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Research Article

# Metabolomic Study on Indonesian Green Tea (*Camellia Sinensis* L.) Cultivation

**Dinar S. C. Wahyuni[1]*, Raissa. K. Putri[1], Anik Arniyanti[1], Sasha H. Audita[1], Syaiful Choiri[1], Saptono Hadi[1], Rita Rakhmawati[1], Estu R. Nugraheni[1], Soerya D. Marliyana[2], Yudi Rinanto[3], Ari Satia Nugraha[4] and Ahmad Ainurofiq[1]**

## ABSTRACT

**Background:** Indonesia has been one of the largest tea producers in the world with one of the reputable plantations in Ngargoyoso region, Java Island in which the plantation specified in five green teas variants, TRI 2024, TRI 2025, Gambong, Kiara and Chinruan. Despite their premium quality, there were limited metabolites information related to varieties, altitude, and plucking position existed in Ngargoyoso. **Objective:** this research aimed to investigate metabolite profiling affected by varieties, plucking positions and altitudes in the tea plantation grown in Ngargoyoso, Indonesia. **Material and Methods:** Green tea (*Camellia sinensis* L.) grown in the plantation are five varieties TRI 2025, Gambong, TRI2024, Kiara and Chinruan in Ngargoyoso. **Results:** $^1$H NMR metabolomics protocol successfully identified TRI 2025 variety to have higher levels of theanine, catechins and caffeine than other varieties. The young leaves plucking position showed a higher level of catechin, EGCG, caffeine, theobromine, and L-glutamine than the older ones. While green tea at 1159 masl contains a higher level of theanine, L-glutamin, α-glucose, β-glucose, quinic acid, and succinic acid than those at 899 masl. This makes the TRI 2025 variety at the young position can be recommended to be the best quality in taste and benefits. **Conclusion:** Present study might suggest the tea quality based on metabolites profiling both for taste and benefits.
**Keywords:** Tea leaves; *Camellia sinensis*; metabolite profiling; $^1$H-NMR; Indonesia.

**Dinar S. C. Wahyuni[1]*, Raissa. K. Putri[1], Anik Arniyanti[1], Sasha H. Audita[1], Syaiful Choiri[1], Saptono Hadi[1], Rita Rakhmawati[1], Estu R. Nugraheni[1], Soerya D. Marliyana[2], Yudi Rinanto[3], Ari Satia Nugraha[4] and Ahmad Ainurofiq[1]**

*[1]Active Pharmaceutical Discovery and Development Research Group, Department of Pharmacy, Universitas Sebelas Maret Jl. Ir. Sutami 36A Surakarta 57 126, Central Java, INDONESIA.*

*[2]Chemistry Department, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret, Jl. Ir. Sutami 36A Surakarta 57 126, Central Java, INDONESIA.*

*[3]Biology Education Program, Faculty of Teacher Training and Education, Universitas Sebelas Maret, Jl. Ir. Sutami 36A Surakarta 57 126, Central Java, INDONESIA.*

*[4]Drug Utilisation and Discovery Research Group, Faculty of Pharmacy, University of Jember, Jl. Kalimantan I/2, Jember, 68121, East Java, INDONESIA.*

**Correspondence**

**Dinar S. C. Wahyuni**

Active Pharmaceutical Discovery and Development Research Group, Department of Pharmacy, Universitas Sebelas Maret Jl. Ir. Sutami 36A Surakarta 57 126, Central Java, INDONESIA.

E-mail: dinarsari_cw@staff.uns.ac.id

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## INTRODUCTION

Green tea (*Camellia sinensis* L.) is one of the most widely consumed beverages in the world, due its taste and beneficial health effects[1]. The latter has been reported such as antioxidant[2], anti-cancer[3,4], anti-inflammatory[5], and antihyperlipidemic[6]. Those activities are affected by chemical composition of green tea. The major metabolites of interest are the polyphenols such as catechin derivates, the typical amino acids such as theanine and the alkaloid such as caffeine. Those compounds are strongly related to the quality of tea[7]. Theanine as a typical amino acid in tea is responsible for the tea taste[8]; Catechins and caffeine are associated to its benefit as astringent properties in tea[9].

The metabolites of tea depends on geography[10], genetic strain[11], plant stages[12] and the plucking positions[13]. Particular tea varieties grown in Korea showed higher amount of amino acids and catechin compounds which related to in the rich-taste; higher amount of epigallocatechin-3-O-gallate and epigallocatechin-3-O-(3-O-methyl)-gallate were associated with rich tea varieties[11]. Higher content of catechin gallate, epigallocatechin gallate, and total catechins in the oolong showed significantly higher at a high altitude[14]. Moreover, young leaves showed the higher content of caffeine, theanine, and gallic acid than the older ones[13]. Thus, study of metabolite profile of tea is important to determine its chemical composition. Little knowledge of metabolomic study especially tea plantation in Indonesia.

Metabolomic as a large-scale analysis is an appropriate method to reveal biochemical components. It is a comprehensive, unbiased, and high-throughput analytical method so that it could provide all information about metabolite[15]. Currently, proton nuclear magnetic resonance ($^1$H-NMR) is a favored method to analysis a metabolome[10] due to its simple and non-destructive method[15]. Moreover, $^1$H-NMR method cover a wide range of metabolites like primary and secondary metabolites[16]. It has been used to analysis biochemical profiling in grape berry[17], coffee[18], tomato[19], carrot[20] and green tea varieties[11]. Therefore, we aimed to investigate metabolite profiling affected by varieties, plucking positions and altitudes in the tea plantation grown in Ngargoyoso, Indonesia. $^1$H-NMR is simultaneously applied with multivariate analysis including Principle Component Analysis (PCA) and Orthogonal partial least square discriminant analysis (OPLS-DA) to do chemical profiling. Moreover, one-dimensional $^1$H-NMR coupled with two-dimensional NMR techniques was applied to do the identification.

## MATERIALS AND METHODS

### Materials and Reagents

Reagent used were $KH_2PO_4$ (E-Merck), 3-(Trimethylsilyl) propionic acid-$d_4$ sodium salt (Santa Cruz Biotechnology, Dallas, TX), methanol-$d_4$ ($CD_3OD$) 99.8% for spectroscopy (Merck, Germany), deuterium oxide ($D_2O$) >99.9% atom for spectroscopy (Merck, Germany) and Sodium deuteroxide (Santa Cruz Biotechnology, Dallas, TX). Samples were collected from commercial green tea plantation located at Ngargoyoso (070 36’ 23.6” S, 1110 07’ 02.9” E), Middle Java, Indonesia.

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## Extraction of Plant Material

Green tea (*Camellia sinensis* L.) leaves were harvested during rainy season on May 2016. TRI 2025, Gambong, TRI2024, Kiara and Chinruan were five varieties grown in Ngargoyoso. The harvesting method in this tea plantation has been conducted using two plucking position young leaves (first and second youngest leaves) and old leaves (third and fourth leaves). Meanwhile, this plantation has two altitudes from 899 masl and 1159 masl. Each sample was in three replicates for $^1$H-NMR measurement. Fresh leaves were directly collected and kept in nitrogen liquid for temporary until the next treatment. Leaves were ground into fine size under liquid $N_2$ and freeze-dried. Dried samples (30mg) was weighed and added into a 2-ml microtube. The standard metabolomic procedure were followed[21], samples were extracted with 1.5 ml of a mixture of $KH_2PO_4$ buffer (pH 6.0) in $D_2O$ containing 0.05% TMSP and methanol-*d4* (1:1).

## NMR Analysis

$^1$H-NMR spectra were recorded at 25$^0$C on 400 MHz (Agilent, Universitas Sebelas Maret). $D_2O$ was used as the internal lock. Each $^1$H-NMR spectra comprise of 128 scans requiring 6 min and 48 s s acquisition time with the following parameters: 0.29 Hz/point, pulse width (PW) of 90$^o$ (6.8 µs), and relaxation delay (RD) of 2s.

$^1$H-NMR spectra were recorded at 25$^0$C on 400 MHz Agilent spectrometer. $D_2O$ solvent was used as the internal lock. Each $^1$H-NMR spectra consisted of 128 scans requiring 56 sec and 48 s acquisition time with the following parameters: 0.29 Hz/point, pulse width (PW) of 90$^o$ (6.8 µs), and relaxation delay (RD) of 2s. Eight scans per 64 increments for F1 (chemical shift axis) and eight scans for F2 (spin-spin coupling constant axis) using spectral widths of 64 Hz and 4807.7 Hz, respectively were applied in J-resolved NMR spectra. Both dimensions were multiplied by sine-bell functions (SSB = 0) before double complex Fourier transformation. $^1$H-$^1$H correlated COSY spectra were acquired with a 1.0 s relaxation delay and 4807.7 Hz spectral width in both dimensions. Further metabolites identification in samples applied MestRenova (11.00).

