Discover Plants

Research

Evaluation of the anthocyanin content in Sri Lankan tea cultivars

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Abstract
Purple tea, produced from harvestable shoots of *Camellia sinensis*, which is a specialty tea, has shown a growing interest in the consumer market and is slowly gaining recognition in many countries. The objective of the study was to evaluate the potential of tea genetic resources from Sri Lanka for the production of purple tea for the first time. Tea anthocyanins, including pelargonidin, delphinidin, malvidin, and cyanidin, were quantified using high-performance liquid chromatography(HPLC) following acid hydrolysis. The quantification of anthocyanidins was carried out in fresh tea leaves and in purple tea of the five tea cultivars. The results revealed that delphinidin and cyanidin were detected in all five tea cultivars, and malvidin was not detected in any of the five tea cultivars; however, pelargonidin was detected only in TRI 26 and TRI 2043 tea cultivars. The highest anthocyanidin content, which was 0.847 mg/g, was recorded in the fresh tea leaves of the TRI 5006 tea cultivar, and the lowest anthocyanidin concentration was detected in the fresh tea leaves of the TRI 3055 tea cultivar, which was 0.044 mg/g. Malvidin was not detected in the tested tea cultivars; further research on additional tea cultivars is required to confirm this. In addition, the contents of caffeine, gallic acid, catechins, and total polyphenols were also determined. According to the results, TRI 5006, TRI 26, and TRI 2043 tea cultivars could be utilized as potential tea cultivars for purple tea production in consideration of their higher anthocyanidin content and polyphenolic profiles.

Keywords Anthocyanidins · HPLC · Purple tea · Tea cultivars · Tea leaves

1 Introduction

Tea is considered an imperative and a popular beverage that is produced from the tender shoots of *Camellia sinensis* [1]. The demand for tea is one of the most rapidly expanding in the world currently, and as increasing demand and recognition in tea develop worldwide, more specialized teas are being produced from it. Specialty teas such as oolong, yellow, and white teas are highly desired by the consumers nowadays [2]. These varieties of tea have been categorized based on the preparation and the degree of oxidation/fermentation throughout the tea processing [3].

Recently, a purple-colored tea cultivar has attracted the attention of numerous researchers. Purple tea is renowned for both ornamental and therapeutic purposes owing to its vibrant purple color and high anthocyanin content. Though anthocyanins exist in many tea cultivars, purple tea has the highest concentration, which has a strong association with the extent to which it is purple. There are two primary reasons behind the purple transformation in tea plants. Primarily in response to

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unfavorable environmental factors, anthocyanins accumulate in the tissues of the buds and leaves. In addition, a genetic component leads significant amounts of anthocyanins to accumulate, giving high-anthocyanin-content tea cultivars [4]. Purple tea, which is a specialty tea, has shown a growing interest in the consumer market and is slowly gaining recognition in many countries, such as Kenya, China, and Japan. Purple tea brew, as the name implies, has a distinctive and attractive purple color due to the presence of the pigment anthocyanin. Processing of purple tea is carried out by following both green tea and black tea processing methods from anthocyanin-rich tea cultivars [5]. Furthermore, the chemistry of tea leaves greatly varies depending on the tea cultivar, geology, climate, propagation method, processing method, and the age of the tea plant [3, 6]. Anthocyanin-rich tea products possess an economic value and, furthermore, have acquired more attention worldwide for their potential therapeutic and nutritional benefits.

Anthocyanins are glycosylated polyphenolic compounds that belong to the flavonoid family and impart purple, red, orange, blue, and violet color phenotypes in plants [7]. Anthocyanins are water-soluble cyanic pigments accumulated in the central vacuole of the plant cell and present in diverse parts of the plant, including the root, stem, peel, and leaves [8]. The presence of more functional groups, which include hydroxyl and methoxy, in the chemical structure results in producing red and blue colors [9]. Anthocyanins are susceptible to degradation since they are highly unstable. Structural modifications such as glycosylation and acylation and environmental factors such as temperature, light, oxygen, pH, and enzyme activity affect the stability of anthocyanins. Furthermore, the presence of complex compounds such as other co-pigments, flavonoids, sugars, and metal ions also influences its stability [10]. Besides anthocyanins, which are in the form of a glycoside, it can be present as anthocyanidins, which are the aglycone form. In tea cultivars that are rich in anthocyanidins, the main types of anthocyanidins present are delphinidin (blue-reddish or purple pigment), cyanidin (reddish-purple or magenta pigment), pelargonidin (orange pigment), and malvidin (purple pigment). Recently, petunidin (a dark red or purple pigment) was also identified as an anthocyanidin present in tea. Malvidin and petunidin are methylated pigments [11].

The Tea Research Institute of Sri Lanka (TRI) has preserved approximately 600 tea germplasm accessions, and 70 of them have been recommended for commercial tea cultivation. The availability of high-quality planting materials with the appropriate characteristics is the main factor influencing the sustainability and economic success of the Sri Lankan tea industry. Though, owing to its lack of genetic foundations, the Sri Lankan tea industry is more vulnerable to both biotic and abiotic influencing factors. A wide variety of exotic tea cultivars are needed and introduced on a regular basis to sustain tea cultivation. The evaluation of functional properties as well as biochemical features is an essential component in determining and recognizing prospective parental tea cultivars to expedite the breeding of improved tea cultivars [12–14]. As a result, the metabolic profile of Sri Lankan tea cultivars, except for anthocyanin content, has been evaluated in our previous studies. Although there is a surge in the global demand for purple tea among the consumers, Sri Lanka, being one of the leading tea manufacturing countries, has not initiated producing purple tea on a commercial scale. In light of the above, screening of anthocyanidin-rich tea cultivars across the country is essential to identify tea cultivars having the potential of producing purple tea in the future. The objective of the study was to evaluate the potential of tea genetic resources from Sri Lanka to produce purple tea. Based on the leaf morphology, five tea cultivars were selected for this study. The quantification of anthocyanidins was carried out in fresh tea leaves and in purple tea of the five tea cultivars for the first time in Sri Lankan tea cultivars. Characterization of tea anthocyanins was done using HPLC following acid hydrolysis for the first time. In addition, the present study evaluated the anthocyanins along with total polyphenol, caffeine, and catechin contents in both tea leaves and purple tea. Furthermore, in terms of scope and importance, this research established the foundation to generate new tea hybrids that could be employed to diversify and improve purple tea germplasms. Subsequently, it will enhance the variety of tea products to boost the economic expansion of the tea industry in Sri Lanka by expanding the market for purple tea consumption.

