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Variation of elastase, collagenase, tyrosinase enzyme inhibitory and antioxidant potential of different tea cultivars

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Abstract
Sri Lankan tea germplasm consists of over 600 accessions, and currently, 70 cultivars are recommended for commercial cultivation. However, the skin whitening and anti-aging effects of Sri Lankan tea cultivars/accessions have not been reported. In this study, tea leaves and black tea of fifteen tea cultivars were tested for their anti-oxidative and enzyme inhibitory potentials against elastase, collagenase, and tyrosinase enzymes for the first time. All samples were evaluated for their total polyphenol, caffeine, and individual catechin contents, and the results significantly differed among tea cultivars. The antioxidant potential of black tea and tea leaves significantly varied among the tea cultivars used. The enzyme inhibitory potential on tyrosinase, elastase, and collagenase inhibitory potential in black tea were varied among the tea cultivars, with the IC_{50} ranged from 208.82 ± 5.18 to ≥ 1200 ppm, 36.71 ± 6.38 to ≥ 600 ppm, and 786.31 ± 2.33 to ≥ 1300 ppm, respectively. None of the tested tea cultivars resulted in strong collagenase inhibitory potential. This study concludes that, among the tested tea cultivars, black tea manufactured using tea cultivars TRI 4004 and TRI 4049 and both tea leaves and black tea manufactured from TRI 4053, TRI 3017, and TRI 4061 exhibited remarkable anti-aging and skin-whitening properties. The findings of this study could be utilized for the selection of tea cultivars/accessions for the manufacture of speciality tea with functional properties and also to promote Sri Lankan tea in the global market.

Keywords Anti-aging · Antioxidant potential · Black tea · Skin whitening · Tea cultivars

1 Introduction

Tea, besides water, is one of the most widely consumed non-alcoholic beverages globally. The immature leaves and buds from *Camellia sinensis* L O (Kuntze) (tea plant) are used to produce all tea types. Various types of production processes can be used to produce different types of tea such as white tea, green tea and black tea etc. [1]. Currently, green tea makes up 20–22% of the world's tea production, black tea accounts for 76–78% and white is still in its infancy [2]. Regardless of the type, tea comprises a diverse array of phytochemicals such as polyphenols, alkaloids, saponins, pigments, polysaccharides, and

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free amino acids. On a dry weight basis, tea leaf contains 35% of polyphenols, 2.5–4.0% caffeine, and 1–3% theanine [3, 4]. However, the quantities of tea polyphenols vary with the degree of enzymatic oxidation that occurs during the production process [3]. These phytochemicals have a physiological effect on humans and it has been discovered that they are responsible for the numerous health benefits of tea including, anti-inflammatory properties, anti-oxidative properties, weight loss, relief from metabolic syndrome, a lower risk of non-communicable diseases and cognitive enhancement [5].

The increasing demand for skincare products globally is driving research studies on the cosmetic applications of natural background in the scientific literature. Novel substances that can positively and safely impact on skin are explored by researchers. Substances that are, super active with high quality are specifically focused. Currently, tea extracts are becoming more popular as dietary supplements and ingredients in cosmetics due to their wealthy composition in this regard [6]. The antioxidant potential [7], anti-aging characteristics, anti-cellulite (slimming) effects [8], photo-protective capabilities [9, 10], improvement of skin conditions [11], and improved skin microcirculation properties [12] in tea extracts have made them a vital natural source in cosmeceutical industry [13]. Aging is a biological process that is dynamic, gradual, undesirable, and tragically irreversible. The elastin and collagenous fibers in human skin are responsible for its tightness and firmness. Collagen degrades with both intrinsic and extrinsic influences and the elastase enzyme is responsible for the fragmentation of elastin fibers in the dermis, leading to wrinkles in the skin [14, 15]. Elastase, collagenase and tyrosinase present in humans are some of the major enzymes which are targeted in skin care formulations [16]. As a matter of fact, tea polyphenols show significant enzyme-binding abilities; thus, they can act as natural enzyme inhibitors. Natural enzyme inhibitors are pivotal in treating diseases with minimum side effects. Elastase and collagenase enzymes are involved with the breakdown of elastin and collagen proteins respectively. Collagen causes the tightness of the skin while elastin is responsible for the firmness and elasticity [17]. The natural production of these proteins declines with age and is further exacerbated by excessive exposure to sunlight. Moreover, the over-activation of elastase and collagenase enzymes also hydrolyze them causing further reduction of their concentrations [18]. The reduced amounts of collagen and elastin in the skin fasten up skin aging and cause skin sagging and wrinkles. Thus, the inhibitors of elastase and collagenase are considered beneficial for maintaining a youthful, healthy appearance. Tyrosinase enzyme is involved with the synthesis of melanin, the main coloring pigment found in human skin that gives the color to the hair and eyes as well. Unfortunately, overexposure to sunlight stimulates excessive melanin production as a result of radical activated reaction series [19]. This could lead to pigmentation diseases and age-related hyperpigmentation [20]. Ergo, tyrosinase inhibitors are beneficial in limiting the synthesis of melanin in the skin and advantageous for the development of natural skin-whitening solutions. Furthermore, these enzyme inhibitors may also optimize brain and tissue function during aging, and act on biological systems recognized to prolong lifespan [21, 22].

Tea Research Institute of Sri Lanka currently maintains about 600 tea germplasm accessions. The continued availability of high-quality planting materials with desired qualities is very important for the sustainability and profitability of the tea industry in Sri Lanka. However, Sri Lankan tea cultivation is more susceptible to biotic and abiotic stressors due to its limited genetic base. In order to maintain tea cultivation, it is necessary to regularly introduce a variety of exotic tea cultivars. To speed up the breeding of high-quality tea cultivars, the assessment of biochemical characteristics is an essential stage in the selection and identification of viable parental groups. Therefore, the metabolite diversity of each accession has been reported. While the inhibitory potentials of elastase, tyrosinase, and collagenase in teas have been documented in literature, cultivar-specific variations in these biological effects have not been documented yet [7, 8, 11]. Moreover, there are hardly any study reporting the variations of biological potential in consideration of skin care potential in Sri Lankan tea cultivars/accessions. There prevails a great need to discover the anti-aging and skin whitening properties of Sri Lankan tea germplasm for the tea breeding program. Therefore, the aim of this study was to identify potential Sri Lankan tea cultivars with anti-aging and skin-whitening potential. 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. In addition, tea cultivars with potential anti-aging and skin-whitening properties could be used in the development of skincare formulations.

2 Material and methods

2.1 Chemicals and equipment

Standard caffeine, gallic acid, (+)-catechin (C), epicatechin (EC), epigallocatechin (EGC), epigallocatechin 3-gallate (EGCG), epicatechin-3-gallate (ECG), ascorbic acid, EDTA ≥ 99%, ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), $FeSO_4.6H_2O$, 2, 4, 6-Tripyridyl-S-triazine (TPTZ), acetonitrile (purity 99%, HPLC grade), tyrosinase obtained from mushroom ≥ 1000 units/mg solid (9002-10-2), L-tyrosine ≥ 98%, Porcine pancreatic elastase (39445-21-1), N-Succinyl-Ala-Ala-Ala-p-nitroanilide (AAAPVN),

Collagenase from *Clostridium histolyticum* (Type I-S, 0.2–1.0 FALGPA units/mg solid, ≥ 125 CDU/mg solid) (9001-12-1), N-[3-(2-furyl) acryloyl]-Leu-Gly-Pro-Ala (FALGPA), Tricine buffer, calcium chloride, sodium chloride, sodium potassium tartrate ≥ 99%, 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^+$ ≥ 98%, HPLC), 2,2-azobis (2-amidinopropane) dihydrochloride (AAPH), potassium persulfate, dimethyl sulfoxide (DMSO), sodium acetate trihydrate, methanol, hydrochloric acid, sodium hydroxide, monosodium dihydrogen orthophosphate, disodium monohydrogen orthophosphate, ascorbic acid, Folin—Ciocalteu's phenol reagent, sodium carbonate, acarbose tablets (ACARB 25—Orchid Healthcare, India), donepezil hydrochloride, and orlistat were purchased from Sigma Aldrich, Germany. Deionized water was obtained from the Milli-Q system (USA).

2.2 Preparation of tea samples

Fifteen tea cultivars (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) were chosen from high-elevation regions at the Tea Research Institute of Sri Lanka. Fresh tea leaves were collected from each tea cultivar. 100 g of collected fresh leaves were freeze-dried, ground into a powder, and extracted with methanol. The obtained extract was then evaporated to dryness using a rotary evaporator which gave the methanolic extract of tea leaves and referred to as 'tea leaves' in the following sections. Next, 500 g of the collected fresh leaves were subjected to miniature manufacture at the Biochemistry Division, TRI, Sri Lanka which resulted approximately 100 g of black tea. After brewing the black tea samples according to the ISO 3103 (Tea, preparation of liquor use in sensory tests), the brews were freeze-dried and referred as 'black tea' in the following sections. Then, for testing, these tea leaves and black tea were used from each cultivar.

