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DETERMINATION OF CATECHIN CONTENTS IN S3A3 AND TV18 TEA CULTIVAR USING HPLC METHOD

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Abstract

Conventional characterization of catechin content in fresh tea leaves refers to the quantification of (-)-EGCG, (-)- ECG, (-)- EGC, (-)- EC, and (+)- C for their medicinal benefits. For the two tea cultivars S3A3 and TV18, being cultivated in NE India, the article targets a detailed catechin profile and a comparative assessment with the best findings in the literature. The investigations affirmed that the chosen tea cultivar leaves possessed higher constitution of galloyl catechins (EGCG & ECG). Also, while EC and C are in low concentrations, EGC concentration has been very high. Henceforth, both S3A3 and TV18 can be customized as promising sources for the development of functional tea beverage products.
Keywords: *fresh tea leaves, catechin content.*

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1. Introduction

The diversity of nutritional tea constituents is highly promising from the perspective of medicinal benefits. Compared to china tea, biochemical profilin g of fresh tea shoots of Assam and Combod tea has been inferred in terms of higher polyphenol and lower pigment constitution. Thereby, tea polyphenols refer to about 35% of the total dry weight of the fresh tea leaves (Astill et al., 2001; Higdon & Frei, 2003; Lee et al., 2014; Namal Senanayake, 2013; Vuong et al., 2010). These are majorly characterized as Flavan-3-ols group (Catechin, Theaflavins & Thearubigin) and greater than 90% constitution as catechins (Higdon & Frei, 2003; Lee et al., 2014; Vuong et al., 2010; Zhang et al., 2018). In due course of the oxidation as Oolong and black tea, the catechins in the fresh tea leaves undergo transformation as theaflavins and thearubigins.

Being the predominant polyphenols in the tea leaves (>80%), catechins qualify as a suitable biochemical marker for the identification of quality tea cultivars (Gulati et al., 2009). Thus, with increasing catechin content, the medicinal value of tea leaves enhances significantly (Sabhapondit et al., 2012). From a detailed profiling perspective, major constituent catechins in tea leaves are (-) - Epigallocatechin gallate (EGCG), (-) – Epigallocatechin (EGC), (-) - Epicatechin gallate (ECG), (-) - Epicatechin (EC) and (+) - Catechin (C)(Vuong et al., 2010, 2011). Previous studies also affirmed the relationship between the catechin profile with its antioxidant prowess and other medicinal properties. The results indicated that tea extract with higher percentage of EGC, EGCG, and ECG had significantly higher antioxidant activities(Nain et al., 2022).

Several factors such as oxidation stage, cultivar type, geographical location, and climate potentially influence the catechin composition of tea leaves(Astill et al., 2001; Wei et al., 2011). Relevant prior art confirms that the Assam and Combod type tea cultivar leaves possess a higher constitution of galloyl catechins and epigallocatechin in comparison with the China tea cultivars that possess better +(-) catechin profile(Sabhapondit et al., 2012). Thereby, both Assam and Combod tea leaves possess similar quantities of total catechins in comparison with other alternate cultivars and about 20 – 30 % of their dry weight. Comparatively, China tea possesses only 14 – 17 % total catechins on a dry basis. These variations in the catechin content in Assam and Combod tea are due to the combinatorial influence of the tree variety, geographical location, soil type, and other environmental factors(Wei et al., 2011).

It was also observed that the method of drying, temperature, and duration of drying caused significant impact on the total phenolic content and antioxidant properties of the fresh tea leaves. Previous studies reported the use of different drying methods such as oven drying, super-heated steam drying, tray drying, freeze drying and their effect on the phenolic profile. Prior studies reported that freeze drying resulted in better retention of total phenolic concentrations in comparison to the other drying methods (Roslan et al., 2020). While in comparison to conventional oven drying, tray drying below 70°C resulted in better drying rates and constitution of phenolic compounds (Harbourne et al., 2009). However, freeze drying due to its cost expensive and unscalable nature, was not highly preferred for drying of tea leaves in general. Tray drying at low temperatures (below 70°C) had been preferred for drying of many medicinal herbs, coffee leaves and tea prior to the polyphenol extraction (Ngamsuk, S. Huang, T. Hsu, 2020).

