PHARMACOLOGICAL PROPERTIES AND HEALTH BENEFITS OF TEA SELECTED FROM THREE GROWING REGIONS OF KENYA

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(Biochemistry)

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2017

Pharmacological Properties and Health Benefits of Tea Selected from three Growing Regions of Kenya

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A Thesis Submitted in Partial Fulfilment for the Degree of Masters of Science in Biochemistry in the Jomo Kenyatta University of Agriculture and Technology.

2017

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

This thesis is dedicated to my wife Mary, my sons Bencarson and Dan, daughter Angela, my parents, brothers and sisters.

ACKNOWLEDGMENTS

I am profoundly grateful to my principal supervisor Dr. Johnson Kinyua for his wise counsel and guidance throughout the course of this work, including the availing of the research funding advanced to him by the Research, Production and Extension of Jomo Kenyatta University of Agriculture and Technology. I would also like to express my deep appreciation to my other supervisors Prof. Daniel Kariuki and Prof. Gabriel Magoma for their invaluable suggestions given throughout the study. Am greatly indebted to Jomo Kenyatta University of Agriculture and Technology as my registering Institution and the Vice Chancellor of JKUAT, for allowing me to use the University research facilities. Special thanks go to the entire Biochemistry Department staff for their invaluable assistance. My gratitude goes to Prof. Esther Magiri, Dr. Joseph Ng'ang'a, Dr. Steven Ger, Prof. Naomi Maina, the late Prof. Venny Nyambati, Dr. Jane Ngaira, Prof. Fred Wamunyokoli, Mr. Naftaly Njogu and my colleagues Thadeus Mose and Shadrack Kimani for their able useful assistance, discussion and encouragement through out my postgraduate course. I am deeply grateful to my parents, brothers and sisters for their untiring patience, support and encouragement throughout my postgraduate course.

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ABBREVIATIONS AND ACRONYMS

ANOVA	-	Analysis of variance
α	-	Alpha
APS	-	Acute phase proteins
BSA	-	Bovine serum albumin
C	-	Catechins
ºC	-	Degrees centigrade
C.V	-	Calculated value
DPI	-	Day post infection
GC	-	Gallo catechins
EC	-	Epicatechin
EGC	-	Epigallo catechin
ECG	-	Epicatechin-3- gallate
EGCG	-	Epigallocatechin-3-gallate
GFP	-	Green fluorescent protein
GrTE	-	Green tea
G6PDH	-	Glucose-6-phosphate dehydrogenese
gm	-	Grams
g	-	Centrifugal force
HCL	-	Hydrochloric acid
HPLC	-	High Performance Liquid Chromotography
hr	-	Hour

L	-	Litres
Log_{10}	-	Logarithm of base 10
IP	-	Intraperritoneal route
JKUAT	-	Jomo Kenyatta University of Agriculture and Technology
kg	-	Kilogrames
ml	-	Mililitres
mg	-	Milligrams
Msc	-	Master of science
Mw	-	Molecular weight
mM	-	Milimolar
M	-	Molar concentration
NAD+	-	Nicotinamide Adenine Dinucleotide Oxidized form
NADH	-	Nicotinamide Adenine Dinucleotide Reduced form
NADP+	-	Nicotinamide Adenine Dinucleotide Phosphate Oxidized form
NADPH	-	Nicotinamide Adenine Dinucleotide Phosphate Reduced form
ND	-	Not done
PAL	-	Phenylalanine ammonia lyase
P^H	-	Log hydrogen ion concentration
PBS	-	Phosphate buffered saline
PCV	-	Packed cell volume
RPE	-	Research Production and Extension
SEM	-	Standard Error of means

Sec	-	Seconds
SDH	-	Shikimate dehydrogenase
T.V	-	Tabulated value
UV	-	Ultra violet
µl	-	Microlitres
V/V	-	Volume by volume
W/V	-	weight by volume
%	-	Percentage

ABSTRACT

This study evaluated the phytochemical compounds, antimicrobical activities, antiplasmodium and anti-inflammatory effects of three types of tea; green, orthodox and black Kenyan tea in order to determine their pharmacological value. Tea sampling was done from Kangaita (Green and orthodox tea), Ngere and Nyankoba (Black tea). Phytochemical screening of the three extracts of tea showed the presence of cardiac glycosides, alkaloids, saponins, flavonoids, terpenes and tannins. Green tea lacked anthraquinones, orthodox tea lacked cardenolides and phenolics. The effect of tea on *Plasmodium berghei* infection in mice and and antimicrobial activity was determined. There was a significant difference in parasitemia and PCV levels ($p < 0.05$) on day 11 between the infected mice given tea extracts (10mg/ml) and the infected control. Tea produced a significant ($p < 0.01$) elevation of parasite's induced hypoproteinemia as compared to infected control. The *in-vitro* antimicrobial activities of the three aqueous extracts of tea was done using clinical isolates of *S. aureus, S. typhimurium, E. coli, S. faecalis,* and, *C. albicans* by agar well diffusion. The three extracts of tea inhibited *S. aureus* at concentrations ranging from 100-200mgml^{-1} with zones of inhibition of 10.0±0.0, 4±0.2, and 6.25±0.0 respectively; green and orthodox tea extracts on *E. coli* and *S. faecalis* at 100-400mgml^{-1}. Black tea MIC on *C. albicans* was 100mgml^{-1}, on *E. coli*, at 200-400mgml-1 and a MIC of Green and black tea on *S. typhimurium* was 200mg\ml. It is evident from the findings of this study that tea; genus Camellia, have important health phytochemicals. If further explored tea phytochemicals may provide alternative therapeutic remedies addressing problems of drug resistance disease management and prevention.

CHAPTER ONE

INTRODUCTION

1.1 Introduction

Tea is one of the most widely consumed beverage. Its world wide prominence is attributed to its pleasant flavor combined with it stimulating effects and health benefits. There are many types of tea, including green tea, black tea and oolong tea and each has several sub-classifications (Eden,1931). Recently, green tea has been used in ice cream, candy soft drinks etc. All of them are prepared from *Camellia sinensis* (L) theacea and its varieties by different manufacturing processes. A surveillance done continuously for one year at elementary school proved that those who continuously drink large amount of green tea have less tooth decay (Onishi *et al.*, 1981a). It has also been reported that the green tea extract contained many active substances for dental carries prevention (Onishi *et al.*, 1981_b). The active principles have not yet been thoroughly defined, although several polyphenolic compounds in the green tea have been reported as moderate antibacterial principles (Sekanaka, 2000, Sakanaka *et al*., 1989_b), against *Streptococcus mutans* a bacterium responsible for causing dental carries (Hamada & Slade, 1980). Interestingly, the anti- bacterial activity of polar substances in green tea has not yet been investigated (Mogotlane *et al*., 2007). With this in mind, green tea, orthodox tea and black tea compounds were tested against a selected fungus, gram positive and gram negative bacteria. The crude extracts were used in the assay.

1.2 Varieties of Tea

Camellia sinensis (L) O. Kurtze, belongs to the family theuceae and is the only widely used species processed to generate tea beverages. There are over 300 species reported under the genus Camellia and most of them easily hybridize with tea (Takeda , 1990). Tea is thought to have been discovered in the mid- west main land China (Yunnan) the year 2737 BC by the emperor Shen Nung, (Bokuchava *et al*.,1968; Haro *et al*.,1995), from where dispersal extended towards the south/west to India, to the south of Cambodia, to the east towards Eastern China, Taiwan, Korea and Japan. This dispersal resulted in a varietal speciation process and the evolution of three distinct taxa, Var. *assamica*, Var. *cambodiensis* syn Var *Lasiocalyx* and Var. *sinensis* respectively (Yamaguchi *et al*, 1999). From the above taxa, other *Camellia sinensis* varieties have been described and they include Var. Waldenae (Hu) (Chang & Batholomew.,1982) Var. *dehungensis* (Chang & Batholomew.,1982) and *C. sinensis* ssp, *Buisanensis* (sasaki) (LU & Yong 1987). The contribution of these new varieties to the gene pool of cultivated tea is however unknown (Magoma *et al*,. 2000). The species or varieties and their subsidiary forms intercross with each other. Within hybrid populations there is generally dominance of features which bias the populations toward *Assamica* or *Sinensis*.

1.3 Economic importance of tea

Tea is a major foreign exchange earner and a source of livelihood for millions of people in the world (Owour *et al*.,2001; Obwoge *et al*.,2016). In Kenya, the tea sector is leading among the various agricultural activities. It is the source of livelihood for over 3

million people and it contributes over 20% of total foreign exchange earnings (M'Imwere, 1999). In 2016 for example, Kenya exported 480.3 million Kg of tea which resulted in foreign earnings of 120 billion Kenyan shillings (FAO, 2015). The major importers of the Kenyan tea are United Kingdom, Pakistan and Egypt representing 29.0%, 25.4% and 17.7% respectively (KTDA website). Generally the Kenyan tea sector is very important since its world market share (17% is the third behind India, and Sri Lanka (FAO, 2015, Mutai , 2002, Annual bulletin of statistics 1999).

1.4 World wide cultivation of tea

Tea is mostly cultivated through much of the tropical world especially in Eastern, Central and Southern Africa, the Indian subcontinent, Malaysia, Indonesia, Vietnam, Papua New Guinea, China and some Latin American countries (Weatherstone , 1992). The cultivated forms of *Camellia sinensis* are derived from two main varieties, *Camellia sinensis, Var. sinensis* and C*amellia sinensis, var. assamica*. The third variety *Camellia assamica ssp, Insiocalyx syn Cambondiensis* is considered a sub species of *C. sinensis,Var. assamica* (Banerjee, 1992). The *assam* varieties are grown widely in India, Sri Lanka and east Africa and are for manufacture of fermented (black) tea while the China types which are mainly grown in China and Japan are for making of non-fermented (green) and semi-fermented (Oolong and Pouchang) teas. However, tea being a highly out crossing crop, all the above taxa freely interbreed resulting in acline extending from extreme china Assam types to those of Assam origin (Wight, 1959). The hybridization has been extreme that it is often debated if the original C. *sinensis, C. assamica ssp,* lasiocahyx still exist (Magoma *et al* .,2000; Visser, 1969).

1.5 Origin of Tea

The earliest documented use of tea is in China where the small leafed *sinensis* type was established as a commercial crop by 793 AD (Sealy, 1958). The large leafed type *assamica* was initially grown for use as vegetable rather than as beverage in countries such as Burma, Thailand, Laos and Assam in North East India (Sealy, 1958). However, tea cultivation outside its native range was reported as early as 801 AD in Yeinsan, Japan (Weatherstone, 1992). In Europe, the use of tea was first reported in 1545 (Weatherstone, 1992) and it quickly became an important commodity of trade between China and Europe particularly England and Holland by mid1750, It was wide spread in tea houses around London (Weatherstone, 1992). However, commercial tea reached Eastern Europe by 1650.

1.6 Statement of the Problem

The continued use of tea as a beverage has gained world wide prominence due to the quality of its phytochemicals and other related tea compounds, such as polyphenols. The study aims to elucidate the anti-microbial activities, anti-*Plasmodium* properties, anti-inflammatory properties and other phytochemical aspects associated with crude extracts of tea selected from three growing regions of Kenya. These aspects had been perceived to be more in green tea than in black tea hence the tendency to influence market trends(Venessa & Williams 2009). Tea polyphenols (flavor) and their oxidative products are increasingly being indentified with a number of diverse pharmaco therapeutic effects such as reduction of the risk of heart disease and cancer in humans (Venesa & William, 2009).

1.7 Justification

Emerging scientific data from pharmacological and physiological studies continue to show that tea has beneficial effects on human health. A number of *in vitro* studies have shown that tea helps the immune response by acting as anti-allergic, anti-viral and anti-bacterial agent (Karori *et al*., 2010 ; 0bwoge *et al*.,2014). It has been said that those who continuously drink large amount of green tea have less tooth decay. (Onishi *et al.*, 1981_b). In Kenya and Africa in general there are very few if any other products derived from tea other than the leaf products such as ready to drink teas, tea concentrates, flavoring agents, wines, confectionery, antiseptic soaps and many other products are produced and sold in other countries at premium prices, where tea is not even grown. Ortho-quinones have distinct antiseptic properties and by their action, the rolled tea leaf is largely rendered free from bacteria and fungi. Therefore there is need for research work along that area for example the possibility of using this antiseptic property of tea to develop a disinfectant or food preservative (Orchandas, 2010). In order to remain competitive and ensure sustained growth, the tea industry in Kenya must continually seek to improve the quality . Product development from this should also follow alongside that of black tea, and this requires a lot of research particularly focusing on consumer acceptance and its health potential. This study aims to establish the anti-microbial activity, the anti *plasmodium berghei* and the effect on inflammation of Kenyan green, black, and orthodox teas. The findings from this work will improve the consumption and marketing of Kenyan tea with tea trade now thriving due to medicinal associated catechins for example in 2003 China exported 800 tons of tea polyphenols,

300 tons of tea pigments (theaflavins and thearubigins), 10 tons of L-theanine and appreciable amount of saponins (Wan *et al*.,2004)

1.8 Null Hypothesis (Ho)

Tea has no anti microbial, anti *Plasmodium berghei*, and anti- inflammatory properties.

1.9 Broad objective

To determine the, phytochemical profile, antimicrobial activity , ant*i-plasmodial*, and antinflammatory effects of tea

1.10 Specific objectives

1) To determine the categories of phytochemicals in green, black and orthodox teas.
2) To evaluate the antimicrobial activity of green, orthodox and black tea on clinical isolates of *Escherichia coli*, *Staphylococus aureas, Streptococus faecalis, Salmonella typhimurium, and Candida albicans.*
3) To determine the effect of green, black and orthodox teas on *Plasmodium berghei* infection in mouse model

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction of tea in Africa

The first tea plant appeared at the Cape of Good Hope in South Africa in 1687 but actual planting on the African continent was in the vicinity of Durban in the second half of the 19th century (Wilson, 1962). In 1878, tea was planted near Blantyre Malawi, with successful results. Subsequently tea growing as a commercial venture was started in 1891 at Mlanje, Malawi. After the successful establishment of tea in Malawi, tea growing spread to Mozambique, Zimbabwe and Tanzania during the 1920's. However, it is not clear whether the source of planting material in these countries was from Malawi or elsewhere (Wilson, 1962).

2.1.1 Introduction of tea in Kenya

The first recorded case of tea in Kenya was by the Caine brothers who imported dark-leafed, "Manipuri" hybrid seed from Assam in 1904 and 1905 to establish a plantation at Limuru (Matheson, 1950). In 1912, *sinensis* seed was imported from Sri Lanka to establish a plantation of tea with high quality and yield (Matheson, 1950). However, over the next twelve years, interest in tea growing was low although several small plantations were established at Limuru, Kericho and Kaimosi (Matheson, 1950). It was not until 1925 when serious planting began after several companies became interested. These companies followed advice given by the Howland brothers in 1924 urging the use of quality seed of light coloured leaf Assam type or the manupuri types for drought resistance. The planting expanded rapidly and by 1929, there were 2162 ha of tea in

Kenya (Greenway, 1945). By 1964, the acreage increased to 23,062 ha and currently, the acreage is approximately 114,00 ha (MImwere,1999).

2.1.2 Tea plant secondary metabolites

The major metabolites in tea, which include the catechins and the aromatic amino acids, are formed through intermediary glucose metabolism, starting with the pentose shunt pathway followed by the shikimate pathway and finally the prephenelate pathway. The metabolites derived from the shikimate pathway can constitute up to 60% of the dry weight of some plant tissues and the accumulation of these metabolites is dependent on the flux from the pentose pathway (Schnarren *et a.l*, 1995). The shikimate dehydrogenase (SDH) is involved in the conversion of dehydroshikimate to shikimic acid, an essential precursor in the formation of catechins and aromatic amino acids.