## Statistical analysis

The $^1$H NMR spectra were automatically reduced to ASCII files. Spectral intensities were scaled to internal standard and reduced to integrated regions of equal width (0.04) corresponding to the region of δ 0.0–9.98. AMIX software for bucketing was performed by scaling on internal standard. Principal component analysis (PCA) with Pareto scaling was performed with the SIMCA-P software (version 15.0, Umetrics, Umeå, Sweden). The ANOVA and LSD Test for the $^1$H-NMR signals was performed by SPSS.

# RESULTS AND DISCUSSION

## Proton Signal Assignment and Chemical Identification

$^1$H-NMR spectra of green teas were analyzed using 2D NMR technique such as J-resolved and COSY (Correlated Spectroscopy). 1H-NMR

**Table 1. $^1$H-NMR chemical shifts (δ) and coupling constants (Hz) of Camellia sinensis L. metabolites identified by references and using 1D and 2D NMR spectra ($CD_3OD$–$KH_2PO_4$ in $D_2O$, pH 6.0). Signals in bold were used to do quantification. [a]Abbreviations, s = singlet, d = doublet, t = triplet, dd = double-doublet.**

| Metabolite | δ H (Multiplicity [a]) |
|---|---|
| Amino acid | |
| Alanine | 3,80 ppm (*q; J* = 7,6 Hz; H-3); **1,50 ppm (*d; J*= 7,14 Hz; H-4)** |
| Threonine | 3,06 ppm (*d; J*= 6,71 Hz; H-3); **1,31 ppm (*d; J*= 7,03 Hz; H-5)** |
| Theanine | 3,21 ppm (*q; J*= 7,18 Hz; H-8); **1,13 ppm (*t; J*= 7,24 Hz; H-9)** |
| L-Glutamine | 3,76 ppm (*t; J*= 9,58 ; H-3); **2,14 ppm (*m;* H-4)**; 2,48 ppm (*t; J*=3,43 ; H-5) |
| Glucose | |
| α-Glucose | **5,20 ppm (*d; J*= 4,01 Hz; H-1)**; 3,52 ppm (*dd; J*= 3,37 & 10,02 Hz; H-2); 3,76 ppm (*t; J*= 9,6 Hz; H-3); 3,45 ppm (*t; J*= 9,41 Hz; H-4) |
| β-Glucose | **4,60 ppm (*d; J*= 8,06 Hz; H-1)**; 3,21 ppm (*t; J*= 8,46 Hz; H-2); 3,71 ppm (*t; J*= 8.64 Hz; H-3); 3,45 ppm (*t; J*= 9,41 Hz; H-4) |
| Fructose | 4,07 ppm (*d; J*= 9,62 Hz, H-3); 4,04 ppm (*dd; J*= 3,01 Hz; H-4) |
| Sucrose | **5,42 ppm (*d; J*= 3,88 Hz, H-1)**; 3,53 ppm (*dd; J*= 9,83 & 3,28 Hz; H-2); 3,76 ppm (*t; J*= 9,15 Hz; H-3); 3,45 ppm (*t; J*= 9,30 Hz, H-4); 3,67 ppm (*s;* H-1'); 4,19 ppm (*d; J*= 8,92 Hz; H-3') |
| Organic acid | |
| Succinic acid | **2,39 ppm (*t; J*= 7,39 Hz; H-3 & H-4)** |
| Phenolic | |
| Catechin | 4,64 ppm (*d; J*= 7,17 Hz; H-2); 4,13 ppm (*td; J*= 7,5 Hz & 5,5 Hz; *H-3);* 2,82 ppm (*dd; J*= 15,49 & 4,81 Hz; H-4*);* 2,53 ppm (*dd; J*= 16,28 & 7,29 Hz; H-4*);* 5,96 ppm (*d; J*= 3,22 Hz; H-6); 6,05 ppm (*d; J*= 2,93 Hz; H-8); **6,90 ppm (*d;J*= 7,38 Hz; H-2')**; 6,88 ppm (*brs;* H-5'); 6,80 ppm (*dd; J*= 6,23 & 1,30 Hz; H-6') |
| Epicatechin (EC) | 4,27 ppm (*m;* H-3); 2,90 ppm (*dd; J*= 16,67 & 3,77 Hz; H-4*);* 2,73 ppm (*dd; J*= 18,57 & 1,97 Hz; H-4*);* 6,03 ppm (*d; J*= 3,08 Hz; H-6); 6,08 ppm (*d; J*= 3,08 Hz; H-8); 7,01 ppm (*brs;* H-2'); 6,88 ppm (*brs;* H-5'); **6,88 ppm (*brs;* H-6')** |
| Epicatechin-3-gallate (ECG) | 3,03 ppm (*dd; J*= 17,53 & 4,44 Hz; H-4*);* 2,73 ppm (*dd; J*= 18,57 & 1,97 Hz; H-4); 6,05 ppm (*d; J*= 2,93 Hz; H-6); 6,08 ppm (*d; J*= 2,94 Hz; H-8); 6,99 ppm (*d; J*= 3,29 Hz; H-2'); **6,80 ppm (*d; J*= 9,01 Hz; H-5')**; 6,89 ppm (*d; J*= 1,60 Hz; H-6') |
| Epigallocatechin (EGC) | 2,90 ppm (*dd; J*= 16,67 & 3,77 Hz; H-4); 2,73 ppm (*dd; J*= 18,57 & 1,97 Hz; H-4); 5,96 ppm (*d; J*= 3,22 Hz; H-6); 6,03 ppm (*d; J*= 3,12 Hz; H-8); **6,60 ppm (*s;* H-2' & H-6')** |
| Epigallocatechin-3-gallate (EGCG) | 5,05 ppm (*s;* H-2); 5,55 ppm (*s;* H-3); 3,03 ppm (*dd; J*= 17,53 & 4,44 Hz; H-4); 2,90 ppm (*dd; J*= 16,67 & 3,77 Hz; H-4); 6,08 ppm (*d; J*= 2,94 Hz; H-6); 6,05 ppm (*d; J*= 2,93 Hz; H-8); **6,61 ppm (*s;* H-2' & H-6')**; 6,97 ppm (*s;* H-2" & H-6") |
| Quercetin | 6,99 ppm (*dd; J*= 7,50 & 2,51 Hz; H-6'); 6,80 ppm (*d; J*= 8,84 Hz; H-5'); **7,23 ppm (*d; J*= 2,64 Hz; H-2')** |
| Quinic acid | **1,90 ppm (*dd; J*= 9,99 & 13,46 Hz; H-2);** 4,12 ppm (*dt*; H-3); 3,53 ppm (*dd; J*= 4,05 & 11,05 Hz; H-4); 4,00 ppm (*dt; J*= ; H-5); 2,06 ppm (*dd; J*= 2,98 & 14.14 Hz; H-6) |
| Gallic acid | **7,01 ppm (*s;* H-3 & H-5)** |
| Alkaloid | |
| Caffeine | **7,83 ppm (*s;* H-8)** |
| Theobromine | **7,87 ppm (*s;* H-8)** |

spectrum is classified into three parts; amino acids appear around δ 2.0 – δ 0.5 ppm, organic acid at δ 3.0 – δ 2.0 ppm, sugars at δ 5.0 – δ 3.0 ppm, and phenolic at δ 7.5 – δ 6.0 ppm. Metabolites present in the green tea extract are carbohydrates (α-glucose, β-glucose, fructose, sucrose), amino acids (alanine, theanine, threonine, L-glutamine), organic acid (succinic acid), alkaloid (caffeine, theobromine), and phenolic (catechin, EC, ECG, EGC, EGCG, gallic acid, quinic acid, quercetin). The detailed signals assignment are listed in Table 1.

