ANTIMICROBIAL ACTIVITY OF KENYAN TEA PRODUCTS AGAINST *Salmonella typhimurium* AND *Escherichia coli*AND ITS POTENTIAL EFFECT ON SPOILAGE MICROORGANISMS IN MEAT.

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2015

DECLARATION

I hereby declare that this project report is my original work and it has not been submitted to any other institution of Higher learning for examination

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Acronyms

TPC: Total Plate Counts

OD: Optical Density

Z.O.I: Zone of Inhibition

Z.D.I: Zone Diameter of Inhibition

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Abstract

Meat is a highly perishable food due to its highly nutritive value for microbes. It is therefore prone to the growth of various microorganisms which causes adverse health effects as well as food spoilage leading to economic loss.Previous studies have shown that tea possesses antimicrobial activity against many pathogenic microorganisms including Gram-positive bacteria such as *Listeria spp*. and *Staphylococcus aureus*. Green tea and black tea contain compounds such as catechins, theaflavins and polyphones which according research are potentially antimicrobial. The aim of this study was to establish tea as a potential preservative for food vulnerable to spoilage such as meat.

The tea extracts were prepared through general infusion method. Moreover the antimicrobial activity was determined using the agar-well diffusion method and paper disc diffusion methods. The growth inhibition by green tea and black tea extracts against *Salmonella typhi* and *Escherichia coli* was determined by measurement of the diameter of inhibition halo. The potential preservation of meat was determined through total plate count analysis on samples treated with green tea and black tea extracts and control samples without any treatment.

Four tea blends of green tea and black tea were selected from the major tea processing companies in kenya. The blends were labelled A (Unilever Tea), B (Finlay tea), C (KTDA) and D (Sasini tea). Tea extracts were obtained from these blends and were tested against *Salmonella typhimurium* and *Escherichia coli* as well as other spoilage microorganisms in meat.

The results showed that Black tea extract from blend B (Finlay tea) showed highest zone (11.67±0.33) mm of inhibition while tea extract from blend D (Sasini tea) showed the lowest zone (9.67±0.88) mm of inhibition. Against *Escherichia coli*, black tea extract from blend A (Unilever tea) showed had the highest (13.67±0.33) mm inhibition zones while extract from blend D (Sasini tea) showed the lowest (11.67±0.33) mm inhibition zones. Green tea extracts

from blend A (Unilever tea) and D (Sasini tea) showed highest growth inhibition against *Salmonella typhi* with measurements of 14.67 mm and 14.33 mm respectively while tea blend B (Finlay tea) showed the lowest growth inhibition. Against *Escherichia coli*, extract from blend C (KTDA) showed highest inhibition (16.00 ± 0.58) mm while the lowest was from blend B (Finlay tea) with measurement of (14.67 ± 0.33) mm. There was significant reduction ($P<0.05$) of the microbial counts between control samples and samples treated with green tea and black tea extract. However, on comparing the microbial counts between 4hrs and 8 hrs after treatment, the results showed that there was no significant difference at ($P>0.05$). i.e. (3.8 cfu/ml and 4.4 cfu/ml) and (3.7 cfu/ml and4.4 cfu/ml) respectively. This was also observed in the innoculated meat samples where meat sample treated with green tea extract had (4.8 cfu/ml and 4.7 cfu/ml) and that treated with black tea extract had 6.0 cfu/ml in both 4hrs and 8hrs after treatment.

Generally there was a higher antimicrobial activity of both green tea and black tea against *E. coli* as compared to *S.typhi*.For instance, black tea extract from blend C (Sasini tea) had a higher inhibition against *S.typhi* as compared to *E.coli*.

Polyphenolic compounds are the major components found in tea and are the major contributors to the antimicrobial effect of tea. Against both microorganisms, the relationship was stronger in black tea than in green tea. The effect of black tea and green tea was much stronger against *E.coli* than *S.typhi*.

Meat spoilage usually begins from the surface going inwards. Through surface treatment using the tea extracts on the meat samples, there was reduction in microbial growth of the spoilage microorganisms in the meat samples. This shows that tea extracts have an antimicrobial effect on the spoilage microorganisms that are found on meat.

It can be concluded that both green tea and black tea extracts from the different tea blends showed no significant difference in their antimicrobial activity against both *S.typhi* and *E.*

coli with blend B (Finlay tea) and C (KTDA tea) showing similar growth inhibition against *E.coli*. However, the data may offer the consumer a choice of tea brands with very high antimicrobial properties against *S.typhi* and *E.coli*.

The strong relationship between polyphenol levels and antimicrobial activity is an indication that the antimicrobial activity of tea is determined by the levels of polyphenols in the tea product.

Since the meat samples treated with green tea and black tea extracts showed lower microbial counts compared to the control, they could be used for controlling growth of spoilage microrganisms of foods such as meat.

CHAPTER ONE

1.1Introduction

1.1.1 Background information

Traditional system of medicine has used plants widely as a source of drugs for treatment of diseases. Indian literature mentions use of many plants in treatment of various human illnesses. India alone has been found to possess about 45,000 different plant species of which several possess medicinal properties. The high cost of synthetic drugs used for treatment and their side effects has resulted in the shift to herbal medicine. The herbal medicines are used for the treatment of the same ailments that would have been treated with the synthetic drug. (Namita *et al.*, 2012).

Tea beverage is one of the most ancient and popular beverages consumed around the world.it is obtained from the leaf of the plant "*Camellia sinensis*". It was first discovered in China (Chinese *Camellia sinensis*) and Southeast parts of Asia. Today tea species are cultivated all over the world, predominantly in the tropical and subtropical regions.

