BIOCHEMICAL DIVERSITY OF TEA GERMPLASM AND POTENTIAL FOR PROCESSING OF DIVERSIFIED TEA PRODUCTS

MUTUKU AUGUSTINE MUTHIANI

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DECLARATION AND RECOMMENDATION

DECLARATION

This thesis is my original work and has not, wholly or in parts, been submitted for examination in any institution for the award of any degree.

Signature:_____ Date:_____

Augustine Muthiani

RECOMMENDATION

This thesis has been submitted with our approval as supervisors for examination according to Egerton University regulations.

Signature:_____ Date:_____

Dr. Stephen Karori Mbuthia
Department of Biochemistry and Molecular Biology,
Egerton University, P.O Box 536 - 20115, EGERTON.

Signature:_____ Date: _____

Dr. John Kanyiri Wanyoko
Tea Processing and Value Addition Programme,
Tea Research Institute (TRI), P.O Box 820-20200, KERICHO.

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DEDICATION

This work is dedicated to my loving parents Mr. and Mrs. Mutuku, my siblings and Purity Ngina, whose support, encouragement and prayers have seen me through my entire study period.

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ABSTRACT

Tea industry in Kenya forms the largest agribusiness contributing up to 4% of the country's gross domestic product and over 26% of total foreign exchange earnings. However, significant revenue is lost when tea is sold in undiversified form. Therefore, this study aimed at determining the biochemical diversity in Kenyan tea clones and their potential for diverse product development. Samples were obtained from 197 tea clones conserved in Kericho and Kangaita substation and assayed for total polyphenols, catechins, antioxidant activities, caffeine, chlorogenic acid, anthocyanins and theanine using Reverse Phase High Performance Liquid Chromatography (RP-HPLC) and spectrophotometric protocols. The influence of different growing regions, Kangaita and Timbilil, on the levels of catechins and caffeine was also done. The data was subjected to analysis of variance using GENSTAT-C statistical software and the Least Significant Difference (LSD) test used to separate the means. There was a significant clonal difference ($P \leq 0.05$) in total polyphenols (16.4%- 30.9%) and total catechins (9.46%-25.42%) among the 197 clones assayed. Sixteen (16) clones ready to be released for commercial cultivation, recorded a significant ($P \leq 0.05$) polyphenol content (mean value of 28.11%), higher than that of standard reference clone, TRFK 6/8 (27.4%), an indication of their suitability in the development of high quality black teas. Fifteen clones were suitable for the manufacture of theaflavin-3, 3'-digallate rich black tea based on their high levels of ECG and EGCG. Clones TRFK 301/5 and TRFK 301/4 had a high EGC/EC and low EGCG/ECG ratios and were suitable for manufacture of less astringent green teas, while clones TRFK 687/1 and 73/7 which had the least caffeine contents at 1.96% and 2.04%, respectively therefore could be utilized in manufacturing low-caffeine beverages. Among the purple colored clones, TRFK 306/1 had the highest anthocyanins content of 1319 mg/l though not significantly different making it suitable for manufacture of anthocyanin-rich beverages or extracts. Remarkable high values of antioxidant activity in 79 clones (90.97 %) compared to the reference clone TRFK 6/8 suggest that these clones can be exploited for their potential health benefits. Clones assayed for chlorogenic acid and theanine showed that clones AHP SC 31/37 and TRFK 6/8 had the highest contents of 0.131% and 1.7%, respectively, and are suitable for chlorogenic and theanine rich teas. On the regional comparison study, clones in Kangaita had significantly higher ($P \leq 0.05$) total and individual catechins with mean total catechins content of 18.7% compared to 16.2% in Timbilil. Clones in Timbilil however had significantly high caffeine contents (mean value 4.2%) compared to those in Kangaita (3.9%). The observed chemical differences based on clones and regions show that Kenyan teas have a remarkable diversity in biochemical attributes and thus suitable for development of diversified tea products with remarkable geographical indications.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANS	Anthocyanin synthase
BHA	Butylated hydroxyl anisole
BHT	Butylated hydroxytoluene
BT	Black tea
CHS	Chalcone synthase
CHI	Chalcone isomerase
C4H	Cynnamate-4-hydroxylase
CTC	Cut, tear and curl
4CL	4-coumarate: CoA ligase
DFR	Dihydroflavonol 4-reductase
DPPH	2, 2-diphenyl-1-picrylhydrazyl radical
EC	Epicatechin
ECG	Epicatechin gallate
EGC	Epigallocatechin
EGCG	Epigallocatechin gallate
F3H	Flavanone 3- hydroxylase
FLS	Flavone synthase
F3’H	Flavonoid-3’-hydroxylase
F3’5’H	Flavonoid 3’, 5’-hydroxylase
FLS	Flavonol synthase
GABA	Gamma-amino butyric acid
GDP	Gross Domestic Product

Gp 41	Glycoprotein 41
GSH	Glutathione
GT	Green tea
HEK 293 WT cells	Human Embryonic Kidney 293 wild type cells
LCR	Leucocyanidin 4-reductase
LDH	Lactate dehydrogenase
PAL	Phenylalanine ammonia lyase
PPO	Polyphenol oxidase enzyme
TFs	Theaflavins
TRs	Thearubigins
TRI	Tea Research Institute
RP C6	Reverse phase carbon-6
RP C18	Reverse phase carbon-18
HPLC	High Performance Liquid Chromatography

CHAPTER ONE
INTRODUCTION

1.1 Background Information

Tea processed from *Camellia sinensis* is the most common and widely consumed non-alcoholic beverage worldwide (Cabrera *et al*., 2003). The popularity of this beverage is attributed to its attractive aroma, refreshing taste and also its potential health benefits (Hodgson and Croft, 2010). In recent years, consumers of tea have become conscious of health benefits of tea and are demanding products from tea that are pharmacologically active (Nyirenda *et al*., 2006). Kenya is the third largest producer of tea (after China and India) supplying 22% of the world's black tea (ITC, 2015). A larger proportion (95%) of the Kenyan tea is exported with little or no product diversification to countries such as Pakistan, United Kingdom, Egypt, Afghanistan and Sudan and only 5% is consumed locally (ITC, 2015). From the trade, tea contributes enormously to Kenya's economy by being the largest agribusiness and the top foreign exchange earner. This has a significant effect in the economy since a contribution of over 26% of total foreign exchange earnings and over 4% of Gross Domestic Product is achieved (Anonymous, 2015) with the industry earning the country about 129 billion shillings (ITC, 2015). The tea industry is also a source of livelihood for 4 million people directly and indirectly along its value chain (Anonymous, 2012). Owing to the low level of mechanization in its field operations, the industry offers direct employment to millions in tea estates and factories as well as in its other subsidiary businesses.

The tea industry is one of the profitable agro-enterprises in Kenya and this has seen the industry expand greatly over the 80 years of its existence and the country is now the largest single exporter of tea (Wachira and Ronno, 2005; Owuor and Obanda, 2007; Anonymous, 2016). This increase in tea acreage coupled with the increased production per unit area due to improved crop husbandry, has created a problem of overproduction in a scenario where the conventional market outlets (including local market) are not expanding. The situation is made worse by the fact that teas from Kenya are processed almost entirely as black Cut, Tear and Curl (CTC) and is sold in bulk. This has caused a glut in the market with the price of tea remaining quite unstable, either stagnating or decreasing (Owuor and Obanda, 2007; Anonymous, 2016). For example; annual average unit prices for processed black tea at the Mombasa auction decreased from an average high of US$ 3.18 per Kg in 2012 to a low of 2.54 in 2013 and US$ 2.14 in 2014 (Wachira and Ronno, 2005; Anonymous, 2014). This is

despite the fact that production costs (inform of farm inputs and labor costs) of growing tea in Kenya had considerably increased by about 317% between 1992 and 2003. The tea farmers' net profit has consequently decreased from a high of about US$ 0.21 per kg in 1993/94 to the present US$ 0.03 per kg green leaf (Wachira and Ronno, 2005; Anonymous, 2014).

Owing to its history, the East African tea market chiefly sells its tea as a generic product that is blended with other teas of less quality grown elsewhere to produce some of the popular blends in the world (TBK, 2014). Kenyan tea is therefore rarely sold as a finished branded product and thus a very good tea is almost unknown to tea drinkers in the outside world (Anonymous, 2010). The generic black tea exported from Kenya is added value through blending and packaging, branding or even further processing before it is distributed to the consumers through retail and niche markets worldwide. Much of the value addition is carried out mainly in Europe thus denying the country the much needed jobs and extra economic returns (Agritrade, 2011). In Japan and China, where the predominant product is green tea, the market outlets have been greatly expanded by products diversification and value addition to include fast moving consumer goods and health products ("functional foods") thereby enhancing profitability and sustainability of their native tea enterprises (Anonymous, 2010). There is therefore need to change the way tea from Kenya is processed and marketed to ensure that tea farmers get a fair return from their farming enterprises. This will among others entail value addition and branding of raw tea and the development of diversified tea products such as ready to drink (RTD) teas, teas with high functional components for sale as health products and raw material for fast moving consumer and environmentally friendly home and industrial cleaning agents, deodorizers, antimicrobial agents among others.

To achieve the foregoing, it will be necessary to first screen the available Kenyan tea clones for the levels of biomolecules and to determine the clones suitable for the development of novel diversified tea products. In this study, determination of the levels and profiles of the bioactive molecules (catechins, total polyphenols, caffeine, theanine, chlorogenic acid, anthocyanins and antioxidant activities) in 197 tea clones was carried out to aid in selecting suitable clones with desired levels of the biomolecules to be used as raw materials in the manufacture of high value teas and diversified tea products. In addition, the influence of the two Tea Research Institute's growing regions (Timbilil and Kangaita) on the levels of catechins and caffeine was also observed so as to guide in the production of region-specific clones and thereby ensure that high levels of these compounds are realized during tea production. Selection of clones for commercial farming places a lot of emphasis on high-

yielding properties rather than quality. This study further aimed at characterizing biomolecules for development of products with specific consumer preferences such as low or high caffeine, high or low epigallocatechin gallate (EGCG) rich green tea, theflavin-3, 3'-digallate rich black tea among others. This is desirable in the industry since the resulting tea products will be marketed based on their intrinsic health-enhancing properties. It is envisaged that this shift will consequently increase product demand both in the international and local market thus translating to a highly sustainable tea industry as well as a healthy nation.

There is already an effort by the International Standards Organization (ISO) to introduce characterization of teas in the tea trade based on levels and profiles of polyphenols. Similarly, as consumers increasingly become aware of the functional health potential of tea, the beverage will be marketed more based on the level of bioactivity rather than yield. Technological advancements based on the use of biosensors have already been made to facilitate the rapid screening of tea samples for antioxidative capacity (Milardovic *et al*., 2006). It is anticipated that the identification and characterization of novel biomolecules with potential human health enhancing properties in diversified Kenyan teas will contribute to widening of the market for the Kenyan tea as well as to diversification of its applications. These will then translate to higher returns to sector stakeholders (Tsalwa and Theuri, 2016) and the Government of Kenya (GOK), improve the human health and also the livelihoods of tea farmers.

1.2 Statement of the Problem

Tea production in Kenya has expanded rapidly from the 18.1 thousand metric tons produced in 1963 to more than 400 thousand metric tons produced in 2014. This expansion has not been matched by expansion in the per capita as well as total consumption in the domestic and international tea markets. The per capita consumption of tea in Kenya has declined (from 0.8 kg/ year in the 1980s to the current 0.4 kg/ year) and therefore, over 95% of Kenyan tea is exported as a generic non branded product. Despite its relatively high quality, tea from Kenya is then added value by blending with other cheap and low quality teas grown elsewhere. This process is done mainly in Europe thus denying the country the much needed jobs and extra economic returns. The situation is made worse by the fact that Kenya produces only one line of product; black CTC tea and little product diversification has been done which has seen the demand and prices for our principal product decrease over the years. Because of the above factors and a glut in the black tea market, tea farmers are receiving less return from their tea enterprises. In the face of the increasing production costs,

this situation is not sustainable and there is need for appropriate interventions to determine tea clones suitable for the production of high value tea (with high levels of health-enhancing functional components) and diversified tea products. This will allow Kenyan teas to be marketed as a "functional food" which is expected to boost returns from our tea industry.

1.3 Objectives

1.3.1 General objective

To determine the biochemical diversity of tea germplasm and their potential for processing of diversified tea products.

1.3.2 Specific objectives

i. To profile 197 Kenyan tea clones for individual and total catechins, caffeine, total polyphenols, antioxidant activity and anthocyanins in the purple colored tea clones.
ii. To determine the levels of L-theanine and chlorogenic acid in the fresh leaves of 15 selected tea clones.
iii. To compare the influence of two growing regions (Kangaita and Timbilil) on the levels of catechins and caffeine in the fresh leaves of 60 tea clones.

1.4 Null hypotheses

1. There are no significant differences in the levels of total and individual catechins, caffeine, total polyphenols, antioxidant activities in the fresh leaves of 197 Kenyan tea clones and anthocyanins in the purple colored clones.

2. There are no significant differences in the levels of L-theanine and chlorogenic acid in the fresh leaves of 15 selected tea clones.

3. There are no significant differences in the levels of catechins and caffeine between Kangaita and Timbilil regions in the fresh leaves of 60 tea clones.

1.5 Justification

Emerging scientific data from pharmacological and physiological studies continue to show that tea has beneficial effects on human health. In particular, it has been shown to protect against cancer and cardiac diseases (Babu and Liu, 2008) as well as exhibit anti-viral (Khan and Mukhtar, 2007), anti-inflammatory (Karori *et al*., 2008) and anti-mutagenic properties (Butt and Sultan, 2009). Despite this retinue of potential positive effects on health,

the state of research on these aspects is limited particularly for Kenyan tea products. Much of the research activities on health aspects of tea have been carried out using green tea which is produced mainly in the Far East. Additionally, information on chlorogenic acid as a tea component is grossly lacking and therefore this study provides useful insight regarding it's availability in tea clones and its potential application as a tea quality marker. The Inter-Governmental Group on tea under the auspices of FAO's committee on Common Commodity has recognized that the beneficial health aspects of tea can be used to market the product. Research on this domain would help Kenya diversify her tea products and characterize her teas and therefore contribute to their branding and marketing. It is anticipated that this form of marketing would make the Kenya tea industry more sustainable and contribute to enhanced returns to tea producers and all stakeholders in the tea value chain including farmers, consumers, transporters, warehouse operators, investors and employees. A sustainable and highly profitable tea sector would contribute to Kenya's vision 2030. It is anticipated that the identification and characterization of novel biomolecules with potential human health enhancing properties in diversified Kenyan teas will contribute to widening of the market for the Kenyan tea as well as to diversification of its applications. These will then translate to higher returns to sector stakeholders and the Government of Kenya (GOK), improve the human health and also the livelihoods of tea farmers.

CHAPTER TWO
LITERATURE REVIEW

2.1 Tea

Tea is a perennial crop manufactured from the young tender leaves of the plant *Camellia sinensis* (Cabrera *et al*., 2003) which has the two varieties China tea (*Camellia sinensis* var. *sinensis*) and the Assam tea (*Camellia sinensis* var. *assamica*) (Banerjee, 1992). The China tea is characterized by small semi-erect leaves while the Assam tea has relatively larger and horizontally held leaves. The tea plants are propagated from seed and cutting and they require at least 127 cm (50 inches) of well distributed rainfall a year, an altitude range of 1500-2700m above sea levels and prefer acidic soils (Rolfe and Cave, 2003). Tea plants grow mainly in warm (tropical and subtropical) climates but can thrive at altitudes ranging from sea level to 2,100 m. The best high-quality teas, however, are produced by plants grown at higher altitudes where the leaves mature more slowly and yield a richer flavor (Pruess and Harney, 2006). Depending upon the altitude, a new tea plant may take about 4 to 12 years to bear seed and from two and a half to 5 years to be ready for commercial picking/harvesting, but once productive, it can provide tea leaves for close to a century (Duke, 1983). Tea plants produce abundant foliage but only the flush comprising of the young two leaves and apical bud at the top of each young shoot are picked (Guang, 2007). The growth of new shoots can occur every week at lower altitudes but at higher altitudes, they take several weeks resulting in the production of better-flavored teas. If left undisturbed e.g. in the wild, a tea plant will grow into a tree reaching a height of up to 16 m (52 ft) but cultivated plants i.e. on tea plantations (called gardens or estates), are constantly pruned to waist height of about 1 m to encourage new growth and for ease of plucking (Mondal, 2007).

Tea is reported to have originated in ancient China where it was used as a medicinal plant before it was discovered and became popular as a beverage during the Tang dynasty (618 to 906 A.D.) (Ahmad and Mukhtar, 1999). The plant was introduced in Kenya in 1903 by a white settler, G. W Caine, in the present day Limuru (Watts, 1999). Since then, tea cultivation has expanded tremendously in volume and in the tea growing regions mainly the East (Kiambu, Thika, Maragua, Muranga, Nyambene, Meru, Nyeri, Kerinyaga, Embu) and West (Kericho, Sotik, Kisii, Nyamira Bomet, Nandi) of the Rift valley. In the country, tea production is divided into the small scale sector (with average holdings ranging from less than one hectare to twenty hectares) under the Kenya Tea Development Agency (KTDA) which accounts for 60% of the total production and the large (multinational) sector

accounting for the remaining 40% (Anonymous, 2015). The large sector is organized under the Kenya Tea Growers Association (KTGA) and includes companies like Finlay Tea, Unilever Tea and Eastern Produce Limited. It's sold mainly through the Mombasa auction with the largest buyers being the United Kingdom, Pakistan and Egypt. The country stands out as a producer of high quality tea owing to the fact that the crop is grown free of agrochemicals with only fertilizer used for soil replenishing purposes. Furthermore, these regions fall along the equator and as such they enjoy 12 hours of sunlight throughout the year. This ensures consistency both in quality and quantity of the resulting tea products (Anonymous, 2015). In the past few years, cultivation of the crop has expanded to include even marginal areas initially considered unsuitable for the crop (Wachira *et al*., 2002). Therefore there exist small to large differences in climatic and environmental conditions between the different growing regions, which consequently affects the quality of the resulting tea products (Owuor *et al*., 2009; Owuor *et al*., 2010b; owuor *et al*., 2010a). It is therefore imperative that the level of influence by the different environmental factors on tea biochemicals be monitored for development of region-specific clones which will ensure realization of high levels of these biomolecules as the tea industry moves towards product diversification. This investigation is necessary as it will confirm or negate the long-held belief that clones selected for their superior quality attributes in one region maintain their quality status in other tea growing regions in Kenya.

2.2 Tea value addition and diversification

Value addition in tea refers to pre-production modifications done on tea with an aim of increasing the quality of the resulting tea products which in turn enhances the product's market value (TBK, 2014). Diversification on the other hand involves modifying production processes to include manufacture of different products in addition to the primary products with an aim of diversifying their applications. Though a commonly known phenomena, little efforts have been made towards diversification of our Kenyan tea products even in the face of decreasing returns to the tea industry (Anonymous, 2014). Owing to the significant contribution to Kenya's economy, the tea industry needs to consider diversification of her tea products so as to increase demand for the diversified tea products which will consequently increase returns to producers. Value addition will include production of teas rich in specific biochemical compounds such as EGCG-rich green teas, less astringent (EGCG-low) green teas, caffeine rich green and black teas, low caffeine green and black teas and black teas rich in specific theaflavin fractions such as theaflavin-3, 3'-digallate rich black tea. With the

increasing awareness of the health benefits associated with tea biochemicals by consumers (Nyirenda *et al*., 2006), these tea products will be marketed and sold based on their intrinsic quality value. Diversification of our tea products will include manufacture of green, white, purple, oolong and ready to drink teas in addition to the majorly produced black CTC tea. This venture will also include extraction of the major bioactive chemicals in tea such as caffeine, total polyphenols, catechins, EGCG and anthocyanins. These will be utilized as raw materials in the pharmaceutical (fortification of drugs and antimicrobial agents), cosmetic (deodorizers, health care products and lotions) and food industries (as natural preservatives and colorants) due to their potential health benefits such as anti-oxidant (Butt and Sultan, 2009), anti-inflammatory (Karori *et al*., 2008) and cardiovascular-promoting benefits (Stangl *et al*., 2007). The value addition process must be preceded by a full characterization of the Kenyan tea clones for their biochemicals to identify which clones are suitable for the value addition and diversification endeavor.

2.3 Types and processing of tea

Variations in environmental conditions such as altitude, climate and soils result in tea leaves with distinct chemical composition. The tea processing method, however, is more important in developing the individual characteristics of the predominant types of teas; green, black and oolong (Cabrera *et al*., 2006). Other types include the specialty white and the purple teas derived from specific tea cultivars (Zuo *et al*., 2002) (Appendix VII).

Green tea is popular in China and is made by briefly steaming the just harvested leaves, rendering them soft and pliable and preventing oxidation of the catechins by the Polyphenol Oxidase (PPO) enzyme (McKay and Blumberg, 2002). The steaming process essentially maintains the polyphenols in their monomeric forms such as the individual catechins monomers (Joubert *et al*., 2008). After steaming, the leaves are rolled, then spread out and "fired" (dried with hot air or pan-fried in a wok) until they are crisp. Green tea is the least processed, thus provides the most antioxidant polyphenols, notably a catechin called Epigallocatechin-3-gallate (EGCG) which is responsible for most of the health benefits linked to green tea (Khan and Mukhtar, 2007).

Black teas are processed from fresh tea leaves which are first withered to reduce their moisture content (to about 50%), soft the leaves to enable rolling and also help in the production of compounds responsible for tea flavor development. They are then subjected to crushing or rolling, disrupting the cellular compartment and bringing phenolic compounds into contact with polyphenol oxidases which initiates the aeration (oxidation) of the leaf

catechins after which they are fired (McKay and Blumberg, 2002). The aeration process (earlier known as fermentation) causes the catechins to polymerize and form larger and highly complex polyphenols: the theaflavins (TFs) and thearubigins (TRs) (Wang *et al*., 2000; Grove and Lambert, 2009), which give the black tea its quality characteristics i.e. (reddish brown color, brightness and taste) (Muthumani and Kumar, 2007). TFs are homogenous substances, which give a yellow red coloration in aerated black tea and contribute to the brightness of tea liquor. They act as oxidizing agents to produce the chemically heterogeneous substances called TRs that are responsible for the color, body and taste of tea.

Oolong tea is manufactured mainly in Taiwan and made by withering the leaves to a moisture content of about 60% then partially aerating the leaves (i.e. a shorter aeration period than black tea) before being fired (McKay and Blumberg, 2002). It falls midway between green and black teas in its quality characteristics with a greenish-brown color and richer flavor and aroma than that of green tea, but more delicate than that of black tea (Duthie and Crozier, 2003; Del Rio *et al*., 2004; Wang *et al*., 2008).

White tea is a rare specialty tea that gets its name from the silvery buds harvested from the plants to produce the tea, as well as a particular post-harvest processing that raises small silvery hairs on the dried buds (Santana-Rios *et al*., 2001). White tea leaves are picked and harvested before they fully open, usually done when the buds are still covered by fine white hair. It is a product of partial steaming and air-drying of the hairy tips and this unique processing preserves most of the catechins in white tea (Xu and Chen, 2002). White tea contains a higher proportion of the buds that are covered with fine "silvery" hairs that impart a light white colour to the product.

Purple tea is a new tea variety that is propagated by grafting and cutting as opposed to seedling. It has been under development for the last 25 years in Kenya and is more resistant to frost, disease, drought, and pests. It was primarily developed for tea health products and is rich in antioxidants and thus has higher medicinal properties than the green and black teas (Kamunya *et al*., 2009b). It is rich in anthocyanin (a flavonoid), which pigments the leaves a purplish color (Kerio *et al*., 2012). It is processed in a way similar to green tea. The differences between the various processes of tea manufacture result in differences in the polyphenol profile between the black, green, oolong and white tea as shown in Figure 1.

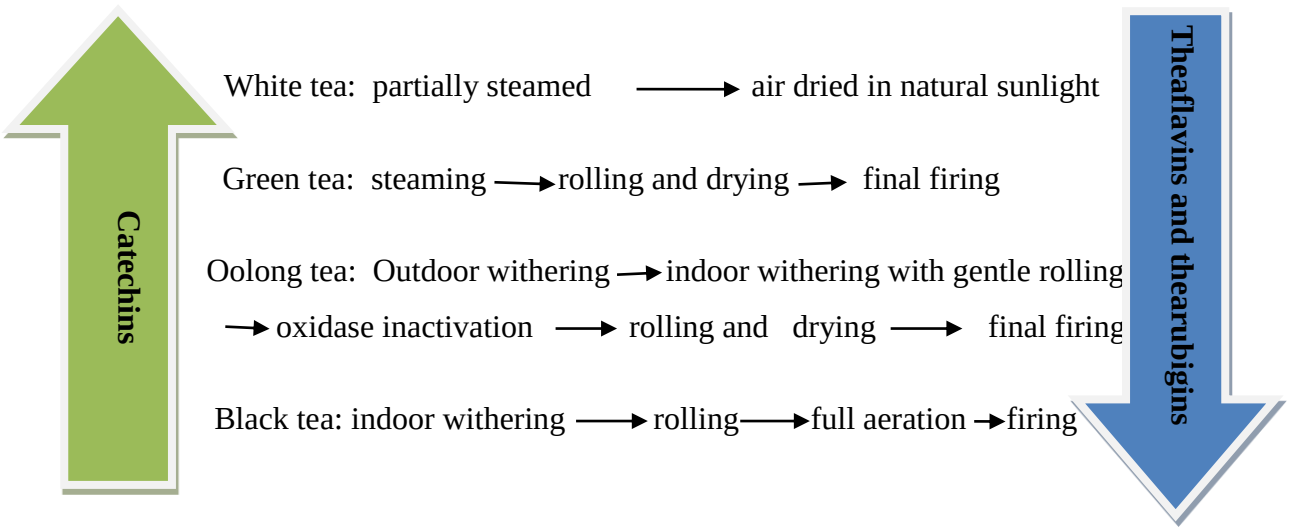


Figure 1: Effects of tea processing on Polyphenol content of the resulting tea types.

Tea processing in Kenya majorly focuses on the manufacture of black CTC teas with no product diversification at the processing level. This tea is therefore exported as a raw material to blend low quality teas from other countries at the expense of Kenyan tea brands (TBK, 2014). Despite the potential health benefits associated with green tea consumption, Kenya is yet to embrace the practice of diversifying its tea products. With the increasing consumer awareness of the health benefits associated with other tea types such as green and white teas, Kenya should open up a new line of processing high quality green and white teas which will be sold as health-enhancing beverages. This move is expected to increase the demand for our tea products which will in turn improve returns to producers leading to a more sustainable enterprise. Additionally, with the increasing global tea production, there is a lot of competition (Van der wall, 2008) for the available major tea markets whereby only producers of high quality teas and diverse tea products are able to get fair returns from tea exports. For example, Sri Lanka whose tea production is lower than Kenya attracts high demand for their tea product owing to their large scale value addition thereby fetching high tea prices (TBK, 2014). Kenya, grossly lacking in value addition and diversification of her tea products has not been spared from the declining demand as evidenced by the dwindling returns from the total sales of tea. Thus, for the Kenyan tea industry to revitalize the demand for its tea products, a lot of effort is necessary geared towards shifting the industry from the traditional marketing methods (based on high yields) to marketing based on the intrinsic health-promoting properties of the products. Although some research aspects on value addition are being addressed, none of the efforts as yet have considered identifying the potential clones in terms of their chemical composition. The value addition process must be

preceded by a full characterization of the Kenyan tea clones for their biochemicals to identify which clones are suitable for the value addition and diversification endeavor.