2.3 Quantification of total polyphenol content (TPC)

Samples of tea leaf extract/freeze dried black tea brew weighing 0.200 ± 0.001 g were measured and transferred into 10 mL extraction tubes along with 5 mL of 70% hot methanol. Samples were mixed by vertexing using a vortex mixer (Lab-Line, USA) and maintained at 70 °C for 10 min. The samples were allowed to reach room temperature and were centrifuged for ten minutes at 3500 rpm. The resulting supernatant was transferred into graduated tubes. After repeating the above process, the obtained extracts were combined in a volumetric flask and filled to the mark (10 mL) with 70% methanol. The extract was diluted by adding 1 mL of the extract to a volumetric flask (100 mL), and adding distilled water up to the mark, the solution was thoroughly mixed. In a test tube 1 mL of this sample and 5 mL of 10% Folin-Ciocalteu's phenol reagent were mixed together and into this solution, 4 mL of 7.5% sodium carbonate solution was added within three to eight minutes. This mixture was incubated for one hour at room temperature. Using a UV–Vis spectrophotometer (Shimadzu, Japan) the optical density of each solution was determined at 765 nm. The calibration plot was constructed using gallic acid ranging in concentration from (10 to 60) ppm. The results were reported as a percentage by mass on a dry matter basis (w/w %) [23]. Dry matter content was determined: tea samples weighing 5 g were precisely measured and placed in previously measured aluminium moisture cans. These cans were then dried in a Memmert UND300 oven (Germany) at 103 ± 2 °C for six hours until a constant weight was achieved. The percentage dry matter content of the samples was subsequently quantified based on the differences in weight before and after drying [24].

2.4 Quantification of individual catechins and caffeine

The tea extract that was used in 2.3 was employed here. In a graduated tube 1 mL of the extract was diluted four times with a stabilizing solution. The solution consisted of 10% v/v acetonitrile with 500 µL mL^{-1} of ascorbic acid and EDTA. These solutions were mixed well, filtered and added to HPLC vials. The main catechins and caffeine found in the tea extracts were identified by High-performance liquid chromatography (HPLC) analysis [25].

2.4.1 HPLC instrumentation

The instrumentation included a Diode Array Detector (DA Detect G1315D) and an Agilent Technologies 1260 HPLC system. A Phenyl-Hexyl security guard cartridge (4 mm × 3.0 mm) from Phenomenex was used with a Luna Phenyl-Hexyl column (250 × 4.6 mm, 5 µm particle size). The injection volume and flow rate for the standards and samples were 10 µL and 1.0 mL/min, respectively. Mobile phase A consisted of 9% v/v acetonitrile, 2% v/v acetic acid, and 20 µg/mL EDTA, and mobile phase B consisted of 80% v/v acetonitrile, 2% v/v acetic acid, and 20 µg/mL EDTA. The sample ran for 45 min

under a binary gradient, which consisted of 10 min at 100% mobile phase A, followed by 15 min of a linear gradient of 68% A and 32% B, which was held for 10 min. Prior to the subsequent injection, mobile phase-A was reset to 100% and allowed to equilibrate. The column temperature was 35 °C. Catechins were detected at 278 nm UV, with peak areas/retention times compared to a caffeine standard.

2.5 ABTS assay

Methanol was used to dissolve tea samples, and a concentration gradient was prepared. A freshly prepared ABTS solution and 50 mM phosphate buffer saline (PBS, pH 7.4) were used as reagents. The PBS was made by dissolving 0.77 g of monosodium dihydrogen orthophosphate, 5.18 g of disodium monohydrogen orthophosphate, and 1.46 g of sodium chloride. All the salts were dissolved in 500 mL of distilled water and the pH value of the final solution was adjusted to 7.4. The fresh ABTS radical solution was prepared by mixing 10 mg of ABTS with 2.5 mL of 2.5 mM potassium persulfate solution and it was incubated for 16 h at room temperature. This solution was then diluted sevenfold with PBS. Subsequently, 110 µL of PBS and 40 µL of the test sample were introduced into a microtiter plate well. The absorbance of the plate was measured before any reaction, at a wavelength of 734 nm. Then, 50 µL of the diluted ABTS solution was dispensed into each well. The plate was incubated for 10 min at room temperature to allow the reaction between the test sample and ABTS to begin. After the incubation, the absorbance was read again at 734 nm to measure the ABTS radical scavenging ability of the sample [26].

2.6 FRAP (Ferric reducing antioxidant power) assay

A 1000 ppm tea sample solution was prepared. The FRAP working solution was made by mixing 300 mM acetate buffer, 10 mM TPTZ solution, and 20 mM $FeCl_3 \cdot 6H_2O$ in a ratio of 10:1:1. An aliquot of 1.5 mL of this solution was combined with 50 µL of the sample and 150 µL of deionized water. The solution was vortexed, and 200 µL of this was mixed with the sample in a 96-well microplate. The absorbance at a wavelength of 593 nm was then measured after the microplate was incubated in the dark for 30 min. An aqueous solution of known $FeSO_4.6H_2O$ concentration was used for calibration, and the results were represented as mmol Fe(II) per gram on a dry weight basis [27].

2.7 ORAC (oxygen radical absorbance capacity) assay

75 mM potassium phosphate buffer (PB) was prepared using an aliquot (14.60 mL) of 1.0 M KH2PO4 stock solution and 22.90 mL 1.0 M K2HPO4 were mixed, then the pH was adjusted to 7.40 and topped up in a 500.0 mL volumetric flask with deionized water. Trolox standards ranging from 200 µM to 0.75 µM were prepared using 75 mM PB solution and 20 mM trolox stock solution. Fluorescein working solution was prepared using Stock 1: 0.045 g of fluorescein was dissolved in 100.0 mL of 75 mM PB (36 ng/mL) and Stock 2: 500 µL of Stock 1 was dissolved in 100.0 mL of PB. Working solution: 1.6 mL of stock 2 was diluted in a 100.0 mL volumetric flask with 75 nM PB. 20.0 µL of sample was added into 96-well plates. 160.0 µL of fluorescein was added. 20.0 µL of AAPH solution was added. Pre-incubated at 37 °C for 5 min. The decay of fluorescein was scanned for 2 h in a 1 min interval at 37 °C (Ext. 494 nm, Em. 535 nm). The area under the curve for samples/trolox was recorded [28].

2.8 Anti-elastase assay

Tris–HCl buffer (0.2 mM, pH 8.0) was used for the experiment. The porcine pancreatic elastase enzyme (PE), elastase was dissolved in sterile water to create a 3.33 Units/mL stock solution. AAAPVN was dissolved in phosphate-buffered saline to create the substrate solution of 1.6 mM. Before adding the substrate and initiating the reaction, the test extracts were pre-incubated with the enzyme for fifteen minutes. In a total volume of 250 µL, the final reaction mixture comprised 100 µL of the test extract (concentration gradient from 62.5 ppm to 1000 ppm), 1 µg/mL PE (25 µL), 0.8 mM AAAPVN (25 µL), and the buffer (100 µL). EGCG (250 µM, or 0.114 mg/mL) and distilled water were used as the positive and negative controls respectively. Immediately following the addition of the substrate, the absorbance was measured continuously for 20 min within the wavelength range of 381–402 nm (determined by pre-screening scans), using a micro plate reader (BioTek, EPOCH 2, USA) [17]. The percentage inhibition was calculated using the following equation.

$$\text{Percentage inhibition} = \left[\left(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}\right)/\left(\text{Abs}_{\text{control}}\right)\right] \times 100$$

2.9 Anti-collagenase assay

The assay was carried out in a buffer solution consisting of 50 mM Tricine (pH 7.5), 400 mM sodium chloride, and 10 mM calcium chloride. Both the enzyme and the substrate were used after dissolving in this buffer. The collagenase enzyme, which is derived from *Clostridium histolyticum* at a concentration of 0.8 Units/mL was utilized. The substrate was N-[3-(2-furyl) acryloyl]-Leu-Gly-Pro-Ala (FALGPA) which had a concentration of 2 mM. The test sample extract (concentration gradient from 62.5 ppm to 1000 ppm) dissolved in the buffer was previously incubated with the enzyme for 15 min, followed by the addition of the substrate. The final reaction mixture had a total volume of 150 µL, consisting of the Tricine buffer (75 µL), 0.8 mM FALGPA (25 µL), 0.1 units of collagenase (25 µL), and 25 µL of the test sample. The positive control was epigallocatechin gallate (EGCG) at a concentration of 250 µM (0.114 mg/mL), while the negative control was distilled water. The absorbance measurements were taken at 335 nm and 410 nm, and continuously monitored for twenty minutes using a micro plate reader (BioTek, EPOCH 2, USA) [17]. The percentage inhibition was calculated using the following equation.