There are three different categories of tea according to their chemical processing i.e. Green tea (non fermented), semi-fermented tea and black tea (fermented).The compounds predominantly found in tea are known as polyphenols. The polyphenols account for up to 30% of the dry weight in young tea leaves.The processing of tea usually results in the changing of the polyphenolic component of the tea. Green tea contains 30-40% polyphenol extracted from water while black tea contains 3-10% extractable polyphenols. (Archana & Abraham, 2011).The polyphenols found in tea are divided into two main groups namely

i. Crude catechins- predominantly found in green tea and

ii. Crude theaflavins- which include theaflavins and thearubigins and predominantly found in the black tea.

It has been documented that green tea contains catechin and polyphenols which are highly sensitive to oxidation process. These have been found to posses antibacterial and antiviral action as well as ant carcinogenic and ant mutagenic properties. These compounds could be responsible for the inhibition of pathogens. The antibacterial effects of the tea polyphenols extracted from Korean green tea (*C. sinensis*) against clinical isolates of *Staphylococcus aureus* were evaluated (Archana and Abraham., 2012). Toda *et al*. (1991) found that extracts of tea inhibited and killed a large number of Gram-positive and Gram- negative bacteria including V. cholerae. The inhibitory as well as the bactericidal activity of tea extracts against various pathogenic bacteria causing several infections has been reported (Toda *et al.,* 1989)

Meat, an excellent source of protein in human diet is highly susceptible to microbial contaminations which can cause its spoilage and food borne infections in human, resulting in economic and health losses (komba *et al.,* 2012). Although muscles of healthy animals do not contain microorganisms, meat tissues get contamination (Ercolini *et al.,* 2006). Fresh meat is generally inhabited by a great diversity of microbes, but depending on factors such as pH, composition, textures, storage temperature, and transportation means of raw meat, different types of microbes may become dominant (Ercolini *et al.,* 2006). Organizations such as food and Agricultural organization (FAO) of the United Nation and the World Health Organization (WHO) state that illness due to contaminated food is perhaps the most widespread health problem and an important cause of reduced economic productivity (Kaferstein, 2003). Raw meat may harbor many important pathogenic microbes i.e. *Salmonella spp., Campylobacter jejuni, Yersinia enterocolitica, E.coli, S. aureus* and to some extent *Listeria monocytogenes* making the meat a risk for human health, as without the proper handling and control of these pathogens food borne illnesses may occur (Norrung *et al.,* 2009).

1.2 Statement of the problem

The slaughtering and butchering of food animals for meat and meat products provide bacteria with an opportunity to colonize the meat surfaces. A wide range of microorganisms coming from different sources are introduced to surfaces which contain abundant nutrients and which have a high water availability. This results in contamination of the meat product.A number of studies have reported outbreak of infections due to consumption of contaminated foods such as meat.(Iroha *et al.,* 2011). However, few community based reports provide evidence of several outbreak caused by *Salmonella, Shigella, E.coli* and *Listeria spp* in different parts of the world.(Zweifer *et al.,* 2008).Microbial contamination of raw meat may occur during harvesting and subsequent processing. Through the process, contamination may be introduced onto the edible product from the hide, gastrointestinal tract, workers, and enviroment. The presence of such bacteria in food items plays a role in food-borne illnesses through consumption of the contaminated meat. (Iroha *et al.,* 2011). Tea has been reported to possess antimicrobial activity (Mandal *et al.,* 2011). Research from tea produced in other countries has shown that both green tea and black tea (fermented) have been found to have antimicrobial properties (Mandal *et al.*,2011).The beef industry acknowledges that microbial contamination may occur; however, it has taken multiple actions to reduce the potential for contamination and it has incorporated, scientifically proven antimicrobial interventions that can be applied individually or in combination to reduce pathogens on the carcass surfaces. There is inadequate documentation as to whether the antimicrobial property of tea can be applied onmeat whichis susceptible to spoilage microorganisms like *Escherichia coli* and *Salmonella typhi,* which are attributed to its spoilage.The current study was designed to assess whether tea can also be used to control growth of these bacteria that cause meat spoilage.

1.3 Justification

Tea is available and commonly consumed in every household. It is mainly produced for consumption and may also be used for food preservation as an alternative to techniques such as heat treatment and use of artificial chemical preservatives. The potential advantage of tea is not only due to cost but the fact that its readily available and utilized in form of beverages.

1.4Aim

The aim of this study was to establish tea as a potential preservative for food vulnerable to spoilage such as meat.

1.5Purpose

The purpose of the study was to demonstrate the antimicrobial properties of the kenyan tea products.

1.6Objectives

1.6.1Overall objective

The overall objective was to determine the antimicrobial properties of tea extracts and its potential as a meat preservative.

1.6.2Specific objectives

i. To extract tea and determine presence of antimicrobial activity.

ii. To determine the levels of phenolic compounds attributed to the antimicrobial activity in the tea products.

iii. To determine whether tea can be used to preserve meat.

1.7Research Questions

i. Do the differently processed Kenyan tea products possess antimicrobial activity?

ii.Is there relationship between poyphenol compounds and antimicrobial activity?

iii. Can tea be used to preserve meat?

CHAPTER TWO

2.1 Literature Review

Tea was first discovered in China about 2000 years ago. It is the plant species that produces Chinese tea using its leaves and buds. Different countries have different common names that refer to the tea beverage. The Chinese refer to it as 'cha'. Russians refer to it as 'Chai', Italy refer to it as 'te', US refer to it as 'tea' while the Indians refer to it as 'Chha' (Namita *et al.*, 2012). The tea plant can be described as an evergreen tree usually trimmed to below six feet. It has strong roots, green leaves that are 4-15cm long and 2-5cm broad/wide. The young leaves are usually light green in color while the older leaves are dark green in color. It grows well at high altitudes particularly in highland areas.

