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Characterization and Quantitation of Anthocyanins of the Pigmented Tea Cultivar TRI 2043 (*Camellia sinensis* L.) from Sri Lanka

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Abstract: Tea leaves are rich in diverse bioactive compounds. The tea accession TRI 2043 is unique due to its pigmented leaves caused by anthocyanins, clonal origin, resistance to blister blight disease, and high pubescence density. Because of its peculiarity, TRI 2043 is used to produce high-quality silver tip tea, a premium type of tea that commands high prices. This study was carried out to clarify and elucidate the types of anthocyanins in this particular accession. Four different anthocyanin species were identified and quantitated as cyanidin-3-*O*-β-D-galactoside and delphinidin-3-*O*-β-D-galactoside equivalents for leaf blades and stems of the cultivar TRI 2043. The characterization was performed by comparison with commercially available reference substances and further confirmed using ion mobility high-resolution time-of-flight-mass spectrometry (IMS-HRTOF-MS). Quantitation was carried out using ultra-high-performance liquid chromatography ultraviolet–visible detection (UHPLC-UV-vis) with cyanidin-3-*O*-β-D-glucoside as an internal standard. *E*- and *Z*-geometric isomers of 6-*p*-coumaroyl derivates of delphinidin and cyanidin-3-*O*-β-D-galactopyranosides were observed, and collision cross section (CCS) values were determined for all four different anthocyanidin species. The content of anthocyanins in leaf blades of cultivar TRI 2043 was 856.32 ± 41.56 µg/g dry weight, with cyanidin being the more abundant anthocyanin (69.8%). Conversely, the stem material contained an anthocyanin amount of 459.5 ± 44.7 µg/g dry weight, with a higher content of delphinidin (69.6%). In summary, an enrichment strategy using analytical membrane chromatography was established to fully elucidate and quantify the anthocyanin profile of plant samples such as the special tea variety TRI 2043.

Keywords: anthocyanins; extraction; enrichment; membrane chromatography; quantitation; collision cross section; IMS-(HR)TOF; *Camellia sinensis* L.; pigmented tea

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1. Introduction

Tea (*Camellia sinensis* L.) is the most widely consumed beverage in the world and is cultivated in more than 60 countries. Fresh leaves are extremely rich in polyphenolic compounds, and the total polyphenols, including flavan-3-ols (catechins), in tea range from 20 to 30% of the dry weight [1]. Polyphenols are major non-nutrient components in tea. They exhibit pharmacological effects and health benefits such as antioxidant, antimicrobial, and anticarcinogenic properties [2].

Among plant pigments, anthocyanins are ubiquitous natural flavonoid glycosides that occur across a number of plant families [3]. There are six naturally occurring anthocyanidins,

namely cyanidin, delphinidin, pelargonidin, petunidin, peonidin, and malvidin, which impart a wide spectrum of colors to flowers, fruits, and vegetables [4]. Anthocyanins have also been shown to play a role in plant defense against insects and pathogens [5]. As a result of the potential health benefits, interest in anthocyanin-rich purple tea has increased [6].

Tea varieties with high anthocyanin content can be used to produce "purple teas". Hence, tea breeders in different tea-growing countries have developed anthocyanin-rich purple tea varieties. Recent biochemical profiling studies have identified several anthocyanidins and quantitated them in purple-colored tea varieties bred in China, India, and Kenya [7–9]. TRI 2043, a popular anthocyanin-rich tea cultivar (cf. Figure 1) developed and recommended for commercial cultivation by the Tea Research Institute (TRI) of Sri Lanka, is used for the production of specialty tea called silver tips or white tea, which gives a pale color to the brew [10,11]. This category of is priced higher than conventional teas, especially in the Middle East, due to its high caffeine and flavan-3-ol content [12]. The silvery white color observed in silver tips/white teas is caused by the high amount of pubescence present in the apical buds of special cultivars such as TRI 2043.

Figure 1. Red tea cultivar TRI 2043.

