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Polyphenolic composition and antioxidant activity of Kenyan Tea cultivars

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Abstract
Polyphenolic fractions in tea are potent bioactive molecules. In this study, the polyphenolic composition of 25 different types of Kenyan tea cultivars was determined using the HPLC and the Folins Ciocalteus spectrophotometric methods. Total Polyphenols, Total Catechins, individual catechins and Antioxidant Activity were significantly ($P<0.05$) different among tea varieties, with green tea had the highest levels of Total Polyphenols ranging from (19.70-26.12%), TC (8.51%-17.60%), individual catechins, and AA (86.65-94.50%). *In vitro* bioassay carried out using 2, 2'-diphenyl picrylhydrazyl radical showed epigallocatechin gallate was the most potent catechin and the most potent in antioxidant activity ($r=0.968$***). Epigallocatechin ($r=0.659$***, $p<0.001$), Epicatechigallate ($r=0.454$*, $p<0.001$) and Epicatechin (EC) ($r=0.780$***, $p<0.001$) showed significant ($p<0.05$) antioxidant activity. Black tea contained high levels of Theaflavins and Thearubigins (2.072% to 17.12%), respectively) which accounted for its antioxidant activity ($r=0.803$*** and $r=0.859$***, respectively). Gallic acid also showed significant ($r=0.530$*) contribution to the antioxidant activity in black tea. Data obtained from this study reveals that different Kenyan tea cultivars have different polyphenolic composition which imparts on their unique biochemical qualities. Green and white tea products are rich in catechins, black tea products are rich in TFs and TRs while purple teas are rich in anthocyanins.

Keywords: Catechins, EGCG, Theaflavins, Thearubigens, Anthocyanins, Antioxidant Activity.

1. Introduction
Tea, from *Camellia sinensis* L.O Kuntze is one of the most widely consumed beverages in the world and it was first introduced in Kenya in1904 by the British settlers. The crop has expanded to cover an area of around 157,720 ha in the highlands East and West of the Great Rift Valley in Kenya [1]. The tea plant is an evergreen bush that grows to 15 m high in the wild, and 60–100 cm under cultivation. Tea in cultivation forms a table from which the young leaves are harvested through and a cyclic pruning is carried out after every 3 to 4 years and commercial harvesting is carried out either by hand or machine [2].
Young leaves of tea are processed into different types of products, the predominant ones being black, green, white and oolong tea. Green tea is mainly consumed in China, Japan and the Middle East, while black tea is mostly consumed in India, Sri-Lanka, European countries and regions of Africa. Popularity of tea is due to its aroma, pleasant taste and medicinal benefit [3]. Tea from Kenya tea is better perceived since it is grown free of agrochemicals in an ideal environment that naturally deters to pests and attack by plant diseases. This pleasant natural condition guarantees the consumer the safest and most refreshing sought after health drink in the world. Phytochemicals and functional components in tea are receiving a lot of attention due to their potential benefits in health when consumed as part of a varied diet on a regular basis and at effective levels. Many nutraceuticals, functional foods and naturally occurring polyphenols have physiological and pharmacological activities including their well characterized antioxidant properties [4, 5]. Since the scientific community and food industry communities share a common goal of extending the quality of human life, through development of viable options for the management of chronic diseases through the use of nutraceuticals, functional foods have become a potential starting point. This is because functional foods are fairly affordable, readily available and extremely active, have profound effect on cell metabolism and often demonstrate few side effects [6]. It is evident that nutraceuticals offer a selective advantage over synthetic drugs necessitating need to investigate their usefulness to human health.

Despite the use of tea in food and drinks, it has increasingly been put to many uses. For example, numerous environmentally friendly industrial cleaning agents, deodorizers and anti-microbial agents have been formulated using tea [7, 8]. However, data to support the view that tea is pharmacologically active has been generated particularly using green tea, which is widely consumed in Asia [9, 10]. As a result, green tea has been widely marketed as a health product since the chemical composition is well characterized. However, the much information on the chemical composition of black aerated or fermented tea, the principle type of tea product consumed in Kenya is based broadly on the theaflavins and thearubigens but none of the theaflavins fractions. In addition, information on the chemical composition of purple tea, a novel product released recently by the Tea Research Institute (TRI) is lacking. There is need therefore to conduct systematic research on the phytochemical composition of Kenyan tea cultivars especially black tea and purple teas in order to generate requisite data which can be used to understand the pharmacological activity of tea.

2.1 Tea Products and Determination of Flavonoid Levels

2.1.1 Tea samples

The tea samples were sourced from Tea Research Institute (TRI), Timbilil Estate, Kericho (latitude 0˚ 22'S, longitude 35˚ 21'E, altitude 2180 m above mean sea level and processed at the TRFK miniature factory as described by Karori *et al.,* [5].

2.1.2 Biochemical profiling of the tea extracts based on Catechins

A modified method of Zuo *et al.,* [11] which is based on high performance liquid chromatography was used to assay for the tea catechins of as described by Kerio *et al.,* [12].

2.1.3 Determination of total polyphenols in the tea extracts

The Folin-Ciocalteu phenol reagent method was used to determine total polyphenols in the tea extracts according to ISO (BS ISO 14502-1: 2005(E)).

2.1.4 Analysis of the content of total theaflavins and individual theaflavin fractions content in the tea samples

Black, green, purple and white teas were also assayed for total theaflavins (TF) using the flavognost method of Hilton and Palmer Jones [13] whereas individual theaflavins fraction ratio were determined as described by Kilel *et al.,* [14].

2.1.5 Spectrophotometric determination of total thearubigins in the tea samples

Total thearubigins (TRs) were determined in the tea samples using the method of Roberts and Smith [15].

2.1.6 Determination of total monomeric anthocyanin content

The total monomeric anthocyanin content in the processed aerated, unaerated purple tea samples was determined in duplicate using the pH differential method Kerio *et al.,* [12].

2.1.7 Determination of antioxidant activity of tea

The stable DPPH (2,2-diphenyl-1-picrylhydrazyl) radical was used for determination of free radical scavenging of tea extracts using a modified method of Brand-Williams *et al.,* [16]

2.1.8 Statistical analysis

All statistical analysis was carried out using MSTAT-C statistical software. ANOVA was used to determine the means, coefficient of variation and any differences between the samples. Least Significance Difference (LSD) was used separate means. The probability limit was set at $p \leq 0.05$ significant level. Results of the parameter determined were expressed as a mean of the triplicate determination.

