IN VITRO STUDIES OF THE EFFECTS OF PURPLE TEA (*Camellia sinensis*) EXTRACTS ON SELECTED HUMAN CANCER CELL LINES AND MULTI-DRUG RESISTANT BACTERIA

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I56/83056/2015

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN MICROBIOLOGY

UNIVERSITY OF NAIROBI

2019

DECLARATION

This research project is my original work and has not been presented for a degree or any other award in any other University.

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DEDICATION

I dedicate this study to my parents David Koskei and Eunice Silbah Koskei, siblings and friends for their continuous support and encouragement. God bless you all.

ACKNOWLEDGEMENT

First, I acknowledge the Almighty God for giving me strength, guidance and good health throughout the research period. My sincere gratitude goes to the University of Nairobi especially my supervisors Prof. Francis B. Mwaura and Dr. Maina Wagacha for their invaluable guidance and support from the onset of research proposal to the final completion of this project.

Special thanks to the Kenya Medical Research Institute (KEMRI) for allowing me to conduct part of this study in their research laboratories. I am greatly indebted to Prof. Elijah Maritim Songok for the support and guidance offered throughout the research period.

My sincere gratitude goes to the University of Manitoba Queen Elizabeth II Jubilee Diamond Scholarship Foundation for the scholarship; and allowing me to conduct this study in their laboratories. Special thanks to Dr. Denice Bay and Dr. Gilbert Arthur for their guidance and invaluable assistance in ensuring that this study was done to acceptable standards and within the stipulated time.

I would also like to thank my family for the support, encouragement and assistance which they offered me throughout the study. I also extend my appreciation to my close friends for their moral support during the study period.

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LIST OF ABBREVIATIONS AND ACRONYMS

ACA: American Cancer Association

AICR: American Institute for Cancer Research

ATCC: American Type Culture Collection

CCR: Colorectal Cancer

CDC: Center for Disease Control

DDP: Cis-diamminedichloroplatinum

EC: Epicatechin

ECG: Epicatechin-3-gallate

EGC: Epigallocatechin

EGCG: Epigallocatechin-3-gallate

GTCs: Green Tea Catechins

HCCs: Hepatocellular Carcinomas

HPV: Human Papilloma Virus

IARC: International Agency for Research on Cancer

IC_{50}: Half Maximal Inhibitory Concentration

ICC: Intrahepatic Cholangiocarcinoma

IV: Intravenous

KNCO: Kenya Network of Cancer Organization

MBC: Minimum Inhibitory Concentration

McAb: Monoclonal Antibody

MDR: Multidrug Resistant

MIC: Minimum Inhibitory Concentration

MMPs: Metalloproteinases

MPHS: Ministry Of Public Health and Sanitation

MRSA: Methicilin Resistant *Staphylococcus aureus*

NCI: National Cancer Institute

NHPs: Natural Health Products

NPs: Natural Products

VAP: Ventilator Associated Pneumonia

WHO: World Health Organization

ABSTRACT

The objective of this study was to determine the anti-proliferative properties of crude extracts of purple tea on breast cancer (JIMT1), cervical cancer (HeLa), prostate cancer (PC3), liver cancer (HepG2) and ovarian cancer (A2780 cisplatin sensitive and resistant) cell lines; and to determine the anti-bacterial properties on multi-drug resistant strains of *Klebsiella pneumonia* DSM 26371, *Pseudomonas aeruginosa* DSM 102274, *Escherichia coli* DSM 22311, *Shigella sonnei* DSM 25715, *Staphylococcus aureus* DSM 102265 and *Acinetobacter baumannii* DSM 105126. Purple tea (*Camellia sinensis)* leaf samples were obtained from Tumoi Tea farm in Nandi County. Specific phytochemical tests were performed to screen aqueous and organic extracts of purple *Camellia sinensis* leaves for the presence of various bioactive compounds. The phytochemical screening revealed the presence of phenols, alkaloids, steroids, flavonoids and tannins in all extracts except those of ethyl acetate. Aqueous and ethanol extracts contained the highest number of bioactive compounds and were therefore selected for further studies. Anti-proliferative assay performed indicated that aqueous extract inhibited 50% of the total cancer cells in the following decreasing order: $A2780_{s,}$ JIMT1, $A2780_{cp,}$ HeLa, PC3 and HepG2. Ethanol extracts also showed inhibitory effects in the decreasing order: $A2780_{s,}$ $A2780_{cp,}$ JIMT1, PC3, HeLa and HepG2. Both aqueous and ethanol extracts exhibited highest anti-proliferative activity against $A2780_s$ ovarian cancer cell line with IC_{50} values of 36.84µg/ml and 56.54µg/ml, respectively. Both aqueous and ethanol extracts also showed higher activities against A2780cp ovarian cancer cell and JIMT1 breast cancer cell with IC_{50} values of 75.97µg/ml and 93.52µg/ml; and 72.09µg/ml and 116.73µg/ml, respectively. Aqueous and ethanol extracts showed lowest anti-proliferative activity against HepG2 liver cancer cell lines with IC_{50} values of $1.4*10^4$µg/ml and 463.6µg/ml, respectively. However, ethanol extract enhanced the growth of HeLa, PC3 and HepG2 cancer cells at concentrations of 0-125 µg/ml, 0-100 µg/ml and 0-150 µg/ml, respectively, before showing its inhibitory effect. Aqueous extract completely inhibited $A2780_s$, $A2780_{cp}$ and JIMT1 cancer cell lines at concentrations of 75µg/ml, 200µg/ml and 125µg/ml, respectively. Complete cell inhibition was also exhibited by ethanol extract on $A2780_s$ and $A2780_{cp}$ at concentrations of 100µg/ml and 200µg/ml. For antibacterial susceptibility test, micro-broth serial dilution and spot plating methods were used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) after incubation at 37°C for 24 h, respectively. All multi-drug resistant strains of bacteria tested were sensitive to aqueous purple tea extract with MIC values

ranging from 0.0064 mg/ml to 6.4 mg/ml and MBC values ranging from 0.0064 mg/ml to 12.8 mg/ml. The highest antimicrobial activity was recorded against methicillin resistant-*Staphylococcus aureus* (0.0064mg/ml-0.032mg/ml), followed by *Shigella sonnei* and *Pseudomonas aeruginosa* (1.6 mg/ml- 3.2 mg/ml). These results show that both aqueous and ethanol extracts of purple *Camellia sinensis* have various bioactive compounds with varying degrees of anti-proliferative and antimicrobial activities; and may be a promising source of new anticancer and antibacterial agents for treatment of various types of cancer and infections caused by multidrug resistant bacteria, respectively. Therefore, further studies should aim at isolating individual bioactive compounds and determination of the most active compounds so as to maximize their potential use as chemotherapeutics.

CHAPTER ONE: INTRODUCTION

1.1 Background of the study

Cancer remains one of the most dreaded diseases and major cause of deaths globally (Jemal *et al.,* 2009). Every year, tens of millions of people are diagnosed with cancer worldwide, and over a half of the patients ultimately die from it (Ma *et al.,* 2006). In developing countries, cancer is rated as the 3rd leading cause of death after infectious and cardiovascular diseases (WHO, 2013) with infection-related cancers being predominant (Nagai *et al.,* 2017). According to Globocan, it is predicted that by 2020, between 15 and 17 million new cases of cancer will be reported yearly; and 60% of the cases will be from developing countries (Lopez-Gomez *et al.,* 2013). The economic effect of cancer is hefty and is rising. The total yearly economic cost of cancer globally in 2010 was projected at about US$ 1.16 trillion (WHO, 2018).

Cancer is a disease where typical body cells transform into tumour cells in a several stage process that mainly develops from a pre-cancerous lesion to a malignant tumour. These alterations occur as a consequence of the interaction between an individual's genetic factors and three sets of outside agents, comprising of: physical carcinogens, for example ultraviolet and ionizing radiation; chemical carcinogens, for instance asbestos, constituents of tobacco smoke, aflatoxin (a food contaminant), and arsenic (a drinking water contaminant); and biological carcinogens, like infections from particular viruses, bacteria, or parasites (WHO, 2018). An estimated total number of 1.9 million (17.8%) of worldwide cancer cases were associated with different infectious agents in 2002 (Parkin *et al.,* 2006); *Helicobacter pylori* infection is well-known to raise the risk of developing stomach cancer (Uemura *et al.,* 2001). The link between socioeconomic status and acquiring *H. pylori* infection has been supported in numerous studies. From published data, the incidence of *H. pylori* infections among children from developed and developing countries vary from 8.9 percent to 72.8%, respectively; the re-infection rate is also considerably higher in the latter (Magalhaes *et al.,* 2006).

Several characteristics displayed by cancer cells have been documented which involve; genetic and molecular modifications such as transformation, deregulation of apoptosis, proliferation, invasion angiogenesis, and metastasis. Apoptosis is defined as a programmed cell death which occurs in normal cells through a series of molecular steps. Angiogenesis is blood vessels being

created a fresh from pre-existing blood vessels, and metastasis is when cancer cells spread from where they were first to another body part (Fimognari *et al.,* 2011). Other factors such as infectious agents, nutrition, lifestyle, genetics and environment are reported to play a vital part in progression of cancer (Ma *et al.,* 2006). According to a review done by Hanahan and Weinberg (2000), there are six basic defects that occur in a normal cell leading to their transitioning into cancerous phenotypes and promoting growth of the transformed cells. These defects are normally caused by aberrant signaling cascades involving numerous molecules and can be targeted by chemo-preventive agents which can either prevent the mutagenic initiation of the carcinogenic process or further progression of lesions already formed (Hanahan *et al.,* 2000).

In 2015, WHO reported that since time immemorial human beings have been utilizing plant products to treat various diseases (WHO, 2015). In developing countries, many millions of people living in rural areas use herbal medicine, traditional treatment and traditional herbalist as the main source of health care (WHO, 2015). Presently, a lot of research is being done for discovery of antitumor and antibacterial agents from natural products. Edible plants are to a greater extent being considered as sources of anticancer and antibacterial drugs that can not only improve the efficacy of conventional medicine but reduce side effects of chemotherapy and strengthen the immune system to battle cancer and bacterial infections (Koh *et al.,* 2015).

1.2 Problem statement

Every year, tens of millions of people are diagnosed with cancer worldwide, and more than half of the patients in the long run die from it (Ma *et al.,* 2006). In Kenya incidences of cancer have been rising at an alarming rate. Recent reports indicate that cancer is the third primary cause of deaths after infectious and cardiovascular diseases (KNCO, 2011- 2016). Annually, it is estimated that about 28,000 incidences of cancer and over 22,000 mortality cases occur (Afolayan, 2004). Over 60% of those affected are in their productive ages (below 70 years) and the risk of getting cancer below 75 years is 14% and that of dying is about 12% (KNCO, 2011-2016). Among women in Kenya, breast cancer and cervical cancer are the most common accounting for 23% of all cancer cases recorded and 25 per 100,000 people respectively. On the other hand, prostate cancer is the commonly known type of cancer afflicting men, comprising of 15.6% of all the cases of cancer observed. Leukaemia (Blood cancer) and lymphomas are most

common among children (Korir *et al.,* 2015). Cancer of the digestive tract such as that of the liver and reproductive tract such as ovarian cancer has also been noted to be on the rise (Korir *et al.,* 2015).

Currently, the available techniques for cancer treatment involve surgical procedure, radiation, chemotherapy, and /or bone marrow or blood stem cells transplant. However, reports indicate that each of these treatment techniques weakens the immune system and leaves the patients vulnerable to infections more than healthy individuals of similar age (Gudiol *et al.,* 2014) or suffer from side effects such as resistant tumors, appetite loss, nausea and vomiting, fatigue, sore mouth, loss of hair, weight gain, early menopause, bleeding, and diarrhea (Esmaeili-Mahani *et al.,* 2014). Bacterial infections, especially blood stream infections are some of the infections that cancer patients suffer from as a result of weakened immune system (Gudiol *et al.,* 2014). In recent years, the occurrence of antimicrobial resistance has become a major concern globally, and poses serious threats to every person, but cancer patients are at a specific risk (Gudiol *et al.,* 2014), because with no functioning antimicrobials for infections' prevention and treatment, use of the currently available techniques in cancer treatment will be very risky as the patients may die as a result of infections by these antibiotic resistant bacteria (WHO, 2017).

1.3 Justification

Regardless of developments in diagnosis, surgical procedures, patient care, and adjuvant treatments, patients still face a lot of challenges in Kenya because cancer services are very limited and available only in the capital city, Nairobi and large cities. Cancer patients have to travel from across the country to these cities so as to access treatment. To make matters worse only one public and one private health facility provide radiotherapy services in the country with a capacity to handle 3,800 patients in a year out (Korir *et al.,* 2015). Therefore, patients referred from other hospitals have to wait for several months before being attended to leading to the progression of most types of cancer into stage four (Korir *et al.,* 2015). In spite of the accessibility and affordability of cancer screening services (CDC, 2012), a disproportionate number of individuals especially in Kenya do not go for screening or further treatment because of limited or no access to health insurance to cater for the high cost of treatment and as a result suffer higher rates of deaths (Edwards, 2010).

Naturally occurring phytochemicals present in human diet have been reported as potent sources of chemo-preventive agents (Artun *et al.,* 2017). Furthermore, medicinal plants have been recognized as possible sources of novel compounds of therapeutic value that can play a crucial part in drug design and development (Bisi-Johnson *et al.,* 2017). Research shows that approximately 30% of cancer deaths are accounted for by diet, which is comparable to the figure accounted for by smoking (AICR, 1997). A favorable approach to averting the development of cancer and microbial infections, therefore, involves change in dietary habits to involve regular consumption of foods of plant origin containing anticancer and anti-inflammatory phytochemicals (Béliveau *et al.,* 2007). Consumption of tea has been attributed to many health benefits including; anti-cancer (Lecumberri *et al.,* 2013), and anti-microbial capabilities (Bancirova, 2010). Therefore, this study aimed at investigating the anticancer and antimicrobial activities of purple *C. sinensis* (purple tea) against a range of selected human cancer cell lines and pathogenic bacteria including antibiotic resistant bacterial strains.

1.4 Research questions

i. Do purple tea extracts have any phytochemical constituents with anti-proliferative and anti-bacterial activities?
ii. Are the purple tea extracts active against the selected cancer cell lines?
iii. Are the purple tea extracts active against the selected antibiotic resistant bacteria?

1.5 Objectives

1.5.1 Broad objective

The broad objective of this study was to evaluate the anti-proliferative and anti-bacterial properties of purple tea's crude extracts on selected cancer cell lines and antibiotic resistant bacteria.

1.5.2 Specific objectives

The specific objectives of this study were:

i. To determine the phytochemical constituents present in the aqueous, methanol, ethanol and ethyl acetate crude extracts of purple tea.

ii. To determine the anti-proliferative properties of purple tea's aqueous and ethanol crude extracts on breast, prostate, cervical, ovarian and liver cancer cell lines.

iii. To determine the anti-bacterial properties of purple tea's aqueous crude extracts on multi-drug resistant strains of *Klebsiella pneumonia*, *Pseudomonas aeruginosa, Escherichia coli*, *Shigella sonnei*, *Staphylococcus aureus* and *Acinetobacter baumannii*.