2.1.1 Scientific classification

The Binomialname for the tea plant is *Camelliasinensis*which characterized by Genus:*Camellia*and Species*C.sinensis.* It is found under theFamilyTheaceae which belongs to the KingdomPlantae. The family Theaceae is distributed throughout tropical and subtropical areas. Of all its plant species, the camellia genus, *C. sinensis*, is the most important commercially.

Tea has been cultivated and consumed in China and Southeast Asia for more than 2000 years (Balentine *et al,* 1997). A book titled "Ch'a Ching" meaning 'Tea Classic' talks about the various kinds of tea, their cultivation and manufacturing in China. Its origin is reported to be an indefined area to the south east of the Tibetan plateau that includes sea-Chuan,Burma,SiamandAssamintheNorth EastIndia. Factors such as genotype,typeofsoil,altitudeandclimaticconditionsoftheareainwhichitisgrown are said to influence the flavor and characteristics of the tea. (Owuor and Obanda, 1995).Processingmethodsalsoaffecttheflavorandcharacteristics,asdoestheblendingofdifferentt easfromdifferentculturesandgeographicregions.

China and India mostly use the tea as a medicinal plant, and has been consumed for generations as Ayurveda, Unani and Homeopathy Green Tea. (Namita *et al.*, 2012)

TeawasfirstintroducedinKenyafrom IndiabyacolonialsettlerG.WCainein1903.Currently teaistheleadingexportcropinKenya. Intheworld, Kenyaisthethirdlargestproducerofblacktea afterIndiaandSriLanka. Teaismainlygrowninseveral districtswhichincludeKericho,Bomet,Nandi,Kiambu, Thika,Maragua,Muranga,Sotik,Kisii,Nyamira, Nyambene,Meru,Nyeri, Kirinyaga,Embu,Kakamega, NakuruandTrans-nzoia.Intheseareasthecropenjoys 80%favorableweatherpatterns. (Gesimba *et al*., 2005)**.**

2.1.2 Types of tea.

Tea is the second most commonly drunk liquid after water and the most common beverage. The most common types of tea are black tea and green tea.

Black tea

This is one of the varieties processed from the *C.sinensis* plant. It is formed as a result of oxidation process during fermentation of the tea leaves. It is generally stronger in flavor than green tea and oolong tea.(Owuor and Obanda, 1995). In the production of black tea, the leaves are rolled which disrupts cellular compartmentation and brings into contact polyphenol oxidases and the phenolic compounds.(Del rio *et al.,* 2004). The fermentation process converts catechins into Theaflavins and thearubigins which are the common polyphenols in black tea.(Owuor and Obanda, 1995).

Green tea

Green tea according to Namita *et al.*(2012) is a non-fermented tea and mainly contains more catechins that are strong anti-oxidant.It is produced when the young leaves are rolled and steamed to minimize oxidation process. This is because the steam destroys the oxidase enzymes which are responsible for the oxidation process.(Del rio *et al.*, 2004).

2.2 Methods of tea extraction.

There are different methods of extraction of tea depending on the type of solvents used during the extraction process.

Methanolic extraction: This involves the use of methanol as the solvent for extraction of the polyphenols in the tea. In this extraction process the tea powder is macerated in the methanol solvent to produce crude extracts with a wide range of the active compounds.Archana & Abraham. (2009)

Aqueous extraction: This involves use of water as a solvent of extraction. Here the powdered tea is soaked in distilled water for a period of time and concentrated using a rotavapour before any form of analysis is done.Archana & Abraham. (2009)

2.3 Chemistry of tea

Teaisreportedtocontainnearly 4000bioactivecompoundsofwhichonethirdis attributed topolyphenols. Other compoundsare alkaloids (whichincludecaffeine,theophyllineandtheobromine),aminoacids,carbohydrates,proteins,chlorophyll,volatileorganiccompounds,mineralsandtraceelements.Polyphenols foundinteaaremostlyflavonoids, flavanols,flavandiols andphenolicacids;Thesecompoundsmayaccountforupto30%ofthedry matterinyoungtealeaves.

The polyphenols that occur in tea are divided into two main groups namely the crude catechins, theaflavins and thearubigins. Catechins are mainly found in green tea and are divided into four main constituents namely: Epigallocatechin gallate (EGCG), Epicatechin gallate (ECG), Epigallocatechin (EGC) and Epicatechin. (Diane*etal.,*2007)

Theaflavins are mainly found in black tea. They are formed during fermentation process as a result of the oxidation of catechins. Theaflavins are divided into four main constituents namely: theaflavin, theaflavin-3-gallate, theaflavin-30-gallate and theaflavin-3, 30-gallate. They are well soluble dimeric flavonoids of bright orange to red color and are determinants of tea quality. (Ostadalova *et al.,* 2011). Thearubigins are the most abundant phenolic fraction of black tea, and they are largely oxidized polymer, difficult to define and unstable substance with a wide molecular weight. They also occur as a result of oxidation of catechins during the fermentation process. They are found mainly in black tea. They are heterogeneous mixtures of red-yellow to orange-brown soluble and insoluble oxidation products.(Ostadalova *et al.,* 2011).Itisimportanttohaveanunderstandingofthechemicalcompositionofdifferentteasandthevarianceincompositionofchemicalsinthedifferentteas in ordertoeventuallyunderstandthemechanismsbywhichmicrobialgrowthisinhibited.

Figure 1 shows the major phenolic compounds and alkaloids found in tea and their molecular structures.