3. Results and Discussion

3.1 Chromatographic and Spectrophotometric Analysis

3.1.1 Tea polyphenols

This study compared the total polyphenols levels in tea samples processed from Kenyan germplasm using the Folins-Ciocalteus method. The green (unaerated), black (aerated), white and purple teas samples analyzed differed significantly in the levels of total polyphenols ($p \leq 0.05$). Kenyan green teas were rich in total polyphenols with their levels ranging from the highest amount of 26.1% for cultivar Ejulu-L to the lowest of 19.7% for cultivar TRFK 430/12 as shown in Table 1. Black teas had a lower total polyphenol concentration compared to green teas with cultivar Ejulu-L having 21.2% and cultivar TRFK 301/4 the lowest value of 19.7%. It was however evident, that some black teas from Kenya had a higher polyphenolic concentration than green teas. Cultivar TRFK 6/8, a high quality Kenyan genotype used in this study as an internal standard for black tea quality, recorded a total polyphenol content of 25.13% and 20.72% for unaerated and aerated tea respectively, which was not significantly different ($p \leq 0.05$) from tea cultivar Ejulu-L. Total polyphenol content of aerated and anaerated teas processed from purple coloured leaf cultivars was 20.03% and 21.90% respectively while white teas processed from plucked shoots of cultivar AHP S15/10 was 22.43% (Table 1).

Polyphenols which are constituents of secondary metabolism in plants play a role in plant defense mechanism against insects, birds and animals. This study revealed that these chemicals are retained almost intact in unaerated processed teas. Unaerated Green tea is made without enzymatic auto-oxidation of polyphenols, since the enzyme polyphenol oxidase is inactivated by heat during the early stages of processing [17]. This process ensures that polyphenols present in green tea are nearly the same as those found in fresh tea leaves. In a broad sense, green tea polyphenols consist of simple and complex compounds, the large majority of which are the flavonoid monomers catechins, catechin gallates and flavonols [18, 19].

Polyphenols occurring in black tea usually consist of residual green tea polyphenols such as catechins flavonols and oxidation products of green tea polyphenols such as theaflavins and thearubigins [19]. Some catechins and catechin gallates may be epimerized or degallated during the processing of black tea. Most of the catechins and their gallates undergo known enzymatic oxidation to form more polymeric polyphenols that are characteristic of black tea, namely theaflavins and thearubigins [19, 20]. Therefore, the amount of polyphenols in green tea is higher than that of black teas since the auto-oxidation process results in a significant conversion of green tea polyphenols to highly polymerized substances such as theaflavins which contribute

to the characteristic bright orange color of black tea and thearubigens which are more chemically heterogeneous and tend to be brownish-red [20, 21]. However, despite this observation, the exact contribution of inter-flavonoid linkages and the general structure of thearubigins to the above quality parameters remains ambiguous and their structures remain speculative. There is need therefore to elucidate the thearubigins structure to help in optimizing the tea processing parameters which might contribute to the customer's needs.

Table 1: Total polyphenols (TP) (%) and Total Catechins (TC) (%) levels of different tea products used in this study

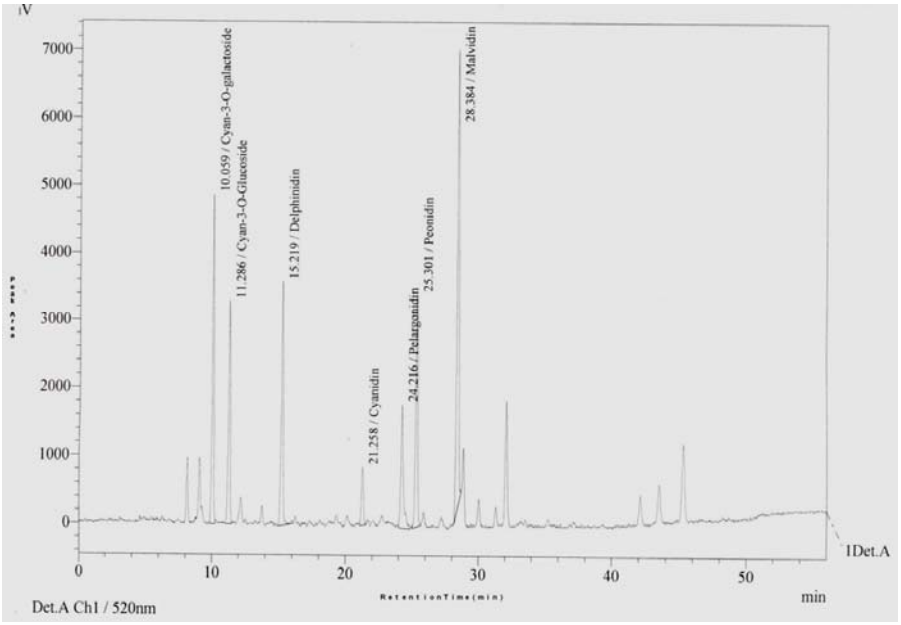
Clone	Green Tea (TP)	Black Tea (TP)	Green Tea (TC)	Black Tea (TC)
TRFK 301/4	22.64^{def}	14.96^{h}	16.14^{cdef}	2.650^{kl}
TRFK 301/5	22.07^{defg}	15.91^{gh}	16.42^{bcde}	3.515^{hijkl}
TRFK 303/216	21.42^{def}	16.41^{fgh}	15.78^{fgh}	2.635^{l}
TRFK 303/231	25.61^{ab}	18.85^{cde}	15.41^{ghi}	3.680^{hijkl}
TRFK 303/577	23.21^{def}	23.21^{def}	16.60^{bc}	7.215^{ab}
TRFK 303/745	20.85^{ghi}	18.35^{de}	11.26^{m}	4.305^{efgh}
TRFK 337/138	20.85^{ghi}	19.76^{abcd}	17.60^{a}	6.335^{bc}
TRFK 371/3	24.75^{ab}	20.38^{abc}	14.93^{i}	4.25^{efghi}
TRFK 430/3	22.74^{de}	18.22^{def}	14.20^{j}	3.820^{ghij}
TRFK 430/4	19.98^{hi}	17.56^{efg}	9.52^{n}	3.225^{ijkl}
TRFK 430/12	19.70^{i}	16.15^{gh}	11.90^{l}	3.900^{fghij}
TRFK 430/63	21.26^{fgh}	15.98^{gh}	15.31^{hi}	3.700^{ghijk}
TRFK 430/90	23.25^{cd}	19.65^{abcd}	15.89^{efg}	8.115^{a}
TRFK 524/170	21.84^{defg}	17.56^{efg}	11.90^{l}	3.82^{ghij}
TRFK 524/48	22.70^{de}	17.52^{efg}	16.39^{bcde}	3.665^{hijkl}
BBK35	24.55^{bc}	21.03^{a}	16.04^{def}	3.760^{ghij}
EPKC12	22.03^{defg}	20.38^{abc}	12.14^{l}	5.215^{de}
EPK D 99/10	21.59^{efg}	17.39^{efg}	14.15^{j}	3.150^{jkl}
EJULU-L	26.12^{a}	21.22^{a}	16.53^{bcd}	5.850^{cd}
AHPS 15/10	22.43^{def}	18.94^{bcde}	15.99^{ef}	5.000^{de}
EPK TN 14/3	21.92^{defg}	17.94^{efg}	10.82^{m}	3.565^{hijkl}
TRFK 6/8	25.13^{ab}	20.72^{ab}	16.85^{b}	6.775^{bc}
TRFK 31/8	21.94^{defg}	20.73^{ab}	14.31^{j}	4.755^{efg}
TRFK 31/11	22.85^{de}	20.68^{abc}	12.77^{k}	4.890^{def}
TRFK 100/5	23.07^{d}	19.09^{bcde}	15.91^{efg}	3.555^{hijkl}
TRFK 306/1	21.90defg LSD=1.436 CV=3.07% Mean=22.7	20.03abcd LSD=1.871 CV=4.87% Mean=18.6	8.51o LSD=1.436 CV=1.80% Mean=14.3	5.235de LSD=1.871 CV=11.46% Mean=4.48

