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Research

Evaluation of antioxidant, acetylcholinesterase, lipase, α-amylase, xanthine oxidase, and α-glucosidase enzyme inhibitory activities of Sri Lankan tea cultivars

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Abstract
Tea, from *Camellia sinensis*, is second only to water in global consumption. Tea germplasm in Sri Lanka consists of over 600 tea cultivars, and currently, 70 of them are recommended for commercial tea cultivation. However, the biological activities of Sri Lankan tea cultivars have not been documented. The present study investigated the in vitro enzyme inhibitory activities, viz., lipase, α-amylase, xanthine oxidase, α-glucosidase, and acetylcholinesterase, as well as antioxidant activity in black tea and tea leaves harvested from 15 different tea cultivars during the major seasons in Sri Lanka. The remarkable α-glucosidase, xanthine oxidase, and acetylcholinesterase inhibitory activities in black tea and tea leaves of tea cultivars were observed, with the IC_{50} of black tea ranging from 5.77 to 104.10 ppm, 0.55 to 36.75 ppm, and 28.37 to 184.06 ppm, respectively. Additionally, some tea cultivars have exhibited moderate α-amylase and lipase inhibitory activities. The radical scavenging activities of DPPH (1,1-diphenyl-2-picrylhydrazyl) and nitric oxide on black tea and tea leaves were exhibited with the IC_{50} of black tea ranging from 14.03–67.23 ppm and 76.22 to 251.35 ppm, respectively.

Keywords Antioxidant activity · Black tea · Enzyme inhibitory activities · Tea cultivars · Tea leaves

1 Introduction

Tea (*Camellia sinensis*) is second only to water in global consumption. Young tea shoots(bud and the first two leaves) produce various kinds of tea based on the tea manufacturing techniques [1]. Tea contains many phytochemicals, such as alkaloids, polyphenols, saponins, sugars, pigments, and amino acids. Additionally, there have been many research studies that have shown the importance of these phytochemicals in maintaining human health [2]. Furthermore, tea leaves contain polyphenols up to 35%, 2.5–4.0% of caffeine, and 1–3% of theanine based on their dry weight [3]. Over the past few decades, numerous investigations have been conducted to emphasize the positive health effects of tea, including antioxidant properties and anti-inflammatory, weight loss, relief from metabolic syndrome, a lower risk of non-communicable diseases, and cognitive enhancement [4].

Globally, diabetes and Alzheimer's diseases are significant health issues. These two degenerative diseases deteriorate with age and are linked with multiple conditions. Progressive memory loss, cognitive impairment, and personality changes are the symptoms of Alzheimer's disease. The inadequate amount of the neurotransmitter acetylcholine near

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the synaptic cleft is the major reason for Alzheimer's disease. Acetylcholine is inhibited by the acetylcholinesterase (AChE) enzyme. Therefore, the availability of acetylcholine could be increased using the AChE inhibitors, thereby facilitating nerve impulse transmission. Recently, Alzheimer's disease has been treated with AChE inhibitors at a symptomatic stage. Nevertheless, in certain instances, employing AChE inhibitors in conjunction with diet could be beneficial in treating Alzheimer's disease [5, 6]. Extensive research studies revealed that Alzheimer's disease is strongly related to diabetes. Diabetes, obesity, and oxidative stress are some of the modifiable risk aspects for Alzheimer's disease, according to recent studies [5].

The phytochemicals, including polyphenolic compounds, have been identified as possessing glycemic regulatory, xanthine oxidase (XO) inhibitory activity, and anti-inflammatory and antioxidant activities that allow for potential treatment for Alzheimer's and gout disease, type 2 diabetes mellitus (T2DM) and obesity. Furthermore, animal studies have suggested that bioactive polyphenols may be directly involved in reducing obesity and hyperglycemia [7, 8]. Consequently, a large number of phytochemicals have been evaluated to investigate their potential use as a preventive measure [9, 10]. In consideration of the interconnections of Alzheimer's disease, obesity, and T2DM, common herbal therapeutic and preventive measures are productive. Polyphenols in black tea exhibited anti-obesity activities by lipid digestion and inhibition of sugar, thereby reducing the calorie intake. Additionally, these polyphenols can attenuate the process of lipogenesis while at the same time enhancing lipolysis and decreasing lipid accumulation [11]. The national germplasm of tea belongs to the Tea Research Institute (TRI), Sri Lanka and currently maintains about 600 accessions, and the diversity of phytochemicals present among them has been documented [12]. Nevertheless, research studies on the health effects of various tea cultivars remain inadequate. Thus, taking into account the health benefits of various tea cultivars, the goal of this study was to encourage Ceylon tea on the international market. Therefore, in this study, the potential health benefits in relation to glycemic regulation, anti-obesity activities, xanthine oxidase, and acetylcholinesterase inhibitory activities of tea cultivars were evaluated in two major seasons.

2 Material and methods

2.1 Chemicals

α-Glucosidase (*Saccharomyces cerevisiae*), lipase (*Candida rugosa*), acetylcholinesterase (*Electrophorus electricus*), α-amylase (porcine pancreas), xanthine oxidase (Bovine Milk), 1,1-diphenyl-2-picrylhydrazyl, p-nitrophenyl butyrate, para-nitrophenyl-α-D-glucopyranoside, sodium potassium tartrate, acetylthiocholine iodide, 3,5-dinitro salicylic acid, 5,5′-Dithiobis(2-nitrobenzoic acid), xanthene, sodium nitroprusside, ascorbic acid, Griess reagent, dimethyl sulfoxide, disodium monohydrogen orthophosphate, sodium chloride, glacial acetic acid, donepezil hydrochloride, hydrochloric acid, monosodium dihydrogen orthophosphate, sodium bicarbonate, acetonitrile, methanol, sodium acetate trihydrate, and sodium hydroxide were purchased from Germany (Sigma Aldrich). Orlistat, acarbose, and allopurinol tablets were purchased from Orchid Health Care, India, and MP-Biomedicals, USA. Spectrophotometric readings were taken on a microplate reader (EPOC T, Germany).

2.2 Preparation of tea extracts/samples

Young shoots of tea accessions/cultivars were harvested from high elevations (≥ 1200 m) during the dry and wet seasons in the germplasm at the Tea Research Institute of Sri Lanka. Dry season: Months-January–March and August–September; Average temperature: 28–30 °C; Average rainfall: less than 100 mm; Sunshine duration: 7 to 8 h. Wet season: Months-April–July and October–December; Average temperature: 20–22 °C; Average rainfall: more than 150 mm; Sunshine duration: 4.9 to 6.4 h. Tea leaves of each tea cultivar (150 g) were freeze-dried, and powdered, and 10 g of tea sample was used to prepare methanol extract. Methanol (100 mL) was added to the freeze-dried tea samples, sonicated for 15 min, and evaporated using a rotary evaporator (EYELA, USA). From the same batch of fresh tea leaves (500 g of each sample), 100 g of black tea was prepared using a miniature manufacturer facility at the Biochemistry Division, TRI. According to ISO 3103:2019, a standard black tea cup was prepared by weighing 2 g of black tea accurately, adding 100 mL of boiled water, and brewing for 5 min (ISO 3103:2019 -Preparation of tea liquor for use in sensory tests). The filtered tea brew was freeze-dried. These extracts were employed for further biological analysis. Conditions of miniature manufacture system of black tea (Teacraft, UK): withering: the difference between wet and dry bulb: 4 °C, relative humidity: 75%, rolling: orthodox roller (15 min), maceration: twice in roller, sieving: mesh no. 8 (2.057 mm), fermentation: cupboard, dry bulb

(25 °C), wet bulb (25 °C), relative humidity (100%), drying: fluid bed dryer, inlet temperature (125 °C), outlet temperature (95 °C), duration (18–21 min), removal of fiber: fiber mat.

