Antimicrobial Properties of Polyphenolic Extracts from Selected Kenya's Specialty Tea Camellia Sinensis on Pathogenic Bacteria and Fungi

Kipsura Jemutai Emily

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ANTIMICROBIAL PROPERTIES OF POLYPHENOLIC EXTRACTS FROM SELECTED KENYA'S SPECIALTY TEA *Camellia sinensis* ON PATHOGENIC BACTERIA AND FUNGI

KIPSURA EMILY JEMUTAI

A THESIS SUBMITTED TO THE BOARD OF GRADUATE STUDIES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE CONFERMENT OF THE DEGREE OF MASTER OF SCIENCE IN MICROBIOLOGY OF THE UNIVERSITY OF KABIANGA

UNIVERSITY OF KABIANGA

NOVEMBER, 2025

DECLARATION AND APPROVAL

DECLARATION

This thesis is my original work and has not been presented before for the conferment of a degree or award of a diploma in this or any other institution.

Signature_____ Date: _____

Kipsura Jemutai Emily

PGC/MIC/008/16

APPROVAL

This thesis has been submitted with our approval as university supervisors.

Signature_____ Date: _____

Prof. Joel Koech

Department of Biological Sciences

University of Kabianga

Signature_____ Date: _____

Dr. Robert Koech

Tea Research Institute (TRI-KALRO)

Signature_____ Date: _____

Dr. Christine Bii

Centre for Microbiology Research, (CMR-KEMRI, NAIROBI)

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DEDICATION

To my wonderful and supportive parents Mr. and Mrs. Philip Sura, my husband Eutycus Wenanga, my son Leroy Wenanga, my brothers, sisters, nephews and nieces and in-laws.

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ABSTRACT

Tea *Camellia sinensis* has received attention due to its high levels of polyphenols that are associated with human health properties such as anti-inflammatory, antioxidant, antibacterial and antifungal effects. However, microbial resistance has reduced the effectiveness of several conventional antimicrobial agents currently used to manage bacterial and fungal infections. Therefore, the development of new, natural antimicrobial products from plant sources with higher potency and fewer side effects is critical in overcoming the emerging challenge of antibiotics resistance. This study evaluated the antimicrobial activity of specialty tea extracts that include oolong, purple, yellow, green and black orthodox teas. They were extracted using hot distilled water, methylene chloride and ethyl acetate. Selected pathogenic bacteria strains included Penicillinase-Methicillin antimicrobial resistance *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853. Fungal strains included *Candida albicans* ATCC 90028 and clinical isolate *Trichophyton mentagrophytes*. Total polyphenols content was analyzed using Folin-Ciocalteu method. Individual catechins and caffeine content were profiled using High Performance Liquid Chromatography (HPLC-PDA) machine and antioxidant activity was determined by DPPH assay. All experimental data were analyzed using analysis of variance (ANOVA) to determine statistically significant differences among treatment means at 95% confidence level ($p< 0.05$). For post hoc analysis, Duncan's Multiple Range Test (DMRT) was applied to separate group means and identify significant differences. Water extracts had the highest total yield (82.2%) while methylene chloride had the least (2.63%). Ethyl acetate extracts contained highest polyphenol content (11.4% -78.1%) and catechin content (46.6% - 75.9%), contrary to, methylene chloride extracts which had low total catechin contents (1.2% to 4.5%). Caffeine was predominantly in methylene chloride extracts (29.97% - 58.99%). Antimicrobial activity was tested using disc diffusion method. Ethyl acetate extracts showed the strongest antimicrobial activity, with an inhibition zone of 23mm against methicillin-penicillinase resistant *staphylococcus aureus* ATCC 25923 at 10 mg/ml concentration. Water and residual extracts also had a moderate inhibition zone of 18mm and 14mm, respectively, while methylene chloride exhibited weak activity (8mm) at 10mg/ml concentration. Ethyl acetate extracts also showed antimicrobial activity against *Candida albicans* ATCC 90028 and clinical isolate *Trichophyton mentagrophytes* with an inhibition zone of 12mm and 16mm respectively, at 1mg/ml concentration. The lowest dilution concentration of 0.31mg/ml exhibited zone of inhibition of 9mm for methicillin -penicillinase resistant *Staphylococcus aureus* ATCC 25923. However, no antimicrobial activity was observed against *Pseudomonas aeruginosa* ATTC 27853. The data confirm that the choice of solvent significantly impacts both composition and bioactivity. Ethyl acetate was most effective solvent for extracting bioactive compounds. Kenya specialty teas demonstrated broad-spectrum of antimicrobial potential, supporting their use as natural alternatives antimicrobial agents.

TABLE OF CONTENTS

DECLARATION AND APPROVAL ..... **i**

COPYRIGHT ..... **ii**

DEDICATION ..... **iii**

ACKNOWLEDGEMENTS ..... **iv**

ABSTRACT ..... **v**

TABLE OF CONTENTS ..... **vi**

LIST OF TABLES ..... **xii**

LIST OF FIGURES ..... **xiii**

LIST OF PLATE ..... **xiv**

LIST OF ACRONYMS AND ABBREVIATIONS ..... **xv**

CHAPTER ONE **1**

INTRODUCTION **1**

1.1 Overview 1

1.2 Background Information 1

1.3 Statement of the Problem 6

1.4 Objectives of the study 6

1.4.1 General Objective 6

1.4.2 Specific Objectives 7

1.5 Hypothesis 7

1.6 Justification 7

1.7 Significance of the Study 8

1.8 Scope of the Study 8

1.9 Assumptions 9

CHAPTER TWO 11

LITERATURE REVIEW 11

2.1 Introduction 11

2.2 Review of Related Literature 11

2.2.1 Tea and Health 11

2.2.2 Tea Phytochemicals 14

2.2.3 Catechins (Flavanols) 15

2.2.4 Theaflavins and Thearubigins 18

2.2.5 Anthocyanins 20

2.2.6 Processing and Different Types of Tea 24

2.2.6.1 White Tea 26

2.2.6.2 Green Orthodox Tea 26

2.2.6.3 Oolong Tea 27

2.2.6.4 Black Orthodox Tea 28

2.2.6.5 Purple Orthodox Tea 29

2.2.6.6 Yellow Tea 30

2.2.7 Antioxidant Properties of tea polyphenols 31

2.2.8 Antimicrobial Activity of Tea Compounds 32

2.2.9 Infectious Diseases Burden 33

2.2.10 Pathogens Bacteria and Fungi 35

2.2.10.1 *Staphylococcus aureus* 36

2.2.10.2 *Escherichia coli* 37

2.2.10.3 *Pseudomonas aeruginosa* 37

2.2.10.4 *Trichophyton mentagrophytes* 38

2.2.10.5 *Candida albicans* 39

2.3 Conceptual Framework 39

2.4 Identification of the Knowledge Gap 40

CHAPTER THREE 43

MATERIALS AND METHODS 43

3.1 Introduction 43

3.2 Research Design 43

3.3 Location of Study 44

3.4 Chemicals and Reagent 45

3.5 Sample and Sampling Procedures 45

3.5.1 Preparation of Tea Samples 45

3.6 Laboratory Analysis 47

3.6.1 Extraction 47

3.6.2 Fractionation 47

3.6.3 Polyphenol Analysis on Tea Samples 50

3.6.4 Total Polyphenols Estimation in Tea Extracts 50

3.6.5 Determination of Catechins in Tea Extracts 51

3.6.6 Evaluation of Antioxidant Activity in Tea Extracts 53

3.7 Antimicrobial Assays 54

3.8 Data Analysis 55

CHAPTER FOUR **56**

RESULTS AND DISCUSSION **56**

4.1 Introduction 56

4.2 Total Polyphenolic Content and Antioxidant Activity of Extracts. 56

4.2.1 Total catechins content 58

4.2.2 Individual catechins levels 59

4.2.3 Antioxidant activity 61

4.2.4 Correlation coefficient analyses between Biochemical Parameters 62

4.3 Antibacterial Activity of Tea Extracts 63

4.4 Antifungal Activity of Tea Extracts 70

4.5 Positive Correlation between the Diameters of Inhibition Zones and Different Biochemical Parameters of Tea 72

4.6 Discussion 74

4.6.1 Total Polyphenol Content and Antioxidant activity of the Extracts 74

4.6.2 Antibacterial Activity of Tea Extracts 77

4.6.3 Antifungal Activity of Tea Extracts 79

4.6.4 Correlation Between Biochemical Composition and Antimicrobial Activity 80

CHAPTER FIVE **81**

SUMMARY, CONCLUSION AND RECOMMENDATION **81**

5.1 Introduction 81

5.2 Summary 81

5.3 Conclusions 82

5.4 Recommendations 83

5.5 Suggestions for Future Research 83

REFERENCES 85

APPENDICES 98

Appendix I: The gallic acid calibration curve for measuring the total phenolic content. 98

Appendix II: Total Catechin Content (%) in tea extracts by tea type and solvent ... 99

Appendix III: Individual Catechins Content (%) of Tea Extracts by Tea type and Solvent 100

Appendix IV: A chromatograph representation of elution order of the catechins in tea sample using HPLC-PDA Machine. 101

Appendix V: Antioxidant activity (%) of Tea Extracts 102

Appendix VI: Dose-dependent activity of tea extracts against resistant *S. aureus* 103

Appendix VII: Dose dependent activity of tea extracts against Escherichia coli determined by zone inhibition (mm) 104

Appendix VIII: Mean inhibition zones (mm) for antifungal activity of tea extracts against T. mentagrophytes and C. albicans. 105

Appendix IX: Disc diffusion assays 106

Appendix X: Extracts and solutions 108

Appendix XI: Tea extracts 109

Appendix XII: Letter of approval by BGS for fieldwork.....110

Appendix XIII: NACOSTI Research License.....111

Appendix XIV: Publication.....112

LIST OF TABLES

Table 2.1: The six most frequently occurring anthocyanins found in nature.....21

Table 4.1: Total Polyphenolic Content (%) of Tea Extracts by Tea type and Solvent 58

Table 4.2: Linear correlation between biochemical parameters of the analyzed tea samples.....62

Table 4.3: Mean inhibition zones (mm) for antibacterial activity of tea extracts against S. aureus and E. coli 65

Table 4.4: Correlation coefficients between biochemical parameters of the tea extracts and the diameters of inhibition zones (IZDs) for resistant S. aureus ATCC 25923 73

Table 4.5: Correlation coefficients between the diameters of inhibition zones (IZDs) and the biochemical parameters of different tea extracts for E. coli ATCC 25922. 73

Table 4.6: Correlation coefficients between the diameters of inhibition zones (IZDs) and the biochemical parameters of different tea extracts for T. mentagrophytes. 74

LIST OF FIGURES

Figure 2.1: Catechins chemical composition Camellia Sinensis.(Anand et al., 2015) 15

Figure 2.2: The phenylpropanoid pathway in the production of catechins (Rehan, 2021) 17

Figure 2.3: Chemical structure of (a) theaflavins and (b) thearubigins (Sen et al., 2022) 19

Figure 2.4: The basic structure of anthocyanins (Qi et al., 2023) 21

Figure 2.5: A schematic overview for anthocyanin biosynthesis pathway (He et al., 2017) 22

Figure 3.1: Experimental research design used in this study. 44

Figure 3.2: Diagrammatic representation of the extraction and fractionation process..... 49

Figure 4.1: Total polyphenol content (%) of tea extracts by tea type and solvent. ... 57

Figure 4.2: Total catechin content (%) by tea type and solvent type. 59

Figure 4.3: Individual catechin content 60

Figure 4.4: Antioxidant activity in all tea types 61

Figure 4.5: Antibacterial activity of specialty tea extracts. 64

Figure 4.6: Dose -dependent antibacterial activity of all ethyl acetate extracts from specialty teas against Staphylococcus aureus. 67

Figure 4.7: Dose-dependent antibacterial activity of ethyl acetate tea extracts against Escherichia coli ATCC 25922. 69

Figure 4.8: Mean zone inhibition (mm) showing antifungal activity of tea extracts against T. mentagrophytes and C. albicans ATTC 90028. 71

LIST OF PLATE

Plate 2.1: Green tea Timbilil field at Tea Research Institute, Kericho (Tea Research Institute- Kericho, 2020). 25

Plate 2.2: Purple tea Timbilil field at Tea Research Institute, Kericho (Tea Research Institute-Kericho,2020). 25

Plate 2.3: White tea prepared at Tea Research Institute, Kericho (Tea Research Institute-Kericho, 2020). 26

Plate 2.4: Green orthodox tea at Tea Research Institute, Kericho (Tea Research Institute-Kericho, 2020). 27

Plate 2.5: Oolong tea at Tea Research Institute-Kericho (Tea Research Institute-Kericho,2020). 28

Plate 2.6: Black orthodox tea at Tea Research Institute-Kericho (Tea Research Institute-Kericho,2020). 29

Plate 2.7: Purple orthodox tea at Tea Research Institute-Kericho (Tea Research Institute-Kericho,2020) 30

Plate 2.8: Yellow tea at Tea Research Institute-Kericho (Tea Research Institute-Kericho, 2020). 31

Plate 4.1: Inhibitory concentration of tea extracts against S. aureus ATCC 25923, resistant to methicillin and penicillinase. 66

Plate 4.2: Dose-dependent activity of tea extracts against methicillin and penicillin resistant S. aureus ATCC 25923. 68

Plate 4.3: Dose- dependent activity of tested tea extracts that inhibit the growth of antibacterial-resistant E. coli ATCC 25922. 70

Plate 4.4: The dose dependent activity for C. albicans ATCC 90028 and T. mentagrophytes against tested extracts. 72

LIST OF ACRONYMS AND ABBREVIATIONS

ATCC	American Type Culture Collection
AMR	Antimicrobial Resistance
CTC	Cut Tear and Curl
EC	Epicatechin
ECG	Epicatechin gallate
EGC	Epigallocatechin
EGCG	Epigallocatechin gallate
GDP	Gross Domestic Production
IZDs	Inhibition zone diameters
KALRO	Kenya Agricultural and Livestock Research Organization
KEMRI	Kenya Medical Research Institute
TF	Theaflavins
TR	Thearubigins
TRI	Tea Research Institute

CHAPTER ONE

INTRODUCTION

1.1 Overview

This chapter introduces the study by highlighting the global challenge of antimicrobial resistance (AMR) and the urgent need for alternative, natural antimicrobial agents. It emphasizes the significance of polyphenolic compounds found in tea (*Camellia sinensis*), particularly those in specialty teas produced in Kenya, as promising sources of antimicrobial activity. The chapter outlines the rationale for focusing on Kenyan specialty teas that includes black, oolong, green, yellow, and purple orthodox which are based on their varying fermentation levels and biochemical diversity. It also presents the research problem, objectives, justification, and scope of the study, establishing a strong foundation for investigating how different solvent extracts influence the polyphenolic composition and bioactivity of these teas. The chapter concludes by presenting a conceptual framework that connects the variables under investigation, providing a theoretical basis for exploring the antimicrobial properties of the tea extracts

1.2 Background Information

Tea (*Camellia sinensis*) is one of the most consumed beverages globally after water and is widely known for its medicinal and nutritional value (Hazra et al., 2017). The health properties of tea are largely attributed to its abundance of bioactive phytochemicals particularly polyphenols that accounts 33% of the total tea phytochemicals. These include catechins, theaflavins, thearubigins, phenolic acids and anthocyanins, which exhibit potent antioxidant, anti-inflammatory, antibacterial, and antifungal properties (Bag et al., 2022). The consumption of tea has been associated with reduced risks of

chronic and infectious diseases, including cardiovascular disorders, cancer, and microbial infections (Samanta, 2022). Tea beneficial health are linked to high free radical scavenging ability of its phenolic compounds, which protect the body from damage caused by oxidative stress induced by free radicals (Tang et al., 2019).

Kenya is a tropical country in East Africa, and has diverse climatic and geographical regions. Kenya's tea production regions are in two major areas by the Great Rift Valley. The western region includes the highlands of the Mau escarpment in Mount Elgon, Kericho and Kisii highlands. On the eastern side of Rift Valley are the cooler Aberdare Highlands, Nyambene Hills, and Mount Kenya region (Recha, 2019). Tea is cultivated on the slopes of these highlands at altitudes from 1,500m to 3,100m above sea level. Kenya, which ranks third globally in tea production after China and India (Karuri, 2020), is renowned for its high-quality black CTC tea. A large portion of the Kenyan tea around 95% is exported to countries such as Egypt, Pakistan and United Kingdom, while only 5% is used locally (Karuri, 2021). It has increased gaining global attention for its specialty teas, which include purple, white, yellow, oolong, and orthodox black and orthodox green teas.

Specialty teas are premium category of tea products distinguished by their unique processing techniques and distinct organoleptic qualities which include flavor, aroma and appearance (Mishra et al., 2020). Specialty teas are characterized by their premium quality leaves, typically composed of whole, unbroken leaf. These leaves are often hand plucked to maintain consistency and ensure superior quality. In addition, specialty teas exhibit tastes profiles such as floral, fruity or earthy notes which are strongly influenced by agro ecological factors including climate, altitude, and soil composition of the cultivation region. Their production is usually limited to small batches, highlighting the use of artisanal and traditional methods. They are categorized based on the degree of

fermentation during processing, which plays a critical role in determining their phytochemical composition and bioactivity. These teas have gained recognition for its unique flavors and high sale value in tea value addition sector (Besky, et al., 2020).

In Kenya, specialty tea production is a relatively recent but rapidly expanding segment of tea industry. While the country has long been known for producing black CTC (cut-tear-curl) tea for export, the Tea Research Institutes and private tea sectors have introduced orthodox manufacturing methods to produce a wide range of specialty teas (KTDA, 2020). Green orthodox tea, which is unfermented, retains high levels of monomeric catechins such as epigallocatechin gallate (EGCG), epicatechin (EC), and epicatechin gallate (ECG). These catechins are powerful antioxidants and contribute significantly to green tea's antimicrobial effects.

Black orthodox tea, fully fermented, contains reduced catechin levels due to enzymatic oxidation, resulting in the formation of theaflavins (TF, TF-3-G, TF-3'-G, TF-3,3'-DG) and thearubigins, which impart color, astringency, and demonstrate strong antimicrobial properties and controls cholesterol levels (Imran et al., 2020). Oolong tea, which is semi-fermented, possesses a balanced mixture of both catechins and their oxidation products, giving it intermediate biochemical properties between green and black tea.

Purple tea, a Kenyan innovation, is rich in anthocyanins, particularly delphinidin and cyanidin, which are responsible for its purple pigmentation and associated with antioxidant and antifungal properties (Wachira et al., 2016). White tea, derived from young silvery hairy buds and minimally processed, retains delicate catechins and flavonols, whereas yellow tea, which undergoes a unique "sealed yellowing" process, has a softer flavor profile and a slightly altered catechin structure. These specialty teas

were selected due to their diverse fermentation levels and distinct phytochemical profiles, which are hypothesized to influence their antimicrobial potency.

The production of specialty teas in Kenya aligns with national agricultural diversification strategies aimed at improving farmer incomes and increasing the country's share in the global premium tea market. Additionally, their high content of bioactive compounds has attracted research interest in their pharmacological potential, particularly in the search for natural alternatives to synthetic antimicrobials.

Despite the increasing recognition of these teas for their functional health benefits, their antimicrobial potential remains underexplored, particularly within Kenya. At the same time, antimicrobial resistance (AMR) has emerged as a global crisis. According to World Health Organization , communicable diseases accounted for approximately 20% of all global deaths, with lower respiratory infections, diarrheal diseases, malaria, tuberculosis, and HIV/AIDS leading the burden (WHO, 2018). In Kenya, the Ministry of Health (2018) highlighted that these diseases remain among the top ten causes of morbidity and mortality, particularly in rural and peri-urban settings where access to healthcare is limited (MOH, 2018).

The rising prevalence of multidrug-resistant organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA), extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli*, multi-drug resistant *Pseudomonas aureginos*a, azole-resistant *Candida albicans* and terbinafine-resistant *Trichophyton mentagrophyte*, resistant further exacerbates treatment challenges, that prolongs illness and increase the health care cost (WHO, 2020). *S. aureus* is a gram-positive bacterium commonly found on human skin and mucosa. It is associated with a wide range of infections, from skin abscesses to life threatening conditions such as pneumonia and bacteremia. The

emergence of methicillin-resistant S. aureus (MRSA) has made treatment challenging due to multiple beta-lactam antibiotics resistance (Lade et al., 2022). *E. coli*, a gram-negative bacterium is part of the normal human flora but includes pathogenic strains that cause diarrheal illnesses and urinary tract infections. Extended spectrum beta-lactamase producing *E. coli* has become a global concern due to resistance to penicillin's and cephalosporins (Padmini et al., 2017). *Candida albicans* is an opportunistic dimorphic fungus that causes oral thrush and vaginal candidiasis, and in low immunity patients such as diabetes, organ transplant patients, it can lead to systemic candidemia. *T. mentagrophytes*, a dermatophyte fungus, is implicated in tinea infections affecting the skin, hair and nails. It is transmitted through direct contact or contaminated surfaces. It is common in warm, humid environments with emerging reports of antifungal resistance in chronic cases ((Singh et al., 2019).

Conventional antimicrobials, while still essential, they have become increasingly ineffective due to widespread of antibiotic infection resistance. Consequently, plant-derived antimicrobials have gained scientific attention as viable alternatives. Tea polyphenols have demonstrated mechanisms such as disruption of microbial membranes, inhibition of virulence factor expression, interference with DNA synthesis, and formation of biofilm and suppression of quorum sensing (W. Liu et al., 2020). This growth suppression support in infection control and also reduce overdependence on conventional antibiotics, which is a major contributor to the development of drug-resistant strains. Published studies have majorly focused on green or black tea, with limited evidence available on the antimicrobial efficacy of Kenya's specialty teas.

This study, therefore, aimed to assess the antimicrobial properties of polyphenolic extracts from selected Kenyan specialty teas, focusing on their efficacy against clinically relevant bacteria and fungi, namely *Staphylococcus aureus, Escherichia coli*,

Pseudomonas aeruginosa, *Candida albicans*, and *Trichophyton mentagrophytes*. Extracts were prepared using both aqueous and organic solvents to obtain a broad spectrum of phytochemicals. By profiling these extracts and evaluating their activity, the study identified potential natural antimicrobial agents that are affordable, acceptable, accessible, particularly in low resource settings.

1.3 Statement of the Problem

Globally infectious diseases caused by bacterial and fungal pathogens remain a major health concern, contributing to high morbidity and mortality rate (santé, 2018). The problem is further compounded by the emergence of antibiotic resistant strains, which make available conventional frontline antibiotics ineffective. According to the World Health Organization (WHO,2018) diseases such as lower respiratory tract infections, diarrheal diseases and tuberculosis remain among the leading causes of death (https://communitymedicine4all.com/2017/02/01/). In Kenya, resistance to commonly prescribed antibiotics has been reported, exacerbating the burden of infectious diseases. These multidrug resistant organisms have been linked to persistent infections in both hospital and community settings. While black and green CTC teas have been studied for their antimicrobial activities, there is limited scientific evidence on the efficacy of Kenya's specialty teas. Given their rich polyphenolic content, these teas may offer novel antimicrobial solutions. Therefore, this study seeks to fill the knowledge gap by evaluating the antimicrobial activity of selected Kenya specialty tea extracts.

1.4 Objectives of the study

1.4.1 General Objective

To determine antimicrobial activity of extracts from selected Kenya's specialty tea cultivars on selected pathogenic bacteria and fungi.

1.4.2 Specific Objectives

1. To determine the polyphenols composition and antioxidant activity of extracts from black orthodox, oolong, green orthodox, yellow orthodox and purple orthodox teas processed from green and purple leaf tea cultivars.
2. To determine antibacterial activity of polyphenols extracts from black orthodox, oolong, green orthodox, yellow orthodox and purple orthodox teas as processed from green and purple leaf tea cultivars against *Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus*
3. To evaluate antifungal activity of polyphenols extracts from black orthodox, oolong, green orthodox, yellow orthodox and purple orthodox teas as processed from green and purple leaf tea cultivars against *Candida. albicans* and *Trichophyton mentagrophytes*.

1.5 Hypothesis

1. There are no significant variations in polyphenolic composition of extracts of orthodox (green, purple and black), oolong and yellow tea extracts processed from green and purple leaf cultivars.
2. There are no significant variations in antibacterial efficacy of these polyphenolic extracts.
3. There are no significant variations in antifungal activity of these polyphenolic extracts.

1.6 Justification

Kenya's tea sector is important to the national economy, contributing highly to export earnings and employment. However, overreliance on black CTC tea and fluctuating international prices have caused economic value. As part of tea diversification,

specialty teas are being promoted for their added health value and higher market prices. These teas are rich in bioactive compounds that may serve as alternative antimicrobial agents. Research on the antimicrobial potential of Kenya's specialty teas is limited. Exploring these teas for their health benefits can promote their consumption, support public health and improve tea sector commercial value. Additionally, addressing AMR through natural alternatives aligns with global health goals.

1.7 Significance of the Study

The study will generate scientific evidence on the antimicrobial properties of Kenya's specialty teas, contributing to public health strategies of combating antimicrobial resistance. It will also support the Kenya tea industry by promoting value addition and diversification. The findings may promote further research and utilization of tea-based compounds in antimicrobial drug development.