Means within a column followed by the same letter are not significantly different at p≤0.05 according to DMRT. Purple tea; TRFK 306/1; White tea; AHPS 15/10

3.1.2 Total catechin content

Results of the total catechins levels in green, black and white tea products processed from the 25 tea cultivars assayed in this study are presented in Table 1. The tea cultivars produced tea products that differed significantly (p≤0.05) in total catechin content. Non-aerated (green) teas contained significantly (p≤0.05) higher amounts of total catechins of 17.60% to 8.51% than aerated (black) teas which ranged from 8.115% to 3.150%. These results demonstrated clearly that the degree of auto-oxidation ''fermentation'' during the manufacturing process had an influence on the catechin content of the final product. During this processing, the polyphenol oxidase enzyme catalyzes the oxidation of catechins into quinones by molecular oxygen [20, 22]. The quinones generated from the oxidation of the B-ring in the dihydroxylated catechins condense with the quinones from the B-ring of the trihydroxy related catechins to form theaflavins. Since there is a difference in the reduction potential, quinones will also take part in the redox equilibrium at fermentation and this explains the depletion of catechins at different rates [19, 20]. White tea comes from the same plant *Camellia sinensis* as the case of green and black teas. It is processed from the young buds and/or young leaves and the descriptive term "white" stems from the high proportion of silvery buds harvested from the plants to produce the tea. The buds to manufacture this type of tea are picked then rapidly steamed and dried, without fermentation, rolling or roasting. Minimal processing not only protects the delicate, light and slightly sweet flavor of white tea, but also enables the retention of high levels of phytochemicals [18, 19]. This explains why white tea had high levels of total catechins despite being processed only the bud. The composition of tea leaves varies significantly with shoot maturity and season. Young leaves are composed of polyphenols in the following order EGCG > EGC > ECG > EC. As for mature leaves, it is EGC > EGCG > ECG > EC while old leaves composition was EGC > EGCG > EC > ECG. It is therefore important to use young leaves and obey plucking standards to achieve optimum quality especially for black tea. However, since mature and old tea leaves possess high amounts of EGC, it would be advisable to research more on ways they can utilized for further applications.

Purple leaf coloured cultivar TRFK 306/1 recorded a very low concentration of total catechins of only 8.51% for processed green tea. In order to establish why the purple teas recorded low levels of catechins, an analysis of the total monomeric anthocyanin content and a fractionation of anthocyanin by HPLC were carried out. Results from this experiment revealed that purple tea was instead rich in anthocyanins which are important phytochemicals found in the novel purple-pigmented cultivars and not catechins as earlier thought. Anthocyanins are a sub-class of flavonoids synthesized via the phenylpropanoid pathway and are wide spread in the animal kingdom where they present diverse biological and biochemical interests [23]. The anthocyanidins fractions in the processed unaerated and aerated tea products from the purple coloured tea cultivar 306/1 were identified and quantified by HPLC using pure anthocyanidin /anthocyanin standards. The order of elution of the anthocyanidin/anthocyanin was cyanidin-3-O-galactoside < cyanidin-3-O-glucoside < delphinidin < cyanidin < pelargonidin < peonidin < malvidin with malvidin being the most predominant anthocyanin as shown in a representative HPLC chromatogram of aerated tea from a purple tea cultivar (Figure 1). The results obtained from this study collaborated with those of Kerio *et al.,* [12].



Retention time, min

Fig 1: A representative HPLC chromatogram of processed black tea sample from purple colored cultivar TRFK 306/1
KEY: X-axis = Retention time (min) Y-axis = Peak area

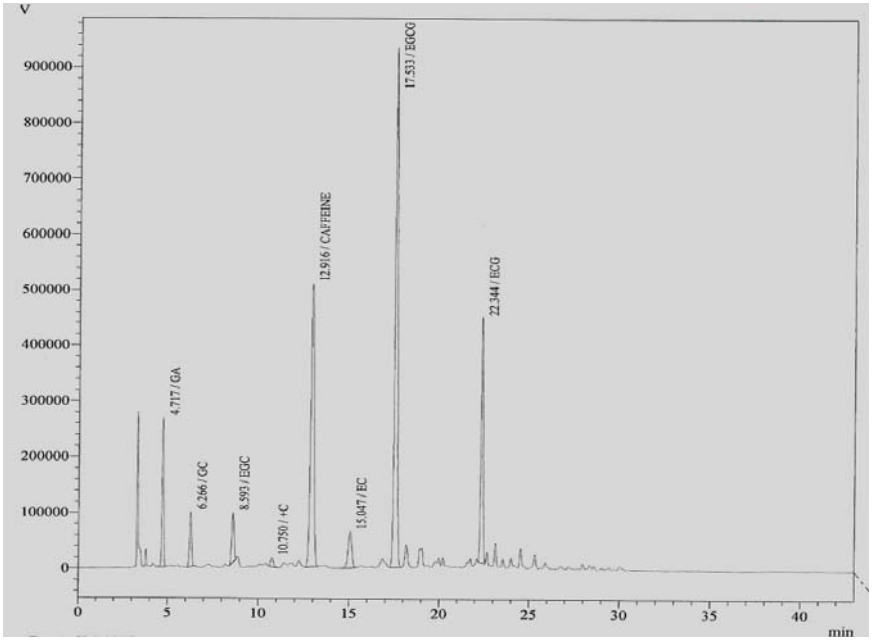
However, it was noted that anthocyanidins levels were significantly higher ($p \leq 0.05$) in unaerated than in aerated tea. This can be attributed to the tea processing procedures where conversion of fresh tea leaf to the aerated tea decreases the total monomeric anthocyanins. Kerio *et al.*, [12] observed similar results while characterizing anthocyanins in Kenyan teas and attributed this to anthocyanin degradation during the manufacture of black teas. Although no mechanism has been developed so far on the anthocyanin degradation, studies in other plants such as strawberry have been attempted [24]. In their work Liu *et al.*, [24], found out that anthocyanins were rapidly degraded by PPO in the presence of other polyphenol compounds such as catechins. For example, cyaniding-3-O-rutinoside is degraded by PPO in the presence of (-)-epicatechin and this is responsible for the formation of dehydroepicatechin A [12, 24]. The coupled oxidation reactions can be used to explain the sudden reduction of anthocaynins in the aerated teas. However, this is not the case in the unaerated teas since PPO is deactivated by steaming freshly plucked tea leaf and therefore the formation of the reactive O-quinones is stopped and subsequently, anthocyanins are not degraded. This is a hypothesis which needs further studies to establish the exact cause of degradation.