## Multivariate data analyses (MvDA) and relative quantification of various varieties

PCA scoring plot and Hierarical Clustering Analysis (HCA) were used to study the pattern of the five varieties. The former resulted five varieties were clustered into 2 groups by PC1 36% (Figure S1). TRI 2025 was located in the positive quadrant of PC1; whereas TRI 2024, Kiara, and Chinruan varieties were grouped in the negative quadrant of PC1. On the other hands, Gambong was distributed in all quadrants (Figure S1). It is in accordance with the internal information which explained that Gambong was a hybrid cultivar between TRI 2024 and TRI 2025. Thus, Gambong inherits the properties of both parents. Further analysis was proceed by excluding Gambong varieties to establish a clear separated groups.

The PCA scoring plot of four culitvars resulted a clear separation in both large groups, namely group 1 (TRI 2025) and group 2 (TRI 2024, Kiara, Chinruan) (Figure 1). The similarities of the four varieties can be seen in the HCA dendogram (Figure 2). The height of Y-axis is to measure of closeness of individual data point, the shorter Y-axis is the closer the similarity is. Scoring plot of PCA that TRI 2024, Chinruan, and Kiara varieties were relatively similar in metabolite profiling; whereas TRI 2025 varieties exhibited the least similarity to other varieties. The loading plot of PCA resulted positive values belong to metabolites associated to group 1 (TRI 2025) which was higher of relative concentrations of EGC, ECG, caffeine, and gallic acid. Besides, negative value showed metabolites related to group 2 (TRI 2024, Chinruan, Kiara) which were higher of relative concentration of β -glucose (Figure 3). β-glucose, EGC, ECG, gallic acid, and caffeine were the metabolites significantly different between group 1 (TRI 2025) and group 2 (TRI 2024, Kiara, Chinruan) (Figure 4). The β-glucose content in group 2 was quarter times higher than group 1 varieties. EGC, ECG, gallic acid, and caffeine in group 1 (TRI 2025) varieties were half times higher than group 2 (TRI 2024, Chinruan, Kiara).

Comparing all five varieties, theanine content were two times higher in average in Kiara and TRI 2025 varieties than other varieties (Figure 5). It has a role as a unique "umami" flavor in tea[8]. As consequent, this made Kiara and TRI 2025 varieties can be recommended as varieties for flavor-rich tea production. Gallic acid and epicatechin galat were significanly half times higher in Gambong. It might contribute to adstringets activity[9], however the caffeine abundance was not significant among varieties.

## Multivariate data analyses (MvDA) and relative quantification of different plucking position

The PCA scoring plot of plucking position resulted that the metabolites were relatively clustered into 2 groups. Young and old leaves were located in the positive and negative quadrant of PC2, respectively (Figure S2). Further analysis OPLS-DA was used to determine the metabolites that are responsible for separation. The OPLS-DA scoring plot of of two leaves position resulted that 56% of total separation variations is caused by leaves position. Young and old leaves was located in the positive and negative quadrant, respectively (Fig 6).

The loading plot of two green tea leaves position (Figure 7) showed that the distinguishing metabolite between the two are catechin, EGCG, quinic acid, caffeine, theobromine, L-glutamine, α-glucose, β-glucose, and sucrose. Catechin, EGCG, caffeine, theobromine, and L-glutamine content in young leaves were significantly higher than older leaves. In the other hands, quinic acid, α-glucose, β-glucose content in older leaves were higher than young leaves (Fig 8). It is in line with previous study by Lee et al.[13] reported the young leaves of green tea collected in South Korean had a higher caffein and less glucose and sucrose content. Moreover, the abundance of caffein, EGCG, teobromin and glutamine showed higher in the young green tea leaves grown in China[7]. However, catechin and EGCG grown in South Korea showed a different pattern with this current study[13]. This different result might be due to the different season of harvesting periode which had been done in rainy season[14].

## Multivariate data analyses (MvDA) and relative quantification of different plucking position

The PLS-DA scoring plot of two altitudes resulted the total variation was 52,15% (PC = 31,53%, PC2 = 20,62%). 899 masl and 1159 masl was located in the negative and positive quadrant, respectively (Figure S3). Further analysis OPLS-DA has been done to obtain clear separation. The scoring plot of of two altitude resulted that 31,53% of total separation variations (Fig 9).

The loading plot of two green tea altitude (Figure 10) showed that theanine, L-glutamin, α/β -glucose, quinic acid, and succinic acid content at 1159 masl were sightly higher than those at 899 masl. In the other hands, ECG, EGC, and, gallic acid in 899 masl were significantly higher than 1159 masl (Figure 11). Metabolite profiling of green teas grown in other location showed different pattern depends on its natural conditions. Lee at al.[22] reported the contents of amino acids such as leusin, isoleusin, valin, alanine and threonine and phenolic compounds such as EGCG, CG and total catechins were higher at 1950 masl; the teanin was higher at 1200 masl grown in South Korea. In the other hands, the content of phenolic compouds such as EC, ECG and EGCG were higher at 212 masl meanwhile EGC was highest at 1020 masl in green tea grown in China[14]. The different result of metabolite profiling in green teas might due to the different climates in each location which leads to different metabolites produces by plants.

Amino acid is the main key in determining the quality of green tea which gives a sense of "umami"[23]. Besides, catechins and caffeine compounds can be used as a support to determine the quality of green tea. The catechin derivative content has been known as an antioxidant compound in tea[24] and is a compound that affects the quality of tea (bitter taste)[9], while caffeine has been known to function as a psychostimulant to increase alertness[25]. As a result, this makes the young leaves of TRI 2025 as a recommended cultivar both taste and benefits due to its high level of amino acid and catechins as well as caffeine, respectively. Plants grown at 1159 masl is suggested to have good quality of green tea due to its high levels of amino acid theanine, besides green tea grown at 899 masl is recommended to generate good quality of green tea due to its high levels of catechin. Therefore, these results may provide useful information to the tea plantation grower to produce the best quality of green tea leaves especially for green tea plantation in Indonesia.

# CONCLUSION

The metabolite profiling of different tea varieties and its environmental influences grown in Indonesia have not been reported yet. Among 5 green tea varieties, TRI 2025 variety contain higher levels of theanine, catechins and caffeine than other varieties which makes it as the flavor-rich tea. Besides, the youngest leaves contain higher level of catechin, EGCG, caffeine, theobromine, and L-glutamine. Meanwhile tea grown at 1159 masl contain higher level of theanine, L-glutamin, α-glucose, β-glucose, quinic acid, and succinic acid than 899 masl. This

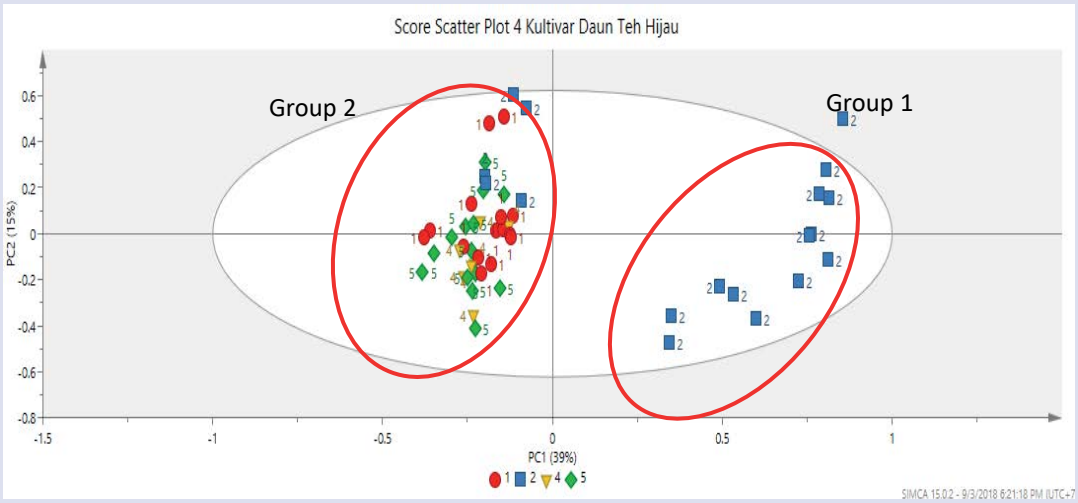


**Figure 1.** PCA scoring plot of four green tea varieties (*Camellia sinensis* L.) i.e. TRI2025 (■), TRI2024 (●), Kiara (▼), Chinruan (♦).