2.3 Quantification of total polyphenol content

Samples of tea weighing 0.200 ± 0.001 g were measured and transferred into 10 mL tubes along with 5 mL of 70% hot methanol. Samples were mixed by vertexing (Lab-Line) and maintained at 70 °C for 10 min. Before being centrifuged for 10 min at 3500 rpm, the samples were permitted to cool down to room temperature. The supernatant that was obtained was subsequently poured into graded tubes. The aforementioned procedure was repeated, and the resultant extracts were mixed with 10% methanol to fill a volumetric flask to the mark. One milliliter of the extract was added to a 100 mL volumetric flask, and distilled water was then added to the mark; the solution was thoroughly mixed. One milliliter of this component and five milliliters of 10% Folin-Ciocalteu's phenol reagent were combined in a test tube, and in three to 8 min, four milliliters of 7.5% sodium carbonate solution was added to this mixture. This mixture was allowed to incubate for 1 h at room temperature. Using a UV–Vis spectrophotometer (Shimadzu, Japan), the optical density was determined at 765 nm. The calibration plot was constructed using gallic acid ranging in concentration from 10 to 60 mg L^{-1}. The results were reported as a percentage by mass on a dry matter basis (w/w%) [13]. Dry matter content was determined using ISO 1573:1980. Tea samples weighing 5 g were precisely measured and placed in previously measured aluminum moisture cans. These cans were then dried in an oven (Memmert) at 103 ± 2 °C for 6 h until a constant weight was achieved. The dry matter content of the samples was subsequently quantified based on the differences in weight before and after drying [14].

2.4 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radicle scavenging assay

A tea sample (100 µL) in methanol and DPPH (0.2 mM, 100 µL) was transferred into a microtiter plate (96 wells), incubated (25 °C, 30 mins). The absorbance (515 nm) was measured using a microplate reader [15].

2.5 α-Amylase inhibitory assay

5.31 M Sodium potassium tartrate in 2 M NaOH (8 mL) and water (deionized, 12 mL) were used for the preparation of the enzyme substrate; 3,5-dinitro salicylic acid (DNSA) reagent (96 mM). α-Amylase (8 U/mL, 100 µL) with the tea extract was incubated (25 °C, 30 min), and 100 µL of starch (0.5% (w/v)) solution was transferred to it and incubated (25 °C, 3 min). Thereafter, the enzyme substrate,and 100 µL of DNSA, was transferred to it and incubated in the water bath (90 °C, 15 min), and distilled water (900 µL) was added to it before cooling the solution. The same steps were used to prepare the positive control (acarbose), negative control, and blank solution. The absorbance was measured at 540 nm [15].

2.6 α-Glucosidase inhibitory assay

The enzyme, α-glucosidase (0.401 units/mL), was dissolved in phosphate buffer (pH 6.5, 30 mM). The enzyme (25 µL), 100 µL phosphate buffer, and 20 µL of tea sample were incubated (37 °C, 5 min). Thereafter, 50 µL of 0.996 M para-nitrophenyl-α-D-glucopyranoside was transferred to it and incubated (37 °C, 30 min). The wavelength used to measure the absorbance was 410 nm. As a positive control, acarbose was utilized [15].

2.7 Lipase inhibitory assay

The tea extract (2.4 mg) in phosphate-buffered saline (7.4 pH, 300 µL) and p-nitrophenyl butyrate (10 µL) in 10 mL of acetonitrile solution were prepared. 10 mg of lipase in 10 mL of phosphate-buffered saline (50 µL), 100 µL of phosphate-buffered saline, and the tea extract (25 µl) were transferred into a microtiter plate (96 well) and incubated (37 °C, 15 min). Then 25 µL of phosphate-buffered saline was transferred and incubated (37 °C, 30 min). The absorbance of the reaction mixture was taken at 400 nm. Orlistat was employed as the positive control [15].

2.8 Acetylcholinesterase inhibitory assay

To measure the acetylcholinesterase inhibitory activity of tea extracts, a modified Ellman's method was utilized. 100 µL of phosphate buffer (8.0 pH), 50 µL of 0.2 U/mL acetylcholinesterase enzyme in phosphate buffer (8.0 pH), and the tea sample (25 µL) were transferred to the microtiter plate and incubated (room temperature, 10 min). A solution of 15 mM acetylthiocholine iodide (25 µL) in deionized water, 3 mM DTNB (50 µL, pH 7.0 phosphate buffer), and 0.1 M sodium bicarbonate (0.2 mg/mL) were added to the microtiter plate. Donepezil hydrochloride was employed as a positive control [16].

2.9 Nitric oxide (NO) radical scavenging activity

Tea extracts in 1:99 DMSO, distilled water, and sodium nitroprusside (1 mL, 5 mM) in phosphate-buffered saline were incubated under a light intensity (220 mW/cm^2) lamp with white compact fluorescent light (29 °C, 120 min). Griess reagent (2 mL) was transferred to this reaction mixture and shaken well. Griess reagent was not added to the sample blank or negative control. The readings were taken at the wavelength of 546 nm [17].

2.10 Xanthine oxidase (XO) inhibitory activity

Tea extract in DMSO (10 µL), phosphate buffer (pH 7.4, 50 mM, 150 µL), and 0.003 units of xanthine oxidase enzyme in phosphate buffer (20 µL) were transferred to 96-well plates, incubated (10 min, room temperature), and the absorbance was recorded at 295 nm. Then 0.1 mM xanthine (20 µL) was added to it as the substrate, and absorbance readings were taken for 15 min at every 1-min interval. The positive control used in this experiment was allopurinol. The percentage inhibition of the tea samples was calculated in comparison with blank (DMSO) using the equation given below [18, 19].

$$\text{Percentage Inhibition} = 100 - \left[(\text{Optical Density of test compound}/\text{Optical Density control}) \times 100\right]$$

The percentage inhibition of antioxidant and enzyme inhibitory activities except xanthine oxidase was determined using the following equation prior to the calculation of the IC_{50}.

$$\text{Inhibition}(\%) = [(\text{Absorbance of control} - \text{Absorbance of sample})/\text{Absorbance of control})] \times 100$$

2.11 Statistical analysis

The SAS software (version 9.1) was utilized to conduct the statistical analysis, and at a 95% confidence interval (*p*-value less than 0.05), significant differences were considered. The Duncan multiple-range test was employed for the calculation of the mean separation. The mean values and standard deviation are provided. The IC_{50} (half maximal inhibitory concentration) values were computed by one-site binding competition of Graph Pad Prism software using averaged observations at 4–5 different levels. Heatmap generation and hierarchical clustering were carried out using the ClustVis web tool.

3 Results and discussion

The present study was focused on a comparison of the enzyme inhibitory activities of acetylcholinesterase, lipase, α-amylase, xanthine oxidase, and α-glucosidase enzymes and the potential antioxidant abilities in freeze-dried black tea brew and tea leaves that were harvested from different tea cultivars during two seasons grown under the same conditions. Rational application of the existing germplasm in breeding programs is primarily based on comprehension, knowledge, and understanding of the appropriate characteristics and genetic diversity of the tea germplasm collection. Chemical and physical characterization of the tea germplasm is a vital initiative toward the appropriate application of genetic resources in plant breeding programs. Sri Lanka currently maintains about 600 accessions, and the diversity of phytochemicals present among them has been documented. Based on the metabolic profile, 15 tea cultivars were selected for this study [1, 12].