3.1.3 Catechin fractions

The catechins identified in tea cultivars were EC, EGC, ECG and EGCG. The two main gallated catechins present were EGCG and ECG while the others were non-gallated. Beside the main peaks identified, several minor peaks were also detected, which indicated that other unidentified catechin compounds existed in the tea extracts (Figure 2). There was however great similarity in the HPLC chromatographic pattern which indicated the close similarity in catechin profiles in the teas studied.

Catechin fractions assayed in this study were statistically different ($p<0.05$) as shown in Table 2. The results obtained revealed that black (aerated) teas had lower catechin levels than the green and white (non-aerated) teas (Table 2 and 3). Individual catechins varied significantly ($p<0.05$) among the teas with EGCG, GC and EGC levels being the highest and +C, ECG and EC being less abundant. These results are similar to those of Karori *et al.,* [5]. The reduction in the

catechin content in black teas compared to the green tea due to the monomeric flavan-3-ols undergoing polyphenol oxidase-dependent polymerization. This results in the formation of theaflavins, thearubigins, bisflavanols and other complex oligomers [25, 26, 27].



Retention time, min

Fig 2: A representative high performance liquid chromatogram of green tea cultivar

Table 2: Individual catechin (%) levels of different green tea products analyzed

Individual Catechins					
Clone	**EGCG%**	**EGC%**	**EC%**	**ECG%**	**C%**
TRFK 301/4	3.800^{m}	2.725^{j}	2.745^{b}	4.585^{ab}	2.690^{a}
TRFK 301/5	3.105^{n}	4.000^{fg}	3.280^{a}	4.180^{c}	1.850^{b}
TRFK 303/216	4.870^{hi}	5.770^{bc}	1.960^{cde}	2.035^{ijkl}	1.075^{de}
TRFK 303/231	5.555^{def}	4.410^{ef}	1.485^{fghij}	2.465^{ef}	0.5150^{hij}
TRFK 303/577	5.185^{fgh}	3.695^{gh}	1.775^{cdefg}	7.215^{ab}	1.240^{cd}
TRFK 303/745	3.830^{lm}	3.370^{hi}	1.355^{ghijk}	1.455^{n}	1.250^{cd}
TRFK 337/138	5.725^{cde}	4.040^{fg}	1.440^{fghijk}	2.275^{fghi}	1.280^{cd}
TRFK 371/3	5.525^{def}	4.585^{e}	1.770^{cdefg}	1.990^{kl}	0.9600^{ef}
TRFK 430/3	4.990^{ghi}	5.130^{d}	1.725^{cdefg}	3.820^{ghij}	0.3200^{j}
TRFK 430/4	3.000^{n}	2.600^{j}	1.275^{hijkl}	1.665^{mn}	1.295^{cd}
TRFK 430/12	3.920^{klm}	3.225^{i}	1.460^{fghij}	2.650^{e}	0.6400^{ghi}
TRFK 430/63	5.040^{ghi}	5.790^{bc}	1.695^{defg}	$2.275f^{fghi}$	0.5100^{hij}
TRFK 430/90	5.305^{fg}	3.215^{i}	1.870^{cdef}	2.185^{ghijk}	0.7250^{fgh}
TRFK 524/170	3.660^{m}	3.470^{hi}	1.600^{efghi}	2.380^{fg}	0.785^{fg}
TRFK 524/48	6.030^{bc}	5.715^{bc}	1.935^{cde}	2.295^{fgh}	0.4200^{ij}
BBK35	5.825^{cd}	6.075^{ab}	1.835^{cdef}	3.400^{d}	0.7050^{fgh}
EPKC12	4.235^{kl}	3.250^{i}	1.555^{efghij}	2.385^{fg}	0.7100^{fgh}
EPK D 99/10	4.305^{jk}	5.530^{cd}	1.475^{fghij}	1.992^{l}	0.9150^{ef}
EJULU-L	6.625^{a}	5.800^{bc}	2.145^{c}	4.775^{a}	1.310^{cd}
AHPS 15/10	5.855^{cd}	5.540^{cd}	1.635^{efghi}	2.380^{fg}	0.5800^{ghij}
EPK TN 14/3	3.755^{m}	3.560^{hi}	1.225^{ijkl}	1.915^{l}	0.3650^{j}
TRFK 6/8	6.420^{ab}	6.255^{a}	2.120^{cd}	4.415^{bc}	1.1495^{c}
TRFK 31/8	5.350^{efg}	5.710^{bc}	1.025^{kl}	1.865^{lm}	0.3600^{j}
TRFK 31/11	4.885^{hi}	4.005^{fg}	1.135^{jkl}	2.350^{fg}	0.4250^{ij}
TRFK 100/5	4.695^{ij}	5.405^{cd}	1.365^{ghijk}	2.025^{jkl}	0.4900^{hij}
TRFK 306/1	2.58^{o} LSD=0.4119 CV=4.18% Mean=4.771	1.490^{k} LSD=0.4119 CV=4.56% Mean=4.398	0.8450^{l} LSD=0.432 CV=12.41% Mean=1.682	0.975^{o} LSD=0.2437 CV=4.61% Mean=2.601	0.4750^{hij} LSD=0.2605 CV=14.20% Mean=0.899

Means within a column followed by the same letter are not significantly different at $p \leq 0.05$ according to DMRT.

White tea which is predominantly manufactured from the young apical hairy bud only, showed high levels of EGCG and ECG that are present in higher amounts in fresh young leaves. This result corroborates the findings of Saijo *et al.,* [28] who determined the chemical constituents of young tea leaves and the change occurring during leaf development. The decrease in the gallic acid esters of catechin such as EGCG and ECG during leaf development means that there is a slow biosynthesis of gallic acid moiety in each catechin gallate compared with dry matter production. Since catechin biosynthesis is slower than dry matter production from young leaves to the less young leaves, it is apparent that there is no weight increase in the less young and mature leaves and as a result catechin moves to other young leaves or are metabolized to other products. This accounts for the change in catechin levels in various leaf developmental stages and hence the levels of residual catechins in tea manufactured from different leaf ages as exemplified in the differences in catechin levels between white tea and the other types of teas in this study. Consequently, because of the different rates of growth among different cultivars, clones will accumulate varying amounts of catechins in their leaves [4]. Cultivar AHP S15/10, from which white tea is processed, is a fast growing clone compared to cultivar TRFK 6/8 from which green and black teas were produced [29]. The lower total polyphenol content in the white tea than in the green teas in our assay can be ascribed to the fast growth of cultivar AHP S15/10 compared to other cultivar such as TRFK 6/8.