CHAPTER TWO: LITERATURE REVIEW

2.1 Definition and risk factors of cancer

According to WHO (2018), cancer can be defined as a disease where normal cells grow rapidly into abnormal cells that propagate past their typical confines, and which can then enter adjacent body parts and disseminate to other organs by a mode known as metastasis. Metastases are a key cause of deaths from cancer (WHO, 2018). Several habits or lifestyles usually adopted in life are documented as part of the risk factors for the development of numerous types of cancer. Basic risk elements for cancer development consist of eating a diet rich in saturated fat and low in fresh fruit and vegetables, lack of physical exercises, usage of tobacco and consuming alcohol particularly in extreme amounts (National Cancer Institute, 2007; Unwin and Alberti, 2006). Particular known risk factors for development of various types of cancers consist of tobacco usage encompassing chewing and smoking tobacco for lung cancers, bladder, mouth, cervix, pancreas, kidney, oesophagus and larynx; bleaching of skin for skin cancer; consumption of alcohol for liver, oesophagus, breast, mouth and throat cancers; poor diet specifically low fibre and high fat diets for colon, prostate and uterus cancers; contact with harmful chemicals or radiation for leukaemia, and breast, thyroid, skin, lung and stomach cancers.

Some bacteria and viruses are also documented as risk factors for cancer, for instance hepatitis B or C for liver cancer, human papilloma virus for cervical cancer, human T-cell leukaemia/lymphoma virus for leukaemia or lymphoma, and *Helicobacter pylori* for stomach cancer; use of oestrogens for hormone replacement therapy may raise the chances of developing cancer of breast; and genetic pre-dispositions in cases such as ovary, breast, colon and prostate and melanomas cancers (National Cancer Institute, 2007). Use of hard drugs are also associated with development of cancer e.g. lung cancer and marijuana use (Han *et al.,* 2010), amphetamines, cocaine and non-Hodgkin's Lymphoma (Nelson *et al.,* 1997). Use of marijuana and cocaine by parents considerably raises the risk of their children developing rhabdomyosarcoma (Grufferman *et al.,* 1993).

2.2 Types of cancer

Cancers are named according to the site where they start to develop and the type of cells they are made of, even if they metastasize to other body parts. For example, a cancer that starts in the

lungs and metastasizes to the liver is still called lung cancer (WHO, 2018). The types of cancers focused on in this research study are reviewed in subsequent sections.

2.2.1 Breast cancer

Among women all over the world, breast cancer is the most commonly diagnosed type of cancer and primary cause of deaths, accounting for 23% of the total cancer cases and 14% of the cancer deaths (Jemal *et al.,* 2011). In less developed countries, women are less than half likely to develop breast cancer by the age of 75 years compared to those from more developed countries (WHO, 2015). According to statistics reported by Kenyan Network of Cancer Organizations (KNCO), in 2013, cancer of breast was the top type of cancer in women, with 34 out of 100,000 women suffering from breast cancer. Currently, the major challenges faced involve late detection, diagnosis, and treatment of breast cancer (Esmaeili-Mahani *et al.,* 2014). The techniques used for breast cancer treatments involve surgery, chemotherapy, and/or radiotherapy. Regrettably, some patients develop resistant tumors, appetite lose, nausea and vomiting, general body weakness, sore mouth, loss of hair, weight gain, early menopause, lowered immunity, bleeding, and diarrhea (Esmaeili-Mahani *et al.,* 2014). Therefore, there is a renewed interest in finding new and effective therapeutic agents against breast cancer from plants used as foods, vegetables, fruits, or spices, rich in bio-nutrients or bioactive phytochemicals ((Esmaeili-Mahani *et al.,* 2014).

2.2.2 Cervical cancer

Cervical cancer is the most frequent genital malignancy in women and is ranked the 2nd top cause of deaths after breast cancer worldwide (Geetha *et al.,* 2013). Approximately half a million new cases are reported yearly and most of which occur in developing countries (Ertem, 2009). According to Nairobi Cancer Registry, cervical cancer accounts for 21% of cancers among women (Korir *et al.,* 2015), causing about 2,500 deaths annually in Kenya (Ferlay *et al.,* 2012). Over the past years, numerous approaches have been developed for clinical use and a number of new anticancer agents introduced (Geetha *et al.,* 2013). Naturally occurring phytochemicals found in human diet have been reported as potent sources of chemo preventive agents (Artun *et al.,* 2017). Eating a lot of vegetables and fruits has also for a long time been linked to prevention of cancer development (Rao, *et al.,* 2004). However, the main challenge associated with current

anticancer agents is lack of specificity as they also kill normal cells (Geetha and Santhy, 2013). Therefore, the search for new, safe, economic and site-specific anticancer drugs continues.

2.2.3 Ovarian cancer

Ovarian cancer is the most deadly gynaecological malignancy responsible for approximately 4% of all female cancer cases and the 6th most frequently occurring cancer (Sak, 2015). It is ranked as the 5th top reason for cancer deaths in women universally (Oronsky *et al.,* 2017). The age-adjusted prevalence of ovarian cancer in Kenya is 40.1 per 100,000 and the mortality is 21.8 per 100,000 women (Rosen *et al.,* 2017). Failure to detect ovarian cancer at an early stage is the primary cause of this high mortality rate. Difficulties are faced in detection because of lack of effective screening procedures and specific signs and symptoms at the initial stages of the disease (Chen *et al.,* 2013). Surgical cyto-reduction followed by combination of chemotherapy using taxane (paclitaxel) and platinum (cisplatin) are currently used in managing ovarian cancer. However, 70-80% of the patients respond to the first-line chemotherapy, but over 80% of them will recur within two years with chemo resistant phenotype and ultimately die of it. Emergence of chemoresistance is a key challenge to effective treatment of recurrent ovarian cancer (Sak, 2015). Therefore, research focusing on finding new treatments to overcome chemoresistance and natural drugs with low toxicity to healthy cells is of great interest to researchers.

2.2.4 Prostate cancer

Cancer of the prostate is among the most common cancers afflicting men and a main reason for cancer-associated deaths globally (Gan *et al.,* 2017). It is the 2nd top reason for cancer deaths in males after lung cancer (Qian *et al.,* 2017). In Kenya, cancer of the prostate is the most common cancer type afflicting men, comprising of 15.6% of all the cases of cancer observed in Kenya (MPHS, 2011). Age is reported as a key contributing factor in the development of cancer of the prostate, since men suffering from cancer of the prostate are between 65 and 80 years, but is infrequent in men below 40 years (Alotaibi *et al.,* 2017). Genetics is the other contributing factor, as documented data indicate that men of African-American origin are at a considerably greater risk of getting prostate cancer than white men and is ranked the 4th common reason for deaths in African-American men (Alotaibi *et al.,* 2017). Of nineteen per cent of black men, (1 in 5) with prostate cancer, about 5% will die from this disease (Jazayeri *et al.,* 2017). Several

epidemiological studies show an inverse link between great intake of fruits and/or vegetables fruits and cancer occurrences (Askari *et al.,* 2014). Some studies show that diet high in products of tomato and lycopene have a preventive result against cancer of the prostate (Etminan *et al.,* 2004), however, contradictory findings have also been reported (Wilson *et al.,* 2012). These therefore explain why the search for new anticancer agents from naturally occurring bioactive compounds in plants has been a great area of interest to scientists.

2.2.5 Liver cancer

Cancer of the liver is the 5th type of cancer, responsible for 9.1% of all deaths connected with cancer globally (Wong *et al.,* 2017). Most liver cancer cases (83%) were reported in less developed countries in 2012 with several (75-90%) of primary cancers of the liver cancers being hepatocellular carcinomas (HCCs), with intrahepatic cholangiocarcinoma (ICC) responsible for most of the other cancer subtypes (Center *et al.,* 2011). Nearly 70-80% of HCCs grow in patients suffering from liver cirrhosis (Sakamaki *et al.,* 2017). Hepatocellular carcinomas mainly develop as a result of chronic viral hepatitis B or C infection, iron superfluous, aflatoxin exposure, obesity, alcohol-related cirrhosis and probably non-alcoholic fatty liver disease (Koh *et al.,* 2015). In 2011, a report by the Ministry of Public Health and Sanitation in Kenya, noted that cases of liver cancer were on the rise and could be traced to Hepatitis B and C viruses (Korir *et al.,* 2015). Common treatments for liver cancer involve surgery, chemotherapy, and/or radiotherapy but severe side effects and toxicity to adjacent healthy tissues have been reported. Therefore, current research mainly focuses on finding alternative therapeutic agents from plant sources that are selective with minimal side effects (Koh *et al.,* 2015).

2.2.6 Colorectal cancer

Cancer of the colon commonly known as Colorectal cancer (CCR) is reported globally as the 3rd most common type of cancer globally affecting men and women, the 2nd principal reason for cancer related deaths, and the leading reason for gastrointestinal cancer-linked deaths. The threat of suffering from this cancer is associated with intestinal inflammatory disease, bad alimentary habits, smoking, cysts, aging and genetic factors. About 90% of the patients suffering from colorectal cancer are more than 50 years of age, with a median age of 64 years; although, the disease is graver in patients who develop this disease at younger years (Granados-Romero *et al.,*

2017). According to the ACA (American Cancer Association), colorectal cancer was responsible for more than 49,700 deaths in the United States in 2015. Early diagnosis and treatment are some of the aims to decrease the mortality. Currently, a patient's prognosis is predicted using the survival rate. Diagnosis of a closest relative with colorectal cancer or colonic polyps earlier than the age of 60, or diagnosis of 2 or more closest relatives with cancer or polyps at whichever age, make the patient to be considered to have a positive familial history. Several methods have been devised for identifying colorectal cancer, for instance DNA stool test, immunochemical test of stool, the guaiac test, colonoscopy, barium enema and sigmoidoscopy (Granados-Romero *et al.*, 2017).

2.3 Global burden of cancer

Despite advances in cancer treatment, there are a lot of problems faced in the prevention, early detection, diagnosis, treatment and palliation resulting in high cancer figures (Sloan *et al.*, 2007). From various cancer statistics, cancer has had major impacts on societies worldwide. In 2012, cancer was ranked among the primary causes of mortality and morbidity universally, with roughly 8.2 million cancer related deaths and 14 million new cases; and the sum of new cases in the next two decades predicted to rise to 22 million (NCI, 2012). The most common cancers diagnosed amongst men are prostate, liver, stomach, colorectal and lung cancer and in women, ovarian, breast, cervix, colorectal, stomach and lung cancer (WHO, 2012). In the same year in Africa 847, 000 new cases of cancer (which is 6% of the world total) and 591,000 related deaths (approximately 7.2% of the world total) in the 54 African nations were reported (Parkin, 2014). Whereas the cancer profiles frequently vary distinctly from one region to the other, liver, Kaposi sarcoma and prostate cancer were the most common type of cancers afflicting men and breast cancer and cervical cancer in women (Parkin, 2014). The dramatic increase in figures indicates that an immediate action has to be taken in order to curb this disaster (WHO, 2014).

2.4 Burden of cancer in Kenya

In Kenya, cancer disease is ranked the 3rd cause of deaths after infectious and cardiovascular disease (MPHS annual report, 2011). In 2005, approximately 18,000 deaths were due to cancer and most of the people who died were in their productive years (below 70 years) (MPHS annual report, 2011). Amongst women in Kenya, cancer of the breast and cervical cancer have been

described as the most common accounting for 23% of all cancer cases recorded and 25 per 100,000 people respectively. The age-adjusted occurrence of ovarian cancer in Kenya was reported to be 40.1 per 100,000 and the mortality is 21.8 per 100,000 women (Rosen *et al.,* 2017). On the other hand, cancer of the prostate is the commonest type of cancer afflicting males, comprising of 15.6% of all the cases of cancer observed in Kenya. Leukaemia (Blood cancer) and lymphomas are most common among children. Cancer of the digestive tract such as that of the liver has also been noted to be on the rise (Korir *et al.,* 2015).

In the recent past, incidences of cancer have been on the rise and the government of Kenya has identified provision of cancer services as priority area needing urgent intervention (MPHS annual report, 2011). This rise has greatly been aggravated by ill-equipped and limited cancer health care systems, high cost of treatment, limited specialized cancer personnel, poor legal framework to address cancer prevention, low level of public awareness and insufficient cancer research in Kenya (MPHS annual report, 2011). Currently, the available techniques for cancer treatment, involve use of chemotherapy, radiotherapy and surgery, which are costly and may subject the patients to severe side effects such as hair loss, thrombocytopenia, lack of appetite, anemia, peripheral neuropathy, cardiac damage among others (Miller *et al.,* 2016). Regardless of advancements in diagnosis, patient care, surgical procedures, and adjuvant treatments, cancer metastasis resistant to conventional treatments remain a significant cause of mortality and morbidity of hospitalized patients (Gupta *et al,* 2006). Therefore, these two major drawbacks in cancer treatment; resistance to conventional therapies and negative side effects have stimulated a lot of research towards finding a safe and better way of cancer treatment and management (Chen *et al.,* 2014).

2.5 Role of bacteria in cancer development and treatment

Traditionally, infections caused by bacteria have not been reported as major causes of cancer (Correa, 1997). Though, lately, there is an increasing link between bacterial infections and cancer development by means of two modes: production of carcinogenic bacterial metabolites and induction of chronic inflammation (Chang and Parsonnet, 2010). Several bacteria have been implicated as oncogenic agent, however, *H. pylori* was the first bacterium designated as a sure cause of cancer in humans by the IARC (International Agency for Research on Cancer). As

cancer continues its ascent as the top reason for deaths in advanced nations, it is paramount to understand the lasting effects of bacteria as a probable means of preventing the growth of cancer (Chang and Parsonnet, 2010).

2.5.1 Gastric cancer and *Helicobacter pylori*

Helicobacter pylori, inhabits the gut of about 50% of the total human's population globally. It is a Gram-negative with a spiral rod shape. In 1994, the IARC (International Agency for Research on Cancer) reported *H. pylori* as a certain bacterial oncogenic agent in human due to the vast confirmation linking its infection and cancer (IRCA, 1994). Over 60% of the total stomach cancer cases are due to *H. pylori* infections corresponding to more than 5.5% of all cancers in the world (Parkin, 2006). A group study conducted in Japan to assess both diffuse-type and intestinal-type cancers showed that only *H. pylori* patients suffered from stomach cancer, indicating an infinite risk proportion (Uemura *et al.*, 2001). Chronic *H. pylori* infections are characterized by chronic inflammation. The development of atrophic gastritis, dysplasia, intestinal metaplasia, and chronic gastritis, results in the growth of gastric adenocarcinoma. Patients with serious atrophic gastritis together with intestinal metaplasia develop intestinal-gastric cancer. Atrophic gastritis is caused by *H. pylori* infection (Park *et al.*, 2015). A study done by Hansson *et al.*, (1996) displayed a link between gastric ulcer and high risk of gastric cancer. Patients with gastritis ulcers suffered from corpus-predominant gastritis and atropic gastritis which progressed to gastric cancer. *Helicobacter pylori* infection treatment may reduce or prevent precancerous lesions but may not cause any change in more advanced lesions (De Vries *et al.*, 2009). The practice of vaccination in children to prevent *H. pylori* infections is proposed as a way to avert cancer of the gastric (Agarwal, 2008). However, for advanced lesion, it is necessary to come up with a treatment option that has both anti-cancer and antibiotic effect.