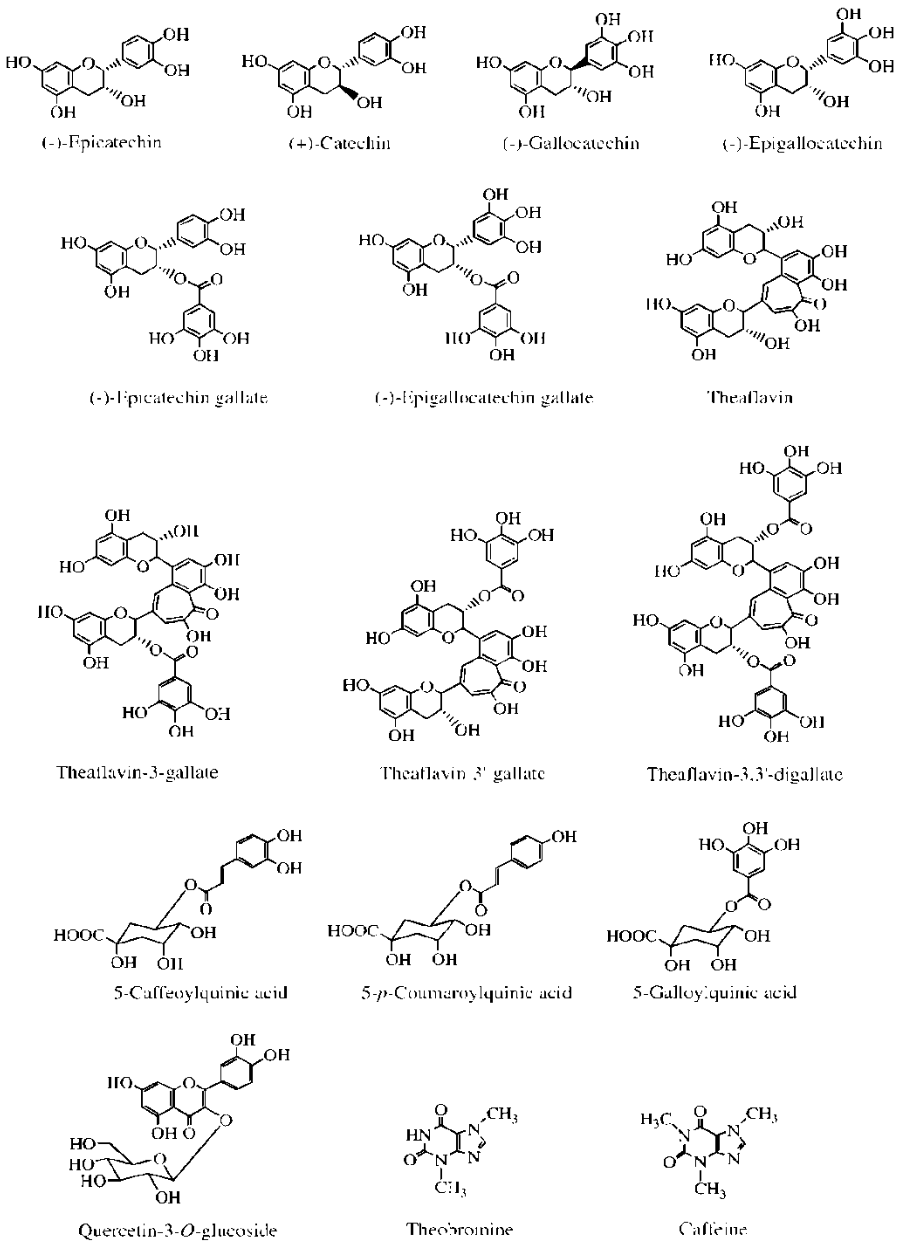

Figure 1: Structures of the major phenolic compounds and alkaloids in Tea. Source; Del Rio *et al* (2004).

2.4 Antimicrobial property of tea.

Green tea

Green tea has been reported to possess antimicrobial activities against various enviromental isolates (Kumar *et al.*,2006).

BacterialspeciessuchasEscherichiacoli(IFO3301),*Streptococcusmutans*(IFO13955),*Helicobacterpylori*(ATCC6919),*Propionibacteracnes*(ATCC6919),*Bacillusstaerothermophilus*(ATCC12980)and*Staphylococcusaureus*(ATCC25923)are inhibited by green tea in vitro (Yamamoto*etal*,1997).

Si *et al*(2006) assayed four compounds i.e. epicatechingallate(ECG),epigallocatechingallate(EGCG),epicatechin(EC),andcaffeine(CN)through abioassay-guidedfractionationtechnique and confirmed thestructuresofthesepolyphenolsbymassspectrometry.They reported that ofthefourcompounds,ECGandEGCGwerethemostactive,particularlyEGCGagainst*S.aureus* . Hence theactivityofteapolyphenols,particularlyEGCGonantibiotics-resistantstrainsof*S.aureas*,suggeststhatthesecompoundsarepotentiallynatural antimicrobialalternatives.

Research done by Rozoy *et al.*, (2013) showed that epigallocatechingallate(EGCG), a major catechin in green tea, had antimicrobial effect against *Brochothrix thermosphacta*,*Pseudomonasasputida*and also*Escherichiacoli*which is microorganisms that havebeenassociatedwithmeatspoilage.

Black Tea.

Black tea is also known to possess antimicrobial activity. Polyphenols that are present in black tea have been shown to possess antibacterial action. There are research data showing strong antibacterial activity of theaflavinagainst eight clinical isolates of *S. maltophilia* and *A.baumannii*.(Kelly & Haswell, 2011).