Table 3: Individual catechin (%) levels of different black tea products analyzed

Individual Catechins					
Clone	**EGCG%**	**EGC%**	**EC%**	**ECG%**	**C%**
TRFK 301/4	0.2950^{j}	0.5050^{j}	0.4250^{bcdefg}	0.8900^{defgh}	0.0650^{d}
TRFK 301/5	0.6050^{ghij}	0.9450^{hij}	0.6050^{b}	0.9700^{defg}	0.3900^{cd}
TRFK 303/216	0.8600^{efgh}	0.6700^{ij}	0.2600^{fg}	0.6850^{fghij}	0.1650^{cd}
TRFK 303/231	0.8100^{efgh}	1.590^{efghi}	0.1750^{g}	0.9650^{defg}	0.2400^{cd}
TRFK 303/577	1.330^{ab}	4.065^{a}	0.5050^{bcdef}	1.095^{cde}	0.4150^{cd}
TRFK 303/745	0.9000^{defgh}	2.530^{cde}	0.2^{efgh}	0.4950^{ij}	0.1150^{d}
TRFK 337/138	1.010^{bcdef}	2.965^{bcd}	0.5550^{bcde}	1.020^{def}	0.3950^{cd}
TRFK 371/3	1.010^{bcdef}	1.260^{ghij}	0.3400^{cdefg}	1.100^{cde}	4.25^{efghi}
TRFK 430/3	0.9150^{cdefgh}	1.515^{fghi}	0.4200^{bcdefg}	0.8350^{efghi}	0.1350^{d}
TRFK 430/4	0.5550^{hij}	1.655^{efgh}	0.3200^{defg}	0.4250^{ij}	0.2500^{cd}
TRFK 430/12	0.8450^{efgh}	1.695^{efgh}	0.5800^{bcd}	0.6900^{fghij}	0.2200^{cd}
TRFK 430/63	0.7050^{fghi}	1.955^{efg}	0.4350^{bcdefg}	0.4350^{ij}	0.3250^{cd}
TRFK 430/90	1.295^{abcd}	3.805^{ab}	1.300^{a}	1.210^{bcd}	2.235^{a}
TRFK 524/170	0.3450^{ij}	1.150^{ghij}	0.5200^{bcdef}	0.9850^{defg}	0.7300^{bcd}
TRFK 524/48	0.7250^{fghi}	1.450^{fghij}	0.5100^{bcdef}	0.6900^{fghij}	0.8900^{abcd}
BBK35	0.5700^{hij}	1.975^{efg}	0.2950^{efg}	0.8750^{defg}	1.045^{abcd}
EPKC12	1.010^{bcdef}	2.055^{defg}	0.4850^{bcdef}	1.105^{cde}	5.215^{de}
EPK D 99/10	0.7950^{efgh}	1.130^{ghij}	0.4850^{bcdef}	0.6900^{fghij}	0.7450^{bcd}
EJULU-L	1.315^{ab}	3.190^{abc}	0.5900^{bc}	1.810^{a}	1.545^{abc}
AHPS 15/10	1.000^{bcdefg}	2.065^{defg}	0.2750^{fg}	0.5350^{hij}	1.415^{abcd}
EPK TN 14/3	0.7900^{efgh}	1.550^{fghi}	0.3200^{defg}	0.6300^{ghij}	0.9000^{abcd}
TRFK 6/8	1.315^{ab}	3.830^{ab}	0.6050^{b}	1.405^{bc}	1.900^{ab}
TRFK 31/8	1.485^{a}	4.755^{efg}	0.3400^{cdefg}	0.8250^{efghi}	1.450^{abcd}
TRFK 31/11	1.170^{abcde}	2.375^{cdef}	0.4700^{bcdef}	0.8750^{defgh}	1.255^{abcd}
TRFK 100/5	0.8150^{efgh}	1.730^{efgh}	0.4700^{bcdef}	0.6900^{fghij}	0.8900^{abcd}
TRFK 306/1	1.300^{abc} LSD=0.3962 CV=21.15% Mean=0.914	5.235^{de} LSD=0.9682 CV=24.02% Mean=1.956	0.4300^{bcdefg} LSD=0.2605 CV=27.29% Mean=0.461	1.505^{ab} LSD=0.3684 CV=19.71% Mean=0.901	1.185^{abcd} LSD=1.401 CV=15.59% Mean=0.795

Means within a column followed by the same letter are not significantly different at $p \leq 0.05$ according to DMRT.

3.1.4 Gallic acid

The results on gallic acid (3, 4, 5-trihydroxybenzoic acid) are presented in Table 4. The results show significant ($p \leq 0.05$) differences in gallic acid content between the black (aerated), green (non-aerated) and white (non-aerated) teas. This can be attributed to the fermentation reaction where a considerable quantity of EGC, EC, EGCG and ECG are oxidized to form theaflavins and their gallates and gallic acid is an important molecule in this reaction. Despite the formation of free gallic acid during fermentation through the process of degallation of EGCG, the enhanced utilization of gallic acid in the formation of TFs and TRs contributes to the decline of gallic acid though Muthumani and Kumar [30] in their study argued that the decline is not significant. The formation pathways of gallic acid have been shown to include the hydration of epigallocatechin gallate and degradation from the dimer of epigallocatechin gallate [31]. However, since these catechins are vital in black tea formation, the hydration that leads to the formation of gallic acid is therefore suppressed at the expense of theaflavins and thearubigin formation. The levels of gallic acid and individual catechins in the black tea have been shown to decrease with an increase in fermentation temperature and time for different clones [19, 20, 27].

Table 4: Gallic acid (GA) (%) levels of different tea products analyzed

Clone	Green Tea	Black Tea
TRFK 301/4	1.075^{a}	0.1350^{n}
TRFK 301/5	0.4500^{jkl}	0.1550^{mn}
TRFK 303/216	0.5300^{ghi}	0.2350^{jlk}
TRFK 303/231	0.5250^{ghi}	0.2900^{hij}
TRFK 303/577	0.5900^{efg}	0.3450^{fgh}
TRFK 303/745	0.4650^{ijkl}	0.3050^{ghi}
TRFK 337/138	0.6250^{def}	0.4250^{de}
TRFK 371/3	0.4850^{hijk}	0.2750^{ijk}
TRFK 430/3	0.4900^{hij}	0.2750^{ijk}
TRFK 430/4	0.4100^{l}	0.1700^{lmn}
TRFK 430/12	0.4500^{jkl}	0.1850^{lmn}
TRFK 430/63	0.8650^{b}	0.5350^{bc}
TRFK 430/90	0.4800^{hijk}	0.4600^{d}
TRFK 524/170	0.7600^{c}	0.4800^{cd}
TRFK 524/48	0.7750^{c}	0.3600^{efg}
BBK35	0.8000^{bc}	0.6050^{a}
EPKC12	0.6650^{d}	0.6250^{a}
EPK D 99/10	0.4250^{jkl}	0.1650^{mn}
EJULU-L	0.8200^{bc}	0.4750^{cd}
AHPS 15/10	0.6750^{d}	0.4600^{d}
EPK TN 14/3	0.5650^{fg}	0.210^{klm}
TRFK 6/8	0.7800^{c}	0.5950^{ab}
TRFK 31/8	0.5450^{gh}	0.3800^{ef}
TRFK 31/11	0.5700^{fg}	0.2800^{hijk}
TRFK 100/5	0.6400^{de}	0.3150^{fghi}
TRFK 306/1	0.4200^{kl} LSD=0.05613 CV=4.20% Mean=0.353	0.4200^{de} LSD=0.05613 CV=7.18% Mean=0.611

Means within a column followed by the same letter are not significantly different at p≤0.05 according to DMRT.