2.5.2 *Escherichia coli* and *Staphylococcus aureus* in breast cancer development

Enterobacteriaceae and *Staphylococcus* are two taxa found in greater quantity in breast cancer patients than in healthy controls. In 2014, Urbaniak *et al.*, conducted a study using tissue samples obtained from 71 women: 45 had cancerous tumors, 13 noncancerous tumors, and 23 without tumors. An examination of breast tissues surrounding the tumors was conducted and bacteria linked with the tissues were amplified and assessement done using 16S rRNA gene sequencing.

The results obtained showed that breast cancer patients had greater amounts of *Staphylococcus aureus* and *Escherichia coli*, whereas the women with no cancer exhibited greater number of *Streptococcus* species and *Lactobacillus*, among other differences. Certain strain isolates of *E. coli* harboured a *pks* pathogenicity island in their genome, which are associated with some types of colon cancer because they can cause double-stranded DNA breaks in the neighbouring host cells. A high quantity of DNA breaks also raise chances for a not well repaired break increasing the chances for development of a cancerous cell as a result of mutations (Urbaniak *et al.*, 2014). Further research needs to be done to establish the link between *S. aureus* and *E. coli* mechanisms of action and breast cancer development since there is little documented evidence.

2.6 Antibiotic resistance and cancer treatment

The most common complications in patients suffering from cancer and hematopoietic stem cell transplant receivers are bacterial infections. In modern years, the occurrence of antimicrobial resistance has become a major problem globally, and poses a threat to every person, but cancer patients are at a greater risk (Gudiol *et al.*, 2014). Currently, the available techniques for cancer treatment involve surgical procedure, radiation, chemotherapy, and /or bone marrow or blood stem cells transplant. All these treatment techniques, however, weakens the immune system and leaves the patients vulnerable to infections more than healthy individuals of a similar age (Gudiol *et al.*, 2014). Without access to effective bacterial infection treatments, the use of these techniques in cancer management and treatment would not have been possible. However, with the rise of antibiotic resistance terrible impediments, economically, socially, and medically may occur, lest actual and exceptional universally coordinated actions are promptly taken (Laxminarayan *et al.*, 2013).

In February 2017, the WHO released its major list of antimicrobial resistant pathogens consisting of twelve families of bacteria which are a great threat to human health (WHO, 2017). The WHO report noted with concerns that new modes of antibiotic resistance are evolving and disseminating universally, threatening the ability to cure common infectious diseases, leading to persistent sickness, disability, and demise (WHO, 2017). The report further explained that with no functioning antimicrobials for infections prevention and treatment, critical medical techniques such as cancer chemotherapy, radiotherapy and organ transplantation, major surgery, for instance

caesarean sections or hip replacements and diabetes management become extremely risky (WHO, 2017).

2.6.1 Bacteria belonging to the family Enterobacteriaceae

2.6.1.1 *Escherichia coli*

E. coli is carbapenem-resistant and ESBL-producing bacteria (CRE). It was mentioned in the WHO list under the critical category of pathogens requiring more investigation and development of alternative antibiotics. This bacterium is gram-negative and non-spore forming. It has become resistant to the available medicines for cure of infections of the urinary tract such as fluoroquinolone. In some nations worldwide, this antibiotic is ineffectual in over half of the patients (WHO, 2017).

2.6.1.2 *Klebsiella pneumonia*

Klebsiella pneumoniae is a gram-negative bacterium that is broadly disseminated in the environment and is greatly reported as a causative agent for invasive infections in hospital settings, mostly in immune-compromised patients such as cancer patients (Wyres and Holt, 2016). Antibiotic resistance in *K. pneumonia*e is rising, resistance to carbapenemases and beta-lactamases is of particular interest and has been well-characterized as aggravating the threat of infection (Lee *et al.,* 2016). In 2017, WHO listed *K. pneumonia* as one of the critical priority pathogens requiring search for new antimicrobial agents (WHO, 2017).

2.6.1.3 *Shigella sonnei*

Shigella species is a gram-negative bacterium that causes shigellosis. *Shigella sonnei* strain biotype G is resistant to six classes of antibiotics, including ciprofloxacin, cotrimoxazole, ampicillin/amoxicillin and azithromycin (Puzari *et al.,* 2017). This means that there is no recommended oral antibiotic available for these infections and intravenous (IV) antibiotics (through the vein), provided in the hospital is recommended instead (Puzari *et al.*, 2017). In the WHO listing, it was categorized under the ‘medium’ priority pathogens requiring new antimicrobial agents (WHO, 2017).

2.6.2 *Staphylococcus aureus*

Staphylococcus aureus, vancomycin-intermediate and methicillin-resistant belonging to the family Staphylococcaceae was listed under the high priority category of pathogens requiring the development of alternative antibiotics. This bacterium is a gram positive and coccoid in shape. It is widespread and a regular cause of infections in hospitals and the community. It is estimated that patients with MRSA are 60% most likely to die than patients with a non-resistant infection (WHO, 2017).

2.6.3 *Helicobacter pylori*

Clarithromycin-resistant-*Helicobacter pylori* belonging to the family Helicobacteracae, was listed under the high priority category of pathogens requiring development of new antimicrobial agents (WHO, 2017). This bacterium is a gram-negative, spiral, rod shaped organism. It infects and inhabits 50% of the total population worldwide and was the first bacterium to be named as definite human oncogenic agent due to evidence linking it's infection with stomach cancer (IRCA, 1994).

2.6.4 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa belongs to the family Pseudomonadaceae. It is a common gram-negative bacterium associated with nosocomial and health-associated infections (HAIs) in hospitalized patients (Raman *et al.*, 2018). The WHO listed carbapenem-resistant *P. aeruginosa* as a critical priority pathogen that urgently need novel treatment options (WHO, 2017). Rising incidences of multidrug-resistant (MDR) *P. aeruginosa* in HAIs and among hospitalized patients is a great public health concern (Raman *et al.,* 2018). Poor outcomes including high resource use and costs, morbidity, and mortality are linked to MDR *P. aeruginosa* infections in the healthcare settings (Raman *et al.*, 2018).

2.6.5 *Acinetobacter baumannii*

Acinetobacter baumannii belonging to the family Moraxellaceae, is a gram-negative nosocomial pathogen that causes severe infections such as ventilator-associated-pneumonia (VAP), bloodstream infection, in addition to wound and urinary tract infection, meningitis and soft tissue infection in ICU settings. This bacterium is linked to high mortality, prolonged length of ICU

and hospital stay, extended mechanical ventilation and therefore greater overall costs (Nowak *et al.,* 2017). In 2017, the WHO placed *A. baumannii* as a critical priority pathogen that urgently need new antimicrobials (WHO, 2017).

2.7 Overview on conventional drugs/antitumor agents used in cancer treatment

According to WHO, cancer is the 2nd top reason for deaths universally, and is estimated to account for about 9.6 million deaths in 2018. Internationally, roughly 1 in 6 deaths is as a result of cancer (WHO, 2018). Currently, the available techniques for cancer treatment involve use of conventional anticancer drugs, surgical procedure, radiation, chemotherapy, and /or bone marrow or blood stem cells transplant which present major shortcomings such as weak immune system and lack of solubility which limit their usage in treatment of cancer (Gudiol *et al.,* 2014). Discoveries of chemotherapeutic agents, alongside the extraordinary scientific and technological advances have permitted understanding of cell biology of human cancer cells and thus the occurrence of targeted therapy. Though the use of targeted therapy drugs have had exceptional successes in particular types of cancer, new therapies are unlikely to substitute cytotoxic agents in the predictable future (Dos Santos *et al.,* 2013). Hence, the continued search for new safe, effective and readily available chemotherapeutic agents. Some of the conventional drugs or antitumor agents used in cancer treatment include the following:

2.7.1 Cisplastin

Cisplatin (cis-diamminedichloroplatinum, DDP) is one of the successfully and extensively applied chemotherapeutic agents for solid tumors treatment. Being a platinum-based compound that forms intra- and inter-strand adducts with DNA, it induces cell cycle arrest and apoptosis in several cancers (Cohen *et al.,* 2001). Regrettably, several patients suffering from these malignancies ultimately relapse and become drug resistant (refractory) to chemotherapy. The resistance is either intrinsically, for instance, witnessed in patients with lung, prostate and colorectal cancer or develops as a result of cisplatin chemotherapy as frequently observed in ovarian cancer patients (Rabik *et al.,* 2007). Cancer cells develop resistance to cisplastin through various ways which include (a) changes in drug transport causing low intracellular cisplatin buildup, (b) an heightened drug detoxification system resulting from increased amounts of intracellular scavengers such as metallothioneins and/or glutathione (c) alterations in DNA repair

including amplified nucleotide excision repair, inter-strand crosslink repair or loss of mismatch repair, (d) alterations in DNA damage tolerance mechanisms, and lastly (e) alterations in the apoptotic cell death pathways (Huang *et al.,* 2017). In lung cancer lines, the level of Gluthatione-S-Transferase-π isoenzyme expression is considerably linked to intrinsic resistance to cisplatin (Huang *et al.,* 2017).

2.7.2 Taxanes

Taxanes are reported as part of the most potent classes of compounds used to treat cancer. The tubulin/microtubule complex is confirmed as a clinically valuable antitumor target. Examples of chemotherapeutics that work through perturbation of tubulin polymerization consist of docetaxel (Taxotere®), paclitaxel (Taxol®), vinblastine, and discodermolide. Docetaxel, a semi-synthetic derivative of paclitaxel and vinblastine aggregates tubulin and cause microtubule depolymerisation, different from the other three compounds that stabilize microtubules (Huang *et al.,* 2017).

2.7.3 Anthracyclines

Doxorubicin is the most potent and extensively used anthracyclinic antibiotic which works by inhibiting the formation of nucleic acids. It has a very thin therapeutic index because it leads to several adverse side-effects like myelosuppression and cardiotoxicity. Therefore, a lot of effort is directed into targeting doxorubicin to cancer tissues, promoting its safety and effectiveness (Minotti *et al.,* 2004).

2.7.4 Paclitaxel

A microtubule-stabilizing agent, Paclitaxel, works by promoting polymerization of tubulin resulting in death of cells by distracting the dynamics essential for division of cells. It is active against several types of cancers, including breast cancer, small and non-small cell lung cancer, melanoma, head and neck cancer, multiple myeloma, ovarian cancer, colon cancer, and Kaposi's sarcoma. In clinical use high rates of severe responses to the drug such as myelo-suppression, neurotoxicity and allergic reactions are indicated. Because its clinical administration is hindered by low solubility in water, excipients such as ethanol and Cremophor EL (polyethoxylated castor

oil) are used in the pharmaceutical drug formulation of the current clinical administration (Singla *et al.,* 2002).

2.7.5 Arsenic trioxide

Arsenic trioxide was said to be a groundbreaking antitumor agent. However, it also displayed toxicity to normal tissue. In an effort to increase its therapeutic effectiveness and lower its toxicity levels, arsenic trioxide-loaded albuminutes immuno-nanospheres targeted with monoclonal antibody (McAb) BDI-1 have been established and its precise cytotoxic effect against bladder cancer cells (BIU-87) studied (Zhou *et al.,* 2005).

2.7.6 Butyric acid

Sodium salts of butyric acid have been used in cancer treatment. Butyric acid is a short-chain fatty acid which is also found naturally occurring in the colon of human. It controls cell growth through precise modulation of the expression of oncogenes for example H-ras, c-fos and c-myc, and several genes involved in the activation of apoptosis like bcl-2 and p53. The clinical use of the sodium salt of butyric acid is narrow because of its short half-life of about 5 min (Serpe *et al.,* 2004). Currently butyric acid is considered as therapeutic agent in the treatment of colorectal cancer and hemoglobinopathies.

2.8 Past and current research on natural products with potential use in cancer treatment

Natural health products (NHPs) and natural products (NPs) play a crucial part in the innovation and development of drugs for human diseases treatment (Newman *et al.,* 2012). In the Native American, Chinese and Indian cultures, traditional medicines have made use of several products from natural sources, including dozens extracts from plant and spices (Ganesan, 2008). Scientific studies conducted to determine the legality of these natural products has confirmed they possess potential anticancer effects (Ganesan, 2008).

An extract obtained from *Podophyllum peltatum,* commonly known as the Mayapple, was customarily applied by Native Americans to fight cancers of the skin, other cancers and multitude of illnesses. Podophyllotoxin, the main constituent of this extract was leading in a chain of bioactive anticancer agents called podophyllins (Mann, 2002). The active component in

turmeric, curcumin, has been extensively investigated for its anticancer properties. *Curcuma longa* (turmeric), was broadly used in Ayurvedic medicine and the chemo-therapeutic values, associated to the presence of bioactive compound curcumin, involve the ability to inhibit growth of tumors in various cancer types (Surh *et al.*, 2007).

Currently studies are mainly directed to the innovation of novel and additional active chemo-therapeutic agents that possess slight to no related toxicity. Recently, emphasis has been placed on NHPs and herbal inventions, mostly in plant forms and other biological sources worldwide. Since time immemorial, NHPs are used by various people of diverse cultures for treatment of many ailments; some of which incessantly offer novel medicinal uses and fascinating anecdotal proof, which calls for more studies. In the present day, there are several natural health products catergorized as traditional medicine, for instance, the Indian herbs Ashwagandha (*Withania somnifera*), Neem (*Azadirachta indica*), and Tulsi (*Ocimum sanctum*). Such kinds of herbal plants have displayed an implausible variety of treatments for illnesses both in ancient and present times (Pattanayak *et al.,* 2010).

Tulsi, similarly known as "Holy Basil," health benefits have been studied in the past years, which consist of but not limited to treatments for malaria, diabetes, pain, asthma, arthritis, cancer, bronchitis, and many microbial infections (Prakash and Gupta, 2005). A study on Tulsi, stated that, eugenol phenolic compound is primarily responsible for the health benefits of Tulsi (Prakash and Gupta, 2005). However, other research done suggests that additional compounds present in Tulsi are also responsible for the health benefits displayed. The phyto-chemicals apigenin, rosmarinic acid, luteolin, myretenal, carnosic acid and β-sitosterol; play a role in the decreasing chemically induced cancers by maintaining anti-oxidative, inducing apoptosis and antiangiogenic effects (Baliga *et al.,* 2013).

Neem leaves are reported to have an extremely similar kind of pharmacological effects to Tulsi and is termed a "living pharmacy" in one study (Atawodi *et al.,* 2009). Documented benefits of neem include, decrease in; inflammation, development of diabetes, oxidative stress, microbial infection, proliferation of cancer cells and growth of tumors. These chemo-preventive benefits are attributed to the present bioactive components within neem such as Azadirone, Nimbolide,

Nimbidin and the Polysaccharides GIa and GIb (Atawodi *et al.*, 2009). A study conducted on *Withania* extract, in 2013 showed its effectiveness against metastatic cancer of breast. The ethanol extract was effective in inhibiting the spread of breast cancer cells in a spheroid invasion assay, while preventing the spread of breast tumors to the lymph nodes and lungs in animal models. This medicinal plant was reported to enhance "general health of patients," when applied together with chemotherapy, as well as enhance the cytotoxicity of chemotherapy in breast cancer patients promoting the quality of life of breast cancer patients (Biswal *et al.,* 2012).