2 Materials and methods

2.1 Chemicals

Acetonitrile (HPLC grade), methanol, ethyl acetate (ACS reagent, ≥ 99.5%), and hydrochloric acid were purchased from Fischer Scientific, UK. Formic acid was purchased from VWR, USA. Folin-Ciocalteu phenol reagent (BDH, France), sodium carbonate (Fisher Scientific, UK), ethylene diamine tetra acetic acid(EDTA), HPLC-grade acetonitrile, ascorbic acid, caffeine standard, gallic acid, ascorbic acid, and catechin standards including (−)-epigallocatechin (970-74-1), (−)-epigallocatechin-3-gallate (95-989-51-5), (+)-catechin (7295-85-4), epicatechin-3-gallate (95-989-51-5), and (−)-epicatechin (490-46-0), as

well as the anthocyanidins including delphinidin (528-53-0), pelargonidin (134-04-3), cyanidin (528-58-5), and malvidin (643-84-5), were purchased from Sigma-Aldrich, Germany.

2.2 Sample preparation for biochemical analysis

Five tea cultivars, TRI 2043, TRI 3055, TRI 5006, TRI 26, and DUN 7 were used. The tea cultivars are grown in the St. Coombs estate, TRI, which is located 1394 m above sea level. This area receives an average of 2500 mm of rainfall annually, with average maximum and minimum temperatures of 22.8 °C and 14.2 °C, respectively. Sample collection was done in October 2023. Approximately 100 g of the two apical leaves and bud, after being harvested, the bud and first two leaves from the five common tea cultivars were transported to the lab in an icebox at 4 °C. Samples were kept at − 80 °C (Fisher—1920CV, USA) immediately after receipt for a minimum of 6 h to undergo pre-freezing. Next, the samples were freeze-dried (Lab-Conco® Corporation, MS, USA) for 24 h. Using a laboratory grinder, the freeze-dried samples were ground until a fine powder was obtained. For further examination, the samples were placed in triple-laminated aluminum foil packets and kept at room temperature. The wet season (July and August 2023) occurred while the sampling was conducted. The mass loss at 103 °C (Memmert UN260, USA) was used for calculating the dry matter content.

2.3 Processing of purple tea

Two leaves and a bud were harvested and transported into a rattan basket from the tea cultivars TRI 2043, TRI 3055, TRI 26, TRI 5006, and DUN 7 (approximately 250 g) in the St. Coombs estate, TRI. The fresh weight of the tea leaves was determined. Next, the tealeaves were steamed at 100 °C for 1 min in a steamer for enzyme deactivation. Then the tea leaves were cooled on a withering trough for about 5–10 min to remove the surface moisture. Next, the tea leaves were pan-fried at low heat using a fryer pan to dry and roll the tea leaves. The tea leaves were tossed by hand until the tea leaves rolled and appeared dried. In the meantime, the tea leaves were gathered into a white cotton cloth to form a ball, and the excess liquid was squeezed out to even out the moisture content in the tea leaves; thus, it ensures uniform moisture loss from the tea leaves of the sample. Finally, the tea leaves were spread out in the oven and dried at 100 °C for about 1 h before storing.. The dry weight of the sample was measured after oven drying, and the cooled samples were stored in airtight, triple-laminated aluminum packages for further analysis.

2.4 Quantification of the anthocyanins

2.4.1 Anthocyanin extraction

The extraction protocol mentioned in Kerio et al. [15] was followed with minor modifications [15]. Optimization was carried out using the TRI 2043 tea cultivar. A freeze-dried sample (1 g) was weighed into a 250 mL conical flask and was mixed with 50 mL methanol/HCl (2N) (99:1 v/v), and the flask was covered with foil. It was stirred for 1 h at room temperature (23 °C) using a magnetic stirrer (LabTech®LMS-3006, Korea), followed by sonication for 2 min (at room temperature). The resultant solution was centrifuged at 4033 g-force for 10 min. The supernatant was transferred to a boiling flask, and methanol was evaporated by using a rotary evaporator (BUCHI rotavapor R-20, Switzerland). The extract was filled up to 10 mL. It was transferred to a 15 mL falcon tube and was centrifuged (Eppendorf 5804R, Germany) at 2552 g-force for 3 min. Finally, it was passed through a nylon membrane filter (0.45 µm, HYUNDAI MICRO®, Korea) and was stored in an ice bath until further analysis [15].

2.4.2 Anthocyanin purification and acid mediated hydrolysis

Anthocyanin purification was done following the protocol mentioned in Wrolstad, 2005 [16]. The SPE SUPELCO Discovery® DSC–18 (3 mL) cartridge was conditioned through the sorbent bed (C18:RP–18, ODS, Octadecyl) using two column volumes of methanol. The remaining methanol was removed by passing three column volumes of acidified distilled water (0.01% v/v HCl). The cartridge has been loaded with two mL of the anthocyanin extract. Two column volumes of acidified distilled water were used for washing the cartridge. Two column volumes of ethyl acetate were employed to wash the cartridge once more. Finally, the anthocyanin pigments were eluted using two column volumes of acidified methanol (0.01% v/v HCl) [16].