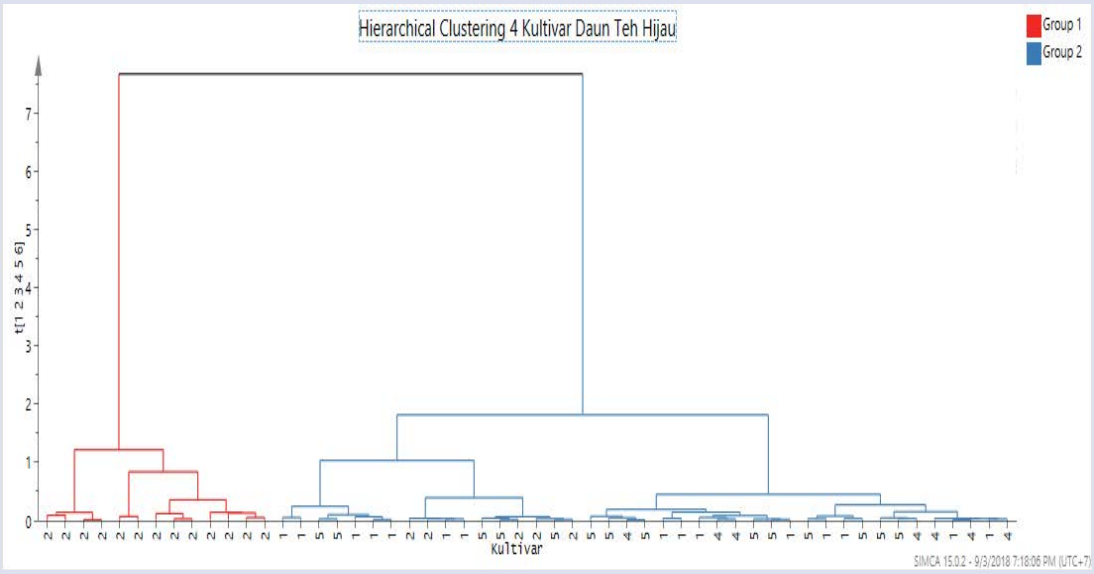


**Figure 2.** Hierarchical Clustering Analysis of four green tea varieties (*Camellia sinensis* L.) i.e. TRI2025 (2), TRI2024 (1), Kiara (4), Chinruan (5).

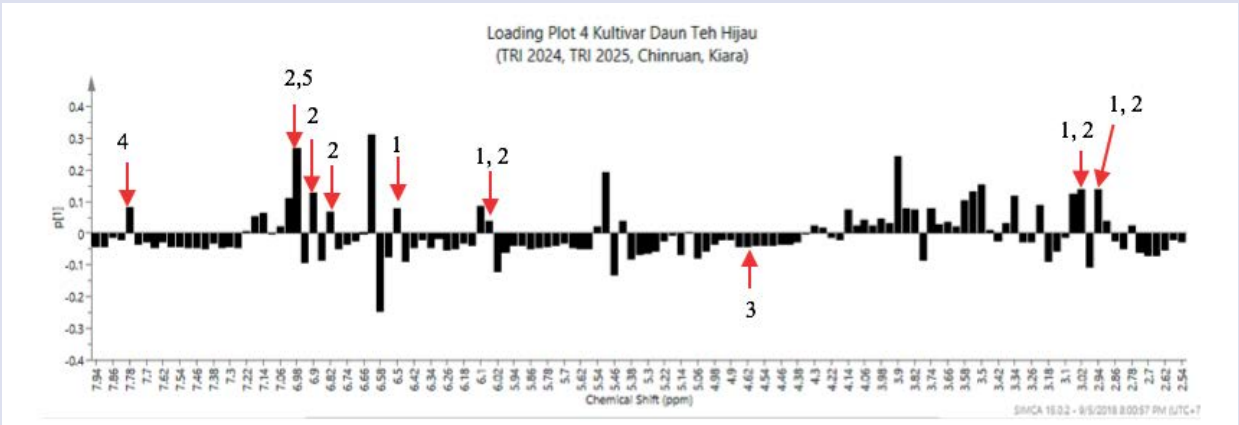


**Figure 3.** Loading plot of PCA of TRI2024, TRI2025, Kiara, Chinruan indicated chemical shift that responsible to PCA scoring plot separation. (1) EGC, (2) ECG, (3) β-glucose, (4) caffeine, (5) gallic acid

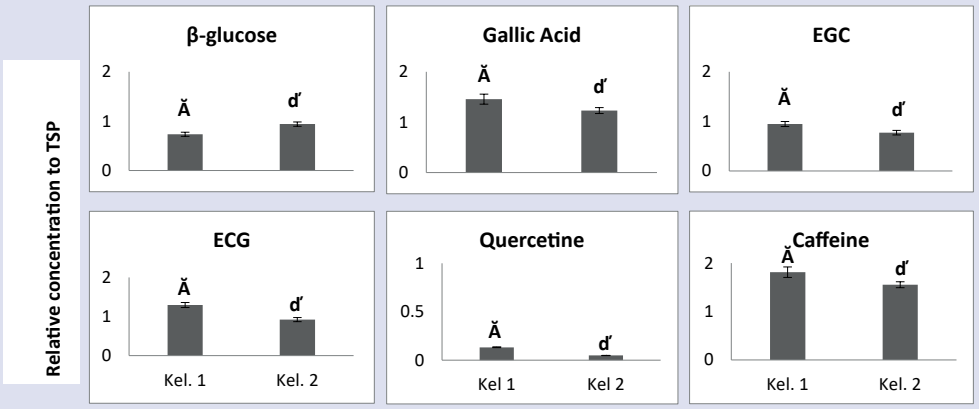


**Figure 4.** Relative quantification of metabolites in two groups of *Camellia sinensis* L. Difference in letters shows a significant value at 95% confidence level.

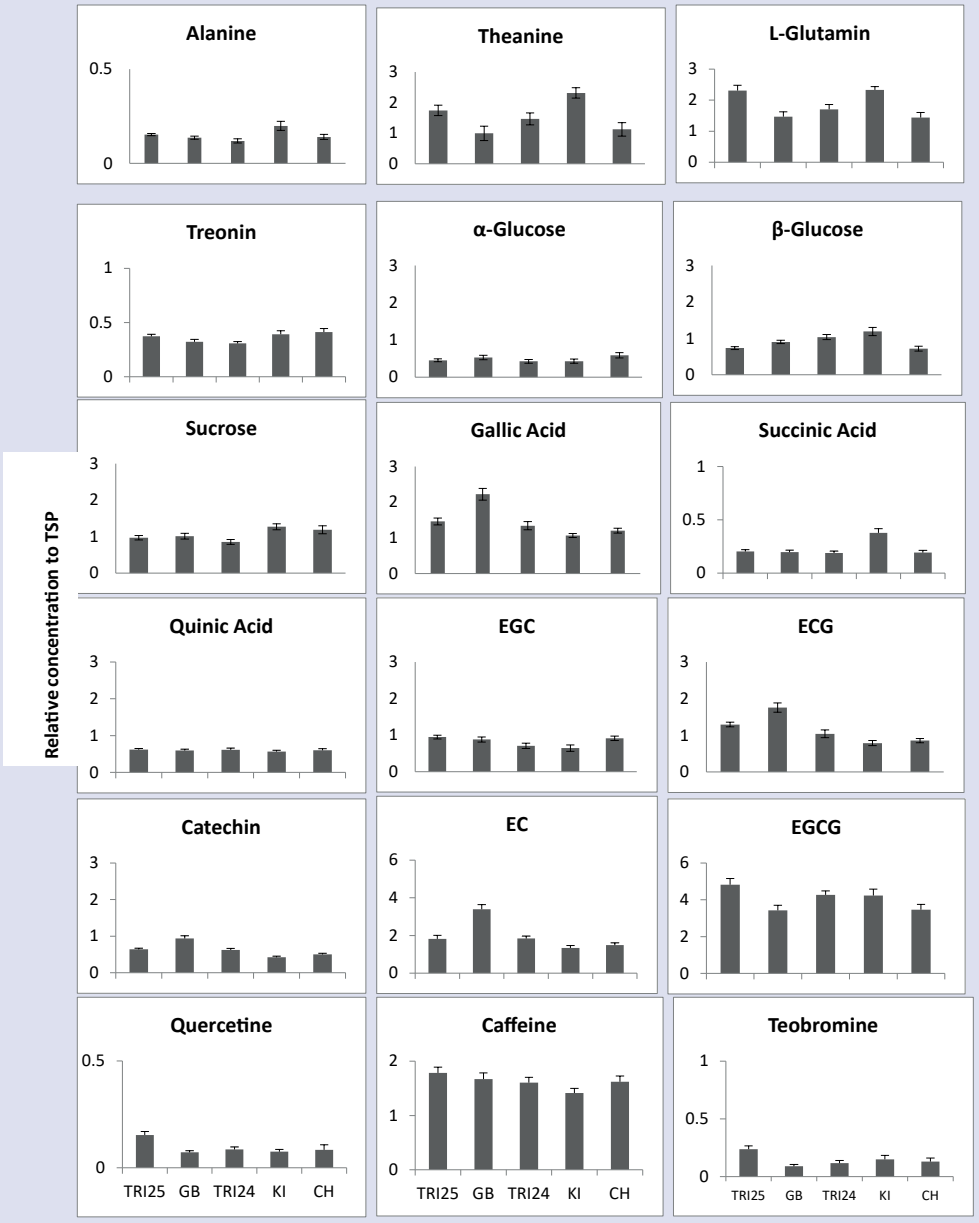


**Figure 5.** Relative quantification metabolites in five varieties of Camellia sinensis L. Difference in letters shows a significant value at 95% confidence level.