Another example that has been applied for years is the extracts of dandelion (*Taraxacum officinale*), commonly recognized for its therapeutic properties. In many traditional and modern herbal medicines, the dandelion species has been used and reported globally. Different parts of dandelion are applied in treatment of various diseases; the root is used for gastro-intestinal illnesses and the leaves as a digestive stimulant and diuretic. All parts of dandelion are used in hepatitis and anorexia treatment too; though certain reports about to this plant have not been confirmed (Yarnell *et al,* 2010; Schütz *et al.,* 2006). Research indicates that dandelion root possess an extensive range of properties including the anti-inflammatory, prebiotic, antiangiogenic, and antineoplastic properties. However, some contradicting information has been published on this dandelion. Studies also show that dandelion root extracts' possess a dose and time dependent selective efficacy against numerous types of cancer. Research on the mechanism of action of the root extracts of dandelion in cancer cells are ongoing, and emphasis are placed on the determination of the most probable apoptotic pathway that make them selective to cancer cells. Some reports state that dandelion root extract targets the apoptosis death-receptor mediated extrinsic pathway and its mode of action is reliant on the caspase-8 activation (Chatterjee *et al.*, 2011; Moo-Puc *et al.,* 2013; Ovadje *et al.,* 2013).

2.9 Tea as a natural source of phenolic compounds

Tea is the most universally consumed drink as green, black, or Oolong tea (Mukhtar, 2000) and has for long been appreciated for its valuable effects on human health (Weisberger, 2000). These types of tea differ based on the processing of the harvested leaves. Black tea is fermented, green and purple tea are non-fermented and oolong tea is semi-fermented. Among all of the different tea varieties, much attention has been given to green tea because of its most significant

anticancer and anti-inflammatory properties. It has specific polyphenolic compounds, (−)-epigallocatechin (EGC), (−)-epigallocatechin-3-gallate (EGCG), (−)-epicatechin (EC) and (−)-epicatechin-3-gallate (ECG), (Khan, 2007).

Table 1: Phytochemical compounds present in aqueous and ethanol extracts of green *C. sinensis*

Chemical group test	Aqueous Extract	Ethanol Extract
Phenolic	+	+
Alkaloids	+	+
Flavonoids	+	+
Steroids	+	-
Saponins	+	-
Tannins	+	+
Glycosides	-	-
Terpenoids	+	-

(+) indicates presence of phytochemical compound; (-) indicates absence of phytochemical compound (Kangogo *et al.,* 2014).

2.9.1 Taxonomic Hierarchy (Integrated Taxonomic Information System, n.d.) of *Camellia sinensis* (tea) (Kangogo *et al.,* 2014)

Kingdom	Plantae – plantes, Planta, Vegetal, plants
Sub-kingdom	Viridiplantae
Infra-kingdom	Streptophyta – land plants
Super division	Embryophyta
Division	Tracheophyta – vascular plants, tracheophytes
Sub-division	Spermatophytina – spermatophytes, seed plants
Class	Magnoliopsida
Super order	Asteranae
Order	Ericales
Family	Theaceae
Genus	*Camellia*
Species	*Camellia sinensis*

2.9.2 Description of purple tea (*Camellia sinensis*)

Purple tea is a new variety of tea developed and currently cultivated in Kenya. It is a product of 25 years of cloning research by the Tea Research Foundation of Kenya (TRFK) (Yagi *et al.*, 2009). This variety of tea is said to poses more health benefits than green tea. Green tea's health benefits are associated to its composition, majorly polyphenols identified as catechins. Catechins is a collection of nearly 30 different kinds of phenolic compounds mostly including epicatechin-3-gallate (ECG), epigallocatechin-3-gallate (EGCG), epicatechin (EC) and epigallocatechin (EGC). Compared to green and black tea, purple tea has moderately lower levels of caffeine. In addition to the common polyphenols present in other teas, purple tea has some unique combination with high levels of special type of polyphenol 1,2-di-O-galloyl-4,6-O-(S)-hexahydroxydiphenoyl-β-D-glucose (GHG), a hydro-lysable tannin and anthocyanidins (malvidin, peralgonodin and cyanidin 3-O-galactoside). Compared to normal tea, purple tea contains high levels of anthocyanins (135-fold) and anthocyanidins (3.5-fold). This pigment is water-soluble and present in numerous plants in different percentage including red grapes and berries such as blueberry. Purple tea is approximated to contain a 1.5% of anthocyanin compared to 0.1% in blueberries. Anthocyanins play a major role in protecting plants against different stresses. The free-radical scavenging rate of purple tea is high, that is, 52% compared to 34% for green tea and 28% for black tea. The high level of anthocyanin is associated to its greater antioxidant activity. Preliminary studies have displayed some health benefits of anthocyanin rich purple tea (Khan *et al.*, 2018).

Figure 1: Fresh hand picked purple tea leaves

Figure 2: Processed purple tea leaves available commercially

2.10 Role of tea in cancer treatment and prevention

2.10.1. Growth inhibition of cancer cells

A lot of research has been done on tea with animal and plant cell experiments but the anticancer activity of green tea and tea catechins has received much attention (Cabrera *et al.*, 2006). In a research done by Isemura *et al.* (1993), EGCG a key catechins component of green tea prevented cancer cells from adhering to the cell layers of endothelial and attaching to fibrolectin (Ogata *et al.*, 1995) and laminin (Suzuki *et al.*, 2001), the two components of the endothelial basement membrane (Kurosawa *et al.*, 1985; Yamaguchi *et al.*, 1985). Using *in vitro* and *in vivo* experimental models, tea infusion from green tea was reported to have anti-metastatic effect on cancer cells (Sazuka *et al.*, 1995). Growth colonies of lung cells of mouse Lewis carcinoma cells reduced in number in an unforeseen system when peroral administration of green tea was done. Other experiments done with basement membrane reconstituted artificially showed that tea infusion prepared from green tea and its component catechins inhibited the growth of cancer cells across the basement membrane. Taniguchi *et al.* (1992) performed an experiment using mouse B16 melanoma cell lines that showed that EGCG also prevented the lung metastasis hence consistent with the above findings.

In cancer metastasis, the destruction of the basement membrane comprising of type IV collagen is important since it enhances the spread of cancer cells from its original site to other body parts. However, green tea catechins are reported to prevent enzyme collagenases or matrix metalloproteinases (MMPs). An example is EGCG, observed to be a strong inhibitor for MMP-9 and MMP-2 derivatives of cancer cells (Sazuka *et al.*, 1997; Maeda-Yamamoto *et al.*, 1999) and MMP-3, stromelysin (Isemura *et al.*, 2000). An experiment performed using affinity chromatography proved that EGCG could bind directly to MMPs to exhibit inhibitory activity (Sazuka *et al.*, 1997) since it gets attached to some proteins such as fibronectin in blood plasma (Sazuka *et al.*, 1996; Sazuka *et al.*, 1998). It was also discovered that EGCG repressed the gene expression of MMPs in some other experiments that were done later (Isemura *et al.*, 1999; Maeda-Yamamoto *et al.*, 2003).

2.10.2 Induction of apoptosis

A key mode of action of certain anti-tumor drugs is initiating apoptosis. Apoptosis is a programmed cell death where abnormal cells receive a signal to die (Skladanowski *et al.*, 1991; Gunji *et al.*, 1993). However, in cancer cells, cells that should die fail to receive a signal leading to relentless cell division and formation of tumors. Epigallocatechin-3-gallate (EGCG), a key catechins of green tea appears to have some anti-tumor mechanisms which include induction of apoptosis by H_2O_2 production (Yang *et al.*, 2000), inhibition of cell cycle progression (Ahmad *et al.*, 2000), suprression of nuclear factor kappa B (NF-κB) (Fujiki *et al.*, 1998; Fujiki *et al.*, 2002), initiation of the mitogen-activated protein kinase cascade (Saeki *et al.*, 2002) and binding to 67 kDa laminin receptor (Tachibana *et al.*, 2011). Hibasami *et al.* (1996) working with human leukemia Molt 4B cells obtained the first results showing that catechins induce apoptosis. Epigallocatechin-3-gallate led to the development of apoptopic bodies and degraded DNA into nucleosomal units in human lymphoma U937 cells (Saeki *et al.*, 1999). Other findings done *in vitro* also showed that EGCG increased apoptosis and reduced the number of aberrant cryptic foci. It also promoted the action of Sulindac drug in an azoxymethane-induced model of colonic carcinogenesis (Gupta *et al.*, 2006; Ohishi *et al.*, 2002). Besides when green tea infusion was administered orally in autochthonous transgenic mouse prostate adenocarcinomas, prostate cancer development was prevented and apoptosis occurred.

In addition, a proposal was made by Hayakawa *et al.* (2001), on the involvement of EGCG in the direct binding to Fas, in order to induce signal transduction for apoptosis. Fas, is a death receptor protein found on the cells' surface and Fas-Fas ligand system operates in the apoptotic cascade. Caspase 8 activities increase, caspase 8 fragmentation and inhibition of DNA ladder development by caspase 8 inhibitor occurred when human monocytic leukemia U937 cells were subjected to EGCG. Hence, suggesting the participation of Fas-mediated cascade in the EGCG-stimulated apoptosis in U937 cells. Further evaluation through affinity chromatography showed the attachment between EGCG and Fas, suggesting that EGCG-attaching Fas on the cell surface activates the Fas-aided apoptosis in U937 cells. Affinity chromatography with EGCG attached on Sepharose 4B confirmed its importance in finding out the EGCG-binding proteins as used to confirm those in serum (Sazuka *et al.*, 1996). The technique was effectively applied in many

studies conducted later to determine proteins that play a part in EGCG-mediated growth prevention and cancer cells apoptosis (Ermakova *et al.*, 2005; Shim *et al.*, 2008).

According to Hayakawa (2001) and Ohata *et al.* (2005), green tea contains high amount of EGCG which initiates apoptosis in cancer cells by a process involving cell cycle arrest. Since previous research shows that purple tea contains higher amounts of EGCG than green tea (Yagi *et al.*, 2009), same mechanism of initiation of apoptosis by EGCG in cancer cell can be applied when purple tea extract is used. In a study conducted by Oguni *et al.* (1989) in Japan, the rate of death from stomach cancer in males of Nakakawane town was about $1/5^{th}$ of the average for Japanese males overall. This was considered a lower death rate and associated with green tea consumption. Later, it was reported that consumption of tea did not correlate to the risk of stomach cancer. Other studies however, indicated that the consumption of green tea among Japanese women lowered the danger of distal gastric cancer (Sasazuki *et al.*, 2004) and stomach cancer (Kang *et al.*, 2010). This observed difference may be due to consumption of different varieties of tea, lifestyle, cancer etiology, and genetic factors. Sun *et al.* (2002), suggested that new epidemiological studies should consider the amount of tea polyphenols in urine, plus epicatechin and epigallocatechin, and their corresponding metabolites so as to obtain additional reliable data on the link between cancer risk and tea consumption.

2.10.3 Role of tea in bacterial infections and treatment

Since ancient times, drinking tea as a beverage has been considered a health-promoting habit and current medical research on tea has been providing scientific basis of this belief. With each and every new study reported in scientific literature, the proof supporting the health benefits of drinking tea becomes stronger (Singhal *et al.*, 2017). Green tea has gained a lot of popularity globally due to scientific findings that show the health potentials of this tea (Singhal *et al.*, 2017; Cabrera *et al.*, 2006). Purple tea has however been gaining popularity and overtaken green tea because it has been reported to have more polyphenols than green tea (Yagi *et al.*, 2009). In a study conducted to investigate the antimicrobial activity of green tea aqueous extract against standard ATCC strains like *Escherichia coli* 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, and clinical isolates of Multidrug Resistant *Pseudomonas aeruginosa* (MDRPA) and Methicillin Resistant *Staphylococcus aureus* (MRSA), the results

indicated that aqueous crude extract of green tea has a noteworthy antibacterial action against both standard ATCC bacterial strains and extremely resistant clinical isolates of MRSA and MDRPA (Dubey *et al.*, 2016). There is also growing proof that catechins such as EGC, EGCG and ECG found in green and in purple tea, have antibacterial activity (Yam *et al.*, 1997).

2.10.4 Safety and efficacy of tea in treatment of cancer and bacterial infection

In a clinical trial done by Bettuzzi *et al.* (2006) to evaluate the efficacy and safety of green tea and its catechins, the results obtained indicated their effectiveness in preventing growth of cancer in numerous experimental models. Thirty patients were given three capsules treatment containing 200mg of catechin daily for one year, only one tumor was identified among the thirty-green tea catechins (GTCs) treated men while in 30 placebo-treated men 9 cancers were found. Moreover, the total prostate-specific antigen greatly remained the same between the two groups, but men subjected to GTCs exhibited figures continually lower with regards to placebo-treated ones. These were the first ever results showing the safety and activity of green tea catechins in treating premalignant lesions leading to the development of prostate cancer. A secondary observation was also made indicating that the use of green tea catechins lower urinary tract symptoms, an indication that these catechins may also be useful in treating symptoms of benign prostate hyperplasia (Bettuzzi *et al.*, 2006).

A randomized placebo-controlled trial using Polyphenon E® (PolyE®) containing 200mg of EGCG, a proprietary blend of decaffeinated GTCs, comprising of 400 mg EGCG daily, was administered with food to 97 men with severe prostatic intraepithelial neoplasia (HGPIN) and/or unusual minor acinar proliferation. The results showed that daily consumption of a standardized, decaffeinated, catechin mixture with 200 mg EGCG BID ingested for one year built-up in the plasma and was endured and did not cause any adverse effects with baseline severe prostate intraepithelial neoplasia (HGPIN) (Kumar *et al.*, 2016). The use of 15% polyphenol E ointment, a defined extract of green tea catechins in treatment of skin genital warts, caused by infection of HPV (human papilloma viruses), was effective and safe (Tzellos *et al.*, 2011). Thus, the majority of the studies done to determine the safety and efficacy of tea and its catechins show that they are safe, and can be used in management and treatment of several infections and diseases.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Description of the study and source of purple tea

This study was carried out at the Kenya Medical Research Institute (KEMRI) and at the Cell and Tissue Culture Laboratory and Antimicrobial Resistance Laboratory, University of Manitoba, Canada. The purple tea samples were obtained from Tumoi Tea farm which is owned and managed by small-scale Kenyan tea farmers in Nandi Hills, Kenya. The farm lies in an altitude of 2065 metres above sea level, latitude of 0.10307^0 S, 35.17637^0 E. This region has an ideal climate for growing tea which includes well spread rainfall ranging between 1200 mm and 1400mm per annum, long sunny days and tropical, volcanic red soils (Kigen *et al.*, 2016).

