

Phytochemical Composition of Selected Tea Clones Grown in Two Agroecology Regions of Rwanda

Rugema Grace*, Kipkorir Koskei*, and Owaga Eddy*

ABSTRACT

Tea leaves contain diverse groups of phytochemical compounds, which contribute to the quality and health benefits of tea. Polyphenols and caffeine are important indicators of tea quality. This study explored the variation in total polyphenol, catechin, and caffeine content across tea clones grown in two regions with different agroecological characteristics. A completely randomized block design was used. The samples were collected from two distinct agroecological zones in Rwanda. Total Polyphenol content was analyzed by the International Organization for Standardization (ISO 14502-1-2005) procedure using ultraviolet (UV) spectrometry. Catechins, gallic acid, and caffeine were analyzed using High-Performance Liquid Chromatography HPLC. The data obtained were statistically analyzed using Minitab software, and means were compared using Tukey's test at a significance level of 5%. The results showed significant differences among the tea clones from the two different growing regions. Tea clones grown in Mata generally had lower levels of polyphenols, catechins, and gallic acid than those grown in Rubaya. However, caffeine showed a different pattern: it was higher in the tea clone from Mata than in the tea clone from Rubaya. This Difference indicates the impact of agroecological conditions such as altitude, temperature, and rainfall on the composition of tea. It was concluded that tea grown in different regions of Rwanda had distinct variations in polyphenol, catechin, and caffeine levels. The results provide insights into the potential for region-specific tea branding and tea product diversification based on polyphenol and caffeine profiles. Therefore, there is a need to evaluate tea clones selected for quality in other tea-growing regions of Rwanda to ensure that farmers use clones that can produce high-quality tea.

Keywords: Agroecology, catechins, Rwanda, tea clones.

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Dedan Kimathi University of Technology,
Kenya.

*Corresponding Author:

e-mail: rugemagrace02@gmail.com;

e-mail: kipkorir.koskei@dkut.ac.ke;

e-mail: eddy.owaga@dkut.ac.ke

1. INTRODUCTION

Tea leaves (*Camellia sinensis*) were used for tea production. It is the most widely consumed beverage worldwide [1]. It contains a wide range of health-beneficial bioactive compounds including catechins, polyphenols, and flavonoids. These compounds impart tea with a remarkable flavor, fragrance, and related health benefits, such as protection against cancer, obesity, and hypertension [2]. In addition to its health benefits, tea is also important for the economies and livelihoods of tea-producing countries [3].

Tea leaves are distinguished by their remarkable content of bioactive compounds, which are predominantly responsible for the unique sensory and health beneficial

properties of tea and account for its popularity as a beverage [4], with bioactive compounds largely belonging to the polyphenol group, accounting for 25% to 35% on a dry weight basis [5]. The major tea polyphenols are catechins and their derivatives epicatechin (EC), simple catechins (C), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and epigallocatechin-3-gallate (EGCG). Apart from being the most biologically active biomolecules in tea, catechins are also important precursors of black tea theaflavins and thearubigins and are therefore important quality markers [6].

Caffeine, an alkaloid in the methyl xanthine family, is a naturally occurring substance found in the leaves, seeds, and fruits of over 63 plant species worldwide [7].



In tea, caffeine accounts for approximately 1.5%–4.5%, which contributes to the bitter taste of tea [8]. Tea leaves contain phenolic acids, including gallic acid, constituting approximately 1% on a dry matter basis, and act as a crucial precursor of galloylated catechins, which are the major and characteristic metabolites and an important contributor to tea taste [9].

Changes in tea composition depend on many factors such as the variety of tea leaves, growing environment, and processing conditions [10]. Mutuku *et al.* [11] reported that the quality performance of tea clones varies with region owing to variations in the biomolecules synthesized in tea.

Currently, in Rwanda, over 28,000 hectares are planted with tea across the Northern, Western, and Southern provinces at altitudes between 1550 m and 2500 m. These regions feature an ideal climate and well-distributed rainfall ranging from 1200 mm to 1400 mm annually [12].

Tea clones BBK35, TRFK100/5, BBK10, and TRFK6/8 were grown in Rwanda. They originated from Kenya as planting materials, with the assumption that the clone will retain the same characteristics in different growing agroecological zones of Rwanda. However, these assumptions have not been proven in relation to the influence of the different growing conditions in Rwanda. Several studies have shown that tea clones respond differently to different growing regions [13]. A previous study reported that changes in air temperature affect the bioactive components of tea clones [14].