In fresh tea leaves, EGCG catechins constitute the highest concentration among all catechins. Being a potent antioxidant, previous studies confirmed on its application as a cardio-protective agent (Legeay et al., 2015; Ogawa et al., 2016; Tian et al., 2021). Also, prior studies have affirmed its role as a novel anticancer substance for the treatment of breast cancer (Mittal et al., 2004; Ping et al., 2020; Kelly & Owusu-Apenten, 2015). Also, the galloyl catechins (EGCG & ECG) exhibited their potential to inhibit the micellar solubility of cholesterol and as a potent anti-atherosclerosis agent (Liu et al., 2017; Miura et al., 2001; Ogawa et al., 2016). Besides the galloyl catechins, EGC and EC also exhibited antioxidant, antimutagenic and anti-cancer characteristics (Gadkari & Balaraman, 2015; Zhang et al., 2018).

Only a few researchers explored the catechin profile study of fresh tea leaves. Among these, one or two investigations addressed the catechin profile characteristics of the tea cultivars of NE India. Among them, most researchers targeted the catechin profile of the five catechin types that exist in a major proportion. These refer to EGC, EGCG, ECG, C and EC. Other than these, works have also been done on Gallocatechin (GC), and Catechin gallate (CG) concentrations along with the other catechins (Zhang et al., 2018).

The catechin profile in tea leaves varies extensively with cultivar type and geo-environmental conditions. Adopting ISO 14502 2005 method for the determination of catechin profile of fresh tea leaves, (Wei et al., 2011) deployed C18 column for the HPLC based analysis of five types of catechins (EGC, EGCG, ECG, C and EC) in three cultivars being cultivated at ten different locations in China. It was inferred by the authors that among five catechin types, the Catechin (C) followed with Epicatechin (EC) did vary significantly with the location. Adopting gradient elution method with C12 reverse-phase column, (Zhang et al., 2018) targeted the determination of total catechin content of twenty-eight green tea samples (24 from Sri Lanka and 4 from China). The authors concluded that the total catechin content varied from 143.6 – 282.6 mg/g. Thereby, the highest total catechin content was found in Gunpowder extra special cultivar.

In comparison with other tea cultivars grown in other regions of the world, the tea cultivars grown in northeast India possess higher concentrations of galloyl catechins and EGC. (Sabhapondit et al., 2012) studied the catechin profile of the mentioned five types of catechins constituted in the fresh tea leaves of seven combod tea cultivars, ten China tea cultivars, and ten Assam tea cultivars. Adopting ISO 14502-2:2005 method, the authors inferred that the total catechin content varied as 142.1 – 274 mg/g and S3A3 tea cultivar possessing the highest constitution of mentioned catechins.

Considering the fact that the tea cultivars grown in northeast India do have variant catechin profiles due to geo-environmental conditions and very few works being addressed for the catechin profile of fresh tea leaves of northeast tea cultivars, this work targets the evaluations of two commonly cultivated cultivars S3A3 and TV18 for their catechin profile characteristics. Tray drying method was adopted for the drying of the fresh tea leaves prior to the bioactives extraction due to its efficient drying rates, scalability, and inexpensive attributes. Thereby, their utility for functional beverage development has been envisaged.

2. Materials and methods

2.1 Materials

Fresh tea leaf samples (three leaves and a bud) were procured from Mazbat Tea Estate (26°79´N, 92°28´E, 118 MSL), a finest region that hosts good quality CTC and green tea cultivars in the spring season first flush (March, 2021). For the conducted investigations, an Assam type (S3A3) and a Combod type (TV18) tea cultivar were selected. The primary catechin standards namely (-) - Epigallocatechin gallate (EGCG), (-) - Epigallocatechin (EGC), (-) - Epicatechin gallate (ECG), (-) - Epicatechin (EC) and (+) - Catechin (C) were purchased from Sigma Aldrich. Further, acetonitrile (HPLC grade), acetic acid (HPLC grade), EDTA and ascorbic acid were also supplied by Sigma Aldrich.

2.2 Extraction of fresh tea leaves

The tea leaves were dried at 60°C for 3 hours and 15 mins. The preliminary experiments concluded that the drying at the mentioned conditions favoured negligible loss of catechins. Thereafter, the dried tea leaves were grounded using a mortar & pastel. The tea leaves were sieved and it was found that 87% of the particles passed through the 355-micron sieves of US mesh size 45. Subsequently, the grounded tea leaves were stored in sealed pouches in a deep freezer and were subjected to further analysis.