3.2 Plant collection and extraction of the purple tea bioactive compounds

3.2.1 Sample collection and processing

Purple tea (*Camellia sinensis,* TRFK, 306/1 clone), leaves grown in Tumoi Tea farm in Nandi Hills, Nandi County, Kenya were collected in April 2017. Young, tender shoot tea leaves, comprised of two unfolded younger leaves with a bud were harvested randomly from the farm in the morning (8-9am), at noon (12-1pm) and evening (5-6pm), and combined into a composite by mixing all the tea leaves. Young tender leaves are plucked depending on the quality desired; if older leaves are harvested they tend to express more substantial bitterness and astringency which is not preferred. The tea leaves were sampled at the three time regimes because it is believed that the amount of polyphenols in the tea leaves vary throughout the day (Turkmen *et al.*, 2009). The 20 kg tea leaves samples were processed at a privately operated factory known as Tumoi Tea Factory in Nandi Hills, Nandi County, Kenya. The processing entailed slight withering, pan-firing (to inactivate the polyphenol oxidase enzyme), tight rolling, drying and packaging. Processed leaves were weighed and stored in air tight plastic bags at room temperature (23 ± 2°C) until further analysis/extraction (Ogutu *et al.*, 2012).

3.2.2 Preparation of purple tea crude extracts

3.2.2.1 Aqueous extraction

Aqueous extraction of purple tea crude extracts involved the procedures described by Ogutu *et al.* (2012). Briefly, 100g of the powdered tea was weighed and transferred into a 1000ml conical flask. Then, 500ml of double distilled water was added and heated up to 80 $^{\circ}$C while stirring

using a magnetic stirrer for 1hr 30min. The suspension was left to cool at room temperature, and then filtered first using gauze sponges then twice using Whatman^{TM} no. 1 filter paper with 10µm pore size. Forty millilitres of the filtrate was measured into 50ml test tubes, balanced using a weighing balance and then centrifuged for 10min at 2000rpm in a small bench centrifuge (HERMILE, Labortechnik, Z 32 HK, Wehingen, Germany). The filtrated was filtered again using Whatman^{TM} no.1 filter paper with 10µm pore size and stored in -80 $^{\circ}$C freezer to freeze the filtrate before drying. The frozen filtrate was dried using a lyophilizer (Model Alpha 1-4, Martin Christ, Germany) weighed and stored at -20°C in a refrigerator until use (Ogutu *et al.,* 2012).

3.2.2.2 Organic extraction

Briefly, 100g of the dried fine powdered tea was weighed using an electrical top balance and put in a conical flask. One litre of the respective organic acid (100%); ethanol, methanol and ethyl acetate, were added to cover the plant material completely and left to stand for 72 h then filtered first using gauze sponges then twice using Whatman^{TM} No. 1 filter paper with 10µm pore size. The filtrate was concentrated using a rotary evaporator (Buchi water bath 8-480, Butch laboratechn IK AG, Switzerland) in a water bath at 50 $^{\circ}$C. The concentrated extract was weighed, labelled and stored at -20 $^{\circ}$C until use (De Sousa *et al.,* 2010).

3.3 Phytochemical screening of purple tea crude extracts

Qualitative phytochemical screening of purple tea extracts was done using the following standard procedures.

3.3.1 Test for phenols

One millilitre of the crude extract was put in a test tube and 1-2 drops of 2% $FeCl_2$ added. A blue, red, green or purple coloration showed the presences of phenols (Harborne, 1998).

3.3.2 Test for steroids: Leibermann Burchard reaction

Two millilitre of the crude extract was transferred into a test tube and 5 ml of chloroform added. The sample was mixed with 2 drops of acetic acid and two drops of concentrated sulphuric acid added gentle along the side of the test tube. Observation of a blue greenish ring showed the presence of steroids (Tariq and Reyaz, 2012).

3.3.3 Test for glycosides

One millilitre of the crude extract was measured into a test tube and 2 ml of chloroform added then mixed, 2 ml of concentrated sulphuric acid was carefully added and hand-shaken gently using. A red brown colour showed the presences of steroidal ring (glycine portion of glycosides) (Sofowara, 1993).

3.3.4 Test for alkaloids

One millilitre of the crude extract was mixed with 1% of Hydrochloric acid in a test tube and heated gently. Few drops of Mayer's or Wagner reagent were added in a test tube. Observation of a precipitate showed the presence of alkaloids (Trease and Evans, 1997).

3.3.5 Test for flavonoids

One millilitre of the crude extract was added in a test tube and 5 ml of dilute ammonia and 2 ml concentrated Sulphuric acid added and shaken. The presence of yellow colour showed the presence of flavonoids (Harborne, 1998).

3.3.6 Test for terpenoids

One millilitre of the crude extract was put into a test tube and 2 ml chloroform added then shaken. The sample was evaporated to dryness and about 2 ml of concentrated sulphuric acid added and heated for about 2 minutes. A greyish colour indicated the presence of terpenoids (Tariq and Reyaz, 2012).

3.3.7 Test for saponins

One millilitre of the extract was put into a test tube and 5 ml of water added then mixed. Observation of stable foam indicated the presence of saponins (Trease and Evans, 1997).

3.3.8 Test for tannins

Two millilitre of distilled water was put into a test tube; 2 ml of the crude extract was added and heated until it boiled. About 1% of $FeCl_3$ was added drop wise and observation made. A brownish coloration indicated the presence of tannins (Harborne, 1998).

3.4 Source, maintenance and preparation of cell lines for anti-proliferative tests

The anti-proliferative effect of aqueous and ethanol extracts of purple *C. sinensis* were tested on the following human cancer cell lines; breast cancer (JIMT1); cervical cancer (HeLa); prostate cancer (PC3); liver cancer (HepG2); and ovarian cancer (A2780 cisplatin sensitive and resistant). The cancer cell lines have their origin in Manassas, Virginia, U.S.A but are regularly maintained at the Cell and Tissue Laboratory, Department of Medical Genetics and Biochemistry, University of Manitoba in Canada. The standard procedure outlined by De Los Reyes *et al.* (2016), was followed to grow the breast, cervical, prostate and liver cancer cells in Dulbecco's Modified Eagle Medium (1X) (DMEM, Gibco®, U.S.A) containing 10% fetal bovine serum (FBS, Gibco®, U.S.A) and 3mL of 1x penicillin/streptomycin antibiotic (Gibco®, U.S.A). The ovarian cells were cultured in Dulbecco's Modified Eagle Medium/F12+GlutaMAX^{TM-1}(1X) (DMEM, Gibco®, U.S.A) containing 5% fetal bovine serum (FBS, Gibco®, U.S.A) and 3mL of 1x penicillin/streptomycin antibiotic (Gibco®, U.S.A) and incubated at 37 $^{\circ}$C, 5% CO_2 and 100% humidity. When the cells achieved 80-100% confluence, visualized using a microscope, the old media was suctioned out, monolayers washed with phosphate-buffered saline (PBS, Ph 7.4, Gibco®, U.S.A), trypsinized with 0.05% Trypsin-EDTA (Gibco®, U.S.A) and re-suspended in fresh complete media. Then 50 µL of the cell suspension was pipetted and added into each of the five accuvettes with 10 mL of Isoton II and counted using a coulter counter. The average number of cells counted was calculated and 100 µL of the cells seeded into the 96-well microtiter plates (Falcon^{TM}, U.S.A) using a final inoculation density of 7.5 x 10^3 cells/well. The test plates were further incubated overnight at 37 $^{\circ}$C, 5% CO_2 and 100% humidity until complete cell attachment was achieved (De Los Reyes *et al.*, 2016). The cells were used for anti-proliferation assays.

3.5 Anti-proliferative assays

To the monolayers on the 96-well microtiter plates, 100 µL of sterile double distilled water and ethanol used to prepare the purple tea extract was added to the first column which acted as the control; for test using aqueous extract double distilled water was added and for ethanol extract, ethanol was added. To the other columns, 100 µL of the plant extract was added at two-fold serial dilutions to make final concentrations of 200, 150, 125,100, 75, 50, 25 and 12.5µg/mL.

The cells were further incubated at 37 °C, 5% CO_2 and 100% humidity for 48-72 hours, when the cell viability assay was performed (De Los Reyes *et al.,* 2016).

3.6 Cell viability assay

The cytotoxicity of both aqueous and ethanol extracts of purple *C. sinensis* was determined using PrestoBlue® (Molecular Probes®, Invitrogen, USA). This assay is based on the presence of mitochondrial reductase enzyme in viable cells which reduces the resazurin dye (blue and non-fluorescent) in the reagent to resorufin (red and highly fluorescent). The degree of conversion is directly proportional to the number of metabolically active cells and inversely proportional to the level of cell inhibition. After 48-72 hours of incubation, the plates were taken out of the incubator and 20 µl of the PrestoBlue added into each well. The amount of PrestoBlue dye added was determined by calculating 10% of the total volume of each well, which in this case is 200 µl (10% of 200 µl = 20 µl). The plates were further incubated at 37 °C, 5% CO_2 and 100% humidity for 2-3 hours. The wells with no plant extract served as the negative controls while wells containing media only were used to correct for background absorbance. An Absorbance Micro-Plate Reader, (SpectraMax M2^{e,} Molecular Device) was used to measure absorbance at 570nm and normalized to 600nm values (reference wavelength). The percentage cell viability for each sample was calculated using the absorbance reading using the equation by De Los Reyes *et al.* (2016).

$$\text{Cell viability (\%)} = \frac{(\text{Absorbance of treated sample} - \text{Absorbance of blank})}{(\text{Absorbance of negative control} - \text{Absorbance of blanks})} \times 100\%$$

3.7 Antibacterial assay

3.7.1 Microbial test strains

Bacterial cultures were purchased from the Leibniz-Institut- DSMZ - German collection of microorganisms and cell cultures (https://www.dsmz.de/). Antimicrobial resistant bacteria strains selected for AST were: *Klebsiella pneumonia* DSM 26371, *Pseudomonas aeruginosa* DSM 102274, *Escherichia coli* DSM 22311, *Shigella sonnei* DSM 25715, *Staphylococcus aureus* DSM 102265, *Acinetobacter baumannii* DSM 105126. Three antimicrobial susceptible reference strains of *Escherichia coli* (DSM 787, DSM 301 and DSM 1103) were also included in the study.

All bacterial strains used in AST were sub-cultured from the cryopreserved dimethylsulfoxide stocks grown in trypticase soy broth (TSB) media for 24 hour at 37 °C.

3.7.2 Minimum inhibitory concentration

A two-fold serial dilution micro-plate method was used to determine the minimum inhibitory concentrations (MIC) of the aqueous purple tea extract against the five clinical strains of bacteria in triplicate. This method was used because of its sensitivity, simplicity, reproducibility, rapidity and low cost (Elisha *et al.,* 2017). Briefly, a two-fold serial dilution of 10 mg/ml stock of tea extract in sterile ddH_2O was added into wells of 96 well microtitre plates (300 µl capacity/ well). An aliquot of 90 µl of tea extract serial dilutions ranging from 0.1 mg/ml to 12.8 mg/ml were tested against all five multidrug resistant bacterial species listed in Table 1 as well as three *E. coli* antimicrobial susceptible reference strains grown in TSB. All bacterial cultures were grown in triplicate at 37 °C with overnight shaking (18 hours) and normalized to an optical density 600 nm (OD_{600nm}) of 1.0 unit in sterile TSB before addition to microtitre plates at 10^{-3} and 10^{-6} culture dilutions in TSB. Plates were incubated in a shaking incubator for 24 hours growth at 37°C and OD600nm values were measured using a ThermoFisher Multiskan spectrum 96 well UV-Vis microplate reader. Minimum inhibitory concentration values were defined as the lowest concentration of tea extract that inhibited growth (based on lowest OD_{600nm}) from the negative control wells containing only tea extract and TSB (Elisha *et al.,* 2017).

3.7.3 Minimum bactericidal concentration

Minimum bactericidal concentration values of the purple tea extract were determined for all antimicrobial susceptibility test experiments performed by TSB agar spot plating cultures. 1 µl of each 24 hours AST 96 well culture was spotted onto TSB agar using an ethanol dipped flame sterilized Boekel 48-pin steel replicator. Spot plating was performed in triplicate for each bacterial strain tested at each tea extract dilution. Agar spot plates were incubated for 24 hours at 37 °C and MBC values were visually defined as the lowest concentration of tea extract that prevented colony growth formation at the spot site (Mah, 2014).

3.8 Study limitations

The purple colour of the aqueous extract prevented broth micro dilution AST results at high concentrations due to the absorbance of the extract in the visible OD_{600nm} region at concentrations of extract above 6.4 mg/ml. As a result MBC values are more accurate.

3.9 Data analysis

Linear regression and statistical analyses were done using SPSS V 23.0. For anti-proliferative assay, a dose-response curve was drawn so as to obtain the IC_{50} values in µg/ml by extrapolation, which is the concentration of each extract required to inhibit the growth of cells by 50%. One-way Analysis of Variance (ANOVA) was conducted to determine significant differences among treatment, followed by Tukey's multiple comparison post hoc tests, to compare different pairs of data sets. For antibacterial assay, the AST OD_{600nm} and spot plate colony formation results were reported as mean values with standard deviations (SD) determined from three replicates. The statistical significance of these values when appropriate were determined using to one-way analysis of variance (ANOVA) calculations and differences between variables were determined by unpaired two-tailed Student's *t*-test assuming unequal variances. The results were considered significant at $p \leq 0.05$.

CHAPTER FOUR: RESULTS

4.1 Phytochemical compounds of purple *Camellia sinensis*

Specific qualitative tests were conducted to screen for the presence various compounds in the aqueous and organic extracts of purple tea *Camellia sinensis* leaves. Table 2 shows a summary of the compounds present in the aqueous and organic extracts of purple *Camellia sinensis* leaves. The aqueous extract had the highest range of phytochemicals, followed by ethanol and methanol; and finally ethyl acetate.

Table 2: Phytochemical compounds of aqueous and organic extracts of purple *C. sinensis*

Chemical group test	Ethanol Extract	Methanol Extract	Ethyl acetate Extract	Aqueous Extract
Phenolic	+	+	-	+
Alkaloids	+	+	-	+
Flavonoids	+	+	-	+
Steroids	+	+	+	+
Saponins	-	-	-	+
Tannins	+	+	-	+
Glycosides	-	-	-	-
Terpenoids	-	-	+	+

(+) indicates presence of phytochemical compound; (-) indicates absence of phytochemical compound

4.2 Anti-proliferative properties of purple tea crude extracts

In vitro anti-proliferation properties of aqueous and ethanol crude extracts of purple *C. sinensis* were determined at different concentrations; 12.5µg/ml, 25µg/ml, 50 µg/ml, 75µg/ml, 100µg/ml, 125µg/ml, 150µg/ml and 200µg/ml to compare their extent of anti-proliferation effects. The extracts showed concentration dependent cytotoxicity against the cancer cell lines. Reduction in cell count was observed with increase in the concentration of the extracts for some cell lines whereas for others the cell count increased at lower concentrations up to certain points where the count started decreasing. The IC_{50} values of the extracts are presented in Table 3. The aqueous extract inhibited 50% of the total cancer cells in the following decreasing order: $A2780_{s,}$ JIMT1, $A2780_{cp,}$ HeLa, PC3 and HepG2. Ethanol extracts also showed inhibitory effects in the decreasing order: $A2780_{s,}$ $A2780_{cp,}$ JIMT1, PC3, HeLa and HepG2. Compared to other cell lines, both aqueous and ethanol extracts exerted the highest cytotoxic activity against $A2780_s$ ovarian cancer cell line with IC_{50} values of 36.84µg/ml and 56.54µg/ml, respectively. Both aqueous and

ethanol extracts also had higher activities against A2780cp ovarian cancer cell which are resistant to standard anticancer drug, cisplastin, and JIMT1 breast cancer cell with IC_{50} values of 75.97µg/ml and 93.52µg/ml, and 72.09µg/ml and 116.73µg/ml, respectively. Aqueous and ethanol extracts showed lowest anti-proliferative activity against HepG2 liver cancer cell lines with IC_{50} values of $1.4*10^4$µg/ml and 463.6µg/ml.