Information on the main polyphenol content of tea from various regions could be useful for improving the quality, diversifying products, and developing strategies for tea quality enhancement. In Rwanda, little research has been conducted to evaluate the phytochemical composition of tea clones grown in various ecological regions.

This study aimed to determine the levels of total polyphenols, catechins, gallic acid, and caffeine in four common tea clones cultivated in the western and southern agroecological zones of Rwanda.

2. METHODOLOGY

2.1. Description of Study Site

This study was conducted in two ecological zones. One tea plantation estate was selected in each zone. Mata tea estate was chosen from the central plateau south province of Rwanda, and Rubaya tea estate was selected from the Congo Nile watershed, West Province. Detailed information is presented in Table I.

2.2. Experimental Design and Sample Preparation

A completely randomized block design with three replicates was used for this study. Four common tea clones, TRFK6/8, BBK35, BBK10, and TRFK100/5, were obtained from the two different tea-growing regions, as indicated above. The sites for sampling were carefully selected, the plants were of the same age, and all sites had mature tea clones planted in 2008. Tea plants at each site were pruned in April 2021 so that all plants were in the same pruning cycle life.

Approximately 1 kg of fresh tea leaves from each clone, consisting of two leaves and a bud, was randomly plucked manually, in replicates, by professional pluckers within an area of 0.02 Ha from approximately 260 tea plants grown in each study site. The samples were quickly dried in a microwave oven at 105°C for five minutes to a moisture content of 4%–3.0% to prevent oxidation. Dried tea leaves were ground to the finest powder using an electric coffee blender and stored in aluminum-coated airtight paper bags until tea extraction. Further analysis was performed at the Dedan Kimathi University of Technology and Tea Research Foundation of Kenya.

2.3. Tea Extract Preparation

Before extraction, the dry matter of each sample was determined following the procedure described by the International Organization for Standardization (ISO). The tea was extracted according to the procedure outlined in [16]. Finely ground tea samples were weighed $0.200\text{ g} \pm 0.001\text{ g}$ into extraction tubes, and 5 ml of 70% hot methanol/water was added. The tubes were then stoppered and mixed thoroughly by vortexing. The samples were incubated in a water bath at 70°C for 10 min with vortexing after 5 min and 10 min, cooled at room temperature, and then centrifuged at 3500 rpm for 10 min. The supernatant was then carefully decanted into a graduated tube. The extraction steps were repeated, resulting in two extracts. The two extracts were combined and made to 10 mL using a cold 70% methanol/water extraction mixture.

2.3.1. Analysis of Total Polyphenols (TPs)

The procedure outlined by the International Organization for Standardization (ISO 14502:205) and Folin–Ciocalteu phenol reagent were used. From the tea extract, 1 mL was pipetted into a 100 mL volumetric flask and made up to the mark with distilled water. One milliliter of the diluted sample was mixed with 5 mL of 10% Folin–Ciocalteu's phenol reagent and 4 mL of 7.5% sodium carbonate solution, which was kept in dark place

TABLE I: STUDY SITE [15]

Location	Mata tea estate	Rubaya tea estate
Agroecological zone	Central plateau/South province	Congo-Nile Ridge/West province
Latitude	2° 34' 34" South	1° 46' 38" South
Longitude	29° 34' 23" East	29° 30' 15" east
Altitude	1700 m–2000 m	2400 m and 2500 m
Temperature	18°C–21°C	10°C and 18°C
Rainfall	1100 mm and 1300 mm	1300 and 1600 mm
Soils	Humus, Inceptisols pH: 4.3–5.5	Humus-bearing kaolisols pH: 3.6–4.4

for 1 h before spectrometric reading at 765 nm wavelength. A calibration curve was established using gallic acid as the standard, with a concentration range of 10–50 µg/mL. The gallic acid standard solution was prepared by transferring 1 mL, 2 mL, 3 mL, 4 mL, and 5 mL, corresponding to 10 mg, 20 mg, 30 mg, 40 mg, and 50 mg of anhydrous gallic acid, respectively, into a 100 mL volumetric flask and filling it with distilled water.