2.4.3 HPLC analysis of anthocyanidins

The anthocyanidin compounds were separated using a Supelco Ascentis® C18 reverse-phase column (25 cm × 4.6 mm, 5 µm). There was a 1 mL/min flow rate. The volume of injection was 20 µL, and the column temperature was kept at 35.0 °C. Anthocyanidin compounds were identified using the spectral profile provided by the DAD detector (190–700 nm), along with the retention time and co-chromatography (spiking) with anthocyanidin standards. The solvent gradient was 97% mobile phase A (water/acetonitrile/formic acid, 87/3/10 (v/v/v)) and 3% mobile phase B (acetonitrile) from 0 to 45 min., 75–70% mobile phase A in mobile phase B over one minute, and 97% mobile phase A in mobile phase B at 3 min. Anthocyanidin content of the samples (mg/g) was quantified separately using the calibration curves. The calibration curves were generated for cyanidin, delphinidin, and pelargonidin at 5 different concentrations (0.125–5 ppm). The stock solutions of 1000 ppm were made by dissolving the standards in the mobile phase A [15, 16].

2.5 Quantification of total polyphenol content (TPP) of freeze-dried tea leaves and purple tea

After being weighed, tea samples weighing 0.200±0.001 g have been placed into 10 mL extraction tubes with 5 mL of 70% hot methanol (70 °C). A vortex mixer (Lab-Line, USA) had been employed to mix the samples, and they were left at 70 °C for ten minutes. After allowing the samples to cool down to room temperature, they were centrifuged at 2552 g-force for 10 minutes. Gradient tubes have been filled with the obtained supernatant. The extracts were mixed together in a volumetric flask and filled to the brim (10 mL) with 70% methanol after the previously mentioned procedure was repeated. 1 mL of the extract was then transferred to a 100 mL volumetric flask, and distilled water was added until the mark was reached. The mixture was subsequently thoroughly mixed. 1 mL of this sample and 5 mL of 10% Folin-Ciocalteu's phenol reagent have been mixed in a test tube. Within three to eight minutes, 4 mL of a 7.5% sodium carbonate solution was added to the mixture. At room temperature, this combination was incubated for an hour. The optical density of the solution was measured at 765 nm using a UV-Vis spectrophotometer (Shimadzu, Japan). Gallic acid with a concentration range of 10–60 ppm was used for developing the calibration plot. The findings have been given as a dry matter basis (w/w%) percentage by mass [17]. Dry matter content was determined by following the ISO 1573:1980 protocol. First the weighing containers were left in the oven for 1 hour at 103 °C with the lids open and were cooled in the desiccator. After cooling, the weighing containers were weighed with the lid fixed to the nearest 0.001 g using an analytical balance to obtain the empty crucible weight. Next, 4 g of the test sample was measured. Finally, the weighing containers were heated with the lid open alongside the bottle at 103 °C for 6 h and were cooled in the desiccator, and the weight of the container with the sample was determined [18].

2.6 Quantification of catechins and caffeine of tea leaves and purple tea

The tea extract used in 2.5 was utilized. 1 mL of the extract was diluted four times with a stabilizing solution in a graduated tube. The solution contained 500 ppm of ascorbic acid, EDTA, and 10% v/v acetonitrile. After mixing thoroughly and filtration, these solutions were placed into HPLC vials. By using HPLC analysis, the major catechins and caffeine present in the tea extracts were determined. A diode array detector and an Agilent Technologies 1260 HPLC system were employed. A Luna Phenyl-Hexyl column (250 × 4.6 mm, 5 µm particle size) was utilized in conjunction with a Phenyl-Hexyl security guard cartridge (4 mm × 3.0 mm) from Phenomenex. The standards and samples had injection volumes of 10 µL and flow rates of 1.0 mL/min, respectively. Nine percent v/v acetonitrile, two percent v/v acetic acid, and twenty micrograms of EDTA comprised mobile phase A, while eighty percent v/v acetonitrile, two percent v/v acetic acid, and twenty micrograms of EDTA formed mobile phase B. During the 45-minute run, the sample was subjected to a binary gradient comprising 10 mins at 100% mobile phase A, 15 mins of a linear gradient of 68% A and 32% B, and 10 mins of holding. After being reset to 100%, mobile phase A was given time to equilibrate before the next injection. 35 °C was the column temperature. When compared to a caffeine reference, catechins' peak areas and retention periods were measured at 278 nm UV [19].

2.7 Statistical data analysis

Each of the determinations was carried out three times. To ascertain statistical differences within groups, the data were subjected to a one-way ANOVA, which was followed by Duncan's multiple range test for the mean separation. A p-value of less than 0.05 was taken to be statistically significant. Minitab 17 software and IBM SPSS version 27.0 were employed for statistical analysis.

3 Results and discussion

The Tea Research Institute of Sri Lanka has preserved approximately 600 tea germplasm accessions, and 70 tea cultivars have been recommended for commercial tea cultivation. As this is the preliminary study, based on the morphological characteristics of the leaves, five tea cultivars were selected for this study (see Fig. 1). Three highly pigmented tea cultivars, one moderately pigmented tea cultivar, and one non-pigmented tea cultivar have been used in this investigation. The architecture and agronomic traits of the selected tea cultivars are given in the supplementary file.

Purple tea was prepared in Yan et al. [4] with slight modifications. The process involved the following basic steps: steaming, cooling, pan-frying, pressing, and oven drying, after the tea leaves were harvested (see Fig. 2) [4].

In this study, characterization of tea anthocyanins was done using HPLC following acid hydrolysis. The optimization of the extraction of anthocyanins using the TRI 2043 tea cultivar was carried out according to Kerio et al. [15] with slight modifications [15]. However, several modifications to the original protocol were done for the detection of the required anthocyanidins in the samples and to yield uniform results from HPLC. Anthocyanins are soluble in water and alcohols; however, they are insoluble in non-polar organic solvents. The basic skeleton of anthocyanins is usually glycosylated by one or more polar side chains and thus exhibits strong polarity. Therefore, ethanolic or methanolic solutions are considered as ideal solvents for the extraction of anthocyanins from tea samples [20]. Hence, following the theory and the original protocol, methanol with HCl (99:1 v/v) was used as the solvent in the present study. In addition, it is recommended to use acidic solvents because they denature the cell membranes and simultaneously facilitate the dissolution of the anthocyanin pigments. Furthermore, acids such as formic, acetic, tartaric, citric, and hydrochloric acid stabilize