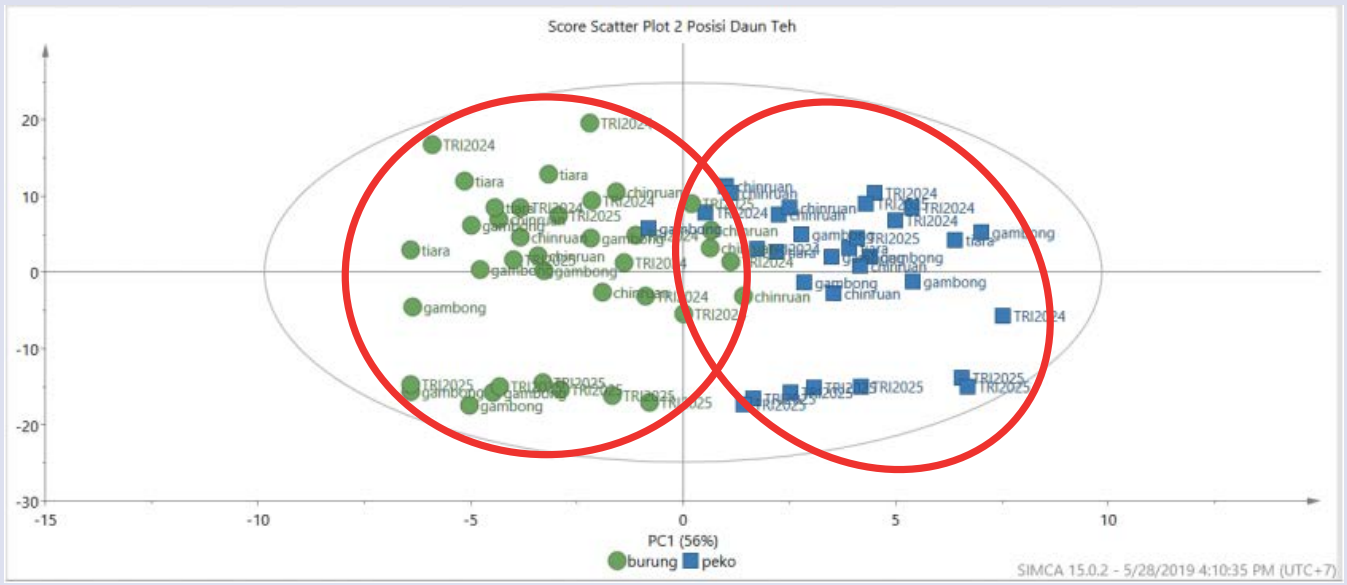


**Figure 6.** Scoring plot of OPLS-DA between young ( ■ ) and old leaves ( ● ) *of* Camellia sinensis L. grown in Ngargoyoso, Indonesia

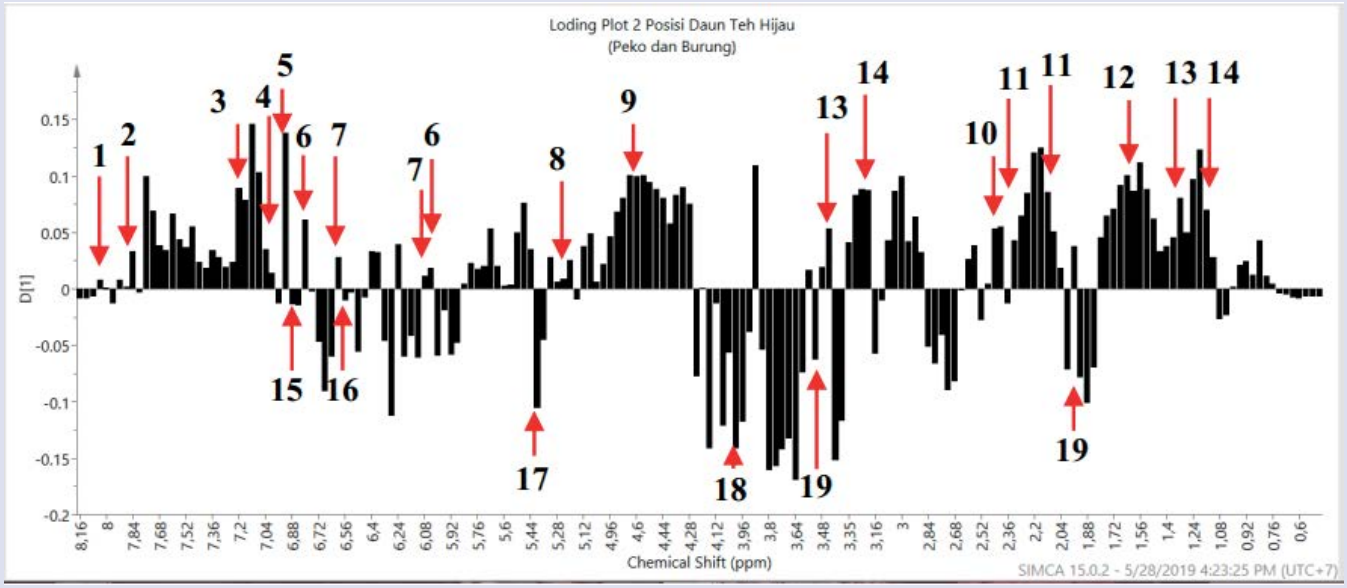


**Figure 7.** Loading plot of OPLS-DA of Peko and Burung indicated chemical shift that responsible to OPLS-DA scoring plot separation. (1) Theobromine, (2) Caffeine, (3) Quercetin, (4) Gallic Acid, (5) EC, (6) ECG, (7) EGCG, (8) α-Glucose, (9) β-Glucose, (10) Succinic Acid, (11) L-Glutamine, (12) Alanine, (13), Threonine, (14) Theanine, (15) Catechin, (16) EGC, (17 Sucrose, (18) Fructose, (19) Quinic Acid