Research by Friedman *et al* (2006) found that theaflavins: theaflavin-3, 3'-digallate, theaflavin-3'-gallate, and theaflavin-3-gallate have antimicrobial activity against *Bacillus cereus.* Microorganisms such as *Listeriamonocytogenes* tend to affect ready to eat products. However tea has shown antibacterial activity against this microorganism.

A comparison of Chitosan-coated plastic films incorporating black tea extracts and Chitosan-coated films without black tea showed growth inhibition of *Listeria monocytogenes* while the one without black tea did not inhibit growth of *Listeria monocytogenes.*(Vodnar, 2012).

2.5 Mechanism of antimicrobial activity

The catechins and to some extent caffeine which are the main components of the Camellia sinensis extracts are the compounds attributed to the antimicrobial activity of the tea. Gallated catechins i.e. Epigallocatechin (EGC) and Epicatechin gallate (ECG) have been reported to deactivate proteins and disrupt the bacterial lipid bilayer(Ikigai*etal*,1993). It does this by changing the membrane morphology and fluidity. The caffeine present in the tea is responsible for inhibition of the normal cell division thus inhibiting spore germination and growth of microbes.(AnejaandGianfagna,2001).

2.6 Microbial spoilage in meat

Meat is one of the major source of proteins (animal protein) and quality vitamins for humans, therefore essential for growth repair and maintenance of body cells. Meat is a highly perishable food due to its highly nutritive value for microbes. It is therefore prone to the growth of various microorganisms which causes adverse health effects as well as food spoilage leading to economic loss. (Al-Allaf *et al.,* (2009).

The leading causes of illnesses and death in most developing countries is food-borne pathogens. The major contributing factors include poor hygiene practices, handling and lengthy food supply procedures (Hedberg *et al.*, 1992). Few reports have provided evidence of outbreaks of food-borne illnesses caused by various microorganisms. Studies donehoweverby (Zweifer *et al.,*2008) showed several outbreaks caused by *Shigella spp*, *E. coli* and *Listeria spp*.

Iroha et al. (2011) carried out studies on contamination of meat and found out that most of the samples they tested were contaminated by *Pseudomonas aeruginosa*, *S. typhi, Shigella dysentriae* and *Staphylococcus aureus.*

The method of slaughtering the animal has been found to be the major source of contamination. A comparison between traditional and modern methods of slaughtering showed that the traditional method which involves using knives, minimized contamination as compared to the mechanized system of slaughter.(Iroha *et al.*, 2011).

Since meat is vulnerable to microbial spoilage, the current study was done to find out whether tea can be used to control growth of some of the microorganisms in meat, as preservative for meat and meat products.

CHAPTER THREE

3.1 Research methodology

3.1.1 Study design

This study employed the experimental study design applying quantitative data collection methods through laboratory analysis.

3.1.2 Sampling

This study employed the use of purposive sampling and simple random sampling techniques. Through purposive sampling, four tea brands were selected from the major tea processing companies in kenya. The brands were labelled A(Unilever Tea), B(Finlay tea), C(KTDA) and D(Sasini tea). The sampling of the different tea blends was done in accordance with the International sampling standards for tea (ISO 1839:1980). A total of 16 samples of tea products were used. 8 samples of green tea and 8 samples of black tea. A total of 5 meat samples were obtained from local butcheries and were obtained 8 hours after slaughter. The test microorganisms *Salmonella typhimurium* (ATCC 14028) and *Escherichia coli* (ATCC 25922) were obtained from Kenya Bureau of Standards (KEBS) laboratories (No:RCT00000084174)

3.2 Research tools and equipment

3.2.1 Research methods

The methods used were laboratory analysis which involved extraction of tea and microbial analysis.

3.2.2Tea extraction and test for its antimicrobial activity.

This involved use of test organisms *Salmonella typhimurium*(ATCC14028) and *Escherichia Coli* (ATCC 25922). Hence the variable here was the antimicrobial activity which was determined invitro using cultural growth inhibition.

Tea Extraction: This was done according to the general infusion methodas described by (Owuor and Obanda, 1995).

Antimicrobial activity: Salmonella typhimurium(ATCC 14028).

The cultural growth inhibition was used to determine the antimicrobial activity using agar-well diffusion inhibition test and paper disk diffusion inhibition test according to (Archana & Abraham(2009) &Al-Allaf *et al.*,(2009).

Antimicrobial activity: Escherichia coli(ATCC 25922).

The cultural growth inhibition was used to determine the antimicrobial activity using agar-well diffusion inhibition test and paper disk diffusion inhibition test (Archana And Abraham, (2009) &Al-Allaf *et al.*,(2009).

3.2.3 Determination of total polyphenols.

This involved the use of the Folin-Ciocalteu method (ISO 14502-1:2005) for the analysis of the polyphenol levels in the different tea samples.

3.2.4 Determination of antimicrobial activity of tea against spoilage microorganisms in meat.

The third specific objective was to determine whether tea can be used to preserve meat. The data were obtained through the determination of the Total Plate Count.

Isolation of spoilage microorganisms in meat: 20g of the meat sample was weighed asceptically and placed in a sterile stomacher bag and 180ml of sterile saline solution added to it. The mixture was homogenised in a stomacher circulator at 230rpm for 10 minutes. it was transfered into a steril flask for further analysis.

Antimicrobial activity of the tea extracts on spoilage microorganisms from meat: Fixed volume (0.1ml) of the homogenate containing the spoilage microorganisms was aseptically introduced into the petri dishes containing nutrient agar and evenly spread using a sterile 'L' rod. Wells were aseptically drilled using a sterile cork borer. Fixed volume(2ml) of the tea extracts were introduced into the wells. The plates were incubated at 37^{o}c for 24hours. The antimicrobial activity was determined by measuring the clear zones around the wells.