These include B 275, KP 204, PLLG, TRI 2023, TRI 2026, TRI 2043, TRI 3017, TRI 3041, TRI 3055, TRI 4004, TRI 4042, TRI 4049, TRI 4052, TRI 4053, and TRI 4061. Buds and the first two leaves were harvested during the wet and dry spells from 15 tea cultivars. Methanolic extraction of freeze-dried tea leaves (hereafter referred to as tea leaves) and freeze-dried

black tea infusion (hereafter referred to as black tea) were prepared. Antioxidant and anti-inflammatory activities were carried out using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and nitric oxide radicle scavenging assays, respectively. The uric acid-lowering activity and the anti-obesity activity were assessed by xanthine oxidase and lipase inhibitory assays, respectively. In addition, the regulation of glycemic index through inhibition of carbohydrate digestion was studied using α-glucosidase and α-amylase inhibitory assays. Also, the neuroprotective potential was determined by an acetylcholinesterase enzyme inhibitory assay. The findings showed that enzyme inhibitory and antioxidant activities of tea leaves and black tea varied significantly across tea cultivars.

3.1 Total polyphenol (TPP) content

A large number of chemical constituents are present in the fresh tea leaves, but the most important are a group of compounds classified as polyphenols. Tea polyphenols undergo a series of changes during black tea processing and are responsible for the characteristic color and taste of black tea. According to the literature, the secondary metabolites, including caffeine, catechins, polyphenols, theobromine, and gallic acid, present in the leaves of Sri Lankan tea cultivars varied significantly [1]. According to the results, the polyphenols of black tea and tea leaves were significantly varied, ranging from 19.36% to 34.89% and 29.83% to 47.86%, respectively. The highest TPP was found in the tea leaves of cultivar TRI 2026 (44.21 ± 1.89%) and TRI 3017 (47.86 ± 2.11%) in tea leaves harvested from dry and wet seasons, respectively. In black tea, the highest TPC was resulted from the cultivar TRI 4042 (28.00 ± 1.52%) and TRI 3055 (34.89 ± 1.2) in black tea manufactured from dry and wet seasons, respectively. The total polyphenol concentration varied among tea cultivars and showed substantial variances between various tea extracts.

3.2 1-Diphenyl-2-picrylhydrazyl radicle scavenging activity

The DPPH radical scavenging assay is usually performed to estimate the potential antioxidant activity of food [20]. In this study, DPPH radical scavenging assays were performed to quantify the antioxidant potential of black tea and tea leaves. The results were expressed in IC_{50} (half-maximal inhibitory concentration), and the lower the IC_{50} value, the higher the biological activity. According to the findings, both black tea and tea leaves exhibited high antioxidant activity compared to the positive control, ascorbic acid (IC_{50} = 22.27 ppm). The DPPH radicle scavenging assay exhibited IC_{50} values for tea leaves: 6.61–36.47 ppm and for black tea: 14.03–67.23 ppm, regardless of the season. However, the strongest DPPH radical scavenging potential was resulted from the black tea of PLLG (14.03 ± 0.09 ppm) in the dry season and TRI 3055 (18.33 ± 1.05 ppm). Furthermore, the highest DPPH radical scavenging potential was resulted from the tea leaves of TRI 3041 (13.14 ± 1.44 ppm and 6.61 ± 0.79) in both seasons. The IC_{50} values of the tea samples are shown in Table 1. Radical scavenging activity on DPPH of black tea and tea leaves significantly ($p \leq 0.05$) differed among the tea cultivars utilized in this study. The literature revealed that the theaflavins that are formed during black tea processing possess antioxidant activity comparable to those of catechin derivatives. Theaflavins were found to be more effective and 10 times faster at radicle scavenging of superoxide than epigallo-catechin-3-gallate (EGCG) [20]. Catechins are transformed into theaflavins and thearubigins during the enzymatic oxidation process of black tea manufacturing [20].

3.3 Nitric oxide (NO) radical scavenging activity

Nitric oxide is formed enzymatically from the amino acid, L-arginine in a number of tissues using the nitric oxide synthase [21]. The nitric oxide (NO) is produced by organisms across all phyla, from bacteria to humans, and it has a broad spectrum of biological processes [22]. NO is a physiological mediator that is involved in the pathogenesis of several inflammatory disorders. NO synthesis and release accelerate the development of the inflammatory reaction; therefore, inhibition of NO contributes to the decrease and further progression of inflammatory symptoms. The scavenging of NO radicals is one of the mechanisms involved in the inhibition of the propagation of the inflammatory response. Literature revealed that nitric oxide synthase, which is called pro-inflammatory enzyme, synthesizes nitric oxide, known as a potent inflammatory mediator. Nevertheless, extreme NO synthesis is recognized as the main root cause of neurodegenerative diseases [23]. According to the findings, the radical scavenging activity on NO of black tea and tea leaves significantly varied from IC_{50} 76.22 to 251.35 ppm and 38.75 to 417.00 ppm, respectively (see Table 1). High NO radical scavenging activity was exhibited by tea leaves of tea cultivars TRI 4004 and TRI 4061 in the dry spell with IC_{50} of 60.88 and 38.75 ppm, respectively, compared to the positive control, ascorbic acid (IC_{50} = 66.2 ppm). The results of this study indicated a remarkable

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Table 1 Antioxidant activity of tea leaves and black tea