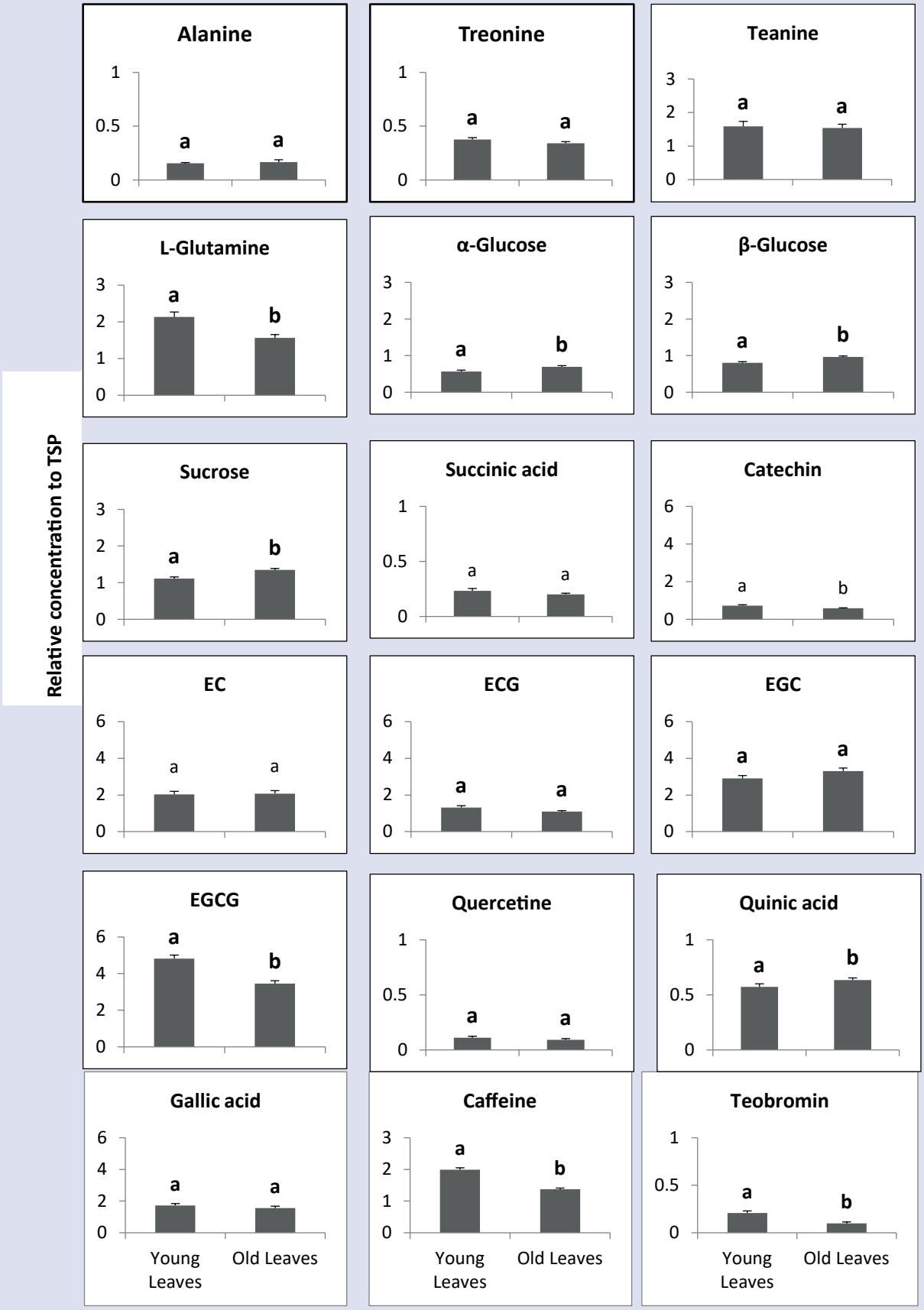


**Figure 8.** Relative quantification graphs of metabolites in two leaves position of *Camellia sinensis* L. Difference in letters shows a significant value at 95% confidence level.

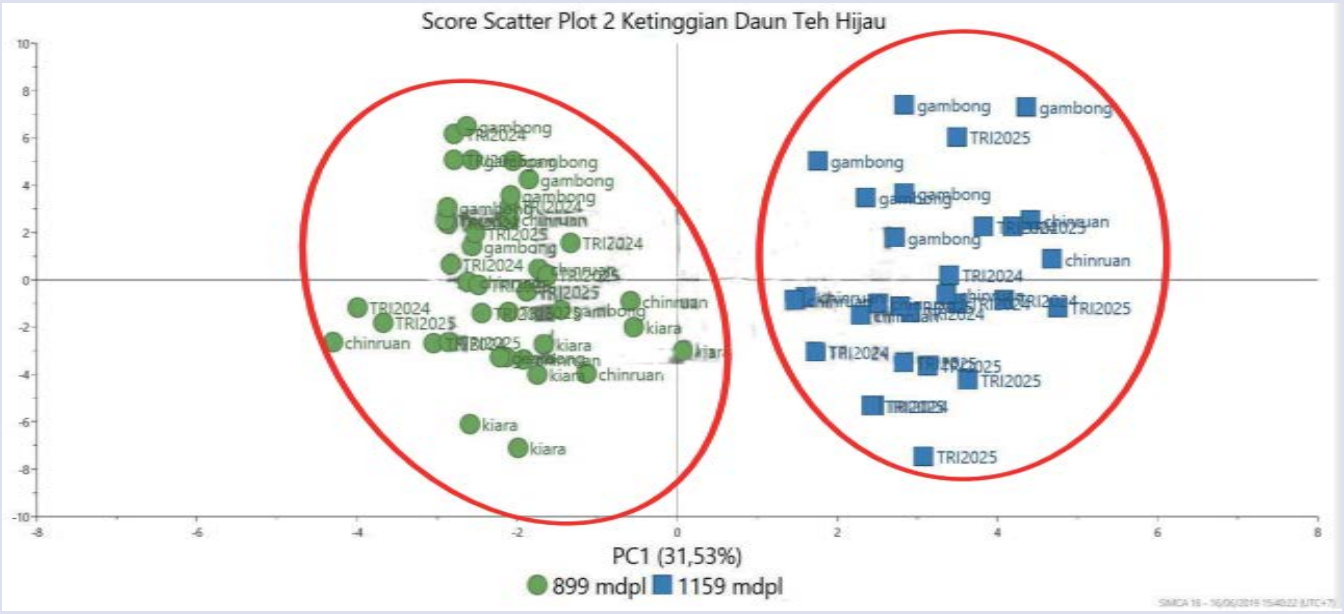


**Figure 9.** Scorig plot of OPLS-DA between 1159 masl (■) and 899 masl (●) of *Camellia sinensis* L. grown in Ngargoyoso, Indonesia.

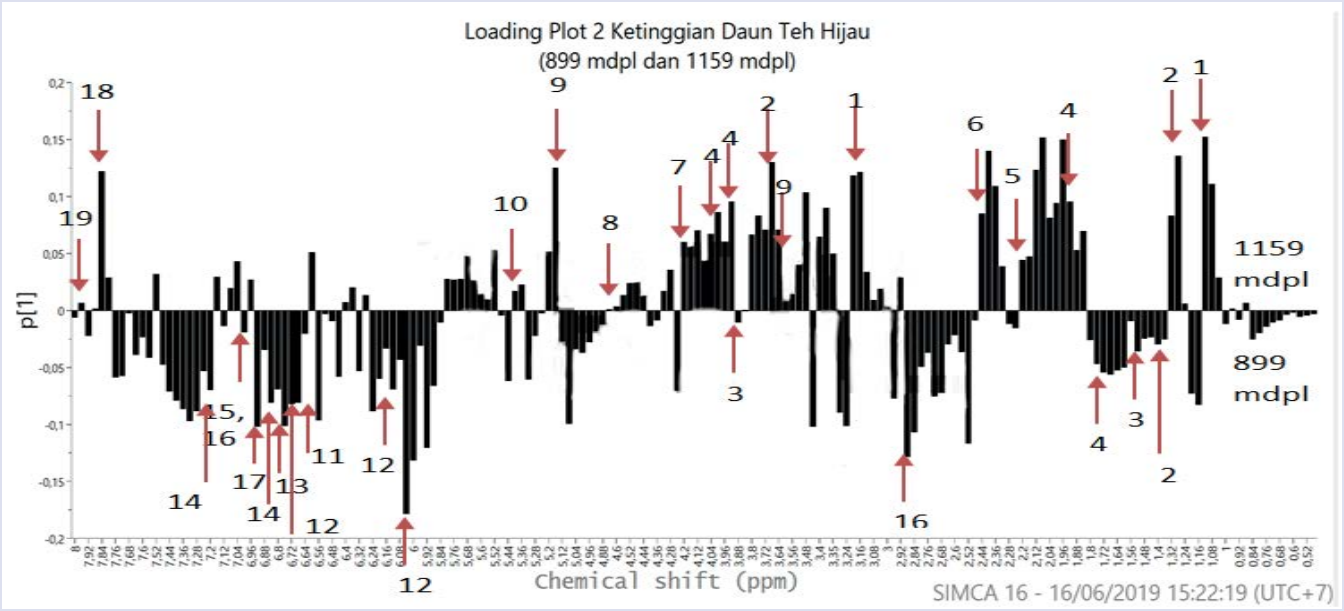


**Figure 10.** Loading plot of OPLS-DA between 899 masl and 1159 masl indicated chemical shift that responsible to OPLS-DA scoring plot separation. (1) Theanine, (2) Threonine, (3) Alanine, (4) Quinic Acid, (5) L-Glutamine, (6) Succinic Acid, (7) Fructose, (8) β-Glucose, (9) α-Glucose, (10) Sucrose, (11) EGCG, (12) EGC, (13) ECG, (14) Quercetin, (15) Gallic Acid, (16) Catechin, (17) EC, (18) Caffeine, (19) Theobromine.