Fig. 1 Young tea shoots of the five tea cultivars

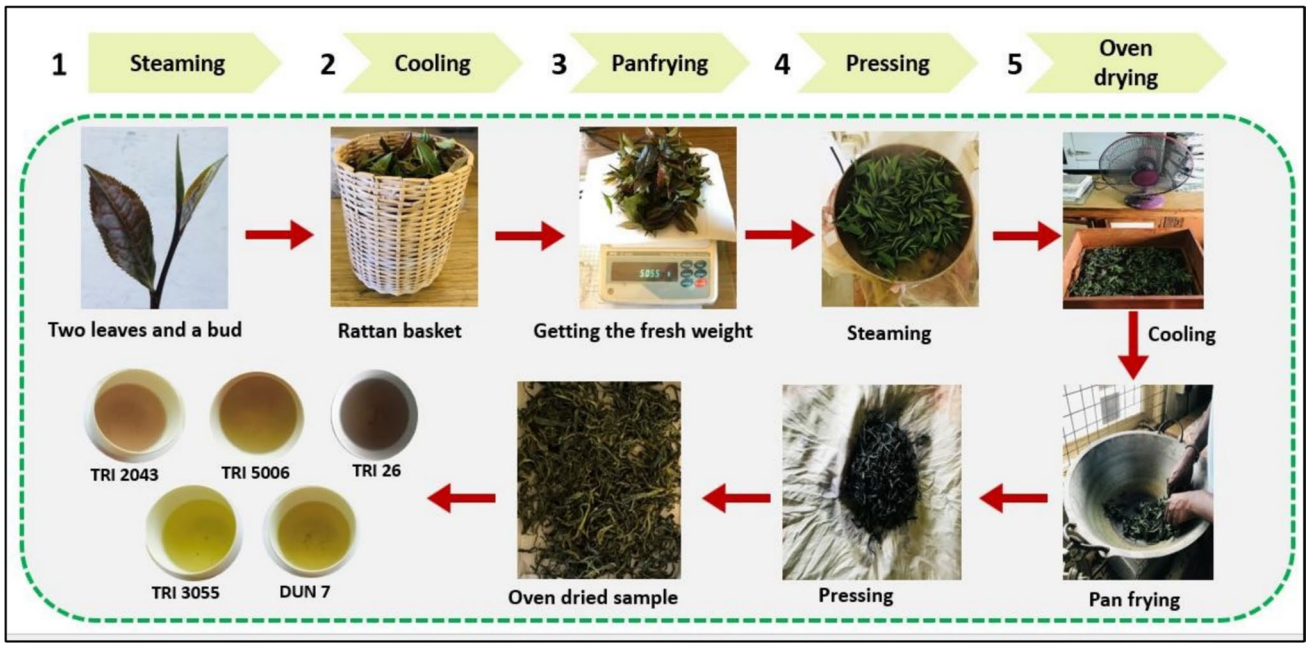

Fig. 2 Processing steps of purple tea

anthocyanins; nevertheless, high concentrations change the native form of the pigment by interrupting the associations made with metals, co-pigments, or other factors in the plant tissue [21].

Instead of filtering the extract based on the original protocol, a centrifugation step for 10 min at 4033 g-force was introduced to reduce the time of extraction, thus increasing the efficiency when handling a higher number of samples. To make sure that a clear filtrate is obtained for purification, another brief centrifugation step for 3 min at 2552 g-force was carried out after re-dissolving the concentrated anthocyanin extract in 10 mL of distilled water. Solid Phase Extraction (SPE) C18 cartridges were utilized in a purification procedure. Polyphenols and anthocyanins have an affinity for the C18 reverse-phase column, where they will compete for the binding sites and get adsorbed to the column. Ethyl acetate was used to elute the polyphenols that were bound to the column, while sugars and acids that were bound were eluted by washing the column with acidified water. The obtained extracts were hydrolyzed immediately after purification. Acid-mediated hydrolysis was done according to the protocol mentioned in Lai et al. [22]. Although the quantification of anthocyanins is tedious, it is desirable for the functional evaluation of these bioactive substances. According to the results, it was evident that acid hydrolysis simplifies the HPLC chromatogram by removing the sugar groups and the attached acyl groups from the anthocyanin molecule to yield the six basic anthocyanidins (pelargonidin, cyanidin, delphinidin, malvidin, peonidin, and petunidin). The quantification of anthocyanins is more complex compared to the quantification of anthocyanidins using HPLC–DAD because all the reference compounds are not available in the market, while the identification of all anthocyanins in a single HPLC–DAD is very difficult [23]. Recent HPLC methods have incorporated acids into the mobile phase to facilitate an acidic environment, which ensures the stability of anthocyanins. It has been found that the anthocyanin compounds are mobilized in their cationic flavylium form, possessing the highest absorbance at 520 nm [24, 25]. Initial HPLC analysis was done with the standards of pelargonidin, cyanidin, malvidin, and delphinidin (see Fig. 3). In addition, the elution sequence of the anthocyanidins determined from the present study was in agreement with similar studies that have been conducted on tea and other plants [26].

In this study, the limit of detection (LOD) values for delphinidin chloride, cyanidin chloride, and pelargonidin chloride were 0.354, 0.006, and 0.279 mg/g, respectively. Limit of quantification (LOQ) values for delphinidin chloride, cyanidin chloride, and pelargonidin chloride were 1.073, 0.491, and 0.846 mg/g, respectively. According to the results, the highest delphinidin and cyanidin contents were recorded in TRI 5006, and the lowest were recorded in TRI 3055 in fresh tea leaves. However, pelargonidin was not detected in TRI 3055, TRI 5006, and DUN 7 tea cultivars. Delphinidin and cyanidin were detected in all the samples, and pelargonidin was not detected in the TRI 3055, TRI 5006, and DUN 7 tea cultivars (see Table 1 and Fig. 4). The total anthocyanidin content was highest in TRI 5006, where the fresh leaves contained 0.847 mg/g and the purple tea contained 0.639 mg/g. However, the lowest anthocyanidin content was seen in a non-pigmented tea cultivar, TRI 3055, where the fresh leaves yields 0.044 mg/g and 0.029 mg/g in the purple tea, respectively. The extracted

