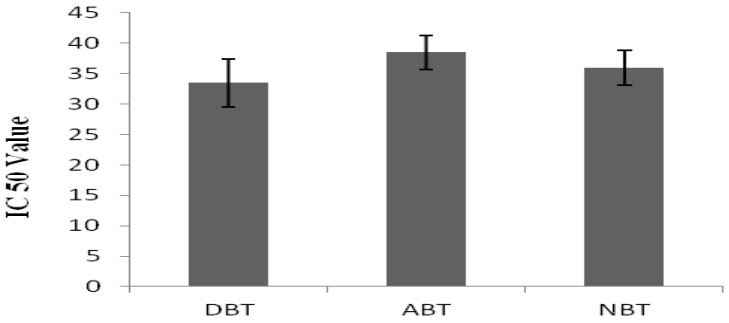
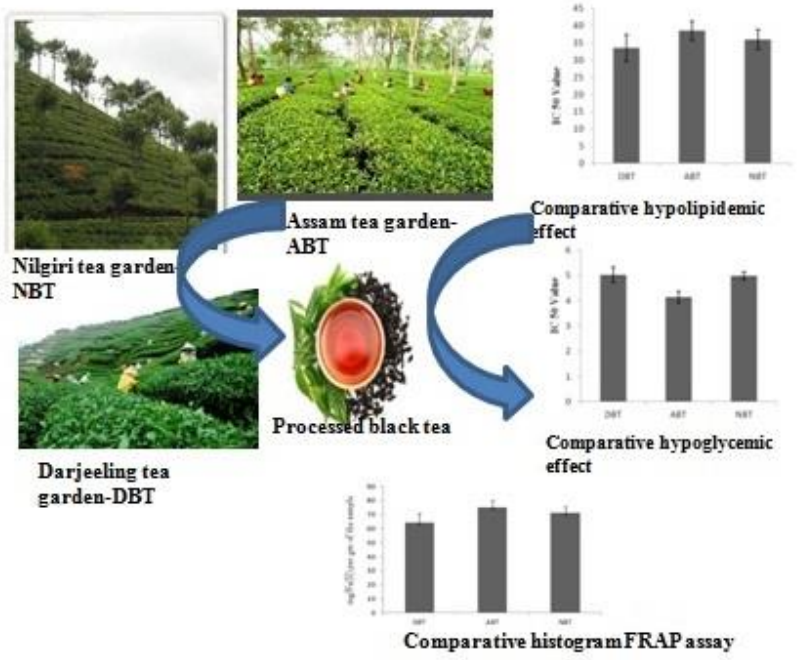


Figure 5. Comparative histogram of hypolipidemic effect of three different varieties of black tea studied by pancreatic lipase inhibitory assay



Tea is a source of several potent pharmacologically active molecules including the dietary poly phenols, benzotropolone compounds viz. theaflavins (3-6%), thearubigins (12-18%), small amounts of theaflagallins, methyl xanthenes like caffeine, theobromine, theophylline, flavonol glycosides viz. myricetin, kaempferol, quercetin, phenolic acids, amino acids viz. L-theanine; carbohydrates, proteins and minerals like Cr, Zn, Se, Mn etc [2, 16-18]. Geographical distributions, climatic changes, soil variations greatly influences the concentrations of the secondary metabolites which in turn is responsible for the variations in pharmacological profile of plants in general and here tea in particular [19, 20].

CONCLUSION

Black tea is a widely accepted and largely consumed beverage in Indian context. India earns a huge amount of foreign exchange due to tea export. This paper made a comparative study of health aspects of three different varieties of tea grown in three different regions of India.

Author's contributions

S.K. and B.D. developed the central theme of the research article; K.B. carried out the experimentations in consultations with P.K. and G.S. The manuscript was written by S.K. and B.D. with the valuable suggestions and inputs of other three authors.

Conflict of interests

No conflict of interest exists.