1.8 Scope of the Study

This study focused on evaluating the antimicrobial properties of Kenya's specialty tea extracts. Tea samples were collected from the Tea Research Institute (TRI) in Kericho, where a randomized complete block design (RCBD) was applied using five tea cultivars: TRFK 895/20, TRFK 914/30, TRFK 791/2, TRFK 791/1, and a second-generation purple leaf tea cultivar. Each cultivar was replicated three times and harvested from the experimental tea fields at Timbilil Estate. The freshly plucked tea leaves were processed into different tea types using a miniature factory setup at TRI. The scope of the study included both the biochemical profiling of tea extracts at the TRI laboratory. Antimicrobial susceptibility testing against selected pathogenic microorganisms. These included both Gram-positive (Methicillin-penicillinase resistant *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*), as

well as fungal strains (*Candida albicans* and *Trichophyton mentagrophytes*) conducted at the Centre for Microbiology Research (CMR-KEMRI) in Nairobi. The entire processing and laboratory analysis was conducted of a period of four months. This research aimed to explore the potential of specialty teas as alternative sources of antimicrobial agents for managing infections caused by pathogenic bacteria and fungi, thereby contributing to the search for natural solutions in combating microbial resistance.

1.9 Assumptions

This study was conducted under the assumption that all tea samples collected from the Tea Research Institute (TRI) were correctly identified, uniformly harvested, and processed under standardized conditions to ensure consistency in quality across different cultivars and specialty tea types. It was assumed that the bioactive compounds within the tea leaves particularly polyphenols and catechins remained stable during processing and extraction, and that they were primarily responsible for the observed antimicrobial effects. The selected test organisms, including *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Trichophyton mentagrophytes*, were presumed to be clinically relevant representatives of common pathogenic microbes causes infectious disease. The methods used to assess antimicrobial activity, such as agar well diffusion and minimum inhibitory concentration (MIC) determination, were assumed to be appropriate, sensitive, and reproducible for evaluating plant-based extracts. It was also assumed that experimental conditions such as incubation temperature, time, and extract concentrations were adequately controlled, and that the laboratory equipment used in both biochemical and microbiological analyses was properly calibrated and functioning optimally throughout the research period. Finally, it was presumed that the

antimicrobial activity observed was due to the tea extracts alone and not influenced by external contaminant.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

The chapter outlines a review of existing literature related to the study. The chapter looks at the infectious diseases and how it has been exacerbated by development of resistance by disease pathogens to the frontline antibiotics. Additionally, it discusses the different types of tea from tea plant *Camellia sinensis*, highlighting their distinct biochemical composition and how these influences their physiological and antimicrobial activities. The chapter aimed to identify existing knowledge gaps in this study.

2.2 Review of Related Literature

2.2.1 Tea and Health

Tea is widely known for its therapeutic potential, primarily due to the presence of polyphenolic compounds. Recent studies have shown that these polyphenolic compounds are associated with a reduced risk of coronary heart disease (Vastrad et al., 2022), prevention of cancer (Liu et al., 2018), and improvement in oral health (Li et al., 2019), as well as anti-obesity effect (Xu et al., 2023). Research further demonstrates that black tea supplementation enhances blood flow in the brachial artery, thereby supporting cardiovascular function (Schreuder et al., 2014).

Additionally, experimental studies using animal models have indicated that tea flavonoids play a role in inducing apoptosis, making them potentially effective in fighting cancers of the colon, esophagus, and prostate (Sheikh et al., 2020). Similarly, mouth rinsing with green tea extract (0.61%) has shown protective effects on dental

enamel erosion and abrasion, comparable to the effects of fluoride (250ppm). The consumption of black tea has also been associated with favorable modulation of gut microbiota, promoting the production of short-chain fatty acids like propionate and acetate. These acids activate AMP-activated protein kinase, which regulates lipid metabolism and helps balance energy intake and expenditure, thereby supporting obesity in humans (Rothenberg et al., 2018). In addition, tea consumption enhances insulin activity (Fu et al., 2017), promotes skin regeneration, prevents respiratory diseases, and supports physiological functions, such as antioxidant (Tong et al., 2019) and antimicrobial activity.

The clinical trial studies have shown that tea polyphenols act as an anti-diabetic agent by exerting insulinotropic effect (stimulating the production of insulin). For instance, epigallocatechin gallate (EGCG), a key catechin in green tea, has been found to stimulate insulin secretion following meals (Wan et al., 2024). In animal model experiments, green tea polyphenols were also found to enhance the wound healing process (Xu et al., 2021), and stimulate aged keratinocytes to initiate DNA synthesis. This reaction was possibly leading to renewed cell division and skin regeneration.

Theophylline, an alkaloid found in tea, has been used to treat respiratory conditions like chronic bronchitis and asthma by relaxing and opening the airways in the lungs (Palai et al., 2023). Additionally, hydroxyl groups in flavanol structure of tea polyphenols contribute to their antioxidant properties through hydrogen bonding (Vishnoi et al., 2018). Black tea contains theaflavins that exhibit strong free radical scavenging activity. Among them, theaflavin-3,3'-digallate has the highest antioxidant capability, followed by theaflavin-3'-gallate and theaflavin-3-gallate, due to the presence of hydroxyl groups and the gallic acid moiety.

Tea's natural antioxidant properties have contributed to its incorporation into commercial food and beverage products such as cereals, biscuits, cakes, and dietary supplements. Additionally, tea extracts have been added to dairy products, confectionery, instant noodles, and ice cream to enhance their health appeal to consumers (Rashidinejad, 2022). Tea polyphenols have demonstrated antibacterial effects and have also been used as raw materials in nanomaterial production. For instance, in a recent study using Pu-erh tea, the polyphenols damaged *Escherichia coli* cell membranes (Huang, 2023). Furthermore, white, and green tea extracts exhibited antimicrobial activity by inhibiting *Helicobacter pylori*, while preserving beneficial gut microbes thus reduce the risk of gastric cancer (Mohammed, 2020).

In addition, L-theanine, the predominant amino acid in tea, accounting about fifty percent of the total amino acid in tea leaves (Too et al., 2016). It has been associated with pharmacological activity such as lowering blood pressure (Li et al., 2019), and regulates neurotransmitters such as norepinephrine, dopamine, and serotonin levels in the brain (Dietz and Dekker, 2017). It also produces neuro-protective and cognitive enhancing actions in the brain (Deb et al., 2019).

Caffeine an alkaloid present in tea (*Camellia Sinensis*), stimulates the central nervous system and relaxes the respiratory and cardiac muscles (Tang et al., 2019).It is commonly acknowledged for its ability to increase alertness and attentiveness (Dietz and Dekker, 2017). However, in some individuals, consuming tea may lead to caffeine withdrawal symptoms such as headaches, anxiety, gastrointestinal irritation, and difficulty sleeping (Sharma et al., 2023).

Therefore, to address this, decaffeination of tea has been done by using ethyl acetate and methylene chloride solvents (Bermejo et al., 2016). Today, the caffeine extracted

during tea decaffeination is utilized in energy drinks, soft drinks, and pharmaceutical products as an active ingredient or adjuvant in formulations like paracetamol (Monteiro et al., 2019).

2.2.2 Tea Phytochemicals

Phytochemicals are naturally occurring biochemical compounds within plants. Among these are secondary metabolites, compounds that plant produce not for growth, development, or reproduction. They are synthesized by plants for ecological roles like defense against pests, attract pollinators, and adaptation to environmental stress (Divekar et al., 2022). Moreover, secondary metabolites play a role in producing phytoalexins, which are secondary compounds generated in response to pathogenic attacks, like fungal or bacterial infections. These phytoalexins function as signaling molecules in plant-microbe interactions, serving as protectants for the plant (Tiku, 2020).

Phytochemicals are generally categorized into five sub-classes: phenolics, carotenoids, alkaloids, organosulfur compounds, and nitrogen-containing compounds. Of these, phenolics and carotenoids are the most extensively studied. The primary phytochemicals in tea are polyphenols. It is well-established that tea contains approximately 4,000 bioactive compounds, with polyphenols making up about one-third of these compounds (Nataraj et al., 2016).

Polyphenols are molecules with benzene rings bonded to multiple hydroxyl groups, which has drawn significant research interest owing to their natural antioxidant effects (Prabhu et al., 2021). Tea polyphenols include phenolic acids, flavonoids, stilbenes, and tannins, with flavonoids being the predominant subgroup. Flavonoids are classified by their structure consisting of two phenyl rings (A and B) and a pyranoid ring (C). They

are classified into flavanols (catechins), anthocyanidins, flavonols (quercetin, kaempferol), flavones and isoflavones (Shi et al., 2023). The distribution of these compounds in tea leaves depends on plant variety, growth stage, and processing method (Wong et al., 2022).

2.2.3 Catechins (Flavanols)

Flavanols, also termed as catechins or flavan-3-ols are immensely discovered for their antioxidant related effects in human health (Almanza-Aguilera et al., 2021). Catechins accounts for 60 to 80 percent of tea polyphenols (Baldi et al., 2020). Major catechins found in tea are epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), catechin (C), and epicatechin (EC) and smaller quantities of catechin gallate (CG), gallocatechins (GC), and gallocatechin gallate (GCG) as shown in Figure 2.1 below. Processing methods such as dry heating or steaming inactivate the polyphenol oxidase (PPO) enzyme, preserving catechin content which positively correlates with green tea quality (Koch et al., 2018).

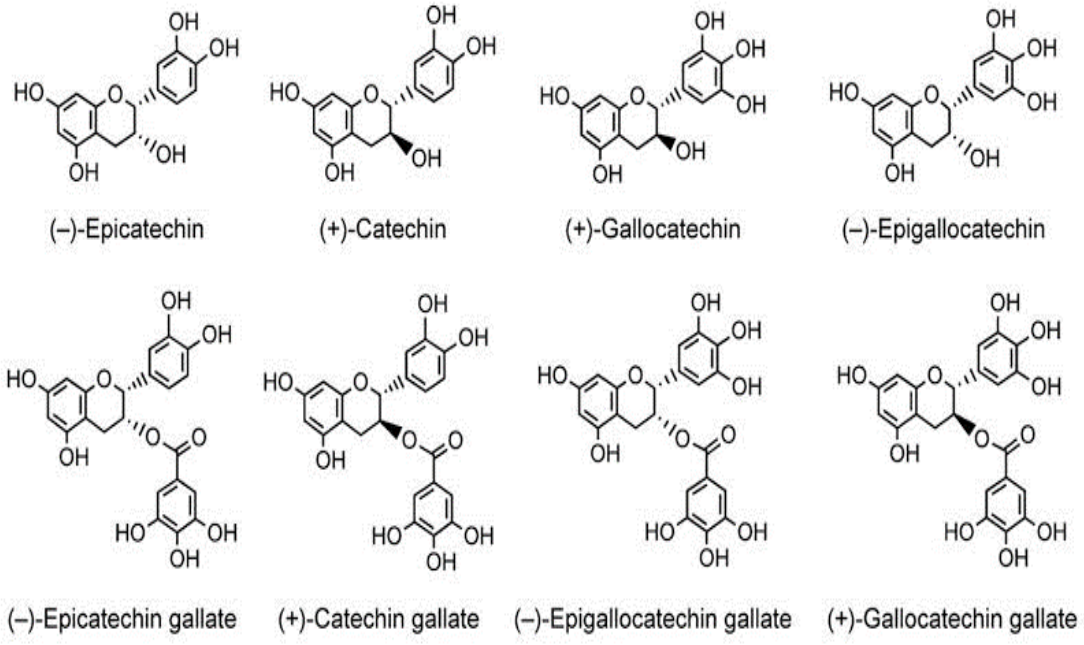


Figure 2.1: Catechins chemical composition Camellia Sinensis.(Anand et al., 2015)

The phenylpropanoid (PP) and flavonoid pathway (FL) synthesize catechins (Rehan, 2021). This biosynthesis of catechins is more prominent in growing tea shoots than in mature leaves, which accounts for the higher catechin content in young leaves. In the initial step of the pathway (**Figure 2.2**) the conversion of phenylalanine to cinnamic acid is catalyzed by phenylalanine ammonia lyase (PAL) enzyme, which is further hydroxylated by the enzyme cinnamate 4-hydroxylase (C4H) to 4-coumaric acid. Subsequently, 4-coumaric acid is then activated to form 4-coumaroyl-CoA by 4-coumaroyl CoA ligase (4CL) enzyme which is a central precursor for various flavonoids, including flavanols. Another key step is the enzymatic conversion of 4-coumaroyl-CoA into naringenin chalcone synchronized by enzyme chalcone synthase (CHS). Naringenin chalcone then acts as substrate for the formation of naringenin by chalcone isomerase (CHI) enzyme. It further hydroxylated to dihydroflavonols through enzymatic reduction, catalyzed by enzyme flavanone 3-hydroxylase (F3H). The enzyme acts as an intermediate in flavanols biosynthesis. These dihydroflavonols are subsequently converted into leucoanthocyanidin by the enzyme dihydroflavonol reductase (DFR). It acts as a substrate to produce flavan-3-ols, the fundamental structure of catechins, with enzyme leucoanthocyanidin reductase (LAR) which is essential in the process (Rehan, 2021).

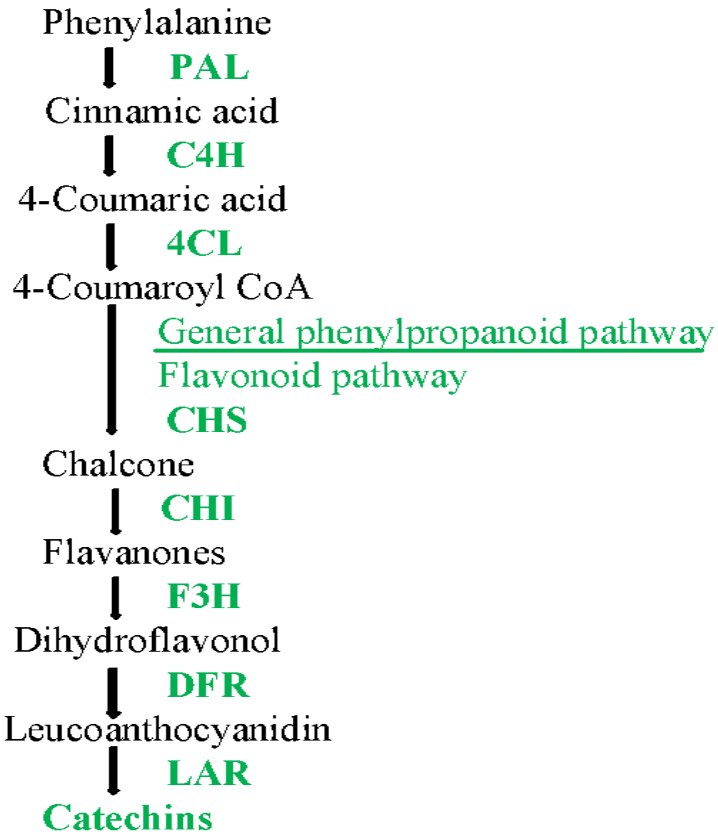


Figure 2.2: The phenylpropanoid pathway in the production of catechins (Rehan, 2021)

PAL, *phenylalanie ammonia-lyase*; C4H, *cinnamate 4-hydroxylase*; 4CL, *4-coumaroyl CoA ligase*; CHS, *chalcone synthase*; CHI, *chalcone isomerase*; F3H, *flavanone 3-hydroxylase*; DFR, *dihydroflavonol reductase*; LAR, *leucoanthocyanidin reductase* (Rehan, 2021).

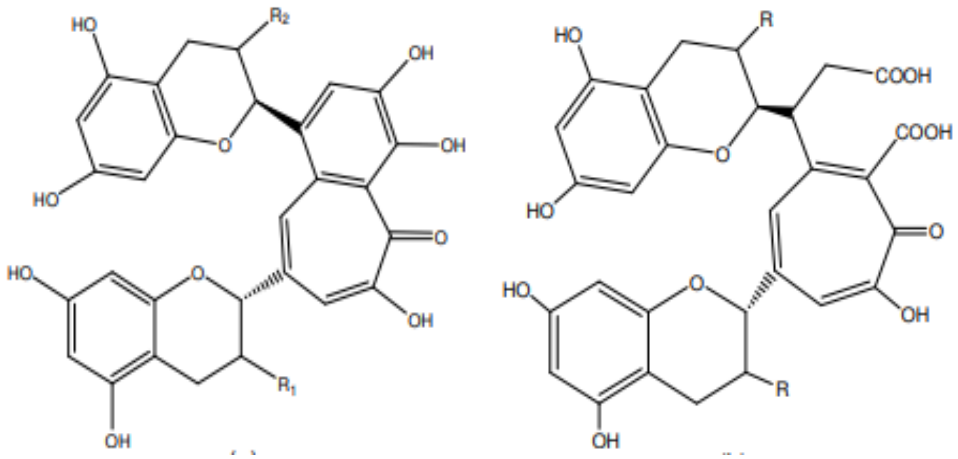
Specific catechin compounds, such as epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), and epigallocatechin gallate (EGCG), are produced through a series of enzymatic reactions and modifications. Additionally, the shikimate pathway contributes to the formation of gallic acid, which is presumed to be esterified to flavan-3-ols in the final stages of tea catechin biosynthesis. Among these catechins, epigallocatechin gallate (EGCG) constitutes a significant proportion of up to 50% of total flavanols and is particularly potent in antioxidant activity (Negri et al., 2018). EGCG and ECG have demonstrated the most potent antioxidant effects, with EGC, EC,

C, and GA showing slightly lesser activity. (Martini, 2016), antidiabetic effect (J. Zhong et al., 2016), antibacterial effect (Boran et al., 2015), antiviral effects , anticancer (Liu et al., 2018) and neuroprotective effects (Rani et al., 2023).

2.2.4 Theaflavins and Thearubigins

Theaflavins (TFs) and thearubigins (TRs) are polyphenolic compounds unique to fermented teas like black tea and semi-fermented teas such as oolong tea. These compounds are not present in high quantities in non-fermented teas and are formed during the oxidation phase of tea processing. Theaflavins are synthesized through oxidative polymerization of catechins under the enzyme polyphenol oxidase (PPO) that is naturally present in tea leaves (Teng et al., 2017). These specific catechins includes epicatechin gallate (ECG), epicatechin (EC), epigallocatechin (EGC), and epigallocatechin gallate (EGCG).

The primary theaflavins include simple theaflavins (TF), theaflavin-3-gallate (TF-3-G), theaflavin-3'-gallate (TF-3'-G), and theaflavin-3,3'-digallate (TF-3,3'-DG), each differing in the number and position of gallate groups (Tu, 2023). Each type of theaflavin results from the oxidative coupling of specific catechin molecules. Plain TF is formed by the coupling of EC and EGC. TF3 is produced through the coupling of EC and EGCG with a gallate group attached to the 3-position of the C-ring. TF3' is synthesized by coupling ECG and EGC, with the incorporation of a gallate group attached to the 3'-position of the B-ring. TF3,3' the most structurally complex theaflavin, is formed through the oxidative coupling of ECG and EGCG, with the attachment of two gallate groups. One attached on the C-ring and another on the B-ring. These theaflavins exhibit a more complex molecular structure compared to their precursor catechins, characterized by multiple phenolic rings and oxygen atoms as shown in Figure 2.3 below (Sen et al., 2022).



(a) **(b)**

Theaflavin: R1=R2=OH
Theaflavin-3-gallate: R1=Galloyl; R2=OH
Theaflavin-3'-gallate: R1=OH; R2=Galloyl
Theaflavin-3,3'digallate: R1=R2=Galloyl

R=Galloyl or other groups

Figure 2.3: Chemical structure of (a) theaflavins and (b) thearubigins (Sen et al., 2022)

A distinctive element of the TFs structure is the seven-membered benzotropolone ring, which is critical their biochemical activity (H.-F. He, 2017). Theaflavins are responsible for the bright reddish color and astringent taste of black tea. In their pure form, they have a strong astringency, causing a dry and rough sensation in the mouth. Among them, TF-3,3'-DG is considered the most astringent, exhibiting the lowest taste threshold relative to other theaflavins (Ghosh et al., 2016).

Beyond sensory properties, theaflavins possess biological activity, including strong antioxidant and cholesterol lowering effects (Cai et al., 2021). Their presence serves as a useful indicator of black tea quality, which is often correlated with the original catechin content in green tea (Akuli et al., 2016). Thearubigins are structurally complex heterogeneous group of high molecular weight polyphenols. They are formed from further oxidation and polymerization of catechins and theaflavins during fermentation. Their exact composition varies depending on tea variety and processing conditions

(Teshome, 2019). It is documented that thearubigins form through reactions involving epitheaflavic acid which is a product of gallic acid oxidation that interacts with theaflavins to produce a spectrum of polymeric compounds (Abudureheman et al., 2022). Thearubigins further contributes to the reddish-brown color and complex flavor profile of the tea liquor (Aaqil et al., 2023). Due to thearubigins structural complexity and diversity, they remain a challenging area to study in tea chemistry (Long et al., 2023).

2.2.5 Anthocyanins

Naturally occurring, anthocyanins are water-soluble phenolic compounds that serve as pigments and colorants. They are found throughout various tissues of higher plants, including flowers, fruits, seeds, stems, and leaves, as well as in their derivatives like juices, tea, and red wines (Riaz et al., 2016). Tea cultivars of *Camellia sinensis* with a purple hue have been found to be high in anthocyanins and are considered promising candidates for specialty tea production (Patel et al., 2022). These colorants are responsible of the red, violet, orange and blue, pigmentation in plants (Shen et al., 2018). These classes of flavonoid are produced via the phenylpropanoid pathway (Figure 2.5) and they play a key role in absorbing light, attract insects to flowers for pollination and protect plant cells from UV radiation damage ((Gao et al., 2023).

The structure of anthocyanin is mainly composed of C6-C3-C6 as the basic C skeleton (Figure 2.4). Of the reported 500 different anthocyanins compounds isolated from plants,(Fernandes et al., 2014), six are the most predominant anthocyanins in tea cultivars (Table 2.1). They include cyanidin, delphinidin, pelargonidin, peonidin, petunidin, and malvidin (Qi et al., 2023). Cyanidin, delphinidin and pelargonidin which are non-methylated anthocyanidins are the most common in nature, being found in 80% of pigmented leaves,50% in flowers, and 69% in fruits (Khoo et al., 2017).

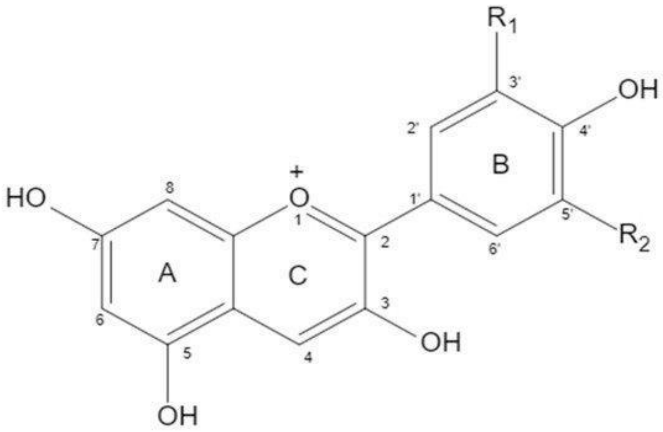


Figure 2.4: The basic structure of anthocyanins (Qi et al., 2023)

Table 2.1
The six most frequently occurring anthocyanins found in nature (Qi et al., 2023)

Anthocyanin	R_1	R_2
Cyanidin	OH	H
Delphinidin	OH	OH
Pelargonidin	H	H
Peonidin	OCH3	H
Petunidin	OCH3	OH
Malvidin	OCH3	OCH3

Tea products rich in anthocyanin have increased attention worldwide due to their economic growth in production and value addition products (Yang et al., 2023). These bioactive compounds are highly soluble in water and gives an attractive and distinctive purple colour liquor to tea infusions(Yan et al., 2024). The anthocyanin biosynthesis process commences with the conversion of phenylalanine into cinnamic acid through the action of phenylalanine ammonia-lyase (PAL) in phenylpropanoid pathway. Cinnamic acid undergoes its first transformation to coumaric acid via the enzyme action cinnamate-4-hydroxylase (C4H) and then further transformed into 4-coumaroyl CoA by 4-coumaroyl CoA ligase (4CL). The enzyme Chalcone synthase (CHS) then condenses 4-coumaroyl CoA with malonyl CoA to produce naringenin chalcone, later, chalcone isomerase (CHI) converts it into naringenin. Dihydroflavonols, such as

dihydroquercetin and dihydrokaempferol, are formed by flavonoid 3'-hydroxylase (F30H) and flavanone 3-hydroxylase (F3H) respectively. In the final phase of this biosynthesis pathway involve the formation of leucocyanidins, cyanidins, and anthocyanins, mediated by dihydroflavonol reductase (DFR), anthocyanidin synthase (ANS), and UDP-glucose: flavonoid-3-O-glycosyltransferase (UFGT). In the cytosol, anthocyanins are synthesized, after which they are transported and stored in the vacuole through specific transporters (Wang et al., 2017).