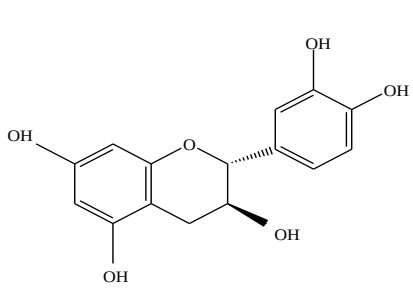
2.4 Chemical composition of tea

The chemical composition of tea is complex and includes mainly polyphenols, alkaloids (caffeine, theophylline and theobromine) (Graham, 1992), proanthocyanidins and amino acids (leucine, phenyloalamine, valine, threonine and L-theanine). Tea also contains carbohydrates, Vitamins (E and the B complex vitamins; riboflavin, biotin, niacin, pantothenate, inositol), chlorophyll, minerals, trace elements and other unidentified compounds (Wu and Wei, 2002). Polyphenols however constitute the most interesting group and are the main bioactive molecules in tea (Cabrera *et al*., 2003). Flavan-3-ols are the major class of polyphenolic compounds in tea with the catechins being the predominant form (Balentine *et al*., 1997). Theaflavins and Thearubigins are the other forms of flavan-3-ols and are the predominant compounds in black tea (Cabrera *et al*., 2003). The other classes of the flavonoids include the flavonols (quercetin, myricetin and kaempferol) and flavones (apigenin) (Scharbert *et al*, 2004). The levels of these tea components in green tea leaves particularly the catechins, theanine, caffeine, anthocyanins and chlorogenic acid determine the quality of the final product. The tea clones differ in their composition of these biologically important molecules, thus selection of the appropriate raw materials for the manufacturing process should be carefully done to ensure that the quality of the desired product is achieved. Additionally, there is a potential market for the extracted tea constituents which will be utilized in the pharmaceutical, food and cosmetic industries due to their health-promoting benefits. This study thus seeks to find the levels of these tea biochemicals with an aim of identifying clones suitable for the manufacture of diverse products apart from the black CTC tea, the major tea product in Kenya.

2.4.1 Catechins

Catechins are the major types of tea polyphenols found in higher concentrations in green and white teas (Peterson *et al*., 2005). They can comprise up to 30% of the dry weight in a freshly picked tea leaf (Lu *et al*., 2009) and are formed through intermediary glucose metabolism comprising the pentose pathway, the shikimate pathway and the flavanoid pathway (Appendix I) (Magoma *et al.*, 2003). Quinic acid and gallic acid moiety are formed through the shikimate pathway in which carbohydrates are presumed to be the precursor to shikimic acid. Several types of catechins such as epicatechin, epigallocatechin, epicatechin

gallate, epigallocatechin gallate (Figure 2) are present in teas in significant quantities (Balentine *et al*., 1997).



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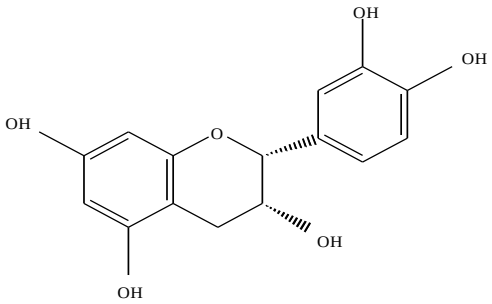

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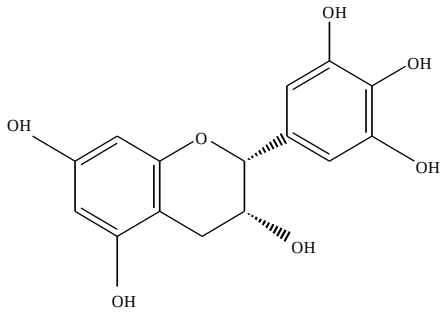

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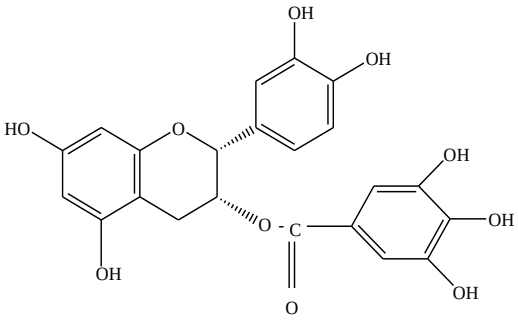

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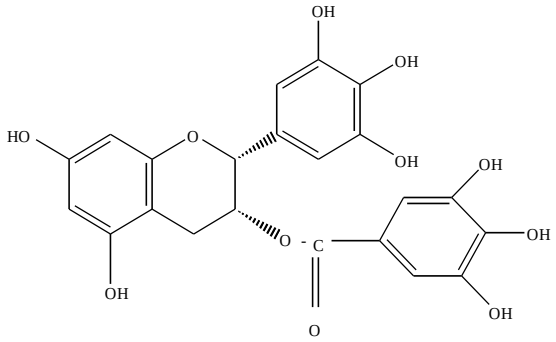

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Figure 2: Structures of the major individual catechins in tea; catechin (1), epicatechin (2), epigallocatechin (3), epicatechin gallate (4) and epigallocatechin gallate (5). (Robertson, 1992).

Catechins act as antioxidants *in vitro* by sequestering metal ions and by scavenging reactive oxygen and nitrogen species (Wiseman *et al*., 1997; Frei and Higdon, 2003). This ability to scavenge for free radicals is due to possession of a phenolic hydroxyl group attached to the flavan-3-ol structure. Although black tea as opposed to green tea has substantially lower levels of catechins owing to its oxidative preparation (Lee *et al*., 2002;

Hamza, 2009), they do exhibit significant antioxidant abilities due to possession of the oxidation products, theaflavins and thearubigins. A research by the U.S. Department of Agriculture suggested that the levels of antioxidants in green and black tea do not differ greatly, as green tea has an oxygen radical absorbance capacity (ORAC) of 1253 while black tea has an ORAC of 1128 (measured in μmol TE/100 g). The polyphenol's antioxidant capacity has been reported to be both chemoprotective and therapeutic (Rietveld and Wiseman, 2003; Karori *et al*., 2007). Their ability to inhibit the effects of various mutagens in cultured cell lines has also been observed (Cai *et al*., 2002). Tea polyphenols may also work indirectly as antioxidants through their effects on the activity of transcription factors and enzymes (Higdon and Frei, 2003). The antioxidant properties of green tea and its constituent catechins have been evaluated in a number of diseases associated with reactive oxygen species, such as cancer and cardiovascular and neurodegenerative diseases (Vanessa and Williamson, 2004). They have also been shown to possess beneficial physiological effects such as being hypocholesterolemiant (Yang and Koo, 2000), anti-atherosclerotic (Chyu *et al*., 2004), antidiabetic, antibacterial, anti-inflammatory, and anti-HIV activities (Muktar and Ahmad, 2000; Yang *et al*., 2002; Dona *et al*., 2003; Khan and Mukhtar, 2007). Despite the retinue of the health benefits associated with the catechins compounds, no efforts have been done to identify clones with appropriate levels of catechins for use in development of high value and diversified products in Kenya.

2.4.2 L-Theanine

L-theanine also known as γ-glutamylethylamide (Figure 3) is a non-protein amino acid and an analogue of glutamic acid (an excitatory neurotransmitter) found almost exclusively in tea leaves, but primarily in green tea (Nathan *et al.*, 2006). Tea is thus the major source of L-theanine in the diet.

Theanine constitutes between 0.5 to 2% of the dry weight of tea, which results in around 25–60 mg per 200 ml serving of liquid tea (Nobre *et al*., 2008). It's the principal amino acid component constituting about 50% of the total free amino acids in tea (Ekborg-Ott *et al*., 1997; Juneja *et al*., 1999; Thippeswamy *et al*., 2006). The amount of theanine in tea is used as a quality indicator as it is responsible for the green teas' umami taste (Balentine *et al*., 1998). It readily crosses the blood-brain barrier and is thought to influence the Central Nervous System (CNS) through a variety of mechanisms, including effects on neurotransmitters.

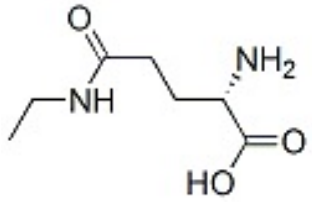

Figure 3: Structure of L-theanine

Animal studies have shown that it increases the release and concentration of the "feel good" neurotransmitter, dopamine which can lead to an increase in mood and concentration (Yokogoshi *et al*.,1998; Yamada *et al*.,2005), an inhibition of glutamate reuptake and blockade of glutamate receptors in the hippocampus; (Kakuda *et al.,* 2002) increases in GABA concentrations; decreases in norepinephrine levels, possibly as a result of increased GABA; (Kimura and Murata, 1980) and both increases in serotonin in the stratum, hippocampus, and hypothalamus and suppression of the generalized release of serotonin (Nathan *et al.,* 2006). It has an effect of increasing alpha brain waves associated with a relaxed yet alert state. In addition, L-theanine appears to antagonize the stimulatory effects of caffeine, which may contribute to its effects on lowering blood pressure (Eschenauer and Sweet, 2006). A study by Siamwala *et al*., (2011) established that L-theanine promotes nitric oxide (NO) production in endothelial cells leading to vasodilation in the arteries; results that are suggestive of L-theanine mediated vascular health benefits of tea. Research shows that theanine on its own is a useful sleep aid which may account for the relaxing, stress reducing quality of drinking tea despite the caffeine content of the leaf. This it does by modulating caffeine's psychoactive effects, such as insomnia, nervousness or headaches. A study done by Kimura *et al*., (2007) to examine the ability of L-theanine to reduce psychological and physiological stress responses showed that its intake resulted in a reduction in the heart rate and salivary immunoglobulin A responses to an acute stress task. These results were suggestive that its intake (orally) could cause anti-stress effects via the inhibition of cortical neuron excitation.

These effects on the CNS would predict that L-theanine might have a role in the regulation of anxiety due to effects on serotonin and GABA; the enhancement of cognitive performance due to increases in monoamines, and the possibility of neuroprotective effects due to antagonistic effects on glutamate. In the tea market, green teas with high theanine content fetch significantly high prices due to its contribution to taste and the potential health benefits. Identification of theanine-rich clones for the manufacture of high quality green tea is therefore an important part of value addition which this study sought to accomplish.

2.4.3 Caffeine

Caffeine (1, 3, 7 – trimethyl xanthine) is a naturally occurring and somewhat bitter like plant alkaloid belonging to a group of purine-based compounds collectively referred to as methylxanthines (Wolfrom and Welsch, 1990) shown in Figure 4. It is found mainly in coffee, tea and cocoa and possesses central nervous system stimulant properties. It constitutes about 3% of tea's dry weight, translating to between 30 mg and 90 mg per 250 ml cup depending on type, brand and brewing method (Zhang *et al*., 2002). The level of oxidation of the tea leaves does not however impact the caffeine level. It easily crosses the blood-brain barrier and its effects on the brain include a general increase in neurotransmitter activity by blocking the inhibitory action of adenosine (a neuromodulator which causes a calming effect) (Davis *et al*., 2003. It is usually associated with improved performance of tasks that require sustained effort and attention (Bonnet and Arand, 1994; Lorist *et al.*, 1994; Nawrot *et al*., 2003; Heckman, 2010). Other studies have shown some specific effects of caffeine on speed of response (Ruijter *et al.*, 1999; Ruijter *et al.*, 2000) and on feelings of well-being and improved moods (by increasing dopamine levels in the blood), bronchial dilation (Doherty and Smith, 2005), pain relief, energy, motivation for work, self-confidence, alertness, concentration (Mumford *et al.*, 1994) and also diuretic activity (Maughan and Griffin, 2003; Grandhi *et al*., 2007).

Tea may also contain trace amounts of the alkaloids; theophylline and theobromine (with weaker stimulating effect than coffee). Theophylline has only two methyl groups but with profound effects on the heart-rate and breathing than caffeine. Theobromine on the other hand possess stimulatory effects but weaker than caffeine (Yang and Landau, 2002).

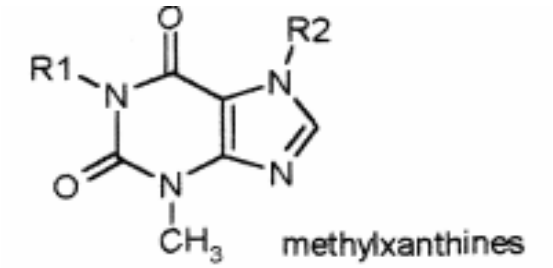

Figure 4: Structure of methylxanthines (Caffeine: R1=R2=CH3, Theobromine: R1=H, R2= CH3, Theophylline: R1=CH3, R2=H)

Some consumers are sensitive to caffeine due to the side effects associated with caffeine intake. Decaffeination process offered a better alternative for such consumers but due to its associated loss of attributes such as aroma, the tea industry seeks a more suitable approach. In line with this challenge, this study purposed to utilize a quality-friendly

approach of identifying those clones with low caffeine levels to be used in the manufacture of caffeine low beverages. On the other hand, some consumers highly regard tea for its psychoactive effects (Yang *et al*., 2007b) brought about by caffeine. Therefore, this study also purposed to identify the caffeine rich clones suitable for the manufacture of high caffeine teas or extracts for use in the pharmaceutical industries (Kerrigan and Lindsey, 2005).

2.4.4 Anthocyanins

Anthocyanins are plant pigments found in many plant species such as berries, grapes and are members of the flavonoid group of phytochemicals ingested in food as components of complex mixtures of flavonoid components. They are glycoside moieties of anthocyanidins derived from the flavylium (2-phenylbenzopyrilium) cation (Figure 5). There are several anthocyanidins described in nature but the commonly found in fruits and vegetables include; cyanidin, pelargonidin, petunidin, delphinidin, peonidin, malvidin, cyanidin-3-*O*-galactoside and cyanidin-3-*O*-glucoside (Table 1). They have been found to be the largest and most important group of water soluble pigments found in nature and they contribute to the attractive colours of fruits, vegetables and flowers imparting red, orange, purple, violet and blue colours (Feild *et al*., 2001).

Table 1: The five most common anthocyanins in nature based on their substitution patterns on the R_1, R_2 and R_3 groups.

Anthocyanins	**R_1**	**R_2**	**R_3**
Cyanidin	OH	OH	H
Delphinidin	OH	OH	OH
Malvidin	OCH_3	OH	OCH_3
Pelargonidin	H	OH	H
Peonidin	OCH_3	OH	H

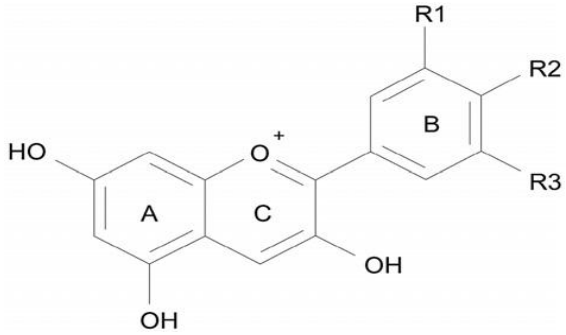

Figure 5: The basic structure of the anthocyanin pigment (the flavylium cation)

The different anthocyanins found in nature differ in their substitution patterns in the B-ring (hydroxylation and methylation), which also affects their biological activities. They are important due to their free radical scavenging and antioxidant capacities. A study by Khalid *et al*., (2013) showed that the anthocyanins significantly raised brain glutathione levels, implying boost in brain antioxidant capacity. Like tea catechins, they exhibit a wide range of biological effects such as antioxidant (Bae and Suh, 2007; Choi *et al*., 2007; Orak, 2007), anti-inflammatory (Arli and Cau, 2007; Dai *et al*., 2007), antimicrobial (Heinonen *et al*., 2007; Viskelis *et al*., 2009), antiartherosclerotic (Mazza, 2007), anticarcinogenic (Wang and Stoner, 2008) activities and induction of apoptosis in cancer cells (Hafeez *et al*., 2008) as well as with the chemoprotection of cells against oxidative stress-induced apoptosis and neuroprotective effects. A recent study carried out on anthocyanin rich tea germplasm revealed that malvidin was the most predominant in Kenyan processed black and green tea (Kerio *et al*., 2012). An *in vitro* study conducted to assay for the attenuation of *t*-butylhydroperoxide induced oxidative stress in HEK 293 WT cells by tea catechins and anthocyanins showed that LDH leakage (an indication of cell cytotoxicity) was lowered in a dose dependent manner demonstrating that tea anthocyanins were effective cytoprotectants against oxidative stress and that anthocyanins increased the GSH concentration in the *t*-BHP treated cells (Kerio *et al*., 2011).

2.4.5 Chlorogenic acid

Chlorogenic acid (figure 6) is a naturally occurring chemical compound (an ester of caffeic and quinic acid) found mainly in sunflower seeds, green coffee beans, potatoes, fruits and wheat. It has been shown to exhibit strong antioxidant activities and thus able to protect against cancer, cardiovascular and neurodegenerative diseases (Cho *et al*., 2009). It also possesses antibacterial, antifungal, antiviral, anti-hypertensive (Watanabe *et al*., 2006), anti-obesity (by slowing fat absorption and activating metabolism of extra fat) and anti-diabetic

(by inhibiting the enzyme glucose-6-phospatase hence reducing blood sugar level) activities (Van Dijk *et al*., 2009). Despite the health benefits associated with chlorogenic acid, information on the levels of chlorogenic acid found in Kenyan tea is grossly lacking. This biochemically important molecule thus provides a potential avenue for the value addition and diversification process expected to increase demands for our tea products. Therefore, this study sought to find the levels of chlorogenic acid contained in Kenyan tea clones to identify clones suitable for manufacture of high chlorogenic acid teas and extracts.

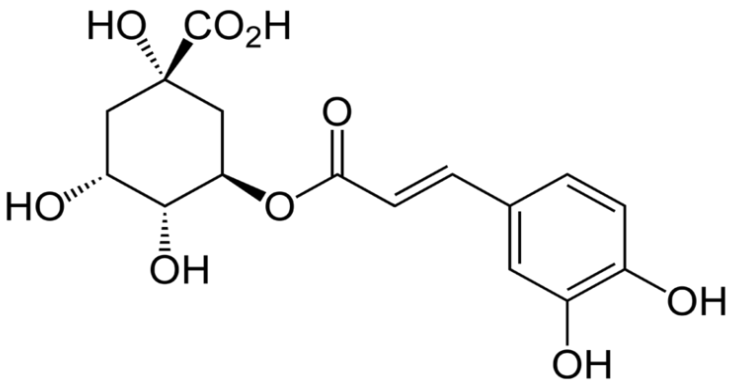

Figure 6: Structure of chlorogenic acid

2.5 Health benefits of tea

Consumption of tea has been attributed to various health benefits that may be related to the high content of bioactive molecules (such as polyphenols), which have been reported to possess antioxidant, anti-inflammatory (Karori *et al*., 2008) and antiviral activities (Khan and Mukhtar, 2007). They have also been shown to have the ability to modulate activity of detoxification enzymes such as glutathione peroxidase and glutathione reductase (Mandel *et al*., 2006), stimulate immune function and decrease platelet aggregation (Lampe, 2003; Frankel and Finley, 2008). In particular, consumption of Green Tea (GT) has been associated with low incidence of chronic pathologies in which oxidative stress is involved, such as cancer (Chung *et al*., 2003; Butt and Sultan, 2009) and cardiovascular diseases (CVDs); (Stangl *et al*., 2007; Babu and Liu, 2008). The tea polyphenols act synergistically with ascorbic acid to strengthen the capillary blood vessels and also exhibit an anti-atherosclerosis action. Tea polyphenols particularly with the galloyl moiety have been demonstrated to inhibit the entry of the HIV virus by binding to the gp41, which is essential for the HIV-1 attachment to the target cells (Liu *et al*., 2005). An in vitro study by Chandra *et al*., (2007) to evaluate the anticancer effect of green and black tea polyphenols alone and in combination

with bovine milk lactoferrin (bLF) on oral carcinoma cells (human tongue squamous carcinoma (CAL-27) and normal human gingival fibroblast (HGF) cells) was carried out. It was observed that both the green and black tea polyphenols preferentially inhibited the growth of CAL-27 cells in a dose-dependent manner and that the green tea polyphenols were found to be more effective than the black tea polyphenols. Also, the combination of the green tea polyphenols and bLF (1:2 ratios) exhibited synergistic inhibition of CAL-27 cells.

Among all tea polyphenols, epigallocatechin gallate (EGCG) is responsible for much of the health-promoting ability of GT (Khan *et al*., 2006). In general, GT has been found to be superior to black tea (BT) in terms of health effects, owing to the higher content of EGCG (Cabrera *et al.*, 2006), although the role of thearubigins and theaflavins contained in BT have not been properly investigated. In vitro and animal studies provide strong evidence that polyphenols derived from tea possess bioactivity to delay the onset of risk factors associated with disease development (Cabrera *et al*., 2006; Yang *et al*., 2007a). Its regular consumption has also been linked with protection against harmful UV radiation, maintenance of skin structure and functions (Heinrich *et al*., 2011) and also reduced risks of cognitive impairment where it has been shown to delay or prevent the onset of dementia (Ng *et al*., 2008; Chen *et al*., 2010).

The ability of tea polyphenols to scavenge for free radicals is due to possession of phenolic hydroxyl groups attached to the flavan-3-ol structure and has been associated with teas' therapeutic action against free radical- mediated diseases thereby attracting tremendous research interest (Amie *et al*., 2003). Free radicals are constantly generated due to environmental pollutants, radiation, chemicals, toxins, physical stress and the oxidation process of drugs and food. They are associated with the etiology of chronic and degenerative diseases disorders in humans including cancer, artheroscerolosis, arthritis, ischemia, Central Nervous System (CNS) injury, Alzheimer's, Parkinson's disease (Mandel and Youdim, 2004), diabetes, gastritis, dementia, renal disorders and Acquired Immune Deficiency Syndrome (AIDS) (Pourmorad *et al*., 2006). Many plant phenolics have been reported to have antioxidant properties that are even much stronger than vitamins E and C (Rice-Evans, 1999) and other synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxytoluene (BHT) (Chen and Wan, 1994). In addition, currently available synthetic antioxidant like butylated hydroxyl anisole (BHA), butylated hydroxytoluene (BHT) and gallic acid esters have been suspected to cause negative health effects and hence the increased quest to obtain natural antioxidants with broad-spectrum action. Tea has also been shown to promote oral health by preventing the demineralization of the enamel

(protective coat) thus preventing dental decay by both pathogenic microorganisms and commensal organisms. This has been attributed mainly to the mineral composition of tea and especially fluoride (Hamilton-Miller, 2001).

However, despite the remarkable benefits associated with tea, Kenya still sells her tea in bulk (95%) with little or no diversification and value addition. This has led to a decrease in demand for our non-branded bulk tea. Consequently the tea prices have either stagnated or in some cases declined (Anonymous, 2008; Anonymous, 2016) despite the increase in cost of production leading to decreased returns to the producers. This problem can be mitigated by screening the available Kenyan tea clones for their suitability in production of high value and diversified tea products such as teas rich in the specific bioactive components to be sold as health-enhancing products. Given the myriad of tea's potential health benefits as shown by previous research works (Cabrera *et al*., 2006; Babu and Liu, 2008; Karori *et al*., 2008; Butt and Sultan, 2009;), the resulting tea products can thus be marketed more based on their health-enhancing properties. The tea for export which is usually of high quality is used to blend other low quality teas grown elsewhere and as such, a high quality tea beverage is almost unknown to tea consumers. Further, this blending is done outside the country therefore denying Kenyans the much needed jobs (Agritrade, 2011). This lack of diversification in our tea products has led to a decline in demand and consequently a decrease in returns to producers which calls for urgent interventions to make the industry a successful venture. Traditional marketing of tea has put emphasis more on yield than quality whereby only the high yielding clones which are of average quality based mainly on polyphenolic composition are released for commercial farming. In this study, tea cultivars from the East and west of rift were analysed to determine suitable tea clones with high levels of the bioactive molecules and select high value teas with high functional components and diversified tea products for enhanced product development. It is envisaged that results from this work will enable Kenyan teas to be marketed as functional foods to widen the shrinking market and increase demand, both locally and internationally. In other countries where this model of marketing tea as a health product has been embraced, such as in Japan, tea has been able to outcompete trendy soft drinks and offer handsome return to the producers. Similarly in those countries, tea is being used in the manufacture of numerous fast moving consumer goods such as health care products like soaps, shampoos, domestic and industrial cleaning agents, confectionaries, liquors and wines, oral health care products, drug supplements and high value extracts for industrial use such as dyes, stabilizers among others. Research on health potential of Kenyan black tea can contribute to an increase of market for Kenyan tea.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sample collection

About 500 g of two leaves and a bud were obtained from each of the 197 tea cultivars (see appendix II) conserved at the Timbilil station (0°26´S, 37°15´ E) and Kangaita experimental centre (0°30´S, 37°16´E) of the Tea Research Institute (TRI). The cultivars used in this study comprised of those released for commercial cultivation based on their superior agronomic characteristics (93 clones) and also the test cultivars under TRI breeding programme (104 clones). The rationale was that most of the unreleased clones could be of high quality despite their inferior agronomic characteristics and thus screening of all the clones would give a better profile of their biochemical (quality) diversity necessary for the diverse product development endeavor. Out of the 197 clones sampled, 60 clones grown in both the Kangaita and Timbilil regions (appearing in both regions) were used for the regional (geographical) comparison study. Fifteen clones selected from the initial 197 clones based on the optimal EGCG and ECG levels suitable for manufacture of theaflavin-3, 3- digallate rich black tea were also assayed for their levels of chlorogenic acid and theanine.

3.2 Sample preparation

The samples were first steamed for one to two minutes immediately after plucking, dried in a micro-wave and pulverized with a grinder into a fine powder to be used in screening for the following biomolecules; total polyphenols, catechins, caffeine, anthocyanins, chlorogenic acid, theanine and antioxidant activities. All assays were carried out using established and published wet chemistry and High Performance Liquid Chromatography (HPLC) and spectrophotometric protocols at the TRI. Appropriate clones with high leaf concentrations of biochemicals were then selected for use as raw material in the processing of diversified tea products.

3.3 Screening of the tea samples for levels of the bioactive molecules.

3.3.1 Catechins

Two grams of the sample was placed on a preweighed moisture dish and left for 16 h at 103ºC in the oven to dry for the determination of dry matter. Of these, 0.2 ± 0.001 g was weighed into an extraction tube. Five milliliter of 70% v/v methanol/water pre-warmed to 70

˚C was dispensed into the sample as an extraction mixture and vortexed. Heating of the extraction tube was continued in the water bath (Digital water bath LWB-122D, Korea) for 10 minutes with mixing in the vortex mixer (VM-1000, Taiwan) after every 5 minutes. The extraction tubes were then removed from the water bath and allowed to cool. The tubes were then placed in a centrifuge (HSCEN-197, MRC) at 3500 rpm for 10 minutes. The supernatant was decanted into a graduated tube and the extraction procedure repeated. The extracts were combined and made up to 10 ml with cold methanol/water mixture. One milliliter of the sample extract was transferred into a graduated tube and diluted to 5 ml with a stabilizing solution (10% v/v acetonitrile with 500 µg/ml EDTA and ascorbic acid). The solution was further filtered through a 0.45 µm nylon membrane filter. A 20 µl aliquot of this solution was injected into HPLC for analysis.

A high performance liquid chromatography method (Kilel *et al.*, 2013) was used to assay for the tea catechins. A Shimadzu LC 20 AT HPLC system fitted with a SIL 20A auto sampler and a SPD-20 UV visible detector with a class LC 10 chromatography workstation was used for analysis of the prepared samples. A Gemini 5 µM C6- Phenyl, 250mm x 4.6 mm (Phenomenex, Torrance, CA, USA) separation column with a Reodyne precolumn filter disk was used. The sample was degassed before injection into the HPLC system. A gradient elution was employed using the following solvent systems: Mobile phase A (acetonitrile/acetic acid/double distilled water- 9/2/89 v/v/v), mobile phase B (acetonitrile/acetic acid/double distilled water- 80/2/18 v/v/v). The mobile phase composition for a binary gradient condition was started at 100% solvent A for 10 minutes then over 15 minutes a linear gradient to 60% mobile phase A, 32% mobile phase B and held at this composition for 7 minutes. The condition was reset to 100% mobile phase A and then allowed to equilibrate for 10 minutes before the next injection. The flow rate of the mobile phase was 1 ml/minute and the temperature in the column was maintained at 35 ± 0.5ºC.

The identification of individual catechins was carried out by comparing the retention times and UV-absorbance of unknown peaks with peaks obtained from the mixed known catechin standards under the same conditions. The quantification of catechins was performed at 278nm and achieved using a caffeine standard with a calibration curve R^2=0.9984 in conjunction with the consensus individual catechin relative response factor (RRF) values with respect to caffeine calculated on a dry matter basis. Total catechin as percentage by mass on a sample dry matter basis was given by the summation of individual catechins.

% Total catechins = [%ECG + %EC + %EGCG + %EGC + %C] content

Since caffeine is used as a standard in the HPLC identification and quantification of catechins, caffeine levels were determined alongside the individual catechins by comparing the retention times of the samples to those of authentic standards.