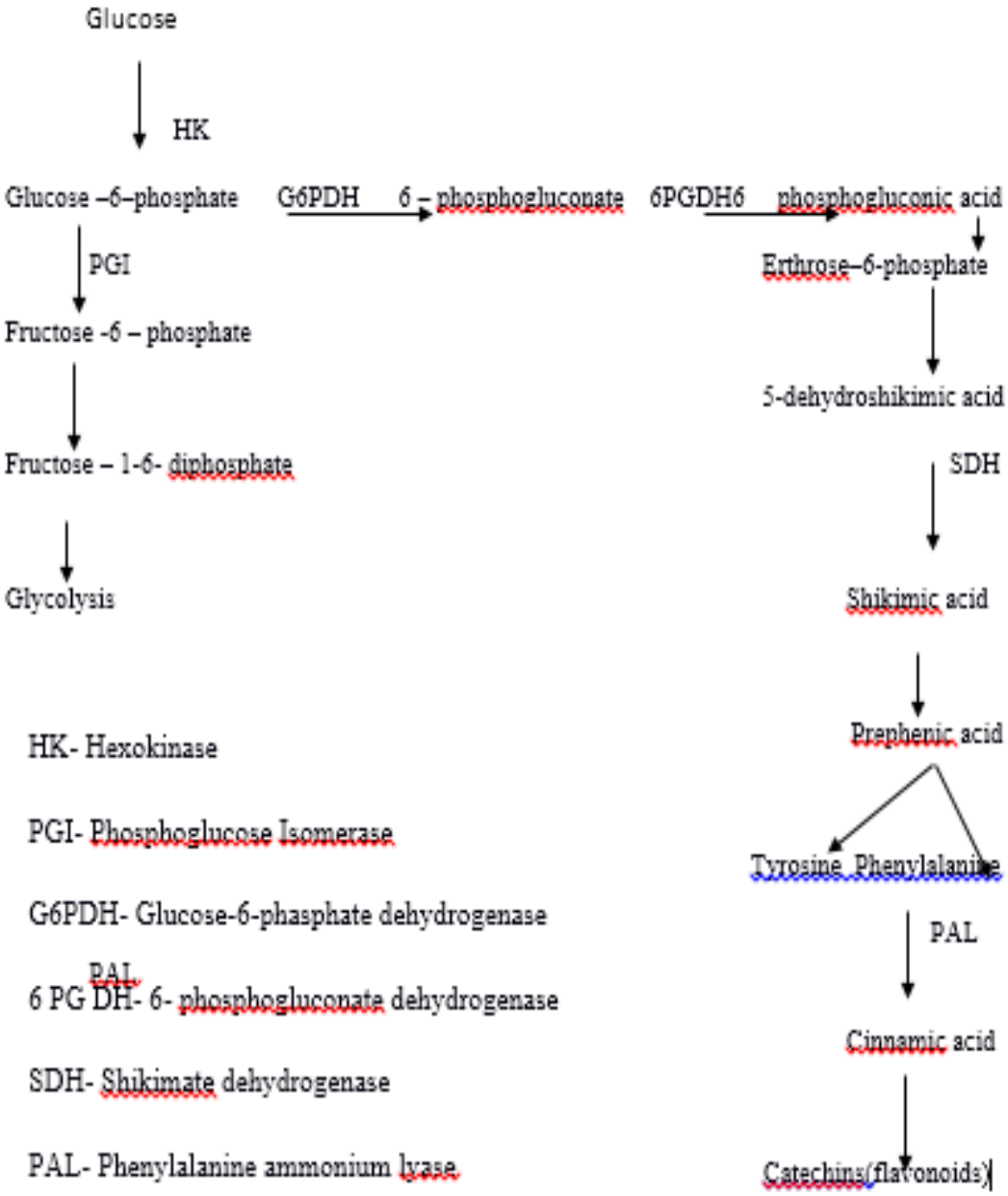


Figure 2.1: Flow of carbon compounds in Cammelia and the pentose phosphate pathway towards the productions of Flavonoids (metabolites) (Schnarren *et al.*, 1995).

The activity of SDH in tea is greater in growing shoot than in mature leaves (Sanderson, 1966). However the activities of the initial shikimate pathway enzymes are not always related to the Flavonoid contents (Saijo and Takeo, 1979).

2.1.3 Regulation of the biosynthesis of secondary metabolites

Phanylalanine ammonia lyase (PAL) plays a major role as the rate limiting step indetermining the flux into total phenolics since there is a qualitative relationship between this enzyme and phenyl propanoid accumulation (Bate *et al*., 1994). Glucose - 6-phosphate dehydrogenese catalyses the first committed and late limiting step of the pentose pathway and is known to be affected by NADPH/NADP ratio, pH, magnesium and the substrate concentration in the chloroplast (Copeland and Turner. 1987., Schaewen *et al*., 1995). It has been shown that the pentose flux increases in tandem with the shikimate pathway to provide sufficient secondary metabolites in response to microorganism attack (Herrman, 1995). The genes for the initial enzymes in phenyl propanoid pathway appear to be induced early on in a coordinated manner implying the occurrence of a multi gene families and metabolic channeling (Hahebrock and Scheel,1989; Channeling *et al.*, 1992). However the existence and role of the multi-gene families has yet to be established. Analysis of the expression of some of the enzymes involved in the biosynthesis of tea catechins like G6PDH, 6PGDH, and SDH may yield information on the pathway.

2.1.4 Occurrence of secondary plant metabolites

The phenolic compounds in plant have many roles; their occurrence as polymers serves as a structural role while their colored nature makes plants possessing them attractive to insects (Maher *et al.*, 1994). The specific biochemical role of phenolic in plants is their defense responses which are either constitutive or inducible (phytoalexins). Constitutive phenolics are important in non-specific defense mechanisms. Plants with high concentrations of phenolic compounds are generally unpalatable to herbivores due to their astringent taste and ability to inhibit digestive enzymes such as amylase. Specific defense is displayed when plants with high levels of a specific phenolic compound inhibit a specific pathogen e.g. transgenic tobacco plants with reduced levels of chlorogenic acid are more susceptible to virulent fungal pathogens such as *Cercospora nicotianae* (Maher *et al*., 1994). The inducible deposition of phytoalexins usually occurs as a defense response and results in the general enhancement of lignin and lignin- like polymer deposition associated with microbial attack (Rhodes & Parr, 1996).

2.2 Pharmacological importance of catechins

The role of plant phenolics and particularly the tea catechins on human health are many and could generally be linked with their ability to inhibit enzymes or act as reducing agents (Andrian and Bolwell, 2000).

2.2.1 Catechins and alleviation of hypertension

The catechins present in tea have been shown to be important in reducing serum cholesterol and alleviating hypertension and vascular disorders. They achieve this by

inhibiting cholesterol absorption through precipitation and enhancing the decomposition of the cholesterol that is deposited on artery walls (Matsubare., 1985; Yildizogle-Ari., 1991). The gallated catechins, epicatechin gallate (ECG) and epigallocatechin gallate (EGCG) have been demonstrated to be more effective than the free catechins (Ohtsura, 1991; Ikeda, 1992).

2.2.2 Catechins and alleviation of cancer

The major tea catechins, epicatechin (EC), (-)epigallocatechin (EGC), (-) epigallocatechingallate (EGCG) and (-), epicatechingallate (ECG) have been shown to have anti-carcinogenic activity (Zheng *et al.*,1996). The anticarcinogenic property of tea catechins has been demonstrated in the anticarcinogenic effects of tea on *Salmonella typhimurium* assay using catechin and EGCG (Hayatsu *et al.*, 1992). The mechanism of anti-carcinogenic action of these catechin is through the inhibition of pro-oxidant enzymes like cytochrome P_{450} which activate pre carcinogens to carcinogens and also through the stimulation of carcinogen detoxifying enzymes like quinine reductase and glutathione s- transferase (Stoner and Mukltar, 1995). Catechin has also been reported to be an effective inhibitor of tyrosine Kinase a key enzyme in modulating signal transduction pathway important in carcinogenesis (Sharma *et al.*, 1994), plant phenolic compounds have also been shown to interact with estrogen and testosterone receptors, therefore, the plant phenolics could be used in protection against hormone dependent cancers like breast and prostate cancer (Ingram, *et al.*, 1997; Palhck *et al.*, 1997).

2.2.3 Catechins and Inflammation

The antinflammation effect of the major tea catechins has been demonstrated in a number of studies (Sugiyama, 1995). An *in vivo* study to determine the effect of tea extracts on an animal model of male swiss albino mice infected with *Tryponosoma brucei brucei* isolate KETRI 2710 had been done. The catechins have been shown to achieve this role through the inhibition of lipooxygenase there by interfering in the production of postaglandins, the major inflammatory agents, from arachidonic acid. They prevent the formation of leukotrenes. Erythrocytes use only the lipoxygenese pathway. Most antinflammatory drugs act by producing phospholipase inhibitor. Asprin inhibits the cycloxygenese pathway therefore preventing the formation of prostonoids resulting in peptic ulcers.

2.2.4 Catechins and caffeine in tea quality

The main polyphenols found in tea are the catechins (+), catechin(c), (-) epicatechin(EC), (-)epigallocatachin(EGC), (-) epigallocatechingallate (EGCG) and (-) epicatechingallate (ECG). During the fermentation stage in tea manufacturing process, the catechins are oxidized by oxygen in reactors catalysed by polyphenol oxidase to form theaflavins and thearubigins. These fermentation products, the initial catechins and their complexation with caffeine give tea its quality attributes (Obanda *et al.*, 1997). The urigallated catechins EC and EGC have bitterness with astringency (Sanderson. 1976). The caffeine gives tea its briskness and the creamy characteristic usually seen when drinking black tea (Millin *et al.* 1969). The total catechins content has to indicate the quality potential of tea clones, with high content being related to high quality

(Obanda *et al.*, 1992). However, the relationship is not very conclusive. Therefore the composition of individual catechins could be a major determinant in the realization of black tea quality.

2.2.5 The chemistry of black tea processing

The constituents which influence taste, flavor and colour of tea include polyphenolic bodies, caffeine, non-caffeine nitrogenous compounds, pectic substances, sugars, minerals and other compounds jointly or separately (Werkhoven *et al*., 1974). For quality production, tea leaf consisting of two leaves and a bud is considered the normal and best material for tea manufacture. Such leaf contains 74-77% moisture and 23-26% dry matter. About half the dry matter is insoluble in water and is made up of crude fiber, cellulose, proteins, fats and others (Werthoren *et al*., 1974). The soluble part includes about 30 polyphenolic bodies, over 20 amino acids, caffeine, sugar and organic acids (Harler, 1963). Traces of a number of substance which may be associated with what is known as essential oil responsible for aroma of tea, have been recorded (Harler,1963). In green tea leaves the total polyphenols makes up about 30% of the dry matter and contains mainly of bodies called flavanols or catechins, six of which are present in fresh tea leaf as major components. The catechin makes up 1/5 of the dry matter. The polyphenolic body in the tea shoots decrease in quality from bud to stalk (Harler, 1963). The black tea manufacturing process involves disruption of the cellular integrity of green tea shoots, thus facilitating the mixing of the polyphenols held in the cell vacuole with the cytoplasmic enzyme polyphenol oxidase. The chemical and biochemical reactions which then follow result in the uptake of atmospheric oxygen and the

formation of pigment, hot water soluble, polyphenolic compounds, characteristic of black tea

2.2.6 Green leaf polyphenols

Young green shoots are extremely rich in polyphenolic compounds, the largest group being the catechins (flavan-3-ols) which constitute up to 30% of the dry weight of the materials (Roberts, *et al*., 1962). *Camellia sinensis* is unique in its range of Flavonoid and have also been found in manufactured green and black teas but not fresh green tea and are therefore considered to be recemization or epimerization, caused by the drying process (Roberts., 1962). More recently other related compounds (-)-epicatechin -3,5 digallate (Coxon *et al*., 1972) have been reported in fresh green leaf but their contribution to the quality characteristics of black tea remains uninvestigated. Climatic and agronomic factors affect both the flavanol content of the green tea shoot, and the composition of the resultant black tea liquor (Hillon *et al*., 1973, Ramaswamy, 1964, Robertson, 1983).

2.2.7 Polyphenol oxidase

Polyphenol oxidase, the enzyme responsible for the oxidation of polyphenol has been reported by various scientists (Christine *et al*, 2008.,Roberts, $1950_{b,}$). Its isolation and purification was successfully done by Gregory and Bendall (Gregory *et al.*, 1966) and its location in the tea plant suggested by several scientists (Bukachara *et al.*, 1970, Gregory & Bendau. 1966, Harler, 1963, Oparin, 1941). A demonstration of the location of tea

polyphenol oxidase has been done by several scientists who have identified it to be in the cell cytoplasm (Gregory & Bendau, 1966).

2.2.8 Constituents other than the polyphenols

Other constituents include caffeine, chlorophyll, inorganic constituents, pectins and other enzymes. Caffeine levels increases during loading and transportation of the plucked leaf and during withering. Caffeine imports a very slight bitter taste but it gives tea drink its refreshing and stimulating character. It is also important in forming cream, the precipitate that appears when a tea infusion cools (Williamso, 1992). Chlorophyll (green) is transformed during aeration to pheophorbide (coppery red) or chlorophyllide (black) or pheophorbide (black) by the action of chlorophylase enzyme during fermentation. The intensity of blackness of made tea is considered to be a function of the amount of chlorophyl in the fresh leaf (Wickremasinghe and Perera, 1960). In regard to inorganic constitutes it is noteworthy that (i) Potassium is the element with the highest concentration (ii) the tea plant is a greater accumulator of both alluminium and manganese and (iii) a part from deficiencies in major elements, deficiencies in minor elements such as copper, zinc and sulphur may occur incidentally (Williamson,1992). Pectins are present in large quantities in tea shoots. They break down in the course of processing to form pectic acid and methylalcohol. The resultant pectic acid then forms a coating on the mercerated and aerating leaf, partially inhibiting the process of polyphenol oxidation and prevents over aeration. The pectic acid is thought to form a kind of varnish on the outside of leaf during the early stages of drying (Williamson, 1992). Besides polyphenol oxidase, a number of enzymes activities take place during

tea processing. After the shoots are detached from bush the different systems start to react with the corresponding substrates. The brake down of the proteins and pectin are brought about by protease and pectinase, respectively. Other enzyme mediated reactions include changes in, caffeine, amino acids, phosphates development of aroma and breakdown of chlorophyll (Deb, 1969).

2.3 The manufacture of black tea

Withering is the first stage of processing. This process partially dehydrates the plucked tea content from 70-80% water to 45-75%. There are also other chemical changes that take place. This process is sometimes prolonged (16-20hrs) by the unpredictable weather patterns (Williamson, 1992) and these results into plucked tea leaves going to waste as steyawalt processing in many of the Kenyan tea factories

The most important characteristic components of tea are the polyphenols in the leave sap which undergo a series of chemical changes during the aeration process initiated after the weathered leaf has been incarnated during rolling. Some of the polyphenols converted into polyphenolic compound, which have been found to change during aeration are epigallocatechin (EGC), epigallocatechuingallate (EGCG), epicatechingallate (EGCG) and epicatechin gallate (ECG). The rate of conversion of this phenol is afunction of temperature, oxygen concentration and their qualities. The oxidation of polyphenols upon exposure to air is very slow unless brought above by the activity of the appropriate enzyme e.g. polyphenol oxidase or catechol oxidase. The basis of aeration is to bring oxygen and substrate together by rupturing the membrane so that the polyphenols can diffuse into the cytoplasm. In conventional tea manufacture,

this process is brought about during rolling wringing action on the weathered leaf tissues and by mechanical disruption of its cells. The enzyme polyphenol oxidase, oxidizes the polyphenollic bodies to arthoquinones. The arthroquinones by dimerization condense to bis-flavanols which in turn condense to theaflavins which are yellow bodies. An additional oxidation transforms t arubigins which are red and brown bodies with tannin properties. Finally, some thearubigins are precipitated by leaf proteins to form insoluble bodies which are lost to the tea infusion. The reactions do not proceed to completion during normal manufacture and the finished black tea contains both thearubigins and theaflavins.

2.4 Phytochemicals

2.4.1 Alkaloids

These are heterocyclic nitrogenous compounds known to have microbicidal effects. Some key examples are berberine, morphine and codeine. Salamargine is a glycoalkaloid from berns of *solanum khasinum* has been found to have antidiarrheal effects (Cowan, 1999). Ditterpenoid alkaloids is isolated from plants of Ranuclulaceae family have been found to have antimicrobial properties (Ramar and Ponnal, 2008).Tea alkaloids are; theophylline, theobromine and caffeine. Theophylline is used in therapy of respiratory diseases such as obstractive pulmonary disease and asthma. As a member of the xanthine family, it bears structural and pharmacological similarity to theobromine and caffeine. Theophylline blocks the action of adenosine an inhibitory transmitter that induces sleep, contracts the smooth muscles and relaxes the cardiac muscles. The main actions of theophylline involve: relaxing bronchial smooth muscle, increasing; hearth

muscle contractractibility and efficiency, heart rate ,renal blood flow, blood pressure, anti-inflammatory effects and central nervous system stimulatory effects mainly on the medullary respiratory center.a. Alkaloids have also anticancer and antimalarial effects.

2.4.2 Flavones, flavonoids and flavanols

Flavones are phenolic compounds consisting of one carbonyl group. Flavonoids have two carbonyl groups while flavonols in addition have a hydroxl (3-oH) group, thus yielding flavanols. They are known to be synthesized by plants in response to microbial infections and are effective substances against a wide range of micro-organisms (Ramar and Ponnal , 2008). Their activity is due to their ability to complex extracellular and soluble proteins and also bacterial cell wall. Some are more lipophillic flavonoids and disrupt microbial membranes such as catechin found in green tea and phloretin in apples (Cowan, 1999). They are; antioxidants and have anti-inflammatory, anti-cancer, anti-diabetes mellitus, and treats cardiac disorders among others.

2.4.3 Tannins

Tannin is a general descriptive name for a group of polymeric substances capable of tanning leather or precipitating gelatin from solution, a property known as astringency, found in plants in park, wood, leaves, fruits (Scalbert, 1991). Many human physiological activities such as stimulation of phagocytic cells, host mediated tumour activity and a wide range of antinfective actions have been assigned to tannins (Haslam, 1996). One of their molecular actions is to complex with proteins through non specific forces such as hydrogen bonding and hydrophobic effects as well as covalent bond formation.