Monitoring parameter: Total plate count

20g of meat samples of two sets of samples were each weighed and put in two separate sets of A and B. Set A was left as the fresh sample while set B was innoculated using the spoilage microorganisms cultured from homogenate prepared earlier (culture contained 10^{7} cfu/ml).Both sets of meat samples were subjected to surface treatment using fixed volume (2ml) of the tea extracts. Both sets were stored at room temperature (25^{o}c). Both sets of samples were analysed immediately, 4hrs after treatment and 8hrs after treatment.

3.3 Statistical data analysis

Descriptive statistical tests including ANOVA and Duncan's multiple range testfor the variables was carried out using Genstat 12th edition and Microsoft Excel. Pearson's correlation analysis was performed to determine the relationship between polyohenol levels and antimicrobial activity of the tea products.

Chapter Four: Results

4.1Presence of antimicrobial activity

The results show that against *Salmonella typhimurium* (Table 1), black tea extract from blend B (11.67±0.33)mm showed highest zone of inhibition while tea extract from blend D (9.67±0.88)mm showed the lowest zone of inhibition. Against Escherichia coli, black tea extract from blend A (13.67±0.33)mm showed had the highest inhibition zones while extract from blend D showed the lowest (11.67±0.33)mm.

Table 1. Mean Zone Diameter of Inhibition(mm) of black tea against *S.typhi* and *E.coli*.

BLACK TEA		
Sample	**Mean Zone of Inhibition(mm)**	
	Salmonella typhimurium	***Escherichia coli***
Tea blend A	10.67 ± 0.58^{a}	13.67 ± 0.33^{a}
Tea blend B	11.67 ± 0.33^{b}	13.33 ± 0.88^{a}
Tea blend C	10.00 ± 0.00^{ab}	13.33 ± 0.67^{a}
Tea blend D	9.67 ± 0.88^{ab}	11.67 ± 0.33^{b}

KEY:
Mean ± Standard Error of Mean(S.E.M)

Means with the same superscript lettera,b are not significantly different at ($p<0.05$).

n= 8
Tea blend A- Unilever tea
Tea blend B- Finlay tea
Tea blend C- KTDA
Tea blend D- Sasini tea

Green tea extract (Table 2) from blend A (Unilever tea) and D (Sasini tea) showed highest growth inhibition against *Salmonella typhi* with measurements of 14.67mm and 14.33mm respectively while tea blend B showed the lowest growth inhibition. Against *Escherichia coli*, extract from blend C showed highest inhibition(16.00 ± 0.58)mm while the lowest was from blend B with measurement of (14.67 ± 0.33)mm.

Table 2. Mean Zone Diameter of Inhibition(mm) of green tea against *S.typhi* and *E.coli*

GREEN TEA		
Sample	**Mean Zone of Inhibition(mm)**	
	Salmonella typhimurium	***Escherichia coli***
Tea blend A	14.67 ± 0.33^{a}	15.67 ± 0.88^{a}
Tea blend B	12.00 ± 0.58^{b}	14.67 ± 0.33^{ab}
Tea blend C	13.67 ± 0.33^{ab}	16.00 ± 0.58^{a}
Tea blend D	14.33 ± 0.33^{a}	15 ± 0.00^{ab}

KEY:

Mean± Standard Error of Mean (S.E.M)

Means with the same superscript lettera,b are not significantly different at ($p<0.05$.)

n= 8.

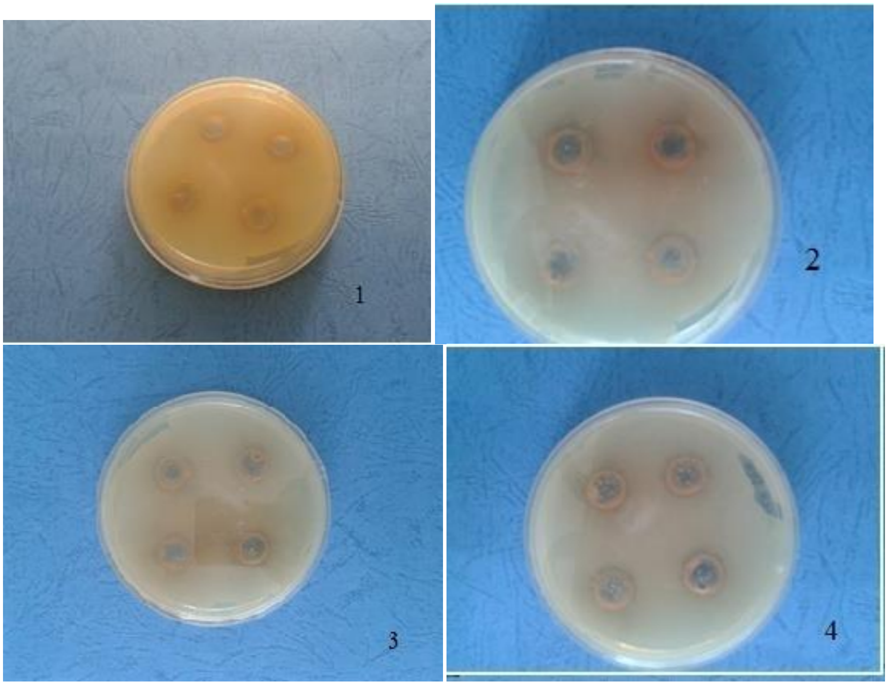

Figure 2: Inhibition zones of Green tea and Black tea against S.typhi and E.coli: 1. Green tea against *S.typhi*, 2. Black tea against *S.typhi*, 3. Green tea against *E.coli*, 4. Black tea against *E.coli*.