3.3.2 Estimation of total Polyphenols

The Folin-Ciocalteu phenol reagent method was used to determine total polyphenols as described previously (Pourmorad *et al.*, 2006). The reagent was used because it contains phospho-tungistic acid as oxidants. One milliliter of the sample extract was transferred to a 100 ml volumetric flask, diluted to the mark with distilled water and mixed. One milliliter of the diluted sample extract was transferred in duplicate into separate tubes. Five (5 ml) of 10% (v/v) Folins-Ciocalteu (Sigma Aldrich) diluted with distilled water was pipetted into each tube and mixed. Within 3-8 minutes after addition of the Folins-Ciocalteu phenol reagent, 4ml of 7.5% w/v sodium carbonate solution was added to each tube, stoppered and mixed well. The mixture was allowed to stand at room temperature for 60 minutes before their absorbance was measured at 765nm using a CE 393 Cecil digital grating spectrophotometer. A calibration curve was obtained for gallic acid over a concentration range of 10µg/ml to 60µg/ml. The Optical Density readings of the test samples were referenced to the calibration curve to determine the total polyphenols content of the tea samples.

3.3.3 Chlorogenic acid

Five grams of each sample was weighed into a conical flask and 45ml of aqueous methanol (50:50 v/v) added and magnetically stirred for 40 minutes. The resulting mixture was filtered and the residue washed with 10ml of 50% methanol. The first filtrate and the washed residue were combined and condensed to approximately 25ml by a rotavapor at 50°C. After cooling, the liquid was transferred into a 50ml volumetric flask and diluted with 50% aqueous methanol to the volume. An aliquot of the diluted sample was filtered through a 0.45 µm Millipore filter and 10µl of the filtrates were assayed by HPLC.

The analysis was carried out on a chromatography system equipped with a high precision pump (Shimadzu model LC-10-ATVP) operating at 327nm, and a phoenix Luna C18 (250×4.6 mm i.d.; 5µm particle size; 100 Å pore size). A mixture of acetonitrile and 0.5% aqueous phosphoric acid (11.5:88.5 v/v) was used as the mobile phase with the flow rate at 1.0 ml/minute and injection volume of 20 µl. A stock solution was prepared by accurately weighing 25mg/ml of HPLC grade methanol. From the stock, five additional standards with concentrations of 0.0784, 0.05488, 0.0392, 0.02352, 0.00784 and 0.00392

mg/ml were prepared by appropriate dilution of the stock solution. The standard solutions were injected twice into the chromatograph to obtain the peak values. A calibration curve was drawn by plotting the peak areas against the concentration of the chlorogenic acid standard. The chlorogenic acid content of the test tea samples was determined by the corresponding calibration curve and expressed as milligram of chlorogenic acid per gram of the respective tea clone.

3.3.4 Theanine

One gram of a finely ground sample was weighed in a 200 ml beaker and 100 ml of boiling double distilled water added. The mixture was allowed to brew for 5 minutes on a magnetic stirrer after which it was filtered. The tea infusion was allowed to cool then made up to volume and filtered using a 0.45 µm membrane before HPLC analysis. A phenomenex column Aqua 250 x 4.6 mm internal diameter was used. A gradient elution was employed with a thermostatically controlled column compartment and an ultraviolet detector set at 210nm. An injection volume of 20 µl and a flow rate of 1 ml/ minute were used with the following solvent system; mobile phase A composed of double distilled water and mobile phase B composed of 100% acetonitrile. Analysis time was 10 minutes with 100% mobile phase A, and then the next 8 minutes was wash time with 20% mobile phase A and 80% mobile phase B. The next 20 minutes was conditioning with 100% mobile phase A before the next injection. To calculate theanine content, a theanine calibration graph was drawn, using concentration of theanine in the working solutions against theanine peak area of the sample.

3.3.5 Anthocyanins

Five grams of grounded tea samples was weighed into 250 ml conical flasks, covered with foil to prevent photo degradation and mixed with 50 ml Methanol/ formic acid at a ratio of 99:1 v/v. The sample was magnetically stirred for four hours at room temperature at a speed of 900rpm. The resultant solution was filtered and methanol and formic acid removed using a rotary evaporator (Bunchi rotavapour R-300, Switzerland) under reduced pressure at 35˚C. The residue was reconstituted to 10 ml with distilled water and passed through a 0.45µm membrane filter. The extracts were then passed through RP C_{18} Solid Phase Extraction (SUPELCO, SPE) cartridges previously activated with acidified methanol (10% HCL/MeOH). The anthocyanins were adsorbed into the column while sugars, acids and other water soluble compounds were washed out using 0.01% HCL in distilled water. The anthocyanins were then recovered using acidified methanol (10% formic acid/methanol v/v). The cartridges were washed with ethyl acetate (Fischer Scientific, UK) to remove phenolic

compounds other than anthocyanins. The purified extracts were stored at -10˚C until further analysis.

Qualitative and quantitative analyses of the tea extract and anthocyanin profiles of the tea extracts was carried out by high-performance liquid chromatography (Kerio *et al*., 2013). Briefly, 1 ml of the anthocyanin sample was pipetted into separate tubes and diluted to 2 ml with mobile phase A solution (87:3:10 water/acetonitrile/formic acid v/v/v), filtered and then loaded into 2 ml vials. A Shimadzu LC 20 AT HPLC fitted with a SIL 20A auto sampler and a SPD-20 UV–visible detector with a class LC10 chromatography workstation with UV detection at 520 nm was used for analysis of the prepared samples. A Luna TM 5 μM, C18, 25 cm × 4.6 mm internal diameter (Phenomenex, Torrance, CA, USA) column fitted with a Rheodyne precolumn filter of 7335 model was used. Mobile phase solutions were filtered through a 0.45 μm nitrocellulose filter on a membrane filter disk and degassed before injection into the HPLC system. Gradient elution was employed for analysis using the following solvent: the eluents were mobile phase A (water/acetonitrile/formic acid at a ratio of 87/3/10 v/v/v) and mobile phase B (100% HPLC grade acetonitrile). The flow rate of the mobile phase was set at 1 ml/minute, column temperature at 35±0.5°C, and injection volume at 20 μl. Chromatographic conditions were set as follows: 3% mobile phase B in mobile phase A at the time of injection, 25% mobile phase B in mobile phase A at 45 minutes, 30% mobile phase B in mobile phase A at 46 minutes, and 3% mobile phase B in mobile phase A at 47 minutes. The conditions were then reset to 3% mobile phase B for 10 minutes before the next injection to allow for equilibration.

Identification of individual anthocyanins was carried out by comparing the retention times from sample chromatographs and absorbances of unknown peaks with the peaks obtained from the individual and mixed standards under similar conditions. The standards used were cyanidin-3-O-galactoside, cyanidin-3-O-glucoside, cyanidin chloride, delphinidin chloride, petunidin chloride, pelargonidin chloride, and malvidin chloride purchased from Sigma Aldrich, (London, UK). Quantification of anthocyanins was performed at 520 nm using external anthocyanin standards each with its determined calibration curve. The identified individual anthocyanin content calculated on a dry matter basis was determined by the formula:

$$\frac{A_{sample} - A_{intercept} \times V \times d \times 100}{Slope_{anthocyanin} \times m \times DM}$$

Where,

A_{sample} is the peak of the individual anthocyanin in the test sample

$A_{intercept}$ is the peak area at the point the individual anthocyanin calibration line intercepts the y-axis

$Slope_{anthocyanin}$ is the individual anthocyanin calibration line slope

V is the sample extraction volume

d is the dilution factor

m is the mass, in grams of the sample test portion

DM is the dry matter content in percentage

3.3.6 Antioxidant activity

The DPPH radical scavenging activity as previously described (Karori *et al*., 2007) was used whereby 5g of tea was infused in 100 ml boiling distilled water and put on a magnetic stirrer for 10 minutes. The extract was filtered through a nylon mesh followed by filtration through filter paper (Whatman no. 54/0.45µm). Aliquots of the extracts were kept frozen at -18˚C. Ten milliliters of the infusion was then dried to constant weight in pre weighed moisture dishes at 103˚C for over 12 hrs (in duplicate). The weight of the soluble solids was taken and expressed as mg/ml. The soluble solids of the extracts were standardized to give stock solutions of 50mg/100ml. A methanolic solution 50µl of the antioxidant was then mixed with 2ml DPPH solution (6×10^{-5}M made with 80% methanol) in a cuvette and the absorbance read at 517nm after 15-20 minutes incubation. The percentage inhibition against DPPH was obtained as outlined in the formula below;

% inhibition = $\frac{A_B - A_A}{A_B} \times 100$ where A_B is the absorbance of the blank sample and A_A is the absorbance of tested sample after 15minutes.

3.4 Data analysis

All determinations (levels of total polyphenols, total and individual catechin fractions, caffeine, anthocyanins, antioxidant activity, chlorogenic acid and theanine) for the 197 clones were done in triplicates and the data subjected to one-way analysis of variance using GENSTAT statistical software packages. A Principal Component Analysis (PCA) plot was also adopted for the clonal variation study to generate a cluster of the clones based on quality.

The segregation criterion for the low, medium and high performing clones was based on the levels of the biochemicals in clone TRFK 6/8, used as the standard reference clone for black tea quality. In the regional comparison study, a two-way analysis of variance was adopted to determine if there were significant interactions between growing sites and clones and also significant differences in catechins and caffeine levels of the 60 clones grown in Kangaita and Timbilil sites. The least significant differences Test (LSD) was used to separate the means for all the determinations.

CHAPTER FOUR

RESULTS

4.1 Clonal variation

4.1.1 Total polyphenol

Results for total polyphenols in the 197 clones are presented in Figures 7, 8 and Appendix II**.** Clones screened for phytochemicals had significant variations (P≤0.05) in total polyphenol content. Clone BBK 152 recorded the highest polyphenol content of 30.9%, while clone TRFK 833/1 was the lowest performing with 16.4%. The mean polyphenolic content was 25.5% with variation being generally continuous, an indication of polygenic influence and presence of wide range of selection for cultivars with varying potential of making different products (Figure 7). The Principal Component Analysis (PCA) plot (Figure 7) and Appendix II show that most of the low polyphenolic content are largely of the Chinary type (sinensis), while the medium and high polyphenolic content are predominantly assamica with a few chinary and cambod types clustering in this group.

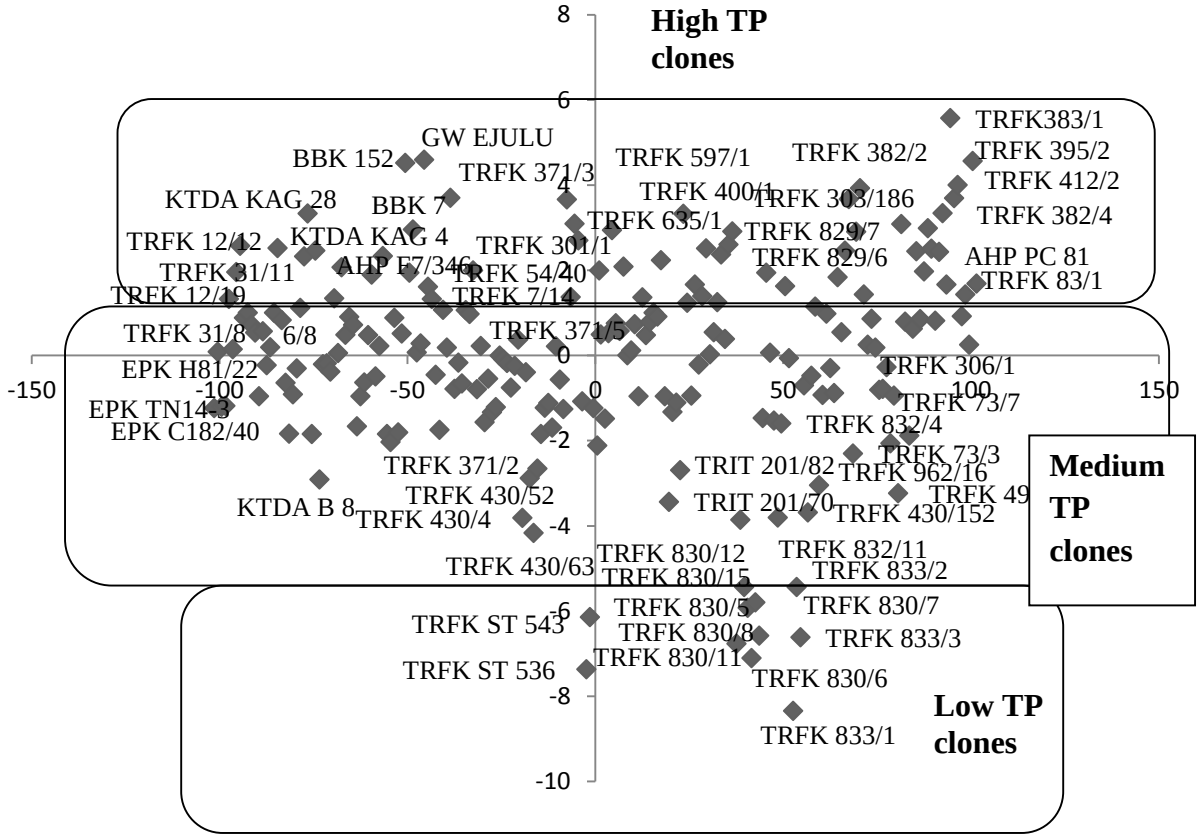

Figure 7: Principle component analysis plot of variation in Total polyphenols (TP) among the 197 tea clones.

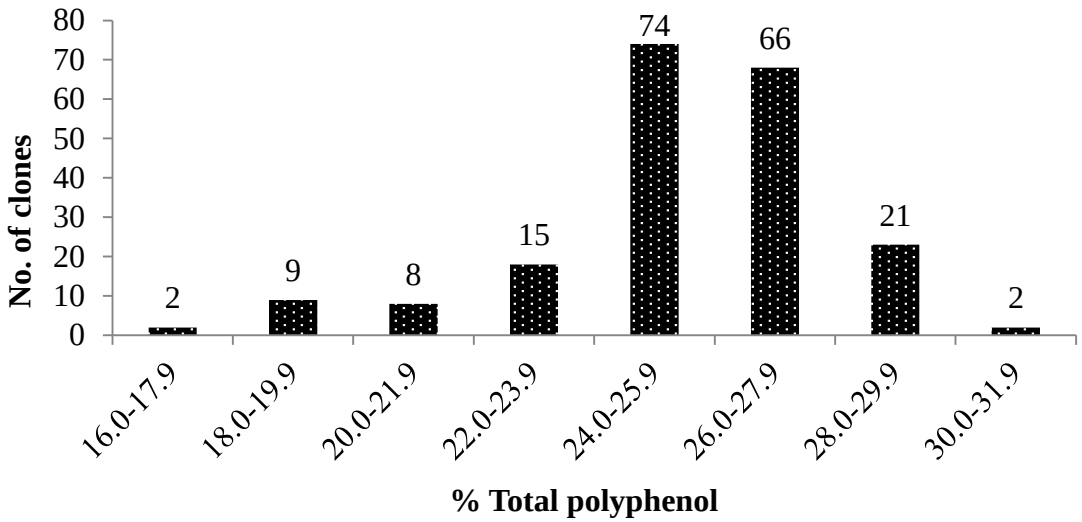

Figure 8: Histogram showing the levels (%) of total polyphenols contained in the sampled 197 clones.

4.1.2 Catechins

Results for catechins levels in the 197 clones are shown in Figures 9, 10 and Appendix II. The total catechin (TC) levels varied significantly ($P \leq 0.05$) among the clones with an observed mean value of 19.64%. Clone AHP F7/346 had the highest catechins content of 25.42% followed by clones TRFK 301/3 (24.82%), TRFK 11/52 (24.45%), TRFK 31/8 (24.38%), TRFK 381/5 (24.28%), TRFK 100/5 (23.96%), BBK 7 (23.80%) and TRFK 382/2 (23.79%) while clone TRFK 657/1 had the lowest with 9.46%. The principal component analysis (PCA) plot (Figure 9) and Appendix II show that most of the low catechin content are mainly of the Chinary type (sinensis), while the medium and high catechin content are predominantly assamica with a few chinary and cambod types clustering in this group.

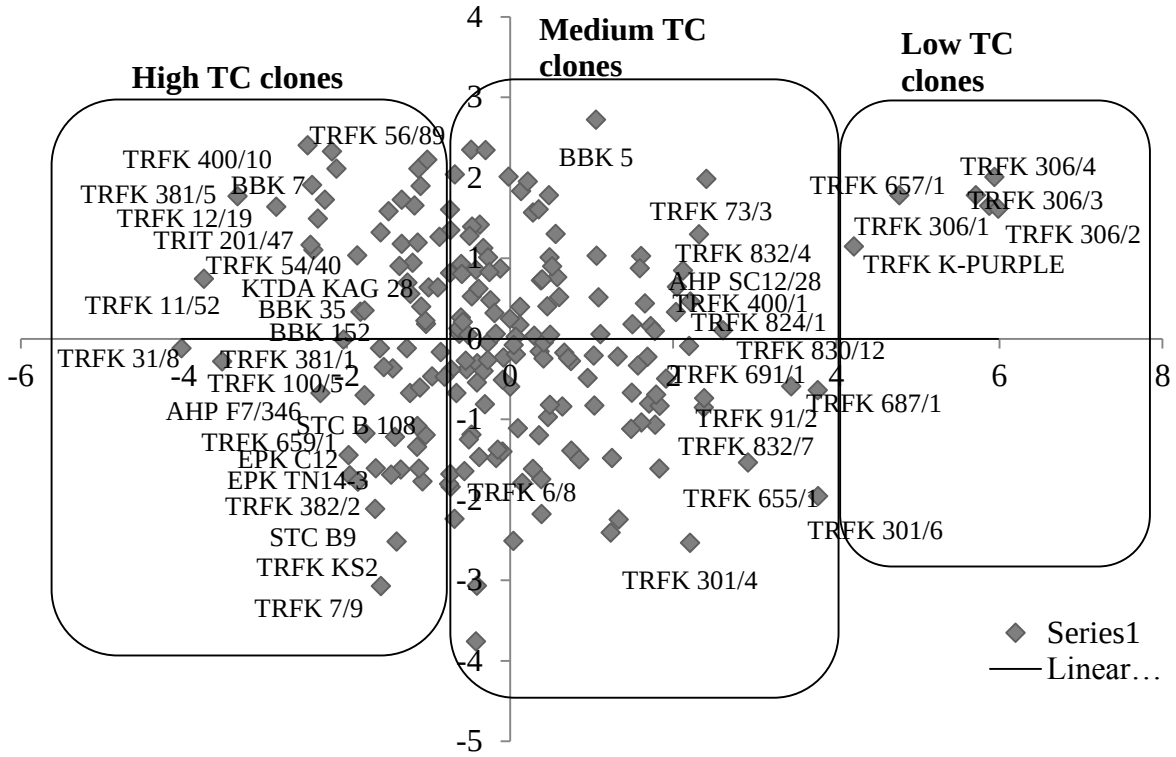

Figure 9: Principle component analysis plot on variation in Total Catechins among the 197 clones.

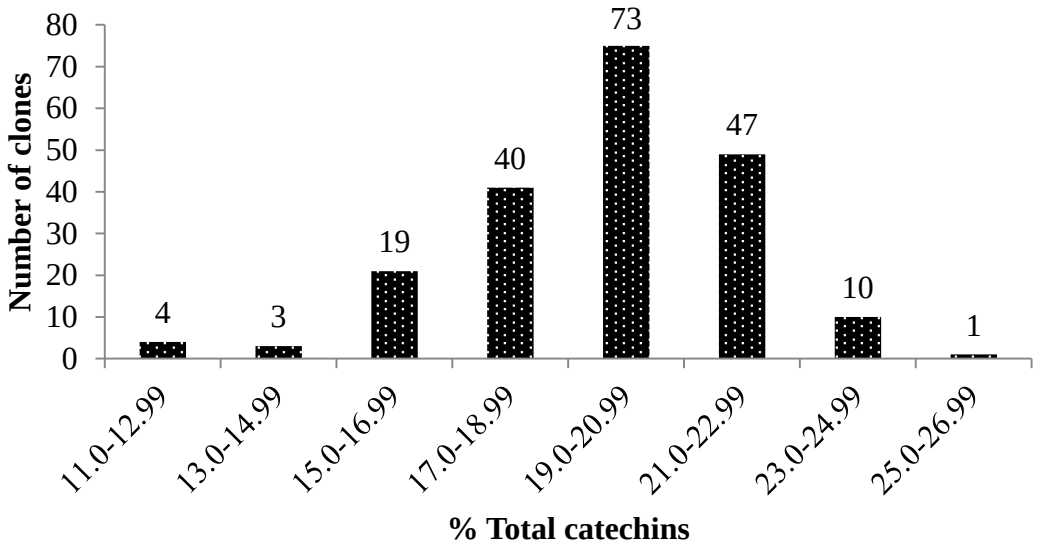

Figure 10: A histogram showing the number of clones and their specific quantities (%) of total catechins among the 197 studied clones.

The individual catechin levels from HPLC analysis exhibited an elution pattern where the non-gallated catechins; epigallocatechin (EGC), catechin (+C) and epicatechin (EC) eluted before the gallated catechins; epigallocatechin gallate (EGCG) and epicatechin gallate (ECG). Non-gallated catechins (EGC, +C and EC) are polar and hence bound less tightly to the non-polar C18 column and eluted in highly polar mobile phase A (9% acetonitrile), while the gallated catechins (ECG and EGCG) eluted last since they bind more tightly to the column and are eluted upon increase of the non-polar mobile phase B (80% acetonitrile). The elution time in minutes of the catechins fractions were as follows EGC; 8.3 minutes, +C: 10.5 minutes, EC: 15.5 minutes, EGCG: 19.9 minutes, ECG: 25 minutes while caffeine used as a standard was eluted at 12.8 minutes (Appendix V).

The levels of the individual catechins levels varied significantly ($P \leq 0.05$) among the studied clones except for the simple catechins (+C). epigallocatechin gallate (EGCG) levels were significantly higher ($P \leq 0.05$) at 7.86% followed by epigallocatechin (EGC) at 6.46% and epicatechin gallate (ECG) at 2.93%. epicatechin (EC) and catechin (+C) were present in significantly lower amounts with mean values of 1.87% and 0.53% respectively.

4.1.2.1 Epigallocatechin gallate

Results on EGCG fraction in various clones are shown in Figures 11, 12 and Appendix II. Clones TRFK 306/2 and TRFK 381/5 recorded the lowest and highest EGCG levels at 3.63% and 11.68% respectively. In addition to clone TRFK 381/5, other high EGCG rich clones as shown in the PCA results (Figure 11) include TRFK 31/8(11.09%), TRFK 12/19(11.09%), TRFK 11/4(10.69%), TRFK 100/5(10.62%), TRFK 31/11(10.82%), TRFK 56/89(10.86%), BBK 5(10.67%), BBK 7(11.05%), TRFK 400/10 (11.25%), TRFK 11/52 (11.41%). Among these, clones TRFK 400/10, TRFK 381/5 and TRFK 11/52 have not been released to farmers for commercial cultivation due to their below average yields. Their release for cultivation will however provide an excellent source of this most biologically potent catechin fraction in green tea for manufacture of EGCG-rich green teas and extracts. The distribution of the clones (Figure 12) shows that most of the clones are of average to high quality while the distribution from low to high EGCG clones show the potential diverse products that can be developed such as EGCG rich or less astringent (EGCG low) tea products.

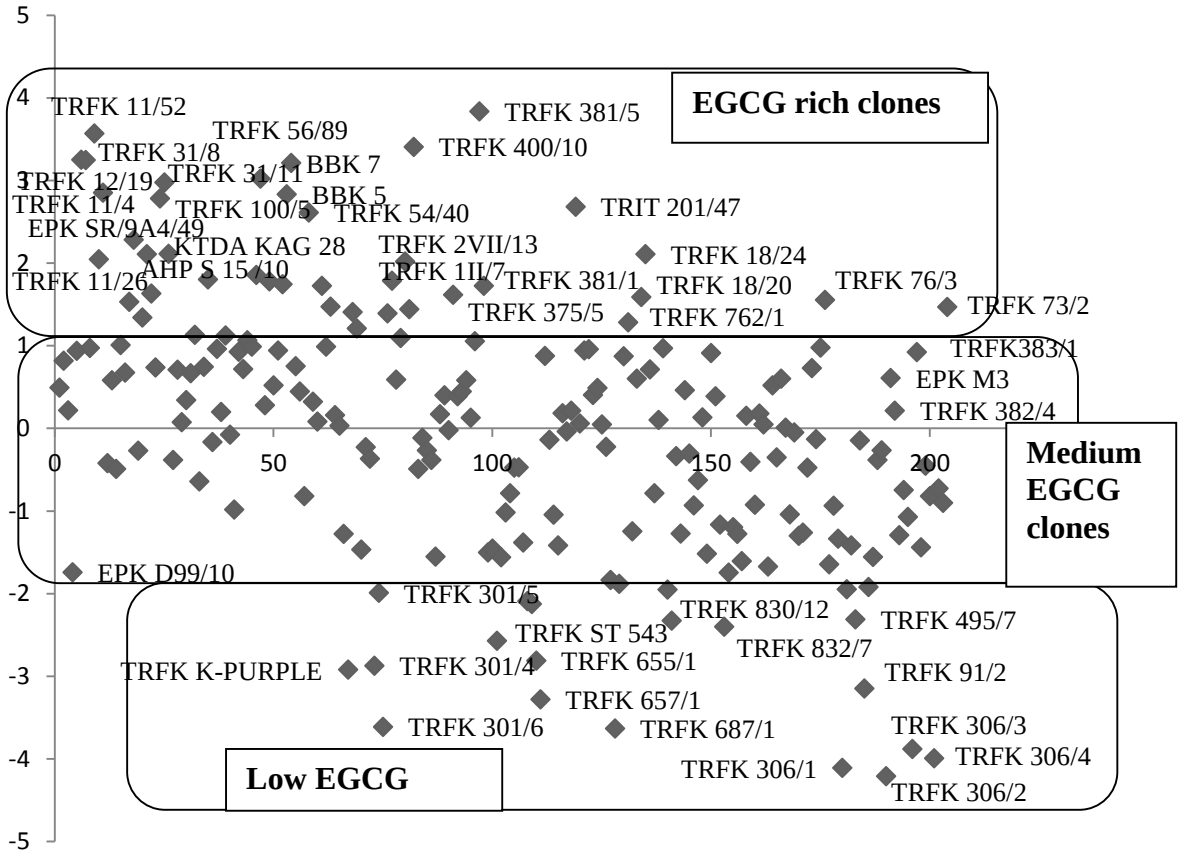

Figure 11: Principle component analysis plot of variation in EGCG among the 197 tea clones

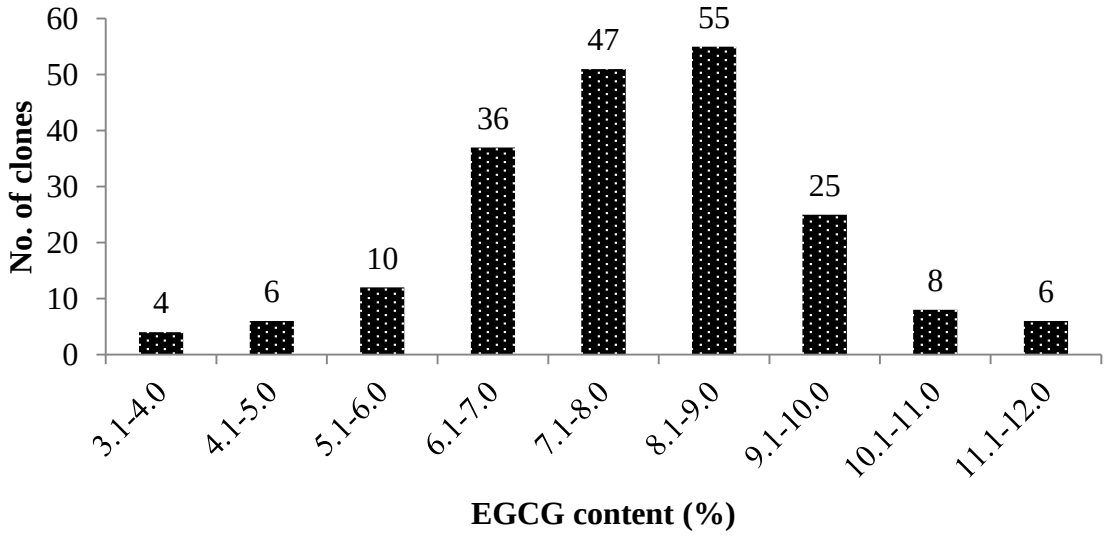

Figure 12: A Histogram showing the number of clones containing specific amounts of EGCG in the studied 197 clones.