Tea cultivars from around the world have been reported to produce different anthocyanin species ranging from cyanidin and delphinidin [9] to petunidin [13] and pelargonidin, peonidin and malvidin [14]. Several studies performed on anthocyanin-rich tea shoots focused on the isolation and characterization of anthocyanins as well as the effects of tea anthocyanins on oxidative stress [8]. The TRI 2043 variety, which came to the market in 1958, is one of the most promising new varieties. This *lasiocalyx* hybrid originated out of about 600 tea germplasm accessions, including introductions, estate selections, improved cultivars, and non-beverage type accessions maintained at the Tea Research Institute of Sri Lanka [15]. TRI 2043, with a dense pubescence in the unfolded bud, was proven to be highly resistant to leaf blight disease and contains anthocyanins, which have been identified as cyanidin and delphinidin types after acid hydrolysis [5,16]. Despite having a high antioxidant capacity, TRI 2043 had the lowest content of flavan-3-ols, especially epigallocatechin gallate, compared to other TRI varieties [10]. Therefore, the antioxidant activity and blister blight leaf disease resistance of TRI 2043 may be due to its anthocyanins. The content of flavan-3-ols, other phenolic compounds, and macronutrients such as bound nitrogen, phosphorus, and potassium varies depending on the season. The highest content is recorded in summer, while the lowest content was observed during monsoon season [17].

This study was conducted to fully elucidate the spectrum of anthocyanins present in the pigmented tea variety TRI 2043. Since anthocyanins are minor constituents in tea, achieving full elucidation and quantitation of the occurring anthocyanins requires the

preparation of an enriched extract. To accomplish this goal, an optimization of the proper extraction and enrichment protocols to determine anthocyanin content in tea cultivar TRI 2043 was necessary. For the identification of anthocyanins, the extract is analyzed by high-resolution mass spectrometry combined with ion mobility as an additional source of structural information.

2. Materials and Methods

2.1. Plant Material

The fresh two leaves and apical bud samples from ten bushes of the cultivar TRI 2043 were collected (standard system of plucking tea in Sri Lanka) at the Tea Research Institute of Sri Lanka (Talawakelle, Sri Lanka), immediately plunged into liquid nitrogen, and then freeze-dried and stored at −20 °C. The freeze-dried samples were transported to Germany in sealed bags for further analysis. The leaf blades and stems were then manually separated and analyzed.

2.2. Chemicals

For identification and quantitation, cyanidin-3-*O*-β-D-glucoside chloride (purity ≥ 98.00%), cyanidin-3-*O*-β-D-galactoside chloride (purity ≥ 95.00%), and delphinidin-3-*O*-β-D-galactoside chloride (purity ≥ 95.00%) were purchased from Phytolab (Vestenbergsgreuth, Germany). Amberlite™ XAD-7HP was obtained from Sigma-Aldrich Co. (St. Louis, MO, USA), and Sartobind® S strong acidic cation exchanger MA 75 was obtained from Sartorius Stedim Biotech GmbH (Göttingen, Germany). Double-deionized water (Nanopure®, Werner GmbH, Leverkusen, Germany) was used for extraction and UHPLC-DAD analysis. Water (LC-MS grade) and acetonitrile (UPHLC-MS grade) were purchased from TH. Geyer GmbH & Co. KG (Renningen, Germany). Dichloromethane (HPLC grade), methanol (HPLC grade), and formic acid (LC-MS grade) were purchased from Fisher Scientific (Loughborough, UK). Acetonitrile (HPLC grade) for UHPLC-DAD analysis was purchased from Honeywell Specialty Chemicals (Seelze, Germany). Formic acid (ACS Reagent 99–100% purity) and ethyl acetate (HPLC grade) were obtained from VWR Chemicals (Radnor, PA, USA).