2.4.4 Quinones

They are compounds having aromatic rings with two ketone substitution and are ubiquitoes in nature. These compounds are responsible for the browning reactions in cut or injured fruit and vegetables. Quinones have been shown to have antimicrobial activity with hypericin and anthraquinone from cassia, italic examples (Cowan, 1999). Quinones are known to complex irreversibly with nucleophillic amino acids in proteins leading to in activation of proteins and loss of functions. Quinones have a large industrial application in the production of hydrogen peroxide. Derivertives of quinones are common biological molecules, some serve as electron acceptor in electron transport chain as those in photosynthesis (plastoquinone phylloquinone).They are ; anti-tumour, anti-microbial, anti-parastic, anti-cardiac diseases and inhibits PGE2 biosynthesis. They are used to make dyes such as alizarin, battery charger carrier, reagents in organic chemistry such as benzoquinone and chronil.

2.5 Micro organisms evaluated in the antimicrobial activity of tea

These are enormous species of bacteria and fungi. Some bacteria used in this research include *Staphylococcus aureus, Salmonella typhimurium, Escherichia coli, Streptococcus faecalis,* one fungus*, Candida albicans* has been used. Black tea is a rich source of dietary flavan-3-ols. Flavanoids have been shown to have (a) direct antibacterial activity, (b) synergistic activity, and (c) the ability to suppress bacterial virulence factors in numerous *in vitro* and a limited number of *in vivo* studies (Cowan, 1999). In the *in vivo* studies , the finding is that oral quercetin protects guinea pigs against *Helicobacter pylori* an *Staphylococcus spp*. Green and black tea boiled water

extracts inhibited the growth of *Salmonella typhimurium1402*/84 and *Escherichia coli(* sera *et al.,* 2016*).*

2.5.1 *Staphylococcus aureus*

Staphylococcus species are gram positive cocci. *Staphylococcus aureus* causes boils, styes, pustules, impetigo infection of wounds (cross-infection) ulcers and burns, oesteomylitis, martitis, scapticaemia, menegitis pneumonia and pleural empyema, toxic skin exfoliation. Antibiotics with activity against *Staphylococcus aureus* include penicilines, macrolides, fusidic acid, vancomycin and cephalosporic.

2.5.2 *Escherichia coli*

Escherichia coli belong to the gram positive rods referred to as enterobacteria. They are naturally found in the intestinal tract, in the soil and water. *Escherichia coli* causes watery diarrhea in infants and adults. It also causes vomiting, dysentery, fever and colitis with blood mucus and pus cells in faecal specimens. Antimicrobial agents that are used to treat *Escherichia coli* are sulphanomides, trimethoprime, contrimoxazole nalidixic amocycline, caphalosporins and aminoglycosides (Monica, 2000).

2.5.3 *Candida albicans*

Candida albicans fungus is the commonest cause of candidiosis. The yeast is a common commensal of gastrointestinal tract. Most candida infections are opportunists occurring in debilitated persons. It also causes vaginal thrash (Monica, 2000).

2.5.4 *Streptococcus faecalis*

Streptococcus faecalis is the most prevalent species cultured from humans. It causesclinical infections in humans. They are hardy facultative anaerobes that can grow in many environments. They are Gram positive cocci that grow in chain. They hydrolyze esculin in presence of bile; grow in 6.5% sodium chloride, demonstrate pyrroldonyarylamidase and leucine amino peptidase and react with group D antiserum and accounts for 95% isolate.

2.5.5 Salmonella typhimurium

Salmonella typhimurium is a pathogen Gram negative rod shaped bacteria predominantly found in the intestinal lumen (Scalbert, 1991). Its toxicity is due to an outer membrane consisting largely of lipopolysacchrides (LPS) which protect the bacteria from the environment. The LPS is made up of an O-antigen a polysaccharide core and a lipid A which connects it to the outer membrane. The LPS is made up of two phosphorylated glucosamines which are attracted to fatty acids. These phosphate groups determine the bacteria toxicity. *Salmonella typhimurium* infect by coming in direct contact with non- phagocytic cells. This contact induces the formation of appendages on the bacteria cell surface. The appendages formed are shorter than flagella but thicker than both flagella and pill (Garcia-Del portillo *et al,* 2007). They cause the host cytoskeleton to re-arrange which allows the bacteria to enter the cell. *Salmonella typhimurium* causes gastroenteritis in human and other mammals. When the bacterial cell enters epithelial cells lining the intestine, they cause host cell a raffling which temporally damages the microvilli on the surface of the cell. This causes a rush of white

blood cells into the mucosa which throws off the ratios between absorption and secretion and leads to diarrhea. In mice *Salmonella typhimurium* causes symptoms resembling typhoid fever in humans (Roseberger *et al*.,2000).

2.5.6 Rodent *Plasmodium* parasites

In research aimed at developing strategies for eradicating human malaria, rodent malaria parasites are used as laboratory models (Carlto, 2001). There are four African rodent malaria parasites namely *Plasmodium berghei, Plasmodium yoelli, Plasmodium chabondi* and *Plasmodium vincket* (Smith and Parsons, 1996). Rodent parasites often represent the only practical means towards *in vivo* experimentation (Janse & Waters, 1995). However, in absence of sufficient number of rodents, similarly non human primate parasite model exist but pharmacokinetic evaluation are much more expensive especially monkey model that can sustain infection by *Plasmodium falciparum* monkeys, morqui monkeys and squirrel (Ridley 2002). The species that infect non human primate models include; *Plasmodium knowlesi, Plasmodium vivax, Plasmodium simiovale,* an *Plasmodium youngei*. Many plasmodium species infect more than one primate host species. Primates which get infected with *Plasmoium* include;howler monkeys, spider monkeys, morique monkeys, titi monkeys an squirrel monkeys. *Plasmodium berghei* was originally discovered by Vincke and Lips in 1948 and infects hamsters, rats and mice (Janse and Waters, 1995). At least five isolates (strains of *Plasmodium berghei*) have since been isolated namely ANKA, SPII, NKb5, LUKA and K173 (Janse and Waters, 1995). All life stages of the different isolates have a similar morphology and duration of development. The isolates also show a comparable

sensitivity to antimicrobial drugs and no variation in isoenzyme (Carto, 2001, Jane and Waters, 1995). Generation of transgenic ANK strains of *Plasmodium berghei* expressing exogenous proteins green fluorescent protein (GFP) and /or luciferase has broad possibilities for *in vivo* drug screening (Dekoning ward, 2000). *Plasmodium berghei* has successfully been used in drug testing investigation discovery (Peters., 1991). The other rodent parasites play invaluable role in malaria research. *Plasmodium berghei* infections are rapidly lethal to laboratory rodents hampering studies on the *in-vivo* generation and selection of antigenic variations (Janse and Waters, 1995). *Plasmodium yoelli*, is extnsively in studies on biology of liver stage antigens and their role in immunity and vaccine development (Janse and Water, 1995).

2.5.7 Life cycle of *Plasmodium*

The *Plasmodium* parasite has a complex life cycle (Fig 2), involving development in female anopheles mosquitoes and in human presenting a major obstacle in the design of control measures (CDC 2007).

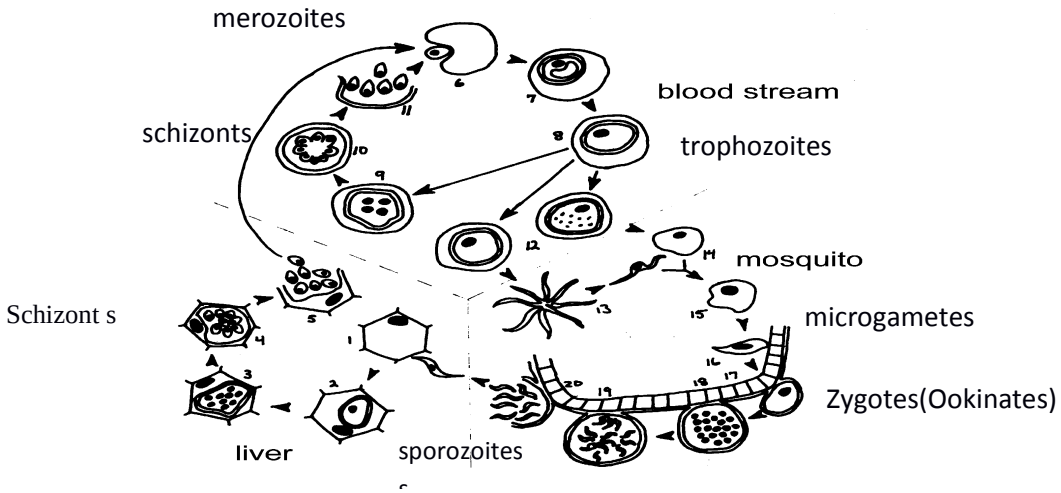


Figure 2.2: Life cycle of *Plasmodium berghei* (Barry, 2005*)*

Infection is initiated after malaria parasite infected female anopheles mosquito inoculates sporozoite into the human host. Sporozoite infect hepathocytes, mature into schizoites and then rapture to release merozoites, which infect erthrocytes. The parasite undergo asexual multiplication (erthryocytic schizogony) after which erythrocytes bursts and releases new merozoites capable of remitiating new erthrocytic schzogony. Some parasites in newly infected erythrocytes differenciate into male (microgametocytes) and female (Macrogametocytes) which anopheles mosquito ingests during blood meal (Barry, 2005). The gamete differentiates to form a zygote which then differentiate into a motile and elongated Okinetes. Okinetes invade the midgut wall of the mosquito, develop into Ooctes, that grow and raptures to release sporozoites. The sporozoites migrate to the salivary glands ready to initiate new infection. Despite the similarities in the life cycles between malaria parasites, *Plasmodium berghei* shows specific features such as strong preference for invading reticulocytes and asynchronity of blood stages in short (23-23-hr) and then the period of development of gametocytes is also short with sporozoites developing into mature schizoites with 43-52 hr after invasion of hepotocyte (Janse & Water, 1995).

2.5.8 Serum proteins as markers of inflammation

Inflammation is the body's response to injury or infection and it can be classified as either acute or chronic. Generally inflammatory markers include both mediators and inhibitors of inflammation, as well as scavengers or potentially dangerous substances such as toxins. Serum levels of certain proteins change during acute phase response; that increase are called positive acute phase response; those that decline are called the

negative acute phase proteins. By definition an acute phase protein changes by at least 25% during inflammation (Mungatana *et al*., 2007). The following protein changes are noticed during the inflammatory response; a rapid fall in serum albumin, prealbumin and trasferin levels as well as Retinoblastoma (RBB), however they are expected to return to normal as the inflammatory resolves. It is clear that these negative acute phase reactants are affected by factors other than intake. The reason for this alternating in protein concentrations are complex ; but likely due to increase in synthesis of immune mediators during time of stress and decreased need for other proteins that are not essential for immune function, There is also an increase in al and a2 globulin levels. In chronic inflammatory conditions and some maligneamia the level of al globulin levels remains the same but a2 globulin is increased leading to diffuse increase in gamma-globulins. The most widely used indicator of inflammation is C-reactive protein (CRP) because of its ability to change rapidly with changing conditions ,its wide availability ,its sensitivity as a marker of inflammation. Chronic inflammation can arise after bacterial or as a result of autoimmune response. In autoimmune response the autoimmune response is triggered when there is no stimuli and the immune system attacks itself. Extra protein is often released from the site of inflammation. These proteins can easily be detected in the blood stream and are therefore referred as to inflammatory markers. Perhaps the most commonly used marker of inflammation is C - reactive protein (CRP). This protein is synthesized in the liver and despite being a minor plasma protein, levels are drastically increased within 6 hours after the onset of inflammation. The final increase can sometimes be so much as 60-fold furthermore CRP is more specific than some of the commonly used markers of inflammation such as erythrocyte sedimentation rate (ESR).

In significant bacterial infection CRP levels are unusual below 10mg/l except in neonates where 10 – 40mg/l typically represents mild inflammation, levels between 40 – 200 mg/l represents significant acute inflammation or bacterial infection. In burns or serious bacterial infections levels may raise up to 300gm/l or high.

Cytokines are not generally tested because of limited availability, lack of standardization of serum levels and high cost. This is done by monitoring the trend of proteins during the experimental period.

Its important to note that the main factor affecting protein concentration in patients is the rate of transcapillary escape into interstitial fluid. This transcapillary escape of albumin is markedly increased in diseases (as part of the systemic inflammatory response syndrome leading to decreased plasma albumin concentration. Postoperative patients with severe infection will have low plasma protein concentrations.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Tea samples

1. Green (non oxidized), obtained commercially from Kangaita tea factory in Kirinyaga county.
2. Orthodox tea (partially oxidized), from kangaita tea factory in Kirinyaga county.
3. Black tea(oxidized) was obtained from Ngere tea factory in Muranga county.
4. Black tea (oxidized) was obtained from Nyankoba tea factory,Nyamira county.

The choice of the locality/factory of sample collection was determined according to convenience.

3.1.1 Reagents

Reagents used were of analytical grade from Oxoid, Aldrich, Sigma, and BDH. They were obtained from a local dealer Kobian. The reagents were transported and stored in the laboratory according to the manufacture s specifications.

3.1.2 The microorganisms used for in vitro antimicrobial assays of tea

Staphylococcus aureus, Salmonella typhimurium, Escherichia coli, Streptococcus faecalis, and, *Candida albicans* were obtained from Medical microbiology laboratory JKUAT stock cultures.

3.1.3 Qualitative determination of Phenolics

To 2ml of aqueous tea extract, 1ml of 1% ferric chloride solution was added. Blue colour formation was an indication of phenols (Martinez, 2003)

3.1.4 Qualitative determination of Flavonoids

The 2g tea powder was extracted in 10ml water. To 2ml filtrate few drops of concentrated HCL followed by 0.5g magnesium turnings was added. After 3min pink colour formation indicated the presence of flavonoids (Jigna & Sumitra. 2007).

3.1.5 Qualitative determination of terpenes

To 2ml of aqueous extract, 5mg chloroform, 2ml acetic anhydride, concentrated HCL were added carefully to form a layer. Redish brown colour of interface was an indication of terpenes (Harborne, 1973).

3.1.6 Qualitative determination of cardiac glycosides

To 2ml alcoholic filtrate, 1ml glacial acetic acid and 1-2 drops of ferric chloride were added followed by 1ml of concentrated sulphuric acid. A presence of brown ring at the interface indicated the presence of cardiac glycosides. A violet ring also appeared below the brown ring (Trease & Evans, 1989).

3.1.7 Qualitative determination of cardenolides

To 4g of each sample was extracted in the test tube with 80% ethanol, and appropriately labeled. They were divided into two portions for Kedde's test and Keler-Killian's test. For Kedde's test, few drops of 10% lead acetate were added to each of the tubes,

followed by few drops of distilled water and chloroform. The contents were evaporated to dryness in a water bath. 5% sodium hydroxide was added to each residue and then 2% of 3-5 dinitrobenzene acid. For Keller-Killian's test, few drops of 10% lead acetate, water and chloroform were added to each test sample. The mixture was evaporated to dryness in the water bath and subsequently a few drops of concentrated sulphuric acid were added. For Keller-Killian's test, a brown ring indicated the presence of cardenolides, while for Kedde's test a brown to purple colour was indicative of cardenolides.

3.1.8 Qualitative determination of anthraquinones

To 1g of each tea sample was shaken with 10ml of ferric chloride solution with 5ml of 6 M HCL. Each mixture was heated in a water bath for 10-15min, filtered and allowed to cool. The filtrate was extracted with chloroform and shaken gently. The clear layer at the base was pipette into test tubes and 2ml each of ammonia sulphate added. An observation of a delicate pink rose indicated the presence of anthraquinones.

3.1.9 Qualitative determination of saponins

To 5ml of each plant extract was placed into a test tube and diluted with 5ml of distilled water. The mixture was shaken vigorously for 2min persistent appearance of foam lasting for 5min or the forming of emulsion when olive oil was added confirmed the presence of sponins.

3.1.10 Qualitative determination of tannins

To 2ml of aqueous extract 2ml of 5% $FeCl_3$ was added. Formation of a yellow precipitate indicated the presences of tannins (Jigna & Sumitra, 2007).

3.1.11 Determination of alkaloid content

The 2.5% of the tea powder was extracted using 100ml 20 % acetic acid in ethanol. The solution was covered for almost 4hours. The filtrate was concentrated to 25ml, through evaporation in a boiling water bath at 90^0 c. 3M ammonium hydroxide was added step wise to attain precipitation. The whole solution was kept as such so that precipitate settled. The collected precipitate was washed with dilute ammonium hydroxide and finally filtered. The filtrate was discarded and pellets obtained, dried and weighed (Okwu & Josiah, 2006).

3.1.12 Determination of saponin content

The 10g of sample was mixed with 100ml of 20% aqueous ethanol. The mixture was kept for 4 hours on water bath shaker at 55^{o}C. Residue was again re- extracted in the same manner. The combined extract was concentrated to 40ml over water bath at 90^{o}C. Concentrate obtained was transferred into a separating funnel and 10ml of diethyl ether was added to it. After shaking vigorously, aqueous layer was recovered and ether layer discarded. The process was repeated. To the aqueous layer n- butanol was added. The whole mixture was washed in separating funnel twice with 10ml 5% aqueous Nacl and the upper part was retained and heated in water bath until evaporation. Then it was dried in oven to constant weight (Obadon & Ochuko, 2001; Edeoga *et al.*, 2005).