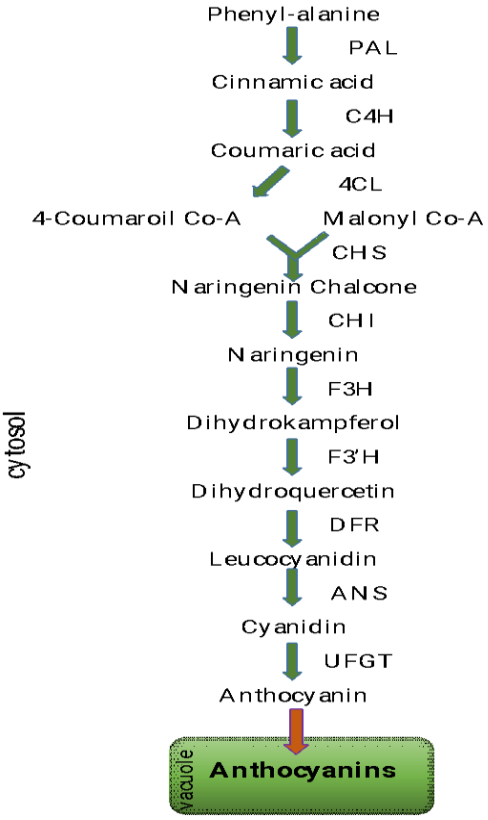


Figure 2.5: A schematic overview for anthocyanin biosynthesis pathway (He et al., 2017)

PAL: Phenylalanine ammonialyase, **C4H**: Cinnamate-4-hydroxylase, **4CL**: 4-Coumaroyl CoA ligase, **CHS**: Chalcone synthase, **CHI**: Chalcone isomerase, **F3H**: Flavanone 3-hydroxylase **F3'H**: Flavonoid 3'-hydroxylase, **DFR**: Dihydroflavonol reductase, **ANS**: Anthocyanidin synthase **UFGT**: UDP-glucose: flavonoid-3-O-glycosyltransferase (He et al., 2017).

Anthocyanins are glucosides of the anthocyanidins that are bonded to a sugar moiety such as glucose, galactose, and rhamnose. An aromatic A ring is joined to an oxygen-containing heterocyclic C ring, which is further connected to a third aromatic ring, the B ring, through a carbon-to-carbon bond (B. Li et al., 2021). The main differences between anthocyanins are the nature and the number of bonded sugars to their structure, number of hydroxyl groups, the aromatic or aliphatic carboxylates bonded to the sugar in the molecule and position of these bonds (Wallace and Giusti, 2015).

Anthocyanins have unsaturated double bonds and easily oxidizable groups, which make them highly unstable. Their degradation is primarily influenced by external factors such as pH, temperature, light, and oxygen, while secondary factors include enzymes, metal ions, and ascorbic acid (Oancea, 2021). In acidic conditions, anthocyanins appear red, but in a basic environment, they turn blue. However, they become unstable in alkaline conditions and are prone to breaking down into dark brown oxidized substances (Khoo et al., 2017).

Anthocyanins have shown potential health benefits in numerous in vitro and in vivo studies. These bioactive compounds possess antimicrobial and anti-parasitic properties, as they can inhibit the replication and growth of certain Gram-negative and Gram-positive bacteria, as well as parasites (Sun et al., 2018; Ma et al., 2019).These molecules have also act as natural antioxidant by scavenging free radicals that causes oxidation of cellular components such as proteins, nucleic acid, and lipid peroxidation (Bendokas et al., 2020) and thus reduce the risk of long-term diseases, including neurodegenerative disorders like Alzheimer's disease (AD) and Parkinson's disease (PD), cardiovascular disorders (Martín et al., 2017) and cancer diseases (Masci et al.,

2016; Forbes-Hernández et al., 2017). The notable anthocyanins antioxidant properties are primarily attributed to their conjugated rings and hydroxyl groups (Gonçalves et al., 2018). Additionally, anthocyanins help regulate (Lai et al., 2016; Noordin et al., 2019) and protect the eyes by producing retinal pigments and enhancing blood circulation in the retina's capillaries. This leads to improved night vision and provides protection against oxidative damage and diabetic retinopathy (Khoo et al., 2017).

2.2.6 Processing and Different Types of Tea

The processing process during tea production play a key role in determining the quantitative and qualitative composition of bioactive compounds, especially polyphenols in the final product (Sun et al., 2022).The major steps used in tea processing include withering, steaming or pan frying, rolling, oxidation/fermentation and drying that lead to tea chemical profiling which impacts the color, flavor and health benefits of tea (Kumar et al., 2018).

Based on the extent of fermentation, teas are categorized into unfermented (green, white, yellow, purple), semi-fermented (oolong tea), fully fermented (black tea) and post-fermented tea (Pu-erh) tea. In unfermented teas, the leaves are quickly pan-fried or steamed after plucking to inactivate polyphenol oxidase enzyme that is responsible for oxidation/fermentation. This process preserves catechins in green tea, such as EGCG and anthocyanidins in purple tea, resulting in high levels of these unoxidized flavonoids. As a result, unfermented teas retain strong antimicrobial and antioxidant properties (Salman et al., 2022). Semi-fermented teas on the other hand, are partially fermented tea. They undergo moderate fermentation and this causes partial transformation of catechins into theaflavin (TFs) and thearubigins (TRs) imparting a balance of green and black tea characteristics. These teas contain both catechins and

oxidized polyphenols, providing a broad spectrum of biological activities and complex flavor (Ng et al., 2018).

In fully fermented tea, the leaves are fully oxidized, allowing oxidation reaction of catechins in to theaflavins. This gives tea its golden-orange color and brisk flavor while thearubigins contribute to dark brown color. While catechins concentrations are reduced, black tea maintains bioactivity of antioxidant and antimicrobial effects due to presences of these oxidation products (Nibir et al., 2017). Thus, the fermentation level, tea variety and method of processing determine the final phytochemical profile of tea and also modulate its sensory attributes such as color, aroma and taste (Wong et al., 2022).

Plate 2.1: Green tea Timbilil field at Tea Research Institute, Kericho (Tea Research Institute- Kericho, 2020).

Plate 2.2: Purple tea Timbilil field at Tea Research Institute, Kericho (Tea Research Institute-Kericho,2020).

2.2.6.1 White Tea

A rear specialty tea in the market which acquires its name from a certain cultivar, as well as specific post-harvest preparation which gives silvery hairs that look like an upright feather after the bud's dries. White tea leaves are harvested and collected while they are still very young, before they fully open. This is usually done when the buds are still coated with fine silvery white hairs (Sanlier et al., 2018). White tea processing consists of partial steaming and air-drying or sun-drying under a shade for a period of two days (Tan et al., 2017). This tea variety has a greater concentration of buds, which are coated in delicate silvery hairs, giving the resulting brew a distinctive color that is either yellow or pale green. The unique handling of white tea preserves majority of the catechins in white tea. In addition, the tea type is more sensitive in aroma and taste (Ning et al., 2016).

Plate 2.3: White tea prepared at Tea Research Institute, Kericho (Tea Research Institute-Kericho, 2020).

2.2.6.2 Green Orthodox Tea

The process of making green orthodox tea involves quickly pan-firing or steaming freshly harvested tea leaves, making them soft and pliable while preventing oxidation (Kumar et al., 2018). After being steamed, the leaves are rolled, arranged, and subsequently dried by pan-frying using hot air until they become crisp. It remains the

most well-known type of tea from China. The tea liquor is an ordinarily green color, its flavor ranges from grassy (pan fried tea), toasty notes to fresh steamed greens, complemented by a gentle, vegetable-like astringency (Ahmed & Stepp, 2013).

Plate 2.4: Green orthodox tea at Tea Research Institute, Kericho (Tea Research Institute-Kericho, 2020).

2.2.6.3 Oolong Tea

Oolong tea a captivating and unique variety to produce requires a time-intensive process (Kosińska and Andlauer, 2014). The tea type uses all five basic steps. Rolling and oxidizing steps is done more than once. This tea ranges between 8 to 80 percent oxidized (estimated roughly by looking on the leaf the amount of red or brown colored during processing). The withered leaves are delicately rolled and left to rest for oxidation over a set period. Afterwards, they were rolled, and then allowed to rest and oxidized over and over. During this type of tea processing, the made tea produces a "panting" or beautiful layering of flavor and aroma (Sheibani et al., 2016). Oolong regularly has considerably a flavor that is complex than white or green tea, with a smooth astringency and fruity rich flavor. As a result of its smooth yet rich flavor profiles, oolong tea is perfect for people introduced first-hand in tea drinking. It is a traditional tea from China, particularly popular in the southern regions (Ng et al., 2018).

Plate 2.5: Oolong tea at Tea Research Institute-Kericho (Tea Research Institute-Kericho,2020).

2.2.6.4 Black Orthodox Tea

Black orthodox tea uses each of the five fundamental steps and undergoes full oxidation. These stages follow a linear sequence, implying that they are typically not repeated in the course of a single batch. The fresh leaves are initially wilted to lower their moisture content, and then subjected to a rolling machine (Deb and Jolvis Pou, 2016). This process breaks down the cellular structure, enabling phenolic compounds to react with polyphenol oxidases, thereby triggering the oxidation of catechins in the leaves. Commonly referred to as fermentation, this oxidation leads to the polymerization of catechins into more complex polyphenols, known as theaflavins (TFs) and thearubigins (TRs) (Imran et al., 2018), which give the tea type its quality characteristics. This is then followed by a firing process. It is a product that is made within a day and its liquor ranges between deep red to dark brown color (Ho et al., 2018). This tea type offers delicate flavor and at times a noteworthy astringency.

Plate 2.6: Black orthodox tea at Tea Research Institute-Kericho (Tea Research Institute-Kericho,2020).

2.2.6.5 Purple Orthodox Tea

Purple tea is processed similarly to green tea, with the main difference being the distinctive color, which comes from the purple-hued tea leaves of this specific cultivar. Purple tea introduces a completely new variety of tea to the global market, characterized by its unique liquor color and numerous medicinal properties (Rashid et al., 2014;Joshi et al., 2015). The purple shade of the tea leaves is due to the presence of anthocyanins, which not only enhance the color but also offer additional health advantages. This tea offers a remarkable new option for tea consumers worldwide, alongside black, green, and white teas, boasting significant medicinal properties that are beneficial for human health (Joshi et al., 2017).

Plate 2.7: Purple orthodox tea at Tea Research Institute-Kericho (Tea Research Institute-Kericho,2020)

2.2.6.6 Yellow Tea

Huangcha, or yellow tea, is a distinctive, lightly oxidized tea that is only produced in China. The color yellow in Chinese symbolize power, royalty and prosperity (Xu et al., 2018). It is valuable rear type of tea, increasable achieved fame in recent years due to its unique characteristic pleasant mellow taste and well-known medicinal advantages. These include anti-inflammation, anti-cancer and oxidation-preventing properties (Kujawska et al., 2016). Yellow tea is partially oxidized with enticing characteristics as green tea. Initial procedure of these two teas is similar, however, yellow tea processing includes an extra step called “sealed yellowing” (during which leaf is piled, then covered or wrapped, and kept damp at temperatures between 25 and 35 degrees Celsius) until it turns yellow. Yellow tea’s unique flavor is the result of the combined actions of thermochemical reactions and exogenous enzymes, which yield a mellower taste than that of other teas. This tea is less widely studied (Wei et al., 2020).

Plate 2.8: Yellow tea at Tea Research Institute-Kericho (Tea Research Institute-Kericho, 2020).

2.2.7 Antioxidant Properties of tea polyphenols

Reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, and hydroxyl radicals, are byproducts of normal metabolism and can accumulate due to environmental stress. In healthy individuals, a balance exists between ROS and endogenous antioxidant systems. However, when this balance is disrupted, oxidative stress may occur, leading to cellular damage to DNA, lipids, and proteins, and contributing to chronic diseases such as cancer, cardiovascular disease, and neurodegeneration (Kıran et al., 2023).

There is increasing scientific interest in the use of natural antioxidants, particularly plant polyphenols, which are considered safe alternatives for mitigating oxidative stress (Akbari et al., 2022). Tea polyphenols, especially catechins and theaflavins, have demonstrated potent antioxidant activities due to their hydroxyl groups structures and capability to donate electron(Dangles, 2012). The galloyl group in EGCG and ECG enhances their ability to scavenge radicals, making them effective against superoxide and hydroxyl radicals (J. He et al., 2018).

The antioxidant capacity of teas varies with processing. Green and yellow teas, which are minimally fermented, retain high catechin levels, while black teas contain more theaflavins due to enzymatic oxidation. Purple teas are rich in anthocyanins, another class of flavonoids with strong potential to scavenge radicals (Kerio et al., 2013; Rashid, et al., 2014).

Given their role in protecting against oxidative damage, antioxidants derived from tea are relevant to this study's aim of evaluating Kenyan specialty teas for its potential to promote human health and their application as natural alternatives in addressing antimicrobial resistance.

2.2.8 Antimicrobial Activity of Tea Compounds

Antimicrobial activity of tea *Camellia sinensis* has increased recognition as result of presence of bioactive compounds such as catechins, theaflavins, thearubigins, and anthocyanins. These compounds exhibit antimicrobial effect against a broad spectrum of gram-positive and gram- negative bacteria as well as yeast and fungi (Fazal and Rauf, 2015). Among the bioactive compounds, epigallocatechin gallate (EGCG), the predominant catechin in green tea, has been extensively studied for its ability to inhibit enzyme activity, damage microbial membranes, and interfere with nucleic acid synthesis (Xu et al., 2017).

Scientific data have reported inhibitory effect on pathogenic bacteria *Pseudomonas aeruginosa* and *Bacillus cereus* by green tea extracts (Parvez et al., 2019). Catechins found in green tea diminish the heat resistance of spore-forming thermophilic bacteria like *Clostridium thermoaceticum* and *Bacillus stearothermophilus*, which can cause sour spoilage in milk pasteurization and other beverages found in vending machines(Ali et al., 2017). Theaflavins polyphenols in black tea have demonstrated potent

antimicrobial effect against Methicillin resistant *Staphylococcus aureus* and multi drug resistant *Pseudomonas aeruginosa*, through disruption of bacterial outer membranes and inhibition of virulence factor expression (Reygaert, 2018).

Theaflavins polyphenols found in black tea, have also shown antifungal activity against candida albicans and aspergillus fumigatus, by imparting cell lysis through cell wall integrity and disruption of spore germination (Betts et al., 2013; Manso et al., 2021). Polyphenols in tea have demonstrated mechanisms of antimicrobial action. These compounds are able to disrupt the cytoplasmic membrane of microorganisms by interrupting the lipid bilayer, increasing membrane permeability and causing leakage of macromolecules and ions (Zhong et al., 2023).

In addition, tea polyphenols flavanols have demonstrated inhibition of essential microbial enzymes, such as ATP synthase and DNA gyrase thus impairing microbial metabolism and replication (Renzetti et al., 2020). The polyphenols phenolic acids, gallic acid, also have shown metal-chelating agent against iron and zinc ions (Fe^{2+}, Zn^{2+}), which deprives bacteria and fungi of essential cofactors required for growth, enzyme activation and virulence, (Kola et al., 2021) resulting to loss of proton and cell lysis. Furthermore, catechins particularly ECG and EGCG have demonstrated to suppress bacterial quorum sensing system inhibiting communication among bacterial populations such as motility, toxic production and biofilm formation (Qais et al., 2019;Hao et al., 2021). This effectiveness of anti-quorum sensing, offers a promising approach to mitigate bacterial persistence and antibiotic resistance.

2.2.9 Infectious Diseases Burden

Infectious diseases have continued to impose a heavy burden on global public health, especially in low and middle income countries. According to the World Health

Organization, infectious diseases accounted for over 15% of global deaths annually, with lower respiratory infections, diarrheal diseases, tuberculosis, HIV/AIDS, and malaria ranked among the top (WHO, 2018). In sub-Saharan Africa, these diseases have caused mortality, accounting 65% of deaths particularly due to high prevalence of preventable and treatable diseases such as respiratory infections (Global Burden of Disease Study, 2018; World Health Organization, 2018a). This has affected children under five and immunosuppressed patients (WHO, 2018) being the most vulnerable groups.

This burden of emerging and re-emerging pathogens, that included fungal organisms such as *Cryptococcus neoformans*, *Candida albicans*, and dermatophytes like *Trichophyton* species (Bongomin et al., 2017; WHO, 2020). These opportunistic infections have gained prominence in immunocompromised patients, especially the patients under HIV/AIDS, chemotherapy and organ transplants (Bongomin et al., 2017). In addition, multidrug resistant bacterial pathogens including extended spectrum beta- lactamase producing Escherichia coli (Iliyasu et al., 2018), methicillin resistant Staphylococcus aureus (Garoy et al., 2019) and multidrug resistant Pseudomonas aeruginosa (Ribeiro et al., 2019). They have caused frequent infectious diseases such as skin infections, urinary tract infections, and gastrointestinal tract diseases, resulting to high health crisis burden global especially in hospitals and other healthcare facilities where inadequate infection control promote their spread.

In Kenya, communicable diseases have remained among the top ten causes of outpatient visits and hospital admissions posing a persistent public health challenge. According to the Ministry of Health (MOH., 2018), disease such as upper respiratory tract infections (pneumonia), diarrheal illnesses, and skin infections consistently was ranked in the top ten causes of morbidity across health facilities, particularly in peri-urban and rural

settings. These consistencies are closely linked to factors such as limited access to clean water, poor sanitation, overreliance of antibiotic treatment, and inadequate diagnostic infrastructure capacity. Such conditions increased disease transmission and also the development of antimicrobial resistance (AMR). In the same time, the MOH reported increasing resistance of pathogens to commonly prescribed antibiotics, like beta-lactams and tetracyclines (MOH., 2018). This resistance of pathogens to first line antibiotics has caused prolonged hospital stay, increased healthcare costs and rising death rates among vulnerable patients such as immunocompromised patients and children under five years (MOH, 2018; WHO, 2018).

Given these systemic challenges, there is an urgent need to identify affordable, easily accessible and safe antimicrobial alternatives that can complement conventional treatments especially in low resource settings. Tea *Camellia sinensis*, widely consumed across Kenya and highly known for its rich bioactive compounds such as polyphenols, presents a promising natural antimicrobial compound. The investigation of Kenya's specialty teas as complementary therapeutic agents. This aligns with global health strategies promoting the integration of locally available natural products into infection prevention

2.2.10 Pathogens Bacteria and Fungi

The study focused on selected bacterial and fungal pathogens that are common clinical infections in humans, exhibited antibiotics resistance patterns, and prevalence in both hospital facilities and community areas. These included methicillin and penicillinase resistant gram-positive bacteria *Staphylococcus aureus*, gram-negative bacteria *Escherichia coli*, fungal *Candida albicans* and clinical isolate fungal *Trichophyton mentagrophytes*.

The primary difference between gram-positive and gram-negative bacteria lies in their cell wall structures (Mai-Prochnow et al., 2016). Gram-negative bacteria have a cell wall with an outer membrane and a thin layer of peptidoglycan. In the outer membrane, the lipid-protein bilayer includes lipopolysaccharides, phospholipids, and lipoproteins. The complex molecules known as lipopolysaccharides include Lipid A, an O-polysaccharide and a core polysaccharide. Lipid A operates as an endotoxin, released specifically when gram-negative bacteria die, while the O-polysaccharide serves as an antigen for distinguishing various gram-negative bacterial species (Caroff and Novikov, 2019). Unlike gram-negative bacteria, gram-positive bacteria have a cell wall composed entirely of a thick peptidoglycan layer. Additionally, teichoic acid, that is unique to gram-positive bacteria, plays a role in regulating cation movement across the cell and maintaining cell wall rigidity. As a result, the additional barrier in gram-negative bacteria provides greater resistance to antimicrobial agents compared to the more permeable cell wall of gram-positive bacteria (Miller, 2016).

2.2.10.1 Staphylococcus aureus

Staphylococcus aureus a gram-positive bacterium, often produces golden-yellow colonies when grown on agar plates. This species is non-spore forming, non-motile, and a facultative anaerobe, meaning it can generate ATP through both aerobic and anaerobic respiration. It is commonly found as a normal flora of human conditions, such as the nose (specifically the anterior nares and nasopharynx) and skin, due to its ability to thrive across a broad temperature range (70°C to 48.5°C) (Kadariya et al., 2014). S. *aureus* is a pathogen responsible for a wide range of infections, from skin folliculitis and wound infections to server illnesses like bacteremia, pneumonia, and osteomyelitis (Ghalehnoo, 2018). Transmitted via respiratory secretions or person to person by fomites, air or unwashed hands of health care workers, especially in

nosocomial settings. Furthermore, chronic wounds are often connected to the presence of *S. aureus* biofilm, according to research findings (Roy et al., 2020). The biofilm formation influences gene regulation in keratinocytes, thereby producing cytokines that play a role in mediating the host's immune response, which in turn contributes to the infection of the healing wound. Furthermore, S. *aureus* is responsible for foodborne illnesses, leading to around 240,000 cases annually (Kadariya et al., 2014). This type of food poisoning occurs when foods containing enterotoxins released by dead *S. aureus* are consumed. The key indicators of staphylococcal food poisoning are severe vomiting, intense nausea, and abdominal cramps.

2.2.10.2 Escherichia coli

E. coli, a Gram-negative bacterium, is a member of the Enterobacteriaceae family. This bacillus-shaped microorganism that form no spores and is a facultative anaerobic microbe. While most strains of E. coli are harmless, they commonly reside in the intestinal flora of humans and other animals, and can also be found in the female genital tract. However, some strains are pathogenic and cause diseases such as diarrheal diseases, urinary tract infections and bacteremia which is bloodstream infection (Smith et al., 2014). Some strains have acquired resistance through extended spectrum beta-lactamase genes, thus result to treatment complications.

2.2.10.3 Pseudomonas aeruginosa

A typical gram-negative opportunistic bacterium, *P. aeruginosa* is encapsulated, aerobic, and rod-shaped. Capable of causing severe disease in humans with weakened natural defenses such as acute lung injury, as well as acute respiratory distress syndrome. This bacterium is one of the main pathogens responsible for hospital-acquired infections leading to life threatening due to its high resistance to multi drug antibiotics (Pachori et al., 2019). Infections by this bacterium is triggered by several

virulence factors like production of exotoxin, secretion of enzymes such as protease, possession of lipopolysaccharide, flagella, pyocyanin and pili on their cell wall. Pyocyanin present in this pathogen catalyzes the production of hydrogen peroxide and superoxide which causes tissue damage leading to inflammation.

P. aeruginosa's pathogenesis enables adhesion, influences or disrupts host cellular pathways, and focuses on the extracellular matrix. These microbe forms biofilm that protects it from antibiotics and the host immune system. Biofilms also enhances its ability to adhere and survive in environmental surfaces like soil, medical equipment and airways inpatients with prolonged respiratory infections (Moradali et al., 2017).

2.2.10.4 Trichophyton mentagrophytes

T. mentagrophytes is a common dermatophyte, belongs to family Arthrodermataceae, commonly form white to cream color colonies with a powdery to the granular surface. Normally inhabits soil , moist environment (pools, locker rooms, bathrooms), humans (anthropophillic) and animals (zoophilic) (Greywal and Friedlander, 2018).Transmission occurs through contact with fungi spores, direct contact with an infected person or animal, or by direct contact with infested particles of hair, nails and skin of the host on fomites (Vanathi, 2015).

The pathogen has the ability to secrete a variety of proteases that enable it to invade keratinous substrates. Enabling the pathogen to penetrate the host tissue, to obtain nutrients and survive. It has been suggested that dermatophytes secrete proteases in response to components present on the skin during tissue invasion. It has been identified five different keratinolytic enzymes which play a role in the pathogenesis of infections in both humans and animals (Chinnapun, 2015). Furthermore, the fungal causes spectrum of dermatophytosis such as tinea pedis (athlete's foot), tinea corporis (arms

and legs infection), tinea capitis (hair and scalp infection) and tinea unguium (fungal infection of the nail).

2.2.10.5 Candida albicans

Candida albicans is a diploid fungus capable of growing in both yeast and filamentous forms. Its colonies typically appear cream-colored, glistening, and occasionally waxy. This fungus is a normal part of the human microflora, residing on the skin, mucous membranes, and within the reproductive and digestive tracts of both humans and animals (Conti and Gaffen, 2015). While generally harmless as a commensal organism, *Candida albicans* can become an opportunistic pathogen in immunocompromised or immunologically deficient individuals under optimal environmental conditions. It causes superficial infections such as oral thrush and vaginal candidiasis and can lead to life threatening systemic infections like candidemia especially in intensive care unit patients(Mohammed et al., 2017).Its resistant to azole antifungals including fluconazole that has been increasingly reported (Berkow and Lockhart, 2017).

2.3 Conceptual Framework

The conceptual framework for this study was based on the hypothesis that the polyphenolic composition of Kenyan specialty teas using different polarity solvents for extraction and tea type based on different fermentation levels determines their antimicrobial efficacy against selected bacterial and fungal pathogens. Tea types, which includes black, oolong, green, yellow, and purple, orthodox varieties, served as an independent variable that influenced the biochemical profile of the leaves, particularly in terms of polyphenol and catechin content. The solvent used for extraction, including water (polar), ethyl acetate (semi-polar), methylene chloride (non-polar), and the aqueous phase fraction, that represented another independent variable affecting the

efficiency of phytochemical recovery. These two factors together influence the polyphenolic content specifically total polyphenols, catechins (EGCG, ECG, EGC, EC, +C), which served as a mediating variable that showed the extract's biological activity. The dependent variables include antioxidant activity, evaluated via the DPPH radical scavenging assay. Antimicrobial activity, assessed through the zone of inhibition in agar disc diffusion assays against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Trichophyton mentagrophytes*. Together, these variables illustrated how biochemical richness driven by tea type and extraction method translates into functional bioactivity relevant to natural antimicrobial.