Cultivar	Radical scavenging activity ($IC_{50,}$ ppm) of tea leaves ± SD				Radical scavenging activity ($IC_{50,}$ ppm) of black tea ± SD			
	DPPH		Nitric oxide		DPPH		Nitric oxide	
	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
B275	$21.21^{c} \pm 0.17$	$10.31^{b,c,d} \pm 0.56$	$156.95^{c} \pm 7.00$	$182.40^{c,d,e} \pm 1.07$	$22.51^{b} \pm 0.47$	$23.77^{e,f} \pm 3.41$	$249.35^{a} \pm 2.00$	$251.35^{a} \pm 0.07$
KP204	$14.28^{e,f} \pm 1.01$	$13.72^{b} \pm 0.60$	$123.05^{d,e,f} \pm 6.2$	$197.70^{b,c,d} \pm 2.16$	$16.22^{f,g,h} \pm 1.95$	$46.92^{c} \pm 2.57$	$112.45^{d,e} \pm 3.57$	$115.50^{b,c,d} \pm 3.21$
PLLG	$25.96^{b} \pm 3.52$	$14.24^{b} \pm 1.89$	$203.70^{b} \pm 5.32$	$172.65^{d,e,f,g} \pm 2.21$	$14.03^{h} \pm 0.09$	$42.87^{c} \pm 1.94$	$89.42^{e} \pm 6.12$	$97.46^{c,d} \pm 0.77$
TRI2023	$15.56^{e,f} \pm 1.36$	$7.85^{c,d} \pm 0.68$	$71.93^{g,h} \pm 2.27$	$206.05^{b,c,d} \pm 2.45$	$17.45^{e,f,g} \pm 0.50$	$29.66^{d,e} \pm 1.90$	$94.91^{e} \pm 2.28$	$171.95^{a,b} \pm 2.91$
TRI2026	$19.98^{e,f} \pm 2.22$	$11.35^{b,c} \pm 4.11$	$166.70^{c} \pm 3.79$	$158.25^{e,f,g} \pm 3.40$	$15.14^{g,h} \pm 0.39$	$48.24^{c} \pm 0.92$	$229.7^{a,b} \pm 3.79$	$149.95^{a,b,c,d} \pm 1.74$
TRI2043	$16.54^{d,e,f} \pm 1.1$	$36.47^{a} \pm 3.36$	$152.70^{c,d} \pm 7.49$	$143.75^{f,g,h} \pm 2.65$	$16.04^{g,h} \pm 0.36$	$67.23^{a} \pm 3.31$	$244.05^{a} \pm 2.49$	$148.55^{a,b,c} \pm 2.10$
TRI3017	$27.44^{b} \pm 1.38$	$9.98^{b,c,d} \pm 0.65$	$92.14^{f,g,h} \pm 7.21$	$226.25^{a,b} \pm 0.07$	$42.51^{a} \pm 2.09$	$60.77^{a,b} \pm 1.87$	$155.70^{c} \pm 1.23$	$125.55^{a,b,c,d} \pm 4.45$
TRI3041	$13.14^{f} \pm 1.44$	$6.61^{d} \pm 0.79$	$75.76\ g^{h} \pm 5.99$	$116.50^{h,i} \pm 2.91$	$15.46^{g,h} \pm 0.15$	$44.03^{c} \pm 0.67$	$94.99^{e} \pm 5.99$	$154.80^{a,b,c} \pm 3.68$
TRI3055	$17.67^{c,d,e} \pm 4.2$	$13.99^{b} \pm 2.62$	$100.00^{f,g} \pm 1.22$	$212.85^{b,c} \pm 2.73$	$21.81^{b} \pm 2.41$	$18.33^{f} \pm 1.05$	$164.00^{c} \pm 1.12$	$131.15^{a,b,c,d} \pm 3.40$
TRI4004	$19.87^{c,d} \pm 1.79$	$11.59^{b,c} \pm 3.17$	$60.88^{h,i} \pm 4.99$	$255.20^{a} \pm 1.74$	$18.06^{d,e,f} \pm 0.77$	$55.72^{b} \pm 0.17$	$161.70^{c} \pm 4.99$	$76.22^{d} \pm 2.49$
TRI4042	$13.83^{e,f} \pm 0.75$	$12.06^{b,c} \pm 0.61$	$138.25^{c,d,e} \pm 0.3$	$141.25^{g,h} \pm 2.85$	$20.33^{bc,d} \pm 0.43$	$45.01^{c} \pm 2.72$	$204.00^{b} \pm 0.35$	$134.10^{a,b,c,d} \pm 2.72$
TRI4049	$14.99^{e,f} \pm 1.38$	$7.72^{c,d} \pm 1.56$	$82.87^{g,h} \pm 0.89$	$100.68^{i} \pm 2.68$	$17.02^{e,f,g} \pm 0.78$	$33.00^{d} \pm 0.65$	$247.65^{a} \pm 0.87$	$188.30^{a} \pm 2.76$
TRI4052	$28.36^{a,b} \pm 4.40$	$35.76^{a} \pm 2.19$	$118.55^{e,f} \pm 2.27$	$117.10^{h,i} \pm 2.97$	$19.99^{b,c,d} \pm 0.65$	$28.91^{d,e} \pm 1.40$	$101.10^{d,e} \pm 2.27$	$138.35^{a,b,c,d} \pm 2.65$
TRI4053	$31.67^{a} \pm 4.11$	$12.38^{b,c} \pm 3.54$	$417.00^{a} \pm 4.04$	$177.55^{d,e,f} \pm 2.49$	$19.18^{c,d,e} \pm 0.90$	$29.54^{d,e} \pm 0.96$	$135.65^{c,d} \pm 4.04$	$115.00^{b,c,d} \pm 3.85$
TRI4061	$21.19^{c} \pm 4.12$	$10.92^{b,c,d} \pm 3.86$	$38.75^{i} \pm 3.32$	$159.55^{e,f,g} \pm 0.77$	$21.44^{b,c} \pm 0.53$	$33.42^{d} \pm 0.94$	$148.40^{c} \pm 3..32$	$126.50^{a,b,c,d} \pm 3.67$

Means with the same letter within a column are not significantly different at 0.05 probability level

SD Standard deviation

nitric oxide radical scavenging activity in black tea, and it can be concluded that regular black tea consumption implies a wide range of therapeutic advantages against numerous diseases and disorders.

3.4 Acetylcholinesterase inhibitory activity

The results revealed that some of the tea cultivars possessed anticholinesterase inhibitory activity. Black tea produced from tea cultivars TRI 2023 and PLLG harvested from the wet season exhibited acetylcholinesterase inhibitory activities with IC_{50} values of 379.01 ± 9.04 and 146.83 ± 4.32 ppm, respectively, while black tea from tea cultivar KP 204 harvested from wet and dry seasons exhibited 140.17 ± 5.14 and 96.79 ± 6.05 ppm, respectively. Furthermore, black tea of TRI 3055 showed acetylcholinesterase inhibitory activity with IC_{50} values of 37.94 ± 5.14 ppm harvested from the dry season, and the rest of the tea cultivars showed acetylcholinesterase inhibitory activity higher than 400 ppm. Tea leaves of TRI 2043, TRI 3041, and TRI 4061 harvested from the wet season exhibited remarkable acetylcholinesterase inhibitory activity with IC_{50} values of 184.06 ± 5.98, 137.13 ± 5.38, and 34.51 ± 5.24 ppm, and the rest of the tea cultivars showed acetylcholinesterase inhibitory activity with IC_{50} values higher than 400 ppm (see Table 2). Several research studies have been undertaken to investigate the relationship between human cognitive function and the consumption of tea [6, 24]. Additionally, some scientists claimed that black and green tea extracts can be used to treat Alzheimer's disease and other impairments of age-related memory. According to Baranowska-Wójcik and coworkers, green and black tea have inhibited the human acetylcholinesterase enzyme with IC_{50} of 0.03 and 0.06 mg/mL, respectively [6]. Previous studies have linked frequent tea consumption to improved cognitive abilities in the elderly and a decreased risk of Alzheimer's disease and other types of neurodegenerative disorders [25]. The results of this study could further support the beneficial effects of tea on neurodegenerative diseases.

3.5 Xanthine oxidase (XO) inhibitory activity

The rate at which xanthine transforms into uric acid is determined by the XO inhibitory activity. Uric acid is a colorless compound that absorbs UV light at a wavelength of 295 nm [26]. Interestingly, Sri Lankan black tea exhibited remarkable XO inhibition towards black tea and tea leaves. The results revealed that XO inhibition of black tea and tea leaves possessed IC_{50} of 0.55–36.75 ppm and 0.60–17.26 ppm, respectively (see Tables 2 and 3). The positive control, allopurinol, inhibited the activity of XO with an IC_{50} of 1.51 ppm. Some tea cultivars showed high XO inhibition, which is comparable to allopurinol. Literature indicated that Sri Lankan white tea also possessed a high XO inhibitory activity with an IC_{50} value in the range of 2.51–48.96 ppm [15]. Furthermore, Dew et al. (2005) revealed that aflavin-3–3′-digallate and black tea exhibited remarkable in vitro XO inhibitory activity [27, 28]. It is also stated that theaflavins are potent XO inhibitors [29]. In this study, it is clearly shown that tea leaves and black tea exhibited XO inhibitory activity and varied among the tea cultivars used.Furthrmore, the results revealed that apart from tea cultivars TRI 3055, TRI 2026, and PLLG, the rest of the tea cultivars showed remarkable XO inhibitory activity in dry and wet spells. Interestingly, two tea cultivars, PLLG and TRI 2026, exhibited high XO inhibitory activity in the dry spell, whereas low XO inhibitory activity in the wet spell. Furthermore, the black tea of 15 cultivars exhibited high XO inhibitory activity in both dry and wet spells. The highest XO inhibitory activity was observed by tea cultivars TRI 2026, PLLG, TRI 2043, and TRI 3017 regardless of the season. Figure 1 illustrates the xanthene oxidase activity of black tea and tea leaves of 15 tea cultivars during dry and wet spells.