3.1.13 Determination of phenolic content

The 0.5g of sample powder was extracted with 10ml of 80% ethanol. Supernatant was evaporated to dryness and extract in 5ml water. Different aliquots 0.1 -1ml were pipette into test tubes and final volume made to 3ml by water. 0.5ml of Folin's reagent (sodium 1, 2 nephthoquinone-4-sulfonate) followed by 20% Na_2CO_3 equal to 2ml, were added respectively. Tubes were vortexed and kept in boiling water for one minute. After cooling, absorbance was read at 650nm against the blank. A standard curve was plotted using different concentrations of 1000mg catechol or gallic acid equivalence (Malick & Singh,1980).

3.1.14 Determination of tannin content

The 2g of tea was extracted thrice in 70% acetone. After centrifuging the sample, supernatant was removed. Different aliquots were taken and final volume made to 3ml was adjusted by distilled water. The solution after voltexing was mixed with 1ml of 0.016M $K_3Fe(CN)_6$ followed by 1ml of 0.02M $FeCl_3$ or 0.10M HCL. Voltex was repeated and test tubes kept as such for 15min. 5ml of stabilizer (3:1:1 ratio of water, H_3PO_4 and 1% gum Arabic) was added. Followed by revortexing, and absorbance was read at 700nm against the blank. Standard curve was plotted using various concentration of 0.016 M gallic acid (Graham, 1992).

3.1.15 Determination of carbohydrate content

5g of plant powder was extracted with 80% ethanol. Extract was dissolved in10ml water. Different aliquots were prepared and final volume made to 1ml by water. 5ml of

90% concentrated H_2SO_4 was added followed by shaking and incubation for 40min at room temperature. 1ml of 5 % phenol was added to each tube and absorbance read at 490nm. Standard curve using different concentrations of 25mg of glucose was plotted (Knulnoven *et al*., 1984).

3.1.16 Determination of protein content

1g tea was extracted using 10ml water, added with few drops of triton X100. The supernatant was extracted in acetone and the pellet obtained was dissolved in 0.1M NaoH. Aliquots were prepared and final volume made to 1ml by distilled water, 5ml of copper reagent was added to tubes, mixed well and incubated for 10min. 1ml of Folin's reagent was mixed. Tubes were incubated for 30min at room temperature and absorbance read at 700nm. Standard curve was prepared using 50mg BSA (Lowry *et al*., 1951).

3.1.17 Determination of lipid content

1g tea sample was dissolved in ether and stirred for 1 hour. The mixture was centrifuged, dried and dissolved in ethanol. 0.1ml of alcohol was taken as a blank, olive oil was used as standard and test samples as unknown respectively. 2ml of concentrated H_2SO_4 and 5ml of phosphor valin reagent were added and mixed well, incubated for 30min. Absorbance was read at 540nm (Gnai *et al.*, 2005).

3.2 Determination of anti-microbial effect of tea extracts

Four human pathogenic bacteria made of two gram positive and two gram negative were used for agar well diffusion assays. One yeast *Candida albicans* was used for antifungal

assay. The agar well diffusion method was used to test the tea extract for antimicrobial activity. Nutrient agar and potato dextrose agar were used for the bacteria and fungal cultures respectively. Bacteria cultures were incubated at 37^{o}C for 18-24 hours, and 72hours for fungi. 0.1ml of tea extracts at varying concentrations (50mg/ml^{-1}, 100mg/ml^{-1},200mg/ml^{-1}, 400mg/ml as well as antibiotic 4 mg/ml solutions were loaded into wells). Control experiments were set up using cefadroxil and streptomycin for both the bacteria and fungi. Distilled water was used as negative control. According to pharmacological and biometric specifications, the antimicrobial studies were do. 3ne in triplicate and diameters of zones of inhibition from the edge of wells measured in mm as indication of activity.

3.2.1 The minimum inhibitory concentrations (MIC)

The /minimum inhibitory concentrations (MIC) of plant extracts were determined using the broth dilution method as described by Salim & Washington, (1999). 1ml of extract solution at conce.ntrations of 800mg/ml^{-1} was added to 1ml of nutrient broth and subsequently transferred to make solution of varying concentrations of 400mg/ml, 200mg/ml,100mg/ml and 50mg/ml in different test tubes. Then 1ml of bacterial and fungal suspensions and 0.1ml of plant extacts at different concentration and incubated at 37^{0}C for 24 hours for bacterial and 48 hours for fungi. The test tube with the concentration of plant extract at which no detectable growth (no turbidity) was observed was considered as the MIC.

3.2.2 Mice used for in vivo studies

Swiss white (male) mice weighing 20-30g, aged 6-8 weeks old were used. They were acquired from Chiromo campus university of Nairobi. The animals were kept in the experimental laboratory when the temperature was 25^0C. They were fed for two weeks on mice pellets and water for acclimatization

3.2.3 *Plasmodium berghei* isolate used for infection experiments

Plasmodium berghei ANKA isolate cryopreserved at Biochemistry laboratory was used. It 3.is a protozoan parasite which causes malaria in certain rodents. It causes symptoms which are to a certain extent comparable to those of celebral malarial in patients infected with human malarial parasite Plasmodium faiciparum. The parasite was a donation from KEMRI. The parasite was propagated and maintained in clean mice 5 days before the commencement of the research.

3.2.4 Clearance for the methods used

Animal care protocols and procedures used in the current study were reviewed and approved by the institutional animal care and use committee.

3 .2. 5 Determination of tea extract consumption by mice

Preliminary evaluation tests were done to ascertain whether the test animals could voluntarily drink water 10g/l sucrose and various concentrations of green tea extract (GrTE) (0-20g/l) (Karori *et al*., (2010). The mice were acclimatized for 2 weeks during which each mouse was treated once using 0.1ml of 1 % ivermectin to exclude any helminthes infections. The animals were then randomly allocated into 5 groups each of

6 mice per group with each group being housed separately, over a period of 10 days. Each group was subjected to either:

Group-1;Water with 10g/l sucrose (Control)

Group-2;Water supplement with 10g/l sucrose + 5g/l GrTE

Group-3;Water supplement with 10g/l sucrose + 10g/l GrTE

Group-4;Water supplement with 10g/l sucrose + 15g/l GrTE

Group-5;Water supplement with 10g/l sucrose + 20g/l GrTE

The test animals were closely observed thoroughly daily for 10 days on the consumption of tea water for toxic signs and symptoms. PCV was determined using the standard micro- haematocrit method.

3.2.6 Body weights measurements of mice treated with various dosages of green tea

The animals were weighed daily for 11 days. Their overall health and general well being was observed and recorded daily. Significant weight loss (more than 2 fold loss compared to water 10g/l sucrose control over the 10 days closing period) was considered a key indicator of declining health due to toxicity.

3.2.7 Innoculation of the mice with *Plasmodium berghei* ANKA

The mice were inoculated with 0.2ml of the stabilate on phosphate saline glucose (PSG) buffer intra peritoneal. The mice were bled on the tail vein once daily for 3 weeks and blood examined by wet film for the presence of parasites, centrifuged in haematocrit

centrifuge for PCV determination and serum obtained from blood for proteins estimation (Lowry method) spectrophotometrically.

3.2.8 Determination of effect of tea extracts on mice infection

Green tea, orthodox tea and black tea extracts (10g/ml) were evaluated *in-vivo* for *Plasmodium berghei* activity using intraperitoneal innoculation of each mouse with $1.0X10^4$ parasites (0.2ml). Treatment was administered 24 hours later. A total of 105 mice eight weeks old adults and healthy were used for the experiments. The mice were randomly divided into seven equal groups (n= 15/ group) and subjected to one of the following treatments; green tea, orthodox tea, black tea at 10g/l (administered orally) 0.1ml of anti- inflammatory drug (dexamethasone) equivalent to 0.2mg per mouse (IP), water only (infected), water only non-infected (placebo) (administered orally) , and pyremethamine as a reference drug (intramuscular) IM,. Except the placebo group, animals in other groups were infected with *Plasmodium bherghei*. All the extracts were freshly prepared in distilled water. Each group of mice was contained in a mice cage. Mice were checked daily during the 18 days of treatment to estimate the number of parasites in their tail blood in a wet blood film except the reference control group when the sampling was necessitated by death of the animals on day 5. The absolute number of parasites per milliliter of blood was taken as log using the rapid matching method for estimating the host's parastemia according to Herbert and Lumsden, 1976. At higher level of parasites this was achieved by matching microscopic fields of wet blood films against charts and when fewer parasites were present by counting the number of parasites in 5, 10 or 20 such microscopic field for the assessment of effect of tea extract,

the level of paraistemia (expressed as log of absolute number of parasites per milliliter of blood) in the animal was compared to the control animals. Animals were checked daily for parasites in tail vein for 18 days. Animals that survived to the end of the experiment, with no parasites in the blood were considered cured. During the period, PCV, weight and serum albumin concentration of mice were also determined and recorded.

3.3 Quantification of *Plasmodium berghei*

Two micro liter of blood was taken from the cut tail vein of a mouse by a scapel blade by capillary tube, dropped onto a glass slide and covered in a cover slip. The slide was examined using phase contrast/dark ground microscopy at x400 magnification to determine the presence of *Plasmodium berghei*

Forty five micro liters of staining solution was pipetted into two tubes. Five micro liters of blood was added to the first tube, and then mixed well. A fresh capillary tube was used to transfer further 5µl of diluted blood from first tube to second tube and mixed well. The capillary tubes were allowed to stain for 5 min. The coverslip was carefully attached to the improved neuber counting chamber before filling slide of the counting chamber with a capillary tube and allowing the contents to settle for 2-3 min. *Plasmodium berghei* were then counted in areas C,c,F,e,, contained in a circle as shown below.

The microscope was first adjusted using the 10x objectives lens until the grid was seen at the centre then switched to the 40x objective lens. *Plasmodium berghei* has a very

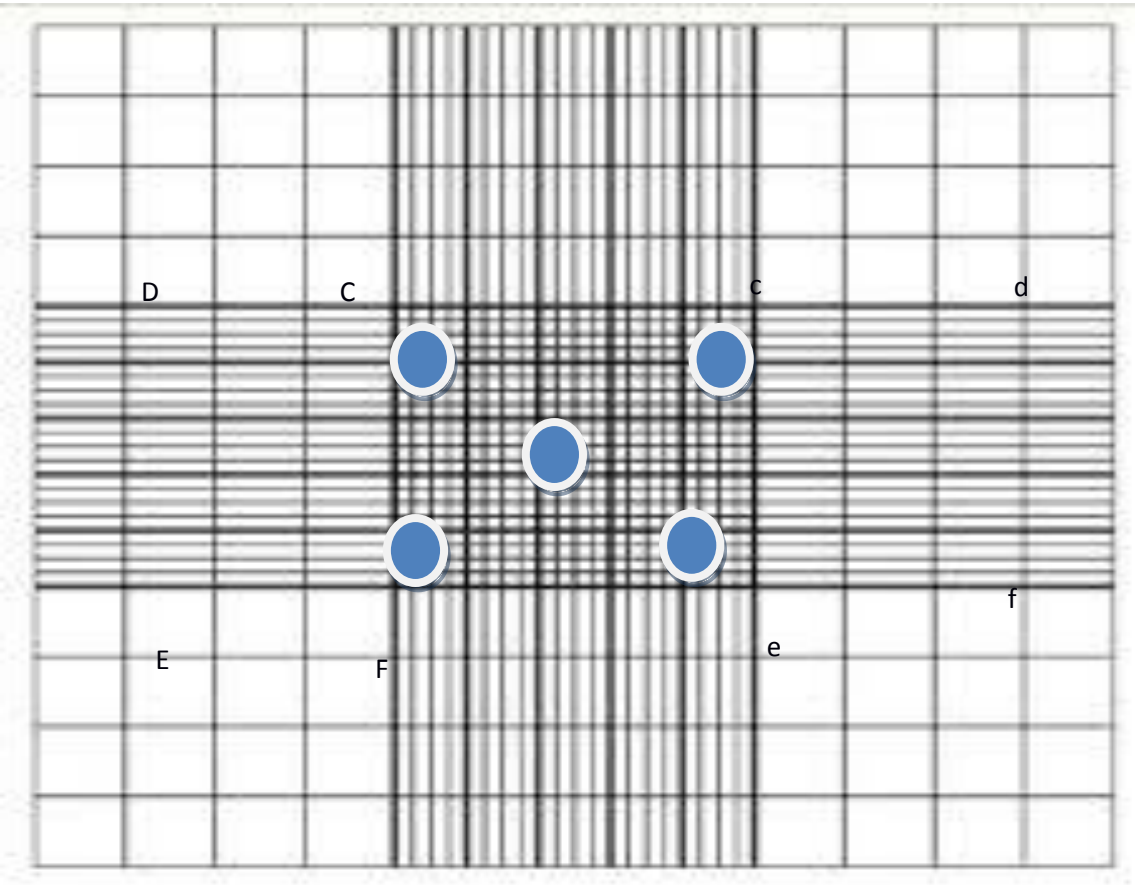
district shape under a microscope which makes it easy to see. The appearance of the grid is shown in figure 7. A haemocytometer has two grids. Parasites were counted in specific squares of both grids under the 40x objective lens, and then the parasite concentration calculated (Herbert and Lumsden, 1976). The drawing below shows the squares which the parasites were counted in one grid i.e. the circled areas (the four corner squares and the centre square). The cells were counted in a total of 10 squares for each sample. The following formula was used to calculate cell concentration: $^{n}/_{10}$ x dilution factor x 250,000 parasites/ml where n = the total number of parasites in the 10 squares and 250,000 factors represents the number of squares (which are actually cubes) in a ml.

For example where n=4

$$\frac{4}{10} * 100 * 250{,}000 = 10{,}000{,}000 parasites$$

Log_{10} 10,000,000 parasites = 7.0

A B a b



H G h . g

Figure 3. 2: Haemocytometer count chart.

The circled areas where counting of parasite was done. The dilution factor 1/100 was taken into account during the calculation.

3.3.1 Determination of PCV

This is the fraction of whole blood volume that consists of red blood cells. The blood was collected in heparinized capillary tubes which were sealed immediately. The capillary tubes with blood were then centrifuged in a microcentrifuge for 5 min at 10000g. After centrifugation the height of the red blood cell column was measured by use of haematocrit reader and compared to the total height of the column of the whole blood. The % Pcv of the total blood volume occupied by RBC mass in the haematocrit which depend mostly on the number of RBCs was calculated. The reference values are 42-52% for males and 36-48% for females. The haematocrit is usually about 3 times haemoglobin value. The average error haematocrit is about 1-2%. The haematocrit may be changed by altitude and position as in the case of haemoglobin. Thus decrease in PCV against the reference indicates the presence of infection.

3.3.2 Determination of serum protein concentration in mice.

To 10ml (0.01) of sample protein; .099ml of distilled water and 3ml of reagent D (alkaline copper solution) were added and mixed thoroughly. The mixture was allowed to stand for 15min at room temperature before adding reagent E (Folin-Ciocalteu reagent); and allowing the mixture to stand for 30min at room temperature. The protein standard was prepared starting with the blank to 100µg in 1 ml of distilled water and the contents treated as in test sample. The absorbance was measured at 670nm against the

blank. The protein concentrations of the test sample were calculated using the standard plot (lowry *et al*.,1951).

3.3.3 Statistical analysis

Data obtained from PCV, serum protein values and parastemia was analyzed using STATS view statistical programme (SAS) and significance of difference between the means determined by ANOVA. A P value of <0.05 was considered to be statistically significant.

CHAPTER FOUR

RESULTS

4.1 Tea samples

The sample information of quantity and locality of collection tea used in the experiments is presented in table 4.1 below. They were obtained from three different ecological regions of Kenya. The three samples had undergone the same process of manufacture except the oxidation /aeration in which they differ. The processes are; leaf blending, withering, merceration, ,oxidation/aeration and drying.

Table 4.1: Sample information of tea studied.

NAME	LOCALITY OF COLLECTION	QUANTITY
Green tea	Kirinyaga	150g
Orthodox tea	Kirinyaga	150g
Black tea	Murang'a	150g
Black tea	Namira	150g

4.1.2 Phytochemical screening

The three types of tea in comparison were found qualitatively to contain Flavonoids, terpenes, saponins, tannins, cardiac glycosides as common metabolites. However the specific test employed gave negative results on anthraquinones for green tea, phenolic and cardenolides for orthodox tea unlike black tea which gave positive tests for all the metabolites tested (table 4.2).

Table4.2: Phytochemical constituents of crude extract of tea samples

	Green tea	Orthodox tea	Black tea(Muranga)	Black tea(Nyamira)
Phenolics	+	-	+	+
Flavonoids	+	+	+	+
Terpenes	+	+	+	+
Cardia glycosides	+	+	+	+
Cardenolides	+	-	+	+
Anthraquinones	-	+	+	+
Alkaloids	+	+	+	+
Saponins	+	+	+	+
Tannins	+	+	+	+

+ Presence of secondary metabolite

- Absence of secondary metabolite

4.1.3 Quantitative determination of phytochemicals

Quantitative estimation of bioactive constituents of orthodox, green, and black tea are summarized in table 4. 3. In addition estimation of carbohydrates, lipids, and proteins are shown in the same table 4.3 below. Results suggests that all the teas contain good phenolic content. Alkaloid content increased according to the oxidation status of the tea extract.