2.4 Identification of the Knowledge Gap

Antibiotics medications have been used globally for several decades. However, the ongoing use of these drugs to treat infections has led to the development of resistance among infectious agents that has caused critical threat to public health and global disease control efforts (Hoffman et al., 2015). Antimicrobial resistance (AMR), defined as the ability of microorganism agents such as bacteria and fungi to withstand the effects of antimicrobial medication that once inhibited them, has progressively compromised treatment of infectious diseases (Salam et al., 2023). AMR is present and rising both in first line antibiotics as well as second line treatment (Florescu et al., 2017). The World Health Organization (WHO, 2018) ranked AMR among the top ten threats to global health, which estimated that drug-resistant infections for high death rates. Similarly, the Kenyan Ministry of Health (MOH, 2018) reported increased incidences of antimicrobial resistance, especially in tertiary referral hospitals, that resulted to lengthy hospital stays to patients, increased healthcare costs and treatment failures. The main factors that contribute to AMR includes inadequate sterilization,

improper prescription of antibiotics drugs, insufficient infection prevention, self-medication, poor food handling, counterfeit medications and lack of controlled healthcare environment (Mbogori, 2016).

Research from WHO reports have indicated elevated resistance threshold against antimicrobial drugs. Clinically significant bacterial pathogens such as *Staphylococcus aureus, Escherichia coli*, and *Klebsiella pneumoniae* have developed multi-drug resistance (Pirvanescu et al., 2014).In Kenya, isolates of *S. aureus* from hospital-acquired and community demonstrated methicillin resistance. This has been difficult to treat using conventional beta-lactams, such as penicillins and cephalosporins. In *E. coli*, the presence of extended-spectrum beta-lactamases has reported beta-lactam antibiotics such as penicillins and cephalosporins ineffective, thus narrowing therapeutic options (Organization, 2018a; Tornberg-Belanger et al., 2022). Fungal pathogens have also been reported to be resistant to antifungal drugs. *Candida albicans,* a common cause of opportunistic fungal infections, has demonstrated increasing resistance to azole antifungals, particularly fluconazole. (Keniya et al., 2018). In Kenya, the Antimicrobial Resistance Surveillance Report (MOH, 2018) documented a rise prevalence of multi-drug resistance strains.

The growing ineffectiveness of conventional antimicrobials has triggered an urgent search for alternative antimicrobial agents. Natural plant-based compounds particularly polyphenol extracts from medicinal and dietary plant like *Camellia sinensis*, have attracted attention due to their broad spectrum of antimicrobial activity. Tea plant *Camellia sinensis* polyphenols such as catechins, anthocyanins, theaflavins and flavonols, act through multiple mechanisms which includes biofilm inhibition, membrane disruption, metal ion chelation, and interference with microbial quorum sensing that are strategies that reduce the risk of resistance prevalence.

Given the high-rise crisis of antimicrobial resistant pathogenic microbes has led to critical search for safe, cost effective, available, and sustainable antimicrobial agents. Natural products, particularly plant-based polyphenols have gained attention as viable alternatives. Tea plant *Camellia Sinensis* is a promising plant derived antimicrobial agent due to its broad antimicrobial spectrum, and high availability. In Kenya, tea is a major agricultural product and among the leading tea exporter worldwide. Exploiting specialty teas for their antimicrobial potential offers both public health benefits and enhances the economic value of tea industry. Despite the recognized benefits of black and green CTC teas, limited research has been conducted on antimicrobial properties of Kenya's specialty teas Such as oolong, yellow, purple, green orthodox and black orthodox teas. This study seeks to bridge that knowledge gap.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

This chapter includes the specific site of the study area, the sample collection and processing and the test microorganisms. It also describes the preparation of the test samples and the laboratory analysis methods used in the study including the data analysis and presentation methods.

3.2 Research Design

The Figure 3.1 presents the study design used in this research. The schematic summarized the sequential procedures undertaken, starting with selection and collection of specialty tea samples from the Tea Research Institute-Kericho. It further outlined the preparation and freeze drying of tea samples, extraction and quantification of polyphenols, antioxidant and antimicrobial assays, data analysis, and interpretation of results.

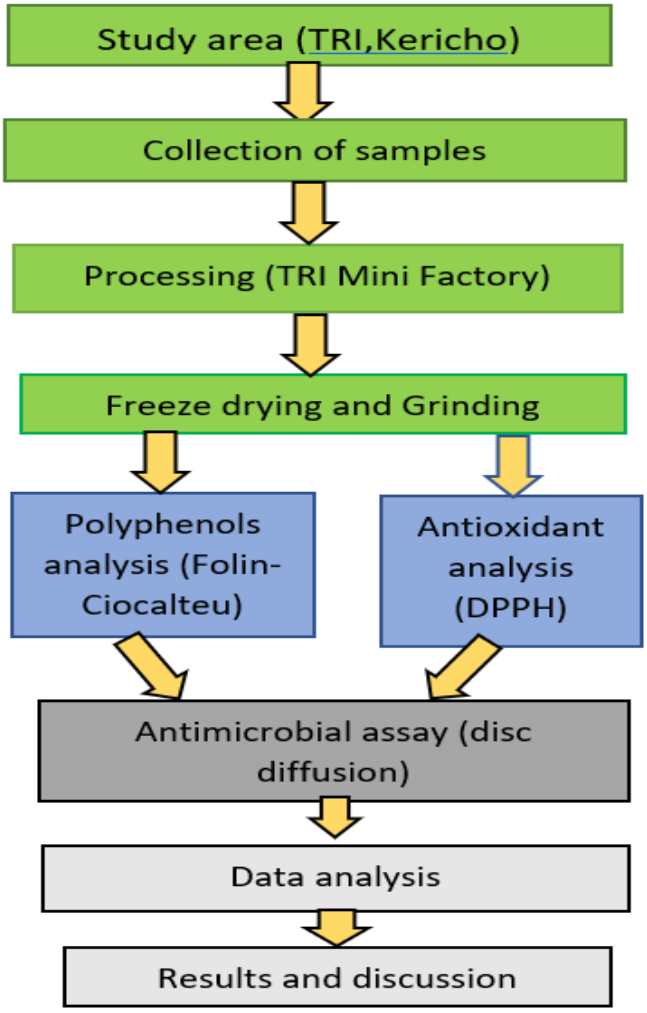


Figure 3.1: Experimental research design used in this study.

3.3 Location of Study

Tea samples were collected from the Tea Research Institute (TRI), Kericho. Tea Research Institute (TRI)-KALRO, Kericho, is located in the western highlands of Kenya at a latitude of 0° 22' S, longitude 35° 21' E, and an altitude of 2180 meters above sea level. Fresh tea leaves were hand plucked at Timbilil Estate experimental fields using a randomized complete block design (RCBD). These included five tea cultivars: TRFK 895/20, TRFK 914/30, TRFK 791/2, TRFK 791/1, and a second-generation purple leaf tea cultivar (Experiment number 2019/63). Each cultivar was replicated three times. The freshly plucked tea leaves were processed into black orthodox, oolong

tea, green orthodox, yellow orthodox, and purple orthodox using a miniature factory setup at TRI.

The microorganisms used in the tests included ATCC bacteria and fungi isolates from KEMRI Centre in Nairobi. Microorganisms consisted of methicillin and penicillin-resistant *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Candida albicans* ATCC 90028, and *Trichophyton mentagrophytes* respectively.

3.4 Chemicals and Reagent

Chemicals: Acetic acid, Acetonitrile, Chloramphenicol, DMSO (Dimethyl Sulfoxide), DPPH ((2, 2-diphenyl-1-picrylhydrazyl)), EDTA (Ethylene diamine tetra acetic acid), Ethyl acetate ,Folin-Ciocalteu's phenol, Methylene chloride, Sodium carbonate, **Standards:** Ascorbic acid, Caffeine, Epigallocatechin gallate (EGCG), Catechin (+C), Epicatechin (EC),Gallic acid (GA), Epicatechin gallate (ECG), Epigallocatechin (EGC) ,**Agar Media**: Muller Hinton Agar, Potato Dextrose Agar, **Suppliers:** Finar India Ltd through Kobian, Kenya suppliers and Sigma Chemical Co., US through Kobian, Kenya Limited.

3.5 Sample and Sampling Procedures

3.5.1 Preparation of Tea Samples

Sample collection and preparation at Tea Research Institute -Kericho Timbili field experiment.

Fresh two leaves and a bud of green and purple colored tea leaves were collected from the tea field, placed in brown carrier bag and stored in cooler boxes to maintain the fresh green leaves state and transported to TRI miniature tea factory. After which the

leaves were spread out in thin layer on a mesh tray and exposed to warm air for withering or until the moisture content had dropped to about 55% -60%.

For oolong tea, fresh tea leaves were sun withered for 30min before being withered for 1hr indoors. After which the leaves were tossed using a bamboo basket for 3-5 minutes, then indoor wither for 2hr, tossed again for 3min, indoor wither for 1hr and finally tossed for 6min before fixing with a pan fryer to obtain a moisture content of 60%. After that, they were rolled with a roller machine and dried for 1hr at 110^0C using a fluid bed drier and finally milled using a coffee miller (Model-Moulinex, Type AR11) and stored in a sealed aluminum bags awaiting analysis.

Green orthodox and purple orthodox were processed using fresh green and purple leaves. The leaves were indoor withered for 4hr, steamed for 1min, indoor withered again to achieve a moisture content of 60%, rolled with a roller machine, after which they were dried at 70^0C using a fluid bed drier. A coffee grinder was used to grind the tea, which was then stored in airtight aluminum bags until it was needed for analysis.

Yellow orthodox tea preparation, green leaves were withered for 1hr indoors, steamed for 6min, followed by indoor withering again to achieve a moisture content of 60%, rolled with a roller machine, and sealed yellowed for 12-18hr, during which the leaves were piled, wrapped in a muslin cloth, and kept damp at 26^0C until it turned yellow. They were then processed with a roller machine and dried at a low temperature of 70°C with a fluid bed dryer and milled with a coffee miller before being stored in sealed aluminum bags awaiting analysis.

Black orthodox tea was processed using fresh green tea leaves that were withered for 16hrs indoors, rolled and oxidized for 3hrs.The next phase was drying using a fluid bed

drier set at 120^0C. To prepare for examination, the tea was ground with a coffee mill and stored in sealed aluminum bags until analysis.

3.6 Laboratory Analysis

3.6.1 Extraction

Solvent extraction and partitioning were used to recover various components from the test samples following a multistep procedure based on Mariem (Mariem et al., 2014). Initially, 50 grams of milled tea leaf samples were measured with a Shimadzu BL-3200 HL analytical balance (manufactured in Japan) and transferred into 1-liter thermos flasks. Five hundred milliliters of hot distilled water were added to the flasks, and the mixture was shaken using a scientific mechanical shaker for one hour.

The mixture was then filtered through a sterile cotton wool plug and Whatman No. 2 filter paper to remove coarse particles, and allowed to cool to room temperature. The residues were re-extracted by repeating the procedure, resulting in up to two extracts. The filtrate was transferred to a round-bottom flask for freeze-drying and then placed in a deep freezer. Later, the round bottom flask was loaded to the freeze-drying machine at pressures below 30 Torr and temperatures below -50ºC (MRC Freeze drier; Model–FD5-8B). Lyophilization took two days, then the total water extract was weighed and calculated to get the extraction yield percentage and the total water extract was kept in airtight containers at -20^0C for further analysis.

3.6.2 Fractionation

The filtrate was fractionated with solvents of progressively increasing polarity, starting from non-polar to polar, as illustrated in Figure 3.1. An equal volume of dichloromethane was added to the filtrate, which was then transferred to a separating funnel (1:1 v/v). The mixture was vigorously shaken and allowed to stand until the

organic and aqueous phases separated. The organic phase, which was at the bottom (dichloromethane), was then transferred to a rotary evaporator for evaporation. Meanwhile, the aqueous phase was reserved for further fractionation steps. The separation process was repeated on the aqueous phase until the organic phase became completely clear. Solvent selection was based on polarity differences to optimize the extraction of diverse bioactive compounds from the tea samples. Water, methylene chloride and ethyl acetate were chosen to provide a wide polarity range and capture both hydrophilic and lipophilic constituents.

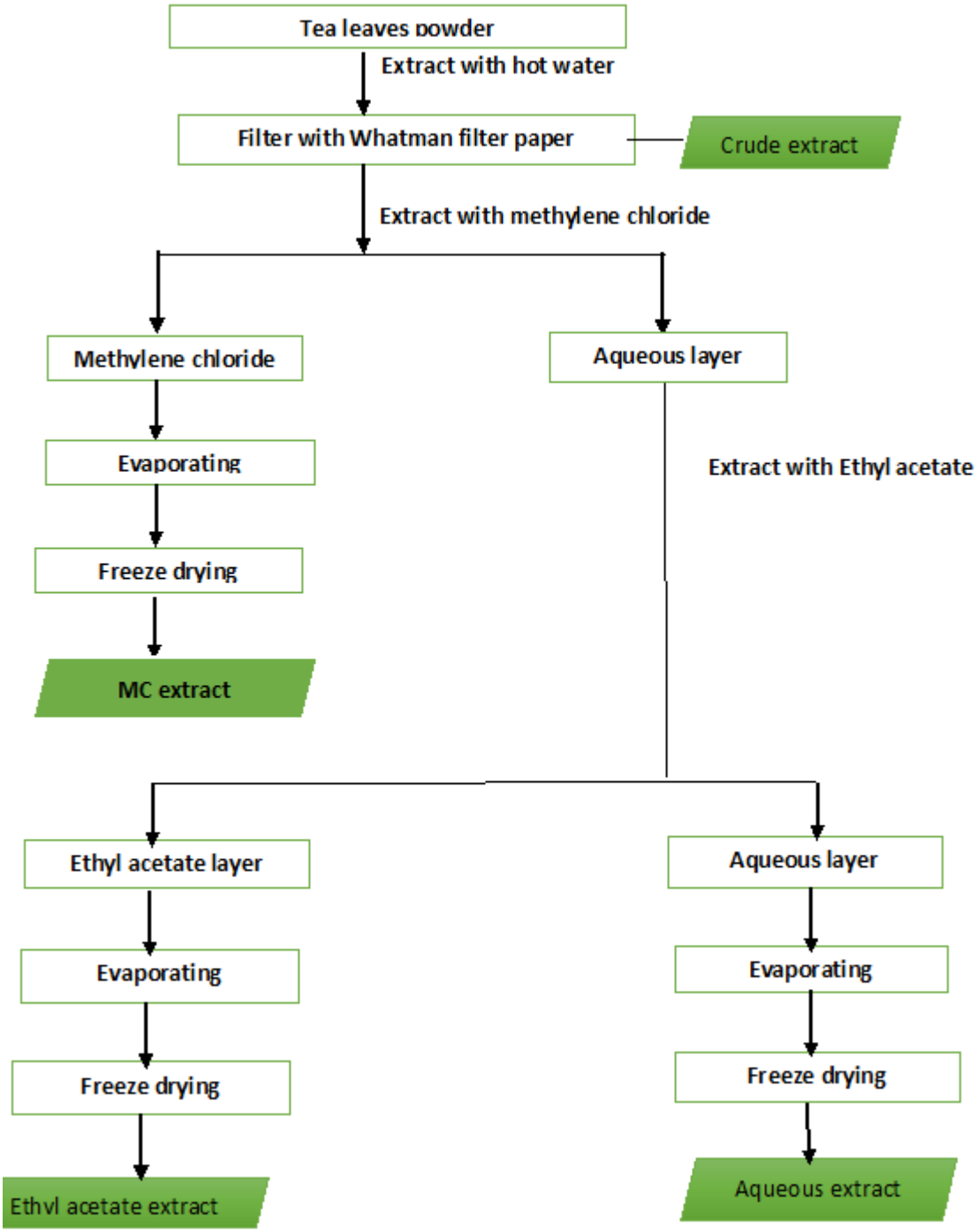


Figure 3.2: Diagrammatic representation of the extraction and fractionation process.

To absorb any leftover water in the organic phase, anhydrous sodium sulfate was applied at the end of each step. The separated organic phase (dichloromethane) was then evaporated, freeze-dried, and stored at 4°C for future analysis. The fractionation process proceeded by mixing the aqueous phase with organic solvents of progressively

higher polarity. The other applied organic solvent was ethyl acetate. Fractionation phase was evaporated, freeze dried, weighed and calculated to get the extraction yield percentage and extract was kept at 4^{o}C until analysis (Ayoola et al., 2008). The residual aqueous phase was lyophilized and reserved for further analysis. Figure 3.1 provides a summary of the extraction and fractionation process.

$$\text{Percentage yield extraction (\%)} = \frac{\text{dry weight of extract (g)}}{\text{original weight of plant used (g)}} \times 100$$

3.6.3 Polyphenol Analysis on Tea Samples

A sample weighing two grams was transferred to a pre-weighed moisture dish, which had been calibrated, and then placed in an oven at 103°C for five hours to ensure complete drying for the determination of dry matter the extraction and fractionation procedures (ISO-7513). The process was required since the biomolecules of interest was expressed quantitatively in percentage dry weight. Extract sample, 0.1g was weighed in to a 10ml one- mark volumetric flask. Carefully 5ml of hot water was transferred in to the sample, the mixture was vortexed and allowed to cool to room temperature. One milliliter of acetonitrile was added to the mixture and diluted to the mark with distilled water and vortexed.

3.6.4 Total Polyphenols Estimation in Tea Extracts

Determination of the total phenolic content in the sample was measured in accordance with ISO-14502-2:2005. From the test sample extract of 10ml, one milliliter was carefully transferred in to a one-mark 100ml volumetric flask, which was filled to the mark with distilled water and mixed. Gallic acid standard solutions were prepared by transferring 1ml, 2ml, 3ml, 4ml and 5ml from stock standard solution (1mg/ml) corresponding to 10, 20, 30, 40 and 50 ug/ml gallic acid, into 100ml volumetric flasks

and filled to the mark with distilled water. Afterwards, one milliliter of the diluted tea samples and gallic acid standards were transferred in to test tubes in duplicates. Ten percent of five milliliter of a mixture of dilute Folin-Ciocalteu and distilled water (10 % v/v) was pipetted in to each tube containing test sample and mixed, after which addition of 4 ml of a 7.5% sodium carbonate solution was added to each tube after 4-8 minutes. Then the mixture was stoppered and mixed. The test sample was stood at room temperature for an hour. Samples and standards were analyzed for optical densities in a 1 cm cell on a spectrophotometer, calibrated to a wavelength of 765 nm. A concentration range from 10 µg/ml to 60 µg/ml was used to generate the calibration curve. The polyphenol content of the test samples was quantified by comparing their optical density readings to the gallic acid calibration curve.

3.6.5 Determination of Catechins in Tea Extracts

The analysis of tea catechins was carried out using high-performance liquid chromatography, as specified in ISO-14502-2:2005, an International Organization for Standardization procedure. Following the procedure detailed in section 3.5.2, the tea extracts were prepared. Subsequently, 1 ml of the extract was introduced into a graduated tube and made up to a total volume of 5 ml, using a stabilizing solution composed of ascorbic acid, acetonitrile (10% v/v), and EDTA (500 µg/ml).

Once the solution had been filtered using a 0.45 µm membrane filter, a 20 µl aliquot was injected into the instrument for analysis. A Shimadzu LC 20 AD HPLC system equipped with a SIL 20AC auto-sampler and an SPD-M20A photodiode array detector, along with L-solution chromatography software, was utilized for sample analysis. The separation was performed by use of Gemini 5 µm C6-Phenyl column (250 mm x 4.6 mm, Phenomenex, Torrance, CA, USA) with a Phenomenex Security Guard 4 mm x 3.0 mm Phenyl cartridge. The gradient elution was achieved through the application of

the specified solvent systems: Mobile phase A (acetonitrile/acetic acid/EDTA/double-distilled water in a 9/2/0.2/88.8 v/v/v/v ratio) and mobile phase B (acetonitrile/acetic acid/EDTA/double-distilled water in an 80/2/0.2/17.8 v/v/v/v ratio). The gradient system gradient started with 100% mobile phase A for 10 minutes, followed by a linear gradient to 68% mobile phase A and 32% mobile phase B over 15 minutes, then held for 10 minutes. After resetting the condition to 100% mobile phase A, the system was equilibrated for 10 minutes prior to the next injection. The mobile phase flow rate was fixed at 1 ml/min, with the column temperature controlled at 35 ± 0.5ºC. The binary gradient began with 100% mobile phase A for 10 minutes, followed by a linear transition over 15 minutes to 68% mobile phase A and 32% mobile phase B, which was maintained for 10 minutes. Afterward, the system was returned to 100% mobile phase A and allowed to equilibrate for 10 minutes before the next injection. A flow rate of 1 ml/min was maintained for the mobile phase, with the column temperature regulated at 35 ± 0.5ºC.

To identify the individual catechins, the retention times of unknown peaks were compared with those of known standards including catechin, gallic acid, epicatechin, epicatechin gallate, epigallocatechin gallate, and epigallocatechin, provided by Sigma Aldrich, UK, all under the same chromatographic conditions. The measurement of catechin levels was achieved by use of relative response factor (RRF) values for each catechin compared to caffeine, expressed on the basis of dry weight. The catechin concentration, calculated as a percentage by mass based on dry matter, was found by adding the quantities of all individual catechins.

%Total catechins= (%EGC+%C+%EC+%EGCG+%ECG) content

This equation represents the sum of the percentages of individual catechins: epicatechin gallate (ECG), epicatechin (EC), epigallocatechin gallate (EGCG), and catechin (C).

3.6.6 Evaluation of Antioxidant Activity in Tea Extracts

The free radical scavenging activity of tea extracts was measured using the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, according to a modified technique based on the approach of Brand-Williams et al. (1995). The ability of antioxidants to neutralize stable DPPH radical is measured by this assay (Sánchez-Moreno, 2002). A 0.5-gram sample of tea extract was weighed and combined with 10 ml of boiled double-distilled water, then thoroughly mixed using a vortex mixer. Afterward, the mixture was left to cool at room temperature. Preparation of the stock solution involved adjusting the soluble solids extract to contain 50mg per 100ml of 50% methanol. A sample of 50µl from the tea solution was mixed with 2 ml of DPPH solution (6×10^{-5} M, prepared in 80% methanol) and analyzed using a cuvette. Absorbance at 517 nm was measured with a spectrophotometer until it stabilized, with intervals for readings set between 15 and 30 minutes. Measurements were conducted in triplicate. Absorbance data were utilized to compute the percentage of DPPH radical inhibition as per Yen and Duh's (1994) method.

DPPH inhibition, expressed as a percentage, is calculated as follows:

$$\% \text{ Inhibition} = [(AB - AA) / AB] \times 100$$

Where:

- AB is the absorbance of the blank sample (50 µl of double-distilled water and 2 ml of DPPH).
- AA is the absorbance of the test sample measured after 15 minutes.

3.7 Antimicrobial Assays

The antimicrobial activity of specialty tea extracts was evaluated using the agar disc diffusion method, following Clinical and Laboratory Standards Institute (CLSI, 2012) guidelines. Test microorganisms included *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans* and *Trichophyton mentagrophytes*. Each strain was standardized to 0.5 McFarland standard (1.5×10^8 CFU/mL) prior to inoculation. Sample tea extracts were dissolved in 5% Dimethyl Sulfoxide (DMSO) to prepare a stock solution of 10mg/ml. DMSO was used as the solvent for reconstituting tea extracts in antimicrobial assays. This is due to its ability to effectively dissolve both polar and non-polar bioactive compounds, such as catechins and flavonoids, which have limited solubility in water. At the working concentration (5%), DMSO is biologically inert and does not interfere with microbial growth, ensuring accurate measurement of antimicrobial activity. Two-fold serial dilutions were made to obtain concentration of 5mg/ml, 2.5mg/ml, 0.63mg/ml, 0.31mg/ml, and 0.16mg/ml. Sterile 6mm paper discs were carefully and aseptically impregnated with 10µL of each concentration in a sterile test tube that contained the different dilution concentrations, and allowed to air dry under aseptic conditions.

Muller Hinton Agar for bacteria and Potato Dextrose Agar for fungi plates were inoculated with standardized microbial suspensions using sterile cotton swabs. Tea extract impregnated discs were carefully placed onto the agar surface using sterile forceps. Chloramphenicol (60ug/disc) and Nystatin (30ug/disc) were used as positive controls for bacterial and fungal isolates respectively. DMSO 5% impregnated discs were used as negative controls. Plates inoculated with bacteria were incubated at 37^0C for 24 hours and 37^0C for48hours for *Candida albicans*. *Trichophyton mentagrophytes* was incubated at 30^0C for 96 hours. Antimicrobial activity was assessed by measuring

the diameter of the zone of inhibition (ZOI) around the disc in millimeters. A zone of inhibition measuring exactly 6mm (the diameter of the paper disc) was interpreted as no inhibition. This indicates that the microbial growth reached the edge of the disc with no observable antimicrobial effect. Each experiment was performed in triplicate and results were expressed as mean standard deviation.