2.3. Sample Preparation

The freeze-dried tea samples were first separated into leaf blades and stems and then ground using a laboratory mill (IKA-Werke, Staufen im Breisgau, Germany). For the powdered leaf blade samples and stem powder, approximately 200 mg was used for the extraction procedure. The samples were macerated in 20 mL of a mixture of water, methanol, and formic acid (49.5/49.5/1; $v/v/v$) while being stirred at 800 rpm and room temperature for 12 h. For quantitation replicates, 500 µL of a cyanidin-3-*O*-β-D-glucoside solution with a concentration of 20 mg L^{-1} was added as an internal standard. The extract was filtered, and the extraction was repeated two more times. Afterwards, the methanol of the combined extracts was removed using a rotary evaporator at 200 mbar and 40 °C. The aqueous solution was then extracted three times using 15 mL of dichloromethane to remove alkaloids, such as caffeine and theobromine, as well as lipids, waxes, and chlorophyll. The aqueous phase was subsequently extracted three times using ethyl acetate to separate most of the flavanols. Prior to submitting the extract to the Amberlite™ XAD-7HP adsorption column (15 mm × 150 mm), the remaining ethyl acetate was removed using a rotary evaporator at 40 °C and 200 mbar. The microporous resin was conditioned using methanol, followed by water. The extract was loaded in and washed with water to remove free sugars, organic acids, and amino acids and eluted with methanol. Since formic and acetic acid cannot be removed easily using a rotary evaporator, no acids were added to the methanol. The solvent was evaporated using a rotary evaporator at 40 °C and 200 mbar. This extract, which still contains flavonols, was further purified using a Sartobind® S MA 75 membrane-modified method according to Juadjur and Winterhalter [18]. The membrane was conditioned with 40 mL McIllvain buffer (pH 3.5) at a flow rate of 10 mL/min. After

the membrane was conditioned, the extract, which was solved in 20 mL water containing 0.1% acetic acid, was loaded onto the membrane. The membrane was washed with 60 mL methanol to elute flavonols and other remaining phenols. The elution of anthocyanins was performed using 40 mL of 1 M sodium chloride/methanol (1:1; *v*/*v*). After the removal of methanol by rotary evaporator at 40 °C and 200 mbar, the residue was loaded onto an Amberlite™ XAD-7HP resin column to remove the sodium chloride following the method described above with methanol as eluent. Most of the methanol was removed using a rotary evaporator at 200°mbar and 40 °C; the aqueous residue was diluted with water and freeze-dried afterwards. The freeze-dried extract was then dissolved in 1 mL water/acetonitrile/formic acid (95/4/1; *v*/*v*/*v*), filtered through a 0.2 µm PTFE syringe filter, and stored at 4 °C until measured. For quantitation, the extraction was performed in triplicates for the leaf blade and stem powder, with cyanidin-3-*O*-β-D-glucoside as an internal standard. The extraction procedure for anthocyanins in the cultivar TRI 2043 is summarized in Figure 2.