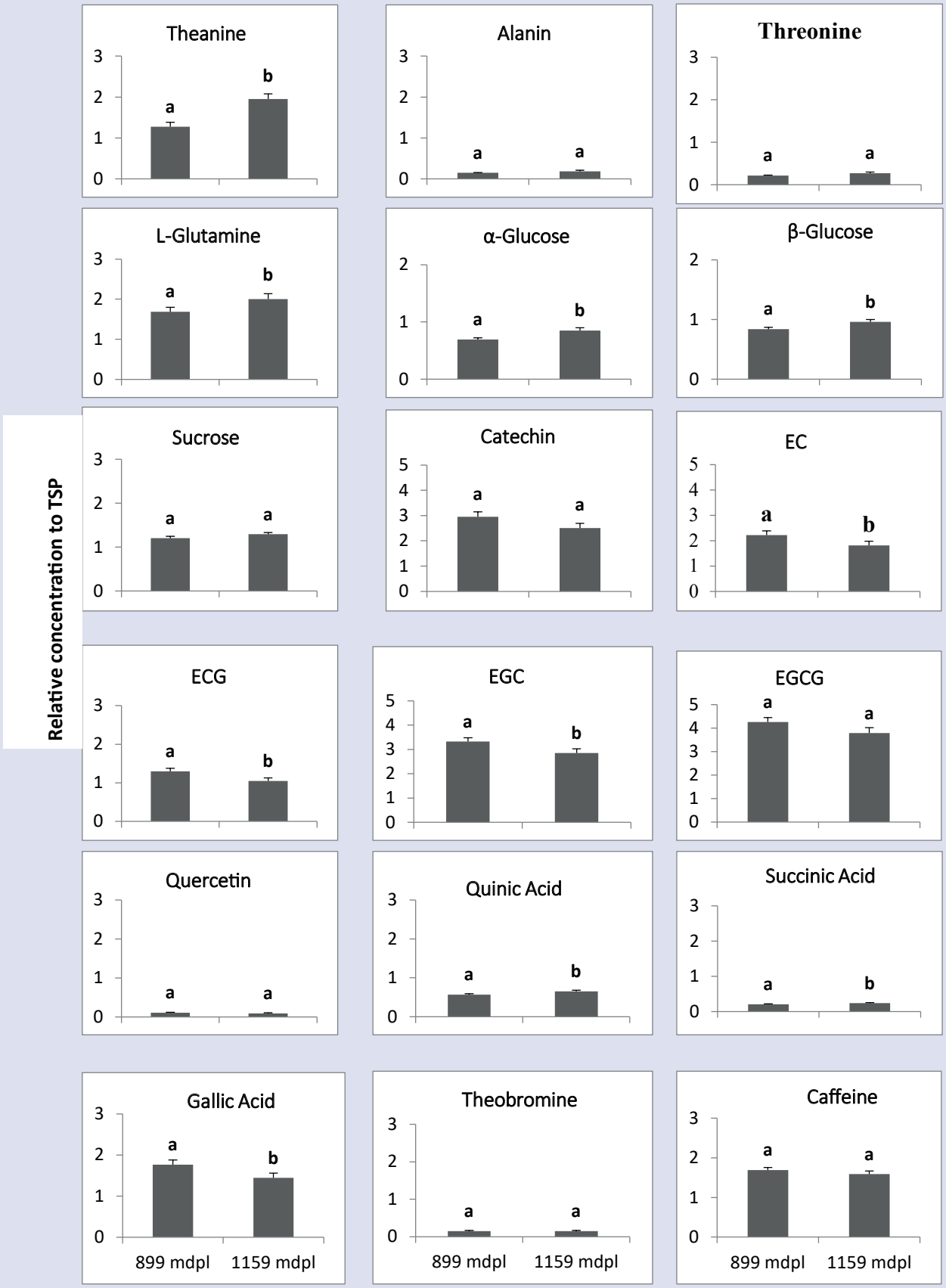


**Figure 11.** Relative quantification graphs of metabolites in two altitude of *Camellia sinensis* L. Difference in letters shows a significant value at 95% confidence level.

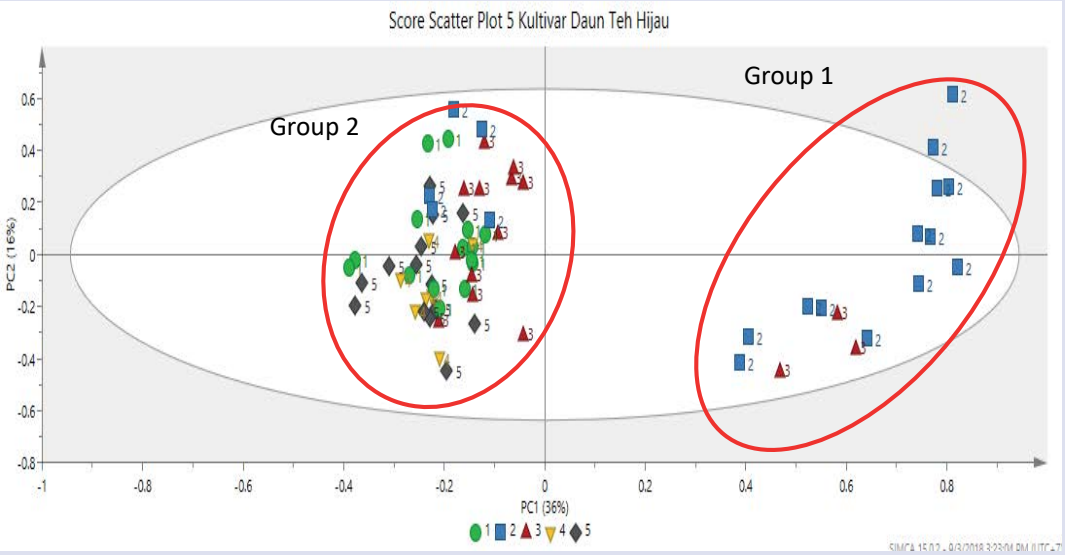


**Figure S1**. PCA scoring plot of five green tea cultivars (*Camellia sinensis* L.) i.e. TRI2024 (●), TRI2025 (■), Gambung (▲), Kiara (▼), Chinruan (♦) grown in Kemuning, Indonesia.

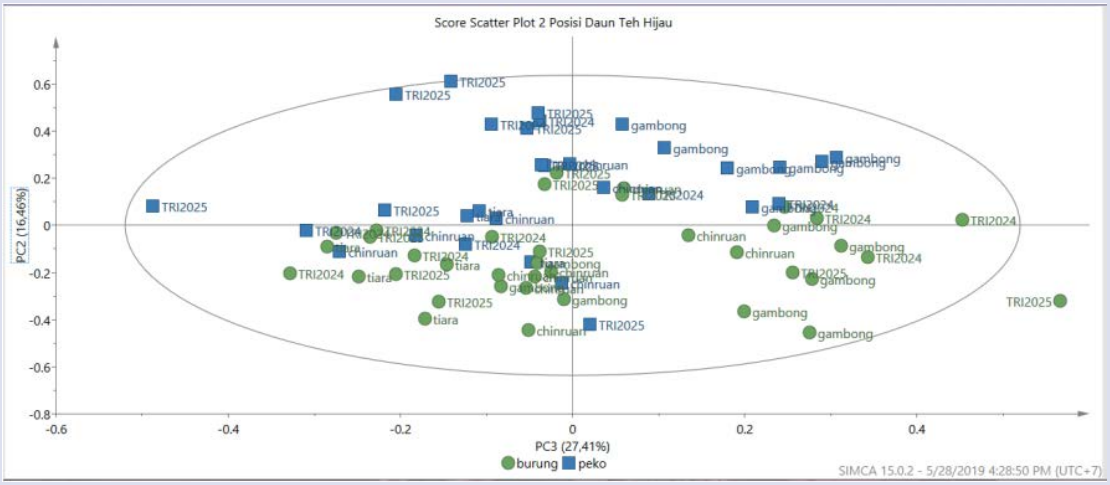


**Figure S2**. PCA scoring plot between young (■) and old leaves (●) of *Camellia sinensis* L. grown in Kemuning, Indonesia.