REFERENCES

[1]. https://www.teacoffeespiceofindia.com/tea/tea-origin
[2]. Sen, G.; Bera, B. Black tea as a part of daily diet: A boon for healthy living. IJTS *9(2–3)*, 2013, 51-59.
[3]. Skotnicka, M.; Chorostowska-Wynimko, J.; Jankun, J.; Skrzypczak-Jankun, E. The black tea bioactivity: an overview. Cent. Eur. J Immunol. *36(4)*, 2011, 284-292.
[4]. Bhandari, K.; De, B.; Adiki, S.K.; Katakam, P.; Saha, G.; Mitra, A. Safety profiling of black tea extract and its ameliorative effect in alleviating oxidative stress, neurotransmitter depletion and cognitive performance in colchicine induced Alzheimer rats. IJOPILS *5(4)*, 2017, 86-107.
[5]. Shalini, D.; Sudha, G. Antioxidative activity of various tea extracts (*Camellia sinensis*). J. Bio. Sci. Res. *1(1)*, 2010, 271-278.
[6]. Banu, S.S.; Majula, A.S.; Latha, M.T.; Ravi, T.K. De Novo drug design and synthesis of certain Indole derivatives and screening for their Xanthine oxidase inhibitory activity. Int. J. Pharm. Tech. Res. *2(4)*, 2010, 2128-2138.
[7]. Shonisani, N. Effect of brewing temperature and duration on quality of black tea (*Camellia sinensis*) and equal combination of bush tea (*Athrixia phylicoides* DC) and black tea. Mini Dissertation, University of Limpopo 2010.
[8]. Taheri, M.; Sairi, R.; Giahi, M.; Ghafoori, H. Screening antioxidant activity of extracts from different tea samples. Pharmacologyonline *2*, 2011, 674-683.
[9]. Xu, J.Z.; Yeung, S.Y.V.; Chang, Q.; Huang, Y.; Chen, Z.Y. Comparison of antioxidant activity and bioavailability of tea epicatechins with their epimers. J. Nutri. *91*, 2004, 873-881.
[10]. Patel, G.; Gauni, B.; Mehta, K.; Patel, B.N. Comparative study of antioxidant capacity of raw powder and waste black tea by FRAP assay. Res. J. Recent. Sci. *3*, 2014, 42-44.
[11]. Subramanian, R.; Azmawi, Z.M.; Sadikun, A. *In vitro* α-glucosidase and α-amylase enzyme inhibitory effects of *Andrographis paniculata* extract and andrographolide. Acta. Biochimica. Polonica. *55(2)*, 2008, 391-398.
[12]. Basak, S.S.; Candan, F. Chemical composition and *in vitro* anti-oxidant and anti diabetic activities of *Eucalyptus camaldulensis* Dehnh, Essential oil. J. Iran. Chem. Soc. *7(1)*, 2010, 216-226.
[13]. Inthongkaewa, P.; Chatsumpunb, N.; Supasuteekul, C.; Kitisripanyac, T.; Putalunc, W.; Likhitwitayawuida, K.; Sritularaka, B. Glucosidase and pancreatic lipase inhibitory activities and glucose uptake stimulatory effect of phenolic compounds from *Dendrobium formosum*. Rev. bras. farmacogn. *27*, 2017, 480–487.
[14]. Chedda, U.; Kaikini, A.; Bagle, S.; Madhav, S.; Sadhana, S. *In vitro* pancreatic lipase inhibition potential of commonly used Indian spices. IOSR. J. Pharm. 6(10), 2016, 10-13.

[15]. Senapaty, S.; Yadav, R.P.; Paul, N.S.; Chavan, R. Evaluation of *In-Vitro* Pancreatic Lipase Inhibitory Activity of Fenugreek Seeds Extracts. Int. J. Pharm. Sci. Health Care *4(4)*, 2014, 1-7.

[16]. Li, S.; Lo, C.Y.; Pan, M.H.; Lai, C.S.; Ho, C.T. Black tea: chemical analysis and stability. Food Funct 2013, 10–18.

[17]. Luczaj, W.; Skrzydlewska, E. Antioxidant properties of black tea. Prev. Med., 40(6), 2005, 910-918.

[18]. Sharangi, A.B.; Siddiqui, M.D.W.; Aviña, J.E.D. Black Tea Magic: Overview of Global Research on Human Health and Therapeutic Potentialities. JTSR. *4(1)*, 2014, 1-16.

[19]. Skotnicka, M., Chorostowska-Wynimko, J., Jankun, J., Skrzypczak-Jankun, E. The black tea bioactivity: an overview. Cent. Eur. J. Immunol. 36(4), 2011, 284-292.

[20]. Ahmed, S.; Stepp, J.R.; Orians, C.; Griffin, T.; Matyas, C.; Robbat, A.; Cash, S.; Xue, D.; Long, C.; Unachukwu, U.; Buckley, S.; Small, D.; Kennelly, E. Effects of Extreme Climate Events on Tea (*Camellia sinensis*) Functional Quality Validate Indigenous Farmer Knowledge and Sensory Preferences in Tropical China. PLoS One, *9(10)*, 2014, e109126.