4.1.2.2. Epicatechin gallate

The clonal variations in ECG levels are shown in Figures 13, 14 and Appendix II. Clone STC M3 had the lowest ECG content of 1.03%, while TRFK 301/6 had the highest with 6.04% followed by clones TRFK 832/8 (5.64%), TRFK 655/1 (5.23%), TRFK 301/4 (5.16%), TRFK 301/3 (4.98%), TRFK 301/1 (4.84%), TRFK 824/1 (4.60%), TRFK 598/1 (4.53%) and TRFK 831/1 (4.50%) as shown in the PCA results (Figure 13). Among the ECG-rich clones, only TRFK 301/4 is grown commercially. In Figure 14, most clones tend towards the low ECG range indicating that ECG is one of the most limiting individual catechins fractions. Thus, the relatively few clones with high ECG levels are of significant importance since their levels in the green leaf determines the possible combinations of ECG with the other catechins fractions to form the respective theaflavin molecules in black tea.

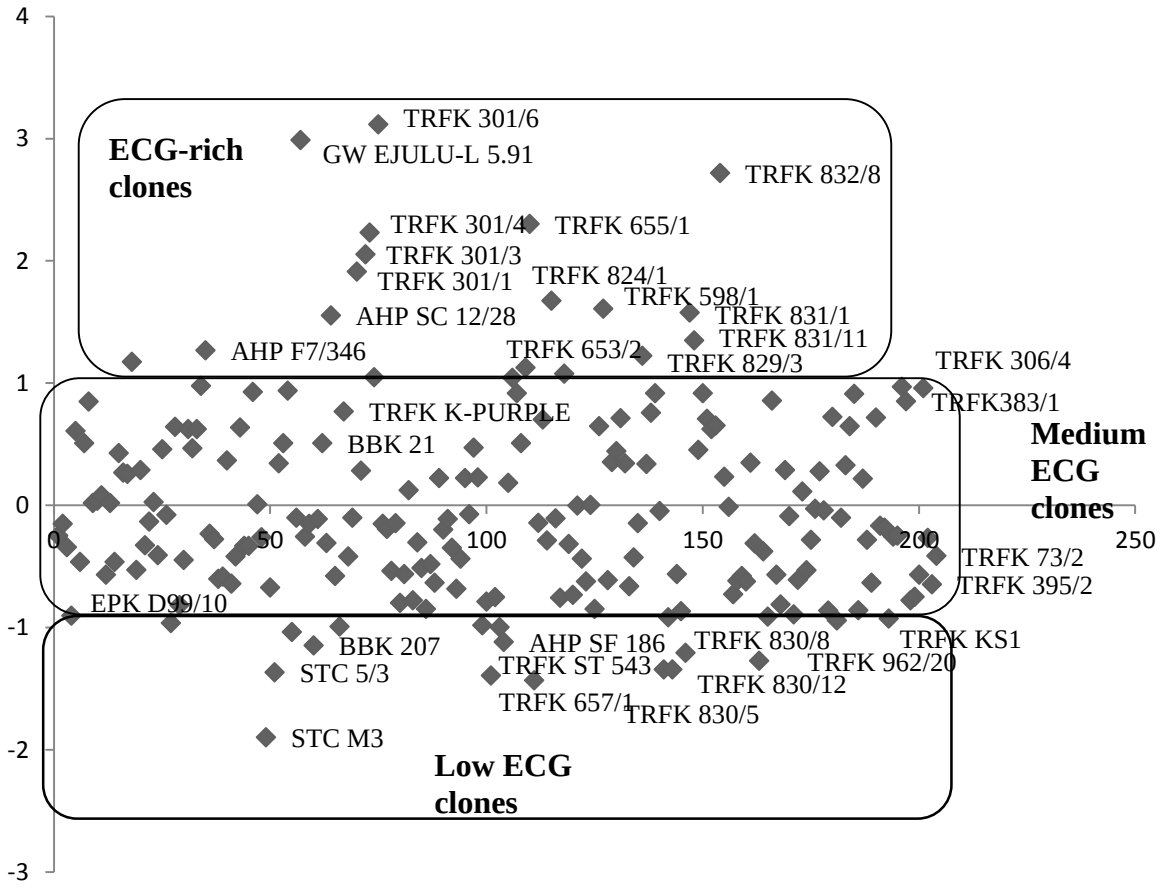

Figure 13: Principle component analysis plot of variation in ECG among the 197 clones

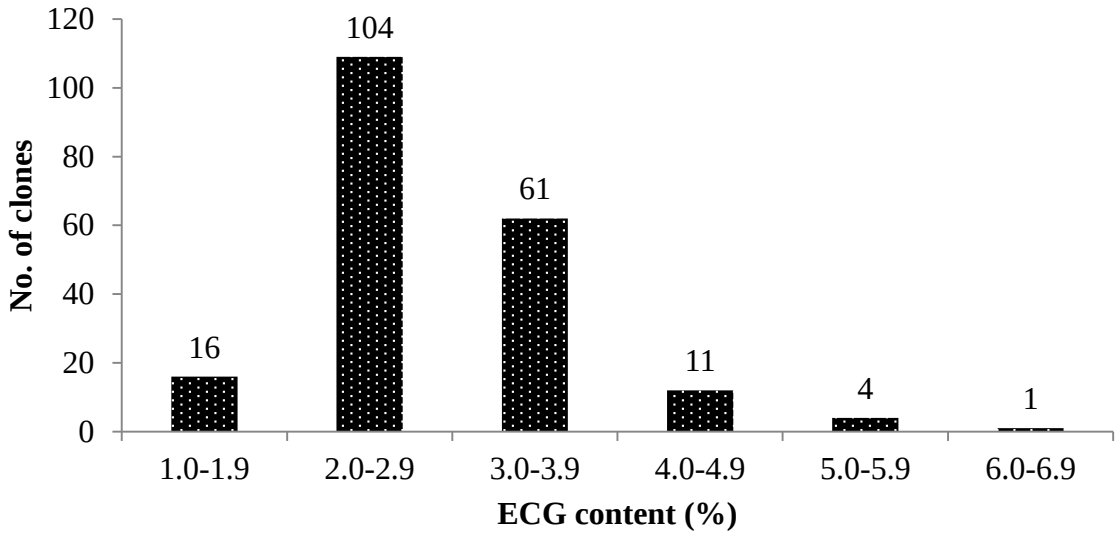

Figure 14: A histogram showing number of clones containing specific amounts (%) of ECG among the studied 197 clones

4.1.2.3 Epigallocatechin

The levels of EGC in different clones are shown in Figures 15, 16 and Appendix II. Clones TRFK 306/4 and TRFK 7/9 had the lowest and highest amounts of 1.74% and 9.94% respectively. Other high performing clones included EPK D99/10 (9.80%), STC B9 (9.11%), TRFK KS2 (9.33%), TRFK KS1 (9.10%), TRFK 412/2 (8.50%), TRFK 382/2 (8.84%), TRFK 301/3 (8.67%), AHP M2/10/51 (8.63%), TRFK 100/5 (8.60%), KTDA B1 (8.57%),TRFK 31/8 (8.77%), EPK C12 (8.77%), EPK C182/40 (8.54%), EPK H81/22 (8.83%) and EPK TN 14-3 (8.70%) as shown in the PCA analysis results (Figure 15). Clones TRFK KS2, TRFK KS1, TRFK 412/2, TRFK 382/2, TRFK 301/3, and TRFK 100/5 are yet to be released for commercial cultivation. In Figure 16, more than half of the clones possessed EGC levels higher than the mean EGC level (6.46%) indication that most of the assayed clones are of high quality.

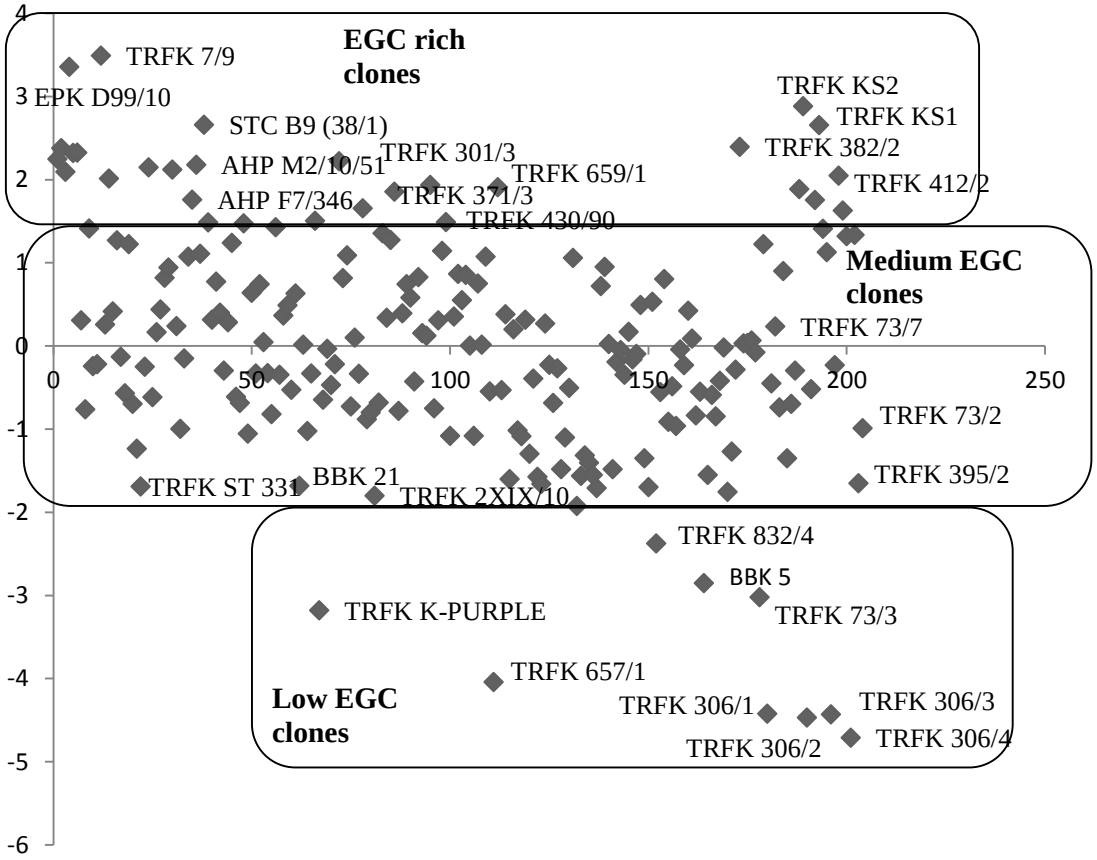

Figure 15: Principle component analysis plot of variation in EGC among the 197 tea clones

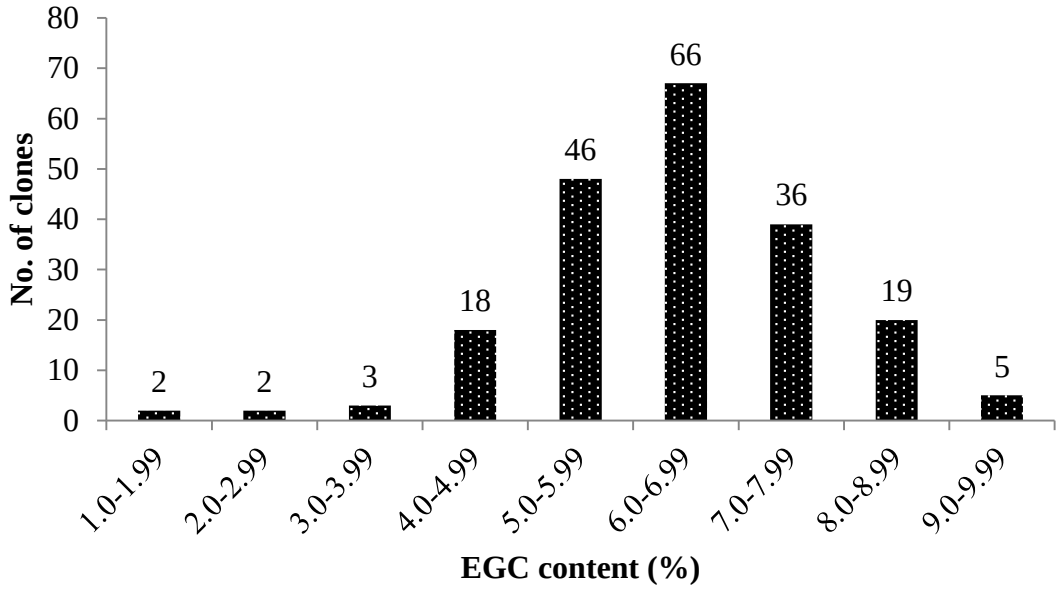

Figure 16: A histogram showing number of clones containing specific amounts of EGC among the studied 197 clones

4.1.2.4 Epicatechin

Results for clonal variations in EC levels are shown in Figure 17 and Appendix II. Clone TRFK 301/6 possessed the highest EC level of 5.41 while TRFK 420/13d contained the lowest with 0.77. Apart from TRFK 301/6, other EC rich clones include TRFK 655/1 (4.60%), TRFK 301/5 (4.24%), TRFK 301/1 (4.15%), TRFK 301/4 (3.81%), TRFK 91/1 (3.44%), TRFK 653/2 (3.14%) and TRFK 301/3 (3.12%). Only clones TRFK 301/5 and TRFK 301/4 have been released for commercial growing. The EC fraction is the most limiting fraction as evidenced in figure 17 whereby only few clones possessed high EC levels. The high-EC clones determine the possible combinations with the other catechins fractions to form black tea theaflavins.

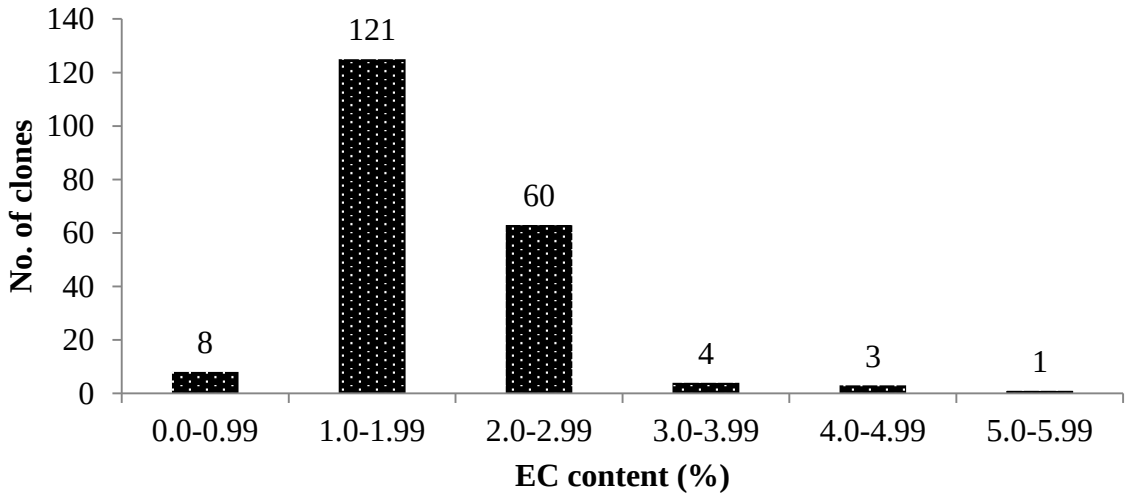

Figure 17: A histogram showing the variation in EC content among the studied 197 clones

4.1.2.5 Catechin (+C)

Results for the levels of simple catechin (+C) among the clones are shown in Figure 18 and Appendix II. No significant difference was observed amongst the clones in the contents of simple catechin (+C) but clones TRFK ST 536 and TRFK 306/1 had the lowest and highest contents of 0.21% and 1.52% respectively. Other high +C clones include TRFK 306/2(1.44%r), TRFK 306/3(1.48%), EPK SR/18V/49 (0.84%), BBK 152(0.80%), TRFK 371/3(0.80%), TRFK 371/2(0.76%) and TRFK 73/5 (0.76%).

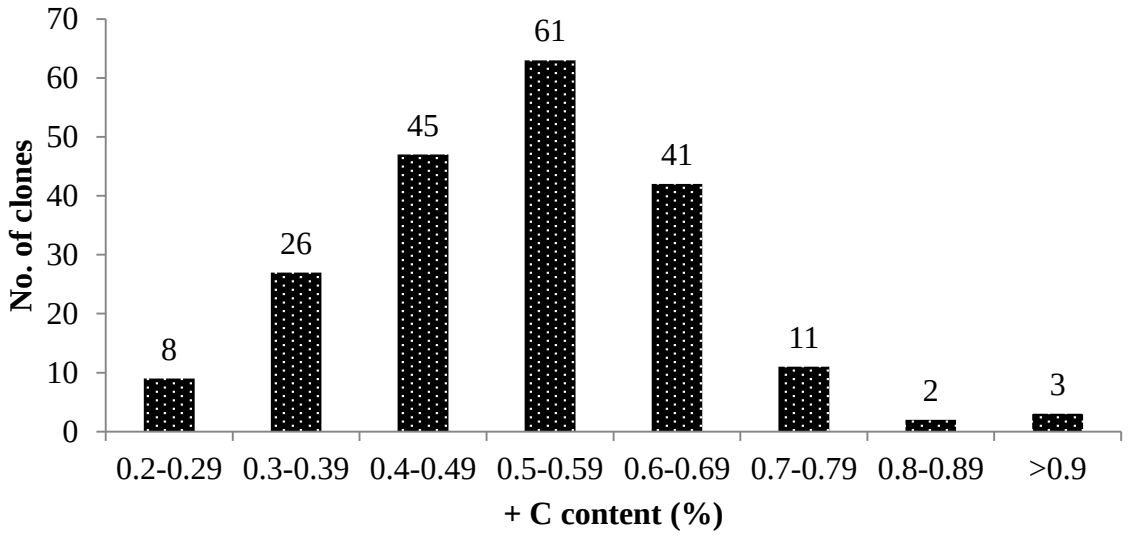

Figure 18: A histogram showing number of clones containing specific amounts of +C among the studied 197 tea clones

4.1.3 Caffeine

Results presented in Figures 19, 20 and Appendix II indicate that caffeine levels varied significantly ranging between 1.96% and 4.37% for clones TRFK 687/1 and TRFK 371/5, respectively, with an average of 3.23% across the population. Other high caffeine containing clones included TRFK 12/12 (4.26%), TRFK 381/5 (4.23%), TRFK 400/7 (4.14%), TRFK 371/3 (4.11%), TRFK 830/6 (4.11%), TRFK 430/52 (4.10%), TRFK 11/52 (4.09%), STC B9 (4.04%), TRFK 829/3 (4.04%) and TRFK 381/1 (3.95%) as shown in PCA results (Figure 19). Only TRFK 12/12, STC B9 and TRFK 371/3 have been released for commercial growing. On the other hand, low caffeine clones other than TRFK 687/1 include TRFK 73/7(2.04), TRFK 830/12 (2.14%), TRFK 306/2 (2.18%), TRFK 306/4 (2.18%), TRFK 306/1 (2.21%), AHP PC 81 (2.25%), TRFK 306/3 (2.31%) and TRFK 830/5 (2.38%).

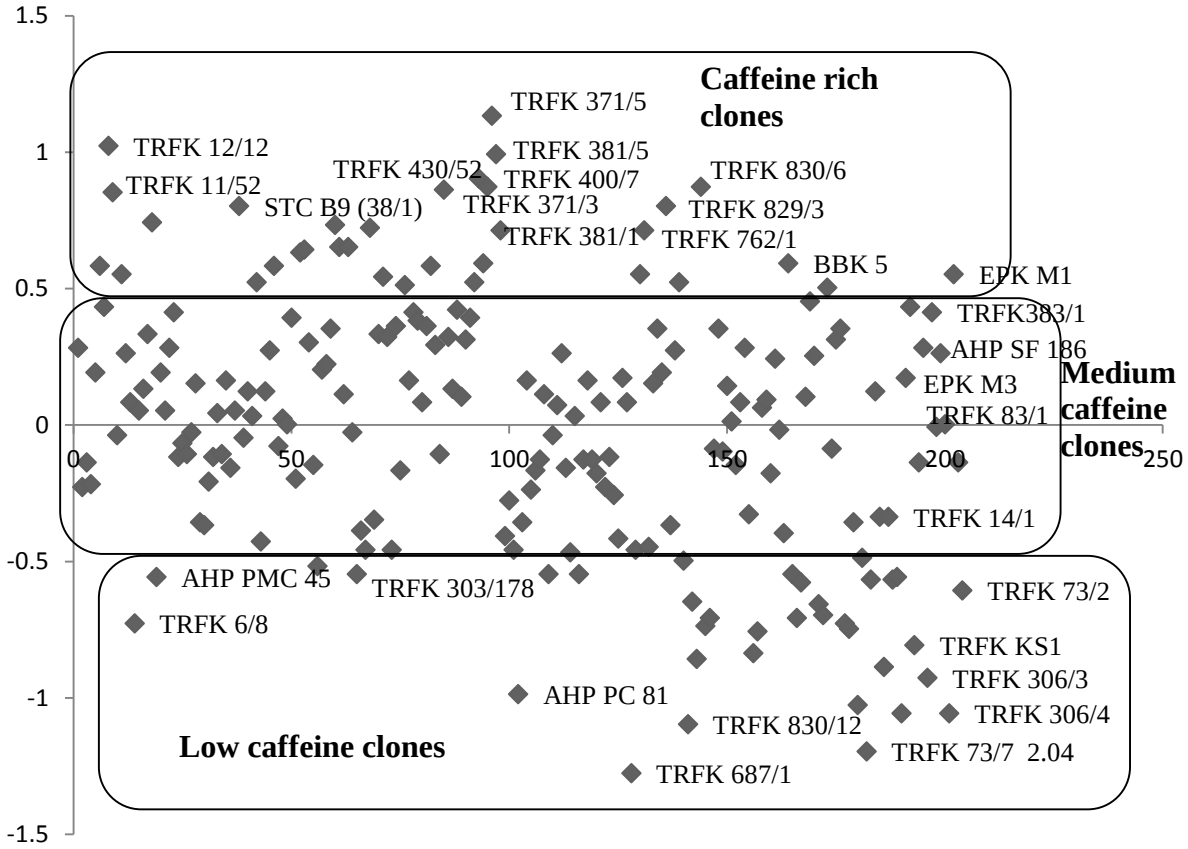

Figure 19: Principle component analysis plot of variation in caffeine among the 197 tea clones

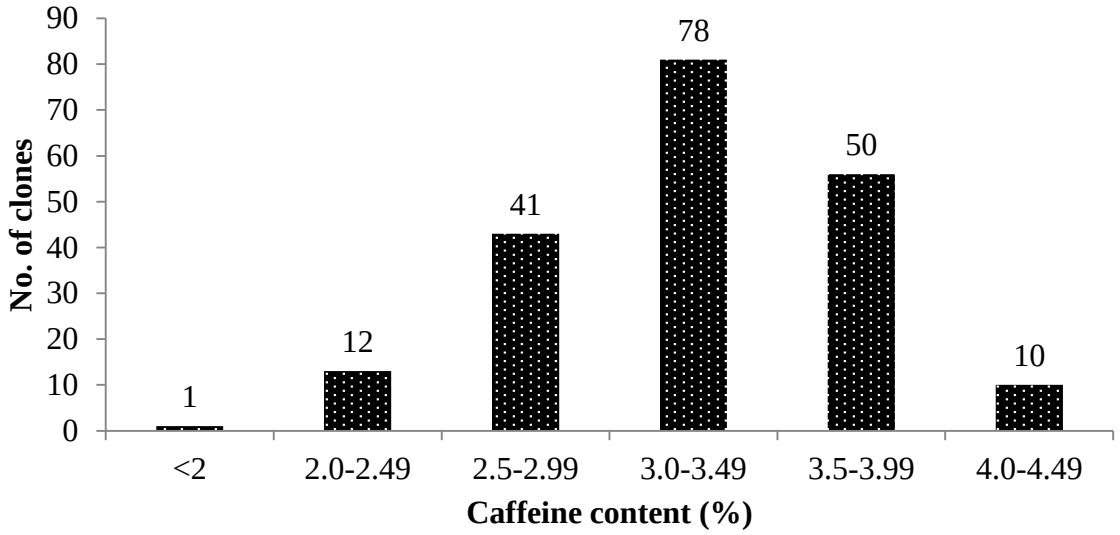

Figure 20: A histogram showing number of clones containing specific amounts of caffeine among the studied 197 tea clones

4.1.4 Anthocyanins

Results on total anthocyanins and anthocyanins profiles among the four purple colored clones are presented in Table 2. The total anthocyanins levels varied though not significantly ($p \leq 0.05$) among the four clones of purple tea studied. Clone TRFK 306/1 had the highest content of 1319 mg/l followed by TRFK 306/2 with 1260 mg/l, TRFK 306/3 with 1168 mg/l while TRFK 306/4 had the least amounts of anthocyanins with a value of 986 mg/l. The levels of the anthocyanin fractions were not significantly different ($p \leq 0.05$) among the clones except for the malvidin levels. The anthocyanidin pigment, Malvidin was present in highest amounts in the purple tea clones followed by peonidin, kuromanin, pelargonidin, cyanidin, iadein and lastly delphinidin which was also observed by Kerio *et al*., (2012).

Table 2: Contents in mg/l of total anthocyanins and anthocyanin fractions in the four purple colored tea clones.

Clone	**Iadein**	**Kuromanin**	**Delphinidin**	**Cyanidin**	**Pelargonidin**	**Peonidin**	**Melvidin**	**T.anthc**
306/1	36.6	81.5	17.9	62.1	64.3	464.0	593.0	1319.4
306/2	33.2	81.2	16.5	58.3	66.8	420.0	583.5	1259.6
306/3	33.6	70.3	16.4	60.5	59.1	398.6	529.4	1168.0
306/4	36.4	67.5	15.4	58.2	58.5	318.7	431.0	985.6
CV (%)	10	8.8	4.1	3.4	6.7	7.8	6.3	6.3
LSD ($p \leq 0.05$)	11.1	21.1	2.1	6.5	13.23	99.7	106.9	237.1

4.1.5 Antioxidant activity

The results for the antioxidant activities of the studied clones are presented in Figure 21 and Appendix II. The studied clones exhibited relatively strong antioxidant activities ranging from 84.7% for clone TRFK ST 536 to 91.93% for clone TRFK 463/63 which varied significantly among the clones ($P \leq 0.05$). The distribution of the clones in Figure 21 shows that most (three quarter) of the clones possess above average antioxidant activities an indication that these clones are of high quality.

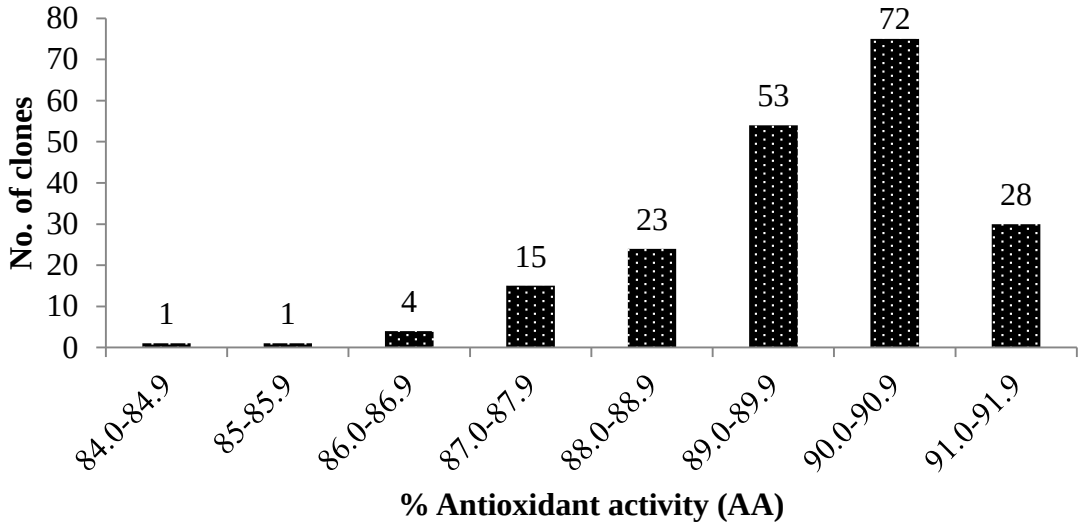

Figure 21: A histogram showing number of clones containing specific antioxidant activities among the studied 197 tea clones

4.1.6 Chlorogenic acid

The chlorogenic acid results are shown in Figure 22. Significant differences ($p \leq 0.05$) in the contents of chlorogenic acid among the 15 clones analysed for this parameter were observed. Clones AHP SC 31/37, TRFK 831/11 and TRFK 6/8 had the highest content of 0.131%, 0.067% and 0.066% respectively while clone TRFK 301/6 possessed the lowest amounts of 0.05%.