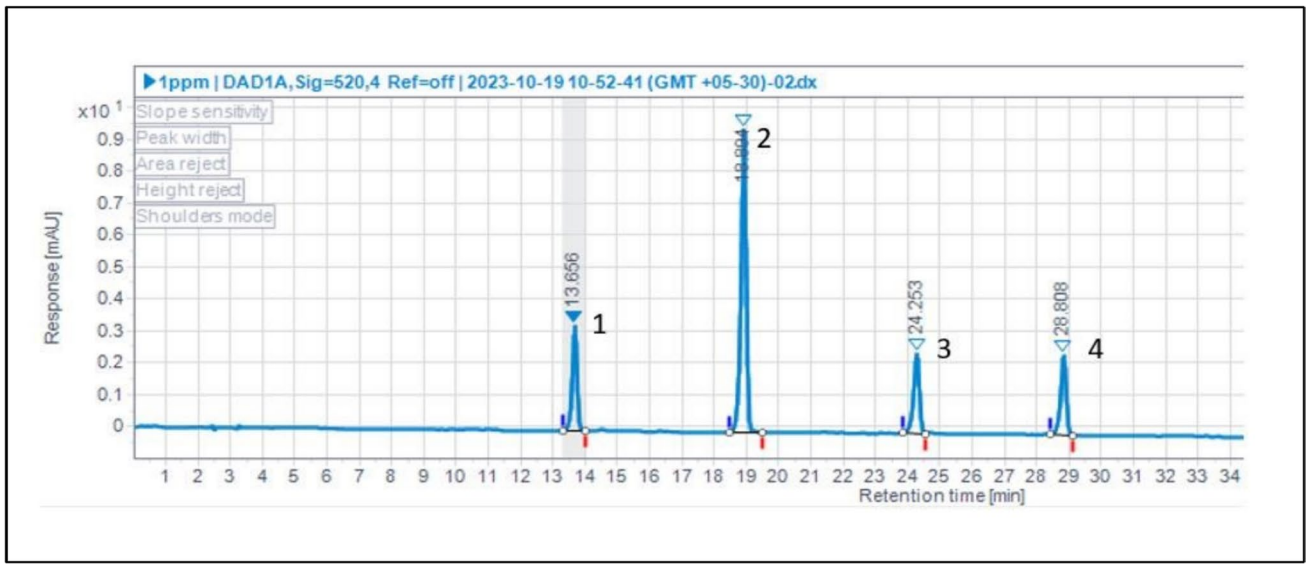

Fig. 3 HPLC chromatogram for delphinidin (1), cyanidin (2), pelargonidin (3) and malvidin (4)

anthocyanins of the TRI 3055 tea cultivar appeared green in color and were different from the rest of the tea cultivars. In addition, it is a non-pigmented tea cultivar and is not a potential tea cultivar for the manufacturing of purple tea.

And also, the anthocyanidin contents of the purple tea and fresh tea leaves varied significantly among the tea cultivars used. However, in contrast to our study, a similar study conducted in India has shown that the total anthocyanidin contents in their tea cultivars range from 1645.38 ppm to 4792.64 ppm. In addition, they have shown higher levels of individual anthocyanidins compared to the present study, where levels of pelargonidin and cyanidin are 123.18–912.80 ppm and 221.21–1650.86 ppm, respectively [27]. Moreover, Kenyan tea cultivars have depicted 62.76 ppm of delphinidin, 48.01 ppm of cyanidin, and 121.37 ppm of pelargonidin in processed unaerated tea from purple tea cultivars [15]. The variation of the anthocyanidin contents in different tea cultivars in these regions could be attributed to different environmental and geographic conditions in addition to the different methods followed during manufacturing processes. Research done on Kenyan and Chinese Zijuan tea cultivars has detected the presence of malvidin [15, 28]. Furthermore, the predominant anthocyanidin in purple-colored tea cultivars TRA St. 817 and TRA P7 in India was malvidin, which accounts for 1911.30 ppm and 1330.63 ppm, respectively [28]. According to the present study, malvidin was not detected. However, it cannot be concluded that malvidin is not present in Sri Lankan purple tea germplasms since only five tea cultivars were tested in the current study, which is not sufficient to draw a conclusion. Therefore, it is required to increase the number of tea cultivars tested. The results further elucidate that the environment and the genotype could affect the composition of anthocyanins, which needs to be researched further.

Furthermore, the anthocyanidin contents of the purple tea in each tea cultivar are reduced compared to the fresh leaves, and according to the results of one-way ANOVA, there is a statistically significant ($p \leq 0.05$) difference. Hence, it suggests that anthocyanidin degradation is possible due to its highly unstable nature, thus either being lost or oxidized during the processing of tea. Therefore, it is essential to use tea cultivars that contain high amounts of initial anthocyanin content during the manufacturing process to have a considerable amount of the anthocyanin in the made tea. In the anthocyanin-rich Zijuan tea cultivar in China, the presence of eight anthocyanins was identified from the study done by Lv et al. [28]. Pelargonidin-3,5-diglucoside, delphinidin, cyanidin, peonidin, pelargonidin, cyanidin-3-glucoside, malvidin, and cyanidin-3-glucoside are the most abundant. However, a similar study done by Jiang et al. [29] on the same 'Zijuan' tea cultivar has indicated the presence of only 4 types of anthocyanins [29]. In an attempt by Lai et al. [22] on the Chinese tea cultivar 'Ziyan,' delphinidin (708 µg/g) was the most abundant [22]. In Kenyan tea, according to the study done by Kerio et al. [15], malvidin (81.23 ppm and 233.01 ppm) was the most abundant in black tea and green tea. Furthermore, another study done in Kenya on the purple tea variety TRFK 306 elucidated cyanidin as the most prevalent (1755.60 ppm) and delphinidin (122.85 ppm) as the least prevalent [15, 30]. Nevertheless, it is worth noting that these research studies demonstrate the composition of anthocyanins is greatly affected by the genotype as well as the processing and climatic conditions, which accounts for the observed variations. Differences in anthocyanidin content between Sri Lankan and Kenyan tea cultivars may reflect genetic, environmental, or methodological factors.