Both green tea and black tea extracts from some of the different tea blends showed no significant difference($p<0.05$) in their antimicrobial activity against both *S.typhi* and *Ecoli* with blend B (Finlay tea) and C (KTDA) showing similar growth inhibition of 13.33 mm against *E.coli*. This was also observed in blend A(Unilever tea) and blend D(Sasini tea) of green tea against *S.typhi*.

4.2Levels of phenolic compounds attributed to the antimicrobial activity in the tea products.

The results showed that there was a relationship between the polyphenol levels and the antimicrobial effect in both*E.coli* and *S.typhi*. (Table 3) showed there is a strong positive correlation between polyphenol compounds in black tea and inhibition of *E.coli* ($r=0.7833$). Howeverthere is a weak positive correlation between polyphenol compounds in both black and green tea and *S.typhi* ($r=0.5642$). There was no significant difference($P>0.05$) between polyphenol levels in green tea and black tea and the antimicrobial activity against *E.coli*. There was also no significant difference($P>0.05$) between polyphenol levels in both green tea and black tea and the antimicrobial activity against *S.typhi*.

Table 3. Relationship between polyphenol levels and antimicrobial activity of the tea products.

Coefficient of correlation (r)		
	black tea	**green tea**
E.coli	0.7833	0.5670
S.typhi	0.5642	0.3785

r = coefficient of correlation showing the relationship between polyphenol levels of the tea products and their antimicrobial effect.

The results in (Table 4) show that 61.35% of the variability of antimicrobial activity is accounted for by polyphenol levels in black tea against *E.coli* while for green tea, it was only 32.15%. Against *S.typhi*, variability was accounted for by 31.83% in black tea and 14.32% in green tea. The low percentages show that there may be other components of tea that influence antimicrobial activity against these two microorganisms.

Table 4. Coefficient of determination measuring proportion of variation in antimicrobial activity that is explained by polyphenol levels.

Coefficient of determination (r^2) %		
	black tea	**green tea**
E.coli	61.35	32.15
S.typhi	31.83	14.32

r^2= coefficient of determination

4.3Potential effect of tea on spoilage microorganisms in meat.

This study showed that there is antimicrobial activity of both green tea and black tea against spoilage microorganisms found in meat. However, green tea showed a higher antimicrobial effect than the black tea. Results showed that there was antimicrobial activity of the tea extracts against the spoilage microorganisms. Green tea had a larger zone of inhibition (10.33±0.58)mm as compared to black tea with a zone of inhibition (8.33±0.58)mm.

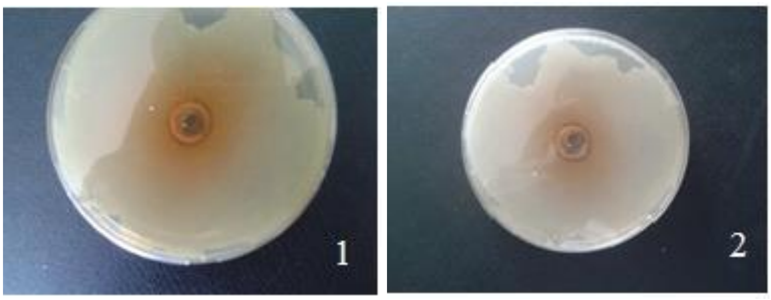

Figure 3: Inhibition zones of black tea and green tea against spoilage microorganisms in meat: 1. Black tea against spoilage microorganisms in meat, 2. Green tea against spoilage microorganisms in meat

There was significant reduction ($p<0.05$) of the microbial counts between control samples and samples treated with green tea and black tea extracts. This study showed that both green tea and black tea can reduce growth of the spoilage microorganisms through surface treatment on meat. Colony forming units in fresh meat treated with green tea and black tea were lower than that of the control. There was no significant difference ($p>0.05$) in the reduction of microbial counts four hours after treatment and eight hours after treatment with (3.8cfu/ml and 4.4cfu/ml) and (3.7cfu/ml and4.4cfu/ml) respectively at the temperature 25^{o}C . This was also observed in the innoculated meat samples where meat sample treated with green tea extract had (4.8cfu/ml and 4.7cfu/ml) and that treated with black tea extract had 6.0 cfu/ml in both 4hrs and 8hrs after treatmentrespectively.

Table 5: Total Plate Count(TPC) in meat against green tea and black tea on fresh meat samples (log_{10} cfu/ml).

Fresh meat sample			
	0hrs after treatment	**4hrs after treatment**	**8hrs after treatment**
Control	6.5 ± 0.58^{a}	6.6 ± 0.88^{b}	6.8 ± 0.53^{b}
Green tea	3.8 ± 1.20^{a}	3.8 ± 0.67^{b}	3.7 ± 0.15^{b}
Black tea	4.6 ± 1.15^{a}	4.4 ± 0.58^{b}	4.4 ± 0.33^{b}

P value: 0.05
Replicates: 3
Means with the same superscript lettera,b are not significantly different at ($p>0.05$).
Dilution: 7

Table 6: Total Plate Count(TPC) in meat against green tea and black tea on innoculated meat samples (log_{10} cfu/ml).

Innoculated meat sample			
	0hrs after treatment	**4hrs after treatment**	**8hrs after treatment**
Control	10.8 ± 2.03^{a}	12.3 ± 0.33^{b}	13.0 ± 0.58^{b}
Green tea	5.0 ± 0.58^{a}	4.8 ± 1.15^{a}	4.7 ± 0.58^{a}
Black tea	6.2 ± 1.73^{a}	6.0 ± 0.58^{b}	6.0 ± 1.13^{b}

P value: 0.05
Replicates: 3
Means with the same superscript lettera,b are not significantly different at (p>0.05.)
Dilution: 7

Chapter five:Discussion

5.1 Presence of antimicrobial activity

Tea has been known to possess antimicrobial properties against various microorganisms. Thiswas seen in the bacteria isolates that showed sensitivity through growth inhibition by the different tea blends. This assessment of the antimicrobial activity was determined through measurement of inhibition zones.