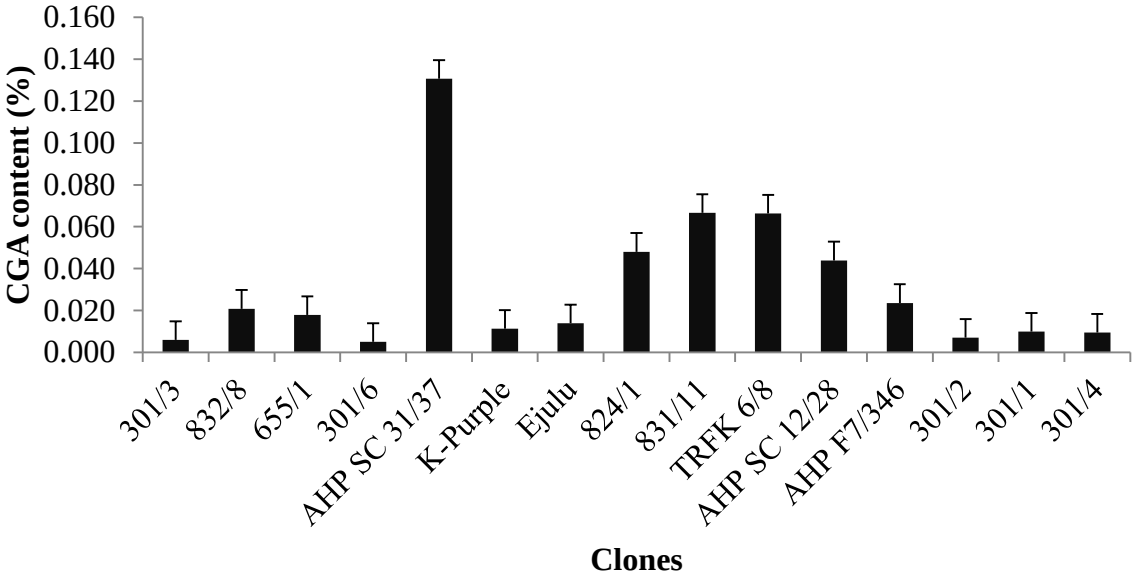

Figure 22: Variation in chlorogenic acid among the 15 tea clones assayed. CV% 14.3, LSD at 5% level 0.0098, F pr. < 0.001

4.1.7 Theanine

Significant differences (P≤ 0.05) in theanine composition among the 15 clones assayed were observed (Figure 23). Clones TRFK 6/8, TRFK 831/11, TRFK 655/1, TRFK 301/1 and 301/3 had the highest theanine content of 1.7%, 1.57%, 1.51%, 1.46% and 1.26% respectively while clone AHP SC 12/28 had the lowest with 0.32%.

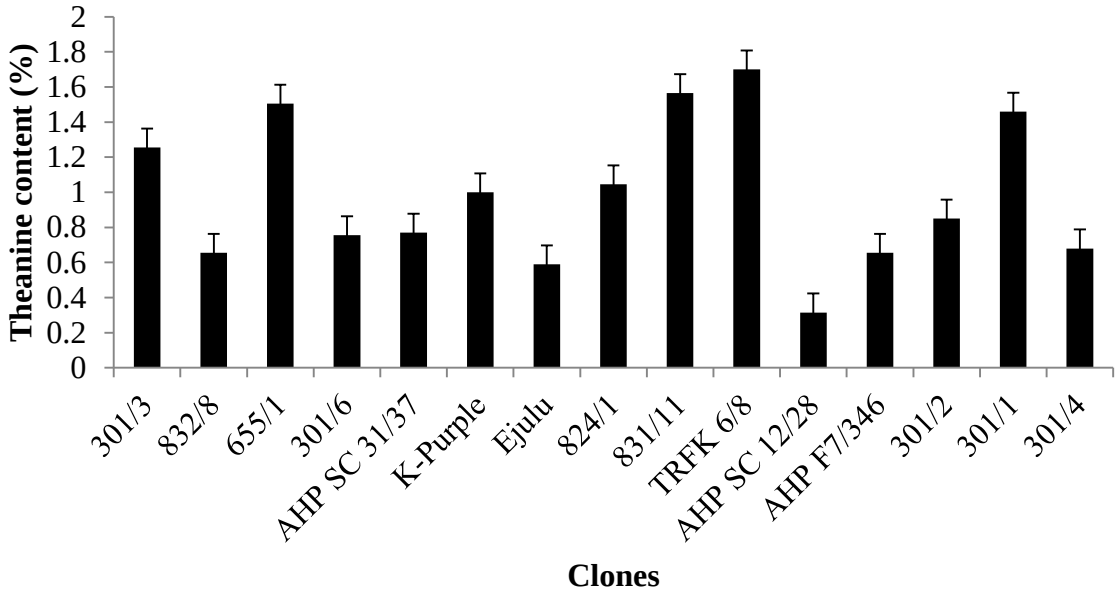

Figure 23: Differences in percentage theanine composition between the 15 assayed clones. CV% 14.7, LSD at 5% level 0.3105, F pr. <.001

4.2 Regional comparison

Results from the regional comparison analysis revealed significant differences (P ≤ 0.05) in the levels of catechins (total and individual) and caffeine between the two study sites; Kangaita (Kirinyaga) and Timbilil (Kericho). In this study, the soil compositions between the two regions slightly differ with Kangaita being more sandy, loamy, porous and strongly acidic while Timbilil is composed of clay, loamy, less porous and slightly acidic soil. However, both sites received similar agronomical inputs and therefore, all the observed differences were attributed to the underlying climatic differences between the two sites.

4.2.1Total catechins

Cultivars from Kangaita experimental station had a significantly higher total catechin content with a mean value of 18.7% compared to 16.2% observed for Timbilil clones as shown in Figure 24 and Appendix III. However, 10 clones namely; BBK 35, TRFK K-Purple,

TRFK 301/5, TRFK 430/90, TRFK 91/1, AHP SC 31/37, TRIT 201/16, TRIT 201/44, TRIT 201/47 and TRIT 201/82 from Timbilil performed better than Kangaita clones.

4.2.2 Epigallocatechin gallate

Clones from Kangaita region had significantly high EGCG values with a mean value of 8.0 % compared to 6.7% observed in the clones from Timbilil as shown in Figure 25 and Appendix III. However, BBK 35, TRFK K Purple, TRFK 400/10, TRFK 430/90, AHP SC 31/37, TRIT 201/16, TRIT 201/44, TRIT 201/47, TRIT 201/50, TRIT 201/55, TRIT 201/70, TRIT 201/73, TRIT 201/75 and TRIT 201/82 from Timbilil had higher EGCG levels.

4.2.3 Epicatechin gallate

Results for regional comparison (Figure 26 and Appendix III) showed that clones from Kangaita had significantly higher ECG levels (mean value of 3.3%) than clones from Timbilil (mean value of 2.5%) with exceptions of the clones BBK 35, AHP SC 31/37, TRIT 201/16, TRIT 201/44, TRIT 201/50, TRIT 201/73 and TRIT 201/82.

4.2.4 Epigallocatechin

Kangaita clones had a significantly high mean value of EGC (5.2%) compared to Timbilil clones (5.0%) as shown in Figure 27 and Appendix III. However clones EPK H 81/22, TRFK 31/8, TRFK 12/12, KTDA KAG 4, AHP F7/346, MICHI (51/10/20), TRFCA SFS 150, STC M3, BBK 5, TRFK 303/577+, GW EJULU-L, BBK 35, BBK 21, AHP SC 12/28, TRFK K PURPLE, TRFK 301/1, TRFK 301/3, TRFK 301/4, TRFK 301/5, TRFK 301/6, TRFK ST 543, TRFK 306/1, TRFK 306/3, TRFK 91/1, AHP SC 31/37, TRIT 201/47 and TRIT 201/82 performed better.

4.2.5 Epicatechin

Significant difference was observed for EC levels with Kangaita clones having a high mean value of 1.5% compared to 1.4% in the Timbilil clones (Figure 28 and Appendix III). However, clones KTDA KAG 4, AHP F7/346, MICHI (51/10/20), TRFCA SFS 150, STC M3, BBK 5, TRFK 303/577+, BBK 35, TRFK 301/1, TRFK 301/3, TRFK 301/4, TRFK 301/5, TRFK 301/6, TRFK 306/1 and TRIT 201/47 in Timbilil performed better. Clones such as EPK C 182/40, BBK 5, BBK 35, BBK 21, TRFK purple, AHP SF186, TRFK 306/3 and TRIT 201/47 had no significant difference in EC between the two sites an indication of their ability to resist regional influence on EC levels.

4.2.6 Catechin (+C)

Results for the variations in simple catechins between the two regions among the studied clones (Figure 29 and Appendix III) showed significantly high levels in Kangaita (mean value 0.7338) compared to those in Timbilil which had a mean value of 0.592 with clones KTDA KAG 4, STC M3, TRFK 301/3, TRFK 306/1, TRFK 306/4, TRFK 91/1, AHP SC 31/37 and AHP CG28V929 performing better in Timbilil.

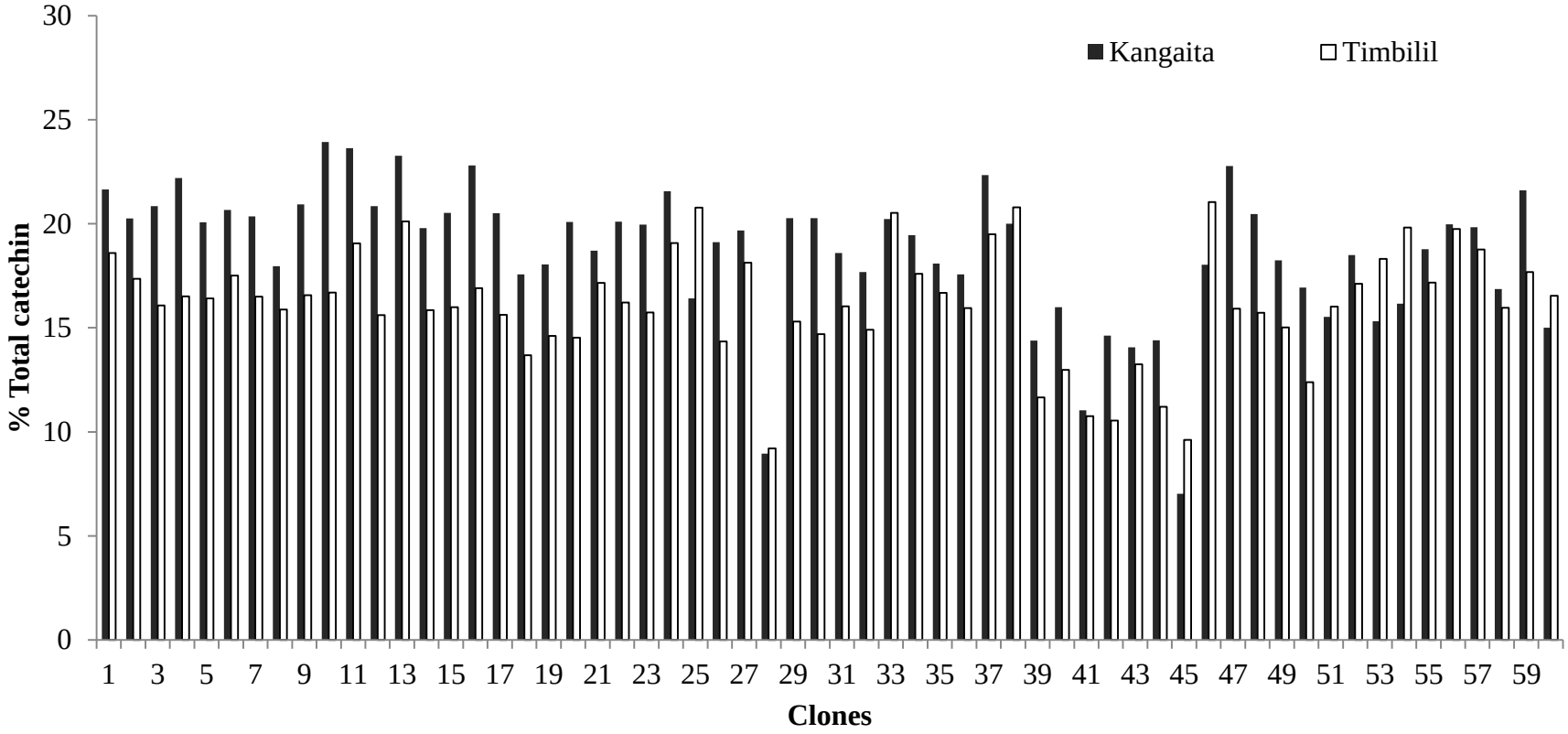

Figure 24: A comparative representation of contents of total catechins between the tea clones grown in Kangaita and Timbilil regions

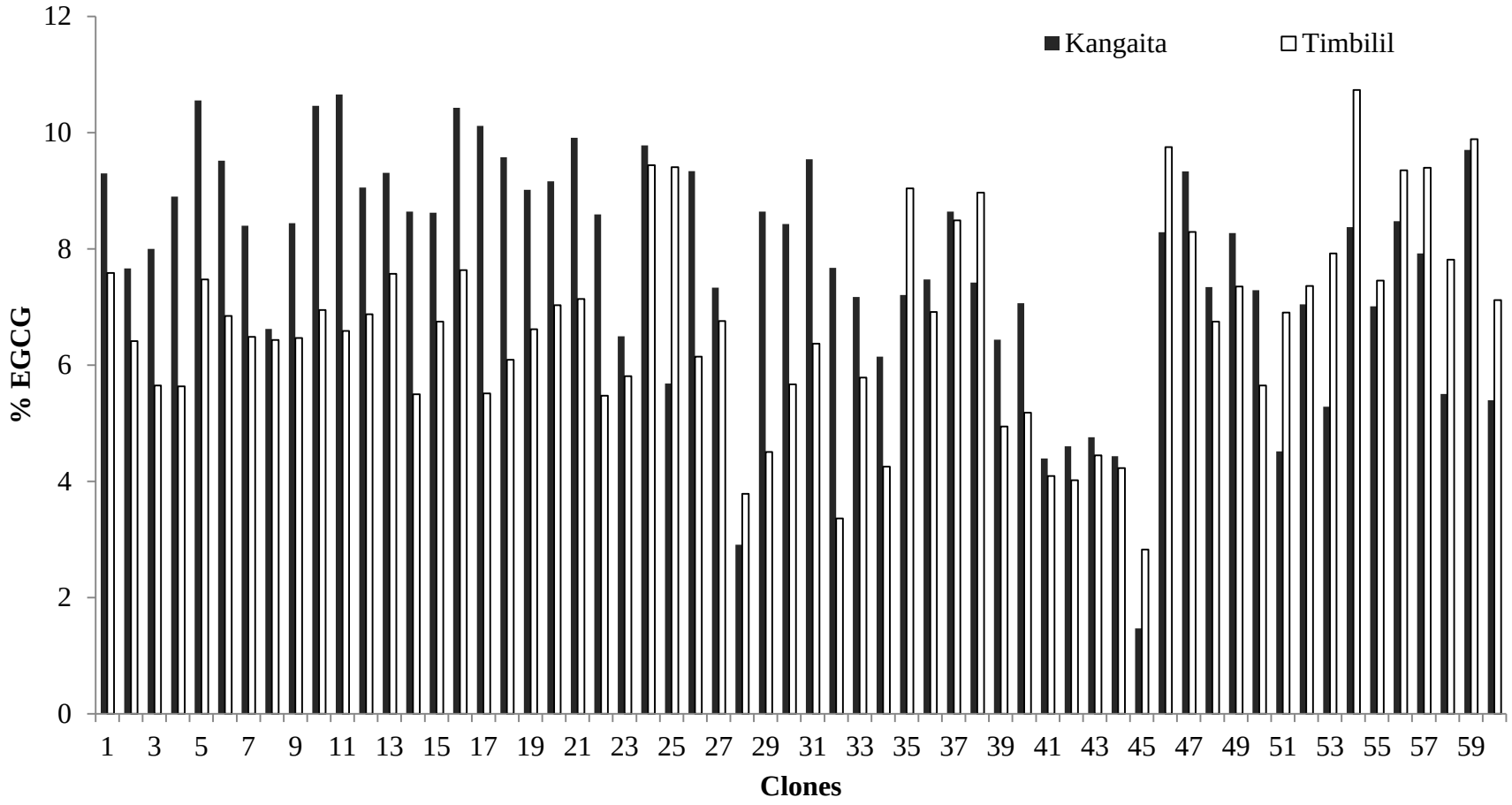

Figure 25: Comparison of EGCG levels in the 60 tea clones grown in Kangaita and Timbilil regions

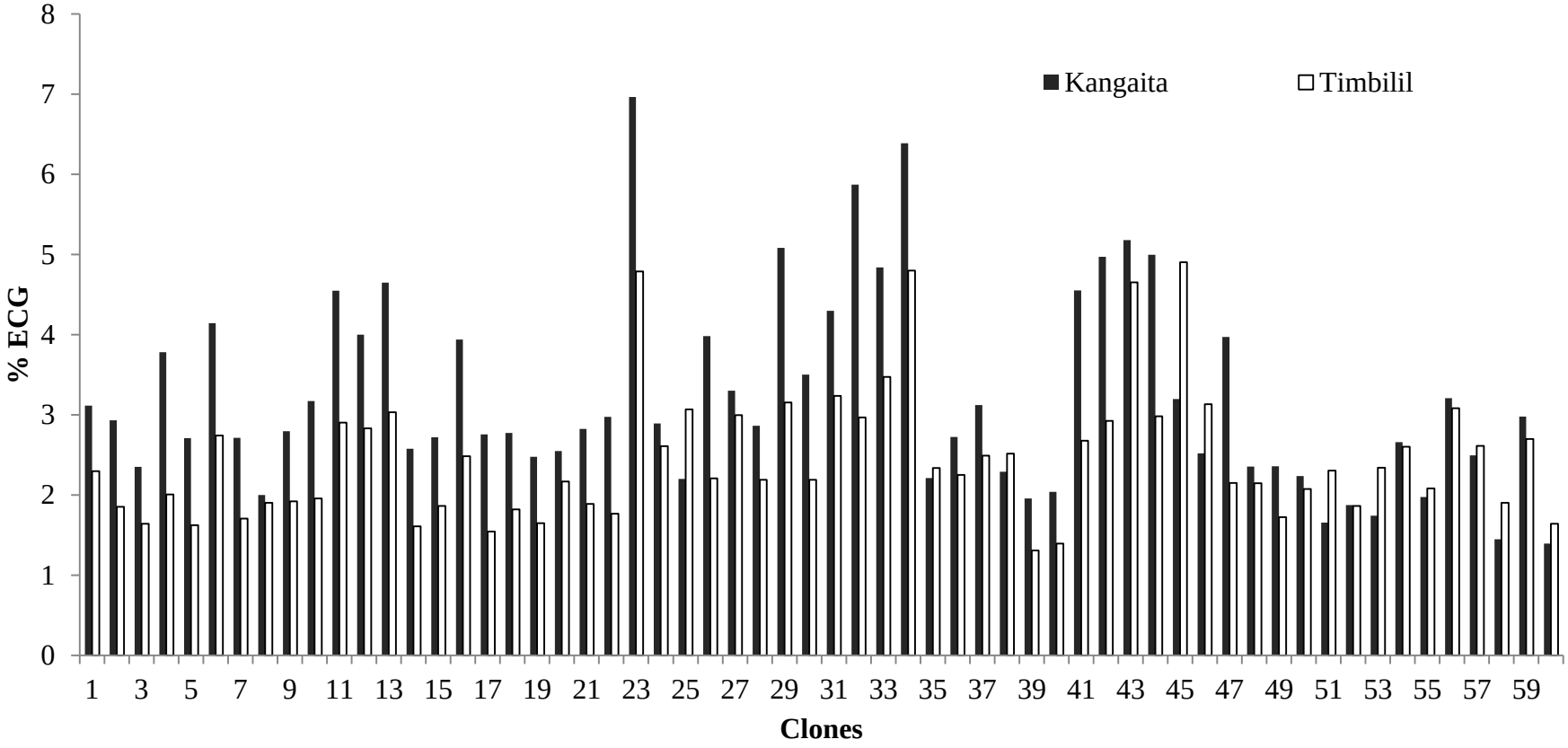

Figure 26: Comparison of ECG contents (%) among the 60 tea clones grown in Kangaita and Timbilil regions.

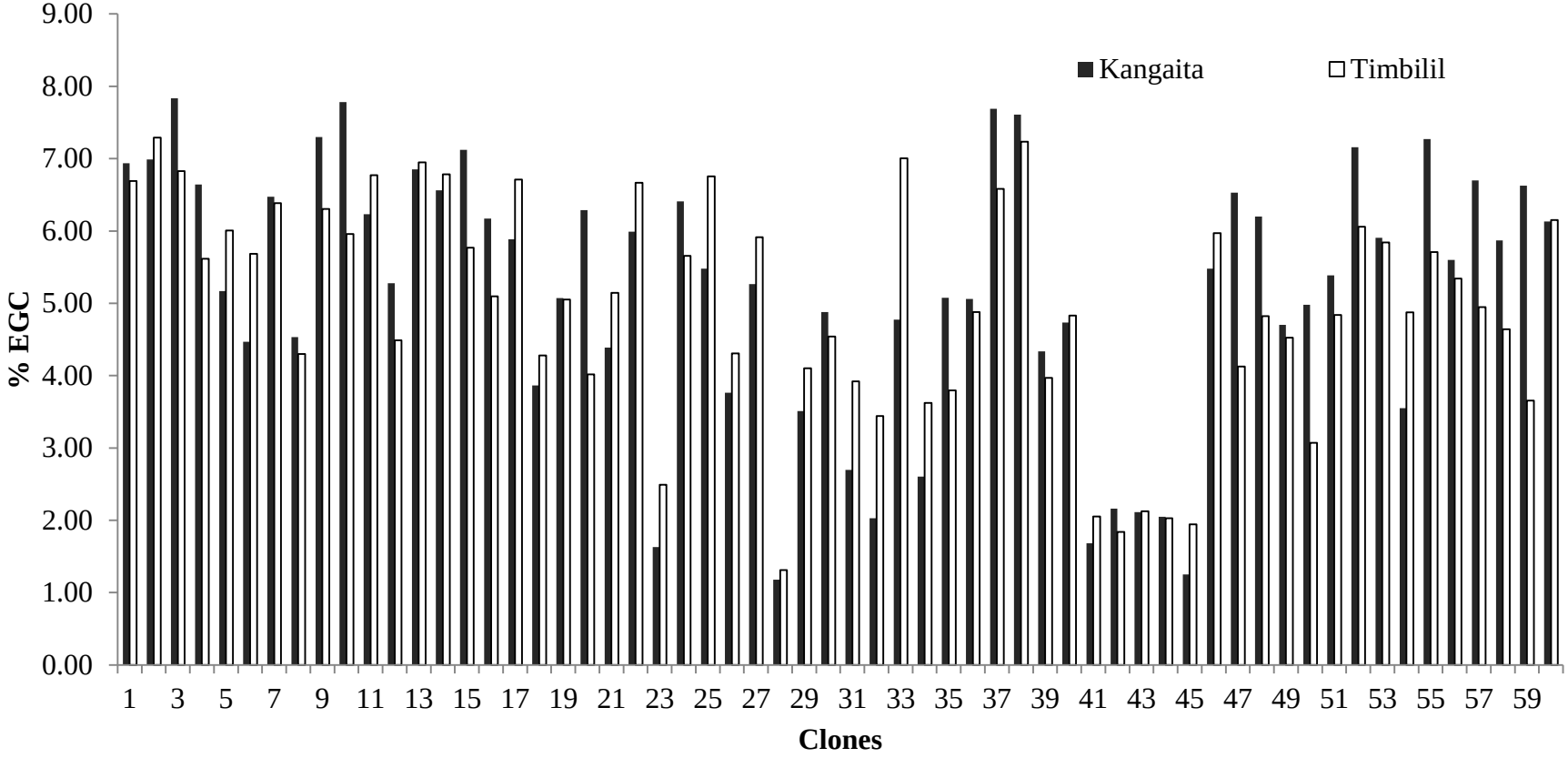

Figure 27: Comparison of EGC contents in the 60 tea clones grown in Kangaita and Timbilil regions

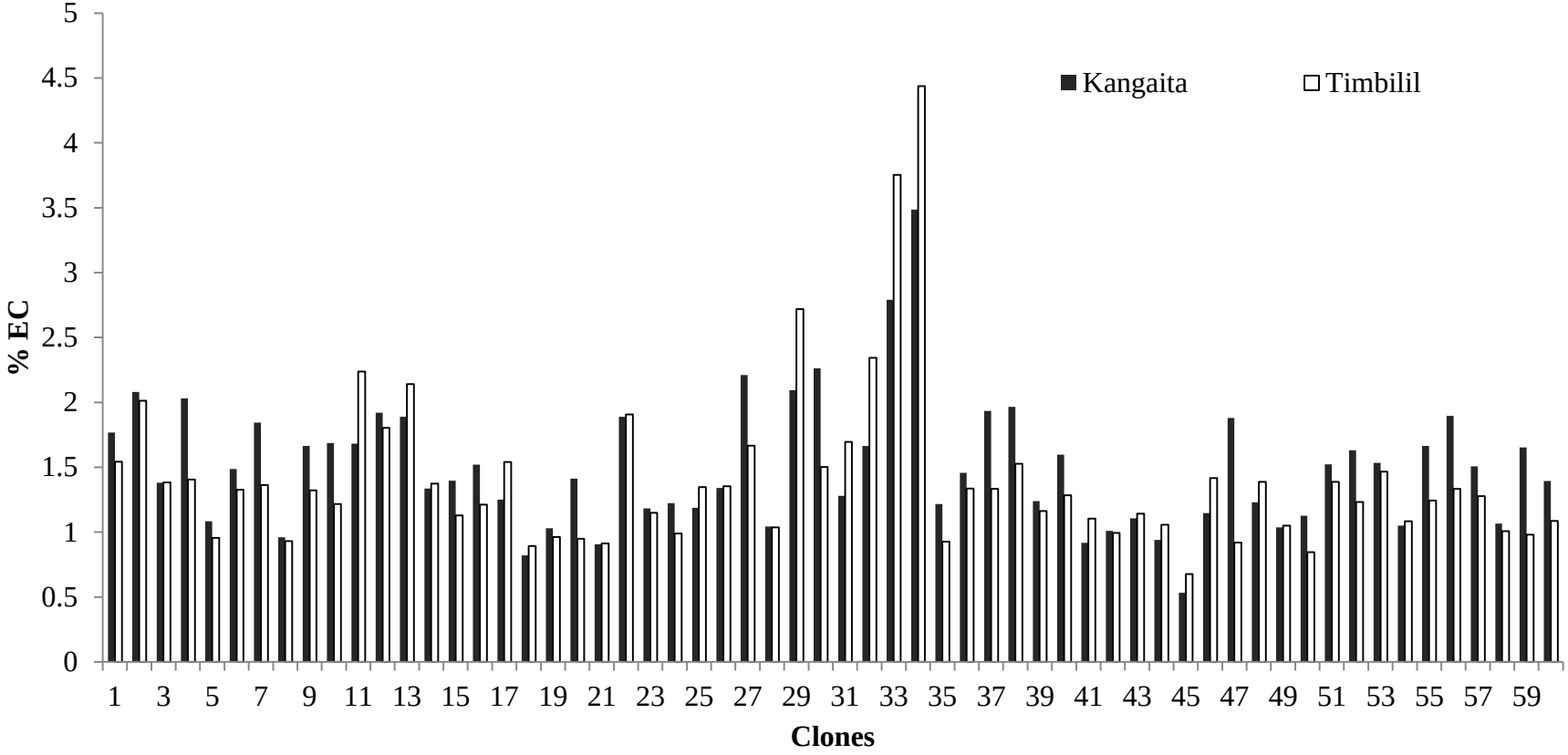

Figure 28: Differences in EC contents among the 60 tea clones grown in Kangaita and Timbilil regions.