3.6 α-Glucosidase and α-amylase inhibitory activities

The recent data suggests that around 346 million people globally suffer from type 2 diabetes mellitus, and regular high postprandial glucose increments in the blood raise the chance of developing impaired glucose tolerance. One of the root causes of postprandial blood glucose is the increased hydrolysis of starch by enzymes including α-amylase and α-glucosidase. Several research studies indicated that polyphenols have the potential to inhibit the α-amylase enzyme, whose mechanism is similar to that of acarbose, which could be used to manage type 2 diabetes mellitus [30]. Even though Alzheimer's disease and type 2 diabetes mellitus have long been recognized as unavoidable ailments of life, recent evidence has revealed that dietary and routine lifestyle modifications have the potential to control the onset of these circumstances. Thus, the determination of α-glucosidase and α-amylase enzymes inhibitory potential of Sri Lankan black tea along with the acetylcholinesterase inhibition would be important to promote tea as a healthy beverage.

Except for tea cultivars including TRI 4061, PLLG, and TRI 2023, the rest of the tea cultivars used in this study exhibited IC_{50} of α-amylase that was less than 900 ppm. The results were expressed in IC_{50} (half-maximal inhibitory concentration),

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Table 2 Enzyme inhibitory activities and total polyphenol contents of tea leaves

Cultivar	Inhibitory activity ($IC_{50,}$ ppm) of tea leaves									Total polyphenol contents (%)	
	Xanthene oxidase		Acetylcholine esterase	Lipase		α-Glucosidase		α-amylase			
	Dry	Wet	Dry	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
B275	$7.32^{b,c} \pm 0.47$	$4.32^{e,f,g} \pm 0.16$	$71.12^{b,c} \pm 1.53$	$954.18^{b} \pm 12.58$	$387.84^{e} \pm 10.41$	$106.67^{a,b} \pm 1.05$	$62.45^{c} \pm 3.63$	≥ 900	≥ 900	$35.50^{f,g,h} \pm 0.54$	$39.96^{e,f} \pm 1.36$
KP204	$5.89^{c,d} \pm 0.75$	$3.93^{f,g} \pm 0.43$	≥ 400	$849.88^{c,d} \pm 14.20$	$296.05^{f} \pm 1.32$	$29.53^{e,f,g} \pm 1.80$	$4.54^{f} \pm 0.79$	≥ 900	≥ 900	$42.78^{b} \pm 1.20$	$40.91^{d,e} \pm 2.00$
PLLG	$3.59^{e} \pm 0.56$	$22.32^{b} \pm 0.14$	$28.37^{e} \pm 0.60$	$769.07^{d,e} \pm 9.43$	$228.57^{f} \pm 9.07$	$54.27^{d} \pm 1.02$	$86.35^{b} \pm 0.41$	$257.4^{a} \pm 3.98$	$280.1^{a} \pm 7.09$	$32.60^{h,g} \pm 1.69$	$37.79^{f,g} \pm 1.58$
TRI2023	$1.31^{f} \pm 0.02$	$36.75^{a} \pm 0.23$	$41.13^{d,e} \pm 2.43$	$645.73^{g} \pm 5.56$	$1009.56^{a} \pm 7.48$	$37.60^{d,e,f} \pm 2.14$	$22.69^{d,e} \pm 2.04$	≥ 900	$221.13^{b} \pm 6.34$	$39.69^{c} \pm 1.32$	$39.61^{e,f} \pm 1.99$
TRI2026	$8.16^{b} \pm 0.28$	$8.09^{d} \pm 0.14$	$44.23^{d,e} \pm 5.66$	$1000.69^{a,b} \pm 13.57$	$389.93^{e} \pm 7.99$	$17.52^{f,g,h} \pm 3.97$	$33.55^{d} \pm 2.39$	≥ 900	≥ 900	$44.21^{a,b} \pm 1.89$	$38.44^{f} \pm 0.89$
TRI2043	$5.81^{c,d} \pm 0.62$	$4.04^{f,g} \pm 0.29$	≥ 400	≥ 1100	$117.98^{g} \pm 7.68$	$6.18^{h} \pm 0.40$	$5.57^{f} \pm 3.13$	≥ 900	≥ 900	$35.82^{d,f,e} \pm 1.54$	$30.19^{h} \pm 1.27$
TRI3017	$6.49^{c,d} \pm 0.03$	$5.87^{e} \pm 1.09$	$28.83^{e} \pm 2.39$	$1063.36^{a} \pm 12.36$	$489.49^{c,d} \pm 6.87$	$51.26^{d} \pm 1.16$	$105.60^{a} \pm 1.43$	≥ 900	≥ 900	$29.83^{g} \pm 1.78$	$47.86^{a} \pm 2.11$
TRI3041	$5.51^{d} \pm 0.54$	$3.87^{f,g} \pm 1.87$	$43.66^{d,e} \pm 3.94$	≥ 1100	$434.69^{d,e} \pm 6.22$	$74.65^{c} \pm 0.75$	$13.90^{e,f} \pm 1.44$	≥ 900	≥ 900	$45.79^{a} \pm 2.03$	$38.84^{e,f} \pm 1.77$
TRI3055	$15.61^{a} \pm 1.82$	$10.63^{c} \pm 0.38$	≥ 400	$913.33^{b,c} \pm 7.62$	$519.74^{c} \pm 8.65$	$95.98^{b} \pm 2.44$	$3.65^{f} \pm 0.74$	≥ 900	≥ 900	$38.41^{c,d,e} \pm 1.02$	$44.57^{b,c} \pm 1.99$
TRI4004	$7.04^{b,c} \pm 0.04$	$2.95^{g,h} \pm 0.70$	$87.80^{a,b} \pm 2.08$	≥ 1100	$761.92^{b} \pm 2.85$	$35.89^{d,e,f} \pm 1.04$	$13.90^{e,f} \pm 3.45$	≥ 900	≥ 900	$36.09^{g,h} \pm 1.64$	$40.16^{d,e} \pm 1.25$
TRI4042	$6.18^{c,d} \pm 0.28$	$2.25^{h,i} \pm 0.54$	≥ 400	≥ 1100	$112.74\,g \pm 5.36$	$7.41^{h} \pm 0.11$	$12.03^{e,f} \pm 3.27$	≥ 900	≥ 900	$34.94^{f,g} \pm 2.31$	$42.81^{b,c,d} \pm 1.4$
TRI4049	$3.37^{e} \pm 0.39$	$7.57^{d} \pm 0.40$	$108.87^{a} \pm 4.45$	≥ 1100	$514.52^{c} \pm 5.11$	$48.08^{d,e} \pm 4.02$	$21.87d^{e} \pm 4.36$	≥ 900	≥ 900	$37.45^{d,e,f} \pm 1.89$	$33.91^{h} \pm 1.78$
TRI4052	$1.59^{f} \pm 0.07$	$1.32^{i} \pm 1.17$	≥ 400	$397.78^{f} \pm 12.33$	$293.08^{f} \pm 1.91$	$11.06^{g,h} \pm 0.14$	$9.21^{e,f} \pm 0.82$	≥ 900	≥ 900	$42.69^{b} \pm 2.14$	$35.91^{g,h} \pm 1.98$
TRI4053	$8.17^{b} \pm 0.72$	$5.46^{e,f} \pm 0.15$	≥ 400	$711.34^{e,f} \pm 13.18$	$406.44^{e} \pm 1.51$	$123.20^{a} \pm 2.05$	$59.92^{c} \pm 2.16$	≥ 900	≥ 900	$41.52^{b} \pm 1.02$	$44.41^{b,c} \pm 1.88$
TRI4061	$0.55^{f} \pm 0.48$	$1.75^{h,i} \pm 0.17$	$55.43^{c,d} \pm 5.82$	$832.06^{c,d} \pm 12.30$	$383.47^{e} \pm 9.67$	$56.47^{c,d} \pm 1.60$	$11.23^{e,f} \pm 0.54$	$119.10^{b} \pm 5.34$	≥ 900	$34.63^{f,g} \pm 1.11$	$45.05^{b} \pm 1.46$