3.1.5 Total theaflavins and total thearubigens levels of black, green and white tea products

There was a significant difference in the total TFs and TRs levels for Kenyan teas. Black tea had the highest levels of total TFs and total TRs which ranged from 2.072% to 17.12%, respectively (Table 5). However, results from the present study clearly showed that TRs were present in green tea products and black and white (unaerated) tea products from the purple coloured leaf tea clones. Further observations revealed that in green and white teas, TRs were formed in the presence of low levels of TFs unlike in black tea where the TFs levels were slightly higher. Black tea therefore has high levels of TFs and TRs that are the main fermentation products as evident in table 5 below.

The variation in the polyphenolic composition of the different tea products resulted from the leaf maceration during manufacturing. The rolling and cutting of the tea shoots in non-orthodox manufacture causes a release of polyphenol oxidase which interacts with phenolic compounds, one simple catechin and one gallocatechin, to produce, theaflavins and thearubigins that possess a benzotropolone skeleton [32, 33]. Owuor and Obanda [34] investigated the use of green tea flavan-3-ols in predicting black tea quality potential and revealed that a correct balance of the trihydroxylated flavan-3-ols and dihydroxylated flavan-3-ols was necessary to ensure maximum formation of the theaflavins. The trihydroxylflavan-3-ols are oxidized faster during the fermentation phase of black tea processing explaining the high levels of EGCG and EGC in green tea and the subsequent reduction in black tea. Theaflavins are further oxidized to form thearubigins that are heterogeneous in nature and contribute significantly towards taste, color and body of tea [13, 19 & 35].

Results from the present study however clearly showed that TRs were present in green tea. Further observation revealed that in green tea, TRs were formed in the presence of low levels of TFs unlike in black tea where the levels were almost similar. This may suggest that theaflavins are not the only source of thearubigins. Wilson and Clifford [36] explained the factors affecting the formation and degradation of theaflavins and thearubigins in black tea and observed that maximum synthesis of theaflavins occurs when oxygen is in excess to support benzotropolone ring formation. However, under limiting oxygen concentration, polyphenol oxidase, which has a high affinity for the substrate, has a preferential demand for oxygen and theaflavins formation is suppressed at the expense of catechin quinone formation. This competition for oxygen is particularly noticeable during the early stages of fermentation when the concentration of the catechins is at its highest and enzyme turnover is unimpeded by substrate availability. This occurs during green tea manufacture since the enzyme is active before deactivation through steaming. For this reason, high enzyme activity in an already low oxygen concentration creates almost total anaerobiosis, which suppresses benzotropolone ring formation. Consequently as a result of this, thearubigens are formed, mainly from gallocatechins since the simple catechins are unable to react in benzotropolone ring formation. Moreover, it might be possible to minimize thearubigins formation by deactivating the enzyme immediately after plucking through a steaming procedure although this is hardly achievable during commercial tea processing. Further research is desirable to explain in details the existence of these thearubigins in green tea and the importance of steaming during tea processing [14, 19, 26, 37, 39, 40].

Table 5: Total theaflavins and total thearubigins (%) levels of black, green, purple and white tea products processed from different cultivars

Clone	Green Tea	Black Tea		
	TF%	TR%	TF%	TR%
TRFK 301/4	0.8500^{cde}	9.705^{def}	0.96^{kr}	13.11^{hij}
TRFK 301/5	0.6750^{defghij}	10.02^{cde}	1.555^{jklm}	15.55^{bcd}
TRFK 303/216	0.5950^{efghij}	9.775^{cdef}	1.275^{qr}	14.59^{cdef}
TRFK 303/231	0.6500^{efghij}	8.700^{hi}	1.665^{defg}	13.680^{hijkl}
TRFK 303/577	1.025^{bc}	11.48^{b}	1.525^{klmn}	15.16^{bcde}
TRFK 303/745	0.8000^{cdefgh}	9.835^{cdef}	1.655^{efgh}	13.41^{fghij}
TRFK 337/138	0.6800^{defghij}	8.880^{hi}	1.800^{b}	16.26^{ab}
TRFK 371/3	0.5750^{fghij}	12.15^{a}	1.685^{cdef}	15.54^{bcd}
TRFK 430/3	0.6550^{defghij}	6.935^{k}	1.280^{q}	14.12^{efgh}
TRFK 430/4	1.685^{a}	9.74^{def}	1.750^{bc}	16.02^{ab}
TRFK 430/12	0.8450^{cde}	8.865^{ij}	1.395^{p}	12.35^{j}
TRFK 430/63	0.5500^{ghij}	8.68^{hi}	1.475^{no}	12.74^{ij}
TRFK 430/90	0.6850^{defghij}	9.280^{fgh}	1.605^{ghij}	13.22^{ghij}
TRFK 524/170	0.4800^{ij}	9.800^{cdef}	1.21^{qr}	14.07^{efghi}
TRFK 524/48	0.5350^{hij}	10.14^{cde}	1.415^{op}	14.00^{efghi}
BBK35	0.6200^{efghij}	8.980^{ghi}	1.590^{hijk}	13.30^{fghij}
EPKC12	0.8100^{cdefg}	10.18^{cd}	1.725^{cd}	14.56^{cdefg}
EPK D 99/10	0.9200^{bcd}	12.00^{ab}	1.695^{cde}	17.12^{a}
EJULU-L	1.135^{b}	10.37^{c}	1.735^{bc}	16.03^{ab}
AHPS 15/10	0.7200^{defghi}	10.17^{cd}	1.505^{mn}	13.40^{fghij}
EPK TN 14/3	0.8250^{cdef}	10.15^{cd}	1.515^{lmn}	12.72^{ij}
TRFK 6/8	1.150^{b}	12.28^{a}	2.072^{a}	16.13^{ab}
TRFK 31/8	0.6750^{defghij}	9.600^{def}	1.470^{no}	14.28^{defgh}
TRFK 31/11	0.7350^{defghi}	9.535^{efg}	1.625^{fghi}	13.90^{efghi}
TRFK 100/5	0.4450^{j}	8.05^{j}	1.580^{ijkl}	13.86^{efghi}
TRFK 306/1	0.5350^{hij} LSD=0.2685 CV=3.43% Mean=0.764	8.865^{hi} LSD=0.6144 CV=3.06% Mean=9.767	1.240^{qr} LSD=0.0651 CV=2.25% Mean=1.538	15.68^{bc} LSD=1.372 CV=4.61% Mean=14.48

Means within a column followed by the same letter are not significantly different at p≤0.05 according to DMRT.