$$\text{Percentage inhibition} = \left[\left(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}\right)/\left(\text{Abs}_{\text{control}}\right)\right] \times 100$$

2.9.1 Anti-tyrosinase assay

The tea sample was dissolved in a pH 6.5 solution of 50 mM phosphate buffer. At first, the inhibitory potential of a 333.3 µg/mL tea sample was assessed. A concentration gradient (from 62.5 ppm to 1000 ppm) of the sample was prepared, and from the sample 70 µL was combined with 30 µL of the enzyme (333 Units/mL). This mixture was allowed to stand at 37 °C for ten minutes. Following incubation, 110 µL of 2 mM L-tyrosine was introduced to initiate the reaction. The reaction solution was reincubated at 37 °C for 30 min. Subsequently, the absorbance was determined at a wavelength of 492 nm. Kojic acid was used as the positive control while the negative control was distilled water [29]. The percentage inhibition was calculated using the following equation.

$$\text{Percentage inhibition} = \left[\left(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}\right)/\text{Abs}_{\text{control}}\right] \times 100$$

2.10 Statistical analysis

The reported mean values are presented as mean ± standard deviation. The IC_{50} values were calculated using the GraphPad Prism one-site binding competition model, using the averaged measurements obtained from four to five different concentrations. Minitab 17 software package was employed. The results were determined to be statistically significant when $p \leq 0.05$ (95% confidence level). Tukey's test was employed to determine significant differences between the mean values and Dunette's test was used to compare the mean values of the samples with the positive controls.

3 Results and discussion

The results of this study explain the inhibitory potentials of elastase, collagenase and tyrosinase enzymes and antioxidant potentials of fifteen tea cultivars. Half of the collected fresh tea leaves were extracted into methanol and freeze-dried (hereafter referred to as tea leaves). The other half was prepared as black tea brew and freeze-dried (hereafter referred to as black tea). ABTS radical scavenging assay, FRAP and ORAC assays were used to determine the antioxidant potential of the samples since the antioxidant potential of dermatology remedies plays a significant role in skincare. Elastase and collagenase inhibitory assays were used to determine the anti-aging property while tyrosinase inhibitory assay was performed to assess the skin-whitening ability of the samples (Supplementary 1).

3.1 Total polyphenolic content (TPC)

It is known that polyphenols have the ability to protect the human body from oxidative stress. Thus, those are not only vital for nutrition but also possess a pharmacological effect as well [30]. Literature reported that tea polyphenols tend to vary both qualitatively and quantitatively with different processes used during tea manufacturing which directly influence its bioactivities [31]. The results of the quantification of the total polyphenols of the tea leaves and black tea are presented in Table 1. The total polyphenolic contents of black tea and tea leaves significantly varied among the tea cultivars used. According to the findings, the highest TPC was found in the tea leaves of cultivar PLLG (29.36 ± 1.96%) and TRI 2026 (28.39 ± 1.78%) which had no significant difference. In black tea, the highest TPC was resulted by cultivar TRI 3055 (22.75 ± 2.58%), TRI 4042 (21.98 ± 1.36%) and TRI 4052 (21.54 ± 1.69%) which had no significant difference with each other. Additionally, the total polyphenol contents of black tea varied from 17.17 to 22.75%, and this was comparable to the literature data, where the total catechin content of black tea ranged from 8.3 to 24.8% [32]. It is revealed that the total catechin content in the fresh tea leaves obtained in the dry season from the germplasm were 144.98, 162.57 and 167.44 mg g^{-1} and in the wet season 150.26, 168.82 and 173.70 mg g^{-1} in China, Assam and Cambod types, respectively [33]. Therefore, the results obtained in this study were comparable to the literature data. It is reported that when comparing tea leaves and black tea, the TPC of black tea is lower than that of tea leaves in all tested tea cultivars [3]. This could be attributed to the conversion of the tea polyphenols into thearubigins and theaflavin during the oxidation/aeration process of black tea [34]. Furthermore, this also may be due to the fact that the extraction solvent has an effect on extracting bioactive chemicals from plant materials [35]. Since methanol was employed in this study to obtain the tea leaf extract, it might be rich with a lot of high polar polyphenols.

3.2 Individual catechins, caffeine and gallic acid contents

Table 1 also presents results obtained after the quantification of caffeine, gallic acid, and major tea catechins [catechin (C), epicatechin (EC), epigallocatechin (EGC), epigallocatechin 3-gallate (EGCG), and epicatechin-3-gallate (ECG)] performed for the tea leaves. Tea contains up to 30% of polyphenols in dry weight [3]. The caffeine and individual catechin contents of black tea and tea leaves significantly varied among the tea cultivars used. Catechins and flavanols are predominant in tea leaves, and the most prevalent catechin, EGCG, constitutes 50–80% of the total catechins, which has been attributed to the health benefits of tea [27, 36]. This study also showed that EGCG (varied from 2.56 to 11.82%), is the major catechin in all analyzed samples of tea leaves. It is followed by the quantities of ECG and EGC which are the second most prevalent catechins in some tea cultivars and lower levels of catechin and epicatechin were observed. Furthermore, the total catechin contents of tea leaves varied from 9.00 ± 0.25 to 19.84 ± 1.05%, and this was comparable to the literature data [31]. It is claimed that the catechin levels were in the range of 7.91–20.07% in the fresh leaf [37].

Caffeine, which is identified as 1,3,7-trimethylxanthine, is a methylated xanthine alkaloid derivative naturally present in tea. Caffeine confers the bitterness, contributing to the acidity in tea. Thus, it impacts the taste of tea and ultimately determines its quality characteristics. Caffeine is a central nervous system stimulant, thus refreshing and enhancing the alertness after consuming a cup of tea [38].According to the results of this study, caffeine content ranged from 2.08 to 3.20% (see Table 1). In China, caffeine content varied between 12 mg g^{-1} and 59 mg g^{-1} in the tea germplasm [39] whereas in Kenyan tea germplasms the caffeine content was found to be between 19.6 and 43.7 mg g^{-1} [40]. However, the study done by Kottawa-Arachchi et al. claims that the caffeine content was 20.18 mg g^{-1}, 32.68 mg g^{-1}, and 31.26 mg g^{-1} in China and Assam and Cambod tea germplasms in Sri Lanka [33].

3.3 Evaluation of antioxidant potential

3.3.1 ABTS assay

The ABTS assay measures the extent to which antioxidants scavenge the ABTS radical cation, which reacts quickly with hydrogen/electron donors, including polyphenols. The IC_{50} of the in vitro ABTS radical scavenging potential of the tea samples is presented in Table 2. The findings exhibited that, ten tea cultivars had no significant difference with the positive control, ascorbic acid IC_{50} 22.27 ± 1.01 mgL^{-1}. Tea leaves and black tea exhibited ABTS radical scavenging potential with the IC_{50} values in the range of 12.13 ± 1.38–60.19 ± 6.11 and 14.28 ± 1.43–82.44 ± 4.99 ppm, respectively (see Table 2).