Means with the same letter within a column are not significantly different at 0.05 probability level, SD = Standard deviation

Table 3 Enzyme inhibitory activities and total polyphenol contents of black tea

Cultivar	Inhibitory activity ($IC_{50,}$ ppm) of black tea										Total polyphenol contents (%)	
	Xanthene oxidase		α-Glucosidase		Acetylcholine esterase		Lipase		α-amylase			
	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	WET	Dry	Wet
B275	$5.15^{e} \pm 0.27$	$2.44^{h,i} \pm 0.27$	$24.57^{d,e} \pm 1.11$	$28.16^{e} \pm 1.90$	≥ 400	≥ 400	≥ 1100	≥ 1100	≥ 900	≥ 900	$25.09^{g} \pm 1.88$	$25.89^{g} \pm 2.11$
KP204	$9.95^{b} \pm 0.57$	$9.91^{b} \pm 0.56$	$17.08^{e} \pm 2.39$	$65.73^{b} \pm 4.19$	≥ 400	≥ 400	≥ 1100	≥ 1100	≥ 900	≥ 900	$19.36^{k} \pm 1.29$	$24.40^{j} \pm 2.01$
PLLG	$2.74^{f,g} \pm 0.22$	$2.25^{h,i} \pm 0.23$	$23.50^{d,e} \pm 2.79$	$21.97^{e,f,g} \pm 2.9$	≥ 400	$146.83^{b} \pm 4.32$	≥ 1100	≥ 1100	$357.25^{a} \pm 9.99$	$370.23^{a} \pm 8.03$	$21.46^{j} \pm 1.97$	$23.76^{l} \pm 1.89$
TRI2023	$4.64^{e} \pm 0.56$	$7.16^{d,e} \pm 0.59$	$47.53^{b} \pm 2.45$	$22.52^{e,f,g} \pm 1.3$	≥ 400	$379.01^{a} \pm 9.04$	≥ 1100	995.93	≥ 900	$197.1^{b} \pm 7.12$	$20.56^{k} \pm 1.25$	$26.37^{g} \pm 1.55$
TRI2026	$0.60^{h} \pm 0.83$	$5.06^{g} \pm 0.83$	$35.00^{c,d} \pm 0.91$	$44.94^{d} \pm 0.47$	≥ 400	≥ 400	≥ 1100	≥ 1100	≥ 900	≥ 900	$23.74^{h} \pm 1.04$	$24.16^{k} \pm 1.68$
TRI2043	$2.47^{f,g} \pm 0.32$	$2.98^{h} \pm 0.31$	$108.53^{a} \pm 3.21$	$11.16^{l,j} \pm 0.29$	≥ 400	≥ 400	≥ 1100	136.90	≥ 900	≥ 900	$26.06^{f} \pm 1.97$	$27.33^{c} \pm 1.52$
TRI3017	$1.86^{g} \pm 0.09$	$2.07^{h,i} \pm 0.09$	$23.80^{d,e} \pm 0.62$	$5.77^{j} \pm 0.24$	≥ 400	≥ 400	≥ 1100	≥ 1100	≥ 900	≥ 900	$26.29^{e} \pm 2.04$	$28.91^{b} \pm 1.48$
TRI3041	$6.36^{d} \pm 0.22$	$17.26^{a} \pm 0.23$	$32.32^{c,d} \pm 3.66$	$104.10^{a} \pm 2.78$	≥ 400	≥ 400	≥ 1100	≥ 1100	≥ 900	≥ 900	$26.46^{d} \pm 1.44$	26.85e ± 2.00
TRI3055	$6.84^{c,d} \pm 0.71$	$1.71^{i} \pm 0.71$	$33.65^{c,d} \pm 2.36$	$19.88^{f,g,h} \pm .39$	37.94 ± 5.14	≥ 400	≥ 1100	≥ 1100	≥ 900	≥ 900	$26.44^{d} \pm 1.69$	$34.89^{a} \pm 1.21$
TRI4004	$7.69^{c} \pm 0.20$	$2.82^{h,i} \pm 0.23$	$32.32^{c,d} \pm 3.75$	$104.10^{a} \pm 2.75$	≥ 400	≥ 400	≥ 1100	≥ 1100	≥ 900	≥ 900	$21.97^{i} \pm 1.46$	$22.30^{n} \pm 1.64$
TRI4042	$14.97^{a} \pm 0.74$	$5.88^{f,g} \pm 0.74$	$33.03^{c,d} \pm 3.16$	$25.17^{e,f} \pm 0.24$	≥ 400	≥ 400	≥ 1100	≥ 1100	≥ 900	≥ 900	$28.00^{a} \pm 1.52$	$23.40^{m} \pm 1.78$
TRI4049	$14.85^{a} \pm 0.62$	$7.91^{c,d} \pm 0.63$	$26.79^{d,e} \pm 1.16$	$52.72^{c} \pm 0.81$	≥ 400	≥ 400	≥ 1100	≥ 1100	≥ 900	≥ 900	$23.86^{h} \pm 1.26$	$25.12^{i} \pm 1.79$
TRI4052	$10.88^{b} \pm 0.4$	$8.35^{c} \pm 0.39$	$42.52^{b,c} \pm 3.01$	$15.10^{h,i} \pm 1.32$	≥ 400	≥ 400	≥ 1100	≥ 1100	≥ 900	≥ 900	$27.65^{b} \pm 1.74$	$27.03^{b} \pm 1.57$
TRI4053	$6.21^{d} \pm 0.66$	$7.71^{c,d,e} \pm 0.6$	$28.27^{d} \pm 1.33$	$51.49^{c} \pm 2.94$	≥ 400	≥ 400	≥ 1100	≥ 1100	≥ 900	≥ 900	$21.72^{i} \pm 1.23$	$26.53^{f} \pm 1.67$
TRI4061	$3.11^{f} \pm 0.22$	$6.73^{e,f} \pm 0.22$	$29.37^{d} \pm 2.52$	$17.83^{g,h} \pm 1.61$	≥ 400	≥ 400	696.12 ± 7.9	≥ 1100	$159.33^{b} \pm 9.44$	≥ 900	$26.64^{c} \pm 107$	$22.78^{n} \pm 1.25$

Means with the same letter within a column are not significantly different at 0.05 probability level, SD = Standard deviation