Table 3: IC_{50} values of purple *C. sinensis* aqueous and ethanol extracts on selected cancer cell lines.

Type of cancer	Cell line	Aqueous (µg/ml)	Ethanol (µg/ml)
Ovarian	$A2780_s$	36.84	56.54
	$A2780_{cp}$	75.97	93.52
Cervical	HeLa	265.21	433.70
Breast	JIMT1	72.09	116.73
Prostate	PC3	373.09	184.81
Liver	HepG2	$1.4 * 10^4$	463.6

cp-cisplastin resistant, s- cisplastin sensitive

All cancer cell lines were cultured in presence of aqueous and ethanol purple tea extracts, the percentage of viable cells determined using PrestoBlue® and a dose-response curve drawn so as to obtain the IC_{50} values in µg/ml by extrapolation.

All measurements are means of individual data obtained from the experiment done in triplicate

In figure 1 the ethanol extract enhanced the growth of HeLa, PC3 and HepG2 cancer cells at concentrations of 0-125 µg/ml, 0-100 µg/ml and 0-150 µg/ml, respectively, before showing its inhibitory effects. Aqueous extract completely inhibited $A2780_s$, $A2780_{cp}$ and JIMT1 cancer cell lines at concentrations of 75µg/ml, 200µg/ml and 125µg/ml, respectively. Ethanol extract exhibited complete cell inhibition on $A2780_s$ and $A2780_{cp}$ at concentrations of 100µg/ml and 200µg/ml. Aqueous extract enhanced the growth of HepG2 cancer cells at all concentrations (0-200 µg/ml).

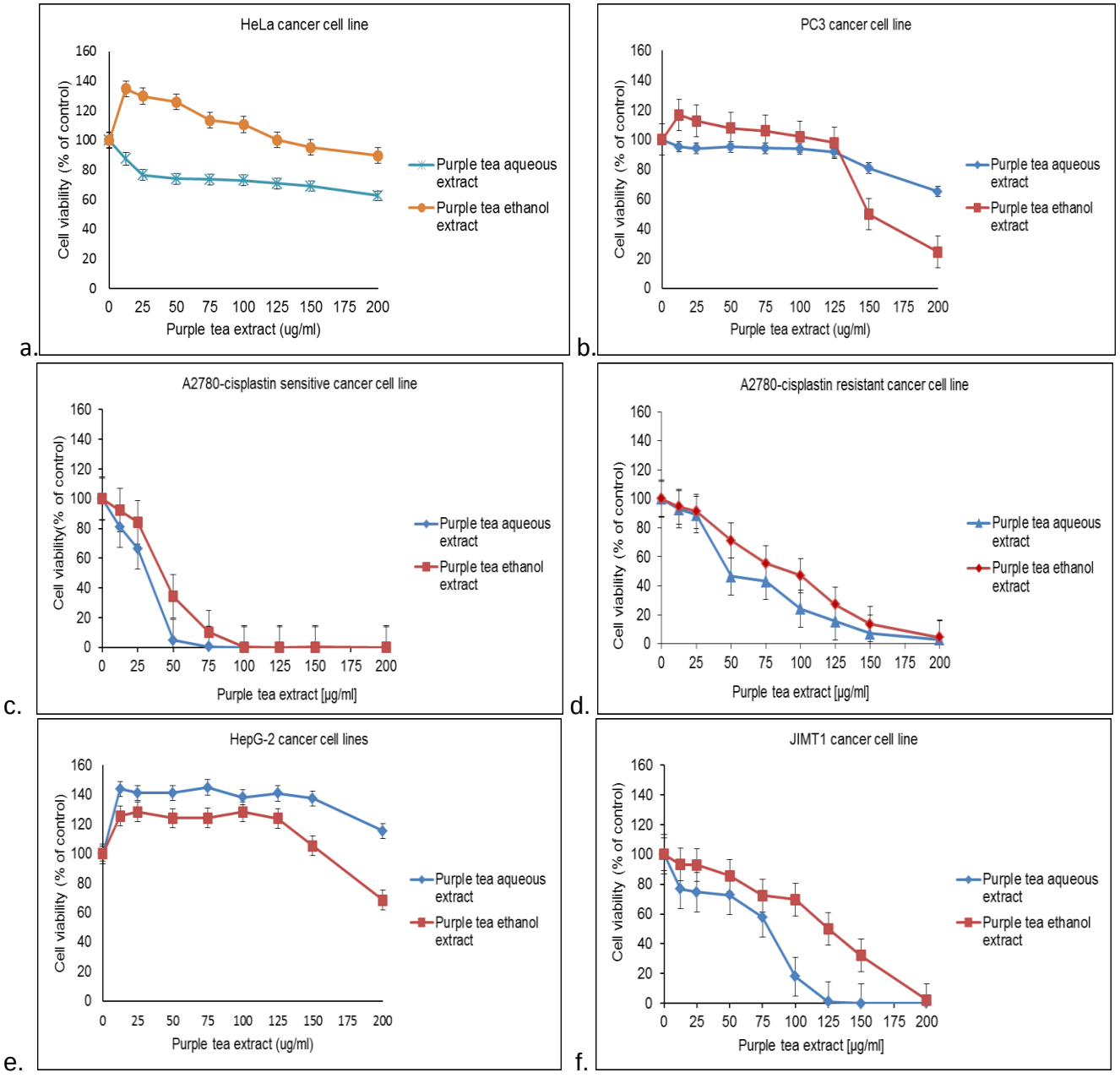


Figure 3: Anti-proliferative effect of purple *C. sinensis* extracts on various cancer cell lines. All cancer cell lines were cultured in presence of aqueous and ethanol purple tea extracts, the percentage of viable cells determined using PrestoBlue® and a dose-response curve drawn.

All measurements are means of individual data obtained from the experiment done in triplicate.Error bars represent standard errors of the means.

4.3 Minimum inhibitory concentration of the purple tea aqueous extract

There was a decline in the number of viable bacteria as the concentration of purple tea aqueous extract increased (Figure 2 and 3). A sharp decline was reported when the test was done at 10^{-6} dilution. The MIC values determined for aqueous purple tea extracts from antimicrobial susceptibility test bacterial cultures normalized to OD600nm 1.0 unit (approximately 1 x10^{9} CFU/ml) and diluted to 10^{-3} and 10^{-6} demonstrated similar tea extract susceptibility values as compared to the reference *E. coli* strains within a 2-fold error range associated with serial dilutions used. Only *S. aureus* demonstrated a significant reduction in MIC value to the lowest concentration of purple tea extract tested 0.0064 mg/ml (Figure 2).

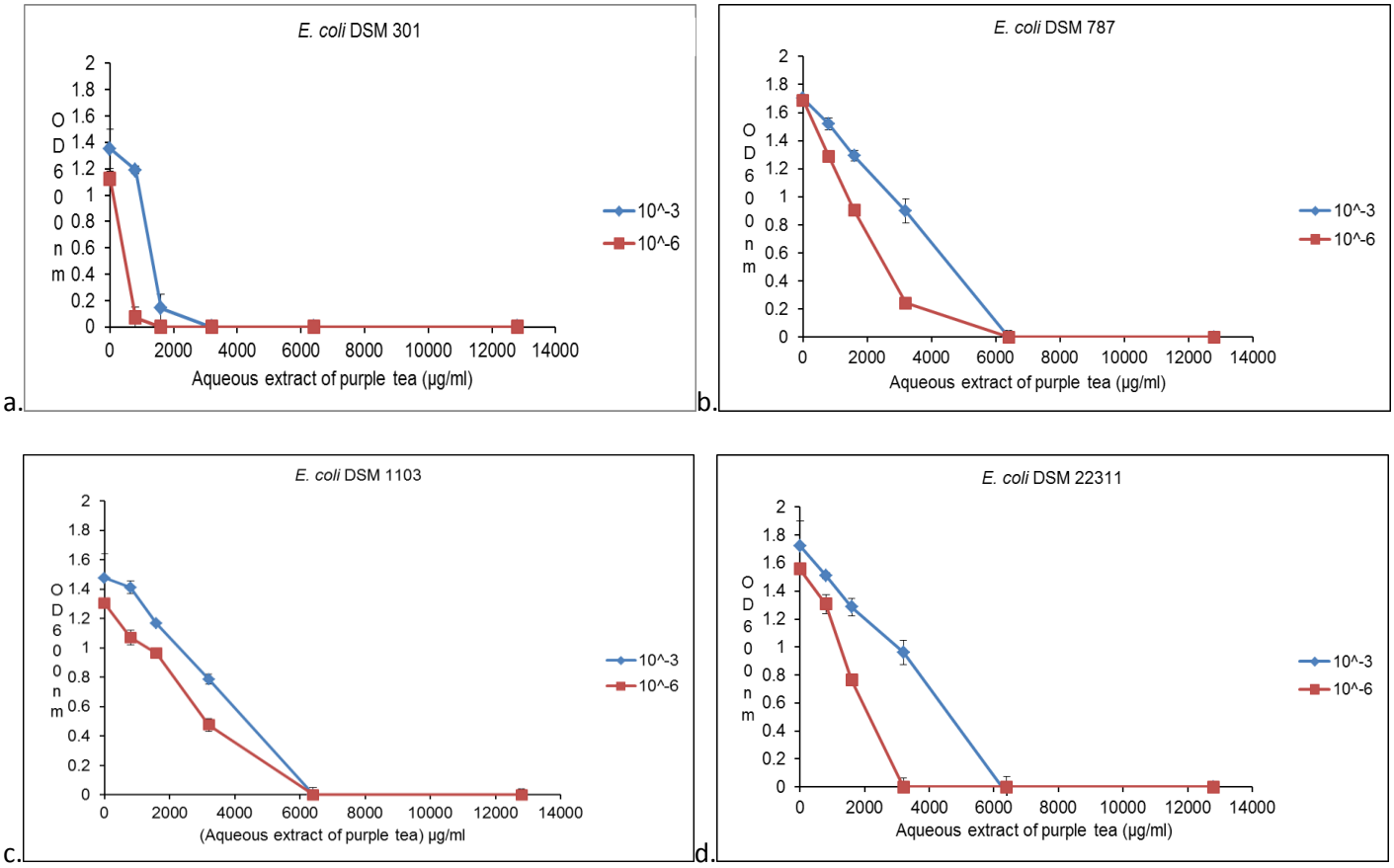
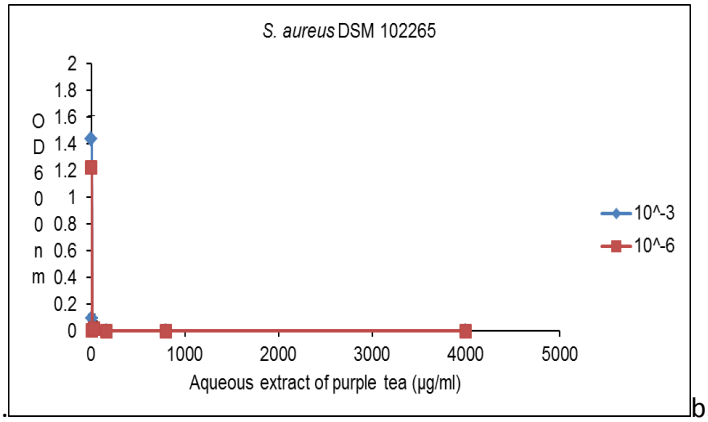


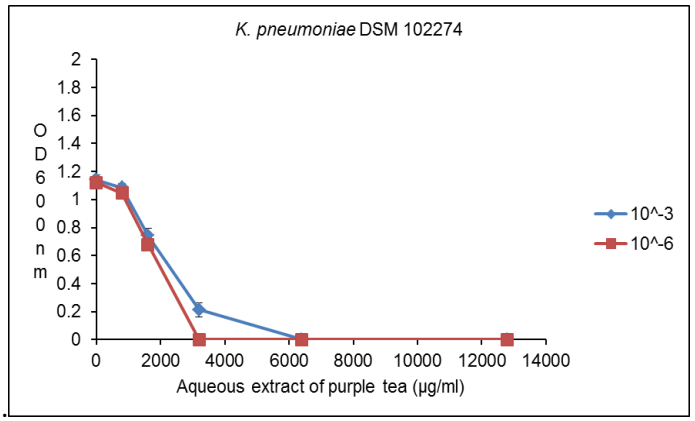
Figure 4: Mean minimum inhibitory concentration values of aqueous purple tea extract against a.) *E. coli* DSM 301, b.) *E. coli* DSM 787, c.) *E. coli* DSM 1103 and d.) *E. coli* DSM 22311 bacteria at 10^{-3} and 10^{-6} dilutions in trypticase soy broth for 24 hour at 37 $^{\circ}$C.

All measurements are means of individual data obtained from the experiment done in triplicate. Error bars represent standard errors of the means.

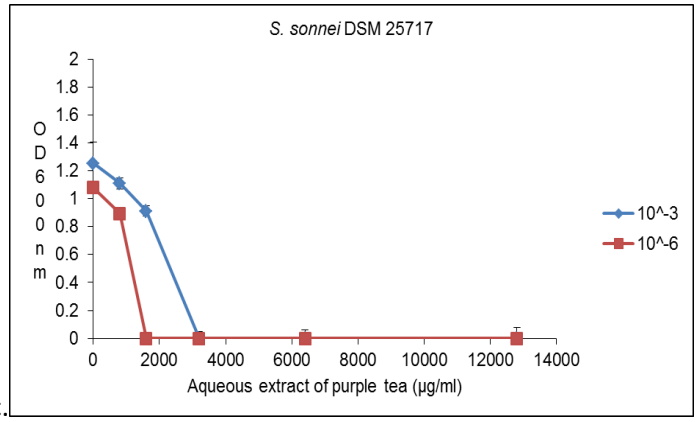
a.



b.



c.



OD-optical density

Figure 5: Mean minimum inhibitory concentration values of aqueous purple tea extract against multi-drug resistant bacteria a.) *S. aureus*, b.) *K. pneumonia* and c.) *S. sonnei* at 10^{-3} and 10^{-6} dilutions in trypticase soy broth for 24 hour at 37 $^{\circ}$C.

All measurements are means of individual data obtained from the experiment done in triplicate. Error bars represent standard errors of the means.