The total polyphenol content was quantified using a standard curve generated using gallic acid as a reference standard and expressed as the amount of gallic acid equivalent. The total polyphenol content was expressed as a percentage of the dry matter mass.

2.3.2. Analysis of Catechins, Gallic and Caffeine

The quantitative analysis of catechins was carried out using high-performance liquid chromatography (HPLC), in accordance with the procedure described by ISO 14502:205.1 mL. The tea extract was transferred into a graduated tube and diluted to 5 mL with a stabilizing solution (10% v/v acetonitrile with 500 µg/mL ethylene diamine tetra acetic acid (EDTA) and ascorbic acid). The solution was filtered through a 0.45 µm nylon membrane filter and a 20 µL aliquot of this solution was injected into the HPLC for analysis.

The HPLC system was a Shimadzu LC 20 A series fitted with an SIL 20A auto sampler, an SPD-20 UV-Visible detector, a class LC10 chromatography workstation, and UV detection at 278 nm, using a Gemini C6 ODS column (250 mm × 4.6 mm) (Phenomenex Inc. Torrance CA, USA) fitted with a Gemini C6 ODS (4.0 mm × 3.0 mm) (Phenomenex Inc. Torrance CA, USA) guard column. The column temperature was maintained at 35°C. The mobile phase conditions were as follows: A, 9:2:89 v/v/v (Acetonitrile: Acetic acid: EDTA) and mobile phase B, 80:2:18 v/v/v (acetonitrile: acetic acid: EDTA) at a flow rate of 1 mL/min, and sample injection volume of 20 µL used. The conditions for the binary gradient were set at 100% solvent A for 10 min, followed over 15 min a linear gradient to 68% mobile phase A, 32% mobile phase B, and held at this composition for 10 min. conditions were again reset to 100% mobile phase A and allowed to equilibrate for 10 min before the next sample injection.

Individual catechins were identified by comparing the retention times of the unknown peaks with those identified from the peaks obtained from the mixed catechin standards. Catechin and caffeine quantifications were performed using a caffeine calibration curve, together with the consensus relative response factors (RRFs) with respect to caffeine, calculated on a dry matter basis. The total catechin content of the teas, as a percentage by mass on a sample dry matter basis, was determined by the summation of individual catechins, as follows:

$$\% \text{ Total Catechins} = \% (\text{ECG} + \% \text{ EC} + \% \text{ EGCG} + \% \text{ C} + \% \text{ EGC})$$

$$\text{Caffeine}\% = \frac{(\text{A sample} - \text{A intercept}) \times \text{RRF}_{\text{std}} \times \text{V} \times \text{D} \times 100}{\text{Slope caffeine} \times 1000 \times \text{DM}}$$

where

A sample – peak area of the individual component in the test sample.

A intercept – peak area at the point of interception on y-axis.

Slope caffeine – caffeine calibration line slope.

V – sample extraction volume

D – dilution factor.

M – mass in grams of test sample.

DM – dry matter content of test sample

2.4. Data Analysis

Data analysis was performed by using one way ANOVA, and the comparison of means was performed by Tukey's test at a significance level of 5% using Minitab19 statistical software.

3. RESULTS AND DISCUSSION

3.1. Total Polyphenol Content

Polyphenols are secondary metabolites that play a role in plant defence mechanisms. In tea leaves, polyphenols account for 25% to 35% of the dry weight basis [5]. The main polyphenol components of fresh leaves are catechins, flavonols, and phenolic acids, such as gallic and cinnamic acids, which [17] are important traits for tea breeders that provide a basis for the selection, improvement, and management of tea quality [18].

Table II shows the total polyphenol content of the fresh tea leaf samples. The results were significantly different among tea clones from the same region and across two distinct growing regions. In Rubaya, clones TRFK100/5 and TRFK6/8 had higher levels of polyphenols at 26.36% and 25.79%, respectively). This was followed by BBK35 with 24.49% and BBK10 with the lowest level of 23.03%). In Mata, clones TRFK6/8 and TRFK100/5 had the highest levels of 25.30% and 24.75%, respectively which was followed by BBK10 with 24.44% and BBK35 having the lowest level of 23.07%).