3.8 Data Analysis

All statistical analyses were carried out using the SAS version 9.1 package. Polyphenol content experimental data were analyzed using analysis of variance (ANOVA) to determine statistically significant differences among treatment means at 95% confidence level ($p < 0.05$). For post hoc analysis, Duncan's Multiple Range Test (DMRT) was applied to separate group means and identify significant differences. The results were illustrated through tables. Antimicrobial activity of bacteria and fungi for each experiment was performed in triplicate and results were expressed as mean standard deviation. Post hoc analysis, Duncan's Multiple Range Test (DMRT) was applied to separate group means and identify significant differences. The results were illustrated through tables. The relationship between the levels of polyphenolic compounds and its antibacterial and antifungal activity was expressed as tables using correlation analysis.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Introduction

This chapter presents the findings of the study in relation to its specific objectives. It highlights the phytochemical composition, antioxidant activity, and antimicrobial potential of polyphenolic extracts obtained from selected Kenyan specialty teas (*Camellia sinensis*). The results are structured by objective and tea type, and compared across four extract types that includes water, methylene chloride, ethyl acetate, and the aqueous phase extract that each representing different solvent polarities. The aqueous phase extract refers to the remaining aqueous fraction collected after successive extraction with methylene chloride and ethyl acetate. This was included to assess whether any bioactive compounds remained after sequential solvent extraction of methylene chloride and ethyl acetate. The results are presented first, followed by a discussion of their significance, referencing relevant literature. Emphasis is placed on how fermentation level, solvent polarity, and biochemical profile affect extract activity against selected bacterial and fungal pathogens. This analysis supports the problem statement, which emphasizes the need for natural antimicrobial in addressing the global threat of antimicrobial resistance (AMR).

4.2 Total Polyphenolic Content and Antioxidant Activity of Extracts.

The polyphenolic profile of Kenyan specialty tea extracts varied significantly ($p \leq 0.05$) based on both the type of tea and the solvent used. Ethyl acetate extracts exhibited the highest total polyphenols content across all tea types, especially in green orthodox (91.0%), yellow orthodox (91.9%), and purple orthodox (90.4%) teas. Water extracts showed moderate levels (36.2%-38.3%), as well as aqueous phase extracts (12.2%-

18.0%), while methylene chloride extracts had the lowest polyphenol content, with black orthodox recording as low as 9.3% as shown in Table 4.1. The statistical comparison using Duncan's Multiple Range Test (DMRT) indicated that the observed differences were significant, as indicated by the superscript letters in Table 4.1. A visual summary of these results is provided in Figure 4.1, where the bars represent mean values standard deviation. These aqueous phase extracts referred to as the final aqueous portion remaining after sequential extraction with methylene chloride and ethyl acetate. This was also analyzed to assess the recovery of any remaining bioactive compounds that may not have been solubilized.

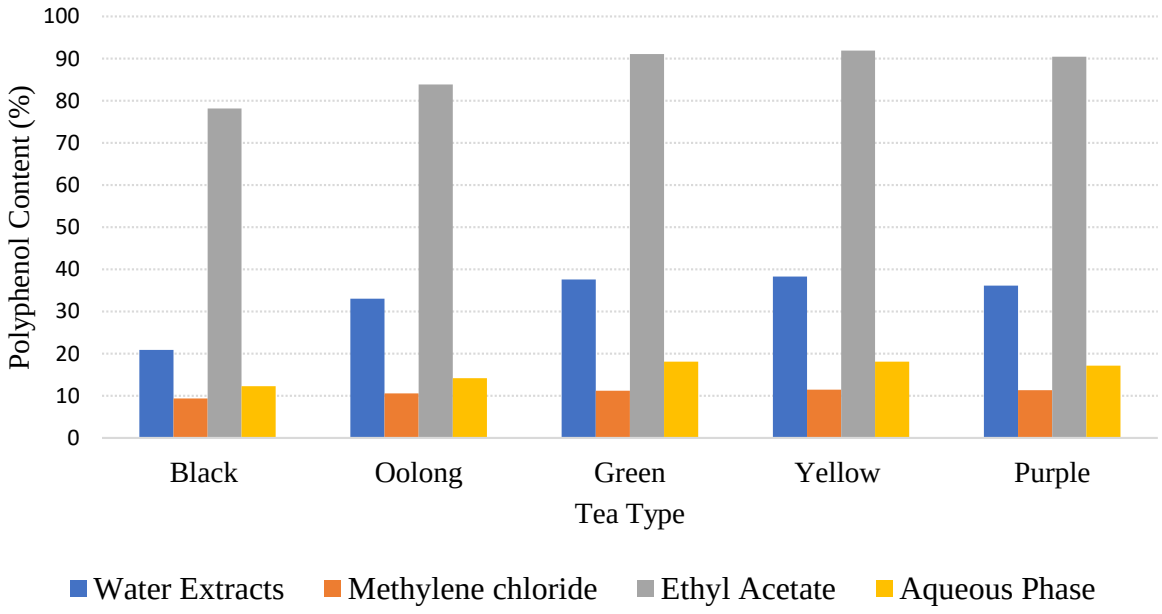


Figure 4.1: Total polyphenol content (%) of tea extracts by tea type and solvent.

The bar chart compares the polyphenol content of five Kenyan specialty orthodox teas extracted using three different solvents: water, methylene chloride, ethyl acetate, and aqueous phase. Ethyl acetate consistently yielded the highest polyphenol content across all tea types, followed by water extracts and aqueous phase extracts. Methylene chloride recorded the lowest polyphenol yields.

Table 4.1

Total Polyphenolic Content (%) of Tea Extracts by Tea type and Solvent

Tea Type	Tea Extract	Polyphenol (%) (Mean ± SD)
Black orthodox	Water	20.89 ± 0.27^{b}
	Methylene chloride	9.32 ± 0.28^{l}
	Ethyl acetate	78.13 ± 0.15^{c}
	Aqueous phase	12.24 ± 0.10^{j}
Mean		30.14
Oolong	Water	33.01 ± 0.18^{f}
	Methylene chloride	10.56 ± 0.03^{kl}
	Ethyl acetate	83.83 ± 0.21^{b}
	Aqueous phase	14.18 ± 0.05^{i}
Mean		35.40
Green orthodox	Water	37.57 ± 0.23^{dc}
	Methylene chloride	11.20 ± 0.25^{k}
	Ethyl acetate	91.07 ± 0.23^{a}
	Aqueous phase	18.09 ± 0.24^{h}
Mean		39.48
Yellow orthodox	Water	38.28 ± 0.02^{d}
	Methylene chloride	11.44 ± 0.15^{k}
	Ethyl acetate	91.09 ± 0.12^{a}
	Aqueous phase	18.07 ± 0.27^{h}
Mean		39.92
Purple orthodox	Water	36.15 ± 0.03^{f}
	Methylene chloride	11.30 ± 0.09^{ik}
	Ethyl acetate	90.41 ± 0.24^{a}
	Aqueous phase	17.16 ± 0.18^{h}
Mean		38.76
Total mean		34.94

Note. Aqueous phase extracts refer to the final aqueous layer remaining after sequential extraction with methylene chloride and ethyl acetate.
Values are expressed as mean ± standard deviation. Means within the same column followed by the same superscript letters are not significantly different at $p \leq 0.05$, according to Duncan's Multiple Range Test (DMRT).

4.2.1 Total catechins content

The concentrations of total catechins differed significantly ($p \leq 0.05$) across different tea types and solvent extracts. A graphical representation in Figure 4.2 illustrates that ethyl acetate extracts consistently yielded the highest catechin content. This was especially recorded in green and yellow orthodox teas, with concentrations of 75.9%, and 73.9%

respectively as shown in Appendix II. Water extracts showed moderate levels, ranging from 12% to 28%, while methylene chloride extracts had the lowest catechin content (1.2% -4.6%). Aqueous phase extracts contained low amounts (5.4% - 8.8%), indicating some retention of catechins after solvents extraction. The detailed statistical comparison is presented in Appendix II.

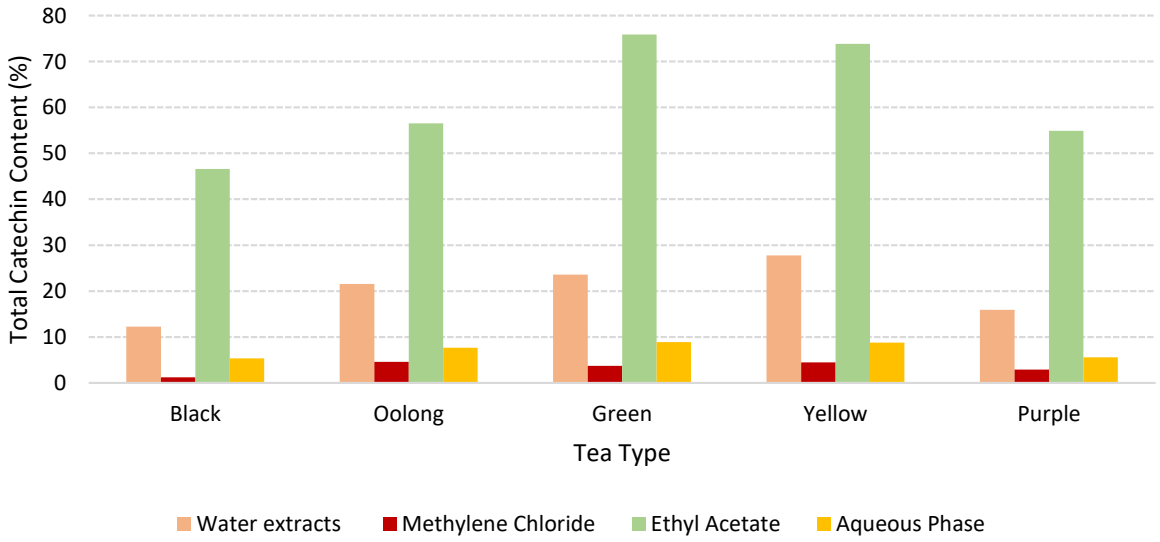


Figure 4.2: Total catechin content (%) by tea type and solvent type.

The figure above shows the total catechin mean values ± standard deviation across five types of tea which are black, oolong, green, yellow and purple orthodox teas and four extracts that include water, methylene chloride, ethyl acetate, and aqueous phase.

4.2.2 Individual catechins levels

Individual catechin profiling included gallate catechins, specifically epigallocatechin gallate (EGCG) and epicatechin gallate (ECG), and non-gallated catechins included epigallocatechin (EGC), epicatechin (EC), and catechin (+C). As illustrated in Figure 4.3, ethyl acetate extracts particularly from green and yellow orthodox teas, exhibited significantly higher ($p \leq 0.05$) concentrations of individual catechins. A detailed statistical comparison between the tea types and extraction solvents is provided in

Appendix III. Ethyl acetate green orthodox, the major catechins detected were EGCG (34.3%), EGC (14.6%), and EC (13.4%). Similarly, yellow orthodox contained EGCG (36.3%), EGC (12.8%), and EC (13.1%) (Appendix III). Among all extracts, EGCG was the most abundant catechin, with the highest levels observed in yellow orthodox (36.3%), followed by green orthodox (34.3%), purple orthodox (23.59%), oolong tea (23.3%) and black orthodox (17.87%). A representation of elution order of the catechins in tea sample (reference sample green orthodox) is shown in Appendix IV.

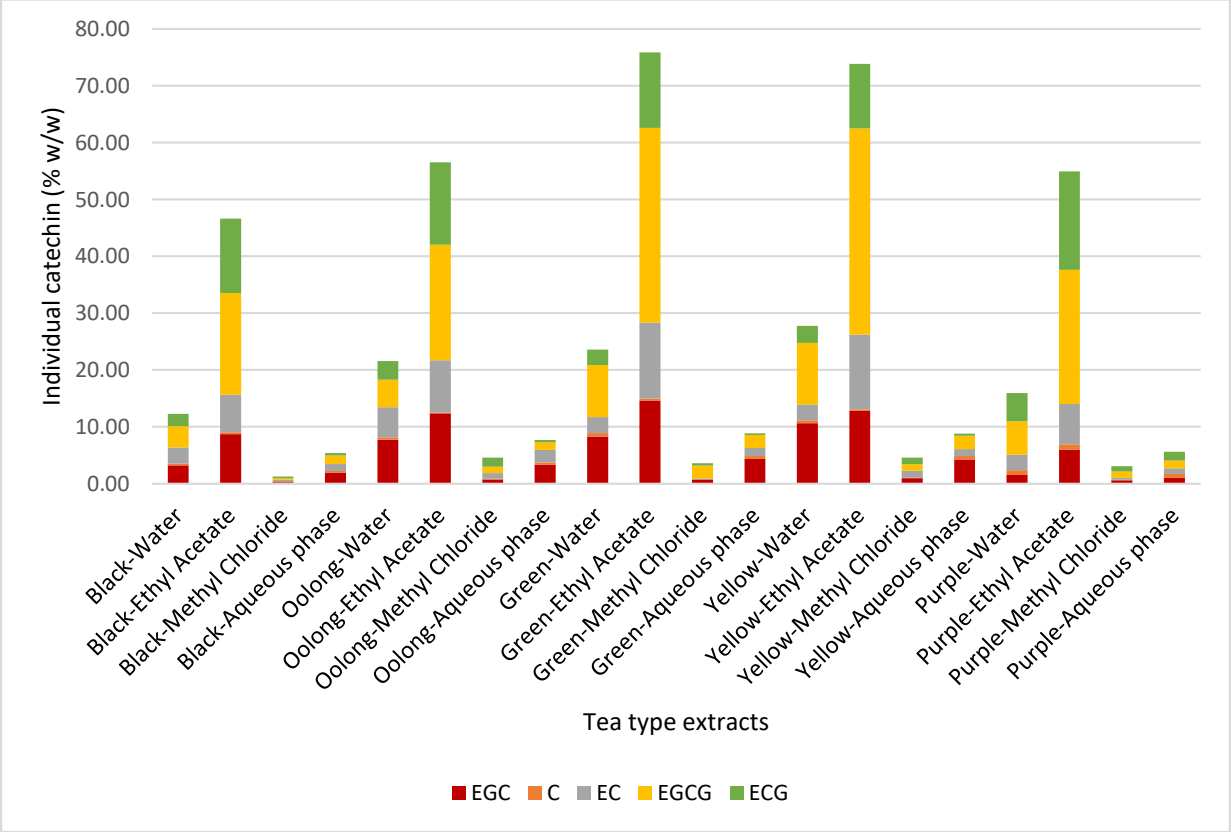


Figure 4.3: Individual catechin content

Individual catechin concentrations (EGC, C, EC, EGCG and ECG) in the four extracts (water, methylene chloride, ethyl acetate and aqueous phase) of the five orthodox tea types (black, oolong, green, yellow and purple). Bars represent mean values ± standard deviation (n=3).

4.2.3 Antioxidant activity

The antioxidant activity of the tea extracts, measured using radical scavenging assay. Ethyl acetate extracts consistently exhibiting the highest antioxidant activity across all tea types while methylene chloride demonstrated the least as shown in Figure 4.4. Green orthodox tea extract with ethyl acetate showed the highest antioxidant activity (93.18%), while the methylene chloride extract of black orthodox tea recorded the lowest (59.47%). Water extracts also recorded high levels of antioxidant activity especially yellow and purple orthodox which was not significantly different to ethyl aetate extracts (Figure 4.4). A detailed comparison of the antioxidant activity of all tea types and extraction solvents is illustrated in Appendix V. The values ranged from 59.47% to 93.18%, with ethyl acetate extracts consistently exhibiting the highest antioxidant percentages across all tea types. The results indicated that solvent polarity strongly influences the efficiency of the antioxidant extraction, with ethyl acetate and water being more effective than methylene chloride.

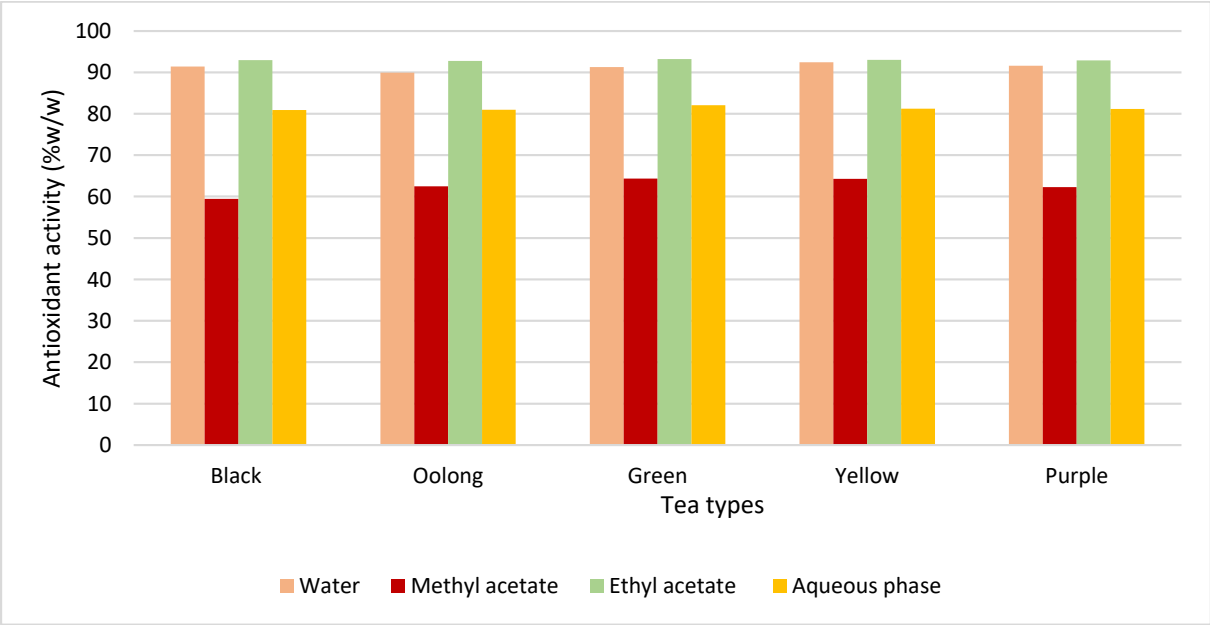


Figure 4.4: Antioxidant activity in all tea types

Antioxidant activity in black, oolong, green, yellow and purple orthodox teas and extraction solvents (water, methylene chloride, and ethyl acetate). Aqueous phase refers to the final aqueous layer remaining after sequential extraction with methylene chloride and ethyl acetate.

4.2.4 Correlation coefficient analyses between Biochemical Parameters

A multivariate correlation matrix was statistically analyzed to determine the relationship between biochemical parameters of the analyzed samples. This includes total polyphenols, total catechins, Individual catechins, and antioxidant (Table 4.2**)**. The polyphenol content (**A**) exhibited a strong positive correlation with total catechins (**B**) (r= 0.9801***), Epicatechin (**G**) (r= 0.9327***), Epigallocatechin gallate (**H**) (r= 0.9565***), Epigallocatechin (**D**) (r= 0.8137***), Epicatechin gallate (**I**) (r= 0.9672***), and antioxidant activity (**J**) (r= 0.6625**). Antioxidant activity (**J**) was also significantly correlated with total catechins (**B**) (r= 0.6524**), Epigallocatechin (**D**)(r= 0.7191***), Epicatechin (**G**) (r= 0.6049**), Epigallocatechin gallate (**H**) (r= 0.6009**), and Epicatechin gallate (**I**) (r= 0.5627*).

Table 4.2

Linear correlation between biochemical parameters of the analyzed tea samples

	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
A	1.000	0.9801***	-0.4100	0.8137***	0.2357	-0.4078	0.9327***	0.9565***	0.9672***	0.6625**
B		1.0000	-0.4566	0.8760***	0.1494	-0.4048	0.9729***	0.9880***	0.9143***	0.6524**
C			1.0000	-0.3640	0.4912	-0.2969	-0.4102	-0.4897	-0.4234	0.1147
D				1.0000	0.0706	-0.4936	0.8443***	0.8346***	0.6765**	0.7191***
E					1.0000	-0.4459	0.0796	0.1310	0.2194	0.4342
F						1.0000	-0.3814	-0.3609	-0.3182	-0.8695
G							1.0000	0.9625***	0.8586***	0.6049**
H								1.0000	0.8817***	0.6009**
I									1.0000	0.5627*
J										1.0000

* There is a significant correlation at the $p \leq 0.05$ level

** There is a significant correlation at the $p \leq 0.05$ level

*** There is a significant correlation at the $p \leq 0.05$ level

4.3 Antibacterial Activity of Tea Extracts

The antibacterial activity of the tea extracts was evaluated using agar disc diffusion assays with a two-fold dilution series from 10mg/ml. Ethyl acetate extracts exhibited the strongest antibacterial effects, particularly, green orthodox ethyl acetate showed inhibition zones 23.33mm for *S. aureus* ATCC 25923 and 19.33mm for *E. coli* ATCC 25922 at 10mg/ml concentration (Table 4.3). Water extracts showed moderate activity of 16.67mm for *S. aureus* and 12.67mm for *E. coli*. Aqueous phase extracts exhibited weak activity with inhibition zones (11.33mm to 14.67mm) against *S. aureus* while methylene chloride extracts were largely ineffective. A 6mm inhibition zone was recorded, which corresponds to the disc diameter and is interpreted as no inhibition. *S. aureus* was the most susceptible bacterium, while *E. coli* showed reduced inhibition, and *P. aeruginosa* displayed complete resistance across all tea extracts. The comparative activity is visualized in Figure 4.5 below, showing more clearly across tea types, and extracts solvents.

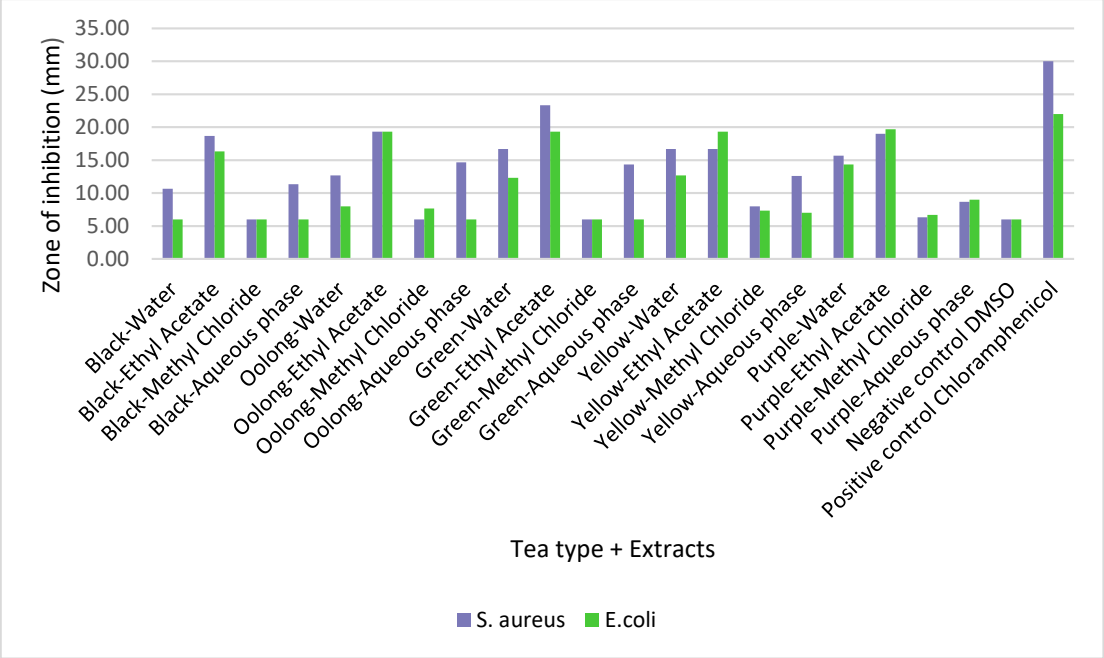


Figure 4.5: Antibacterial activity of specialty tea extracts.

Comparative antibacterial activity of tea extracts against methicillin and penicillin resistance *S. aureus* ATCC 25923 and antibiotic resistant *E. coli* ATCC 25022.Ethyl acetate extracts exhibited antibacterial activity against S. aureus across all tea types (black, oolong, green, yellow, purple).

Table 4.1:

Mean inhibition zones (mm) for antibacterial activity of tea extracts against S. aureus and E. coli

Type of tea	Tea extracts	*S. aureus*	*E. coli*
Black orthodox	Water	10.67^{h}	6.00^{j}
	Methylene Chloride	6.00^{j}	6.00^{j}
	Ethyl acetate	18.67^{c}	16.33^{c}
	Aqueous phase	11.33^{h}	6.00^{j}
Mean		**11.67**	**8.58**
Oolong	Water	12.67^{g}	8.00^{g}
	Methylene Chloride	6.00^{j}	7.67^{gh}
	Ethyl acetate	19.33^{c}	19.33^{b}
	Aqueous phase	15.67^{de}	6.00^{j}
Mean		**13.42**	**10.25**
Green orthodox	Water	16.67^{d}	12.33^{e}
	Methylene Chloride	6.00^{j}	6.00^{j}
	Ethyl acetate	23.33^{b}	19.33^{b}
	Aqueous phase	14.33^{ef}	6.00^{j}
Mean		**15.08**	**10.92**
Yellow orthodox	Water	16.67^{d}	12.67^{e}
	Methylene Chloride	8.00^{i}	7.33^{ghi}
	Ethyl acetate	16.67^{d}	19.33^{b}
	Aqueous phase	12.60^{g}	7.00^{ghi}
Mean		**13.49**	**11.58**
Purple orthodox	Water	15.67^{de}	14.33^{d}
	Methylene Chloride	6.33^{j}	6.67^{ij}
	Ethyl acetate	19.00^{c}	19.67^{b}
	Aqueous phase	8.67^{i}	9.00^{f}
Mean		**12.42**	**12.42**
Negative control	DMSO	6.00^{j}	6.00^{j}
Positive control	Chloramphenicol	30.00^{a}	22.00^{a}
Total Mean		**14.58**	**11.68**

Note:

DMSO-Dimethyl sulfoxide.