4.4 Minimum bactericidal concentration of the purple tea aqueous extract

Minimum bactericidal concentration values were obtained by visually identifying the lowest concentration of the tea extract that prevented colony growth formation at the spot site (Figure 4). The positive control wells containing bacterial cultures, growth media and sterile double distilled water showed maximum growth whereas the negative control wells containing growth media and sterile double distilled water showed no growth.

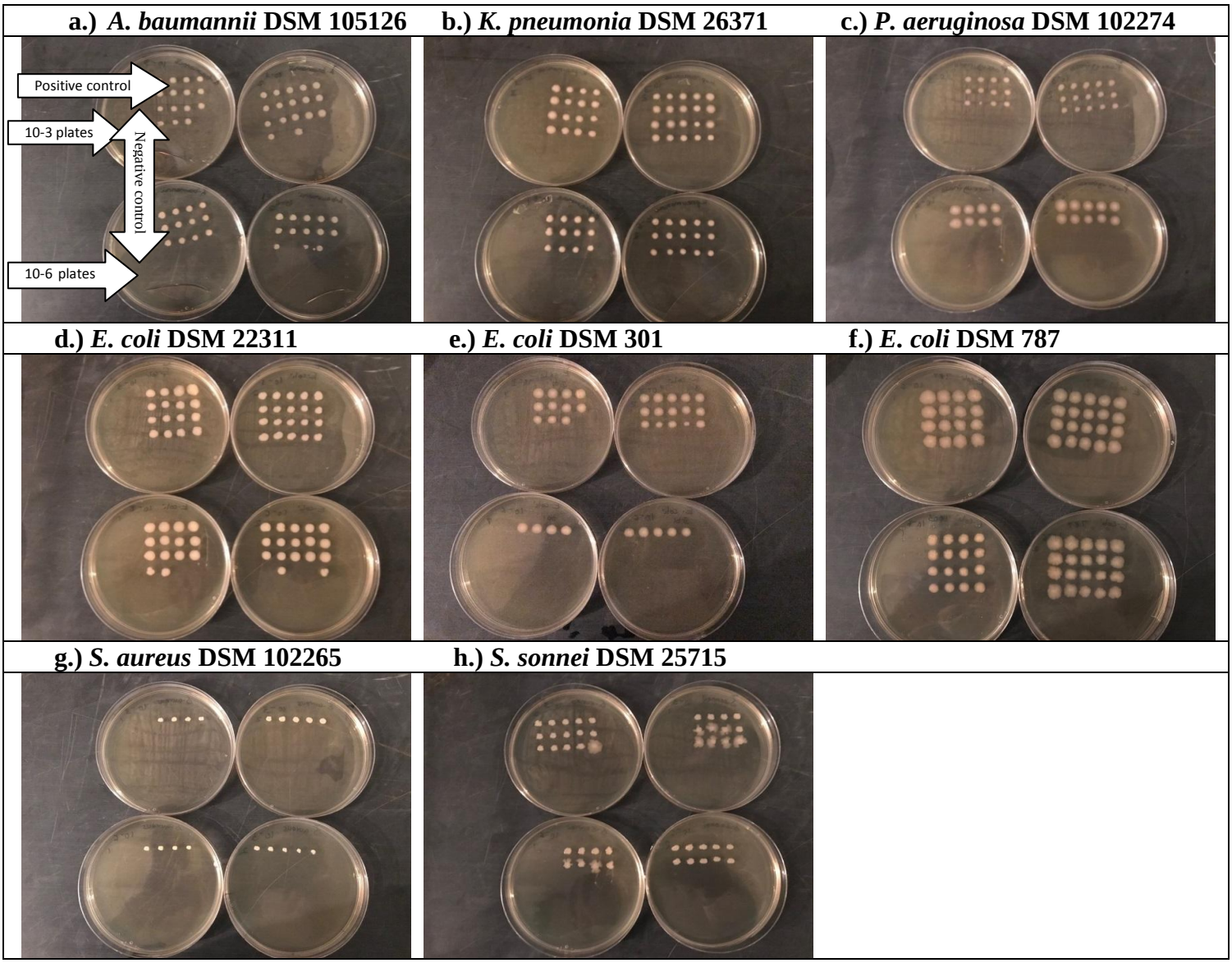


Figure 6: Bactericidal activity of aqueous purple tea extract against multi-drug resistant bacteria: a.) *A.baumannii* DSM 105126, b.) *K. pneumoniae* DSM 26371, c.) *P. aeruginosa* DSM 102274, d.) *E. coli* DSM 22311, e.) *E. coli* DSM 301, f.) *E. coli* DSM 787, g.) *S. aureus* DSM 102265 and h.) *S. sonnei* DSM 25715 at 10^{-3} and 10^{-6} dilutions in trypticase soy broth for 24 hour at 37 ºC.

Minimum bactericidal concentration results determined from MIC culture plates demonstrated that at 10^{-3} dilutions, all of the Gram-negative species examined were with a 2 fold error of the *E. coli* DSM 1103 susceptible reference strains when exposed to aqueous purple tea extract. The only exception was *S. sonnei* and *P. aeruginosa* which had a significant 4 fold reduction in MBC value suggesting that these species were slightly more susceptible to the tea extract (Table 4). As

observed for MIC results, the MBC of *S. aureus* was significantly reduced compared to the Gram-negative species indicating that it is susceptible to aqueous extracted purple tea compounds.

Table 4: Mean minimum bactericidal concentration (mg/ml) values determined from AST cultures exposed to 2 fold serial dilutions of aqueous purple tea extracts

Microbial strain	10^{-3} dilution (Fold change difference from *E. coli* DSM 1103)	10^{-6} dilution (Fold change difference from *E. coli* DSM 1103)
E. coli DSM 1103	12.8	6.4
E. coli DSM 787	6.4	6.4
E. coli DSM 301	6.4	6.4
S. aureus DSM 102265	0.032(400)	0.0064(10^3)
S. sonnei DSM 25715	3.2(4)	1.6(4)
K. pneumoniae DSM 26371	6.4(2)	3.2(2)
P. aeruginosa DSM 102274	3.2(4)	1.6(4)
A. baumannii DSM 105126	6.4(2)	3.2(2)
Contamination control (media and ddH_2O)	No growth	No growth
Positive control (culture, media and ddH_2O)	Maximum growth	Maximum growth

MBC-minimum bactericidal concentration, ddH_2O-double distilled water

Spot plating was performed in triplicate for each bacterial strain tested at each tea extract dilution. Agar spot plates were incubated for 24 hours at 37 $^{\circ}$C and MBC values were visually defined as the lowest concentration of tea extract that prevented colony growth formation at the spot site.

4.5 Correlation between viability of cancer cells and concentration of purple tea extracts

The linear regression analysis was performed to show the influence of purple tea extracts on different cancer cell lines. The independent variable was the concentration of purple tea while the dependent variable was the respective cancer cell line. The model summary shows the R squared, the adjusted R squared and the standard error of estimate. R-squared is a statistical measure of how close the data are to the fitted regression line. It is also known as the coefficient of determination, or the coefficient of multiple determinations for multiple regressions. The adjusted R^2 is the percentage of the variance in the dependent described uniquely or jointly by the independent variable. The findings are presented in the following Table 5. There was a

correlation between the percentage cell viability of breast, ovarian, liver, prostate and cervical cancer cells and the concentration of purple tea extracts as demonstrated in Table 5. From the model in Table 5, concentration of purple tea extracts influenced the viability of the cancer cells. The purple tea extracts had significant effect on the cancer cell lines, which explain the 88.6%, 84.3%, 71.1% and 89.1% of variations in breast, ovarian, prostate and cervical cancer cell lines, respectively. Furthermore, the correlation coefficients for breast, ovarian, prostate and cervical cancer cell lines were 94.9%, 92.9%, 86.4% and 95.1%, respectively, indicating a strong negative correlation between the two variables. However, for liver cancer cells, only 2% of the variations in cell viability were explained by the increase in the concentration of purple tea.

Table 5: Correlation between viability of cancer cells and concentration of purple tea extracts

Cancer cell line	R	Correlation coefficient %	R square	Adjusted R square	CV%	Standard error of the estimate
Breast	0.949[a]	94.9	0.900	0.886	88.6	11.424
Ovarian	0.929[a]	92.9	0.862	0.843	84.3	15.004
Liver	0.356[a]	35.6	0.127	0.002	2	16.510
Prostate	0.864[a]	86.4	0.747	0.711	71.1	11.242
Cervical	0.951[a]	95.1	0.904	0.891	89.1	3.670

R- Correlation coefficients, CV% - coefficient of variation percentages

The association between the percentage of viable cells and increase in the concentration of purple tea extracts was determined by performing a linear regression analysis.

Regression coefficients were calculated to determine whether the value of dependent variable will increase or decrease with increase in the independent variable. A positive coefficient indicates that as the value of independent variable increases, the mean of the dependent variable increases and vice versa. From the regression model presented in Table 6, ovarian cancer cell line had the highest regression coefficient at 0.519, followed by breast 0.474, prostate 0.267 and cervical 0.156. Liver cancer cell line had the lowest coefficient at 0.087. Therefore, a unit increase in purple tea concentration would lead to a decrease in breast, ovarian, liver, prostate and cervical cancer cells by a factor of 0.474, 0.519, 0.087, 0.267 and 0.156, respectively. The

regression coefficients for all the cancer cell lines except liver were statistically significant ($p \leq 0.05$).

Table 6: Relationship between dependent (cancer cell line) and independent variable (purple tea)

Cancer cell Line		Unstandardized Coefficients	Standard error	Standardized coefficients	t-statistic	Significance
		B		Beta		
Breast	(Constant)	81.294	6.201		13.110	0.000
	Purple tea	-0.474	0.060	-0.949	-7.940	0.000
Ovarian	(Constant)	83.800	8.144		10.290	0.000
	Purple tea	-0.519	0.078	-0.929	-6.620	0.000
Liver	(Constant)	131.034	8.961		14.622	.000
	Purple tea	-0.087	0.086	-0.356	-1.008	0.347
Prostate	(Constant)	112.300	6.102		18.405	0.000
	Purple tea	-0.267	0.059	-0.864	-4.543	0.003
Cervical	(Constant)	106.497	1.992		53.470	0.000
	Purple tea	-0.156	0.019	-0.951	-8.133	0.000

$p \leq 0.05$-regression coefficients are statistically significant

Regression coefficients were calculated to determine whether the value of dependent variable will increase (+) or decrease (-) with increase in the independent variable.

CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Phytochemical properties of purple *Camellia sinensis*

Qualitative screening of phytochemicals compounds in the purple *C. sinensis* methanol, ethanol, ethyl acetate and aqueous extracts was performed to determine the presence of various compounds associated with anticancer and antimicrobial properties. The phytochemical screening revealed the presence of phenols, alkaloids, flavonoids, steroids and tannins in all extracts except those of ethyl acetate. Saponins were only present in the aqueous extract whereas terpenoids were present in both ethyl acetate and aqueous extract. Glycosides were absent in all the extracts, while ethyl acetate extracted steroids and terpenoids. Aqueous and ethanol extracts were selected for this study because they contained high presence of bioactive compounds. Earlier studies indicate that successful extraction of bioactive solvents is to a great extent dependent on the type of solvent used for extraction (Tiwari *et al.,* 2011). Different solvents have different polarities and extract specific bioactive compounds in plants. Polar solvents such as water and ethanol yield highest amounts of crude extracts and record highest presence of phytochemicals (Tiwari *et al.,* 2011), as seen from the results obtained in this study.

The presences of phytochemical constituents in plants have led to the use of medicinal plants in treatment and management of diseases (Nostro *et al.,* 2001). Phytochemicals occur naturally and play a major role in the defense and protection of plants from various diseases (Doss *et al.,* 2012; Padmini *et al.,* 2011). Research indicates that some of these phytochemical constituents play a part in the prevention and treatment of certain ailments in humans and animals (Ngbede *et al.,* 2008). Studies show that these bioactive compounds which are also found in *C. sinensis* leaves (Table 1) serve as valuable starting materials for medicine development (Lister *et al.,* 2001). Purple tea is a new cultivar of *C. sinensis* in Kenya, developed by TRFK (Tea Research Foundation of Kenya) for 25 years and is reported to have high levels of phytochemicals than any other variety of tea (Kerio *et al.,* 2012).

Literature shows that bioactive compounds from plants such as phenols, alkaloids, flavonoids, terpenoids, saponins, steroids, tannins and glycosides (Table 1) possess an essential source of pharmacological effects that serve as new anti-infections, antioxidant and anti-cancer agents

(Tariq *et al.*, 2012). Phenols and flavonoids have been reported to have many biological effects such as anti-oxidant, free radical scavenging abilities, anti-inflammatory and anticancer effects (Thamaraiselvi *et al.*, 2012). Studies on alkaloids have exhibited multiple pharmacological properties, including anti-diabetic, anti-protozoal and cytotoxicity (Akindele *et al.*, 2007) and anti-inflammatory effects (Malairajan *et al.*, 2006). Tannins have been reported to have remarkable toxicity against both bacteria and fungi (Banso *et al.*, 2007). On the other hand, steroids are known for their insecticidal, analgenic properties, cardiotonic and central nervous system activities, antimicrobial and anti-inflammatory activities (Argal *et al.*, 2006).

5.1.2 Anti-proliferative properties of purple *Camellia sinensis* on selected cancer cell lines

The results obtained from this study showed that both aqueous and ethanol extracts exhibited a significantly high anti-proliferative activity against $A2780_s$ and $A2780_{cp}$ ovarian cancer cell line with IC_{50} values of 36.84µg/ml and 56.54µg/ml; and 75.97µg/ml and 93.52µg/ml, respectively. These results are of great significance because ovarian cancer is one of the most fatal female malignancies accounting for most deaths compared to other gynaecological cancers (Sak, 2015). The treatment failure is associated with resistance to conventional chemotherapeutic drugs and their toxic side effects (Sak, 2015). Therefore, purple tea's ability to prevent growth of both chemo-sensitive and chemo-resistant ovarian cancer cell lines may be useful in suppressing progression of ovarian cancer and targeting drug resistance which is significant for improving prognosis and increasing overall survival (Sak, 2015).

Significant reduction in the number of viable JIMT1 breast cancer cells was also observed when treated with both aqueous and ethanol purple tea extracts, IC_{50} of 72.09µg/ml and 116.73µg/ml, respectively. Aqueous purple tea extract completely inhibited the growth of breast cancer cells at a concentration of 125µg/ml. An earlier study done on 4TI breast cancer cells also showed the ability of aqueous purple tea infusion to inhibit 4TI breast cancer cells with an IC_{50} of 29.27µg/ml (Mbuthia *et al.*, 2017). The phytochemical groups identified in the purple tea extracts may be responsible for the anti-proliferative properties of these extracts on the breast cancer cell line. The differences in the IC_{50} values could be due to the fact that different solvents extract different amounts of bioactive compounds which have different mechanisms of actions (Reygaer, 2014).