From the results obtained, it is clear that all four tea clones were rich in total polyphenols. This finding is in agreement with research done in Kenya by Karori *et al.* [19]. The authors reported that *Assam* tea clones grown in Kenya are rich in polyphenols. The high polyphenol content was expected because all clones planted in Rwanda originated from Kenya. These clones were selected as tea germplasms based on their high total phenol [20].

TABLE II: THE LEVEL OF TOTAL POLYPHENOL (TP%) FRESH TEA FROM RUBAYA AND MATA

Tea Sstate/Region	Tea clone	TP%
Rubaya/Western region	TRFK6/8	25.79 ± 0.41 ^{ab}
	BBK35	24.49 ± 0.74 ^c
	BBK10	23.03 ± 0.05 ^d
	TRFK100/5	26.36 ± 0.45 ^a
Mata/Southern region	TRFK6/8	25.30 ± 0.50 ^{bc}
	BBK35	23.07 ± 0.60 ^d
	BBK10	24.44 ± 0.92 ^c
	TRFK100/5	25.04 ± 0.71 ^{bc}

Note: Means within a column preceded by the same superscript letter(s) are not significantly different ($P \leq 0.05$).

Most tea clones grown in Rubaya showed higher levels of total polyphenols than those grown in Mata, except for clone BBK10. This difference could be associated with environmental factors, such as altitude and temperature, which vary in the Rubaya and Mata tea plantation regions.

Rwanda is characterized by a high degree of agroecological diversity owing to its topography [15]. Rubaya tea plantations are located along the Congo Nile bridge in the western region of Rwanda, with high hills and altitudes varying from 2400 m to 2500 m above sea level, and cool temperatures varying between 10°C and 18°C. The Mata tea plantation is situated in the southern part of the central plateau region with a low altitude of between 1700 m and 2000 m and warm temperatures varying between 18°C and 20°C [15].

The high level of total polyphenols in clones from Rubaya compared to that from Mata could be associated with variations in environmental conditions, such as altitude and temperature. The present findings are in agreement with those reported by Özdemir *et al.* [21] who indicated that altitude and temperature influenced the growth rate of tea plants and the level of polyphenols. The shoots of tea grown in high-altitude regions grow and mature slowly and, in the process, accumulate high amounts of polyphenolic compounds, whereas tea grown in low-altitude regions grows quickly and produces low levels of phenolic compounds. Furthermore, the present study found that the tea clones did not show the same pattern in the two different growth stages. The differences in clone performance may be associated with genetics. Turkmen *et al.* [17] reported that the level of total polyphenols in different clones was genetically controlled. These findings are similar to those of a previous study by Leonida *et al.* [22]. They established that TRFK6/8 is a benchmark for high-quality black tea in Kenya, and further connected the ability of different tea clones to accumulate nutrients from soils, which influences the synthesis of bioactive compounds in tea leaves.

3.2. Gallic Acid and Catechins Composition in Fresh Tea Leaves for Different Clones Grown in Two Growing Regions

Gallic acid (GA) known as 3,4,5-trihydroxybenzoic acid is an important phenolic acid in tea leaves which act as precursor of gallate catechins the major characteristic metabolites in tea [9]. Gallic acid is an important contributor to antioxidant activity of tea [23].

The results in Table III indicate that the level of gallic acid was significantly different among the tea clones grown in the two different growing regions. In Rubaya, clone BBK10 exhibited a significantly higher level of gallic acid at 0.56% than the other clones, followed by TRFK6/8 having 0.45%). Clones BBK35 and TRFK100/510 exhibited the lowest levels of 0.39% and 0.40%, respectively. In Mata, clones BBK10 and TRFK6/8 had the highest significant levels of gallic acid with levels of 0.36% and 0.38%, respectively. Clones BBK35 and TRFK100/5 had the lowest concentrations of 0.26% and 0.29%, respectively.

The level of gallic acid obtained in the present study was in the range with the level of gallic acid reported in Kenya by Cherotich *et al.* [24] indicated that the level of gallic acid (GA) in fresh tea leaves ranged from 0.4%–1.04% on a dry-weight basis. The present study found that the tea clone grown in Rubaya had higher levels of gallic acid than the tea clone grown in Mata. For instant clones BBK10, 0.56% in Rubaya and 0.39% in Mata. This variation can be attributed to the variation in environmental factors between the two regions. This shows that environmental conditions in Rubaya favor the accumulation of gallic acid compared to that in the Mata region. The Rubaya region has the lowest temperature range of 10°C–18°C [15].