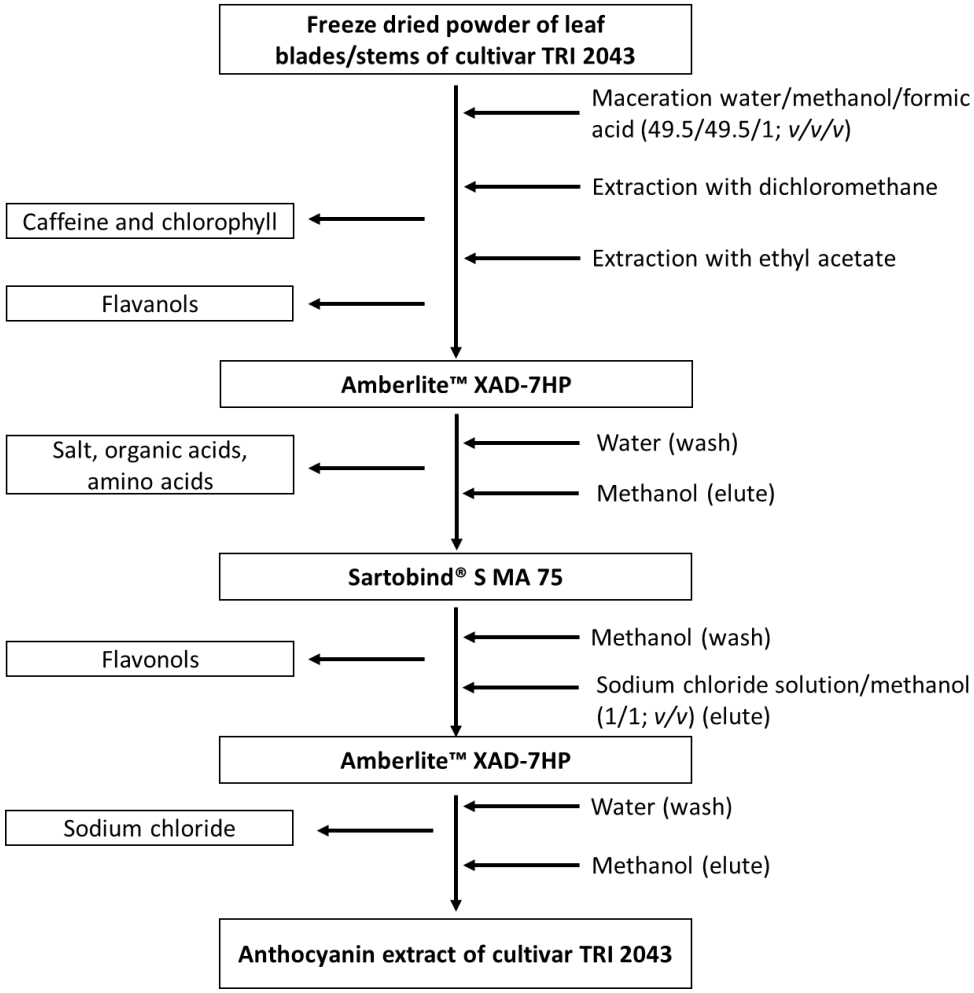

Figure 2. Enrichment strategy of anthocyanins in cultivar TRI 2043.

For the quantitation of anthocyanins, a calibration curve of cyanidin-3-*O*-β-D-galactoside and delphinidin-3-*O*-β-D-galactoside with cyanidin-3-*O*-β-D-glucoside as an internal standard was prepared. The calibration curve was recorded at seven different concentrations, 50, 25, 10, 5, 2.5, 1, and 0.5 mg L^{-1}, with the concentration of the internal standard set to 10 mg L^{-1}. Each concentration of the standard solutions and TRI 2043 extracts was analyzed in duplicate. The concentration of anthocyanins in cultivar TRI 2043 was reported as micrograms of cyanidin-3-*O*-β-D-galactoside for the cyanidin species and delphinidin-3-

O-β-D-galactoside for the delphinidin species (equivalents for the coumaoryl derivates), respectively, per gram of dry weight (DW).

2.4. Ultra-High-Performance Liquid Chromatography (UHPLC) Analysis for the Quantitation of Anthocyanins

Liquid chromatographic analysis was performed on an Agilent 1290 Infinity II System (Agilent Technologies, Waldbronn, Germany) equipped with a binary solvent manager, an autosampler, a column heater, and a diode array detector. The used column was a C18 Kinetex core–shell column (2.1 mm i.d. × 100 mm, 1.7 µm). The column heater was set at 40 °C, and the flow rate was 0.3 mL/min. The mobile phases were (A) water/formic acid (95:5; *v*/*v*) and (B) acetonitrile/formic acid (95:5; *v*/*v*). The chromatographic conditions were 3% B initial conditions, at 20 min 20% B, at 25 min 95% B, at 27 min 95% B, and at 28 min 3% B until 30 min was reached. The injection volume was 5 µL, and the detection wavelength was 520 nm. The software used for operating the system and evaluating the analyses was OpenLAB Version 3.4 (Agilent Technologies, Waldbronn, Germany).

2.5. UHPLC Ion Mobility Spectrometry Time of Flight (TOF) Mass Spectrometry for Qualification of Anthocyanins

The liquid chromatographic system used was an Agilent 1290 Infinity, including the same parts as the system described above; parameters were analogous to the method described above. Chromatographic conditions were also analogous to the parameters described above, except that only 0.1% formic acid was used as an additive in mobile phases A and B. The mass spectrometer system was a timsTOF (Bruker Daltonik, Bremen, Germany). The mass spectrometer settings were scan range, m/z 20–1300; 1/k0 0.45–1.45 V·s/cm^2; ramp time, 100 ms; capillary voltage, 4500 V; nebulizing gas pressure, 2.2 bar (N_2); dry gas flow rate, 10 L/min (N_2); nebulizer temperature, 220 °C; and collision energy, 10 eV. The mass spectrometer and trapped ion mobility were calibrated using ESI-L Low Concentration Tuning Mix (Agilent Technologies, Waldbronn, Germany). The system operating software was Bruker Compass HyStar Version 6.2 and otofControl Version 6.2 (Bruker Daltonik, Bremen, Germany). The analyses were evaluated using Bruker Compass DataAnalysis Version 5.3 (Bruker Daltonik, Bremen, Germany).