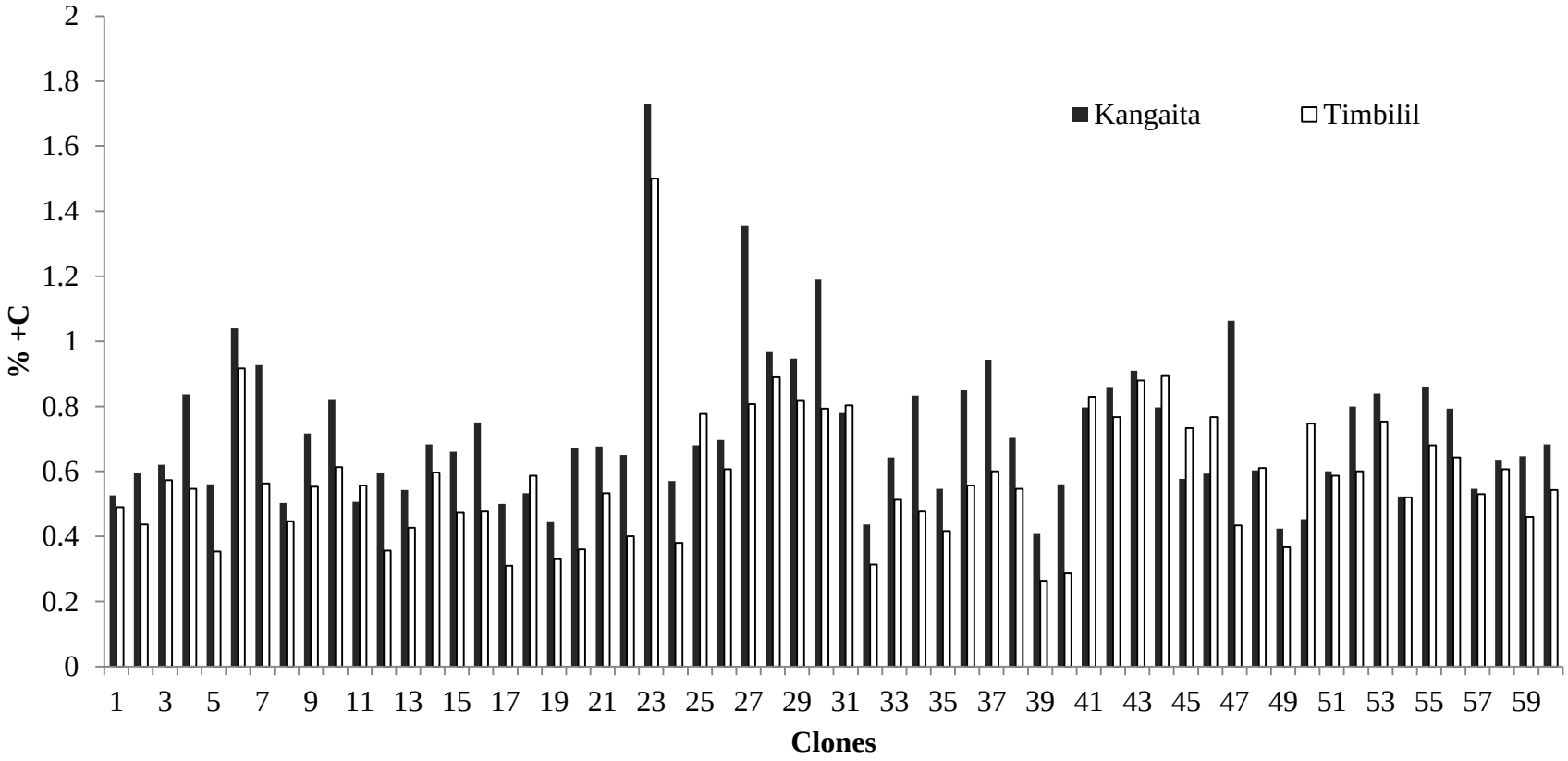

Figure 29: Comparison of simple catechin (+C) content among the 60 tea clones grown in Kangaita and Timbilil

4.2.7 Caffeine

Caffeine analysis results for the two regions (Figure 30 and Appendix III) showed an interesting pattern, different from that observed for individual catechins. A significant difference was observed with Timbilil clones containing high caffeine levels (mean value of 4.18%) compared to Kangaita clones (mean value of 3.85%) as depicted in Figure 19. However, clones EPK H 81/22, EPK C182/40, AHP F5/222, AHP F7/346, MICHI (51/10/20), AHP MNI 1/96, TRFCA SFS 150, STC M3, BBK 5, TRFK 303/577+, TRFK 54/40, BBK 21, AHP SC 12/28, TRFK K-PURPLE, TRFK 301/6, TRFK 400/1, AHP PC 81, AHP SF 186, AHP CG28V929 and TRIT 201/43 in Kangaita performed better. Some clones such as TRFK 31/8, STC M3, STC M1, TRFK 54/40 and TRFK 301/6 in the two regions exhibited no significant differences in caffeine contents indicating their suitability for cultivation in regions with varied environmental conditions.

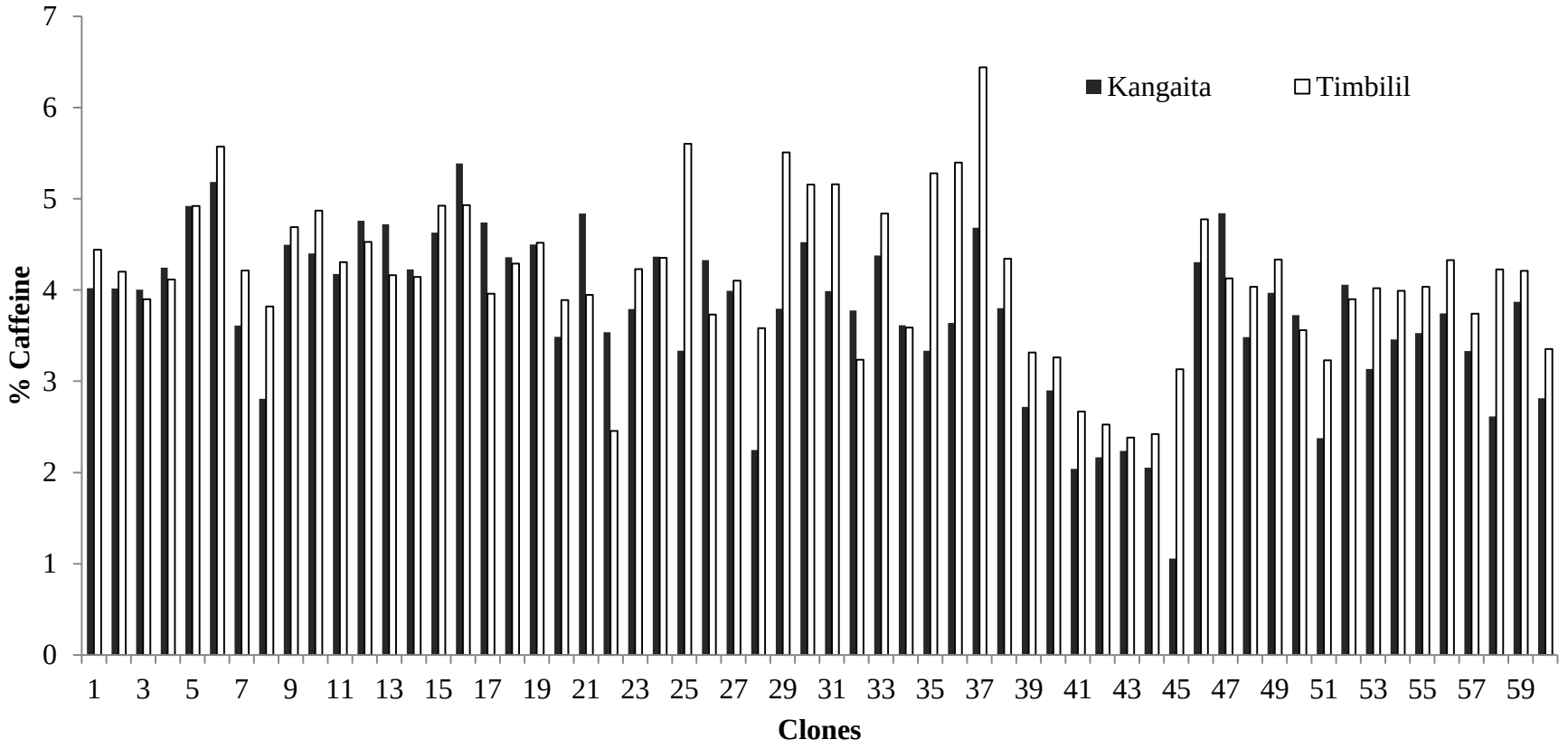

Figure 30: Comparison of caffeine contents among the 60 tea clones grown in Kangaita and Timbilil

CHAPTER FIVE

DISCUSSION

5.1 Clonal variation

Tea stands out as a principal source of polyphenolic compounds in the human diet owing to its widespread use as the most commonly consumed beverage. Most interestingly, there has been an increased quest for plant-derived polyphenolic antioxidants owing to their role as potential protectants against chronic diseases. The polyphenolic contents of the studied clones were observed to be slightly different compared to those documented in the clonal catalogue at the TRI (Wachira *et al*., 2012). This variation could be due to gradual climatic changes and changes in the durations of rains, cold and dry seasons which might eventually influence synthesis and accumulation of the polyphenol compounds (Owuor *et al*., 1991; Owuor, 1994). Sampling times could also be responsible for the observed differences since the leaf samples for this study were collected during the dry season which contributes to increased synthesis of polyphenols (Cherotich *et al*., 2013). The increased synthesis is useful for protection against the prevailing harsh environmental conditions such as harmful sun rays.

The clones in low polyphenolic content cluster would be highly suitable for processing high quality green orthodox or CTC teas, while those in medium and high polyphenolic cluster could be exploited for Oolong and black orthodox/CTC teas. The low polyphenolic content cluster include TRFK St 543 (cv. Yutakamidori) and TRFK St 536(cv. Yabukita), the most popular green tea cultivars in Japan, which were introduced in Kenya in 2001 (Anonymous, 2002). The observed differences in the polyphenolic composition among the tea clones in this study can be attributed to several factors such as the cultivars genotype and geographical origin of the clones (Lin *et al.*, 2003, Kamunya *et al*., 2009a). Clones with high polyphenolic content such as BBK 152 are of Assamica origin while those with low polyphenol content such as TRFK 833/1 are of Chinary origin. Previous studies have shown the superiority of the Assam teas compared to the Chinary and Cambod tea varieties due to differences in their ecological origins and this asserts the contribution played by geographical origin of the clones to their polyphenol composition (Harbowy and Balentine, 1997; Wachira and Kamunya, 2005; De Costa *et al*., 2007). In addition, the individual clone's genetic constitution is also of great importance in contributing to the observed polyphenolic composition. Notably, a total of 46 clones had phenolic composition higher than TRFK 6/8

(27.4%), a clone used as an internal reference standard because of its proven high quality. However, 16 clones have not been released to farmers for commercial utilization as they are still undergoing further observations. The clones include TRFK 301/1, TRFK 381/5, TRFK 381/1, TRFK 635/1, TRFK 400/1, TRFK 382/2, TRFK383/1, TRFK 655/1, TRFK 829/3, TRFK 829/6, TRFK 829/7, TRFK 77/1, TRFK 412/2, TRFK 395/2 and TRFK 395/2. Such clones can be exploited to generate diverse high value tea products for use as tea raw material for extraction and inclusion in cosmetic products and development of polyphenol rich tablets to be sold as pharmacologically active products. This area has not been exploited in Kenya notwithstanding its potential to generate significant revenue to the industry.

Usually, the yield of tea clones is prioritized before they are released for commercial cultivation because farmers too value quantity that would ideally translate to high returns. However, with most tea growing countries increasing their tea acreage and other non-tea growing regions/countries adopting the practice (Wachira *et al*., 2002), the world tea market has been flooded due to the high production. This has consequently led to low tea prices necessitating the producing countries like Kenya to reconsider their marketing strategies to ensure that they stand out on the market front. Tea polyphenolic content being an important quality parameter provides an excellent point of consideration as a high total polyphenolic tea contains relatively high individual polyphenols, notably the catechins. Therefore, utilization of high polyphenol-containing clones even with their low average yields will heighten demand of these tea products owing to their health promoting abilities. All the TRIT (Tea Research Institute of Tanzania) clonal series can be considered below average in relation to black tea quality based on their low polyphenol composition. However, they would be highly suitable for green orthodox processing. TRFK 830 and TRFK 833 clonal series are of relatively low quality with a total phenolic composition of less than 22% and should not be used if a high phenolic rich tea product is to be manufactured.

The observed differences in the total phenolic content between the studied cultivars is an important trait for tea breeders and tea manufacturers as well since it allows for selection of potential clones with desired polyphenolic content either for propagation or manufacture of polyphenolic rich teas and extracts.

Catechins form a very interesting group of health-promoting compounds in tea and accounts for two thirds of the total polyphenolic content in tea plants. The major catechins in tea include catechins (+ C), epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG). In this study, the sampled teas contained EGCG fraction in largest amounts followed by EGC and ECG while C and EC were present in

significantly lower amounts. Similar results were obtained in studies done by Karori *et al* (2014); Karori *et al*., (2007) and Ender *et al*., (2004). Usually, tea clones contain EGC and EGCG in high levels with the EGCG levels accounting for up to 50% of the total catechins content. A change in the levels of these two catechin fractions is expected to greatly affect the total catechin composition of the tea leaves. The purple leaf colored clones (306/1, 306/2, 306/3, 306/4) had significantly lower catechin levels compared to the green leaf cultivars since the purple teas contain a unique compound called anthocyanins which give them the purple colour (Kerio *et al*., 2012). Synthesis of both compounds (anthocyanins and catechins) has been shown to share the same substrate, anthocyanidins (Harbowy and Balentine, 1997). In the flavonoid synthetic pathway, anthocyanins are synthesized prior the catechins and act as precursors in catechins formation. This negative correlation between levels of catechins and anthocyanins is believed to be due to the upregulation of the gene coding for the enzyme anthocyanin synthase, responsible for synthesis of anthocyanins rather than the one for leucoanthocyanin reductase in the catechins biosynthetic pathway (Kerio *et al*., 2012). This observation is believed to be due to response to some environmental stimuli such as high temperatures since both compounds are secondary metabolites derived from the general phenyl propanoid pathway (Punyasiri *et al.,* 2004).

The low catechins clones would be highly suitable for processing less astringent green orthodox or CTC teas, while those in high catechin cluster could be exploited for high quality green teas or catechins extracts. Ninety (90) clones recorded a TC content (21.69%) greater than the standard reference clone, TRFK 6/8 (20.28%). In green tea manufacture, a prescreening procedure to ascertain suitability of the clones in terms of high catechins has not been standardized locally. Instead, all the available clones are combined indiscriminately with the only measure taken being the steaming process that maintains the catechins in their monomeric form. This way, even clones with very low catechins levels are used resulting in a green tea beverage whose quality is not uniform and thus affecting the cup quality. Since catechins are the major constituents and quality markers of green tea, it is important that only the total catechin-rich clones such as AHP F7/346 (25.42%), TRFK 301/3 (24.82%), TRFK 11/52 (24.45%), TRFK 31/8 (24.38%), TRFK 381/5 (24.28%), TRFK 100/5 (23.96%), BBK 7 (23.80%) and TRFK 382/2 (23.79%) are used in manufacture of catechins-rich green tea. In addition, extracts from these clones can be exploited in the pharmaceutical industry for fortification of drugs or in the manufacture of health promoting catechins-rich products which can be sold in form of pills.

The chemical composition of the fresh tea leaves and specifically the individual catechins contributes to the composition of the resulting black tea. During the aeration, the individual catechins fractions react together to form respective theaflavin products in this manner EC + EGC =TF, EC + EGCG= TF-3-g, ECG + EGC= TF-3'-g, ECG + EGCG= TF-3, 3'-dg (Obanda *et al.,* 2001). The correct balance and amount of dihydroxy flavan-3-ol (EC and ECG) and trihydroxy flavan-3-ol (EGC and EGCG) are therefore necessary to ensure maximum formation of the theaflavins (Wright *et al.,* 2002). It's possible to optimize the production of tea products that are rich in specific biomolecules by utilizing clones with the desired combination of catechins fractions. In this study, clones such as GW EJULU, TRFK 301/6, TRFK 832/8, TRFK 655/1, TRFK 301/3, TRFK 824/1, TRFK 831/1, AHP SC 31/37, AHP SC 12/28, TRFK K-purple, TRFK 6/8, AHP F7/346, TRFK 301/2, TRFK 301/1 and TRFK 301/4 can be used for developing high quality black tea product rich in theaflavin-3, 3'-digallate (T-3, 3'-DG). During the selection, a lot of emphasis was based on high ECG levels rather than EGCG since it is the most limiting catechin during the reaction. The ratio between the two fractions ECG: EGCG was maintained at a minimum level with the levels of the clones as follows; 5.91:7.02, 6.04:4.23, 5.64:6.09, 5.23:5.03, 4.98:7.47, 4.60:6.42, 4.50:7.21, 3.21:7.79, 4.48:8.00, 3.69:4.92, 2.46:7.35, 4.19:9.64, 3.21:7.61, 4.84:6.37 and 5.16:4.97 respectively. This is the reason why some clones e.g. TRFK 381/5 with high EGCG amounts were not selected due to a wide EGCG: ECG ratio. Clones TRFK 301/6, TRFK 655/1 and TRFK 301/1 possessed high EC and EGC levels and are suitable in manufacture of black teas with simple theaflavin fraction. The 301 clonal series especially 301/1, 301/3, 301/5 and 301/6 possessed generally higher levels of EC and ECG, the most limiting compounds during black tea formation making them perfect for manufacture of black teas with the various theaflavin fractions. The diversification process requires several approaches to ensure that resulting products meet the different consumer tastes. Kenya, being a major producer of black tea will benefit from utilization of these clones as the resulting products will be sold as high quality and branded black teas, a strategy expected to boost demand for our products consequently increasing returns to producers.

Clones TRFK 301/5 and TRFK 301/4 with high EGC/EC ratios of 7.54:4.24 and 7.26:3.81 respectively but relatively low EGCG/ECG ratios were also selected for manufacture of less astringent and bitter green teas. EGCG and ECG contribute to most of green tea's bitter and astringent taste which has a pleasant taste to some consumers (Scharbert *et al.,* 2005). In Kenya where the main product is black tea, green tea production is yet to grow despite the potential health benefits associated with the product. The awareness on its

benefits offers a potential market which is poorly exploited in Kenya. Production of high quality green tea products therefore which will be marketed predominantly as a health drink will open up a new line of diversification envisaged to place the country in the forefront of the tea market. Since the EGCG fraction possesses much of green tea's potency, several clones not yet released for commercial cultivation such as EPK SR/9A4/49, BBK 5, BBK 7, TRFK 400/10, TRFK 381/5 and TRIT 201/47 should be utilized. The clones can also be exploited as raw materials for extraction and sold as nutritional and pharmaceutical supplements inform of tablets, capsules or health drinks. Green tea extracts can also be used in cosmetic products such as moisturizers, hair care products and sunscreens where they act as preservatives in the products and also help prevent skin damage by the sun due to their antioxidative activities.

Caffeine belongs to a group of purine-based compounds collectively known as methyl xanthenes and contributes to the tea's bitter taste/astringency. Synthesis and accumulation of caffeine in the tea plant is genotype-dependent (Obanda and Owuor, 1997). Age/maturity of the tea crop has also been shown to affect the caffeine levels with the levels decreasing with age (Dev Choudhury *et al*., 1991). It is synthesized as an important secondary metabolite acting as a chemical protectant against invasion by pests and parasites (Ames *et al*., 1990). The presence and levels of purine alkaloids mainly caffeine in tea has long been considered an important quality indicator due to its psycho-active; mood and cognitive-enhancing properties (Yang *et al*.,2007b; Nagatomo and Kubo, 2008) although its consumption has been associated with adverse health effects such as anxiety, headaches, tremor, hypertension and insomnia necessitating development of decaffeinated beverages. Selection of low caffeine tea clones such as TRFK 306 clonal series, TRFK 687/1, TRFK 73/7 and TRFK 830/12 offers a better alternative to caffeine sensitive consumers since the decaffeination process could result in loss of attributes such as aroma. However, clones endowed with high caffeine levels such as TRFK 12/12, TRFK 11/52, STC B9, TRFK 430/52, TRFK 400/7, TRFK 371/3, TRFK 371/5, TRFK 381/5, TRFK 381/1, TRFK 829/3 and TRFK 830/6 should be utilized in manufacture of caffeine-rich teas. Owing to its pharmacological properties, caffeine has been often included in some over the counter analgesics (Kerrigan and Lindsey, 2005) and also added as an ingredient in most commercial soft drinks in the market such as cola, chocolates and energy drinks (Klosterman, 2006). Therefore extraction of caffeine from the caffeine-rich clones for use as health supplement or to fortify foodstuff provides a viable market for diversified tea products. This line of value addition ought to be embraced since production

and marketing of food stuffs with important dietary components are being determined solely by differing consumer tastes, a strategy that will serve to increase demand for our tea products hence returns.

Anthocyanins are a group of flavonoid compounds that contribute to the attractive colours of fruits, vegetables and flowers imparting red, orange, purple, violet and blue colours (Feild *et al*., 2001; Kerio *et al*., 2012). They have been greatly used in the food industries as natural food colorants (He and Giusti, 2010), preservatives and in the manufacture of cosmetics such as soaps and shampoos but recently many studies are focusing on their nutritional value (Choi *et al*., 2007). Research studies have supported their role in the prevention of chronic diseases (Mazza, 2007 further increasing their use as nutritional and pharmacological supplements. In particular, their radical scavenging and antioxidant capacity coupled with the fact that they are water soluble diversifies their use in food industries mainly to prevent lipid peroxidation which has been shown to contribute to deterioration in food quality and unpleasant odors. They have been shown to protect the tea plant against harmful UV radiation (Jansen *et al.,* 1998) and therefore their levels could be influenced by the prevailing environmental/growing conditions.

Tea is a potent antioxidant. In green teas, antioxidant activity is attributed to the polyphenolic composition and mainly the catechins in tea but in purple teas, both the catechins and anthocyanins are responsible for the activities in these purple colored teas. In this study, the low polyphenolic content (18.2%) of clone TRFK ST 536 led to its low antioxidant activity. This trend, however was not observed for the clone TRFK 463/63 which had a below average polyphenolic composition of 24.2% but recorded the highest antioxidant capacity. Additionally, high catechin content did not necessarily translate to a higher radical scavenging ability, an indication that other non-phenolic chemical compounds apart from the catechins could be contributing to the antioxidant activity or acting synergistically with the phenolics. In this study, a total of 79 clones performed better with a mean antioxidant capacity of 90.97% compared to the standard reference clone, TRFK 6/8 which recorded a radical scavenging ability of 90.39%.

From the correlation analysis (Appendix VI), the EGCG fraction possessed the strongest antioxidant activity (r = 0.055) compared to other fractions but there was no significant difference. It was followed by ECG (r= 0.033) then EGC(r =0.021) fraction while the total catechins content also showed contribution to the antioxidant activity ($r = 0.050^{NS}$). A previous study by Karori *et al*., (2014) similarly showed EGCG as the strongest antioxidant

catechin fraction. His work however differed with the present study in the pattern of antioxidative strength of the EGC and ECG fractions whereby he found EGC possessing strong activity than ECG. The total polyphenolic content of the studied clones showed a significant ($P \leq 0.001$) positive correlation to the antioxidant activity (0.209**).

Several structural characteristics are considered important for the observed catechin's antioxidant activities including a 3'4'-dihydroxy-catechol or a 3'4'5'-trihydroxylcatechol in the B ring, hydroxyl groups at positions 5 and 7 in the A ring and a gallic acid group at position 3 of the C ring (Frei and Hidgson, 2003). The radical scavenging activities of catechins thus depends on their level of hydroxylation with the gallated catechins EGCG and ECG possessing the highest number of hydroxyl groups (8 and 7 respectively) hence responsible for much of the antioxidant activity in green tea. These hydroxylation patterns enables these polyphenolic compounds to donate hydrogen atoms (possible mechanism shown below) to scavenge for the free radicals and therefore the many the hydroxyl groups the higher the observed scavenging abilities.

Polyphenols-OH $+R^{\bullet} = Pol\text{-}O^{\bullet} + RH$

Where, $R^{\bullet}$ is the free radical, RH is the reduced stable molecule and $Pol\text{-}O^{\bullet}$ is a less reactive flavonoid compound.

These results are consistent with those of Guo *et al*, (1999) who investigated the scavenging effects of tea catechins with electron spin resonance and found that the scavenging abilities of gallated catechins (EGCG and ECG) are stronger than that of the non-gallated catechins (EGC, EC and C). Similar observations that catechin's scavenging abilities increase with the level of hydroxylation in the structure were reported by Rice-Evans (2001). This observation is attributable to the presence of a gallate group (contributing three hydroxyl groups) attached at position three of the gallated catechins. Similarly, EGC exhibited more free radical scavenging ability than EC which could be explained by the extra hydroxyl group attached at the 5' position in the B ring of their structure. From the correlation analysis results, the total catechin content and the catechin fractions showed a positive correlation with the observed antioxidant activities except for the EC and C fractions which showed a negative correlation though not statistically significant. In purple coloured teas, several parameters are believed to be responsible for the antioxidant efficiency of anthocyanins. These include the level of hydroxylation in the molecule, the catechol moiety in the B-ring,

the oxonium ion structure in the C-ring, the hydroxylation and methylation pattern and to the acylation by phenolic acids (Prior and Wu, 2006).

Tea polyphenols and specifically the catechins have been shown to possess antioxidant activities even stronger than that of plant derived vitamins such as vitamins C and E (Rice-Evans *et al*., 1995). Artificial antioxidants in the market such as butylated hydroxyanisole and butylated hydroxytoluene are facing consumer rejection due to their associated safety concerns such as adverse side effects. In the food industries, natural antioxidants are being used to prevent lipid peroxidation occurring in food products which causes declining quality and shortened shelf life. Tea polyphenols and other bioactive molecules such as caffeine and theanine in high quality tea beverages and food products are considered cost-effective and therefore readily available to consumers who are demanding for natural food products with health-promoting benefits. The use of these bioactive components in the food industry as functional ingredients (nutraceuticals) will serve to appeal consumers thus widening their market potential. They can therefore be extracted, purified and developed as food supplements as well as drugs to treat and prevent chronic disorders and diseases such as cancer and cardiovascular diseases.

Chlorogenic acid is a phenolic acid found mainly in green coffee beans but also in other plants although in smaller quantities such as tea, sunflower seeds, potatoes, fruits such as apples, pears, eggplant and blueberries (Farah and Donangelo, 2006). With scanty published information available on its availability in the tea plant, this study aimed at establishing quantities synthesized by the tea plant and their importance in diverse product development. Studies on chlorogenic acid have been primarily on sources such as coffee and thus there is scarce information on its availability in tea (Olthof *et al*., 2001). Results obtained in this study revealed that clones AHP SC 31/37, TRFK 831/8 and TRFK 6/8 had the highest quantities (0.13%, 0.07% and 0.06% respectively) and are thus suitable in manufacture of high chlorogenic acid teas. Further, the chlorogenic acid can be extracted and the resulting products sold as health enhancing nutraceuticals with anti-glycemic and anti-obesity benefits. Previous studies on chlorogenic acid in coffee have shown that it possesses health benefits such as reduction of the relative risk of cardiovascular disease (Ranheim and Halvorsen,2005), diabetes type 2 (Salazar-Martinez *et al*., 2004), Alzheimer's disease (Lindsay *et al*., 2002), antibacterial (Almeida *et al*., 2006), anti-inflammatory activities (Santos *et al*.,2006), antihypertensive effect in humans (Watanabe *et al*., 2006), inhibitory effect on fat accumulation and body weight in mice and humans (Shimoda *et al*.,2006), modulation of glucose metabolism in humans (Van Dijk *et al*., 2009) and reduces risk of liver

diseases such as cirrhosis and liver cancer (Tverdal and Skurtveit, 2003; Gelatti *et al*., 2005). These health benefits derived from chlorogenic acid have necessitated its extraction from green coffee beans and they are currently being sold as health products in the market. Given its potential health benefits, inclusion of chlorogenic acid as a novel quality marker in tea may help in branding our tea products, further increasing the demand for our tea. Indeed, further research studies should be carried out using relevant animal models to establish if these comparably low levels of chlorogenic acid in tea do exhibit significant physiological benefits in humans.