An inhibition zone of 6mm equals the disc diameter and is considered as no antibacterial activity.

Values are expressed as mean ± standard deviation. Means within the same column followed by the same letter (superscript) is not significantly different at $p \leq 0.05$, according to Duncan's Multiple Range Test (DMRT).

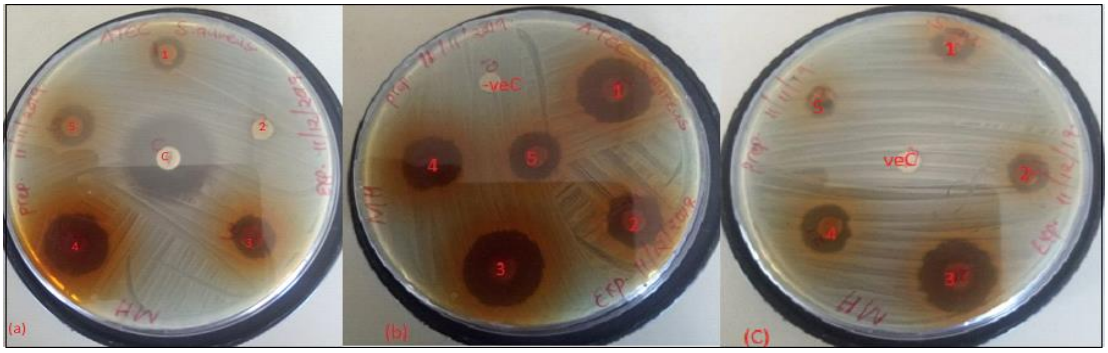


Plate 4.1: Inhibitory concentration of tea extracts against S. aureus ATCC 25923, resistant to methicillin and penicillinase.

Plate 4.1 (a): Inhibitory concentration of tested tea extracts against *S. aureus* ATCC 25923, resistant to methicillin and penicillin; (1) methylene chloride extract- yellow orthodox, (2) methylene chloride extract - green orthodox, (3) residual extract - yellow orthodox, (4) Ethyl acetate extract -black orthodox, (5) water extract - oolong tea and (6) chloramphenicol as positive standard control (C). **Plate 4.1(b)**; (1) ethyl acetate-purple orthodox, (2) residual-oolong tea, (3) ethyl acetate-green orthodox, (4) water-purple orthodox, (-ve C) negative standard control and (5) water-green orthodox. **Plate 4.1 (c)**; (1) water- purple orthodox, (2) green orthodox Japan, (3) ethyl acetate –oolong tea, (4) water- black orthodox, (5) matcha tea, and (-ve C) negative standard control.

The inhibition zones progressively decreased with each dilution, which indicated a strong dose- dependent inhibitory trend as shown in Figure 4.6 and Plate 4.2 for methicillin and penicillin resistant *S. aureus* ATCC 25923 (10mg/ml to 0.16 mg/ml). The two-fold dilution series that started at 10mg/ml concentration, were effective as low as 0.32mg/ml concentration for ethyl acetate extracts. At this concentration, green orthodox tea extract exhibited weak antibacterial activity (9mm). Ethyl acetate extracts at 0.32mg/ml were significantly high compared to both water and aqueous phase extracts at the same concentration, as detailed in Appendix VI. Figure 4.6 shows dose-

dependent activity of all ethyl acetate tea extracts against the resistant *S. aureus* ATCC 25923 as determined by the zone of inhibition (mm).

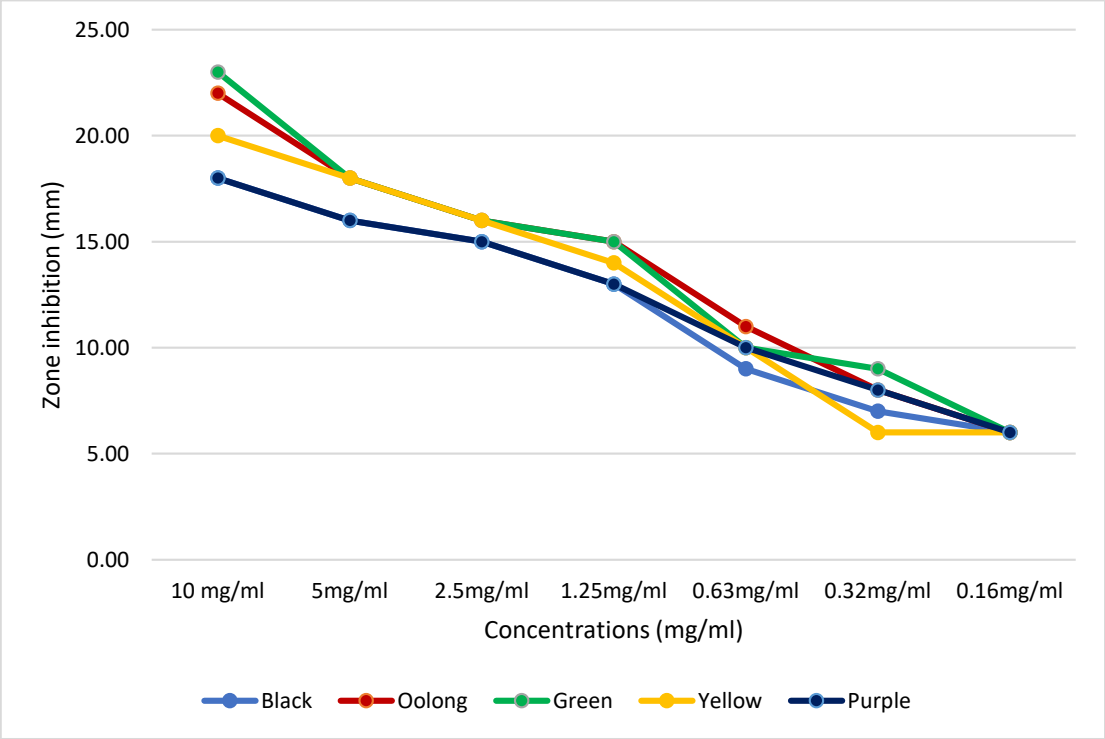


Figure 4.6: Dose -dependent antibacterial activity of all ethyl acetate extracts from specialty teas against Staphylococcus aureus.

The inhibition zones were measured using the agar disc diffusion method across a concentration range from 10mg/ml to 0.16mg/ml. A 6mm zone of indicates no antibacterial activity. Green orthodox showed the highest activity at all concentrations.

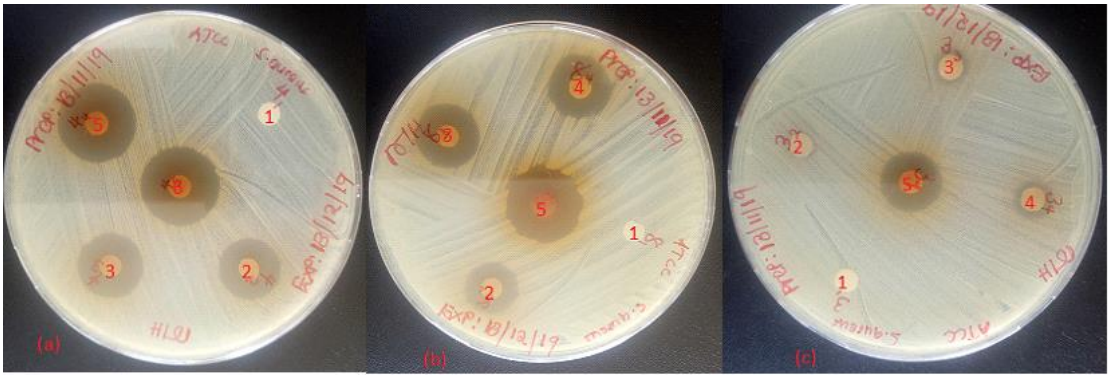


Plate 4.2: Dose-dependent activity of tea extracts against methicillin and penicillin resistant S. aureus ATCC 25923.

Plate 4.2 (a) Dose-dependent activity of green orthodox ethyl acetate extracts against methicillin and penicillin *S. aureus* ATCC 25923; **Plate 4.2(b)** Black orthodox ethyl acetate extract. **Plate (c)** Yellow orthodox aqueous phase extracts.

Escherichia coli ATCC 25922 exhibited low sensitivity to tested tea extract as compared to *S. aureus*. The inhibition zones showed decrease with each dilution (10mg/ml- 0.16mg/ml), that indicated a strong dose-dependent inhibitory trend, as shown in Plate 4. 3 and Figure 4.6**.** Moderate antibacterial activity was exhibited as low as 11mm inhibition zone at 1.25mg/ml concentration of ethyl acetate green orthodox (Figure 4.7). A detailed presentation of inhibition trends across all extracts and concentrations is provided in Appendix VII.

The dose-dependent pattern indicated that even the lower concentration of the tested tea extracts inhibited visible bacterial growth. According to these results, higher concentrations of the extract showed larger inhibition zones at 10mg/ml concentration. In the case of methylene chloride extracts, no detection of antibacterial activity was exhibited against the bacterium. Inhibition zone of 6mm, equivalent to the disc diameter, was considered indicative of no antibacterial activity as there was no measurable zone of clearance beyond the disc edge. Green orthodox ethyl acetate

extracts demonstrated high antibacterial activity at lower concentrations compared to other tea types.

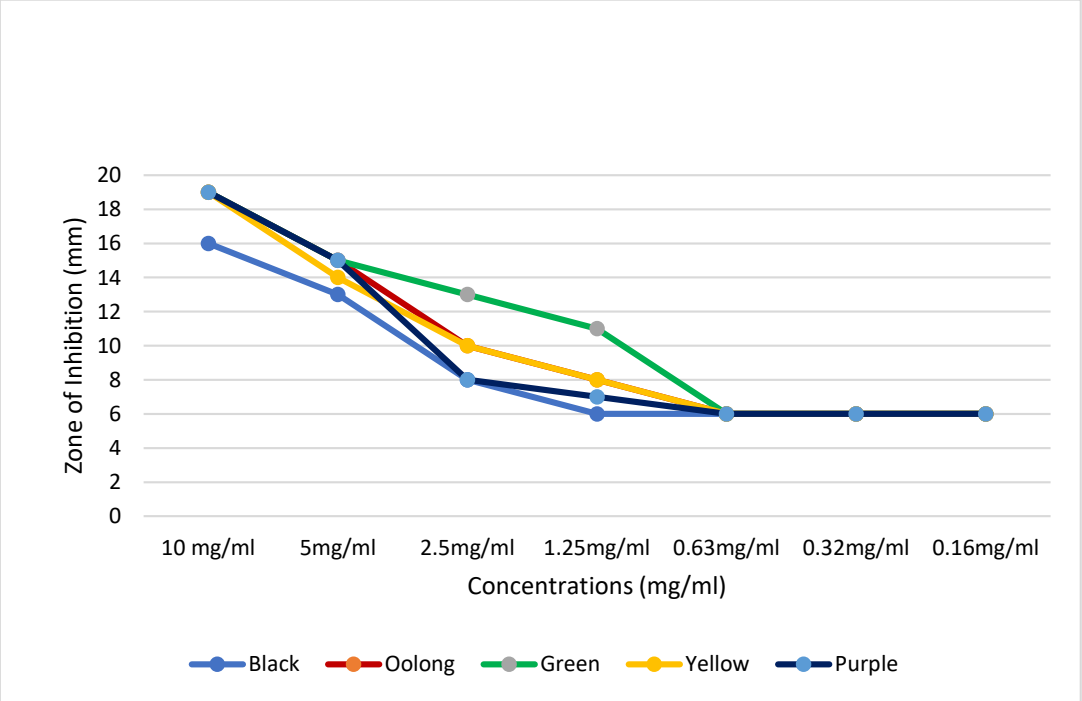


Figure 4.7: Dose-dependent antibacterial activity of ethyl acetate tea extracts against Escherichia coli ATCC 25922.

Inhibition zones were measured using the agar disc diffusion across concentrations ranging from 10mg/ml to 0.16mg/ml. Oolong, green, yellow, and purple orthodox tea extracts exhibited the highest activity at 10mg/ml (19mm), while all extracts showed no detection inhibition (6mm) at concentrations of 0.63mg/ml and below. The data demonstrated a strong dose-dependent inhibitory trend, with green tea extract maintaining activity at lower concentrations compared to the other tea type.

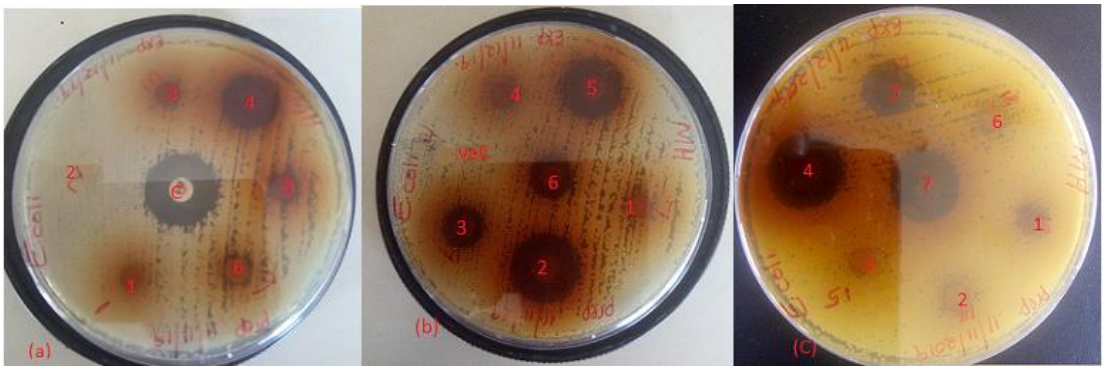


Plate 4.3: Dose- dependent activity of tested tea extracts that inhibit the growth of antibacterial-resistant E. coli ATCC 25922.

Plate 4.3 (a): (1) water extract - black orthodox, (2) methylene chloride-black orthodox, (3) methylene chloride-oolong tea, (4) ethyl acetate-black orthodox, (5) water extract-oolong tea, (6) methylene chloride-yellow, and (C) Chloramphenicol used as positive control. **Plate 4.3 (b)**: (1) methylene chloride- purple orthodox, (2) ethyl acetate-purple orthodox, (3) water extract-purple orthodox, (4) residual extract-purple orthodox, (5) ethyl acetate-yellow orthodox, (6) water extract-green orthodox and (-veC) negative control. **Plate 4.3 (c)**: (1) residual extract-yellow orthodox, (2) residual extract-purple orthodox, (3) water extract-oolong tea, (4) ethyl acetate-green orthodox, (5) water extract-yellow orthodox, (7) ethyl acetate-oolong tea.

4.4 Antifungal Activity of Tea Extracts

The antifungal activity of the tea extracts against *Trichophyton mentagrophytes* and *Candida albicans* ATCC 90028 pathogens was ineffective in most extracts. The highest activity was observed in ethyl acetate extract of black orthodox, 16.33mm for *T. mentagrophytes* as shown in Figure 4.8 and Appendix VIII. Ethyl acetate of green orthodox also showed moderate antifungal activity (10.67mm for *T. mentagrophytes* and 12.33mm for *C. albicans* ATCC 90028). The dose dependent activity for antifungal activity was only effective at 10mg/ml concentration (Plate 4.4). Other tea extracts

showed no inhibitory activity which was based on 6mm that represent the diameter of the discs used in agar disc diffusion assay.

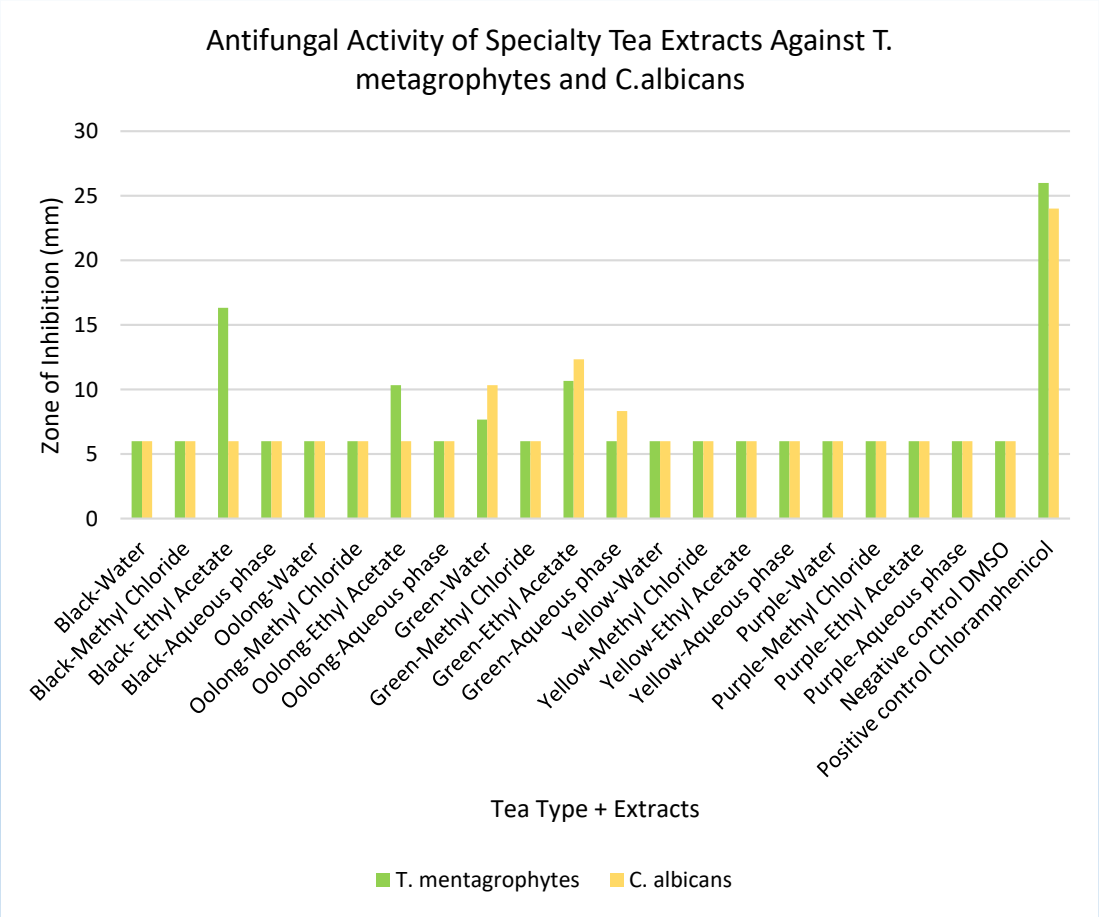


Figure 4.8: Mean zone inhibition (mm) showing antifungal activity of tea extracts against T. mentagrophytes and C. albicans ATTC 90028.

Ethyl acetate extracts of green and black teas demonstrated the highest antifungal activity against *C. albicans* and *T. mentagrophytes*, respectively. Dimethyl Sulfoxide as negative control, while Nystatin was the positive control. A zone diameter 6mm indicates no antifungal inhibition.

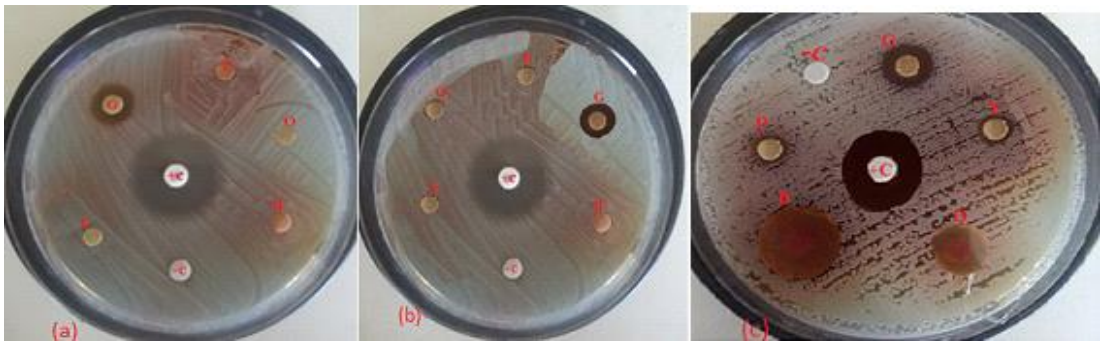


Plate 4.4: The dose dependent activity for *C. albicans* ATCC 90028 and *T. mentagrophytes* against tested extracts.

Plate 4.4(a): Ethyl acetate tea extracts against *C. albicans* ATCC 90028. (G) ethyl acetate-green orthodox, (P) ethyl acetate-purple orthodox, (O) ethyl acetate-oolong tea, (B) ethyl acetate-black orthodox, (-ve C) DMSO as negative control, (Y) ethyl acetate-yellow orthodox (Y) and (+ve C) nystatin as positive control. **Plate 4.4 (b)**: (G)water extract- green orthodox, (B), water extract-black orthodox (-ve), negative control, (Y)water extract-yellow orthodox, (O)water extract-oolong, and (P)water extract-purple orthodox. **Plate 4.4(c)**: Ethyl acetate tea extracts against *T. mentagrophytes*. (G) ethyl acetate-green orthodox, (Y) ethyl acetate-yellow orthodox, (O) ethyl acetate-oolong, (B) ethyl acetate- black orthodox, (P) ethyl acetate-purple orthodox, (-ve C) negative control and (+ve C) nystatin as positive control.

4.5 Positive Correlation between the Diameters of Inhibition Zones and Different Biochemical Parameters of Tea

Results on the correlation between the zone of inhibition measurements for *S. aureus* ATCC 25923 with polyphenol content, individual catechins and antioxidant activity of different tea types extracts as illustrated in Table 4.4. A significant correlation was observed between the diameter of the inhibition zone (**A**) and total polyphenols (**B**) (r= 0.829***), antioxidant activity (**C**) (r= 0.830***), Epigallocatechin (**D**) (r= 0.841***),

Epicatechin (**F**) (r= 0.772***), Epigallocatechin gallate (**G**) (r= 0.773***), and Epicatechin gallate (**H**) (r= 0.745***).

Table 4.2
Correlation coefficients between biochemical parameters of the tea extracts and the diameters of inhibition zones (IZDs) for resistant S. aureus ATCC 25923

	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
A	1	0.829***	0.830***	0.841***	0.41	0.772***	0.773***	0.745***

Note: **A** *have a correlation coefficient of 1 with themselves.*

****Correlation significant at p≤ 0.001 level*

The study examined the relationship between the inhibition zone diameters of antibacterial-resistant *E. coli* ATCC 25922 and the biochemical parameters of the tea extracts tested **(Table 4.5).** The results showed a strong correlation between the diameters of inhibition zones (**A**) and biochemical parameters of the tea extracts tested: polyphenol content (**B**) (r= 0.964***), antioxidant content (**C**) (r=0.624**), Epigallocatechin (**D**) (r= 0.753***), Epicatechin (**F**) (r= 0.867***), Epigallocatechin gallate (**G**) (r= 0.915***), and Epicatechin gallate (**H**) (r= 0.940***).

Table 4.3

Correlation coefficients between the diameters of inhibition zones (IZDs) and the biochemical parameters of different tea extracts for E. coli ATCC 25922.

	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
A	1	0.964***	0.624**	0.753***	0.316	0.867***	0.915***	0.94***

Note: **A** *have a correlation coefficient of 1 with themselves.*

** *The correlation significance at p≤ 0.01 level*

****The correlation significant at p≤ 0.001 level*

The correlation matrix for inhibition zones diameters (**A**) for *T. mentagrophytes* were significantly associated with polyphenol content (**B**) (r= 0.582*), Epigallocatechin (**D**) (r= 0.491*), Epicatechin (**F**) (r= 0.487*), Epigallocatechin gallate (**G**) (r= 0.470*), and Epicatechin gallate (**H**) (r= 0.602**).

Table 4.4

Correlation coefficients between the diameters of inhibition zones (IZDs) and the biochemical parameters of different tea extracts for T. mentagrophytes.

	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
A	1	0.582*	0.35	0.491*	-0.108	0.487*	0.47*	0.602**

Note: **A** *have a correlation coefficient of 1 with themselves*

**Correlation significant at p≤0.05 level*

*** Correlation significance at p≤ 0.01 level*

The results indicated that total polyphenols content, epigallocatechin, epicatechin, epigallocatechin gallate, and epicatechin gallate were determined to be the most potent antimicrobial biochemicals in the tea extracts examined. Therefore, growth inhibitory activity was higher in tea extracts containing these bioactive compounds.