3. Results and Discussion

The extraction of TRI 2043 material was performed to remove other highly abundant components, e.g., caffeine and flavanols (cf. Figure S1 and Table S1) [19–21], using the method of Sun et al. with slight modifications [22]. For further removal of components such as flavonol–glycosides, e.g., quercetin and kaempferol derivates (cf. Figure S2 and Table S2) [19–21], the extraction was followed by a membrane chromatographic approach using the modified method of Juadjur and Winterhalter [18]. The resulting extract was enriched with anthocyanins and still contained some tannin derivates, such as strictinin (cf. Figure S3 and Table S3) [19–21], which had been isolated before from a green tea cultivar [23]. Due to its tannin structure, it is difficult to completely remove strictinin without using elaborate methods [24].

3.1. Identification

The anthocyanins were identified based on their elution order and UV data by comparing the TRI 2043 cultivar extract with the purchased reference substances. Figure 3 shows the UHPLC chromatograms of the analysis of a mixture of the reference substances, the extract of leaf blades and stems of cultivar TRI 2043 as well as the structures of the anthocyanins occurring in the cultivar.

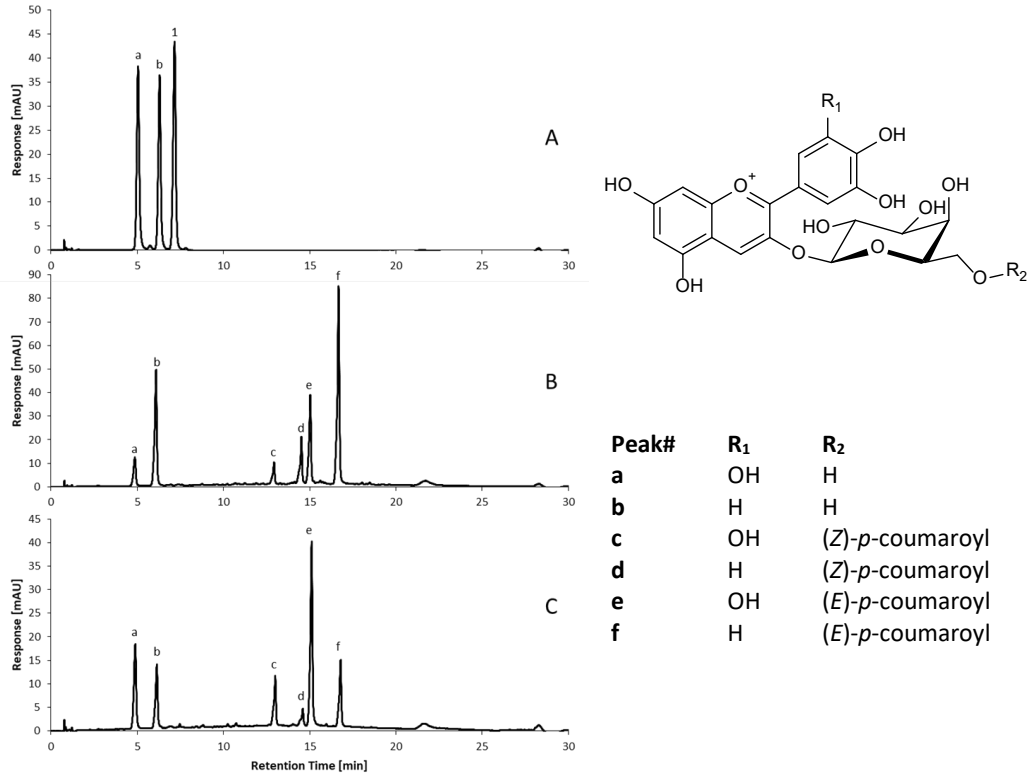

Figure 3. UHPLC chromatogram at 520 nm and structures of the anthocyanin species: (**A**) mixture of the commercial reference substances with a concentration of 10 mg L^{-1}, (**B**) the leaf blade extract of cultivar TRI 2043, (**C**) the stem extract of cultivar TRI 2043. Anthocyanins a-b were identified by comparison with the commercial reference substances, and c-f were tentatively identified by means of literature data [9]. Components: (1) cyanidin-3-*O*-β-D-glucoside (a) delphinidin-3-*O*-β-D-galactoside (Del-Gal); (b) cyanidin-3-*O*-β-D-galactoside (Cy-Gal); (c) delphinidin-3-*O*-β-D-(6-(*Z*)-*p*-coumaroyl)galactopyranoside (Del-Z-Cou-Gal); (d) cyanidin-3-*O*-β-D-(6-(*Z*)-*p*-coumaroyl)galactopyranoside (Cy-Z-Cou-Gal); (e) delphinidin-3-*O*-β-D-(6-(*E*)-*p*-coumaroyl)galactopyranoside (Del-E-Cou-Gal); (f) cyanidin-3-*O*-β-D-(6-(*E*)-*p*-coumaroyl)galactopyranoside (Cy-E-Cou-Gal).

For further confirmation, the samples were spectrometrically investigated by IMS-HRTOF-MS, which yielded high-resolution mass spectral data in conjunction with fragmentation and ion mobility data in the form of collision cross section values (cf. Table 1).