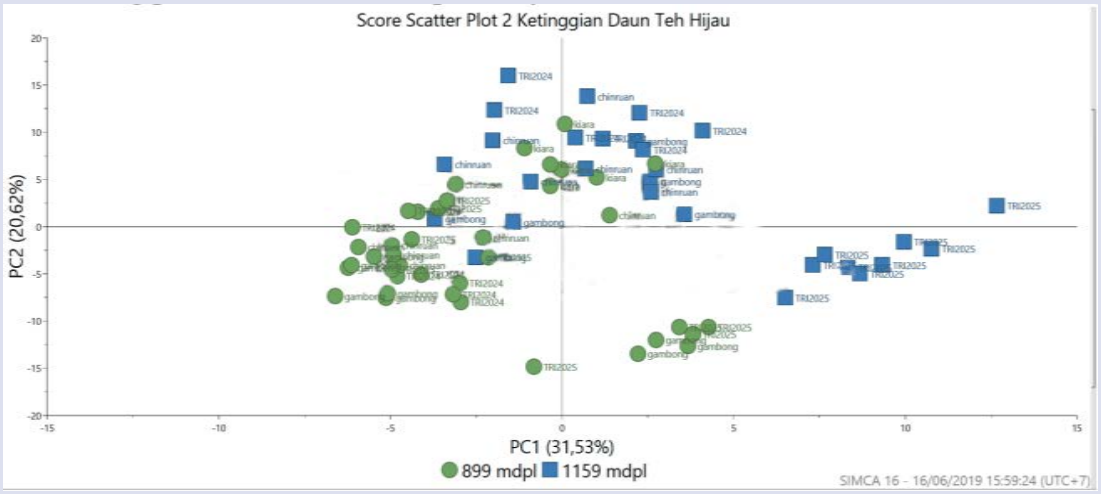


**Figure S3.** PLS-DA scoring plot between 1159 masl (■) and 899 masl (●) of *Camellia sinensis* L. grown in Kemuning, Indonesia.

resulted green tea from TRI 2025 varieties at youngest leaves can be recommended to be the premium quality in green tea, both for taste and benefits. Green tea grown at higher and lower location showed as flavor-rich tea due to the higher of theanine and as health benefits due to the higher of catechins. This present study results the clear metabolite profiling of various green tea varieties and its environmental influences grown in Indonesia.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## REFERENCES

1. Tarachiwin L, Ute K, Kobayashi A, Fukusaki E. 1H NMR based metabolic profiling in the evaluation of Japanese green tea quality. Journal of agricultural and food chemistry. 2007;55(23):9330–6.
2. Anesini C, Ferraro GE, Filip R. Total polyphenol content and antioxidant capacity of commercially available tea (Camellia sinensis) in Argentina. Journal of agricultural and food chemistry. 2008;56(19):9225–9.
3. Ravindranath MH, Saravanan TS, Monteclaro CC, Presser N, Ye X, Selvan SR, et al. Epicatechins purified from green tea (Camellia sinensis) differentially suppress growth of gender-dependent human cancer cell lines. Evidence-Based Complementary and Alternative Medicine. 2006;3(2):237–47.
4. R Conde V, G Alves M, F Oliveira P, M Silva B. Tea (Camellia sinensis (L.)): a putative anticancer agent in bladder carcinoma? Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents). 2015;15(1):26–36.
5. Chattopadhyay P, Besra SE, Gomes A, Das M, Sur P, Mitra S, et al. Anti-inflammatory activity of tea (Camellia sinensis) root extract. Life sciences. 2004;74(15):1839–49.
6. Suriyamoorthy P, Mary MRF, Subrhamanian H, Kanagasapabathy D. Anti hyperlipidemic effect of aqueous extract of Aegle marmelos and Camellia sinensis in oil fed hyperlipidemic rats. Int J Pharm Pharm Sci. 2014;6(2):338–41.
7. Le Gall G, Colquhoun IJ, Defernez M. Metabolite profiling using 1H NMR spectroscopy for quality assessment of green tea, Camellia sinensis (L.). Journal of Agricultural and Food Chemistry. 2004;52(4):692–700.
8. Ekborg-Ott KH, Taylor A, Armstrong DW. Varietal differences in the total and enantiomeric composition of theanine in tea. Journal of Agricultural and Food Chemistry. 1997;45(2):353–63.
9. Chen Q, Zhao J, Guo Z, Wang X. Determination of caffeine content and main catechins contents in green tea (Camellia sinensis L.) using taste sensor technique and multivariate calibration. Journal of food composition and analysis. 2010;23(4):353–8.
10. Lee J-E, Lee B-J, Chung J-O, Kim H-N, Kim E-H, Jung S, et al. Metabolomic unveiling of a diverse range of green tea (Camellia sinensis) metabolites dependent on geography. Food chemistry. 2015;174:452–9.
11. Ji H-G, Lee Y-R, Lee M-S, Hwang KH, Kim E-H, Park JS, et al. Metabolic phenotyping of various tea (Camellia sinensis L.) cultivars and understanding of their intrinsic metabolism. Food chemistry. 2017;233:321–30.
12. Li C-F, Yao M-Z, Ma C-L, Ma J-Q, Jin J-Q, Chen L. Differential metabolic profiles during the albescent stages of 'Anji Baicha'(Camellia sinensis). PLoS One. 2015;10(10):e0139996.
13. Lee J-E, Lee B-J, Hwang J, Ko K-S, Chung J-O, Kim E-H, et al. Metabolic dependence of green tea on plucking positions revisited: a metabolomic study. Journal of agricultural and food chemistry. 2011;59(19):10579–85.
14. Chen Y, Jiang Y, Duan J, Shi J, Xue S, Kakuda Y. Variation in catechin contents in relation to quality of 'Huang Zhi Xiang'Oolong tea (Camellia sinensis) at various growing altitudes and seasons. Food chemistry. 2010;119(2):648–52.
15. Verpoorte R, Choi YH, Mustafa NR, Kim HK. Metabolomics: back to basics. Phytochemistry Reviews. 2008;7(3):525–37.
16. Dixon RA, Gang DR, Charlton AJ, Fiehn O, Kuiper HA, Reynolds TL, et al. Applications of metabolomics in agriculture. Journal of Agricultural and Food Chemistry. 2006;54(24):8984–94.
17. Ali K, Maltese F, Fortes AM, Pais MS, Choi YH, Verpoorte R. Monitoring biochemical changes during grape berry development in Portuguese cultivars by NMR spectroscopy. Food Chemistry [Internet]. 2011;124(4):1760–9. Available from: http://dx.doi.org/10.1016/j.foodchem.2010.08.015
18. Charlton AJ, Farrington WHH, Brereton P. Application of 1H NMR and multivariate statistics for screening complex mixtures: quality control and authenticity of instant coffee. Journal of agricultural and food chemistry. 2002;50(11):3098–103.
19. Mirnezhad M, Romero-González RR, Leiss KA, Choi YH, Verpoorte R, Klinkhamer PGL. Metabolomic analysis of host plant resistance to thrips in wild and cultivated tomatoes. Phytochemical Analysis. 2010;21(1):110–7.
20. Leiss KA, Cristofori G, van Steenis R, Verpoorte R, Klinkhamer PGL. An eco-metabolomic study of host plant resistance to Western flower thrips in cultivated, biofortified and wild carrots. Phytochemistry. 2013;93:63–70.
21. Lee J-E, Lee B-J, Chung J-O, Hwang J-A, Lee S-J, Lee C-H, et al. Geographical and climatic dependencies of green tea (Camellia sinensis) metabolites: a 1H NMR-based metabolomics study. Journal of Agricultural and Food Chemistry. 2010;58(19):10582–9.
22. Ding Y, Yu H, Mou S. Direct determination of free amino acids and sugars in green tea by anion-exchange chromatography with integrated pulsed amperometric detection. Journal of Chromatography A. 2002;982(2):237–44.
23. Unachukwu UJ, Ahmed S, Kavalier A, Lyles JT, Kennelly EJ. White and green teas (Camellia sinensis var. sinensis): variation in phenolic, methylxanthine, and antioxidant profiles. Journal of food science. 2010;75(6):C541–8.
24. Favrod-Coune T, Broers B. The health effect of psychostimulants: a literature review. Pharmaceuticals. 2010;3(7):2333–61.
25. Kim HK, Choi YH, Verpoorte R. NMR-based metabolomic analysis of plants. Nature protocols. 2010;5(3):536.

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