4.6 Discussion

4.6.1 Total Polyphenol Content and Antioxidant activity of the Extracts

The findings from this study revealed that polyphenolic content, catechin levels, and antioxidant activity was strongly influenced by both solvent polarity and the degree of tea fermentation. The significant variation observed between extraction solvents is attributed to their different polarity and solvent affinity (Altemimi et al., 2017). Ethyl acetate extracts emerged as the most efficient solvent, consistently extracting the highest levels of polyphenols and catechins. This outcome is attributed to its

intermediate polarity, which enables the solubilization of moderately polar bioactive compounds such as flavonoids and catechins (Kristiningrum et al., 2018). Therefore, ethyl acetate was confirmed to be a suitable solvent for isolating catechins from tea extract. In contrast, methylene chloride extracts yielded the lowest polyphenol levels, and catechin levels, affirming that non-polar solvents are less effective in extracting polar phenolic compounds (Aljamali, 2016).

Methylene chloride showed high affinity for caffeine which was significantly ($p \leq 0.05$) high in methylene chloride extract of black orthodox tea compared to all mentioned tea types and predominant biomolecule in methylene chloride extracts. Caffeine's attraction to water is stronger due to dipole interactions resulting from its enhanced polar nature, as well as the hydrogen bonds that form between caffeine and water molecules (Senthilnithy et al., 2014). Caffeine, being an organic substance, has a higher affinity for methylene chloride and readily dissolves in it. Since caffeine and methylene chloride are both organic, caffeine is more strongly attracted to the organic solvent.

Water extracts also demonstrated high polyphenol and catechin content (van Oss & Giese, 2011). This suggested presence of hydrophilic compounds (water-soluble) and water affinity for extracting polar compounds. Although, highly polar, water extracted small amount of the mid-polar compounds compared to ethyl acetate. Aqueous phase extracts recorded moderate values, that indicated incomplete removal of bioactive compounds during extraction. Based on the results, the solvent could effectively extract the target or specific compound of choice among the different biochemical compounds in tea extract. This included polar compounds, mid-polar compounds or non-polar compounds in tea bioactive compounds which can be exploited in pharmaceuticals for its antioxidant activity.

In addition, non- fermented tea type such as green, yellow, and purple orthodox teas demonstrated more polyphenols and catechins content than fermented and less fermented types like black orthodox and oolong tea. This is attributed to deactivation of the enzyme polyphenol oxidase (PPO) during processing of non- fermented teas which is involved in the oxidation and hydrolysis of the chemical compounds (Heaney et al., 2018). As for fermented and semi-fermented tea types, the enzymes polyphenol oxidase (PPO), oxidizes polyphenolic compounds present in tea leaves that leads to formation of theaflavins and thearubigins. In addition, it may also be due to different tea cultivars as they have unique genetic profile, resulting in variations in the composition and concentration of bioactive compounds (Yu et al., 2020). The non-fermented teas are rich in gallated catechins such as EGCG, ECG and phenolic acids due to limited oxidation during processing as shown from the results, which are recognized for their strong antioxidant and antimicrobial properties (Martini, 2016; Nikoo et al., 2018). Purple orthodox tea, although also minimally fermented, contains high levels of anthocyanins, which further contribute to its functional potency.

The antioxidant activity measured by the DPPH assay followed the same pattern, with ethyl acetate extracts showing the strongest radical scavenging effects. This can be attributed by the abundance of phenolic hydroxyl groups in catechins, especially EGCG and EGC, which are highly effective in donating electrons to neutralize free radicals. The structure of EGCG, with its gallated moiety and trihydroxylated B-ring, gives it superior radical scavenging potential. In addition to catechins, theaflavins formed during oxidation of black orthodox tea also contributed to antioxidant activity, due to their dimeric structure and higher number of hydroxyl groups. The elevated antioxidant values in green, yellow, and purple orthodox teas further validate that minimal fermentation helps preserve rich antioxidant molecules that might otherwise degrade

during oxidative processing (Huang et al., 2022). For instance, purple orthodox teas, rich in anthocyanins, demonstrated high scavenging potential attributed to the catechol structures in the B-ring and hydroxylation patterns (Joshi et al., 2017). A strong correlation was observed between total polyphenol content, total catechin, and antioxidant activity that explains the role of phenolic compounds on antioxidant function.

The biochemical content in this study supports the hypothesis that specialty teas offer a rich profile of bioactive polyphenols suitable for antimicrobial research. In addition, the findings support the hypothesis that antioxidant activity is closely tied to tea biochemical composition and is influenced by both the solvent polarity and fermentation period during processing. This phytochemical abundance supports the problem statement, which emphasizes the need for new natural antimicrobial agents in light of the global antimicrobial resistance crisis. Since polyphenols are well documented for their membrane disruption, denature of proteins, and oxidative stress induction activities against microbes, their abundance in these teas directly strengthens the scientific basis for evaluating them as potential antimicrobial agents.

4.6.2 Antibacterial Activity of Tea Extracts

The results in this study indicate that ethyl acetate extracts had the most potent antibacterial activity among all solvent tested. *Staphylococcus aureus* ATCC 25923, a Gram-positive bacterium, known for resistance to methicillin and penicillin was more susceptible to the tea extracts than *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 (Figure 4.5; Table 4.3). *S. aureus* was highly susceptible to ethyl acetate extracts from non- fermented teas, particularly green orthodox at 23mm diameter of the inhibition zone of 10mg/ml concentration. Other extracts, such as water extracts, aqueous phase extracts also showed moderate antibacterial activity except

methylene chloride extracts that recorded weak activity of 8mm inhibition zone for yellow orthodox.

The antibacterial potential of ethyl acetate extracts and water extracts may be due to their high levels of bioactive compounds such as total polyphenols, EGCG, ECG, EC and EGC, which were predominant in non-fermented teas (Figure 4.2; Figure 4.3). This suggests the presence of hydroxyl groups at the 3', 4', and 5' positions on the B ring of epicatechin and catechin molecules. The molecular structure plays a significant role in the antimicrobial action by facilitating hydrogen bonding to essential proteins such as nucleic acid synthesis and electrostatic interactions with bacterial membranes (Rahardiyan, 2019). Moreover, high levels of gallocatechins especially EGC and EGCG have been reported to have potent antibacterial activity as result of both trihydroxyl groups on the B-ring and gallated moiety esterified at carbon 3’ on the C-ring (Nikoo et al., 2018).

The presences of anthocyanins in purple orthodox teas may have also attributed to the observed bioefficacy effect (Joshi et al., 2017). Antibacterial activity was recorded in fermented tea, black orthodox on *S. aureus* with an inhibition zone of 18mm at 10mg/ml concentration (Table 4.3). This may be contributed to the presence of polyphenols content (Table 4.1), total catechins (Figure 4.2). Fermented teas also contain high levels of polymeric catechins known as theaflavins which is considered to be potent for antimicrobial actions due to more hydroxyl groups on its structure compared to simple catechins structure (Goswami et al., 2020; Renzetti et al., 2020).

In addition, water extracts and aqueous extracts showed moderate antibacterial activity and this could be due to presence of these phytochemicals at different concentrations in the tea extracts (Figure 4.2). A significant positive correlation was demonstrated

between antibacterial activity and total polyphenol content, catechin levels and antioxidant activity (Table 4.4).

The strong antibacterial effects of black orthodox, oolong, green, purple and yellow orthodox ethyl acetate extracts support their potential use as natural agents against resistant gram positive pathogens. These findings highlight the selective efficacy of certain tea extracts and suggests the need for targeting gram positive bacteria when developing natural antimicrobials from plant.

4.6.3 Antifungal Activity of Tea Extracts

The antifungal findings affirm that tea polyphenols are effective against *Candida albicans,* a dimorphic yeast. It showed moderate susceptibility with a growth inhibition zone of 12mm at 10mg/ml concentration, likely due to the presence of membranes with ergosterol that interact with polyphenols. The activity observed may be attributed to the ability of abundant catechins such as EGCG, ECG and EGC in green orthodox to disrupt fungal membrane integrity, inhibit biofilm formation, and induce oxidative stress (Figure 4.3). The observed dose-dependent inhibition in *T. mentagrophytes*, a dermatophyte fungus associated with skin infections. Exhibited higher sensitivity, particularly to black orthodox ethyl acetate tea extracts with an inhibition zone of 16mm at 10mg/ml concentration (Figure 4.8; Appendix VIII). This could be attributed by polymeric catechins found in black teas that disrupt fungal membrane and interfere with cell wall integrity. This difference could also be explained by the slower growth and simpler cell wall structure of dermatophytes compared to yeasts (Gow et al., 2017).

The antimicrobial effects of tea involve denaturing membrane proteins, which results in disruption of the outer membrane, inhibition of respiration, and eventual cell lysis (Zhong et al., 2023). Phenolic extracts possess the capability to inhibit RNA synthesis,

DNA, polysaccharides, and proteins in both fungal and bacterial cells. When microbes come into contact with polyphenols, they increase the production of defensive proteins for protection while simultaneously decreasing the synthesis of metabolic and biosynthetic proteins. These also includes carbon and energy metabolism as well as protein synthesis and amino acid (Siddiqui et al., 2016).

Though these findings showed less sensitive of *Candida albicans* to the tea extracts. These findings suggest that polyphenolic compounds in Kenyan specialty teas possess antifungal activity, supporting their potential use in treating superficial fungal infections. The strong antifungal activity of the ethyl acetate fractions again affirms the role of solvent polarity in optimizing the recovery of antifungal agents from tea.

4.6.4 Correlation Between Biochemical Composition and Antimicrobial Activity

Correlation analysis revealed strong positive relationships between inhibition zone diameters and concentrations of total polyphenols, EGCG, ECG, and antioxidant capacity (DPPH). For example, EGCG content showed a high correlation ($r = 0.91$) with inhibition zone diameter in *E. coli*, suggesting that extracts rich in catechin are primarily responsible for antibacterial activity. Similarly, total polyphenols were significantly associated with antimicrobial activity against *S. aureus* and *T. mentagrophytes* (Table 4.4: Table 4.7).

These correlations affirm the biochemical basis of antimicrobial activity in tea extracts, where phenolic content and antioxidant potential act synergistically to inhibit microbial growth. The data support the hypothesis that optimizing extract composition through appropriate tea type and solvent selection enhances antimicrobial efficacy.

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATION

5.1 Introduction

This chapter provides a comprehensive overview of the study's findings in relation to its objectives. It begins with the summary of the key results obtained from the evaluation of polyphenolic extracts of Kenyan specialty teas. The chapter also gives conclusions that directly respond to the research questions. The chapter closes with recommendations for future research, and the application of natural antimicrobial and antioxidant agents, especially in the context of the global fight against antimicrobial resistance (AMR).

5.2 Summary

The study investigated the antimicrobial properties of polyphenolic extracts obtained from selected Kenyan specialty teas (camellia sinensis), including black orthodox, oolong, green orthodox, and purple orthodox teas. Polyphenolic extracts were obtained using solvents of varying polarity which included water (polar), methylene chloride (non-polar) and ethyl acetate (intermediate polar) and the remaining aqueous phase fraction was collected as aqueous phase extract. The total polyphenol, catechin content, antioxidant activity of each extract was quantified. Antimicrobial activity was examined using agar disc diffusion assays against common bacterial and fungal pathogens, which included *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*, *Candida albicans*, and *Trichophyton mentagrophytes*.

The study found that ethyl acetate and water extracts of non-fermented teas (green, yellow, and purple orthodox) had the highest polyphenolic content and demonstrated the strongest dose-dependent antibacterial and antifungal activity, The DPPH assay also

confirmed these extracts had high antioxidant capacity. Gram-positive bacteria and dermatophytic fungi were the most sensitive to the extracts, while P. *aeruginosa* showed complete resistance. Strong correlations were observed between polyphenol, catechin levels, antioxidant activity and antimicrobial activity.

5.3 Conclusions

The polyphenolic composition of tea extracts varied with tea type and solvent. Green orthodox, yellow orthodox, and purple orthodox teas extracted using ethyl acetate and water had the highest levels of total polyphenols and catechins (EGCG, ECG, EC). These high levels correlated strongly with their antioxidant activity. Ethyl acetate proved to be the most effective solvent for extracting bioactive compounds.

The tea extracts exhibited dose-dependent antibacterial activity. Ethyl acetate extracts from green and yellow orthodox teas showed the strongest inhibition against Staphylococcus aureus, while Escherichia coli demonstrated moderate sensitivity. Pseudomonas aeruginosa was resistant to all extracts. Water extracts also demonstrated moderate antibacterial effects. These findings indicate the selective potency of tea polyphenols, especially against gram-positive bacteria.

The tea extracts also demonstrated dose-dependent antifungal activity, particularly against Trichophyton mentagrophytes and Candida albicans. Ethyl acetate extracts of green and black orthodox teas showed moderate inhibition, highlighting their potential in combating fungal pathogens.

Overall, the study provides compelling evidence that Kenyan specialty teas, like green, yellow, and purple orthodox types especially when extracted with ethyl acetate. They are promising sources of natural antimicrobial agents addressing antimicrobial resistance.

5.4 Recommendations

Arising from the findings of this study, the following recommendations are made:

1) Kenyan specialty teas, particularly green, yellow, and purple orthodox types should be promoted as natural sources of antimicrobial and antioxidant compounds.
2) The use of ethyl acetate as an effective extraction solvent for isolating bioactive compounds should be adopted in pharmaceutical and industrial applications.
3) Institutions and policymakers should support the integration of plant-based antimicrobial agents into public health strategies, especially in the context of antimicrobial resistance (AMR).
4) Awareness programs targeting local communities and tea stakeholders should be developed to promote the medicinal potential of specialty teas as a means of adding economic value.

5.5 Suggestions for Future Research

1) Further research should be conducted to isolate, purify, and characterize individual bioactive compounds such as EGCG and anthocyanins to better understand their specific mechanisms of action against pathogens.
2) Broader antimicrobial testing should be carried out using more clinical and drug-resistant strains, to determine minimum inhibitory concentrations and bactericidal and fungicidal effects.
3) The most active extracts such as ethyl acetate and water extracts of green and yellow orthodox teas should be evaluated for cytotoxicity and *in vivo* safety to support their potential development into topical and oral antimicrobial formulations.

4) Kenyan tea breeders and producers should be encouraged to prioritize cultivation of specialty teas with high polyphenol content for both health and commercial value.

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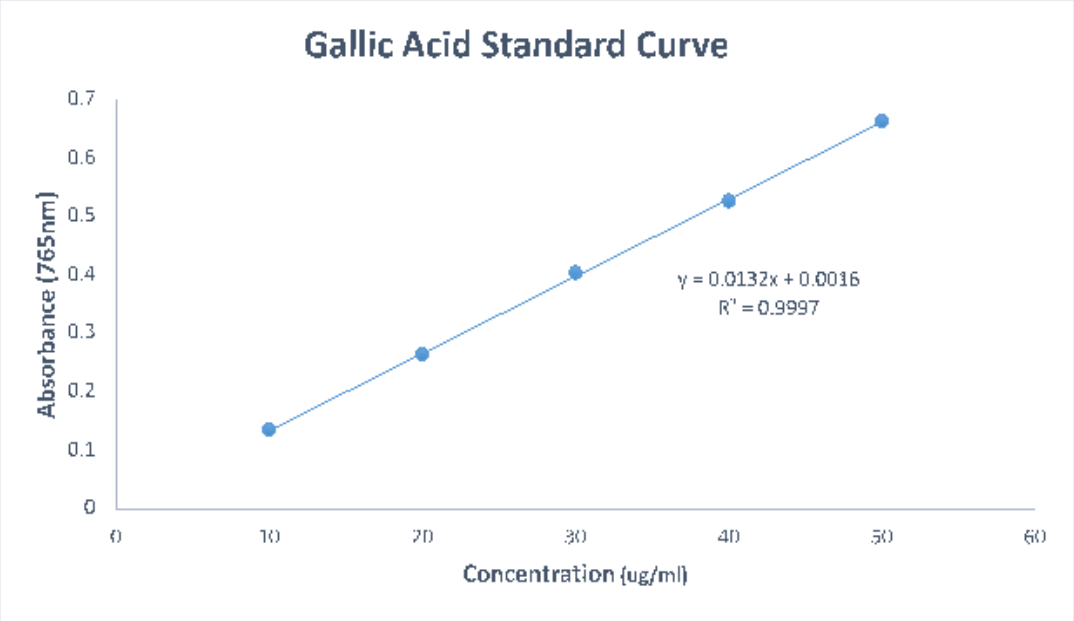
APPENDICES

Appendix I: The gallic acid calibration curve for measuring the total phenolic content.



Appendix II: Total Catechin Content (%) in tea extracts by tea type and solvent

Tea type	Tea extracts	Total Cat (%)
Black orthodox	Water	12.29 ±0.07^{h}
	Methyl Chloride	1.24± 0.03^{o}
	Ethyl Acetate	46.6±4 0.11^{c}
	Aqueous phase	5.36±0.09 ^{k}
	Mean	**16.38**
Oolong	Water	21.56±0.12 ^{f}
	Methyl Chloride	4.57 ±0.06^{l}
	Ethyl Acetate	56.55± 0.16^{b}
	Aqueous phase	7.69± 0.05^{j}
	Mean	**22.58**
Green orthodox	Water	23.60±0.06 ^{e}
	Methyl Chloride	3.74± 0.02^{m}
	Ethyl Acetate	75.89± 0.14^{a}
	Aqueous phase	8.87±0.02^{i}
	Mean	**28.02**
Yellow orthodox	Water	27.75±0.18 ^{d}
	Methyl Chloride	4.50±0.02^{l}
	Ethyl Acetate	73.85±0.10^{a}
	Aqueous phase	8.80±0.01^{i}
	Mean	**28.72**
Purple orthodox	Water	15.94±0.12^{g}
	Methyl Chloride	2.93±0.11^{n}
	Ethyl Acetate	54.92±0.16^{b}
	Aqueous phase	5.60±0.02^{k}
	Mean	**19.85**
	Total mean	**23.11**

Note:

Aqueous phase extracts refer to the final aqueous layer remaining after sequential extraction with methylene chloride and ethyl acetate.

Values are expressed as mean ± standard deviation. Means within the same column followed by the same letter (superscript) is not significantly different at $p \leq 0.05$, according to Duncan's Multiple Range Test (DMRT).

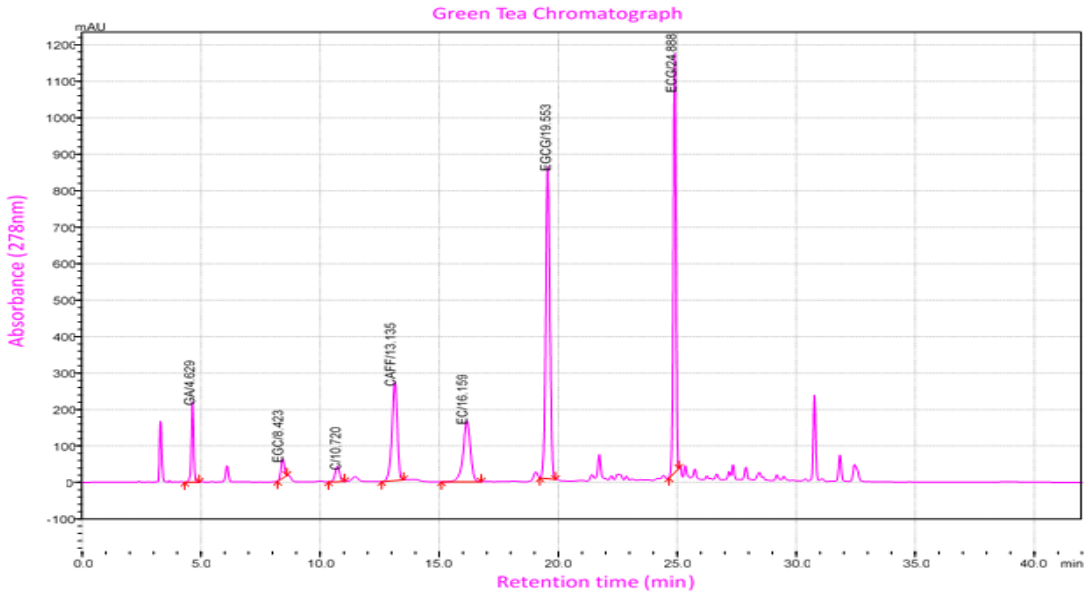
Appendix III: Individual Catechins Content (%) of Tea Extracts by Tea type and Solvent

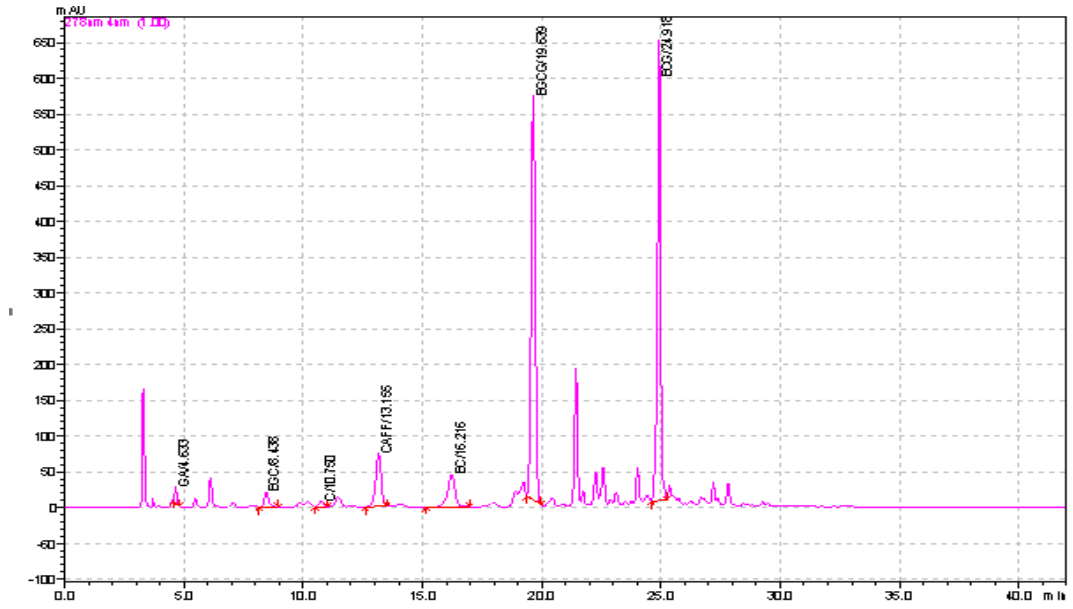
Tea type	Tea extracts	EGC%	C%	EC%	EGCG%	ECG%
Black orthodox	Water	3.23^{j}	0.30^{gh}	2.85^{g}	3.74^{j}	2.17^{j}
	Methylene Chloride	0.28^{o}	0.19^{jk}	0.27^{m}	0.21^{o}	0.29^{o}
	Ethyl Acetate	8.73^{e}	0.31^{gh}	6.61^{e}	17.87^{e}	13.12^{d}
	Aqueous phase	1.95^{k}	0.26^{hi}	1.26^{i}	1.50^{l}	0.39^{o}
	Mean	**3.55**	**0.27**	**2.75**	**5.83**	**4.00**
Oolong tea	Water	7.73^{g}	0.42^{f}	5.31^{f}	4.82^{i}	3.28^{g}
	Methylene Chloride	0.73^{mn}	0.13^{kl}	0.99^{jkl}	1.11^{n}	1.61^{k}
	Ethyl Acetate	12.38^{c}	0.11^{l}	9.21^{c}	20.34^{d}	14.51^{b}
	Aqueous phase	3.38^{j}	0.35^{fg}	2.21^{h}	1.37^{lm}	0.38^{o}
	Mean	**6.06**	**0.25**	**4.43**	**6.91**	**4.95**
Green orthodox	Water	8.28^{f}	0.61^{d}	2.84^{g}	9.12^{g}	2.75^{i}
	Methylene Chloride	0.72^{mn}	0.20^{ij}	0.80^{l}	1.20^{mn}	0.82^{n}
	Ethyl Acetate	14.64^{a}	0.32^{gh}	13.37^{a}	34.27^{b}	13.29^{c}
	Aqueous phase	4.36^{i}	0.52^{e}	1.41^{i}	2.28^{k}	0.30^{o}
	Mean	**7.00**	**0.41**	**4.61**	**11.72**	**4.29**
Yellow orthodox	Water	10.63^{d}	0.52^{e}	2.76^{g}	10.87^{f}	2.96^{h}
	Methyl Chloride	0.97^{m}	0.11^{l}	1.21^{ijk}	1.13^{mn}	1.14^{l}
	Ethyl Acetate	12.80^{b}	0.29^{h}	13.11^{b}	36.27^{a}	11.38^{e}
	Aqueous phase	4.23^{i}	0.63^{d}	1.24^{ij}	2.31^{k}	0.40^{o}
	Mean	**7.16**	**0.39**	**4.58**	**12.64**	**3.97**
Purple orthodox	Water	1.53^{l}	0.82^{b}	2.75^{g}	5.85^{h}	4.99^{f}
	Methyl Chloride	0.57^{no}	0.12^{l}	0.30^{m}	1.13^{mn}	0.98^{m}
	Ethyl Acetate	6.00^{h}	0.91^{a}	7.12^{d}	23.59^{c}	17.30^{a}
	Aqueous phase	1.03^{m}	0.69^{c}	0.99^{kl}	1.37^{lm}	1.53^{k}
	Mean	**2.28**	**0.64**	**2.79**	**7.99**	**6.20**
	Total mean	**5.21**	**0.39**	**3.83**	**9.02**	**4.68**

Note: *Aqueous phase extracts refer to the final aqueous layer remaining after sequential extraction with methylene chloride and ethyl acetate.*

Means within the same column followed by the same superscript letter is not significantly different at p≤ 0.05, according to Duncan's Multiple Range test (DMRT).