The comparison of the retention times, as well as CCS values of the extracts, show that delphinidin-3-*O*-β-D-galactoside and cyanidin-3-*O*-β-D-galactoside are present in the cultivar TRI 2043 sample. The *p*-coumaroyl hexoside species were tentatively identified based on the high-resolution mass data in conjunction with data reported by Saito et al. and Maeda-Yamamoto et al. [13,25]. To the best of our knowledge, the CCS values for the four *p*-coumaroyl galactopyranoside species have not been reported yet. Likewise, the CCS values of different hydroxycinnamoyl glycosyl derivates of cyanidin in red cabbage have been reported very recently [26]. The determination of the *E* and *Z* isomeric structure for the *p*-coumaroyl galactopyranosides was based on the observation that *E*-isomers (*trans*-configuration) have been shown to have larger CCS values compared to *Z*-isomers (*cis*-configuration) [27]. These larger molecules show a significant difference in CCS values for the steric isomers (*E* and *Z*) of Δ 11.4 $Å^2$ (5.04%) for the delphinidin- and Δ 12.3 $Å^2$ (5.50%) for the cyanidin *p*-coumaroyl galactopyranoside species compared to the galactoside species. Since the observed differences are above 5% between those molecules, with the same exact mass, we determined the lower CCS value compounds as *Z*-isomeric forms, while the higher ones would be the *E*-isomeric form of the compound. The CCS values of cyanidin-3-*O*-β-D-glucoside (201.9 $Å^2$) and cyanidin-3-*O*-*β*-D-galactoside (201.0 $Å^2$) show

a small difference of about 0.45%, with galactose tending to have a lower CCS value compared to glucose. This tendency was described before by Corinti et al. and thus strengthens the confidence in our observation [28]. In this case, separation in the ion mobility dimension shows poorer separation compared to the chromatographic approach. However, the inclusion of CCS values is promising regarding the high reproducibility of ion mobility [29,30]. Due to the increase in information, CCS values of anthocyanins can be used in conjunction with retention time to further strengthen the confidence in separating and identifying glucose and galactose species. Furthermore, CCS values can be used as a tool to distinguish different structural isomers, whereas this is not possible with high-resolution masses.

Table 1. Anthocyanins identified in leaf blades and stems of cultivar TRI 2043 as well as the commercial standards by UHPLC-ESI-TIMS-HRTOF-MS.