Table 1 Individual catechin and total polyphenolic contents (%) of tea cultivars

Tea Cultivar	GA	C	EC	ECG	EGC	EGCG	TC	Caffeine	TPP of TL	TPP of BT
B 275	$0.045 \pm 0.00^{e,f}$	$0.24 \pm 0.10^{c,d}$	$1.61 \pm 0.05^{c,d}$	2.29 ± 0.23^{d}	$4.52 \pm 0.25^{b,c}$	7.49 ± 0.06^{g}	$16.32 \pm .0.38^{d,e}$	$2.72 \pm 0.11^{d,e,f,g}$	$23.69 \pm 1.23^{f,g}$	$17.93 \pm 1.21^{g,h}$
KP 204	0.09 ± 0.00^{a}	$0.24 \pm 0.06^{c,d}$	$2.15 \pm 0.02^{a,b,c}$	2.49 ± 0.21^{d}	4.11 ± 0.05^{c}	5.85 ± 0.04^{h}	$14.70 \pm 0.32^{e,f}$	2.08 ± 0.23^{j}	$25.96 \pm 2.36^{c,d}$	$20.96 \pm 1.25^{b,c,d}$
PLLG	$0.045 \pm 0.01^{e,f}$	$0.24 \pm 0.08^{c,d}$	$2.24 \pm 0.01^{a,b}$	3.21 ± 0.04^{b}	4.42 ± 0.01^{c}	8.51 ± 0.12^{d}	$18.61 \pm 0.15^{a,b}$	$3.21 \pm 0.63^{a,b}$	29.36 ± 1.96^{a}	17.93 ± 2.36^{h}
TRI 2023	0.035 ± 0.00^{f}	$0.24 \pm 0.06^{c,d}$	$1.76 \pm 0.04^{c,d}$	1.62 ± 0.00^{e}	4.37 ± 0.01^{c}	5.57 ± 0.26^{h}	13.57 ± 0.19^{f}	$2.40 \pm 0.74^{g,h,i}$	21.17 ± 2.13^{h}	$19.46 \pm 1.78^{d,e,f}$
TRI 2026	$0.08 \pm 0.00^{a,b}$	$0.24 \pm 0.07^{c,d}$	$2.25 \pm 0.01^{a,b}$	3.26 ± 0.05^{b}	$4.57 \pm 0.23^{b,c}$	8.49 ± 0.59^{d}	$18.83 \pm 0.25^{a,b}$	$3.20 \pm 0.39^{a,b}$	$28.39 \pm 1.78^{a,b}$	$17.69 \pm 2.25^{g,h}$
TRI 2043	$0.05 \pm 0.01^{d,e}$	0.53 ± 0.09^{a}	$2.15 \pm 0.01^{b,c}$	$2.64 \pm 0.06^{c,d}$	1.36 ± 0.25^{i}	2.56 ± 0.23^{i}	9.00 ± 0.25^{g}	2.20 ± 0.58^{i}	18.58 ± 1.11^{i}	$18.14 \pm 1.24^{f,g,h}$
TRI 3017	$0.075 \pm 0.01^{a,b}$	$0.17 \pm 0.07^{e,f}$	$1.26 \pm 0.07^{d,e}$	2.51 ± 0.01^{d}	2.67 ± 0.25^{g}	9.81 ± 0.21^{c}	$16.41 \pm 0.16^{d,e}$	$3.09 \pm 0.94^{b,c,d}$	$26.23 \pm 1.69^{c,d}$	$19.46 \pm 1.60^{e,f,g}$
TRI 3041	$0.05 \pm 0.01^{d,e}$	$0.17 \pm 0.03^{e,f}$	1.26 ± 0.02^{e}	2.54 ± 0.01^{d}	2.32 ± 0.36^{h}	10.51 ± 0.89^{b}	$16.49 \pm 0.04^{d,e}$	$3.00 \pm 0.96^{b,c,d,e}$	$24.81 \pm 2.31^{d,e,f}$	$19.96 \pm 1.24^{d,e}$
TRI 3055	0.065 ± 0.01^{c}	$0.17 \pm 0.01^{e,f}$	$1.72 \pm 0.72^{c,d}$	$3.11 \pm 0.40^{b,c}$	3.00 ± 0.18^{f}	11.82 ± 0.99^{a}	19.84 ± 1.05^{a}	$2.80 \pm 0.32^{c,d,e,f}$	$27.05 \pm 2.69^{b,c}$	22.75 ± 2.58^{a}
TRI 4004	$0.05 \pm 0.01^{d,e}$	0.25 ± 0.01^{c}	$2.11 \pm 0.08^{b,c}$	4.44 ± 0.05^{a}	3.10 ± 0.85^{f}	$8.26 \pm 0.65^{d,e,f}$	$18.18 \pm 0.62^{b,c}$	$3.15 \pm 0.21^{a,b,c}$	$25.0 \pm 1.96^{d,e,f}$	$20.92 \pm 2.56^{b,c,d}$
TRI 4042	$0.045 \pm 0.00^{e,f}$	$0.15 \pm 0.02^{f,g}$	$2.13 \pm 0.01^{a,b,c}$	$2.61 \pm 0.10^{c,d}$	5.81 ± 0.90^{a}	$8.31 \pm 0.21^{d,e}$	$19.42 \pm 0.39^{a,b}$	$2.76 \pm 0.62^{d,e,f,g}$	$27.44 \pm 1.69^{b,c}$	$21.98 \pm 1.36^{a,b}$
TRI 4049	$0.045 \pm 0.00^{e,f}$	$0.20 \pm 0.01^{d,e}$	$1.62 \pm 0.04^{c,d}$	2.49 ± 0.09^{d}	3.89 ± 0.25^{d}	$7.49 \pm 0.33^{e,f,g}$	15.96 ± 0.66^{e}	$2.65 \pm 0.39^{e,f,g,h}$	$22.74 \pm 1.25^{g,h}$	$18.49 \pm 1.21^{e,f,g,h}$
TRI 4052	$0.045 \pm 0.01^{e,f}$	0.11 ± 0.02^{g}	2.46 ± 0.08^{a}	$2.80 \pm 0.0^{b,c,d}$	4.68 ± 0.05^{b}	$8.24 \pm 026^{d,e,f}$	18.32 ± 0.25^{b}	3.51 ± 0.45^{a}	$24.82 \pm 1.11^{d,e,f}$	$21.54 \pm 1.69^{a,b,c}$
TRI 4053	0.055 ± 0.00^{d}	0.12 ± 0.01^{g}	$2.13 \pm 0.03^{a,b,c}$	3.36 ± 0.02^{b}	3.52 ± 0.02^{e}	8.35 ± 0.98^{d}	$17.64 \pm 0.92^{b,c,d}$	$3.21 \pm 0.12^{a,b}$	$24.11 \pm 1.20^{e,f,g}$	$17.17 \pm 1.36^{g,h}$
TRI 4061	$0.045 \pm 0.00^{e,f}$	0.34 ± 0.01^{b}	$1.53 \pm 0.02^{c,d,e}$	3.26 ± 0.06^{b}	3.85 ± 0.25^{d}	$7.80 \pm 0.21^{e,f,g}$	$16.79 \pm 0.34^{c,d,e}$	$2.43 \pm 0.36^{f,g,h,i}$	$25.63 \pm 2.10^{c,d,e}$	$20.22 \pm 2.44^{e,f}$

The values in the table are given as mean ± SD of triplicates. The means, that do not share the same subscription letter (within a column), are significantly different at 95% confidence ($\alpha = 0.05$)

GA: Gallic acid; C: Catechin; EC: Epicatechin; ECG: Epicatechin gallate: EGC: Epigallocatechin: EGCG: Epigallocatechin gallate: TC: Total catechin: TPC: Total polyphenol content; TL: Tea leaves; BT: Black tea

According to a report, black tea brew has an IC_{50} value of 88.18 ppm for ABTS radical scavenging; the results of this research line up with this value [41]. In the case of black tea, four tea cultivars also had no significant difference with the ascorbic acid highlighting their strong antiradical potentials similar to ascorbic acid. However, the strongest ABTS radical scavenging potential was resulted by the black tea of TRI 4061 (14.28 ± 1.43 mg L^{-1}) which was significantly lower value than ascorbic acid and significantly different ($p \leq 0.05$) from other tea cultivars as well. Furthermore, tea cultivars, PLLG, TRI 2023, TRI 3041 and TRI 4004 displayed strong ABTS radical scavenging potential in both tea leaves and black tea. Moreover, in this assay, the radical scavenging ability of black tea samples seemed to be higher than the tea leaves. Even though tea catechins are known for their antioxidant ability, they get transform into theaflavins and thearubigins during the enzymatic oxidation process of black tea manufacturing. However, theaflavins also exhibit antioxidant properties similar to those of catechins and are ten times faster and more effective at scavenging superoxide radicals than EGCG [26]. Henceforth, the higher antioxidant potential resulted in black tea samples might be caused by theaflavins and they might be more effective in scavenging ABTS radicals than catechins.

3.3.2 Assay

FRAP assay measures the ability of an antioxidant to transfer one electron to reduce ferric ion (Fe^{3+}) in the FRAP reagent into ferrous ion (Fe^{2+}) [42]. The FRAP values obtained for the tea samples are presented in Table 2 as $FeSO_4$ equivalents. In this assay also, ascorbic assay was used as the positive control (39.09 ± 3.12 mmol Fe^{+2}/g of the sample). Moreover, tea leaves of cultivar B275, KP204, TRI2026, TRI2043, TRI3017, TRI4004 and black tea of cultivar TRI4052 resulted significantly higher FRAP values than ascorbic acid (see Table 2) i.e., their antioxidant potential is greater than ascorbic acid. Tea leaves and black tea exhibited FRAP values in the range of 20.57 ± 1.60–71.53 ± 5.17 mmol Fe^{+2}/g and 14.79 ± 2.14 -46.17 ± 3.90 mmol Fe^{+2}/g, respectively. The antioxidant potential of 30 tea infusions from green, black, oolong, white, yellow, and dark teas was assessed using the Trolox equivalent antioxidant capacity assay and ferric-reducing antioxidant power (FRAP) assays ranging from 504.80 ± 17.44 to 4647.47 ± 57.87 µmol Fe^{2+}/g dry weight (DW) and 166.29 ± 24.48 to 2532.41 ± 50.18 µmol Trolox/g DW, respectively [43]. The results obtained in this study were comparable to the literature data. Unlike ABTS assay, almost all tea cultivars resulted higher FRAP values for their tea leaves than black tea due to the FRAP values have found to be significantly decrease with oxidation of tea leaves [44].