This study showed that there was a higher antimicrobial activity of both green tea and black tea against *E. coli* as compared to *S.typhi*.For instance black tea extract from blend C (KTDA) had a inhibition of 10mm gainst *S.typhi* and 13mm against *E.coli*. This was also seen in the green tea where extract form blend C (KTDA) had an inhibition of 13.67mm against *S.typhi* and 16mm against *E.coli*. Research doneby Archana & Abraham(2011) on tea grown in india showed a similar picture where green tea showed an inhibition zone of 12mm against *E.coli* and 6mm against *S.typhi*. Funmilayo *et al.*,(2012) determined that there was antimicrobial effect of tea grown in Nigeria against pathogens such as *Staphylococcus aureus* and *Bascillus subtilis*.

The differences in antimicrobial activities of *C. sinensis* extract might be related to the degree of susceptibility of the cell wall of the test bacterial strains, kind of solvent used in the extract preparation, and different methodology adopted in the determination of antimicrobial activity. (Turkmen *et al.*, 2007). There were no significant differences ($p > 0.05$) in the antimicrobial activity of some of the black tea blends that were analysed against both *S. Typhi* and *E.coli*. The same was also observed in the green tea blends that were analysed. For instance against *S.typhi*, tea blend A(Unilever tea) was not significantly different ($p > 0.05$)to tea blend D (Sasini tea). Tea blend B (Finlay tea) and D (Sasini tea) were not significantly different ($p>0.05$) against *E.coli*.

5.2 Polyphenol levels attributed to the antimicrobial activity of the tea products.

This employed the use of coefficient of correlation and coefficient of determination (r^2) to determine the relationship between polyphenol levels on the tea products and their antimicrobial effect.

The growth inhibition of the microorganism is attributed to the polyphenols that are present in the tea. They usually play an important role in the inhibition. However these antimicrobial activities are selective depending on a variety of factors such as concentration and bacterial species. (Kumar *et al.,* 2012). Polyphenolic compounds are the major components found in tea. Studies have shown that these compounds are the major contributors to the antimicrobial effect of tea. The active substances found in the tea are supposed to reduce the growth and development of the microorganisms they inhibit, (Wang *et al.*, 1992). Research done by Friedman *et al.*, (2006) showed the antimicrobial effect of the different polyphenolic compounds i.e. theaflavins in the black tea and green tea catechins against *Bacillus cereus.* Against both microorganisms the relationship was stronger in black tea than in green tea. This shows that the effect of black and green tea is much stronger against *E.coli* than *S.typhi*. This is a clear indication that the polyphenols influence the antimicrobial effect of tea products. The study showed that there was no significant difference ($p < 0.05$) between the polyphenol levels in both black tea and green tea and antimicrobial activity against both *S.typhi* and *E.coli*. This shows that both green tea and black tea had a similar antimicrobial effect against both microorganisms.

5.3 Potential effect against spoilage microorganisms in meat.

Meat is one of the most widely consumed products. It is the main edible part of the mammal. However, it is also affected by microbial growth, hence making it a major cause of food-borne illnesses. This study entailed finding out whether both green tea and black tea has potential antimicrobial effect against the spoilage microorganisms in meat.

Meat spoilage usually begins from the surface going inwards. It is for this reason that the study applied use of surface treatment using the tea extracts on the meat samples.
The presence of bacteria in meat is common and has been reported from different parts of the world (Kinsella*et al*., 2008). There has been an increase in antibiotic- resistant bacteria strains particularly salmonella in foods and this has caused a great concern in relation to food safety and public health as a whole (Hollingsworth and Kaplon, 1997).
However other methods have been usedto control microbial growth including the use of biological means. Al-Allaf *et al*., 2009., for example used lactic acid bacteria to inhibit growth of other microorganisms in minced meat. Agaoglu *et al*.,2006 investigated the effects of different spices on meat and found that some of the spices that were used showed antimicrobial activity against the microorgaisms such as *Staphylococcus aureus, Escherichiacoli, Enterococcus faecalis.*Both green tea and black tea showed significant reduction ($p > 0.05$) of microbial as compared to control sample. There was also significant reduction ($p > 0.05$) in the innoculated sample. This shows that both green tea and black tea have great antimicrobial effect against spoilage microorganisms in meat.

Chapter six:Conclusion and Recomendation.

6.1 Conclusion

It can be concluded that both green tea and black tea extracts from the different tea blends showed great antimicrobial activity against both *S.typhi* and *E. coli* with blend B (Finlay tea) and C (KTDA) showing similar growth inhibition of against *E.coli*.

The strong relationship between polyphenol levels and antimicrobial activity is an indication that the antimicrobial activity of tea is determined by the levels of polyphenols in the tea product.

Since the meat samples treated with green tea and black tea extracts showed lower microbial counts compared to the control, they could be used for controlling growth of spoilage microrganisms of foods such as meat.

6.2 Recommendation.

The data obtained from his study may offer the consumer a choice of tea brands with very high antimicrobial properties against *S.typhi* and *E.coli*.The potential antimicrobial activity of tea against spoilage microorganisms found in meat as documented in this study are recommended as a basis for further studies on whether both green tea and black tea can be used to preserve meat hence increasing its shelf-life.

Chapter seven
7.0 References.

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