For the extraction of catechins and subsequent HPLC analysis of the total catechin profile, the ISO 14502-2:2005 method was followed with minor modifications. Firstly, 0.2 g of dried tea leaves of each cultivar was taken in a test tube. To this, 5 mL of 70% methanol was added and the mixture was kept for 30 min to equilibrate. Subsequently, the grounded tea leaves were extracted at 70°C for 10 mins. In due course of such extraction, the solution in the test tube was mixed using a vortex mixer (Tarsons Spinix vortex shaker, Cat. 3020) for an intermittent duration of 5 mins. Thereby, the samples were centrifuged for 12 mins at 3500 rpm (Sigma 2-16P). Following this, the supernatant was separated and the remaining solid particles were again subjected to extraction for 10 mins at 70°C with intermittent mixing after every 5 min interval. After final centrifugation at 3500 rpm for 12 mins, the obtained supernatant was mixed with the prior obtained extract solution. Finally, the total extracted solution was subjected to HPLC analysis for the determination of catechin profiles.

2.3 HPLC analysis for catechin determination

The HPLC analysis was carried out using Shimadzu High-performance Liquid Chromatography system equipped with Luna 5 µm Phenyl-Hexyl column (250 × 4.6 mm). Thereby, as per the ISO method, the samples were detected using a UV detector at 278(Sabhapondit et al., 2012). A gradient elution method was followed for the catechin profile analysis.

The HPLC analysis involved the following steps. Using a stabilizing solution, the extracted solution was first subjected to four times dilution. After dilution, the extract solution was filtered using a 0.45 µm syringe filter. Subsequently, a manual injector was used to inject 20 µL of the solution into the HPLC system. Both mobile phases A and B being used in the HPLC analysis were prepared by following the procedure delineated in the ISO method. For the first 10 minutes duration, 100 % mobile phase A was eluted. Thereafter, for the next 15 min, mobile phase B elution was gradually increased to 32% (68% of mobile phase A). Subsequently, for the next 10 mins, the elution ratio was kept constant. Thereafter, mobile phase A elution was once again increased to 100 % (0% mobile phase B). For the entire analysis, the flow rate was maintained constant at 1 mL/min.

3. Results & Discussion

As per the ISO method, standards curves were prepared for the five catechin constituents of the tea leaves. For these curves, Table 1 summarizes intercept, slope, and R^2.

Table 1. Slope, intercept, R square values of standard curves

Standard	Intercept	Slope	R square
EGCG	984428	29225	0.99
ECG	431191	42180	0.99
EGC	182396	7954	0.96
EC	313047	17862	0.98
C	3894	15616	0.99

Affirming good fitness, the R^2 values for the standard curves have been obtained as 0.99, 0.99, 0.96, 0.98, 0.99 for (-)- epigallocatechin gallate (EGCG), (-)- epicatechin gallate (ECG), (-)- epigallocatechin (EGC), (-) epicatechin (EC), and (+)- Catechin (C) cases respectively.

For the S3A3 tea cultivars, the HPLC chromatogram has been illustrated in Fig. 1. Thereby, the peaks for EGC, C, EC, EGCG, ECG have been analyzed to be at elution times of 9.12, 14.92, 20.13, 21.43 and 25.83 min, respectively. Thus, the S3A3 tea cultivar has been affirmed to constitute a major concentration of the galloyl catechins. Similarly, the HPLC chromatogram of the TV18 tea cultivars was also analyzed to indicate the retention times of EGC, C, EC, EGCG, ECG as 9.30, 15.77, 19.36, 21.78 and 26.13 min, respectively as can be clearly seen in Fig. 2.