3.3.3 ORAC assay

The ORAC assay determines the antioxidant capacity by observing the free radical-induced damage to a fluorescent probe, which leads to a gradual decrease in fluorescence intensity over time. The ORAC values obtained for the tea samples are presented in Table 2 as Trolox equivalents (TE). The tea leaves of all tested tea cultivars resulted in significant antioxidant capacity. The highest value was resulted by TRI 4053 (270.27 ± 7.74 µmol TE/g) which is significantly different ($p \leq 0.05$) from other tested samples. Apart from them, B 275 and TRI 4052, black tea of other tea cultivars resulted in significantly higher ORAC values than the positive control. Tea leaves and black tea exhibited ORAC values in the range of 59.37 ± 5.36–270.27 ± 7.74 µmol TE/g and 31.30 ± 2.38 – 261.84 ± 10.11 µmol TE/g, respectively (see Table 2). The highest value was resulted by TRI 3017 (261.84 ± 10.11 µmol TE/g) which is significantly different from other tested samples. There was hardly any study have reported the ORAC values to express the antioxidant potential of Sri Lankan tea cultivars. Hence the results of this study are important. The antioxidant potential of ABTS, FRAP, and ORAC on black tea and tea leaves significantly varied among the tea cultivars used, and remarkable antioxidant potential were observed in tea leaves and black tea. It is reported that ORAC values of two black tea samples were 66.05 ± 13.56 µM TE/mL and 34.38 ± 7.20 µM TE/mL [45] and these values support the findings of our investigation.

3.4 Enzyme inhibitory potential

3.4.1 Elastase inhibitory potential

Excessive effect of the elastase enzyme accelerates the degradation of the elastin fiber network in the skin leading to a loss of skin elasticity and the formation of wrinkles [8]. To combat this, elastase inhibitors are introduced which are capable of maintaining skin elasticity and enhancing anti-aging properties. The elastase inhibitory potential of black tea and tea leaves significantly varied among the tea cultivars used. The IC_{50} values for elastase inhibitory potential are presented in Table 3. Among the tea leaf samples, TRI 2043 showed the lowest IC_{50} value (427.25 ± 0.97 ppm), which was

Table 2 Antioxidant potential of tea leaves and black tea of tea cultivars

Cultivar	ABTS Radical scavenging potential ($IC_{50,}$ mg L^{-1})		FRAP (mmol Fe^{+2}/g of the sample)		ORAC (μM Trolox equivalents/g of the sample)	
	Tea leaves	Black tea	Tea leaves	Black tea	Tea leaves	Black tea
B 275	$22.24 \pm 1.47^{d,e,A}$	$38.63 \pm 4.32^{b,c,d}$	$66.57 \pm 5.50^{a,b}$	14.79 ± 2.14^{e}	99.66 ± 6.34^{f}	31.30 ± 2.38^{g}
KP 204	$19.98 \pm 2.84^{d,e,f,A}$	$35.43 \pm 3.41^{c,d}$	$66.57 \pm 4.64^{a,b}$	17.59 ± 2.05^{e}	$188.49 \pm 6.65^{c,d}$	118.83 ± 6.69^{c}
PLLG	$20.93 \pm 2.95^{d,e,A}$	$16.05 \pm 1.88^{f,A}$	$40.89 \pm 3.36^{d,e,f,A}$	$45.52 \pm 3.62^{a,A}$	$65.22 \pm 7.55^{h,I,A}$	$45.31 \pm 3.75^{f,g,A}$
TRI 2023	$24.50 \pm 1.78^{c,d,A}$	$30.15 \pm 2.63^{c,d,e,A}$	$32.88 \pm 2.70^{f,g,A}$	$28.72 \pm 2.57^{c,d}$	236.18 ± 5.47^{b}	67.37 ± 7.41^{d}
TRI 2026	$16.54 \pm 1.69^{d,e,f,A}$	$37.14 \pm 2.38^{b,c,d}$	$58.27 \pm 4.93^{b,c}$	19.24 ± 2.40^{e}	$61.87 \pm 6.45^{h,I,A}$	$59.99 \pm 6.12^{d,e,f,A}$
TRI 2043	39.84 ± 3.82^{b}	$33.83 \pm 2.79^{c,d}$	71.53 ± 5.17^{a}	$34.56 \pm 3.99^{b,c,A}$	$87.79 \pm 8.32^{f,g}$	116.74 ± 8.06^{c}
TRI 3017	$19.06 \pm 2.15^{d,e,f,A}$	82.44 ± 4.99^{a}	$52.08 \pm 3.22^{c,d}$	19.03 ± 2.11^{e}	144.43 ± 6.16^{e}	261.84 ± 10.11^{a}
TRI 3041	$16.28 \pm 2.41^{e,f,A}$	$23.69 \pm 1.85^{e,f,A}$	$37.91 \pm 2.65^{e,f,A}$	17.20 ± 1.86^{e}	$82.37 \pm 5.15^{f,g,h}$	$60.87 \pm 8.33^{d,e,f,A}$
TRI 3055	60.69 ± 6.11^{a}	$31.25 \pm 5.14^{c,d,e}$	21.92 ± 1.81^{g}	$38.79 \pm 3.67^{a,b,A}$	174.84 ± 6.09^{d}	70.16 ± 4.27^{d}
TRI 4004	$19.86 \pm 3.27^{d,e,f,A}$	$28.80 \pm 3.09^{d,e,A}$	$53.51 \pm 5.76^{b,c,d}$	$33.51 \pm 2.85^{b,c,A}$	$74.78 \pm 6.47^{g,h,i}$	$63.85 \pm 4.74^{d,e,f,A}$
TRI 4042	$12.13 \pm 1.38^{f,A}$	$32.58 \pm 2.80^{c,d,e}$	21.66 ± 8.15^{g}	$43.91 \pm 3.16^{a,A}$	151.81 ± 6.46^{e}	146.02 ± 4.00^{b}
TRI 4049	31.72 ± 2.34^{c}	46.07 ± 2.93^{b}	$47.42 \pm 4.01^{c,d,e,A}$	$32.16 \pm 2.59^{b,c,A}$	201.00 ± 8.77^{c}	74.28 ± 5.62^{d}
TRI 4052	44.55 ± 4.53^{b}	$35.87 \pm 4.73^{c,d}$	$44.58 \pm 3.40^{d,e,f,A}$	46.17 ± 3.90^{a}	$59.37 \pm 5.36^{i,A}$	35.67 ± 2.91^{g}
TRI 4053	$21.39 \pm 2.16^{d,e,A}$	$40.24 \pm 3.38^{b,c}$	20.57 ± 1.60^{g}	$22.71 \pm 2.39^{d,e}$	270.27 ± 7.74^{a}	$47.71 \pm 3.27^{e,f,g,A}$
TRI 4061	$41.81 \pm 2.36^{b,A}$	14.28 ± 1.43^{f}	$45.30^{c,d,e,f,A} \pm 4.47$	$38.08 \pm 1.25^{a,b,A}$	$185.15 \pm 8.24^{c,d}$	$41.44 \pm 2.73^{g,A}$
Positive control	Ascorbic acid 22.7 ± 1.01		Ascorbic acid 39.09 ± 3.12		Trolox 49.92 ± 3.00	

The values in the table are given as mean ± SD of triplicates. The means that do not share the same lowercase superscription letter (between cultivars), are significantly different at 95% confidence (α = 0.05). Those that are not labeled with the uppercase superscription letter 'A' are significantly different from the positive control at 95% confidence (α = 0.05)

significantly different ($p \leq 0.05$) from the other samples. However, all the tea leaf samples from the tested tea cultivars were significantly different from the positive control, EGCG (IC_{50} 110.00 ± 2.41 ppm). However, black tea samples of TRI 4049 (IC_{50} 36.71 ± 6.38 ppm), TRI 4053 (IC_{50} 91.37 ± 2.80 ppm), and TRI 4004 (IC_{50} 99.80 ± 4.84 ppm) resulted in significantly lower values than EGCG, highlighting the high elastase inhibitory potential of those samples (see Table 3). The ability of Sri Lankan low-grown orthodox Orange Pekoe (O.P.) grade black tea as a skin anti-aging through the inhibition of elastase potential has already been reported [46]. Additionally, the present study has demonstrated that Sri Lankan O.P grade tea exhibits mild anti-elastase potential in vitro.

3.4.2 Collagenase inhibitory potential

Collagenase enzyme is also involved with the hydrolysis of dermal matrix protein, collagen. Excessive hydrolysis of collagen can lead to skin damage and the formation of wrinkles [46]. Therefore, collagenase inhibitors are beneficial in maintaning healthy skin and reducing the visible effects of aging. Most of the tea cultivars resulted in weak collagenase inhibition, as they did not achieve 50% inhibition of the enzyme even at the highest tested concentration i.e. 1000 ppm. However, tea leaves of TRI 3041 exhibited an IC_{50} of 724.33 ± 2.05 ppm, while the black teas had IC_{50} values ranging from 786.31 ± 2.33 to 1214.49 ± 16.35 ppm (see Table 3). All of these were significantly different ($p \leq 0.05$) from the positive control, EGCG (IC_{50} 118.34 ± 3.05 ppm). Although this study found only mild collagenase inhibition for black tea, Sri Lankan low-grown orthodox O.P. grade black tea has been reported to have promising anti-collagenase potential in vitro, which was superior to EGCG (IC_{50} 112.12 ± 0.93 µg mL^{-1}) [46].