Table 4.3: Quantitative determination of phytochemicals in tea

Tea extract	Alkaloid	Phenol	Tannin	Saponnin	Carbohydrates	Lipids	Proteins
Green tea	1.64± 0.02	0.0624±0.05	0.425±0.025	2.323±0.002	0.802±0.001	0.004±0.002	0.442±0.025
Orthodox tea	1.80±0.04	0.023±0.01	0.443±0.003	1.442±0.001	0.682±0.002	0.613±0.025	0.338±0.001
Blacktea	2.02±0.02	0.056±0.002	0.208±0.002	1.65±0.004	0.710±0.001	0.348±0.001	0.468±0.024

Results are mean ± SD of triplicate determination on the basis of dry weight in mg.

4.1.4 Anti microbial studies

4.1.4.1 Antimicrobial Assays

The results showed that the three tea aqueous extracts had antimicrobial activity against *Staphylococcus aureus, Salmonella typhimurium, Escherichia coli, Streptococcus faecalis, Candida albiccans* except Orthodox tea which had no activity on *Salmonella typhimurium* and *candida albicans* The various tea extracts gave varied zones of inhibition as shown in table 4.4 . The standard antibiotics used were streptomycin and cefadroxil and they also exhibited activity against the test micro-organism.

The activity of the different tea extracts against selected micro-organisms is summarized in table 4.5.

The diameters of zones of inhibition of various tea extracts against selected micro-organisms are shown in the plates; 4.1, 4.2, 4.3, 4.4, 4.5, 4.6 and 4.7 .

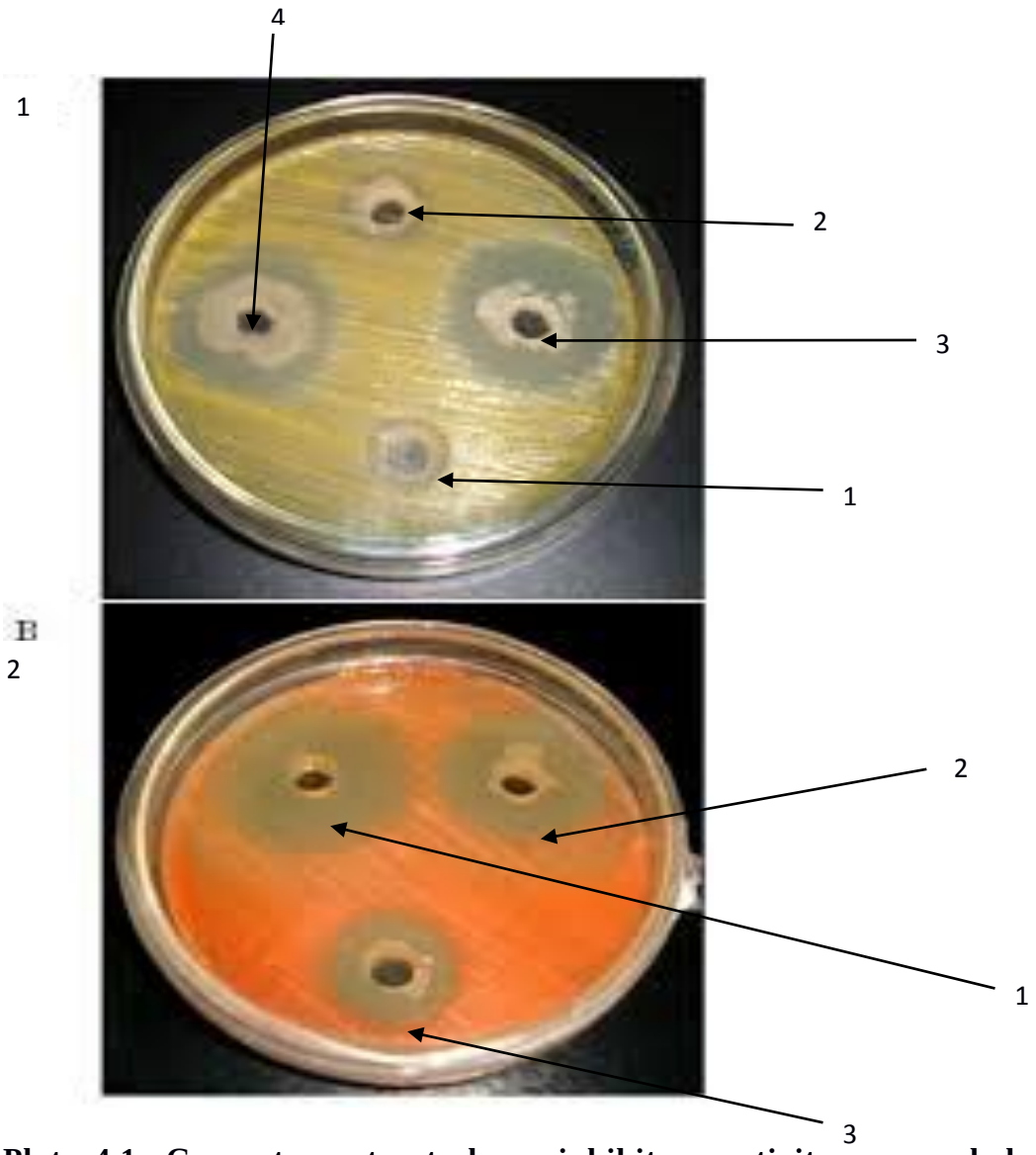


Plate 4.1; Green tea extract shows inhibitory activity **phylococcus aureus** and gives a clear path around the ext4act wells of 1,2,3,4 with different concentrations of 50mg/ml, 100mg/ml, 200mg/ml and 400mg/ml respectively

Plate 4.2; Orthodox tea extract shows inhibitory activity on Staphylococcus aureus and gives a clear path around the extract wells of 1,2,3 with different concentrations of 100mg/ml, 200mg/ml and 400mg/ml respectively

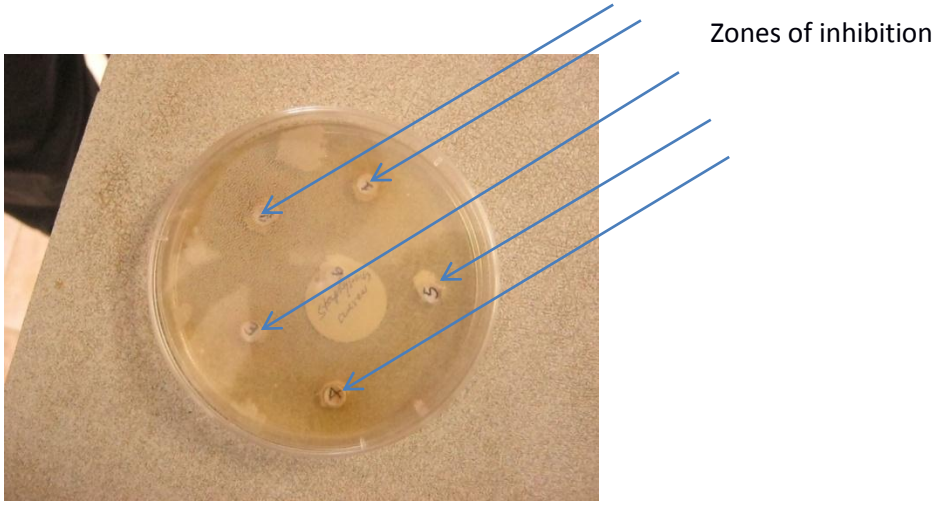


Plate 4.3: Black tea extract (Muranga) shows inhibitory activity on Staphylococcus aureus and gives a clear path around the extract wells of 1,2,3,4 with different concentrations of 50mg/ml, 100mg/ml, 200mg/ml and 400mg/ml respectively. The standard antibiotics streptomycin is represented in well 5, which cefadoxil is represented at well 6 at the centre of the plate.

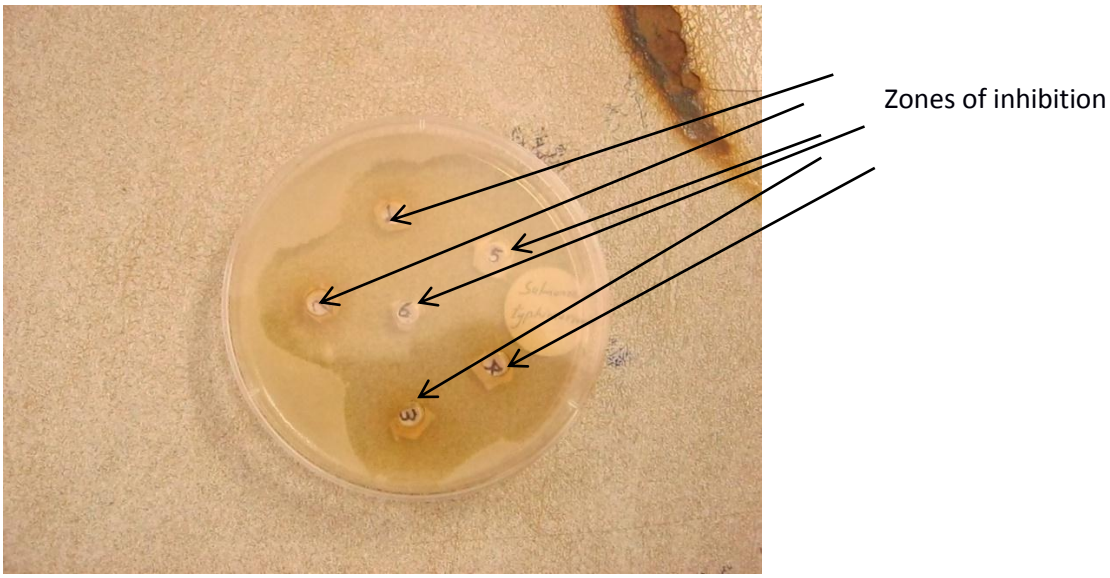


Plate 4.4 : Black tea extract (Muranga) shows inhibitory activity on Escherichia coli, and gives a clear path around the extract wells of 1,2,3,4 with different concentrations of 50mg/ml, 100mg/ml, 200mg/ml and 400mg/ml respectively. The standard antibiotics streptomycin is represented in well 5, which cefadoxil is represented at well 6 at the centre of the plate.

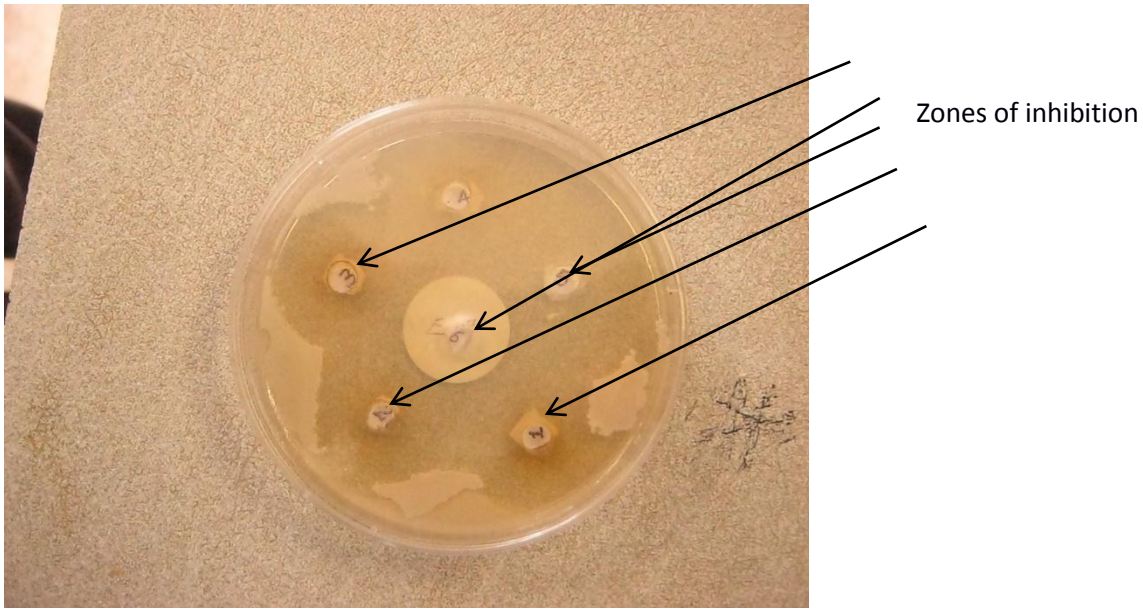


Plate 4.5: Black tea extract (Murang'a) shows inhibitory activity on Salmonella typhimurium, and gives a clear path around the extract wells of 1,2,3,4 with different concentrations of 50mg/ml, 100mg/ml, 200mg/ml and 400mg/ml respectively. The standard antibiotics streptomycin is represented in well 5, which cefadoxil is represented at well 6 at the centre of the plate.

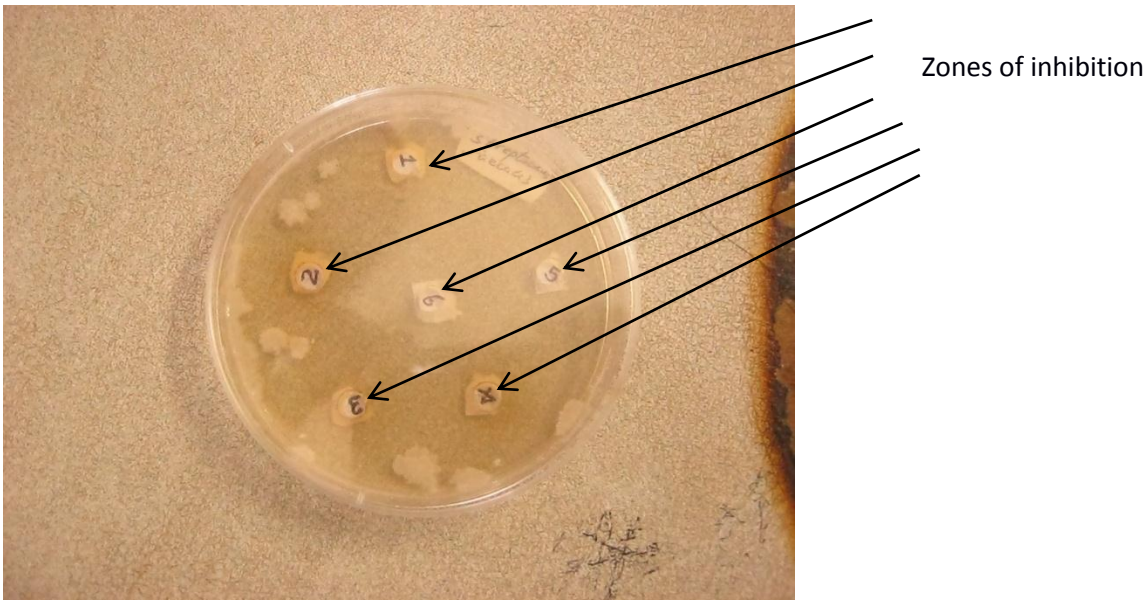


Plate 4.6: Black tea extract (Murang'a) shows inhibitory activity on Streptococcus faecalis and gives a clear path around the extract wells of 1,2,3,4 with different concentrations of 50mg/ml, 100mg/ml, 200mg/ml and 400mg/ml respectively. The standard antibiotics streptomycin is represented in well 5, which cefadoxil is represented at well 6 at the centre of the plate.

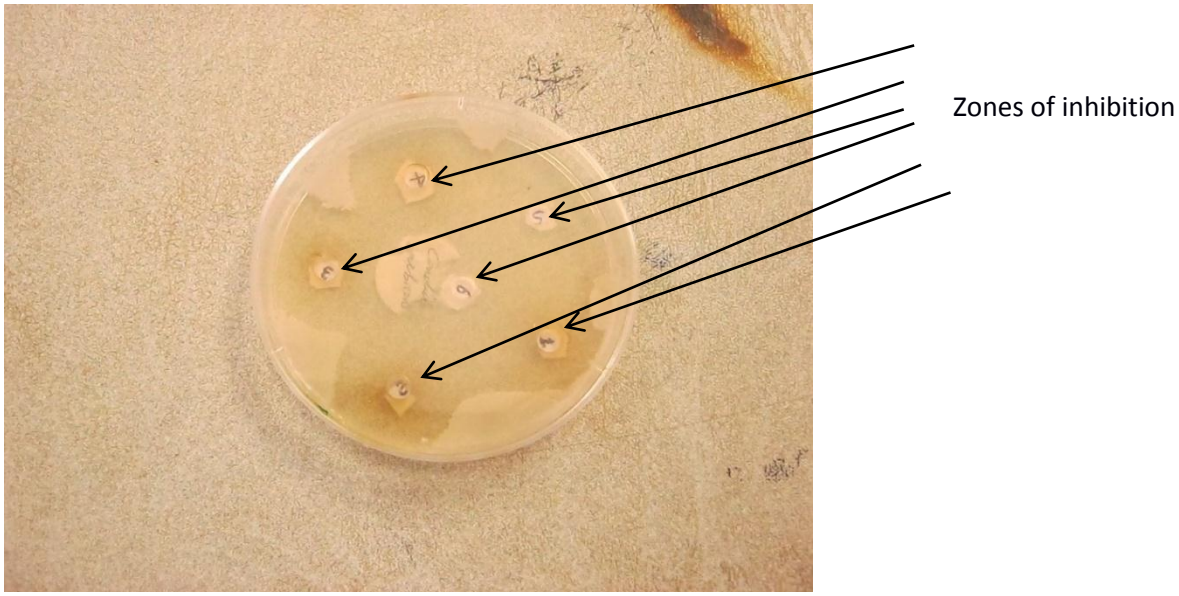


Plate 4.7: Black tea extract (Murang'a) shows inhibitory activity on Candida albicans. And gives a clear path around the extract wells of 1,2,3,4 with different concentrations of 50mg/ml, 100mg/ml, 200mg/ml and 400mg/ml respectively. The standard antibiotics streptomycin is represented in well 5, which cefadoxil is represented at well 6 at the centre of the plate.