This condition in Rubaya can decrease the growth rate of tea leaves while increasing the accumulation of phenolic acids, such as gallic acid, [25] in the Mata region; the temperature is warmer, at 18°C and 21°C [15]. Hence, the climate of low temperature combined with rainfall in Mata promotes the growth rate of tea while decreasing the accumulation of phenolic acids, such as gallic acid. Similar results have been reported by Cherotich *et al.* [24] and Xu *et al.* [26].

Catechins (flavan-3-ols) are the main polyphenolic compounds in fresh tea leaves ranging from 10%–30%. It contributes to the bitter taste and astringency of tea and can be used to select good tea cultivars [8].

The present study found significantly different total catechin (TC) levels among tea clones in the two growing regions. In Rubaya, clones TRFK6/8, BBK10, and TRFK100/5 exhibited the highest levels of total catechins, at 23.23%, 21.83%, and 19.47%, respectively. In Mata, TRFK6/8, BBK10, and TRFK100/5 had lower levels of 18.96%, 16.86%, and 16.075%, respectively.

In addition to total catechins, the study also compared the levels of individual catechin fractions, such as epigallocatechin, simple catechins, epicatechins, epigallocatechin

TABLE III: GALLIC ACID AND CATECHINS IN FRESH TEA CLONES GROW IN THE RUBAYA AND MATA

Region	Clone	Gallic acid	EGC %	+C%	EC%	EGCG %	ECG %	Total catechins
Rubaya/Western region	TRFK6/8	0.45 ± 0.02 ^b	6.54 ± 0.20 ^a	0.59 ± 0.09 ^a	3.13 ± 0.0 ^a	9.91 ± 0.22 ^b	3.06 ± 0.02 ^{de}	23.23 ± 0.17 ^a
	BBK35	0.39 ± 0.02 ^c	3.50 ± 0.20 ^d	0.36 ± 0.04 ^{ab}	3.23 ± 0.02 ^a	7.25 ± 0.40 ^e	4.04 ± 0.60 ^b	18.37 ± 0.90 ^c
	BBK10	0.56 ± 0.03 ^a	5.51 ± 0.40 ^b	0.38 ± 0.18 ^{ab}	2.50 ± 0.07 ^b	8.68 ± 0.15 ^d	4.76 ± 0.02 ^a	21.83 ± 0.07 ^{ab}
	TRFK100/5	0.40 ± 0.01 ^c	2.53 ± 0.20 ^{ef}	0.51 ± 0.2 ^{ab}	2.10 ± 0.00 ^c	11.04 ± 0.32 ^a	3.29 ± 0.03 ^{cd}	19.47 ± 0.03 ^b
Mata/Southern region	TRFK6/8	0.36 ± 0.01 ^d	4.45 ± 0.30 ^c	0.30 ± 0.02 ^b	2.06 ± 0.05 ^c	8.76 ± 0.20 ^d	3.43 ± 0.07 ^{cd}	18.96 ± 0.20 ^{bc}
	BBK35	0.26 ± 0.06 ^e	3.61 ± 0.50 ^d	0.46 ± 0.05 ^{ab}	1.68 ± 0.14 ^d	9.19 ± 0.21 ^{bc}	3.74 ± 0.07 ^{bc}	18.68 ± 0.10 ^{bc}
	BBK10	0.38 ± 0.09 ^{cd}	2.83 ± 0.10 ^e	0.30 ± 0.0 ^b	1.71 ± 0.06 ^d	7.82 ± 0.15 ^e	4.21 ± 0.10 ^{ab}	16.86 ± 0.40 ^d
	TRFK100/5	0.29 ± 0.03 ^e	2.10 ± 0.15 ^f	0.48 ± 0.03 ^{ab}	2.08 ± 0.15 ^c	8.82 ± 0.36 ^d	2.61 ± 0.09 ^e	16.075 ± 0.10 ^d

Note: Means within a column preceded by the same superscript letter(s) are not significantly different ($p \leq 0.05$). EGC, epigallocatechin; C = Catechin; EC = Epicatechin; EGCG = Epigallocatechin gallate; ECG, epicatechin gallate.

gallate, and epigallocatechin. The results showed that the level of the catechin fraction was significantly different between the tea clones in the same region and across the different growing regions.