However, in the present study tea leaves were collected from high altitudes, whereas Ratnasooriya et al., (2017) collected those from low altitudes of Sri Lanka, which may be attributed to the discrepancy in results as geographical location has a direct effect on the types and concentrations of phytoconstituents in tea [46, 47]. Considering the TPC, it has been reported 17.04 ± 0.42% (w/w) in the black tea whereas in the present study, the TPC of the black teas of PLLG, TRI 4042 and TRI 4004 were found to be 17.93 ± 2.36%, 21.98 ± 1.36% and 20.92 ± 2.56% respectively. Even though the TPC contents of the two studies were closely related, the specific metabolites such as EGCG and EC mainly responsible for the inhibition of collagenase in tea might vary due to the differences in the fresh sample [46, 47].

Table 3 Enzyme inhibitory potential of tea leaves and black tea of tea cultivars

Cultivars	IC_{50} of elastase inhibitory potential (ppm)		IC_{50} of Tyrosinase inhibitory potential (ppm)		IC_{50} of collagenase inhibitory potential (ppm)	
	Tea leaves	Black tea	Tea leaves	Black tea	Tea leaves	Black tea
B275	482.50 ± 3.16^{c}	146.42 ± 5.40^{i}	923.92 ± 4.18^{c}	$1064.14 \pm 4.79^{a,b,c}$	≥ 1300	≥ 1300
KP204	≥ 600	151.58 ± 7.26^{i}	1200.00 ± 0.00^{a}	1069.70 ± 4.18^{a}	≥ 1300	≥ 1300
PLLG	≥ 600	413.75 ± 4.16^{c}	653.88 ± 1.27^{h}	$730.78 \pm 5.12^{d,e}$	≥ 1300	≥ 1300
TRI 2023	≥ 600	390.65 ± 2.39^{d}	903.92 ± 4.68^{d}	653.62 ± 4.16^{e}	≥ 1300	≥ 1300
TRI 2026	466.90 ± 4.73^{d}	190.23 ± 6.69^{h}	≥ 1200	≥ 1200	≥ 1300	≥ 1300
TRI 2043	427.25 ± 0.97^{g}	$374.71 \pm 4.11^{f,g}$	≥ 1200	≥ 1200	≥ 1300	≥ 1300
TRI 3017	≥ 600	444.63 ± 2.11^{b}	≥ 1200	≥ 1200	≥ 1300	≥ 1300
TRI 3041	433.98 ± 2.16^{f}	365.21 ± 0.68^{g}	1042.04 ± 3.68^{b}	$1046.40 \pm 3.12^{a,b,c}$	724.33 ± 2.05	786.31 ± 2.33
TRI 3055	≥ 600	$384.63 \pm 2.18^{d,e,f}$	714.82 ± 2.62^{g}	661.59 ± 2.99^{e}	≥ 1300	1214.49 ± 16.35
TRI 4004	≥ 600	99.80 ± 4.84^{j}	≥ 1200	208.82 ± 5.18^{f}	≥ 1300	≥ 1300
TRI 4042	≥ 600	$387.82 \pm 2.18^{d,e}$	≥ 1200	$1001.22 \pm 3.43^{a,b,c,d}$	≥ 1300	≥ 1300
TRI 4049	≥ 600	36.71 ± 6.38^{k}	770.82 ± 2.49^{f}	611.45 ± 6.42^{e}	≥ 1300	≥ 1300
TRI 4052	468.52 ± 2.15^{d}	≥ 600	≥ 1200	644.85 ± 7.27^{e}	≥ 1300	≥ 1300
TRI 4053	455.65 ± 4.16^{e}	91.37 ± 2.80^{j}	≥ 1200	$923.57 \pm 4.61^{b,c,d,e}$	≥ 1300	≥ 1300
TRI 4061	546.75 ± 2.16^{b}	$377.00 \pm 4.13^{e,f,g}$	817.99 ± 4.18^{e}	$844.14 \pm 4.18^{c,d,e}$	≥ 1300	≥ 1300
Positive control	EGCG $110.00^{A} \pm 2.41$		Kojic acid $2.18^{A} \pm 0.23$		EGCG $118.34^{A} \pm 3.05$ mg L^{-1}	

The values in the table are given as mean ± SD of triplicates. The means that do not share the same lowercase superscription letter (between cultivars), are significantly different at 95% confidence (α = 0.05). Those that are not labeled with the uppercase superscription letter 'A' are significantly different from the positive control at 95% confidence (α = 0.05)

3.4.3 Tyrosinase inhibitory potential

Tyrosinase enzyme is associated with the initial steps of the melanin biosynthetic pathway [16]. Excessive effect of tyrosinase causes several skin pigmentation disorders and age-related hyper pigmentation [20]. Therefore, tyrosinase inhibitors are vital as skin-whitening agents. The tyrosinase inhibitory potential of black tea and tea leaves significantly varied among the tea cultivars used. The IC_{50} values for tyrosinase inhibitory potential are presented in Table 3. A previous study has reported that the highest tyrosinase inhibitory potential was shown by white tea followed by green tea and black tea [27, 34]. However, during our study, the tea leaves of six cultivars and black tea of eight cultivars showed moderate potential since they resulted in IC_{50} values below 1000 ppm and they were significantly different ($p \leq 0.05$) from the positive control koijic acid (IC_{50} 2.18 ± 0.23 ppm) (see Table 3). Those were all significantly different from each other as well.

In the case of black tea, the lowest IC_{50} was given by TRI 4004 (IC_{50} 208.82 ± 5.18 ppm) which resulted in the strongest tyrosinase inhibitory potential. Korkmaz et al. 2019 stated that the tyrosinase inhibitory potential was related to the hydroxyl group present in the polyphenols such as koijic acid, gallic acid, benzoic acid, chlorogenic acid etc. Some also stated that the catechin derivatives like EGCG, ECG, and GCG, exhibited much higher inhibitory potential, suggesting a flavan-3-ol skeleton combined with a galloyl moiety has a major role in the optimum tyrosinase inhibition potential [34, 48]. In either case, the tyrosinase inhibitory potential in tea is related to the polyphenols present in the extract. Based on our results both tea leaves and black tea of the mentioned cultivars have resulted in moderate tyrosinase inhibitory potential and TRI 4004 being the strongest tyrosinase inhibitor.

3.5 Correlation analysis of bioactivities

Pearson's correlation coefficients (r) for bioactivities of fifteen tea cultivars are presented in Table 4. Even though it is documented that the TPC content of tea leaves and black tea is responsible for their antioxidant and enzyme-inhibitory potential, a strong correlation was not detected among them. Similarly, no strong correlation was detected between any of the assays performed for the tea leaves samples. However, black tea samples had several significant correlations. First, the results of ABTS radical scavenging assay showed a strong significant ($p \leq 0.05$) positive correlation with ORAC values ($r = +0.754$). Then, FRAP value showed strong significant positive correlations with elastase inhibitory assay ($r = +0.516$), collagenase inhibitory assay ($r = -0.549$) and tyrosinase inhibitory assay ($r = -510$) (see Table 4). Based on these results, we could say that the TPC of tea leaves and black tea do not have a direct effect on their antioxidant and enzyme inhibitory potential. However, the enzyme inhibitory properties of black tea samples might be influenced by the ferric-reducing power. Apart from that the ABTS and oxygen radical scavenging potential of black tea samples were also correlated (Supplementary 2).

The extraction method of tea has an effect on its biological potential as they showed different results, between tea leaves and black tea of the same cultivar. It can be clearly observed that the TPC of black tea is lower than that of tea leaves in all tested tea cultivars. The highest TPC was found in the tea leaves of cultivars PLLG and TRI 2026; in black tea, it was cultivars TRI 3055, TRI 4042 and TRI 4052. Among all tested cultivars strongest ABTS radical scavenging effect was shown by black tea of TRI 4061. Apart from that tea leaves of ten tea cultivars and black tea of four tea cultivars exhibited strong ABTS radical scavenging potential. In terms of FRAP assay strongest anti-oxidative potential was shown by tea leaves of some cultivars. Apart from that the tea leaves of six cultivars and black tea seven cultivars resulted in a strong reducing power. In ORAC assay the tea leaves of all tested cultivars resulted in significant antioxidant capacity.