Peak	Compound	Formula	Retention Time [min]	*m/z* $[M]^+$	Mass Error [ppm]	Fragment Ion *m/z*	CCS [Å^2]
Extracts of leaf blades and stems of cultivar TRI 2043							
a	delphinidin-3-*O*-β-D-galactoside	$C_{21}H_{21}O_{12}$	4.86	465.1026	0.3	303	205.3
b	cyanidin-3-*O*-β-D-galactoside	$C_{21}H_{21}O_{11}$	6.09	449.1077	0.4	287	201.2
c	delphinidin-3-*O*-β-D-(6-(*Z*)-*p*-coumaroyl)galactopyranoside	$C_{30}H_{27}O_{14}$	12.96	611.1401	−0.9	303	226.3
d	cyanidin-3-*O*-β-D-(6-(*Z*)-*p*-coumaroyl)galactopyranoside	$C_{30}H_{27}O_{13}$	14.54	595.1445	0.2	287	223.6
e	delphinidin-3-*O*-β-D-(6-(*E*)-*p*-coumaroyl)galactopyranoside	$C_{30}H_{27}O_{14}$	15.05	611.1400	−0.8	303	237.7
f	cyanidin-3-*O*-β-D-(6-(*E*)-*p*-coumaroyl)galactopyranoside	$C_{30}H_{27}O_{13}$	16.69	595.1448	−0.4	287	235.9
Commercial standards							
a	delphinidin-3-*O*-β-D-galactoside	$C_{21}H_{21}O_{12}$	4.86	465.1023	1.0	303	204.9
b	cyanidin-3-*O*-β-D-galactoside	$C_{21}H_{21}O_{11}$	6.11	449.1073	1.2	287	201.0
1	cyanidin-3-*O*-β-D-glucoside	$C_{21}H_{21}O_{11}$	6.96	449.1073	1.2	287	201.9

3.2. Anthocyanin Contents in Leaf Blades

The anthocyanin contents of the cultivar TRI 2043 are as follows: (**a**) Del-Gal 66.68 ± 1.89 µg/g, (**b**) Cy-Gal 210.02 ± 3.27 µg/g, (**c**) Del-Z-Cou-Gal 34.72 ± 2.82 µg/g, (**d**) Cy-Z-Cou-Gal 66.62 ± 5.69 µg/g, (**e**) Del-E-Cou-Gal 156.84 ± 5.96 µg/g, and (**f**) Cy-E-Cou-Gal 321.44 ± 22.89 µg/g dry weight. The sum of anthocyanins in the dry leaf blades of TRI 2043 is 856.32 ± 41.56 µg/g, which is in a comparable order of magnitude as the "Zijuan" from China tea cultivar that has been reported to have 707 ± 28 µg/g of anthocyanins expressed as cyanidin-3-*O*-β-D-glucoside equivalents in dry weight [9]. Another anthocyanin-rich green tea cultivar named "Sunrouge" from Japan showed higher total anthocyanin contents of up to 3090 ± 140 µg/g dry weight [13].

3.3. Anthocyanin Contents in Stems

The anthocyanin contents of the stems from the cultivar TRI 2043 are as follows: (**a**) Del-Gal 102.83 ± 13.38 µg/g, (**b**) Cy-Gal 62.28 ± 6.31 µg/g, (**c**) Del-Z-Cou-Gal 37.15 ± 2.79 µg/g, (**d**) Cy-Z-Cou-Gal 15.04 ± 0.97 µg/g, (**e**) Del-E-Cou-Gal 180.06 ± 16.12 µg/g, and (**f**) Cy-E-Cou-Gal 62.20 ± 6.42 µg/g dry weight. The sum of anthocyanins in the dry stems of cultivar TRI 2043 is 459.56 ± 44.77 µg/g.

Our analyses have shown that the anthocyanin content in leaf blades is almost two-fold higher compared to the stems of cultivar TRI 2043. Additionally, the distribution of the delphinin and cyanidin species varies between the stems and leaf blades, as shown in Figure 4. Stems exhibited a higher tendency towards delphinidin, with delphinidin derivates representing 69.6% of the total anthocyanins. Conversely, the leaf blades contained more cyanidin derivates, accounting for 69.8% of the total anthocyanin content.

The distribution of anthocyanins in the "Sunrouge" green tea cultivar also demonstrates a higher abundance of delphinidin species in all analyzed plant parts, ranging from 71.9% in the first leaf to 88.9% in the stem [13]. On the other hand, the "Zijuan" tea cultivar from China exhibits a distribution that is almost similar to the leaf blades of cultivar TRI 2043, with 39% content of delphinidin and 49% content of cyanidin [9].