Table 4.4: Antimicrobial activity of aqueous tea extracts, zones of inhibition diameters (mm) on four bacteria and a fungi.

Tea extract	Conc. $Mgml^{-1}$	*Staph aureus*	*Escherichia Coli*	*Salmonella typhimurium*	*Strept faecals*	*Cadida albicans*
Green tea	50	-	-	-	-	-
	100	10± 0.0	-	-	12	-
	200	15± 0.0	14± 0.0	5± 0.0	14	1±0.0
	400	20± 0.0	18 ±0.0	18± 0.0	15	1±0.4
Orthodox tea	50	-	-	-		-
	100	-	-	-		-
	200	4 ±0.2	6± 0.0	-	10± 0.0	-
	400	8 ± 0.0	14± 0.0	-	12 ±0.0	-
Black tea (Murang'a)	50	-	-	-		-
	100	-	-	1± 0.0	2±0.0	4±0.2
	200	6.5± 0.0	3.5 ±0.0	3± 0.0	5±0.0	6±0.1
	400	7.4± 0.2	14 ±0.0	4 ±0.0	6±0.2	9±0.1
Black tea (Kisii)	50		-	-	1±0.0	5±0.0
	100	4.0±0.1	1.0±0.2	1±0.0	3±0.0	6±0.1
	200	8.1±0.2	4±0.1	4±0.1	5±0.1	8±0.2
	400	9.2±0.1	16±0.2	5.0±0.2	7±0.2	10±0.4
Streptomycin	4.0mg/ml	20±0.0	10±0.0	15±0.0	10±0.0	25±0.0
-cefadroxil	4.0mg/ml	20±0.0	20±0.0	20±0.0	20±0.0	20±0.0

- Absence of antimicrobial activity.

The antimicrobial assays were done in triplicate and zones of zones of inhibition from the end of the wells measured in mm as indication of activity. Results are mean+-SD of triplicate diameters of zones of inhibition

Table 4.5: Minimum inhibitory concentrations of aqueous extracts of tea on selected micro-organisms.

Tea extract	Conc. $Mgml^{-1}$	*Staph. Aureus*	*Escherichia Coli*	*Salmonella typhimurium*	*Strept. faecalis*	*Candida albicans*
Green tea	400	-	-	-	-	-
	200	-	-	-	-	-
	150	-	-	+	-	-
	100	-	+	+	-	+
	50	+	+	+	+	+
	25	+	+	+	+	+
Orthodox tea	400	-	-	-	-	+
	200	-	-	-	-	+
	150	-	-	+	-	+
	100	-	+	+	-	+
	50	+	+	+	+	+
	25	+	+	+	+	+
Black tea (Murang'a)	400	-	-	+	-	-
	200	-	-	+	-	-
	150	-	-	+	-	-
	100	-	+	+	-	-
	50	+	+	+	+	+
	25	+	+	+	+	+
Black tea (Kisii)	400	-	-	-	-	-
	200	-	-	-	-	-
	150	-	-	-	-	-
	100	-	-	-	-	-
	50	-	+	+	-	-
	25	+	+	+	+	+

- No growth observed

\+ Growth observed

4.1.4.2 Conventional antimicrobial drug sensitivity

Table 4 also presents susceptibility patterns of clinical isolates. The reference isolates *Staphylococcus aureus, Salmonella typhimurium, Escherichia coli, Streptococcus*

faecalis,and Candida albicans were susceptible to streptomycin and cefadroxil. The diameter of inhibition zones for cefadroxil at 4mg/ml concentration was 20mm for all the bacterial isolates and 10mm for C*andida albicans*. Streptomycin inhibited at 4mg/ml concentration inhibited with the following diameter of inhibition; *Staphylococcus aureus* 20mm,*Escherichia coli* 10mm, *Salmonella typhimurium* 15mm, *Streptococcus faecalis* 10mm, *Candida albicans* 25mm.

4.1.4.3 Antimicrobial activity of green tea

Table 4.4 presents the antimicrobial activity of aqueous green tea extract on selected microorganisms. The water extract of green tea inhibited *Staphylococcus aureus* at a concentration of 100mg/ml and inhibition zone of 10mm as shown in plate 1 and the MIC for the organism was greater than 100mg/ml. The same extract inhibited *Escherichia coli* at a concentration of 200mg/ml, an inhibition zone of 14mm the MIC for the organism was greater than 150mg/ml. Green tea extract also inhibited *Streptococcus faecalis* and *Salmonella typhimurium* at a concentration of 200mg/ml with inhibition zone of 12mm and 5mm respectively and their MIC were 100mg/ml and 200mg/ml respectively. The same extract inhibited *Candida albicans* at a concentration of 200mg/ml, inhibition zone of 1mm and had a MIC of the same concentration.

4.1.4.4 Antimicrobial activity of orthodox tea

The antibacterial activity of aqueous orthodox tea shows that the extract inhibited *Staphylococcus aureus* as shown in plate 2 and *Escherichia coli* at a concentration of 200mg/ml with inhibition zones of 4mm and 6mm respectively. Their MIC were

100mg/ml *Staphylococcus aureus* and 150mg/ml for *Escherichia coli*. The extract had no activity for both *Salmonella typhimurium* and *Candida albicans* even at the highest concentration of 400mg/ml used, thus no MIC.

4.1.4.5 Antimicrobial activity of black tea

Black tea from Murang'a and Kisii zones were used for antimicrobial tests (table 4.2). The aqueous black tea from Murang'a inhibited *Staphylococcus aureus as* shown in plate 4.3 and *Escherichia coli* as shown in plate 4 at a concentration of 200mg/ml with inhibition zones of 6.5mm and 3.5mm respectively. The MIC for the organism were 100mg/ml and 150mg/mlrespectively. The same black tea extract inhibited *Salmonella typhimurium* and *Candida albicans* at concentration of 100mg/ml with inhibition zones of 1.0mm and 4.0mm respectively. The same concentration was also the MIC for both microorganism. The same black tea water extract inhibited *Staphylococcus aureus* at 50mg/ml concentration giving zones of 1mm with a MIC of the same concentration.

The antimicrobial activity of the black tea from Kisii zones prevented the growth of *Staphylococcus aureus* at 100mg/ml concentration having an inhibition zone of 1mm, with a MIC of 50mg/ml. The same extract inhibited *Escherichia coli* at 100mg/ml concentration with the 1mm zone of inhibition. The 100mg/ml concentration of the same tea inhibited *Salmonella typhimurium* with a zone inhibition of 1.0mm concentration. The 50mg/ml concentration of aqueous black tea from Muranga inhibited *Streptococcus faecalis* as shown in plate 6 and *Candida albicans* as shown in plate 4.7 with inhibition zones of 1mm and 5mm respectively, however they had a similar MIC of 50mg/ml.

4.2 Tea dosage determination in mice

The determination of the appropriate tea dosage to be adopted in the subsequent experiment was done using green tea extract on swiss male healthy mice for 10 days (appendix i) and indicated a significant difference ($p<0.05$) on daily water intake (table 4.7) but no significant difference on PCV for all the treatments (table 4.8)(Appendix ii).

Table 4.7: ANOVA daily water intake on dosage green tea determination.

C. variation d *f*	s.s	m.s	*f* ratio
4	-333.44	-83.36	10.15
20	-164.11	-8.21	
24			

f = tabulated

$\alpha = 0.05$

f =(4,20) daily water intake= 2.87

Conclusion

Since *f* computed is greater than *f* tabulated at $\alpha = 0.05$ of significant, then we reject the H_o hypothesis, that all dosages are similar.

Table 4.8: ANOVA % PCV level reductions on dosage green tea determination

s.s	d *f*	m.s	*f*
3.2	3	1.1	1
8.82	8	1.1	
12.02	11		

f = tabulated

$\alpha = 0.05$

f =(V_1=3, V_2=8) PCV reduction= 4.8

Conclusion

Since *f* computed is smaller than *f* calculated at $\alpha = 0.05$ of significance. Then we reject the H_o hypothesis that the PCV reductions between tea treatments were not similar.

Of the five different dosages used, 20mg/ml was the most consumed and tolerated concentration (Fig 4.3a&b). The dosage had no toxicity and ensured the highest amount of tea intake thus activity.

20mg/ml was the most consumed and tolerated concentration (Fig4.3a&b). The dosage had no toxicity and ensured the highest amount of tea intake thus activity.

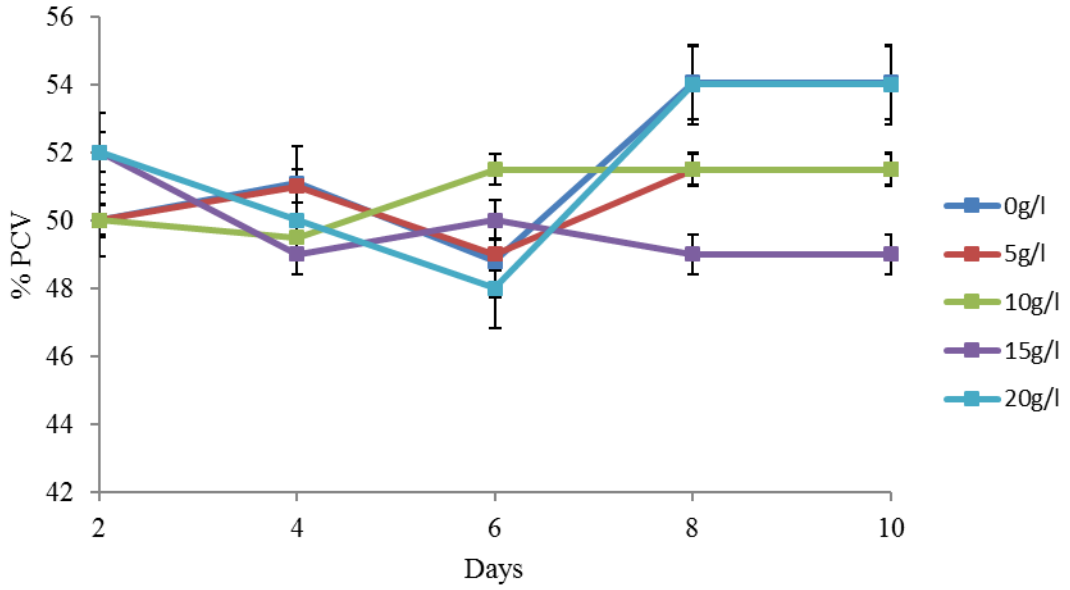


Figure 4.3a: Effect of oral administration of green tea extract on PCV for a period of 10 days on male swiss mice.

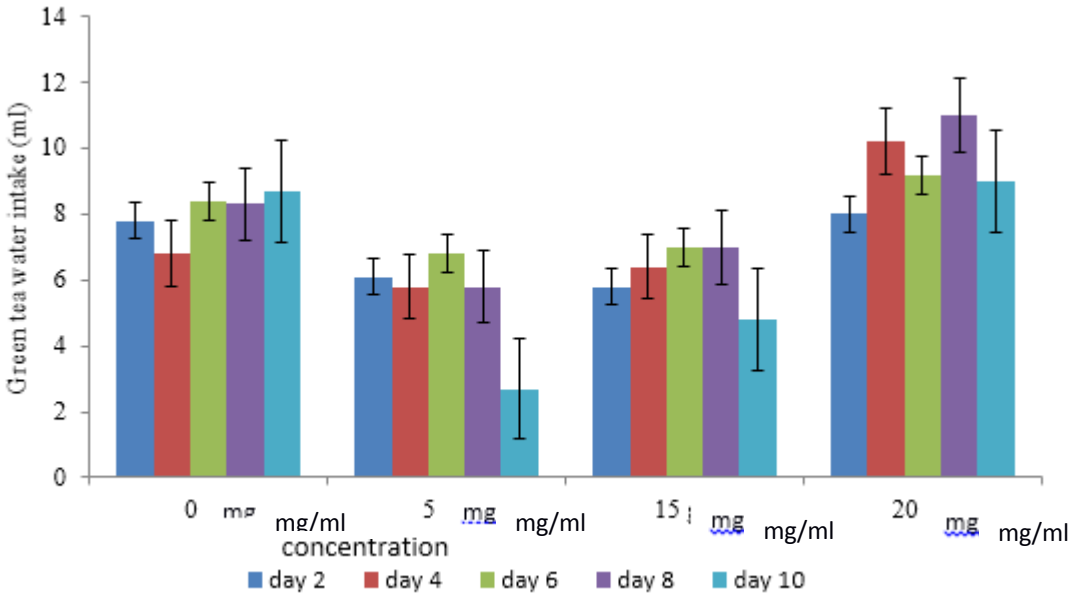


Figure 4.3b: Effect of oral administration of green tea extract on water intake on male swiss weight mice

4.2.2 Effect of tea on parasitemia

To study the effect of tea on *Plasmodium berghei* in mice, the data on mean parasitemia level $\log_{10}$ was recorded for 18 days (Appendix iii).

All the *Plasmodium berghei* infected mice portrayed similar clinical symptoms a pre patent period of five days. The observation of mice on day five after infection was in line with the parasites' known incubation period of 5-10 days. Results on parasitemia levels in control and experimental mice portrayed an exponential increase at similar rates. Day 8 after infection was the peak of parasitemia and the parasites were at similar densities.

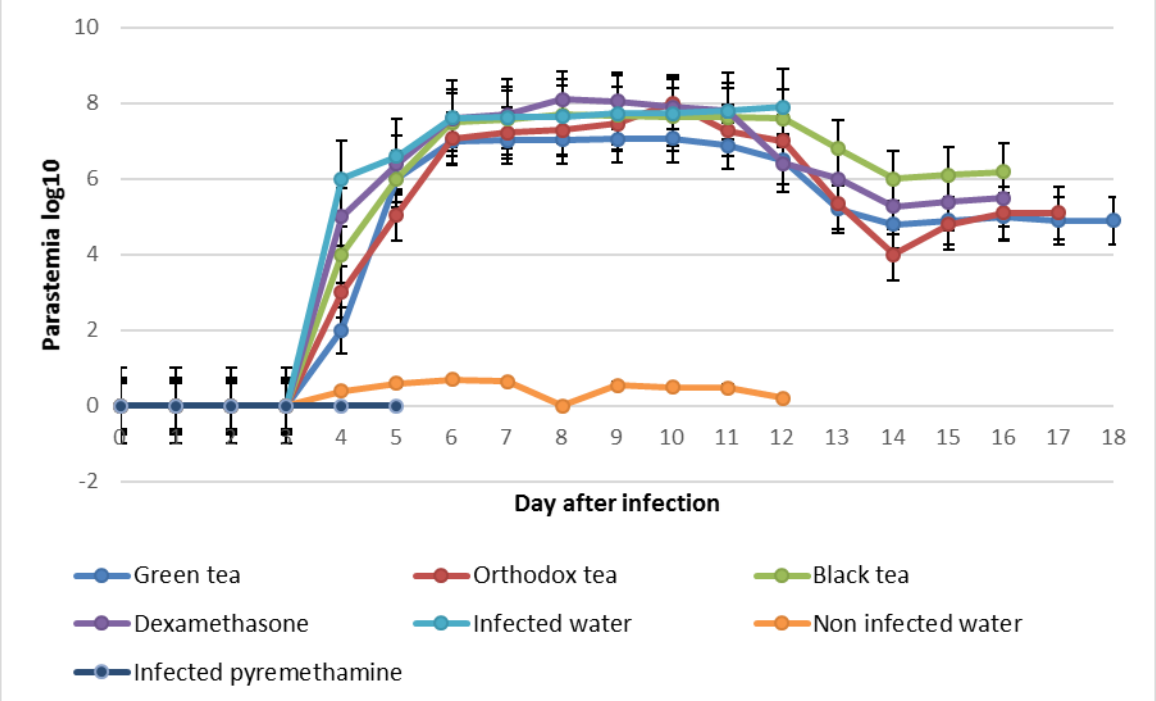


Figure 4.4: Time course of Plasmodium berghei for different treatments plotted as log10 of parasites per milliliter of blood.

Statistical analysis of parasitemic levels declined after parastemic peak on day 2 post infection was significantly different ($P<0.05$) (table4. 9) between the various treatment groups (Table 4.10).

Table 4.9: ANOVA parasitemia decline on day 11 DP_1 between various groups

s.s	d *f*	m.s	*f*
37.3	5	746	-5.3
-290	25	11.11	
-252.7	30		

f = tabulated

$\alpha = 0.05$

f = (V_1=5, V_2=25) parasitemia decline= 2.60

Conclusion

Since *f* computed is greater than *f* tabulated at $\alpha = 0.05$ of significance, then we reject the H_o hypothesis that the parasitemia decline on 11 DP_I between various treatment groups was the same.