The strongest elastase inhibitory potential was shown by the black tea samples of TRI 4049, TRI 4053, and TRI 4004 whereas, tea leaves of TRI 2043 showed moderate inhibition. Most of the tea cultivars resulted in weak inhibition of collagenase enzyme. However, mild effect was shown by tea leaves of TRI 3041 and black teas of TRI 4042, PLLG and TRI 4004. Next, the highest tyrosinase inhibitory potential was resulted by the black tea of TRI 4004. Tea leaves of six cultivars and black tea of eight cultivars showed a moderate tyrosinase inhibitory potential. It is worth mentioning TRI 4053, which has the strongest ORAC (tea leaves) and strongest elastase inhibitory potential (black tea), and black tea manufactured from TRI 4049, which possesses the strongest elastase inhibition and moderate tyrosinase inhibition. Moreover, TRI 3017 and TRI 4061 are also important for their remarkable anti-oxidative properties. Hence, among the tested tea cultivars, black tea of tea cultivars, TRI 4004 and TRI 4049 and both tea leaves and black tea of tea cultivars, TRI 4053, TRI 3017, and TRI 4061 are the best tea cultivars with anti-aging and skin-whitening properties.

The conclusion drawn from the FRAP, ORAC, and ABTS antioxidant assay results is that freeze-dried black tea brews and methanolic extracts of tea leaves of different tea cultivars exhibited remarkable antioxidant capacity. All tea cultivars exhibited weak inhibitory effects against collagenase, with the exception of two tea cultivars, TRI 3041 and TRI 3055.

Table 4 Pearson's linear correlation coefficients (r) within TPC, ABTS, FRAP, ORAC, elastase, collagenase and tyrosinase inhibitory potential of tea leaves and black tea of fifteen tea cultivars

Sample	TPC	ABTS	FRAP	ORAC	Elastase	Collagenase	Tyrosinase
Tea leaves							
TPC							
ABTS	− 0.210 $p = 0.453$						
FRAP	− 0.284 $p = 0.304$	− 0.104 $p = 0.712$					
ORAC	− 0.233 $p = 0.403$	0.083 $p = 0.768$	− 0.459 $p = 0.085$				
Elastase	0.308 $p = 0.264$	− 0.062 $p = 0.826$	− 0.226 $p = 0.418$	0.296 $p = 0.285$			
Collagenase	0.019 $p = 0.947$	0.229 $p = 0.413$	0.126 $p = 0.654$	0.228 $p = 0.414$	0.358 $p = 0.190$		
Tyrosinase	− 0.139 $p = 0.621$	− 0.401 $p = 0.138$	0.252 $p = 0.365$	− 0.153 $p = 0.587$	− 0.327 $p = 0.234$	− 0.018 $p = 0.949$	
Black tea							
TPC							
ABTS	− 0.127 $p = 0.651$						
FRAP	0.412 $p = 0.127$	− 0.417 $p = 0.122$					
ORAC	0.155 $p = 0.580$	0.754 $p = 0.001$	− 0.181 $p = 0.519$				
Elastase	0.415 $p = 0.124$	− 0.051 $p = 0.858$	0.516 $p = 0.049$	0.150 $p = 0.594$			
Collagenase	− 0.157 $p = 0.576$	0.289 $p = 0.297$	− 0.549 $p = 0.034$	− 0.092 $p = 0.744$	− 0.143 $p = 0.611$		
Tyrosinase	− 0.350 $p = 0.201$	0.336 $p = 0.221$	− 0.510 $p = 0.052$	0.425 $p = 0.114$	0.062 $p = 0.826$	0.146 $p = 0.605$	

However, the majority of tea cultivars demonstrated remarkable enzyme inhibitory potential whenever it linked to the elastase and tyrosinase enzymes. Furthermore, compared to the methanol extract of tea leaves, freeze-dried black tea brew exhibited remarkable enzyme inhibitory potential.

Since there are over 600 tea cultivars in the Sri Lankan tea germplasm, only 15 of them were utilized to examine the biological activities in this study due to resource constraints. However, other tea cultivars might possess remarkable anti-aging and skin-whitening properties. To have a comprehensive understanding of the aforementioned described bioactivities, it is preferable to conduct research on a significant number of tea cultivars, in order to find suitable tea cultivars to serve as parents for the future plant breeding program. Several biological activities could be taken into consideration while producing specialty teas. Due to the remarkable biological activities that tea exhibits, future research studies may expand to include new biological activities that will be integrated into plant breeding programs.

4 Conclusions

The tea leaves and black tea samples of the fifteen tea cultivars exhibited anti-oxidative, anti-elastase, anti-collagenase, and anti-tyrosinase properties in varying ratios. The antioxidant and enzyme-inhibitory potential tested on black tea and tea leaves significantly varied among the tea cultivars used. All tested tea cultivars showed remarkable antioxidant potential, and most varieties had moderate to high potential to inhibit elastase and tyrosinase enzymes. The overall findings of this study suggest that one of the best cultivars for manufacturing black tea is TRI 4004. It has strong anti-oxidative properties, mild collagenase inhibitory potential, and a strong potential to inhibit both elastase and tyrosinase enzyme potential. Henceforth, this study concludes that the mentioned tea cultivars have exceptional potential to be used in a skin-whitening and anti-aging formula. Future plant hybridization programs could use these tea cultivars as parents, with the aim of developing new tea cultivars with enhanced health properties.

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Data availability Data is provided within the manuscript and supplementary information files.

Declarations

Ethical approval and consent to participate This is to inform you that in this study, we have not been involved in any animal and human studies. 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.

References

1. Piyasena KN, Hettiarachchi LS, Edirisinghe EN, Abayarathne AA, Jayasinghe WS. Quantification of dynamic changes of sugars during the aeration/oxidation period of black tea processing: a Sri Lankan study. Food Hum. 2023. https://doi.org/10.1016/j.foohum.2023.01.001.
2. Ocieczek A, Pukszta T, Żyłka K, Kirieieva N. The influence of storage conditions on the stability of selected health-promoting properties of tea. LWT. 2023. https://doi.org/10.1016/j.lwt.2023.115029.
3. Balentine DA, Wiseman SA, Bouwens LC. The chemistry of tea flavonoids. Crit Rev Food Sci Nutr. 1997. https://doi.org/10.1080/10408399709527797.
4. Nelum KG, Piyasena P, Ranatunga MA, Jayawardhane SA, Edirisinghe EN, Tharangika HB, Ghouse AS, Abayarathne AA, Jayasinghe WS, Abeysinghe IS, Hettiarachchi LS. Prediction of glucose and sucrose values of black tea samples using NIR spectroscopy and chemometrics. Food Hum. 2023. https://doi.org/10.1016/j.foohum.2023.10.016.
5. Wu D, Chen R, Zhang W, Lai X, Sun L, Li Q, Zhang Z, Cao J, Wen S, Lai Z, Li Z. Tea and its components reduce the production of uric acid by inhibiting xanthine oxidase. Food Nutr Res. 2022. https://doi.org/10.29219/fnr.v66.8239.
6. Koch W, Zagórska J, Marzec Z, Kukula-Koch W. Applications of tea (*Camellia sinensis*) and its active constituents in cosmetics. Molecules. 2019. https://doi.org/10.3390/molecules24234277.
7. Sung H, Nah J, Chun S, Park H, Yang SE, Min WK. In vivo antioxidant effect of green tea. Eur J Clin Nutr. 2000. https://doi.org/10.1038/sj.ejcn.1600994.
8. Rawlings AV. Cellulite and its treatment. Int J Cosmet Sci. 2006. https://doi.org/10.1111/j.1467-2494.2006.00318.x.
9. Mishra AK, Mishra A, Chattopadadhyay P. Herbal cosmeceuticals from ultraviolet B radiation: a review. Trop J Pharm Res. 2011. https://doi.org/10.4314/tjpr.v10i3.7.66.
10. Yarnell E, Abascal K. Herbal sunscreens and ultraviolet protectants. Altern Compl Ther. 2012;18:141–4.
11. Velioglu YS, Mazza G, Gao L, Oomah BD. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. J Agric Food Sci. 1998. https://doi.org/10.1021/jf9801973.
12. Heinrich U, Moore CE, De Spirt S, Tronnier H, Stahl W. Green tea polyphenols provide photoprotection, increase microcirculation, and modulate skin properties of women. J Nutr. 2011. https://doi.org/10.3945/jn.110.136465.
13. Makhamrueang N, Raiwa A, Jiaranaikulwanitch J, Kaewarsar E, Butrungrod W, Sirilun S. Beneficial bio-extract of *Camellia sinensis* var. *assamica* fermented with a combination of probiotics as a potential ingredient for skin care. Cosmetics. 2023. https://doi.org/10.3390/cosmetics10030085.
14. Ratnasooriya WD, Abeysekera WP. In vitro anti-collagenase activity of Sri Pekoe grade black tea. J Chem Res. 2017;7(7):11–5.