In studies done by Sak (2015), on human ovarian cancer cell lines, the polyphenolic catechin epigallocatechin gallate (EGCG) inhibited the growth of both chemosensitive and chemo-resistant ovarian cancer cell lines in a dose and time-dependent manner. Epigallo-catechin gallate, supressed the growth of ovarian cancer cells by inducing apoptosis, cell cycle arrest at G1 or G1/S phase and gene expression regulation (Sak, 2015). Earlier literature also shows that EGCG is the most effective inhibitor of melanoma, glioblastoma and cancers of the breast, lung, colon, pancrease, prostate, mouth and liver cell lines (Adhami *et al.,* 2003). However, epicatechin-gallate (ECG), which is also a component of purple tea, has been reported as a more potent growth inhibitor than EGCG in some ovarian cancer cell lines in other studies (Ravindranath *et al.,* 2006). Breast cancer being the most commonly diagnosed and fatal gynaecological malignancies among women all over the world (Jemal *et al.,* 2011), purple tea serves as a potent source of new bioactive compounds for development of alternative anticancer drugs to help treat both the chemo-sensitive and chemo-resistant tumors.

In certain cancer cell lines such as PC3 and HepG2, both aqueous and ethanol extracts enhanced the growth of the cancer cells first before inhibiting their growth because various bioactive compounds were present in the crude extract some of which may enhance or hinder the growth of cancer cells (Ncube *et al.,* 2008). Another reason could be, various bioactive compounds have different mechanisms of actions for instance, epicatechins have been reported to promote apoptosis, cell cycle arrest and metastasis by impairing angiogenesis, inhibiting metalloproteinases, and reverse multidrug resistance in human cancer (Ravindranath *et al.,* 2006). *In vitro* anticancer action of the various catechins has been reported to differ with the type and stage of malignancy (Ravindranath *et al.,* 2006). Bioactive compounds also target different growth phases of cancer cells; for example, EGCG causes arrest of cell cycle at G1 or G1/S (Ravindranath *et al.,* 2006). In DU145 prostate cancer cells, EGCG prevented proliferation by arresting the cell cycle at G_0/G1-phase (Adhami *et al.,* 2003). This implies that the presence of various compounds in the crude extract each with a different mode of action and may explain why the growth of the PC3 and HepG2 cancer cell lines in this study were first enhanced then inhibited by the purple tea extracts.

Natural products from bioactive plants or their extracts have played a significant role in the discovery of anticancer agents. Approximately 60% of cytotoxic drugs presently used in cancer chemotherapy are obtained from plant sources (Sak, 2015). Plant derived drugs like vinblastine, vincristine, taxol, and camptothecin have been used successfully as chemotherapeutic drugs (Vijayarathna *et al.*, 2012). Plants have almost limitless capacity to generate numerous bioactive compounds with anticancer properties such as phenols, alkaloids, tannins, steroids, saponins, terpenoids, and flavonoids (Vijayarathna *et al.*, 2012). These compounds are present in a variety of food products and have a high potential for use as drug candidates because of their safety, low toxicity and widespread acceptance amongst the public. These facts fascinate many researchers in their quest for new and novel chemotherapeutics from plants (Reed *et al.*, 2005).

Phytochemical screening of both aqueous and ethanol extracts of purple tea indicated the presences of phenols, alkaloids, tannins, terpenoids, steroids, saponins, and flavonoids, which are reported to possess various biological activities (Kumbhare *et al.*, 2012), such as anticancer activities (Geoffrey *et al.*, 2014) and may have contributed to the anti-proliferative effects observed in the various cancer cell lines. Generally, tea has been widely studied for its wide variety of health benefits, including anti-cancer (Lecumberri *et al.*, 2013), and anti-microbial properties (Bancirova, 2010). Green tea and its catechins have been the major focus (Cabrera *et al.*, 2006) because it has high concentrations of polyphenols. However, recent studies show that purple tea has additional and more polyphenols than green tea (Shimoda *et al.*, 2015). In addition to the common polyphenolic compounds present in green tea, such as the most rich catechin called epigallocatechin-gallate, epicatechin, catechin, epigallocatechin, gallo-catechin, epicatechin-gallate, catechin-gallate and gallocatechin-gallate (Cho *et al.*, 2007; Alappat *et al.*, 2015), purple tea is unique in that it also has anthocyanidins (malvidin, peralgonodin and cyanidin 3-*O*-galactoside) and a hydrolysable tannin (Yagi *et al.*, 2009). According to Sak (2015), these polyphenols have variable effects, depending on their concentrations, the compounds they react with and the cell lines (Sak, 2015). Therefore, isolation of individual bioactive compounds and determination of their anti-proliferation effects on several cancer cell lines is warranted for maximum exploitation of their potential anticancer effects.

5.1.3 Antimicrobial properties of purple *Camellia sinensis*

This study was also conducted to determine the antibacterial properties of purple *C. sinensis* against some bacteria that are known to increase the risk of developing some types of cancers and those that cause infections in cancer patients. Antimicrobial resistant bacteria strains selected for antimicrobial susceptibility testing (AST) were as follows: *Klebsiella pneumonia* DSM 26371, *Pseudomonas aeruginosa* DSM 102274, *Escherichia coli* DSM 22311, *Shigella sonnei* DSM 25715, *Staphylococcus aureus* DSM 102265, *Acinetobacter baumannii* DSM 105126. Three antimicrobial susceptible reference strains of *Escherichia coli* (DSM 787, DSM 301 and DSM 1103) were also included in the current study. The MIC values determined for aqueous purple tea extracts from AST bacterial cultures normalized to OD_{600nm} 1.0 unit (approximately 1 $x10^9$ CFU/ml) and diluted to 10^{-3} and 10^{-6} demonstrated similar tea extract susceptibility values as compared to the reference *E. coli* strains within a 2-fold error range associated with serial dilutions used. Only *S .aureus* demonstrated a significant reduction in MIC value to the lowest concentration of purple tea extract tested 0.0064mg/ml. This is consistent with studies conducted by Chan *et al.* (2011), where all tea extracts including aqueous extracts showed inhibitory effects on Gram-positive but not on Gram-negative bacteria (Chan *et al.,* 2011).

Similar to MIC results, MBC values determined from 24 hr AST demonstrated that at 10^{-3} dilutions all of the Gram-negative species examined were with 2 fold error of the *E. coli* susceptible reference strains when exposed to aqueous purple tea extract. The only exceptions were *S. sonnei* and *P. aeruginosa* which had significant 4 fold reduction in MBC value suggesting that these species were slightly more susceptible to the tea extract. As observed for MIC results, the MBC of *S. aureus* was significantly low as compared to the Gram-negative species indicating that it is susceptible to aqueous extracted purple tea compounds. The results obtained from micro-broth tea extract dilution testing demonstrated that aqueous purple tea extracts inhibited MRSA at MIC values of 0.0064mg/ml. Similar studies carried out on green tea extracts, showed that tea polyphenols inhibited the growth of both MSSA and MRSA with MIC values ranging from 5- 18 mg/ml (Cho *et al.,* 2008) an indication that purple tea prevents the growth of these organisms more than green tea.

The observed difference may be attributed to the presence of the same types of catechins compounds and polyphenols, which have been reported to have antibacterial effects (Saikia *et al.,* 2006) in both types of tea but are present in higher amounts in purple tea (Yagi *et al.,* 2009). Furthermore, the amounts of these compounds have been reported to undergo some seasonal changes during the year. Previously published data on phytochemical screening of purple tea (TRFK, 306) shows that, the principal constituents in 50% w/w aqueous ethanol extracted tea include; caffeine (4.4%), EGCG (9.8%), theobromine (1.6%), ECG (5.8%) and GHG (7.4%). However, these percentages underwent some seasonal changes from the month of January to September. The amounts of caffeine, theobromine and ECG were fairly stable ranging between 2.7 to 3.4%, 1.2 to 2.1% and 3.0 to 3.9% respectively. The content of EGCG reduced from February to April and then rose in June with the highest content recorded in September. 1,2-di-galloyl-4,6- hexahydroxydiphenoyl-D-glucose (GHG) content fluctuated between 6.2 to 8.4% (Yagi *et al.,* 2007). The fact that purple tea was collected in the month of April when the level of EGCG, the most potent growth inhibitor (Ravindranath *et al.,* 2006), was present in low amounts due to cooler weather may have played a significant role in the results obtained.

Tea catechins, specifically EGCG and ECG, have antibacterial activity against both Gram-positive and Gram-negative bacteria (Bancirova, 2010). Therefore, the antibacterial activity of purple tea may be due to the effects of catechins which act by damaging the bacterial cell membrane, inhibiting fatty acid synthesis and enzyme activity. Tea catechins have less effect on gram negative bacterial cell membranes because the outer membrane of gram negative bacteria is negatively charged (Ikigai *et al.,* 1993). Many of the antibacterial effects are due to the catechins binding to the bacterial lipid bilayer cell membrane hence damaging the membrane (Sirk *et al.,* 2009). Bacterial cell membrane damage prevents the bacteria from binding to host cells (Sharma *et al.,* 2012), and to each other to form biofilms, which are important in pathogenesis (Blanco *et al.,* 2005). Bacterial membrane damage also prevents the bacteria from secreting toxins (Shah *et al.,* 2008). Inhibition of fatty acid synthesis by green tea has also been found to prevent bacteria from producing toxic metabolites (Sakanaka and Okada, 2004). In the current study, tea extracts showed better activity against gram-positive bacteria. This is consistent with a study conducted to determine the relationship between bacterial structure and antibacterial activity of polyphenols

which concluded that the antibacterial effectiveness of polyphenols is dependent upon the bacterial species (Taguri *et al.,* 2006).

Phytochemical screening of purple tea *C. sinensis* revealed the presence of bioactive compound such as phenols, alkaloids, flavonoids, terpenoids, saponins, steroids, tannins and glycosides which have both anti-microbial and anti-cancer agents (Tariq *et al.,* 2012). Flavonoids particularly catechins have been associated with the ability of *Camellia sinensis* extracts to exert inhibitory effects on bacterial growth. Catechins have the ability to inhibit the action of efflux pumps such as Tet (K) efflux pump and reverse tetracycline resistance in *Staphylococci* (Roccaro *et al.,* 2004). Literature indicates that EGCG can reverse methicillin resistance of MRSA by inhibiting the synthesis of PBP2 (Yam *et al.,* 1998). β-Lactam resistance in *S. aureus* is linked to the mecA gene which encodes a penicillin binding protein (PBP) called PBP2a. PBPs are transpeptidases that play a role in the synthesis of the peptidoglycan layer of the bacterial cell wall. Research shows that tea catechins inhibit the synthesis of PBP2 in MRSA leading to the reversal of resistance to β-lactam drugs (Yam *et al.,* 1998). Tannins have strong, broad spectrum antibacterial properties (Doss *et al.,* 2009). Tannins interfere with bacterial growth by inhibiting extracellular microbial enzymes, depriving them of the substrates required for microbial growth or by the inhibition of oxidative phosphorylation (Scalbert, 1991).

Earlier studies indicate that catechins and polyphenols present in *C. sinensis* leaves have antibacterial effects on both sensitive and multidrug resistant pathogens such as MRSA (Cho *et al.,* 2008). However, their bacterial effects differ depending on several factors such as the type and amount of polyphenols present in the tea. Recent studies show that purple tea has additional and more amounts of polyphenols especially the catechins than any other tea variety (Yagi *et al.,* 2007). Therefore, the better antibacterial effects of purple tea obtained from this study may be attributed to these compounds. Several other factors such as geographical location of the tea farm, soil composition, genotype, harvesting season, handling method, post-harvest treatment and structure of the leaves have also been reported to influence the concentration of the polyphenol content (Lin *et al.,* 2003).

Bacterial infections especially, blood stream infections (BSI) are among the most common complications in immunosuppressed patients with cancer, and are linked with considerable morbidity and mortality and high economic costs (Wisplinghoff *et al.,* 2003). With the occurrence of antimicrobial resistance in recent years treating such kind of infections has become a major problem globally, and cancer patients are the most affected (Gudiol *et al.,* 2014). All cancer patients are generally at risk for BSI but those with chemotherapy-induced neutropenia in whom the condition is profound and prolonged, and those undergoing hematopoietic stem cell transplantation are at the highest risk (Gudiol *et al.,* 2014). In February 2017, WHO released a list of AMR 'priority pathogens' consisting of twelve bacterial families that are critical threats to human health (WHO, 2017). Carbapenem-resistant *Acinetobacter baumannii, Pseudomonas aeruginosa* and various extended spectrum beta-lactamase (ESßL)- producing Enterobacteriaceae as well as, 3rd generation cephalosporin-resistant species including *Klebsiella pneumonia* and *Escherichia coli* were listed as critical priority pathogens for new antimicrobial drug development. These species are commonly isolated from patients in hospitals, nursing homes, and those who require devices such as ventilators and blood catheters (WHO, 2017). Vancomycin intermediate/ resistant and Methicillin-resistant *Staphylococcus aureus* (MRSA) species as well as fluoroquinolone-resistant *Shigella sonnei* are also included in the WHO AMR pathogen listing as 'high' and 'medium' priority species (WHO, 2017). The results obtained from this study, hence, hold a great significance as purple tea proves to be a potential new source of natural compounds with antibacterial activity against multi-drug resistant pathogens.

5.2 Conclusions

Various phytochemical compounds were extracted from purple *C. sinensis* leaves using aqueous, methanol, ethanol and ethyl acetate solvents. Polar solvents like water, methanol and ethanol showed the highest presence of bioactive compounds.

Aqueous and ethanol extracts of purple *C. sinensis* exhibited anti-proliferative effects against $A2780_s$, $A2780_{cp}$, JIMT1, HeLa and PC3, but enhanced the growth of HepG2 cancer cells. The highest effects were observed in $A2780_s$ and $A2780_{cp}$ cancer cells.

Aqueous extract of purple *C. sinensis* also displayed antibacterial effects against antimicrobial resistant *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Shigella sonnei*, *Staphylococcus aureus, Acinetobacter baumannii.* The highest effect was observed in MRSA.

5.3 Recommendations

i. Extraction of phytochemical compounds present in purple tea should be done using different types of solvents, including polar, mid-polar and non-polar solvents to ensure successful extraction of various bioactive compounds.

ii. Isolation of individual bioactive compounds in purple tea extracts and determination of the most active compounds should be done.

iii. Determination of the mode of action of the bioactive compounds from purple tea extracts should also be conducted.

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APPENDICES

Appendix I: Analyses of variance results for various cancer cell lines

Cancer cell line		Sum of squares	Df	Mean square	F-statistics	Significance
Breast	Regression	8227.890	1	8227.890	63.042	.000[a]
	Residual	913.605	7	130.515		
	Total	9141.494	8			
Ovarian	Regression	9866.106	1	9866.106	43.827	.000[a]
	Residual	1575.813	7	225.116		
	Total	11441.919	8			
Liver	Regression	277.085	1	277.085	1.016	.347[a]
	Residual	1908.138	7	272.591		
	Total	2185.223	8			
Prostate	Regression	2607.880	1	2607.880	20.635	.003[a]
	Residual	884.654	7	126.379		
	Total	3492.534	8			
Cervical	Regression	890.713	1	890.713	66.144	.000[a]
	Residual	94.263	7	13.466		
	Total	984.976	8			

Df – degrees of freedom, $p \leq 0.05$- signifcant, $p \geq 0.05$- not signifcant