Appendix IV: A chromatograph representation of elution order of the catechins in tea sample using HPLC-PDA Machine.





Standard sample of tea catechins chromatograph at 278nm
X-axis: Time of Retention (minutes)
Y-axis: Area under the Peak
Compounds: *EC:Epicatechin; ECG:Epicatechin gallate; EGC:Epigallocatechin; C:Catechin; EGCG:Epigallocatechin gallate; and C:Caffeine.*

Appendix V: Antioxidant activity (%) of Tea Extracts

Tea type	Tea extracts	Antioxidant (%)
Black orthodox	Water	91.43^{bcd}
	Methylene Chloride	59.47^{i}
	Ethyl Acetate	92.93^{b}
	Aqueous phase	80.92^{f}
	Mean	**81.19**
Oolong	Water	89.95^{e}
	Methylene Chloride	62.46^{h}
	Ethyl Acetate	92.79^{abc}
	Aqueous phase	80.98^{f}
	Mean	**81.55**
Green orthodox	Water	91.27^{cd}
	Methylene Chloride	64.35^{g}
	Ethyl Acetate	93.18^{a}
	Aqueous phase	82.09^{f}
	Mean	**82.72**
Yellow orthodox	Water	92.43^{abc}
	Methylene Chloride	64.26^{g}
	Ethyl Acetate	93.02^{ab}
	Aqueous phase	81.21^{f}
	Mean	**82.73**
Purple orthodox	Water	91.63^{abc}
	Methylene Chloride	62.28^{h}
	Ethyl Acetate	92.92^{ab}
	Aqueous phase	81.15^{f}
	Mean	**81.99**
	Total mean	**82.04**

Note: *Aqueous phase extracts refer to the final aqueous layer remaining after sequential extraction with methylene chloride and ethyl acetate.*

Means within the same column followed by the same superscript letter is not significantly different at p≤ 0.05, according to Duncan's Multiple Range test (DMRT).

Appendix VI: Dose-dependent activity of tea extracts against resistant *S. aureus*

Tea type	Bacterium	Tea sample conc.(mg/ml)	10	5	2.5	1.25	0.63	0.32	0.16	Minimum Concentration
Black orthodox	*Staphylococcus aureus*	Water	13	11	9	6	6	6	6	2.5mg/ml
		Ethyl Acetate	18	16	15	13	9	7	6	0.32mg/ml
		Aqueous phase	14	10	6	6	6	6	6	5mg/ml
		Methylene Chloride	6	6	6	6	6	6	6	
Oolong		**Tea sample conc.(mg/ml)**	**10**	**5**	**2.5**	**1.25**	**0.63**	**0.32**	**0.16**	**Minimum Concentration**
		Water	15	11	10	9	6	6	6	1.25mg/ml
		Ethyl Acetate	22	18	16	15	11	8	6	0.63mgml
		Aqueous phase	14	9	6	6	6	6	6	5mg/ml
		Methylene Chloride	6	6	6	6	6	6	6	
Green orthodox		**Teas ample conc.(mg/ml)**	**10**	**5**	**2.5**	**1.25**	**0.63**	**0.32**	**0.16**	**Minimum Concentration**
		Water	18	15	11	9	7	6	6	0.63mg/ml
		Ethyl Acetate	23	18	16	15	10	9	6	0.32mg/ml
		Aqueous phase	14	12	10	6	6	6	6	2.5mg/ml
		Methylene Chloride	6	6	6	6	6	6	6	
Yellow orthodox		**Tea sample conc.(mg/ml)**	**10**	**5**	**2.5**	**1.25**	**0.63**	**0.32**	**0.16**	**Minimum Concentration**
		Water	17	15	13	10	8	6	6	0.63mg/ml
		Ethyl Acetate	20	18	16	14	10	6	6	0.63mg/ml
		Aqueous phase	13	11	8	6	6	6	6	2.5mg/ml
		Methylene Chloride	6	6	6	6	6	6	6	
Purple orthodox		**Tea sample conc.(mg/ml)**	**10**	**5**	**2.5**	**1.25**	**0.63**	**0.32**	**0.16**	**Minimum Concentration**
		Water	15	13	12	9	6	6	6	1.25mgml
		Ethyl Acetate	18	16	15	13	10	8	6	0.32mgml
		Aqueous phase	9	7	6	6	6	6	6	5mg/ml
		Methylene Chloride	8	6	6	6	6	6	6	10mg/ml

Note: Label 6mm=no inhibition

Appendix VII: Dose dependent activity of tea extracts against Escherichia coli determined by zone inhibition (mm)

Tea type	Bacterium	Tea sample conc.(mg/ml)	10	5	2.5	1.25	0.63	0.32	0.16	Minimum Concentration
Black orthodox	*Escherichia coli*	Water extracts	6	6	6	6	6	6	6	
		EA extract	16	13	8	6	6	6	6	2.5mg/ml
		Residual extract	6	6	6	6	6	6	6	
		MC extract	6	6	6	6	6	6	6	
Oolong		**Tea sample conc.(mg/ml)**	**10**	**5**	**2.5**	**1.25**	**0.63**	**0.32**	**0.16**	**Minimum Concentration**
		Water extracts	8	6	6	6	6	6	6	10mg/ml
		EA extract	19	15	10	8	6	6	6	1.25mg/ml
		Residual extract	6	6	6	6	6	6	6	
		MC extract	6	6	6	6	6	6	6	
Green orthodox		**Tea sample conc.(mg/ml)**	**10**	**5**	**2.5**	**1.25**	**0.63**	**0.32**	**0.16**	**Minimum Concentration**
		Water extracts	12	6	6	6	6	6	6	10mg/ml
		EA extract	19	15	13	11	6	6	6	1.25mg/ml
		Residual extract	6	6	6	6	6	6	6	
		MC extract	6	6	6	6	6	6	6	
Yellow orthodox		**Tea sample conc.(mg/ml)**	**10**	**5**	**2.5**	**1.25**	**0.63**	**0.32**	**0.16**	**Minimum Concentration**
		Water extracts	13	6	6	6	6	6	6	10mgml
		EA extract	19	14	10	8	6	6	6	1.25mg/ml
		Residual extract	7	6	6	6	6	6	6	10mg/ml
		MC extract	6	6	6	6	6	6	6	
Purple orthodox		**Tea sample conc.(mg/ml)**	**10**	**5**	**2.5**	**1.25**	**0.63**	**0.32**	**0.16**	**Minimum Concentration**
		Water extracts	14	6	6	6	6	6	6	10mg/ml
		EA extract	19	15	8	7	6	6	6	1.25mg/ml
		Residual extract	9	6	6	6	6	6	6	10mg/ml
		MC extract	6	6	6	6	6	6	6	

Note: An inhibition zone of 6mm corresponds to the diameter of the paper disc and indicates no antibacterial activity.

Appendix VIII: Mean inhibition zones (mm) for antifungal activity of tea extracts against T. mentagrophytes and C. albicans.

Type of tea	Tea extracts	*T. mentagrophytes*	*C. albicans*
Black orthodox	Water	6.00^{d}	6.00^{c}
	Methylene Chloride	6.00^{d}	6.00^{c}
	Ethyl acetate	16.33^{b}	6.00^{c}
	Aqueous phase	6.00^{d}	6.00^{c}
	Mean	**8.58**	**6.00**
Oolong	Water	6.00^{d}	6.00^{c}
	Methylene Chloride	6.00^{d}	6.00^{c}
	Ethyl acetate	10.33^{c}	6.00^{c}
	Aqueous phase	6.00^{d}	6.00^{c}
	Mean	**7.08**	**6.00**
Green orthodox	Water	7.67^{d}	10.33^{bc}
	Methylene Chloride	6.00^{d}	6.00^{c}
	Ethyl acetate	10.67^{c}	12.33^{b}
	Aqueous phase	6.00^{d}	8.33^{bc}
	Mean	**7.59**	**9.25**
Yellow orthodox	Water	6.00^{d}	6.00^{c}
	Methylene Chloride	6.00^{d}	6.00^{c}
	Ethyl acetate	6.00^{d}	6.00^{c}
	Aqueous phase	6.00^{d}	6.00^{c}
	Mean	**6.00**	**6.00**
Purple orthodox	Water	6.00^{d}	6.00^{c}
	Methylene Chloride	6.00^{d}	6.00^{c}
	Ethyl acetate	6.00^{d}	6.00^{c}
	Aqueous phase	6.00^{d}	6.00^{c}
	Mean	**6.00**	**6.00**
Negative control	DMSO (0.5%)	6.00^{d}	6.00^{c}
Positive control	Nystatin (30mg)	26.00^{a}	24.00^{a}
	Total mean	**9.61**	**9.04**

Note:

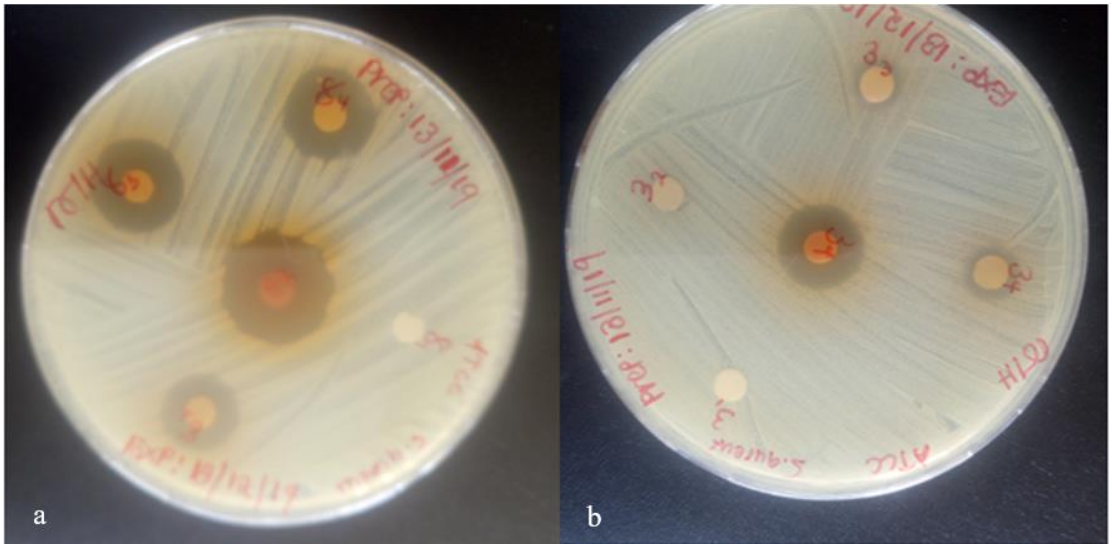
DMSO-Dimethyl sulfoxide.

An inhibition zone of 6mm equals the disc diameter and is considered as no antifungal activity.

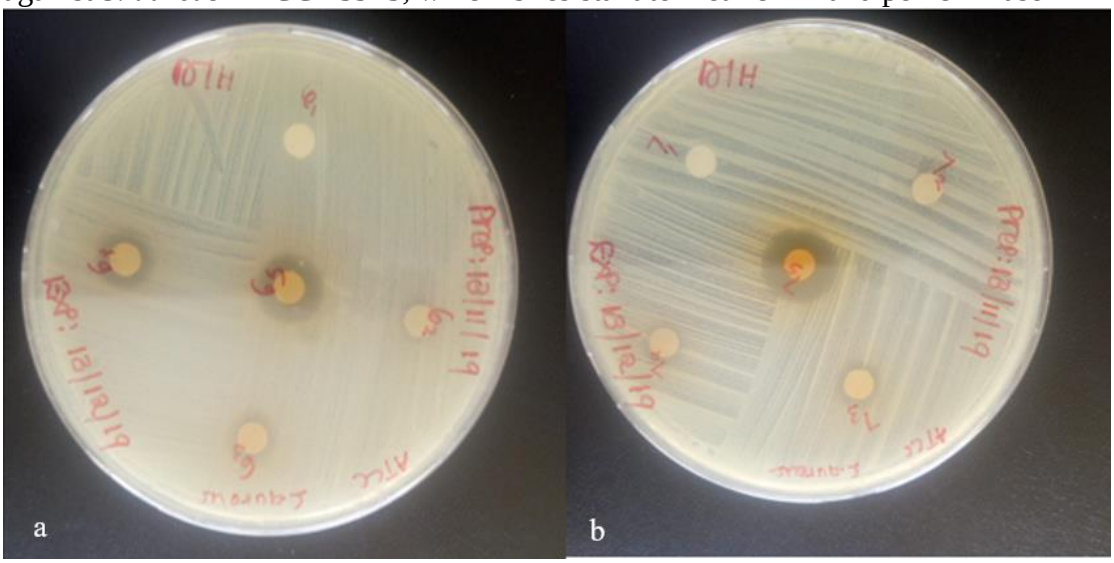
Values are expressed as mean ± standard deviation. Means within the same column followed by the same letter (superscript) is not significantly different at $p \leq 0.05$, according to Duncan's Multiple Range Test (DMRT).

Appendix IX: Disc diffusion assays

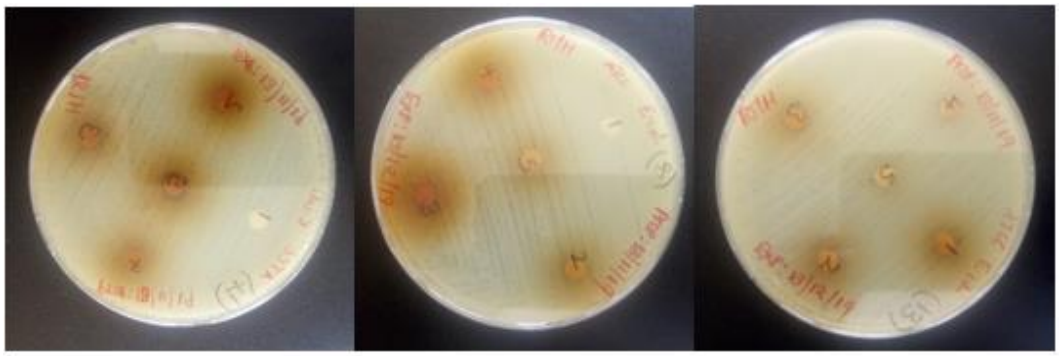
The inhibition area demonstrated by ethyl acetate tea extract (a) and water tea extracts (b) against *S. aureus* ATCC 25923, which is resistant to methicillin and penicillin.



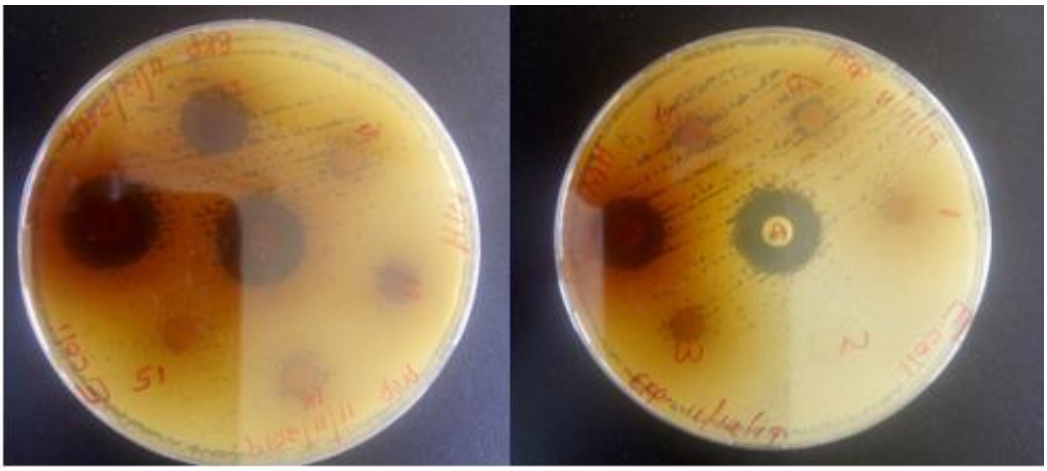
Zone of microbial inhibition shown by water tea extract (a) and residual tea extracts (b) against *S. aureus* ATCC 25923, which is resistant to methicillin and penicillinase



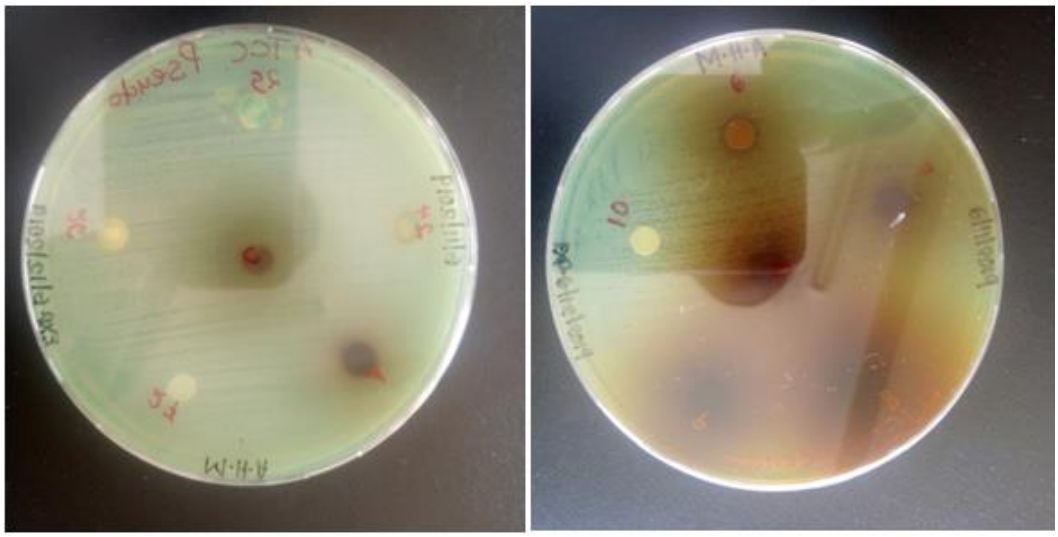
Zone of inhibition exhibited by water tea extracts against *E. coli* ATCC 25922, which is resistant to antibiotics.



Zone of inhibition exhibited by ethyl acetate tea extract against antibiotic resistant E. *coli* ATCC 25922.



Zone of inhibition exhibited by ethyl acetate tea extract against *P. aeruginosa* ATCC 2785



Appendix X: Extracts and solutions
Dissolved tea extracts for catechins and polyphenols analysis

Appendix XI: Tea extracts

Tea extracts of yellow orthodox tea *Camellia Sinensis*

Tea extracts of purple orthodox tea *Camellia Sinensis*

Tea extracts of black orthodox tea *Camellia Sinensis*

APPENDIX XII: letter of approval by BGS for fieldwork

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Tel. No. +254 20 217 2665
Email address:- research@kabianga.ac.ke

P.O BOX 2030-20200
KERICHO, KENYA.

REF: UoK/DIR/RLE/RNA/010/VOL.2/152 8TH JULY, 2019.

DR. CHRISTINE C. BII,
SENIOR RESEARCH OFFICER,
KENYA MEDICAL RESEARCH INSTITUTE,
P. O. BOX 2632- 00200,
NAIROBI.

REF: LETTER OF INTRODUCTION: MR. HENRIK K. RUTO (REG. NO. PGC/MIC/006/16) AND MISS EMILY J. KIPURA (REG. NO. PGC/MIC/008/16)

Mr. Henrik Ruto and Miss Emily Kipura are 2nd year MSc. Students at the University of Kabianga (UoK). They are undertaking their respective projects titled as follows:

1. Antibacterial and antifungal activities of tea seed oil; and
2. Antimicrobial properties of extracts of different cultivars of Kenya's specialty tea *Camellia sinensis* on selected pathogenic bacteria and fungi.

These projects require culturing of micro-organisms for the tests of which, UoK does not have the necessary facility.

This is therefore a request that they are allowed and assisted to use your facility to carry out their work.

Your assistance is very much appreciated.

UNIVERSITY OF KABIANGA
OFFICE OF DIRECTOR
09 JUL 2019
RESEARCH, LINKAGES & EXTENSION

PROF. PETER G. N. NJAGI, PhD
DIRECTOR: RESEARCH, LINKAGES & EXTENSION AND AG. DVC (PR&D)

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Appendix XIII: NACOSTI Research License

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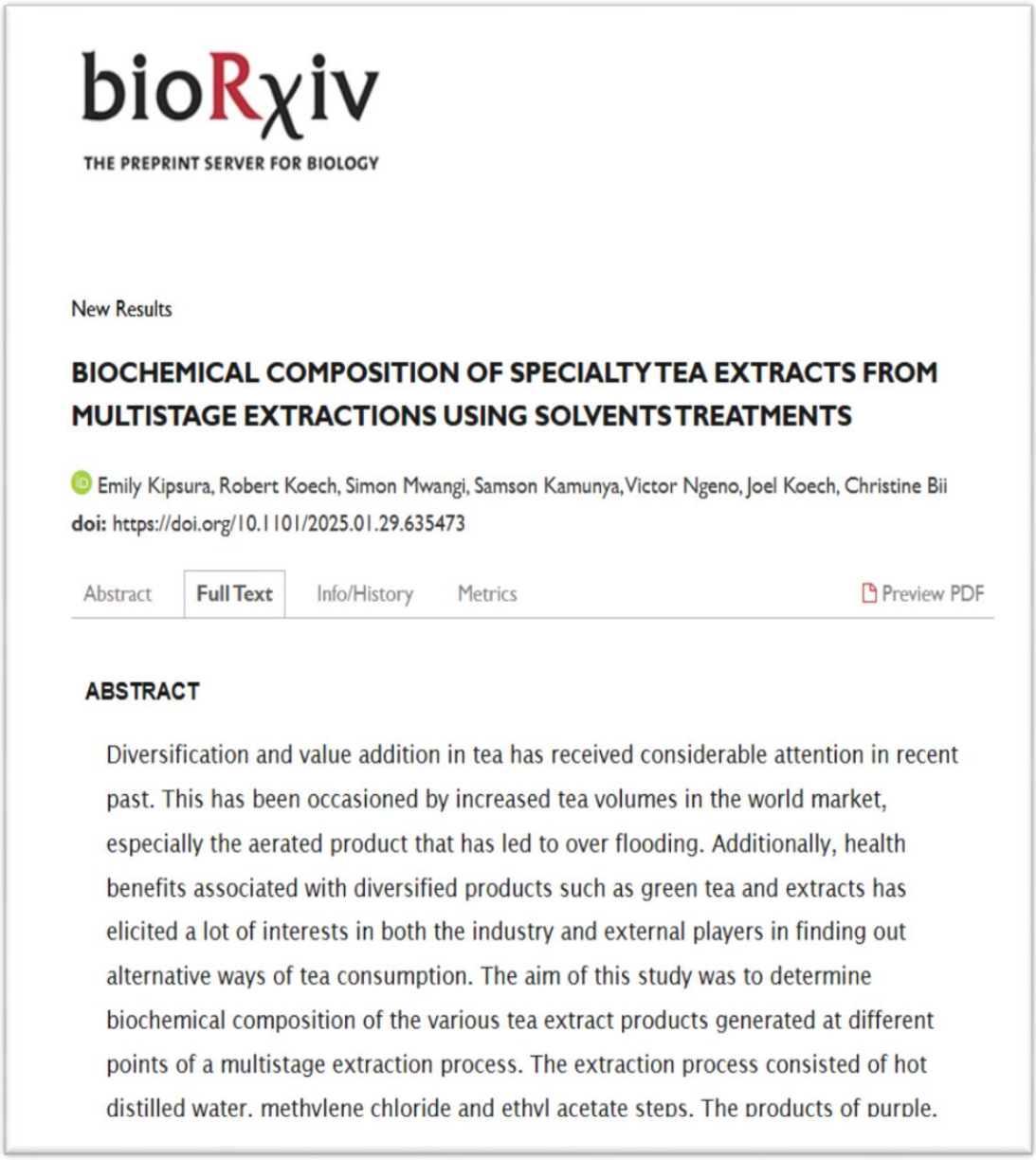
Appendix XIV: Publication



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BIOCHEMICAL COMPOSITION OF SPECIALTY TEA EXTRACTS FROM MULTISTAGE EXTRACTIONS USING SOLVENTS TREATMENTS

Emily Kipsura, Robert Koech, Simon Mwangi, Samson Kamunya, Victor Ngeno, Joel Koech, Christine Bii

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ABSTRACT

Diversification and value addition in tea has received considerable attention in recent past. This has been occasioned by increased tea volumes in the world market, especially the aerated product that has led to over flooding. Additionally, health benefits associated with diversified products such as green tea and extracts has elicited a lot of interests in both the industry and external players in finding out alternative ways of tea consumption. The aim of this study was to determine biochemical composition of the various tea extract products generated at different points of a multistage extraction process. The extraction process consisted of hot distilled water, methylene chloride and ethyl acetate steps. The products of purple,