Theanine is the most physiologically important and abundant amino acid component in tea leaves responsible for giving green tea infusions the sweet umami taste (Balentine *et al*., 1998). Clones with high theanine levels can be utilized as raw materials for production of theanine rich green teas. Owing to its significant contribution to taste and reported health-enhancing benefits particularly on the CNS by inducing a state of mental alertness, relaxation and anti-hypertensive abilities, its content in tea leaves is considered a very important aspect of tea quality. Additionally, green teas with high theanine levels have been shown to command high market prices (Golding *et al*., 2009); further emphasizing the importance of identification of theanine rich tea clones. Interestingly, the tea plant also stands out as the main available source of theanine in the human diet, the second source being the mushroom *Xerocomus badius* (Juneja *et al*., 1999). Previous studies have shown that accumulation of theanine in tea leaves is influenced by sunlight whereby shaded tea plants accumulate more theanine compared to those exposed to sunlight (Kito *et al.*, 1968). Exposure to sunlight converts theanine into its constituent compounds, glutamic acid and ethylamine which is further utilized by the tea plant in synthesis of catechins.

Plants usually produce these diverse chemical compounds as secondary metabolites mainly as defense mechanisms against harmful ultra violet radiation, insects, birds, and animals which would otherwise consume the plant as food (Beart *et al*., 1985). Differences in composition and levels of these biomolecules among the tea clones are responsible for the resulting differences in quality of the manufactured tea products ((Owuor *et al*., 2010a; Owuor *et al*., 2010b). These biomolecules and especially catechins, caffeine and theanine are used as quality indicators in green tea (Le Gall *et al*., 2004). Traditional methods for tea quality assessment depend on professional tea tasters who grade manufactured tea based on appearance (color and color intensity), aroma (sweet, floral, grassy among others) and taste (sweet, bitter and astringent), which at times could be subjective. Further, selection of

suitable clones for commercial cultivation has placed a lot of emphasis mainly on their performance in terms of yield. In the recent years, there has been an increasing need to assess and characterize for the quality of tea by assaying for the bioactive compounds that give tea its unique quality characteristics. Theanine is responsible for the sweet brothy umami taste while both catechins and caffeine give green tea the characteristic astringency and bitterness. Among factors determining the types and levels of these biomolecules include genetic factors such as clones/varieties of the tea bushes and environmental factors such as climate and altitude of the growing region. Other factors such as agronomic factors (farm management, harvesting practices and fertilizer applications) and processing procedures employed are also important but can be controlled. These differences in the green leaf composition allows for selection and manufacture of varying tea products as dictated by consumers/market demands and preferences.

5.2 Regional comparison

There were significant interactions ($p \leq 0.05$) between the growing region and the clones an indication that synthesized catechins and caffeine differ in regions and between the clones. This is because while clonal performance is primarily genetically linked, the growing region and the underlying conditions (Owuor *et al*., 2010a; Owuor *et al*., 2010b; Kwach *et al*., 2013) have some level of influence on the synthesis of the biomolecules as evidenced in this study where the studied clones performed differently in the two study sites. Similar results showing significant interactions between the biochemicals in the assayed clones and sites of production were obtained from a previous study by Kwach *et al*., (2013). This implies that the two factors, region and clone should be taken into consideration during tea production to ensure that high quality of the final product is achieved. From the total catechins results, several clones showed wide variations between the two regions such as EPK C12 with 22.2% in Kangaita and 16.5% in Timbilil, TRFK 100/5 with 23.9% in Kangaita and 16.7% in Timbilil. Catechins are the most biologically active biomolecules in green tea in addition to contributing to green teas astringent taste. Green tea catechins are also important precursors of theaflavins and thearubigins in black teas and are therefore important quality markers. The clones in Kangaita therefore provide the suitable raw materials for production of diversified catechins-rich products such as green teas and catechin extracts. These observations of wide variations in catechins between the two regions imply that clones should be evaluated for their performance in the intended cultivation area to test their suitability before their release. However, in clones such as TRIT 201/55, the variation in the

two regions was not statistically different with 20.0% in Kangaita and 19.8% in Timbilil, an observation consistent with that of a study by Wachira *et al*., 2002 suggesting that irrespective of the region of production, some tea genotypes exhibit resistance to yield variations and thus quality. This observation implies that such clones with least variation between the two growing regions are more stable and thus will have little variations when grown in other tea growing regions of different climatic conditions. Utilization of such clones with adaptations to varied environmental conditions will ensure increased and consistent production of tea with uniform biomolecule composition and therefore produce teas of uniform quality. This observation is similar to a recent study by Omwoyo *et al*., (2014) who found significant differences in chemical quality although based on the micronutrient levels between teas from East and West of the Great Rift Valley. It is clear from the results that most of the clones in Kangaita perform better compared to those in Kericho based on their total and individual catechins. From these results, the influence of regional differences and their underlying climatic conditions on the differential synthesis and accumulation of these bioactive compounds is responsible for quality in tea. This is in agreement with previous studies (Owuor *et al*., 1990a; Owuor *et al*., 2008; Owuor *et al*., 2010a; Owuor *et al*., 2010b; Wei *et al*., 2011) whereby factors such as seasonal climate, altitude, temperature and total rainfall density have been shown to affect the tea's yield, overall growth rate and synthesis and accumulation of the chemical compounds responsible for the quality characteristics.

Altitude has an influence on the growth of the tea plant whereby shoots of teas grown in high altitude regions grow and mature slowly and in the process accumulate high amounts of these polyphenolic catechins (quality markers) and develop a richer flavor (Squire *et al*., 1993; Pruess and Joanna, 2006; Chen *et al*., 2010). This agreed with the present study in caffeine composition. However, this study differs with such observations on catechins levels in that Kangaita has a relatively lower altitude (2020 m) than Kericho (2180 m) yet the tea clones performed better implying that other factors could be contributing to the observed differences. Temperature could have contributed to this observation since Kangaita is located near Mt. Kenya which is relatively cooler throughout the year with a mean annual temperature of 15.5°C compared to 16.6°C in Timbilil. This environment induces a form of dormancy in the rate of shoot growth consequently increasing the accumulation of catechins in these clones. High temperatures on the other hand stimulate a fast shoot growth but with low levels of these biomolecules (Chen *et al*., 2010). Similar results were observed in studies by Mori *et al*., (2005) who reported that elevated temperature reduced flavonoid accumulation and inhibited the gene expression of the enzymes in phenylpropanoid and

flavonoid biosynthetic pathway; chalcone synthase (CHS), flavanone 3- hydroxylase (F3H) and dihydroflavonol 4-reductase (DFR) in grape berries.

Differences in the amounts of rainfall received in the two regions could have played a role in the variation of catechin composition. Kangaita has a mean annual rainfall of 2040 mm with peaks in the months of April/May and October/November while Timbilil receives 2175mm of rainfall spread through the year except in the dry season (December-February). A high precipitation like in Timbilil stimulates a fast shoot growth rate resulting in high yields but producing low quality teas compared to the relatively low precipitation rate in Kangaita, an observation supporting an earlier study (Odhiambo *et al*., 1988). It is worth noting that whereas a slow rate of shoot growth contributes to a high synthesis and accumulation of catechins, the mean productivity decreases owing to the fact that development of pluckable shoots takes much longer thus reducing plucking intensity (Obaga and Ng'etich 1989; Squire *et al*., 1993; Anandacoomaraswamy *et al*., 2000). It's important to note that clonal genetic differences also affects their individual ability to absorb nutrients even under similar agronomic practices and this consequently affects their ability to synthesize and accumulate these bioactive molecules such as catechins (Wanyoko and Njuguna, 1983; Yemane *et al*., 2008). This explains why some clones in Kangaita had relatively low total and individual catechin content compared to the same clones grown in Timbilil even when the conditions in Kangaita seemingly impacted positively on the catechin levels in majority of the clones.

Caffeine analysis showed that growing conditions in Timbilil favored the synthesis and accumulation of caffeine more than Kangaita. A typical example in the variation was observed in clones such as BBK 35 with caffeine levels of 3.3% in Kangaita and 5.6% in Timbilil and clone TRFK 400/10 with 3.3% in Kangaita and 5.28% in Timbilil. Like catechins, synthesis and accumulation of caffeine has been shown to be affected by climatic factors (Suzuki *et al*., 1992). Studies by Lee *et al.,* (2010) and Wang *et al*., (2011) revealed that a high relative humidity stimulated caffeine synthesis in tea plants, observations supported by this study. Timbilil has a relatively high annual rainfall (2175 mm) compared to 2040 mm in Kangaita. Consequently, the high mean temperature in Timbilil (16.6°C) seems to increase synthesis of caffeine in the tea clones compared to those in Kangaita with relatively low mean temperature (15.5°C). This can be explained by the fact that caffeine synthesis by plants is usually in response to adverse climatic conditions such as the relatively high temperature in Timbilil. The high caffeine levels in clones grown in Timbilil could also be attributed to the high altitude of 2180m compared to the relatively low altitude of 2020m in Kangaita. This observation on the influence of altitude on caffeine synthesis concurs with a

study by Owuor *et al*. (1990a) in which they found that a high altitude significantly influenced caffeine synthesis in tea plants by slowing down the rates of shoot growth consequently increasing their accumulation.

The differences in the climatic conditions (rainfall intensity and mean annual temperature) between the two regions are not so wide but significant differences in the levels of catechins and caffeine were observed. This observation corroborates a study by Wachira *et al*., (2002) and refutes earlier assumptions stating that larger climatic differences were necessary for significant quality differences to be observed and that a superior clone selected in one location maintains its desirable attributes within the country. In conclusion, the obtained results imply that it's impossible for tea farmers in different regions to produce tea products of uniform quality. Therefore a high performing clone in one region should be checked for its stability in new regions to ascertain if it maintains relatively high levels of the desired biomolecules. This is desirable since it could lead to development of region-specific clones. Additionally, Kangaita region, favoring synthesis of relatively high quantities of most biomolecules should be considered for growing teas for manufacture of high quality teas or for extraction of biomolecules for use in pharmaceutical and cosmetic industries.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

1. Significant clonal differences in the levels of the assayed biomolecules (catechins, caffeine, total polyphenols, theanine, chlorogenic acid and antioxidant activities were observed except for the simple catechins (+C) and anthocyanins. Different clones exhibited superiority in composition of particular biomolecules making them suitable in manufacture of high value tea products such as high/low astringent green teas, theaflavin-3,3-digallate rich black teas, polyphenols rich black teas and caffeine rich/low black/ green teas.

a. Forty six (46) clones had a total polyphenol composition higher (mean value of 28.11%) than the reference standard clone, TRFK 6/8 (27.4%), making them suitable for the manufacture of high quality black tea.

b. Fifteen clones with optimum ECG and EGCG levels were identified as suitable for the manufacture of theaflavin-3, 3-digallate rich black teas.

c. Clones 301/4 and 301/5 were found to contain low EGCG/ECG but high EGC/EC levels suitable for the manufacture of less bitter/astringent green tea.

3. Significant amounts of theanine and chlorogenic acid were found among the 15 clones assayed, with clones TRFK 6/8 and AHP SC 31/37 recording the highest contents respectively. This makes the clones suitable in the manufacture of theanine rich and chlorogenic rich black or green teas

4. Clones from Kangaita possess significantly high catechin (total and individual) contents compared to those in Timbilil while Timbilil clones contained high caffeine levels than Kangaita clones with a few exceptions.

6.2 Recommendations

1. The selected clones with suitable levels of biomolecules should be utilized in breeding programmes or during manufacture process whereby specific clone(s) with desired chemical combination can be manufactured to suit different consumer preferences.

a) For those individuals sensitive to caffeine due to health issues, clones containing low caffeine levels such as TRFK 687/1 can be used in the manufacture of low caffeine beverages.

b) Clones with low EGCG, ECG and caffeine should be utilized in the manufacture of less astringent green tea products for consumers who desire less astringent green tea beverages among others.

c) Clones with appropriate ratios of the individual catechin fractions should be carefully selected for development of black teas rich in specific theaflavin fractions e.g. Black teas rich in theaflavin-3, 3'-digallate, the most biologically active theaflavin fraction, should be manufactured from the clones with relatively high levels of EGCG and ECG fractions. This will ensure that the resulting black tea products are of high qualities hence able to increase the demand for our black tea consequently increasing returns.

d) Analysis for the other polyphenolic compounds such as quercetin should be done to determine their individual contribution and effectiveness towards tea's antioxidant activities.

2. Further characterization for theanine and chlorogenic acid on the rest of the clones should be undertaken. Screening for Chlorogenic acid should be incorporated in the various tests done on fresh tea leaves and tea products as a novel and important quality marker. Further studies should also be done with appropriate animal models to investigate whether the levels of chlorogenic acid contained in tea exhibit significant physiological effects in humans.

3. For high levels of catechins (total and individual) of the fresh green leaf, clones in TRI's Kangaita substation should be considered. Timbilil clones should however be used when a high caffeine level is desired. All the climatic factors such as altitude, average rainfall and temperature in tea growing regions should be considered during tea production. This will allow for the development of region-specific clones better suited to the underlying climatic conditions which will ensure that optimum levels of these biomolecules are achieved.

Further studies should be done to evaluate the influence of seasonal variation (not done in this study) on the levels of these biomolecules throughout the year. This will give insight on the best season to harvest teas with the highest/optimum concentrations of biomolecules.

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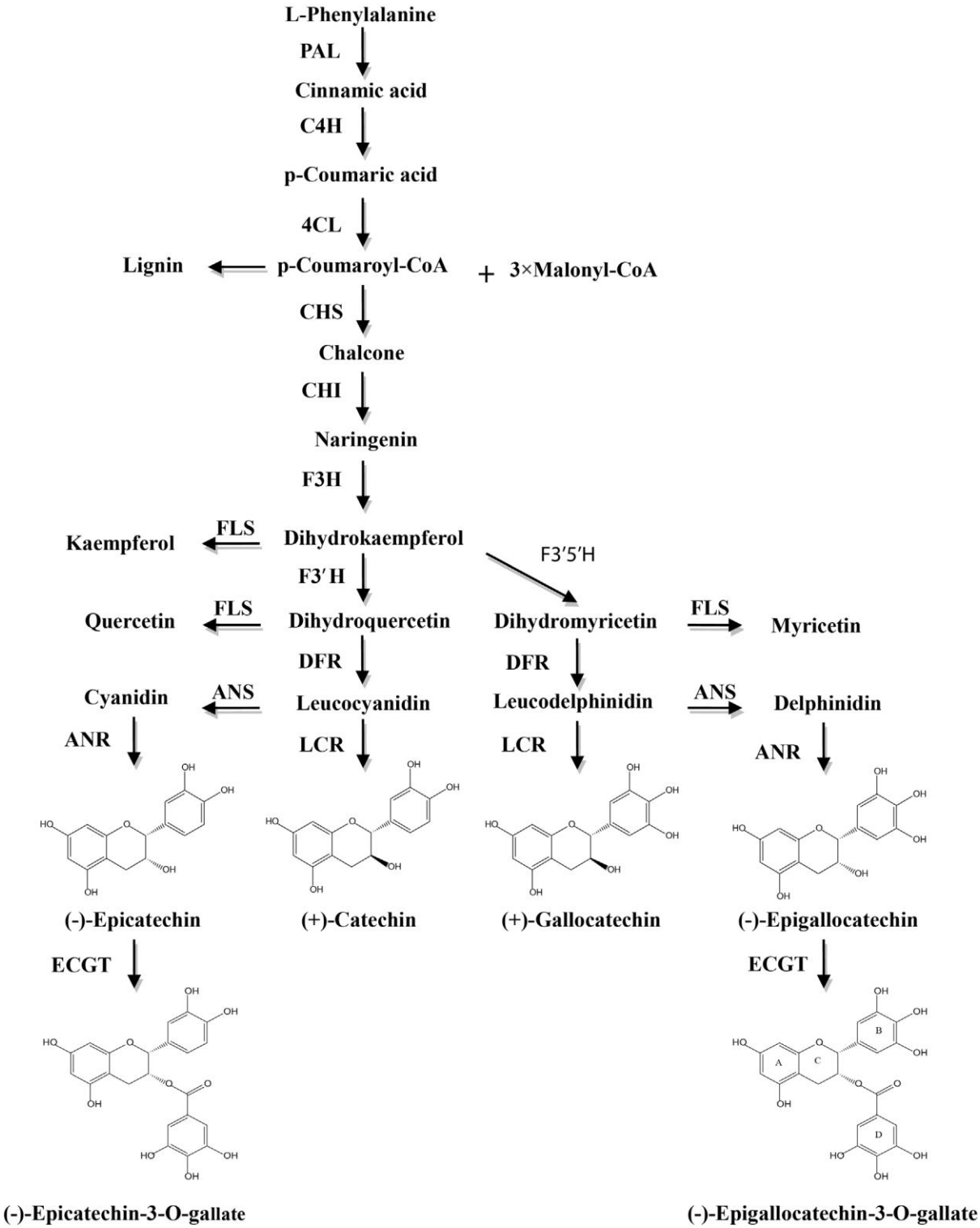
APPENDICES

Appendix I**:** Synthesis of flavonoid compounds in tea plant



Appendix II: Results for clonal variations in the levels of the assayed biomolecules

				Levels of the assayed biomolecules (%)								
No.	**Clone**	**Variety**	**status**	**TP**	**EGC**	**C**	**Caff**	**EC**	**EGCG**	**ECG**	**TC**	**AA**
1	EPK TN14-3	C	R	25.8	8.70	0.51	3.52	1.90	8.33	2.68	22.11	88.16
2	EPK H81/22	C	R	27.1	8.83	0.30	3.01	2.64	8.66	2.77	23.19	89.63
3	EPK C182/40	A	R	25.8	8.54	0.61	3.10	1.88	8.06	2.58	21.56	89.31
4	EPK D99/10	A	R	25.8	9.80	0.78	3.02	2.15	6.10	2.02	20.85	89.95
5	EPK C12	C	R	28.3	8.77	0.66	3.43	2.06	8.78	3.53	23.78	89.86
6	TRFK 31/8	A	R	27.1	8.77	0.66	3.82	1.40	11.09	2.46	24.38	90.03
7	TRFK 12/19	A	R	28.9	6.76	0.67	3.67	1.02	11.09	3.43	22.96	90.26
8	TRFK 12/12	A	R	29.5	5.69	0.77	4.26	2.02	8.81	3.77	21.05	89.55
9	TRFK 11/52	A	NR	27.8	7.86	0.53	4.09	1.71	11.41	2.94	24.45	89.65
10	TRFK 11/26	A	NR	27.9	6.21	0.63	3.20	1.66	9.89	2.96	21.33	89.99
11	TRFK 11/4	A	R	27.6	6.23	0.58	3.79	1.66	10.69	3.01	22.16	89.32
12	TRFK 7/9	C	R	27.4	9.94	0.71	3.50	1.67	7.42	2.36	22.08	89.26
13	TRFK 7/3	C	R	25.9	6.71	0.68	3.32	2.04	8.42	2.94	20.79	90.84
14	TRFK 6/8	A	R	27.4	8.46	0.60	2.51	1.41	7.35	2.46	20.28	90.39
15	EPK SR/IU/49	A	R	26.6	6.86	0.62	3.29	1.94	8.85	3.35	21.61	90.60
16	EPK SR/18V/49	A	R	27.0	7.72	0.84	3.37	1.82	8.51	3.19	22.06	90.65

17	EPK SR/2B1/58	A	R	27.8	6.32	0.53	3.57	1.75	9.37	3.18	21.14	90.71
18	EPK SR/9A4/49	A	R	29.3	5.88	0.70	3.98	2.44	10.12	4.10	23.22	90.59
19	AHP PMC 45	A	R	27.6	7.67	0.66	2.68	1.99	7.57	2.40	20.28	90.79
20	AHP PMC 2	A	R	26.1	5.75	0.71	3.43	1.57	9.18	3.21	20.41	90.60
21	AHP S 15 /10	A	R	24.9	5.21	0.48	3.29	1.92	9.95	2.60	20.15	90.37
22	TRFK ST 331	A	R	25.8	4.76	0.71	3.52	1.41	9.47	2.79	19.13	90.59
23	TRFK 108/82	C	R	26.4	6.20	0.65	3.65	2.22	8.58	2.95	20.59	90.58
24	TRFK 100/5	A	R	27.8	8.60	0.55	3.12	1.68	10.62	2.52	23.96	89.83
25	TRFK 31/11	A	R	29.0	5.83	0.37	3.17	1.71	10.82	3.38	22.10	89.83
26	KTDA KAG 28	A	R	30.0	6.61	0.39	3.13	1.55	9.95	2.85	21.35	90.40
27	KTDA KAG 27	A	R	24.8	6.89	0.48	3.21	1.91	7.46	1.96	18.69	89.84
28	KTDA KAG 4	A	R	29.1	7.27	0.54	3.39	2.74	8.55	3.57	22.64	90.02
29	KTDA B 8	A	R	23.7	7.39	0.32	2.88	1.42	7.91	2.11	19.15	90.11
30	KTDA B 1	A	R	26.4	8.57	0.39	2.87	2.39	8.18	2.48	22.00	90.21
31	AHP PMC 46	A	R	26.4	6.69	0.48	3.03	2.04	8.51	3.55	21.25	90.19
32	AHP PMC 61	A	R	26.2	5.45	0.60	3.12	1.92	8.97	3.39	20.33	90.37
33	AHP B5/63	A	R	27.9	6.30	0.54	3.28	1.73	7.20	3.55	19.31	89.98
34	AHP F5/222	A	R	26.6	7.52	0.30	3.13	2.50	8.58	3.90	22.79	90.11
35	AHP F7/346	A	R	28.6	8.21	0.53	3.40	2.86	9.64	4.19	25.42	90.40

36	AHP M2/10/51	A	R	27.0	8.63	0.57	3.08	1.26	7.67	2.69	20.81	89.08
37	GW CHANGOI 1	C	R	27.4	7.56	0.41	3.29	1.62	8.80	2.65	21.03	89.34
38	STC B9 (38/1)	A	R	27.2	9.11	0.44	4.04	1.91	8.04	2.32	21.81	90.09
39	STC B 108 (38/3)	A	R	24.8	7.93	0.44	3.19	2.14	8.96	2.34	21.80	90.26
40	STC L6 (38/8)	A	R	25.5	6.76	0.70	3.36	1.57	7.76	3.29	20.07	89.32
41	MICHI (5/1/1/20)	A	R	25.8	7.22	0.58	3.27	2.01	6.86	2.28	18.94	89.55
42	BBK 2	A	R	26.9	6.85	0.73	3.76	1.50	8.77	2.51	20.34	90.12
43	AHP S8/38	A	R	28.3	6.15	0.62	2.81	1.93	8.56	3.56	20.82	90.40
44	AHP S7/268	A	R	25.9	6.73	0.56	3.36	1.47	8.91	2.59	20.26	89.79
45	AHP S1/99	A	R	26.6	7.69	0.53	3.51	1.07	8.83	2.59	20.70	89.52
46	AHP MN11/96	A	R	28.7	5.84	0.46	3.82	1.09	9.70	3.85	20.93	89.25
47	TRFK 56/89	C	R	24.5	5.76	0.62	3.16	1.03	10.86	2.93	21.20	89.79
48	TRFCA SFS 150	A		24.3	7.92	0.67	3.26	2.46	8.12	2.66	21.82	88.97
49	STC M3	A	R	27.2	5.39	0.62	3.24	1.22	9.62	1.03	17.87	90.35
50	STC M1	A	R	24.5	7.09	0.62	3.63	1.35	8.36	2.25	19.66	90.57
51	STC 5/3	A	R	26.8	6.12	0.59	3.04	1.49	8.78	1.56	18.52	90.64
52	BBK 152	A	R	30.8	7.19	0.80	3.87	1.76	9.58	3.27	22.59	89.61

53	BBK 5	A	R	28.2	6.49	0.54	3.88	1.73	10.67	3.43	22.86	89.68
54	BBK 7	A	R	29.2	6.12	0.55	3.54	2.21	11.05	3.86	23.80	89.40
55	BBK 10	A	R	26.3	5.63	0.52	3.09	2.07	8.59	1.89	18.83	89.61
56	TRFK 303/577	C	R	26.5	7.88	0.51	2.72	2.55	8.29	2.82	22.04	89.44
57	GW EJULU-L	C	R	30.8	6.10	0.70	3.44	1.15	7.02	5.91	20.88	89.32
58	TRFK 54/40	A	R	27.8	6.81	0.48	3.46	1.71	10.45	2.67	22.11	89.63
59	TRFK 7/14	C	R	27.5	6.94	0.39	3.59	2.56	8.16	2.77	20.82	89.22
60	BBK 207	C	R	25.7	5.92	0.61	3.97	2.17	7.92	1.78	18.38	88.51
61	BBK 35	A	R	24.4	7.08	0.69	3.89	2.05	9.56	2.81	22.19	89.59
62	BBK 21	A	R	27.2	4.77	0.61	3.35	2.29	8.83	3.43	19.92	89.96
63	TRFK 303/259	A	NR	26.3	6.46	0.65	3.89	2.18	9.31	2.62	21.22	89.12
64	AHP SC 12/28	A	R	29.8	5.42	0.48	3.21	2.84	8.00	4.48	21.20	89.52
65	TRFK 303/178	A	R	25.3	6.12	0.45	2.69	2.38	7.87	2.35	19.16	89.14
66	TRFK 303/216	A	R	25.9	7.95	0.52	2.85	1.13	6.56	1.93	18.09	89.06
67	TRFK K-PURPLE	C	NR	25.4	3.27	0.38	2.78	1.61	4.92	3.69	13.86	88.63
68	TRFK 1 II/2	A	NR	27.1	5.80	0.58	3.96	1.86	9.25	2.51	19.98	88.89
69	TRFK 1XV/5	A	NR	27.0	6.41	0.40	2.89	1.98	9.05	2.82	20.65	88.94
70	TRFK 301/1	Cambod	NR	28.0	5.98	0.47	3.57	4.15	6.37	4.84	21.81	89.19
71	TRFK 301/2	Cambod	NR	25.2	6.23	0.50	3.78	2.99	7.61	3.21	20.53	87.67
72	TRFK 301/3	Cambod	NR	26.2	8.67	0.60	3.56	3.12	7.47	4.98	24.82	87.34

73	TRFK 301/4	Cambod	R	24.4	7.26	0.52	2.78	3.81	4.97	5.16	21.72	88.58
74	TRFK 301/5	Cambod	R	25.4	7.54	0.55	3.60	4.24	5.85	3.97	22.13	88.16
75	TRFK 301/6	Cambod	NR	24.6	5.72	0.53	3.07	5.41	4.23	6.04	21.93	88.23
76	TRFK 2VIII/10	A	NR	24.7	6.55	0.41	3.75	2.30	9.23	2.77	21.24	87.74
77	TRFK 1II/7	A	NR	25.9	6.11	0.47	3.40	2.04	9.63	2.73	20.98	87.83
78	TRFK 2XVII/7	A	NR	25.8	8.10	0.38	3.65	2.61	8.43	2.39	21.89	87.76
79	TRFK 2VII/12	A	NR	25.7	5.57	0.58	3.62	1.39	8.93	2.78	19.23	87.82
80	TRFK 2VII/13	A	NR	25.1	5.65	0.55	3.32	1.76	9.86	2.13	19.93	87.89
81	TRFK 2XIX/10	A	NR	25.6	4.65	0.54	3.60	1.32	9.28	2.36	18.14	87.75
82	TRFK 400/10	A	NR	26.2	5.77	0.68	3.82	1.67	11.25	3.05	22.40	87.75
83	TRFK 430/4	C	NR	22.0	7.80	0.35	3.53	2.23	7.35	2.15	19.87	87.61
84	TRFK 371/6	A	NR	25.4	6.79	0.68	3.13	2.69	7.72	2.62	20.49	89.12
85	TRFK 430/52	C	NR	22.9	7.72	0.51	4.10	2.10	7.57	2.41	20.30	90.96
86	TRFK 430/63	C	NR	21.6	8.30	0.35	3.56	2.11	7.46	2.08	20.29	90.96
87	TRFK 371/2	A	NR	23.1	5.67	0.76	3.37	2.08	6.29	2.44	17.22	91.09
88	TRFK 400/4	A	NR	23.9	6.84	0.57	3.66	2.15	8.01	2.29	19.86	90.97
89	TRFK 378/1	A	NR	24.5	7.19	0.48	3.34	1.76	8.24	3.15	20.81	90.96
90	TRFK 371/8	A	R	24.6	7.03	0.55	3.55	1.38	7.82	2.73	19.50	91.12
91	TRFK 375/5	A	NR	24.0	6.02	0.60	3.63	1.90	9.46	2.81	20.79	90.97
92	TRFK 383/4	A	NR	25.9	7.28	0.64	3.76	1.79	8.23	2.58	20.51	90.58