Table 1 Anthocyanidins in the tea leaves and purple tea

Tea cultivars	Concentration (mg/g)							
	Delphinidin chloride		Cyanidin chloride		Pelargonidin chloride		Total Anthocyanidin content	
	Fresh tea leaves (± SD)	Purple tea (± SD)	Fresh tea leaves (± SD)	Purple tea (± SD)	Fresh tea leaves (± SD)	Purple tea (± SD)	Fresh tea leaves (± SD)	Purple tea (± SD)
TRI 26	0.368 ± 0.002^{b}	0.248 ± 0.002^{b}	0.085 ± 0.002^{b}	0.075 ± 0.002^{b}	0.044 ± 0.001^{a}	0.036 ± 0.001^{a}	0.497 ± 0.002^{b}	0.359 ± 0.002^{b}
TRI 3055	0.031 ± 0.001^{e}	0.02 ± 0.001^{e}	0.013 ± 0.001^{d}	0.009 ± 0.001^{b}	Not detected	Not detected	0.044 ± 0.001^{d}	0.029 ± 0.001^{d}
TRI 5006	0.656 ± 0.003^{a}	0.456 ± 0.001^{a}	0.191 ± 0.002^{a}	0.183 ± 0.002^{a}	Not detected	Not detected	0.847 ± 0.002^{a}	0.639 ± 0.002^{a}
TRI 2043	0.076 ± 0.002^{d}	0.05 ± 0.001^{d}	0.04 ± 0.001^{c}	0.035 ± 0.001^{c}	0.039 ± 0.001^{b}	0.027 ± 0.001^{b}	0.155 ± 0.001^{c}	0.112 ± 0.001^{c}
DUN 7	0.193 ± 0.001^{c}	0.185 ± 0.002^{c}	0.044 ± 0.001^{c}	0.032 ± 0.001^{c}	Not detected	Not detected	$0.237 \pm 0.001^{b,c}$	$0.217 \pm 0.001^{b,c}$