Table 4.10: Values (means ± SEM) of log_{10} parasitemia in mice infected with *Plasmodium berghei.*

Treatment	Day 11	Day 14
Infected and green tea	7.10 ± 0.442^{L}	5.60 ± 0.055^{L}
Infected and orthodox tea	7.39 ± 0.458^{L}	5.45 ± 0.032^{L}
Infected and dexamethasore	7.85 ± 0.044^{M}	5.90 ± 0.076^{M}
Infected and black tea	7.63 ± 0.172^{M}	6.80 ± 0.014^{M}
Infected and water only	7.70 ± 0.423^{M}	ND
Non- infected and water only	No parasites	No parasites
Infected and pyrimethemine	5.76 0.588	No parasites
	C.V 5.3 P< 0.05	C.V 1 P< 0.01

Treatments marked the same letter are not significantly different at P<0.05. ND-not done since all mice had died on day 11 after infection. On day 2 post infection mice given tea had a significant reduction in parasitemia levels compared to the ones infected and given water. However the parasitic reduction levels observed between the tea treatment indicates that there were no significant difference (P>0.05) (table3. 11) and table3.10. There was also significant parasitic level reduction by 14 DPI with green tea having the highest reduction in parastemia and significant difference (P<0.01) than other treatments including dexamethasone but not pyrimethamine.

Table 4.11: ANOVA parasitemia decline between inf.GrTE and orthodox TEA

Source of variation	s.s	d *f*	m.s	*f*
Parastemia (SSTr)	1.46	1	1.46	-0.05
Error	-297.96	10	-29.65	
SSE	-296.5	11		

f = tabulated

α = 0.05

f =(V_1=1, V_2=10) parasitemia decline= 4.96

OR: Infec black and Infect Dex

Source of variation	s.s	d *f*	m.s	*f*
Parasitemia (SSTr)	0.2	1	0.2	0.8
Error	2.5	10	0.25	
SSE	2.7	11		

f = tabulated

α = 0.05

f =(V_1=1, V_2=10) parasitemia decline= 4.96

Conclusion

Since *f* computed is greater than *f* calculated (and nearing ratio 1) at α = 0.05 of significance. Then we accept the H_o hypothesis that the parastemia level decline between the treatment is not similar.

4.2.3 The trend of packed cell volume of mice treated with various tea extracts

The trend of packed cell volume was followed along with the parastemia, and thus a companying the events on parastemia above the fall in packed cell volume had occurred on by 7 day post infection (Fig 4. 6). To study the progressive reduction of PCV and effect of various treatments over time the mean change in PCV was carried out daily for 18 days (Appendix iii) analyzed on day11 and 17 (table 12). The results shows that there was significant PCV difference (P<0.05) (table 3.13), between the mice treated using different teas. However, there was no significant difference observed between the tea treatment on day 11 and 17 (table 3.13 and 3.14).

Table 4.12: ANOVA PCV reduction between tea treatments on day 17 and those infected given water only.

Source of variation	s.s	d *f*	m.s	*f*
PCV (SSTr)	520.5	3	173.5	37.8
Error	93.259	20	4.66	
SSE				

f = tabulated

α = 0.05

f =(V_1=3, V_2=20) PCV reduction= 3.10

Conclusion

Since *f* computed is smaller than *f* calculated at $\alpha = 0.05$ of significance. Then we reject the H_o hypothesis that the PVC reductions between tea treatments and those infected given water only were not similar.

Table 4.13a: ANOVA PCV between animal treated using different teas and given water only on day 11.

Source of variation	s.s	d *f*	m.s	*f*
PCV (SSTr)	-587	5	117.4	3.4
Error	103.5	30	34	
SSE		35		

f = tabulated

$\alpha = 0.05$

f =(V_1=5, V_2=30) PCV reduction= 4.17

Conclusion

Since *f* computed is greater than *f* tabulated at $\alpha = 0.05$ of significance. Then we reject the H_o hypothesis that the PVC reductions between mice treated using different teas and those infected and given water only were similar.

Table 4.13b: ANOVA PCV reduction between tea treatments on day 11

Source of variation	s.s	d *f*	m.s	*f*
PCV (SSTr)	253.9	1	253.9	9
Error	281.88	10	28.1	
SSE		11		

f = tabulated

$\alpha = 0.05$

f =(V_1=1, V_2=10) PCV decline= 4.96

Conclusion

Since *f* computed is greater than *f* calculated at $\alpha = 0.05$ of significance. Then we reject the H_o hypothesis that the PVC reductions between treatments on day 11 were different.

Table 4.14: Mean change (means ± SEM) in% PCV of the treated animals and the control group from day 0 to day 11 and 17 post infection.

Treatment	Day 11	Day 17
Infected and green tea	12.0 ±1.33D	22.0 ± 2.24
Infected and orthodox tea	13.5± 1.60D	18.0± 2.60
Infected and dexamethasone	14.5± 1.70D	B
Infected and black tea	15.0± 1.68D	12.0 ± 2.62
Infected and water only	22 ±2.00E	ND
Non- infected and water only	No change in PCV	No change in PCV
Infected any pyremethamine	2 ±1.22	23.02 ± 4.42
	C.V 3.4, P<0.037	C.V 37.8

Treatment marked with the same letters are not significantly different at P<0.05. ND- not done since all mice in this group had died on 11 after infection.

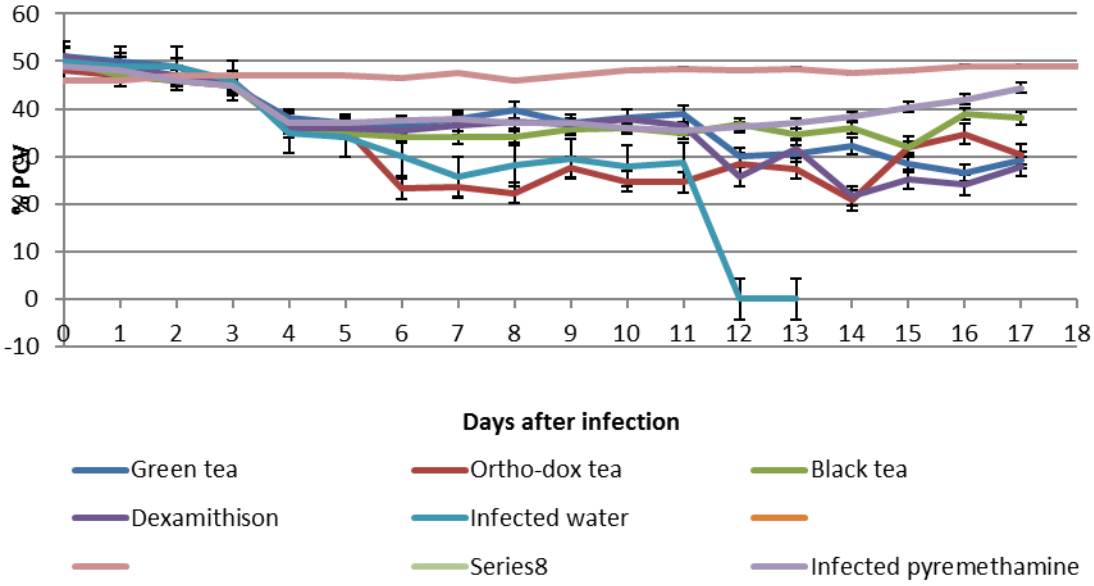


Figure 4. 5: Changes in PCV (means) during the period of study.

4.2.4 Serum protein concentrations

The mice serum protein concentrations were carried out daily from day 0 to 17(appendix iv). However the statistical analysis of data was done on day 11 and day 17 post infection to determine the effect of various tea treatments over time. (4.15).

Table 4.15: Protein concentrations g/dl (means ± SEM) of the treated mice, the infected and non treated mice on day 11 and 17.

Treatment	Day 11	Day 17
Infected and green tea	2.472 ± 0.023Q	2.602 ± 0.14Q
Infected and orthodox tea	2.422 ± 0.042Q	2.300 ±0.18Q
Infected and dexamethasone	2.488 ± 0.094Q	2.568 ±0.09Q
Infected and black tea	2.023 ± 0.462Q	2.00 ± 0.14R
Infected and water only	1.4.4 ±0.201R	NAD
Non- infected and water only	2.846 ±0.245	2.824 ±0.26
Infected any pyrimethamine	2.522 ± 0.348Q	2.742 ±0.1Q
	C.V 13.7, P<0.01	C.V 29 P<0.01

ND- not done since all the mice in this group had died.

Treatments marked with the same letters are not significantly different at P<0.05. Statistical analysis on reduction of mice serum protein (Fig 4. 6) indicates that there was a significant amelioration (P<0.01) of parasite individual hypoproteinemia due to the treatment with various teas (Table 4.15 and 4.16) the placebo (non infected group of mice) had serum protein concentration within the normal range for the whole experiment period. There was a significant reduction in protein concentration on day 11 post infection only in the mice given water (table 4.17) (P<0.01).

Table 4.16: ANOVA Hypoproteinemia due to treatment with various teas, on day 11 and 17.

Day 11

s.s	d *f*	m.s	*f*
29.04	6	4.84	4.58
-36.96	35	1.056	

f = tabulated

$\alpha = 0.01$

f =(V_1=6, V_2=35) Hypoalbuminemia = 3.38

OR Day 17

s.s	d f	m.s	f
8.24	6	1.37	13.7
3.45	35	0.0.9	

f = tabulated

$\alpha = 0.01$

f = (V_1=6, V_2=35) Hypoproteinemia due to treatment with various teas, table 3 = 3.38.

Conclusion

Since f computed is greater than f tabulated at $\alpha = 0.01$of significance. Then we reject the H_o hypothesis that hypoproteinemia is similar within various tea extracts.

Table 4.16: ANOVA Reduction in serum protein concentration on day 11 on mice given water and those given tea.

s.s	d *f*	m.s	*f*
-28.4	1	28.4	4.05
-7.077	10	7.077	

f = tabulated

$\alpha = 0.01$

f =(V_1=1, V_2=10) reduction in albumin = 10.4

Conclusion

Since *f* computed is greater than *f* calculated at $\alpha = 0.05$ of significance. Then we accept the H_o hypothesis that reduction in serum protein concentration on mice given teas and those given water is not similar.

Data analysis on day 17 post infection shows that mice given black and orthodox tea had significantly ($P<0.01$) lower serum protein (table4.17), than animals treated with green tea, pyrimethamine and dexamethasone.

Table 4.17: ANOVA Serum protein concentrations in mice given green tea, back tea, orthodox tea, dexamethazone and pyrimethamine on day 17.

s.s	d *f*	m.s	*f*
1.42	4	2.82	2.2
32.38	25	1.30	

f = tabulated

$\alpha = 0.01$

f = (V_1=4, V_2=25) Albumin concentration = 4.8

Conclusion

Since *f* computed is greater than *f* calculated at $\alpha = 0.01$of significance. Then we accept the H_o hypothesis that black tea treated mice serum was not similar to albumin from green tea, orthodox teas, dexamethazone and pyrimethamine treated mice.

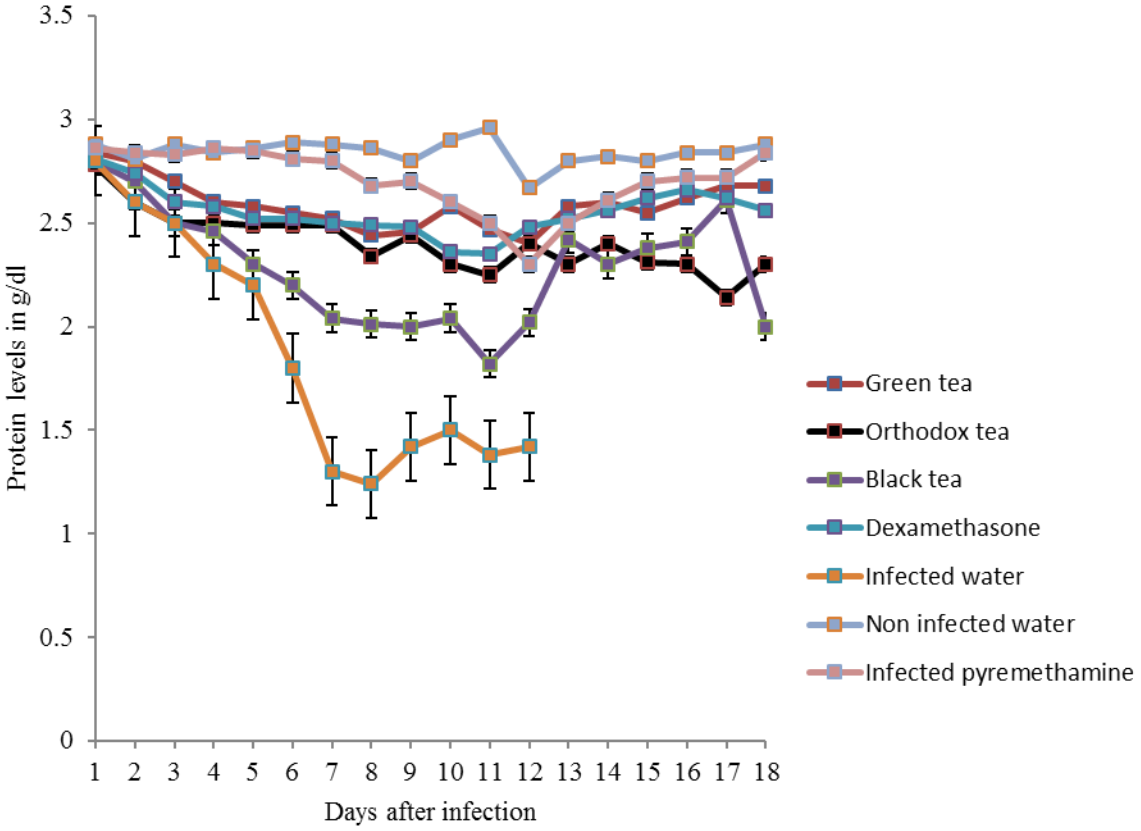


Figure 4.6: Changes in serum protein (means ±SD) concentrations during the period of study

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 DISCUSSION

The presence of the secondary metabolites in tea enhances the pharmacological aspects of tea. The three samples of tea used were green tea non-oxidized tea, orthodox tea partially oxidized tea, and black tea fully oxidized tea. The three types of tea in comparison were found qualitatively to contain flavonoids, terpenes, saponins, tannins, cardiac glycosides as common metabolites. However the specific test employed gave negative results on anthraquinones for green tea, phenolic and cardenolides for orthodox tea unlike black tea which gave positive tests for all the metabolites tested.

Thus the reduction of parasitemia level in mice and inhibition of the microbes by tea can be attributed to the anti*plasmodium berghei* and antimicrobial effects of the polyphenols and other metabolites. The presence of phenolic compounds indicates that the plants are antimicrobial agents (Okwu and Okwu, 2004). This agreed with the findings of Sekanaka. *et al*.,(1989) who reported that several polyphenolic compounds in the green tea have moderate antimicrobial principles. These metabolites have been known to be effective against a wide range of micro-organisms. Flavonoids are phenolic compounds with two carbonyl groups and they are known to be synthesized by plants in response to microbial infections (Ramar and Panal, 2008). The activity of these compounds is due to their ability to complex with extracellular and soluble proteins and also bacterial/ parasite cell wall. Some flavonoids are more lipophilic and disrupt microbial membranes. For example catechins found in tea and phloretin in apples (Cowan,1999).

These probably partly explain why green and orthodox teas inhibited bacteria and reduced parasite levels, compared to black tea since these organisms contain lipids in the cell walls and in their cytoplasms. These catechins are highly hydroxylated. Anthraquinones test show that these compounds were present in both orthodox and black tea but absent in green tea. Anthraquinones have aromatic rings and two ketones.Their absence in green tea is in agreement with earlier work done (Orchanda, 2010) stating that the enzyme polyphenol oxidase in green tea oxidises polyphenolic bodies to ortho-quinones which then by dimerization condense to theaflavins which are yellow bodies.

Quinones have shown to have antimicrobial activity with hypericin, and anthraquinones from *Cassia* (Cowan, 1999). They are also anti-Plasmodium drugs. The mode of action of quinones is by irreversible complexation with nucleophilic amino acids in proteins leading to inactivation of proteins and loss of function. Alkaloids are also present in tea and these are heterocyclic compounds known to have antimicrobial and anti-parasitic effects. These compounds have been found to have anti- diarrheal effects (Cowan, 1999). The inhibition of growth of *Salmonella typhimurium* and other diarrheal associated microbes by tea can be attributed to the presence of alkaloids. Alkaloids have been shown to have numerous functions and among the foremost are their analgesic, antispasmodic and antibacterial effects (Okwo and Josiah, 2006). Thus the big diffrences in MIC between black tea from Muranga >400mg/ml and that from Kisii 100mg/ml against *Salmonella typhimurium* implies that the Nyamira black tea has more alkaloids compared to Muranga black tea and this can be attributed the diffrences

in altitudes. Terpenes were shown to be present in the three samples of tea; green tea, orthodox and black tea. Terpenes have been known to be antiparasitic. For instance terpenoids have shown activity against *Leishmania donovani.* Their mode of action, is by inhibition of DNA isomerase 1 activity (Reys *et al.,* 1995). We can therefore speculate that their presence in tea could have some anti- Plasmodium effect. Antimicrobial activities shown by the three forms of tea are in line with previous antimicrobial studies of tea catechins and theaflavins against the Bacillus species where tea extracts where found to exhibit important inhibitory activity against the growth of certain bacteria (Food *et al.,* 2006). The tea herb also contains saponin which is used to stop bleeding and in treating wounds and ulcers as it helps in red blood cell coagulation (Okwo and Josiah, 2006). So among folkloric remedies tea has been used by nursing mother for sore cracked nipples.