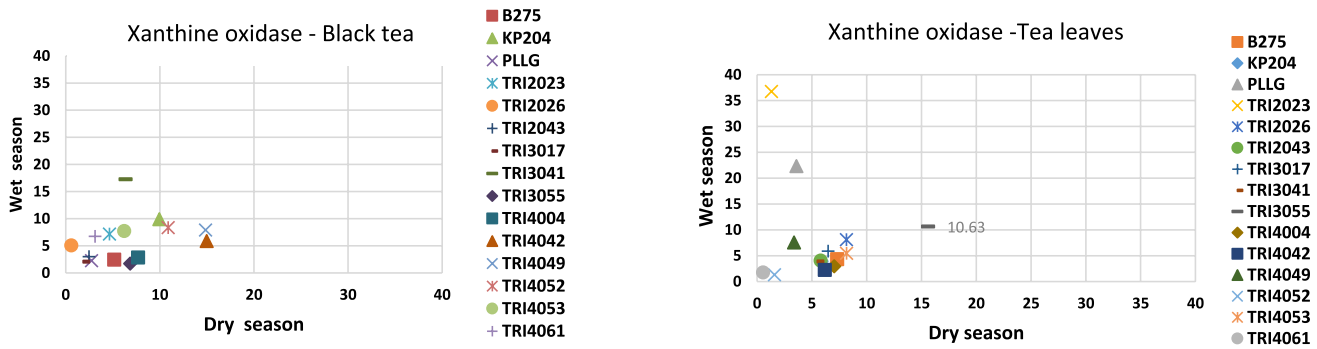

Fig. 1 Xanthine oxidase inhibitory activity of black tea and tea leaves

and the lower the IC_{50} value, the higher the biological activity. Tea cultivars, including TRI 4061, PLLG, and TRI 2023, exhibited α-amylase inhibitory activity in tea leaves, with IC_{50} values of 119.10, 257.40, and 197.10 ppm, respectively. In consideration of the α-glucosidase inhibitory activity, interestingly, all the tea cultivars used in this study exhibited high α-glucosidase inhibitory activity for black tea and tea leaves, with IC_{50} values in the range of 5.77–104.10 and 3.65–106.67 ppm, respectively (see Tables 2 and 3). A literature survey revealed that black tea has a beneficial role in diabetes mellitus. For example, in traditional Chinese medicine, the treatment of diabetes mellitus involves tea, which has been used for centuries as a traditional therapy. According to ethnopharmacological investigations, Sri Lankan indigenous physicians suggest drinking black tea to control blood sugar levels [31]. Moreover, some studies have demonstrated dietary polyphenols are involved in lowering the risk of complications related to diabetes mellitus [32]. Furthermore, the ability of tea polyphenolic compounds to impede the hydrolysis of disaccharides and regulate blood glucose has also been documented [30]. Several plant-based polyphenolic compounds have been mentioned as potential substitutes for acarbose that can modify starch digestion. Tender tea leaves are composed of polyphenols, including flavonoids, flavanols, simple phenolic acids, and flavandiols, which account for 15–20% of the dry weight. Butacnum et al. [33] reported that drinking black tea, which contains tea-polymerized polyphenol, has the potential to lower postprandial blood glucose levels. Moreover, black tea infusion exhibited anti-diabetic, anti-hyperglycemic, and hypoglycemic activities [29]. Thus, the strong α-glucosidase inhibitory activity observed in Sri Lankan tea cultivars suggests a therapeutic benefit against diabetes mellitus.Tea leaves of B 275 and TRI 4053 showed the lowest α-glucosidase inhibitory activity in both seasons. Irrespective of the season, black tea from the tea cultivars TRI 3017, 4061, and PLLG showed the highest α-glucosidase inhibitory activity. However, TRI 4053 showed high α-glucosidase inhibitory activity in the wet season, whereas low in the dry season. In contrast, black tea from the tea cultivar KP 204 exhibited high α-glucosidase inhibitory activity in dry spell compared to wet spell. Figure 2 illustrates the α-glucosidase inhibitory activity of black tea and tea leaves of 15 cultivars during dry and wet spells. The highest α-glucosidase inhibitory activity was observed by tea leaves of TRI 4052, 4042, and TRI 2043, irrespective of the seasons. KP 204 and TRI 3055 showed high α-glucosidase inhibitory activity in wet spell, and TRI 3055 exhibited the lowest α-glucosidase inhibitory activity in dry spell.

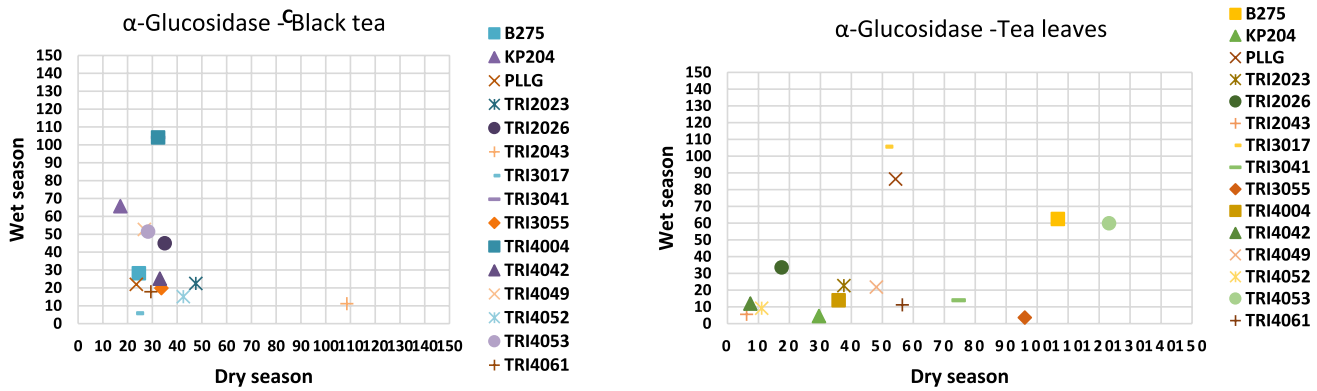

Fig. 2 α-glucosidase inhibitory activity of black tea and tea leaves

3.7 Lipase inhibitory activity

The lipase inhibitory activity of tea leaves was significantly different, with IC_{50} ranging from 112.74 to ≥ 1100 ppm. Even though black tea manufactured from different tea cultivars showed weak lipase inhibitory activity, tea cultivars TRI 2023 and TRI 4061 exhibited moderate lipase inhibitory activity with the IC_{50} of 995.93 and 696.12, respectively (see Tables 2 and 3). Also, the rest of the tea cultivars showed lipase inhibitory activity with IC_{50} values higher than 1100 ppm. The positive control, orlistat, indicated an IC_{50} value of 54.07 ppm. According to previous research studies, methylated epigallocatechin-3-gallate, epigallocatechin-3-gallate, polyphenol metabolites in dark tea, and theaflavins showed properties of weight loss. Polyphenols in green tea are considered as the effective weight loss inducers due to their potent antioxidant effects. It has been revealed that fermented tea polyphenols are as effective as or even superior to tea polyphenols in green tea [34].

3.8 Correlation matrix between total polyphenol contents and biological activities for tea leaves and black tea

The resultant heat map generated for the enzyme inhibitory and antioxidant activities of tea leaves from 15 distinct tea cultivars in wet and dry spells is illustrated in Fig. 3. According to the heat map, tea leaves were categorized into three groups. The first group consisted of tea cultivar TRI 3017, and the second group consisted of five tea cultivars, including TRI 2043, TRI 3055, 4052, 4042, and KP 204. The third and largest group consisted of nine tea cultivars, including B275, PLLG 2, TRI 4061, TRI 2023, TRI 3041, TRI 4053, TRI 2026, TRI 4004, and TRI 4049. Five tea cultivars in the second group exhibited weak anticholinesterase inhibitory activity during the dry season compared to the other nine cultivars. Also, α-amylase and lipase inhibitory activities of tea cultivars were shown by those five cultivars during both dry and wet seasons. In antioxidant assays on DPPH, irrespective of the season, apart from TRI 4052 and TRI 2043, tea leaves from other tea cultivars exhibited high antioxidant activity. TRI 2043, which is used to produce white tea, exhibited higher DPPH radical scavenging activity during the dry spell than during the wet spell. In both seasons, TRI 4052 exhibited low DPPH radical scavenging activity compared to other tea cultivars. Tea cultivars TRI 3017, TRI 4053, and PLLG exhibited higher DPPH radical scavenging during wet spell compared to dry spell. The highest and the lowest nitric oxide radical scavenging activity was observed by tea cultivars TRI 4061 (IC_{50}, 38.75 ± 3.32) and TRI 2023 (IC_{50}, 206.05 ± 2.45), irrespective of the season.