The distribution of individual catechins is an important factor to be considered when selecting high-quality tea clones. It is indicated that even if the total catechins are the same among the tea clones, the distribution ratio among the individual catechin ratios can be different. This ratio can be used as a tool for diversification of tea products [27].

The data showed that For epigallocatechin (EGC), clones TRFK6/8 and BBK10 displayed the highest levels of 6.54% and 5.51%, respectively, in Rubaya. In Mata, clones TRFK6/8 and BBK10 showed the lowest levels of 4.45% and 2.83%, respectively.

The levels of simple catechins (+C) in tea leaves did not exhibit significant differences in tea clones grown within the same region. Comparing between the two growing regions, the difference was observed only for clone TRFK6/8, where it was 0.590% in Rubaya and 0.30% in Mata.

(EC) content was significantly different among tea clones within the same region and across different regions. In Rubaya, clones BBK35 and BBK10 had the highest levels of 3.23% and 2.50%, respectively. In Mata, the levels were 1.68% and 1.75% for clones BBK35 and BBK10, respectively.

Epigallocatechin Gallate (EGCG) levels were significantly different among tea cultivars grown within the same region and across different regions. In Rubaya, clone TRFK100/5 had the highest epigallocatechin gallate level at 11.02%, whereas in Mata, it was 8.82%. The Clone BBK10 was 8.68% in Rubaya and 7.82% in Mata.

The Epicatechin Gallate (ECG) level was significantly different among tea clones grown within one region. However, across the regions, only the tea clone TRFK100/5 showed a significant difference between the two growing regions. In Rubaya, clone TRFK100/5 had the highest level of 3.29%, and the lowest in Mata of 2.61%.

The present study revealed that among the catechins, Epigallocatechin Gallate (EGCG) was dominant, while simple catechins (+C) and EC were present at low concentrations. This finding concurs with those of Nhu-Trang *et al.* [28]. They indicated that epigallocatechin gallate and epicatechin gallate make up approximately 70% of the total catechins, while epicatechin and simple catechin were at low levels.

Most of the individual catechin fractions were high for clones grown in Rubaya but low for clones grown in Mata. Clone BBK35 was an exception because it had a higher level of Epigallocatechin Gallate (EGCG) in Mata than in Rubaya. This was similar to the other phenolic components determined in this study. The variation could be associated with differences in wide climatic conditions, such as temperature, altitude, and rainfall, which may have affected the performance of the clone [29].

In a study by Ahmed *et al.* [30] indicated that low temperatures induced dormancy in the rate of shoot growth. Consequently, the accumulation of biochemical components in the tea leaves increases. High temperatures stimulate fast shoot growth with low levels of biomolecule

accumulation. This report agrees with the findings of the present study, which show that the level of individual catechins is higher in Rubaya tea clones where the temperatures are very low, compared to those from Mata where the temperatures are high.

Furthermore, previous research has reported that elevated temperatures reduce flavonoid accumulation and inhibit the gene expression of enzymes in the phenylpropanoid and flavonoid biosynthetic pathways [31]. In this regard, the elevated temperature in the Mata tea plantation could be the cause of the diminished levels of individual catechins. High-altitude grown tea is exposed to high levels of natural UV radiation, which upregulates flavonoid synthesis [32]. Because Rubaya is located on hills at a high altitude of 2400 m–2500 m, the tea leaves in fields are exposed to high UV radiation, consequently increasing the accumulation of catechins in the tea leaves.

Moreover, the current study revealed that the four tea clones did not show the same change pattern of catechin fraction due to the genetic variation among the clones. The present study found that the catechin fractions were different for each tea clone. Among the four clones grown in the two regions, TRFK6/8 distinguished itself by displaying the highest values for numerous compounds, including EGC, +C, EC, TC, and other clones. Similar results have been reported by Cherotich *et al.* [24] who reported that various tea clones had different levels of catechin fractions, and selected TRFK6/8 as the standard tea clone for black tea in Kenya due to its high quality attributed to its high polyphenol content. It is important to note that genetic differences in tea clones also affect their ability to absorb nutrients even under similar agronomic practices. Consequently, this affects their ability to synthesize and accumulate bioactive molecules. This explains why some clones in Mata had relatively high catechin content compared to the same clones grown in Rubaya, even though the conditions in Rubaya seemingly positively affected the majority of the bioactive components in tea, such as phenols. This is in agreement with a report by Mutuku *et al.* [11] who indicated that the performance of tea clones depended on the growing region. The authors reported that a clone can show high performance in one region but low performance in another.