3.1.6 Theaflavin fractions

Theaflavins present in the assayed samples were fractionated and found to contain the following fractions; theaflavin-3-monogallate, theaflavin-3'-monogallate b, theaflavin-3, 3'-digallate and theaflavin-3, 3- digallate. These fractions were significantly different (p< 0.0001) in all tea cultivars. The fractions differ in structure and previous studies have elucidated them as shown in Figure 3.

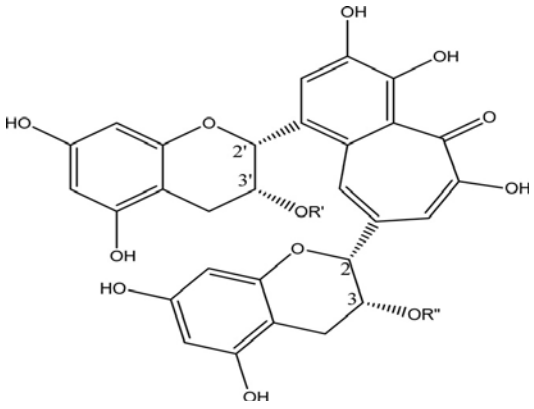


Fig 3: Molecular structure of theaflavins. theaflavin (TF): R'= R''= OH, theaflavin-3-gallate (TF-3-G): R'=H, R''= galloyl; theaflavin-3'-gallate (TF-3'-G): R' galloyl, R'' H; theaflavin-3, 3'-digallate (TF-dG): R'=R''=galloyl.

Green tea catechins are oxidized and dimerized during the manufacture of black tea to form orange red pigments namely theaflavins (TF), which is a mixture of theaflavins (TF1), theaflavin-3-gallate (TF2A), theaflavin-3'-gallate (TF2B) and theaflavin-3,3'-digallate (TF3). These molecules have recently aroused a lot of interest since they are thought to have stronger biological properties than free theaflavins due to the presence of gallic acid residues. In this study, the theaflavin fractions correlated significantly well with each other (Table 6) and this might explain why the activity of black teas observed could not be entirely attributed to the presence of theaflavins alone. The strong correlation of the digillate fractions is a clear indication that they synergize with other polyphenols to enhance the bioactivity. Obanda *et al.,* [20] observed that theaflavin gallate is the most astringent and has been estimated to be 6.4 times more astringent than simple theaflavin, and 2.88 times more astringent than either theaflavin-3-monogallate or theaflavin-3'-monogallate. The formation of a single theaflavin molecule requires a dihydroxy and a trihydroxy flavan-3-ol, as follows:- Epicatechin (EC) + Epigallocatechin (EGC) = simple theaflavin (TF); EC + Epigallocatechin gallate (EGCg) = Theaflavin-3-gallate (TF-3-g); Epicatechin gallate (ECG) + EGC = Theaflavin-3'-gallate (TF-3'- g);ECG + EGCg = Theaflavin-3, 3'-digallate (TF dg) [14]. Since theaflavin fractions are proving to be essential biologically active molecules, it is important to understand how the correct ratio

of dihydroxyl flavan-3-ols to trihydroxyl flavan-3-ols can be utilized to produce value added teas with enhanced quality and biological use.

Table 6: Correlation coefficient matrix analyses between various theaflavin fractions

TF3MG	TF3'MG	TF33DG	TF33'DG	
1.000	0.962***	0.873***	0.961***	TF3MG
	1.000	0.823**	0.908***	TF3'MG
		1.000	0.937***	TF33DG
			1.000	TF33'DG

** - Correlation significant at the p≤ 0.01 level
*** - Correlation significant at the p≤ 0.001 level

3.1.7 Antioxidant activity of green, black, purple and white tea products

The polyphenolic composition of tea and especially its catechins has aroused interest in their potential as radical scavenging compounds. Data on antioxidant capacity is presented in Table 7. Overall, green and white teas' had significantly (p< 0.05) higher antioxidant activity compared to black tea. However, some black teas from cultivars such as Ejulu-L, TRFK 6/8 and TRFK 306/1 had a higher antioxidant activity compared to some unaerated green teas.

Table 7: Percent antioxidant capacity (AA) of green, black and white tea products analyzed

Clone	Green Tea	Black Tea
	AA	AA
TRFK 301/4	89.80^{defgh}	87.55^{de}
TRFK 301/5	88.30^{h}	84.10^{f}
TRFK 303/216	90.55^{cdefgh}	88.90^{cd}
TRFK 303/231	90.75^{cdefgh}	89.15^{bc}
TRFK 303/577	90.85^{cdefg}	89.65^{abc}
TRFK 303/745	90.55^{cdefgh}	88.75^{cd}
TRFK 337/138	91.25^{cdef}	90.00^{abc}
TRFK 371/3	91.35^{cdef}	88.85^{cd}
TRFK 430/3	91.70^{bcde}	88.45^{cd}
TRFK 430/4	89.10^{fgh}	88.70^{cd}
TRFK 430/12	92.05^{abcde}	88.80^{cd}
TRFK 430/63	88.65^{gh}	86.00^{e}
TRFK 430/90	89.60^{efgh}	89.10^{bcd}
TRFK 524/170	91.90^{abcde}	89.55^{abc}
TRFK 524/48	91.15^{cdefg}	89.60^{abc}
BBK35	91.70^{bcde}	89.15^{bc}
EPKC12	91.90^{abcde}	88.90^{cd}
EPK D 99/10	91.90^{abcde}	88.55^{cd}
EJULU-L	94.05^{ab}	91.10^{a}
AHPS 15/10	92.50^{abc}	89.80^{abc}
EPK TN 14/3	89.00^{fgh}	89.60^{abc}
TRFK 6/8	94.30^{a}	91.05^{a}
TRFK 31/8	92.20^{abcd}	88.90^{cd}
TRFK 31/11	92.45^{abc}	89.30^{bc}
TRFK 100/5	91.40^{cdef}	89.70^{abc}
TRFK 306/1	92.35^{abc} LSD=2.505 CV=1.33% Mean=91.21	90.65^{ab} LSD=1.871 CV=11.46% Mean=88.94

[+]Data has been arcsine transformed.
Means within a column followed by the same letter are not significantly different at p≤0.05 according to DMRT.