The resultant heat map generated for the enzyme inhibitory and antioxidant activities of black tea from 15 different tea cultivars harvested from wet and dry spells is indicated in Fig. 4, and black tea samples were categorized into two distinct groups. The first group consisted of two tea cultivars, KP 2024 and TRI 3055, and the second group comprised the other 13 tea cultivars. KP 204 showed weak acetylcholinesterase inhibitory activity during both wet and dry seasons, whereas none of the other cultivars showed acetylcholinesterase inhibitory activity during the dry season.

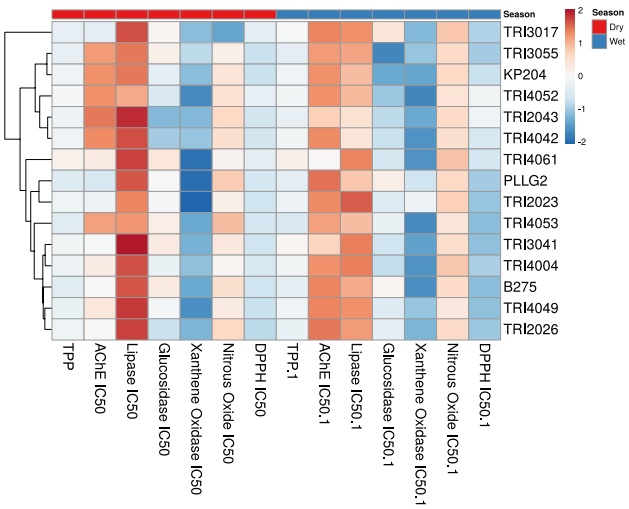

Fig. 3 A heat map depicting correlation matrix between total polyphenol contents and biological activities of tea leaves

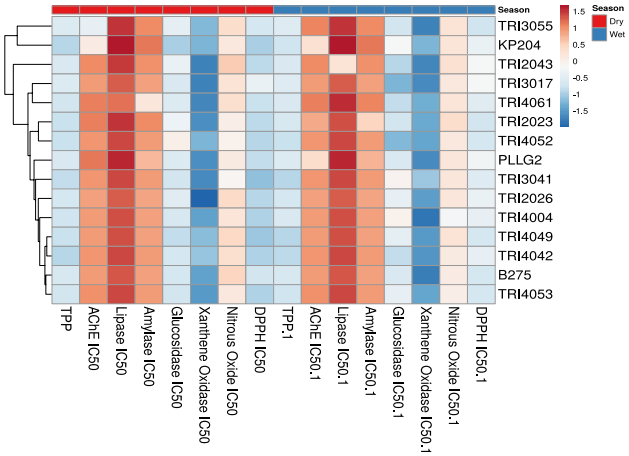

Fig. 4 A heat map depicting the correlation matrix between total polyphenol contents and biological activities of black tea

In addition, tea leaves exhibited significantly high biological activity compared to black tea in consideration of the results of all biological assays tested (see Tables 2 and 3). However, black tea is notable for its bioactivity. This may be because the extraction solvent is one of the most crucial elements influencing the effectiveness of extracting bioactive compounds from plant materials, and methanol also plays an important role in the extraction of phytochemicals [35, 36]. Moreover, it is stated that tea leaves contain higher contents of polyphenols compared to black tea [3]. This study evaluated the in vitro enzyme inhibitory and antioxidant activities of 15 tea cultivars during two major climatic seasons. Tea cultivars were found to have potent inhibition of enzymes such as xanthine oxidase, acetylcholine sterase, and α-glucosidase, and also demonstrated high antioxidant activity. Furthermore, the biological activities of 15 different tea cultivars differed significantly despite the climatic season and extraction protocols. From the resultant correlation matrix generated between total polyphenol content and biological activities for black tea (see Fig. 5), total polyphenol content showed a positive correlation with xanthine oxidase and α-glucosidase inhibitory activities, and DPPH radical scavenging activity of different tea cultivars, indicating high activity with increased TPP content. However, total polyphenol content correlated negatively with nitrous oxide scavenging and acetylcholinesterase activities, indicating low activity in high TPP containing tea cultivars.

The conclusion drawn from the results of antioxidant, xanthine oxidase, and α-glucosidase assays is that freeze-dried black tea brews and methanolic extracts of tea leaves of different tea cultivars exhibited remarkable biological activities. With the exception of lipase inhibitory activity, the tea cultivar PLLG demonstrated remarkable enzyme inhibitory activities towards acetylcholinesterase, α-amylase, xanthine oxidase, and α-glucosidase enzymes. Furthermore, black tea produced from the TRI 2023 tea cultivar also showed the aforementioned biological activities. Only 15 of the tea cultivars

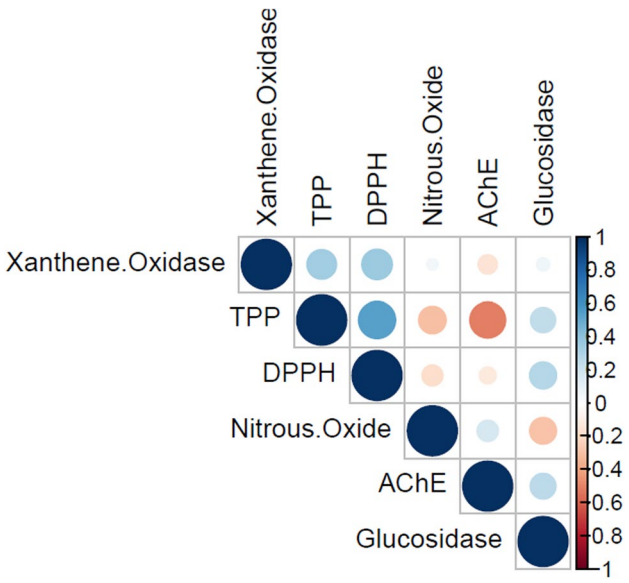

Fig. 5 Correlation matrix between total polyphenol content and biological activities of black tea

in Sri Lankan tea germplasm were used in this study for assessing the biological activities owing to resource limitations. For tea breeders, the discrepancy in biological activities across the tea cultivars is crucial since selecting, developing, and controlling tea quality is based on the chemical characteristics and bioactivities of different tea cultivars. According to the results, some tea varieties have exceptional biological activities. These results could be used to select suitable tea cultivars to act as parents for the future plant breeding programs and to enhance the marketing of Sri Lankan tea in the global marketplace.

4 Conclusions

This study investigated the in vitro enzyme inhibitory and antioxidant activities of 15 tea cultivars during two major climatic seasons in Sri Lanka for the first time. All tested tea cultivars showed remarkable antioxidant potential, and most varieties had moderate to high potential to inhibit xanthine oxidase, acetylcholinesterase, and α-glucosidase enzymes. The strong biological activity observed in Sri Lankan tea cultivars suggests a vast array of therapeutic benefits against many disease conditions. Results revealed that irrespective of the climatic season and extraction protocols, all the biological activities tested were significantly varied among 15 tea cultivars. The findings of this study could be utilized for the selection of tea cultivars/accessions for the manufacture of specialty tea with functional properties and also to promote Sri Lankan tea in the global market.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate The collection of the leaves of *Camellia sinensis* L. O. (Kuntze) used in this study complied with local or national guidelines. Leaves of *Camellia sinensis* L. O. (Kuntze) were collected in the National Tea Germplasm, Tea Research Institute of Sri Lanka. As tea is a commercially grown plant, a special license or special permission is not required to collect the tea leaves.

Competing interests The authors declare no competing interests.

Human and animal rights This is to inform you that in this study, we have not been involved in any animal and human studies.

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