ND Not Detected, *SD* Standard deviation

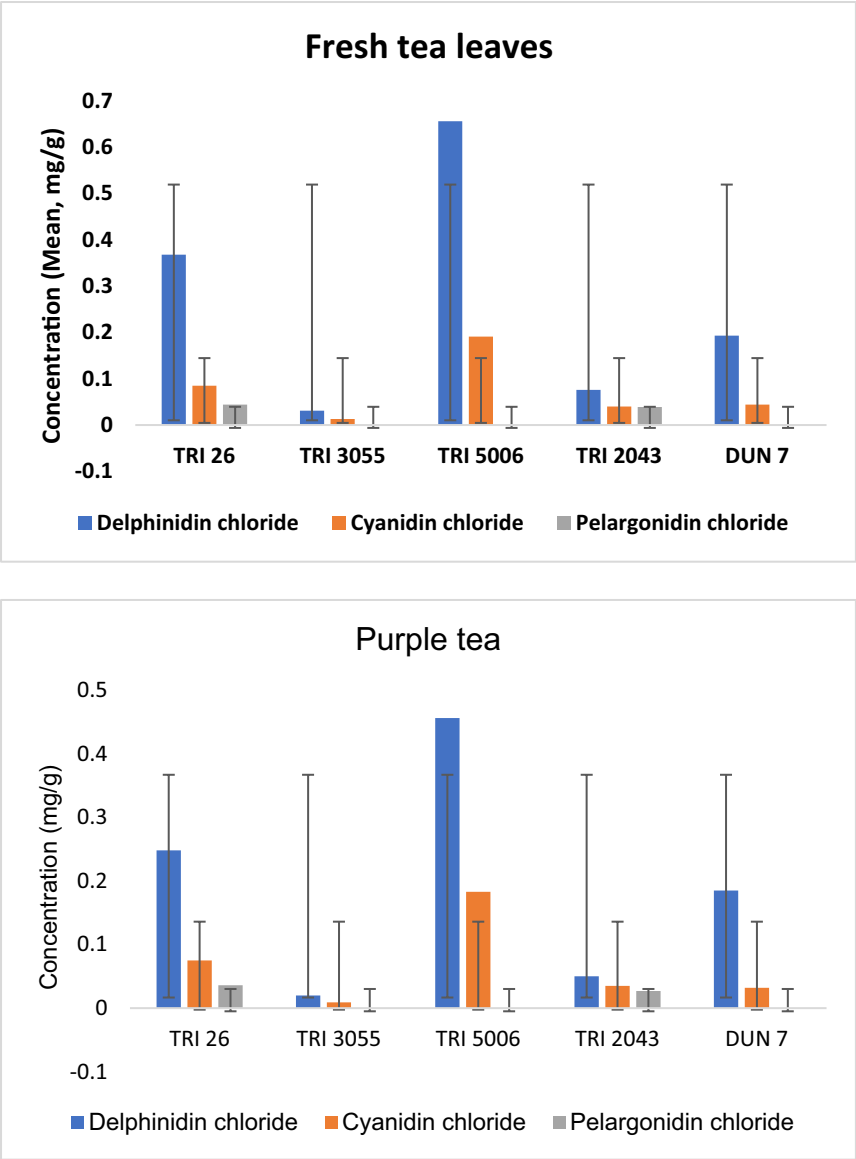

Fig. 4 Variation of anthocyanidin content in the fresh tea leaves and purple tea

Though tea consumption has been linked to health benefits from the beginning of its history, its scientific investigation is being carried out. Tea is a drink that possesses substantial antioxidant and anti-inflammatory qualities, indicating it could assist in combating numerous chronic diseases [13, 14]. However, during the past few years, there has been a surge in comprehensive scientific studies on tea and its biologically active constituents, with a particular focus on its health benefits. The major chemical constituent in tea is polyphenols. Chemically, polyphenols are phenolic compounds that have multiple phenol units. Several bioactivities have been attributed to tea polyphenols. Anticancer, anti-cardiovascular, antioxidant, anti-hyperglycemic, antimicrobial, and anti-obesity activities are some of those. The antioxidant potential of tea polyphenols relates to most of these disease prevention strategies, as they have the potential to stimulate the production of endogenous antioxidant proteins. Henceforth, tea polyphenols are important for disease prevention. However, their use in dietary interventions or alternative treatments must be considered due to their low absorption in the body and potential toxicity risks. The researchers have also confirmed that tea polyphenols have anti-depressive effects [31–34]. Furthermore, caffeine acts as a stimulant to the central nervous system. This improves the mood, sharpens mental clarity and vigilance, delays mental fatigue, enhances alertness, and shortens reaction time [35]. Purple tea was manufactured in this study employing the green tea manufacturing process. Green tea is manufactured with minimal processing of the freshly plucked tea leaves and buds. This allows it to retain a considerable amount of antioxidants, which are mainly the tea polyphenols. Catechin derivatives are the most important polyphenolic compounds present

Table 2 Catechin and total polyphenolic contents (%) of tea leavesfrom different tea cultivars

Tea cultivars	GA	EGC	Cat	EC	EGCG	ECG	Total catechins	Caffeine	TPP
TRI 2043	$0.03^{d} \pm 0.01$	$2.46^{b} \pm 0.06$	$0.55^{a} \pm 0.01$	$2.53^{b} \pm 0.07$	$4.80^{c} \pm 0.76$	$3.66^{a} \pm 0.53$	$14.04^{c} \pm 0.01$	$2.91^{b} \pm 0.18$	$23.40^{c} \pm 0.42$
TRI 26	$0.05^{c} \pm 0.01$	$4.12^{a} \pm 0.19$	$0.15^{d} \pm 0.02$	$2.11^{b} \pm 0.07$	$6.13^{b,c} \pm 0.27$	$2.02^{b} \pm 0.07$	$14.61^{c} \pm 0.62$	$2.63^{b} \pm 0.09$	$24.27^{c} \pm 0.05$
TRI 3055	$0.08^{a} \pm 0.01$	$2.90^{b} \pm 0.11$	$0.35^{c} \pm 0.01$	$1.24^{c} \pm 0.06$	$14.69^{a} \pm 0.50$	$3.34^{a} \pm 0.13$	$22.62^{a} \pm 0.82$	$3.07^{a} \pm 0.12$	$26.74^{a} \pm 0.18$
TRI 5006	$0.03^{d} \pm 0.01$	$2.31^{b} \pm 0.12$	$0.29^{c} \pm 0.13$	$3.14^{a} \pm 0.23$	$5.27^{c} \pm 0.15$	$2.75^{b} \pm 0.13$	$14.81^{b,c} \pm 0.76$	$1.91^{c} \pm 0.12$	$25.31^{b} \pm 0.19$
DUN 7	$0.07^{b} \pm 0.01$	$4.02^{a} \pm 0.02$	$0.46^{b} \pm 0.12$	$2.37^{b} \pm 0.06$	$8.02^{b} \pm 0.11$	$4.11^{a} \pm 0.06$	$19.07^{a,b} \pm 0.39$	$1.65^{d} \pm 0.02$	$25.74^{a,b} \pm 0.29$

The mean ± SD of the triplicate values is shown in the table. At 95% confidence, the means that don't correspond to the same letter (within a column) differ significantly (α = 0.05)

GA Gallic acid, *C* Catechin, *EC* Epicatechin, *ECG* Epicatechin gallate, *EGC* Epigallocatechin, *EGCG* Epigallocatechin gallate, *TPP* Total polyphenol content

Table 3 Ccatechin and total polyphenolic contents (%) of purple tea from different tea cultivars

Tea cultivars	GA	EGC	Cat	EC	EGCG	ECG	Total catechins	Caffeine	TPP
TRI 2043	$0.07^{b} \pm 0.01$	$2.27^{b} \pm 0.08$	$0.37^{a} \pm 0.01$	$2.23^{a,b} \pm 0.35$	$4.81^{c} \pm 0.79$	$3.20^{a} \pm 1.06$	$12.95^{c} \pm 2.11$	$3.05^{b} \pm 0.04$	$22.71^{b} \pm 0.80$
TRI 26	$0.06^{c} \pm 0.01$	$2.94^{b} \pm 0.19$	$0.11^{c} \pm 0.01$	$2.01^{a,b} \pm 0.02$	$6.02^{b,c} \pm 0.29$	$2.13^{b} \pm 0.07$	$13.26^{c} \pm 0.54$	$3.58^{a} \pm 0.10$	$22.98^{b} \pm 0.21$
TRI 3055	$0.10^{a} \pm 0.01$	$2.09^{b} \pm 0.69$	$0.07^{d} \pm 0.00$	$0.85^{c} \pm 0.01$	$15.03^{a} \pm 0.08$	$3.79^{a} \pm 0.04$	$21.93^{a} \pm 0.59$	$3.78^{a} \pm 0.01$	$26.06^{a} \pm 0.36$
TRI 5006	$0.05^{d} \pm 0.01$	$2.37^{b} \pm 0.02$	$0.25^{b} \pm 0.00$	$1.63^{b} \pm 0.03$	$7.08^{b} \pm 0.26$	$3.37^{a} \pm 0.18$	$14.75^{b,c} \pm 0.48$	$2.83^{c} \pm 0.03$	$23.18^{b} \pm 0.60$
DUN 7	$0.03^{e} \pm 0.01$	$4.32^{a} \pm 0.01$	$0.35^{a} \pm 0.00$	$2.39^{a} \pm 0.00$	$7.28^{b} \pm 0.01$	$3.86^{a} \pm 0.01$	$18.23^{a,b} \pm 0.02$	$1.59^{d} \pm 0.01$	$23.21^{b} \pm 0.10$

The mean ± SD of the triplicate values is shown in the table. At 95% confidence, the means that don't correspond to the same letter (within a column) differ significantly (α = 0.05)

GA Gallic acid, *C* Catechin, *EC* Epicatechin, *ECG* Epicatechin gallate, *EGC* Epigallocatechin, *EGCG* Epigallocatechin gallate, *TPP* Total polyphenol content

in green tea. Therefore, in this study, catechin, total polyphenol, and caffeine contents were investigated in the purple tea as well as fresh tea leaves.