93	TRFK 400/7	A	NR	25.1	6.60	0.61	4.14	2.09	8.29	2.24	19.83	90.72
94	TRFK 382/4	A	NR	24.4	6.57	0.50	3.83	1.51	8.42	2.49	19.48	90.25
95	TRFK 371/3	A	R	29.3	8.38	0.80	4.11	2.32	7.97	3.15	22.61	90.77
96	TRFK 371/5	A	NR	27.0	5.70	0.59	4.37	1.34	8.89	2.85	19.36	90.78
97	TRFK 381/5	A	NR	28.7	6.76	0.57	4.23	1.89	11.68	3.40	24.28	90.55
98	TRFK 381/1	A	NR	28.3	7.59	0.56	3.95	1.77	9.56	3.15	22.63	90.16
99	TRFK 430/90	A	R	24.5	7.94	0.40	2.83	2.53	6.34	1.94	19.13	89.87
100	TRFK ST 536	C	NR	18.2	5.37	0.33	2.96	1.59	6.38	2.14	15.78	84.71
101	TRFK ST 543	C	NR	19.4	6.80	0.32	2.78	1.52	5.27	1.53	15.43	86.62
102	AHP PC 81	A	R	24.3	7.31	0.41	2.25	1.04	6.28	2.17	17.20	88.83
103	AHP CG28V929		R	23.4	7.00	0.50	2.88	1.67	6.82	1.93	17.91	87.60
104	AHP SF 186		R	26.0	7.30	0.40	3.40	1.69	7.05	1.81	18.25	88.21
105	TRFK 616/4	A	NR	24.0	6.45	0.45	3.00	1.81	7.36	3.11	19.18	90.79
106	TRFK 632/1	Cambod	NR	26.0	5.37	0.53	3.07	2.60	7.37	3.97	19.82	91.15
107	TRFK 635/1	C	NR	28.4	7.20	0.30	3.11	2.68	6.46	3.84	20.46	91.38
108	TRFK 646/3	C	NR	26.2	6.46	0.55	3.35	1.67	5.75	3.43	17.84	88.54
109	TRFK 653/2	A	NR	26.0	7.52	0.43	2.69	3.14	5.71	4.05	20.85	88.54
110	TRFK 655/1	A	NR	27.5	5.90	0.51	3.20	4.60	5.03	5.23	21.26	88.59
111	TRFK 657/1	A	NR	25.4	2.41	0.24	3.31	0.78	4.56	1.49	9.46	88.11
112	TRFK 659/1	C	NR	25.5	8.36	0.50	3.50	2.15	8.72	2.78	22.50	88.70

113	TRFK 701/2	A	NR	26.1	5.92	0.57	3.08	1.75	7.70	3.63	19.56	89.78
114	TRFK 731/3	C	NR	24.4	6.83	0.45	2.77	1.71	6.79	2.64	18.41	89.56
115	TRFK 824/1	A	NR	26.7	4.85	0.43	3.27	2.57	6.42	4.60	18.85	90.03
116	TRIT 201/16	C	R	25.8	6.65	0.45	2.69	1.75	8.02	2.82	19.67	89.82
117	TRIT 201/43	A	R	26.1	5.43	0.59	3.11	0.82	7.80	2.17	16.81	89.46
118	TRIT 201/44	A	R	26.3	5.36	0.51	3.40	2.49	8.05	4.00	20.41	89.55
119	TRIT 201/47	A	R	26.2	6.76	0.64	3.11	1.78	10.52	2.61	22.29	89.47
120	TRIT 201/50	A	R	27.5	5.15	0.67	3.06	0.78	7.90	2.19	16.68	89.17
121	TRIT 201/55	A	R	24.3	6.06	0.38	3.32	1.29	8.78	2.92	19.42	89.46
122	TRIT 201/70	A	R	21.8	4.87	0.53	3.01	0.78	8.80	2.49	17.46	87.64
123	TRIT 201/73	A	R	23.9	4.79	0.62	3.12	0.80	8.24	2.30	16.74	89.64
124	TRIT 201/75	A	R	24.1	6.72	0.55	2.98	0.99	8.33	2.93	19.51	89.09
125	TRIT 201/82	C	R	22.5	6.22	0.37	2.82	1.57	7.89	2.08	18.11	88.04
126	TRFK 597/1	Cambod	R	28.5	5.76	0.53	3.41	1.57	7.62	3.57	19.04	87.93
127	TRFK 598/1	A	NR	26.4	6.18	0.57	3.32	2.46	6.00	4.53	19.73	86.29
128	TRFK 687/1	A	NR	24.2	4.97	0.24	1.96	1.50	4.21	2.31	13.22	88.45
129	TRFK 691/1	C	NR	26.8	5.35	0.34	2.78	1.90	5.96	3.28	16.81	87.93
130	TRFK 704/2	Cambod	R	24.9	5.94	0.50	3.79	2.01	8.71	3.37	20.52	85.94
131	TRFK 762/1	CA	NR	26.5	7.51	0.40	3.95	1.53	9.12	3.64	22.19	86.60
132	TRFK 18/16	A	NR	25.1	4.89	0.41	3.39	1.96	8.44	2.26	17.96	91.16

133	TRFK 18/20	A	NR	25.6	5.13	0.31	3.59	1.20	9.43	2.50	18.56	91.18
134	TRFK 18/24	A	NR	26.3	5.04	0.39	3.43	2.06	9.95	2.78	20.21	90.82
135	TRFK 829/3	C	NR	27.4	4.90	0.47	4.04	2.21	8.55	4.15	20.27	90.82
136	TRFK 829/4	C	NR	25.4	4.74	0.61	2.87	1.74	7.05	3.26	17.39	91.01
137	TRFK 829/6	C	NR	27.6	7.17	0.65	3.51	1.97	7.94	3.68	21.40	90.95
138	TRFK 829/7	AC	NR	27.9	7.40	0.45	3.76	1.17	8.81	3.84	21.66	90.82
139	TRFK 830/11	C	NR	18.2	6.47	0.40	2.74	1.79	5.89	2.88	17.41	86.45
140	TRFK 830/12	C	NR	21.1	4.97	0.38	2.14	1.16	5.51	1.58	13.60	90.17
141	TRFK 830/15	C	NR	19.5	6.26	0.42	2.59	1.46	7.50	2.01	17.64	89.96
142	TRFK 830/5	C	NR	19.0	6.40	0.44	2.38	1.00	6.57	1.58	15.98	87.19
143	TRFK 830/6	C	NR	17.8	5.10	0.69	3.11	1.58	7.30	2.36	16.02	88.01
144	TRFK 830/7	C	NR	19.1	6.62	0.32	2.50	1.21	7.53	2.06	17.73	90.64
145	TRFK 830/8	C	NR	18.3	6.29	0.48	2.53	1.32	6.91	1.72	16.69	88.14
146	TRFK 831/1	C	NR	23.4	6.35	0.50	3.15	1.96	7.21	4.50	20.51	89.92
147	TRFK 831/11	C	NR	26.8	6.94	0.56	3.59	1.62	7.97	4.27	21.37	90.76
148	TRFK 831/9	C	NR	24.9	5.10	0.53	3.14	1.83	6.32	3.38	17.14	91.07
149	TRFK 832/1	C	NR	23.3	4.75	0.33	3.38	1.56	8.75	3.84	19.22	90.87
150	TRFK 832/11	C	NR	21.0	6.98	0.50	3.25	2.14	8.23	3.63	20.66	90.85
151	TRFK 832/4	C	NR	23.2	4.07	0.40	3.09	2.13	6.67	3.55	16.82	91.55
152	TRFK 832/7	C	NR	26.4	5.89	0.65	3.32	2.17	5.44	3.58	17.72	91.60

153	TRFK 832/8	C	NR	24.7	7.25	0.35	3.52	1.59	6.09	5.64	20.92	91.55
154	TRFK 833/1	C	NR	16.4	5.54	0.30	2.91	1.55	6.64	3.16	15.11	88.75
155	TRFK 833/2	C	NR	19.3	5.96	0.41	2.40	1.58	6.56	2.91	17.41	88.09
156	TRFK 833/3	C	NR	18.1	5.49	0.36	2.48	1.00	6.23	2.20	15.27	88.65
157	TRFK 420/13d	A	NR	24.0	6.41	0.52	3.30	0.77	7.99	2.31	17.99	91.36
158	TRFK 430/152	A	NR	21.0	6.22	0.42	3.33	1.90	7.43	2.35	18.31	91.49
159	TRFK 463/63	C	NR	24.2	6.87	0.34	3.06	1.70	6.91	2.30	18.11	91.93
160	TRFK 503/2	A	NR	25.8	6.54	0.52	3.48	1.69	8.02	3.27	20.02	91.92
161	TRFK 962/16	C	NR	21.6	5.61	0.48	3.22	1.76	7.89	2.61	18.34	91.64
162	TRFK 962/20	C	NR	23.7	5.90	0.43	2.84	1.24	6.17	1.65	15.38	90.60
163	BBK 5	A	R	25.6	3.60	0.45	3.83	1.33	8.36	2.55	16.28	91.61
164	TRFK 73/5	C	NR	24.3	4.90	0.76	2.69	2.09	7.48	2.01	17.24	90.34
165	TRFK 73/4	C	NR	23.7	5.86	0.48	2.53	1.61	8.44	3.78	20.16	91.37
166	TRFK303/35	A	R	26.4	5.60	0.52	2.66	1.63	7.85	2.36	17.94	91.13
167	TRFK 73/1	C	NR	25.1	6.03	0.26	3.34	1.78	6.80	2.11	16.96	90.05
168	AHP SC 31/37	A	R	27.0	6.43	0.44	3.69	1.69	7.79	3.21	19.56	91.40
169	TRFK 400/1	A	NR	28.2	4.69	0.49	3.49	2.43	6.54	2.84	16.99	91.56
170	TRFK 73/3	C	NR	22.2	5.18	0.30	2.58	1.47	6.58	2.03	15.55	90.36
171	TRFK 303/186	A	R	27.4	6.16	0.60	2.54	1.49	7.36	2.31	17.92	89.54
172	TRFK 382/2	A	NR	28.4	8.84	0.55	3.74	2.80	8.57	3.04	23.79	90.67

173	TRFK KS3	A	NR	25.9	6.48	0.52	3.15	1.73	7.71	2.39	18.82	89.70
174	TRFK 52/1	A	NR	24.7	6.48	0.53	3.55	1.07	8.82	2.64	19.52	90.61
175	TRFK 76/3	A	NR	25.3	6.51	0.60	3.59	1.18	9.39	2.90	20.57	91.07
176	TRFK 91/1	A	NR	24.6	6.37	0.34	2.51	3.44	6.19	3.20	19.54	90.84
177	TRFK 73/3	C	NR	23.6	3.43	0.31	2.49	1.91	6.90	2.88	15.43	91.87
178	TRFK 306/1	A	R	24.1	2.02	1.52	2.21	1.17	3.73	3.64	12.09	90.86
179	TRFK 73/7	C	NR	23.4	6.68	0.52	2.04	1.29	6.42	2.82	17.73	90.17
180	TRFK 495/7	A	NR	21.1	5.71	0.36	2.67	2.52	5.53	3.25	17.36	91.42
181	TRFK 91/2	C	NR	25.1	5.10	0.49	2.90	2.17	4.69	3.84	16.27	91.63
182	TRFK 14/1	C	NR	24.9	6.15	0.54	2.90	2.34	6.28	3.14	18.46	90.48
183	TRFK 303/179	A	R	26.7	8.33	0.63	2.67	1.46	7.46	2.64	20.52	91.70
184	TRFK KS2	A	NR	25.1	9.33	0.52	2.68	1.79	7.57	2.29	21.50	90.92
185	TRFK 306/2	A	R	26.2	1.98	1.44	2.18	1.26	3.63	3.64	11.94	90.50
186	EPK M3	A	R	27.2	5.93	0.61	3.41	1.45	8.45	2.76	19.19	89.15
187	TRFK 382/4	A	NR	26.7	8.20	0.53	3.67	1.87	8.05	2.74	21.38	90.47
188	TRFK KS1	A	NR	25.0	9.10	0.64	2.43	2.75	6.55	2.00	21.02	90.89
189	TRFK 77/1	A	NR	27.5	7.58	0.56	3.52	2.43	6.77	2.68	20.00	90.13
190	TRFK 306/3	A	R	25.8	2.01	1.48	2.31	1.18	3.96	3.89	12.53	90.11
191	TRFK383/1	A	NR	29.7	6.22	0.50	3.65	1.31	8.76	3.78	20.56	90.55
192	TRFK 412/2	A	NR	27.8	8.50	0.63	3.23	2.08	6.40	2.15	19.74	90.59

193	TRFK 83/1	C	NR	25.0	7.77	0.62	3.24	1.21	7.02	2.36	18.95	89.80
194	TRFK 306/4	A	R	25.5	1.74	0.73	2.18	0.84	3.85	3.88	11.03	90.41
195	EPK M1	A	R	24.3	7.78	0.62	3.79	1.30	7.11	2.66	19.46	91.21
196	TRFK 395/2	A	NR	28.6	4.80	0.55	3.10	1.84	6.94	2.28	16.39	90.95
197	TRFK 73/2	C	NR	25.7	5.46	0.45	2.63	1.74	9.31	2.51	19.46	91.11
CV (%)				5.4	15.6	30.4	13	33.5	12.4	21.9	9.5	1.7
LSD (P≤0.05)				2.73	1.98	0.31	0.83	1.24	1.92	1.26	3.67	3.09

C- Clones of the Chinary origin

A- Clones of the Assam/Indian origin

AC-Assam chinary hybrid clones

R- Denotes clones already released for commercial cultivation

NR- Denotes test clones not yet released for commercial cultivation

CV%-Refers to the coefficient of variation observed for the specific analysis

Appendix III: Results for differences in catechins and caffeine between the two studied regions

		EGC		C		CAFF		EC		EGCG		ECG		TC	
No.	**Clone**	**K**	**T**	**K**	**T**	**K**	**T**	**K**	**T**	**K**	**T**	**K**	**T**	**K**	**T**
1	EPK TN 14-3	6.94	6.69	0.53	0.49	4.02	4.44	1.77	1.54	9.30	7.58	3.12	2.30	21.64	18.60
2	EPK H 81/22	6.99	7.29	0.60	0.44	4.02	4.20	2.08	2.01	7.66	6.41	2.93	1.85	20.26	17.36
3	EPK C182/40	7.83	6.83	0.62	0.57	4.00	3.90	1.38	1.38	8.00	5.65	2.35	1.64	20.85	16.08
4	EPK C12	6.64	5.62	0.84	0.55	4.24	4.11	2.03	1.41	8.90	5.63	3.78	2.01	22.19	16.51
5	TRFK 31/8	5.17	6.01	0.56	0.35	4.92	4.92	1.08	0.96	10.55	7.47	2.71	1.63	20.08	16.41
6	TRFK 12/12	4.47	5.68	1.04	0.92	5.18	5.57	1.49	1.33	9.52	6.84	4.14	2.74	20.66	17.51
7	TRFK 6/8	6.47	6.38	0.93	0.56	3.61	4.21	1.84	1.36	8.40	6.49	2.71	1.71	20.36	16.50
8	AHP S15/10	4.53	4.30	0.50	0.45	2.81	3.82	0.96	0.93	6.62	6.43	2.00	1.90	17.97	15.88
9	TRFK 108/82	7.30	6.30	0.72	0.55	4.50	4.69	1.66	1.32	8.44	6.47	2.80	1.92	20.93	16.57
10	TRFK 100/5	7.78	5.96	0.82	0.61	4.40	4.87	1.69	1.22	10.46	6.95	3.17	1.96	23.93	16.69
11	KTDA KAG 4	6.23	6.77	0.51	0.56	4.18	4.31	1.68	2.24	10.66	6.59	4.55	2.90	23.63	19.05
12	AHP F5/222	5.28	4.49	0.60	0.36	4.76	4.53	1.92	1.80	9.05	6.88	4.00	2.83	20.84	15.61

13	AHP F7/346	6.85	6.95	0.54	0.43	4.72	4.16	1.89	2.14	9.31	7.57	4.65	3.03	23.26	20.12
14	MICHI (51/10/20)	6.56	6.78	0.68	0.60	4.23	4.14	1.34	1.37	8.64	5.50	2.58	1.61	19.79	15.85
15	AHP S1/99	7.12	5.77	0.66	0.47	4.63	4.92	1.40	1.13	8.62	6.75	2.72	1.87	20.52	15.99
16	AHP MN1 1/96	6.17	5.10	0.75	0.48	5.39	4.93	1.52	1.21	10.43	7.63	3.94	2.49	22.81	16.91
17	TRFCA SFS 150	5.89	6.71	0.50	0.31	4.74	3.96	1.25	1.54	10.11	5.51	2.76	1.55	20.51	15.62
18	STC M3	3.86	4.28	0.53	0.59	4.36	4.29	0.82	0.89	9.58	6.09	2.78	1.82	17.57	13.68
19	STC M1	5.07	5.05	0.45	0.33	4.50	4.52	1.03	0.96	9.01	6.62	2.48	1.65	18.05	14.61
20	STC 5/3	6.29	4.02	0.67	0.36	3.49	3.89	1.41	0.95	9.16	7.03	2.55	2.17	20.09	14.52
21	BBK 5	4.39	5.14	0.68	0.53	4.84	3.95	0.91	0.91	9.91	7.14	2.83	1.89	18.71	17.16
22	TRFK 303/577+	5.99	6.67	0.65	0.40	3.54	2.46	1.89	1.91	8.59	5.47	2.98	1.77	20.10	16.22
23	GW EJULU-L	1.63	2.49	1.73	1.50	3.79	4.23	1.18	1.15	6.49	5.81	6.96	4.79	19.96	15.74
24	TRFK 54/40	6.41	5.66	0.57	0.38	4.37	4.35	1.22	0.99	9.78	9.44	2.89	2.61	21.56	19.07
25	BBK 35	5.48	6.75	0.68	0.78	3.33	5.60	1.19	1.35	5.68	9.41	2.20	3.07	16.41	20.78
26	BBK 21	3.76	4.31	0.70	0.61	4.33	3.73	1.34	1.35	9.34	6.14	3.98	2.21	19.12	14.34
27	AHP SC 12/28	5.27	5.91	1.36	0.81	3.99	4.10	2.21	1.67	7.33	6.76	3.30	3.00	19.68	18.14

28	TRFK K PURPLE	1.18	1.31	0.97	0.89	2.25	3.58	1.04	1.04	2.91	3.79	2.87	2.19	8.96	9.21
29	TRFK 301/1	3.51	4.10	0.95	0.82	3.79	5.51	2.09	2.72	8.64	4.50	5.08	3.16	20.27	15.30
30	TRFK 301/2	4.88	4.54	1.19	0.79	4.52	5.16	2.26	1.50	8.43	5.67	3.50	2.19	20.26	14.70
31	TRFK 301/3	2.70	3.92	0.78	0.80	3.99	5.16	1.28	1.70	9.54	6.37	4.30	3.24	18.60	16.03
32	TRFK 301/4	2.03	3.44	0.44	0.31	3.78	3.24	1.66	2.34	7.67	3.36	5.87	2.97	17.68	14.90
33	TRFK 301/5	4.77	7.00	0.64	0.51	4.38	4.84	2.79	3.75	7.17	5.78	4.84	3.47	20.22	20.52
34	TRFK 301/6	2.60	3.62	0.83	0.48	3.61	3.59	3.49	4.44	6.14	4.25	6.39	4.80	19.46	17.59
35	TRFK 400/10	5.08	3.80	0.55	0.42	3.33	5.28	1.22	0.93	7.21	9.04	2.21	2.34	18.09	16.68
36	TRFK 371/8	5.06	4.88	0.85	0.56	3.64	5.40	1.46	1.34	7.47	6.91	2.73	2.25	17.57	15.95
37	TRFK 371/3	7.69	6.58	0.94	0.60	4.68	6.44	1.93	1.33	8.64	8.49	3.12	2.49	22.34	19.50
38	TRFK 430/90	7.61	7.23	0.70	0.55	3.80	4.34	1.97	1.53	7.42	8.97	2.29	2.52	20.00	20.79
39	TRFK ST 536	4.34	3.97	0.41	0.26	2.72	3.32	1.24	1.16	6.44	4.94	1.96	1.31	14.38	11.65
40	TRFK ST 543	4.73	4.83	0.56	0.29	2.90	3.26	1.60	1.28	7.06	5.18	2.04	1.40	15.99	12.97
41	TRFK 306/1	1.68	2.05	0.80	0.83	2.04	2.67	0.92	1.10	4.39	4.09	4.55	2.68	11.04	10.76
42	TRFK 306/2	2.16	1.84	0.86	0.77	2.17	2.53	1.01	0.99	4.60	4.02	4.97	2.93	14.62	10.54

43	TRFK 306/3	2.11	2.12	0.91	0.88	2.24	2.38	1.11	1.14	4.76	4.45	5.18	4.65	14.06	13.24
44	TRFK 306/4	2.05	2.03	0.80	0.89	2.05	2.42	0.94	1.06	4.43	4.23	5.00	2.98	14.40	11.20
45	TRFK 91/1	1.25	1.94	0.58	0.73	1.06	3.13	0.53	0.68	1.47	2.82	3.20	4.90	7.03	9.62
46	AHP SC 31/37	5.48	5.97	0.59	0.77	4.31	4.77	1.15	1.42	8.28	9.75	2.52	3.13	18.03	21.04
47	TRFK 400/1e	6.53	4.13	1.06	0.43	4.84	4.13	1.88	0.92	9.33	8.29	3.97	2.15	22.78	15.93
48	AHP PC 81	6.20	4.82	0.60	0.61	3.48	4.04	1.23	1.39	7.34	6.75	2.36	2.15	20.46	15.72
49	AHP SF 186	4.70	4.52	0.42	0.37	3.97	4.33	1.04	1.05	8.27	7.35	2.36	1.73	18.24	15.02
50	AHP CG28V929	4.98	3.07	0.45	0.75	3.72	3.56	1.13	0.85	7.29	5.65	2.24	2.08	16.93	12.39
51	TRIT 201/16	5.39	4.84	0.60	0.59	2.38	3.23	1.52	1.39	4.52	6.90	1.66	2.31	15.53	16.02
52	TRIT 201/43	7.16	6.06	0.80	0.60	4.06	3.90	1.63	1.23	7.04	7.36	1.88	1.87	18.50	17.12
53	TRIT 201/44	5.91	5.84	0.84	0.75	3.13	4.02	1.53	1.47	5.28	7.92	1.74	2.34	15.31	18.32
54	TRIT 201/47	3.55	4.87	0.52	0.52	3.46	3.99	1.05	1.08	8.37	10.73	2.66	2.60	16.16	19.81
55	TRIT	7.27	5.71	0.86	0.68	3.53	4.04	1.66	1.24	7.01	7.45	1.98	2.08	18.78	17.17

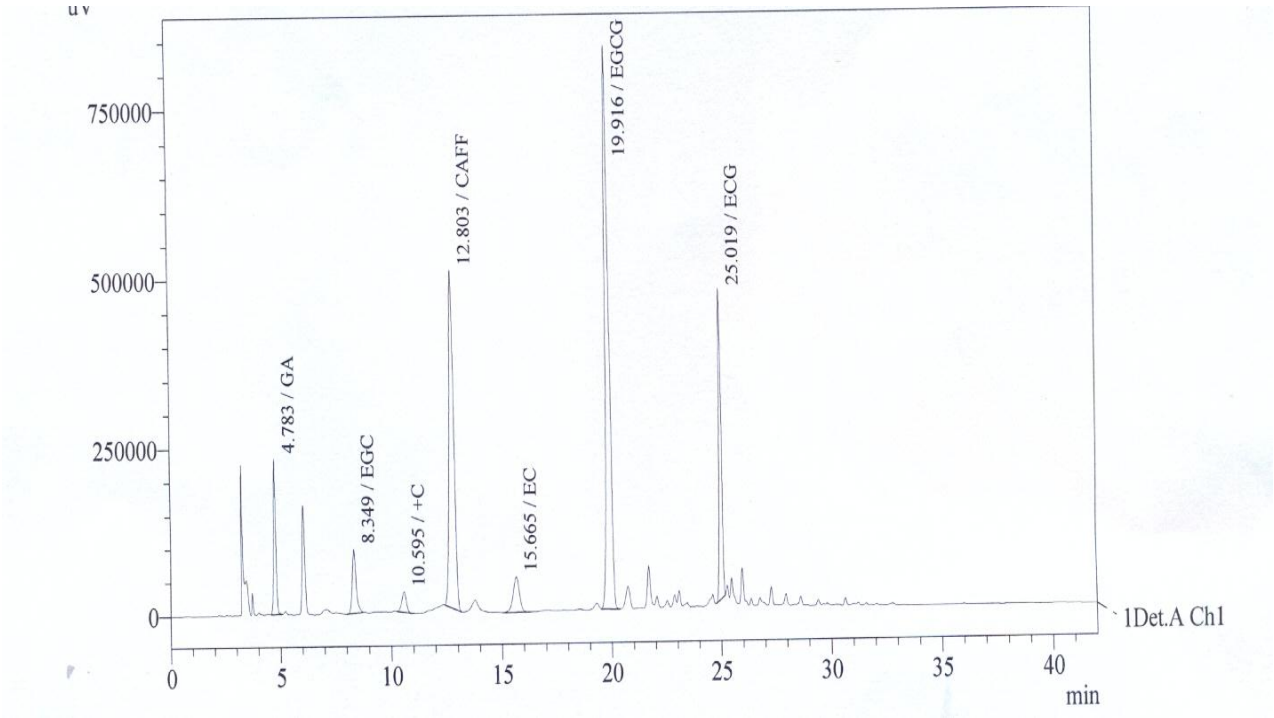
	201/50														
56	TRIT 201/55	5.60	5.34	0.79	0.64	3.74	4.33	1.90	1.33	8.47	9.35	3.21	3.08	19.97	19.75
57	TRIT 201/70	6.70	4.95	0.55	0.53	3.33	3.74	1.51	1.28	7.92	9.39	2.50	2.61	19.83	18.76
58	TRIT 201/73	5.87	4.64	0.63	0.61	2.61	4.23	1.07	1.01	5.50	7.81	1.45	1.90	16.86	15.97
59	TRIT 201/75	6.63	3.65	0.65	0.46	3.87	4.21	1.65	0.98	9.70	9.89	2.98	2.70	21.60	17.68
60	TRIT 201/82	6.13	6.15	0.68	0.54	2.81	3.35	1.39	1.09	5.39	7.12	1.40	1.64	15.00	16.54
CV (%)		10.2		10.7		5.2		5.7		5.1		8.6		5.0	
LSD (P≤0.05)	Region	0.11		0.08		0.04		0.02		0.08		0.05		0.18	
	Clone	0.59		0.01		0.24		0.10		0.43		0.28		0.99	

Appendix IV: Analysis of variance table for the regional variation in total catechins (TC)

Variate: TC

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr**
Rep stratum	2	5.1806	2.5903	3.42	<.001
Rep.* Units* stratum					
Clone	59	2651.0542	44.9331	59.26	<.001
Region	1	566.5071	566.5071	747.08	<.001
Clone.region	59	599.8956	10.1677	13.41	<.001
Residual	238	180.4753	0.7583		
Total	359	4003.1129			

Appendix V: A representative HPLC elution of the individual catechins fractions in clone TRFK 6/8



Key

X- Retention time in minutes

Y- Intensity, µV

GA- Gallic acid

EGC- Epigallocatechin

+C- Simple catechins

Caff- Caffeine

EC- Epicatechin

EGCG- Epigallocatechin gallate

ECG- Epicatechin gallate

Appendix VI: Correlation analysis table of the various assayed biochemicals

	AA	C	TC	EC	ECG	EGC	EGCG
TP	0.209**	0.322***	0.521***	0.160*	0.358***	0.146*	0.375***
EGCG	0.055	0.230**	0.593***	-0.248***	-0.062	0.012	
EGC	0.021	0.256***	0.60***	0.146*	-0.051		
ECG	0.033	0.091	0.425***	0.486***			
EC	-0.061	-0.012	0.365***				
TC	0.050	0.382***					
C	0.123*						

Asterisks *, ** and *** denote significance level at $P<0.05$, $P<0.01$ and $P<0.001$ respectively.

Appendix VII: A plate of the different processed tea types

Purple tea

White tea

Black tea

Green tea

Oolong tea