Caffeine, a purine alkaloid, is an important secondary metabolite in tea plants [33]. It is among the most crucial elements for the quality and functionality of tea. Black tea contributes to the creaming and bitter taste properties of tea beverages [34].

The caffeine levels in fresh tea clones are presented in Table IV. The results showed that caffeine levels were significantly different among clones grown in the same region and across the two growing regions.

In Rubaya, clones BB10 and TRFK100/5 exhibited the highest levels of 4.19% and 4.88%. Clones TRFK6/8 and BBK35 had the lowest levels of 3.44% and 3.55%, respectively. In Mata, clone TRFK100/5 had the highest abundance of 4.63%, followed by BBK 35, having 4.32%). Clones TRFK6/8 and BBK10 had the lowest levels of 4.01% and 3.86%, respectively.

The level of caffeine reported in this study was in the same range, slightly higher compared to the level of caffeine in Kenyan tea, which was at a level of 1.96% and

TABLE IV: CAFFEINE% IN FRESH TEA FROM RUBAYA AND MATA

Region	Clone	Caffeine
Rubaya/Western region	TRFK6/8	3.55 ± 0.08 ^e
	BBK35	3.44 ± 0.01 ^e
	BBK10	4.19 ± 0.02 ^{bc}
	TRFK100/5	4.88 ± 0.08 ^a
Mata/Southern region	TRFK6/8	4.01 ± 0.05 ^{cd}
	BBK35	4.32 ± 0.05 ^b
	BBK10	3.86 ± 0.2 ^d
	TRFK100/5	4.63 ± 0.14 ^a

Note: Means within a column preceded by the same superscript letter(s) are not significantly different ($p \leq 0.05$).

4.37% on similar clones as reported by [20]. This difference could be attributed to variations in sampling season, agricultural practices between Rwanda and Kenya, like fertilizers and pruning. Previous research has reported that environmental and agricultural practices affect caffeine accumulation in tea during growth.

Unlike catechins and total polyphenols, caffeine content differed from that of the other bioactive compounds in this study. Tea clones grown in Mata at a low altitude of 1700 m–2000 m had high levels of caffeine compared to cultivars grown in Rubaya at an altitude of 2400 m–2500 m. Even if the tea in the Rubaya climate favors the accumulation of bioactive components in the present study due to low temperature, high altitude, and abundant rainfall, it does not favor the accumulation of high caffeine content in tea. This finding is consistent with those of Lee *et al.* [35] reported that warm temperatures and abundant rainfall increase growth rate and high levels of caffeine biosynthesis. The accumulation of caffeine in tea grown in Mata could be attributed to the elevated temperatures of 18°C and 20°C and rainfall of 1100 mm–1300 mm; similar results were reported by Cheptot *et al.* [36].

4. CONCLUSION

This study explored the polyphenolic composition of four common clones grown in two agroecological regions in Rwanda. This study revealed significant variations in gallic acid composition among fresh and processed tea leaves, both within selected tea clones and across different growing regions. The results indicate that fresh tea clones from the Rubaya region tend to have higher gallic acid, polyphenol, and catechin contents than those from the Mata region. Furthermore, the study examined individual catechins such as epigallocatechin (EGC), simple catechins (+C), epicatechin (EC), epigallocatechin gallate (EGCG), and epicatechin gallate (ECG) profiles and found that the distribution of catechin profiles was unique for each tea clone grown in the two regions and was significantly different between tea clones. The caffeine content also varied among cultivars and regions where the tea cultivars were grown. Tea from Mata had a higher caffeine content than tea clones grown in Rubaya. The findings revealed that the composition of compounds in tea is influenced by both the tea plant clone and ecological conditions in the growing region. This suggests that Rwandan tea

can be used for tea product diversification and branding based on specific growing regions. Further assessment of other phytochemicals in tea cultivars grown in Rwanda is recommended.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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