Tea extracts were also found to be rich in tannins and they contribute property of astringency. They fasten healing of wounds and inflamed mucous membranes (Okwo and Josiah, 2006). Our results also confirm negligible values of carbohydrates, lipid and proteins. The aqueous tea extracts of green tea, showed significant activity against *Staphylococcus aureus, Escherichia coli, Streptococcus faecalis,* and *Salmonella typhimorium* but had no inhibitory effects against the *Candida albicans*. The diameter of the zones of inhibition of these extracts was found to be remarkably close to that of the control drug streptomycin. The MIC's for green tea were 100mgml^{-} for *Staphylococcus aureus* and *Streptococcus faecalis,* 150mgml^{-} for *Escherichiacoli,* 200mgml- for *Salmonella typhimorium*. However, orthodox tea extract was inactive

against *Salmonella typhimorium* and *Candida albicans,* but showed most significant activity against *Staphylococcus aureus* and *Streptococcus faecalis* and this can be attributed to the fact that they are gram positive bacteria and thus have high content of protein (peptodoglycan) in their cell walls and their cytoplasm hence are easily inhibited by these phytochemicals found in tea hence loss of function. Black tea showed activity against *Candida albicans* and this is probably due to the strong presence of alkaloids in black tea (Adegoke *et al.,*1968) however it had also inhibitory effects on the growth of the three bacterial species, as compared to the orthodox and green tea. MICs for black tea on these bacterial was averagely 200mgml^{-} with average zone of inhibition of 3mm and therefore black tea can be reported as moderately sensitive according to the Kirby-Bauer technique adopted as the official method of the food and drug administration. There is need for further study to ascertain if the yield in these various tea categories could be increased by using stronger fractionating solvents such as ethyl acetone and methyl acetone. These solvents have been reported to be vigorous than other solvents used in crude extraction of plants (Ajaiyoba and Fadare, 2006). Generally, the antibacterial activities of the extracts as reported in the results were stronger and more pronounced than the antifungal activities. Black tea showed high inhibitory activity against *Candida albicans*. The presence of different secondary metabolites in the three types of tea probably offers the therapeutic basis for the antimicrobial activities with other work linking antimicrobial activities with the presence of secondary metabolites (Kisangau *et al.,* 2007; Kubnamwa *et al.,* 2007). This claim is further strengthened by the work of Tiew *et al.,* 2003 where the antifungal properties of sterailiaceae family were reported. Tea could be a source of new antibiotic compounds. Further work is

needed to isolate the secondary metabolites from the extracts studied in order to test specific antimicrobial activity.

In addition the minimum inhibitory concentration (MIC) of tea yielded promising results that are worthy of note. Green tea had low MIC's of $100mgml^{-}$, $150mgml^{-}$, $200mgml^{-}$ and $100mgml^{-}$ for *Staphylococcus aureus*, *Escherichia Coli*, *Streptococcus faecalis*, and *Salmonella typhimorium* respectively. This suggests that they can be gainfully employed in the production of antibiotics as low MIC mean that only a small quantity of the extract will be required to impair bacterial growth. The average minimum MIC of black tea on *Candida albicans* was 100mg/ml a value which is still low enough and thus of great antimicrobial advantage and these findings agrees with work done earlier (Sekanaka *et al*., 1989b, Obwoge *et al*.,2014). The closeness observed in antimicrobial activities demonstrated by green tea and orthodox tea as revealed by values obtained for the MIC also indicate a close relationship in the processing of the two types of tea. The chain of operations in orthodox tea processing include withering, rolling, aeration, sorting and grading while green tea processing lacks the aeration step (Orchandas, 2010). In conclusion, the chemical composition of green tea is similar to that of the leaf, and it contains polyphenolic compounds, which include flavanols, flavadiols, Flavonoids and phenolic acids and account for 30% of the dry weight of green tea leaves (Roberts, 1962). In general antimicrobial activities decreased when the extracts of tea fermentation increased. The antimicrobial activities of various tea extracts with different extents of fermentation varied with test organisms. Green tea exerted the strongest antimicrobial activity followed by the partially fermented orthodox tea, and

lastly by black tea. It was also noted that tea from high altitude exhibited the strongest antimicrobial activity. The results of *in-vivo* studies showed that tea had the ability to lower parasitemia levels. It further showed that green tea and orthodox tea extracts tested were able to stop *Plasmodium berghei* from establishing and multiplying in animals since they contain catechins that are highly hydroxylated compared to black tea that has oxidized polyphenols (Karori *et al.,* 2007). This is probably because they contain catechins (flavan-3-ols) notably Catechins, Epicatechins, Epigallocatechins, Gallocatechins, Epicatechin-3-Gallate and Epigallocatechins-3-Gallate that are highly hydroxylated compared to black tea, that have oxidized polyphenols in the form of Theaflavins and Thearubigins. Etimotic factors and therefore agronomic factors affect both the flavanol content of the green tea shoot (T3) can therefore speculate that high amounts of polyphenols in the reduced form could reduce *Plasmodium berghei* in the host. However due to the presence of other secondary metabolites, there could be synergistic effects in terms of therapeutic effects of tea. There is therefore need for studies towards understanding the mode of action of these active therapeutic compounds and identifying of the drug targets together with the development of parasite specific drug formulations.

Packed cell volume was determined with aid of microhaematocrit reader and the values expressed as percentages (Barbara, 1980), during the dosage determination and the value obtained to which extract was administered in mice orally was more than 45%. The reference values were 42-52% for male and 36-48% for females. The extracts did not affect the packed cell volume values. However there was reduction of total packed

erythrocyte volume early in the infection which might have been due to the binding of the Plasmodium antigens with specific receptors on the erythrocytes giving rise to complexes which elicit antibodies mainly IgM with consequent lysis of the erythrocytes as described(Karori *et* al*.,* 2007, Obwoge *et al.*, 2016). The experimental infected mice given tea extracts showed significantly higher levels of PCV compared to the infected mice given water only, which can be attributed on enhanced resistance to erythrocyte haemolysis conferred by tea. This shows that tea containing polyphenols posses *in vivo* ability to prevent red blood cells from haemolysis which can be accredited to Flavonoids. Furthermore the red blood cells structurally have membranes with high content of polyunsaturated lipids and a rich oxygen supply making them susceptible to lipid peroxidation. This reactive oxygen radicals generated during infections of *Plasmodium berghei* can attack erythrocytes membrane, induce its oxidation and trigger haemolysis (Murray *et al*.,1977). However we can a speculate that the antioxidant activity of tea could have elicited an increase in plasma antioxidant activity leading to a reduction on the vulnerability of red blood cell membrane destruction. From the study, it can be deduced that the taking of tea would decrease the effect of free radical individual oxidative damage of the red blood cells. As expected, the levels of certain plasma constituents (proteins) were to decrease due to damage to the organ or tissues responsible for their synthesis and the mobilization of their structural groups (for instance transamination) in the synthesis of immune mediators during this time of stress. However, the oral administration of tea extracts had a significant ($p<0.01$) prevention of serum protein concentration reduction in *Plasmodium berghei* infected mice thereby indicating a decreased effect on inflammation induced by Plasmodium parasite. The

effect can be due to the presence of Flavonoids. The Flavonoids and evidence for their role in the prevention of many degenerative diseases is emerging (Amie *et al*., 2003). Ability of tea to prevent decline in serum protein concentration and the subsequent antinflammatory effects can be attributed to various properties. These ubiquitous compounds have the ability to exert strong antioxidant effects based in part on their structural characteristics especially the 3:4'-dihydroxylation of the β-ring in the catecthol moiety. These structure configurations of Flavonoids represent the molecular basis for the radical scavaging and reduction of reactive oxygen species, which have been linked with pathogens which cause inflammatory diseases (Hansely *et al.,* 2000).

The green tea contains catechins flava-3-ol which includes Epigallocatechins gallate (EGCG), Epicatechin (EC) with EGCG being the major constituent and also the component with the highest antioxidant property. Catechins undergo major enzymatic transformation to form theaflavins and thearubigins which are the characteristic constituents of black tea but which have less antioxidant capacity (Murray *et al*., 1974). During inflammation toxic oxudates including oxygen radicals, super oxide nitrites are generated. The phenolic hydroxyl substitutions present in the catechins mainly in EGCG act as a potent radical scavengers increasing the capacity of endogenous antioxidant defenses and thereby modulating the cellular redox state (Turray *et al*., 1970., Tas demir *et al.*, 2006). Therefore the study shows that tea flavonoids elevated serum protein concentration afterwards and this is promising for tea as beaverage, being an auxiliary antinflammatory agent in chronic inflammatory diseases implying new immunogenic proteins were synthesized. Inflammation and several other pathological

conditions leading to disease often result from the effect of free radicals, the most important ones being oxygen radicals, superoxide, nitrites and hydroxyl. The efficient radical scavenging property of tea extracts which is due to polyphenol is an important property in the management of degenerative diseases (Murray *et al*., 1974). Among the beneficial effects of tea, the radical scavenging and antioxidant properties of tea polyphenols are the most frequently cited contributors. The evidence supporting an antioxidant function of tea polyphenols is derived from assays of their antioxidant activity *in-vitro* (Tas demir *et al*., 2006., Murrey *et al*.,1974). Although evidence that tea polyphenols are acting directly or indirectly as antioxidant *in vivo* is limited, this study shows clearly the indirect action prospect of tea as anti-inflammatory agent, since the albumin concentration *in vivo* is regarded as an indirect marker of inflammation.

This is because the main factor which affects plasma protein concentration in infected mice is the rate of trascapillary escape into the interstitial fluid. This trascapillary escape of albumin is markedly increased in disease (as part of the systems inflammatory response, leading to a decreased plasma albumin concentration. The more serve the disease the lower the albumin and therefore, the lower the albumin the worse the prognosis.

The parasite causes a severe inflammatory response and extensive tissue damage. During inflammation cytokines are activated leading to the release of acute phase proteins (APPS) which are recognized markers of inflammation. A sustained inflammatory response in critical illness also leads to a prolonged inhibition of synthesis of negative APPS such as albumin. The decline of albumin therefore could be used as a

prognostic marker of inflammation (Eckersall *et al*., 2001., Mungatana *et al.*, 2007., Murata and Yoshika.2004).

Thus animal models offer unique opportunity to study the contribution of the anti-oxidant properties of tea polyphenols to physiological effects during oxidative stress. Black tea, a principal tea product from Kenya, displayed remarkable properties some even comparable to those of green tea. From the results obtained in this study, tea polyphenols and the other secondary metabolites could serve as the models for design of the synthetic drug analogues with greater *in-vivo* activities having more beneficial to human health benefits.

5.2 Conclusion

The green, orthodox and black tea extracts are rich in bioactive phytochemicals notably cardiac glycosides, alkaloids, saponins, flavonoids, terpenes and tannins among others

The differences in antimicrobial activities and anti*plasmodium berghei* effects exhibited by the green, orthodox and black tea extracts can be attributed to the processing of tea. It was evident that tea lowered parasitemia of *Plasmodium berghei.*

Green tea lacks anthraquinones since during the processing, it does not undergo the aeration changes, as the basis of aeration are to bring oxygen and substrates together by rupturing the membrane so that polyphenols can diffuse into the cytoplasm. The enzyme polyphenol oxidase oxidizes the polyphenolic bodies to ortho-quinones (Orchadas, 2010).

The tea phytochemicals elevated protein concentration and this implies that tea is an auxiliary antiflammatory agent in chronic inflammatory diseases

The significantly higher levels of PCV shown by the infected mice given tea extracts in this study, in comparison with the lower PCV level shown by the infected mice given water clearly demonstrates that tea containing phytochemicals possess *in vivo* ability to protect erythrocytes from haemolysis

5.3 Recommendation

1. More research needs to be done towards the separation and fractionation of specific tea compounds and analyzing them using analytical methods.

2..Tea individual polyphenols and other phytochemicals need to be evaluated to gauge their usefulness and be employed in more research to serve as models for the rational design of synthetic drug analogues with higher *in vitro* and *in vivo* activities and more favourable chemical properties.

3.. It is evident that green tea consumption should be given the first preference followed by orthodox tea and then black tea since antimicrobial activity decreases with increase in oxidation status of tea. Likewise the scavenging of radicals by tea polyphenols *in vivo* decreases with increase in oxidation status of tea and thus reducing the capacity of endogenous antioxidant defenses and thereby unmodulating the cellular redox state.

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APPENDICES

Appendix 1: Water intake in dosage determination

Green tea water intake	Day	0g/l	5g/l	15g/l	20g/l
	2	7.8±0.1	6.1±0.2	5.8±0.01	8±0.08
	4	6.8±0.2	5.8±0.06	6.4±0.1	10.2±0.2
	6	8.4±0.1	6.8±0.04	7.0±0.2	9.2±0.2
	8	8.3±0.08	5.8±0.04	7.0±0.1	11±0.2
	10	8.7±0.1	2.7±0.08	4.8±0.2	9±0.1

Appendix 2: PCV at dosage determination

Day	0g/l	5g/l	10g/l	15g/l	20g/l
2	50.0	50	50	52	52
4	51.1	51	49.5	49	50
6	48.8	49	51.5	50	48
8	54.05	51.5	51.5	49	54
10	54.05	51.5	51.5	49	54

Appendix 3: The trend of mean parastemia level in mice treated with various tea/ log 10 parastemia.

Extract name	Days of treatment/ log^{10} parastemia																		
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Green tea	0	0	0	0	2	6.02	7.0	7.02	7.04	7.06	7.07	6.88	6.50	5.20	4.8	4.9	5.0	4.9	4.9
Orthodox tea	0	0	0	0	3	5.06	7.08	7.22	7.30	7.46	8.00	7.28	7.0	5.34	4.01	4.8	5.1	5.1	
Black tea	0	0	0	0	4	6.0	7.50	7.58	7.70	7.68	7.65	7.64	7.6	6.8	6.0	6.1	6.2		
Dexamethasone	0	0	0	0	5	6.4	7.60	7.70	8.10	8.06	7.90	7.80	6.42	6.0	5.28	5.4	5.5		
Infected water	0	0	0	0	6	6.6	7.62	7.64	7.65	7.74	7.74	7.80	7.9	ND	ND	ND	ND	ND	ND
Non infected water	0	0			0.4	0.6	0.705	0.640	0.604	0.550	0.500	0.480	0.20	NP	NP	NP	NP	NP	NP
Infected pyremethamine	0	0	0	0	0	0	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP

Appendix 4: The trend of mean PCV of mice treated with various tea extracts

Extract name	Days of treatment/ % PCV																		
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Green tea	51	50	49	45	38	37	36.52	37.95	39.67	37.04	38	39	29.98	30.55	32.12	28.54	26.46	29.35	
Ortho-dox tea	48	47	46	45	37	36	23.20	23.50	22.32	27.68	24.76	24.54	28.49	27.48	20.77	32	34.66	30.35	
Black tea	50	47	46	45	36	35	34	33.98	34.22	35.76	36	35	36.75	34.58	35.98	32	39	38	
Dexamithison	51	49	47	46	36	36	35.56	36.50	37.32	36.68	37.76	36.54	25.67	31.55	21.74	25.12	24	27.96	
Infected water	50	49	49	46	35	34	30	25.76	28.04	29.52	28.00	28.78	All		had	died			
Non infected water	46	46	47	47	47	47	46.5	47.6	46	47	48	48.5	48	48.5	47.5	48	49	49	49
Infected pyremethamine	49	48	46	45	37	37	37.6	37.98	37	36.98	35.96	35.52	36.2	37	38.4	40.4	42	44.4	

Appendix 5: The trend of serum protein concentration g/dl (means) of the treated animals the infected and non-treated animals

Treatment	Days after treatment/serum protein concentration g/dl																		
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Green tea	2.84	2.80	2.70	2.60	2.58	2.55	2.52	2.44	2.46	2.58	2.47	2.40	2.58	2.60	2.55	2.62	2.68	2.68	
Orthodox tea	2.78	2.60	2.50	2.50	2.49	2.49	2.49	2.34	2.44	2.30	2.25	2.40	2.30	2.40	2.31	2.30	2.14	2.30	
Black tea	2.80	2.70	2.50	2.46	2.30	2.2	2.04	2.01	2.00	2.04	1.82	2.02	2.42	2.30	2.38	2.41	2.61	2.00	
Dexamithisone	2.81	2.74	2.60	2.58	2.52	2.52	2.50	2.49	2.48	2.36	2.35	2.48	2.52	2.56	2.62	2.66	2.62	2.56	
Infected water	2.80	2.60	2.50	2.30	2.20	1.80	1.30	1.24	1.42	1.50	1.38	1.42		ND	ND	ND	ND	ND	ND
Non infected water	2.88	2.81	2.88	2.84	2.86	2.89	2.88	2.86	2.80	2.90	2.96	2.67	2.80	2.82	2.80	2.84	2.84	2.88	
Infected pyremethamine	2.86	2.84	2.83	2.86	2.85	2.81	2.80	2.68	2.70	2.60	2.50	2.30	2.50	2.61	2.70	2.72	2.72	2.84	