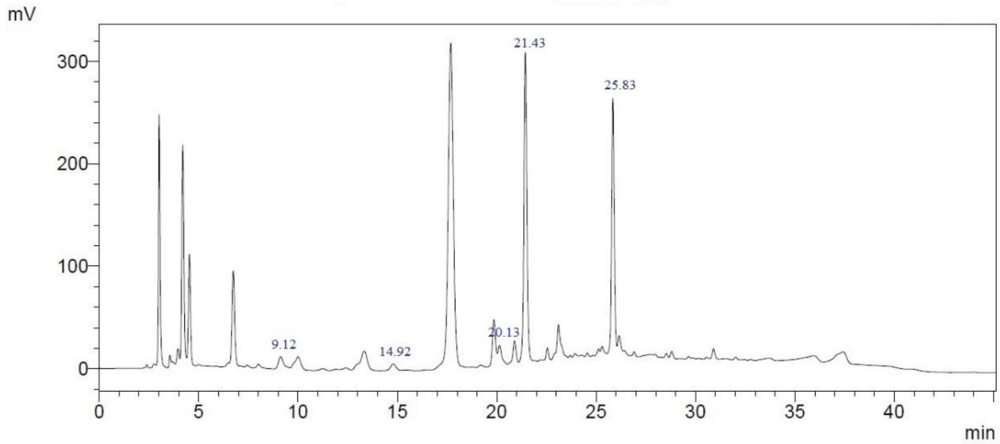

Fig. 1. HPLC chromatogram of S3A3 tea cultivar.

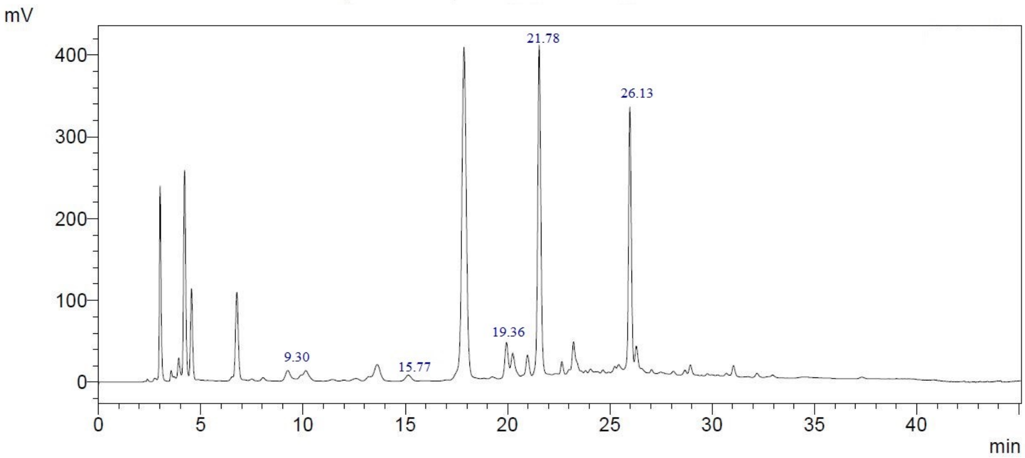

Fig. 2. HPLC chromatogram of TV18 cultivar

Fig. 3 elucidates upon the catechin profile of both cultivars in conjunction with the data being presented in the best available prior art. For the catechin profile analysis, three leaves and a bud were considered for each cultivar plucked in the spring season flush. The results of the unpaired t test indicated significant differences between the two cultivars TV18 and S3A3 in terms of C, EC, and EGC concentration with p values of 0.0011, 0.0088, and 0.0034 respectively. Total catechin concentration, EGCG and ECG concentrations among the two cultivars were not significantly difference as per the analysis with p values of 0.4768, 0.1042, and 0.0760 respectively. The results inferred that the tea cultivars S3A3 and TV18 being commonly cultivated in northeast India possess a better catechin profile in comparison with any other cultivars being studied till date. In this regard, it shall be once again noted that (Wei et al., 2011) indicated that the location, climate and overall environment do significantly influence the catechin constituent profile in the fresh tea leaves. Similar seasonal variance inference has been affirmed for the tea catechin profile of the cultivars being investigated in different countries (Zhang et al., 2018). Previous studies have affirmed that three leaves and a bud possess higher total catechin concentrations and phenolic profile in comparison to two leaves and a bud and one leaf and a bud plucking (Wei et al., 2011). Also, it has been noted that the tea leaves plucked in the first flush during the spring season possess higher phenolic content and better catechin profile in comparison to later flushes of the year (Jayasinghe & Kumar, 2021). These might be among the reasons behind the high concentration of various catechins in the present work. Also, in India and many other major tea producing countries such as China, Bangladesh, climate changes play a major role in the polyphenolic nutrition profile of the tea leaves (Bora et al., 2019; Kfoury et al., 2019). Climate studies reported the average highest and lowest temperature at Mazbat town, Assam as 29.3°C and 16.3°C respectively for the March month during a span of 30 years from 1991-2020 (data collected from Indian Meteorological Department). Previous studies on climate effects on phenolic profile of tea leaves suggested a direct dependence on temperature. Higher temperature in the spring season were correlated with higher concentrations of gallated catechins (EGCG & ECG) while had a negative impact on the EGC concentration. Similar trends were observed for the current study as shown in Table 2. Concentration of EGCG and ECG had increased while EGC concentration reduced in comparison to the prior works on catechin profile study (Sabhapondit et al., 2012). Prior works also included rainfall as one of the significant factors contributing to the change in phenolic profile of the tea cultivars. Changes in rainfall during spring and monsoon seasons contributed to total catechin concentration reduction as well as tea yield (Bora et al., 2019). Average mean rainfall in the Mazbat town were reported as 51.7 mm with an average of 4.4 days of rainfall/month for a span of 30 years ranging from 1991-2020(data collected from Indian Meteorological Department). The stable rainfall and optimal temperatures are major environmental factors contributing to the rich phenolic and catechin profile of the Assam Tea cultivars. Thereby, pertinent variations in the catechin profile characteristics of tea cultivars of North-east India can be understood.