15. Shin JW, Kwon SH, Choi JY, Na JI, Huh CH, Choi HR, Park KC. Molecular mechanisms of dermal aging and antiaging approaches. Int J Mol Sci. 2019. https://doi.org/10.3390/ijms20092126.
16. Chiocchio I, Mandrone M, Sanna C, Maxia A, Tacchini M, Poli FJ. Screening of a hundred plant extracts as tyrosinase and elastase inhibitors, two enzymatic targets of cosmetic interest. Ind Crops Prod. 2018. https://doi.org/10.1016/j.indcrop.2018.06.029.
17. Thring TS, Hili P, Naughton DP. Anti-collagenase, anti-elastase and anti-oxidant activities of extracts from 21 plants. BMC Complement Altern Med. 2009. https://doi.org/10.1186/1472-6882-9-27.
18. Baumann L. Skin ageing and its treatment. J Pathol. 2007. https://doi.org/10.1002/path.2098.
19. Pillaiyar T, Manickam M, Namasivayam V. Skin whitening agents: medicinal chemistry perspective of tyrosinase inhibitors. J Enzyme Inhib Med Chem. 2017. https://doi.org/10.1080/14756366.2016.1256882.
20. Slominski A, Tobin DJ, Shibahara S, Wortsman J. Melanin pigmentation in mammalian skin and its hormonal regulation. Physiol Rev. 2004. https://doi.org/10.1152/physrev.00044.2003.
21. Menendez JA, Joven J, Aragones G, Barrajon-Catalan E, Beltran-Debon R, Borras-Linares I, Camps J, Corominas-Faja B, Cufi S, Fernandez-Arroyo S, Garcia-Heredia A. Xenohormetic and anti-aging activity of secoiridoid polyphenols present in extra virgin olive oil: a new family of gerosuppressant agents. Cell Cycle. 2013. https://doi.org/10.4161/cc.23756.
22. Kim SJ, Park SY, Hong SM, Kwon EH, Lee TK. Skin whitening and anti-corrugation activities of glycoprotein fractions from liquid extracts of boiled sea cucumber. Asian Pac J Trop Med. 2016. https://doi.org/10.5555/20163340218.
23. ISO 14502–1:2005-Determination of substances characteristic of green and black tea-Part 1: content of total polyphenols in tea—colorimetric method using Folin-Ciocalteu reagent.
24. ISO 1573:1980. Tea—Determination of loss in mass at 103 degrees ℃.
25. ISO 14502-2:2005. Determination of substances characteristic of green and black tea Part 2: content of catechins in green tea—method using high-performance liquid chromatography.
26. Piyasena KN, Abayarathne AA, Weerakoon NC, Edirisinghe EN, Jayasinghe WS, Napagoda MT, Hettiarachchi LS, Abeysinghe IS. Chemical and biological characteristics of Sri Lankan white tea. Food Hum. 2023. https://doi.org/10.1016/j.foohum.2023.08.019.
27. Langley-Evans SC. Antioxidant potential of green and black tea determined using the ferric reducing power (FRAP) assay. Int J Food Sci Nutr. 2000. https://doi.org/10.1080/09637480050029683.
28. Dávalos A, Gómez-Cordovés C, Bartolomé B. Extending applicability of the oxygen radical absorbance capacity (ORAC−fluorescein) assay. J Agric Food Chem. 2004. https://doi.org/10.1021/jf0305231.
29. Napagoda MT, Kumari M, Qader MM, De Soyza SG, Jayasinghe L. Evaluation of tyrosinase inhibitory potential in flowers of *Cassia auriculata* L. for the development of natural skin whitening formulation. Eur J Integr Med. 2018. https://doi.org/10.1016/j.eujim.2018.06.005.
30. Korkmaz N, Sener SO, Akkaya S, Badem M, Aliyazicioglu R, Abudayyak M, Oztas E, Ozgen U. Investigation of antioxidant, cytotoxic, tyrosinase inhibitory activities, and phenolic profiles of green, white, and black teas. Turk J Biochem. 2019. https://doi.org/10.1515/tjb-2017-0345.
31. de Mejia EG, Ramirez-Mares MV, Puangpraphant S. Bioactive components of tea: cancer, inflammation and behavior. Brain Behav Immun. 2009. https://doi.org/10.1016/j.bbi.2009.02.013.
32. Hilal Y, Engelhardt U. Characterisation of white tea—comparison to green and black tea. J Verbrauch Leb. 2007. https://doi.org/10.1007/s00003-007-0250-3.
33. Kottawa-Arachchi JD, Ranatunga MA, Amarakoon AM, Gunasekare MT, Attanayake RN, Sharma RK, Chaudhary HK, Sood VK, Katoch R, Banyal DK, Piyasena KG. Variation of catechin and caffeine content in exotic collection of tea [*Camellia sinensis* (L.) O. Kuntze] in Sri Lanka and potential implication in breeding cultivars with enhanced quality and medicinal properties. Food Chem Adv. 2022. https://doi.org/10.1016/j.focha.2022.100108.
34. Hara Y, Luo SJ, Wickremasinghe RL, Yamanishi T. VII. Storage of tea: special issue on tea. Food Rev Int. 1995. https://doi.org/10.1080/87559129509541055.
35. Vural N, Algan Cavuldak Ö, Akay MA, Anlı RE. Determination of the various extraction solvent effects on polyphenolic profile and antioxidant activities of selected tea samples by chemometric approach. J Food Meas Charact. 2020. https://doi.org/10.1007/s11694-020-00376-6.
36. Dias T. White tea (*Camellia sinensis* (L.)): an-tioxidant properties and beneficial health effects. Int J Food Sci Nutr Diet. 2013. https://doi.org/10.19070/2326-3350-130005.
37. Ranasinghe HPP, Rajathewa RMASN, Kottawa-Arachchi JD, Ranathunga MAB, Amarakoon AMT. Bio-chemical evaluation of selected Sri Lankan tea (*Camellia sinensis* L.) cultivars and accessions for production of green tea. In: Proceedings of the International Postgraduate Research Conference 2015 University of Kelaniya, Kelaniya, Sri Lanka, (Abstract). 2015. p. 170, 339.
38. Tfouni SA, Camara MM, Kamikata K, Gomes FM, Furlani RP. Caffeine in teas: levels, transference to infusion and estimated intake. J Food Sci Technol. 2018. https://doi.org/10.1590/1678-457x.12217.
39. Chen L, Zhou ZX. Variations of main quality components of tea genetic resources [*Camellia sinensis* (L.) O. Kuntze] preserved in the China National Germplasm Tea Repository. Plant Foods Hum Nutr. 2005. https://doi.org/10.1007/s11130-005-2540-1.
40. Kamunya SM, Muthiani MA, Wanyoko JK, Wachira FN, Chalo RM, Kimutai S, Khalid R, Karori S. Potential use of Kenyan tea cultivars in development of high value diversified products. Int J Tea Sci. 2016;12(01):30–48.
41. Widowati W, Tjokropranoto R, Damayanti C, Kusuma HS, Handayani T, Rizal R. Potential of black tea (*Camellia Sinensis* (L.) O. Kuntze) extract as anti-oxidant and skin anti-aging. In Proceedings of the 1st International Conference on Emerging Issues in Technology, Engineering and Science. Vol. 1. 2022.
42. Huang D, Ou B, Prior RL. The chemistry behind antioxidant capacity assays. J Agric Food Chem. 2005. https://doi.org/10.1021/jf030723c.
43. Zhao CN, Tang GY, Cao SY, Xu XY, Gan RY, Liu Q, Mao QQ, Shang A, Li HB. Phenolic profiles and antioxidant activities of 30 tea infusions from green, black, oolong, white, yellow and dark teas. Antioxidants. 2019. https://doi.org/10.3390/antiox8070215.
44. Jayasekera S, Molan AL, Garg M, Moughan PJ. Variation in antioxidant potential and total polyphenol content of fresh and fully-fermented Sri Lankan tea. Food Chem. 2011. https://doi.org/10.1016/j.foodchem.2010.09.045.

45. Guzel-Seydim ZB, Seydim AC, Greene AK. Effect of brewing method on quality parameters and antioxidant capacity of black tea. Funct Food Sci. 2021;1(8):1–3.
46. Ratnasooriya WD, Abeysekera WP, Ratnasooriya CT. In vitro anti-hyaluronidase activity of Sri Lankan low grown orthodox orange pekoe grade black tea (*Camellia sinensis* L.). Asian Pac J Trop Biomed. 2014. https://doi.org/10.12980/APJTB.4.2014APJTB-2014-0462.
47. Ahmed S, Griffin TS, Kraner D, Schaffner MK, Sharma D, Hazel M, Leitch AR, Orians CM, Han W, Stepp JR, Robbat A. Environmental factors variably impact tea secondary metabolites in the context of climate change. Front Plant Sci. 2019. https://doi.org/10.3389/fpls.2019.00939.
48. Kosińska A, Andlauer W. Antioxidant capacity of tea: effect of processing and storage. In: Processing and impact on antioxidants in beverages. Academic Press. 2014.