Besides anthocyanins, other constituents such as the total polyphenols, catechins including epicatechin-3-gallate (ECG), (−)-epigallocatechin-3-gallate (EGCG), (+)-catechin (C), (−)-epigallocatechin (EGC), and (−)-epicatechin (EC), as well as caffeine content, contribute to fully harnessing the quality, flavor, and aroma of purple tea if it is to be introduced as a specialty tea [15, 36]. Results revealed that total polyphenol, caffeine, and catechin concentrations in tea leaves and purple tea of different tea cultivars significantly varied (see Tables 2 and 3). The total polyphenol and catechin, as well as caffeine concentrations of the tea leaves, ranged from 23.40 to 26.74%, 14.04 to 22.62%, and 1.65 to 3.07%, respectively. And also, the total polyphenol, total catechin, and caffeine contents of the purple tea ranged from 22.71–26.06%, 12.95–21.93%, and 1.59–3.78%, respectively. In addition, the highest total polyphenolic (26.06±0.36%), total catechin (22.62±0.82%), EGCG (14.69±0.50%), ECG (3.79±0.04%), and caffeine (3.07±0.12%) contents were found in the fresh tea leaves of TRI 3055. Moreover, the highest total polyphenolic (26.74±0.18%), total catechin (21.93±0.59%), EGCG (15.03±0.08%), ECG (3.34±0.13%), and caffeine (3.78±0.01%) contents were found in the purple tea produced from TRI 3055. Interestingly, the lowest anthocyanidin content was seen in TRI 3055. However, the results obtained align with the results reported by Abdel-Aal et al. [11], where the total catechin content of purple tea cultivars was14.0–14.8%, while in green tea cultivars it was 25.9–31.1% [11]. This suggests that elevated anthocyanin contents could lead to reduced levels of catechin derivatives in tea. However, the fluctuation in the catechin content does not consistently decrease with elevated anthocyanin contents. Thus, the catechin content in purple tea-producing tea cultivars is high overall, which contributes to the unique quality of purple tea of having high levels of both catechins and anthocyanins, which enhances its potential health benefits.

Total polyphenol contents in tea leaves account for approximately 30% of the dry weight [36]. Flavonols such as quercetin, myricetin, and kaempferol and flavanols or catechins such as EC, C, EGC, ECG, and EGCG are considered the main classes of polyphenols in fresh leaves [36]. According to reports, total catechin content in tea leaves of the China, Assam, and Cambod types obtained from the tea germplasm during the dry season was 14.49, 16.25, and 16.74%, while during the wet season it was 15.06, 16.88, and 17.37% [12]. The caffeine concentration of tea germplasm in China ranged

from 1.2 to 5.9%. Eventually it was found that the caffeine concentration in Kenyan tea germplasms ranged from 1.96 to 4.37% [37, 38]. Nevertheless, the caffeine concentrations in China, Assam, and Cambod tea germplasms in Sri Lanka were 2.02%, 3.27%, and 3.13%, respectively [12]. The polyphenol and caffeine contents are compatible with the findings of this investigation. Apart from these chemical compounds, tea leaves contain amino acids, carbohydrates, vitamins, proteins, and minerals [39]. It is reported that phenolic profiles of Sri Lankan tea are significantly influenced by a combination of variables like season, climate, soil fertility, and processing processes [40]. In addition, purple tea, which is rich in anthocyanin, has been shown to have various health benefits, such as boosting the immune system, regulating blood glucose levels, and improving gut, mental, and skin health, as well as anti-cancer, immunostimulatory, and antioxidant properties [4, 41]. And also, polyphenols and caffeine also exhibited numerous health aspects. Therefore, tea cultivars with significant amounts of anthocyanins, caffeine, and polyphenols could lead to the production of purple tea with remarkable positive health impacts.

Only five tea cultivars were used in this study owing to resource limitations, since more than 600 tea cultivars are available in Sri Lanka. Therefore, the findings of the study may not be representative of the full genetic diversity of Sri Lankan tea. As this is a preliminary study, our ultimate objective is to screen tea cultivars with appropriate anthocyanin content for producing purple tea. Subsequently, the potential tea cultivar could be utilized as parents for future plant hybridization programs with the goal of developing new tea cultivars for purple tea production. All tea cultivars selected in this study were collected from one location (St. Coombs Estate, TRI) at 1394 m elevation. The anthocyanin content of tea plants can vary significantly based on climate, soil, altitude, and environmental factors. Conducting sampling from multiple locations with different altitudes and climates would have strengthened the study. The primary drawback of this study is that it utilized only five tea cultivars. Although malvidin is not detected in all five samples, an exact conclusion cannot be drawn considering only five cultivars were tested. To provide more reliable results, further research studies with a larger and more varied sample size need to be carried out in the future tea breeding program. The tea cultivars with considerable concentrations of catechins and polyphenols, along with anthocyanins, contribute to their being suitable for the commercial production of purple tea as a healthy beverage.

4 Conclusions

The present investigation has assessed the potential of tea genetic resources from Sri Lanka for the production of purple tea. Tea anthocyanins were characterized using HPLC following acid hydrolysis. According to the current study, delphinidin and cyanidin were detected in all five tea cultivars; however, pelargonidin was detected in some tea cultivars. Furthermore, malvidin was not detected in all five tea cultivars. However, it could not be concluded that malvidin is not present in Sri Lankan purple tea germplasms since only five tea cultivars were tested in the current study, which is not sufficient to draw a conclusion. Therefore, it is required to increase the number of tea cultivars tested. The results further elucidate that the environment and the genotype could affect the composition of anthocyanins, which needs to be researched further. This study provides initial insights into Sri Lankan tea cultivars' anthocyanidin profiles, informing future research.

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Data availability Data will be made available on 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.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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