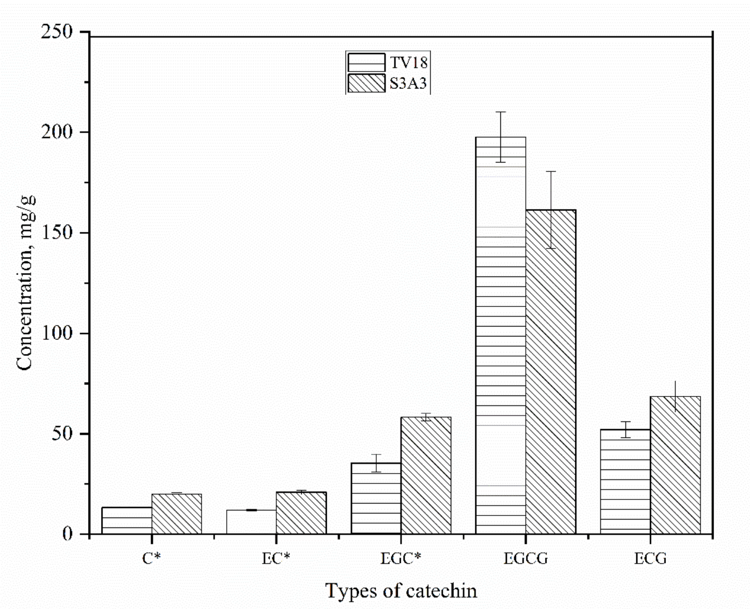

Fig. 3. Catechin profile in S3A3 and TV18 cultivar; asterisks indicate statistically significant differences (unpaired t test)

Among five alternate catechins being investigated, the EGCG has been analysed to have the highest constitution in both cultivars. The HPLC analysis confirmed that comparatively, TV18 tea cultivar possessed higher EGCG (197.77 mg/g) in comparison with the S3A3 (161.45 mg/g). Compared to the most relevant prior art (Sabhapondit et al., 2012), S3A3 and TV18 possessed 9.82 and 20.89% higher constitutions. Further, it shall be noted that till date, no other prior art reported a higher EGCG value than that being prevalent in the TV18 cultivar (163.6 mg/g of dry matter EGCG concentration in TV 18 as per (Sabhapondit et al., 2012).

Among the galloyl catechins being extracted from tea, after EGCG, the ECG is the most extensively investigated. This is due to its multi-nutritional and medicinal properties. Among both cultivars investigated in this work, the S3A3 possessed a better ECG concentration than the TV18. Compared to the best reported literature value (43.6 mg/g), the S3A3 possessed about 57.4% higher ECG concentration (68.63 mg/g). Comparatively, the TV18 possessed a lower ECG concentration (52.14 mg/g or 14.24% lower) in conjunction with the literature reported value of 60.8 mg/g (Sabhapondit et al., 2012).

EGC is one of the dominant catechins in the tea cultivars of NE India. Both S3A3 and TV18 exhibited excellent EGC constitutions. Compared to the literature reported 63.1 mg/g (Sabhapondit et al., 2012), the S3A3 exhibited 58.32 mg/g of EGC concentration and hence a 7.57% reduction in the EGC content. For the TV18 cultivar, the EGC constitution has been 35.35 mg/g which corresponds to 14.03% from the literature reported value (Sabhapondit et al., 2012). The literature also indicated that the S3A1 tea cultivar being commonly cultivated in northeast India also had a high EGC constitution of 62.3 mg/g.