Table 8 presents data on the correlation between tea polyphenols contents and the antioxidant activity of different types of tea products. Total catechins significantly (p<0.001) correlated with antioxidant activity (r=0.909***). EGCG was identified as the most potent antioxidant (r=0.968***, p<0.001). EGC (r=0.659***), EC (r=0.780***), ECG (r=0.454*) and GA (r=0.530*) contents also showed significant influence on the antioxidant activity. Therefore, the antioxidant activity was higher in tea extracts containing high levels of EGCG, EGC, and ECG. These results are similar to those of Gramza *et al.,* [41] and Karori *et al.,* [5].

This antioxidative effect of polyphenols has been attributed to the phenolic hydroxyl groups in their structures that make them potent free radical scavengers [42]. The antioxidative properties of catechins are marked particularly by their ability to inhibit free radical generation, scavenging free radicals and chelate transition metal ions mainly, iron and copper [43]. Nakagawa and Yokozawa [44] showed in their study that green tea extracts significantly impaired nitrogen oxide production in a concentration dependent manner and showed a direct scavenging activity against super oxide anion. On the basis of these results, it appears that the most effective radical scavengers are catechins with a 3', 4' and 5'-trihydroxylated substitution pattern on the B ring and/or hydroxyl group at C-3 position of the catechin structure (Figure 3). This hydroxylation confers a higher degree of stability on the catechin phenoxyl radical by participating in electron delocalisation that is an important feature of the antiradical potential. A study using electron spin resonance showed that the presence of 3', 4', and 5'-trihydroxyl groups attached to the B-ring of the flavan skeleton enhanced the radical scavenging efficiency displayed by catechins, compared to those with 3', 4'-dihydroxyl groups. At the same time the insertion of a galloyl moiety into three positions of the C-ring exerted a synergistic impact on superoxide anion scavenging activity [43, 45]. This explains why radical scavenging is high in the gallocatechins namely EGCG and EGC that are potent antioxidants [42, 46, 47].

To support this observation that EGCG and ECG were potent antioxidants, the findings correlated well with a study conducted in the Republic of Korea that showed that EGC has the highest specific total oxy-radical scavenging capacity against peroxyl radicals, hydroxyl radicals and peroxynitrite, while ECG was the least effective among other catechins [48]. Raza and John [49] reported that tea catechins prevent molecular degradation in oxidative stress conditions by directly altering the subcellular ROS production, glutathione metabolism and cytochrome P_{450} 2E1 activity. These results are promising for the chemotherapeutic use of tea catechins in oxidative stress-related diseases.

Black teas analyzed in this study exhibited some antioxidant activity with a high DPPH radical scavenging activity though less than that of green, white and purple teas. During black tea manufacture, the gallocatechins are first to be oxidized and dimerised to TFs and TRs because of their high oxidation potential and high concentration in the leaves. These major phenolic compounds in black tea also contributed significantly to the radical scavenging activity namely TFs (r=0.803***, p<0.001), TRs (0.859***, p<0.001) and GA (r=0.530*, p<0.05). Interestingly, TFs, which are the major phenolic products in black tea, had a higher radical scavenging activity compared to some of its precursors ECG, EGC and EC (Table 8). This confirms that

conversion of catechins to TFs during black tea process did not affect the radical scavenging potency. These observations are consistent with those of Leung *et al.* [21]; Karori *et al.,* [5] and Wachira *et al.,* [12] who showed that black tea posses more or less the same antioxidant potency as catechins present in green tea. EGCG and EGC contribute significantly to the formation of TFs. These are B ring trihydroxylated catechins, which are oxidized at a much faster rate than the B ring dihydroxylated catechins including EC, ECG and +C due to their lower oxidation potential [24]. TFs formed from this reaction have hydroxyl groups (OH) considered necessary for free radical scavenging activity. These additional groups increase the total number of phenyl hydroxyl groups and make the gallate containing catechins and TFs more able to donate protons due to resonance delocalization thereby expressing the observed antioxidant activity of black tea. Similarly, gallic acid contributed significantly to the radical scavenging activity in black tea because it is a potent hydrogen donator to DPPH.

Table 8: Correlation coefficient matrix analyses between various tea chemical parameters

TP	TFs	TRs	EGC	EGCG	ECG	+C	GA	AA	TC	EC	
1.00	0.818***	0.663***	0.718***	0.803***	0.715**	0.520**	0.626**	0.895***	0.830***	0.681***	TP
	1.00	0.791***	0.732***	0.852***	0.632*	0.452*	0.584*	0.803***	0.808***	0.689***	TFs
		1.00	0.686**	0.843***	0.619**	0.393*	0.378*	0.859***	0.826***	0.733***	TRs
			1.00	0.847***	0.505**	0.291*	0.552**	0.659***	0.856***	0.688***	EGC
				1.00	0.719**	0.444***	0.656**	0.968***	0.956***	0.777***	EGCG
					1.00	0.744*	0.637***	0.454*	0.8300***	0.814***	ECG
						1.00	0.472	0.232*	0.608***	0.690***	+C
							1.00	0.530*	0.685**	0.628**	GA
								1.00	0.909***	0.780***	AA
									1.00	0.895***	TC
										1.00	EC

Additionally, the present study provided evidence of the contribution of TRs towards the antioxidant activity of black tea ($r=0.803^{***}$, $p<0.001$). The antioxidant activity of TRs can be explained by the presence of 3-OH groups, which are more or less esterified by gallic acid in the TRs structure. However, this is a highly speculative hypothesis since to date there is no definite data on TRs structures [21, 50].

Thearubigins are assumed to be formed by the tea plant as a defense mechanism [35]. Plants are thought to utilize the strategy of plant browning as a defense tool. Therefore, the action of a polyphenol oxidase enzyme on phenolic secondary metabolites to produce a brownish coloration is aimed at deterring pest organisms. The tea plant uses a similar process to oxidize flavan-3-ols to thearubigins using tea polyphenol oxidase to gain an evolutionary advantage by deterring pest organisms [51]. Thearubigins account for around 60% to70% of the dry weight of a typical black tea infusion, and any attempt to understand the numerous beneficial health effects of this beverage must take this class of compounds into consideration. However, only a few studies on the biological effects of thearubigins are available. The reason for this lack of knowledge is obvious because TRs have been mysterious for decades, with no clear structural picture and only vague and sometimes contradictory knowledge available. Therefore, there are no defined compounds from the TR fractions that can be used for biological testing, hence no standardized method for obtaining extracts for biological testing. Despite these limitations, TRs are useful molecules that need a detailed study to establish their role in disease prevention. An attempt so far made in this field has established that TRs extracts lowered the expression of superoxide dismutase, a free radical scavenger, in contrast to theaflavins, TR extracts were able to inhibit DNA synthesis in U-937 leukemia cell lines, giving a rationale for the anti-cancer activity of TRs [52]. Lin *et al*., [3] showed that TR extracts were able to block nitric oxide synthase in macrophages and therefore suppress the anti-inflammatory response and multiple stages of carcinogenesis. Data obtained from this study reveals that different tea cultivars have different polyphenolic composition which imparts on their unique biochemical qualities.

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