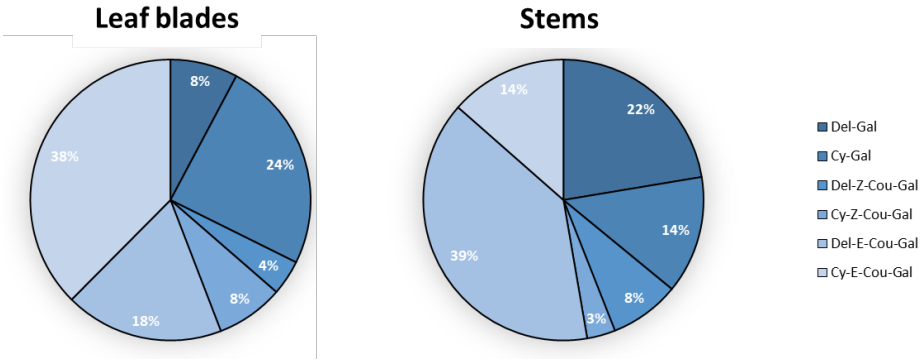

Figure 4. Distribution of anthocyanins in stem and leaf blade material of cultivar TRI 2043.

There is a limited number of studies of tea cultivars containing anthocyanins from around the world [31]. Considering that the stems of the cultivar TRI 2043 have a lower concentration of anthocyanins compared to the leaf blades, they should not be discarded as waste. Alternatively, the stem material could serve as a source for an anthocyanin-rich extract as a nutritional supplement. The utilization of anthocyanin-rich tea extracts from a Taiwan "Purple-Shoot" tea cultivar has demonstrated potential as a chemopreventive dietary agent in relation to colorectal cancer [32]. A very recent study from Kenia tested the effects of purple tea catechins and their antiproliferative activity and synergism with cisplatin [33].

4. Conclusions

According to our results, the TRI 2043 cultivar contains six different anthocyanin species represented by delphinidin– and cyanidin–galactosides as well as coumaroyl galactopyranosides (in two isomeric forms). CCS values were determined for these anthocyanidin species for the first time. During the course of this work, an enrichment strategy for anthocyanins, including analytical membrane chromatography, was established. The sum of anthocyanins in the leaf blades of cultivar TRI 2043 is 856.32 ± 41.56 µg/g and thus two-fold higher than the sum of anthocyanins in the stems of cultivar TRI 2043, which is 459.56 ± 44.77 µg/g. The distribution of anthocyanin species varies between leaf blades and stems, with leaf blades containing 69.8% of the cyanidin species and stems containing 69.6% of the delphinidin species. Also, the stem material of cultivar TRI 2043 could be used as a potential source for an anthocyanin-rich extract for nutritional supplement purposes. The findings of the study could be utilized as a chemotaxonomic method for characterizing tea genetic resources. These would help to identify diverse cultivars with high anthocyanin that are suitable for diversified tea products.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/separations11050157/s1. Section S1: HPLC-DAD chromatograms (280 nm and 520 nm) of a methanolic/aqueous tea extract (Figure S1), an Amberlite™ XAD7-HP tea extract (Figure S2) and a tea extract after membrane chromatography (Figure S3). The largest peaks have been annotated, and the compounds have been tentatively identified using their m/z and fragmentation pattern in comparison with the literature (Tables S1–S3). HPLC-DAD-MS/MS parameters. Section S2: External calibration curve of the cyanidin-3-*O*-β-D-galactoside (Figure S4) and delphinidin-3-*O*-β-D-galactoside (Figure S5).

Author Contributions: Conceptualization, P.H. and P.A.N.P.; methodology, P.H. and R.G.; validation, R.G.; investigation, P.H.; resources, M.K., P.A.N.P. and J.D.K.-A.; data curation, P.H.; writing—original draft preparation, P.H.; writing—review and editing, P.A.N.P., M.K., J.D.K.-A., R.G. and P.W.; visualization, P.H. and R.G.; supervision, R.G.; project administration, P.W. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data that support the findings of this study are available from the corresponding authors upon reasonable request.

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Conflicts of Interest: The authors declare no conflicts of interest.

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