In both S3A3 and TV18, both epicatechin and catechin constitution was found in significant proportions. The reported literature data for the mentioned Assam and Combod type tea indicate a lower constitution of both EC and C. While S3A3 constituted a better EC profile of 20.89 mg/g, the TV18 has been analysed to consist of 11.98 mg/g of EC. Comparatively, the literature reported data refers to 7.7 mg/g of EC for S3A3 and 5.7 mg/g of EC for the TV18 cultivar. Among alternate tea cultivars reported in the prior art, TV23 possessed the highest constitution of EC (19.6 mg/g).

In comparison with EGCG, ECG and EGC, (+-) catechin was found in lower concentration in the tea leaves. Its constitution was analysed to be 20.1 and 13.3 mg/g in S3A3 and TV18 respectively. These values were significantly higher than those being reported for the same cultivars. The literature reported data for the highest catechin concentration corresponds to Fudingdabaicha China tea cultivar being cultivated in the Wuyi province of China (22.7 mg/g). In this regard, it shall be noted that very few studies targeted complete catechin profile of tea cultivars of NE India and most of them deployed green tea for the analysis but not fresh tea leaves.

The literature reported trends for the tea cultivars of NE India affirmed lower epicatechin and catechin constitution as shown in Table 2 (Sabhapondit et al., 2012).

Table 2. Literature comparison table for catechin profile of S3A3 and TV18 cultivar.

Cultivar name	EGCG, mg/g	ECG, mg/g	EGC, mg/g	EC, mg/g	C, mg/g	Total catechin, mg/g	Reference
S3A3	147.0	43.6	63.1	12.5	7.7	274.0	Sabhapondit et al., 2011
S3A3	161.45 ± 19.17, 9.82% ▲	68.63 ± 7.79, 57.4% ▲	58.32 ± 1.81, 7.57% ▼	20.898 ± 1.12, 67.1% ▲	20.1 ± 0.69, 161% ▲	329.39 ± 24.64, 20.21% ▲	This work
TV18	163.6	60.8	31.0	5.7	4.3	265.4	Sabhapondit et al., 2011
TV18	197.77 ± 12.49, 20.89% ▲	52.14 ± 4.05, 14.24% ▼	35.35 ± 4.45, 14.03% ▲	11.98 ± 0.4, 110% ▲	13.3 ± 0.26, 216.2% ▲	310.54 ± 21.80, 17.16% ▲	This work
S3A1	142.5	46.2	62.3	14.6	3.5	269.2	Sabhapondit et al., 2011
TV23	120.1	41.7	39.1	19.8	4.9	220.6	Sabhapondit et al., 2011
Fudingdabaicha	50.8	21.6	17.1	7.1	22.7	119.2	Wei et al., 2011

However, this article inferred that both TV18 and S3A3 exhibit better constitutions of epicatechin and catechin which are well known to be highly sensitive to high temperature and high pH environments. Also, this work considered extraction from dried tea leaves at 60°C for 3.5 h based on several trials and subsequent analysis-based optimization for catechin profiles. The relevant prior art also justified the approach with the observation that the catechins don't change their structure below 70°C (Gadkari & Balaraman, 2015).

4. Conclusions

Several useful inferences have been deduced in the findings of the investigations being reported in this work. Firstly, the tea cultivars of North-east India namely S3A3 and TV18 possessed a higher constitution of Galloyl catechins and total catechins in comparison with the tea cultivars of other locations. The findings affirmed the best catechin profile being reported till date by considering the best reported prior art data. TV 18 cultivar possessed higher EGCG as 197.77 mg/g and S3A3 tea cultivar had higher ECG, EGC, EC, and C content as 68.63, 58.32, 20.90, and 20.1 mg/g respectively. Secondly, the S3A3 and TV18 tea cultivars have been analysed to respectively possess 70% and 80% catechins as (-)- EGCG and (-)- ECG. These have been analysed to facilitate further studies on the medicinal benefits of the tea varieties. Thirdly, comparatively higher constitution of (+)- catechin and (-)-epicatechin has been obtained in the conducted analysis with respect to those being reported in the relevant prior art. S3A3 and TV18 cultivars in the present work possessed 20.71, 11.37% higher concentration of gallated catechins in comparison to the literature reported studies. Thereby, both S3A3 and TV18 can be inferred to be highly competent tea cultivars of the region for the development of functional beverages and products for medicinal and nutritional applications.

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